# Synthesis and Pharmacological Evaluation of Novel Isoquinoline, Indoloquinoline and Quinoxaline derivatives

A Thesis

Submitted for the Degree of

**DOCTOR OF PHILOSOPHY**

**In Chemistry**

By

**RAJNIKANTH**

Dr. Reddy's Institute of Life Sciences

University of Hyderabad Campus

Gachibowli, Hyderabad 500046, India

**August 2016**

***Dedicated to***

***Jehovah***
***&***
***My Family***

# Table of contents

# STATEMENT

I hereby declare that the matter embodied in the thesis is the result of investigation carried out by me in the Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad, India, under the supervision of **Prof. Manojit Pal**.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigators. Any omission, which might have occurred by oversight or error, is regretted.

**Rajnikanth**

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
August 2016

DR. REDDY'S
INSTITUTE OF LIFE SCIENCES

# CERTIFICATE

This is to certify that the thesis entitled ***"Synthesis and Pharmacological Evaluation of Novel Isoquinoline, Indoloquinoline and Quinoxaline derivatives"*** being submitted by ***Mr. Rajnikanth*** to University of Hyderabad for the award of ***Doctor of Philosophy in Chemistry*** has been carried-out by him under my supervision and the same has not been submitted elsewhere for a degree. I am satisfied that the thesis has reached to the standard of fulfilling the requirements of the regulations relating to the nature of the degree.


Manojit Pal, PhD
(Research Supervisor)
Professor and Head-Organic and Medicinal Chemistry
Dean of Academic Affairs (Chem)
Dr. Reddy's Institute of Life Sciences (DRILS)
University of Hyderabad Campus
Gachibowli, Hyderabad 500 046
Telangana, India
Tel.: +91 40 66571500
Fax: +91 40 66571581
E-mail: manojitpal@rediffmail.com
manojitp@drils.org
http://www.drils.org/
Associate Editor -RSC Advances

# *Acknowledgments*

*Though words are seldom sufficient to express gratitude and feelings, it somehow gives me an opportunity to acknowledge those who helped me during the tenure of my study.*

*I humbly owe my sincere gratitude to my supervisor* ***Prof. Manojit Pal*** *for all his guidance, valuable suggestions and unwavering support during the entire course of my research work. His continuous inspiration and constructive guidance has helped me profoundly during the course of my work. It would have been impossible to accomplish this work, without his able guidance, unfailing attention, constant encouragement and wise counsel. I would like to thank the director Dr. Venkateswarulu, former director Prof. Javed Iqbal and all faculties in the DRILS for the kind support that helped me to reach the endpoint of this intellectual journey. Also I would like to thank all administrative staff and analytical staff for their excellent service.*

*I am very thankful to my doctoral committee members Dr. Rajamohan Reddy poondra and Prof. F.A Khan for their critical evaluation and support. I am very much grateful to CSIR (Council of Scientific and Industrial Research), New Delhi, for the award of a Research Fellowship.*

*I feel fortunate to have an unstinting support from my current and past lab mates. My special thanks to Dr. Raju Adepu, Dr. B. Prasad, Dr. Alinakhi, Dr. Vijaya Babu, Dr. Shiva Kumar, Dr. Rambabu, Dr. Dhilli Rao, Dr. Soumita Mukherjee, Dr. Sunder, E.V.V. Shivaji Ramarao, Suresh babu and Yogi srinivas for their great support, constant encouragement and for creating joyful environment during my research work. I sincerely thank to my juniors who worked with me for their dissertation. My best wishes to Vimal Kumar, Atul Kumar Sharma, Chaitayna, Vijay and Nishant. I would like to thank group members of Lab I & Lab III for their friendly affection.*

*Some friends really make difference in our life. I really feel proud and happy to have friends Imthiyaz, Mutthanna, Kishan, Ramesh Reddy, Rajkumar, Raghu, Nilesh, Rajnikanth Rathod and Dr. Ramakanth and I am very gratefulness to my special friend Shivakrishna Reddy is someone who's just always there for me and he understand me a bit more than myself.*

*I take this opportunity to thank M.Sc. faculty members especially, Dr. Sarbani Pal, Prof. jayasree, Prof. P.S.N. Reddy, Jyothi Madam and Dr. Kavitha for their excellent teaching and laid strong foundation to pursue higher studies. I would like to thank all my school teachers, college and undergraduate faculty specially, Mr. Surya Prakash, Mr.Ashok Kumar, Mr. Narayana, Mr. Mallesham. Thanks to all M.Sc., B.Sc., college classmates and schoolmates especially Ramarao, Prakash, Goush, Jagdish, Nanu, Neelabindhu, Manorama and Krishna.*

*Whatever I am and whatever I intend to be in future is because of the goodwill and unstinted support that I have received from my family. I am very grateful to my family members* ***Deenamma*** *(Mother),* ***Linganna*** *(Father), Jaikumar (Brother), Vijay Sagar (Brother) Sujatha (Sister), Vijaya (Sister) Chandrakanth (Brother) George (Brother-in-law), Vijay (Brother-in-law), Vijjakka (Sister-in-law), Jaya babhi (Sister-in-law), Usha (Sister-in-law) and Sweety, Philomina, Pristina, Denny, Srujan, Pandu, Prarthana, Siddhu, Slicy, Sunny. I also thank Ramakrishna reddy and Harikrishna reddy for their continuous support and encouragement.*

*A special thanks and Love to my dearest Wife* ***Srivani*** *who has given a wonderful support and constant encouragement during my research work and Thesis writing. Her prayers really helped me to achieve my goal. God has blessed us with a baby boy my little champ* ***Rithik*** *(bittu) arrival made our family became more brightful.*

*Rajnikanth*

*Dr. Reddy's Institute of Life Sciences*

*August 2016*

# *Biography*

*Mr.* ***Rajnikanth*** *was born in Indervelly, Adilabad Dist., Telangana, India, on 3rd July, 1987. He received his B.Sc. Degree in Maths, Physics and Chemistry from Nalanda Degree College, Kakatiya University, Warangal, Telangana, India in 2007. In 2009 he received M.Sc. degree in Chemistry with specialization in Organic Chemistry from MNR PG College, Osmania University, Hyderabad, Telangana, India. Then, he qualified in CSIR-UGC NET and awarded a Junior Research Fellowship (JRF) from the CSIR, Government of India in December 2010. He also quailified NET Lectureship conducted by joint CSIR-UGC, Government of India in December 2009. In 2011 he started his doctoral research at Dr. Reddy's Institute of Life Sciences, University of Hyderabad under the guidance of Prof. Manojit Pal. During his doctoral programme at Dr. Reddy's Institute of Life Sciences, he has published number of papers in International Journals and also presented posters at national/international symposiums. His areas of research interest include Synthetic Organic Chemistry, Metal catalysed Cascade/Multi Component Reactions and Medicinal Chemistry.*

# Synopsis

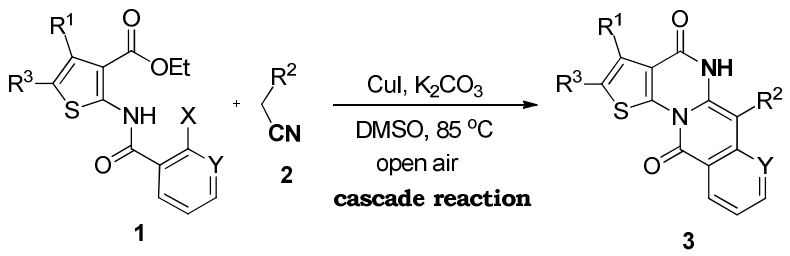
Investigations embodied in this thesis entitled "**Synthesis and Pharmacological Evaluation of Novel Isoquinoline, Indoloquinoline and Quinoxaline derivatives**" comprise five chapters.

## Chapter 1

### Copper catalyzed one pot synthesis of thienopyrimido[1,2-*b*]isoquinolines

(*Chem. Commun.* **2013**, *49*, 190-192)

Over the past years, transition metal catalyzed cascade / domino reactions to construct complex organic molecules is one of the most useful and powerful tools in organic synthesis. Among them, the copper-catalyzed Ullmann-type cross coupling is an important transformation for the synthesis of various *N*-heterocycles *via* domino processes. In this chapter, we have presented design and synthesis of novel thienopyrimido[1,2-*b*]isoquinolines based small molecules as potential inhibitors of PDE4. The synthesis of these compounds were carried out using a new and versatile copper catalyzed cascade reaction under mild conditions without using any co-catalyst, ligand or additive (Scheme 1). The cascade reaction proceeds *via* copper catalyzed Ullmann type C-C bond formation to give an intermediate *in situ*, which subsequently undergo intramolecular nucleophilic addition of NH to CN followed by intramolecular nucleophilic attack by amine to ester group. This allows the formation of a fused ring leading to thienopyrimido[1,2-*b*]isoquinolines. The molecular structure of a representative compound was confirmed unambiguously by single crystal X-ray diffraction study. Some of the synthesized compounds showed promising inhibition of PDE4B when tested *in vitro* at 30 $\mu$M.



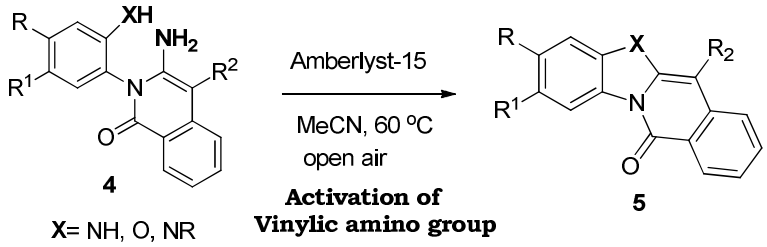

**Scheme 1**: One-pot synthesis of thienopyrimido[1,2-*b*]isoquinoline derivatives.

## Chapter 2

### Amberlyst-15 mediated synthesis of benzimidazo / benzoxazoloisoquinolinones

(*Chem. Commun.* **2013**, *49*, 3570-3572)

In this chapter, we have demonstrated a facile assembly of benzoimidazole or benzoxazole ring with an isoquinolinone moiety *via* a conceptually new and general strategy. The strategy involved Amberlyst-15 mediated activation of vinylic amino group leading to a diverse and unique class of small molecules as potential inhibitors of PDE4. Amberlyst-15 is a heterogeneous catalyst which is inexpensive, commercially available, and non-hazardous in nature. It can be removed easily from the reaction mixture *via* simple filtration. A wide variety of compounds were synthesized using this Amberlyst-15 mediated method in good yields. Several of these compounds showed promising PDE4B inhibition *in vitro* that was supported by *in silico* studies. One of these compounds showed a dose dependent inhibition of PDE4B with an $IC_{50}$ (the half maximal inhibitory concentration) ~ 3 $\mu$M comparable to rolipram's $IC_{50}$ ~ 1 $\mu$M.



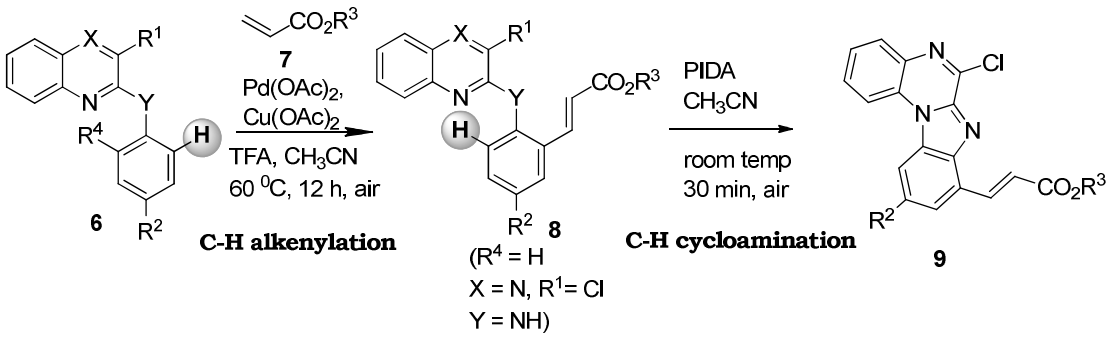

**Scheme 2**: Amberlyst-15 mediated synthesis of compound **5** *via* activation of vinylic amino group.

## Chapter 3

### Synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxalines *via* C-H alkenylation / intramolecular ortho C–H cycloamination

(*RSC Adv*. **2015**, *5*, 70604-70608)

The strategy consisting of directed ortho C-H functionalization followed by converting the directing group into an integral part of the target molecule is of fundamental interest as this may allow easy and quick access to functionalized heteroarenes for their potential applications in organic/medicinal/pharmaceutical chemistry. In this chapter, we have developed a two-step strategy for accessing new chemical entities based on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxaline framework *via* C-H activation methods. The strategy involved use of a quinoxaline moiety as a new directing group for the Pd (or Ru)-catalyzed *ortho* C-H alkenylation of aniline derivatives and subsequent hypervalent iodine(III)-promoted intramolecular *ortho* C–H cycloamination of the resulting *N*-arylquinoxalin-2-amine derivatives. Both the steps were performed under open air and mild conditions. The Pd-catalyzed *ortho* C-H alkenylation of phenol derivatives was also performed successfully when quinoline was found to be an effective directing group. Some of the compounds synthesized were tested for PDE4B inhibitory properties *in vitro* when several of them showed promising inhibition of PDE4B. One of these compounds showed a dose dependent inhibition of PDE4B with an $IC_{50}$ (the half maximal inhibitory concentration) ~ 2.3 $\mu$M comparable to rolipram's $IC_{50}$ ~ 1 $\mu$M.



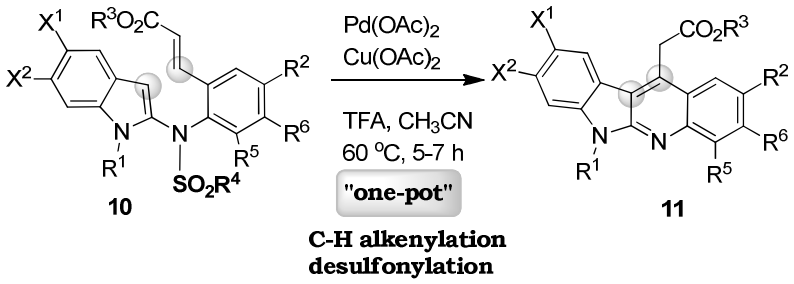

**Scheme 3**: Synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxaline derivatives.

## Chapter 4

## Synthesis of indoloquinoline related to neocryptolepine *via* Pd-catalyzed C–H activation

(*RSC Adv*. **2015**, *5*, 44722-44727)

In recent years, methods involving C-H bond activation and subsequent functionalization have become an attractive area of research in organic synthesis, because it avoids the use of prefunctionalized starting materials, and thereby improving the step-economy of the synthetic route. Especially, transition-metal-catalyzed C-H olefination reactions have been the subject of tremendous research complementing to Mizoroki-Heck reaction because of drawbacks such as, limited availability of expensive aryl halide component, or their cumbersome preparation. Indeed, a remarkable progress has been made in this area where Pd particularly occupied the center stage. However, use of this technology towards the straightforward synthesis of densely functionalized heteroaromatics is not common in the literature and needs further exploration. In this chapter, we have developed a sequential method to synthesize novel indolo[2,3-*b*]quinolines related to neocryptolepine. The strategy involved Pd(II)-catalyzed intramolecular oxidative C3-H alkenylation of an indole ring followed by desulfonylation in the same pot. This straightforward and facile methodology afforded an array of 11-carboxymethyl substituted 6*H*-indolo[2,3-*b*]quinoline derivatives. Several of these compounds showed promising cytotoxicities against cancer cells and apoptosis inducing properties in zebrafish embryos indicating their potential for the treatment of cancer.



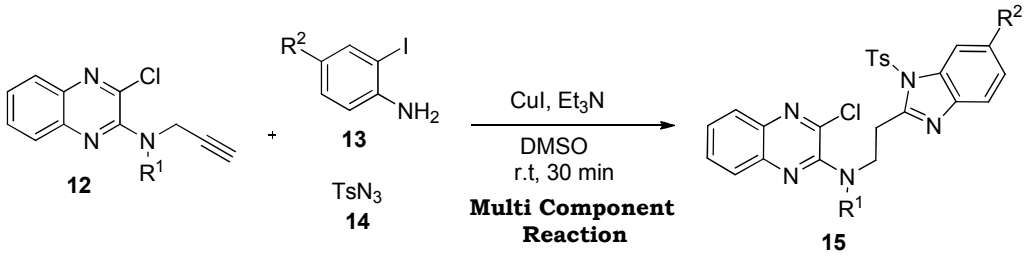

**Scheme 4**: Pd-mediated synthesis of 11-carboxymethyl substituted 6*H*-indolo[2,3-*b*]quinoline derivatives.

## Chapter 5

## Copper-catalyzed one-pot synthesis of hybrid benzo[*d*]imidazoloquinoxalines as potential inducers of apoptosis

(*Org. Biomol. Chem.* **2014**, *12*, 6800-6805)

The hybrid molecules are an agents with two (or more) structural frameworks having different biological functions and dual activity, and can act as two distinct pharmacophores. However, the strategy of hybrid molecule is also used to enhance the pharmacological activities of the individual pharmacophores. In this chapter, we developed an efficient multicomponent reaction involving the reaction of *N*-(prop-2-ynyl)quinoxalin-2-amine derivative with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and $Et_3N$ in DMSO to afford the pre-designed target hybrid molecules containing quinoxaline framework linked with benzimidazole nucleus. The MCR proceeds in the absence of any ligand and / or lateral addition of the catalyst / base affording the product within 30 min in good yields. Some of these compounds showed encouraging apoptosis inducing properties and no significant teratogenicity when tested in zebrafish embryos and therefore seemed to have potential medicinal value.




**Scheme 6:** Synthesis of novel hybrid molecules (**15**) *via* a Cu-catalyzed MCR.

# Abbreviations

| | | |
|---|---|---|
| $^{13}C$ NMR | : | carbon-13 nuclear magnetic resonance spectroscopy |
| $^{1}H$ NMR | : | hydrogen-1 nuclear magnetic resonance spectroscopy |
| $Ac_2O$ | : | acetic anhydride |
| AcOH | : | acetic acid |
| Ar | : | aryl |
| aq | : | aqueous |
| Boc | : | tert-butoxycarbonyl |
| $Br_2$ | : | bromine |
| bs | : | broad singlet |
| $CaCl_2$ | : | calcium chloride |
| cAMP | : | cyclic adenosine mono phosphate |
| CCDC | : | Cambridge Crystallographic Data Centre |
| $CDCl_3$ | : | chloroform-*d* |
| $CF_3CH_2OH$ | : | 2,2,2-Trifluoroethanol |
| cGMP | : | cyclic guanosine mono phosphate |
| $CH_3CN$ | : | acetonitrile |
| $CH_3COCl$ | : | acetyl chloride |
| $CH_3COOH$ | : | acetic acid |
| $CH_3F$ | : | methyl fluoride |
| COPD | : | chronic obstructive pulmonary disease |
| COSY | : | correlation spectroscopy |
| CR | : | component reaction |
| $Cs_2CO_3$ | : | cesium carbonate |
| Cu | : | copper |
| CuBr | : | copper bromide |
| CuCl | : | copper chloride |
| CuI | : | copper iodide |
| $Cu(OAc)_2$ | : | copper acetate |
| $Cu(OTf)_2$ | : | copper triflate |

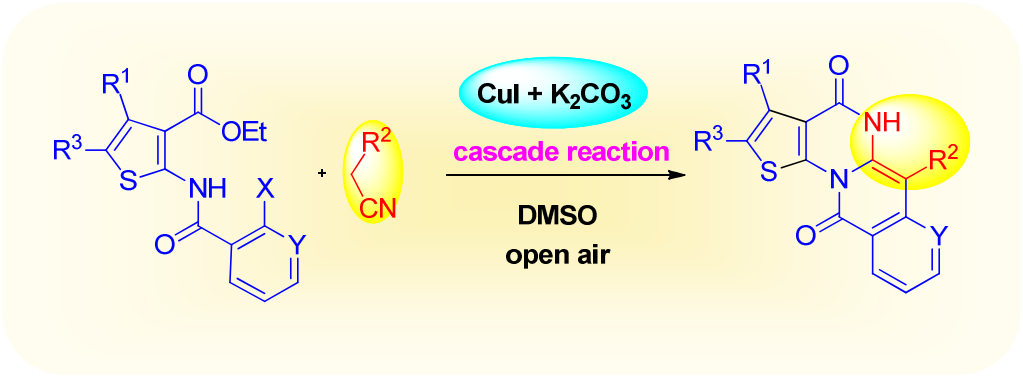
| | | |
|---|---|---|
| d | : | doublet |
| DCM | : | dichloromethane |
| DIPEA | : | *N*, *N*'-diisopropylethylamine |
| DMA | : | *N*,*N*-dimethylacetamide |
| DME | : | dimethoxyethane |
| DMF | : | *N*,*N*-dimethylformamide |
| DMF-DMA or DMFDA | : | *N*,*N*-dimethylformamide dimethyl acetal |
| DMSO | : | dimethyl sulfoxide |
| DMSO-$d_6$ | : | dimethyl sulfoxide-$d_6$ |
| DPE-Phos | : | (Oxydi-2,1-phenylene) bis (diphenylphosphine) |
| Et | : | ethyl |
| $Et_3N$ | : | triethylamine |
| EtOAc | : | ethyl acetate |
| EtOH | : | ethanol |
| Gln | : | glycine |
| h | : | hour(s) |
| HCl | : | hydrochloric acid |
| $H_2O$ | : | water |
| HPLC | : | High performance liquid chromatography |
| $H_2SO_4$ | : | sulfuric acid |
| Hz | : | hertz |
| $I_2$ | : | iodine |
| IBX | : | 2-Iodoxybenzoic acid |
| $IC_{50}$ | : | half maximal inhibitory concentration |
| ICl | : | iodine monochloride |
| i-PrOH | : | isopropanol |
| J | : | coupling constant in Hz |
| KBr | : | potassium bromide |
| KCl | : | potassium chloride |
| $K_2CO_3$ | : | potassium carbonate |
| $KNO_3$ | : | potassium nitrate |
| $LiAlH_4$ | : | lithium aluminiumhydride |

| | | |
|---|---|---|
| m | : | multiplet |
| MCR | : | multicomponent reaction |
| Me | : | methyl |
| MeOH | : | methanol |
| Mg | : | magnesium |
| mg | : | milligram |
| mL | : | milliliter |
| mmol | : | mill mole |
| $N_2$ | : | nitrogen |
| NaCl | : | sodium chloride |
| $Na_2CO_3$ | : | sodium carbonate |
| NaH | : | sodium hydride |
| $NaHCO_3$ | : | sodium bicarbonate |
| NaOH | : | sodium hydroxide |
| NaOAc | : | sodium acetate |
| NaOMe | : | sodium methoxide |
| NaOtBu | : | sodium tertiary butoxide |
| $Na_2SO_4$ | : | sodium sulphate |
| NBS | : | *N*-bromo succinamide |
| NCE | : | new chemical entity |
| $NH_4Cl$ | : | ammonium chloride |
| $NH_2NH_2.H_2O$ | : | hydrazine hydrate monohydrate |
| NOE | : | Nuclear Overhauser Effect |
| Pd/C | : | palladium on carbon |
| $PdCl_2(PPh_3)_2$ | : | Bis(triphenylphosphine)palladium(II) dichloride |
| $Pd_2(dba)_3$ | : | Tris(dibenzylideneacetone)dipalladium(0) |
| $Pd(PPh_3)_4$ | : | Tetrakis(triphenylphosphine)palladium(0) |
| PDE | : | phosphodiesterase |
| $Pd(OAc)_2$ | : | palladium acetate |
| Ph | : | phenyl |
| Phe | : | phenyl alanine |
| $POCl_3$ | : | Phosphoryl chloride |

| | | |
|---|---|---|
| $PPh_3$ | : | triphenyl phosphine |
| Rf | : | retention factor |
| RT (or) rt | : | room temperature |
| s | : | singlet |
| $S_8$ | : | sulphur |
| t | : | triplet |
| TBAB | : | tetra butyl ammonium bromide |
| $^t$Bu | : | tertiary butyl |
| THF | : | tetrahydrofuran |
| TNF | : | Tumor necrosis factor |
| Triton-B | : | benzyl trimethyl ammonium hydroxide |
| Tyr | : | tyrosine |
| UV | : | ultra violet |
| X-Phos | : | 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl |
| δ | : | chemical shift in parts per million |

# *CHAPTER 1*

## Copper catalyzed one pot synthesis of thienopyrimido[1,2-*b*]isoquinolines



## 1.1. Introduction:

Isoquinolinone ring system is a ubiquitous and significant motif of many natural products such as narciclasine,[1] thalifoline,[2] dorianine,[3] ruprechstyril,[4] lycoricidine,[1b] and pancratistatinin[1a, 5] (Figure 1.1), and pharmaceutical candidates that display a wide range of biological activities.[6] Besides, many isoquinoline analogues have also been used as chiral ligands,[7] phosphorescent materials,[8] and fluorosensors.[9]

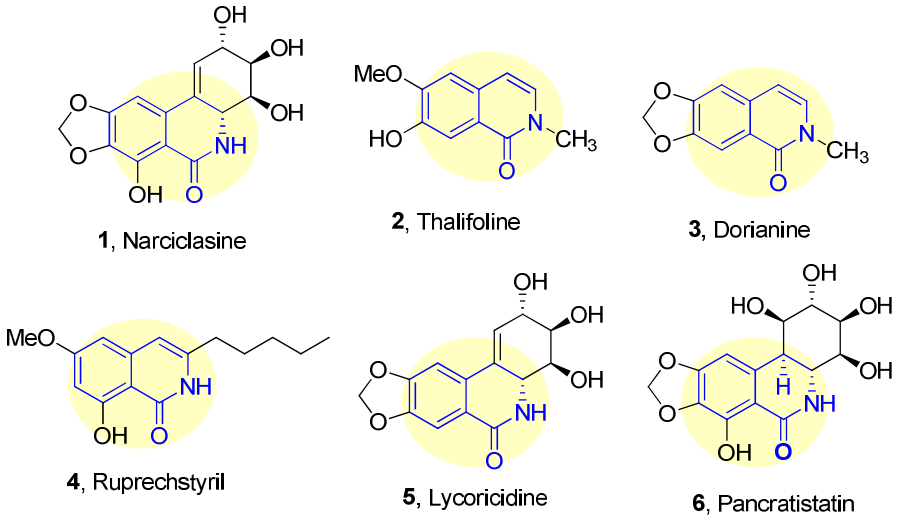



**Fig. 1.1**: Biologically active natural products containing isoquinolinone scaffold

On the other hand, thienopyrimidines and its derivatives have attracted considerable attention because of their wide range of biological activities such as antimicrobial,[10]

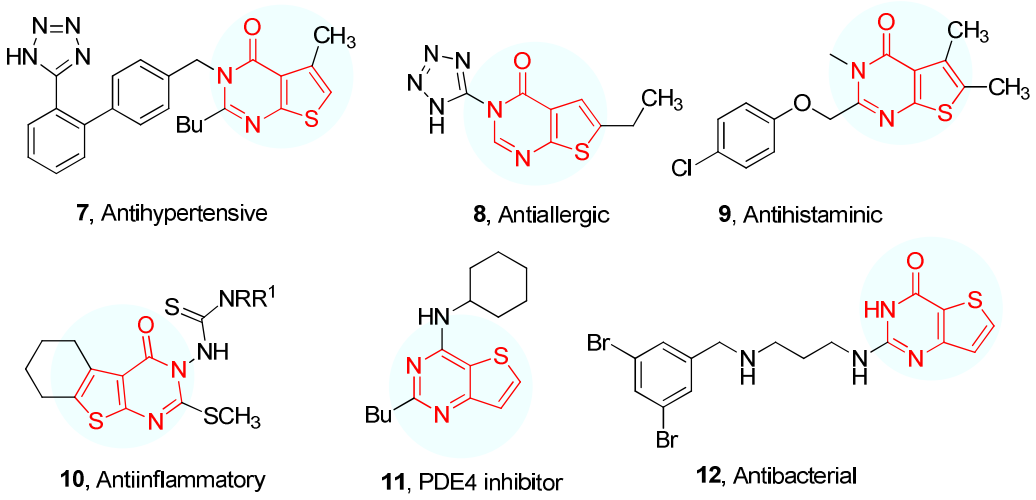



**Fig. 1.2**: Examples for biologically active thienopyrimidine derivatives

analgesic,[11] anticancer,[12] anti-inflammatory,[13] antipyretics,[14] and anti-allergenic effects.[15] Also, some thieno[2,3-*d*]pyrimidines have been identified as potent inhibitors of TGase2 (tissue transglutaminase),[16] selective and potent α1D antagonists,[17] and potent dual 5-HT1A and 5-HT1B antagonists.[18]

Due to their interesting pharmacological properties, compounds containing the thienopyrimidines and isoquinolinone framework have attracted considerable attention in medicinal chemistry, and much effort has been focused on their synthetic methods to construct isoquinoline-fused thienopyrimidines ring systems. However, synthesis of compounds containing the hybrid structure of isoquinoline and thienopyrimidines motifs, namely thienopyrimido[1,2-*b*]isoquinolines derivatives (Figure 1.3), are uncommon in the literature. Consequently, the development of a convenient and efficient synthetic approach towards thienopyrimido[1,2-*b*]isoquinolines derivatives will be valuable for their screening against various biological targets.

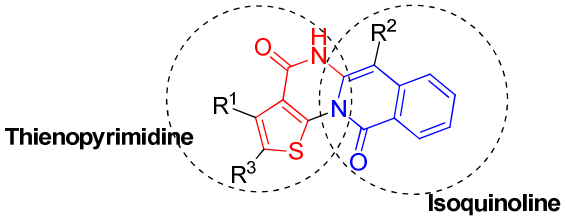


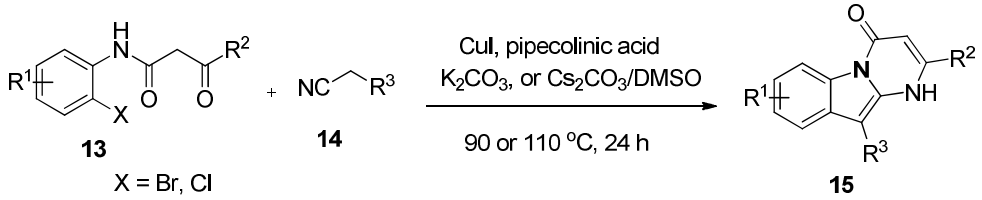

**Fig. 1.3**: Thienopyrimido[1,2-*b*]isoquinolines as a hybrid structure of thienopyrimidine and isoquinolines motifs.

Transition metal catalyzed cascade/domino reactions to construct complex organic molecules is one of the most useful and powerful tools in organic synthesis. Among them, a copper-catalyzed Ullmann-type cross coupling reaction is an important transformation for the synthesis of various *N*-heterocycles *via* domino processes.[19] Recently, great advances have been made on the conceptual evolution of copper catalyzed Ullmann-type reactions by using activated methylene groups to construct polycyclic heteroaromatic systems. The earlier reports on copper catalyzed domino process for synthesis various polycyclic heteroaromatics are discussed below.

## 1.2. Previous work:

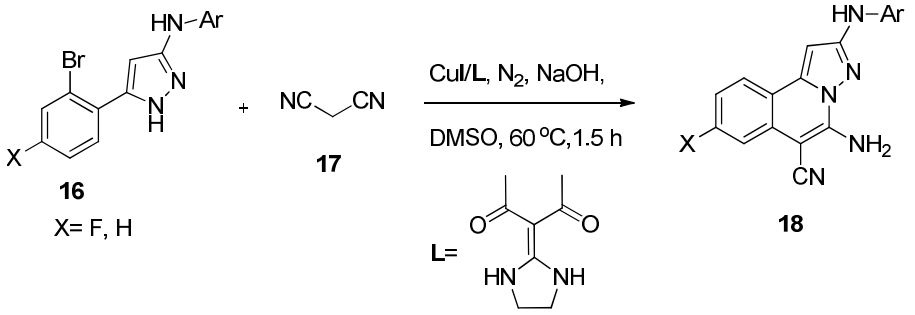
### 1.2.1. Recent examples for synthesis of polycyclic heteroaromatics *via* copper catalyzed domino process:

In 2013, Fu and coworkers developed an efficient copper-catalyzed domino method for the synthesis of 4-oxopyrimido[1,2-*a*]indole derivatives (**15**) *via* the reaction of substituted *N*-(2-halo-phenyl)-3-oxoalkanamides (**13**) with alkyl 2-cyanoacetates (**14**) or malononitrile under mild conditions as shown in Scheme 1.1.[20]




**Scheme 1.1**: Synthesis of 4-oxopyrimido[1,2-*a*]indole derivatives from *N*-(2-halo-phenyl)-3-oxoalkanamides.

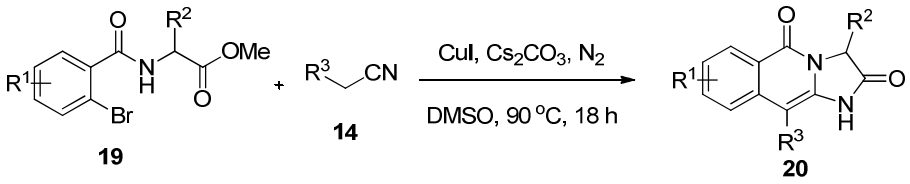
In 2015, Li and coworkers disclosed a novel copper-catalyzed tandem reaction for the synthesis of pyrazolo[5,1-*a*]isoquinolines derivatives (**18**) *via* the reaction of 5-(2-bromoaryl)-*N*-aryl-1*H*-pyrazol-3-amines (**16**) with malononitrile (**17**) as shown in Scheme 1.2. In this method copper(I)iodide acts as a catalyst and first time heterocyclic ketene aminals (HKAs) have been used as ligand.[21]




**Scheme 1.2**: Synthesis of pyrazolo[5,1-*a*]isoquinolines derivatives from 5-(2-bromoaryl)-*N*-aryl-1*H*-pyrazol-3-amines.

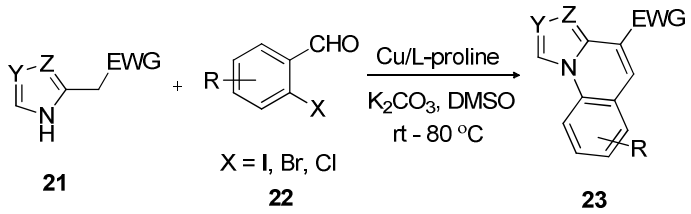
In 2011, Fu and coworkers developed an efficient copper-catalyzed domino method for the synthesis of poly-*N*-heterocycles containing amino acid residues (**20**) *via* the

reaction of substituted 2-halobenzamides (**19**) with alkyl 2-cyanoacetates (**14**) or malononitrile (**17**) without using any ligand or additive as shown in Scheme 1.3.[22]



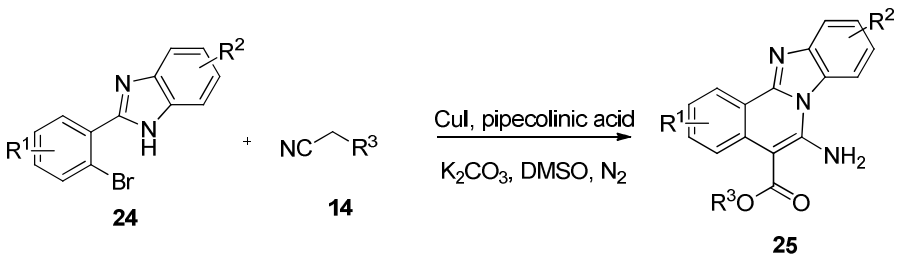

**Scheme 1.3**: Synthesis of poly-*N*-heterocycles from substituted 2-halobenzamides.

In 2010, Ding and coworkers reported the synthesis of aza-fused polycyclic quinolines (**23**) *via* copper-catalyzed cascade reaction of 2-(1*H*-benzo[*d*]imidazole-2-yl)acetonitrile (**21**) with 2-halo benzenealdehyde (**22**) under mild conditions as shown in Scheme 1.4.[19g]



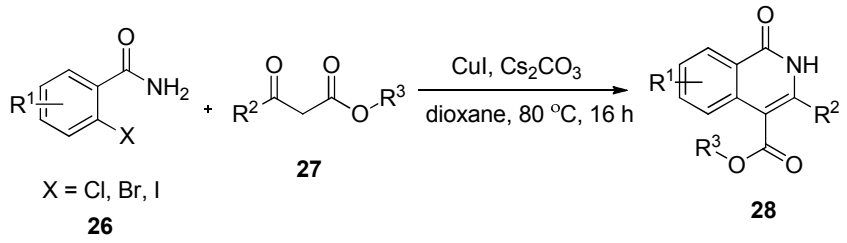

**Scheme 1.4**: Synthesis of aza fused quinolines from 2-(1*H*-benzo[*d*]imidazole-2-yl)acetonitrile.

In 2011, Fu and coworkers reported the synthesis of benzimidazoisoquinoline derivatives (**25**) *via* copper-catalyzed cascade reaction of 2-(2-halophenyl)benzoimidazoles (**24**) with alkyl cyanoacetates (**14**) under mild conditions as shown in Scheme 1.5.[23] This protocol provides corresponding benzimidazoisoquinolines containing amino and carboxylate groups in good to excellent yields.



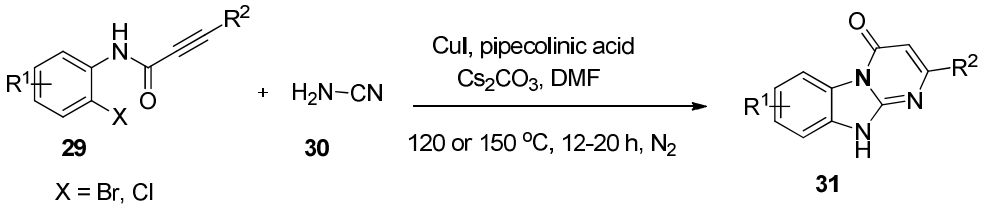

**Scheme 1.5**: Synthesis of benzimidazoisoquinoline from 2-(2-halophenyl)benzoimidazoles

In 2009, Zhao and coworkers reported a copper-catalyzed method for the synthesis of 3,4-disubstituted isoquinolin-1(2*H*)-one derivatives (**28**) *via* the reaction of substituted 2-halobenzamides (**26**) with β-keto esters (**27**) under mild conditions using CuI as a catalyst and without using any ligand or co-catalyst as shown in Scheme 1.6.[24]



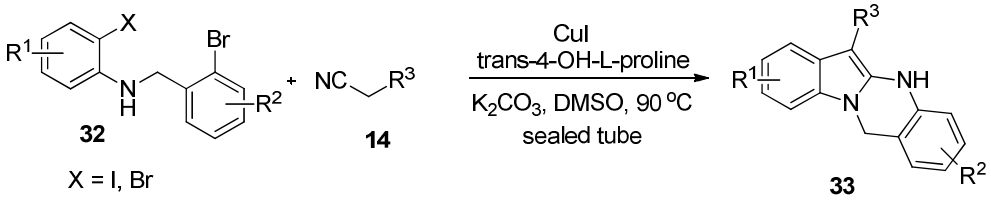

**Scheme 1.6**: Synthesis of 3,4-disubstituted isoquinolin-1(2*H*)-one from 2-halo benzamide.

In 2015, Fu and coworkers developed an efficient copper-catalyzed domino method for the synthesis of benzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones (**31**) *via* the reaction of substituted *N*-(2-halophenyl)-3-alkylpropiolamides (**29**) with cyanamide (**30**) under mild conditions as shown in Scheme 1.7.[25]



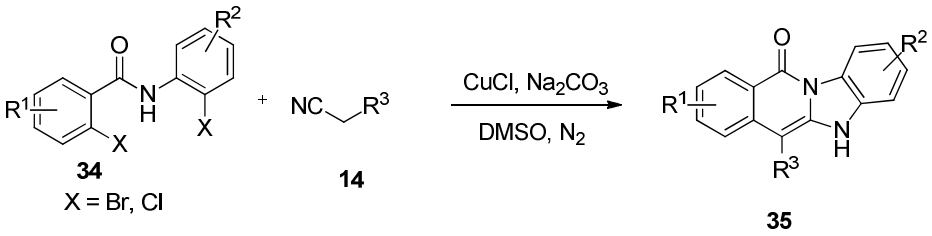

**Scheme 1.7**: Synthesis of benzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones from *N*-(2-halophenyl)-3-alkylpropiolamides.

In 2012, Zhao and coworkers reported a copper-catalyzed domino process for the synthesis of of 5,12-dihydroindolo[2,1-*b*]quinazoline (3**3**) derivatives *via* the reaction of *N*-(2-bromobenzyl)-2-iodoaniline (**32**) with alkyl 2-cyanoacetates (**14**) or malononitrile under mild conditions as shown in Scheme 1.8.[26]




**Scheme 1.8**: Copper-catalyzed domino synthesis of 5,12-Dihydroindolo[2,1-*b*]quinazoline derivatives.

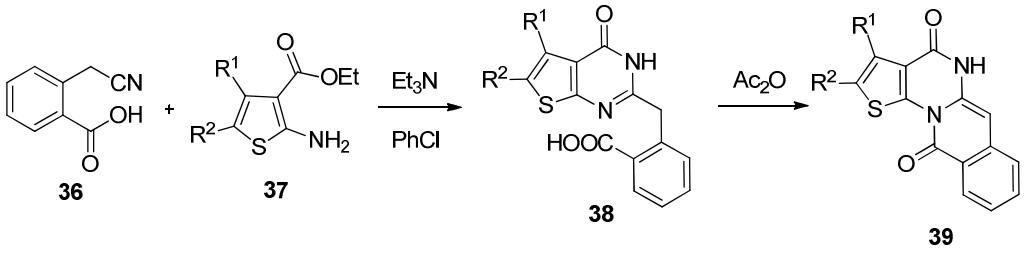
In 2010, Fu and coworkers reported a copper-catalyzed one-pot tandem method for the synthesis of benzimidazo[1,2-*b*]isoquinolin-11-one derivatives (**35**) *via* the reactions of substituted 2-halo-*N*-(2-halophenyl)benzamides (**34**) with alkyl 2-cyanoacetates (**14**) or malononitrile under mild conditions as shown in Scheme 1.9.[27]




**Scheme 1.9**: Copper-catalyzed one-pot tandem synthesis of benzimidazo-[1,2-*b*]isoquinolin-11-ones.

### 1.2.2. Earlier reports for the synthesis of thieno[3',2':5,6]pyrimido[1,2-*b*]isoquinolines:

In 2010, Kovtunenko and coworkers reported the synthesis of thieno[3',2':5,6]pyrimido[1,2-*b*]isoquinoline-4,11(5*H*)-dione (**39**) from 2-cyanomethylbenzoic acid (**36**). Reaction of cyanomethylbenzoic acid (3**6**) with ethyl 3-amino-4-thiophenecarboxylate (**37**) in chlorobenzene afforded compound (**38**). Further the compound (**38**) was heated in acetic anhydride which on cyclization afforded compound (**39**) as shown in Scheme 1.10.[28]

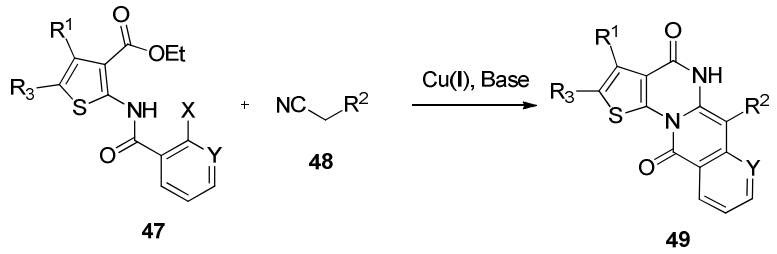



**Scheme 1.10**: Synthesis of thieno[3',2':5,6]pyrimido[1,2-*b*]isoquinoline-4,11(5*H*)-dione from 2-(4-oxo-3,4-dihydrothieno[2,3-*d*]benzoic acid.

## 1.3. Present work:

Our strategy towards synthesis of novel thienopyrimido[1,2-*b*]isoquinolines derivatives, we have developed a new and versatile Cu mediated one pot domino reaction[19d, 29] under mild conditions without using any co-catalyst, ligand or additive

as shown in Scheme 1.11. To synthesize our target molecule, we envisioned a Cu-mediated Ullmann-type[30] coupling of (**47)** with alkyl 2-cyanoacetate (**48**) followed by base promoted intramolecular cyclizations to afford compound (**49).**



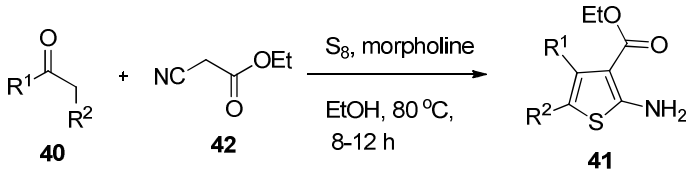

**Scheme 1.11**: Synthesis of thienopyrimido[1,2-*b*]isoquinolines from alkyl 2-(2-halobenzamido)thiophene-3-carboxylate.

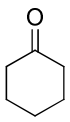
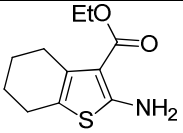
## 1.4. Results and discussion:

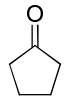
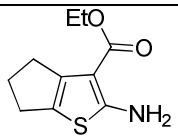
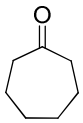
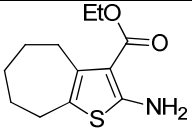
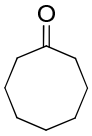
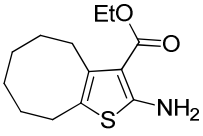
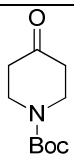
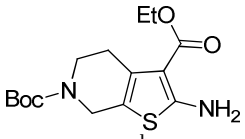
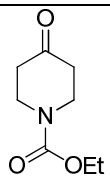
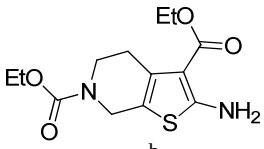
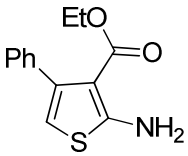
### 1.4.1. Preparation of starting materials:

The key starting material (**41)** required for our study was prepared by using a Gewald reaction[31] (a one-pot cyclocondensation of an $\alpha$-methylene carbonyl compound and $\beta$-substituted aceotonitrile derivatives with elemental sulfur in the presence of a strong Lewis organic base, such as a secondary amine and morpholine, which was first described by Gewald and co-workers in 1960s) to affords the required 2-aminothiophene derivatives. Syntheses of various 2-amino substituted thiophenes were shown in Table 1.1.

**Table 1.1**: Synthesis of 2-amino substituted thiophenes *via* Gewald reaction.[a]




| Entry | Compound (**40**) | Time (h) | Product (**41**) | %Yield[c] |
|---|---|---|---|---|
| 1 |  **40a** | 12 |  **41a** | 73 |

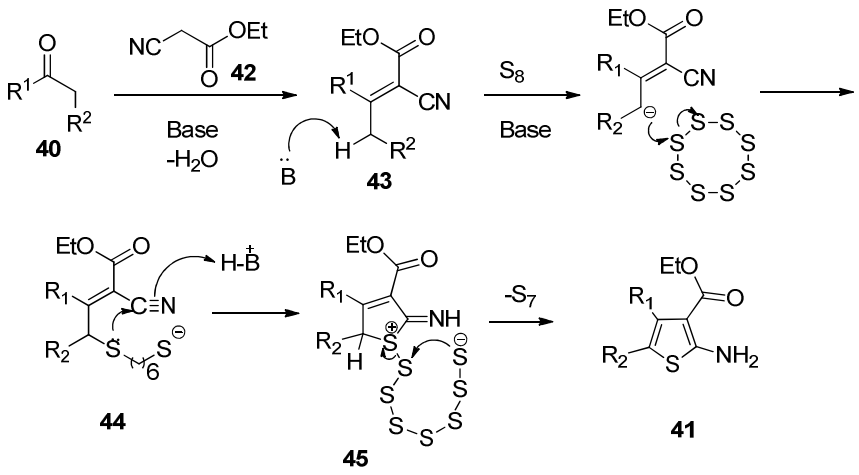
| | | | | |
|---|---|---|---|---|
| 2 |  **40b** | 12 |  **41b** | 78 |
| 3 |  **40c** | 12 |  **41c** | 69 |
| 4 |  **40d** | 12 |  **41d** | 77 |
| 5 |  **40e** | 8 |  **41e**[b] | 80 |
| 6 |  **40f** | 8 |  **41f**[b] | 76 |
| 7 |  **40g** | 12 |  **41g** | 68 |

[a]All the 2-aminothiophene derivatives (**41**) were prepared by using the corresponding ketone with an ethyl cyanoacetate (1.0 equiv) in the presence of elemental sulfur (1.0 equiv), morpholine (1.0 equiv) in EtOH under Gewald reaction conditions. [b]$Et_3N$ (1.0 equiv) was used as a base instead of morpholine. [c]Isolated yield.

**The reaction mechanism of the Gewald reaction:**

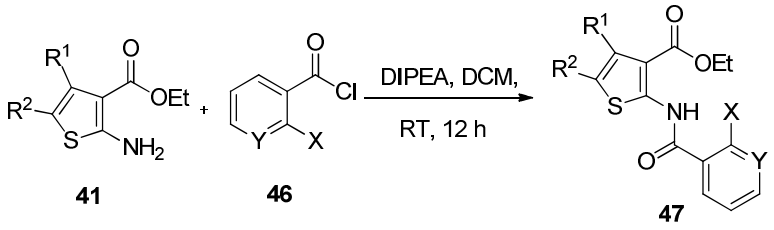
The first step of the Gewald reaction is a Knoevenagel condensation of *β*-substituted acetonitrile (**42**) with an *α*-methylene carbonyl compound (**40**) (ketone or aldehyde) to produce an intermediate (**43**), which on thiolation with elemental sulfur at the γ-methylene group of compound (**43**) afforded the compound (**44**). Finally, the sulfurated compound (**44**) undergoes ring closure *via* nucleophilic mercaptide attack

at the cyano carbon followed by a prototropic rearrangement to afford the 2-aminothiophene (**41**) as depicted in Scheme 1.12.




**Scheme 1.12**: The reaction mechanism of the Gewald reaction.

The compound (**47)** was synthesized from the 2-aminothiophene (**41**) *via* coupling with the corresponding acid chloride (**46**) in the presence of base at room tempatrure as shown in Scheme 1.13.



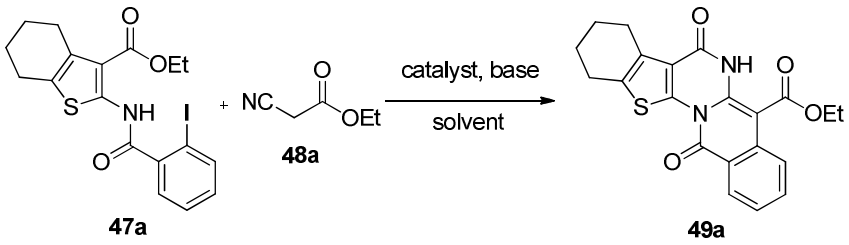

**Scheme 1.13**: Synthesis of compound (**47**).

### 1.4.2. Reaction optimization:

To establish the optimized reaction condition, we commenced our studies by testing the coupling of ethyl 2-(2-iodobenzamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**47a)** with ethyl cyanoacetate (**48a**) under various conditions including catalysts, base and solvents shown in Table 1.2. The reaction was initially performed in the presence of 0.1 equiv. of CuI and 3.0 equiv. of $K_2CO_3$ in DMSO that provided the highest yield of desired product (entry 1, Table 1.2). Effect of bases was investigated by replacing $K_2CO_3$ with $Na_2CO_3$ that decreased the product yield (entry 2, Table 1.2) whereas the effect of $Cs_2CO_3$ was found to be the same as $K_2CO_3$ (entry

3, Table 1.2). Several solvents were tested and DMSO was found to be better than DMF and 1,4-dioxane (entry 5-6, Table 1.2), whereas toluene was not suitable for this reaction (entry 7, Table 1.2). To improve the yield further we changed other copper source like CuBr that was found to be similarly effective like CuI (entry 8, Table 1.2) whereas CuCl provided less yield of product (entry 9, Table 1.2). The reaction did not proceed in the absence of catalyst indicating key role played by the catalyst in the present reaction (entry 4, Table 1.2).

**Table 1.2**: Reaction conditions and optimization.[a]




| Entry[a] | Catalyst | Base | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | CuI | $K_2CO_3$ | DMSO | 75 |
| 2 | CuI | $Na_2CO_3$ | DMSO | 62 |
| 3 | CuI | $Cs_2CO_3$ | DMSO | 74 |
| 4 | - | $K_2CO_3$ | DMSO | 0[c] |
| 5 | CuI | $K_2CO_3$ | DMF | 69 |
| 6 | CuI | $K_2CO_3$ | 1,4-Dioxane | 42 |
| 7 | CuI | $K_2CO_3$ | Toluene | 0 |
| 8 | CuBr | $K_2CO_3$ | DMSO | 72 |
| 9 | CuCl | $K_2CO_3$ | DMSO | 58 |

[a]Reactions were carried out using **47a** (1 mmol), **48a** (1.2 mmol), catalyst (0.1 mmol) and base (3 mmol) in solvent (2 mL) at 85 ºC for 3h under anhydrous conditions.

[b]Isolated yield. [c]No addition of catalyst.

### 1.4.3. Scope of the reaction:

Having identified the optimal reaction condition, we next examined the substrate scope of this Cu-catalyzed domino reaction, and the results are summarized in Table 1.3. Compound (**47**) containing various substituents e.g. $R^1$, $R^3$ representing a fused alicyclic (entry 1-5 and 9-15, Table 1.3) or azaalicyclic ring (entry 6-8, Table 1.3) or hydrogens (entry 18-19, Table 1.3) or $R^1$ = Ph and $R^3$ = H (entry 16-17, Table 1.3) afforded good to acceptable yield of desired products. The other coupling partner **(48a-d)** *i.e.* ethyl cyanoacetate, methyl cyanoacetate**,** malanonitrile and 3-morpholino-3-oxopropanenitrile were tolerated in this reaction. The reaction proceeded well in all these cases afforded the desired products in good yield. All the compounds synthesized were characterized by spectral (NMR and MS) data. Some characteristic signals appeared in $^1$H and $^{13}$C NMR spectra of a representative compound (**49f)** are shown in the following figure (Figure 1.4). The appearance of a peak at δ 12.91 ppm in $^1$HNMR spectra of (**49f)** was due to amidic NH proton, signals at δ 1.80-1.64, δ 1.98-1.88, δ 3.42-3.35 were due to aliphatic cycloheptane ring protons which is attached to thiophene group and peaks at δ 4.54, δ 1.52 were corresponding to ester group. The $^{13}$C signals of carbonyl groups present in compound (**49f)** were observed at δ168.4 (ester), δ158.5 (amidic) and δ155.8 (amidic) as shown in (Figure 1.4).

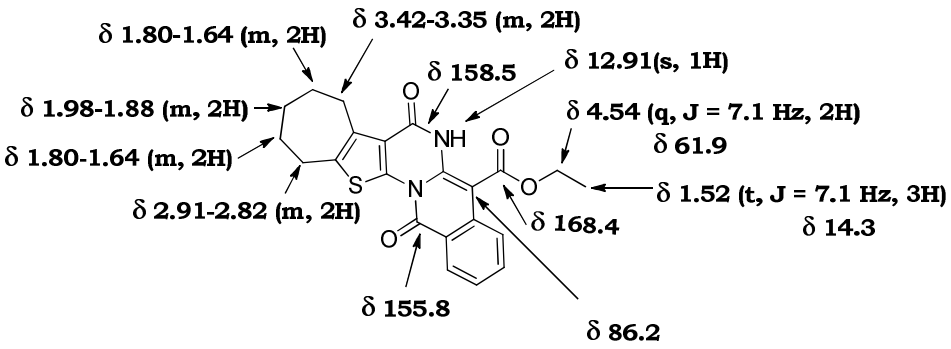



**Fig. 1.4**: Characteristic $^1$H and $^{13}$C NMR peaks of (**49f)**

The molecular structure of a representative compound (**49h)** was further confirmed unambiguously by single crystal X-ray diffraction study (Figure 1.5).

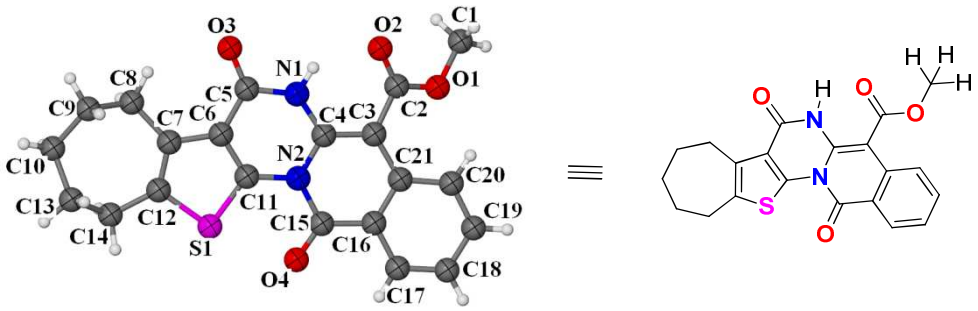
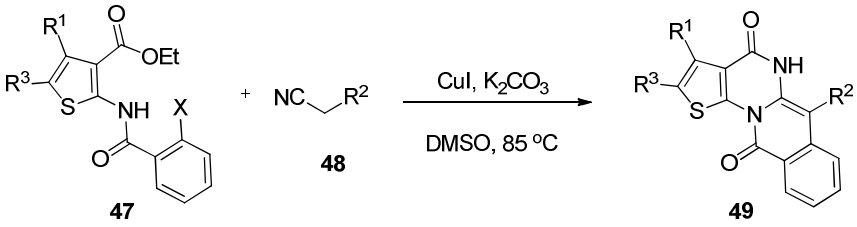
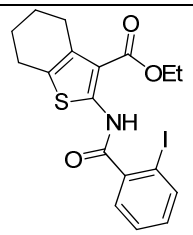
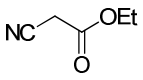
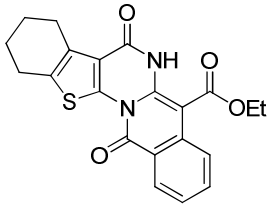
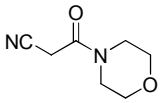
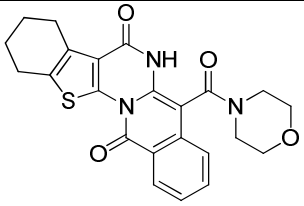
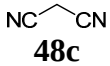
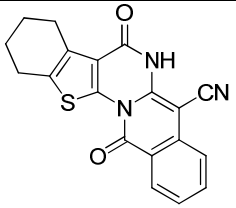
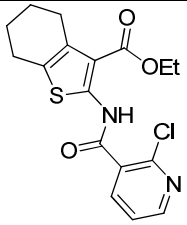
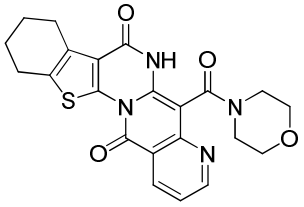
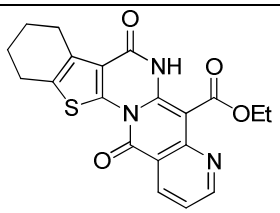
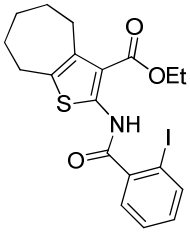
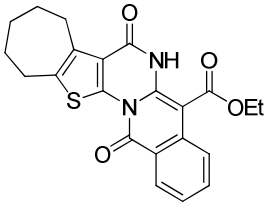
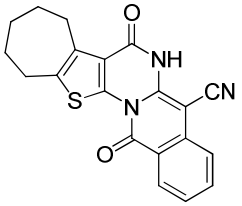
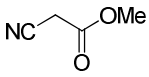
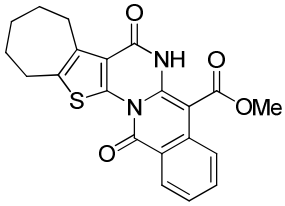
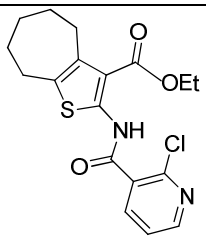
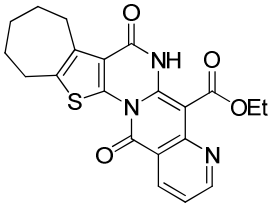
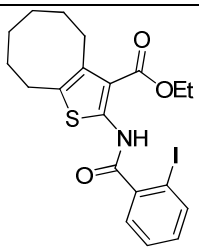
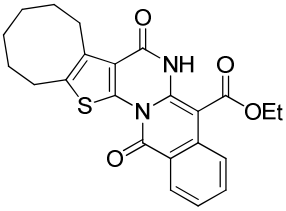


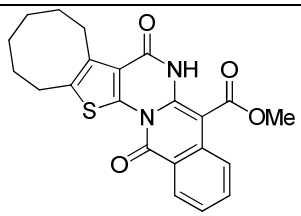
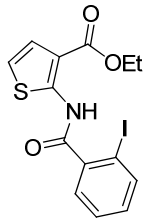
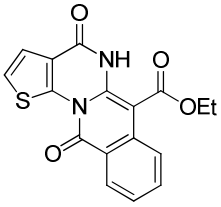
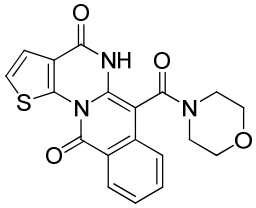
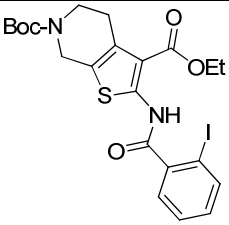
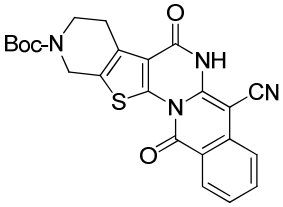
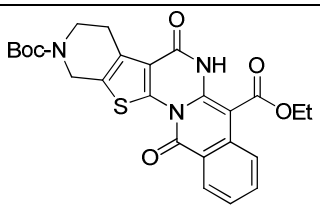
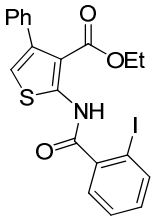
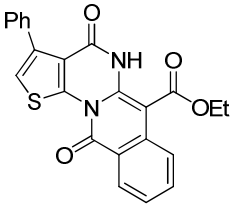
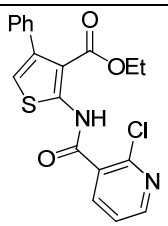
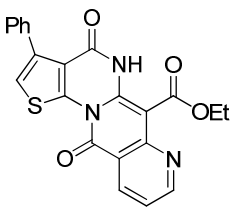

**Fig. 1.5**: ORTEP representation of the (**49h**). Thermal ellipsoids are drawn at 50% probability level.

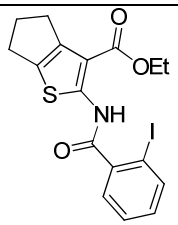
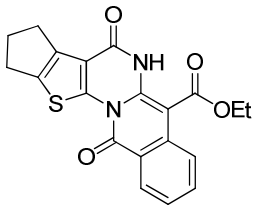
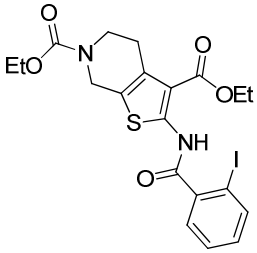
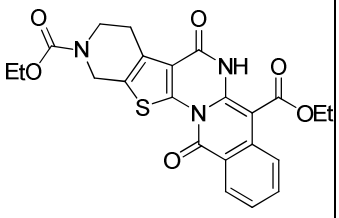
**Table 1.3**: Copper catalyzed synthesis of compound (**49**).[a]




| S.No | Compound (**47**) | Nitrile (**48**) | Time (h) | Product (**49**) | Yield (%)[b] |
|---|---|---|---|---|---|
| 1 |  **47a** |  **48a** | 6 |  **49a** | 75 |
| 2 | **47a** |  **48b** | 7 |  **49b** | 69 |
| 3 | **47a** |  **48c** | 6 |  **49c** | 64 |

| | | | | | |
|---|---|---|---|---|---|
| 4 |  **47b** | **48b** | 8 |  **49d** | 61 |
| 5 | **47b** | **48a** | 6 |  **49e** | 66 |
| 6 |  **47c** | **48a** | 6 |  **49f** | 70 |
| 7 | **47c** | **48c** | 7 |  **49g** | 61 |
| 8 | **47c** |  **48d** | 7 |  **49h** | 72 |
| 9 |  **47d** | **48a** | 9 |  **49i** | 63 |
| 10 |  **47e** | **48a** | 6 |  **49j** | 68 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | **47e** | **48d** | 7 |  **49k** | 69 |
| 12 |  **47f** | **48a** | 7 |  **49l** | 72 |
| 13 | **47f** | **48b** | 8 |  **49m** | 59 |
| 14 |  **47g** | **48c** | 7 |  **49n** | 58 |
| 15 | **47g** | **48a** | 7 |  **49o** | 63 |
| 16 |  **47h** | **48a** | 6 |  **49p** | 70 |
| 17 |  **47i** | **48a** | 8 |  **49q** | 62 |

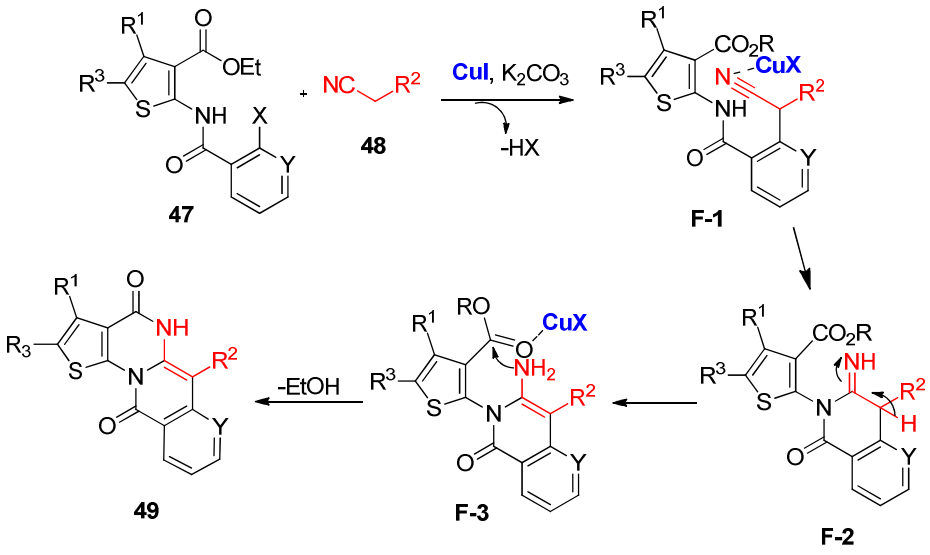
| | | | | | |
|---|---|---|---|---|---|
| 18 |  **47j** | **48a** | 6 |  **49r** | 68 |
| 19 |  **47k** | **48a** | 6 |  **49s** | 71 |

[a]Reactions were carried out using **47** (1 mmol), **48** (1.2 mmol), CuI (0.1 mmol) and $K_2CO_3$ (3 mmol) in DMSO (2 mL) under anhydrous conditions.

[b]Isolated yield.

### 1.4.4. Proposed mechanism:

A plausible mechanism for the copper-catalyzed synthesis of compound (**49**) depicted in Scheme 1.14. First, copper-catalyzed C-arylation of substituted nitrile (**48**) with (**47**) *via* copper catalyzed ullmann type intermolecular C-C bond formation provided **F-1.** Then CuI/base promoted intramolecular nucleophilic attack of NH to CN in **F-1** leads to **F-2** which then tautomerized to **F-3**. Finally, intramolecular amide bond formation within **F-3** afforded the target compound (**49).**




**Scheme 1.14**: Proposed reaction mechanism.

## 1.5. Pharmacology:

### 1.5.1. *In vitro* data:

Some of the compounds synthesized were evaluated for their PDE4 inhibitory properties *in vitro*.[32] Phosphodiesterases (PDE) are enzymes that degrade intracellular cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) into inactive linear 5′ monophosphate (Figure 1.6). Phosphodiesterase 4 (PDE4) is a member of PDE enzyme family, exists in four different isoforms (PDE4A, B, C and D)[33] and it is specific for the hydrolysis of cyclic adenosine monophosphate (cAMP).

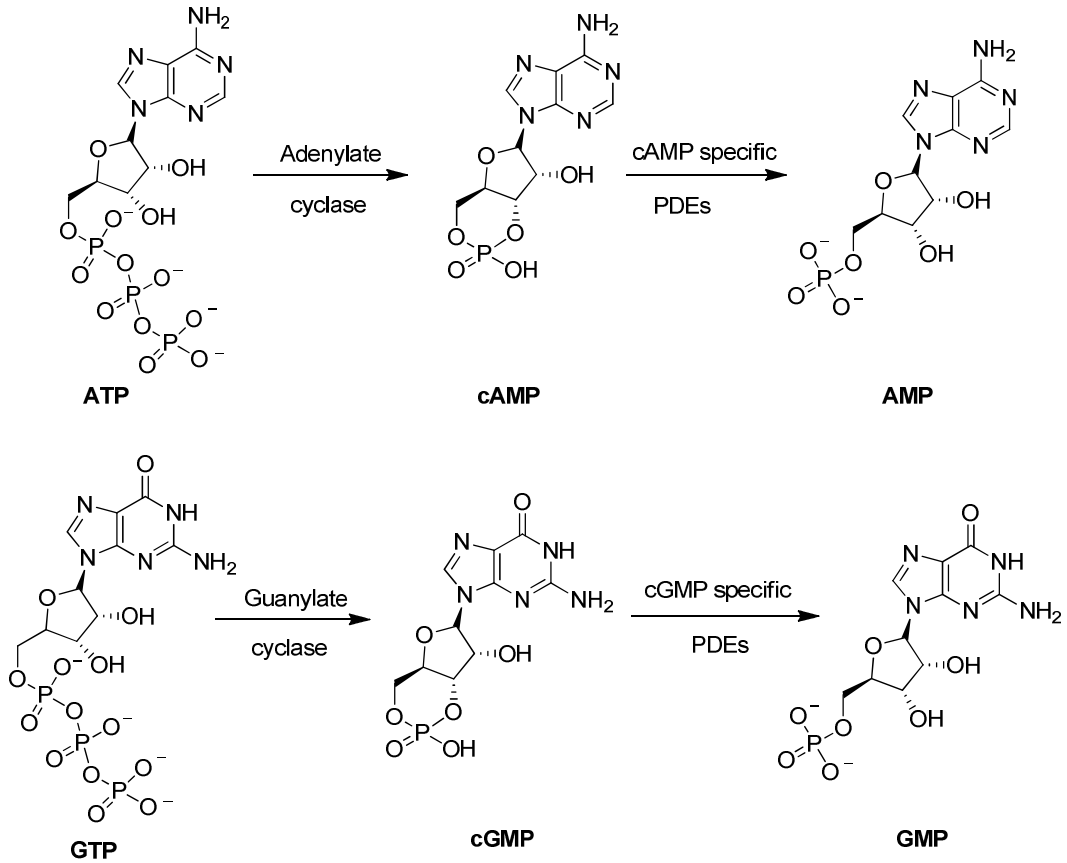



**Fig. 1.6**: Enzymatic conversions of cyclic and linear nucleotides.

The inhibition of cellular responses like production and/or release of pro inflammatory mediators, cytokines, and active oxygen species in inflammatory and immune cells are associated with elevated levels of acyclic adenosine monophosphate.[34] Phosphodiesterase 4 (PDE4) plays a key role in the hydrolysis of acyclic adenosine monophosphate to adenosine mono phosphate. Thus, inhibition of

PDE4 results in an elevation of cAMP in these cells. Hence, PDE4 can be useful for the treatment of inflammatory and immunological diseases including asthma and COPD. Notably, rolipram[35] was the first generation posphodiesterase inhibitor, showed adverse effects such as nausea, and vomiting.

The second-generation inhibitors like cilomilast[36] (Ariflo) and roflumilast has reduced these dose-limiting side effects but their therapeutic index has delayed market launch so far. Recent studies have indicated that PDE4B subtype is linked to inflammatory cell regulation[37] while the PDE4D subtype is implied in the emetic response.[38] Hence, it is necessary to develop next generation PDE4 inhibitors possessing moderate selectivity towards PDE4B over PDE4D. Accordingly, we tested the posphodiesterase inhibiting activity of our synthesized compounds. The compounds were evaluated against PDE4B by using PDE4B enzyme isolated from Sf9 cells14 with rolipram, as a reference compound. Some of the compounds synthesized were tested against PDE4B along with a known inhibitor rolipram using an enzyme based *in vitro* assay.[32] The compound (**49d)** showed (62%) inhibition when tested at 30 $\mu$M. This was further supported by the docking results of (**49d)** with PDE4B protein which was explained in the following section.

### 1.5.2. Docking studies:

The docking studies of molecules were performed using the Maestro, version 9.2.[39] The compounds were sketched in 3D format using build panel and LigPrep module was used to produce low-energy conformers and to refine the structural parameters of molecules. The crystal structure of PDE4B (PDB ID: 3D3P)[40] was obtained from the protein data bank.

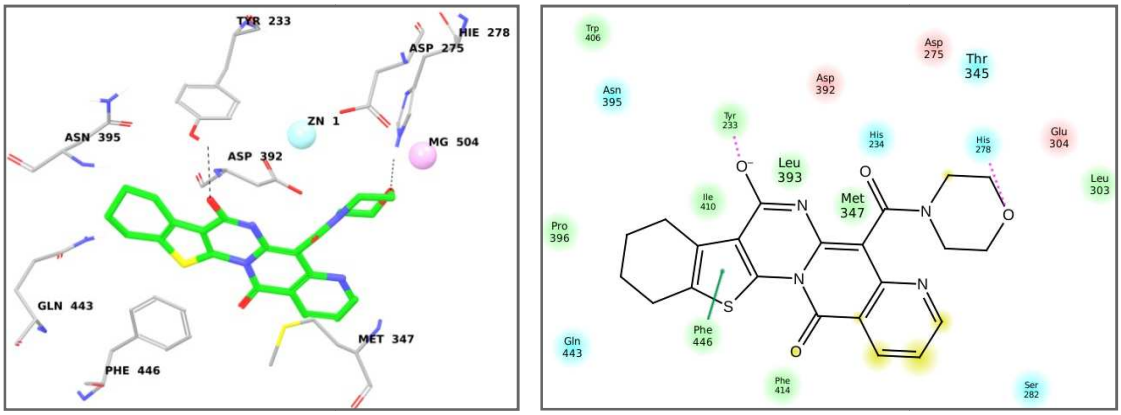



**Fig. 1.7:** Binding mode and interactions molecule (**49d)** with PDE4B.

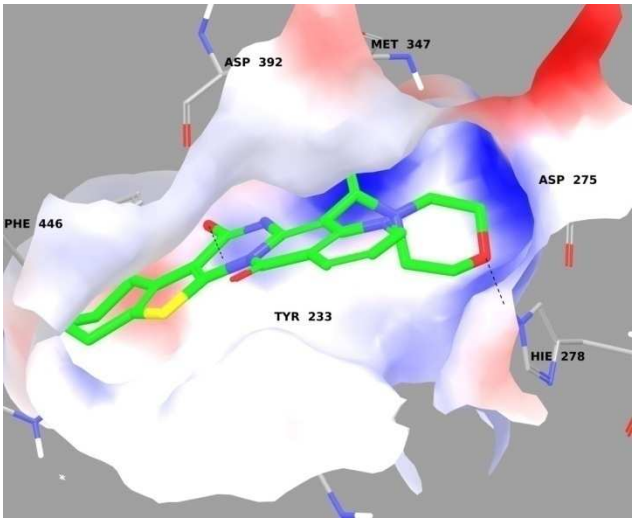



**Fig. 1.8**: Binding orientation of molecule (**49d)** at the active site pocket of PDE4B.

The *in silico* binding studies of a representative compound (**49d)** showed that oxygen atom of morpholine ring and CO group participated in H-bonding with NH of His-278 and OH of Tyr 233 respectively. A pi-pi stacking between the molecule (**49d)** and Phe-446 was also observed (Figure 1.7). The morpholine ring of molecule (**49d)** was found to be well occupied in the partially charged pocket of active site (Figure 1.8).

**Table 1.4:** Glide score and contributing parameters

| Molecule | GScore | LipophilicEvdW | PhobEn | HBond | LowMW |
|---|---|---|---|---|---|
| **49d** | -7.4 | -4.28 | -1.90 | -0.90 | -0.05 |

## 1.6. Conclusion:

In conclusion, we have developed a new and general strategy involving one pot Cu-mediated domino reaction for the synthesis of novel isoquinolino[2,3-*a*]quinazolinones (**49**). The catalyst (CuI) used in this methodology is cheap and readily available. The key starting material (**41)** required for our study was prepared by using a Gewald type of reaction. The cascade reaction proceeds *via* copper catalyzed Ullmann type C-C bond formation to give an intermediate *in situ*, which subsequently undergo intramolecular nucleophilic addition of NH to CN followed by intramolecular nucleophilic attack by amine to ester group. This allows the formation of a fused ring leading to thienopyrimido[1,2-

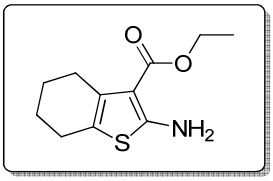
*b*]isoquinolines. A wide range of functional groups were well tolerated under the reaction conditions employed. Some of these synthesized compounds were tested for PDE4B along with a known inhibitor rolipram using an enzyme based *in vitro* assay, a representative compound (**49d**) showed (62%) inhibition of PDE4B. Since COPD and asthma are major health burden worldwide hence the present class of compounds is of further interest. Overall, this is an inexpensive, convenient and efficient copper-catalyzed protocol for the synthesis of isoquinolino[2,3-*a*]quinazolinones (**49**).

## 1.7. Experimental section:

### 1.7.1. Chemistry

**General methods**: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. $^{1}$H NMR and $^{13}$C NMR spectra were recodred in $CDCl_3$ or DMSO-$d_6$ solution by using a 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT- IR spectrometer. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. High-resolution mass spectra (HRMS) were recorded using a Waters LCT Premier XE instrument. Melting points (mp) were determined by using Buchi B-540 melting point appratus and are uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

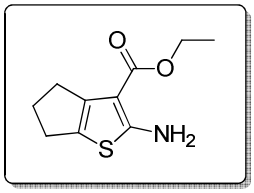
#### 1.7.1.1. Typical procedure for the synthesis of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (41a)



A mixture of cyclo hexanone (1.06 mL, 10 mmol), ethyl cyanoacetate (1.15 mL, 10 mmol), morpholine (0.90 mL, 10 mmol), sulphur (0.32 g, 10 mmol) in ethanol (10 mL) was stirred and refluxed for overnight. After completion of the reaction, the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The crude solid was washed with cold ethanol and filtered though sintered funnel, dried under vacuum. The crude product was dissolved in dichloromethane and washed with brine. The organic layer was collected and concentrated under low vacuum to give the title compound.

Yield: 73% (1.83 g); brown solid; mp: 115-117 °C (lit 116.2-117.2 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 5.93 (s, 2H), 4.25 (q, *J* = 7.3 Hz, 2H), 2.68-2.71(m, 2H), 2.47-2.51 (m, 2H), 1.74-1.80 (m, 4H), 1.33 (t, *J* = 7.3 Hz, 3H).

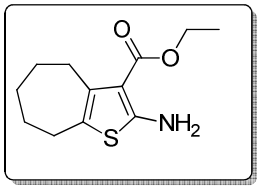
### 1.7.1.2. Ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (41b)



Compound (**41b)** was synthesized from cyclo pentanone following a procedure similar to that of compound (**41a)**.

Yield: 78% (1.79 g); brown solid; mp: 182-184 °C (lit 182.5-183.5 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 5.89 (s, 2H), 4.23 (q, *J* = 7.3 Hz, 2H), 2.81-2.83 (m, 2H), 2.68-2.70 (m, 2H), 2.26-2.30 (m, 2H), 1.40 (t, *J* = 7.3 Hz, 3H).

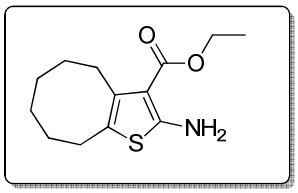
### 1.7.1.3. Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (41c)



Compound (**41c)** was synthesized from cyclo heptanone following a procedure similar to that of compound (**41a)**.

Yield: 69% (1.47 g); light yellow solid; mp: 88-90 °C (lit 89.5-90.5 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ ppm: 5.77 (s, 2H), 4.27 (q, *J* = 6.9 Hz, 2H), 2.97 (t, *J* = 5.5 Hz, 2H), 2.57 (t, *J* = 5.6 Hz, 2H), 1.77-1.83 (m, 2H), 1.58-1.66 (m, 4H), 1.34 (t, *J* = 6.9 Hz, 3H).

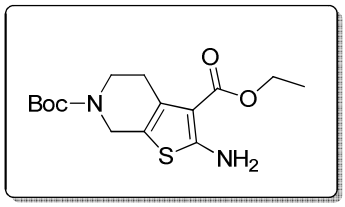
### 1.7.1.4. Ethyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (41d)



Compound (**41d)** was synthesized from cyclo heptanone following a procedure similar to that of compound (**41a)**.

Yield: 77% (1.52 g); brown solid; mp: 48-50 °C (lit 50-51 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 5.91 (bs, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.85–2.81 (m, 2H), 2.68–2.64 (m, 2H), 1.68–1.44 (m, 6H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.30-1.28 (m, 2H).

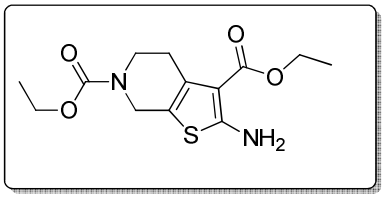
### 1.7.1.5. 6-*tert*-Butyl 3-ethyl 2-amino-4, 5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (41e)



Compound (**41e)** was synthesized from N-Boc-4-piperidone and $Et_3N$ as a base following a procedure similar to that of compound (**41a)**.

Yield: 80% (1.31 g); light yellow solid; mp: 156-158 °C (lit 157-158 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 6.05 (s, 2H), 4.35 (bs, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 3.62 (t, *J* = 4.8 Hz, 2H), 2.78 (bs, 2H), 1.48 (s, 9H), 1.34 (t, *J* = 7.2 Hz, 3H).

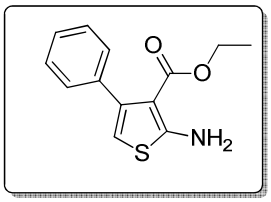
### 1.7.1.6. Diethyl-2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (41f)




Compound (**41f)** was synthesized from ethyl 4-oxopiperidine-1-carboxylate and $Et_3N$ as a base following a procedure similar to that of compound (**41a)**.

Yield: 76% (1.32 g); brown solid; mp: 143-145 °C (lit 144-146 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 6.02 (bs, 2H), 4.40 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.66 (bs, 2H), 2.82 (bs, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

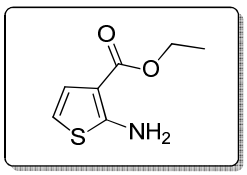
### 1.7.1.7. Ethyl 2-amino-4-phenylthiophene-3-carboxylate (41g)




Compound (**41g)** was synthesized from acetophenone following a procedure similar to that of compound (**41a)**.

Yield: 68% (1.15 g); light green solid; mp: 97-99 °C (lit 97.5-98.5 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.28-7.31 (m, 5H), 6.08 (bs, 2H), 6.06 (s, 1H), 4.10 (q, *J* = 6.9 Hz, 2H), 0.94 (t, *J* = 6.9 Hz, 3H),

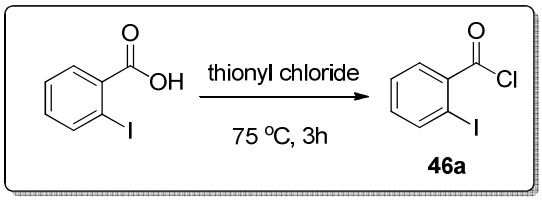
### 1.7.1.8. Ethyl 2-aminothiophene-3-carboxylate (41h)



A mixture of 1,4-dithiane-2,5-dithiol (1.0 g, 6.45 mmol), ethyl cyanoacetate (1.45 mL, 12.90 mmol), triethylamine (1.80 mL, 12.90 mmol) in DMF was heated to 60 °C for 1 h. After completion of reaction, reaction mixture was cooled to room temperature. The mixture was then diluted with ethyl acetate (25 mL), washed with water (3 x 20 mL) followed by brine solution (20 mL). The organic layers were collected, combined, dried over anhydrous $Na_2SO_4$, filtered and concentrated under a reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**41h**).

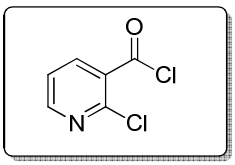
Yield: 71% (0.81 g); off white solid; mp: 46-48 °C (lit 47-48 °C); $^1$H NMR (400 MHz, $CDCl_3$) δ: 6.97 (d, *J* = 5.7 Hz, 1H), 6.24 (d, *J* = 5.7 Hz, 1H), 6.06 (bs, 2H), 4.30 (q, *J* = 6.9 Hz, 2H), 1.38 (t, *J* = 6.3 Hz, 3H).

### 1.7.1.9. Typical procedure for preparation of 2-iodo benzoyl chloride (46a)



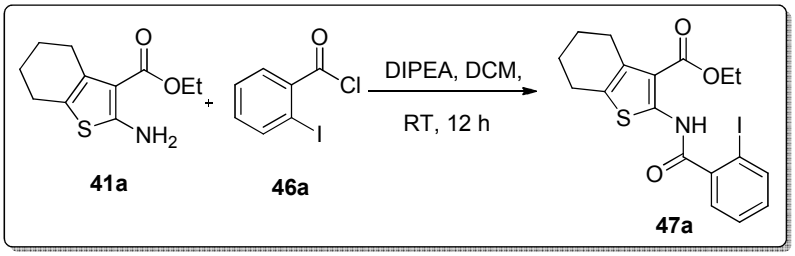

Thionyl chloride (10 equiv.) was slowly added to the 2-iodo benzoic acid (1 equiv.) at 0 ºC and the reaction mixture was heated to 75 ºC for 3h. Then, the reaction mixture cooled to room temperature and excess of thionyl chloride removed under reduced pressure to give desired product which was used further without any purification.

### 1.7.1.10. 2-Chloro nicotinoyl chloride (46b)



Compound (**46b)** was synthesized from 2-chloro nicotinic acid following a procedure similar to that of compound (**46a)**.

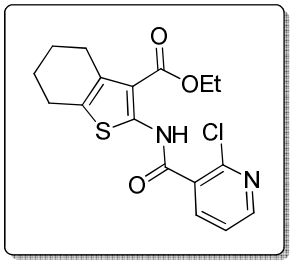
### 1.7.1.11. Typical procedure for preparation of ethyl 2-(2-iodobenzamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (47a)




To a solution of compound (**41a)** (100 mg, 0.44 mmol) in dry DCM (5 mL), DIPEA (0.15 mL, 0.88 mmol) was added at 0 ºC under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.09 mL, 0.66 mmol) was slowly added and the reaction mixture stirred at room temperature for 12 h. After completion of reaction, the reaction mixture diluted with DCM (5 mL), washed with saturated $NaHCO_3$ solution (15 mL), followed by brine solution (10 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound (**47a)**.

Yield: 82% (165 mg); Light yellow solid; mp:109-111 °C; $R_f$ = 0.2 (20% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3228, 2933, 1725, 1663; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.63 (bs, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.16 (t, *J* = 7.4 Hz, 1H), 4.31 (q, *J* = 7.0 Hz, 2H), 2.79 (t, *J* = 5.4 Hz, 2H), 2.69 (t, *J* = 5.4 Hz, 2H), 1.86-1.78 (m, 4H), 1.36 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.5, 165.3, 146.9, 140.6, 140.5, 139.9, 131.9, 131.5, 128.5, 127.5, 112.5, 92.8, 60.6, 26.4, 24.4, 22.9, 22.8, 14.2; MS (ES mass): 455.7 (M+1); HPLC: 93.3%, column: X Bride C-18 150*4.6mm 5µ, mobile phase A: 5mm Ammonium acetate in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/20,2/20, 9/95, 15/95, 17/20,20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 12.2 min.

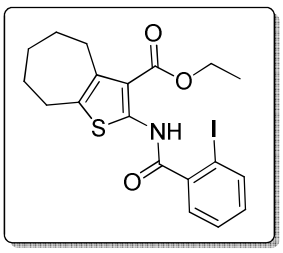
### 1.7.1.12. Ethyl-2-(2-chloronicotinamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (47b)




Compound (**47b)** was synthesized from the reaction of (**41a)** and (**46b)** following a procedure similar to that of compound (**47a)**.

Yield: 77% (124 mg); Yellow solid; mp: 112-114 °C; $R_f$ = 0.2 (40% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3239, 2945, 1730,1658; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.22 (bs, 1H), 8.51 (s, 1H), 8.26-8.10 (m, 1H), 7.40-7.36 (m, 1H), 4.35-4.29 (m, 2H), 2.79 (bs, 2H), 2.68 (bs, 2H), 1.80 (bs, 4H), 1.37-1.34 (m, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.0, 161.0, 151.4, 147.4, 145.9, 139.8, 131.2, 129.7, 127.6, 122.5, 112.9, 60.5, 26.2, 24.2, 22.7, 22.6, 14.1; MS (ES mass): 364.8 (M+1); HPLC: 95.1%, column: Symmetry C-18 75*4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50, 1/50, 4/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.56 min.

**1.7.1.13. Ethyl-2-(2-iodobenzamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (47c)**

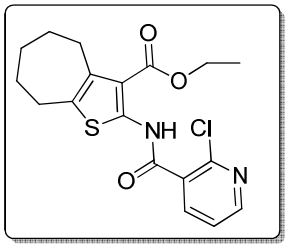



Compound (**47c)** was synthesized from the reaction of (**41c)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 76% (149 mg); Light brown solid; mp: 96-98 °C; $R_f$ = 0.2 (20% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3236, 2986, 1732, 1658; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.52 (bs, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 3.07 (t, *J* = 5.6 Hz, 2H), 2.77 (t, *J* = 5.6 Hz, 2H), 1.89-1.84 (m, 2H), 1.72-1.61 (m, 4H), 1.37 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.5, 165.3, 144.8, 140.7, 140.5, 139.9, 136.8, 131.8,

131.7, 128.4, 113.8, 92.9, 60.8, 32.2, 28.7, 28.3, 27.8, 26.9, 14.2; MS (ES mass): 469.7 (M+1); HPLC: 94.2%, column: Symmetry C-18 75*4.6mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.4 min.

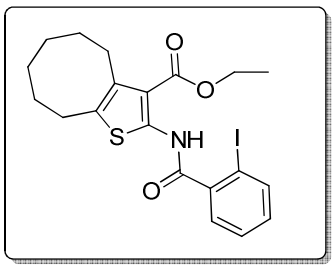
### 1.7.1.14. Ethyl-2-(2-chloronicotinamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (47d)




Compound (**47d)** was synthesized from the reaction of (**41c)** and (**46b)** following a procedure similar to that of compound (**47a)**.

Yield: 72% (113 mg); Light brown liquid; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3189, 2919, 1725, 1662; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 12.08 (s, 1H), 8.54 (dd, *J* = 4.7, 1.8 Hz, 1H), 8.18 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.40 (dd, *J* = 7.6, 4.7 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.09-3.06 (m, 2H), 2.78-2.76 (m, 2H), 1.89-1.84 (m, 2H), 1.71-1.61 (m, 4H), 1.39 (t, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 166.3, 161.2, 151.5, 147.6, 143.9, 140.0, 137.0, 132.1, 130.0, 122.7, 114.4, 60.9, 32.2, 28.6, 28.2, 27.7, 26.9, 14.2; MS (ES mass): 378.8 (M+1); HPLC: 95.4%, column: Symmetry C-18 75*4.6mm 3.5µ, mobile phase A: 0.1% Formic acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50, 1/50, 4/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.56 min.

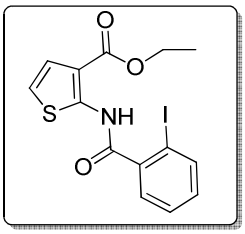
### 1.7.1.15. Ethyl-2-(2-iodobenzamido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (47e)



Compound (**47e)** was synthesized from the reaction of (**41c)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 72% (137 mg); Light brown liquid; $R_f$ = 0.2 (10% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3242, 2912, 1730, 1669; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.70 (s, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.55 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.19-7.13 (m, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 2.92 (t, *J* = 6.2 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 1.66 (d, *J* = 5.4 Hz, 4H), 1.52-1.45 (m, 2H), 1.37 (t, *J* = 7.1 Hz, 3H), 1.34-1.27 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.4, 165.2, 146.4, 143.4, 140.6, 139.8, 133.6, 131.9, 128.5, 128.3, 112.7, 92.8, 60.7, 32.3, 29.8, 26.8, 26.4, 25.5, 25.4, 14.1; MS (ES mass): 483.7 (M+1); HPLC: 94.6%, column: X Bridge C-18 150*4.6 mm ,5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/40, 2/40, 9/98, 16/98, 17/40,20/40; flow rate: 1.0 mL/min; UV 210 nm, retention time 12.28 min.

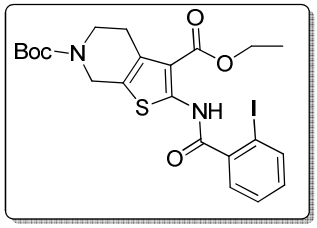
**1.7.1.16. Ethyl-2-(2-iodobenzamido)thiophene-3-carboxylate (47f)**




Compound (**47f)** was synthesized from the reaction of (**41h)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 76% (178 mg); Light brown liquid; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3269, 2925, 1731,1670; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.39 (bs, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.57 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.48-7.44 (m, 1H), 7.28 (d, *J* = 5.7 Hz, 1H), 7.19 (tb, *J* = 7.6, 1.6 Hz, 1H), 6.83 (d, *J* = 5.7 Hz, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:165.5, 165.4, 148.1, 142.1, 139.4, 134.1, 128.7, 128.4, 124.0, 116.5, 113.8, 92.8, 60.8, 14.3; MS (ES mass): 401.7 (M+1); HPLC: 98.4%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/20, 2/20, 9/98, 16/98, 17/20,20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 10.5 min.

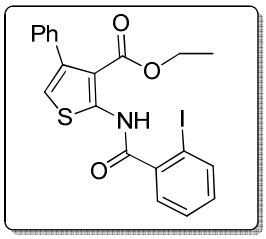
### 1.7.1.17. 6-*tert*-butyl-3-ethyl-2-(2-iodobenzamido)-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (47g)




Compound (**47g)** was synthesized from the reaction of (**41e)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 81% (138 mg); Light yellow solid; mp: 157-159 °C; $R_f$ = 0.2 (35% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3252, 2924, 1731, 1687; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.62 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.55 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 4.55 (s, 2H), 4.33 (q, *J* = 7.2 Hz, 2H), 3.67 (t, *J* = 5.6 Hz, 2H), 2.91 (bs, 2H), 1.49 (s, 9H), 1.37 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.0, 165.4, 154.6, 147.7, 140.7, 140.5, 139.5, 132.2, 128.8, 128.6, 128.3, 112.0, 92.7, 80.2, 60.8, 42.8, 42.7, 28.5 (3C), 28.3, 14.1; MS (ES mass): 554.9 (M-1); HPLC: 94.1%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 5mm ammonium acetate in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 11.8 min.

### 1.7.1.18. Ethyl-2-(2-iodobenzamido)-4-phenylthiophene-3-carboxylate (47h)

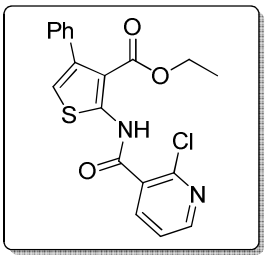



Compound (**47h)** was synthesized from the reaction of (**41g)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 74% (142 mg); Brown solid; mp:147-149 °C; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3198, 2925, 1732, 1655; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.68 (bs, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 6.9 Hz, 1H), 7.47 (t, *J* = 7.3 Hz, 1H), 7.36-

7.28 (m, 5H), 7.24-7.16 (m, 1H), 6.70 (s, 1H), 4.06 (q, *J* = 7.3 Hz, 2H), 0.92 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.8, 165.6, 149.4, 142.0, 140.6, 140.0, 137.5, 132.0, 129.0 (2C), 128.5, 128.3, 127.2 (2C), 126.9, 115.5, 112.4, 92.7, 60.5, 13.3; MS (ES mass): 477.7 (M+1); HPLC: 99.2%, column: Symmetry C-18 75*4.6mm 3.5µ, mobile phase A: 0.1% Tri flouro acetic acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50,0.5/50, 4/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.46 min.

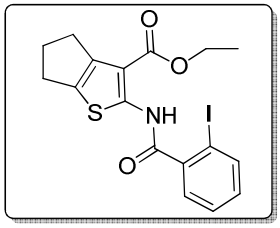
### 1.7.1.19. Ethyl-2-(2-chloronicotinamido)-4-phenylthiophene-3-carboxylate (47i)




Compound (**47i)** was synthesized from the reaction of (**41g)** and (**46b)** following a procedure similar to that of compound (**47a)**.

Yield: 75% (117 mg); white solid; mp:165-167 °C; $R_f$ = 0.2 (70% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3211, 2935, 1724, 1663; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.28 (bs, 1H), 8.58 (dd, *J* = 4.7, 1.9 Hz, 1H), 8.26 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.44 (dd, *J* = 7.6, 4.7 Hz, 1H), 7.40-7.29 (m, 5H), 6.74 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 0.94 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.7, 161.7, 151.8, 148.8, 147.7, 140.3, 140.2, 137.6, 129.6, 129.1 (2C), 127.4 (2C), 127.1, 122.8, 115.9, 113.2, 60.7, 13.4; MS (ES mass): 386.8 (M+1); HPLC: 97.2%, column: Symmetry C-18 75*4.6mm 3.5µ, mobile phase A: 0.1% Formic acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50,1/50,3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.53 min.

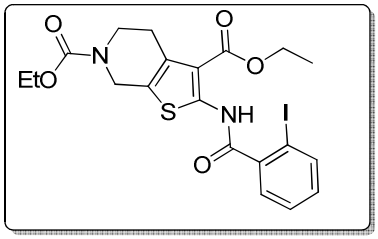
### 1.7.1.20. Ethyl-2-(2-iodobenzamido)-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylate (47j)



Compound (**47j)** was synthesized from the reaction of (**41b)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 80% (141 mg); Light brown liquid; $R_f$ = 0.4 (25% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3230, 2935, 1736, 1670; $^1$H NMR (400 MHz, $CDCl_3$) δ:11.7 (bs, 1H), 7.81-7.78 (m, 1H), 7.49-7.35 (m, 3H), 4.30 (q, *J* = 7.1 Hz, 2H), 2.94-2.86 (m, 4H), 2.43-2.36 (m, 2H), 1.35 (t, *J* = 7.1 Hz, 3H)**;** $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.9, 165.1, 150.8, 142.2, 141.6, 140.5, 139.6, 132.9, 132.4, 128.4, 109.2, 92.9, 60.6, 30.3, 28.9, 28.0, 14.3; MS (ES mass): 441.7 (M+1); HPLC: 91.2%, column: X Bride C-18 150*4.6mm 5µ, mobile phase A: 5mm Ammonium acetate in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/20,2/20, 9/95, 15/95, 17/20,20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 1.69 min.

### 1.7.1.21. Diethyl-2-(2-iodobenzamido)-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (47k)

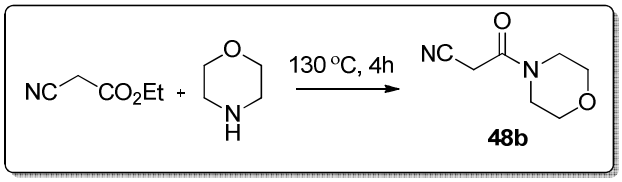


Compound (**47k)** was synthesized from the reaction of (**41f)** and (**46a)** following a procedure similar to that of compound (**47a)**.

Yield: 80% (141 mg); White solid; mp**:** 112-114 °C; $R_f$ = 0.2 (35% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3213, 2935, 1736, 1669; $^1$H NMR (400 MHz, $CDCl_3$) δ: 11.55 (s, 1H), 7.89 (d, *J* = 7.9 Hz, 1H), 7.48 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.11 (dt, *J* = 7.7, 1.4 Hz, 1H), 4.53 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.65 (t, *J* = 5.1 Hz, 2H), 2.86 (s, 2H), 1.30 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.9 (2C), 165.3, 155.3, 147.7, 140.5

(2C), 139.3, 131.9, 128.5 (2C), 128.2, 92.6, 61.5, 60.7, 42.5 (2C), 29.5, 14.5, 14.1; MS (ES mass): 528.7 (M+1); HPLC: 95.5%, column: Symmetry C-18 75*4.6mm 3.5µ, mobile phase A: 0.1% Formic acid in water, mobile phase B: $CH_3CN$, gradient T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention 4.73 min.

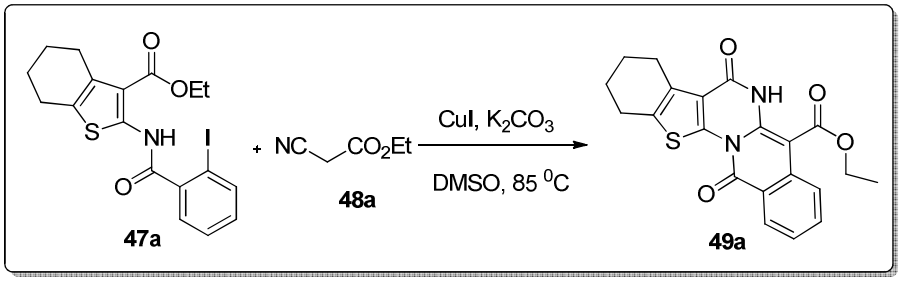
### 1.7.1.22. Typical procedure for preparation of 3-morpholino-3-oxopropane nitrile (48b)




A mixture of ethyl cyanoacetate (1 g, 8.89 mmol) and morpholine (0.87 g, 8.89 mmol) was heated to 130 ºC for 4 h and cooled to room temperature. The solid obtained was washed with ethyl acetate and hexane and filtered off to afford the desired compound (**48**b).

Yield: 75% (1.02 g); brown solid; mp: 81-83 °C; (lit 82-84 ºC); $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 4.01 (s, 2H), 3.60-3.47 (m, 4H), 3.47-3.39 (m, 2H), 3.33-3.27 (m, 2H).

### 1.7.1.23. Typical procedure for preparation of compound (49a)

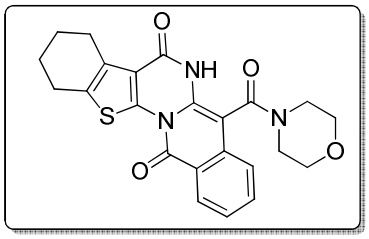



A mixture of compound (**47a)** (100 mg, 0.22 mmol), $K_2CO_3$ (91 mg, 0.66 mmol), ethyl cyano acetate (**48a**) (0.03 mL, 0.31 mmol) and CuI (4.1 mg, 0.022 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions ($CaCl_2$ filled guard tube) for 6 h. After completion of the reaction, reaction mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was

purified by column chromatography using ethyl acetate–hexane to give desired compound (**49a)**.

White solid**;** mp: 232-234 °C; $R_f$ = 0.2 (20% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3096, 2932, 1680, 1638, 1586; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.92 (s, 1H), 8.56 (d, *J* = 8.7 Hz, 1H), 8.50 (dd, *J* = 8.3,1.2 Hz, 1H), 7.73 (t, *J*= 7.2 Hz, 1H), 7.44 (t, *J*= 8.4 Hz, 1H), 4.55 (q, *J* = 7.1 Hz, 2H), 3.05 (dd, *J* = 7.6, 3.5 Hz, 2H), 2.81 (t, *J* = 5.2 Hz, 2H), 1.94-1.82 (m, 4H), 1.51 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.4, 158.5, 155.3, 143.6, 141.5, 134.7, 134.1, 134.0, 130.8, 128.6, 125.3, 125.0, 118.8, 118.7, 86.5, 61.9, 24.8, 23.8, 22.6, 22.1, 14.3; MS (ES mass): 394.9 (M+1).

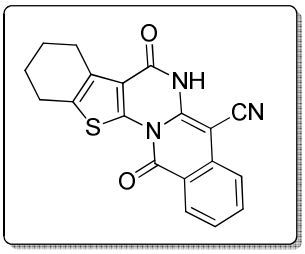
### 1.7.1.24. Compound (49b)




Compound (**49b)** was synthesized from the reaction of (**47a)** and (**48b)** following a procedure similar to that of compound (**49a)**.

Yellow solid; mp: 276-278 °C; $R_f$ = 0.2 (60% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3272, 2941, 1680, 1605; $^1$H NMR (400 MHz, $CDCl_3$) δ: 9.45(s, 1H), 8.52 (d, *J*= 8.4Hz, 1H), 7.73 (t, *J*= 7.2 Hz, 1H), 7.77-7.69 (m, 2H), 4.05-3.15 (m, 8H), 3.08-2.98 (m, 2H), 2.80- 2.04 (m, 2H), 1.95-1.80 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.8, 158.2, 155.2, 141.7, 136.6, 134.2, 134.1, 133.1, 131.0, 129.0, 125.3, 122.4, 118.7, 117.8, 91.7, 66.8 (2C), 46.4, 43.7, 24.6, 23.9, 22.7, 22.1; MS (ES mass): 433.9 (M-1).

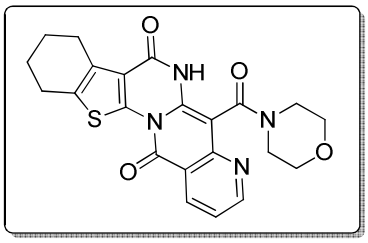
### 1.7.1.25. Compound (49c)



Compound (**49c)** was synthesized from the reaction of (**47a)** and (**48c)** following a procedure similar to that of compound (**49a)**.

White solid; mp: 303-308 °C; $R_f$ = 0.2 (40% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3177, 2934, 2207, 1678, 1606; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.29-8.27 (m, 2H), 7.87 (t, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 2.91-2.86 (m, 2H), 2.77-2.74 (m, 2H), 1.83-1.74 (m, 4H); MS (ES mass): 345.9 (M-1).

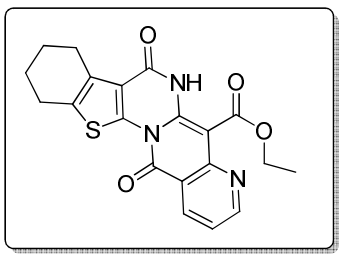
**1.7.1.26. Compound (49d)**




Compound (**49d)** was synthesized from the reaction of (**47a)** and (**48b)** following a procedure similar to that of compound (**49a)**.

Brown solid; mp: 282-284 °C; $R_f$ = 0.2 (80% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3262, 2951, 1686, 1612; $^1$H NMR (400 MHz, $CDCl_3$) δ: 9.77 (s, 1H), 8.96-8.89 (m, 1H), 8.72 (d, *J*=8.4 Hz 1H), 7.38-7.31 (m, 1H), 4.14-3.70 (m, 5H), 3.57-3.30 (m, 2H), 3.28-2.93 (m, 3H), 2.87-2.75 (m, 2H), 1.95-1.83 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.8, 158.2, 155.6, 141.7, 136.6, 134.1, 133.1, 131.0, 129.0, 125.3, 122.4, 118.6, 117.7, 91.7, 66.8 (2C), 44.6, 41.9, 24.9, 23.9, 22.7, 22.1; MS (ES mass): 436.8 (M+1); HRMS (ESI): calcd for $C_{22}H_{21}N_4O_4S$ $(M+H)^+$ 437.1284, found 437.1278.

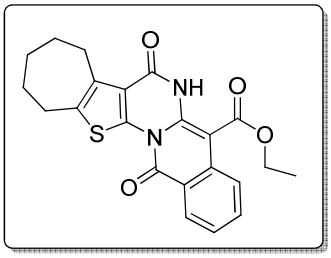
**1.7.1.27. Compound (49e)**




Compound (**49e)** was synthesized from the reaction of (**47b)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Yellow solid**;** mp: 247-249 °C; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3186, 2932, 1688, 1632, 1589; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 12.43 (s, 1H), 9.02-8.99 (m, 1H), 8.69 (dd, *J* = 7.2, 1.4 Hz, 1H), 7.37-7.34 (m, 1H), 4.57 (d, *J* = 7.1 Hz, 2H), 3.04 (t, *J* = 4.8 Hz, 2H), 2.81 (t, *J* = 4.8 Hz, 2H), 1.93-1.84 (m, 4H), 1.48 (t, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.0, 158.5, 155.6, 155.0, 150.6, 144.6, 141.1, 136.5, 134.8, 131.0, 120.1, 118.8, 114.2, 89.7, 62.1, 24.8, 23.9, 22.6, 22.0, 14.2; MS (ES mass): 395.8 (M+1); HRMS (ESI): calcd for $C_{20}H_{18}N_3O_4S$ $(M+H)^+$ 396.1013, found 396.1010.

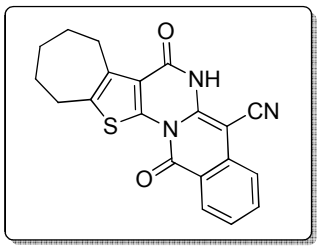
### 1.7.1.28. Compound (49f)




Compound (**49f)** was synthesized from the reaction of (**47c)** and (**48a)** following a procedure similar to that of compound (**49a)**.

White fluffy solid**;** mp: 188-189 °C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3141, 2974, 1682, 1632, 1588; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 12.91 (s, 1H), 8.54 (d, *J* = 8.6 Hz, 1H), 8.45 (d, *J* = 8.5 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 4.54 (q, *J* = 7.1 Hz, 2H), 3.42-3.35 (m, 2H), 2.91-2.82 (m, 2H), 1.98-1.88 (m, 2H), 1.80-1.64 (m, 4H), 1.52 (t, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.4, 158.5, 155.8, 143.4, 139.7, 139.1, 136.3, 134.0, 133.9, 128.5, 125.2, 124.9, 119.1, 118.6, 86.2, 61.9, 32.5, 28.6, 27.8, 27.2, 27.0, 14.3; MS (ES mass): 408.9 (M+1); HRMS (ESI): calcd for $C_{22}H_{21}N_2O_4S$ $(M+H)^+$ 409.1222, found 409.1204.

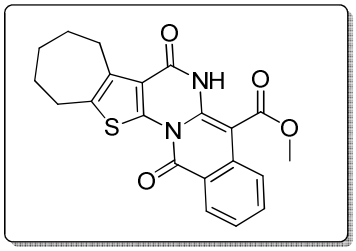
### 1.7.1.29. Compound (49g)



Compound (**49g)** was synthesized from the reaction of (**47c)** and (**48c)** following a procedure similar to that of compound (**49a)**.

White solid**;** mp: 311-313 °C; $R_f$ = 0.2 (40% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3185, 2916, 2849, 2209, 1661, 1548; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.32 (bs, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.87 (t, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 3.28-3.26 (m, 2H), 2.87-2.82 (m, 2H), 1.89-1.82 (m, 2H), 1.64-1.59 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 158.3, 156.9, 144.5, 144.3, 139.9, 138.5, 135.9, 134.3, 128.6, 128.6, 118.8, 118.1 (2C), 115.3, 70.9, 32.4, 28.2, 27.9, 27.2, 26.8; MS (ES mass): 359.9 (M-1); HRMS (ESI): calcd for $C_{20}H_{16}N_3O_2S$ $(M+H)^+$ 362.0963, found 362.0949.

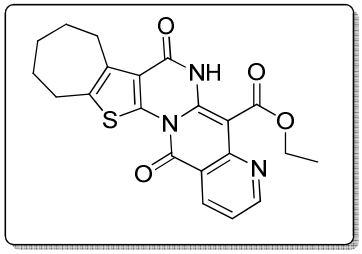
**1.7.1.30. Compound (49h)**




Compound (**49h)** was synthesized from the reaction of (**47c)** and (**48d)** following a procedure similar to that of compound (**49a)**.

Light yellow**;** mp: 239-243°C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3081, 2926, 1682,1650; $^1$H NMR (400 MHz, $CDCl_3$) δ :12.89 (s, 1H), 8.48 (t, *J* = 8.5 Hz, 2H), 7.71 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 1H), 4.06 (s, 3H), 3.41-3.35 (m, 2H), 2.91-2.84 (m, 2H), 1.95-1.89 (m, 2H), 1.77-1.67 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.8, 158.4, 155.7, 143.5, 139.7, 139.2, 136.3, 134.0, 133.7, 128.5, 125.2, 125.0, 119.1, 118.5, 86.0, 52.4, 32.5, 28.6, 27.8, 27.2, 27.0; MS (ES mass): 392.9 (M-1); HRMS (ESI): calcd for $C_{21}H_{19}N_20_4S$ $(M+H)^+$ 395.1066, found 395.1068.

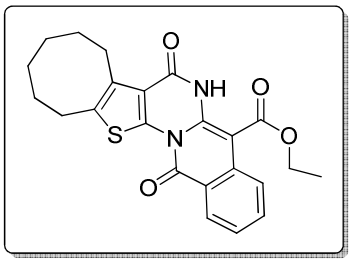
**1.7.1.31. Compound (49i)**



Compound (**49i)** was synthesized from the reaction of (**47d)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Brown solid; mp: 165-167 °C; $R_f$ = 0.2 (60% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3075, 2989, 1689,1643, 1590; $^1$H NMR (400 MHz, $CDCl_3$) δ : 12.47 (bs, 1H), 9.05-8.97 (m, 1H), 8.73 (d, *J* = 6.8 Hz, 1H), 7.40-7.33 (m, 1H), 4.57 (q, *J* = 6.8 Hz, 2H), 3.43-3.35 (m, 2H), 2.93-2.85 (m, 2H), 1.93 (bs, 2H), 1.80-1.65 (m, 4H), 1.48 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.7, 158.3, 155.3, 144.3, 139.2, 138.3, 136.7, 136.5, 126.2, 120.0, 119.1, 114.1, 113.8, 89.0, 63.4, 31.7, 28.5, 27.6, 27.0, 26.9, 14.1; MS (ES mass): 409.9 (M+1); HRMS (ESI): calcd for $C_{21}H_{20}N_3O_4S$ $(M+H)^+$ 410.1175, found 410.1162.

**1.7.1.32. Compound (49j)**

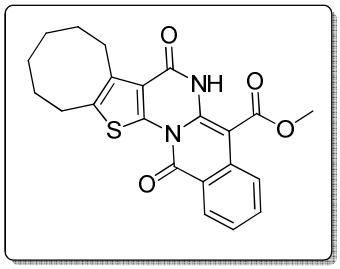


Compound (**49j)** was synthesized from the reaction of (**47e)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Light brown**;** mp: 200-205 °C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3082, 2986, 1680, 1651, 1589; $^1$H NMR (400 MHz, $CDCl_3$) δ : 12.91 (s, 1H), 8.53 (d, *J* = 8.6 Hz, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 1H), 4.54 (q, *J* = 7.1 Hz, 2H), 3.20 (t, *J*= 6.0 Hz, 2H), 2.88 (t, *J*= 5.6 Hz, 2H), 1.80-1.67 (m, 4H), 1.52-1.47 (m, 5H), 1.37-1.27 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.4, 158.5, 155.3, 143.5, 141.0, 137.8, 134.0, 133.9, 133.5, 128.6, 125.3, 125.0,

118.7 (2C), 86.4, 61.9, 32.2, 29.7, 26.0, 25.9, 25.6, 24.2, 14.3; MS (ES mass): 422.8 (M+1); HRMS (ESI): calcd for $C_{23}H_{23}N_2O_4S$ $(M+H)^+$ 423.1379, found 423.1382.

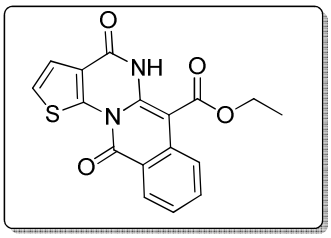
**1.7.1.33. Compound (49k)**




Compound (**49k)** was synthesized from the reaction of (**47e)** and (**48d)** following a procedure similar to that of compound (**49a)**.

Light yellow**;** mp: 224-229 °C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3081, 2993, 1682,1650; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.91 (s, 1H), 8.57-8.44 (m, 2H), 7.73 (t, *J* = 7.2 Hz, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 4.08 (s, 3H), 3.19 (t, *J* = 6.4 Hz, 2H), 2.91 (t, *J* =6.4 Hz, 2H), 1.85-1.67 (m, 4H), 1.65-1.43 (m, 2H), 1.41-1.22 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.9, 158.6, 155.4, 143.6, 139.2, 137.9, 134.1, 133.8, 133.6, 128.6, 125.4, 125.1, 118.8, 118.7, 86.3, 52.4, 32.2, 29.8, 26.0, 25.9, 25.6, 24.3; MS (ES mass): 408.9 (M+1); HRMS (ESI): calcd for $C_{22}H_{21}N_2O_4S$ $(M+H)^+$ 409.1222, found 409.1223.

**1.7.1.34. Compound (49l)**

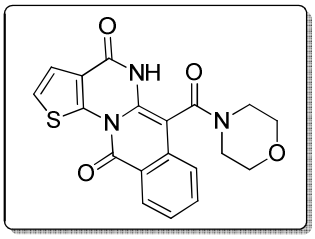



Compound (**49l)** was synthesized from the reaction of (**47f)** and (**48a)** following a procedure similar to that of compound (**49a)**.

White fluffy solid**;** mp: 194-197° C; $R_f$ = 0.2 (40% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3109, 2924, 1681, 1632, 1588; $^1$H NMR (400 MHz, $CDCl_3$) δ : 13.07 (s, 1H), 8.55 (d, *J* = 8.5 Hz, 1H), 8.49 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.74-7.70 (m, 1H), 7.59 (d, *J* = 5.8 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 5.8 Hz, 1H), 4.56 (q, *J* = 7.1 Hz, 2H),

1.53 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.3, 158.2, 154.8, 143.6, 143.2, 134.2, 133.7, 128.5, 125.3, 125.2, 124.6, 121.1, 121.0, 118.5, 87.0, 62.1, 14.3; MS (ES mass): 338.9 (M-1); HRMS (ESI): calcd for $C_{17}H_{13}N_2O_4S$ $(M+H)^+$ 341.0596, found 341.0594.

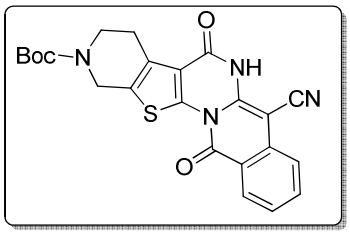
### 1.7.1.35. Compound (49m)




Compound (**49m)** was synthesized from the reaction of (**47f)** and (**48d)** following a procedure similar to that of compound (**49a)**.

Yellow solid**;** mp: 206-210 °C; $R_f$ = 0.2 (80% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3086, 2915, 1678, 1613, 1559; $^1$H NMR (400 MHz, $CDCl_3$) δ : 8.51 (d, $J$ = 8.2 Hz, 1H), 7.77 (t, $J$ = 7.6 Hz, 1H), 7.57 (d, $J$ = 6.0 Hz, 1H), 7.51-7.45 (m, 2H), 7.30 (d, $J$ = 5.8 Hz, 1H), 4.02-3.38 (m, 8H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 163.8, 158.4 (2C), 143.6, 134.6 (2C), 134.1, 128.3 (2C), 125.4, 124.5, 123.2, 121.1, 118.5, 95.1, 66.5, 66.1, 46.9, 42.4; MS (ES mass): 379.9 (M-1); HRMS (ESI): calcd for $C_{19}H_{16}N_3O_4S$ $(M+H)^+$ 382.0862, found 382.0846.

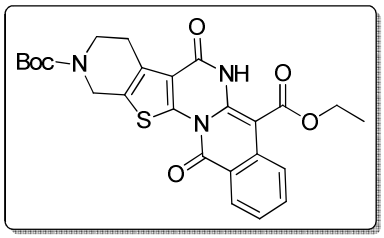
### 1.7.1.36. Compound (49n)




Compound (**49n)** was synthesized from the reaction of (**47g)** and (**48c)** following a procedure similar to that of compound (**49a)**.

Brown solid; mp: 145-147 °C; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3186, 2925, 2215, 1665, 1569; $^1$H NMR (400 MHz, DMSO-*d6*) δ: 8.32-8.30 (m, 1H), 7.91 (t, $J$ = 7.9 Hz, 1H), 7.83 (s, 1H), 7.70 (d, $J$ = 7.6 Hz, 1H), 7.57-7.50 (m, 1H), 4.64 (s, 2H), 3.67-3.60 (m, 2H), 3.00-2.94 (m, 2H), 1.44 (s, 9H); MS (ES mass): 447.0 (M-1);

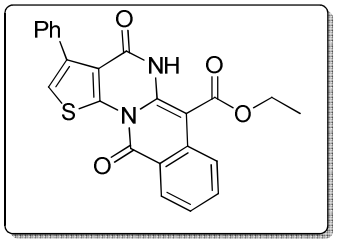
**1.7.1.37. Compound (49o)**




Compound (**49o)** was synthesized from the reaction of (**47g)** and (**48a)** following a procedure similar to that of compound (**49a)**.

White solid**;** mp: 160-163 °C; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3091, 2928, 1689, 1648, 1593; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.98 (s, 1H), 8.57 (d, *J* = 8.0 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.79-7.71 (m, 1H), 7.50-7.41 (m, 1H), 4.68 (s, 2H), 4.56-4.52 (m, 2H), 3.73 (bs, 2H), 3.14 (bs, 2H), 1.57 (bs, 3H), 1.51 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.4, 158.5, 155.1 (2C), 143.4, 138.7 (2C), 138.6, 137.2, 134.3, 133.9, 128.7, 125.4, 125.3, 118.6, 87.0, 80.4, 62.1, 42.1, 42.0, 29.6, 28.4 (3C), 14.3; MS (ES mass): 494.0 (M-1).

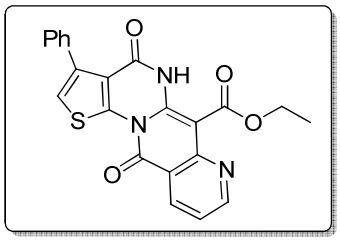
**1.7.1.38. Compound (49p)**




Compound (**49p)** was synthesized from the reaction of (**47h)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Yellow solid**;** mp: 230-232 °C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3087, 2919, 1689, 1644, 1592; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.99 (s, 1H), 8.63-8.57 (m, 1H), 8.57-8.50 (m, 1H), 7.79-7.74 (m, 1H), 7.57-7.54 (m, 2H), 7.49-7.41 (m, 4H), 7.21 (s, 1H), 4.57 (d, *J* = 7.1 Hz, 2H), 1.52 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.2, 158.7, 154.5, 144.8, 143.4, 138.7, 134.6, 134.2, 133.9, 129.4 (2C), 128.6, 127.9, 127.7 (2C), 125.3, 125.1, 122.5, 118.5, 117.5, 86.5, 62.0, 14.2; MS (ES mass): 414.9 (M-1).

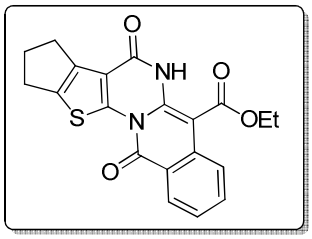
### 1.7.1.39. Compound (49q)




Compound (**49q)** was synthesized from the reaction of (**47i)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Brick red solid**;** mp: 177-179 °C; $R_f$ = 0.2 (60% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3096, 2926, 1680, 1646, 1588; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.37 (s, 1H), 9.03 (d, *J* = 2.9 Hz, 1H), 8.71 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.54-7.51 (m, 2H), 7.45-7.37 (m, 4H), 7.20 (s, 1H), 4.56 (q, *J* = 7.1 Hz, 2H), 1.47 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.6, 158.8, 155.7, 154.1, 150.5, 144.4, 144.3, 138.9, 136.5, 134.3, 130.8, 129.4 (2C), 127.9, 127.7 (2C), 122.4, 120.3, 117.6, 89.7, 62.1, 14.1; MS (ES mass): 417.8 (M+1).

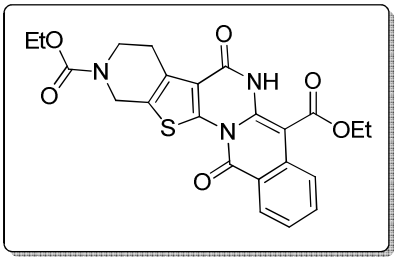
### 1.7.1.40. Compound (49r)




Compound (**49r)** was synthesized from the reaction of (**47j)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Light brown solid; mp: 216-219 °C; $R_f$ = 0.2 (30% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3081, 2993, 1682,1650; $^1$H NMR (400 MHz, $CDCl_3$) δ: 13.00 (s, 1H), 8.55 (d, *J* = 8.7 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.75-7.68 (m, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 4.56 (q, *J* = 7.1 Hz, 2H), 3.11 (t, *J* = 7.2 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H), 2.57-2.48 (m, 2H), 1.52 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.4, 158.4, 155.0, 145.4, 143.7, 141.5, 140.2, 133.9, 133.8, 128.5, 125.3, 125.0, 118.6, 116.0, 86.8, 62.0, 28.9, 28.7 (2C), 14.3; MS (ES mass): 378.9 (M-1).

### 1.7.1.41. Compound (49s)




Compound (**49s)** was synthesized from the reaction of (**49k)** and (**48a)** following a procedure similar to that of compound (**49a)**.

Brown solid**;** mp: 190-192 °C; $R_f$ = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, $cm^{-1}$): 3095, 2935, 1682, 1648, 1589; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.96 (s, 1H), 8.55 (d, *J* = 8.6 Hz, 1H), 8.46 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.77-7.69 (m, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 4.72 (s, 2H), 4.55 (q, *J* = 7.1 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.78 (t, *J* = 5.2 Hz, 2H), 3.15 (s, 2H), 1.52 (t, *J* = 7.1 Hz, 3H), 1.32 (d, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.3, 158.3, 155.5, 154.9, 143.3, 142.3, 134.2 (2C), 133.8, 128.6, 125.4 (2C), 125.2, 118.5, 118.1, 86.9, 62.1, 61.8, 42.2 (2C), 29.6, 14.6, 14.3; MS (ES mass): 395.9 (M-$CO_2$Et+1).

**1.7.2. Single crystal X-ray data for compound (49h).**

Single crystals suitable for X-ray diffraction of (**49h)** were grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data were collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-K*α* radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Bruker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS (Bruker SADABS V2008-1, Bruker AXS.: Madison, WI, USA, **2008**). The crystal structures were solved by direct methods using SHELXS-97 and refined by full matrix least-squares refinement on $F^2$ with anisotropic displacement parameters for non-H atoms, using SHELXL-97 (Sheldrick, G. M.; SHELX-97, Program for Crystal Structure Determination, University of Göttingen, **1997**).

Crystal data of **(49h):** Molecular formula = $C_{21}H_{18}N_2O_4S$, formula weight = 394.44, crystal system = monoclinic, space group = *P*2/c, *a* = 7.09 (1) Å, *b* = 12.017 (16) Å, *c*

= 20.97 (3) Å, $V$ = 1783 (4) Å$^3$, $T$ = 296 K, $Z$ = 4, $D_c$ = 1.469 Mg m$^{-3}$, μ(Mo-Kα) = 0.21 mm$^{-1}$, 17226 reflections measured, 4329 independent reflections, 2496 observed reflections [I > 2.0 σ (I)], $R_1$_obs = 0.071, Goodness of fit =1.04. Crystallographic data (excluding structure factors) for (**49h)** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 902761.

### 1.7.3. Pharmacology

#### 1.7.3.1. PDE4B protein production and purification

PDE4B1 cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 % NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere.[19a] Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4 ºC and supernatant was collected. Supernatant was mixed with Ni-NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris-HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10 % glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 hour at 4 ºC. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4 ºC and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris-HCl pH 8.5, 1 M KCl, 10 mM 2-mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS-PAGE. Eluates containing PDE4B protein were pooled and stored at -80 ºC in 50% glycerol until further use.

**1.7.3.2. PDE4 enzymatic assay**

The inhibition of PDE4 enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 or 0.5 ng commercially procured PDE4D2 enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 µM) for 1 hour. The reaction was halted with stop solution and reaction mix was incubated with detection reagent for 10 minutes in dark. Dose response studies were performed at 13 different concentrations ranging from 200 µM to 0.001 µM. Luminescence values (RLUs) were measured by a Multilabel Plate Reader (PerklinElmer 1420 Multilabel Counter). The percentage of inhibition was calculated using the following formula and the $IC_{50}$ values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, U.S.A). $IC_{50}$ values are presented as mean ± SD.

$$\%\ inhibition = \frac{(RLU\ of\ vehicle\ control - RLU\ of\ inhibitior)}{RLU\ of\ vehicle\ control} X\ 100$$

Some of the synthesized compounds were tested for their PDE4B inhibitory potential *in vitro* at 30 $\mu$M using PDE4B enzyme[7] and rolipram as a reference compound.

**1.7.3.3. Docking Method:**

The docking studies of molecules were performed using the Maestro, version 9.2 (Maestro, version 9.2; Schrodinger, LLC: New York, NY, 2012). The compounds were sketched in 3D format using build panel and LigPrep module was used to produce low-energy conformers and to refine the structural parameters of molecules. The crystal structure of PDE4B (PDB ID: 3D3P) was obtained from the protein data bank. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS-2005 force field. Grids for molecular docking were generated with bound co-crystallized ligand. Compounds were docked using Glide in extra-precision mode (Glide, version 5.7; Schrodinger, LLC: New York, NY, 2012) ,with up to three poses saved per molecule. Ligands were kept flexible by producing

the ring conformations and by penalizing non-polar amide bond conformations, whereas the receptor was kept rigid throughout the docking studies. All other parameters of the Glide module were maintained at their default values. The lowest energy conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with the active sites of PDE4B were determined.

## 1.8. References:


1. (a) Rigby, J. H.; Maharoof, U. S.; Mateo, M. E., *J. Am. Chem. Soc.* **2000**, *122* (28), 6624-6628; (b) Hudlicky, T.; Rinner, U.; Gonzalez, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R., *J. Org. Chem.* **2002**, *67* (25), 8726-8743.
2. Krane, B. D.; Shamma, M., *J. Nat. Prod.* **1982**, *45* (4), 377-384.
3. Glushkov, V.; Shklyaev, Y. V., *Chem. Heterocycl. Compd.* **2001**, *37* (6), 663-687.
4. Pettit, G. R.; Meng, Y.; Herald, D. L.; Graham, K. A.; Pettit, R. K.; Doubek, D. L., *J. Nat. Prod.* **2003**, *66* (8), 1065-1069.
5. Hudlicky, T.; Rinner, U.; Gonzalez, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R., *J. Org. Chem.* **2002**, *67* (25), 8726-8743.
6. (a) Saeed, A.; Ashraf, Z., *Pharm. Chem. J.* **2008**, *42* (5), 277-280; (b) Guastavino, J. F.; Barolo, S. M.; Rossi, R. A., *Eur. J. Org. Chem.* **2006**, *2006* (17), 3898-3902; (c) Asano, Y.; Kitamura, S.; Ohra, T.; Itoh, F.; Kajino, M.; Tamura, T.; Kaneko, M.; Ikeda, S.; Igata, H.; Kawamoto, T., *Bioorg. Med. Chem.* **2008**, *16* (8), 4699-4714; (d) Arnold, N. J.; Arnold, R.; Beer, D.; Bhalay, G.; Collingwood, S. P.; Craig, S.; Devereux, N.; Dodds, M.; Dunstan, A. R.; Fairhurst, R. A., *Bioorg. Med. Chem. Lett.* **2007**, *17* (8), 2376-2379; (e) Croisy-Delcey, M.; Croisy, A.; Carrez, D.; Huel, C.; Chiaroni, A.; Ducrot, P.; Bisagni, E.; Jin, L.; Leclercq, G., *Bioorg. Med. Chem.* **2000**, *8* (11), 2629-2641.
7. (a) Alcock, N. W.; Brown, J. M.; Hulmes, D. I., *Tetrahedron: Asymmetry* **1993**, *4* (4), 743-756; (b) Chen, C.; Li, X.; Schreiber, S. L., *J. Am. Chem. Soc.* **2003**, *125* (34), 10174-10175.

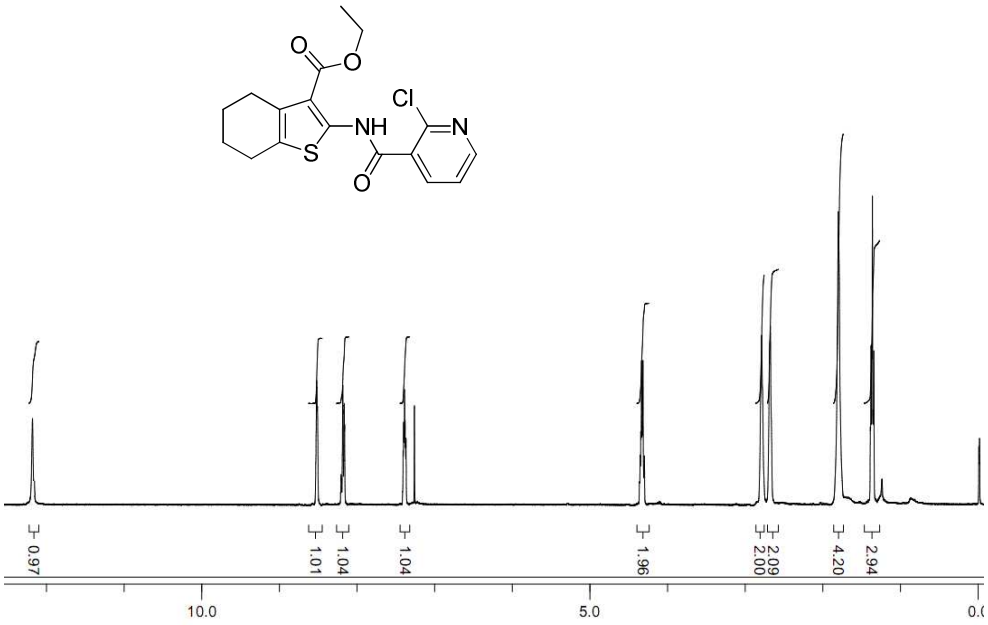
8. (a) Liu, S. J.; Zhao, Q.; Chen, R. F.; Deng, Y.; Fan, Q. L.; Li, F. Y.; Wang, L. H.; Huang, C. H.; Huang, W., *Chem. Eur. J.* **2006**, *12* (16), 4351-4361; (b) Zhao, Q.; Liu, S.; Shi, M.; Wang, C.; Yu, M.; Li, L.; Li, F.; Yi, T.; Huang, C., *Inorg. Chem.* **2006**, *45* (16), 6152-6160.
9. Collado, D.; Perez-Inestrosa, E.; Suau, R.; Desvergne, J.-P.; Bouas-Laurent, H., *Org. Lett.* **2002**, *4* (5), 855-858.
10. Chambhare, R. V.; Khadse, B. G.; Bobde, A. S.; Bahekar, R. H., *Eur. J. Med. Chem.* **2003**, *38* (1), 89-100.
11. Dave, C. G.; Shah, P.; Shah, A.; Dave, K.; Patel, V., *J. Indian Chem. Soc.* **1989**, *66*, 48-50.
12. Baraldi, P. G.; Pavani, M. G.; del Carmen Nunez, M.; Brigidi, P.; Vitali, B.; Gambari, R.; Romagnoli, R., *Bioorg. Med. Chem.* **2002**, *10* (2), 449-456.
13. (a) Elslager, E. F.; Jacob, P.; Werbel, L. M., *J. Heterocycl. Chem.* **1972**, *9* (4), 775-782; (b) Chaykovsky, M.; Lin, M.; Rosowsky, A.; Modest, E., *J. Med. Chem.* **1973**, *16* (3), 188-191; (c) Rodinovskaya, L.; Sharanin, Y. A.; Shestopalov, A.; Litvinov, V., *Chem. Heterocycl. Comp.* **1988**, *24* (6), 658-665; (d) Leistner, S.; Wagner, G.; Guetschow, M.; Glusa, E., *Pharmazie* **1986**, *41* (1), 54.
14. (a) Bousquet, E.; Romeo, G.; Guerrera, F.; Caruso, A.; Amico-Roxas, M., *Farmaco Ed. Sci.* **1985**, *40* (11), 869-874; (b) Bousquet, E.; Guerrera, F.; Siracusa, M.; Caruso, A.; Amico-Roxas, M., *Farmaco Ed. Sci.* **1984**, *39* (2), 110-119.
15. (a) Vieweg, H.; Leistner, S.; Wagner, G.; Boehm, N.; Krasset, U.; Gruppe, R.; Lohmann, D.; Loban, G. *Chem. Abstr.*, **1989**; p 95263; (b) Vieweg, H.; Leistner, S.; Wagner, G.; Boehm, N.; Krasset, U.; Gruppe, R.; Lohmann, D.; Loban, G. *Chem. Abstr.*, **1989**; p 95263.
16. Duval, E.; Case, A.; Stein, R. L.; Cuny, G. D., *Bioorg. Med. Chem. Lett.* **2005**, *15* (7), 1885-1889.
17. Carroll, W. A.; Sippy, K. B.; Esbenshade, T. A.; Buckner, S. A.; Hancock, A. A.; Meyer, M. D., *Bioorg. Med. Chem. Lett.* **2001**, *11* (9), 1119-1121.
18. Kling, A.; Lange, U. E.; Mack, H.; Bakker, M. H.; Drescher, K. U.; Hornberger, W.; Hutchins, C. W.; Möller, A.; Müller, R.; Schmidt, M., *Bioorg. Med. Chem. Lett.* **2005**, *15* (24), 5567-5573.

19. (a) Evano, G.; Blanchard, N.; Toumi, M., *Chem. Rev.* **2008**, *108* (8), 3054-3131; (b) Monnier, F.; Taillefer, M., *Angew. Chem. Int. Ed* **2008**, *47* (17), 3096-3099; (c) Surry, D. S.; Buchwald, S. L., *Chem. Sci.* **2010**, *1* (1), 13-31; (d) Zou, B.; Yuan, Q.; Ma, D., *Angew. Chem. Int. Ed.* **2007**, *46* (15), 2598-2601; (e) Cai, Q.; Li, Z.; Wei, J.; Ha, C.; Pei, D.; Ding, K., *Chem. Commun.* **2009**, (48), 7581-7583; (f) Verma, A. K.; Kesharwani, T.; Singh, J.; Tandon, V.; Larock, R. C., *Angew. Chem. Int. Ed.* **2009**, *48* (6), 1138-1143; (g) Cai, Q.; Li, Z.; Wei, J.; Fu, L.; Ha, C.; Pei, D.; Ding, K., *Org. Lett.* **2010**, *12* (7), 1500-1503.
20. Wang, Y.; Wang, R.; Jiang, Y.; Tan, C.; Fu, H., *Adv. Synth. Catal.* **2013**, *355* (14-15), 2928-2935.
21. Wen, L.-R.; Jin, X.-J.; Niu, X.-D.; Li, M., *J. Org. Chem.* **2014**, *80* (1), 90-98.
22. Liu, T.; Wang, R.; Yang, H.; Fu, H., *Chem. Eur. J.* **2011**, *17* (24), 6765-6771.
23. Lu, J.; Fu, H., *J. Org. Chem.* **2011**, *76* (11), 4600-4605.
24. Wang, F.; Liu, H.; Fu, H.; Jiang, Y.; Zhao, Y., *Org. Lett.* **2009**, *11* (11), 2469-2472.
25. Lou, Z.; Wu, X.; Yang, H.; Zhu, C.; Fu, H., *Adv. Synth. Catal.* **2015**, *357* (18), 3961-3968.
26. Jiang, M.; Li, J.; Wang, F.; Zhao, Y.; Zhao, F.; Dong, X.; Zhao, W., *Org. Lett.* **2012**, *14* (6), 1420-1423.
27. Lu, J.; Gong, X.; Yang, H.; Fu, H., *Chem. Commun.* **2010**, *46* (23), 4172-4174.
28. (a) Zadorozhny, A.; Turov, A.; Kovtunenko, V., *Chem. Heterocycl. Compd.* **2010**, *46* (8), 991-997; (b) Zadorozhny, A.; Kovtunenko, V.; Turov, A., *Chem. Heterocycl. Compd.* **2010**, *46* (8), 957-965.
29. (a) Jiang, M.; Li, J.; Wang, F.; Zhao, Y.; Zhao, F.; Dong, X.; Zhao, W., *Org. Lett.* **2012**, *14* (6), 1420-1423; (b) Lu, J.; Gong, X.; Yang, H.; Fu, H., *Chem. Commun.* **2010**, *46* (23), 4172-4174; (c) Vaillard, V. A.; Rossi, R. A.; Martín, S. E., *Org. Biomol. Chem.* **2011**, *9* (13), 4927-4935.
30. Monnier, F.; Taillefer, M., *Angew. Chem. Int. Ed.* **2009**, *48* (38), 6954-6971.
31. (a) Gewald, K.; Schinke, E.; Böttcher, H., *Chem. Ber.* **1966**, *99* (1), 94-100; (b) Kumar, K. S.; Chamakuri, S.; Vishweshwar, P.; Iqbal, J.; Pal, M., *Tetrahedron Lett.* **2010**, *51* (25), 3269-3273; (c) Sridhar, M.; Rao, R. M.;

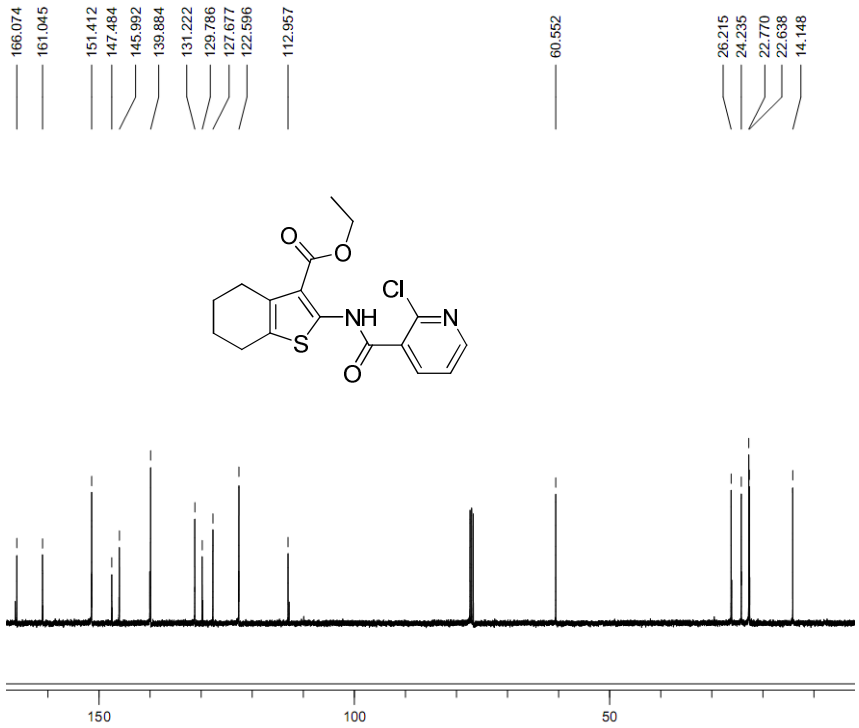
Baba, N. H.; Kumbhare, R. M., *Tetrahedron Lett.* **2007**, *48* (18), 3171-3172; (d) Huang, Y.; Dömling, A., *Molec. Divers.* 2011, *15* (1), 3-33.

32. Wang, P.; Myers, J. G.; Wu, P.; Cheewatrakoolpong, B.; Egan, R. W.; Billah, M. M., *Biochem. Biophys. Res. Commun.* **1997**, *234* (2), 320-324.
33. Houslay, M. D.; Sullivan, M.; Bolger, G. B., *Adv. Pharmacol.* **1998**, *44*, 225-342.
34. Potter, B., 1990.
35. Cheung, W. Y., *Biochemistry* **1967**, *6* (4), 1079-1087.
36. Dastidar, S. G.; Rajagopal, D.; Ray, A., *Curr. Opin. Invest. Drugs.* **2007**, *8* (5), 364-372.
37. Jin, S.-L. C.; Lan, L.; Zoudilova, M.; Conti, M., *J. Immunol.* **2005**, *175* (3), 1523-1531.
38. Robichaud, A.; Stamatiou, P. B.; Jin, S.-L. C.; Lachance, N.; MacDonald, D.; Laliberté, F.; Liu, S.; Huang, Z.; Conti, M.; Chan, C.-C., *J. Clin. Invest.* **2002**, *110* (7), 1045-1052.
39. Schrödinger, M., LLC New York. NY: **2012**.
40. Mimasu, S.; Sengoku, T.; Fukuzawa, S.; Umehara, T.; Yokoyama, S., *Biochem. Biophys. Res. Commun.* **2008**, *366* (1), 15-22.
41. Nakhi, A.; Adepu, R.; Rambabu, D.; Kishore, R.; Vanaja, G.; Kalle, A. M.; Pal, M., *Bioorg. Med. Chem. Lett.* **2012**, *22* (13), 4418-4427.
42. Arya, V., *Ind. J. Chem.* **1972**, *10* (12), 1141-1150.
43. Nankervis, J. L.; Feil, S. C.; Hancock, N. C.; Zheng, Z.; Ng, H.-L.; Morton, C. J.; Holien, J. K.; Ho, P. W.; Frazzetto, M. M.; Jennings, I. G., *Bioorg. Med. Chem. Lett.* **2011**, *21* (23), 7089-7093.

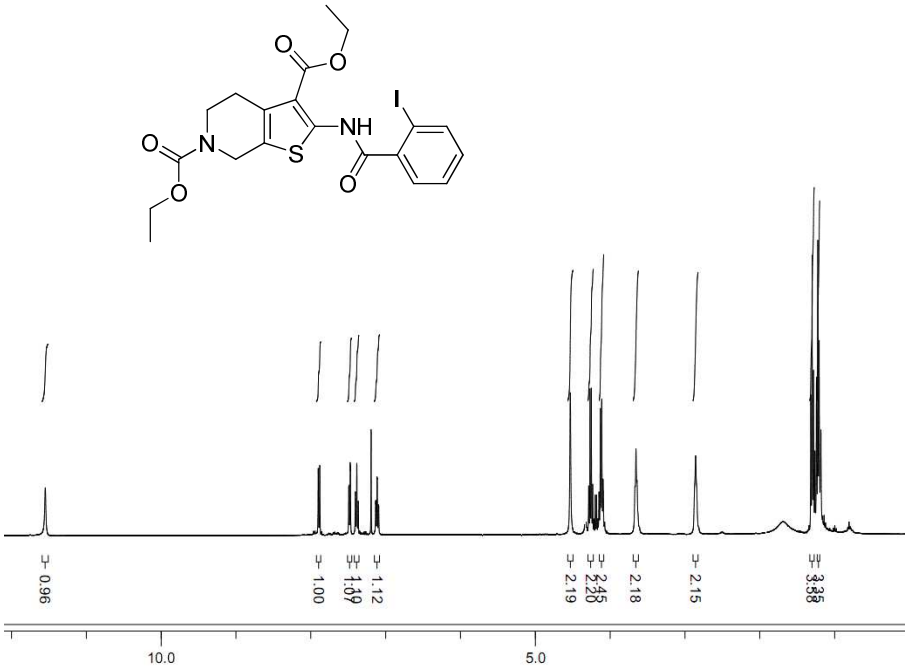
# Appendix



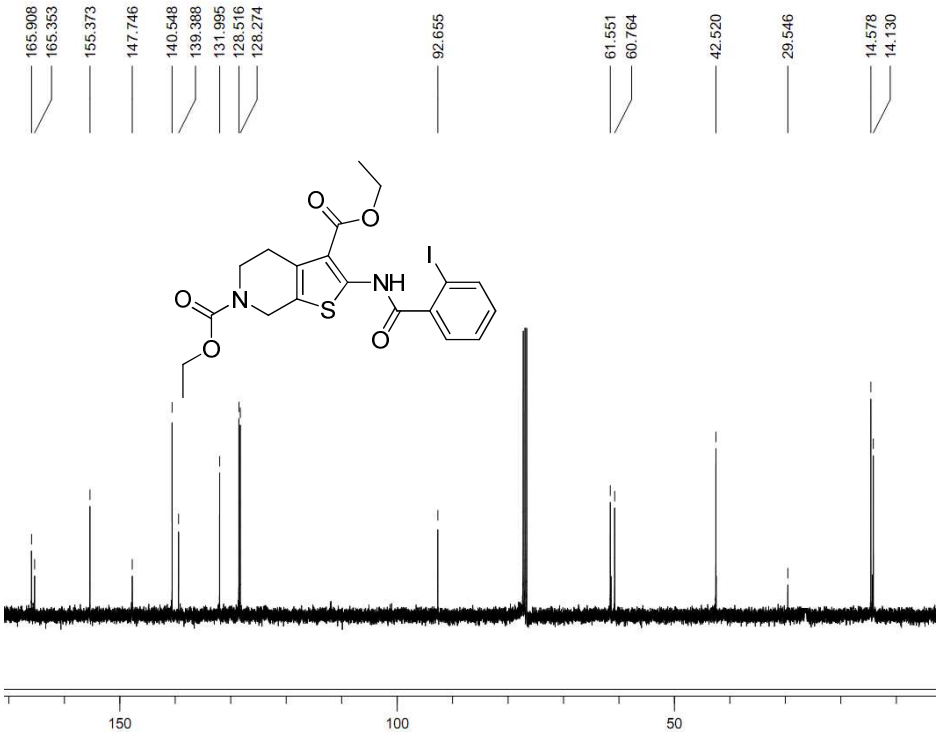

$^{1}$H NMR spectra of compound **47b** ($CDCl_3$, 400 MHz)



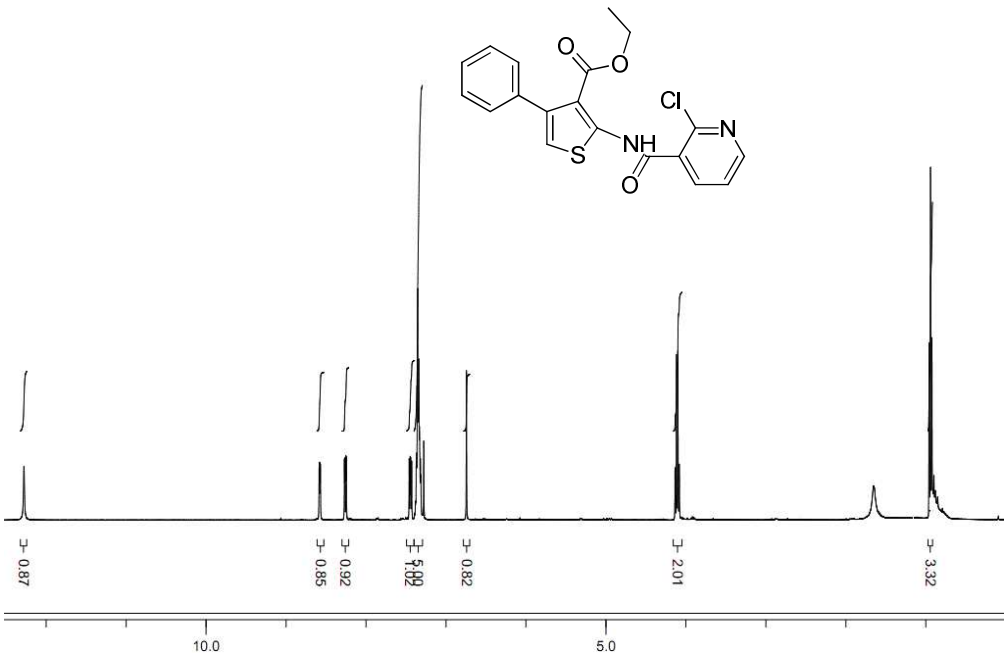

$^{13}$C NMR spectra of compound **47b** ($CDCl_3$, 100 MHz)



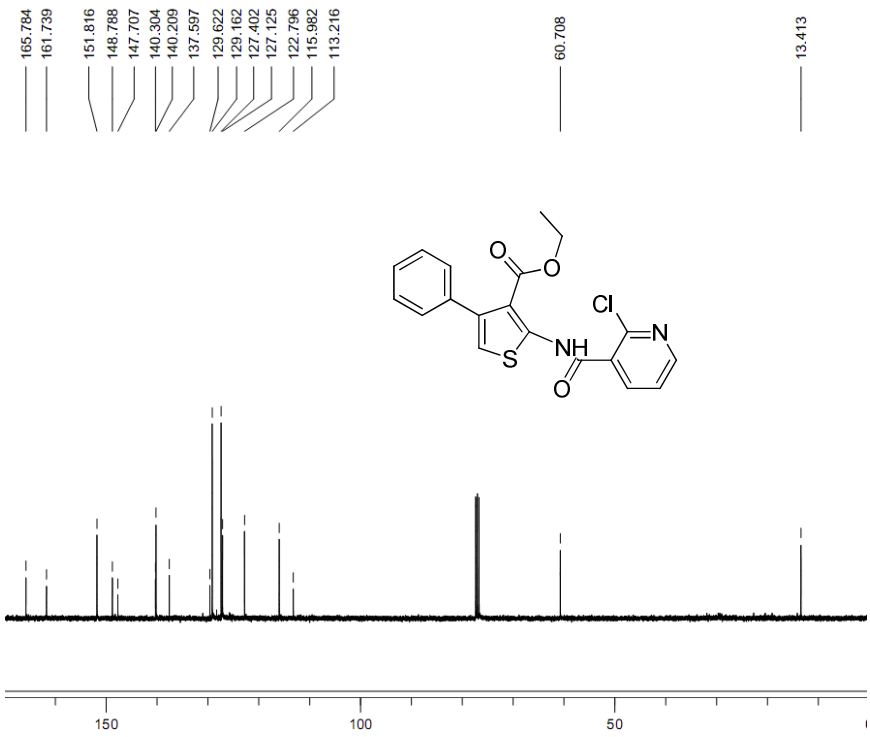

$^{1}$H NMR spectra of compound **47k** ($CDCl_3$, 400 MHz)



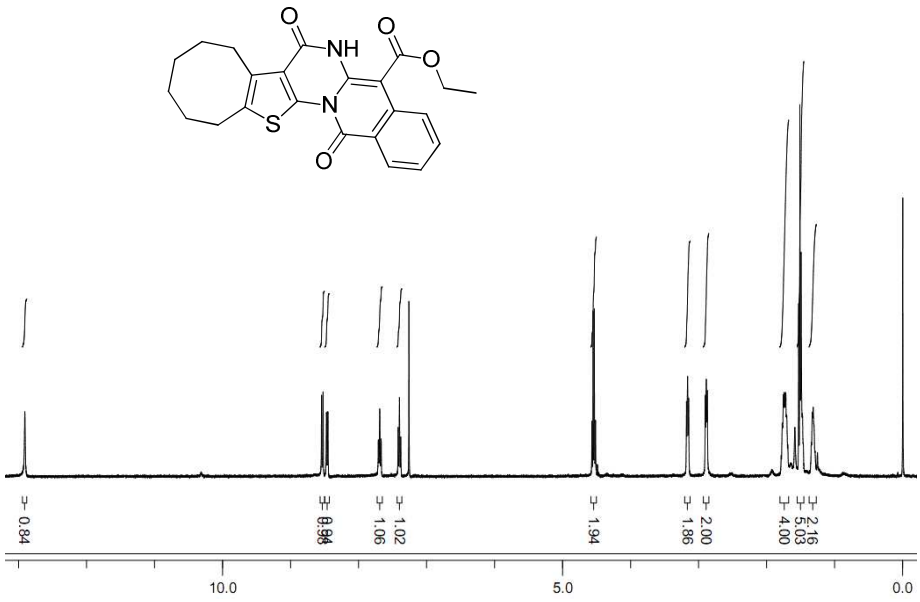

$^{13}$C NMR spectra of compound **47k** ($CDCl_3$, 100 MHz)



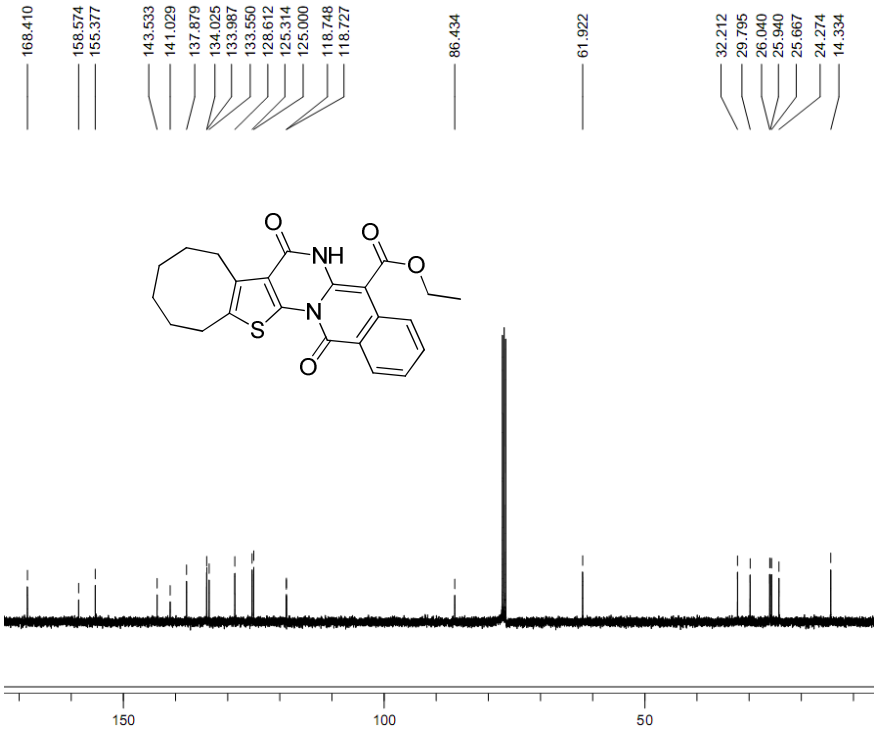

$^{1}$H NMR spectra of compound **47i** ($CDCl_3$, 400 MHz)



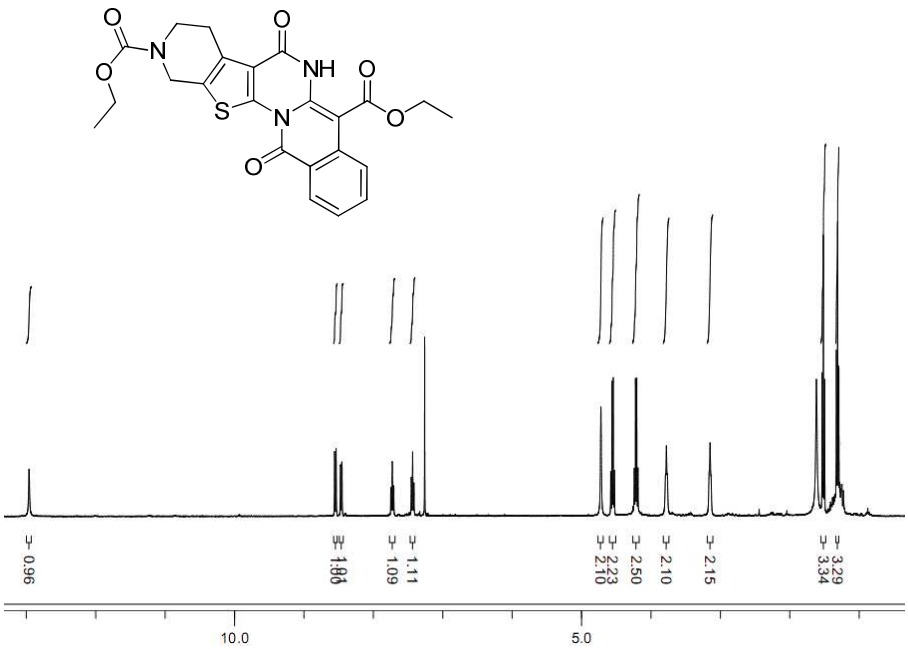

$^{13}$C NMR spectra of compound **47i** ($CDCl_3$, 100 MHz)



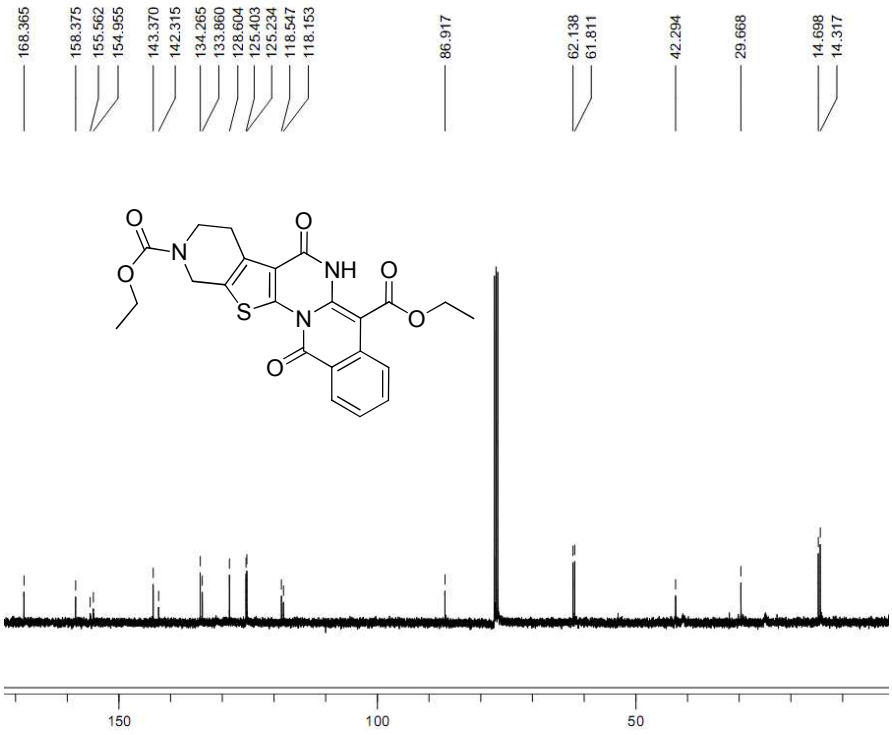

$^{1}$H NMR spectra of compound **49j** ($CDCl_3$, 400 MHz)



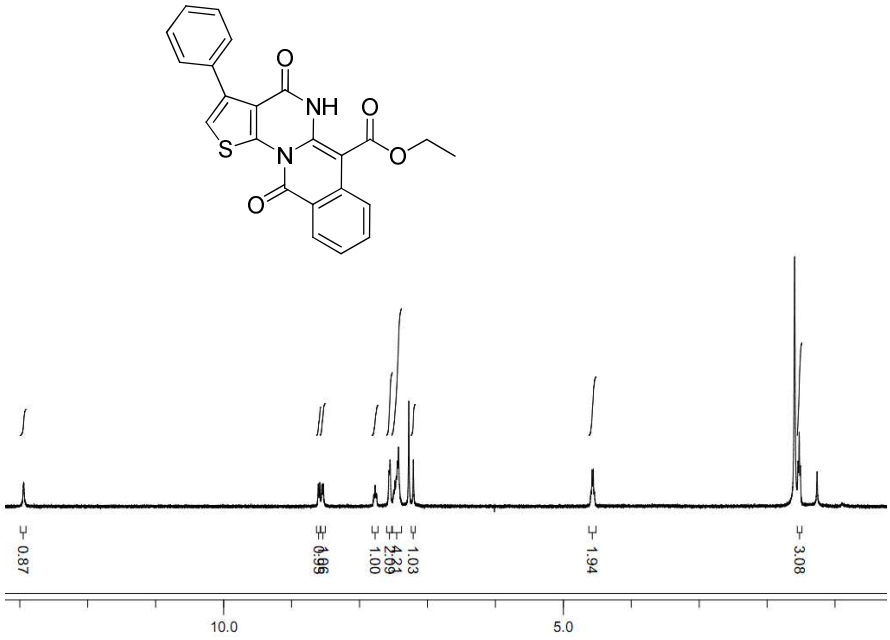

$^{13}$C NMR spectra of compound **49j** ($CDCl_3$, 100 MHz)



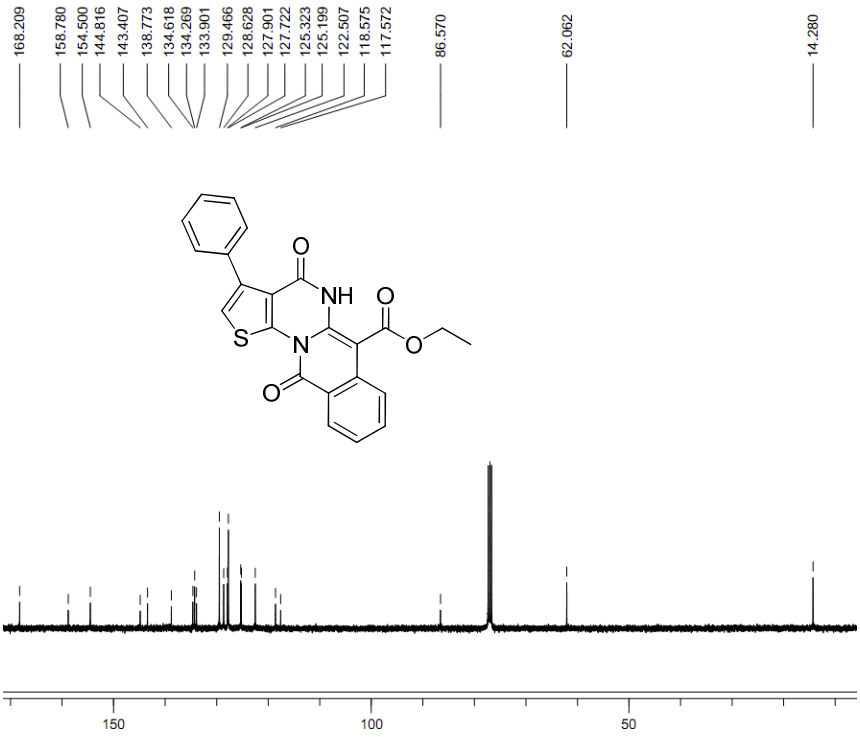

$^{1}$H NMR spectra of compound **49s** ($CDCl_3$, 400 MHz)



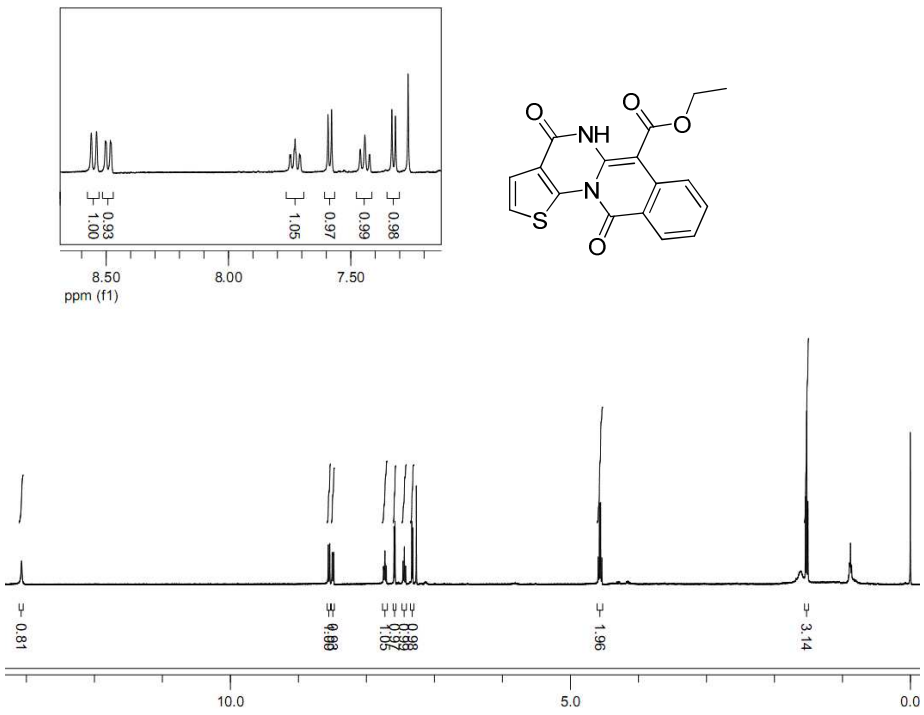

$^{13}$C NMR spectra of compound **49s** ($CDCl_3$, 100 MHz)



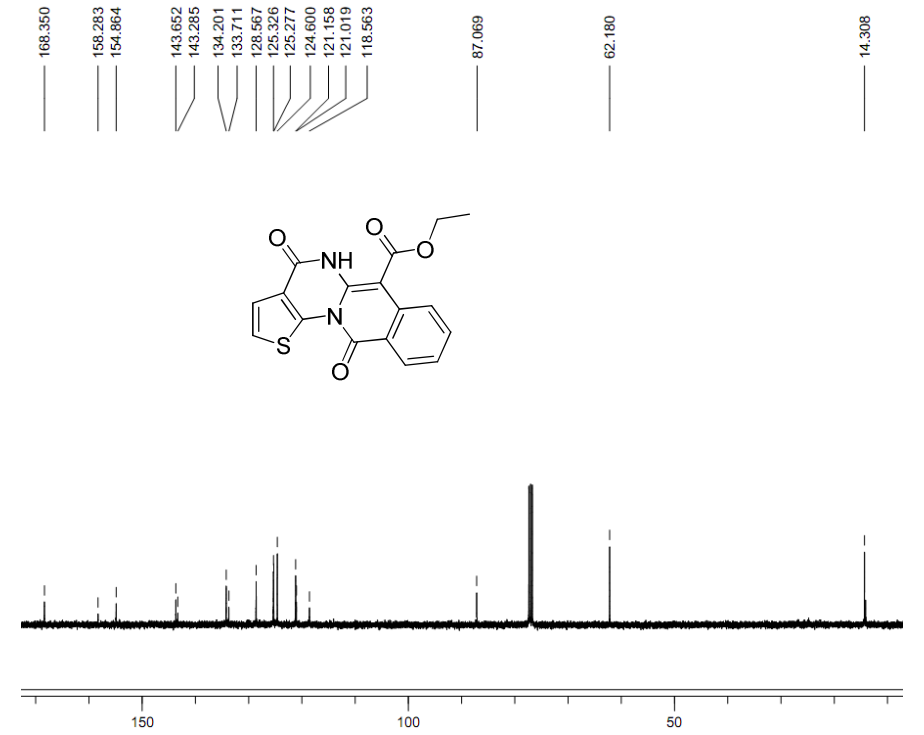

$^{1}$H NMR spectra of compound **49p** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **49p** ($CDCl_3$, 100 MHz)



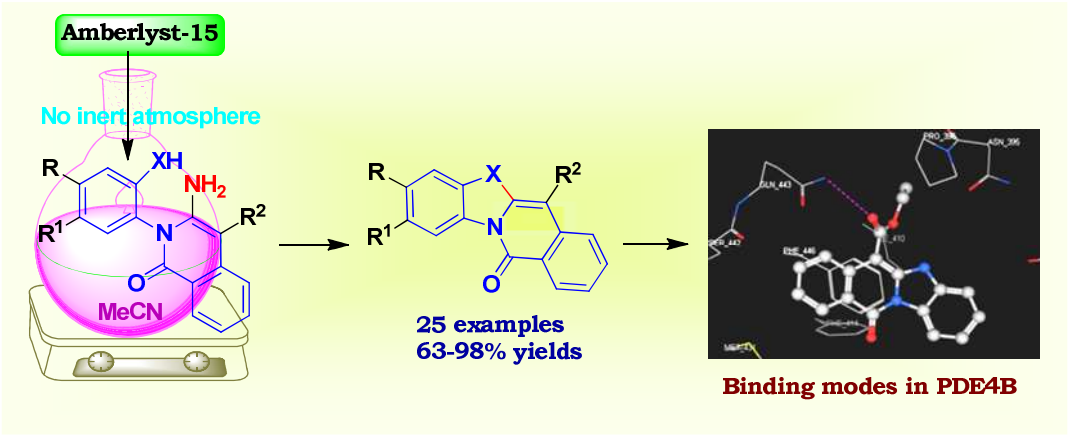

$^{1}$H NMR spectra of compound **49l** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **49l** ($CDCl_3$, 100 MHz)

# CHAPTER 2

# Amberlyst-15 mediated activation of vinylic amino group leading towards the synthesis of benzimidazo/benzoxazoloisoquinolinones



## 2.1. Introduction:

Oxygen and nitrogen containing heterocycles, especially benzimidazole[1] or benzoxazole[2] and isoquinolinone[3] derivatives are omnipresent structural motif owing to their occurrence in numerous natural products and pharmaceutical candidates (**1-8**, Figure 2.1) that exhibit a wide range of biological activities.

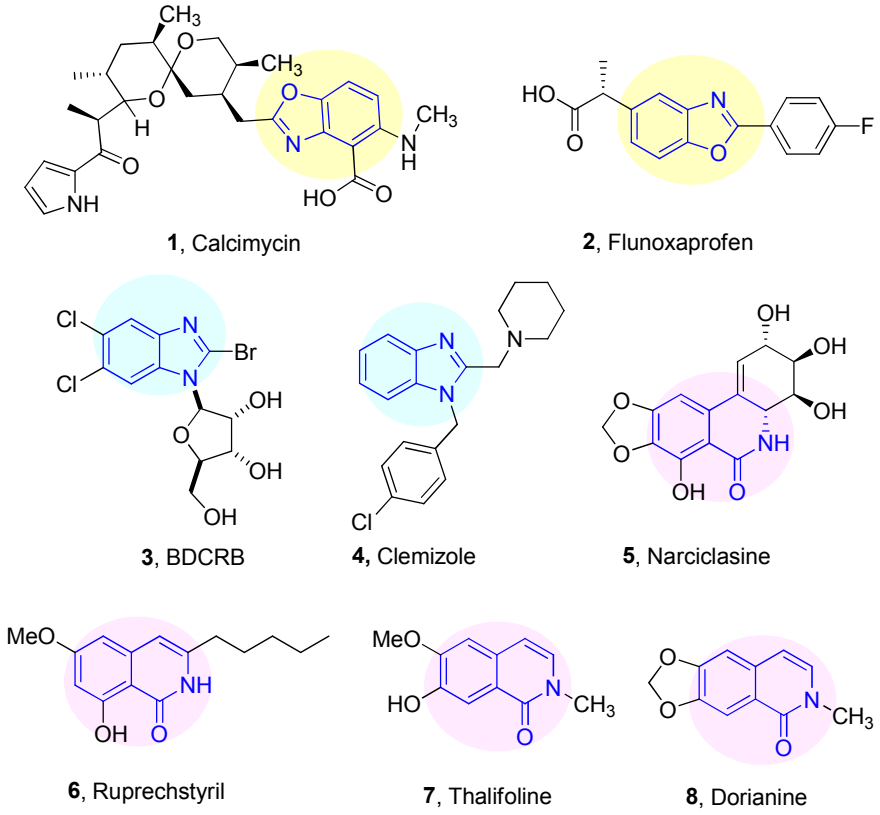



**Fig. 2.1**: Benzoxazole or benzimidazole and isoquinolinone based biologically active scaffolds.

Due to their interesting pharmacological properties, compounds containing the benzimidazole or benzoxazole and isoquinolinone framework have attracted particular attention in medicinal chemistry. However, their combined form *i.e.* (benzimidazo or benzoxazolo) isoquinolinones depicted in Figure 2.2 are largely remained unexplored perhaps due to the limited or no accessibility of this class compounds.[4] This prompted us to explore a new and general method for accessing benzimidazo[1,2-*b*] iso-quinolin-11-one/benzoxazolo[3,2-*b*]iso-quinolin-11-one derivatives. Accordingly, we have developed a protocol mediated by a heterogeneous catalyst *i.e.* Amberlyst-15R

for the synthesis of diverse small organic molecules based on benzimidazo / benzoxolo isoquinolinone framework of potential biological interest.[5]

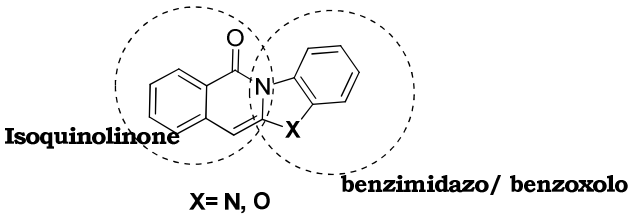



**Fig. 2.2**: Structure of benzimidazo/ benzoxolo isoquinolinones

In recent years economic and environmental concerns have encouraged the application of heterogeneous catalysis in organic synthesis due to the operational simplicity, and environmental compatibility of the procedure and reusability, low cost and nontoxic nature of catalysts and ease of isolation of products. Thus, tremendous upsurge of interest has attracted much attention towards chemical transformation or processes that occur under heterogeneous catalytic conditions. Amberlyst-15 is one of those heterogeneous catalysts that contain a macro reticular polystyrene based ion exchange resin with strongly acidic sulfonic group (Figure 2.3). Thus, it serves as an excellent source of strong acid. It makes reaction processes convenient, environmentally benign, non-corrosive, and more economic. Owing to its interesting properties the cheap and nonhazardous catalyst, Amberlyst-15 has been explored as a powerful catalyst for various organic transformations under mild conditions.

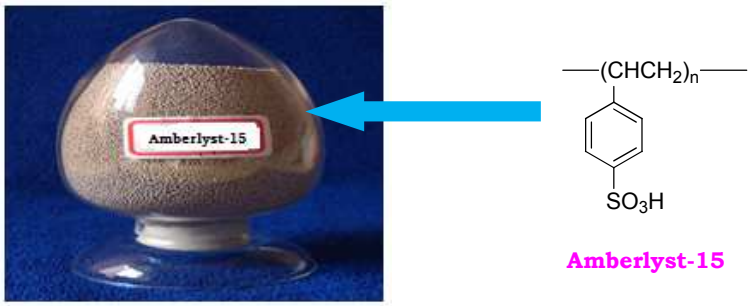
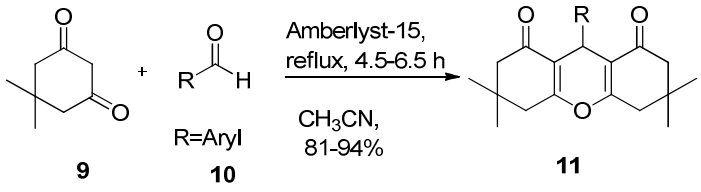



**Fig. 2.3**: **Amberlyst-15**

### 2.1.1. Synthetic application of Amberlyst-15 mediated chemical transformations.

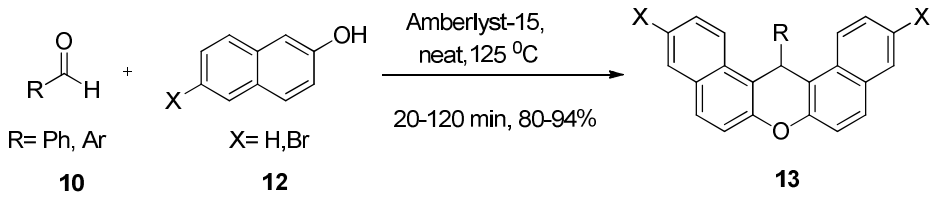
In 2005, Das and co-workers have reported that Amberlyst-15 can act as an excellent catalyst for the synthesis of 1,8-dioxo-octahydroxanthenes (**11**) from aldehydes (**10**) and 5,5-dimethyl-1,3-cyclohexanedione (**9**) in refluxing $CH_3CN$ (Scheme 2.1).[6] Also

they synthesized 1,8-dioxo-decahydroacridines in excellent yields when amines were used along with aldehydes and 5,5-dimethyl-1,3-cyclohexanedione as shown in Scheme 2.1.[6]



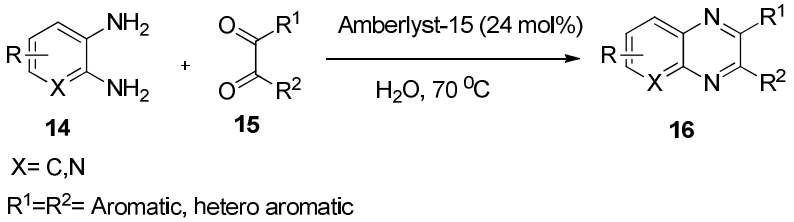

**Scheme 2.1**: Amberlyst-15 catalyzed synthesis of 1,8-dioxodecahydroacridines.

In 2006, Yao and co-workers disclosed the synthesis of biologically active 14-substituted-14*H*-dibenzo[*a*,*j*]xanthenes (**13)** when β-naphthol was reacted with aldehydes in the presence of Amberlyst-15 under a solvent-free condition as shown in Scheme 2.2.[7]



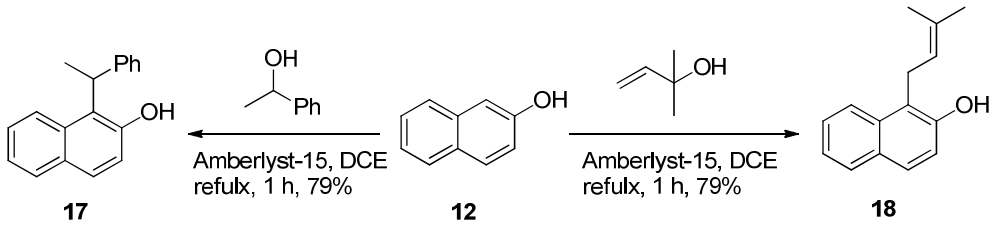

**Scheme 2.2**: Synthesis of 14-substitued-14*H*-dibenzo[*a*,*j*]xanthenes from β-naphthol and aldehydes.

In 2010, Liu and coauthors reported a simple and reliable method for the direct synthesis of quinoxalines (**16)** from diaminobenzene and 1,2-diketone in water at 70 ºC using Amberlyst-15 as a catalyst as shown in Scheme 2.3.[8]



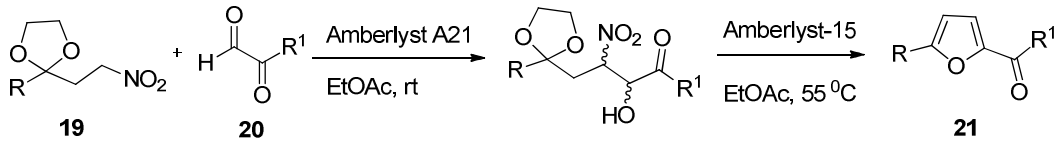

**Scheme 2.3**: Amberlyst-15/$H_2O$ catalyzed synthesis of quinoxalines.

In 2009, Das and coworkers developed benzylation and allylation of naphthols (**12**) by using benzylic and allylic alcohols respectively in the presence of Amberlyst-15 in refluxing 1,2-dichloroethane (DCE) as shown in Scheme 2.4.[9]



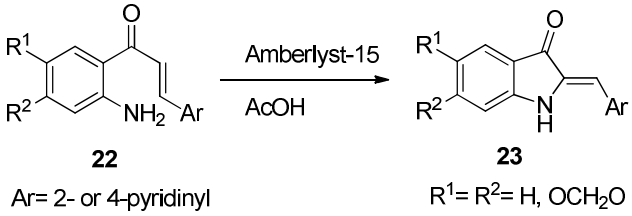

**Scheme 2.4**: Benzylation and allylation of 2-naphthol with Amberlyst-15.

In 2010, Ballini and coworkers demonstrated that Amberlyst-15 can be used for the synthesis of furan derivatives. They synthesized a series of disubstituted furan derivatives (**21)** from functionalized nitroalkane (**19)** that was reacted with aldehydes (**20)** in ethyl acetate in the presence of Amberlyst-A21 and Amberlyst-15 catalysts as shown in Scheme 2.5.[10]



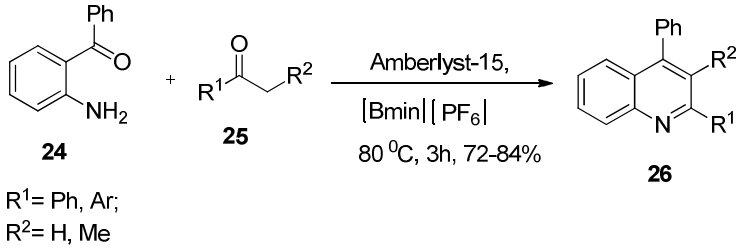

**Scheme 2.5**: Amberlyst-15 catalyzed synthesis of furan derivatives.

In 2008, Abonia and coworkers reported the synthesis of 2-(pyridinylmethylene)indolin-3-one **(23)** from 2'-Aminochalcone (**22)** which underwent intramolecular cyclization in the presence of Amberlyst-15 in AcOH. Unexpectedly, the reaction proceeded through a 5-exo process to give the compound (**23)** as shown in Scheme 2.6.[11]



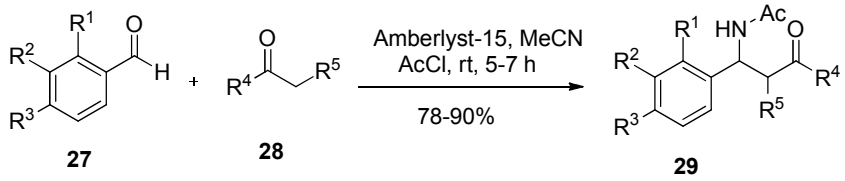

**Scheme 2.6**: Synthesis of 2-(pyridinylmethylene)indolin- 3-ones from 2'-Aminochalcone.

In 2008, Hou and coauthors reported synthesis of quinoline derivatives from 2-aminobenzophenone by using Amberlyst-15 in environmentally benign ionic liquid [Bimn][PF6] as shown in Scheme 2.7.[12]



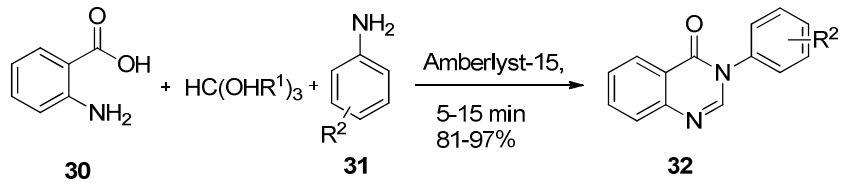

**Scheme 2.7**: Synthesis of quinoline derivatives from 2-aminobenzophenone

In 2006, Das and Reddy developed a simple, mild, and efficient multicomponent reaction for the synthesis of β-acetamido ketones (**29)** using Amberlyst-15 as a reusable catalyst from aromatic aldehydes (**27**), enolizable ketones or keto esters (**28**) in the presence of acetyl chloride (AcCl) in acetonitrile (MeCN) at room temperature as shown in Scheme 2.8.[13] This method offers better yields, shorter reaction times and economic viability compared to the other multicomponent reactions for the synthesis of β-acetamido ketones.




**Scheme 2.8**: Synthesis of β-acetamido ketones from aromatic aldehydes and keto esters.

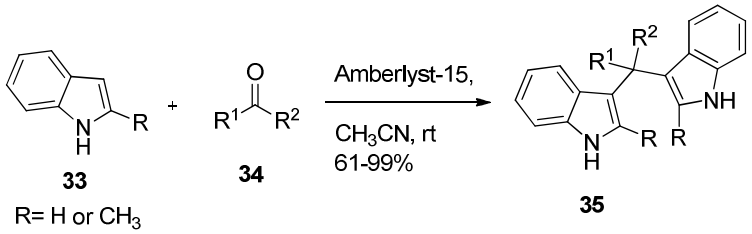
In 2004, Das and Banerjee reported a single-step multicomponent reaction of anthranilic acid, orthoesters and amines leading to the corresponding 4(3*H*)-quinazolines (**32)**, catalyzed by Amberlyst-15 under a solvent-free condition as shown in Scheme 2.9.[14]




**Scheme 2.9**: Synthesis of 4(3*H*)-quinazolines from anthranilic acid, orthoesters and amines.

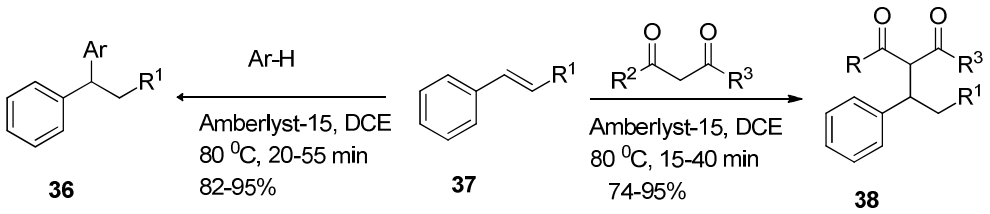
In 2005, Wang and coworkers demonstrated that of Amberlyst-15 can be an effective catalyst for the condensation reactions of indoles (**33)** with aldehydes (**34)** to afford bisindolylalkanes. The reaction was performed by adding Amberlyst-15 to a stirring

solution of indole and carbonyl compounds in acetonitrile at room temperature and the reaction was continued for 4h as shown in Scheme 2.10.[15]



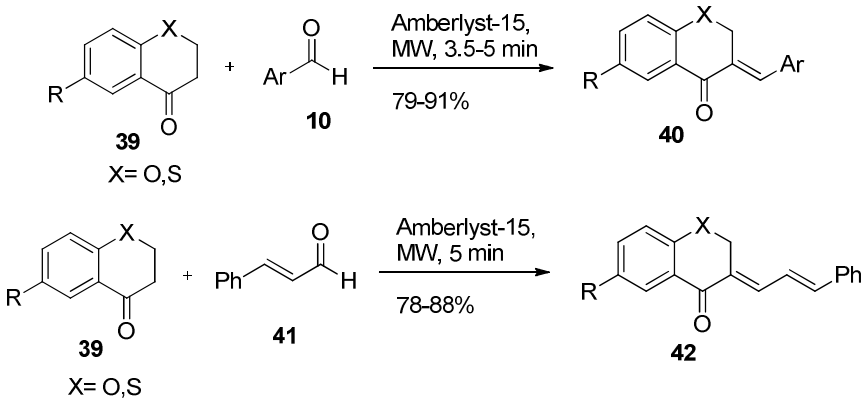

**Scheme 2.10**: Synthesis of bisindolylalkanes from indoles and aldehydes.

In 2009, Das and coworkers developed a metal free C–H functionalization method by using Amberlyst-15 as a catalyst. In this method various styrenes were reacted with different aromatic or 1,3-dicarbonyl compounds in 1,2-dichloroethane (DCE) at 80 ºC The styrenes (**37)** underwent hydroarylation or hydroalkylation reactions to produce the diarylalkanes (**36)** and alkylation products (**38)** respectively as shown in Scheme 2.11.[16]



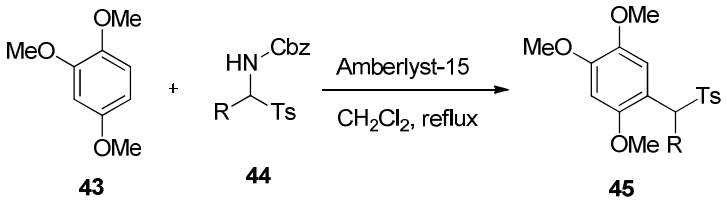

**Scheme 2.11**: Amberlyst mediated C–H functionalization of styrene.

In 2010, Mallik *et al*. reported cross aldol condensation of different aromatic aldehydes (**10**) including cinnamaldehyde (**41**) with chroman-4-ones and 1-thiochroman-4-ones (**39**) in the presence of amberlyst-15 under microwave irradiation in a solvent free condition to afford the corresponding (*E)*-3-arylidene (**40**) and (*E)*-3-cinnamylidene derivatives (**42**), respectively, in high yields as shown in Scheme 2.12.[17]



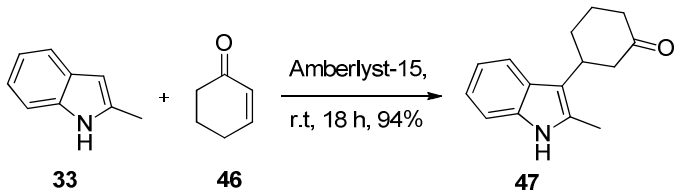

**Scheme 2.12**: Amberlyst mediated cross aldol condensation of aromatic aldehydes.

In 2009, Kadam and coworkers reported that Amberlyst-15 can act as a powerful catalyst for the alkylation of activated arenes or heteroarenes and α-amido sulfones. Friedel-Crafts alkylation of 1,2,4-trimethoxybenzene (**43)** was achieved by using various α-amido sulfones (**44)** in the presence of Amberlyst-15 in refluxing $CH_2Cl_2$ to give the product (**45)** in very good yield as shown in Scheme 2.13.[18]




**Scheme 2.13**: Amberlyst-15-catalyzed Friedel-Crafts alkylation.

Bandini *et al*. reported Michael-type addition of indoles to α,β-unsaturated carbonyl and nitro compounds by using Amberlyst-15 as a reusable catalyst. Michael addition adduct (**47)** was obtained in 94% yield when 2-methylindole was reacted with (**46)** in presence of Amberlyst-15 at room temperature for 18h as shown in Scheme 2.14.[19]




**Scheme 2.14**: Amberlyst-15 catalyzed Michael-type conjugate addition of indoles.

While amberlyst-15 catalyst played an important role in various organic transformations still there is a high demand to explore this catalyst further for

unknown reactions. In this context we have discovered a new application of Amberlyst-15R (a mild acidic resin) for the synthesis of our target molecule (**B**) the design of which is presented in the following section.

### 2.1.2. Design of target B:

Due to our longstanding interest in the identification of novel PDE4 inhibitors[20] we designed our target molecules **B** from a known inhibitor CP-77059 (or methyl 3-(3-benzyl-2,4-dioxo-3,4-dihydropyrido[2,3-*d*]pyrimidin-1(2*H*)-yl)benzoate).[21] We performed some structural modifications of the core framework of CP-77059 as shown in Figure 2.4. Thus the bicyclic ring of CP-77059 was transformed into a tetracyclic ring followed by replacing the *N*-aryl group with an ester moiety to reach the stricture **A**. Indeed, the docking studies as presented in the next section suggested that **A** could be a promising template for further exploration. Therefore, a more generic structure **B** was designed for the generation of library of molecules.

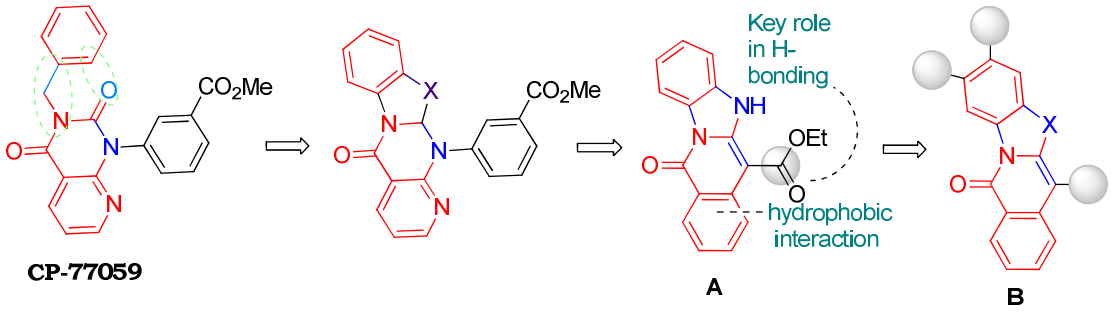



**Fig. 2.4**: Design of **A**/**B** as novel inhibitors of PDE4

### 2.1.3. Protein-Molecular Interactions in the Docking Pose of Molecule (49a) with PDE4B:

The compound **A** and a well known inhibitor of PDE4 e.g. rolipram were docked into the PDE4B protein and their respective Docking scores and interactions were observed. The study showed binding of **A** deep into the active site (docking score -22.07) along with an H-bond interaction of carbonyl oxygen of ester with the side chain amino group of Gln 443 (Figure 2.5). We were particularly encouraged by the results of docking studies of our representative compound **A** with PDE4 enzyme that prompted us to make a plan for the synthesis of our target molecules.

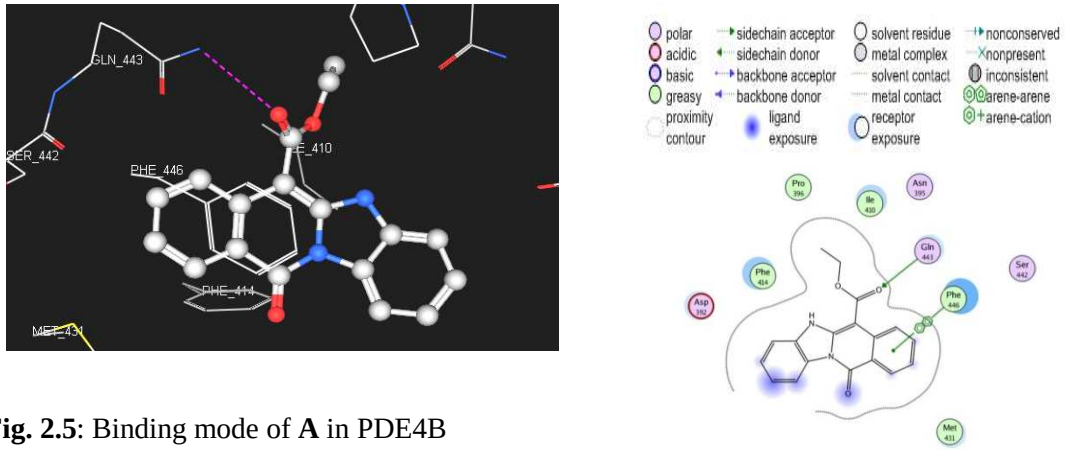



**Fig. 2.5**: Binding mode of **A** in PDE4B (PDB code-1XMY).

The docking Simulation was done with Chemical Computing Group's Molecular Operating Environment (MOE) software 2008.10 Version, "DOCK" application Module.

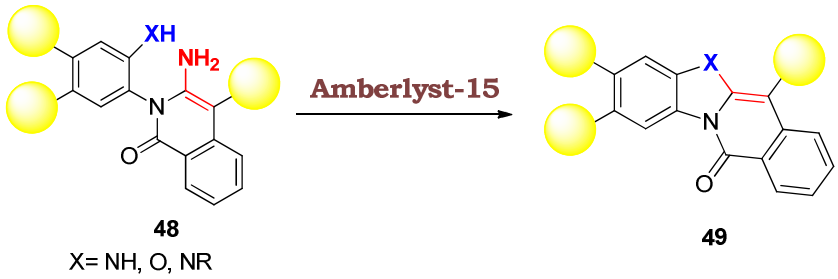
The following Dock scores were obtained after docking with PDE4B protein:-

| **MOE Dock score(K.cal/mol)** | |
|---|---|
| **Molecule** | PDE4B |
| Rolipram | -22.94 |
| **A** | -22.07 |

## 2.2. Results and discussion:

### 2.2.1. Synthetic strategy for target molecule (49):

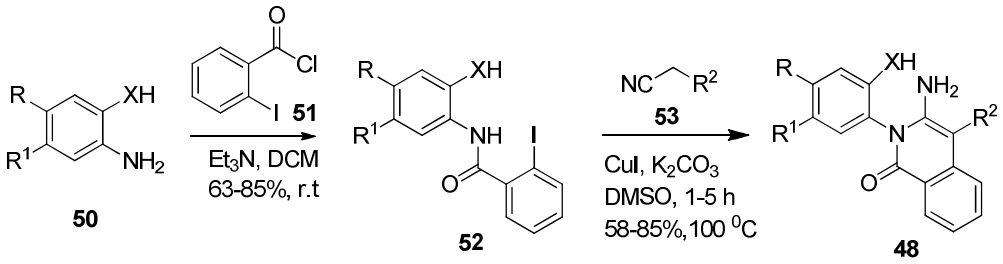
We explored a novel method for the synthesis of our target molecules based on fused benzimidazo/benzoxazoloisoquinolinone. The synthetic strategy involved activation of vinylic amino group of compound (**48)** that could potentially trigger intramolecular cyclization leading to (**49)** (or **B**, Scheme 2.15). We anticipated that the mild acidic resin Amberlyst-15 could play an important role in the synthesis of designed target molecules as shown in Scheme 2.15. Preparation of starting materials and other reaction sequences are described in the following section.




**Scheme 2.15**: Amberlyst-15 mediated activation of a vinylic amino group of (**48)**

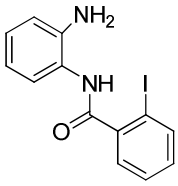
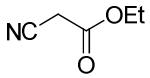
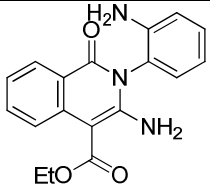
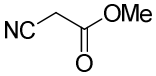
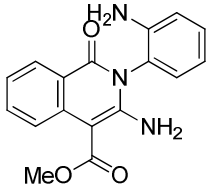
### 2.2.2. Preparation of key starting material (48) & (54):

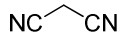
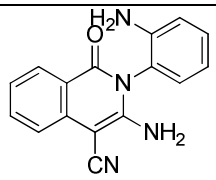
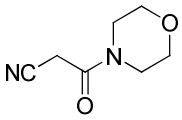
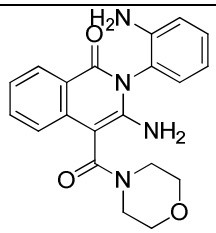
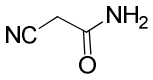
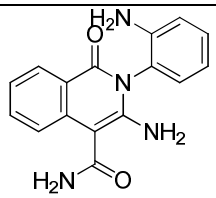
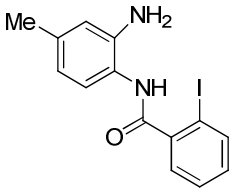
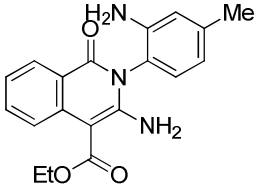
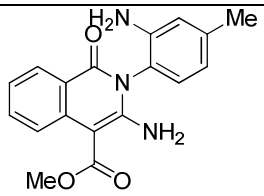
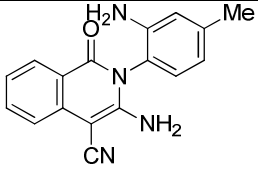
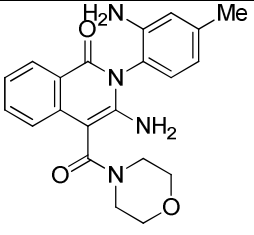
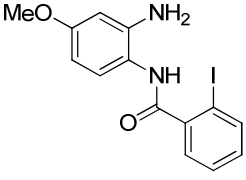
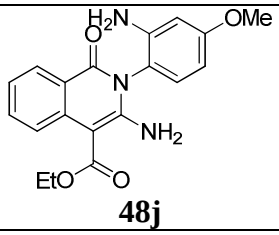
The required key starting material 3-amino-2-aryl-1-oxo-1,2-dihydroisoquinoline (**48**), was synthesized from commercially available benzene-1,2-diamine or 2-aminophenol *via* coupling with 2-iodo benzoyl chloride (5**1**), followed by copper catalyzed cascade reaction (mechanism of this cascade reaction is explained in Chapter 1) as shown in Scheme 2.16.[22] A list of compound synthesized is presented in Table 2.1.

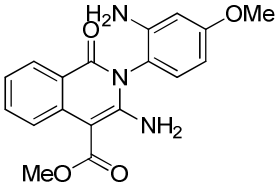
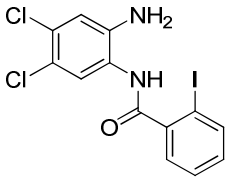
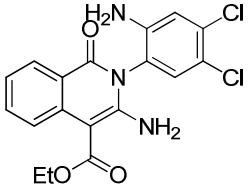
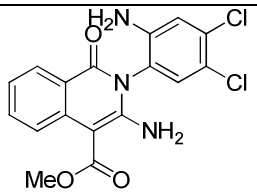
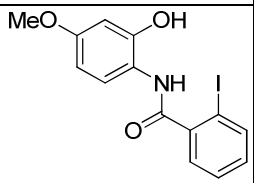
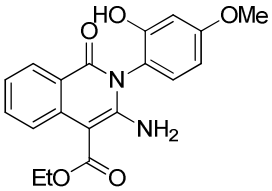
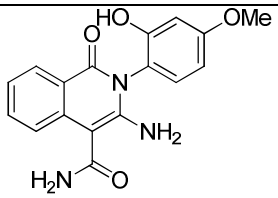
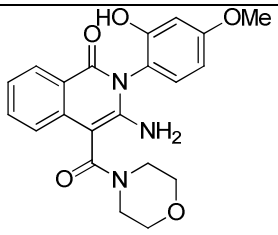
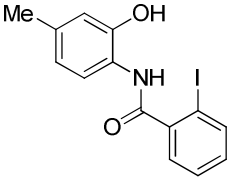
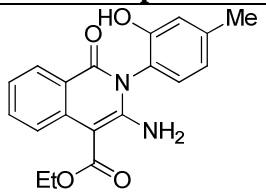



**Scheme 2.16**: Synthesis of key starting material (**48)**.

**Table 2.1:** Synthesis of 3-amino-2-aryl-1-oxo-1,2-dihydroisoquinoline (**48**).[a]

| Entry | Substrate (**52**) | Substrate (**53**) | Time/ h | Product (**48**) | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 |  **52a** |  **53a** | 1.0 |  **48a** | 72 |
| 2 | **52a** |  **53b** | 1.0 |  **48b** | 69 |

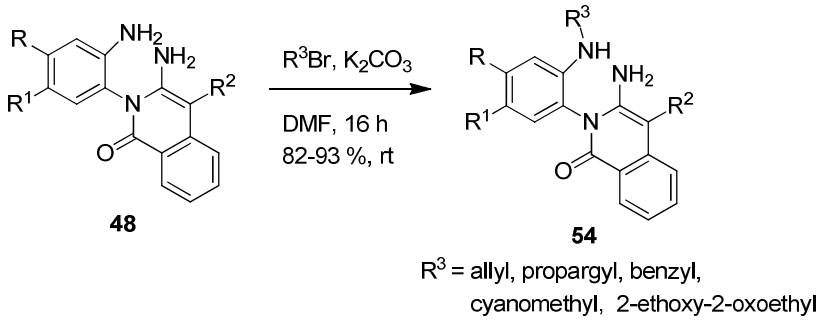
| | | | | | |
|---|---|---|---|---|---|
| 3 | **52a** |  **53c** | 4.0 |  **48c** | 67 |
| 4 | **52a** |  **53d** | 3.0 |  **48d** | 58 |
| 5 | **52a** |  **53e** | 3.0 |  **48e** | 64 |
| 6 |  **52b** | **53a** | 1.0 |  **48f** | 65 |
| 7 | **52b** | **53b** | 1.0 |  **48g** | 64 |
| 8 | **52b** | **53c** | 4.0 |  **48h** | 68 |
| 9 | **52b** | **53d** | 3.0 |  **48i** | 64 |
| 10 |  | **53a** | 3.0 |  **48j** | 83 |

| | | | | | |
|---|---|---|---|---|---|
| | **52c** | | | | |
| 11 | **52c** | **53b** | 4.0 |  **48k** | 83 |
| 12 |  **52d** | **53a** | 1.0 |  **48l** | 85 |
| 13 | **52d** | **53b** | 1.0 |  **48m** | 84 |
| 14 |  **52e** | **53a** | 3.0 |  **48n** | 68 |
| 15 | **52e** | **53e** | 5.0 |  **48o** | 63 |
| 16 | **52e** | **53d** | 5.0 |  **48p** | 60 |
| 17 |  **52f** | **53a** | 3.0 |  **48q** | 65 |

[a]All the reactions are carried out using compound **52** (1 mmol), **53** (1.2 mmol), $K_2CO_3$ (2.0 mmol) and 10 mol% CuI in DMSO (5 mL) at 85 °C under anhydrous conditions.

[b]Isolated yield.

The requisite Ethyl-2-(2-(alkylamino)phenyl)-3-amino-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**54),** was synthesized from (**48)** by selective *N*-alkylation as shown in Scheme 2.17.

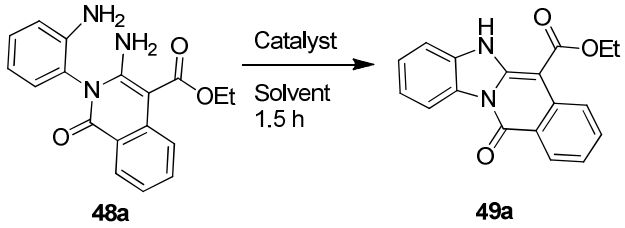



**Scheme 2.17**: Synthesis of key starting material (**54)**.

### 2.2.3. Reaction optimization:

The Amberlyst-15 mediated intramolecular cyclization of ethyl-3-amino-2-(2-aminophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**48a**) was examined initially in a variety of solvents to establish the optimized reaction conditions (Table 2.2).

**Table 2.2**: Reaction conditions and optimization.




| Entry | Catalyst | Solvent | Yield[b] (%) |
|---|---|---|---|
| 1 | Amberlyst-15 | MeCN | 98 (95, 90, 88)[c] |
| 2 | Amberlyst-15 | PEG-800 | 92 |
| 3 | Amberlyst-15 | DMF | 47 |
| 4 | Amberlyst-15 | MeOH | 90 |
| 5 | Amberlyst-15 | $H_2O$ | 75[d] |
| 6 | No cat. | MeCN | No reaction |
| 7 | Amberlite | MeCN | No reaction |

[a]Reaction was carried out using **48a** (1.0 mmol), catalyst (10%, w/w) in solvent (5 mL) at 60 °C. [b]Isolated yield. [c]Catalyst was reused for additional three runs and figures within

parentheses indicate the corresponding yields for each run. [d]The reaction was carried out at 75 °C.

The reaction proceeded well in MeCN, PEG and MeOH (entries 1, 2 and 4, Table 2.2) but not in DMF (entry 3, Table 2.2). Notably, the reaction also proceeded in water affording the product (**49a)** albeit in lower yield (entry 5, Table 2.2). The reaction however, did not proceed in the absence of Amberlyst-15 (entry 6, Table 2.2) indicating the key role played by the catalyst in the present reaction. The reaction also did not proceed in the presence of another catalyst *i.e.* Amberlite (entry 7, Table 2.2). To test the recyclability of the catalyst, Amberlyst-15 was recovered by simple filtration and reused for additional three times when (**49a)** was isolated without significant loss of its yield (entry 1, Table 2.2). Notably, all these reactions do not require the use of any inert atmosphere. Overall, Amberlyst-15 in MeCN was found to be optimum for the preparation of (**49a)**.

### 2.2.4. Scope of the reaction:

We then examined the generality and substrate scope of the present reaction. A range of substituents e.g. Me, OMe, Cl on the *N*-aryl ring and ester, CN, amide on the isoquinolinone ring of (**48)** were well tolerated (Table 2.3). Moreover, the reaction proceeded well irrespective of the nature of participating amino group (e.g. primary or secondary) on the *N*-aryl ring of (**48)**. Secondary amine possessing various $R^3$ groups like allyl, propargyl, benzyl, cyanomethyl or 2-ethoxy-2-oxoethyl participated well in the reaction. The generality of this methodology was demonstrated further by synthesizing benzoxazolo[3,2-*b*]isoquinolin-11-ones (**49v-y**) where phenolic hydroxyl group of *N*-aryl ring of (**48)** participated in the reaction. All the desired products were synthesized in good to excellent yields[7] and well characterized by spectral (NMR, IR and MS) data. Some characteristic signals appeared in $^1$H and $^{13}$C NMR spectra of a representative compound (**49j)** are shown in the following figure (Figure 2.6). The appearance of peaks at δ 12.09 ppm in $^1$HNMR spectra of (**49f)** was due to NH proton, singlet at δ 3.83 is due to methoxy group present in aromatic ring and peaks at δ 4.54, δ 1.52 were corresponding to ester group. The $^{13}$C signals of carbonyl groups present in compound (**49j)** observed at δ166.4 (ester) and δ159.3 (amidic) as shown in (Figure 2.6).

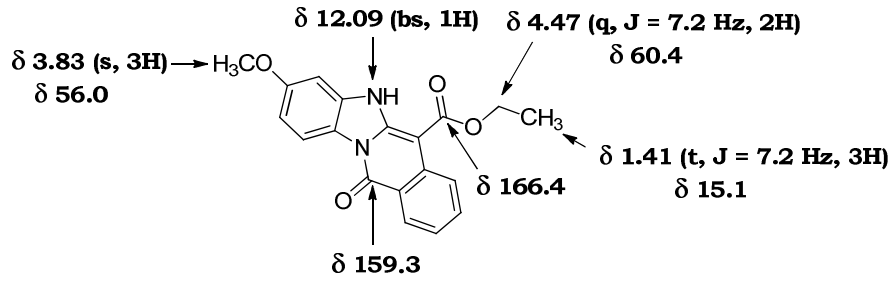
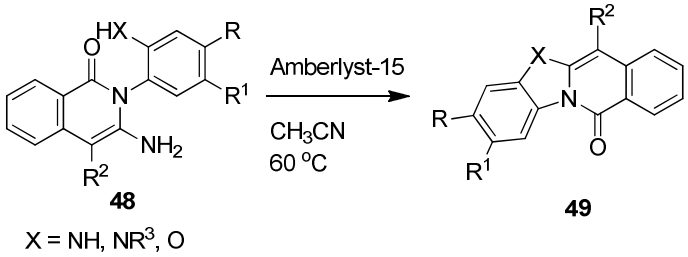
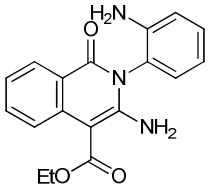
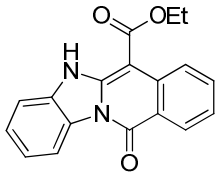
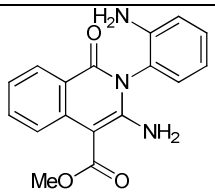
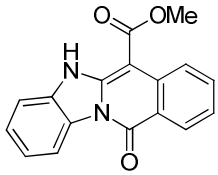
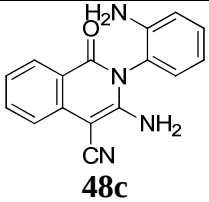
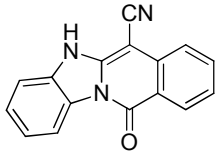
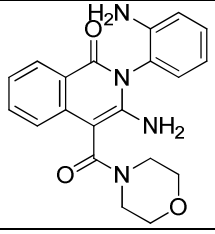
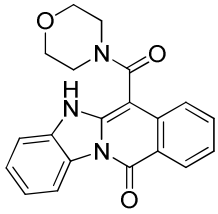
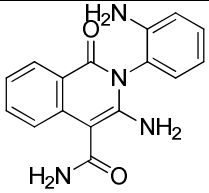
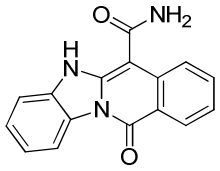
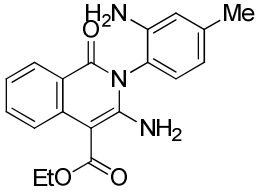
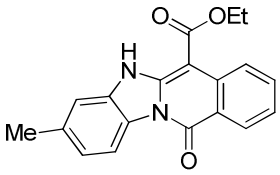
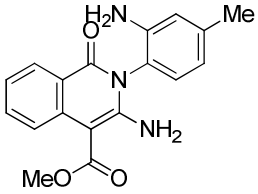
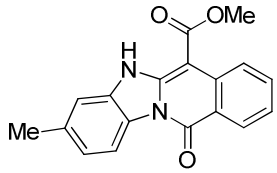
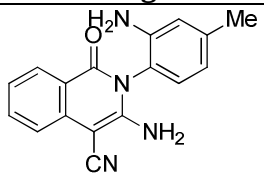
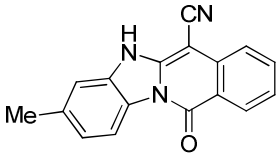
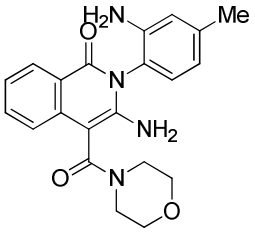
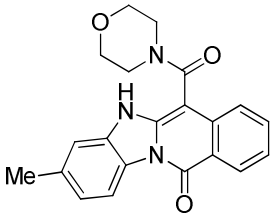
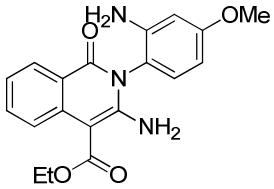
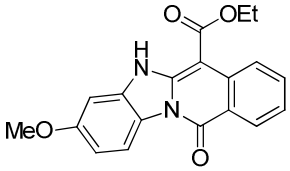
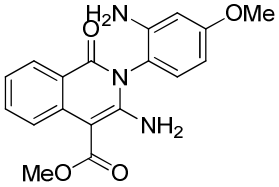
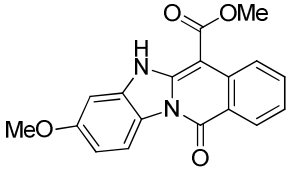
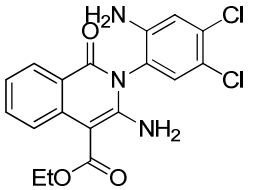
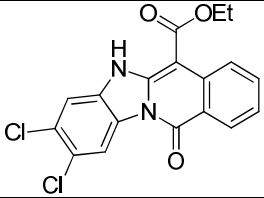


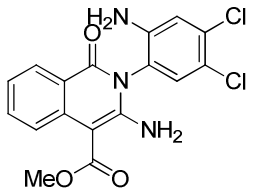
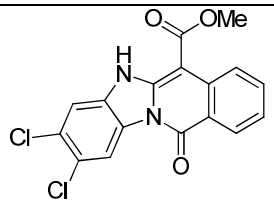
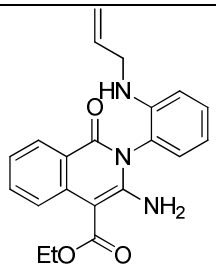
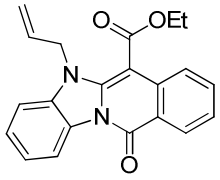
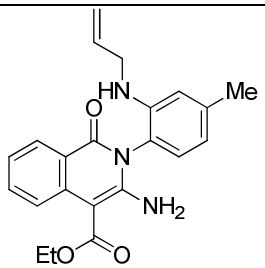
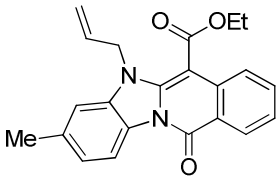
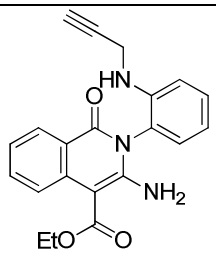
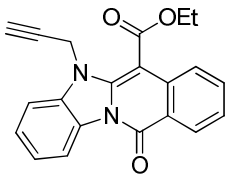
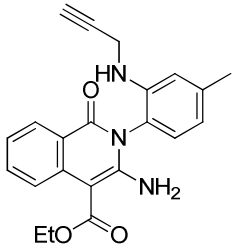
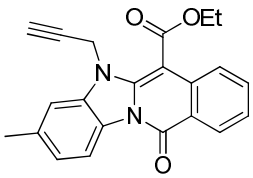
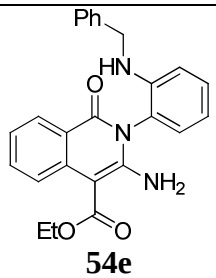
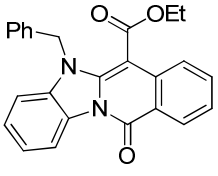

**Fig. 2.6**: Characteristic $^{1}H$ and $^{13}C$ NMR peaks of (**49j)**

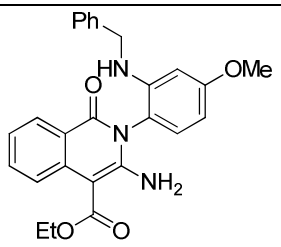
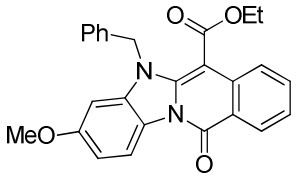
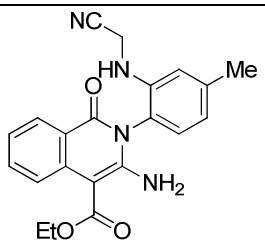
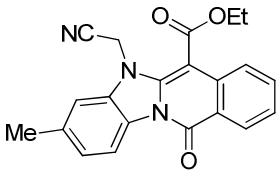
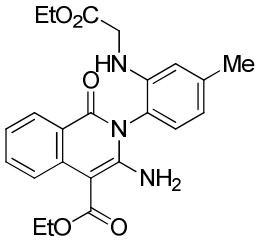
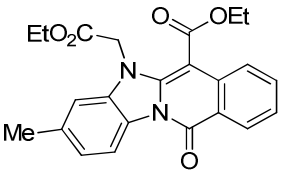
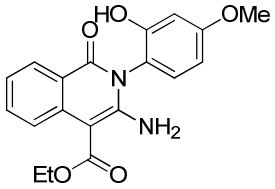
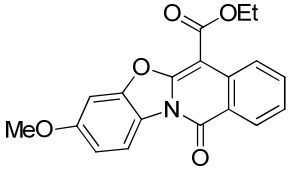
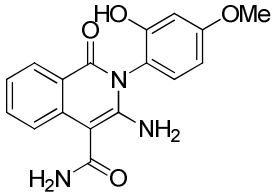
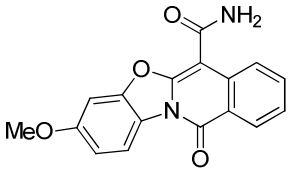
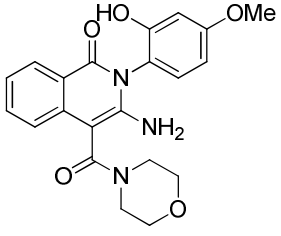
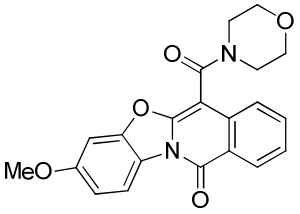
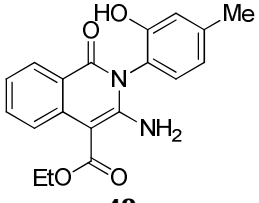
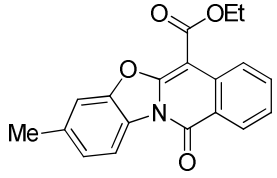
**Table 2.3:** Amberlyst-15 mediated synthesis of benzimidazo[1,2-*b*]isoquinolin-11-ones / benzoxazolo[3,2-*b*]isoquinolin-11-ones (**49**).




| Entry | Substrate (**48**) | Time/h | Product (**49**) | Yield[b] (%) |
|---|---|---|---|---|
| 1 |  **48a** | 1.5 |  **49a** | 98 |
| 2 |  **48b** | 2.0 |  **49b** | 87 |
| 3 |  **48c** | 7.0 |  **49c** | 71 |
| 4 |  | 11.5 |  | 63 |

| | 48d | | 49d | |
|---|---|---|---|---|
| 5 |  **48e** | 8.5 |  **49e** | 65 |
| 6 |  **48f** | 1.5 |  **49f** | 91 |
| 7 |  **48g** | 2.0 |  **49g** | 83 |
| 8 |  **48h** | 7.0 |  **49h** | 75 |
| 9 |  **48i** | 12.0 |  **49i** | 61 |
| 10 |  **48j** | 1.5 |  **49j** | 92 |
| 11 |  **48k** | 2.0 |  **49k** | 91 |
| 12 |  | 2.0 |  | 93 |

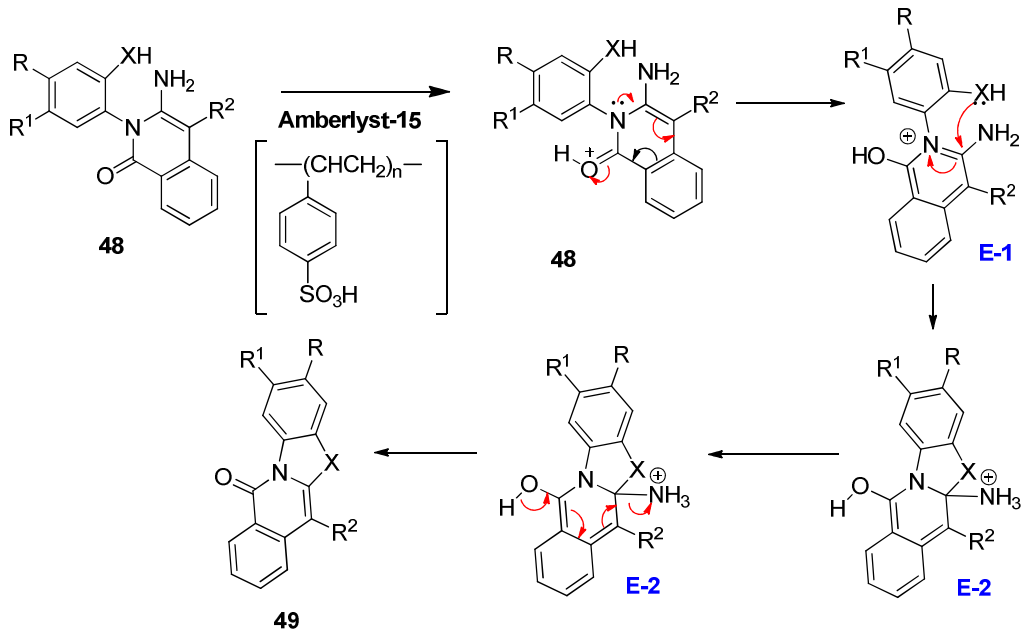
| | **48l** | | **49l** | |
|---|---|---|---|---|
| 13 |  **48m** | 2.0 |  **49m** | 90 |
| 14 |  **54a** | 3.5 |  **49n** | 89 |
| 15 |  **54b** | 3.5 |  **49o** | 91 |
| 16 |  **54c** | 3.0 |  **49p** | 89 |
| 17 |  **54d** | 3.0 |  **49q** | 86 |
| 18 |  **54e** | 4.5 |  **49r** | 95 |

| | | | | |
|---|---|---|---|---|
| 19 |  **54f** | 4.0 |  **49s** | 95 |
| 20 |  **54g** | 5.0 |  **49t** | 96 |
| 21 |  **54h** | 6.0 |  **49u** | 91 |
| 22 |  **48n** | 5.0 |  **49v** | 90 |
| 23 |  **48o** | 8.0 |  **49w** | 85 |
| 24 |  **48p** | 8.0 |  **49x** | 72 |
| 25 |  **48q** | 5.0 |  **49y** | 93 |

[a]All the reactions are carried out using compound **48** (1 mmol) and Amberlyst-15 (10%, w/w) in $CH_3CN$ (5 mL) at 60 °C under anhydrous conditions. [b]Isolated yield

### 2.2.5. Proposed mechanism:

Amberlyst-15 (Scheme 2.18), a macro reticular polystyrene-based ion exchange resin possessing strongly acidic sulfonic groups is known to catalyze various reactions involving carbonyl group.




**Scheme 2. 18**: Proposed reaction mechanism.

Thus, mechanistically (Scheme 2.18), the intramolecular cyclization of (**48)** seemed to proceed *via* a two-step process involving (i) a nucleophilic attack by the -XH moiety of **E-1** on its activated and nearby –C=N- affording the intermediate **E-2** followed by elimination of ammonia to give the desired compound (**49)**. An attempt to isolate the intermediate **E-1** or **E-2** from the reaction of (**48a)** under the condition employed however failed, perhaps due to its rapid participation in the next step leading to **(49a)**.

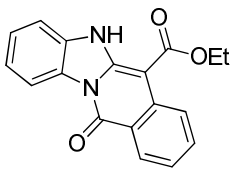
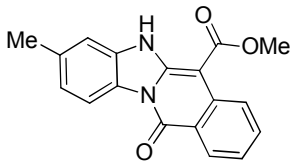
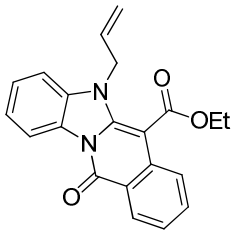
## 2.3. Pharmacology:

### 2.3.1. *In vitro* data:

Some of the compounds synthesized were tested against PDE4B along with a known inhibitor rolipram using an enzyme based *in vitro* assay.[23] The compounds (**49a),** (**49g)**, and (**49n)** showed 61, 63, and 86% inhibition respectively when

tested at 30 $\mu$M.

**Table 2.4**: *In vitro* data of compounds of **49** for inhibition of PDE4B enzyme.

| Entry | Compound No | % of PDE4B inhibition @ 30 µM |
|---|---|---|
| 1 |  **49a** | 61 % |
| 2 |  **49g** | 63 % |
| 3 |  **49n** | 86 % |

A dose response study was carried out using (**49n)** as a representative compound (Figure 2.7).

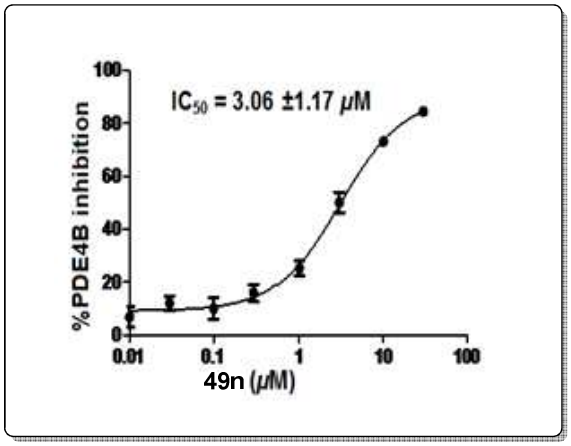



**Fig. 2.7**: Dose dependent inhibition of PDE4B by compound (**49n)**.

The compound (**49n**) showed dose depended inhibition of PDE4B with $IC_{50}$ ~ 3 µM (comparable to rolipram's $IC_{50}$ ~ 1.0 µM). Since COPD and asthma are major health burden worldwide hence the present class of heterocycles could be a new template for the discovery of novel PDE4 inhibitors.

## 2.4. Conclusion:

In conclusion, we have developed a new and general strategy involving Amberlyst-15 mediated activation of vinylic amino group for the synthesis of novel benzimidazo/benzoxazoloisoquinolinones. The catalyst used in this methodology is an inexpensive, recoverable and reusable resin. The key starting materials (**48)** & (**54)** required were prepared *via* Cu-mediated Ullman type of coupling followed by base promoted cyclization of 2-iodobenzamides (**52)** with appropriate cyano derivatives (**53)** in the same pot (followed by selective *N*-alkylation to prepare **54**). A wide range of functional groups were well tolerated under the reaction conditions employed. A series of novel benzoxazolo[3,2-*b*]iso-quinolin-11-ones (**49v–y**) were generated in moderate to excellent yields, synthesis of this class has not been reported earlier. Some of these synthesized compounds were tested for PDE4B along with a known inhibitor rolipram using an enzyme based *in vitro* assay, the representative compound (**49n**) showed dose dependent inhibition of PDE4B with $IC_{50}$ ~ 3 $\mu$M (comparable to rolipram's $IC_{50}$ ~ 1 $\mu$M). Since COPD and asthma are major health burden worldwide hence the present class of compounds is of further interest. Overall, our study indicate that the present benzimidazo/benzoxazoloisoquinolinone framework presented here could be a new template for the discovery of novel PDE4 inhibitors.

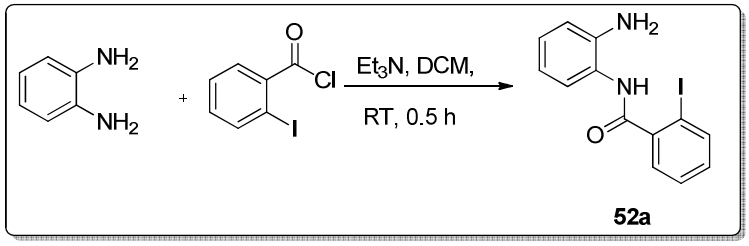
## 2.5. Experimental section:

### 2.5.1. Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. $^{1}H$ NMR and $^{13}C$ NMR

spectra were recodred in $CDCl_3$ or DMSO-$d_6$ solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT- IR spectrometer. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Melting points (mp) were by using Buchi B-540 melting point appratus and are uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

### 2.5.1.1. Typical procedure for preparation of *N*-(2-aminophenyl)-2-iodobenzamide (52a)



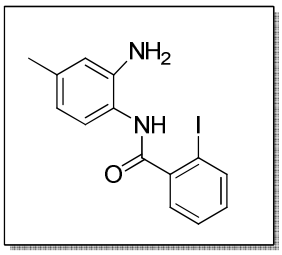

**52a**

To a solution of compound benzene-1,2-diamine (100 mg, 0.92 mmol) in dry DCM (5 mL), triethylamine (0.11 mL, 1.10 mmol) was added at 0 ºC under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.13 mL, 0.92 mmol) was slowly added and the reaction mixture stirred at room temperature for 0.5 h. After completion of reaction, the reaction mixture diluted with DCM (10 mL), washed with saturated $NaHCO_3$ solution (15 mL), followed by brine solution (10 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound (**52a)**.

Yield: 89% (275 mg); light yellow solid; mp: 130-132 °C; $R_f$ = 0.2 (50% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3436, 3352, 3262, 3035, 1644; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 8.0 Hz, 1H), 7.57 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.46-7.38 (m, 3H), 7.18 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.11-7.09 (m, 1H), 6.86 (dd, *J* = 8.8, 1.2 Hz, 2H), 3.96 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.7, 141.8, 140.8, 139.9, 131.4, 128.5, 128.3,

127.6, 125.3, 123.4, 119.5, 118.1, 92.4; MS (ES mass): 338.9 (M+1); HPLC: 95.5%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 3.74 min.

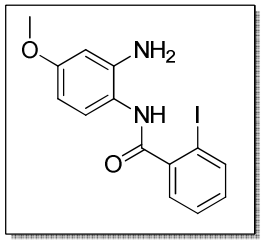
**2.5.1.2. *N*-(2-Amino-4-methylphenyl)-2-iodobenzamide (52b)**




Compound (**52b)** was synthesized from 4-methylbenzene-1,2-diamine following a procedure similar to that of compound (**52a).**

Yield: 95% (270 mg); white solid; mp: 140-142 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3426, 3312, 3139, 3028, 1642; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 7.31 (s, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.17-7.13 (m, 1H), 6.66-6.64 (m, 2H), 3.91 (bs, 2H), 2.28 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.8, 142.0, 140.9, 139.9, 137.7, 131.4, 128.5, 128.3, 125.4, 120.7, 120.3, 118.5, 92.4, 21.0; MS (ES mass): 352.9 (M+1); HPLC: 96.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.01 min.

**2.5.1.3. *N*-(2-Amino-4-methoxyphenyl)-2-iodobenzamide (52c)**

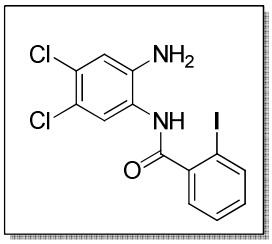



Compound (**52c)** was synthesized from 4-methoxybenzene-1,2-diamine following a procedure similar to that of compound (**52a).**

Yield: 63% (168 mg); white solid; mp: 182-184 °C; $R_f$ = 0.4 (50% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3439, 3356, 3265, 3043, 1638; $^1$H NMR (400 MHz, DMSO-$d_6$) δ:

9.52 (s, 1H), 7.93 (t, *J* = 6.7 Hz, 1H), 7.54 (td, *J* = 7.2, 1.8 Hz, 1H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.21 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 1H), 6.34 (d, *J* = 2.4 Hz, 1H), 6.19 (dd, *J* = 8.2, 2.5 Hz, 1H), 4.96 (bs, 2H), 3.68 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 168.3, 158.6, 144.4, 143.6, 139.3, 131.2, 128.6, 128.4, 127.5, 116.3, 102.3, 101.0, 94.1, 55.3; MS (ES mass): 369.0 (M+1); HPLC: 93.5%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 1.0/50, 9/98, 16/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.47 min.

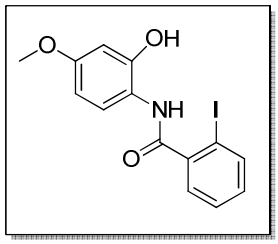
**2.5.1.4. *N*-(2-Amino-4,5-dichlorophenyl)-2-iodobenzamide (52d)**




Compound (**52d)** was synthesized from 4,5-dichlorobenzene-1,2-diamine following a procedure similar to that of compound (**52a).**

Yield: 73% (168 mg); light red solid; mp: 220-222 °C; $R_f$ = 0.3 (30% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3393, 3318, 3220, 3037, 1650; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 9.77 (bs, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.61 (s, 1H), 7.56 (d, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.21 (t, *J* = 7.4 Hz, 1H), 6.95 (s, 1H), 5.42 (bs, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 168.5, 142.9 (2C), 139.3, 131.5, 128.7, 128.5, 128.2, 126.5, 122.9, 116.4, 116.3, 94.2; MS (ES mass): 406.9 (M+1); HPLC: 92.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.66 min.

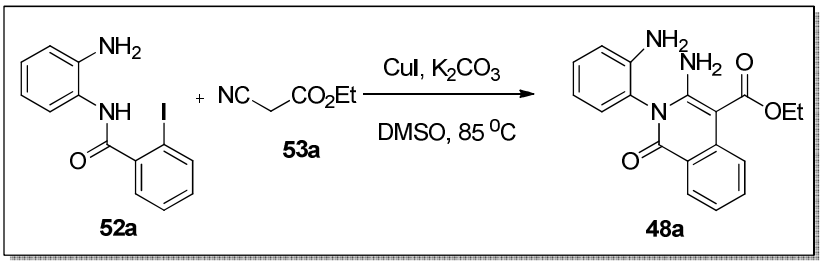
**2.5.1.5. *N*-(2-Hydroxy-4-methoxyphenyl)-2-iodobenzamide (52e)**



Compound (**52e)** was synthesized from 2-amino-5-methoxyphenol following a procedure similar to that of compound (**52a).**

Yield: 87% (230 mg); brown solid; mp: 176-178 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3563, 3277, 2953, 1515; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 7.96 (s, 1H), 7.94-7.92 (m, 2H), 7.62-7.56 (m, 2H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.25-7.15 (m, 1H), 7.01 (dd, *J* = 9.2, 2.8 Hz, 2H), 3.79 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.4, 153.5, 141.9, 140.7, 140.1, 131.9, 128.8, 128.3, 125.7, 119.6, 112.5, 107.4, 92.4, 55.8; MS (ES mass): 369.9 (M+1); HPLC: 95.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.22 min.

### 2.5.1.6. Typical procedure for preparation of ethyl 3-amino-2-(2-aminophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48a)

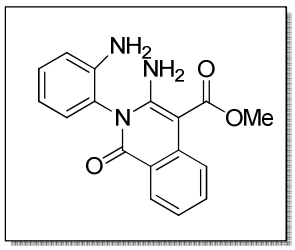



A mixture of compound (**52a)** (100 mg, 0.29 mmol), $K_2CO_3$ (80 mg, 0.58 mmol), ethyl cyano acetate (**53a**) (0.03 mL, 0.34 mmol) and CuI (5.5 mg, 0.029 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions ($CaCl_2$ filled guard tube) for 1h. After completion of the reaction, reaction mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**48a)**.

Yield: 72% (69 mg); white solid; mp: 150-152 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3460, 3322, 3227, 2986, 1645, 1590; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, *J* = 8.6 Hz, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 5.7 Hz, 1H), 7.21 (d, *J* = 7.4 Hz, 1H), 7.09-7.07 (m, 1H),

6.99 (bs, 1H), 6.95-6.91 (m, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 3.71 (s, 2H), 1.47 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.5, 153.2, 136.7, 133.4, 131.1, 129.4, 128.4 (2C), 124.6 (2C), 123.0, 120.0, 119.9, 117.6, 84.0, 60.5, 14.5; MS (ES mass): 324.1 (M+1); HPLC: 99.3%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.46 min.

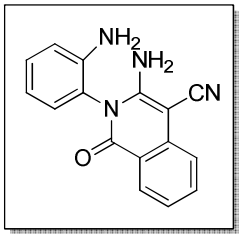
### 2.5.1.7. Methyl-3-amino-2-(2-aminophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48b)




Compound (**48b)** was synthesized from (**52a)** and methylcyanoacetate (**53b**) following a procedure similar to that of compound (**48a).**

Yield: 69% (63 mg); yellow solid; mp: 168-170 °C; $R_f$ = 0.2 (50% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3445, 3356, 3202, 2931, 1636, 1573; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.45 (d, *J* = 8.8 Hz, 1H), 8.35-8.27 (m, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.37-7.29 (m, 1H), 7.23-7.21 (m, 1H), 7.18-7.00 (m, 3H), 6.95-6.92 (m, 2H), 3.96 (s, 3H), 3.71 (bs, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.7, 161.5, 153.3, 143.6, 136.5, 133.5, 131.1, 129.4, 128.4, 124.6, 123.0, 119.9 (2C), 119.8, 117.6, 83.9, 51.3; MS (ES mass): 310.1 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.17 min.

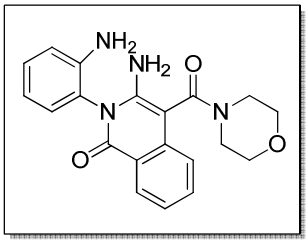
### 2.5.1.8. 3-Amino-2-(2-aminophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carbonitrile (48c)




Compound (**48c)** was synthesized from (**52a)** and malononitrile (**53c**) following a procedure similar to that of compound (**48a).**

Yield: 67% (54 mg); light yellow solid; mp: 134-137 °C; $R_f$ = 0.2 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3463, 3340, 3213, 2968, 2204, 1656, 1566; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.03-7.96 (m, 2H), 7.72-7.66 (m, 2H), 7.45-7.40 (m, 1H), 6.97-6.89 (m, 1H), 6.88 (d, *J* = 8.8 Hz, 1H), 6.63 (t, *J* = 7.6 Hz, 1H), 5.71 (bs, 2H), 5.25 (bs, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 161.1, 152.7, 143.8, 135.9, 134.1, 131.2, 129.1, 128.4, 123.7, 121.6, 119.5, 119.1, 119.0, 117.5, 112.0, 80.3; MS (ES mass): 277.1 (M+1); HPLC: 95.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 3.72 min.

### 2.5.1.9. 3-Amino-2-(2-aminophenyl)-4-(morpholine-4-carbonyl)isoquinolin-1(2*H*)-one (48d)

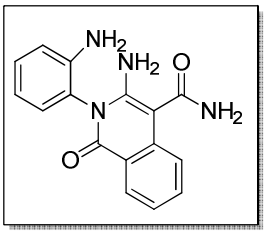



Compound (**48d)** was synthesized from (**52a)** and 3-morpholino-3-oxopropanenitrile (**53d**) following a procedure similar to that of compound (**48a).**

Yield: 58% (62 mg); brown solid; mp: 115-117 °C; $R_f$ = 0.2 (90% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3457, 3341, 3213, 2916, 1652, 1611; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.01 (d, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.2 Hz, 2H), 7.22-7.08 (m, 4H), 6.92 (d, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.65 (t, *J* = 7.2 Hz, 1H), 5.10 (s, 2H), 3.66-3.44 (m, 8H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.7, 162.7, 161.4, 145.9, 144.4, 137.3, 133.1, 130.3, 130.0, 128.1, 121.8, 119.7, 119.6, 116.9, 116.4, 88.9, 66.9, 66.7, 47.0,

45.2; MS (ES mass): 365.0 (M+1); HPLC: 89.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 1/10, 5/95, 10/95, 10.5/10, 12/10; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.15 min.

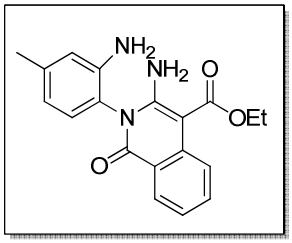
### 2.5.1.10. 3-Amino-2-(2-aminophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (48e)




Compound (**48e)** was synthesized from (**52a)** and 2-cyanoacetamide (**53e**) following a procedure similar to that of compound (**48a).**

Yield: 64% (54 mg); dark brown solid; mp: 122-124 °C; $R_f$ = 0.2 (80% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3457, 3334, 3216, 2924, 1653, 1595; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 8.10 (d, *J* = 7.2 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.43 (bs, 2H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 6.99-6.94 (m, 2H), 6.77 (t, *J* = 7.2 Hz, 1H), 6.31 (s, 2H), 5.09 (s, 2H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ: 170.2, 161.2, 148.7, 145.7, 137.5, 133.0, 130.3, 130.0, 128.1, 123.1, 121.8, 119.8 (2C), 117.2, 116.7, 88.7; MS (ES mass): 294.9 (M+1); HPLC: 91.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/10, 1/10, 5/95, 10/95, 10.5/10, 12/10; flow rate: 1.0 mL/min; UV 235 nm, retention time 3.96 min.

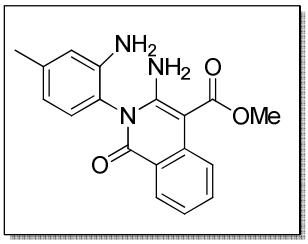
### 2.5.1.11. Ethyl-3-amino-2-(2-amino-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48f)



Compound (**48f)** was synthesized from (**52b)** and ethyl 2-cyanoacetate (**53a**) following a procedure similar to that of compound (**48a).**

Yield: 65% (62 mg); brown solid; mp: 114-116 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3462, 3325, 3229, 2983, 1649, 1595; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, *J* = 8.8 Hz, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 7.62-7.57 (m, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.15 (bs, 2H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 6.8 Hz, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.63 (bs, 2H), 2.33 (s, 3H), 1.46 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.7, 153.4, 143.2, 141.4, 136.7, 133.4, 129.0, 128.4, 124.5, 122.9, 121.1, 119.9, 118.1, 117.3, 83.9, 60.4, 21.3, 14.5; MS (ES mass): 338.1 (M+1); HPLC: 96.3%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.69 min.

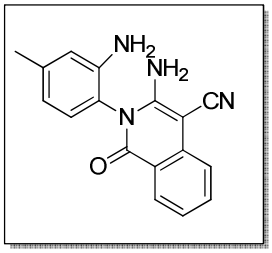
### 2.5.1.12. Methyl-3-amino-2-(2-amino-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48g)




Compound (**48g)** was synthesized from (**52b)** and methyl 2-cyanoacetate (**53b**) following a procedure similar to that of compound (**48a).**

Yield: 64% (58 mg); white solid; mp: 173-176 °C; $R_f$ = 0.2 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3441, 3337, 3218, 2948, 1641, 1575; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 9.6 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.12 (t, *J* =7.6 Hz, 1H), 6.95 (s, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.75 (s, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 3.84 (s, 3H), 3.83 (bs, 2H) 2.25 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.7, 161.7, 153.4, 142.2, 141.5, 136.5, 133.5, 129.0, 128.4, 124.5, 123.0, 121.8, 119.8, 118.8, 117.9, 84.0, 51.3, 21.3; MS (ES mass): 324.1 (M+1); HPLC: 98.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.41 min.

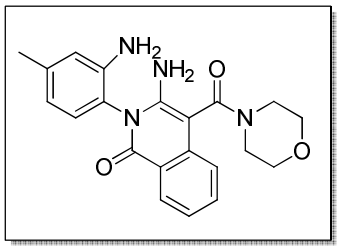
### 2.5.1.13. 3-Amino-2-(2-amino-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carbonitrile (48h)




Compound (**48h)** was synthesized from (**52b)** and malononitrile (**53c**) following a procedure similar to that of compound (**48a).**

Yield: 68% (56 mg); white solid; mp: 228-231 °C; $R_f$ = 0.2 (60% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3447, 3350, 3208, 2958, 2209, 1666, 1558; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.24 (d, *J* = 7.9 Hz, 1H), 7.71-7.65 (m, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 6.98-6.95 (m, 1H), 6.77 (d, *J* = 2.9 Hz, 2H), 4.97 (s, 2H), 3.64 (s, 2H), 2.35 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 161.1, 152.4, 143.1, 141.9, 135.5, 134.3, 128.8, 128.7, 124.2, 121.9, 121.2, 119.4, 118.3, 117.1, 116.7, 67.5, 21.3; MS (ES mass): 291.0 (M+1); HPLC: 97.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 3.72 min.

### 2.5.1.14. 3-Amino-2-(2-amino-4-methylphenyl)-4-(morpholine-4-carbonyl)isoquinolin-1(2*H*)-one (48i)

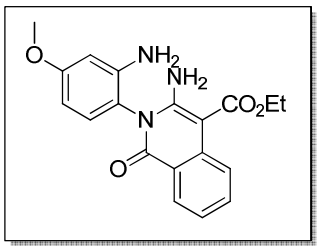



Compound (**48i)** was synthesized from (**52b)** and 3-morpholino-3-oxopropanenitrile (**53d**) following a procedure similar to that of compound (**48a).**

Yield: 64% (68 mg); brown solid; mp: 136-139 °C; $R_f$ = 0.2 (70% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3451, 3341, 3224, 2957, 1654, 1610; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.99 (d, *J* = 8.0 Hz, 1H), 7.57-7.49 (m, 2H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.14-7.05 (m,

1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 8.4Hz, 1H), 6.66-6.61 (m, 1H), 6.47 (d, *J* = 7.9 Hz, 1H), 5.12 (s, 2H), 3.47 (bs, 8H), 2.23 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.7, 164.1, 161.5, 145.5, 144.6, 139.5, 137.2, 133.0, 129.7, 128.1, 128.0, 121.7, 119.7, 118.0, 116.8, 88.8, 66.9, 66.5, 47.0, 42.3, 21.5; MS (ES mass): 379.2 (M+1); HPLC: 92.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 3.27 min.

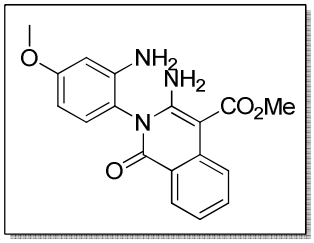
**2.5.1.15. Ethyl-3-amino-2-(2-amino-4-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48j)**




Compound (**48j)** was synthesized from (**52c)** and ethyl 2-cyanoacetate (**53a**) following a procedure similar to that of compound (**48a).**

Yield: 83% (79 mg); light red solid; mp: 170-172 °C; $R_f$ = 0.5 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3444, 3339, 3175, 2986, 1642, 1588; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, *J* = 8.3 Hz, 1H), 8.31 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.60 (td, *J* = 7.4, 1.4 Hz, 1H), 7.22 (t, *J* = 7.1 Hz, 1H), 7.18 (bs, 2H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.50 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 4.44 (q, *J* = 7.12 Hz, 2H), 3.81 (s, 3H), 3.70 (bs, 2H), 1.47 (t, *J* = 7.12 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.9, 161.6, 153.6, 144.6, 136.7, 133.4, 130.2, 128.4, 124.5, 122.9, 119.9, 112.6, 106.1, 102.3, 83.9, 60.5, 55.4, 14.5; MS (ES mass): 354.1 (M+1); HPLC: 95.4%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 2.92 min.

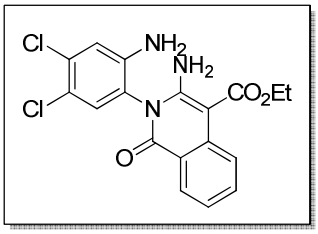
**2.5.1.16. Methyl-3-amino-2-(2-amino-4-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48k)**




Compound (**48k)** was synthesized from (**52c)** and methyl 2-cyanoacetate (**53b**) following a procedure similar to that of compound (**48a).**

Yield: 83% (76 mg); light red solid; mp: 237-239 °C; $R_f$ = 0.5 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3441, 3341, 3209, 2948, 1644, 1578; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.37 (d, *J* = 8.3 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.56 (tb, *J* = 7.4, 1.4 Hz, 1H), 7.40 (bs, 2H), 7.15 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.25 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.20 (bs, 2H), 3.83 (s, 3H), 3.72 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 169.3, 161.6, 161.0, 154.8, 146.9, 137.1, 133.2, 130.8, 128.0, 124.6, 122.4, 120.3, 112.1, 103.6, 100.8, 82.5, 55.3, 51.4; MS (ES mass): 340.1 (M+1); HPLC: 94.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 2.29 min.

### 2.5.1.17. Ethyl-3-amino-2-(2-amino-4,5-dichlorophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48l)

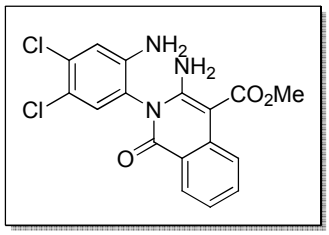



Compound (**48l)** was synthesized from (**52d)** and ethyl 2-cyanoacetate (**53a**) following a procedure similar to that of compound (**48a).**

Yield: 85% (82 mg); light red solid; mp: 162-165 °C; $R_f$ = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3459, 3348, 3218, 2982, 1634, 1580; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, *J* = 8.4 Hz, 1H), 8.28 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.62 (td, *J* = 8.4, 1.4 Hz, 1H), 7.26-7.23 (m, 1H), 7.22 (s, 1H), 7.05 (s, 1H), 6.99 (bs, 2H), 4.44 (q, *J* =

7.1 Hz, 2H), 3.82 (bs, 2H), 1.47 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.1, 161.3, 152.4, 143.3, 136.6, 135.1, 133.8, 130.9, 128.4, 124.7, 123.3, 122.2, 119.6, 118.9, 118.4, 84.4, 60.7, 14.5; MS (ES mass): 392.0 (M+1); HPLC: 98.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 3.96 min.

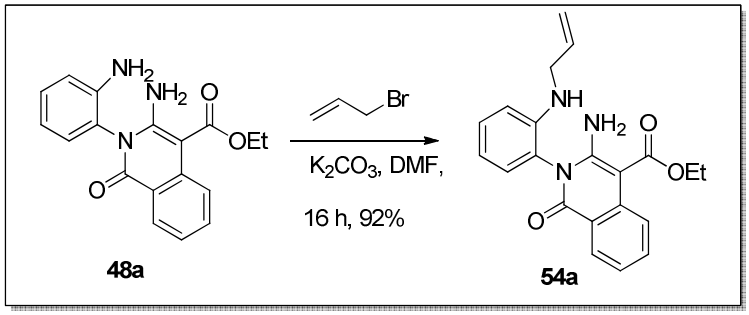
### 2.5.1.18. Methyl-3-amino-2-(2-amino-4,5-dichlorophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48m)




Compound (**48l)** was synthesized from (**52d)** and methyl 2-cyanoacetate (**53b**) following a procedure similar to that of compound (**48a).**

Yield: 84% (78 mg); light red solid; mp: 276-278 °C; $R_f$ = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3448, 3342, 3218, 2990, 1645, 1595; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.38 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.70 (bs, 2H), 7.58 (td, *J* = 7.8, 1.1 Hz, 1H), 7.34 (s, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.02 (s, 1H), 5.72 (bs, 2H), 3.84 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 169.4, 161.3, 154.4, 146.5, 137.3, 133.4, 132.7, 131.8, 128.0, 124.6, 122.4, 120.1, 118.8, 116.9, 116.7, 82.7, 51.4; MS (ES mass): 378.0 (M+1); HPLC: 92.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 3.54 min.

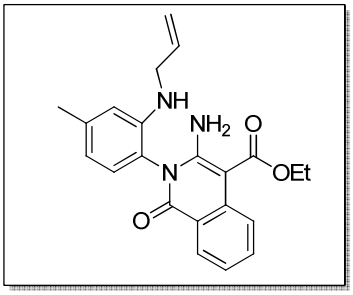
### 2.5.1.19. Typical procedure for preparation of Ethyl 2-(2-(allylamino)phenyl)-3-amino-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54a)




A mixture of compound (**48a)** (100 mg, 0.30 mmol), $K_2CO_3$ (64 mg, 0.46 mmol), and allyl bromide (0.07 mL, 0.61 mmol) in DMF (2 mL) was stirred at room temperature for 16 h. After completion of the reaction, reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (15 mL). The organic layer was washed with brine solution (10 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound (**54a).**

Yield: 92% (103 mg); white solid; mp: 124-125 °C; $R_f$ = 0.5 (50% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3468, 3388, 3289, 2979, 1663, 1592; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.52 (d, *J* = 8.4 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 7.61 (t, *J* = 7.2 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.25-7.21 (m, 2H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.95 (bs, 1H), 6.89-6.83 (m, 2H), 5.90-5.76 (m, 1H), 5.22 (d, *J* = 17.6 Hz, 1H), 5.11 (d, *J* = 10.0 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.87-3.73 (m, 3H), 1.47 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.6, 153.3, 144.1, 136.7, 134.2, 133.5, 131.2, 129.3, 128.5, 124.5, 123.0, 120.0, 119.3, 118.2, 116.4, 112.9, 83.9, 60.5, 45.4, 14.0; MS (ES mass): 364.1 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.26 min.

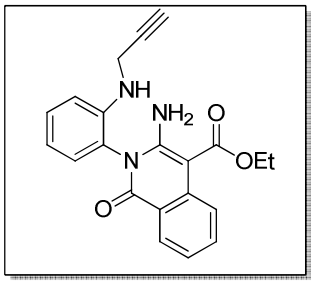
**2.5.1.20. Ethyl-2-(2-(allylamino)-4-methylphenyl)-3-amino-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54b)**




Compound **54b** was synthesized from **48f** and allyl bromide following a procedure similar to that of compound **54a.**

Yield: 89% (99 mg); white solid; mp: 136-138 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3465, 3378, 3287, 2976, 1669, 1594; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, *J* = 8.8 Hz, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 8.4 Hz, 1H), 7.25-7.20 (m, 1H), 7.15 (bs, 2H), 6.94 (d, *J* = 7.6 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 6.65 (s, 1H), 5.85-5.77 (m, 1H), 5.21 (d, *J* = 16.8 Hz, 1H), 5.10 (d, *J* = 10.4 Hz, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 3.77 (d, *J* = 2.4 Hz, 3H), 2.37 (s, 3H), 1.47 (t, *J* = 7.10 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.7, 153.5, 143.7, 141.7, 136.6, 134.3, 133.4, 129.4, 128.9, 128.5, 124.5 (2C), 122.9, 119.2, 116.3, 113.5, 83.8, 60.5, 45.4, 21.8, 14.5; MS (ES mass): 378.2 (M+1); HPLC: 93.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.23 min.

**2.5.1.21. Ethyl-3-amino-1-oxo-2-(2-(prop-2-ynylamino)phenyl)-1,2-dihydroisoquinoline-4-carboxylate (54c)**

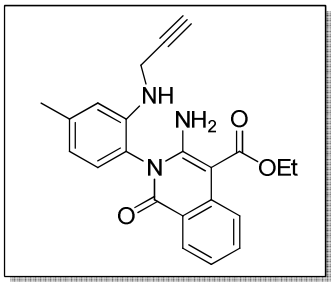



Compound (**54c)** was synthesized from (**48a)** and propargyl bromide following a procedure similar to that of compound (**54a).**

Yield: 93% (103 mg); white solid; mp: 245-247 °C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3452, 3376, 3277, 2971, 2114, 1664, 1595; $^1$H NMR (400 MHz,

$CDCl_3$) δ: 8.51 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 7.63-7.59 (m, 1H), 7.46 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.97 (t, *J* = 7.6 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.99-3.86 (m, 3H), 2.18 (s, 1H), 1.47 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.6, 153.2, 143.3, 136.7, 133.5, 131.2, 129.4, 128.5, 124.6, 123.0, 120.2, 119.9, 119.5, 113.4, 84.0, 80.1, 71.7, 60.5, 33.0, 14.5; MS (ES mass): 362.1 (M+1); HPLC: 94.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 3/95, 10/95, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 236 nm, retention time 3.36 min.

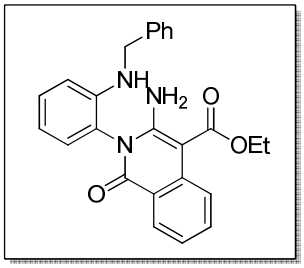
### 2.5.1.22. Ethyl-3-amino-2-(4-methyl-2-(prop-2-ynylamino)phenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54d)




Compound (**54d)** was synthesized from (**48f)** and propargyl bromide following a procedure similar to that of compound (**54a).**

Yield: 82% (91 mg); white solid; mp: 115-117 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3464, 3387, 3289, 2972, 2374, 1649, 1592; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, *J* = 8.4 Hz, 1H), 8.30 (d, *J* = 8.00 Hz, 1H), 7.62-7.58 (m, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.10-7.01 (m, 2H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.81 (s, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 4.43 (q, *J* = 7.2 Hz, 2H), 3.97-3.83 (m, 3H), 2.41 (s, 3H), 2.30 (s, 1H), 1.47 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.7, 153.4, 143.0, 141.5, 136.7, 133.4, 129.1, 128.5, 128.4, 124.5, 122.9, 120.4, 119.9, 117.7, 83.9, 80.2, 71.6, 60.5, 33.0, 21.8, 14.5; MS (ES mass): 376.2 (M+1); HPLC: 95.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 3/95, 10/95, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 3.61 min.

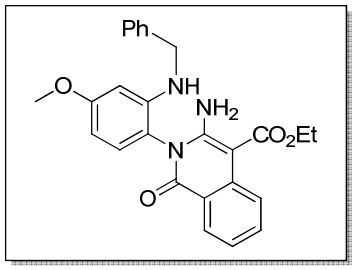
### 2.5.1.23. Ethyl-3-amino-2-(2-(benzylamino)phenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54e)




Compound (**54e)** was synthesized from (**48a)** and benzyl bromide following a procedure similar to that of compound (**54a).**

Yield: 92% (113 mg); white solid; mp: 141-143 °C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3434, 3262, 3064, 2976, 1670, 1640; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.52 (d, *J* = 8.8 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7. 6 Hz, 1H), 7.31-7.29 (m, 4H), 7.26-7.22 (m, 3H), 7.17 (bs, 1H), 7.10 (d, *J* = 7.6 Hz, 2H), 6.88 (t, *J* = 7.6 Hz, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 4.55(q, *J* = 6.8 Hz, 2H), 4.39 (s, 2H), 4.24 (bs, 1H), 1.49 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 161.6, 153.4, 144.1, 138.4, 137.2, 136.7, 133.4, 131.2, 129.3, 128.6, 128.5, 128.0, 127.1, 126.8, 124.6, 123.0, 120.0, 119.4, 118.3, 113.0, 84.0, 60.5, 47.0, 14.5; MS (ES mass): 414.1 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.99 min.

### 2.5.1.24. Ethyl-3-amino-2-(2-(benzylamino)-4,5-dichlorophenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54f)

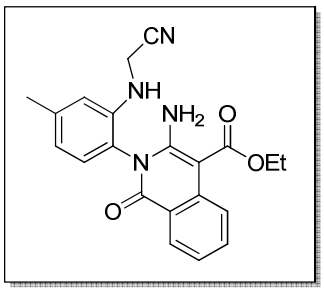



Compound (**54f)** was synthesized from (**48j)** and benzyl bromide following a procedure similar to that of compound (**54a).**

Yield: 87% (109 mg); white solid; mp: 139-142 °C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3381, 3265, 3163, 2976, 1672, 1625; $^1$H NMR (400 MHz, $CDCl_3$) δ:

8.51 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 7.60 (tb, *J* = 8.2, 1.1 Hz, 1H), 7.33-7.27 (m, 4H), 7.26-7.21 (m, 2H), 7.22 (bs, 2H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.41 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.30 (d, *J* = 2.3 Hz, 1H), 4.45 (q, *J* = 7.1 Hz, 2H), 4.36-4.32 (m, 2H), 4.15 (t, *J* = 4.8 Hz, 1H), 3.75 (s, 3H), 1.48 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.4, 161.9, 161.8, 153.8, 145.1, 138.3, 136.6, 133.4, 130.1, 128.7 (2C), 128.5, 127.2, 126.8 (2C), 124.5, 122.9, 120.0, 112.4, 103.3, 99.2, 83.9, 60.5, 55.3, 47.2, 14.5; MS (ES mass): 444.2 (M+1); HPLC: 92.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.41 min.

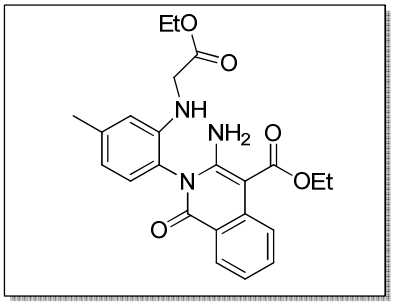
### 2.5.1.25. Ethyl-3-amino-2-(2-(cyanomethylamino)-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54g)




Compound (**54g)** was synthesized from (**48f)** and 2-bromo acetonitrile following a procedure similar to that of compound (**54a).**

Yield: 90% (100 mg); white solid; mp: 216-219 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3427, 3344, 3301, 2915, 2338, 1644, 1524; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.49 (d, *J* = 8.8 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 2H), 6.80 (s, 1H), 4.43-4.37 (q, *J* = 7.6 Hz, 2H), 4.09-4.03 (m, 3H), 2.45 (s, 3H), 1.46 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.2, 161.7, 153.1, 142.1, 141.4, 136.6, 133.6, 129.5, 128.3, 124.6, 123.1, 121.9, 119.7, 118.3, 116.3, 113.7, 84.2, 60.6, 32.0, 21.8, 14.4; MS (ES mass): 377.1 (M+1); HPLC: 93.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.20 min.

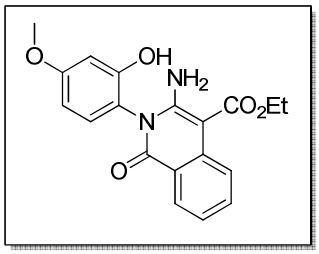
**2.5.1.26. Ethyl-3-amino-2-(2-(2-ethoxy-2-oxoethylamino)-4-methylphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (54h)**




Compound (**54h)** was synthesized from (**48f)** and ethyl 2-bromoacetate following a procedure similar to that of compound (**54a).**

Yield: 88% (110 mg); white solid; mp: 105-112 °C; $R_f$ = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3459, 3263, 2966, 1740, 1677, 1592; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.51 (d, *J* = 8.4 Hz, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 7.61-7.57 (m, 1H), 7.21 (t, *J* = 7.6 Hz, 2H), 7.11 (s, 2H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 7.6 Hz, 1H), 4.42 (q, *J* = 6.8 Hz, 2H), 4.20-4.12 (m, 3H), 3.94 (d, *J* = 6.8 Hz, 1H), 3.86-3.83 (m, 1H), 2.37 (s, 3H), 1.47 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.5, 169.3, 161.8, 153.7, 143.2, 141.1, 136.7, 133.2, 129.2, 128.4, 124.6, 122.8, 119.9 (2C), 117.4, 113.1, 83.8, 61.2, 60.3, 44.9, 21.8, 14.4, 14.0; MS (ES mass): 423.5 (M+1); HPLC: 96.8%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.08 min.

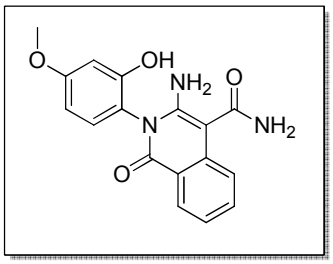
**2.5.1.27. Ethyl-3-amino-2-(2-hydroxy-4-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (48n)**




Compound (**48n)** was synthesized from (**52e)** and ethyl cyanoacetate (**53a**) following a procedure similar to that of compound (**48a).**

Yield: 68% (66 mg); white solid; mp: 167-169 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3639, 3398, 3196, 2923, 1639, 1604; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.50 (d, *J* = 8.4 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.26 (s, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.01 (bs, 2H), 6.87 (d, *J* = 8.8 Hz, 1H), 6.80-6.73 (m, 1H), 6.65 (s, 1H), 4.41 (q, *J* = 6.4 Hz, 2H), 3.70 (s, 3H), 1.45 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.2, 162.7, 153.8, 153.1, 146.9, 137.0, 133.6, 128.2, 124.6, 123.0, 121.2, 119.5, 119.4, 117.7, 113.4, 84.6, 60.5, 55.8, 14.4; MS (ES mass): 355.1 (M+1); HPLC: 95.5%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.32 min.

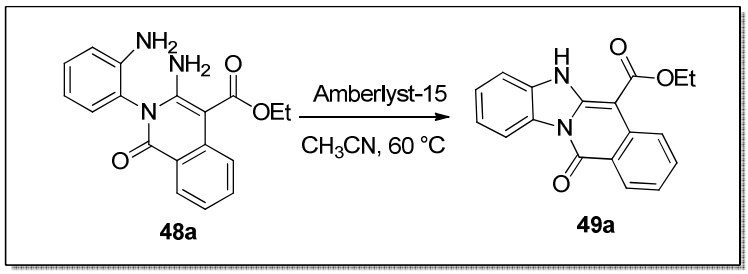
**2.5.1.28. 3-Amino-2-(2-hydroxy-4-methoxyphenyl)-1-oxo-1,2-dihydroisoquinoline-4-carboxamide (48o)**




Compound (**48o)** was synthesized from (**52e)** and 2-cyanoacetamide (**53e**) following a procedure similar to that of compound (**48a).**

Yield: 63% (55 mg); light yellow solid; mp: 174-177°C; $R_f$ = 0.2 (60% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3647, 3427, 3370, 3300, 3188, 2944, 1645, 1602; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 9.48 (bs, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.40 (s, 2H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.94 (s, 2H), 6.73 (s, 1H), 6.38 (bs, 2H), 3.68 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.3, 161.2, 152.9, 149.1, 147.7, 137.4, 133.0, 127.9, 123.1, 122.7, 121.8, 119.5, 117.9, 116.8, 115.3, 88.1, 55.9; MS (ES mass): 326.1 (M+1); HPLC: 91.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 2.74 min.

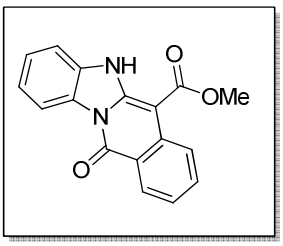
**2.5.1.29. Typical procedure for preparation of 11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (49a)**




To a solution of (**48a) (**100 mg, 0.30 mmol**)** in acetonitrile (5 mL), Amberlyst-15 (10%, w/w) was added and the reaction mixture was allowed to stir at 60 ºC for 1 h. Upon completion of the reaction, the formed solid was filtered and washed with acetonitrile (5 mL) to give desired compound (**49a)**.

Yield: 98% (92 mg); white solid; mp: 321-323 °C (lit[1] 317-319 ºC); $R_f$ = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3317, 2973, 1643, 1601; $^1$H NMR (400 MHz, $CDCl_3$) δ: 12.20 (bs, 1H), 8.82 (d, *J* = 8.2 Hz, 1H), 8.61 (d, *J* = 7.9 Hz, 1H), 8.36 (d, *J* = 7.7 Hz, 1H), 7.72 (m, 2H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.34 (m, 2H), 4.47 (q, *J* = 6.9 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 168.3, 159.9, 146.7, 135.3, 133.2, 130.9, 128.1, 127.3, 126.3, 124.3, 123.1, 122.6, 119.3, 117.0, 109.9, 82.4, 60.8, 14.6; MS (ES mass): 306.9 (M+1); HPLC: 99.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.45 min.

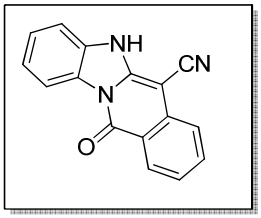
**2.5.1.30. 11-Oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid methyl ester (49b)[1]**




Compound (**49b)** was synthesized from (**48b)** following a procedure similar to that of compound (**49a).**

Yield: 87% (78 mg); white fluffy solid; mp: 335-337 °C (lit[1] 330-333 ºC); $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3320, 2948, 1649, 1600; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 11.21 (s, 1H), 8.76 (d, *J* = 8.0 Hz, 1H), 8.71 (d, *J* = 8.8 Hz, 1H), 8.54 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.73-7.66 (m, 1H), 7.48-7.43 (m, 1H), 7.41-7.32 (m, 3H), 4.04 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.6, 159.9, 146.7, 135.2, 133.3, 130.8, 128.1, 127.8, 126.4, 124.4, 123.2, 122.7, 119.3, 117.0, 109.9, 82.3, 51.6; MS (ES mass): 293.1 (M+1); HPLC: 99.8%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.13 min.

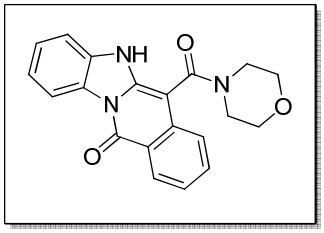
### 2.5.1.31. 11-Oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carbonitrile (48c)




Compound (**49c)** was synthesized from (**48c)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 72% (57 mg); white solid; mp: 290-292 °C (lit[1] 284-287 ºC); $R_f$ = 0.2 (50% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3376, 2921, 2203, 1695, 1619; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 13.4 (bs, 1H), 8.59 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.54-7.48 (m, 2H), 7.45-7.36 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 159.4, 146.8, 136.2, 135.6, 134.5, 130.4, 128.2, 127.8, 127.2, 123.9, 122.7, 122.1, 118.3, 116.4, 111.3, 61.7; MS (ES mass): 258.8 (M-1); HPLC: 95.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.99 min.

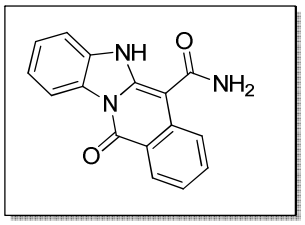
### 2.5.1.32. 11-Oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-(2'-morpholino)carbamide (49d)



Compound (**49d)** was synthesized from (**48d)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 63% (67 mg); light yellow solid; mp: 245-248 °C; $R_f$ = 0.2 (80% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3343, 2961, 1671, 1613; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.88 (s, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.71 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.44-7.42 (m, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.28-7.24 (m, 1H), 3.67-3.48 (m, 8H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.4, 159.1, 140.0, 135.7, 133.5, 133.1, 128.0, 127.7, 126.6, 122.6, 122.4, 121.2, 117.9, 116.2, 110.3, 87.1, 66.8, 66.0, 47.7, 47.4; MS (ES mass): 348.2 (M+1); HPLC: 93.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.17 min.

### 2.5.1.33. 11-Oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carbamide (49e)

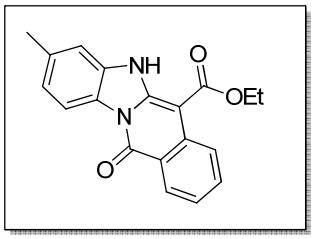


Compound (**49e)** was synthesized from (**48e)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 65% (55 mg); white solid; mp: 266-269 °C; $R_f$ = 0.2 (70% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3362, 2925, 1662, 1557; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.9 (bs, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.73-7.69 (m, 1H), 7.62 (bs, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.2 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 168.1, 159.2, 142.3, 135.9, 133.2, 132.9, 127.6 (2C), 126.6, 123.7, 122.4, 121.3,

117.9, 116.2, 111.1, 88.5; MS (ES mass): 277.7 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.23 min.

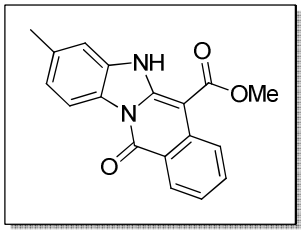
### 2.5.1.34. 3-Methyl-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (4f)




Compound (**49f)** was synthesized from (**48f)** following a procedure similar to that of compound (**49a).**

Yield: 91% (86 mg); white solid; mp: 199-201 °C (lit[1] 202-204 ºC); $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3304, 2920, 1672, 1612; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 11.24 (s, 1H), 8.81 (d, *J* = 8.4 Hz, 1H), 8.65-8.63 (m, 1H), 8.59 (d, *J* = 8.0 Hz, 1H), 7.73 (t, *J* = 7.6 Hz, 1H), 7.41-7.37 (m, 1H), 7.21-7.18 (m, 2H), 4.56 (q, *J* = 7.2 Hz, 2H), 2.53 (s, 3H), 1.56 (t, *J* = 6.8 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.4, 159.8, 146.8, 136.8, 135.4, 133.2, 131.1, 128.0, 125.7, 124.3, 123.6, 123.0, 119.3, 116.5, 110.2, 82.5, 60.7, 21.7, 14.6; MS (ES mass): 321.0 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.76 min.

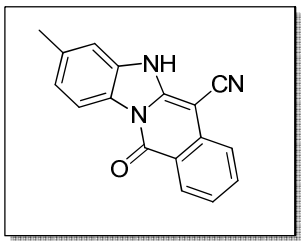
### 2.5.1.35. 3-Methyl-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid methyl ester (49g)




Compound (**49g)** was synthesized from (**48g)** following a procedure similar to that of compound (**49a).**

Yield: 83% (75 mg); light yellow solid; mp: 219-221 °C (lit[1] 213-215 ºC); $R_f$ = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3443, 2924, 1642, 1582; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 11.2 (bs, 1H), 8.46 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.46-7.28 (m, 2H), 7.24-7.21 (m, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 3.91 (s, 3H), 2.33 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 169.3, 154.5, 145.4, 139.8, 137.2, 133.1, 132.5, 129.6, 128.0, 124.6, 122.4, 120.3, 118.3, 117.1, 116.6, 82.3, 51.3, 21.5; MS (ES mass): 307.0 (M+1); HPLC: 90.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.41 min.

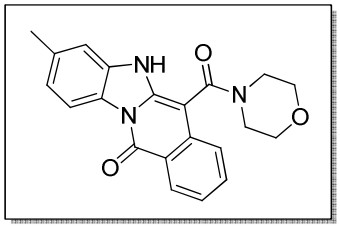
### 2.5.1.36. 3-Methyl-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carbonitrile (49h)




Compound (**49h)** was synthesized from (**48h)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 75% (60 mg); white solid; mp: 358-361 °C; $R_f$ = 0.2 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3261, 2923, 2208, 1687, 1627; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 13.21 (bs, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 8.35-8.29 (m, 1H), 7.86 (t, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.44-7.37 (m, 1H), 7.31 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 2.43 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 158.9, 146.5, 136.9, 135.9, 134.1, 132.7, 128.0, 126.0, 123.3, 121.9, 118.0, 117.2, 115.7, 111.1, 109.9, 62.7, 21.6; MS (ES mass): 274.0 (M+1); HPLC: 97.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.87 min.

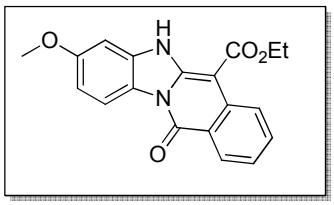
### 2.5.1.37. 3-Methyl-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-(2'-morpholino) carbamide (49i)




Compound (**49i)** was synthesized from (**48i)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 67% (71 mg); light green solid; mp: 264-267 °C; $R_f$ = 0.2 (80% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3271, 2957, 1757, 1663; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.79 (bs, 1H), 8.44 (d, *J* = 7.6 Hz, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.19 (s, 1H), 7.04 (d, *J* = 7.9 Hz, 1H), 3.69-3.43 (m, 8H), 2.42 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 168.1, 159.0, 142.3, 136.3, 135.8, 133.4, 132.7, 127.6, 125.6, 123.7, 122.3, 122.2, 117.9, 115.8, 111.2, 88.5, 66.7, 66.4, 47.9, 47.4, 21.8; MS (ES mass): 359.9 (M-1); HPLC: 91.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 3.84 min.

**2.5.1.38. 3-Methoxy-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (49j)**

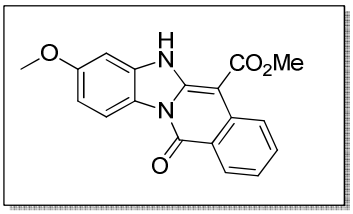



Compound (**49j)** was synthesized from (**48j)** following a procedure similar to that of compound (**49a).**

Yield: 92% (87 mg); white solid; mp: 242-245°C; $R_f$ = 0.6 (35% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3343, 2982, 1630, 1601; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.09 (bs, 1H), 8.85 (d, *J* = 8.4 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.73 (tb, *J* = 8.4, 1.0 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 2.3 Hz, 1H), 6.91 (dd, *J* = 8.4, 2.2 Hz, 1H), 4.47 (q, *J* = 7.2 Hz, 2H), 3.83 (s, 3H), 1.41 (t, *J* = 7.07 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.4, 159.3, 158.5, 146.2, 135.9, 133.7, 133.4, 127.6, 124.5, 123.2, 121.6, 118.6, 117.0, 109.2, 97.0, 82.4, 60.4, 56.0, 15.1; MS (ES

mass): 337.1 (M+1); HPLC: 96.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.61 min.

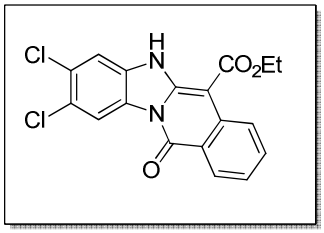
**2.5.1.39. 3-Methoxy-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid methyl ester (49k)**




Compound (**49k)** was synthesized from (**48k)** following a procedure similar to that of compound (**49a).**

Yield: 91% (83 mg); white solid; mp: 245-247 °C; $R_f$ = 0.6 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3326, 2948, 1638, 1605; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.00 (bs, 1H), 8.86 (d, *J* = 8.4 Hz, 1H), 8.44 (dd, *J* = 8.4, 1.4 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.17 (s, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 3.93 (s, 3H), 3.81 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 169.3, 159.2, 158.5, 145.9, 136.0, 133.7, 133.3, 127.5, 124.3, 123.1, 121.6, 118.6, 116.9, 109.1, 96.7, 82.1, 55.9, 51.6; MS (ES mass): 323.0 (M+1); HPLC: 94.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.04 min.

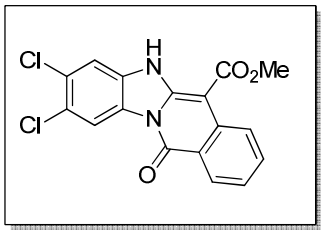
**2.5.1.40. 2,3-Dichloro-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (49l)**




Compound (**49l)** was synthesized from (**48l)** following a procedure similar to that of compound (**49a).**

Yield: 93% (88 mg); white solid; mp: 162-164 °C; $R_f$ = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3316, 2980, 1677, 1640; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 11.27 (bs, 1H), 8.90 (s, 1H), 8.77 (d, *J* = 8.4 Hz, 1H), 8.56 (d, *J* = 8.4 Hz, 1H), 7.74 (tb, *J* = 8.4, 1.1 Hz, 1H), 7.47 (s, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 4.54 (q, *J* = 7.1 Hz, 2H), 1.53 (d, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 168.0, 159.3, 146.7, 135.0, 133.6, 130.4, 130.2, 128.0, 126.8, 126.3, 124.5, 123.6, 119.1, 118.2, 111.1, 83.2, 61.1, 14.5; MS (ES mass): 374.9 (M+1); HPLC: 96.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.98 min.

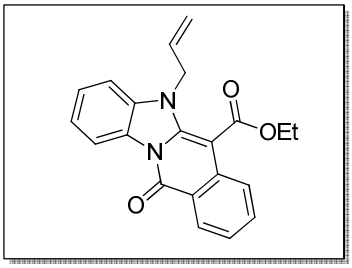
### 2.5.1.41. 2,3-Dichloro-11-oxo-5,11-dihydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6-carboxylic acid methyl ester (49m)




Compound (**49m)** was synthesized from (**48m)** following a procedure similar to that of compound (**49a).**

Yield: 90% (82 mg); light brown solid; mp: 278-280 °C; $R_f$ = 0.6 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3302, 2952, 1678, 1643; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ: 12.17 (s, 1H), 8.81 (d, *J* = 8.4 Hz, 1H), 8.62 (s, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 7.77-7.74 (m, 2H), 7.38 (t, *J* = 7.6 Hz, 1H), 3.94 (s, 3H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ: 166.1, 159.2, 146.0, 135.9, 133.8, 132.4, 128.8, 127.6, 127.2, 124.4, 124.0, 123.5, 118.6, 117.1, 112.7, 82.7, 51.1; MS (ES mass): 360.9 (M+1); HPLC: 96.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.78 min.

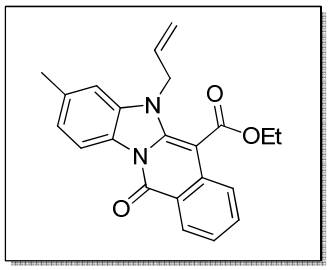
### 2.5.1.42. 11-Oxo-5-allyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49n)




Compound (**49n)** was synthesized from (**54a)** following a procedure similar to that of compound (**49a).**

Yield: 89% (84 mg); white solid; mp: 244-247 °C; $R_f$ = 0.5 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3315, 2926, 1759, 1670, 1597; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.88 (d, *J* = 8.0 Hz, 1H), 8.55 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.69-7.65 (m, 1H), 7.48-7.31 (m, 4H), 5.99-5.90 (m, 1H), 5.32 (d, *J* = 10.4 Hz, 1H), 5.26 (d, *J* = 17.6 Hz, 1H), 4.77 (dd, *J* = 3.6, 2.1 Hz, 2H), 4.48 (q, *J* = 7.1 Hz, 2H), 1.48 (t, *J* = 6.9 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.8, 159.9, 135.6, 134.4, 132.9, 131.1, 127.9, 127.7, 126.0, 124.4, 123.2, 122.6, 122.4, 118.7, 118.4, 117.1, 108.4, 87.2, 61.3, 48.3, 14.3; MS (ES mass): 347.1 (M+1); HPLC: 92.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 3/95, 10/95, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.18 min.

### 2.5.1.43. 3-Methyl-11-oxo-5-allyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49o)

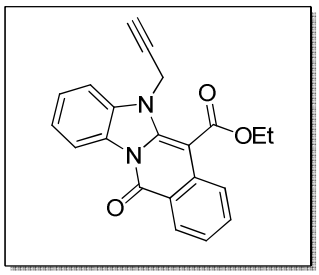



Compound (**49o)** was synthesized from (**54b)** following a procedure similar to that of compound (**49a)**.

Yield: 91% (86 mg); yellow solid; mp: 170-173 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3317, 2936, 1761, 1677, 1595; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.72 (d, *J* = 8.4 Hz, 1H), 8.54 (d, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.68-7.64 (m, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.04 (s, 1H), 5.97-5.89 (m,

1H), 5.31 (d, *J* = 10.4 Hz, 1H), 5.24 (d, *J* = 17.2 Hz, 1H), 4.77-4.73 (m, 2H), 4.48 (q, *J* = 7.1 Hz, 2H), 2.51 (d, *J* = 7.9 Hz, 3H), 1.45 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.9, 159.7, 141.4, 136.4, 135.5, 134.6, 132.7, 131.1, 127.7, 125.7, 123.2, 123.1, 122.6, 118.6, 118.2, 116.6, 108.8, 87.2, 61.3, 48.1, 21.8, 14.3; MS (ES mass): 361.1 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 245 nm, retention time 5.68 min.

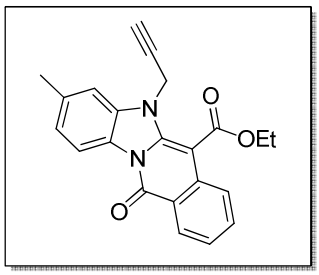
### 2.5.1.44. 11-Oxo-5-propargyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49p)




Compound (**49p)** was synthesized from (**54c)** following a procedure similar to that of compound (**49a).**

Yield:89% (84 mg); white solid; mp: 280-282 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3215, 2983, 2112, 1789, 1674, 1609; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.87 (d, *J* = 8.0 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 7.71-7.67 (m, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 7.41-7.35 (m, 3H), 4.94 (d, *J* = 2.4 Hz, 2H), 4.58 (q, *J* = 7. 2 Hz, 2H), 2.36-2.35 (m, 1H), 1.47 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.5, 159.8, 141.6, 135.4, 133.8, 133.0, 127.9, 127.7, 126.1, 123.6, 123.2, 122.8, 119.1, 117.2, 109.9, 108.2, 87.6, 74.3, 61.4, 36.0, 14.4; MS (ES mass): 345.1 (M+1); HPLC: 99.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 3/95, 10/95, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 250 nm, retention time 3.85 min.

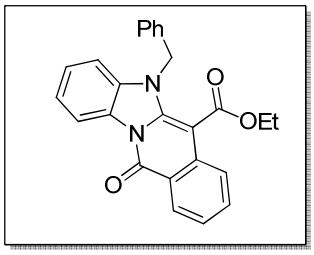
### 2.5.1.45. 3-Methyl-11-oxo-5-propargyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49q)




Compound (**49q)** was synthesized from (**54d)** following a procedure similar to that of compound (**49a).**

Yield: 86% (82 mg); white solid; mp: 293-295 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3218, 2986, 2385, 1693, 1668; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.72 (d, *J* = 8.8 Hz, 1H), 8.54 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 7.69 (t, *J* = 8.4 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 6.8 Hz, 2H), 4.94 (d, *J* = 2.2 Hz, 2H), 4.58 (q, *J* = 7.2 Hz, 2H), 2.54 (s, 3H), 2.35 (s, 1H), 1.50 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.6, 159.7, 141.6, 136.5, 135.4, 134.0, 132.9, 127.7, 125.8, 123.7, 123.5, 123.1, 119.1, 116.8, 108.6, 87.6, 70.2, 74.2, 61.4, 35.9, 21.9, 14.4; MS (ES mass): 359.1 (M+1); HPLC: 94.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 3/95, 10/95, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 250 nm, retention time 4.15 min.

### 2.5.1.46. 11-Oxo-5-benzyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49r)

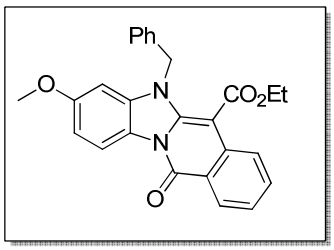



Compound (**49r)** was synthesized from (**54e)** following a procedure similar to that of compound (**49a).**

Yield: 95% (91 mg); white solid; mp: 186-189 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3069, 2981, 1663, 1608; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.92 (d, *J* = 7.6 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.66 (t, *J* = 8.4 Hz, 1H), 7.41-7.28 (m, 6H), 7.15 (d, *J* = 6.8 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 1H), 5.40 (s,

2H), 4.05 (q, *J* = 7.6 Hz, 2H), 1.06 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.7, 159.9, 153.9, 141.2, 135.5, 134.8, 134.7, 132.9, 128.8 (2C), 127.7 (2C), 126.1, 126.0, 123.3, 122.6, 122.5, 118.7, 117.1, 109.9, 108.4, 87.6, 61.3, 49.1, 13.8; MS (ES mass): 397.1 (M+1); HPLC: 95.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 245 nm, retention time 5.66 min.

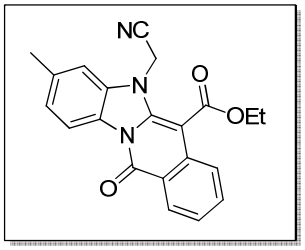
### 2.5.1.47. 3-Methoxy-11-oxo-5-benzyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49s)




Compound (**49s)** was synthesized from (**54f)** following a procedure similar to that of compound (**49a).**

Yield: 95% (91 mg); light yellow solid; mp: 281-284 °C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3084, 2985, 1669, 1645; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.80 (d, *J* = 8.4 Hz, 1H), 8.55 (d, *J* = 8.2 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.64 (tb, *J* = 8.4, 1.0 Hz, 1H), 7.39-7.27 (m, 4H), 7.15 (d, *J* = 7.2 Hz, 2H), 6.87 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 5.36 (s, 2H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 1.06 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.7, 159.5, 158.6, 141.3, 136.0, 135.3, 134.7, 132.6, 128.8 (2C), 127.7, 127.5, 126.0 (2C), 123.3, 122.6, 121.9, 118.7, 117.7, 107.7, 95.0, 88.0, 61.3, 55.8, 49.1, 13.8; MS (ES mass): 427.2 (M+1); HPLC: 97.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.42 min.

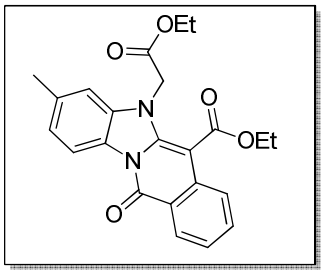
### 2.5.1.48. 3-Methyl-11-oxo-5-cyanomethyl-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49t)




Compound (**49t)** was synthesized from (**54g)** following a procedure similar to that of compound (**49a).**

Yield: 96% (91 mg); white solid; mp: 240-242 °C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3066, 2923, 2338, 1658, 1610; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.72 (d, *J* = 8.0 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.35 (t, *J* = 8.4 Hz, 1H), 7.76-7.72 (m, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.15 (s, 1H), 5.09 (s, 2H), 4.63 (q, *J* = 7.2 Hz, 2H), 2.55 (s, 3H), 1.54 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.2, 161.7, 153.1, 142.1, 141.4, 136.6, 133.6, 129.5, 128.3, 124.6, 123.1, 121.9, 119.7, 118.3, 116.3, 113.7, 84.2, 60.6, 32.0, 21.8, 14.4; MS (ES mass): 360.1 (M+1); HPLC: 93.0%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.20 min.

**2.5.1.49. 3-Methyl-11-oxo-5-(2′-ethoxy-2-oxoethyl)-11-hydro-benzo[4,5]imidazo[1,2-*b*]isoquinoline-6(5*H*)-carboxylic acid ethyl ester (49u)**

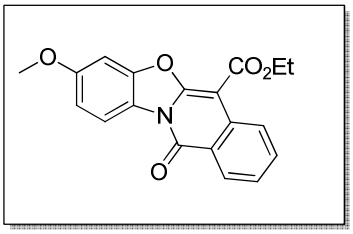



Compound (**49u)** was synthesized from (**54h)** following a procedure similar to that of compound (**49a).**

Yield: 91% (88mg); yellow solid; mp: 169-172 °C; $R_f$ = 0.6 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3379, 2979, 1756, 1683, 1603; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.69 (d, *J* = 8.0 Hz, 1H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.16-8.11 (m, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.96 (s, 1H), 4.81 (s, 2H), 4.48

(q, *J* = 7.2 Hz, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 2.50 (s, 3H), 1.47 (t, *J* = 6.8 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 167.4, 167.4, 166.6, 159.4, 142.6, 136.5, 134.5, 132.7, 127.6, 123.6, 123.3, 123.1, 119.0, 117.3, 116.4, 108.3, 87.0, 61.9, 61.4, 47.9, 21.6, 14.3, 14.0; MS (ES mass): 406.5 (M+1); HPLC: 99.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.35 min.

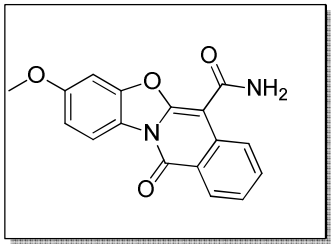
### 2.5.1.50. 3-Methoxy-11-oxo-11-hydro-benzo[4,5]oxazolo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (49v)




Compound (**49v)** was synthesized from (**48n)** following a procedure similar to that of compound (**49a).**

Yield: 92% (87 mg); white solid; mp: 185-188 °C; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3352, 2981, 1689, 1609; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.80 (d, *J* = 8.8 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 2.4 Hz, 1H), 7.78 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.49 (t, *J* = 7. 6 Hz, 1H), 7.41(d, *J* = 8.8 Hz, 1H), 7.00-6.97 (m, 1H), 4.53 (q, *J* = 7.2 Hz, 2H), 3.93 (s, 3H), 1.50 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 164.4, 159.1, 157.1, 141.0, 135.1, 133.7 (2C), 127.6, 127.5, 125.1, 125.0, 120.9, 113.5, 110.7, 101.5, 87.8, 60.9, 56.2, 14.4; MS (ES mass): 338.0 (M+1); HPLC: 94.6%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.45 min.

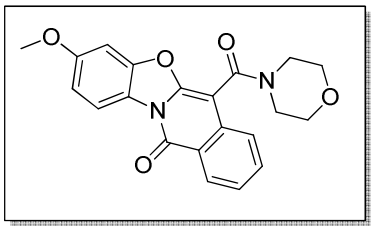
### 2.5.1.51. 3-Methoxy-11-oxo-11-hydro-benzo[4,5]oxazolo[1,2-*b*]isoquinoline-6-carbamide (49w)



Compound (**49w)** was synthesized from (**48o)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 85% (73 mg); white solid; mp: 226-229 °C; $R_f$ = 0.4 (80% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3395, 3315, 2922, 1658, 1610; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.35 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 7.81-7.79 (m, 2H), 7.65 (d, *J* = 9.2 Hz, 1H), 7.50 (t, *J* = 9.2 Hz, 1H), 7.06 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.86 (s, 3H); $^1$C NMR (100 MHz, $CDCl_3$) δ: 164.8, 158.4, 156.7, 141.2, 135.6, 133.6, 131.3, 128.0, 127.5, 125.1, 125.0, 120.9, 112.5, 111.4, 101.8, 79.5, 56.4; MS (ES mass): 309.0 (M+1); HPLC: 97.7%, column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 5.20 min.

**2.5.1.52. 3-Methoxy-11-oxo-11-hydro-benzo[4,5]oxazolo[1,2-*b*]isoquinoline-6-(2'-morpholino) carbamide (49x)**

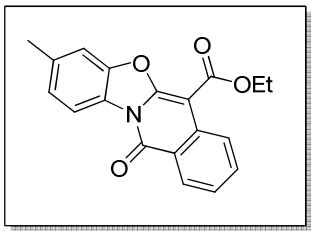


Compound (**49x)** was synthesized from (**48p)** following a procedure similar to that of compound (**49a)** and purification done by column chromatography.

Yield: 72% (69 mg); light green solid; $R_f$ = 0.2 (80% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3392, 3315, 2929, 1651, 1614; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.54 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 2.8 Hz, 1H), 7.80-7.69 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 9.2 Hz, 1H), 6.97 (dd, *J* = 8.9, 2.5 Hz, 1H), 4.07-3.97 (m, 2H), 3.94 (s, 3H), 3.92-3.83 (m, 2H), 3.77-3.67 (m, 1H), 3.65-3.58 (m, 1H), 3.56-3.49 (m, 1H), 3.49-

3.41 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 163.2, 158.7, 157.0, 141.0, 134.8, 133.6, 128.1, 127.9, 125.1, 123.5, 121.3, 113.0, 110.4, 109.9, 101.7, 90.1, 67.1, 66.9, 56.2, 47.6, 47.6; MS (ES mass): 378.6 (M+1).

**2.5.1.53. 3-Methyl-11-oxo-11-hydro-benzo[4,5]oxazolo[1,2-*b*]isoquinoline-6-carboxylic acid ethyl ester (49y)**




Compound (**49y)** was synthesized from (**48q)** following a procedure similar to that of compound (**49a).**

Yield: 93% (88 mg); white solid; $R_f$ = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 2987, 1685, 1602; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.82 (d, *J* = 8.4 Hz, 1H), 8.56 (d, *J* = 7.6 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 7.80 (t, *J* =7.2 Hz, 1H), 7.52 (t, *J* =7.2 Hz, 1H), 7.37 (s, 1H), 7.25 (s, 1H), 4.55 (q, *J* = 7.2 Hz, 2H), 2.54 (s, 3H), 1.52 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.6, 162.2, 158.6, 147.2, 137.3, 135.1, 133.5, 127.6, 125.5, 125.1, 125.0, 121.2, 116.1, 110.8, 109.9, 87.8, 60.8, 21.7, 14.4; MS (ES mass): 322.1 (M+1).

## 2.6. References:

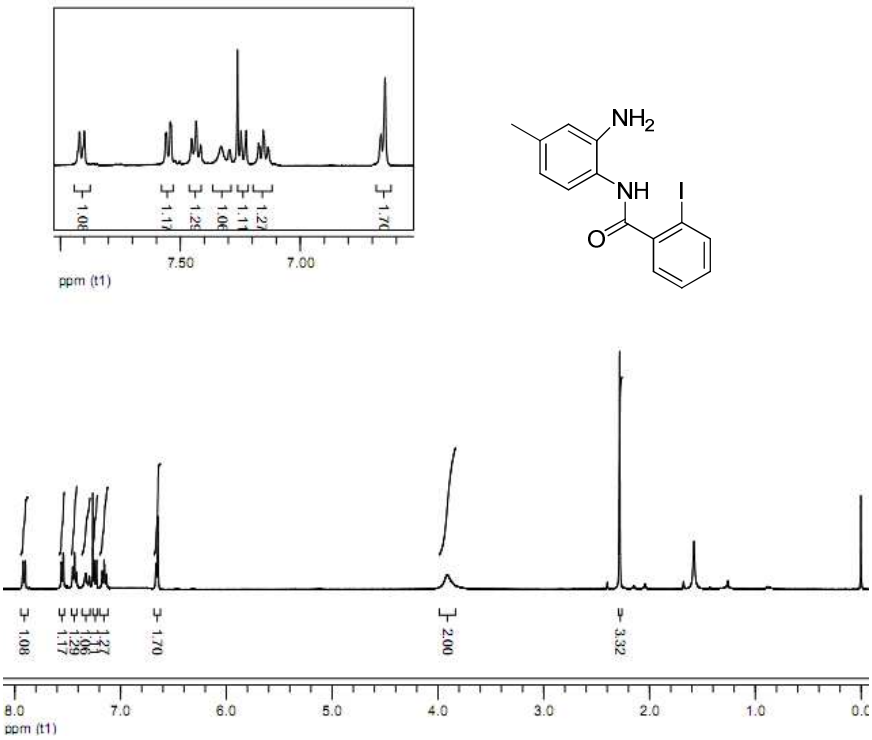

1. (a) Shaharyar, M.; Mazumder, A.; Garg, R.; Pandey, R. D., *Arabian Journal of Chemistry.* **2011**; (b) Shah, K.; Chhabra, S.; Shrivastava, S. K.; Mishra, P., *Med. Chem. Res.* **2013,** *22* (11), 5077-5104.
2. (a) McKee, M. L.; Kerwin, S. M., *Bioorg. Med. Chem.* **2008,** *16* (4), 1775-1783; (b) Potashman, M. H.; Bready, J.; Coxon, A.; DeMelfi, T. M.; DiPietro, L.; Doerr, N.; Elbaum, D.; Estrada, J.; Gallant, P.; Germain, J., *J. Med. Chem.* **2007,** *50* (18), 4351-4373; (c) Huang, S.-T.; Hsei, I.-J.; Chen, C., *Bioorg. Med. Chem.* **2006,** *14* (17), 6106-6119.
3. (a) Croisy-Delcey, M.; Croisy, A.; Carrez, D.; Huel, C.; Chiaroni, A.; Ducrot, P.; Bisagni, E.; Jin, L.; Leclercq, G., *Bioorg. Med. Chem.* **2000,** *8* (11), 2629-2641; (b) Asano, Y.; Kitamura, S.; Ohra, T.; Itoh, F.; Kajino, M.; Tamura, T.;

Kaneko, M.; Ikeda, S.; Igata, H.; Kawamoto, T., *Bioorg. Med. Chem.* **2008,** *16* (8), 4699-4714; (c) Matsui, T.; Sugiura, T.; Nakai, H.; Iguchi, S.; Shigeoka, S.; Takada, H.; Odagaki, Y.; Nagao, Y.; Ushio, Y., *J. Med. Chem.* **1992,** *35* (18), 3307-3319.

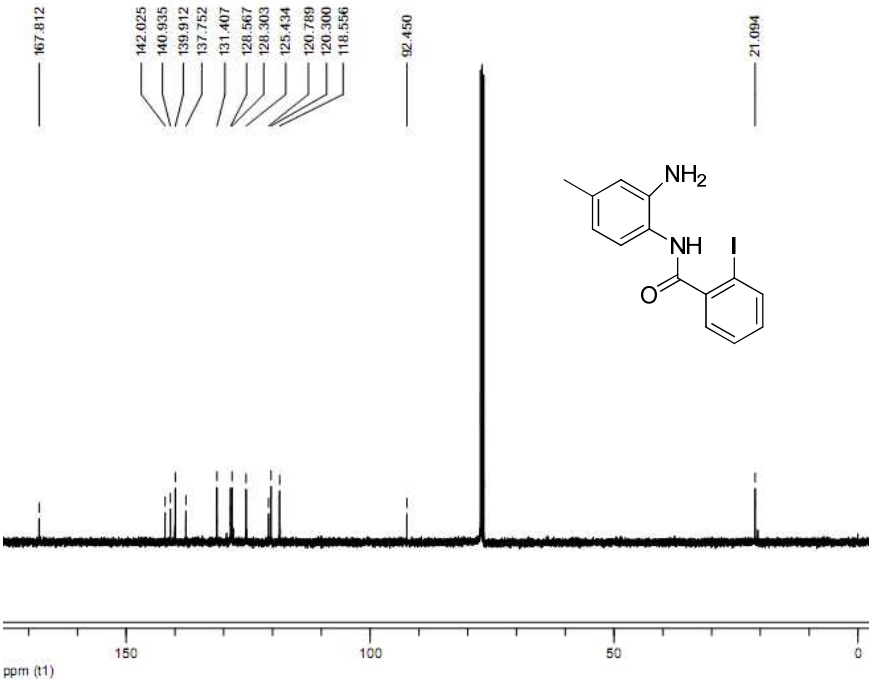
4. Bollini, M.; Casal, J. J.; Alvarez, D. E.; Boiani, L.; González, M.; Cerecetto, H.; Bruno, A. M., *Bioorg. Med. Chem.* **2009,** *17* (4), 1437-1444.
5. (a) Lee, S. H.; Park, D. R.; Kim, H.; Lee, J.; Jung, J. C.; Woo, S. Y.; Song, W. S.; Kwon, M. S.; Song, I. K., *Catal. Commun.* **2008,** *9* (9), 1920-1923; (b) Park, S.; Cho, K. M.; Youn, M. H.; Seo, J. G.; Baeck, S.-H.; Kim, T. J.; Chung, Y.-M.; Oh, S.-H.; Song, I. K., *Catal. Lett.* **2008,** *122* (3-4), 349-353; (c) Song, I. K.; Lee, W. Y., *Appl. Catal. A: Gen.* **2003,** *256* (1), 77-98.
6. Das, B.; Thirupathi, P.; Mahender, I.; Reddy, V. S.; Rao, Y. K., *J. Mol. Catal. A: Chem.* **2006,** *247* (1), 233-239.
7. Ko, S.; Yao, C.-F., *Tetrahedron Lett.* **2006,** *47* (50), 8827-8829.
8. Liu, J.-Y.; Liu, J.; Wang, J.-D.; Jiao, D.-Q.; Liu, H.-W., *Synth. Commun.* **2010,** *40* (14), 2047-2056.
9. Das, B.; Veeranjaneyulu, B.; Krishnaiah, M.; Balasubramanyam, P., *Synth. Commun.* **2009,** *39* (11), 1929-1935.
10. Palmieri, A.; Gabrielli, S.; Ballini, R., *Chem. Commun.* **2010,** *46* (33), 6165-6167.
11. Abonia, R.; Cuervo, P.; Castillo, J.; Insuasty, B.; Quiroga, J.; Nogueras, M.; Cobo, J., *Tetrahedron Lett.* **2008,** *49* (34), 5028-5031.
12. Hou, R. S.; Wu, J. L.; Cheng, H. T.; Xie, Y. T.; Chen, L. C., *j. Chin. Chem. Lett.* **2008,** *55* (4), 915-918.
13. Das, B.; Reddy, K. R., *Helv. Chim. Acta.* **2006,** *89* (12), 3109-3111.
14. Das, B.; Banerjee, J., *Chem. Lett.* **2004,** *33* (8), 960-961.
15. Ke, B.; Qin, Y.; Wang, Y.; Wang, F., *Synth. Commun.* **2005,** *35* (9), 1209-1212.
16. Das, B.; Krishnaiah, M.; Laxminarayana, K.; Damodar, K.; Kumar, D. N., *Chem. Lett.* **2009,** *38* (1), 42-43.
17. Pal, R.; Mandal, T. K.; Guha, C.; Mallik, A. K., *J. Cheminform.* **2012,** *43* (11).
18. Kadam, S. T.; Thirupathi, P.; Kim, S. S., *Tetrahedron* **2009,** *65* (50), 10383-10389.

19. Bandini, M.; Fagioli, M.; Umani-Ronchi, A., *Adv. Synth. Catal.* **2004,** *346* (5), 545-548.
20. Kodimuthali, A.; Jabaris, S. S. L.; Pal, M., *J. Med. Chem.* **2008,** *51* (18), 5471-5489.
21. Lowe III, J. A.; Archer, R. L.; Chapin, D. S.; Cheng, J. B.; Helweg, D.; Johnson, J. L.; Koe, B. K.; Lebel, L. A.; Moore, P. F., *J. Med. Chem.* **1991,** *34* (2), 624-628.
22. Lu, J.; Gong, X.; Yang, H.; Fu, H., Concise copper-catalyzed one-pot tandem synthesis of benzimidazo [1, 2-b] isoquinolin-11-one derivatives. *Chemical Communications* **2010,** *46* (23), 4172-4174.
23. Wang, P.; Myers, J. G.; Wu, P.; Cheewatrakoolpong, B.; Egan, R. W.; Billah, M. M., *Biochem. Biophys. Res. Commun.* **1997,** *234* (2), 320-324.

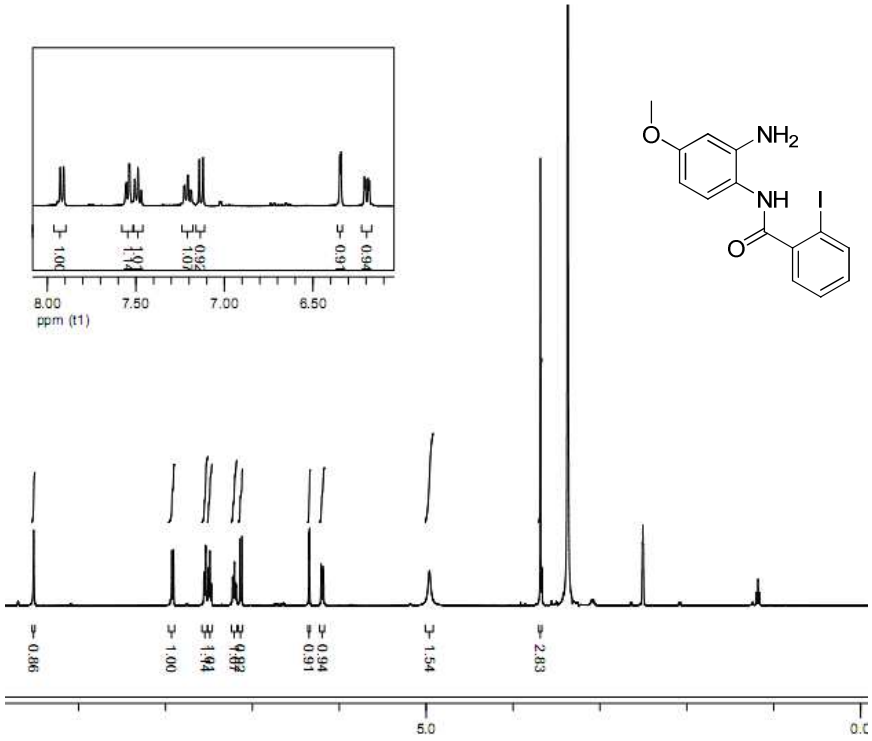
# Appendix



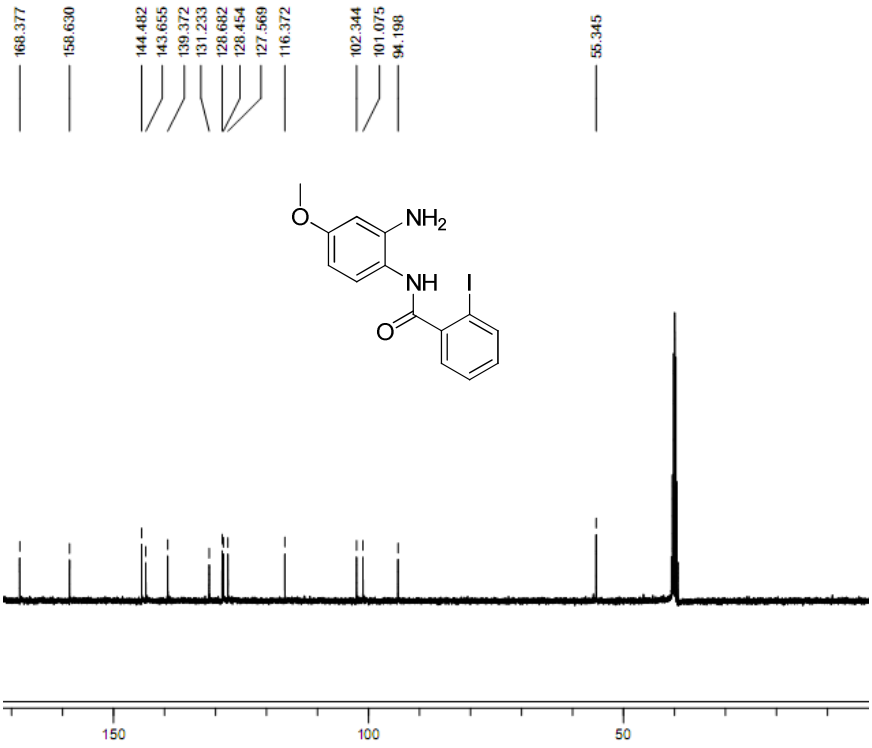

$^{1}$H NMR spectra of compound **52b** ($CDCl_3$, 400 MHz)



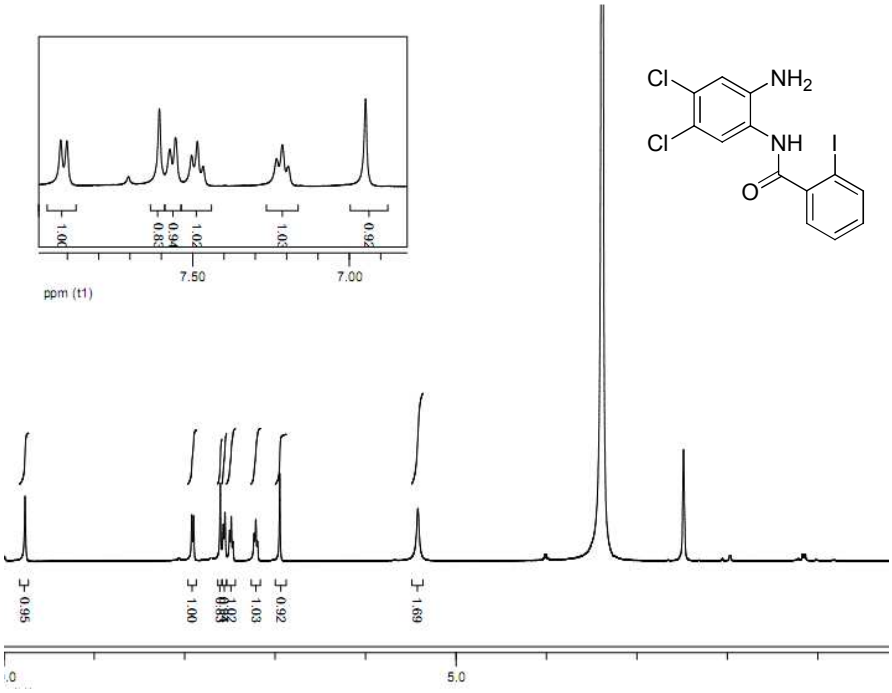

$^{13}$C NMR spectra of compound **52b** ($CDCl_3$, 100 MHz)



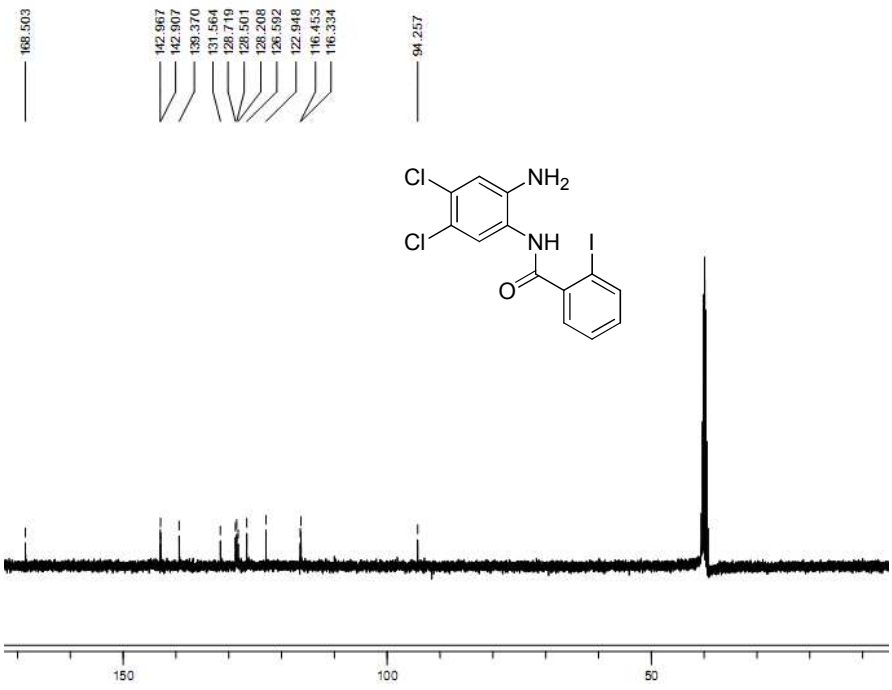

$^{1}$H NMR spectra of compound **52c** (DMSO-$d_6$, 400 MHz)



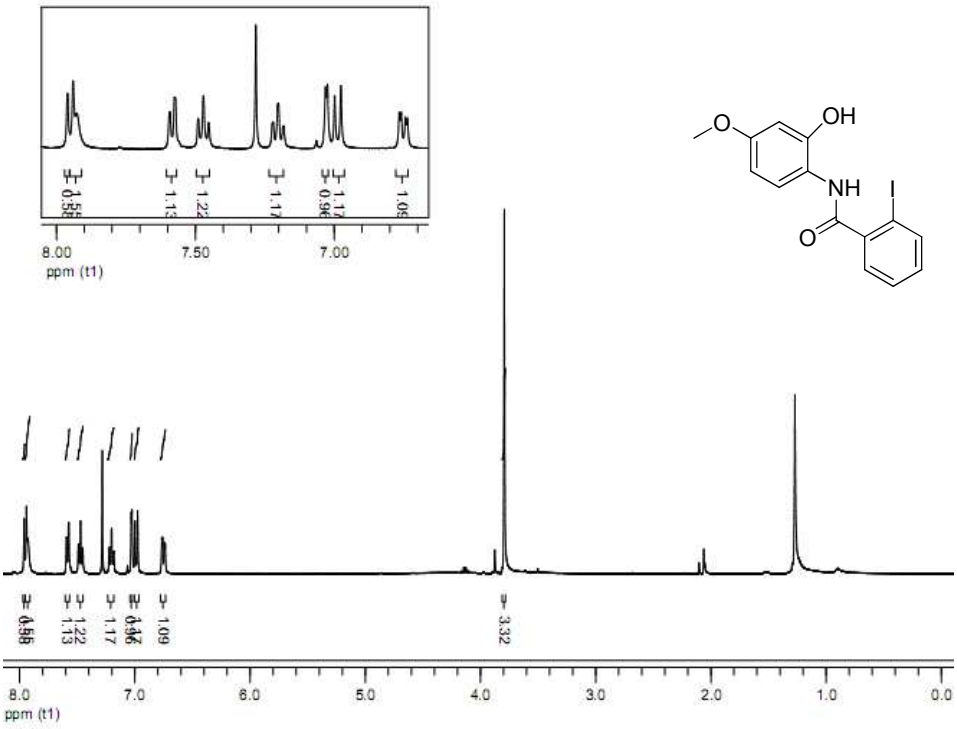

$^{13}$C NMR spectra of compound **52c** (DMSO-$d_6$, 100 MHz)



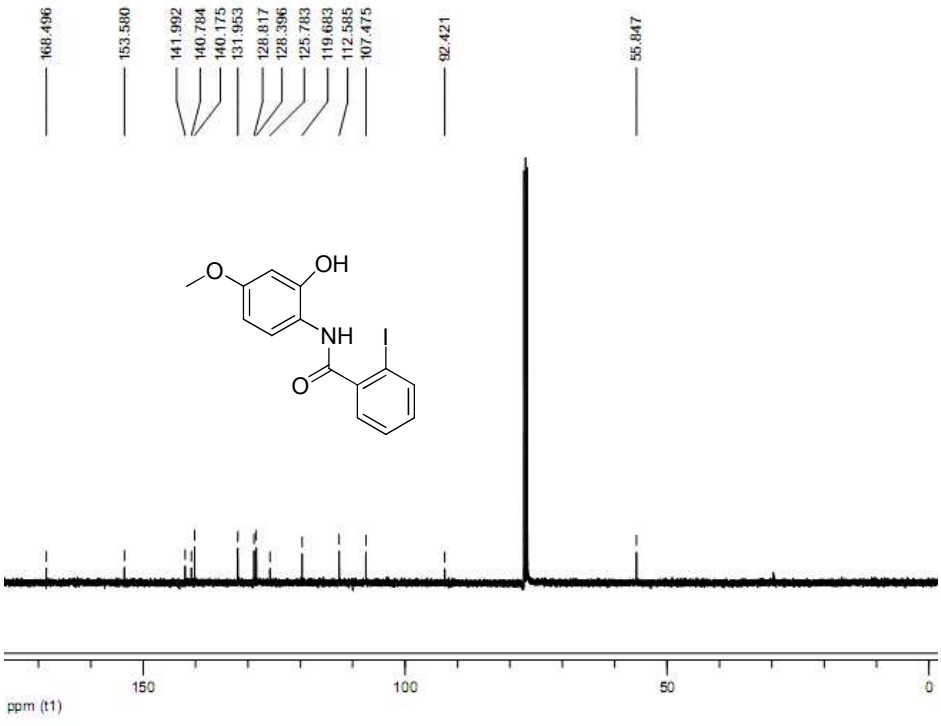

$^{1}$H NMR spectra of compound **52d** (DMSO-$d_6$, 400 MHz)



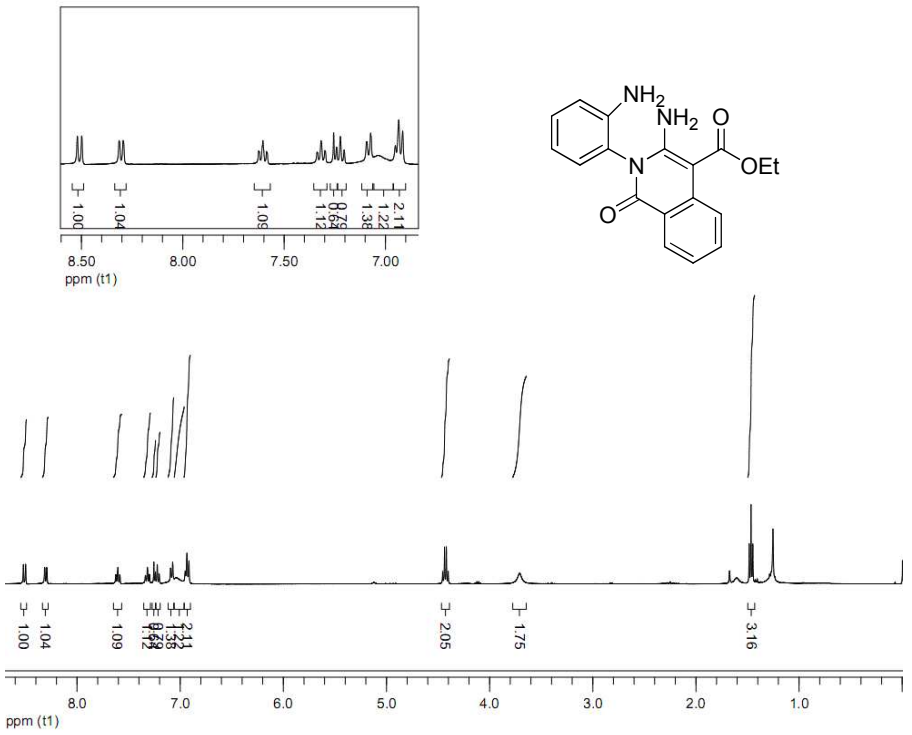

$^{13}$C NMR spectra of compound **52d** (DMSO-$d_6$, 100 MHz)



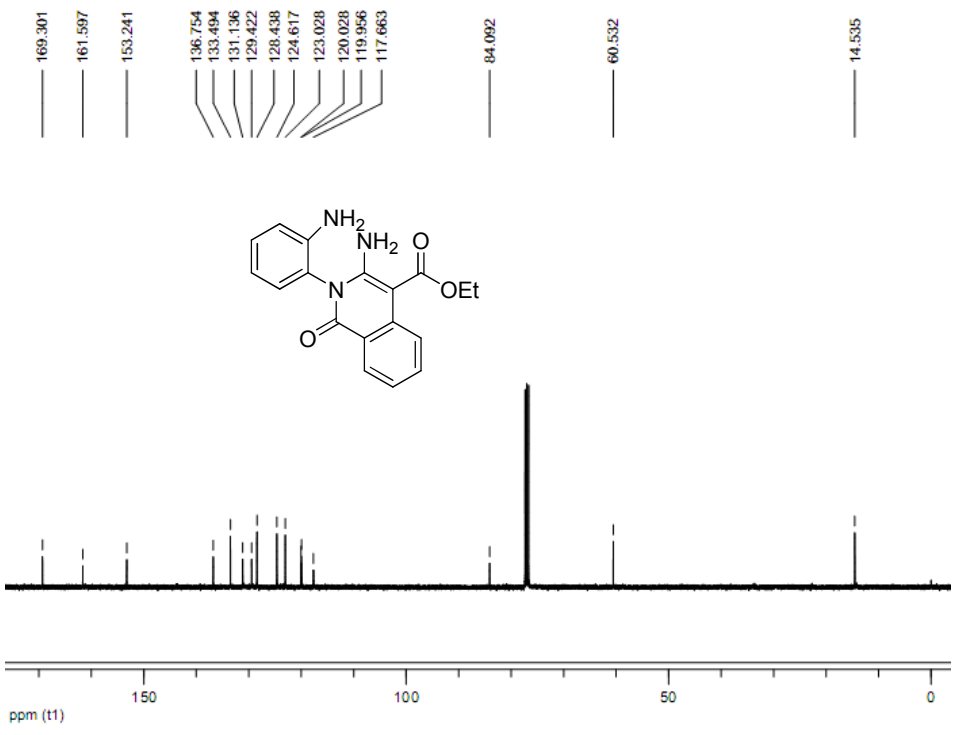

$^{1}$H NMR spectra of compound **52e** ($CDCl_3$, 400 MHz)



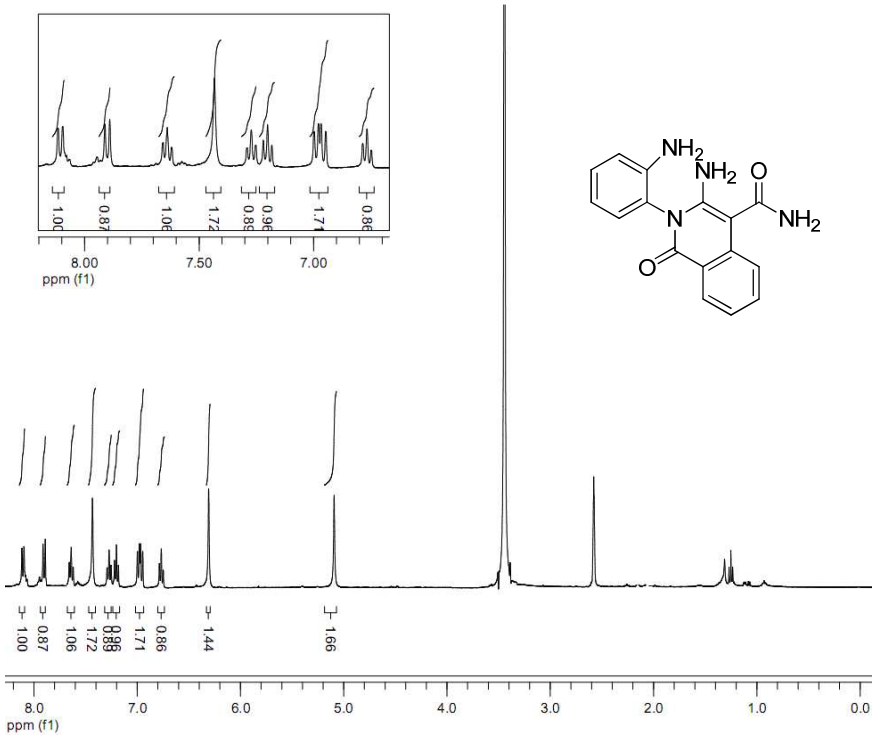

$^{13}$C NMR spectra of compound **52e** ($CDCl_3$, 100 MHz)



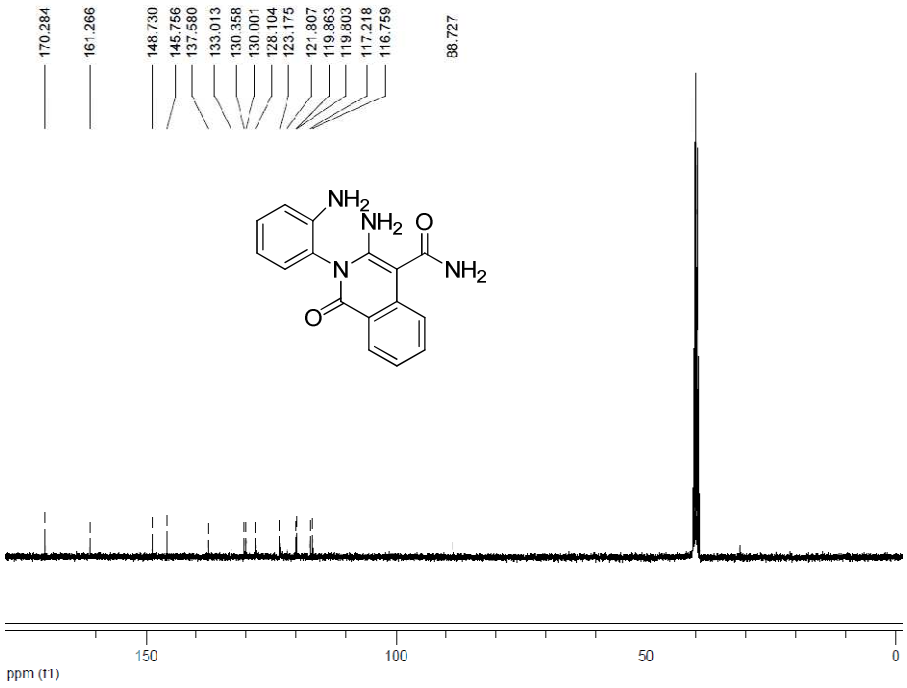

$^{1}$H NMR spectra of compound **48a** ($CDCl_3$, 400 MHz)



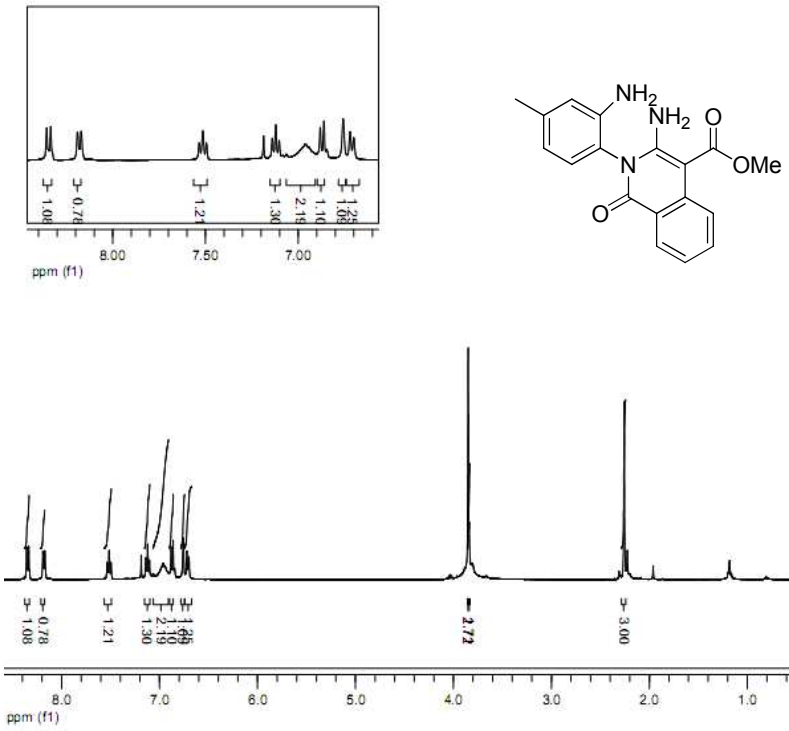

$^{13}$C NMR spectra of compound **48a** ($CDCl_3$, 100 MHz)



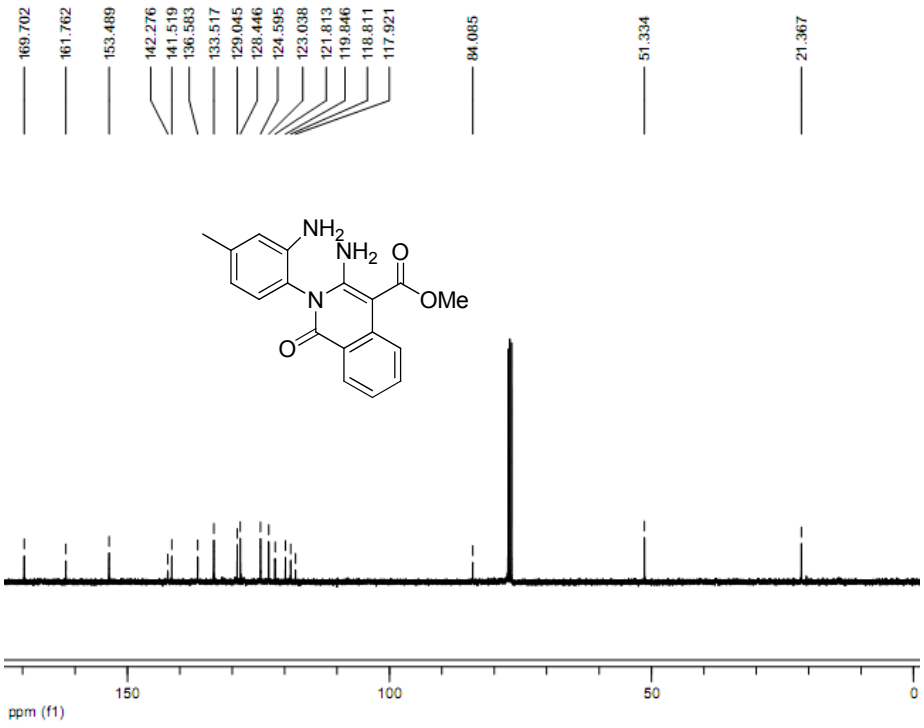

$^{1}$H NMR spectra of compound **48e** (DMSO-$d_6$, 400 MHz)



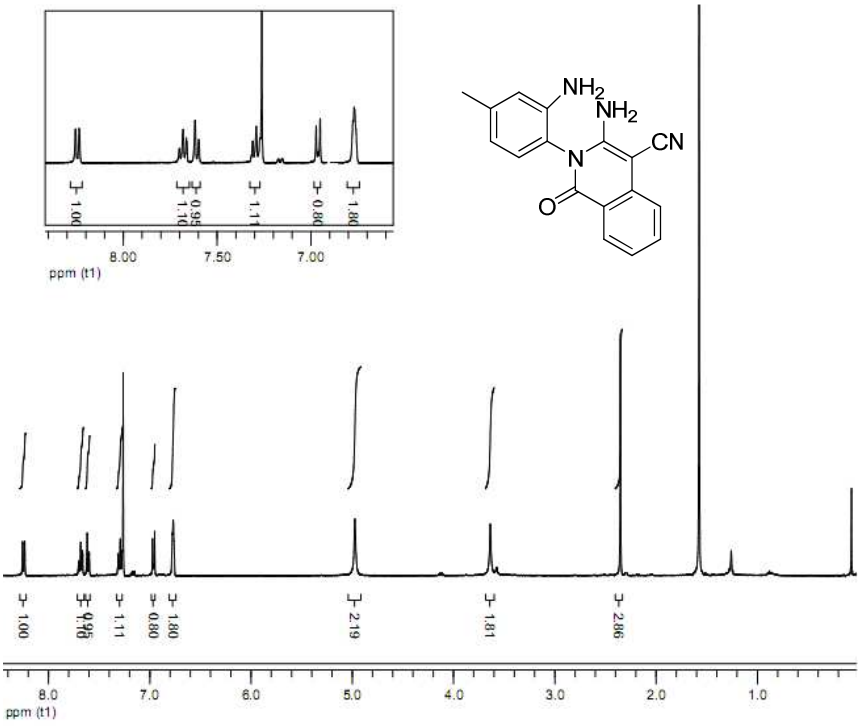

$^{13}$C NMR spectra of compound **48e** (DMSO-$d_6$, 100 MHz)



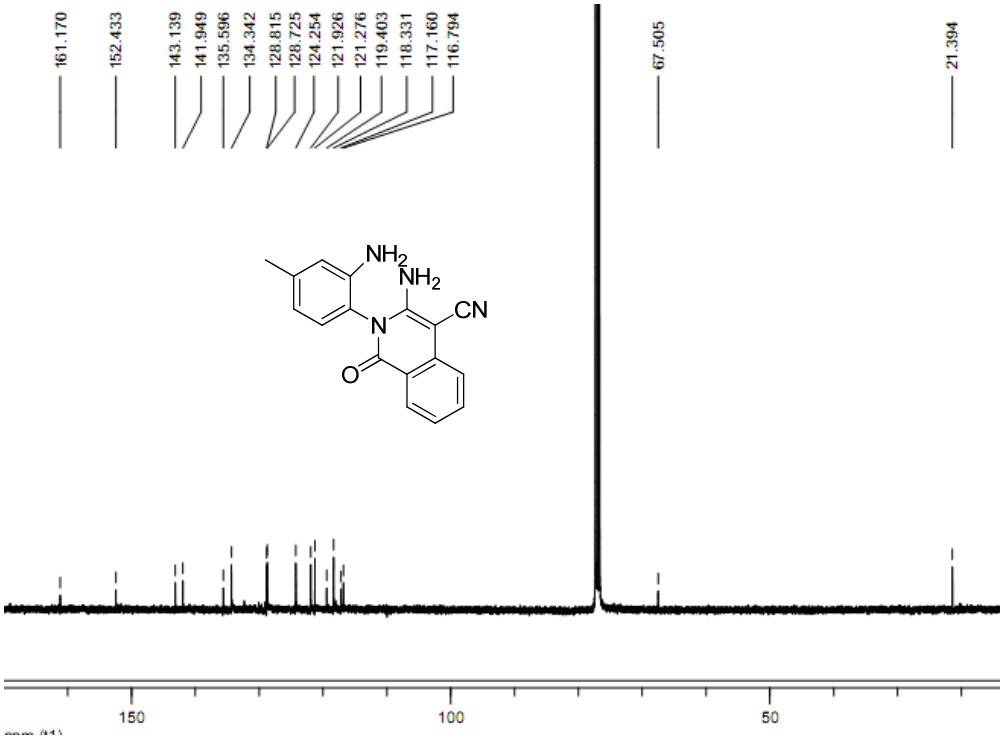

$^{1}$H NMR spectra of compound **48g** ($CDCl_3$, 400 MHz)



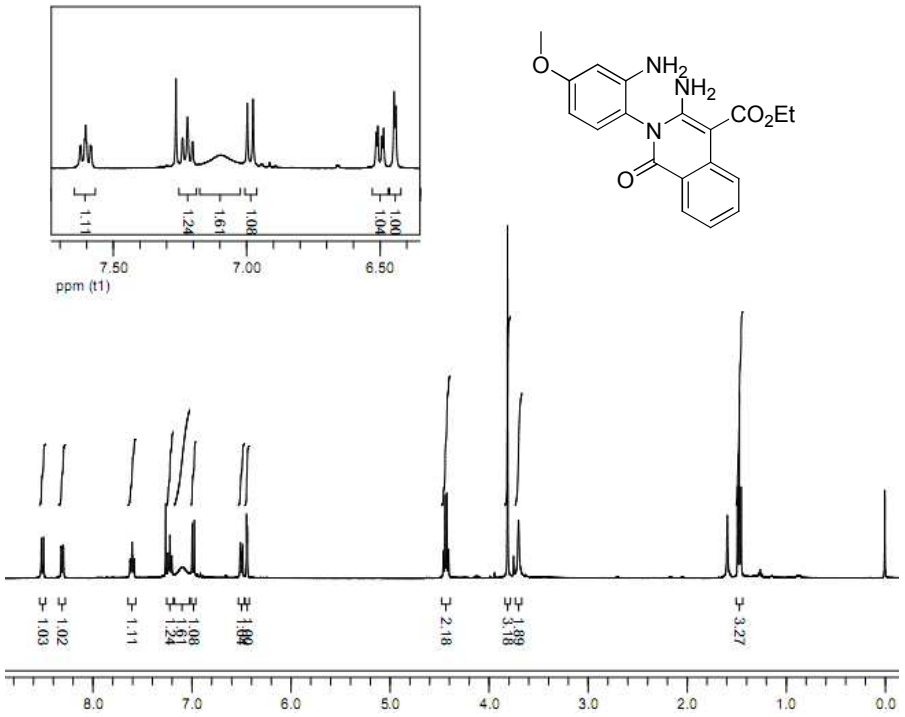

$^{13}$C NMR spectra of compound **48g** ($CDCl_3$, 100 MHz)



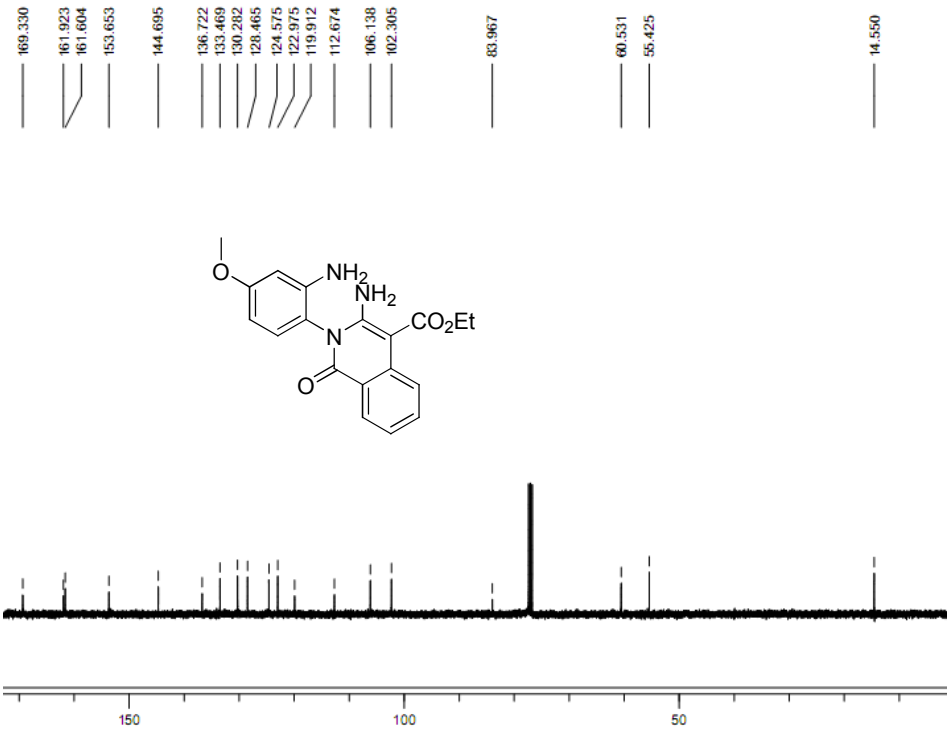

$^{1}$H NMR spectra of compound **48h** (DMSO-$d_6$, 400 MHz)



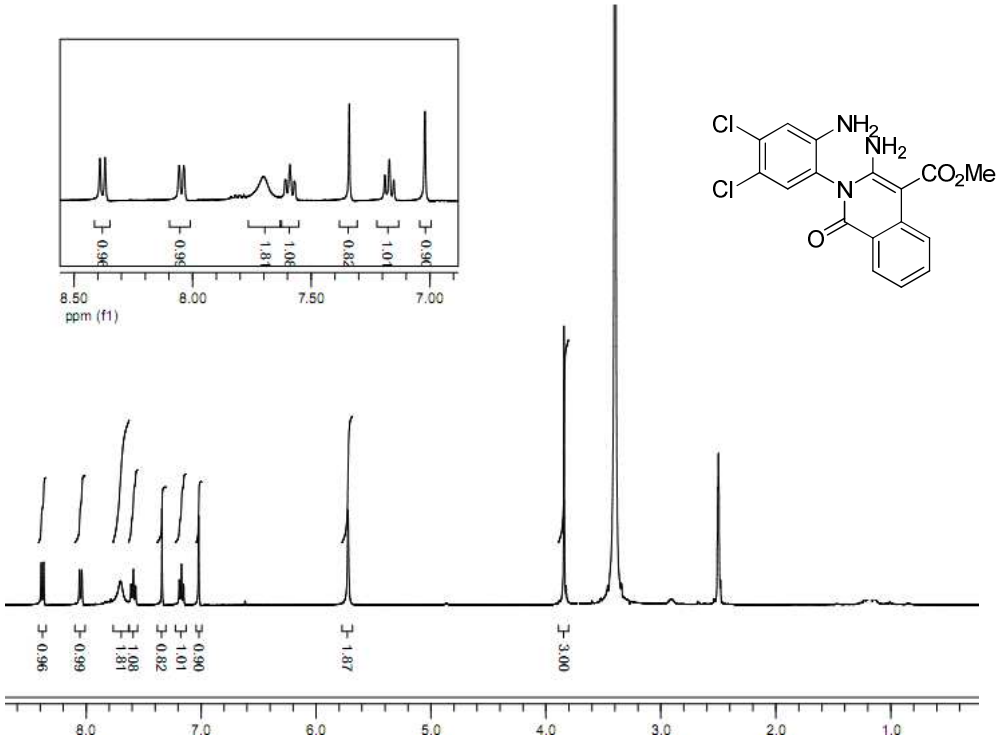

$^{13}$C NMR spectra of compound **48h** (DMSO-$d_6$, 100 MHz)



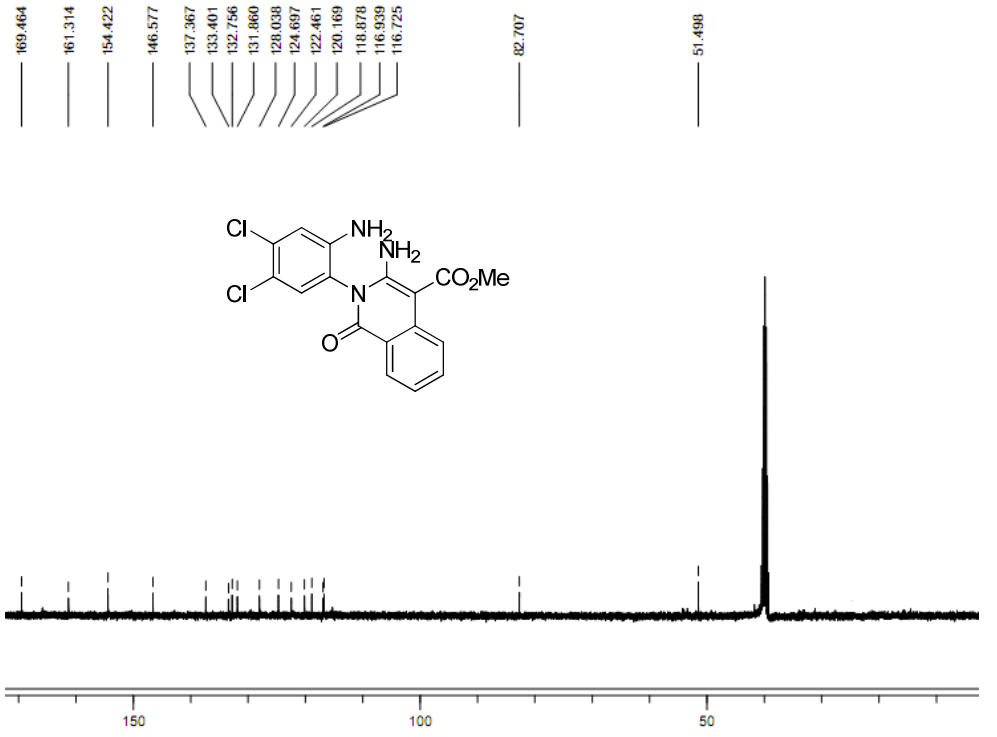

$^{1}$H NMR spectra of compound **48j** ($CDCl_3$, 400 MHz)



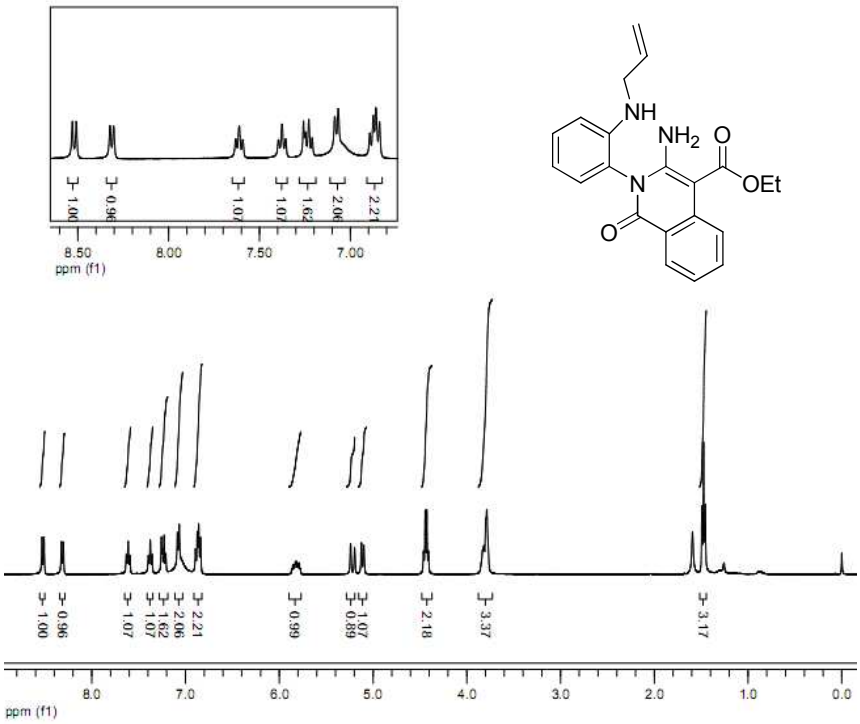

$^{13}$C NMR spectra of compound **48j** ($CDCl_3$, 100 MHz)



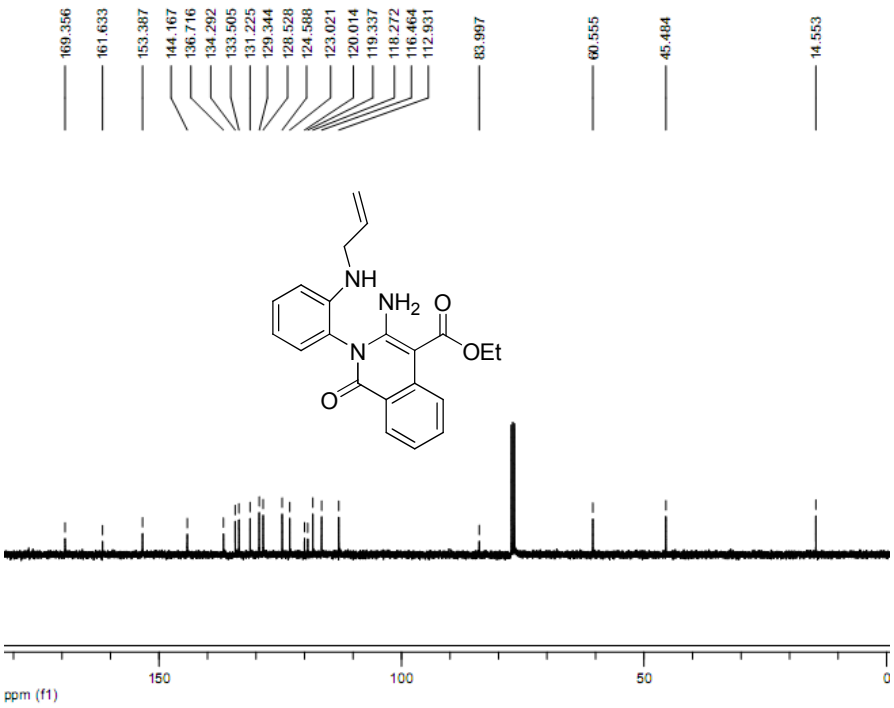

$^{1}$H NMR spectra of compound **48m** (DMSO-$d_6$, 400 MHz)



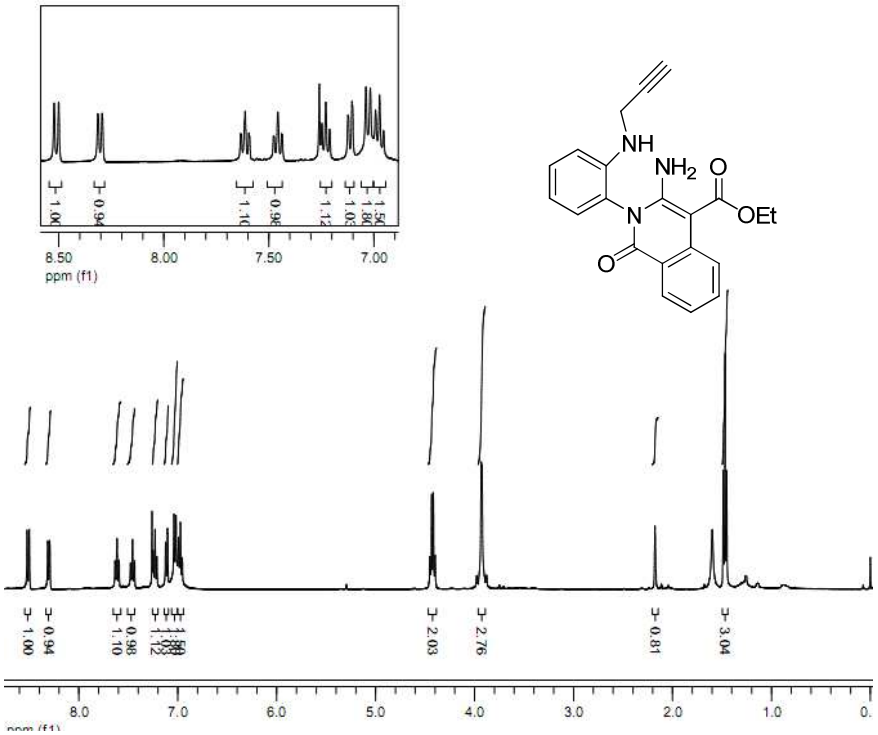

$^{13}$C NMR spectra of compound **48m** (DMSO-$d_6$, 100 MHz)



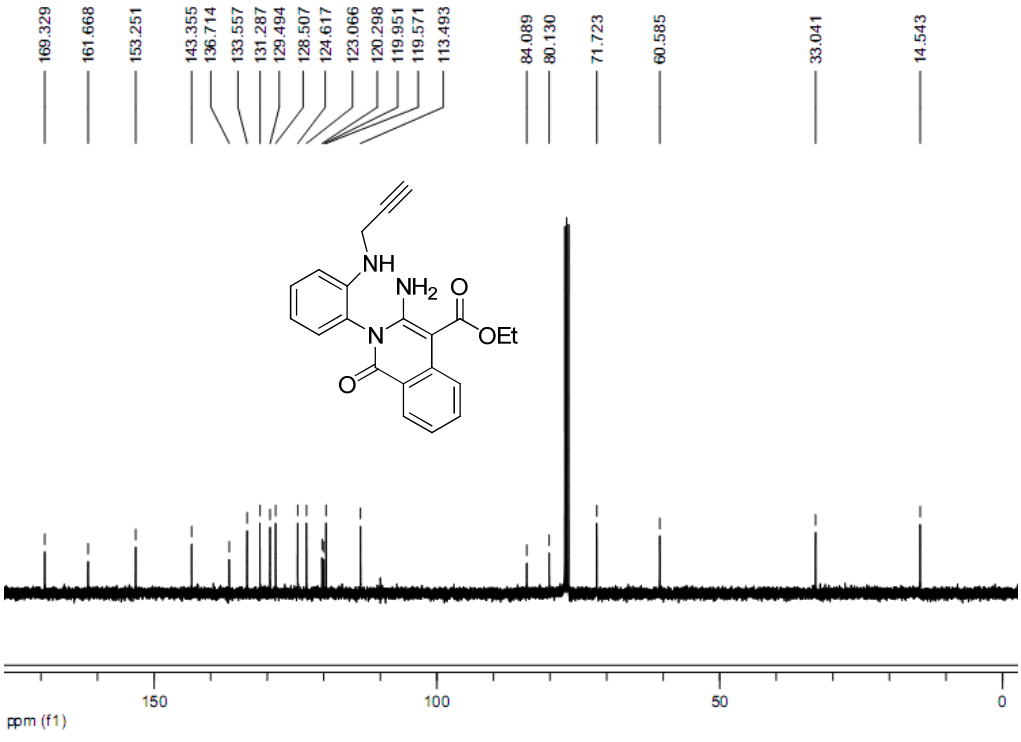

$^{1}$H NMR spectra of compound **54a** ($CDCl_3$, 400 MHz)



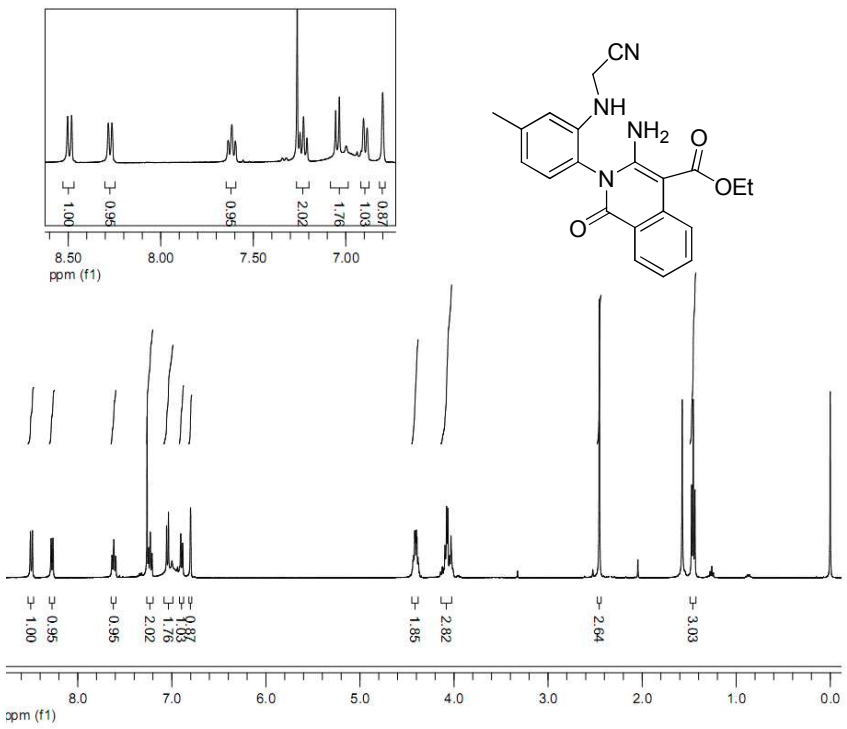

$^{13}$C NMR spectra of compound **54a** ($CDCl_3$, 100 MHz)



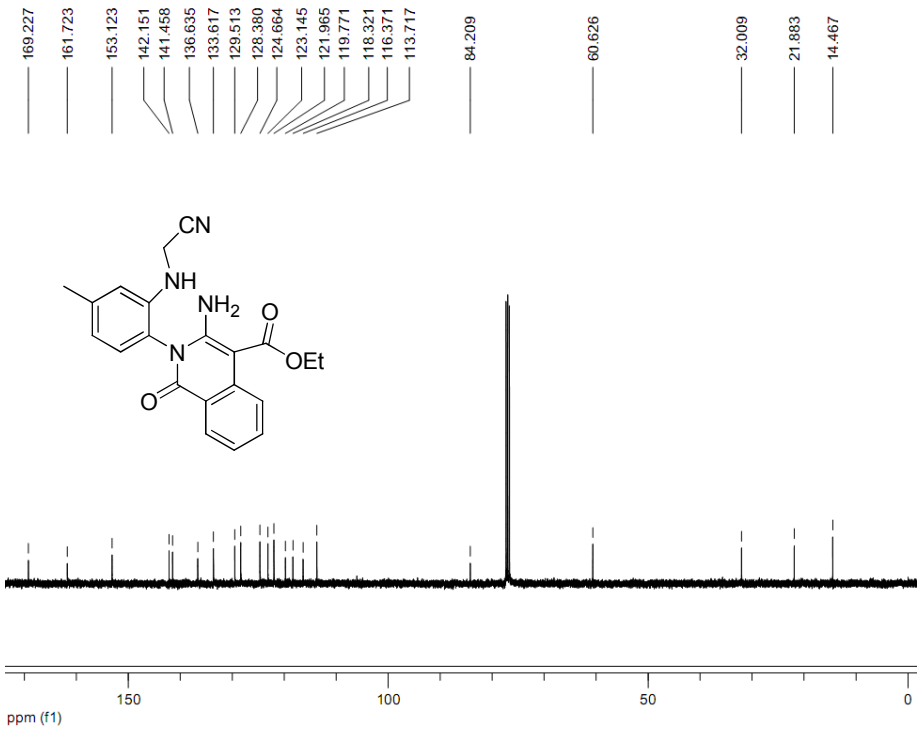

$^{1}$H NMR spectra of compound **54c** ($CDCl_3$, 400 MHz)



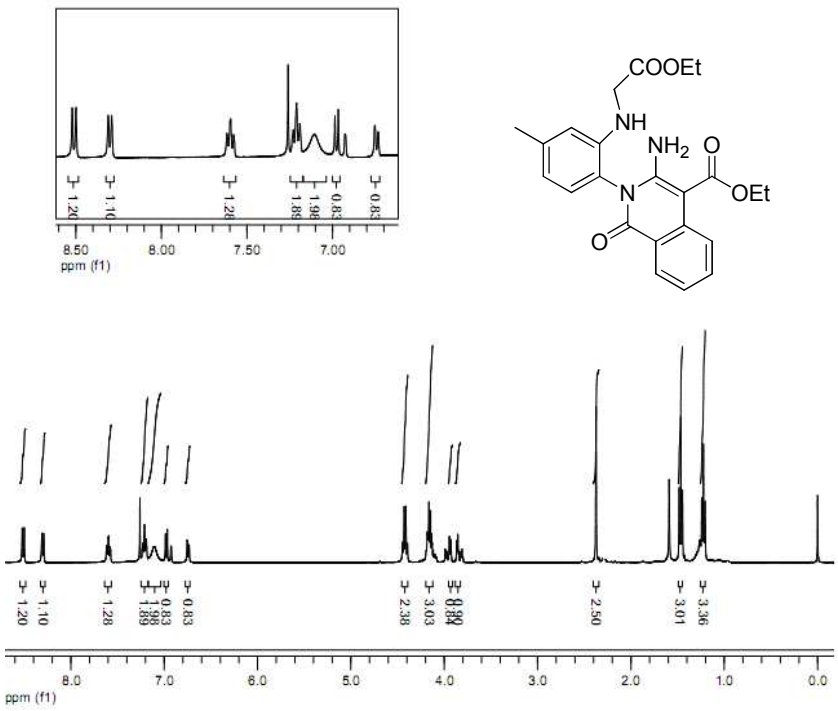

$^{13}$C NMR spectra of compound **54c** ($CDCl_3$, 100 MHz)



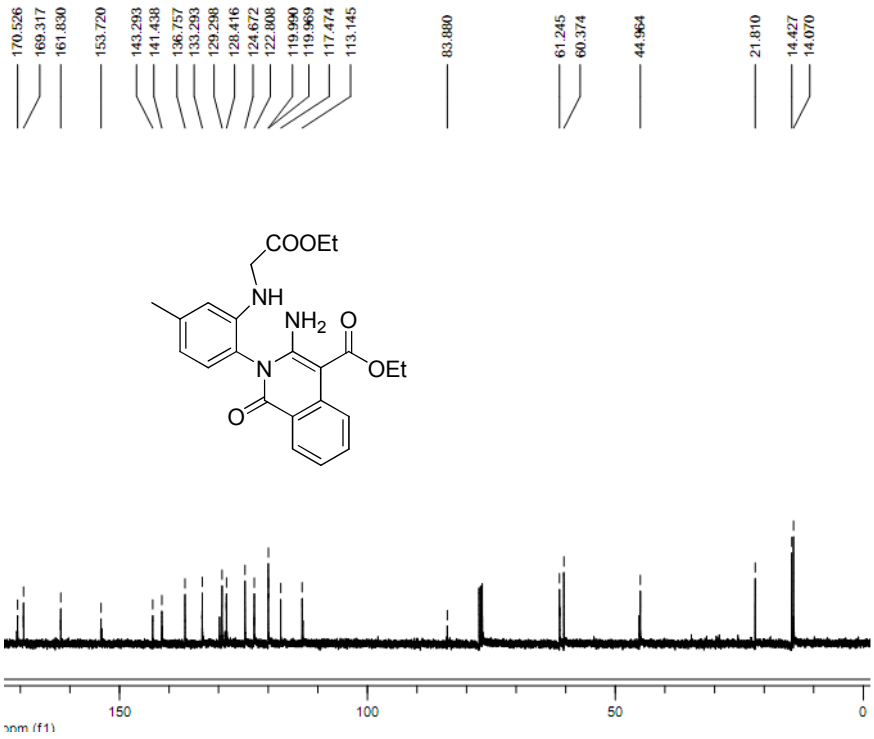

$^{1}$H NMR spectra of compound **54g** ($CDCl_3$, 400 MHz)



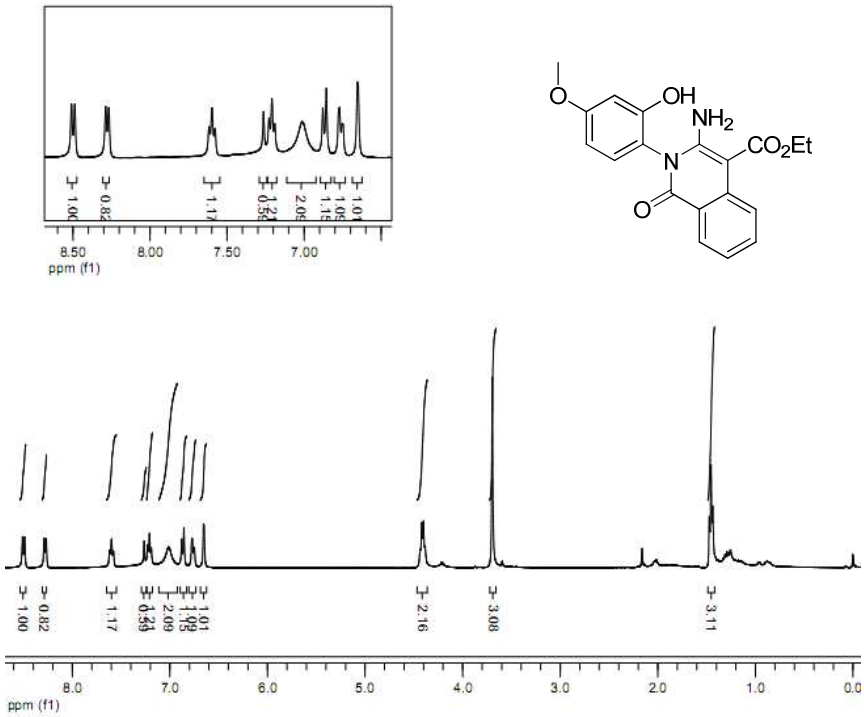

$^{13}$C NMR spectra of compound **54g** ($CDCl_3$, 100 MHz)



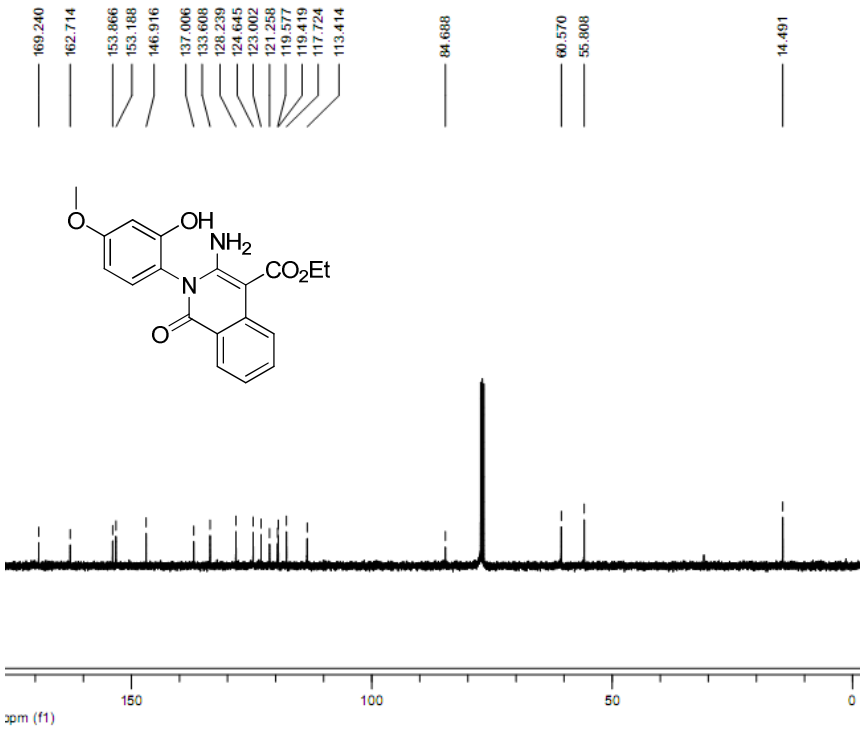

$^{1}$H NMR spectra of compound **54h** ($CDCl_3$, 400 MHz)



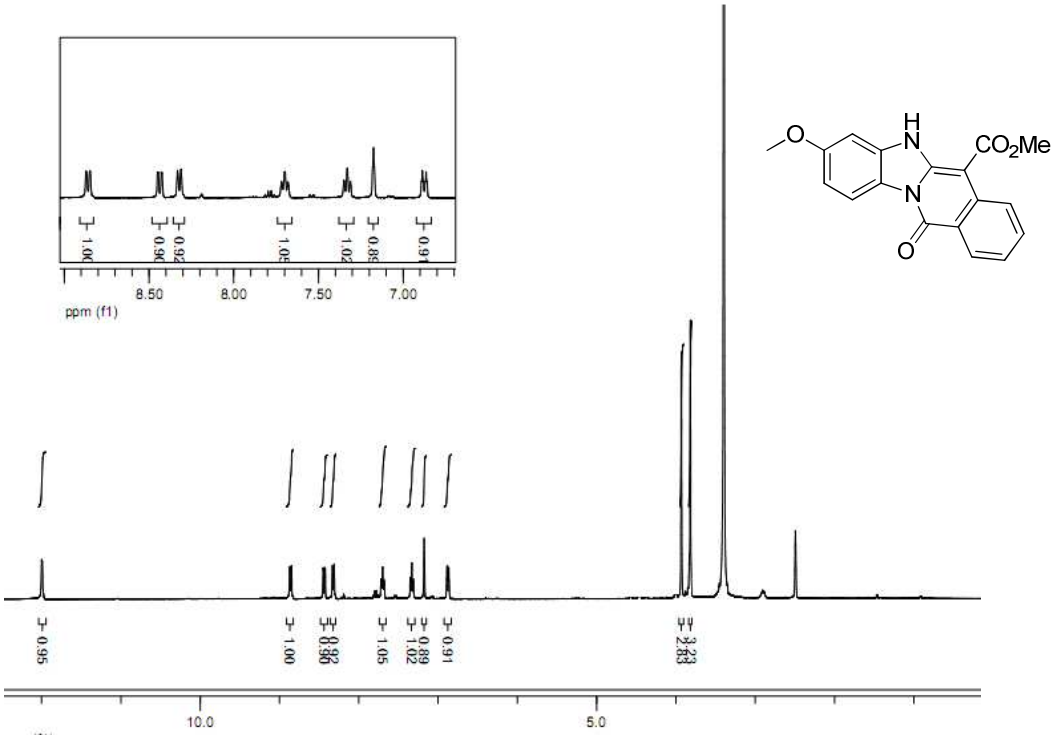

$^{13}$C NMR spectra of compound **54h** ($CDCl_3$, 100 MHz)



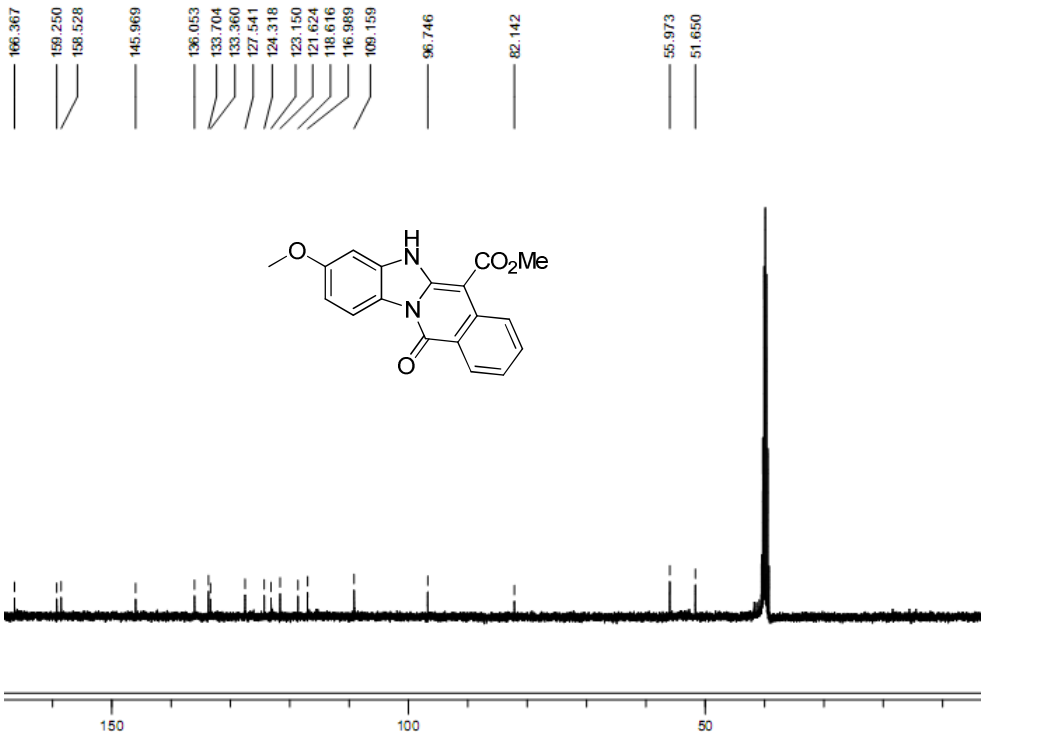

$^{1}$H NMR spectra of compound **48n** ($CDCl_3$, 400 MHz)



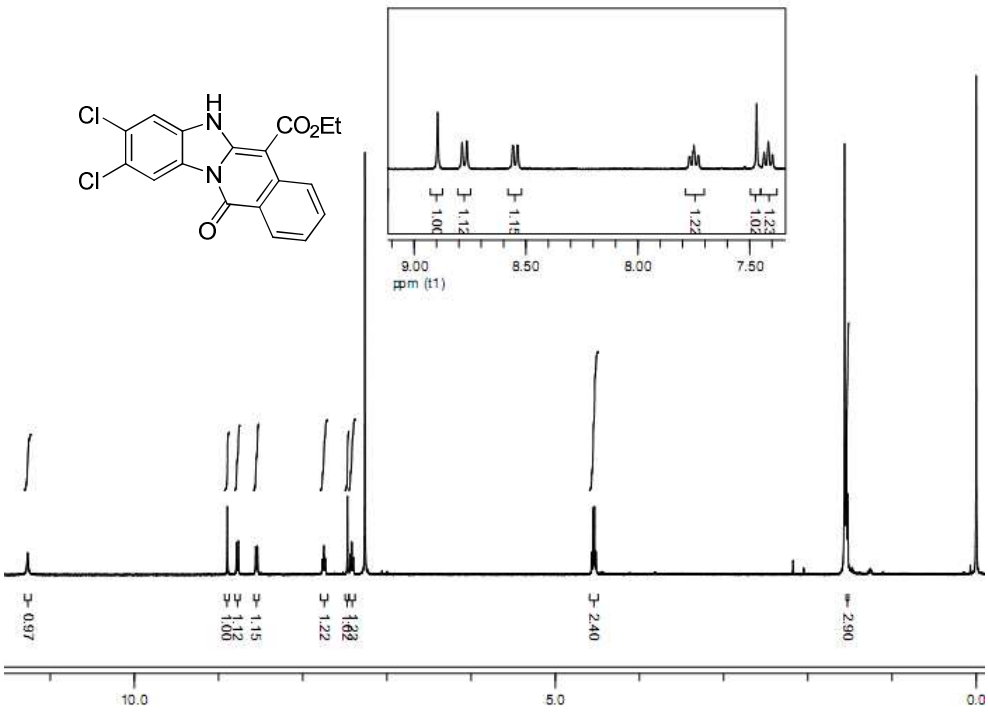

$^{13}$C NMR spectra of compound **48n** ($CDCl_3$, 100 MHz)



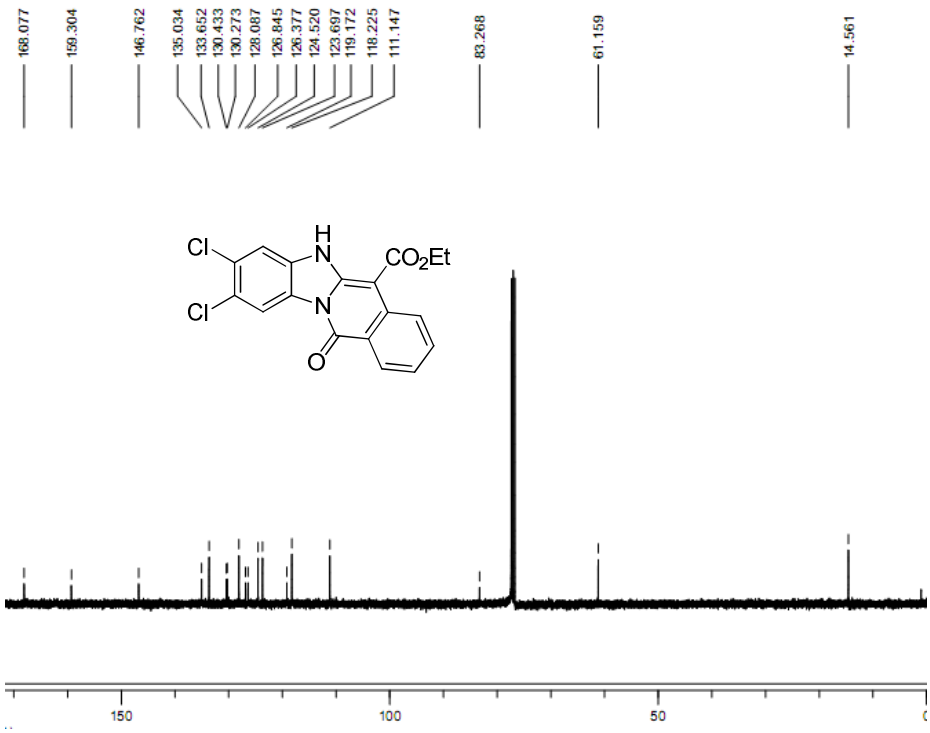

$^{1}$H NMR spectra of compound **49k** (DMSO-$d_6$, 400 MHz)



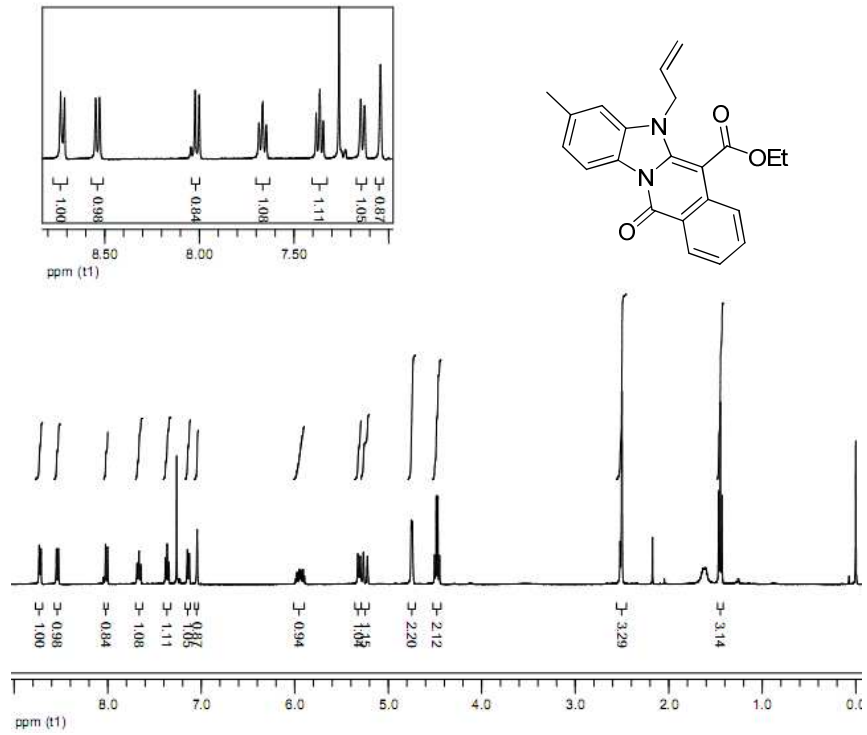

$^{13}$C NMR spectra of compound **49k** (DMSO-$d_6$, 100 MHz)



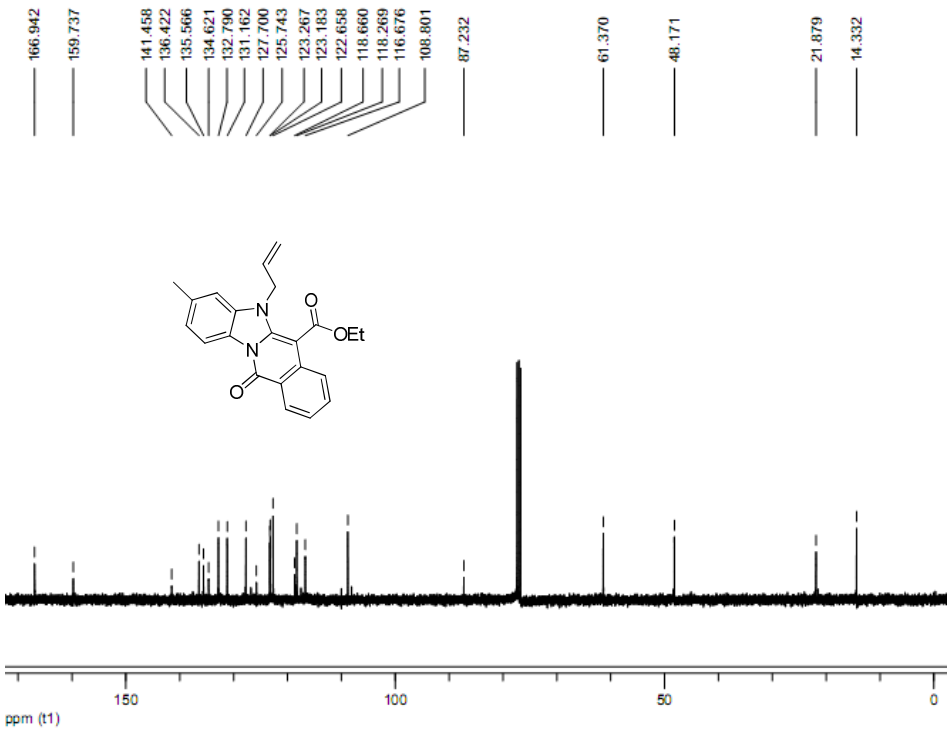

$^{1}$H NMR spectra of compound **49l** ($CDCl_3$, 400 MHz)



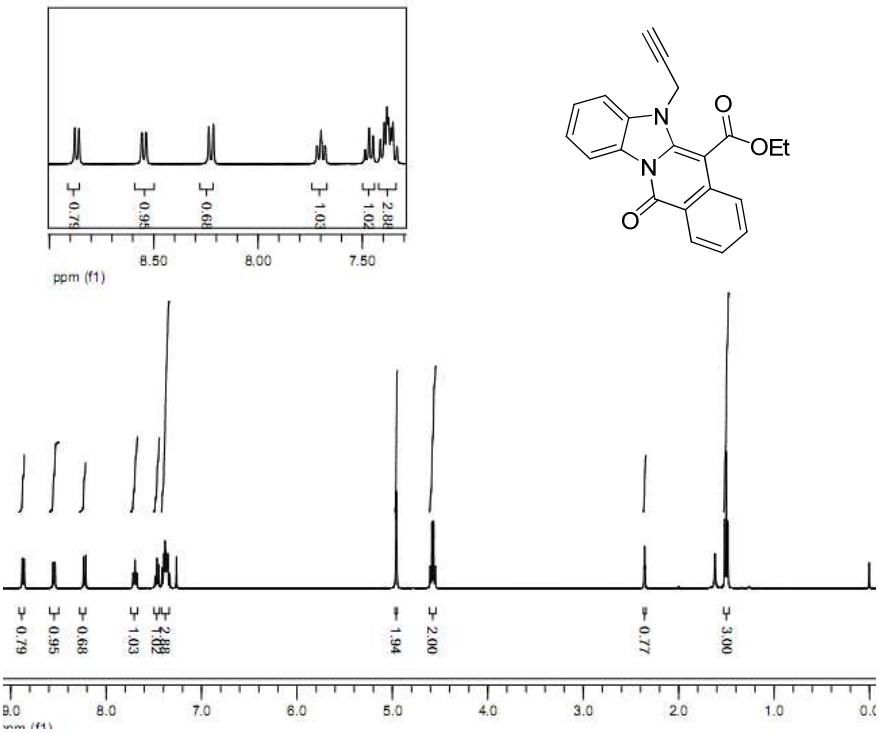

$^{13}$C NMR spectra of compound **49l** ($CDCl_3$, 100 MHz)



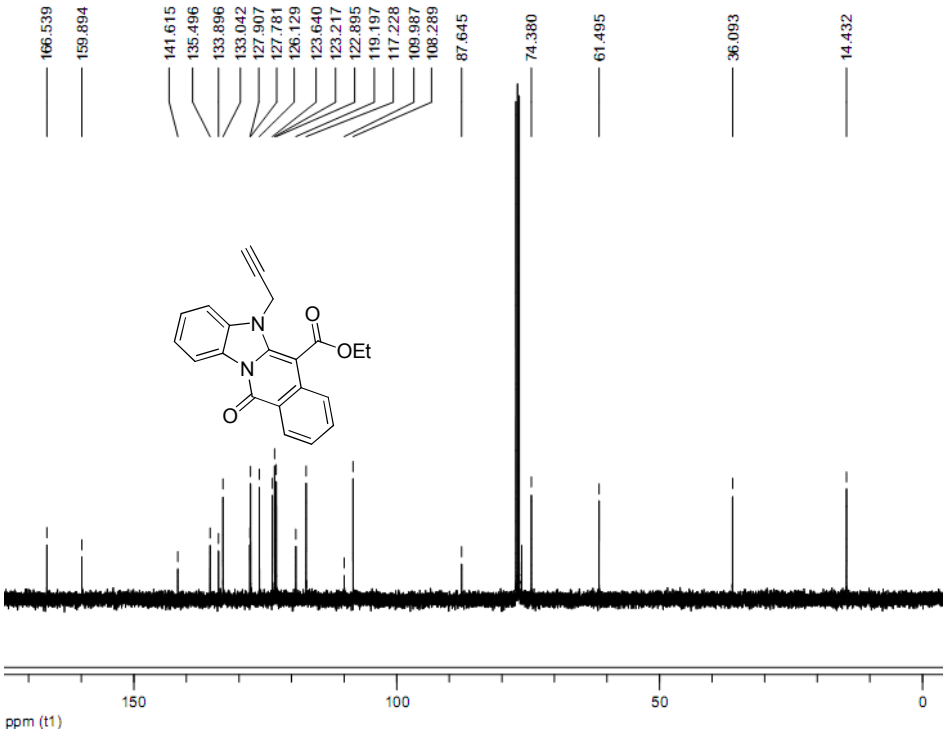

$^{1}$H NMR spectra of compound **49o** ($CDCl_3$, 400 MHz)



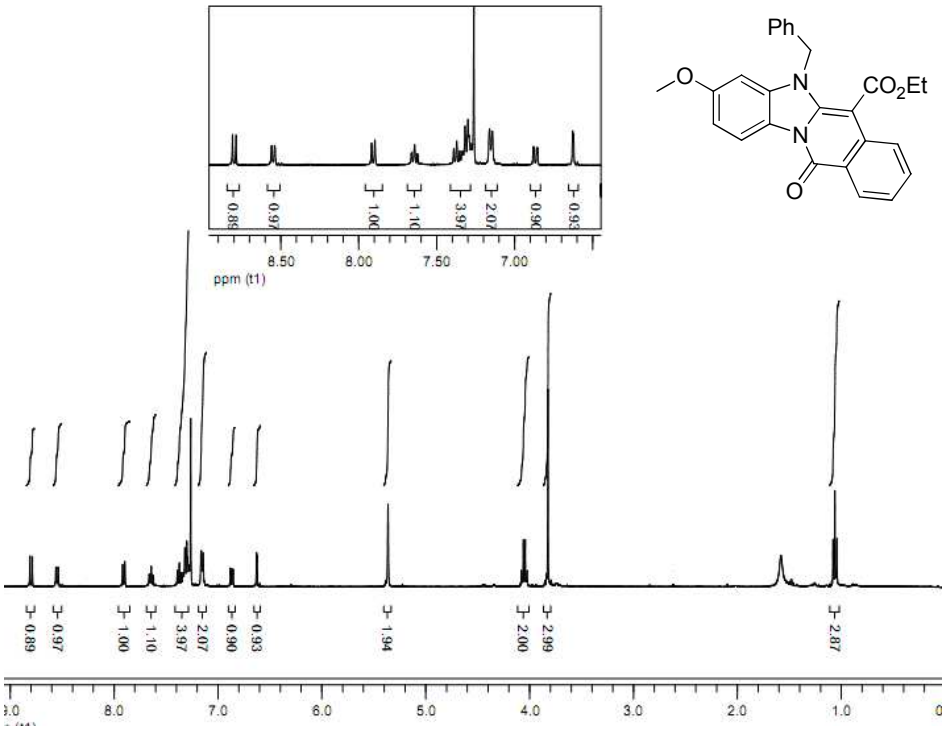

$^{13}$C NMR spectra of compound **49o** ($CDCl_3$, 100 MHz)



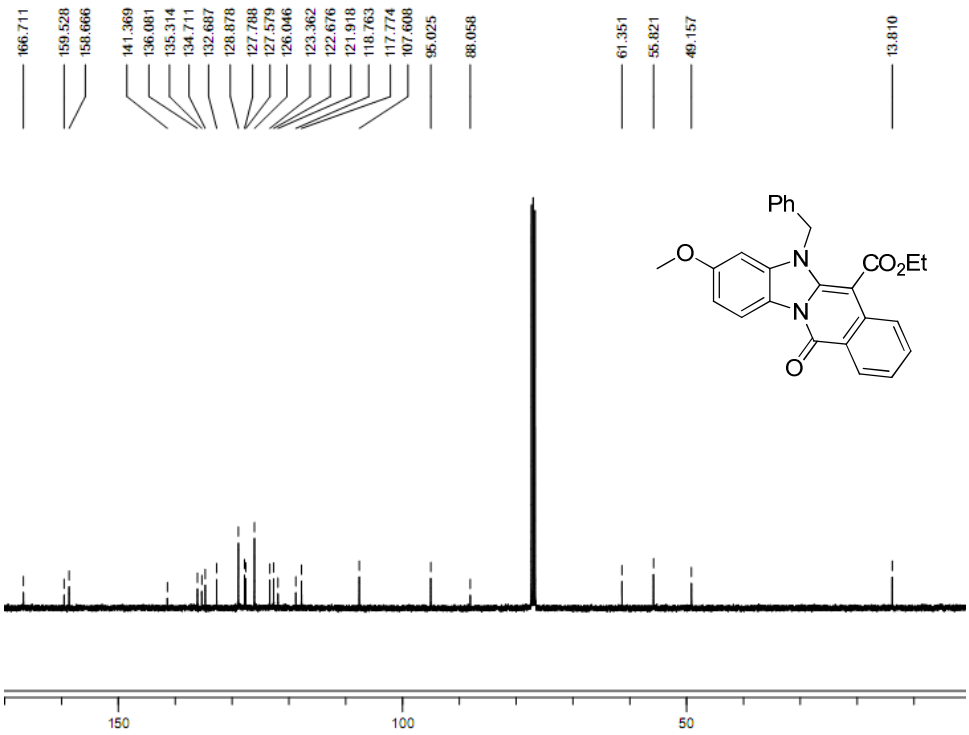

$^{1}$H NMR spectra of compound **49p** ($CDCl_3$, 400 MHz)



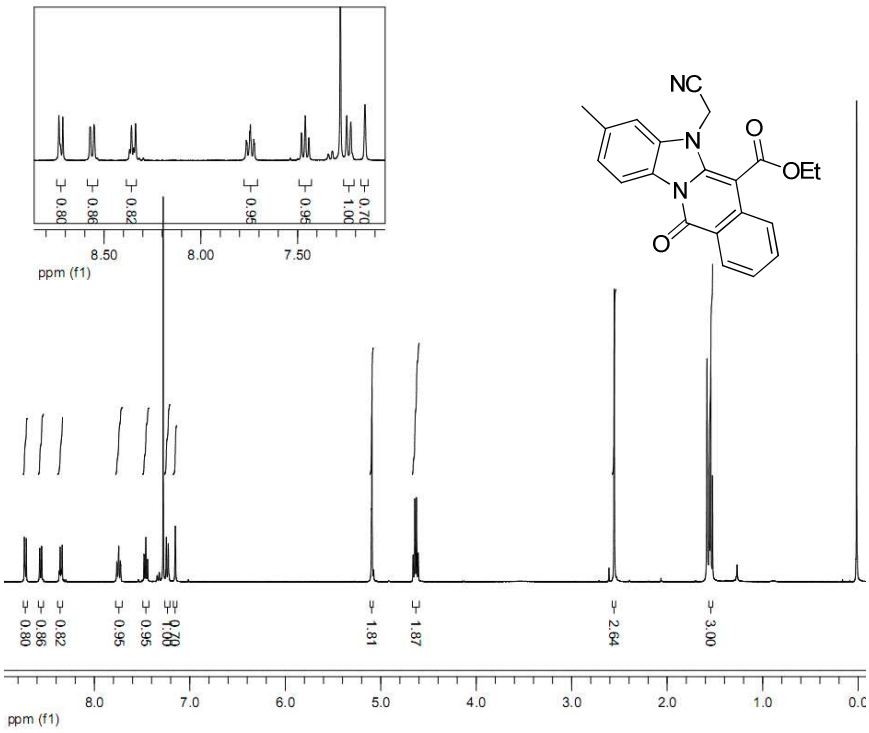

$^{13}$C NMR spectra of compound **49p** ($CDCl_3$, 100 MHz)



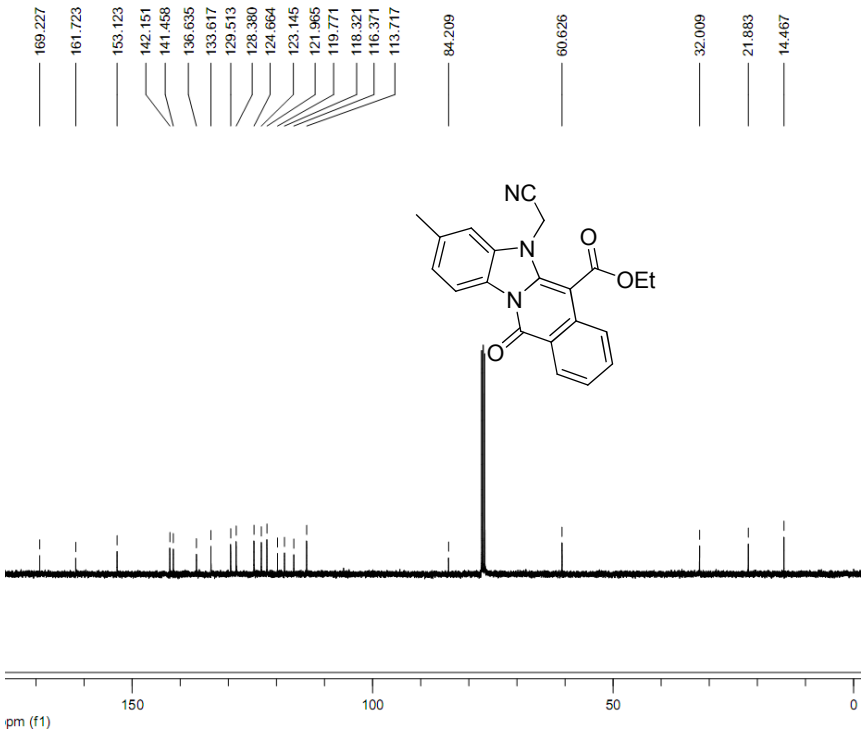

$^{1}$H NMR spectra of compound **49s** ($CDCl_3$, 400 MHz)



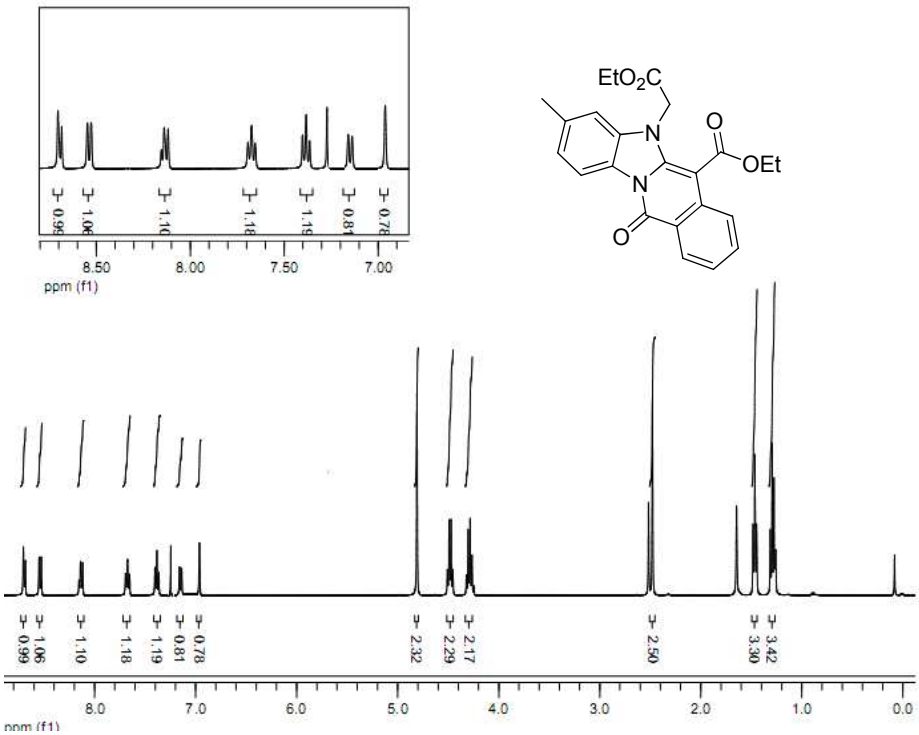

$^{13}$C NMR spectra of compound **49s** ($CDCl_3$, 100 MHz)



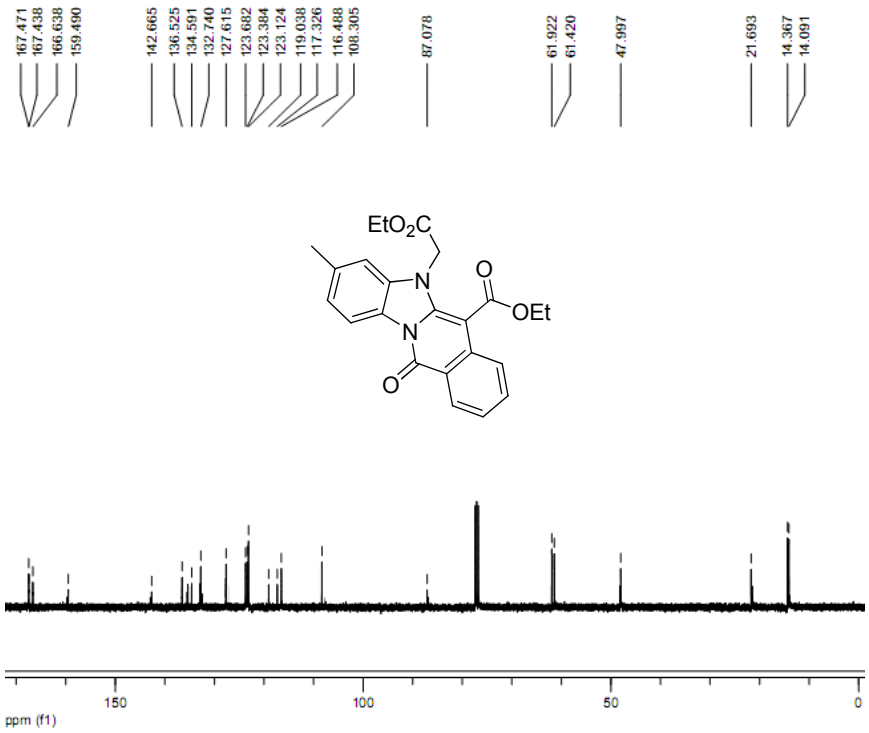

$^{1}$H NMR spectra of compound **49t** ($CDCl_3$, 400 MHz)



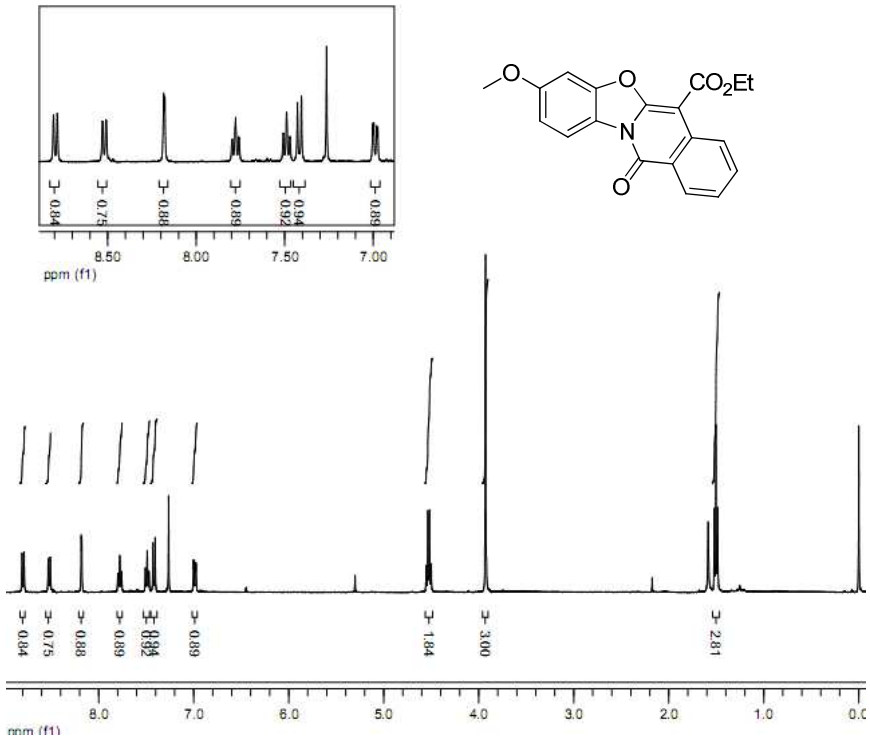

$^{13}$C NMR spectra of compound **49t** ($CDCl_3$, 100 MHz)



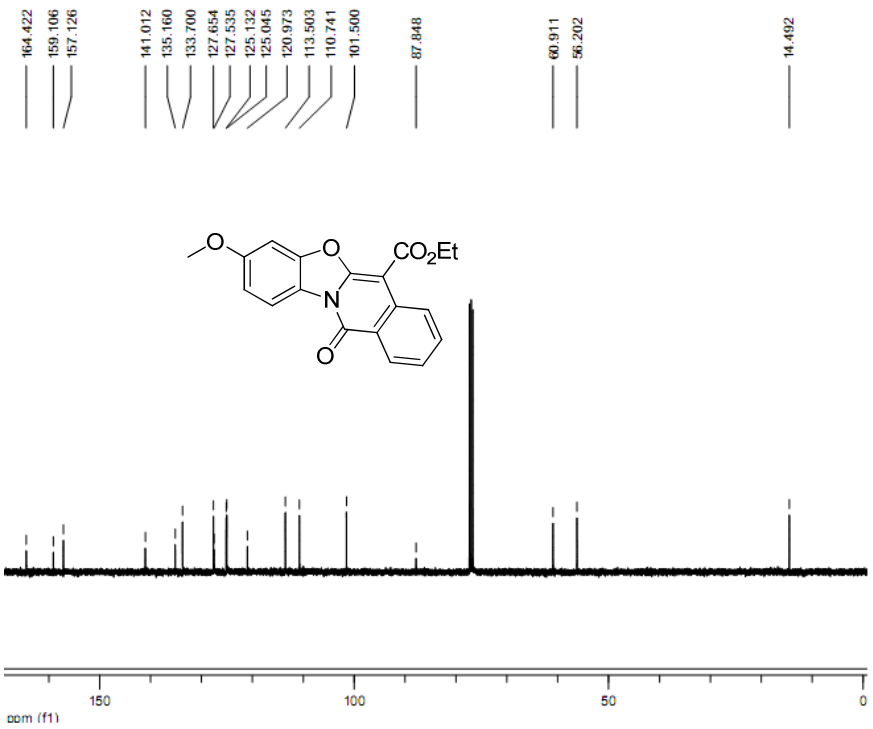

$^{1}$H NMR spectra of compound **49u** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **49u** ($CDCl_3$, 100 MHz)



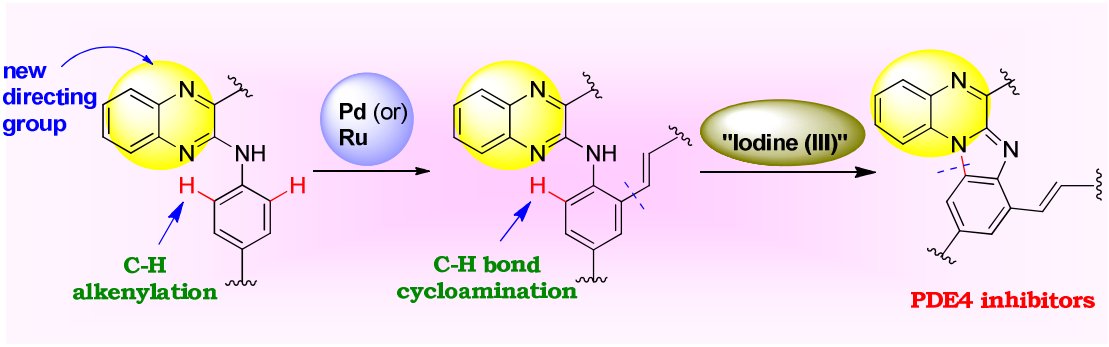

$^{1}$H NMR spectra of compound **49v** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **49v** ($CDCl_3$, 100 MHz)

# *Chapter 3*

## Synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxalines *via* C-H alkenylation / intramolecular ortho C–H cycloamination



## 3.1. Introduction:

Quinoxaline and its derivatives are versatile class of nitrogen containing heterocyclic compounds that are prevalent in many natural products (like echinomycin & triostin-A) and pharmaceuticals possessing a broad spectrum of biological activities.[1] In addition, quinoxaline derivatives also play important roles in materials science such as preparation of luminescent materials and polymers.[2] A few quinoxaline scaffold containing pharmacologically active molecules are shown in (Figure 3.1). Quinacillin (**1**) is highly effective against penicillinase producing strains of *staphylococcus aureus*. AG 1295 (**2**)[3] and AG 1385 (**3**) acted as an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Compound (**4**) is a topoisomerase II inhibitor[4] and compound (**5**) is an inhibitor of p38 alpha MAP kinase.[5] Compound (**6**) is identified as selective antagonist of human A (3) adenosine receptors.[6] On the other hand, benzimidazoles are biologically and medicinally important chemical entities, which have a wide range of biological activities and are often used as enzyme inhibitors[7], drugs[8], and dyes.[9]

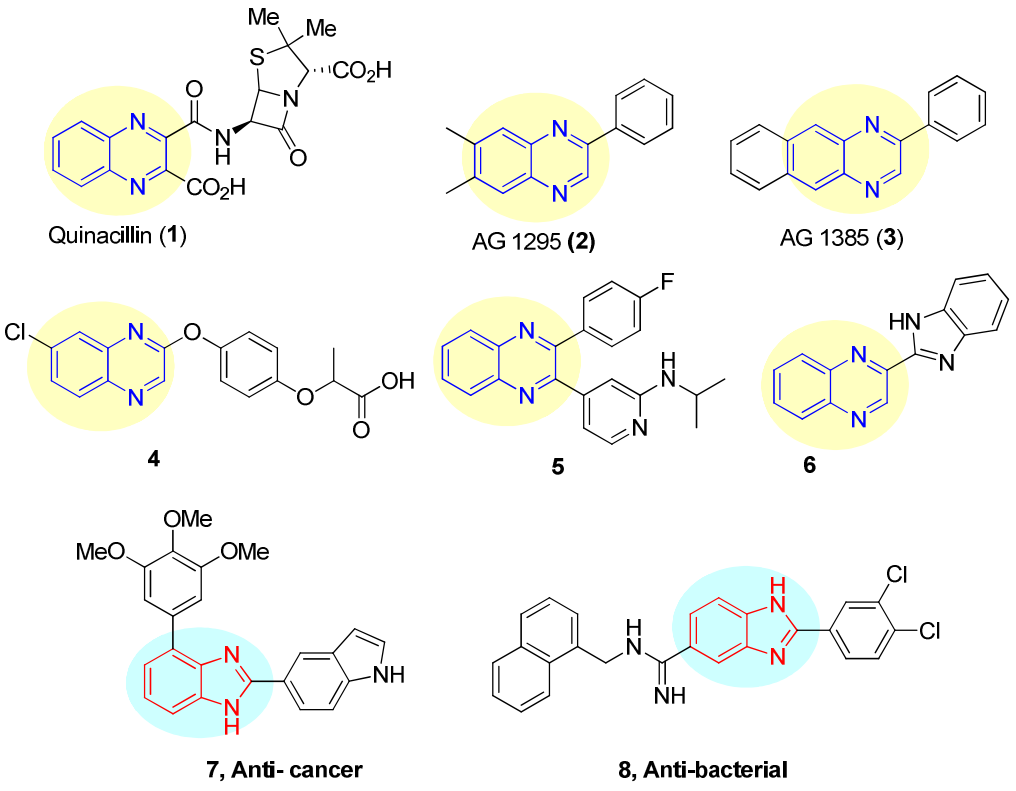



**Fig. 3.1**: Examples of quinoxaline and benzimidazole containing biologically active compounds.

Functionalized alkenes[10] have been explored in the discovery of new drugs[11] e.g. tamoxifen[11a] (Figure 3.2) was used to prevent breast cancer in women in the past. We envisioned that assembly of quinoxaline and benzimidazole framework in a single molecular entity *i.e.* benzo[4,5]imidazo[1,2-*a*]-quinoxaline with an alkenyl moiety attached with it largely remained underexplored. Therefore, the development of a convenient and efficient synthetic approach towards alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines (**A**, Figure 3.2) will be valuable for their screening against various biological targets. The selection of benzo[4,5]imidazo[1,2-*a*]quinoxaline ring was inspired by the outstanding biological and medicinal activities of structurally related imidazo[1,2-*a*]quinoxaline based molecules[12] e.g. EAPB0203 (**B**, Figure 3.2). The core structure of benzo[4,5]imidazo[1,2-*a*]quinoxalines (**A)** *i.e.* the central polynuclear heterocyclic ring can be realized simply by moving and fusioning the benzene ring of 3-methoxy phenyl group with the imidazole ring of EAPB0203 (**B)** as shown in Figure 3.2.

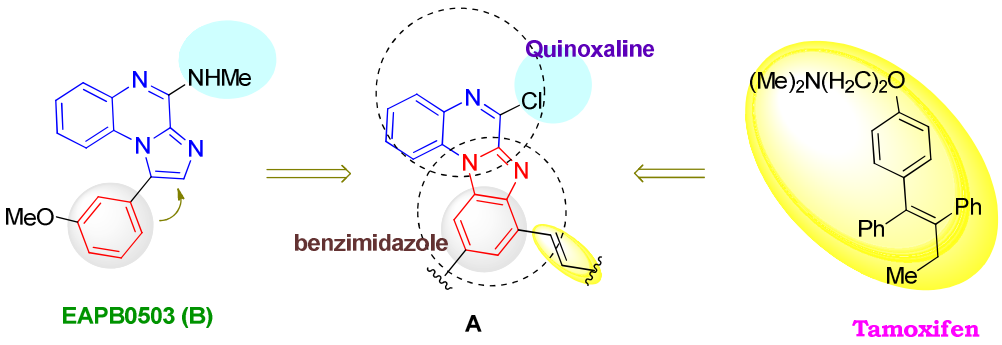



**Fig. 3.2**: New alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines (**A**) from a known imidazo[1,2-*a*]quinoxaline derivative EAPB0203 (**B**) and Tamoxifen.

In recent years, methods involving C-H bond activation and subsequent functionalization has become an attractive area of research in organic synthesis. This is because it avoids the use of prefunctionalized starting materials, and there by improving the step-economy of the synthetic route. Especially, transition-metal-catalyzed C-H olefination reactions have been the subject of tremendous research complementing to Mizoroki-Heck reaction[13] because of drawbacks such as, limited availability of expensive aryl halide component, or their cumbersome preparation. Direct C-H bond olefination (the Fujiwara−Moritani reaction)[14] has attracted huge attention as a greener and alternative approch to Mizoroki-Heck reaction during past several years. While various transition metals including Pd,

Cu, Ni, Co, Rh, and Ru have been used extensively for the C-H functionalization leading to C−C and C−heteroatom bond formation.[15] The Pd-catalyzed chelation-directed $sp^2$ C-H activation has been found to be a highly effective strategy for this purpose. However, selectivity in the activation process can be controlled by suitably positioned directing groups. As a result, a wide range of directing groups has been reported to aid C(Ar)-H activation until recently e.g. pyridine[16], imidazoline[17], pyrazole[18], oxazoline[19], amide[20], oximeether,[21] ketones,[22] hydroxyl,[23] carboxylic acids,[24] 2-pyridylsulfinyl,[25] quinoline[26] etc. However, several of these groups are non-removable and are considered as serious limitations for practical applications of these processes. In our strategy we wondered if any of these groups or a new one could be considered as a pre-installed precursor required for further chemical transformation instead of attempting their removal after C-H activation step. This strategy appeared to be attractive and economical and could avoid the problems associated with the removal of directing groups. A retro synthetic analysis of **A** depicted in (Figure 3.3) suggested that a quinoxaline moiety could serve the purpose and play the role of a directing group in the present case. This idea prompted us to test quinoxaline as a new directing group for the C-H functionalization with *o*-selectivity."

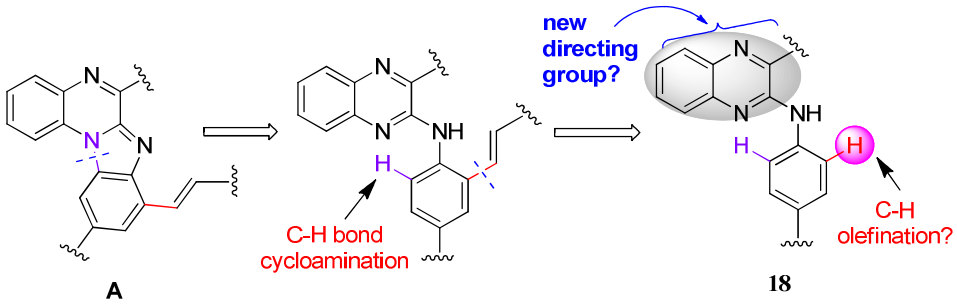
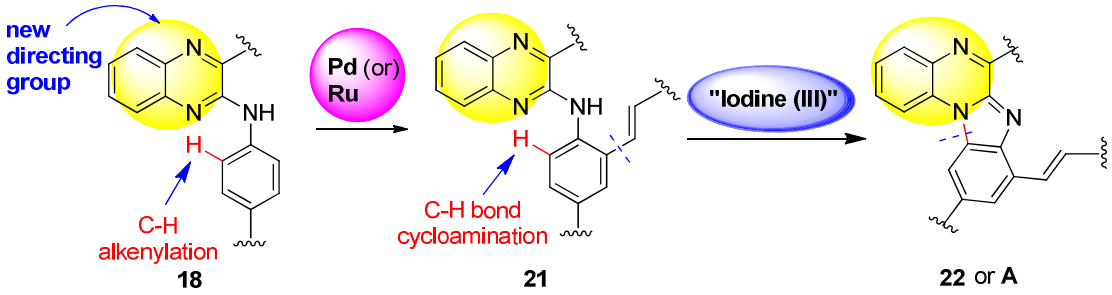



**Fig. 3.3**: Retrosynthetic analysis of compound **A**.

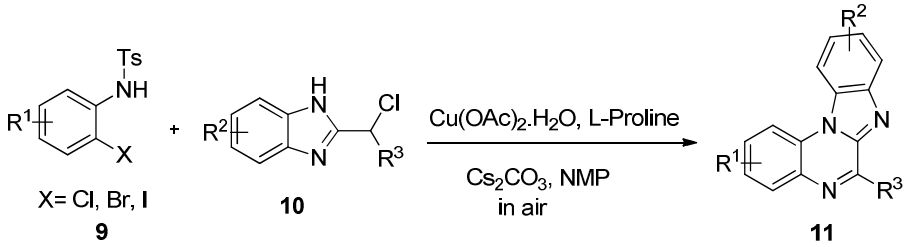
To obtain **A**, we have developed a two-step strategy for accessing new chemical entities based on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxaline framework *via* the Pd (or Ru)-catalyzed *ortho* C-H alkenylation and subsequent hypervalent iodine promoted intramolecular *ortho* C–H cycloamination reaction as shown in Scheme 3.1.




**Scheme 3.1**: Pd-catalyzed *ortho* C-H functionalization followed by intramolecular C–H cycloamination under open air leading to alkenyl substituted *N*-heteroarenes (**22**).

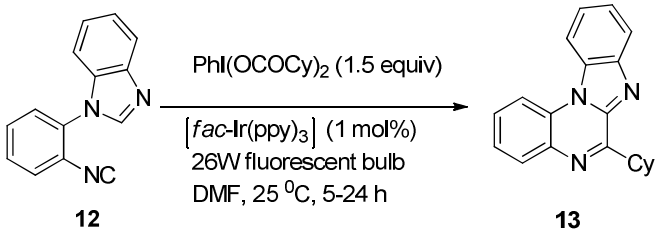
### 3.1.1. Previous reports for the synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxalines derivatives:

In 2013, Ma and coworkers developed a convenient and efficient copper-catalyzed domino process for the construction of benzo[4,5]imidazo[1,2-*a*]quinoxalines (**9**) from *N*-tosyl-2-haloanilines (**7**) and 2-(chloromethyl)-1*H*-benzo[*d*]imidazoles (**8**) under mild conditions as shown in Scheme 3.2. This method provides a variety of benzo[4,5]imidazo-[1,2-*a*]quinoxaline derivatives in good to excellent yields.[27]




**Scheme 3.2**: Synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxalines.

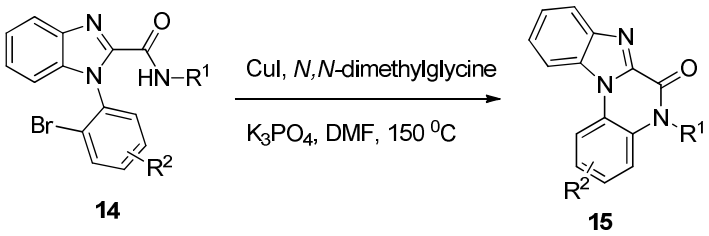
In 2014, Jamison and coworkers developed a mild and facile method for the synthesis of highly functionalized polycyclic quinoxaline derivatives *via* a visible-light-induced decarboxylative radical cyclization of arylisocyanides.




**Scheme 3.3**: Construction of heterocyclic-fused quinoxaline core structures.

This methodology utilizes environmentally friendly radical precursor phenyliodine(III)dicarboxylates as easily accessible catalyst as shown in Scheme 3.3.[28]

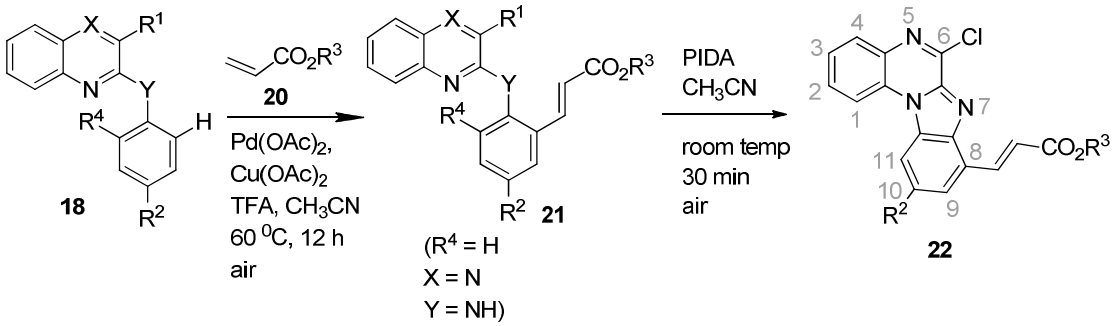
In 2010, Shen and coworkers developed a novel method for synthesis of benzimidazo[1,2-a]quinoxalin-6(5*H*)-one derivatives *via* intra molecular Goldberg reaction. In this method copper(I)iodide acts as catalyst and potassium phosphate as a base as shown in Scheme 3.4.[29]




**Scheme 3.4**: Synthesis of 3-(trifluoromethoxy)benzimidazo[1,2-*a*]quinoxalin-6(5*H*)-ones.

## 3.2. Present work:

Though limited work has been done on the synthesis of benzo[4,5]imidazo[1,2-*a*]quinoxalines, synthesis of alkenyl substituted polynuclear *N*-heteroarenes e.g. benzo[4,5]imidazo[1,2-*a*]quinoxalines[27] is not common in the literature. Herein we report a novel two-step strategy for accessing new chemical entities based on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxaline framework *via* Pd (or Ru)-catalyzed *ortho* C-H alkenylation and subsequent hypervalent iodine(III)-promoted intramolecular *ortho* C–H cycloamination under open air and mild conditions as shown in following Scheme 3.5.

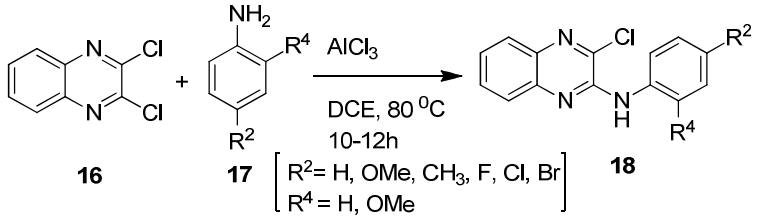



**Scheme 3.5**: Synthesis of alkenyl substituted polynuclear *N*-heteroarenes (**22**).

## 3.3. Results and discussion:

### 3.3.1. Preparation of key starting material (18):

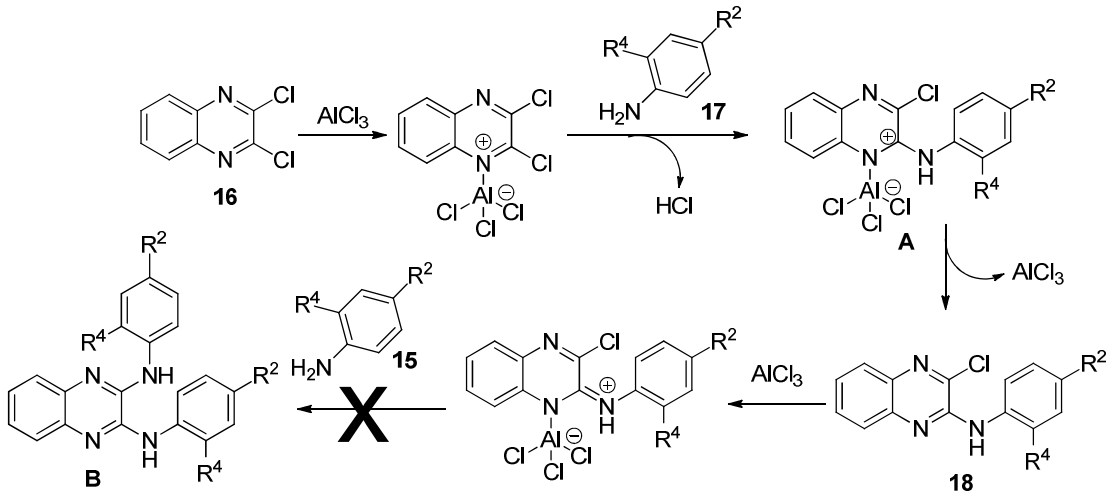
The desired key starting material *i.e.* 3-chloro-*N*-aryl quinoxalin-2-amine (**18**) required for our study was prepared according to our previously reported procedure[30] *via* $AlCl_3$ induced C-N bond formation reaction between 2,3 dichloroquinoxaline **(16)** and substituted anilines (**17)** in dichloroethane at 80 °C as shown as in Scheme 3.6.




**Scheme 3.6**: Synthesis of 3-chloro-*N*-aryl quinoxalin-2-amine (**18**).

### 3.3.2. Proposed mechanism for synthesis of (18) from (16):

The plausible mechanism for conversion of (**16**) to (**18**) is depicted in Scheme 3.7.

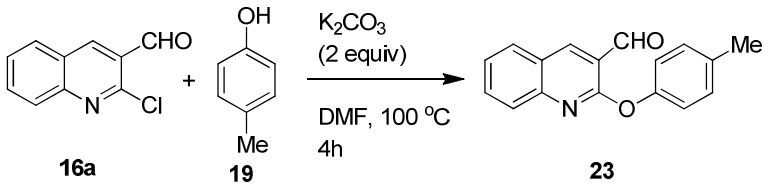



**Scheme 3.7**: Proposed reaction mechanism for the formation of (**18)** *via* $AlCl_3$ induced C–N bond forming reaction between (**16**) & (**17**).

The reaction seemed to involve complexation of $AlCl_3$ with one of the ring nitrogens of (**16**) followed by nucleophilic attack by the amine (**17**) affording intermediate (**A**). Finally release of $AlCl_3$ leading to the desired product (**18**) as shown in Scheme 3.7. Further, a second nucleophilic attack on (**18**) was disfavored perhaps due to the

preferential complexation of $AlCl_3$ with the *N*-1(aided by the adjacent amine group) over *N*-4 of (**16**).

The requisite other starting material 2-(*p*-tolyloxy)quinoline-3-carbaldehyde (**23**) was prepared by heating of 2-chloroquinoline-3-carbaldehyde (**16a**) with substituted phenol (**19**) at 100 °C in presence of potassium carbonate in DMF as shown in Scheme 3.8.[31]

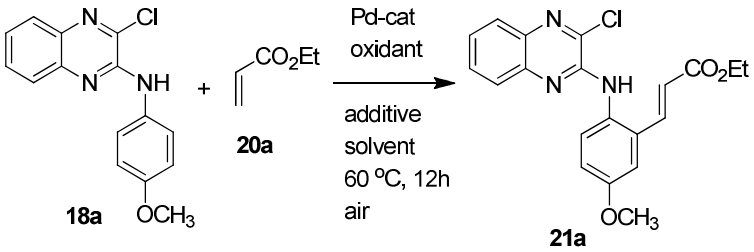



**Scheme 3.8**: Synthesis of 2-(*p*-tolyloxy) quinoline-3-carbaldehyde (**23**).

### 3.3.3. Reaction optimization:

We commenced our studies by testing the coupling reaction of (**18a)** with ethyl acrylate (**20a**) under various conditions such as catalysts, solvents, additives and oxidants as shown in Table 3.1. The reaction was initially performed in the presence of a Pd-catalyst, $Cu(OAc)_2$, trifluoroacetic acid (TFA), in $CH_3CN$ at 60 $^oC$ for 12h under open air. The use of $Pd(PPh_3)_4$, $Pd(dba)_3$, and $Pd(PPh_3)_2Cl_2$ did not afford good yield of (**21a)** (entries 1-3, Table 3.1). Pleasingly, the yield of (**21a)** was improved to 84% with the use of $Pd(OAc)_2$ as the catalyst (entry 4, Table 3.1). Next, we focused on the screening of oxidants, such as $K_2S_2O_8$ and $CuCl_2$ in place of $Cu(OAc)_2$, but the result was discouraging (entries 5 & 6, Table 3.1) as $CuCl_2$ acted as an inhibitor. When the reaction was performed without using any oxidant some progress of reaction was observed (perhaps that was assisted by aerial oxygen) but the yield of (**21a)** was low (entry 7, Table 3.1). $CH_3CN$ was found to be the solvent of choice, whereas other solvents such as DMF, DCE (1,2-dichloroethane), EtOH and toluene furnished (**21a)** in low to moderate yields (entries 8-12, Table 3.1) except 1,4-dioxane. The role of $Pd(OAc)_2$ and TFA was confirmed by performing the reaction in absence of them individually when no or poor yield of (**21a)** was observed (entries 13 & 14, Table 3.1). Indeed, TFA was better compared to other additives e.g. PivOH and $CH_3COOH$ (entries 15 & 16, Table 3.1).

**Table 3. 1**: Reaction conditions and optimization.[a]




| Entry | Catalyst | Additive / Oxidant | Solvent | %Yield[b] |
|---|---|---|---|---|
| 1 | $Pd(PPh_3)_4$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 26 |
| 2 | $Pd(dba)_3$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 35 |
| 3 | $Pd(PPh_3)_2Cl_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 52 |
| 4 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 84 |
| 5 | $Pd(OAc)_2$ | $TFA/K_2S_2O_8$ | $CH_3CN$ | 22 |
| 6 | $Pd(OAc)_2$ | $TFA/CuCl_2$ | $CH_3CN$ | 0 |
| 7 | $Pd(OAc)_2$ | TFA / --- | $CH_3CN$ | 30[c] |
| 8 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | 1,4-Dioxane | 82 |
| 9 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | DMF | 10 |
| 10 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | DCE | 48 |
| 11 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | EtOH | 0 |
| 12 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | Toluene | 72 |
| 13 | --- | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 0[d] |
| 14 | $Pd(OAc)_2$ | --- / $Cu(OAc)_2$ | $CH_3CN$ | 28[e] |
| 15 | $Pd(OAc)_2$ | $PivOH/Cu(OAc)_2$ | $CH_3CN$ | trace |
| 16 | $Pd(OAc)_2$ | $AcOH/Cu(OAc)_2$ | $CH_3CN$ | 19 |
| 17 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 45[f] |
| 18 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 80[g] |

[a]All the reactions are carried out using **18a** (1 mmol), alkene **20a** (1.5 mmol), a Pd-catalyst (5 mol%), an oxidant (1.5 mmol) and an additive (1.2 mmol) in a solvent (2.5 mL) at 60 °C for 12h under open air. [b]Isolated yield. [c]No oxidant. [d]No catalyst. [e]No additive. [f]Performed at 40 °C. [g]Reaction time was 24 h.

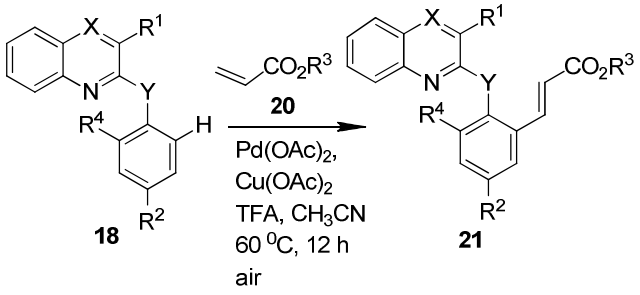
We also investigated the effect of the reaction temperature on the yield of the reaction. When the reaction temperature was decreased to 40 °C the product yield

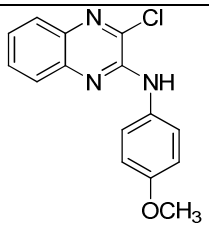
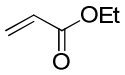
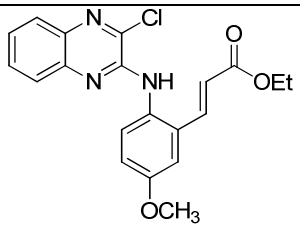
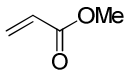
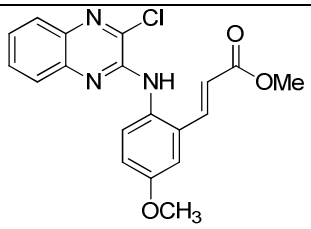
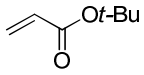
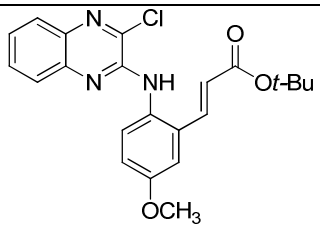
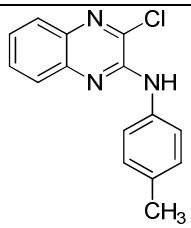
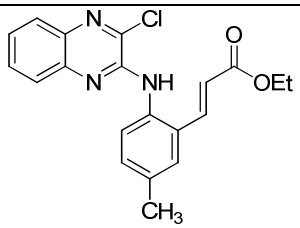
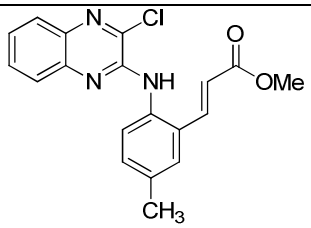
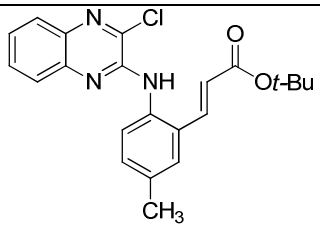
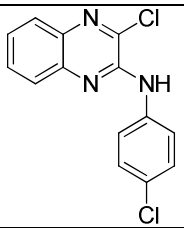
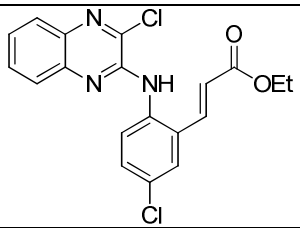
was decreased (entry 17, Table 3.1) whereas increase of temperature (e.g. to 80 ºC) resulted quick evaporation of TFA (bp 72.4 ºC). Moreover, a longer reaction time was also found to be less effective (entry 18, Table 3.1). Notably, the present quinoxaline directed *ortho* C-H alkenylation proceeded well in the presence of a Ru(II) catalyst (vide infra). However, requirement of sealed tube in this case prompted us to proceed with Pd(II)-catalyzed method. Eventually, the optimized reaction conditions were determined as (entry 4, Table 3.1), **18a** (1 mmol), alkene **20a** (1.5 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1.5 mmol) and TFA (1.2 mmol) in $CH_3CN$ (2.5 mL) at 60 °C for 12h under open air.

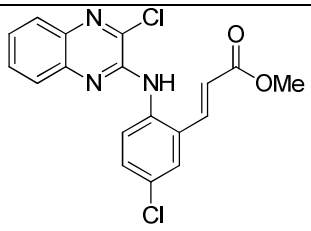
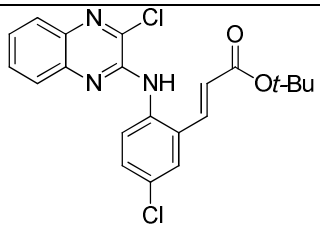
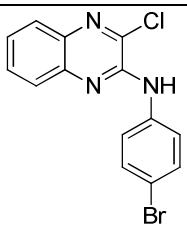
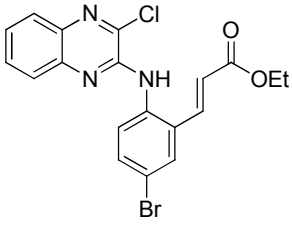
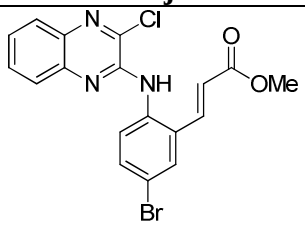
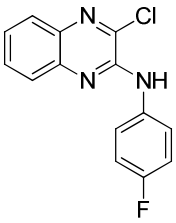
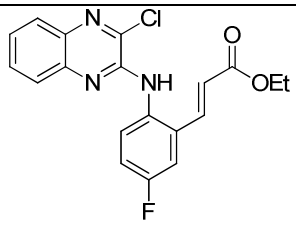
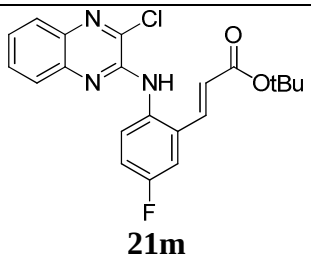
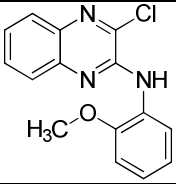
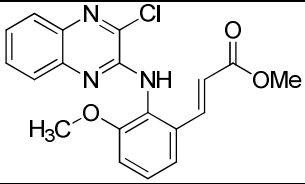
### 3.3.4. Scope of the reaction:

In order to explore the reaction scope, a number of 3-chloro-*N*-aryl quinoxalin-2-amines (**18**) with different substituents were tested and the results were summarized in Table 3.2. A range of substrates e.g. (**18a-g)** carrying substituents like Me, OMe, Cl, F, and Br on the *N*-phenyl ring were employed (Table 3.2). The other coupling partner **(20a-c)** *i.e.* Et, Me, or *t*-Bu ester of acrylic acid were also tolerated well in this reaction. The reaction proceeded well in all these cases affording the corresponding desired product (**21a-n)** in good to acceptable yield. The Pd-catalyzed *ortho* C-H alkenylation of phenol derivatives (**23a-c)** was also performed successfully when quinoline was found to be an effective directing group.

**Table 3. 2: synthesis of compound (21)**.[a]



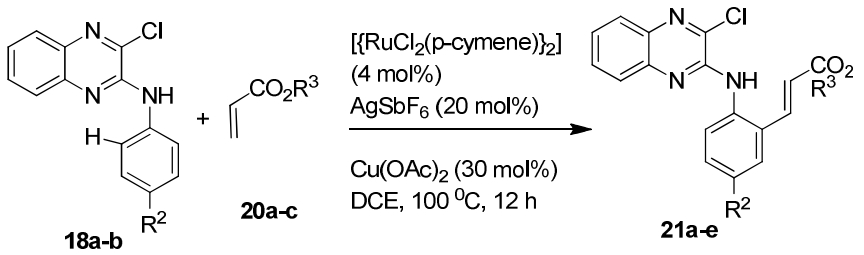
| Entry | Substrate (**18**) | Acrylate (**20**) | Product (**21**) | Yield[b] (%) |
|---|---|---|---|---|
| 1 |  **18a** |  **20a** |  **21a** | 84 |
| 2 | **18a** |  **20b** |  **21b** | 82 |
| 3 | **18a** |  **20c** |  **21c** | 67 |
| 4 |  **18b** | **20a** |  **21d** | 75 |
| 5 | **18b** | **20b** |  **21e** | 80 |
| 6 | **18b** | **20c** |  **21f** | 62 |
| 7 |  | **20a** |  | 77 |

| | | | | |
|---|---|---|---|---|
| | **18c** | | **21g** | |
| 8 | **18c** | **20b** |  **21h** | 71 |
| 9 | **18c** | **20c** |  **21i** | 59 |
| 10 |  **18d** | **20a** |  **21j** | 78 |
| 11 | **18d** | **20b** |  **21k** | 74 |
| 12 |  **18e** | **20a** |  **21l** | 79 |
| 14 | **18e** | **20c** |  **21m** | 74 |
| 15 |  | **20b** |  | 55 |

| | | | | |
|---|---|---|---|---|
| | **18f** | | **21n** | |

[a]All the reactions are carried out using compound **18** (1 mmol), alkene **20** (1.5 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1.5 mmol) and TFA (1.2 mmol) in $CH_3CN$ (2.5 mL) at 60 °C, under air. [b]Isolated yield.

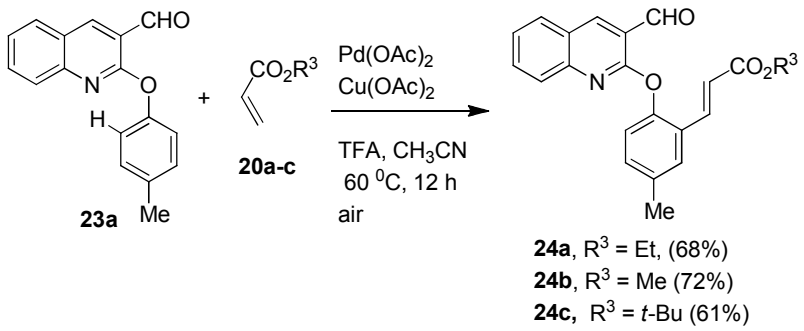
To test the effectiveness of quinoxaline moiety towards Ru(II)-catalyzed *o*-alkenylation we performed a ruthenium catalyzed oxidative coupling of 3-chloro-*N*-aryl quinoxalin-2-amines (**18**), with alkenes (**20a-c)** in the presence of catalytic amounts of $AgSbF_6$ and $Cu(OAc)_2$ under a reported condition[32] when the desired alkene derivatives (**21a-e)** was obtained almost in the same yields (Scheme 3.9) as observed in case of Pd-catalyzed reaction. Notably, the Ru(II)-catalyzed *o*-alkenylation of 3-chloro-*N*-aryl quinoxalin-2-amines (**18**) was carried out in a sealed tube as the reaction did not proceed well when performed in an open reaction vessel.




$R^2$ = OMe, $R^3$ = Me (**21a**; 82%), Et (**21b**; 81%), *t*-Bu (**21c**; 68%)
$R^2$ = Me, $R^3$ = Et (**21d**; 73%), Me (**21e**; 80%)

**Scheme 3.9**: Ru-catalyzed direct *ortho* C-H alkenylation of (**18a-b)**.

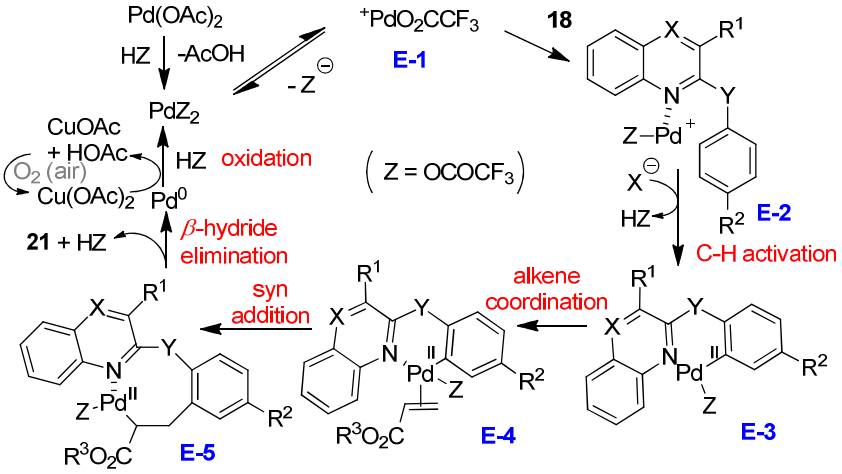
To demonstrate the synthetic utility of this method, the Pd-catalyzed *ortho* C-H alkenylation of a phenol derivative 2-(p-tolyloxy)quinoline-3-carbaldehyde (**23a**) was carried out by coupling with the alkene (**20a-c)** under the condition of entry 4 of Table 3.1. The reaction proceeded smoothly to afford the alkenyl substituted analogs (**24a-c)** in moderate to good yields. It was observed that quinoline was acting as directing group for alkenylation in these cases. While alkenylation of phenol derivatives are known in the literature[33] the use of quinoline moiety as a directing group for this purpose has not been explored earlier. Thus the present strategy of *ortho* C-H alkenylation of phenol is of further interest.




**Scheme 3.10**: Pd-catalyzed *ortho* C-H alkenylation of a phenol derivative (**23a).**

### 3.3.5. Proposed mechanism:

According to the proposed mechanism (Scheme 3.11), the reaction appeared to proceed[34] *via* (i) *in situ* generation of highly electrophilic Pd(II) cationic species **E-1** in TFA, (ii) stabilization of **E-1** by the quinoxaline / quinoline nitrogen aided by the C-2 arylamine / aryloxy moiety (*via* +M effect) in **E-2**, (iii) generation of **E-3** *via* σ-bond formation between the "Pd" center and the proximate *ortho* C-aryl following a C(aryl)-H activation, (iv) alkene coordination with **E-3** to give **E-4,** (v) *syn* addition *via* a 1,2-migratory insertion to afford **E-5**, that undergoes (vi) *β*-hydride elimination to give (**21)** and the $Pd^0$ species, and (vii) finally, oxidation of $Pd^0$ to $Pd^{II}$ by $Cu(OAc)_2$ to complete the catalytic cycle. The $Cu(OAc)_2$ is regenerated from the reduced copper species *i.e.* CuOAc by the aerial oxygen.

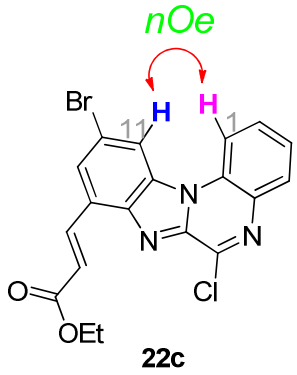



**Scheme 3.11**: Proposed reaction mechanism.

### 3.3.6. The intramolecular C–H cycloamination of (21).

We began our study by examining the intramolecular C–H cycloamination of (**21)**

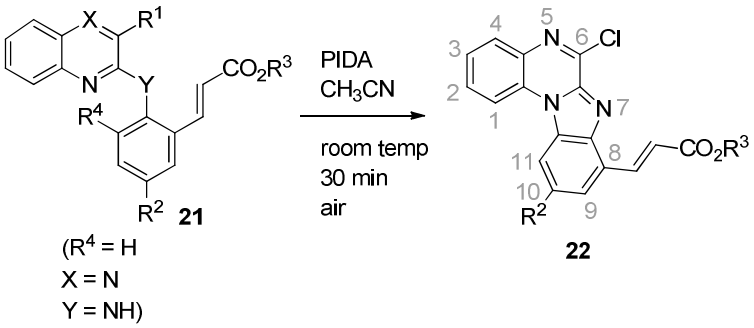
by using a hypervalent iodine(III) reagent[35] such as PIDA [phenyliodine diacetate or $PhI(OAc)_2$] as oxidant at room temperature in acetonitrile as solvent. Delightfully, the desired products *i.e.* alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines (**22**) was obtained in good to excellent yields in a much shorter reaction time. The substituents like Cl, F, Br and alkenyl ester remained intact during this mild and selective oxidative cyclization. All the synthesized compounds *i.e.* (**22a-d)** were characterized by spectral data. The NOE experiment performed using (**22c)** indicated close promiximity of C(1)-H with C(11)-H as they interacted with each other (shown in Figure 3.4 also see in Appendix). The presence of alkenyl moiety with *E*-geometry in compound (**22)** was confirmed by the appearance of a pair of doublets in the region δ8.2-8.1 and δ7.5-7.3 with $J$ = 16 Hz.



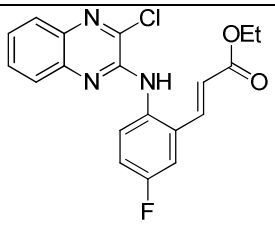
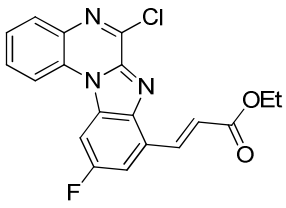
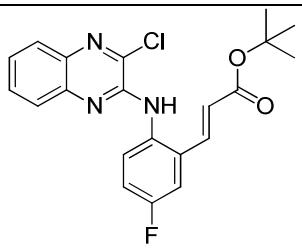
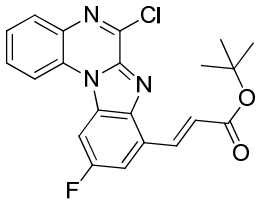
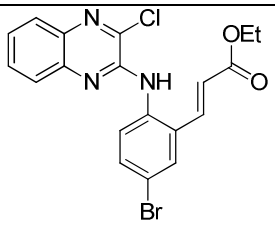
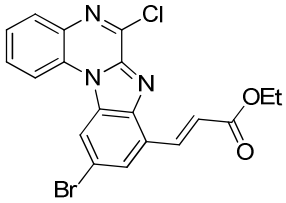
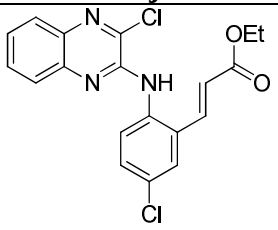
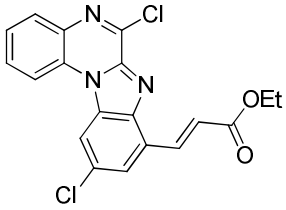

**22c**

**Fig. 3.4:** NOE correlation of C(1)-H with C(11)-H aromatic proton.

**Table 3.3:** Synthesis of compound **(22)**[a]



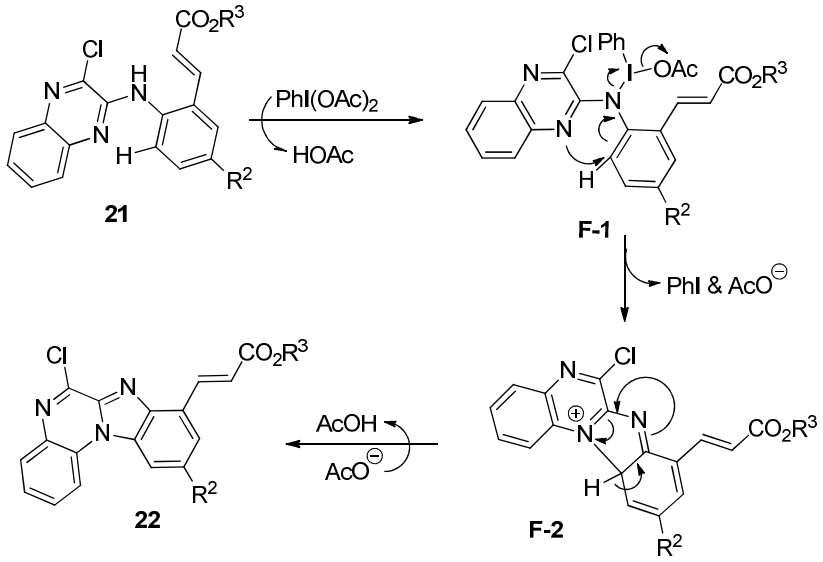

| Entry | Substrate **(21)** | Product **(22)** | Yield[b] **(%)** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 |  **21l** |  **22a** | 90 |
| 2 |  **21m** |  **22b** | 85 |
| 3 |  **21j** |  **22c** | 91 |
| 4 |  **21g** |  **22d** | 82 |

[a]All the reactions are carried out using compound **21** (1 mmol), PIDA (1.5 mmol) in $CH_3CN$ (2.5 mL) at room temparature in 30 min, under air. [b]Isolated yield.

### 3.3.7. Proposed mechanism for the synthesis of (22) from (21):

The plausible mechanism for conversion of (**21**) to (**22**) is depicted in scheme 4.11. The reaction seemed to involve an initial activation of the aniline nitrogen of (**21)** by PIDA that facilitated a nucleophilic attack by the proximate quinoxaline nitrogen atom on the aniline ring of **F-1** affording the cyclic intermediate **F-2** (Scheme 3.12). Deprotonation followed by aromatization of **F-2** afforded product (**22)**.



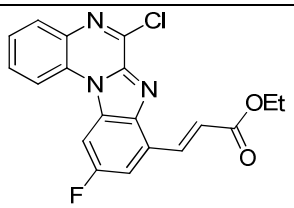

**Scheme 3.12**: Proposed reaction mechanism for the formation of (**22)** *via* intramolecular C–H cycloamination of (**21)** under open air.

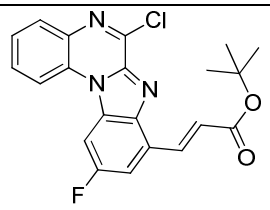
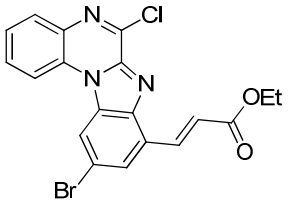
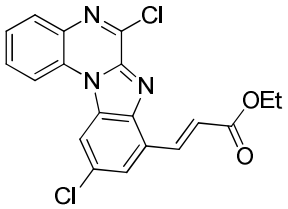
## 3.4. Pharmacology:

### 3.4.1. *In vitro* data:

Owing to the reported PDE4 (phosphodiesterase type 4) inhibitory activities of related imidazo[1,2-*a*]quinoxalines.[36] All the synthesized compounds (**22a-d)** were evaluated for their PDE4 inhibitory properties *in vitro*[38] at 30 µM. Notably, inhibitors of PDE4 are known to be generally useful for the treatment of chronic obstructive pulmonary disease (COPD) and asthma.[37] All these compounds showed promising inhibition of PDE4B and among which, compound (**22d)** showed maximum inhibition *i.e.*, 79%.

**Table 3.4**: *In vitro* data of compounds of (**22)** for inhibition of PDE4B enzyme.

| Entry | Compound No | % of PDE4B inhibition @ 30 µM |
|---|---|---|
| 1 |  **22a** | 69.77±9.69 % |

| | | |
|---|---|---|
| 2 |  **22b** | 42.15±1.23 % |
| 3 |  **22c** | 60.66±3.93 % |
| 4 |  **22d** | 79.50±1.12 % |

A dose response study was carried out using (**22d)** as a representative compound, which showed dose depended inhibition of PDE4B with $IC_{50}$ ~ 2.3 µM (comparable to rolipram's $IC_{50}$ ~ 1.0 µM) (Figure 3.5) indicating potential medicinal value of this class of heterocycles.

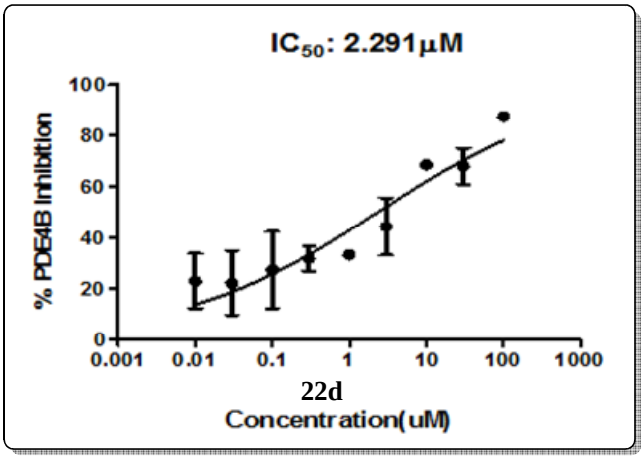



**Fig. 3.5:** Dose dependent inhibition of PDE4B by compound **(22d).**

## 3.5. Conclusion:

In this study, we demonstrated a Pd (or Ru)-catalyzed *ortho* C-H alkenylation and subsequent hypervalent iodine(III)-promoted intramolecular *ortho* C–H cycloamination for accessing new chemical entities based on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines. Both the steps were performed under open air and mild conditions. In this strategy we identified quinoxaline group as a new directing group for the Pd (or Ru)-catalyzed *ortho* C-H alkenylation of aniline derivatives. Some of these synthesized compounds **(22a-d)** were tested for PDE4B along with a known inhibitor rolipram using an enzyme based *in vitro* assay, the representative compound (**22d**) showed dose dependent inhibition of PDE4B with $IC_{50}$ ~ 2.3 µM (comparable to rolipram's $IC_{50}$ ~ 1.0 µM) (Fig 4.4). Since COPD and asthma are major health burden worldwide hence the present class of compounds is of further interest. The Pd-catalyzed *ortho* C-H alkenylation of phenol derivatives was also performed successfully when quinoline was found to be an effective directing group. Overall, our efforts on exploration of new C-H activation strategies for Med Chem purpose would be of further interest.

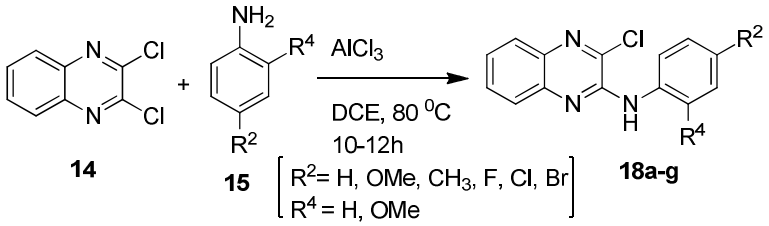
## 3.6. Experimental section:

### 3.6.1. Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. $^{1}H$ NMR and $^{13}C$ NMR spectra were recodred in $CDCl_3$ or DMSO-$d_6$ solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Melting points (mp) were by using Buchi B-540 melting point apparatus and are uncorrected. Chromatographic purity

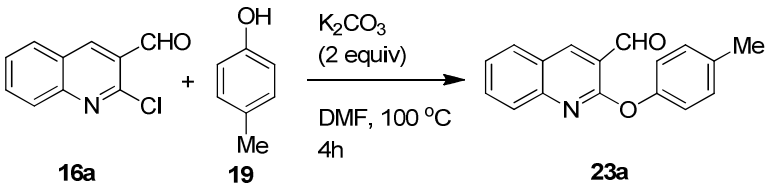
by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

### 3.6.1.1. General Procedure for the preparation of 3-chloro-*N*-aryl substituted quinoxalin-2-amine (18a-g)




A mixture of 2,3-dichloroquinoxaline (**14)** (1.0 mmol), an appropriate amine (**15)** (1.0 mmol) and $AlCl_3$ (1.25 mmol) in 1,2-dichloroethane (5mL) was stirred at 80 °C for 10-12 h under a nitrogen atmosphere. After completion of the reaction, the mixture was cooled to room temperature, poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 × 10 mL). The combined organic layers were washed with cold water (2 × 10 mL), brine (4mL) and dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using ethylacetate/hexane to give the desired product (**18a-g)**.

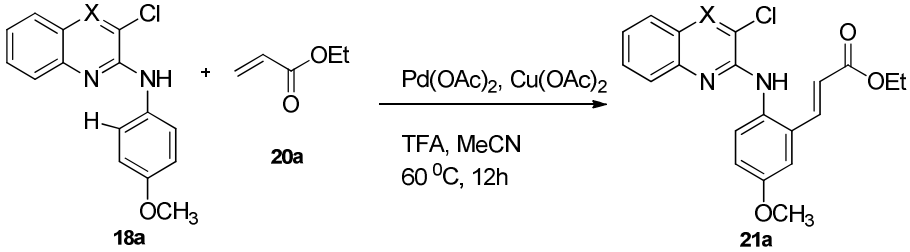
### 3.6.1.2. Typical procedure for the preparation of 2-(*p*-tolyloxy)quinoline-3-carbaldehyde (23a)



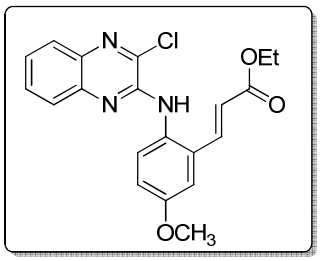

A100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-chloro-3-formylquinoline (**16a)** (1 mmol), phenol (**19)** (1 mmol), anhydrous potassium carbonate (2 mmol) and dimethyl formamide (5 mL). The mixture was heated at 100 $^0$C for 4h and the progress of the reaction was monitored by TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and then poured into chilled water (50 mL) with

continuous stirring followed by neutralization with 1.5N HCl until pH ~ 7 resulted. The solid mass separated was collected by filtration, washed well with water, dried and crystallized from ethylacetate to give compound (**23a)**.

### 3.6.1.3. Typical procedure for the preparation of (*E*)-ethyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methoxyphenyl)acrylate (21a)



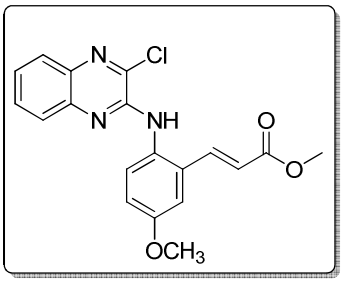

A mixture of 3-chloro-*N*-(4-methoxyphenyl)quinoxalin-2-amine (**18a)** (0.350 mmol), ethyl acrylate **20a** (0.526 mmol) **,** $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (0.526 mmol), TFA (0.42 mmol) in $CH_3CN$ (2.5 mL) was heated at 60 °C in air for 12h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**21a)**.




Yield: 84%; Light yellow; mp: 117-179 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1H$ NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 7.6 Hz, 1H), 7.86-7.84 (m, 2H), 7.69-7.67 (m, 1H), 7.60-7.56 (m, 1H), 7.48-7.44 (m, 1H), 7.25 (s, 1H), 7.15 (d, *J* = 2.8 Hz, 1H), 7.05 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.46 (d, *J* = 16.0 Hz, 1H), 4.23 (q, *J* = 7.2 Hz, 2H), 3.88 (s, 3H), 1.29 (t, *J* = 7.2 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$): 166.4, 157.3, 146.0, 140.4, 139.4, 137.6, 137.3, 130.2, 130.0, 129.8, 127.8, 126.5 (2C), 126.0, 120.9, 116.8, 111.5, 60.6, 55.5, 14.1; MS (ES mass): 384.1 (M+1); HPLC: 98.8%,

Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/50, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 3.8 min.

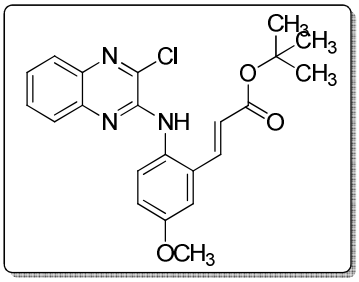
**3.6.1.4. (*E*)-Ethyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methoxyphenyl)acrylate (21b)**




Compound (**21b)** was synthesized from (**18a)** following a procedure similar to that of compound (**21a)**

Yield: 82%; Light yellow; mp: 156-158 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.90-7.84 (m, 3H), 7.69-7.67 (m, 1H), 7.61-7.56 (m, 1H), 7.49-7.45 (m, 1H), 7.23 (s, 1H), 7.16 (d, *J* = 3.2 Hz, 1H), 7.06 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.47 (d, *J* = 16.0 Hz, 1H), 3.89 (s, 3H), 3.78 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 157.3, 146.1, 140.5, 139.7, 137.6, 137.3, 130.2, 129.8, 127.8, 126.6, 126.5, 126.1, 120.5, 116.8, 111.6, 109.9, 55.5, 51.7; MS (ES mass): 370.0 (M+1); HPLC: 98.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 3/20, 8/40, 15/95, 20/95, 25/20, 30/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 255.0 nm, retention time 3.6 min.

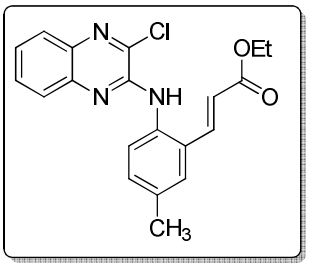
**3.6.1.5. (*E*)-*tert*-butyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methoxyphenyl)acrylate (21c)**



Compound (**21c)** was synthesized from (**18a)** following a procedure similar to that of compound (**21a)**

Yield: 67%; Light yellow; mp: 115-117 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.8 Hz, 1H), 7.86-7.84 (m, 1H), 7.78 (d, *J* = 16.0 Hz, 1H), 7.69-7.67 (m, 1H), 7.60-7.56 (m, 1H), 7.48-7.44 (m, 1H), 7.27 (s, 1H), 7.15 (d, *J* = 2.8 Hz, 1H), 7.04 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.38 (d, *J* = 16.0 Hz, 1H), 3.88 (s, 3H), 1.48 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.6, 157.2, 146.0, 140.5, 138.2, 137.6, 137.2, 130.2, 130.0, 129.7, 127.8, 126.5, 126.3, 126.0, 122.8, 116.6, 111.4, 80.7, 55.5, 28.0; MS (ES mass): 412.1 (M+1); HPLC: 98.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/50, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.1 min.

### 3.6.1.6. (*E*)-Ethyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methylphenyl)acrylate (21d)

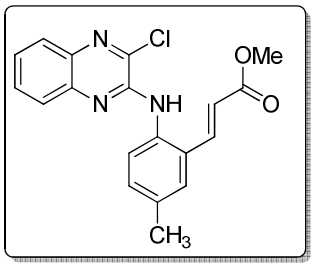



Compound (**21d)** was synthesized from (**18b)** following a procedure similar to that of compound (**21a)**

Yield: 75%; Light yellow; mp: 129-131 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 16.0 Hz, 1H),7.84 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.51-7.46 (m, 2H), 7.41 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.42 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 145.6, 140.4, 139.4 (2C), 137.7, 137.3, 135.1, 134.2, 131.5, 130.3, 128.0, 127.8, 126.6, 126.2, 124.0, 120.8, 60.5, 21.0, 14.2; MS (ES mass): 368.1 (M+1); HPLC: 95.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow

rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.0 min.

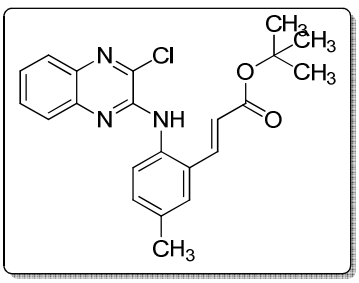
**3.6.1.7. (*E*)-Methyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methylphenyl)acrylate (21e)**




Compound (**21e)** was synthesized from (**18b)** following a procedure similar to that of compound (**21a)**

Yield: 80%; Light yellow; mp: 168-170 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 16.0 Hz, 1H),7.87-7.85 (m, 1H), 7.72-7.70 (m, 1H), 7.62-7.58 (m, 1H), 7.50-7.44 (m, 2H), 7.37 (s, 1H), 7.31-7.29 (m, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 3.79 (s, 3H), 2.41 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.9, 145.6, 140.3, 139.7, 137.7, 137.3, 135.1, 134.2, 131.5, 130.3, 128.0, 127.8 (2C), 126.5, 126.2, 124.0, 120.2, 51.7, 20.9; MS (ES mass): 354.1 (M+1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/95, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.7 min.

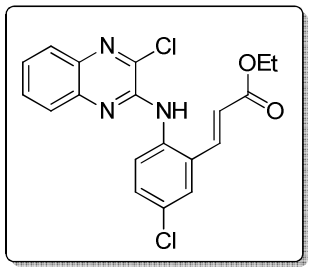
**3.6.1.8. (*E*)-*tert*-butyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-methylphenyl)acrylate (21f)**



Compound (**21f)** was synthesized from (**18b)** following a procedure similar to that of compound (**21a)**

Yield: 62%; Light yellow; mp: 112-114 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.02 (d, *J* = 8.0 Hz, 1H), 7.87-7.84 (m, 1H), 7.80 (d, *J* = 16.0 Hz, 1H), 7.73-7.71 (m, 1H), 7.64-7.58 (m, 1H), 7.49-7.44 (m, 2H), 7.42 (s, 1H), 7.29-7.27 (m, 1H), 6.39 (d, *J* = 16.0 Hz, 1H), 2.39 (s, 3H), 1.49 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.7, 145.6, 140.4, 138.2, 137.2, 134.9, 134.0, 131.2, 130.2, 127.8 (2C), 127.7, 126.5, 126.1, 123.7, 122.6, 120.0, 80.6, 28.0, 20.9; MS (ES mass): 396.1 (M+1); HPLC: 98.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 265.0 nm, retention time 3.1 min.

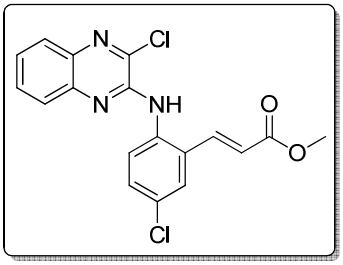
### 3.6.1.9. (*E*)-Ethyl 3-(5-chloro-2-((3-chloroquinoxalin-2-yl)amino)phenyl)acrylate (21g)




Compound (**21g)** was synthesized from (**18c)** following a procedure similar to that of compound (**21a)**

Yield: 77%; Light yellow; mp: 207-209 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.24 (d, *J* = 8.4 Hz, 1H), 7.89-7.83 (m, 2H), 7.76-7.74 (m, 1H), 7.66-7.62 (m, 1H), 7.59 (d, *J* = 2.4 Hz, 1H), 7.54-7.50 (m, 1H), 7.46-7.43 (m, 2H), 6.48 (d, *J* = 16.0 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.0, 145.0, 140.0, 137.8, 137.6, 137.4, 135.2, 130.5, 130.4, 130.3, 128.9, 127.9, 127.3, 126.7, 126.5, 124.6, 122.5, 60.8, 14.1; MS (ES mass): 388.1 (M+1); HPLC: 95.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.2 min.

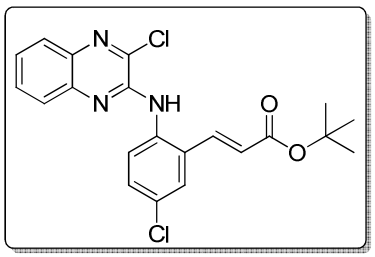
**3.6.1.10. (*E*)-Methyl 3-(5-chloro-2-((3-chloroquinoxalin-2-yl)amino)phenyl)acrylate (21h)**




Compound (**21h)** was synthesized from (**18c)** following a procedure similar to that of compound (**21a)**

Yield: 71%; Light yellow; mp: 201-203 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, *J* = 8.8 Hz, 1H), 7.89-7.83 (m, 2H), 7.75-7.73 (m, 1H), 7.65-7.61 (m, 1H), 7.58 (d, *J* = 2.4 Hz, 1H), 7.53-7.49 (m, 1H), 7.46-7.43 (m, 2H), 6.48 (d, *J* = 16.0 Hz, 1H), 1.56 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 145.0, 140.0, 138.1, 137.6, 137.5, 135.2, 130.5, 130.4, 130.3, 128.9, 127.9, 127.3, 126.7, 126.5, 124.7, 122.0, 51.9; MS (ES mass): 374.0 (M+1); HPLC: 97.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 3.9 min.

**3.6.1.11. (*E*)-*tert*-butyl 3-(5-chloro-2-((3-chloroquinoxalin-2-yl)amino)phenyl)acrylate (21i)**

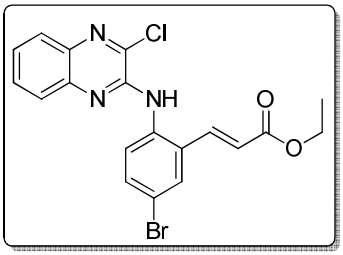



Compound (**21i)** was synthesized from (**18c)** following a procedure similar to that of compound (**21a)**

Yield: 59%; Light yellow; mp: 147-149 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.28 (d, *J* = 8.8Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.79-7.76 (m, 2H), 7.67 (t, *J* = 7.2 Hz, 1H), 7.60 (s, 1H), 7.55-7.44 (m, 3H), 6.43 (d, *J* =

16.0 Hz, 1H), 1.52 (m, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.2, 145.0, 140.1, 137.6, 137.5, 136.6, 135.1, 130.5, 130.2 (2C), 128.9, 127.9, 127.2, 126.6 (2C), 124.5, 124.4, 81.1, 28.1; MS (ES mass): 416.1 (M+1); HPLC: 98.2%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.7 min.

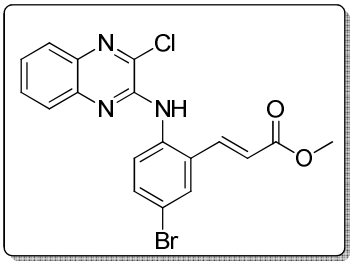
**3.6.1.12. (*E*)-Ethyl 3-(5-bromo-2-((3-chloroquinoxalin-2-yl)amino)phenyl)acrylate (21j)**




Compound (**21j)** was synthesized from (**18d)** following a procedure similar to that of compound (**21a)**

Yield: 78%; Light yellow; mp: 129-131 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.21 (d, *J* = 8.8 Hz, 1H), 7.89-7.82 (m, 2H), 7.76-7.72 (m, 2H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.59-7.57 (m, 1H), 7.54-7.49 (m, 1H), 7.46 (s, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.0, 144.9, 140.0, 137.7, 137.6, 137.5, 135.7, 133.3, 130.6, 130.3, 129.1, 127.9, 126.7, 126.6, 124.6, 122.6, 117.8, 60.8, 14.2; MS (ES mass): 434.0 (M+3); HPLC: 95.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.7 min.

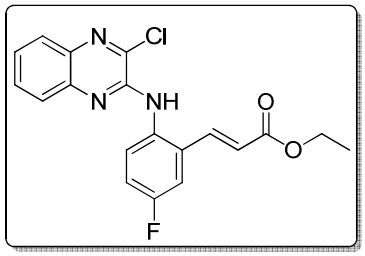
**3.6.1.13. (*E*)-Methyl 3-(5-bromo-2-((3-chloroquinoxalin-2-yl)amino)phenyl)acrylate (21k)**




Compound (**21k)** was synthesized from (**18d)** following a procedure similar to that of compound (**21a)**

Yield: 74%; Light yellow; mp: 125-127 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.19 (d, *J* = 8.8 Hz, 1H), 7.89-7.83 (m, 2H), 7.76-7.72 (m, 2H), 7.66-7.62 (m, 1H), 7.60-7.59 (m, 1H), 7.54-7.50 (m, 1H), 7.45 (s, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 3.81 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.9, 140.0, 138.0, 137.6, 137.4, 135.7, 133.3, 130.5, 130.3, 129.1, 127.9, 126.7, 126.6, 124.7, 122.1, 117.8, 51.9; MS (ES mass): 420.0 (M+3); HPLC: 93.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/10, 2/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.0 min.

**3.6.1.14. (*E*)-Ethyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-fluorophenyl)acrylate (21l)**

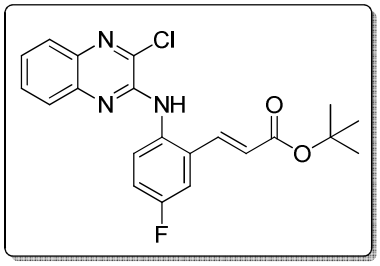



Compound (**21l)** was synthesized from (**18e)** following a procedure similar to that of compound (**21a)**

Yield: 79%; Light yellow; mp: 177-179 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.06 (dd, *J* = 8.8, 5.2 Hz, 1H), 7.88-7.83 (m, 2H), 7.72-7.69 (m, 1H), 7.63-7.59 (m, 1H), 7.52-7.48 (m, 1H), 7.35=7.32 (m, 2H), 7.22-7.17 (m, 1H), 6.46 (d, *J* = 16.0 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 1.30 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.0, 161.1 (C-F *J* = 244.5Hz), 158.7, 145.5, 140.2, 138.1, 137.5 (C-F *J* = 7.8 Hz), 137.4, 132.7 (2C), 130.4, 130.2 (C-F *J* = 7.9 Hz),, 130.1,

127.8, 126.5 (C-F *J* = 7.4 Hz), 126.4, 126.3, 126.2 , 122.1, 117.6 (C-F *J* = 22.7 Hz), 117.4, 113.7 (C-F *J* = 23.2 Hz), 113.5, 60.7, 14.1; MS (ES mass): 372.0 (M+1); HPLC: 99.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 3.8 min.

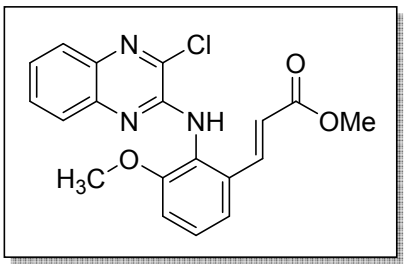
### 3.6.1.15. (*E*)-*tert*-butyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-5-fluorophenyl)acrylate (21m)




Compound (**21m)** was synthesized from (**18e)** following a procedure similar to that of compound (**21a)**

Yield: 74%; Light yellow; mp: 167-169 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1H$ NMR (400 MHz, $CDCl_3$) δ: 8.08 (dd, *J* = 8.8, 5.2 Hz, 1H), 7.88-7.85 (m, 1H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.72-7.70 (m, 1H), 7.63-7.59 (m, 1H), 7.51-7.47 (m, 1H), 7.36 (s, 1H), 7.32 (dd, *J* = 9.2, 2.9 Hz, 1H), 7.22-7.14 (m, 1H), 6.38 (d, *J* = 15.6 Hz, 1H), 1.49 (s, 9H); $^{13}C$ NMR (100 MHz, $CDCl_3$): 165.2, 161.1 (C-F *J* = 244.1Hz), 158.6, 148.8, 145.5, 140.2, 137.5, 137.4, 137.0, 132.6 , 130.4, 130.2, 127.8, 126.5, 126.3, 126.2 (C-F *J* = 8.4 Hz), 126.1, 124.0, 117.4 (C-F *J* = 22.5 Hz), 117.1, 113.6 (C-F *J* = 23.3 Hz), 113.4, 109.9, 80.9, 28.0; MS (ES mass): 400.2 (M+1); HPLC: 99.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 3.0 min.

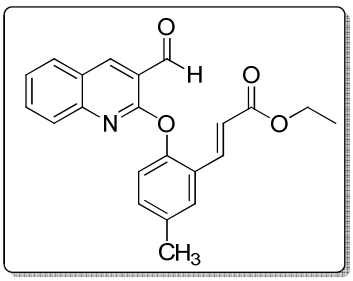
### 3.6.1.16. (*E*)-Methyl 3-(2-((3-chloroquinoxalin-2-yl)amino)-3-methoxyphenyl)acrylate (21n)



Compound (**21n)** was synthesized from (**18f)** following a procedure similar to that of compound (**21a)**

Yield: 55%; Pale yellow; mp: 124-126 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.85-7.83 (m, 1H), 7.72 (d, *J* = 16.0 Hz, 1H), 7.56-7.49 (m, 2H), 7.45-7.41 (m, 1H), 7.36-7.30 (m, 2H), 7.25 (s, 1H), 7.02-7.00 (m, 1H), 6.44 (d, *J* = 16.0 Hz, 1H), 3.85 (s, 3H), 3.63 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 157.3, 146.0, 140.4, 139.4, 137.6, 137.3, 130.2, 130.0, 129.8, 127.8, 126.5 (2C), 126.0, 120.9, 116.8, 111.5, 60.6, 55.5; MS (ES mass): 370.1 (M+1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.8 min.

### 3.6.1.17. (*E*)-Ethyl 3-(2-((3-formylquinolin-2-yl)oxy)-5-methylphenyl)acrylate (24a)

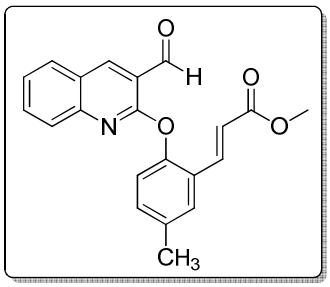


Compound (**24a)** was synthesized from (**23a)** following a procedure similar to that of compound (**21a)**

Yield:68%; pink; mp: 160-162 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 10.71 (s, 1H), 8.78 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H),7.87 (d, *J* = 16.0 Hz, 1H) 7.71-7.70 (m, 2H), 7.55 (s, 1H), 7.50-7.46 (m, 1H), 7.30 (s, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 16.0 Hz, 1H), 4.23-4.15 (m, 2H), 2.45 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 188.5, 166.7, 160.4, 149.6, 148.5, 140.8, 138.5, 135.2, 132.7, 131.8, 129.6, 128.0, 127.8, 127.2, 125.8, 125.2, 122.9, 120.0,

119.7, 60.4, 20.9, 14.2; MS (ES mass): 362.1 (M+1); HPLC: 98.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.9 min.

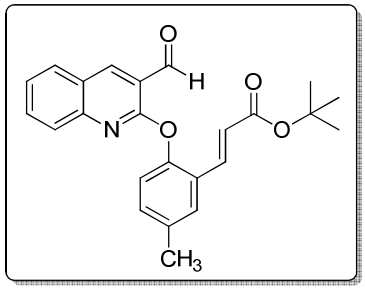
### 3.6.1.18. (*E*)-Methyl 3-(2-((3-formylquinolin-2-yl)oxy)-5-methylphenyl)acrylate (24b)




Compound (**24b)** was synthesized from (**23a)** following a procedure similar to that of compound (**21a)**

Yield:72%; white; mp: 136-138 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 10.71 (s, 1H), 8.79 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 16.0 Hz, 1H), 7.71 (d, *J* = 4.4 Hz, 2H), 7.56 (s, 1H), 7.50-7.46 (m, 1H), 7.31 (s, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.49 (d, *J* = 16.0 Hz, 1H), 3.73 (s, 3H), 2.46 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 188.5, 167.1, 160.4, 149.6, 148.5, 140.8, 138.8, 135.3, 132.7, 131.9, 129.6, 128.1, 127.8, 127.2, 125.8, 125.2, 122.9, 120.0, 119.3, 51.6, 20.9; MS (ES mass): 348.1 (M+1); HPLC: 96.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 3.8 min.

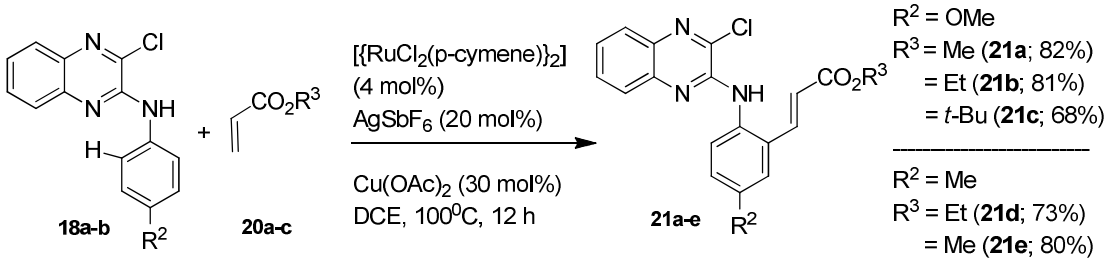
### 3.6.1.19. (*E*)-*tert*-butyl 3-(2-((3-formylquinolin-2-yl)oxy)-5-methylphenyl)acrylateacrylate (24c)



Compound (**24c)** was synthesized from (**23a)** following a procedure similar to that of compound (**21a)**

Yield: 61%; Pink; mp: 126-127 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 10.70 (s, 1H), 8.77 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 16.0 Hz, 1H), 7.70 (d, *J* = 4.6 Hz, 2H), 7.55 (s, 1H), 7.49-7.45 (m, 1H), 7.28 (s, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.40 (d, *J* = 16.0 Hz, 1H), 2.44 (s, 3H), 1.44 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): 188.5, 165.9, 160.4, 149.5, 148.5, 140.7, 137.4, 135.1, 132.6, 131.6, 129.9, 129.5, 127.8, 127.3, 125.7, 125.1, 122.9, 121.4, 119.9, 80.4, 28.0, 20.9; MS (ES mass): 388.0 (M-1); HPLC: 97.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 260.0 nm, retention time 4.2 min.

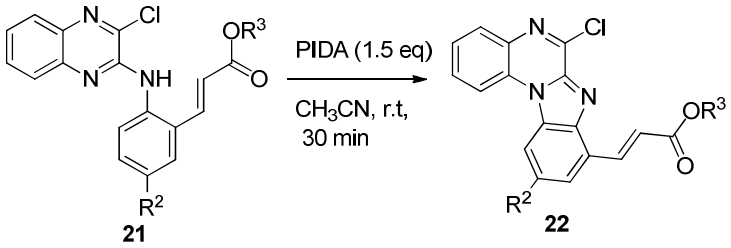
### 3.6.1.20. General procedure for the Ru-catalyzed direct *ortho* C-H alkenylation of (18a-b)




To a mixture of [{$RuCl_2$(p-cymene)}$_2$] (0.04 mmol, 4 mol %), $AgSbF_6$ (0.20 mmol, 20 mol %), $Cu(OAc)_2$ (0.30 mmol, 30 mol %) and 3-chloro-*N*-aryl quinoxalin-2-amine (**18-b)** (1.0 equiv), taken in a sealed tube (fitted with a septum) was added acrylate (**20a-c) (**1.5 equiv) and then dichloroethane (3.0 mL) via a syringe under nitrogen. The mixture was allowed to stir for 5 min at room temperature. Then, the septum was taken off and the reaction mixture was stirred under an open air for an additional 10 min. The tube was covered with a screw cap and the reaction mixture was allowed to stir at 100 °C for 12 h. After completion of the reaction the mixture was cooled to room temperature, transferred to an RB flask and solvent was evaporated. The residue was diluted with ethylacetate (5 mL) and filtered through Celite. The filtrate was washed with water (3 x 15 mL) followed by brine solution (20 mL), dried over anhydrous $Na_2SO_4$, filtered and

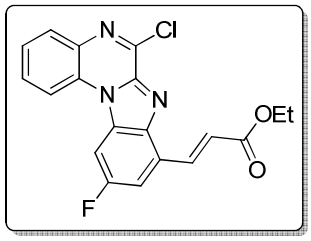
concentrated under vacuum. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using ethylacetate/hexane to give the desired product (**21a-e)**.

### 3.6.1.21. General procedure for the preparation of (*E*)-Alkyl 3-(10-substituted-6-chlorobenzo[4,5]imidazo[1,2-*a*]quinoxalin-8-yl)acrylate (22a-d)




To a solution of (**21)** (1.0 mmol) in acetonitrile (5 mL) was added PIDA (1.5 mmol) and the solution was allowed to stirred at room temperature for 30 min. After completion of the reaction (indicated by TLC), the mixture was extracted with ethylacetate (3 x 10 mL). The combined organic phase was collected, washed with brine and dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**22)**.

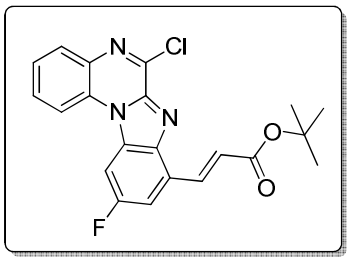
### 3.6.1.22. (*E*)-Ethyl 3-(6-chloro-10-fluorobenzo[4,5]imidazo[1,2-*a*]quinoxalin-8-yl)acrylate (22a)




Yield: 90%; white solid; mp: 221-223 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.34 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 16.0 Hz, 1H), 8.14-8.08 (m, 2H), 7.84-7.79 (m, 1H), 7.68-7.64 (m, 1H), 7.56 (dd, *J* = 9.8, 2.0 Hz, 1H), 7.43 (d, *J* = 16.0 Hz, 1H), 4.36 (q, *J* = 7.2 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 161.4 (C-F *J* = 244.5Hz), 159.0, 144.7, 139.2, 138.7, 138.6, 134.5, 131.3, 131.2, 130.6 (C-F *J* = 40.6Hz), 130.2, 129.7, 129.6, 128.9 (C-F *J* = 9.6Hz), 126.7, 124.3, 114.7 (C-F *J* = 25.6Hz), 114.4 (2C), 102.1(C-F *J* = 29.0 Hz),

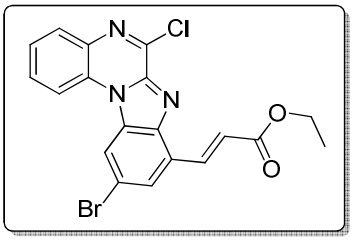
101.8, 60.8, 14.3; MS (ES mass): 370.1 (M+1); HPLC: 97.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20,; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.8 min.

### 3.6.1.23. (*E*)-*tert*-butyl 3-(6-chloro-10-fluorobenzo[4,5]imidazo[1,2-*a*]quinoxalin-8-yl)acrylate (22b)




Yield: 85%; white solid; mp: 198-200 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* = 16.0 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.09 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.84-7.80 (m, 1H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.55 (dd, *J* = 9.8, 1.9 Hz, 1H), 7.32 (d, *J* = 16.0 Hz, 1H), 1.59 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.1, 161.5, 159.0 (C-F *J* = 243.6 Hz), 144.7, 139.3, 139.2, 137.6 (2C), 134.5, 130.5, 130.1, 128.9, 128.2, 126.6, 126.1, 114.4, 114.2, 101.8, 101.5 (C-F *J* = 28.9 Hz), 80.9, 28.2; MS (ES mass): 398.0 (M+1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20,; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.8 min.

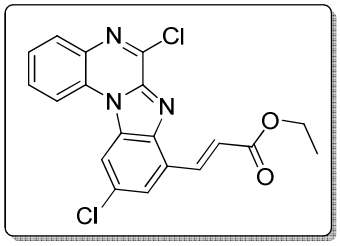
### 3.6.1.24. (*E*)-Ethyl 3-(10-bromo-6-chlorobenzo[4,5]imidazo[1,2-*a*]quinoxalin-8-yl)acrylate (22c)




Yield: 91%; white solid; mp: 209-211 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.55 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.20-8.12 (m, 2H), 7.88 (s, 1H), 7.86-7.82 (m, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 16.0 Hz, 1H), 4.34

(q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 144.6, 141.4, 139.2, 138.5, 134.6, 132.3, 131.0, 130.6, 130.3, 129.3, 128.8, 126.8, 124.4, 118.6, 117.9, 114.6, 60.7, 14.3; MS (ES mass): 431.9 (M+3); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20,; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.8 min.

### 3.6.1.25. (*E*)-Ethyl 3-(6,10-dichlorobenzo[4,5]imidazo[1,2-*a*]quinoxalin-8-yl)acrylate (22d)




Yield: 82%; white solid; mp: 185-187 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.41-8.39 (m, 2H), 8.21 (d, *J* = 16.0 Hz, 1H), 8.15 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.86-7.82 (m, 1H), 7.77 (s, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 16.0 Hz, 1H), 4.35 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 144.7, 138.6, 134.6, 131.9, 131.2, 130.6, 130.3, 129.8, 129.4, 126.8, 126.7, 125.8, 125.7, 124.4, 114.9, 114.6, 60.7, 14.3; MS (ES mass): 385.9 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20,; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.4 min.

## 3.7. References:

1. (a) Malamas, M. S.; Ni, Y.; Erdei, J.; Stange, H.; Schindler, R.; Lankau, H.-J.; Grunwald, C.; Fan, K. Y.; Parris, K.; Langen, B., *J. Med. Chem.* **2011,** *54* (21), 7621-7638; (b) Butini, S.; Budriesi, R.; Hamon, M.; Morelli, E.; Gemma, S.; Brindisi, M.; Borrelli, G.; Novellino, E.; Fiorini, I.; Ioan, P., *J. Med. Chem.* **2009,** *52* (21), 6946-6950; (c) More, S. V.; Sastry, M.; Wang, C.-C.; Yao, C.-F., *Tetrahedron Lett.* **2005,** *46* (37), 6345-6348; (d) Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A., *J. Med. Chem.* **2005,** *48* (6), 2019-2025; (e) Seitz, L.

E.; Suling, W. J.; Reynolds, R. C., *J. Med. Chem.* **2002,** *45* (25), 5604-5606; (f) Sakata, G.; Makino, K.; Kurasawa, Y., *Heterocycles* **1988,** *27* (10), 2481-2515.

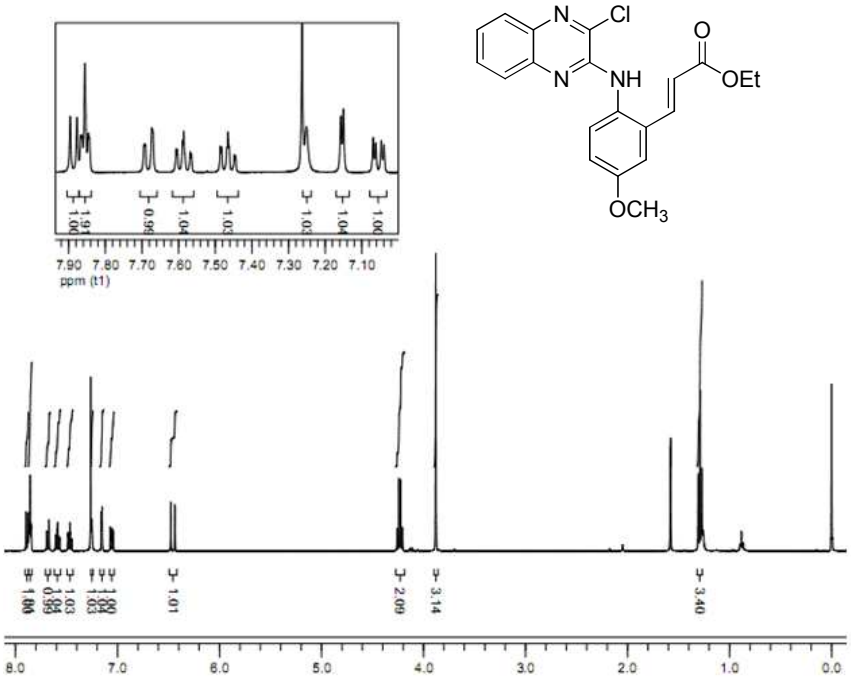
2. Zhang, Y.; Zou, J.; Yip, H.-L.; Chen, K.-S.; Zeigler, D. F.; Sun, Y.; Jen, A. K.-Y., *Chem. Mater.* **2011,** *23* (9), 2289-2291.
3. Kovalenko, M.; Gazit, A.; Böhmer, A.; Rorsman, C.; Rönnstrand, L.; Heldin, C.-H.; Waltenberger, J.; Böhmer, F.-D.; Levitzki, A., *Cancer Res.* **1994,** *54* (23), 6106-6114.
4. Ding, Z.; Parchment, R. E.; LoRusso, P. M.; Zhou, J.-Y.; Li, J.; Lawrence, T. S.; Sun, Y.; Wu, G. S., *Clin. Cancer Res.* **2001,** *7* (11), 3336-3342.
5. Koch, P.; Jahns, H.; Schattel, V.; Goettert, M.; Laufer, S., *J. Med. Chem.* **2010,** *53* (3), 1128-1137.
6. Novellino, E.; Cosimelli, B.; Ehlardo, M.; Greco, G.; Iadanza, M.; Lavecchia, A.; Rimoli, M. G.; Sala, A.; Da Settimo, A.; Primofiore, G., *J. Med. Chem.* **2005,** *48* (26), 8253-8260.
7. (a) Zarrinmayeh, H.; Nunes, A. M.; Ornstein, P. L.; Zimmerman, D. M.; Arnold, M. B.; Schober, D. A.; Gackenheimer, S. L.; Bruns, R. F.; Hipskind, P. A.; Britton, T. C., *J. Med. Chem.* **1998,** *41* (15), 2709-2719; (b) White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T., *J. Med. Chem.* **2000,** *43* (22), 4084-4097; (c) Hauel, N. H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J.-M.; Wienen, W., *J. Med. Chem.* **2002,** *45* (9), 1757-1766.
8. Velık, J.; Baliharova, V.; Fink-Gremmels, J.; Bull, S.; Lamka, J.; Skalova, L., *Res. Vet. Sci.* **2004,** *76* (2), 95-108.
9. Schwartz, G.; Fehse, K.; Pfeiffer, M.; Walzer, K.; Leo, K., *Appl. Phys. Lett.* **2006,** *89* (8), 3509.
10. (a) Schrock, R. R., *Angew. Chem. Int. Ed. Engl.* **2006,** *45* (23), 3748-3759; (b) Grubbs, R. H., *Angew. Chem. Int. Ed. Engl.* **2006,** *45* (23), 3760-3765.
11. (a) KumaráSeerapu, G. P.; SarmaáChennubhotla, K.; LalithaKumar, K., *Chem. Commun.* **2013,** *49* (56), 6268-6270; (b) Kumar, T. B.; Sumanth, C.; Vaishaly, S.; Rao, M. S.; Sekhar, K. C.; Meda, C. L. T.; Kandale, A.; Rambabu, D.; Krishna, G. R.; Reddy, C. M., *Bioorg. Med. Chem.* **2012,** *22* (17), 5639-5647.

12. (a) Khier, S.; Deleuze-Masquéfa, C.; Moarbess, G.; Gattacceca, F.; Margout, D.; Solassol, I.; Cooper, J.-F.; Pinguet, F.; Bonnet, P.-A.; Bressolle, F. M., *Eur. J. Pharm. Sci.* **2010,** *39* (1), 23-29; (b) Deleuze-Masquefa, C.; Moarbess, G.; Khier, S.; David, N.; Gayraud-Paniagua, S.; Bressolle, F.; Pinguet, F.; Bonnet, P.-A., *Eur. J. Med. Chem.* **2009,** *44* (9), 3406-3411.
13. Mizoroki, T.; Mori, K.; Ozaki, A., *Bull. Chem. Soc. Jpn.* **1971,** *44* (2), 581-581.
14. (a) Jia, C.; Kitamura, T.; Fujiwara, Y., *Acc. Chem. Res.* **2001,** *34* (8), 633-639; (b) Zhou, L.; Lu, W., *Chem. Eur. J.* **2014,** *20* (3), 634-642.
15. (a) Ackermann, L.; Vicente, R.; Kapdi, A. R., *Angew. Chem., Int. Ed.* **2009,** *48* (52), 9792-9826; (b) Daugulis, O.; Do, H.-Q.; Shabashov, D., *Acc. Chem. Res.* **2009,** *42* (8), 1074-1086; (c) Colby, D. A.; Bergman, R. G.; Ellman, J. A., *Chem. Rev.* **2009,** *110* (2), 624-655; (d) Lyons, T. W.; Sanford, M. S., *Chem. Rev.* **2010,** *110* (2), 1147-1169; (e) McMurray, L.; O'Hara, F.; Gaunt, M. J., *Chem. Soc. Rev.* **2011,** *40* (4), 1885-1898; (f) Song, G.; Wang, F.; Li, X., *Chem. Soc. Rev.* **2012,** *41* (9), 3651-3678; (g) Arockiam, P. B.; Bruneau, C.; Dixneuf, P. H., *Chem. Rev.* **2012,** *112* (11), 5879-5918.
16. (a) Deng, G.; Zhao, L.; Li, C. J., *Angew. Chem., Int. Ed.* **2008,** *47* (33), 6278-6282; (b) Kalyani, D.; Deprez, N. R.; Desai, L. V.; Sanford, M. S., *J. Am. Chem. Soc.* **2005,** *127* (20), 7330-7331.
17. Oi, S.; Aizawa, E.; Ogino, Y.; Inoue, Y., *J. Org. Chem.* **2005,** *70* (8), 3113-3119.
18. Shabashov, D.; Daugulis, O., *Org. Lett.* **2005,** *7* (17), 3657-3659.
19. (a) Gericke, K. M.; Chai, D. I.; Bieler, N.; Lautens, M., *Angew. Chem., Int. Ed.* **2009,** *48* (8), 1447-1451; (b) Saito, B.; Fu, G. C., *J. Am. Chem. Soc.* **2007,** *129* (31), 9602-9603.
20. (a) Li, B. J.; Tian, S. L.; Fang, Z.; Shi, Z. J., *Angew. Chem., Int. Ed.* **2008,** *47* (6), 1115-1118; (b) Daugulis, O.; Zaitsev, V. G., *Angew. Chem., Int. Ed.* **2005,** *44* (26), 4046-4048.
21. Wang, D.-H.; Wasa, M.; Giri, R.; Yu, J.-Q., *J. Am. Chem. Soc.* **2008,** *130* (23), 7190-7191.
22. Terao, Y.; Kametani, Y.; Wakui, H.; Satoh, T.; Miura, M.; Nomura, M., *Tetrahedron* **2001,** *57* (28), 5967-5974.

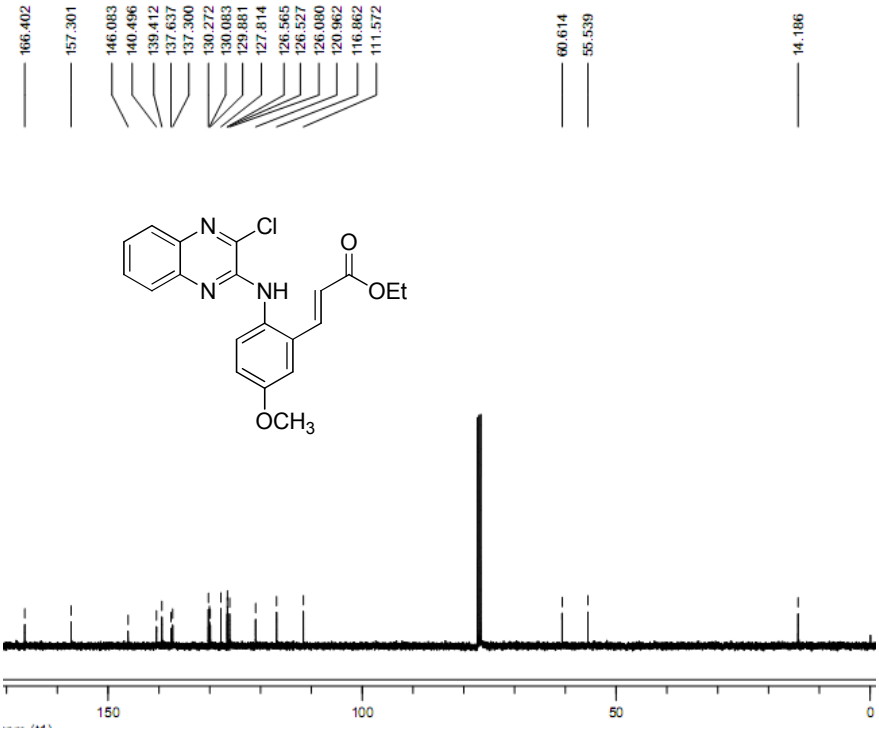
23. (a) Satoh, T.; Kawamura, Y.; Miura, M.; Nomura, M., *Angew. Chem., Int. Ed. Engl* **1997,** *36* (16), 1740-1742; (b) Satoh, T.; Inoh, J.-i.; Kawamura, Y.; Kawamura, Y.; Miura, M.; Nomura, M., *Bull. Chem. Soc. Jpn.* **1998,** *71* (9), 2239-2246.
24. Giri, R.; Yu, J.-Q., *J. Am. Chem. Soc.* **2008,** *130* (43), 14082-14083.
25. Yu, M.; Liang, Z.; Wang, Y.; Zhang, Y., *J. Org. Chem.* **2011,** *76* (12), 4987-4994.
26. Deb, A.; Bag, S.; Kancherla, R.; Maiti, D., *J. Am. Chem. Soc.* **2014,** *136* (39), 13602-13605.
27. Huang, A.; Chen, Y.; Zhou, Y.; Guo, W.; Wu, X.; Ma, C., *Org. Lett.* **2013,** *15* (21), 5480-5483.
28. He, Z.; Bae, M.; Wu, J.; Jamison, T. F., *Angew. Chem.* **2014,** *126* (52), 14679-14683.
29. Meng, T.; Zhang, Y.; Li, M.; Wang, X.; Shen, J., *J. Comb. Chem.* **2010,** *12* (2), 222-224.
30. Prasad, B.; Kumar, K. S.; Babu, P. V.; Anusha, K.; Rambabu, D.; Kandale, A.; Vanaja, G.; Kalle, A. M.; Pal, M., *Tetrahedron Lett.* **2012,** *53* (45), 6059-6066.
31. Mungra, D. C.; Patel, M. P.; Rajani, D. P.; Patel, R. G., *Eur. J. Med. Chem.* **2011,** *46* (9), 4192-4200.
32. (a) Graczyk, K.; Ma, W.; Ackermann, L., *Org. Lett.* **2012,** *14* (16), 4110-4113; (b) Padala, K.; Jeganmohan, M., *Org. Lett.* **2012,** *14* (4), 1134-1137.
33. (a) Dai, H.-X.; Li, G.; Zhang, X.-G.; Stepan, A. F.; Yu, J.-Q., *J. Am. Chem. Soc.* **2013,** *135* (20), 7567-7571; (b) Martínez, C.; Muñiz, K., *ChemCatChem.* **2013,** *5* (12), 3502-3503.
34. (a) Ge, H.; Niphakis, M. J.; Georg, G. I., *J. Am. Chem. Soc.* **2008,** *130* (12), 3708-3709; (b) Liu, Y.; Li, D.; Park, C. M., *Angew. Chem., Int. Ed.* **2011,** *123* (32), 7471-7474; (c) Pankajakshan, S.; Xu, Y. H.; Cheng, J. K.; Low, M. T.; Loh, T. P., *Angew. Chem., Int. Ed.* **2012,** *51* (23), 5701-5705.
35. Zhdankin, V. V., *Arkivoc* **2009,** *1*, 1-62.
36. (a) Parra, S.; Laurent, F.; Subra, G.; Deleuze-Masquefa, C.; Benezech, V.; Fabreguettes, J.-R.; Vidal, J.-P.; Pocock, T.; Elliott, K.; Small, R., *Eur J Med Chem.* **2001,** *36* (3), 255-264; (b) Deleuze-Masquéfa, C.; Gerebtzoff, G.; Subra, G.; Fabreguettes, J.-R.; Ovens, A.; Carraz, M.; Strub, M.-P.; Bompart, J.; George, P.; Bonnet, P.-A., *Bioorg. Med. Chem.* **2004,** *12* (5), 1129-1139.

37. Kodimuthali, A.; Jabaris, S. S. L.; Pal, M., *J. Med. Chem.* **2008,** *51* (18), 5471-5489.
38. Wang, P.; Myers, J. G.; Wu, P.; Cheewatrakoolpong, B.; Egan, R. W.; Billah, M. M., *Biochem. Biophys. Res. Commun.* **1997,** *234* (2), 320-324.

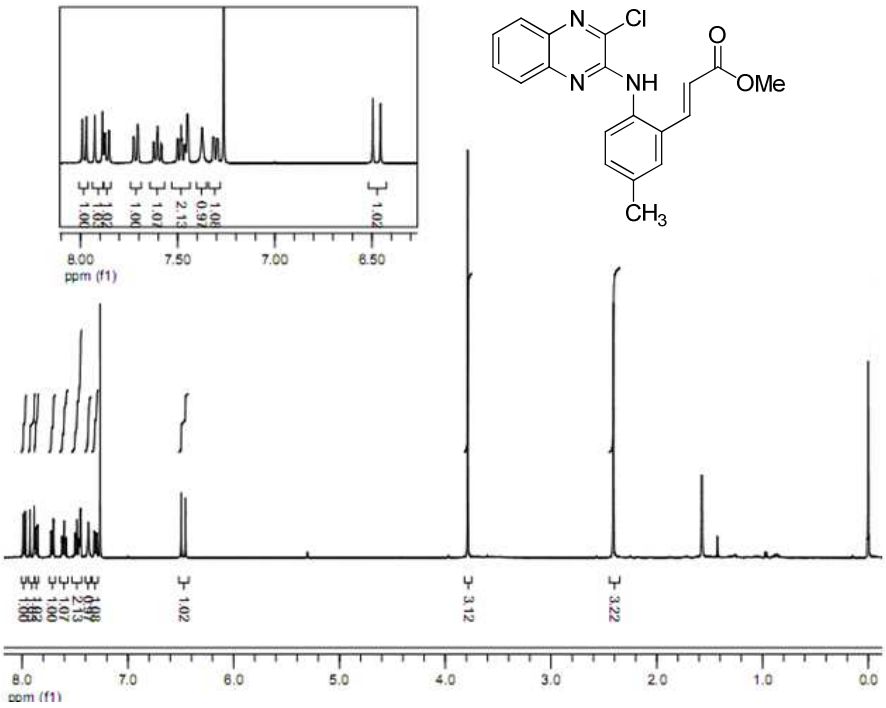
# Appendix



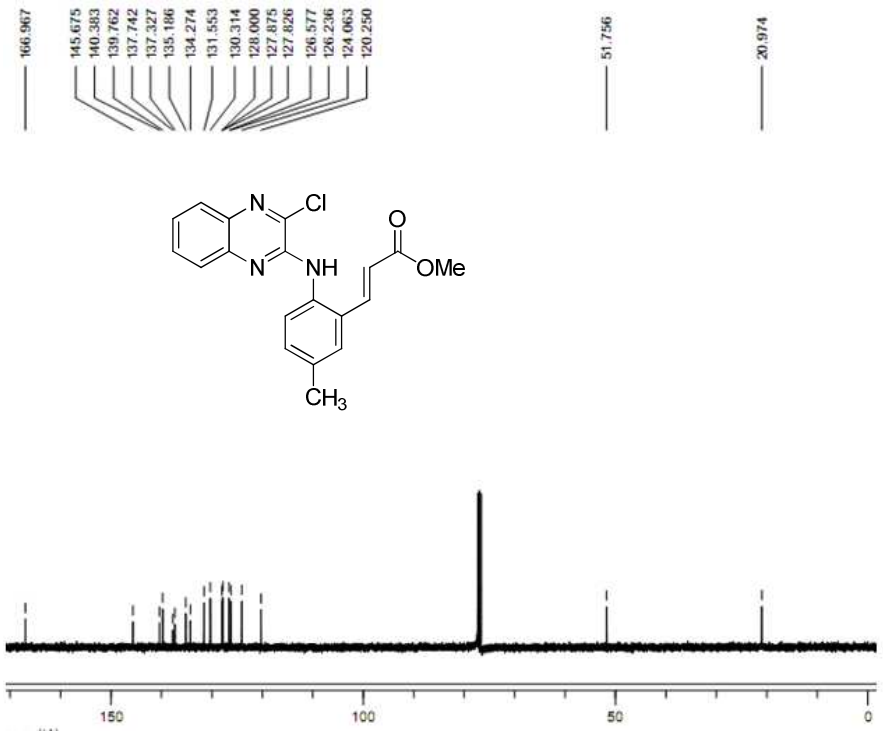

$^{1}$H NMR spectra of compound **21a** ($CDCl_3$, 400 MHz)



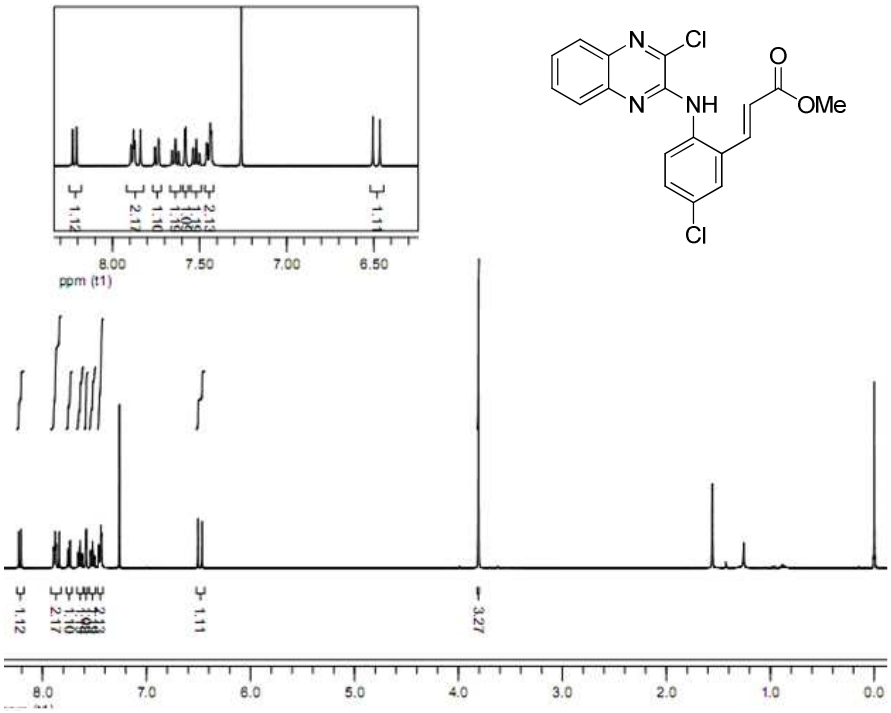

$^{13}$C NMR spectra of compound **21a** ($CDCl_3$, 100 MHz)



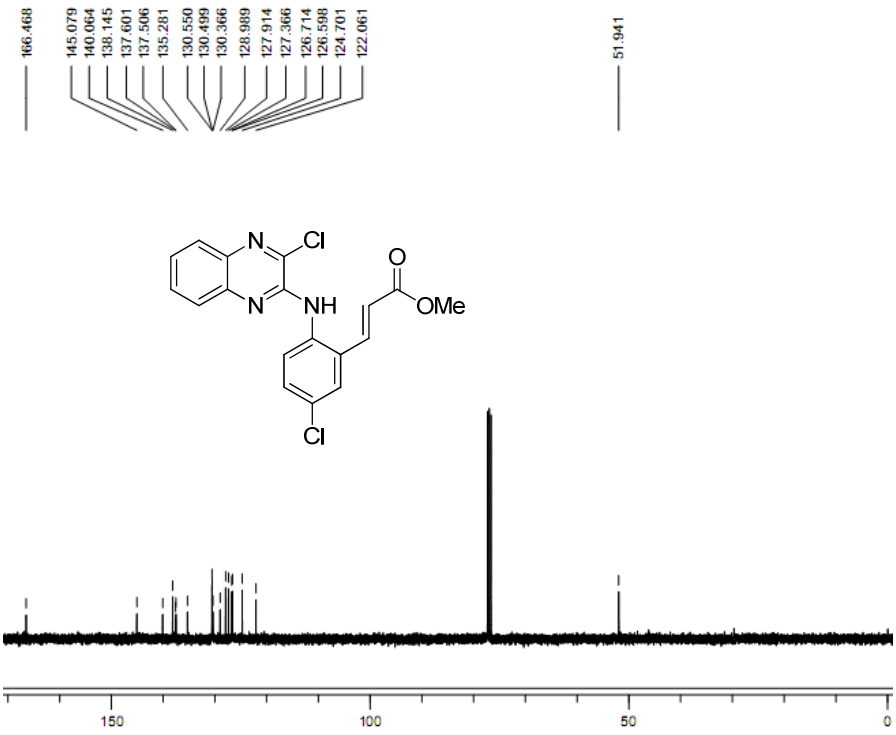

$^{1}$H NMR spectra of compound **21e** ($CDCl_3$, 400 MHz)



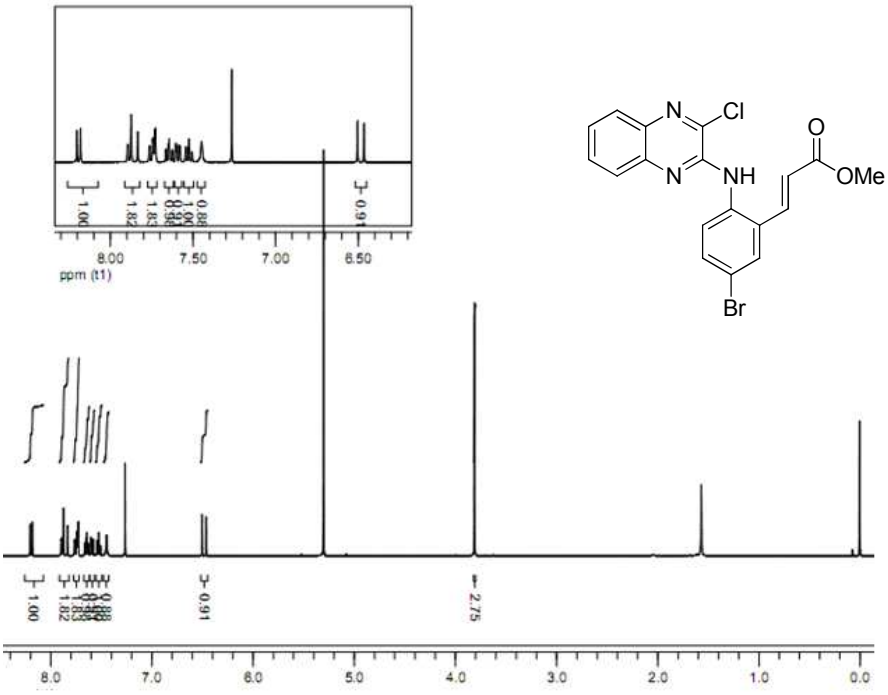

$^{13}$C NMR spectra of compound **21e** ($CDCl_3$, 100 MHz)



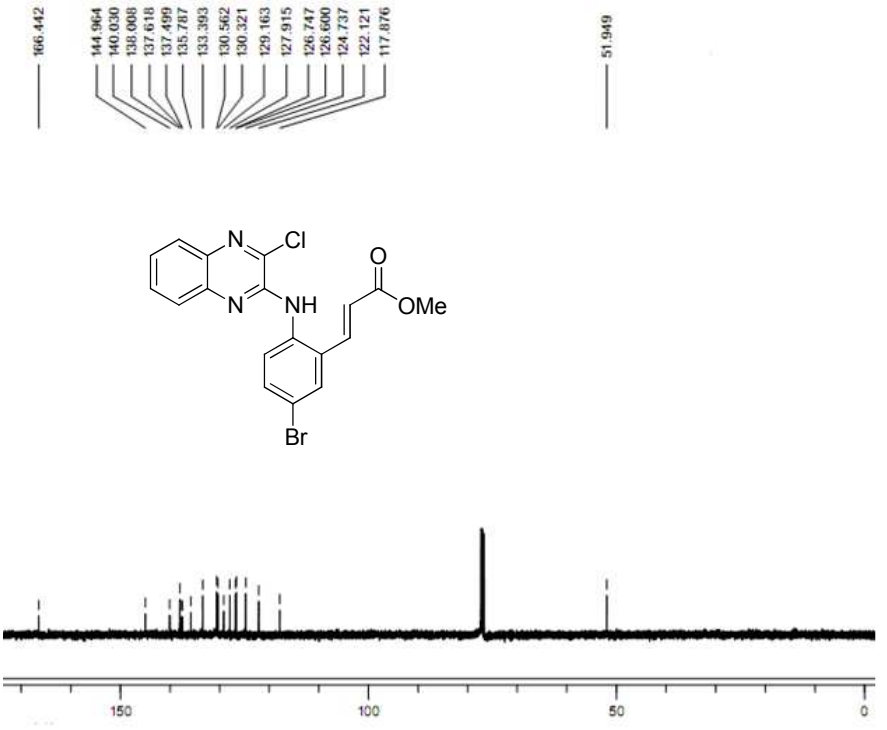

$^{1}$H NMR spectra of compound **21h** ($CDCl_3$, 400 MHz)



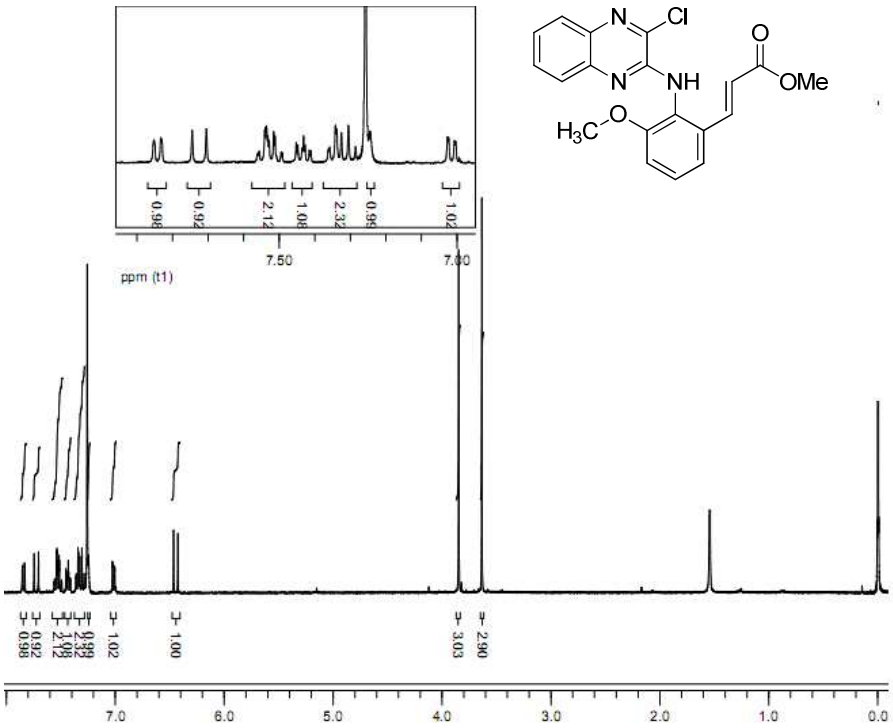

$^{13}$C NMR spectra of compound **21h** ($CDCl_3$, 100 MHz)



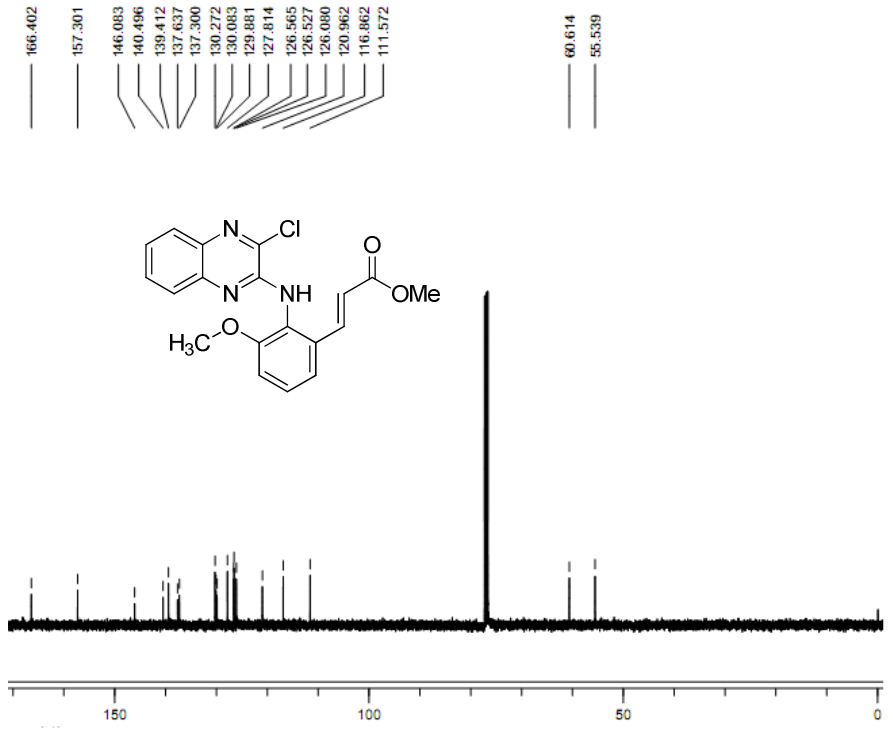

$^{1}$H NMR spectra of compound **21k** ($CDCl_3$, 400 MHz)



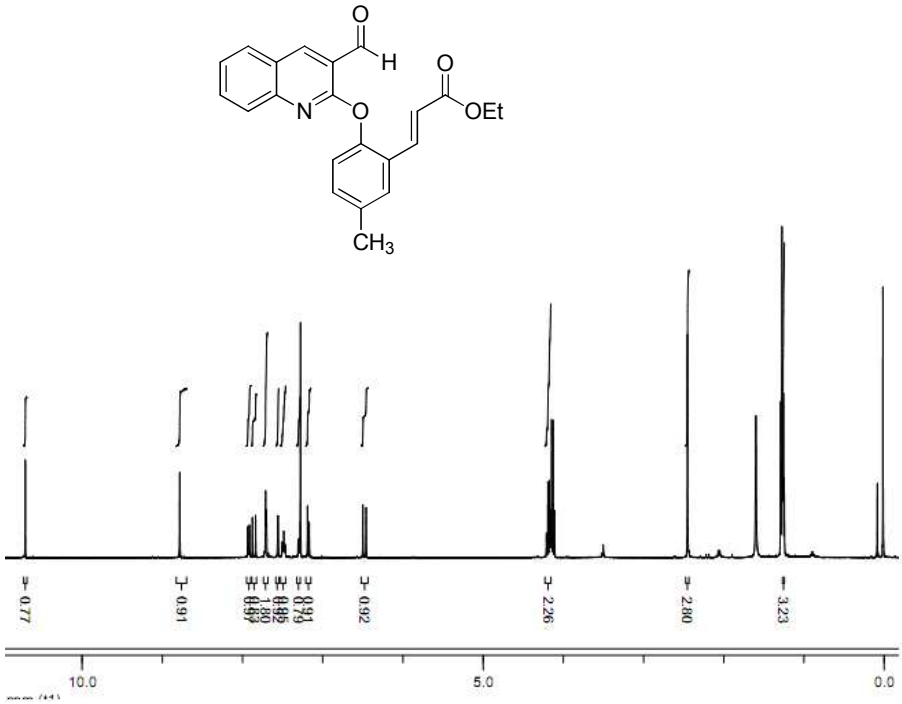

$^{13}$C NMR spectra of compound **21k** ($CDCl_3$, 100 MHz)



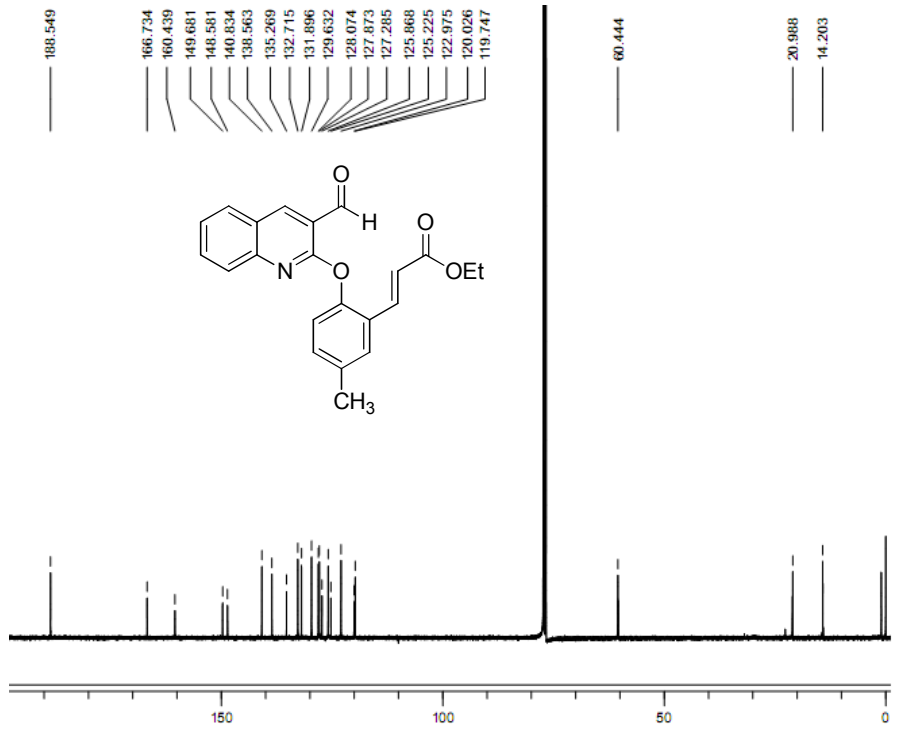

$^{1}$H NMR spectra of compound **21n** ($CDCl_3$, 400 MHz)



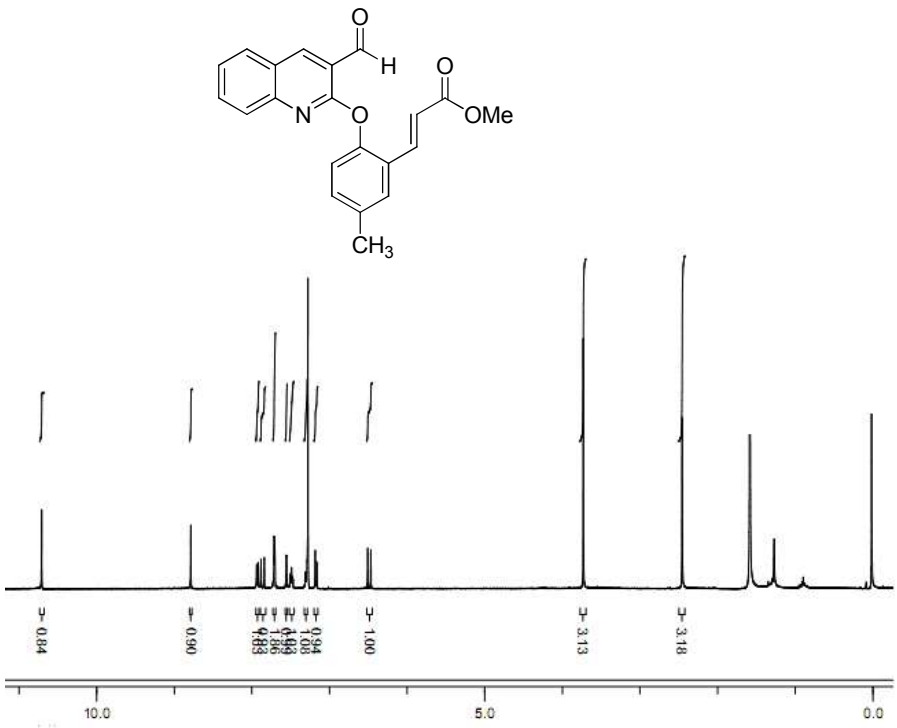

$^{13}$C NMR spectra of compound **21n** ($CDCl_3$, 100 MHz)



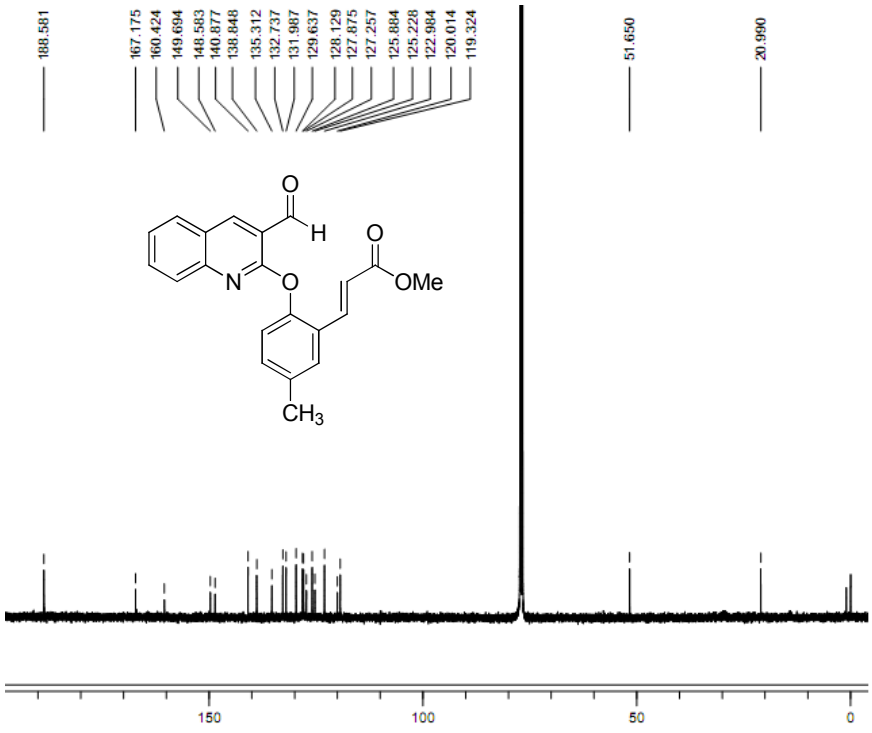

$^{1}$H NMR spectra of compound **24a** ($CDCl_3$, 400 MHz)



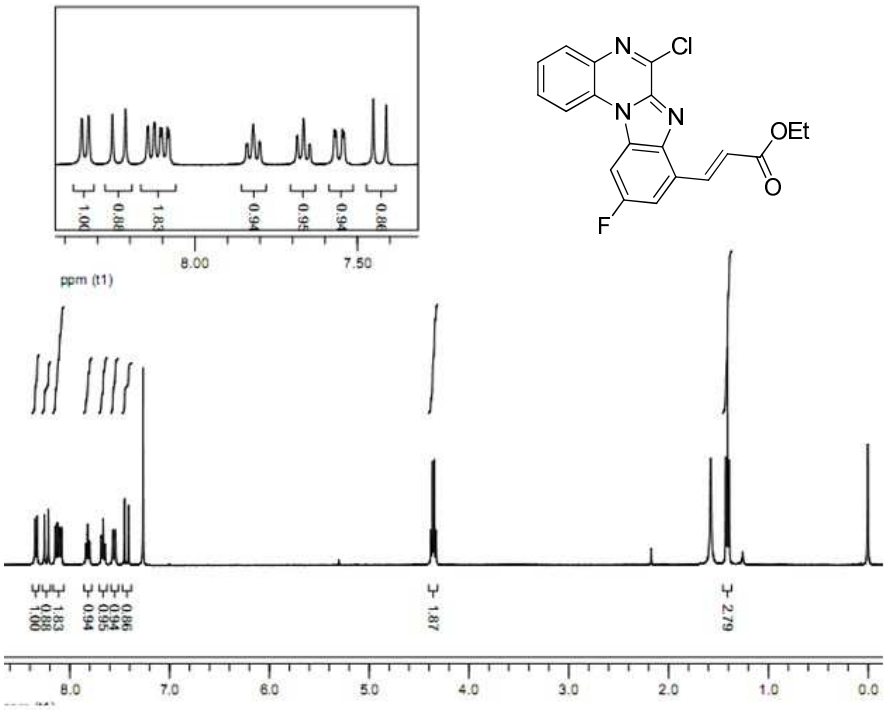

$^{13}$C NMR spectra of compound **24a** ($CDCl_3$, 100 MHz)



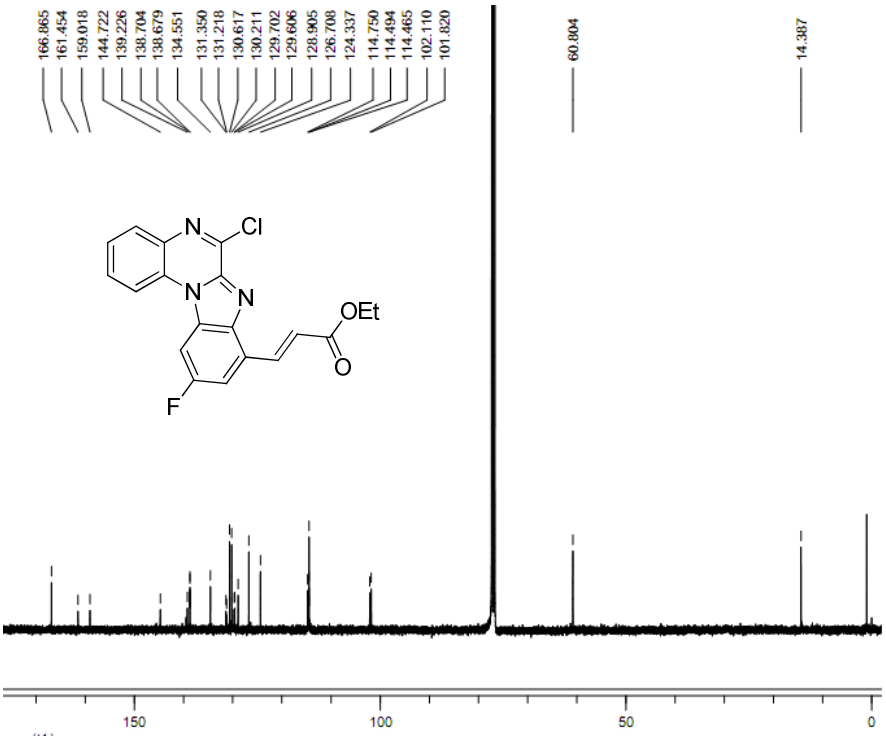

$^{1}$H NMR spectra of compound **24b** ($CDCl_3$, 400 MHz)



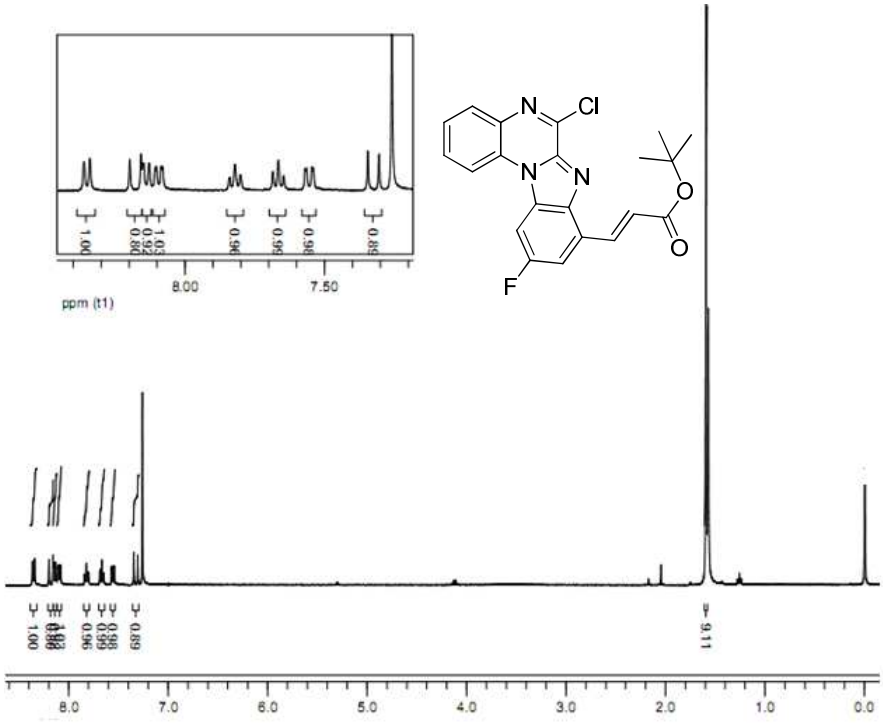

$^{13}$C NMR spectra of compound **24b** ($CDCl_3$, 100 MHz)



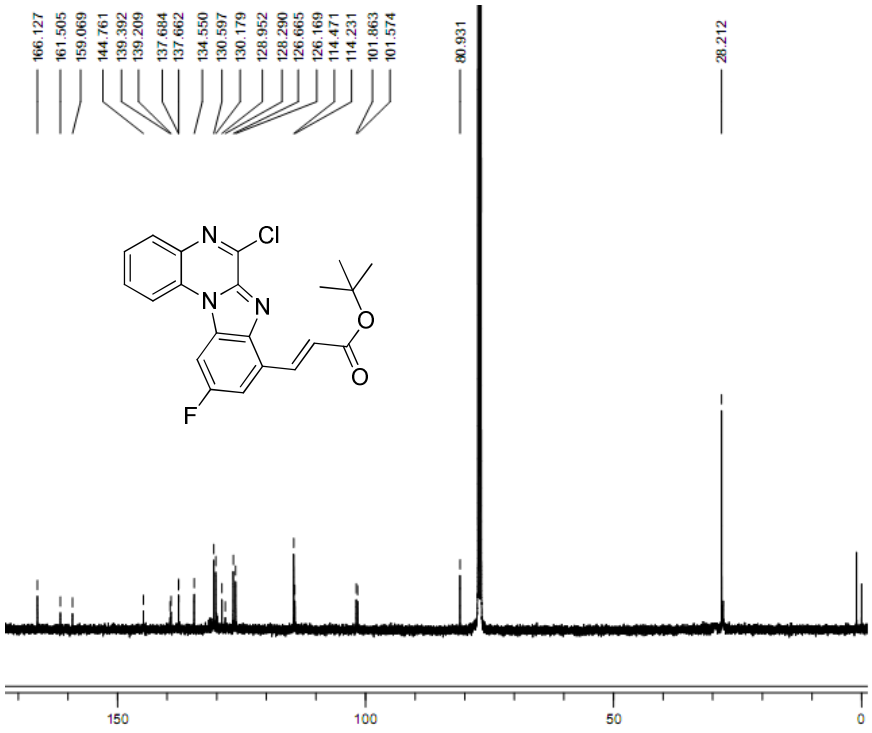

$^{1}$H NMR spectra of compound **22a** ($CDCl_3$, 400 MHz)



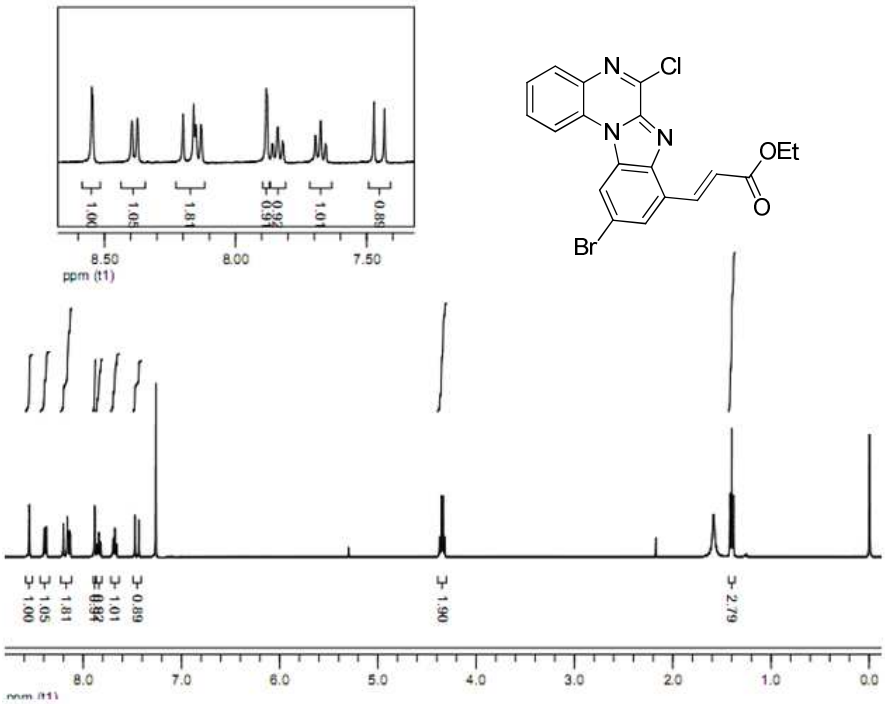

$^{13}$C NMR spectra of compound **22a** ($CDCl_3$, 100 MHz)



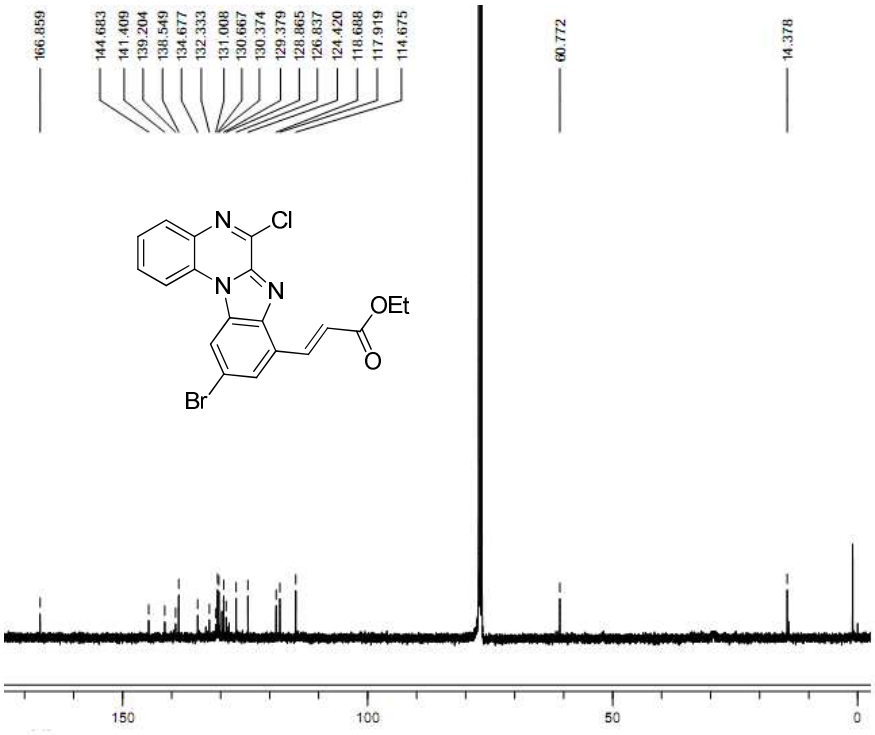

$^{1}$H NMR spectra of compound **22b** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **22b** ($CDCl_3$, 100 MHz)



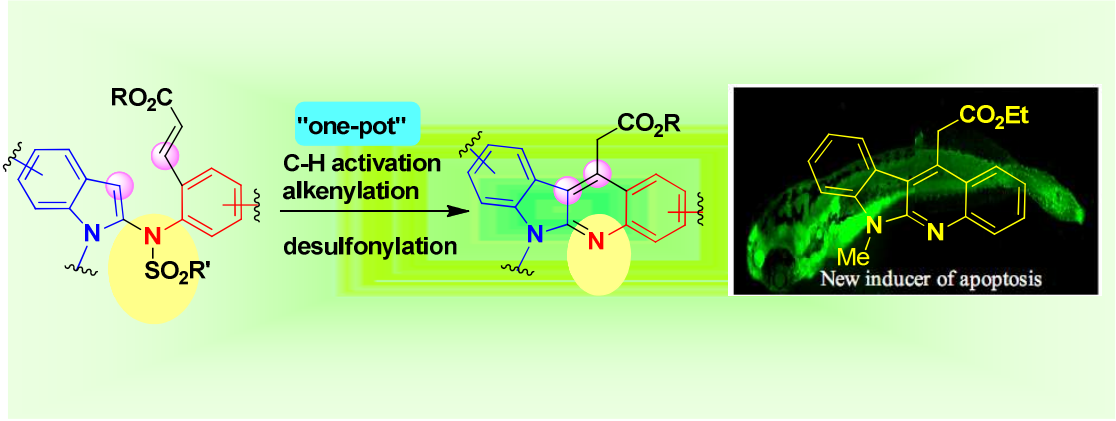

$^{1}$H NMR spectra of compound **22c** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **22c** ($CDCl_3$, 100 MHz)

# *CHAPTER 4*

# Synthesis of novel Indoloquinolines related to neocryptolepine *via* Pd-catalyzed C–H activation



## 4.1. Introduction:

Indoloquinolines are unique natural alkaloids, characterized by an indole and a quinoline fused ring system, found exclusively in a climbing shrub *Cryptolepis sanguinolenta*[1] indigenous to West Africa. During the last few decades the indoloquinoline ring system has been an interesting molecular scaffold for the design of new drugs due to the broad spectrum of biological activities of compounds based on indoloquinoline.[2] Particularly, among the various isolated products containing indoloquinoline structure, Cryptolepine (5-methyl-5*H*-indolo[3,2-*b*]quinoline) (**1**), neocryptolepine (cryptotackieine, 5-methyl-5*H*-indolo[2,3-*b*]quinoline) (**2**) and isocryptolepine (cryptosanguinolentine, 5-methyl-5*H*-indolo[3,2-*c*]quinoline) (**3**) are three examples of the thirteen characterized alkaloids from *Cryptolepis sanguinolenta* (Figure 4.1) and were studied extensively. All these compounds showed broad spectrum of biological properties such as antimalarial, antibacterial and antifungal, anti-inflammatory, and antiplasmodial activities.[3] Further, recent studies have shown that cryptolepine (**1**) and neocryptolepine (**2**) possess linearly arranged tetracyclic planar structures. Thus, they behave as a DNA intercalating agents by inhibiting DNA replication, transcription, and topoisomerase activities.[4] Therefore these molecules are acting as promising anticancer agents in modern healthcare. Moreover, 11-substituted indolo[3,2-*b*]quinolines, displays a range of remarkable pharmacological properties. For example, 11-[2-methoxy-4-(methylsulfonyl)phenyl]amino indoloquinoline (**4**) has shown to exhibit antitumor activity,[5] while both 11-(4-diethylamino)-2-methylbutylamino indoloquinoline (**5**)[6] and 11-(4-hydroxylphenyl)amino indoloquinoline (**6**)[7] have shown promising activity with chloroquine to inhibit a multi-resistant *Plasmodium falciparum* strain (Figure 4.2).[7-8]

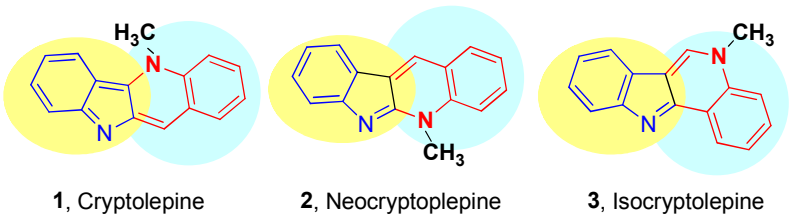



**Fig 4.1**: Examples of bioactive indoloquinoline alkaloids.

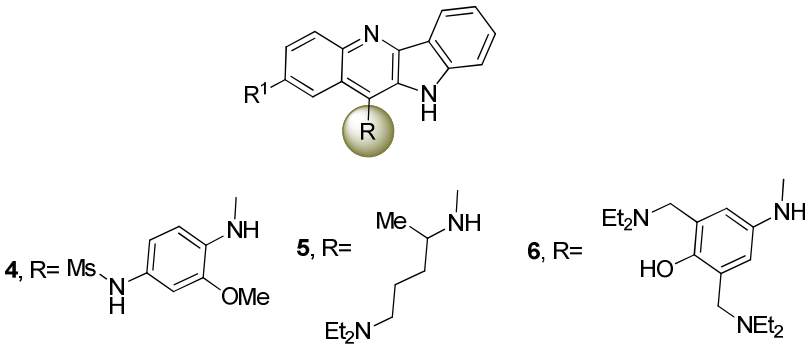



**Fig 4.2**: Examples of bioactive 11-substituted indolo[3,2-*b*]quinolines.

Owing to their remarkable therapeutic potentials, the synthesis of indoloquinoline derivatives has attracted considerable attention of synthetic organic chemists. In addition to that they are considered as attractive templates for new drug discovery[9] especially in anticancer research. In this context, we were particularly interested in design of novel bioactive molecules **B** (Figure 4.3) based on known anti-cancer alkaloid neocryptolepine[10] **A** (5-methyl-5*H*-indolo[2,3-*b*]quinoline, and its synthetic analogue 5,11-dimethyl-5*H*-indolo[2,3-*b*]quinoline[11] (DIMIQ), which showed promising cytotoxic and anticancer activities. The promising cytotoxic effects were also observed with 6-substituted 6*H*-indolo[2,3-*b*]quinolines.[12] Thus we were interested to use the 6*H*- indolo[2,3-*b*]quinoline framework **B** (Figure 4.3) for the discovery of novel apoptotic agents.

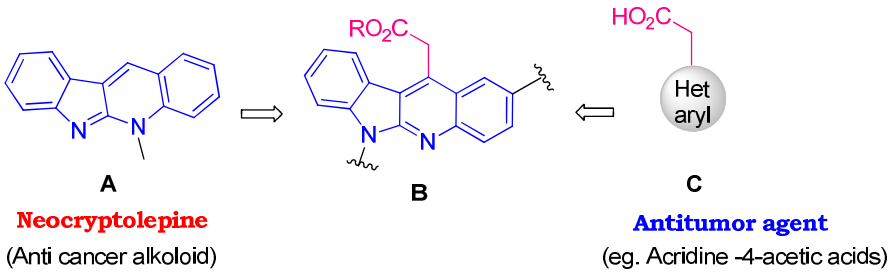


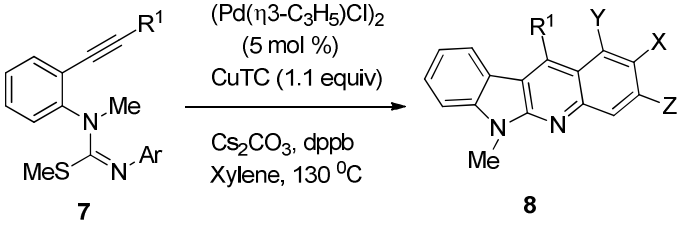

**Fig 4.3**: Design of novel bioactive molecules **B**

The introduction of $-CH_2CO_2R$ moiety at C-11 of **B** was particularly inspired by the fact that certain aryl acetic acids[13] **C** (Figure 4.3) e.g. acridone-4-acetic acids showed potent solid tumor activity via induction of cytokines including tumor necrosis factor (which affected tumor blood flow) and other host-mediated cytotoxicity mechanisms (Figure 4.3). Moreover a recent publication[14] disclosed that the introduction of an ester group to the core structures led to the enhanced

anticancer activities. It is believed to be due to an increase in both the lipophilicity and bioavailability of the corresponding drug.

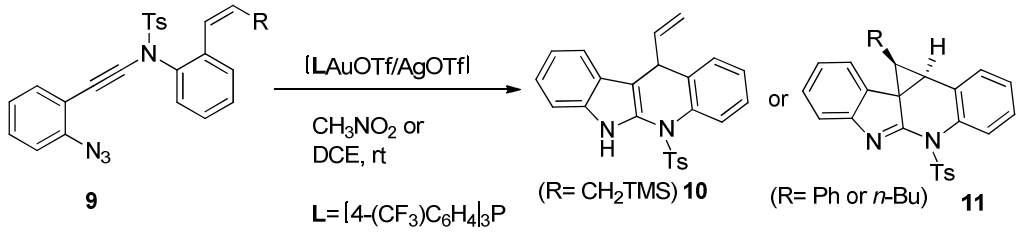
### 4.1.1. Earlier reports for the synthesis of indoloquinoline derivatives:

In 2009, Takemoto and coworkers disclosed Pd-catalyzed annulation of 1,2-dialkylisothioureas (**7)** with 5 mol% $(Pd(\eta 3\text{-}C_3H_5)Cl)_2$ in the presence of CuTC (copper thiophenecarboxylate) and $Cs_2CO_3$ at 130 ºC that provided a wide range of 6-*N*-alkylatedindolo[2,3-*b*]quinolines (**8**) in good yields as shown in Scheme 4.1.[15]



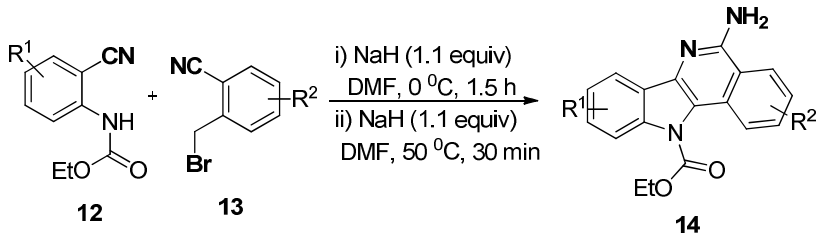

**Scheme 4.1**: Synthesis of 6-*N*-alkylated indolo[2,3-*b*]quinolines (**8)**.

In 2014, Ohno and coworkers developed a novel method for the synthesis of indoloquinolines *via* the gold-catalyzed cascade cyclization of (azido)ynamides. Ynamides bearing an allylsilane gave terminal alkenes (**10)**, whereas ynamides bearing a simple alkene gave cyclopropanes (**11)** as shown in Scheme 4.2.[16]



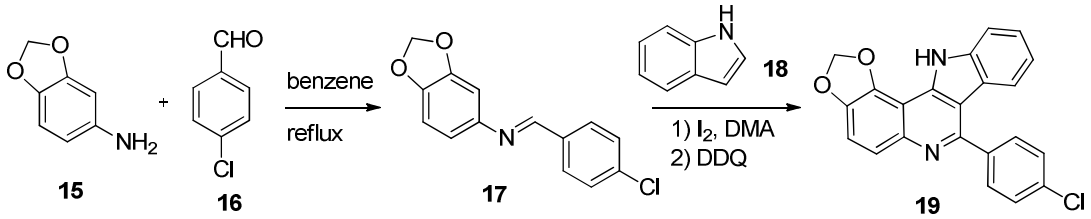

**Scheme 4.2**: Synthesis of indoloquinolines from (azido)ynamides.

In 2015, Kurth and coworkers developed a transition metal free one-pot protocol for the synthesis of 11-*H*-indolo[3,2-*c*]isoquinolin-5-amines (**14)** *via* the atom economical annulations of ethyl(2-cyano-phenyl)carbamates (**12)** and 2-cyanobenzylbromides (**13)**. This method proceeds *via* sequential *N*-alkylation and base promoted cyclization as shown in Scheme 4.3.[17]



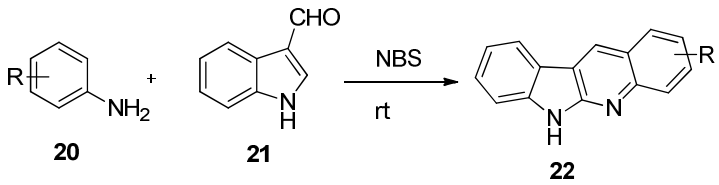

**Scheme 4.3**: Synthesis of 11-*H*-indolo[3,2-*c*]isoquinolin-5-amines from 2-cyanobenzylbromides.

In 2013, Wang and coworkers disclosed the synthesis of indolo[3,2-*c*]quinoline derivatives (**19**) *via* the reaction of schiff base (**17**) with indole (**18**) in the presence of iodine in DMA and subsequent treatment with DDQ to give the desired product (**19**) in good yields as shown in Scheme 4.4.[18]



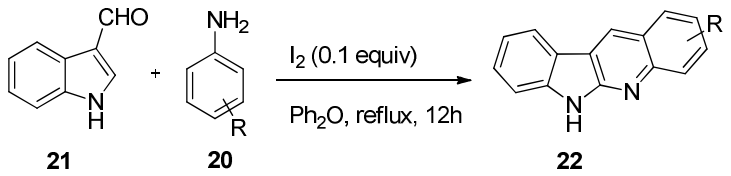

**Scheme 4.4**: Iodine mediated synthesis of indolo[3,2-*c*]quinoline derivatives.

In 2012, Malaekehpoor and coworkers reported *N*-bromosuccinimide catalyzed synthesis of poly cyclic indolo[2,3-*b*]quinoline derivatives (**22)** from various arylamines (**20)** and indole-3-carbaldehyde (**21)** in good to high yields. The reaction proceeded at room temperature under mild conditions as shown in Scheme 4.5.[19]



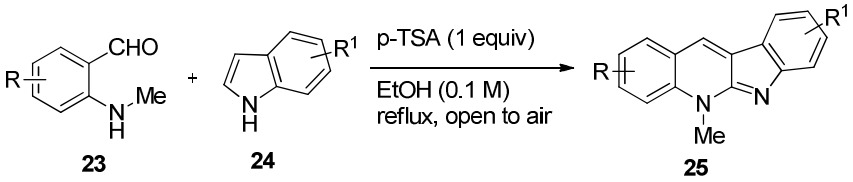

**Scheme 4.5**: Synthesis of indoloquinolines derivatives.

In 2009, Tilve and coworkers reported a one-pot synthesis for linear 6*H*-indolo[2,3-*b*]quinolines *via* the reaction of indole-3-carboxyaldehyde (**21)** with arylamine (**20)** in the presence of a catalytic amount of iodine in refluxing diphenylether as shown in Scheme 4.6.[20]



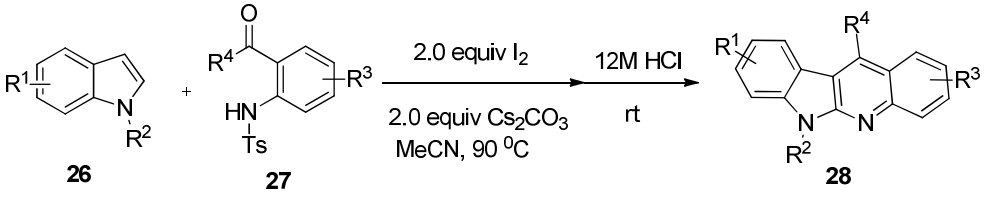

**Scheme 4.6**: Synthesis of 6*H*-indolo[2,3-*b*]quinoline derivatives.

In 2011, Seidel and coworkers reported the synthesis of neocryptolepine and its analogues *via* acid-promoted reaction of secondary amino benzaldehyde (**23**) with indole (**24**). The reaction proceeded through annulation followed by spontaneous oxidation to give compound (**25**) in excellent yields as shown in Scheme 4.7.[21]




**Scheme 4.7**: Synthesis of neocryptolepine and analogues.

In 2012, Liang and coworkers developed a one-pot method for the synthesis of substituted indolo(2,3-*b*)quinolines from indoles. The reaction involves activation of C2 and C3 of indoles by molecular iodine ($I_2$) and base followed by *in situ* reaction with 1-(2-tosylaminophenyl)ketones or 2-tosylaminobenzaldehyde to afford novel C-11-substituted derivatives in moderate to excellent yields as shown in Scheme 4.8.[22]

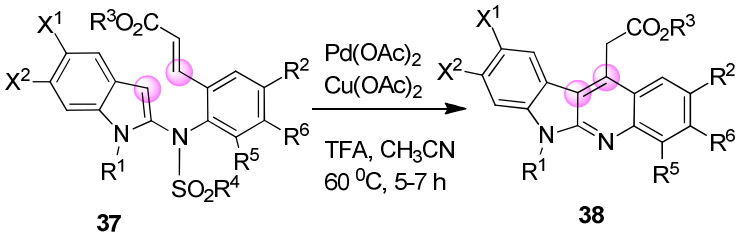



**Scheme 4.8**: One-pot synthesis of indolo(2,3-*b*)quinolines from indole.

### 4.1.2. Present work:

Though several interesting and elegant methods have been reported for the construction of indoloquinoline ring, none of them were suitable for the preparation of our target compounds *i.e.* 11-substituted 6*H*-indolo[2,3-*b*]quinoline derivatives (**B**). A recent one-pot approach[22] though afforded this class of compounds having alkyl/ aryl substituents at C-11 was also found to be

inconvenient for a direct access to **B** (Figure 4.3) (vide infra). However, based on a recent report[23] we have developed a new strategy involving sequential Pd-catalyzed C-H activation-intramolecular alkenylation followed by desulfonation in the same pot to afford the target compound **B** (or **38**) in a straightforward manner (Scheme 4.9).



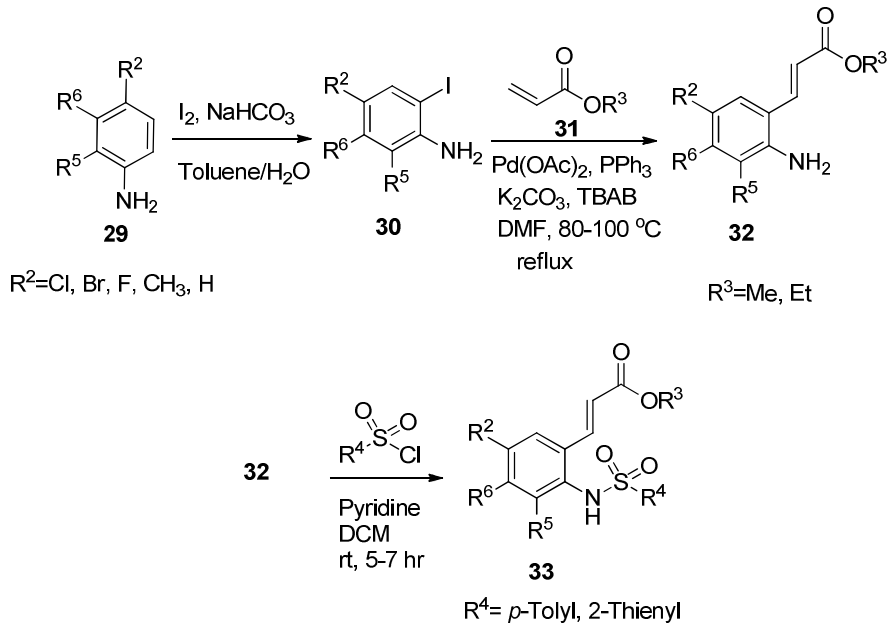

**Scheme 4.9**. Pd-mediated synthesis of 11-carboxymethyl substituted 6*H*-indolo[2,3-*b*]quinoline derivatives.

Recently, transition-metal catalyzed C−H activation[24] has become a hot area of research[25] and found wide applications in forming C−C and C-heteroatom bonds. Indeed, a remarkable progress has been made in this area where Pd particularly occupied the center stage. However, use of this technology towards the straightforward synthesis of densely functionalized heteroaromatics is not common in the literature and needs further exploration.

## 4.2. Results and discussion:

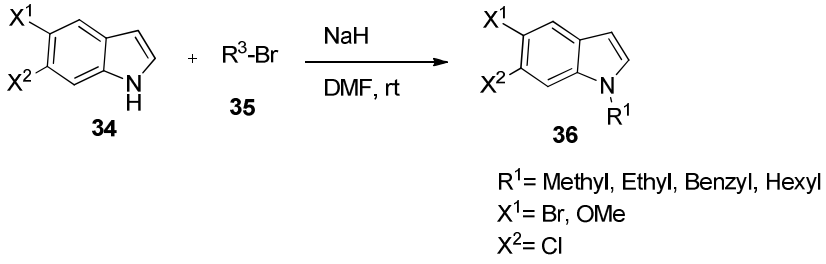
### 4.2.1. Preparation of starting compounds

The requisite starting material (**33)** was synthesized from substituted anilines. Iodination of anilines provided 2-iodo substituted anilines (**30**), which on Heck reaction with various acrylates (**31**) afforded (*E*)-alkyl 3-(2-amino-substituted phenyl)acrylate (**32**). Further on tosylation the compound (**32**) afforded (*E*)-ethyl 3-(2-(sulfonamido)phenyl)acrylate derivatives (**33)** as shown in Scheme 4.10.




**Scheme 4.10**: Synthesis of (*E*)-ethyl 3-(2-(sulfonamido)phenyl)acrylate derivatives (**33)**

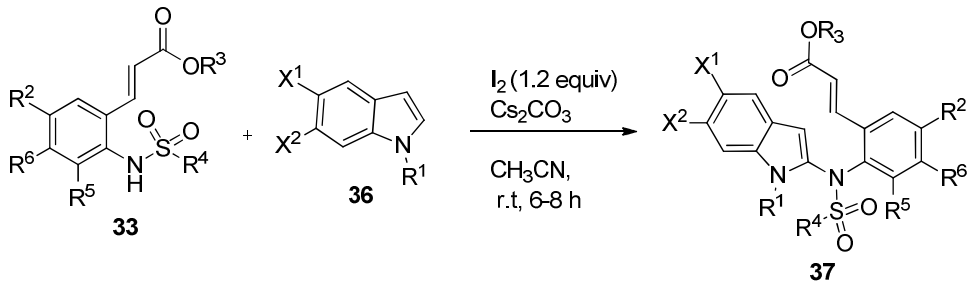
The other *N*-substituted indole derivatives (**36)** were prepared *via* the reaction of indoles with appropriate alkyl bromide/iodides (**35)** in the presence of a base as shown in Scheme 4.11.[26]

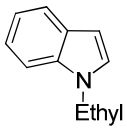
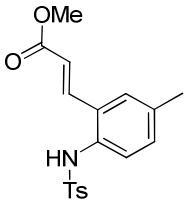
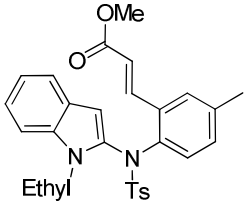
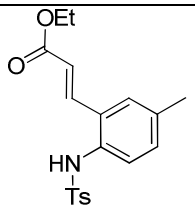
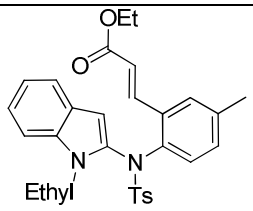
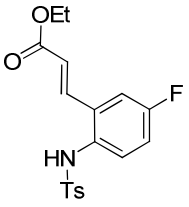
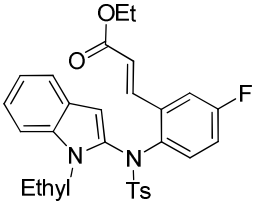
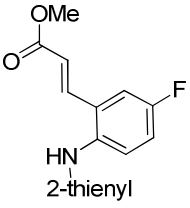
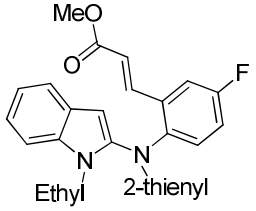
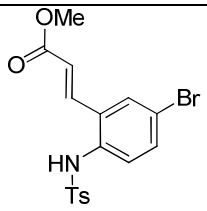
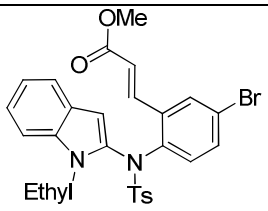


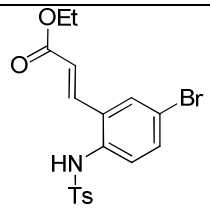
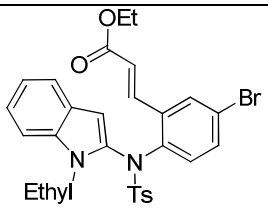
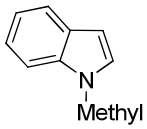
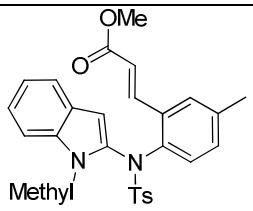
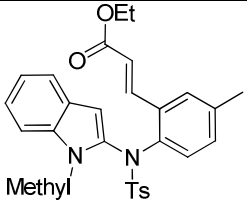
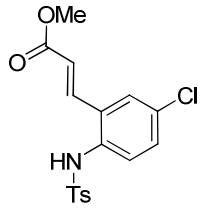
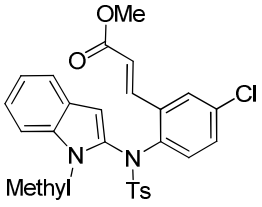
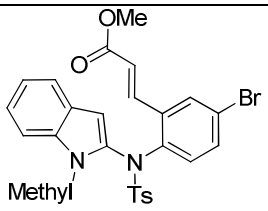
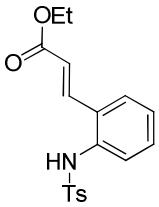
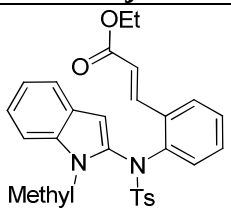
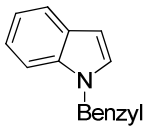
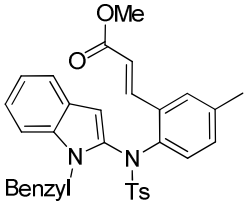

**Scheme 4.11**: Synthesis of *N*-substituted indole derivatives (**36)**

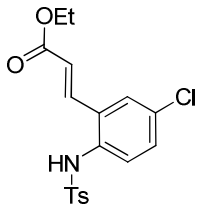
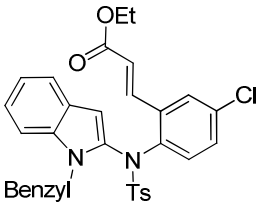
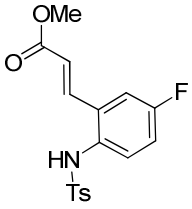
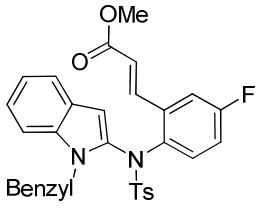
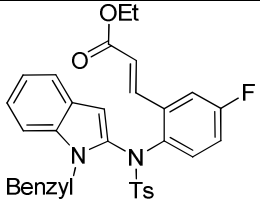
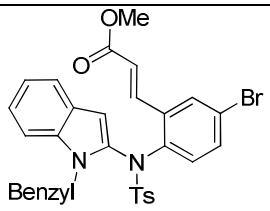
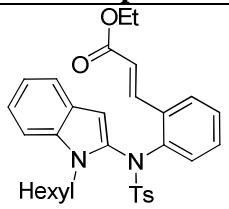
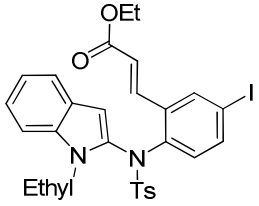
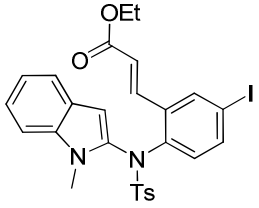
The key starting material *i.e.* (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives **(37)** required for study was prepared by direct C-2 amination of *N*-substituted indoles (**36**) with (*E*)-ethyl-3-(2-(sulfonamido)phenyl)acrylate derivatives (**33)** in the presence of molecular iodine and a base at room temparature.[27] The mild conditions permited a broad set of functionalities both in the indoles and in the compound (**33)**. The reaction afforded a variety of (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives **(37)** in moderate to good yields (Table 4.1).

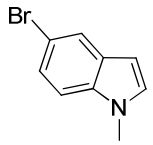
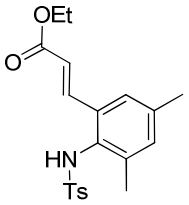
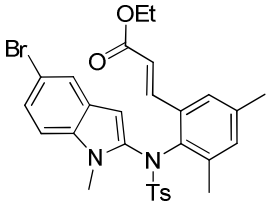
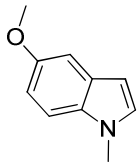
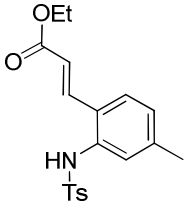
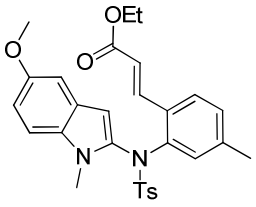
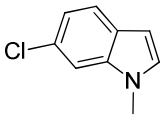
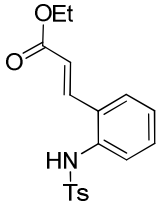
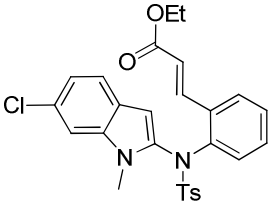
**Table 4.1:** Iodine mediated synthesis of (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives **(37).**[a]




| Entry | Indole (**36**) | Compound (**33**) | Time (h) | Product (**37**) | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 |  **36a** |  **33a** | 6 |  **37a** | 85 |
| 2 | **36a** |  **33b** | 6 |  **37b** | 87 |
| 3 | **36a** |  **33c** | 6.5 |  **37c** | 83 |
| 4 | **36a** |  **33d** | 8 |  **37d** | 68 |
| 5 | **36a** |  **33e** | 7 |  **37e** | 75 |

| | | | | | |
|---|---|---|---|---|---|
| 6 | **36a** |  **33f** | 7 |  **37f** | 80 |
| 7 |  **36b** | **33a** | 6 |  **37g** | 71 |
| 8 | **36b** | **33b** | 6 |  **37h** | 83 |
| 9 | **36b** |  **33g** | 6 |  **37i** | 68 |
| 10 | **36b** | **33e** | 7.5 |  **37j** | 60 |
| 11 | **36b** |  **33h** | 5 |  **37k** | 73 |
| 12 |  **36c** | **33a** | 7 |  **37l** | 84 |

| | | | | | |
|---|---|---|---|---|---|
| 13 | **36c** |  **33i** | 6 |  **37m** | 83 |
| 14 | **36c** |  **33j** | 6.5 |  **37n** | 82 |
| 15 | **36c** | **33c** | 6 |  **37o** | 79 |
| 16 | **36c** | **33e** | 7 |  **37p** | 76 |
| 17 |  **36d** | **33h** | 8 |  **37q** | 55 |
| 18 | **36a** | **33h** | 12 |  **37r** | 73[c] |
| 19 | **36b** | **33h** | 12 |  **37s** | 68[c] |

| | | | | | |
|---|---|---|---|---|---|
| 20 |  **36e** |  **33k** | 6 |  **37t** | 84 |
| 21 |  **36f** |  **33l** | 6 |  **37u** | 87 |
| 22 |  **36g** |  **33h** | 6.5 |  **37v** | 82 |

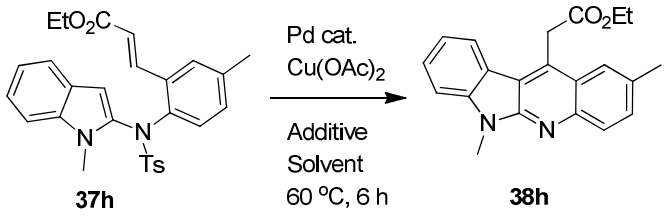
[a]All the reactions were carried out using compound **33** (1.2 mmol), **36** (1.0 mmol), $I_2$ (1.2 mmol) and $Cs_2CO_3$ (1.5 mmol) in $CH_3CN$ (5.0 mL) at room temperature under nitrogen .
[b]Isolated yield. [c]3.0 equiv of $I_2$ was used for 12h.

### 4.2.2. Reaction optimization:

The Pd(II)-mediated intramolecular C-H alkenylation was then examined under various conditions using the compound (**37h)** (Table 4.2). Though the $Pd(OAc)_2$ catalyzed reaction afforded a low yield of desired product (**38h)** (entry 1, Table 4.2) the yield was increased dramatically when trifluoroacetic acid (TFA) was used as an additive (entry 2, Table 4.2). To improve the yield further we changed the Pd catalysts (entries 3-5, Table 4.2) and the additive (entry 6 and 7, Table 4.2). However, (**38h)** was either obtained in low or moderate yield or not formed at all. While $CH_3CN$ was used as a solvent in these reactions, the use of other solvents like DMSO, DMF, toluene and DCE (1,2-dichloroethane) was also examined and found to be less effective (entries 8-11, Table 4.2). Notably, the present synthesis of (**38h)** does not require the use of an inert atmosphere as the yield was not affected whether the reaction was performed under open air or nitrogen (entry 2 vs 12, Table 4.2). The reaction did not proceed in the absence of $Pd(OAc)_2$ alone or $Pd(OAc)_2$ / $Cu(OAc)_2$ indicating key role played by the catalyst and the oxidant in the present reaction. However, the reaction proceeded slowly in the absence of

$Cu(OAc)_2$ when performed under open air affording (**38h)** in 70% yield after 12h. The aerial oxygen played the role of oxidant in this case. Notably, (**38h)** was not formed when (**37h)** was treated with 12M HCl under the reported condition[28] even after 12h indicating inappropriateness of the earlier method in the present case.

**Table 4.2**: Reaction conditions and optimization.

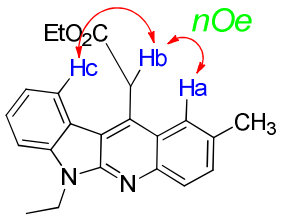



| Entry | Catalyst | Additive | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | $Pd(OAc)_2$ | - | $CH_3CN$ | 22 |
| 2 | $Pd(OAc)_2$ | TFA | $CH_3CN$ | 82 |
| 3 | $PdCl_2$ | TFA | $CH_3CN$ | 67 |
| 4 | $Pd(PPh_3)_2Cl_2$ | TFA | $CH_3CN$ | 9 |
| 5 | $Pd(PPh_3)_4$ | TFA | $CH_3CN$ | 40 |
| 6 | $Pd(OAc)_2$ | Amberlyst | $CH_3CN$ | 0 |
| 7 | $Pd(OAc)_2$ | PTSA | $CH_3CN$ | Trace |
| 8 | $Pd(OAc)_2$ | TFA | DMSO | 30 |
| 9 | $Pd(OAc)_2$ | TFA | DMF | 27 |
| 10 | $Pd(OAc)_2$ | TFA | Toluene | 52 |
| 11 | $Pd(OAc)_2$ | TFA | DCE | 11 |
| 12 | $Pd(OAc)_2$ | TFA | $CH_3CN$ | 81[c] |
| 13 | - | TFA | $CH_3CN$ | 0 |
| 14 | - | TFA | $CH_3CN$ | 0[d] |

[a]Reactions were performed using compound **37h** (0.20 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (0.30 mmol) and TFA (0.24 mmol) in $CH_3CN$ (2.5 mL) at 60 °C for 6h under air. [b]Isolated yield. [c]Reaction was performed under nitrogen. [d]Reaction was performed without $Pd(OAc)_2$ and $Cu(OAc)_2$

### 4.2.3. Scope of the reaction:

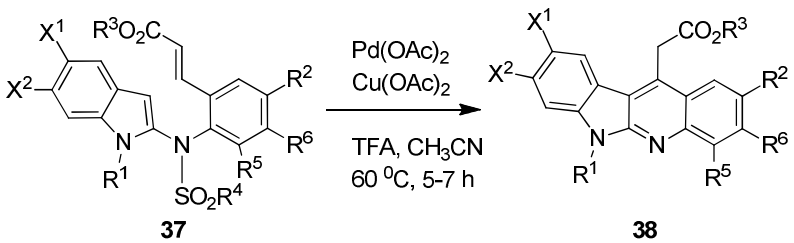
Having identified the optimum condition (entry 2, Table 4.3) we then examined the substrate scope and generality of this method. Thus, a range of (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives (**37**) were employed in the present reaction to give the desired 6*H*-indolo[2,3-*b*]quinoline derivatives (**38**) in acceptable to good yield (Table 4.3). All the compounds were well characterized by $^{1}$H & $^{13}$C NMR, MS & HPLC. The disappearance of certain $^{1}$HNMR signals of (**37)** i.e. a singlet near 6.2-6.5 δ due to the indole C3-H and two doublets near 8.3-8.5 and 6.3-6.4 δ (*J* ~ 16 Hz) due to two *trans* olefinic protons and appearance of a singlet near 4.6-4.7 δ (and a $^{13}$C NMR signal near 35 ppm) due to the 11-carboxymethylene (-$CH_2$COO-) protons indicated the formation of product (**38).** A representative compound (**38b)** was further characterized by $^{1}$H-$^{1}$H COSY, 2D NOESY, DEPT analysis (see Appendix) and the NOE experiment performed using (**38b)** indicated close promiximity of Hc and Ha protons with Hb proton as they interacted with each other (shown in Figure 4.4). In addition to various substituents as $R^1$, $R^2$, $R^3$, $R^5$, $R^6$, $X^1$ and $X^2$ (Table 4), the Pd(0) labile iodo group was also tolerated in this reaction (entry 18 and 19, Table 4.3) which is amenable for further functionalization *via* Pd(0) chemistry.



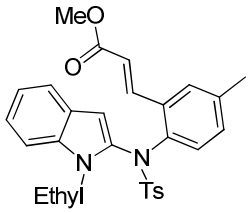
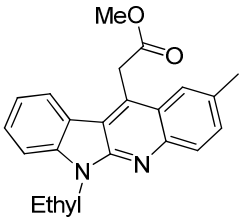
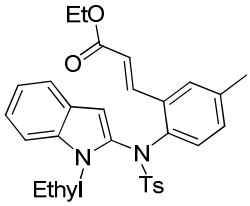
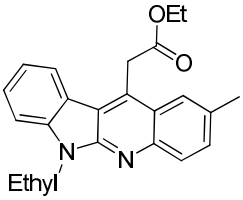
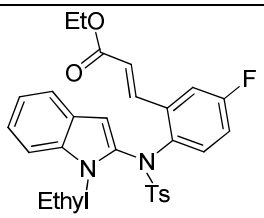
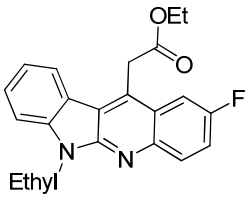
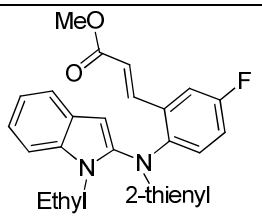
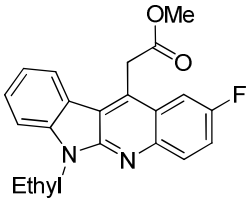
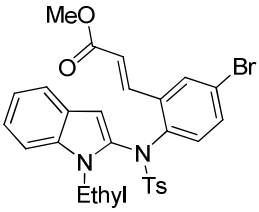
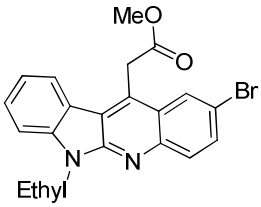
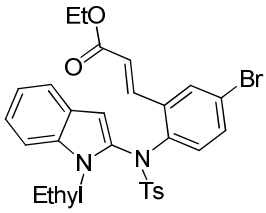
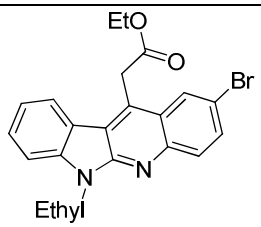

**38b**

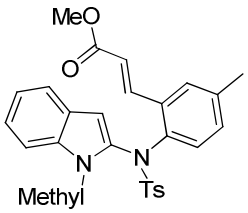
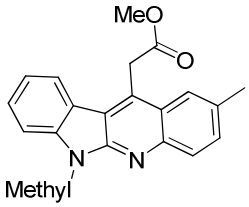
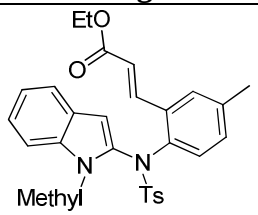
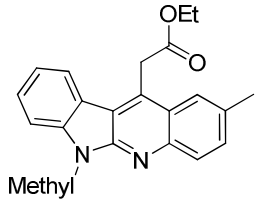
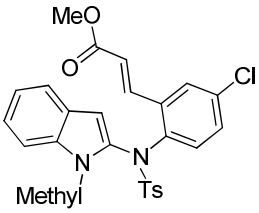
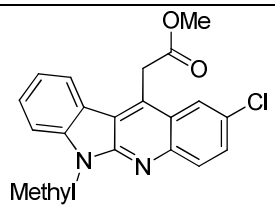
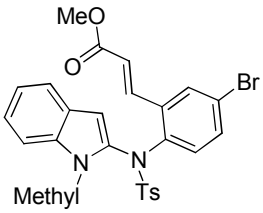
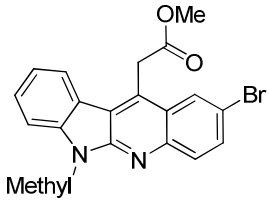
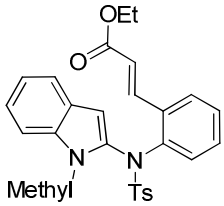
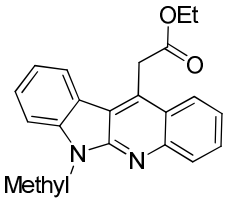
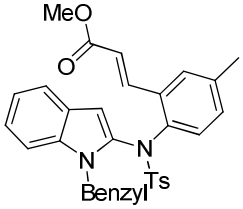
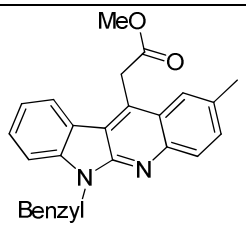
**Fig. 4.4:** NOE correlation of (**38b**).

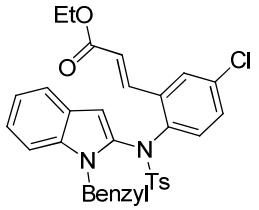
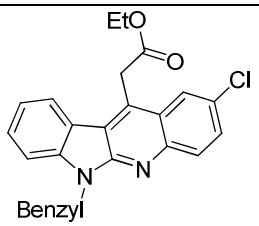
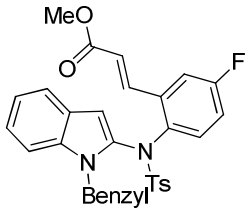
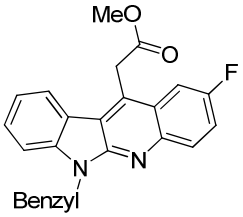
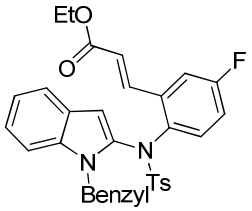
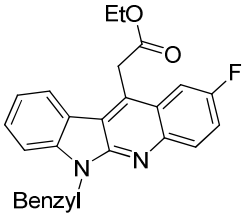
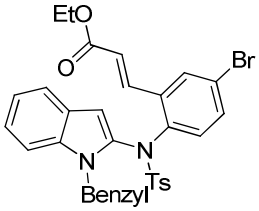
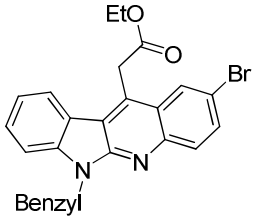
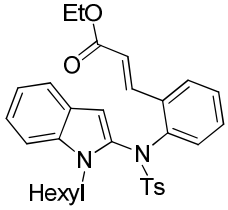
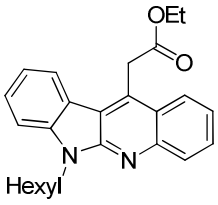
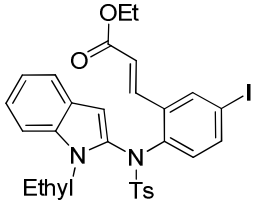
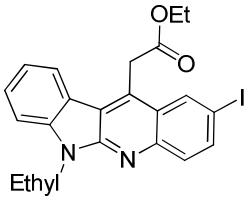
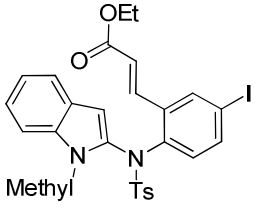
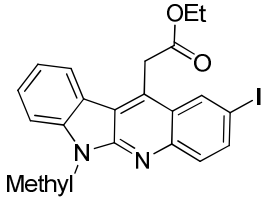
**Table 4.3:** Synthesis of compound **(38)**.[a]

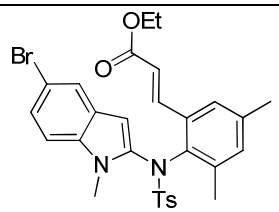
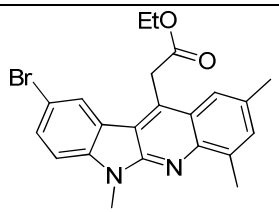
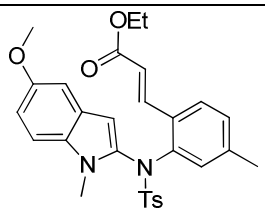
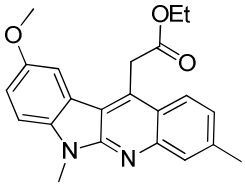
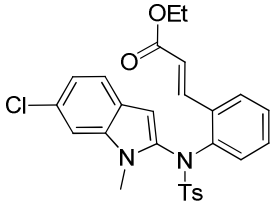
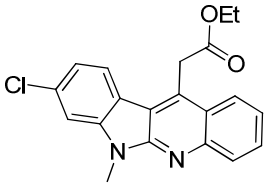



**37** **38**

| Entry | Substrate (**37**) | Product (**38**) | Time (h) | yield[b] (%) |
|---|---|---|---|---|
| 1 |  **37a** |  **38a** | 5 | 86 |
| 2 |  **37b** |  **38b** | 5 | 89 |
| 3 |  **37c** |  **38c** | 6 | 78 |
| 4 |  **37d** |  **38d** | 7 | 52 |
| 5 |  **37e** |  **38e** | 7 | 80 |
| 6 |  **37f** |  **38f** | 7 | 88 |
| | | | | |

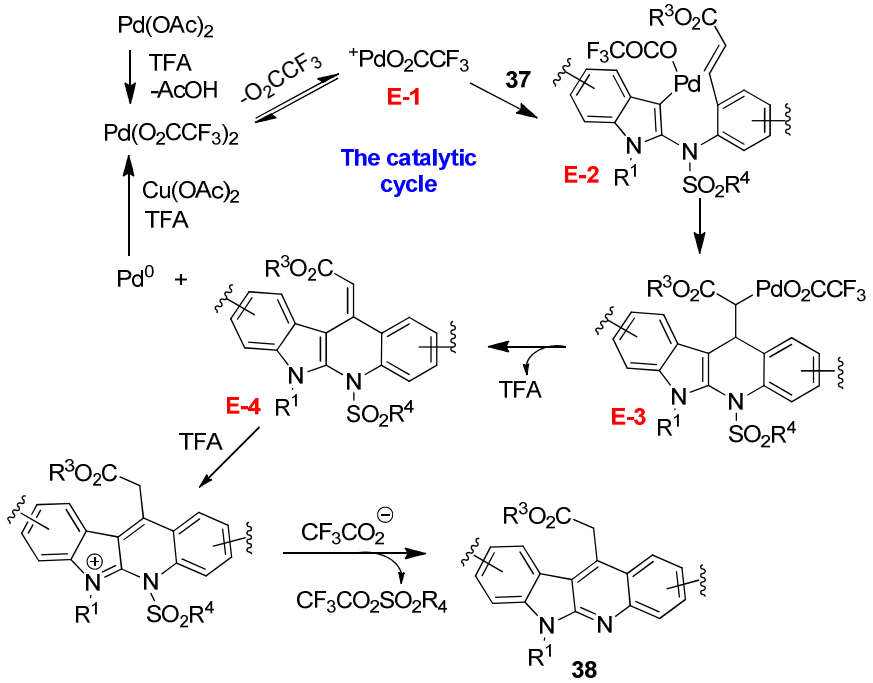
| | | | | |
|---|---|---|---|---|
| 7 |  **37g** |  **38g** | 6 | 75 |
| 8 |  **37h** |  **38h** | 6 | 82 |
| 9 |  **37i** |  **38i** | 6 | 85 |
| 10 |  **37j** |  **38j** | 7 | 77 |
| 11 |  **37k** |  **38k** | 6.5 | 84 |
| 12 |  **37l** |  **38l** | 5 | 89 |

| | | | | |
|---|---|---|---|---|
| 13 |  **37m** |  **38m** | 6 | 75 |
| 14 |  **37n** |  **38n** | 5 | 81 |
| 15 |  **37o** |  **38o** | 5 | 83 |
| 16 |  **37p** |  **38p** | 7 | 77 |
| 17 |  **37q** |  **38q** | 6 | 67 |
| 18 |  **37r** |  **38r** | 7 | 72 |
| 19 |  **37s** |  **38s** | 7 | 69 |

| | | | | |
|---|---|---|---|---|
| 20 |  **37t** |  **38t** | 5 | 87 |
| 21 |  **37u** |  **38u** | 5 | 84 |
| 22 |  **37v** |  **38v** | 7 | 81 |

[a]All the reactions are carried out using compound **37** (1 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1.5 mmol) and TFA (1.2 mmol) in $CH_3CN$ (2.5 mL) at 60 °C, 6h under air. [b]Isolated yield.

### 4.2.4. Proposed mechanism:




**Scheme 4.12**: Proposed reaction mechanism.

As depicted in Scheme 4.12, the reaction seemed to proceed[29] *via* (i) *in situ*

generation of highly electrophilic Pd(II) cationic species **E-1** in TFA, (ii) formation of σ-indole-Pd complexes **E-2** through metalation of indolyl C3-H bond in the presence of **E-1,** this is favored as C-3 of indole ring is the better electrophilic (electron rich) position than the double bond, (iii) intramolecular 6-exo-trig cyclization (via *cis*-arylpalladation to C-C double bond) of **E-2** leading to the intermediate **E-3**, which (iv) undergoes β-hydride elimination to give **E-4** and the $Pd^0$ species, (v) subsequent cleavage of the *N*-(het)arylsulfonyl group in the presence of TFA gives the product (**38)** and (vi) oxidation of $Pd^0$ to $Pd^{II}$ species completed the catalytic cycle.

## 4.3. Pharmacology:

In the life of cell cycle, cell death is an essential part for normal development that continues into adult hood. Cell death may occur *via* at least two broadly defined mechanisms: necrosis or apoptosis. Necrosis is an uncontrolled and accidental cell death due to physical or chemical injury. In contrast apoptosis or programmed cell death (PCD) is an organized cell death program which occurs during development and aging of cells. In apoptosis, cell death occurs in a controlled and regulated fashion and it begins *via* activating a family of proteins known as caspases in the early stages of apoptosis. These caspases further activates proteases/nucleases which breakdown or cleave key cellular components that are required for normal cellular function including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. The caspases also activate other degradative enzymes such as DNAases, which begin to cleave the DNA in the nucleus.

Apoptotic cells display distinctive morphology during the apoptotic process, and this can be seen in (Figure 4.5). The cell then shrinks and develops blebs on its surface. The cytoskeleton is destroyed and nuclear DNA is degraded. Ultimately, the cell breaks apart into membrane-wrapped cellular fragments called apoptotic bodies. The apoptotic bodies are engulfed by macrophages and subsequently removed from the tissue without leading to an inflammatory response. Furthermore, killing of cancer cells by current therapies is largely due to the induction of apoptosis in tumor cells. Since a hallmark of human cancers is their resistance to apoptosis, there is a demand to develop novel strategies that restore the apoptotic machinery in order to overcome cancer resistance. New drugs that could modulate the expression of molecules

involved in the apoptotic pathway with the ability to induce apoptosis in multidrug-resistant or apoptosis resistant tumor cell lines are of great importance in cancer chemotherapy. Therefore the identification of apoptosis inducers represents an attractive approach for the discovery and development of potential anticancer agents.

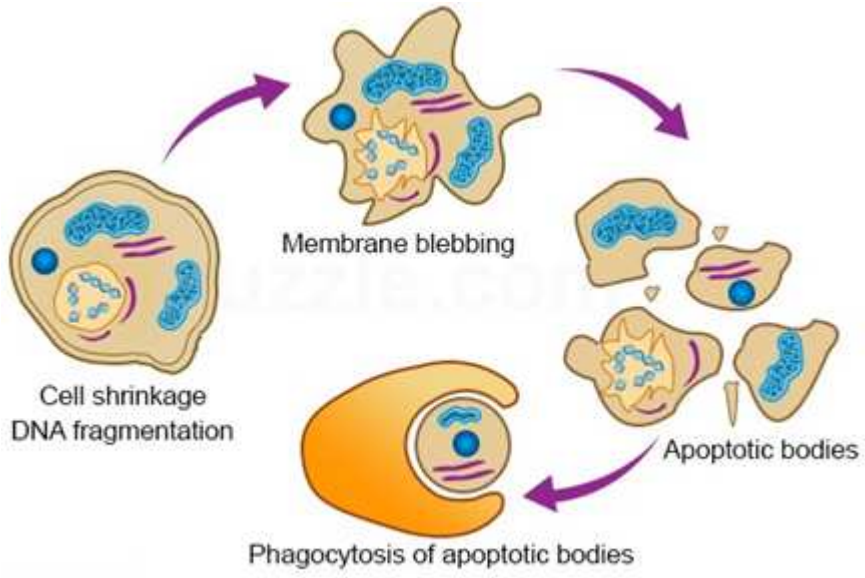



**Fig. 4.5:** Schematic diagram of apoptosis in cell.

In order to assess their potential to induce apoptosis the synthesized compounds were tested in Zebrafish embryos[30] along with a known drug methotrexate at 30 µM. Embryos of zebrafish (*Danio rerio*) are excellent animal models for studying the effects of small molecules in early development, the major strengths of these fish include fast to develop, inexpensive maintenance, its early-stage embryos have a transparent body and high degree of genetic conservation with human. Thus, zebrafish has been an important *in vivo* model for evaluations of potential drugs before embarking on expensive studies in mice and humans.

**Fig. 4.6:** Developing zebrafish embryos

Based on their considerable effects in the present assay of apoptosis compounds (**38k)**, (**38j)** and (**38a)** were further tested at 1, 3, 10 and 30 µM along with

methotrexate (Figure 4.7 & 4.8). While the compound **38k** showed significant increase in apoptotic activity from 1µM to 30µM, whereas **38j** showed significant apoptotic activity at 30µM. In case of **38a** the increased apoptotic activity was seen upto10µM and embryos were found dead at 30µM. These compounds showed NOAEL (No Observed Adverse Effect Level) 10 (**38k**), 3 (**38j**) and 1 µM (**38a**) when evaluated for their potential toxicities like teratogenicity in Zebrafish embryo at a range of 1.0-30 µM with phenobarbital (3 mM) as a positive control (Figure 4.9 & Figure 4.10).[18] The compound (**38k)** showed toxicity at 30µM and found to be safe at 1, 3 and 10µM. The compound (**38j)** was found to be safe at 1 & 3µM and toxic at 10 and 30 µM. In case of compound (**38a)** toxicity was observed at 3 and 10 µM. Embryos were found to be dead at 30µM. Based on overall therapeutic index (TI = NOEL/$EC_{50}$) the compound (**38k)** (TI = 6.26) was found to be promising and is of further interest (Table 4.4).

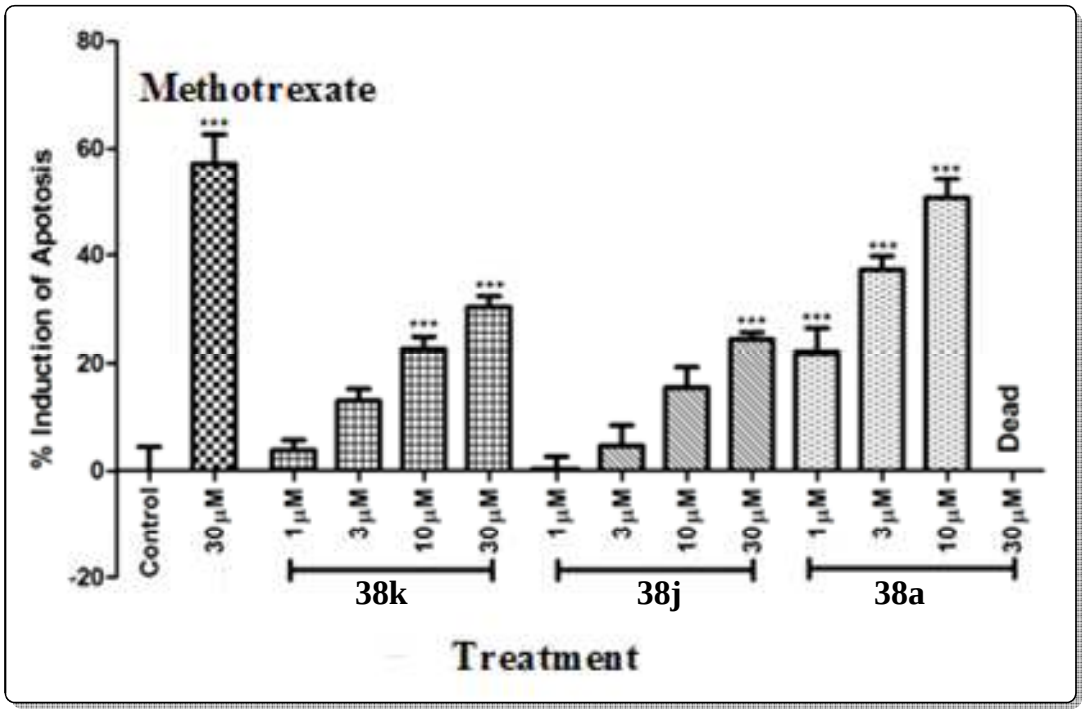



**Figure 4.7.** The percentage induction of apoptosis caused by compounds **38k**, **38j** and **38a** at different concentrations along with Methotrexate. All the statistical analysis was performed using GraphPad Prism® software.

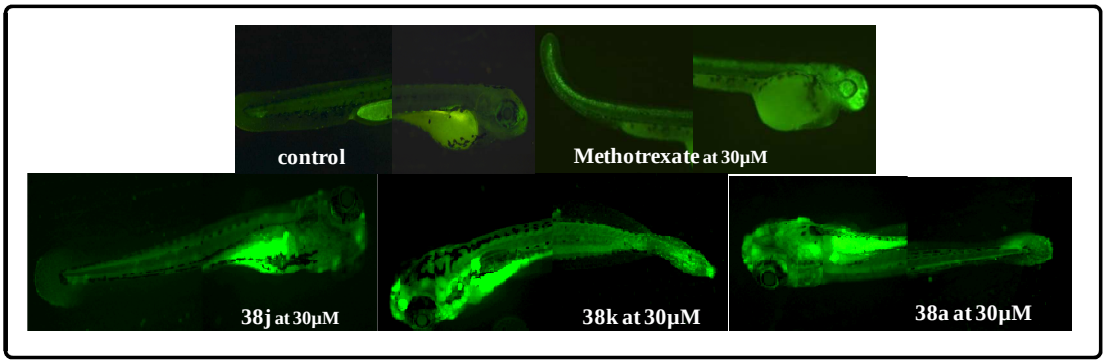



**Figure 4.8.** Representative images of the embryos treated with compounds assayed for apoptosis.

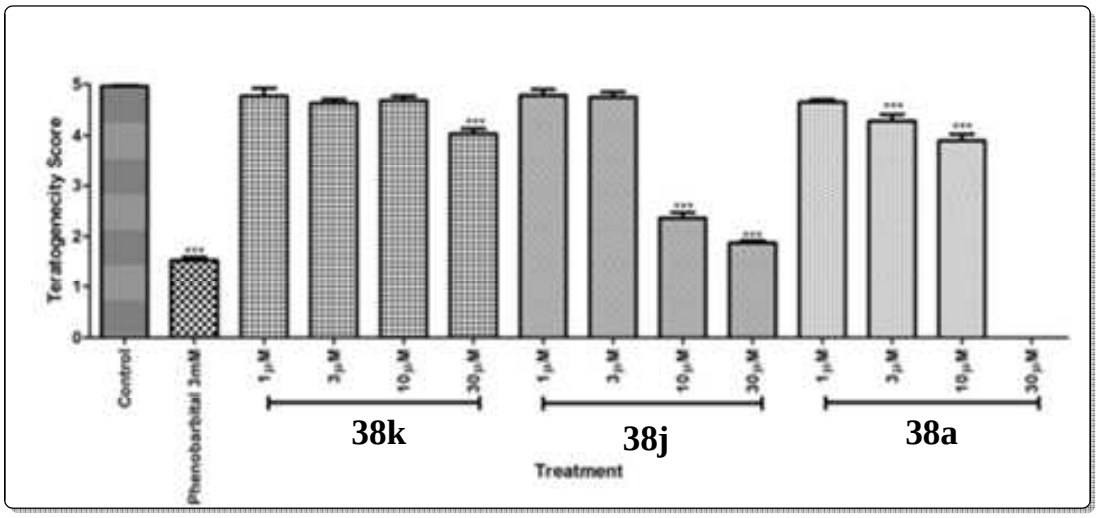



**Figure 4.9.** Results of teratogenicity assay using compounds **38k**, **38j** and **38a**. Statistical analysis for scoring was done using GraphPad Prism® software using two-way ANOVA.

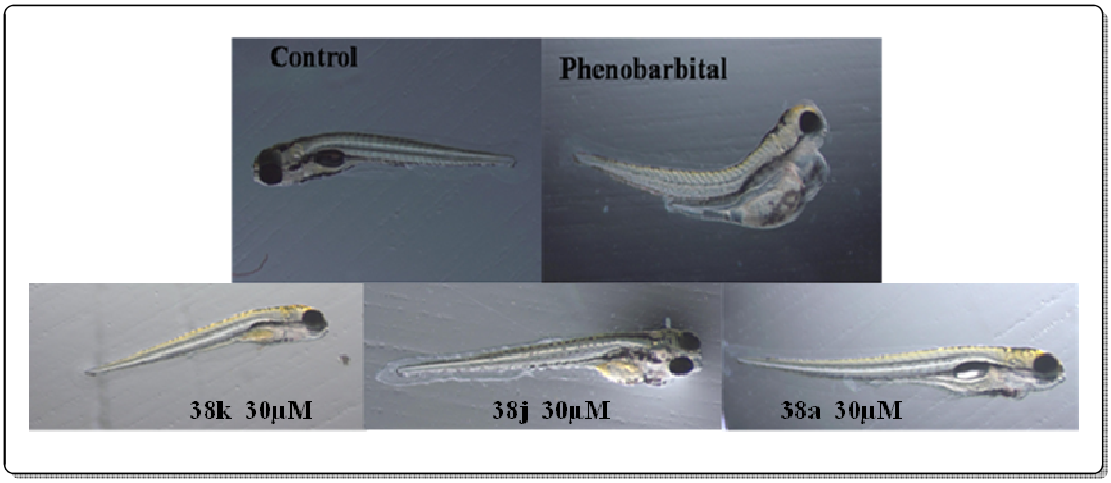



**Figure 4.10**. Representative images of teratogenicity assay

**Table 4.4.** Summary of results in Zebrafish assay

| Pharmacological Evaluations | | | | Test Compounds Data | | |
|---|---|---|---|---|---|---|
| Tests | Endpoint | Positive Control | Parameters | **38k** | **38j** | **38a** |
| Apoptosis | Acridine Orange staining of apoptotic cells | Methotrexate | $EC_{50}$ | 1.59 | 4.18 | 2.20 |
| Teratogenicity | Morphological assessment of Phenotypic changes | Phenobarbital | NOAEL | 10µM | 3µM | 1µM |
| Overall Therapeutic Index | Ratio of NOAEL/EC50 (Overall NOAEL = lowest NOAEL) | - | Therapeutic Index | 6.26 | 0.717 | 0.493 |

Further, the compounds were tested at 10 µM initially for their ability to inhibit the growth of four cancer cells e.g. A549 (lung), Cal27 (oral) MCF-7 (breast) and TZM-BL (cervical) using the sulphorhodamine B (SRB) assay[31] with gemcitabine as a reference compound. Among the active compounds, (**38a)**, (**38b)**, (**38d-k)** and (**38q)** (> 90% inhibition comparable to gemcitabine's 90% inhibition) against lung cancer cells, (**38b,** 40%) and (**38j,** 46%) against oral cancer cells (gemcitabine 92%), (**38d,** (62%), (**38f,** 55%), (**38i,** 57%), (**38j,** 42%) and (**38q,** 63%) against breast cancer cells (gemcitabine 49%), (**38a,** 53%), (**38j,** 63%), and (**38q,** 76%) against cervical cancer cells (gemcitabine 90%) were found to be interesting.

## 4.4. Conclusion:

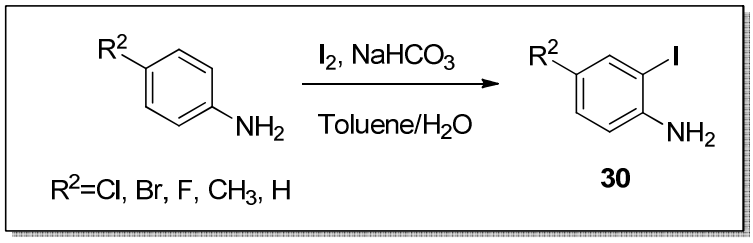
In conclusion, a sequential method has been developed for the first time to synthesize novel indolo[2,3-*b*]quinolines related to neocryptolepine. The strategy involved Pd(II)-catalyzed intramolecular oxidative C3-H alkenylation of an indole ring followed by desulfonylation in the same pot. This straightforward and facile methodology afforded an array of 11-carboxymethyl substituted 6*H*-indolo[2,3-*b*]quinoline derivatives. Several of these compounds showed promising cytotoxicities against cancer cells and apoptosis inducing properties in zebrafish embryos indicating their potential for the treatment of cancer. Overall, these findings could be a new and useful addition to the C-H activation/cascade reaction as well as indoloquinoline chemistry.

## 4.5. Experimental section:

### 4.5.1. Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. $^{1}H$ and $^{13}C$ NMR spectra were recodred in $CDCl_3$ or DMSO-$d_6$ solution by using a 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Melting points (mp) were by using Buchi B-540 melting point appratus and are uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

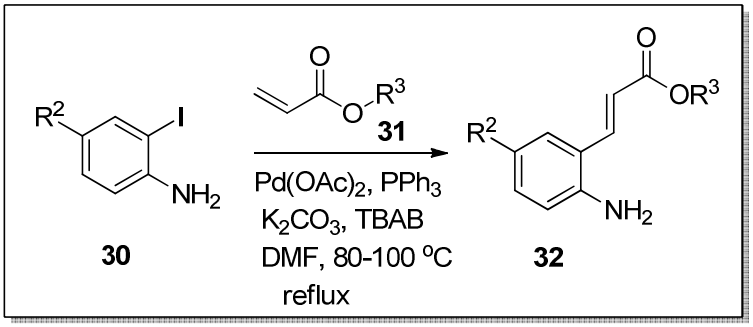
#### 4.5.1.1. General Procedure for the preparation of 4-substitued-2-iodoanilines (30)




A mixture of 4-substituted aniline (1 mmol), iodine (1 mmol) and sodiumbicarbonate (1.5 mmol) in toluene, $H_2O$ (10 mL, 9:1) was stirred at room temperature for 3 hours. After completion of the reaction, the mixture was diluted with ethyl acetate (30 mL), washed with sodium thiosulphate solution (2 x 20 mL), followed by brine solution (20 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by column

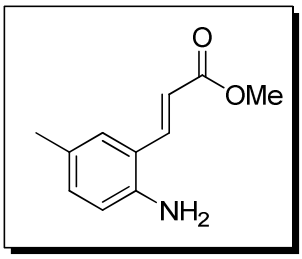
chromatography using ethylacetate–hexane to give the desired compound **30**.

**4.5.1.2. General Procedure for the preparation of (*E*)-Alkyl 3-(2-amino-5-substituted phenyl)acrylate (32)**



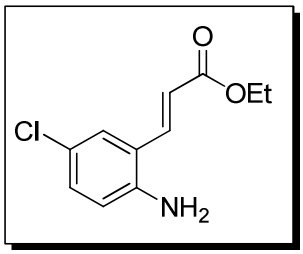

The compound (**32)** was prepared according to a procedure described in the literature[32]

**4.5.1.3. (*E*)-Methyl 3-(2-amino-5-methylphenyl)acrylate (32a)**



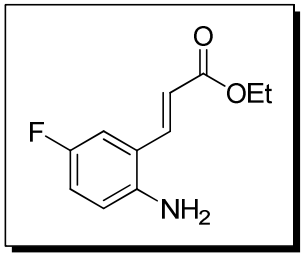

Yield: 75%; yellow solid; mp: 77-78 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 16.0 Hz, 1H), 7.21 (s, 1H), 7.01 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 16.0 Hz, 1H), 3.85 (s, 2H), 3.81 (s, 3H), 2.26 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 167.7, 143.2, 140.3, 132.2, 128.1 (2C), 119.8, 117.3, 116.9, 51.6, 20.3; MS (ES mass): 192.2 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 2.5 min.

**4.5.1.4. (*E*)-Ethyl 3-(2-amino-5-chlorophenyl)acrylate (32b)**



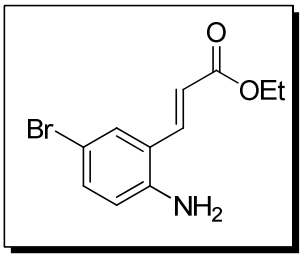

Yield: 66%; yellow solid; mp: 85-87 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 16.0 Hz, 1H), 7.35 (s, 1H), 7.12 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 4.27 (q, *J* = 7.2 Hz, 2H), 4.02 (s, 2H), 1.34 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 143.9, 138.5, 130.8, 127.2, 123.5, 121.1, 119.3, 117.8, 60.6, 14.2; MS (ES mass): 226.1 (M+1); HPLC: 99.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 255.0 nm, retention time 3.4 min.

**4.5.1.5. (*E*)-Ethyl 3-(2-amino-5-fluorophenyl)acrylate (32c)**



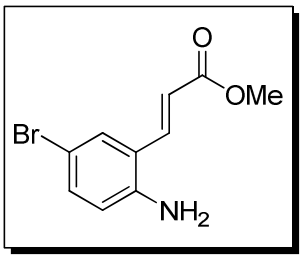

Yield: 83%; yellow solid; mp: 80-82 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 16.0 Hz, 1H), 7.09 (dd, *J* = 9.6, 2.8 Hz, 1H), 6.93-6.89 (m, 1H), 6.66 (dd, *J* = 8.8, 4.8 Hz, 1H), 6.34 (d, *J* = 16.0 Hz, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 3.83 (s, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 157.3 (C-F *J* = 235.7 Hz), 155.0, 141.7, 138.8 , 120.8, 119.3, 118.3 (C-F *J* = 22.6 Hz), 118.0, 117.9 (C-F *J* = 7.6 Hz), 117.8, 113.4 (C-F *J* = 22.6 Hz), 113.2, 60.5, 14.2; MS (ES mass): 210.1 (M+1); HPLC: 99.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5 µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.0 min.

**4.5.1.6. (*E*)-Ethyl 3-(2-amino-5-bromophenyl)acrylate (32d)**



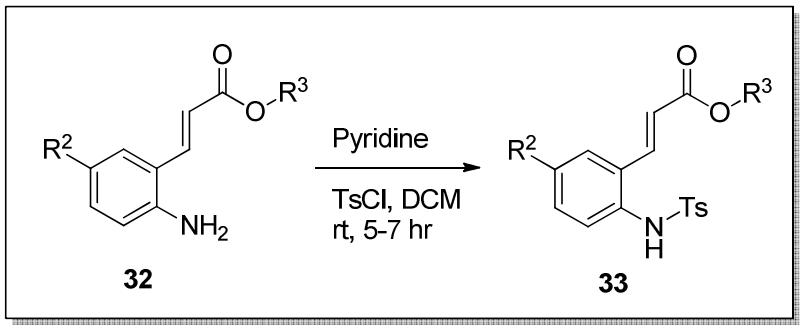

Yield: 58%; yellow solid; mp: 88-90 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 16.0 Hz, 1H), 7.49 (s, 1H), 7.25 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 4.27 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 2H), 1.34 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 144.4, 138.4, 133.6, 130.2, 121.6, 119.4, 118.2, 110.5, 60.6, 14.2; MS (ES mass): 272.1 (M+3); HPLC: 98.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 250.0 nm, retention time 3.5 min.

**4.5.1.7. (*E*)-Methyl 3-(2-amino-5-bromophenyl)acrylate (32e)**




Yield: 69%; yellow solid; mp: 90-92 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 16.0 Hz, 1H), 7.48 (s, 1H), 7.26-7.22 (m, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.34 (d, *J* = 16.0 Hz, 1H), 3.97 (s, 2H), 3.81 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 167.2, 144.4, 138.7, 133.7, 130.2, 121.5, 118.9, 118.2, 110.6, 51.7; MS (ES mass): 257.9 (M+3); HPLC: 96.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 250.0 nm, retention time 3.3 min.

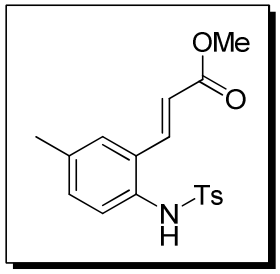
**4.5.1.8. General Procedure for the preparation of (*E*)-Alkyl 3-(5-substituted-2-(4-methylphenylsulfonamido)phenyl)acrylate (33)**




**32** **33**

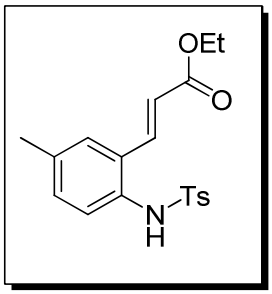
Compounds **33a−33j** were prepared according to a procedure described in the literature[33]

**4.5.1.9. (*E*)-Methyl 3-(5-methyl-2-(4-methylphenylsulfonamido)phenyl)acrylate (33a)**



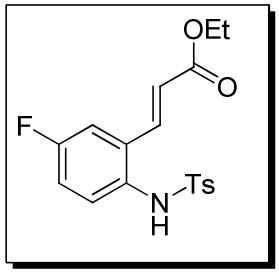

Yield: 95%; white solid; mp: 160-162 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 16.0 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4Hz, 2H), 7.19-7.14 (m, 1H), 6.69 (s, 1H), 6.13 (d, *J* = 16.0 Hz, 1H), 3.80 (s, 3H), 2.38 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.9, 143.8, 139.3, 137.3, 135.8, 132.0, 131.7, 130.6, 129.5 (2C), 127.9, 127.3, 127.2 (2C), 119.7, 51.8, 21.4, 20.9; MS (ES mass): 344.2 (M-1); HPLC: 98.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.4 min.

**4.5.1.10. (*E*)-Ethyl 3-(5-methyl-2-(4-methylphenylsulfonamido)phenyl)acrylate (33b)**




Yield: 97%; white solid; mp: 145-147 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 15.6 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.25 (s, 1H), 7.17 (t, *J* = 8.4 Hz, 3H), 6.70 (s, 1H), 6.10 (d, *J* = 15.6 Hz, 1H), 4.23 (q, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.32 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.6, 143.6, 139.3, 137.2, 135.8, 132.1, 131.6, 130.8, 129.5 (2C), 128.2, 127.3, 127.2 (2C), 119.9, 60.7, 21.4, 20.9, 14.2; MS (ES mass): 358.2 (M-1); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.5 min.

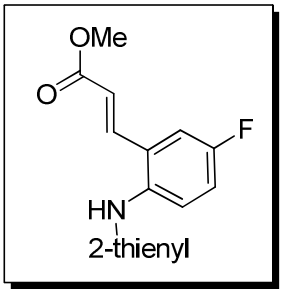
**4.5.1.11. (*E*)-Ethyl 3-(5-fluoro-2-(4-methylphenylsulfonamido)phenyl)acrylate (33c)**




Yield: 91%; white solid; mp: 161-163 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 15.6, 1H), 7.39 (dd, *J* = 7.8, 4.2 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.14 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.09-7.05 (m, 1H), 6.81 (s, 1H), 6.09 (d, *J* = 15.6 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.38 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.2, 162.6 (C-F *J* = 246.6 Hz), 160.1, 144.0, 138.0 (2C), 135.5, 133.3 (C-F *J* = 8.3 Hz), 133.2, 130.7, 130.6 , 129.6, 127.2, 121.4, 117.9, 117.7, 113.3 (C-F *J* = 23.3Hz), 113.1, 60.9, 21.4, 14.2; MS (ES mass): 362.2 (M-1); HPLC: 99.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B):

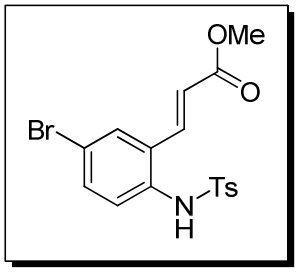
0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.5 min.

**4.5.1.12. (*E*)-Methyl 3-(5-fluoro-2-(thiophene-2-sulfonamido)phenyl)acrylate (33d)**




Yield: 85%; white solid; mp: 171-173 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56-7.54 (m, 1H), 7.50 (d, *J* = 15.8, 1H), 7.41-7.35 (m, 2H), 7.20 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.13-7.05 (m, 1H), 7.01-6.99 (m, 1H), 6.79 (s, 1H), 6.20 (d, *J* = 15.8 Hz, 1H), 3.79 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 162.8 (C-F *J* = 247.4 Hz), 160.3, 138.9, 137.6, 133.4 (C-F *J* = 8.2 Hz), 133.3, 133.0, 132.8, 130.5 (C-F *J* = 8.8 Hz), 130.4, 130.1, 127.5, 121.4, 118.0 (C-F *J* = 22.6 Hz), 117.8, 113.4 (C-F *J* = 23.5 Hz), 113.2, 51.9; MS (ES mass): 342.2 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 260.0 nm, retention time 3.2 min.

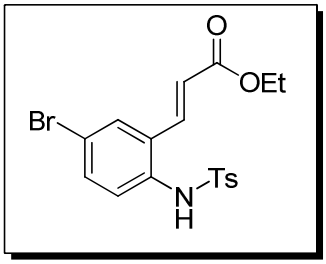
**4.5.1.13. (*E*)-Methyl 3-(5-bromo-2-(4-methylphenylsulfonamido)phenyl)acrylate (33e)**




Yield: 97%; white solid; mp: 192-194 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.58 (s, 1H), 7.56-7.55 (m, 2H), 7.46 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.42 (d, *J* = 15.8 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.72 (s, 1H), 6.13 (d, *J* = 15.8 Hz, 1H), 3.80 (s, 3H), 2.36 (s,3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.2, 137.7, 135.5, 133.7

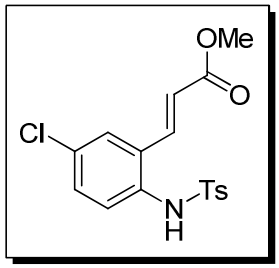
(2C), 132.0, 129.8, 129.7 (2C), 128.7, 127.2 (2C), 121.5, 120.7, 52.0, 21.4; MS (ES mass): 410.1 (M+1); HPLC: 99.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.5 min.

**4.5.1.14. (*E*)-Ethyl 3-(5-bromo-2-(4-methylphenylsulfonamido)phenyl)acrylate (33f)**




Yield: 98%; white solid; mp: 146-149 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.2 Hz, 3H), 7.46 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.38 (d, *J* = 16.0 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.70 (s, 1H), 6.11 (d, *J* = 16.0 Hz, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 2.38 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.2, 144.0, 137.7, 135.6, 133.8, 133.6, 132.4, 129.8 , 129.7 (2C), 129.2, 127.2 (2C), 121.6, 120.7, 61.0, 21.4, 14.2; MS (ES mass): 424.1 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.7 min.

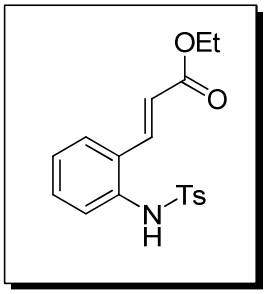
**4.5.1.15. (*E*)-Methyl 3-(5-chloro-2-(4-methylphenylsulfonamido)phenyl)acrylate (33g)**




Yield: 98%; white solid; mp: 149-151 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 16.4 Hz, 1H), 7.41 (s, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.32 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 2H),

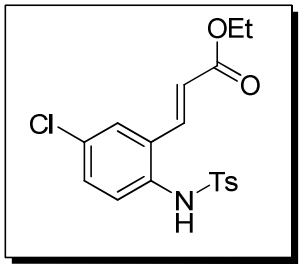
6.77 (s, 1H), 6.13 (d, *J* = 16.4 Hz, 1H), 3.80 (s, 3H), 2.38 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.6, 144.1, 138.1, 135.5, 133.2, 133.0, 132.1, 130.7, 129.7 (2C), 129.0, 127.2 (2C), 126.8, 121.1, 52.0, 21.4; MS (ES mass): 364.2 (M-1); HPLC: 98.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.5 min.

**4.5.1.16. (*E*)-Ethyl 3-(2-(4-methylphenylsulfonamido)phenyl)acrylate (33h)**




Yield: 98%; white solid; mp: 140-143 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 15.6 Hz, 1H), 7.45-7.42 (m, 2H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 2H), 6.72 (s, 1H), 6.13 (d, *J* = 15.6 Hz, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 143.8, 138.9, 135.8, 134.7, 130.8, 130.4, 129.6 (2C), 127.5, 127.2 (2C), 127.1, 127.0, 120.5, 60.8, 21.4, 14.2; MS (ES mass): 344.2 (M-1); HPLC: 99.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.4 min.

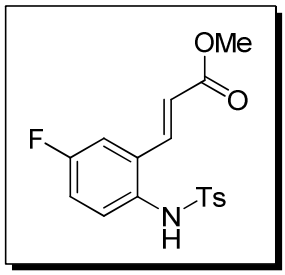
**4.5.1.17. (*E*)-Ethyl 3-(5-chloro-2-(4-methylphenylsulfonamido)phenyl)acrylate (33i)**




Yield: 95%; white solid; mp: 169-171 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 8.4 Hz, 2H), 7.41-7.38 (m, 2H), 7.38-7.36 (m, 1H),

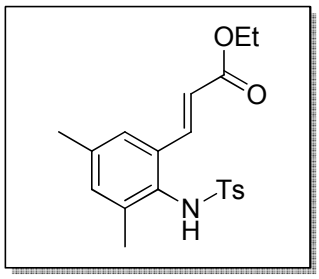
7.32 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.79 (s, 1H), 6.13 (d, *J* = 15.7 Hz, 1H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 1.35 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.1, 144.1, 137.6, 135.6, 133.2, 132.9, 132.0, 130.7, 129.7 (2C), 128.9, 127.2 (2C), 126.8, 121.7, 61.0, 21.4, 14.2; MS (ES mass): 378.2 (M-1); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.6 min.

**4.5.1.18. (*E*)-Methyl 3-(5-fluoro-2-(4-methylphenylsulfonamido)phenyl)acrylate (33j)**



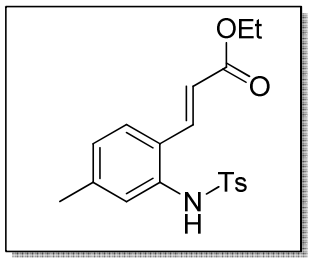

Yield: 93%; white solid; mp: 156-158 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 15.6 Hz, 1H), 7.35-7.32 (m, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.15 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.10-7.03 (m, 1H), 6.45 (s, 1H), 6.11 (d, *J* = 15.6 Hz, 1H), 3.79 (s, 3H), 2.39 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.7, 162.6 (C-F *J* = 246.7 Hz), 160.1, 144.0, 138.5, 138.4, 135.5, 133.4 (C-F *J* = 8.1 Hz), 133.3, 130.7, 130.6, 129.6, 127.2, 120.8, 117.9, 117.7, 113.3 (C-F *J* = 23.3Hz), 113.1, 52.0, 21.4; MS (ES mass): 348.2 (M-1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.3 min.

**4.5.1.19. (*E*)-Ethyl 3-(3,5-dimethyl-2-(4-methylphenylsulfonamido)phenyl)acrylate (33k)**




Yield: 93%; white solid; mp: 142-144 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.52 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 16.0 Hz, 1H), 7.18-7.09 (m, 4H), 6.22 (s, 1H), 6.01 (d, *J* = 16.0 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 2.37 (s, 3H), 2.31 (s, 3H), 2.29 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.8, 143.5, 140.8, 139.3, 137.8, 136.2, 133.8, 133.7, 130.7, 129.5 (2C), 127.4 (2C), 125.2, 118.2, 60.6, 21.4, 21.0, 18.8, 14.2; MS (ES mass): 372.2 (M-1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 3.7 min.

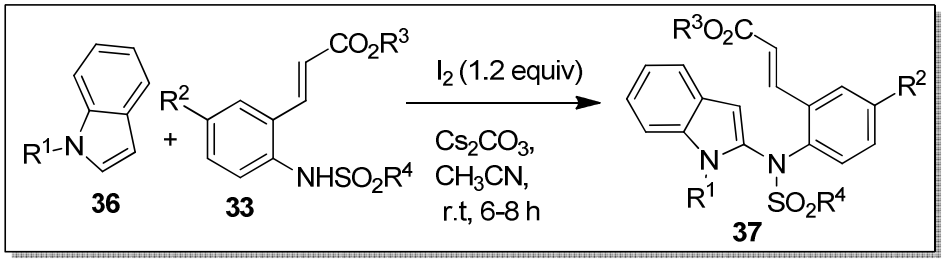
**4.5.1.20. (*E*)-Ethyl 3-(4-methyl-2-(4-methylphenylsulfonamido)phenyl)acrylate (33l)**




Yield: 95%; white solid; mp: 132-134 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 15.6, 2H), 7.26 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 7.6 Hz, 1H), 6.56 (s, 1H), 6.07 (d, *J* = 15.6, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.34 (s, 3H), 1.32 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.6, 143.8, 141.6, 138.7 (2C), 135.7, 134.5, 129.5, 128.2, 128.1, 127.5, 127.2, 126.7, 119.4, 119.3, 60.7, 21.4, 21.3, 14.2; MS (ES mass): 358.2 (M-1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95,

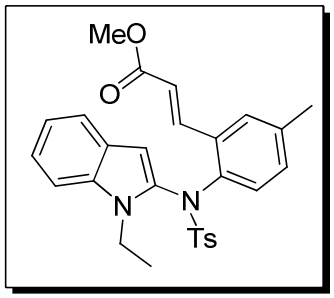
10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 3.7 min.

**4.5.1.21. General Procedure for synthesis of (*E*)-Alkyl 3-(5-substituted-2-(*N*-(1-alkyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)phenyl)acrylate (37)[4]**




To a mixture of (*E*)-alkyl 3-(5-substituted-2-(4-methylphenylsulfonamido)phenyl)acrylate derivative (**36)** (1.0 mmol), $Cs_2CO_3$ (1.5 mmol), $I_2$ (1.2 mmol) in acetonitrile (2.5 mL) added indole derivative (**33)** (1.2 mmol), then stirred at room temperature under nitrogen for 6-8 h. The progress of the reaction was monitored by TLC. Upon completion, the reaction was quenched with a saturated solution of $Na_2S_2O_3$ (5 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were collected, combined washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under a reduced pressure. The residue was purified by column chromatography over silica gel using ethyl acetate–hexane to give the desired product (**37**).

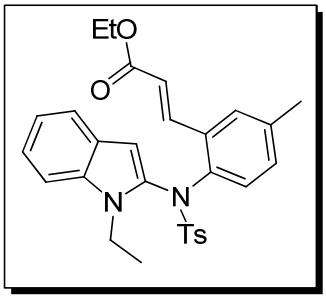
**4.5.1.22. (*E*)-Methyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-methylphenyl)acrylate (37a)**




Yield: 85%; white solid; mp: 180-182 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.47 (d, *J* = 16.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.33-7.27 (m, 3H), 7.24-7.19 (m, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.14-7.09 (m, 1H), 7.10-7.06 (m, 1H), 6.42 (d, *J* = 16.0 Hz, 1H), 6.30 (s, 1H), 4.29 (q, *J* =

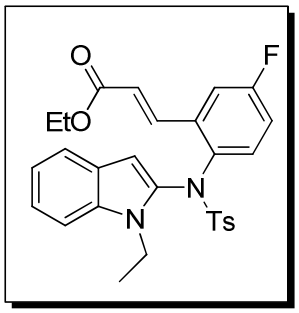
7.2 Hz, 2H), 3.86 (s, 3H), 2.47 (s, 3H), 2.36 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.9, 144.5, 141.0, 138.9, 136.9, 134.4, 134.3 (2C), 133.8, 131.4, 129.9 (2C), 129.3 (2C), 129.2, 127.6, 125.9, 122.6, 121.0, 199.8, 119.4, 109.9, 100.3, 51.7, 37.6, 21.6, 21.1, 14.8; MS (ES mass): 489.2 (M+1); HPLC: 98.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.2 min.

**4.5.1.23. (*E*)-Ethyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-methylphenyl)acrylate (37b)**



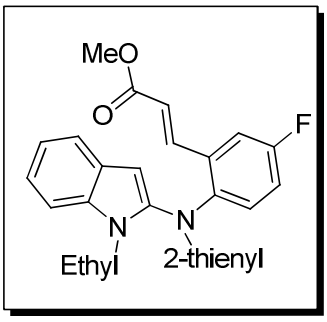

Yield: 87%; white solid; mp: 147-149 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.44 (d, *J* = 16.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.32-7.26 (m, 3H), 7.22 (d, *J* = 7.2 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.13-7.10 (m, 1H), 7.08 (t, *J* = 6.8 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 6.31 (s, 1H), 4.35-4.30 (q, *J* = 7.2 Hz, 2H), 4.30-4.24 (m, 2H), 2.46 (s, 3H), 2.36 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 144.5, 140.6, 138.9, 136.8, 134.4, 134.3 (2C), 133.8, 131.3, 129.9, 129.3 (2C), 129.1 (2C), 127.6, 125.9, 122.5, 121.0, 119.9, 119.8, 109.9, 100.3, 60.5, 37.6, 21.6, 21.1, 14.8, 14.3; MS (ES mass): 503.3 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.4 min.

**4.5.1.24. (*E*)-Ethyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-fluorophenyl)acrylate (37c)**




Yield: 83%; white solid; mp: 156-158 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.41 (d, *J* = 16.0 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.38 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.31-7.27 (m, 4H), 7.25-7.21 (m, 1H), 7.11-7.07 (m, 1H), 7.04-6.99 (m, 1H), 6.40 (d, *J* = 16.0 Hz, 1H), 6.32 (s, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 4.28-4.25 (m, 2H), 2.47 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.0, 163.2 (C-F *J* = 250.0 Hz), 160.7, 144.8, 139.5, 139.4, 137.0, 136.9, 134.0 (C-F *J* = 7 Hz), 133.8, 132.1, 129.5 (2C), 129.1 (2C), 125.8, 122.8, 121.4, 121.0, 119.9, 117.6, 117.4, 113.6, 113.4 (C-F *J* = 22.5 Hz), 109.9, 100.4, 60.7, 37.6, 21.6, 14.9, 14.3; MS (ES mass): 507.2 (M+1); HPLC: 99.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5μm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.2 min.

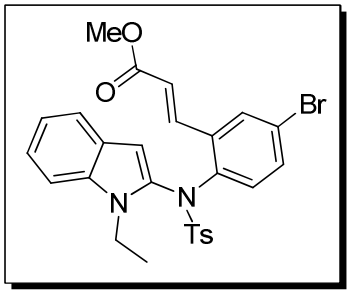
**4.5.1.25. (*E*)-Methyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)thiophene-2-sulfonamido)-5-fluorophenyl)acrylate (37d)**




Yield: 68%; white solid; mp: 173-175 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.41 (d, *J* = 16.4 Hz, 1H), 7.72-7.71 (m, 1H), 7.57-7.55 (m, 2H), 7.40-7.37 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.17-7.15 (m, 1H), 7.12-7.09 (m, 1H), 7.08-7.03 (m, 1H), 6.48 (s, 1H), 6.43 (d, *J* = 16.4 Hz, 1H), 4.28 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.5, 163.4 (C-F *J* = 249.2 Hz), 160.9, 139.6, 137.0, 136.9,

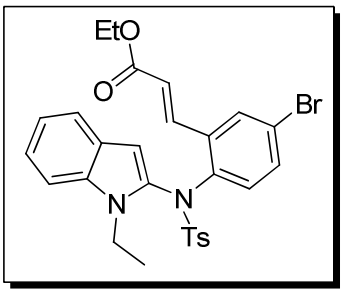
135.1, 134.9 (C-F *J* = 5.6 Hz), 134.9, 134.0, 133.9, 133.5, 131.9, 127.5, 125.8, 123.0, 121.2, 121.1, 120.1, 117.8 (C-F *J* = 23.0 Hz), 117.6, 113.8 (C-F *J* = 23.1 Hz), 113.6, 110.0, 100.5, 51.9, 37.7, 14.8; MS (ES mass): 485.2 (M+1); HPLC: 97.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 220.0 nm, retention time 3.8 min.

**4.5.1.26. (*E*)-Methyl-3-(5-bromo-2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)phenyl)acrylate (37e)**



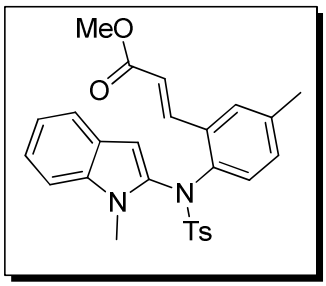

Yield: 75%; white solid; mp: 199-201 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.40 (d, *J* = 16.0 Hz, 1H), 7.83 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.32-7.29 (m, 3H), 7.24-7.20 (m, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 7.11-7.07 (m, 1H), 6.43 (d, *J* = 16.0 Hz, 1H), 6.29 (s, 1H), 4.25 (q, *J* = 7.6 Hz, 2H), 3.87 (s, 3H), 2.47 (s, 3H), 1.20 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 144.9, 139.5, 138.3, 136.6, 134.0, 133.8, 133.6, 133.4, 131.6, 130.1, 129.5 (2C), 129.1 (2C), 125.7, 122.9, 122.8, 121.1, 120.9, 119.9, 109.9, 100.5, 51.9, 37.6, 21.6, 14.8; MS (ES mass): 555.1 (M+3); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.9 min.

**4.5.1.27. (*E*)-Ethyl-3-(5-bromo-2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)phenyl)acrylate (37f)**




Yield: 80%; white solid; mp: 163-165 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.38 (d, *J* = 16.0 Hz, 1H), 7.83 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.43 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.33-7.26 (m, 3H), 7.23 (t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.42 (d, *J* = 16.00 Hz, 1H), 6.29 (s, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 2.47 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.0, 144.9, 139.2, 138.2, 136.7, 134.0, 133.8, 133.6, 133.3, 131.6, 130.1, 129.5 (2C), 129.1 (2C), 125.7, 122.9, 122.8, 121.4, 121.1, 119.9, 109.9, 100.6, 60.7, 37.6, 21.7, 14.9, 14.3; MS (ES mass): 567.2 (M+1); HPLC: 98.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.5 min.

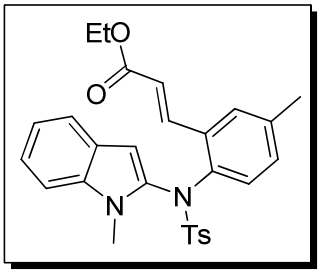
**4.5.1.28. (*E*)-Methyl-3-(5-methyl-2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37g)**




Yield: 71%; white solid; mp: 172-173 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.40 (d, *J* = 16.0 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.45 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.26-7.21 (m, 2H), 7.11 (s, 2H), 7.10-7.06 (m, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 6.28 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 2.48 (s, 3H), 2.35 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.9, 144.5, 141.2, 139.1, 137.2, 135.1, 135.0, 134.6, 134.4, 131.4, 129.8, 129.3 (2C), 129.0 (2C), 127.8, 125.6, 122.6, 120.8, 119.8 (2C), 109.8, 100.2, 51.7, 30.0, 21.6, 21.0; MS (ES mass): 475.2

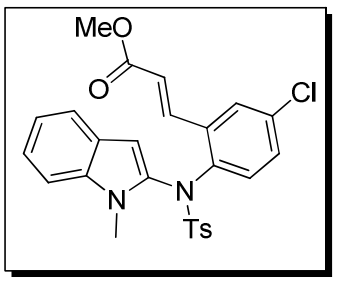
(M+1); HPLC: 99.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 7.8 min.

**4.5.1.29. (*E*)-Ethyl-3-(5-methyl-2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37h)**



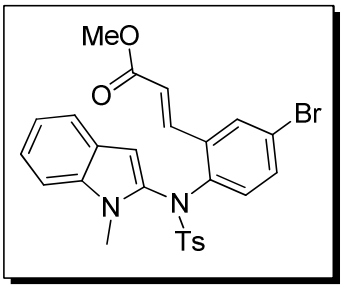

Yield: 83%; white solid; mp: 184-186 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.39 (d, *J* = 16.0 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.45 (s, 1H), 7.32-7.20 (m, 5H), 7.11-7.04 (m, 2H), 6.33 (d, *J* = 16.0 Hz, 1H), 6.30 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 2.47 (s, 3H), 2.35 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.4, 140.8, 139.1, 137.2, 135.2, 135.0, 134.6, 134.5, 131.3, 129.8, 129.3 (2C), 129.0 (2C), 127.7, 125.6, 122.6, 120.8, 120.2, 119.8, 109,2, 100.2, 60.5, 30.0, 21.6, 21.0, 14.3; MS (ES mass): 489.2 (M+1); HPLC: 98.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.3 min.

**4.5.1.30. (*E*)-Methyl-3-(5-chloro-2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37i)**



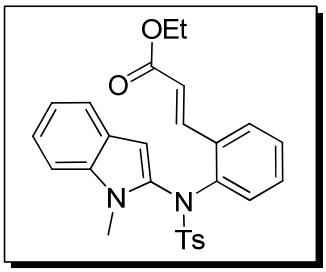
Yield: 68%; white solid; mp: 178-180 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 16.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.57 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.33-7.26 (m, 4H), 7.25-7.22 (m, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 7.11-7.07 (m, 1H), 6.35 (d, *J* = 16.0Hz, 1H), 6.27 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 2.48 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 144.9, 139.8, 138.1, 136.5, 135.1, 135.0, 134.5, 134.2, 131.3, 130.4, 129.5 (2C), 129.0 (2C), 127.3, 125.6, 122.9, 121.3, 120.9 (2C), 109.9, 100.4, 52.0, 30.0, 21.7; MS (ES mass): 495.0 (M+1); HPLC: 96.4%, Column: Symmetry C-18 250 * 4.6 mm, 5µm, mobile phase A: 5mm Ammonium Acetate in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.4 nm, retention time 15.0 min.

**4.5.1.31. (*E*)-Methyl-3-(5-bromo-2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37j)**



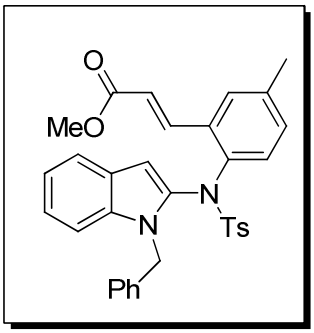

Yield: 60%; white solid; mp: 204-206 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.34 (d, *J* = 16.0 Hz, 1H), 7.77 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.43 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.27 (s, 1H), 7.26-7.24 (m, 1H), 7.12-7.07 (m, 2H), 6.35 (d, *J* = 16.0 Hz, 1H), 6.26 (s, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 2.48 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.8, 139.7, 138.6, 136.8, 135.1, 134.4, 134.2, 133.4, 131.5, 130.3, 129.5 (2C), 129.0 (2C), 125.5, 123.0, 122.9, 121.3, 120.9, 120.0, 109.8, 100.4, 51.9, 30.0, 21.6; MS (ES mass): 541.1 (M+3); HPLC: 97.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.2 min.

**4.5.1.32. (*E*)-Ethyl 3-(2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37k)**




Yield: 73%; white solid; mp: 167-169 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.44 (d, *J* = 16.0 Hz, 1H), 7.66 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.37-7.27 (m, 6H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.12-7.06 (m, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 6.31 (s, 1H), 4.31 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 2.48 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.6, 140.7, 139.7, 135.1, 135.0, 134.9, 134.6, 130.5, 130.1, 129.4 (2C), 129.1, 129.0 (2C), 127.3, 125.6, 122.8, 120.9, 120.5, 119.9, 109.8, 100.4, 60.6, 30.0, 21.6, 14.3; MS (ES mass): 475.1 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.2 min.

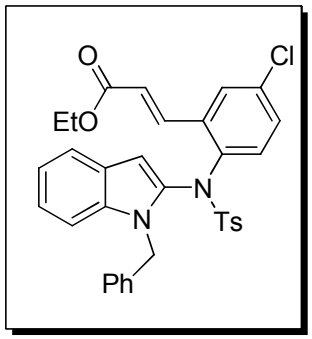
**4.5.1.33. (*E*)-Methyl-3-(2-(*N*-(1-benzyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-methylphenyl)acrylate (37l)**




Yield: 84%; white solid; mp: 161-163 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.18 (d, *J* = 16.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.56-7.54 (m, 1H), 7.29 (d, *J* = 8.0 Hz, 3H), 7.13-7.06 (m, 4H), 7.06-6.98 (m, 4H), 6.64 (d, *J* = 7.2 Hz, 2H), 6.48 (s, 1H), 6.04 (d, *J* = 16.0 Hz, 1H), 5.51 (s, 2H), 3.72 (s, 3H), 2.48 (s, 3H), 2.30 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.5, 144.6, 140.6, 138.9, 136.9, 136.6, 135.4, 134.6 (2C), 134.3, 131.2, 129.9, 129.4 (2C), 129.2 (2C), 128.0 (2C), 127.6, 126.7, 125.9, 125.6 (2C), 122.9, 120.9, 120.2, 119.7, 110.7, 100.8, 51.5, 46.4, 21.7, 21.0; MS (ES mass): 551.2 (M+1); HPLC: 98.7%, Column: Symmetry C-18 75

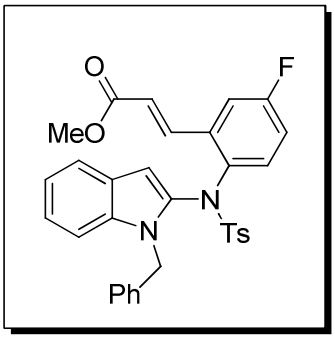
* 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 4.3 min.

**4.5.1.34. (*E*)-Ethyl-3-(2-(*N*-(1-benzyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-chlorophenyl)acrylate (37m)**



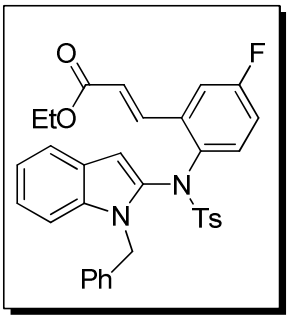

Yield: 83%; white solid; mp: 135-137 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.16 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.59-7.54 (m, 1H), 7.41 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 4.36 Hz, 2H), 7.14-7.06 (m, 3H), 7.05-6.97 (m, 3H), 6.58 (d, *J* = 7.2 Hz, 2H), 6.48 (s, 1H), 6.05 (d, *J* = 16.0 Hz, 1H), 5.48 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.48 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.7, 144.9, 139.0, 137.3, 136.7, 136.6, 134.7 (3C), 134.1, 131.5, 130.0, 129.6 (2C), 129.1, 128.1 (2C), 127.0, 126.9 (2C), 126.8, 125.8, 125.4, 123.2, 121.4, 121.0, 120.3, 110.6, 101.2, 60.5, 46.1, 21.7, 14.3; MS (ES mass): 585.2 (M+1); HPLC: 97.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 215.0 nm, retention time 4.6 min.

**4.5.1.35. (*E*)-Methyl-3-(2-(*N*-(1-benzyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-fluorophenyl)acrylate (37n)**




Yield: 82%; white solid; mp: 139-141 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.12 (d, *J* = 16.4 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.58-7.56 (m, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.23-7.19 (m, 1H), 7.16-7.06 (m, 4H), 7.02 (t, *J* = 7.2 Hz, 3H), 6.96-6.86 (m, 1H), 6.60 (d, *J* = 7.6 Hz, 2H), 6.47 (s, 1H), 6.00 (d, *J* = 16.4 Hz, 1H), 5.51 (s, 2H), 3.75 (s, 3H), 2.48 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.1, 163.2 (C-F *J* = 248.7 Hz), 160.7, 144.9, 139.4, 137.2, 137.1, 136.8, 135.0, 134.7, 134.0, 132.1, 132.0, 129.5 (2C), 129.1, 128.1 (2C), 126.8, 125.8 (2C), 125.4, 123.2, 121.0, 120.9, 120.4, 117.3 (C-F *J* = 22.8Hz), 117.1, 113.6 (C-F *J* = 23.5Hz), 113.3, 110.7, 101.0, 51.7, 46.2, 21.7; MS (ES mass): 555.2 (M+1); HPLC: 98.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 4.1 min.

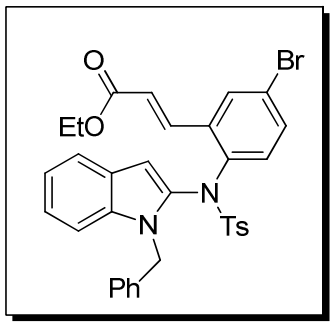
**4.5.1.36. (*E*)-Ethyl-3-(2-(*N*-(1-benzyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-fluorophenyl)acrylate (37o)**




Yield: 79%; white solid; mp: 149-151 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.12 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.57-7.55 (m, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.26-7.22 (m, 1H), 7.13-7.07 (m, 4H), 7.02 (t, *J* = 6.5 Hz, 3H), 6.92-6.87 (m, 1H), 6.60 (d, *J* = 7.6 Hz, 2H), 6.49 (s, 1H), 6.00 (d, *J* = 16.0 Hz, 1H), 5.50 (s, 2H), 4.22 (q, *J* = 7.2 Hz, 2H), 2.47 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.7, 163.2 (C-F *J* = 250.0 Hz), 160.7, 144.8,

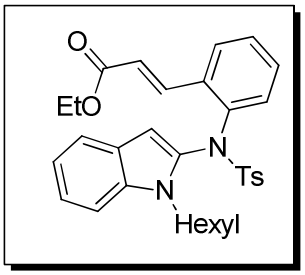
139.2 (2C), 136.7, 134.9 (2C), 134.7, 134.1, 129.5 (2C), 129.1 (2C), 128.1 (2C), 126.8, 125.8, 125.4 (2C), 123.2, 121.4, 121.0, 120.3, 117.2 (C-F *J* = 20.0 Hz), 117.0, 113.5, 113.3, 110.7, 101.1, 60.5, 46.2, 21.7, 14.3; MS (ES mass): 569.1 (M+1); HPLC: 99.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 4.3 min.

**4.5.1.37. (*E*)-Ethyl-3-(2-(*N*-(1-benzyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-bromophenyl)acrylate (37p)**



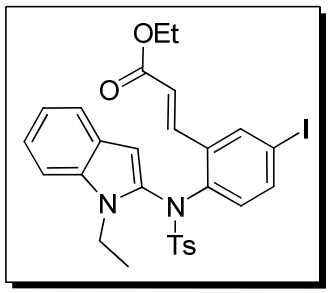

Yield: 76%; white solid; mp: 145-147 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.66-7.60 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 3H), 7.13-7.08 (m, 4H), 7.02 (t, *J* = 7.2 Hz, 3H), 6.58 (d, *J* = 7.6 Hz, 2H), 6.48 (s, 1H), 6.02 (d, *J* = 16.0 Hz, 1H), 5.47 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.48 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 165.7, 144.9, 138.9, 137.8, 136.9, 136.6, 134.7, 134.6, 134.1, 133.0, 131.7, 130.0 (2C), 129.6 (2C), 129.1 (2C), 128.1, 126.8, 125.8, 125.4 (2C), 123.2, 122.8, 121.4, 121.0, 120.3, 110.6, 101.2, 60.5, 46.1, 21.7, 14.2; MS (ES mass): 631.1 (M+3); HPLC: 98.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.6 min.

**4.5.1.38. (*E*)-Ethyl -3-(2-(*N*-(1-hexyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)phenyl)acrylate (37q)**




Yield: 55%; white solid; mp: 125-127 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.45 (d, *J* = 16.0 Hz, 1H), 7.73 (dd, *J* = 7.6, 2.4 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.42-7.32 (m, 3H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.26-7.18 (m, 2H), 7.10-7.06 (m, 1H), 6.45 (d, *J* = 16.0 Hz, 2H), 4.32 (q, *J* = 7.2 Hz, 2H), 4.13-4.04 (m, 2H), 1.56 (s, 2H), 1.40 (t, *J* = 7.2 Hz, 3H), 1.32-1.16 (m, 6H), 0.85 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.3, 144.5, 140.6, 139.0, 134.6, 134.5, 134.1 (2C), 130.5, 130.2, 129.4 (2C), 129.1 (2C), 128.7, 127.1, 125.7, 122.5, 121.0, 119.9, 119.8, 110.0, 100.9, 60.5, 43.0, 31.5, 29.9, 26.5, 22.4, 21.6, 14.3, 13.9; MS (ES mass): 545.2 (M+1); HPLC: 98.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 5.1 min.

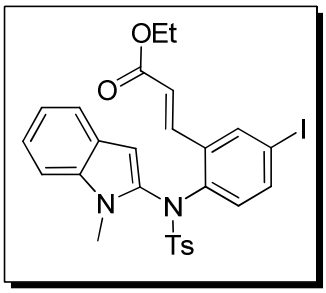
### 4.5.1.39. (*E*)-Ethyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-iodophenyl)acrylate (37r)




Yield: 73%; white solid; mp: 173-175 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 16.0 Hz, 1H), 8.03 (s, 1H), 7.65-7.57 (m, 3H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 3H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.09 (t, *J* = 7.2Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 6.29 (s, 1H), 4.32 (q, *J* = 7.2 Hz, 2H), 4.25 (q, *J* = 6.8 Hz, 2H), 2.47 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 166.0, 144.8, 139.3, 139.1, 139.0, 136.9, 136.2, 134.1, 133.9, 131.7, 129.5 (2C), 129.1 (2C), 125.8, 122.8, 121.3, 121.1,

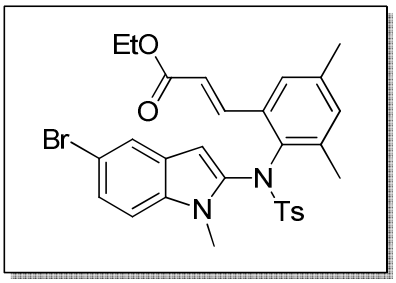
119.9, 109.9 (2C), 100.6, 94.5, 60.7, 37.6, 21.6, 14.9, 14.3; MS (ES mass): 615.1 (M+1); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 2095, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.6 min.

**4.5.1.40. (*E*)-Ethyl-3-(5-iodo-2-(4-methyl-*N*-(1-methyl-1*H*-indol-2-yl)phenylsulfonamido)phenyl)acrylate (37s)**



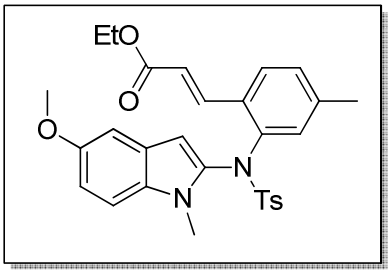

Yield: 68%; white solid; mp: 163-165 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 16.0 Hz, 1H), 7.97 (s, 1H), 7.62 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.29 (s, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.12-7.07 (m, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.33 (d, *J* = 16.0 Hz, 1H), 6.26 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 3.84 (s, 3H), 2.47 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 165.9, 144.8, 139.3 (2C), 139.2, 137.0, 136.3, 135.1, 134.4, 134.3, 131.6, 129.5 (2C), 129.0 (2C), 125.5, 122.9, 121.7, 120.9, 120.0, 109.8, 100.4, 94.7, 60.7, 30.0, 21.6, 14.3; MS (ES mass): 601.1 (M+1); HPLC: 96.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.6 min.

**4.5.1.41. (*E*)-Ethyl 3-(2-(*N*-(5-bromo-1-methyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-3,5-dimethylphenyl)acrylate (37t)**




Yield: 84%; pink solid; mp: 213-215 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 16.0 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.62 (s, 1H), 7.32-7.26 (m, 3H), 7.24 (s, 1H), 7.12 (s, 1H), 7.03 (d, *J* = 8.8 Hz, 1H), 6.62 (s, 1H), 6.25 (d, *J* = 16.0 Hz, 1H), 4.34-4.14 (m, 2H), 3.50 (s, 3H), 2.47 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.4, 144.7, 141.9, 139.7, 139.0, 135.6, 135.4, 135.1, 134.9, 134.8, 133.7, 129.6 (2C), 128.9 (2C), 127.2, 126.1, 125.1, 123.1, 119.6, 113.0, 110.9, 100.3, 60.4, 30.8, 21.6, 20.9, 20.5, 14.4; MS (ES mass): 581.1 (M+1); HPLC: 98.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 5.1 min.

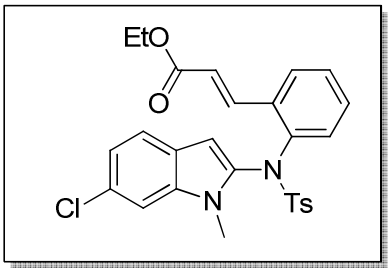
**4.5.1.42. (*E*)-Ethyl 3-(2-(*N*-(5-methoxy-1-methyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-4-methylphenyl)acrylate (37u)**




Yield: 87%; white solid; mp: 180-182 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.20-7.12 (m, 2H), 7.06 (s, 1H), 6.98 (t, *J* = 4.3 Hz, 1H), 6.90 (dd, *J* = 8.8, 2.4Hz, 1H), 6.36-6.26 (m, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 2.46 (s, 3H), 2.30 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.6, 154.2, 144.5, 141.2, 140.6, 139.4, 135.1, 134.6, 131.8, 130.7, 130.3, 129.9, 129.3 (2C), 129.0 (2C), 127.0, 125.8, 119.3, 113.2, 110.7, 102.3, 100.1, 60.5, 55.7, 30.1, 21.7, 21.3, 14.3; MS (ES mass): 519.2 (M+1); HPLC: 94.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in

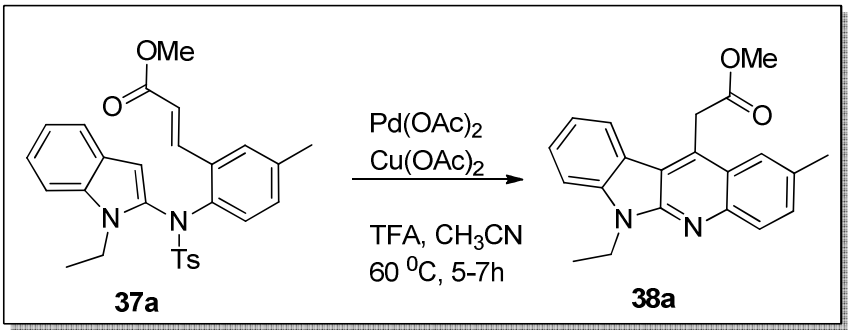
water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 4.2 min.

**4.5.1.43. (*E*)-Ethyl-3-(2-(*N*-(6-chloro-1-methyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)phenyl)acrylate (37v)**




Yield: 82%; white solid; mp: 157-159 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.38 (d, *J* = 16.0 Hz, 1H), 7.69 (dd, *J* = 7.2, 2.0 Hz, 1H),), 7.57 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.38-7.32 (m, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.27 (s, 1H), 7.26-7.22 (m, 1H), 7.05 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 6.31 (s, 1H), 4.30 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 2.47 (s, 3H), 1.38 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.3, 144.8, 140.6, 139.4, 135.6, 135.4, 134.9, 134.3, 130.6, 130.1, 129.5 (2C), 129.2, 129.0 (2C), 128.7, 127.4, 124.1, 121.9, 120.7 (2C), 109.8, 100.6, 60.6, 30.2, 21.7, 14.3; MS (ES mass): 509.2 (M+1); HPLC: 98.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 4.5 min.

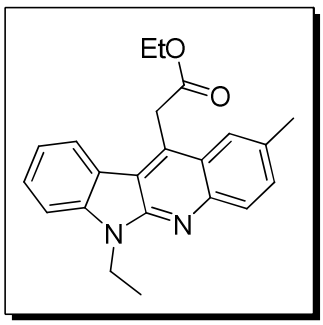
**4.5.1.44. Typical procedure for synthesis of methyl-2-(6-ethyl-2-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38a)**



(*E*)-Methyl-3-(2-(*N*-(1-ethyl-1*H*-indol-2-yl)-4-methylphenylsulfonamido)-5-methylphenyl)acrylate (**37a) (**0.20 mmol), Pd $(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (0.30 mmol), TFA (0.24 mmol) and $CH_3CN$ (2.5 mL) was heated at 60 °C in air for 5h. The progress of the reaction was monitored by TLC. After completion of the reaction, reaction mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**38a)**.

Yield: 86%; light yellow solid; mp: 183-186 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.60-7.55 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 4.69 (s, 2H), 4.60 (q, *J* = 7.2 Hz, 2H), 3.69 (s, 3H), 2.61 (s, 3H), 1.50 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.4, 151.2, 145.2, 141.9, 132.8, 132.6, 130.8, 128.0, 127.6, 123.5, 123.4, 122.6, 120.6, 119.7, 117.1, 108.7, 52.4, 35.9, 34.8, 21.8, 13.6; MS (ES mass): 333.1 (M+1); HPLC: 99.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.0 nm, retention time 3.0 min.

**4.5.1.45. Ethyl-2-(6-ethyl-2-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38b)**

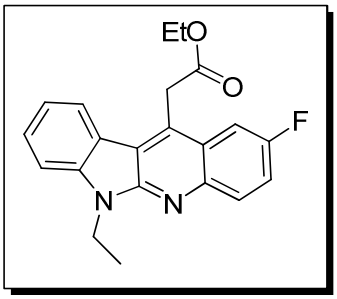



Compound (**38b)** was synthesized from (**37b)** following a procedure similar to that of compound (**38a)**

Yield: 89%; yellow solid; mp: 285-287 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 8.01 (s, 1H), 7.61-7.54 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 4.68 (s, 2H), 4.60 (q, *J* = 7.2 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 2.61 (s, 3H), 1.50 (t, *J* = 7.2

Hz, 3H), 1.20 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.9, 151.2, 145.2, 141.9, 133.0, 132.5, 130.8, 127.9, 127.6, 123.5 (2C), 122.8, 120.6, 119.6, 117.1, 108.6, 61.3, 35.9, 35.0, 21.8, 14.1, 13.6; MS (ES mass): 347.1 (M+1); HPLC: 96.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 3.2 min.

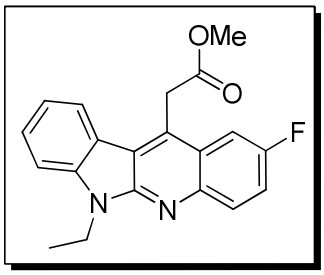
**4.5.1.46. Ethyl-2-(6-ethyl-2-fluoro-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38c)**




Compound (**38c)** was synthesized from (**37c)** following a procedure similar to that of compound (**38a)**

Yield: 78%; orange solid; mp: 133-135 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 9.2, 5.6 Hz, 1H), 7.88 (dd, *J* = 10.8, 2.7 Hz, 1H), 7.65-7.57 (m, 1H), 7.53-7.46 (m, 2H), 7.35-7.31 (m, 1H), 4.63 (s, 2H), 4.62 (q, *J* = 7.2 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 1.51 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.5, 151.2, 143.6, 142.1, 132.9, 130.2 (C-F *J* = 8.8 Hz), 130.1, 128.1, 123.8 (3C), 120.1, 119.8, 118.6 (C-F *J* = 25.6 Hz), 118.3, 117.7, 108.7, 107.5 (C-F *J* = 22.9 Hz), 107.3, 61.5, 35.2, 29.6, 14.1, 13.5; MS (ES mass): 351.2 (M+1); HPLC: 93.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 3.8 min.

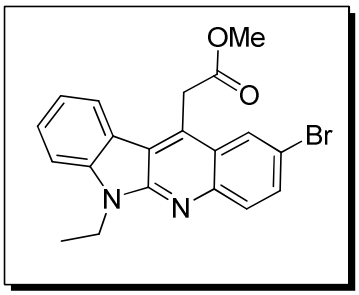
**4.5.1.47. Methyl-2-(6-ethyl-2-fluoro-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38d)**




Compound (**38d)** was synthesized from (**37d)** following a procedure similar to that of compound (**38a)**

Yield: 52%; off white solid; mp: 163-175 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.32 (d, *J* = 8.0 Hz, 1H), 8.13 (dd, *J* = 9.2, 5.6 Hz, 1H), 7.86 (dd, *J* = 10.6, 2.6 Hz, 1H), 7.61 (t, *J* = 7.2 Hz, 1H), 7.55-7.44 (m, 2H), 7.33 (t, *J* = 7.6 Hz, 1H), 4.64 (s, 2H), 4.59 (q, *J* = 7.2 Hz, 2H), 3.70 (s, 3H), 1.50 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.0, 159.8 (C-F *J* = 241.6 Hz), 157.4, 151.2, 143.6, 142.1, 132.7 (2C), 130.2 (C-F *J* = 8.8 Hz), 130.2, 128.2, 123.8 (2C), 120.0, 119.9, 118.6 (C-F *J* = 25.6 Hz), 118.4, 108.8, 107.4 (C-F *J* = 22.8 Hz), 107.2, 52.5, 36.0, 35.0, 13.5; MS (ES mass): 337.2 (M+1); HPLC: 95.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 3.8 min.

**4.5.1.48. Methyl-2-(2-bromo-6-ethyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38e)**

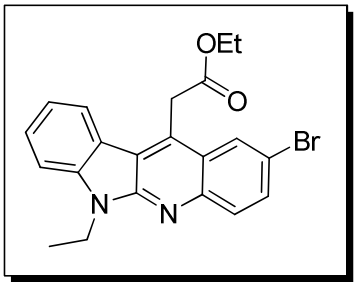



Compound (**38e)** was synthesized from (**37e)** following a procedure similar to that of compound (**38a)**

Yield: 80%; white solid; mp: 186-189 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.77 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.34 (t, *J* = 7.2 Hz, 1H), 4.65 (s, 2H), 4.55 (q, *J* = 7.2 Hz, 2H), 3.71 (s, 3H), 1.51 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:169.9, 151.6, 145.3, 142.1, 132.5, 131.7,

129.9, 128.2, 126.0, 124.7, 123.8, 120.2, 120.1, 117.8, 116.5, 108.9, 52.6, 36.0, 34.7, 13.5; MS (ES mass): 399.1 (M+3); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.0 min.

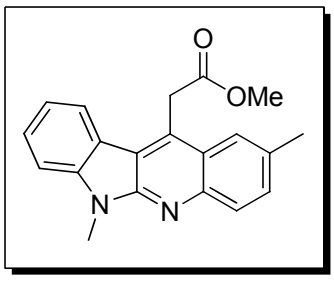
**4.5.1.49. Ethyl-2-(2-bromo-6-ethyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38f)**




Compound (**38f)** was synthesized from (**37f)** following a procedure similar to that of compound (**38a)**

Yield: 88%; yellow solid; mp: 187-189 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.39 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.77 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.64-7.57 (m, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.34-7.30 (m, 1H), 4.63 (s, 2H), 4.58 (q, *J* = 7.2 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 1.50 (t, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.4, 151.6, 145.2, 142.1, 132.7, 131.7, 129.8, 128.2, 126.2, 124.8, 123.8, 120.2, 120.0, 117.8, 116.4, 108.9, 61.5, 36.0, 35.0, 14.1, 13.5; MS (ES mass): 411.1 (M+1); HPLC: 99.2%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.3 min.

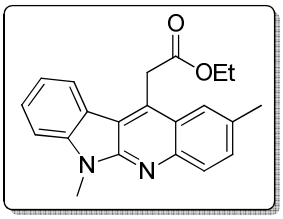
**4.5.1.50. Methyl-2-(2,6-dimethyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38g)**



Compound (**38g)** was synthesized from (**37g)** following a procedure similar to that of compound (**38a)**

Yield: 75%; yellow floppy solid; mp: 208-210 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.28 (d, *J* = 8.0 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 7.99 (s, 1H), 7.61-7.56 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 4.70 (s, 2H), 3.99 (s, 3H), 3.69 (s, 3H), 2.61 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.4, 151.9, 145.1, 142.9, 132.9, 132.7, 130.9, 127.8, 127.7, 123.4, 123.3, 122.7, 120.4, 119.9, 117.1, 108.5, 52.4, 34.8, 27.6, 21.8; MS (ES mass): 319.2 (M+1); HPLC: 98.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 2.9 min.

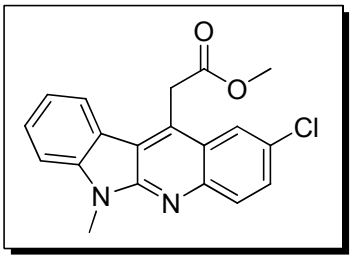
**4.5.1.51. Ethyl-2-(2,6-dimethyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38h)**




Compound (**38h)** was synthesized from (**37h)** following a procedure similar to that of compound (**38a)**

Yield: 82%; yellow solid; mp: 145-147 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 8.8 Hz, 1H), 8.01 (s, 1H), 7.61-7.55 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 4.68 (s, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 2.61 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.8, 151.9, 145.1, 142.9, 133.2, 132.6, 130.9, 129.7, 128.9, 127.8, 127.7, 123.4, 122.8, 119.8, 117.1, 108.5, 61.3, 35.0, 27.6, 21.8, 14.1; MS (ES mass): 333.2 (M+1); HPLC: 94.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 3.0 min.

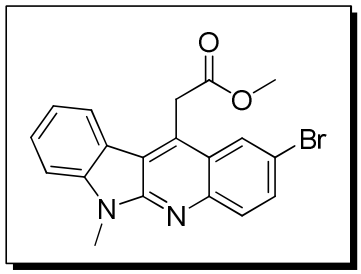
**4.5.1.52. Methyl-2-(2-chloro-6-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38i)**




Compound (**38i)** was synthesized from (**37i)** following a procedure similar to that of compound (**38a)**

Yield: 85%; white solid; mp: 167-169 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 8.0 Hz, 1H), 8.20 (s, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.63-7.60 (m, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.36-7.32 (m, 1H), 4.65 (s, 2H), 3.98 (s, 3H), 3.70 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.8, 152.2, 145.0, 143.1, 132.7, 129.6, 129.3, 128.7, 128.3, 124.1, 123.6, 122.8, 120.3, 120.0, 117.8, 108.8, 52.6, 34.8, 27.6; MS (ES mass): 339.0 (M+1); HPLC: 96.4%, column: Symmetry C-18 250 x 4.6 mm 5μm, mobile phase A: 5 mm Ammonium Acetate in water, mobile phase B: $CH_3CN$, gradient (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 260 nm, retention time 14.4 min.

### 4.5.1.53. Methyl-2-(2-bromo-6-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38j)

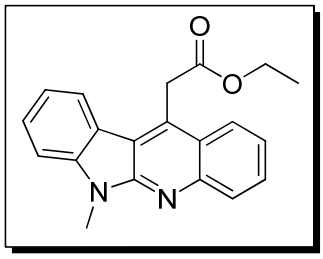



Compound (**38j)** was synthesized from (**37j)** following a procedure similar to that of compound (**38a)**

Yield: 77%; light green solid; mp: 201-203 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.78 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.66-7.60 (m, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 4.65 (s, 2H), 3.99 (s, 3H), 3.71 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:169.8, 152.3, 145.2, 143.1, 132.6, 131.8, 129.8, 128.3, 126.1, 124.7, 123.6, 120.4, 120.0, 117.8, 116.6, 108.8, 52.6, 34.7, 27.6; MS (ES mass): 385.0 (M+3);

HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 280.0 nm, retention time 3.8 min.

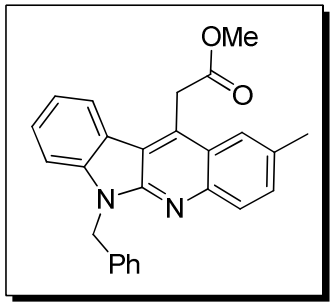
**4.5.1.54. Ethyl-2-(6-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38k)**




Compound (**38k)** was synthesized from (**37k)** following a procedure similar to that of compound (**38a)**

Yield: 84%; off white solid; mp: 145-147 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.33 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.75-7.71 (m, 1H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.54-7.50 (m, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 4.70 (s, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.01 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:169.7, 152.2, 146.7, 143.0, 133.9, 128.6, 128.0, 127.9, 123.9, 123.6, 123.5, 123.1, 120.4, 120.0, 117.2, 108.6, 61.4, 35.1, 27.6, 14.1; MS (ES mass): 319.2 (M+1); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 3.2 min.

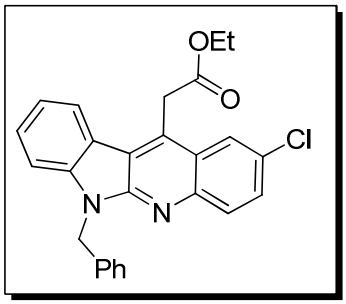
**4.5.1.55. Methyl-2-(6-benzyl-2-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38l)**



Compound (**38l)** was synthesized from (**37l)** following a procedure similar to that of compound (**38a)**

Yield: 89%; yellow solid; mp: 202-204 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 7.6 Hz, 1H), 8.06 (t, *J* = 8.4 Hz, 1H), 8.02 (s, 1H), 7.57 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.51-7.44 (m, 1H), 7.33-7.27 (m, 5H), 7.24-7.19 (m, 2H), 5.77 (s, 2H), 4.71 (s, 2H), 3.71 (s, 3H), 2.62 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.4, 151.7, 145.2, 142.1, 137.2, 133.0, 132.9, 130.9, 128.6 (2C), 128.1, 127.7, 127.2, 127.1 (2C), 123.7, 123.4, 122.7, 120.7, 120.1, 117.0, 109.5, 52.5, 44.8, 34.8, 21.9; MS (ES mass): 395.2 (M+1); HPLC: 97.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 3.8 min.

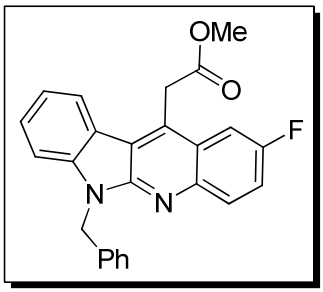
**4.5.1.56. Ethyl-2-(6-benzyl-2-chloro-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38m)**




Compound (**38m)** was synthesized from (**37m)** following a procedure similar to that of compound (**38a)**

Yield: 75%; yellow solid; mp: 154-156 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.33 (d, *J* = 8.0 Hz, 1H), 8.25 (s, 1H), 8.07 (d, *J* = 8.8 Hz, 1H), 7.65 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.34-7.27 (m, 6H), 7.24-7.22 (m, 1H), 5.77 (s, 2H), 4.66 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 1.23 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.4, 152.2, 145.1, 142.3, 137.0, 133.0, 129.8, 129.3, 128.8, 128.6 (2C), 128.3, 127.4, 127.1 (2C), 124.5, 123.7, 122.9, 120.4, 120.3, 117.7, 109.7, 61.6, 44.9, 35.1, 14.1; MS (ES mass): 429.2 (M+1); HPLC: 94.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.6 min.

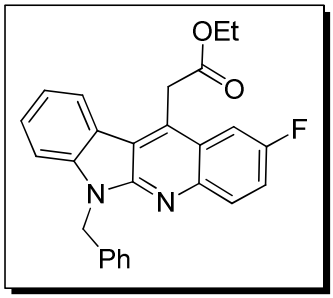
**4.5.1.57. Methyl-2-(6-benzyl-2-fluoro-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38n)**




Compound (**38n)** was synthesized from (**37n)** following a procedure similar to that of compound (**38a)**

Yield: 81%; yellow solid; mp: 171-173 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: ppm 8.33 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 9.2, 5.6 Hz, 1H), 7.88 (dd, *J* = 10.4, 2.8 Hz, 1H), 7.54-7.49 (m, 2H), 7.36-7.27 (m, 6H), 7.25-7.22 (m, 1H), 5.77 (s, 2H), 4.66 (s, 2H), 3.72 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.0, 143.6, 142.3, 137.0, 136.6, 130.4 (C-F *J* = 8.8 Hz), 130.3, 128.7, 128.6, 128.2, 127.3, 127.1, 123.7, 123.6, 120.7, 120.3, 120.1, 118.7, 118.5 ( C-F *J* = 25.5 Hz), 117.6, 109.6, 107.7, 107.5, 107.2, 52.6, 44.8, 35.0; MS (ES mass): 399.1 (M+1); HPLC: 93.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.1 min.

**4.5.1.58. Ethyl 2-(6-benzyl-2-fluoro-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38o)**

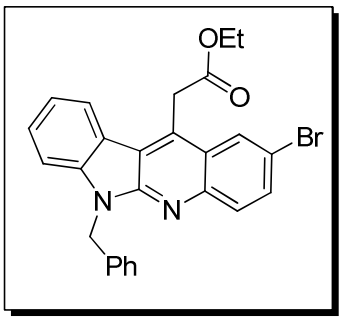



Compound (**38o)** was synthesized from (**37o)** following a procedure similar to that of compound (**38a)**

Yield: 83%; yellow solid; mp: 174-176 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.34 (d, *J* = 8.0 Hz, 1H), 8.12 (dd, *J* = 9.2, 5.6 Hz, 1H),

7.90 (dd, *J* = 10.4, 2.4 Hz, 1H), 7.52-7.47 (m, 2H), 7.34-7.28 (m, 6H), 7.25-7.20 (m, 1H), 5.75 (s, 2H), 4.64 (s, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:169.5, 151.8, 143.6, 142.4, 137.1, 133.2, 130.4, 130.3, 128.6 (2C), 128.2, 127.3, 127.1, 123.7, 120.3, 120.2, 118.7, 118.4, 117.7, 109.6, 107.6, 107.3, 61.5, 44.8, 35.3, 14.1; MS (ES mass): 413.2 (M+1); HPLC: 93.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 10/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.2 min.

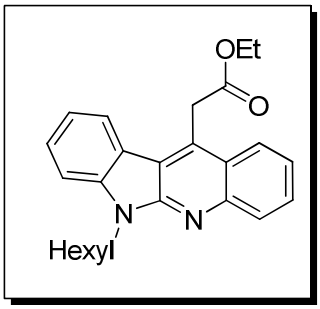
**4.5.1.59. Ethyl 2-(6-benzyl-2-bromo-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38p)**




Compound (**38p)** was synthesized from (**37p)** following a procedure similar to that of compound (**38a)**

Yield: 77%; yellow solid; mp: 166-168 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.42 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.77 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.39-7.28 (m, 6H), 7.24-7.21 (m, 1H), 5.77 (s, 2H), 4.66 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.4, 145.3, 142.3, 136.9, 133.0, 131.8, 130.1, 130.0, 128.7, 128.6, 128.3, 127.4, 127.1, 126.2, 125.1, 123.7, 120.4, 120.3, 117.7, 116.7, 109.9, 109.7, 61.6, 44.9, 35.1, 14.1; MS (ES mass): 473.1 (M+1); HPLC: 90.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 4.8 min.

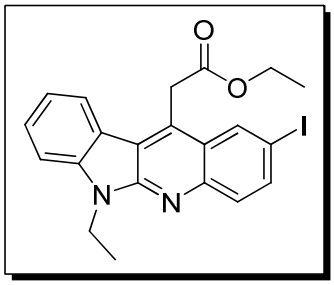
**4.5.1.60. Ethyl 2-(6-hexyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38q)**




Compound (**38q)** was synthesized from (**37q)** following a procedure similar to that of compound (**38a)**

Yield: 67%; light yellow solid; mp: 132-135 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.34 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 8.15 (d, *J* = 8.8 Hz, 1H), 7.75-7.70 (m, 1H), 7.62-7.56 (m, 1H), 7.53-7.59 (m, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.33-7.30 (m, 1H), 4.70 (s, 2H), 4.56-4.51 (t, *J* = 7.2 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 2.00-1.90 (m, 2H), 1.46 (dd, *J* = 10.53, 5.83 Hz, 2H), 1.41-1.35 (m, 2H), 1.33-1.29 (m, 2H), 1.19 (t, *J* = 7.2 Hz, 3H), 0.88 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.8, 152.0, 146.7, 142.4, 133.6, 128.4, 128.3, 127.7, 123.9, 123.6, 123.0, 120.5, 119.7, 117.1, 109.9, 108.9, 61.3, 41.3, 35.1, 31.5, 28.3, 26.7, 22.5, 14.1, 14.0; MS (ES mass): 389.2 (M+1); HPLC: 91.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.0 min.

**4.5.1.61. Ethyl 2-(6-ethyl-2-iodo-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38r)**

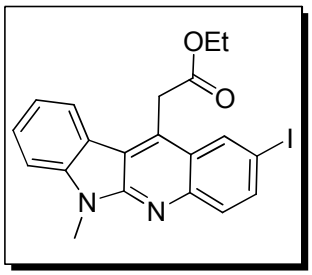



Compound (**38r)** was synthesized from (**37r)** following a procedure similar to that of compound (**38a)**

Yield: 72%; light yellow solid; mp: 159-161 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.93 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 7.65-7.57 (m, 1H), 7.47 (d, *J* = 8.4 Hz, 1H),

7.36-7.29 (m, 1H), 4.63 (s, 2H), 4.59 (q, *J* = 7.2 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 1.50 (t, *J* = 7.2 Hz, 3H), 1.23 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.4, 145.6, 142.1, 139.8, 136.9, 132.8, 132.6, 130.0, 128.2, 125.5, 123.8, 120.3, 120.1, 117.6, 108.9, 87.3, 61.5, 36.0, 35.0, 14.1, 13.5; MS (ES mass): 459.1 (M+1); HPLC: 96.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 280.0 nm, retention time 4.3 min.

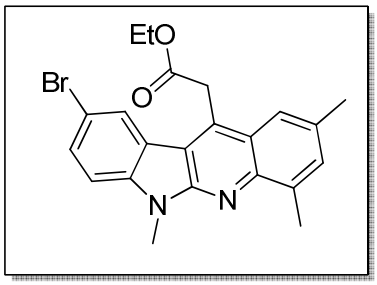
**4.5.1.61. Ethyl 2-(2-iodo-6-methyl-6*H*-indolo[2,3-b]quinolin-11-yl)acetate (38s)**




Compound (**38s)** was synthesized from (**37s)** following a procedure similar to that of compound (**38a)**

Yield: 69%; light yellow solid; mp: 167-171 °C; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 7.98-7.91 (m, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.37-7.31 (m, 1H), 4.63 (s, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 169.3, 152.3, 145.6, 143.1, 137.0, 132.9, 132.7, 129.8, 128.3, 125.5, 123.6, 120.3, 120.1, 117.6, 108.8, 87.4, 61.5, 35.0, 27.6, 14.1; MS (ES mass): 445.1 (M+1); HPLC: 91.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 2/20, 10/95, 20/95, 22/20, 25/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 280.0 nm, retention time 4.2 min.

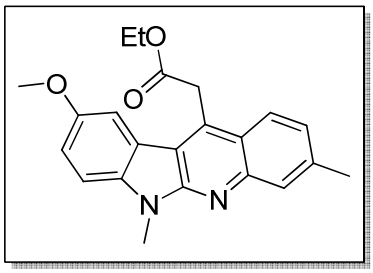
**4.5.1.62. Ethyl 2-(9-bromo-2,4,6-trimethyl-6*H*-indolo[2,3-*b*]quinolin-11-yl)acetate (38t)**




Compound (**38t)** was synthesized from (**37t)** following a procedure similar to that of compound (**38a)**

Yield: 87%; yellow solid; mp: 213-215 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 7.86 (s, 1H), 7.66 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.46 (s, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 4.60 (s, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 3H), 2.85 (s, 3H), 2.56 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 169.7, 150.6, 144.4, 141.4, 135.4, 133.8, 132.3, 131.4, 129.9, 125.9, 123.2, 122.0, 120.6, 115.3, 112.1, 109.6, 61.4, 35.1, 27.3, 21.8, 18.3, 14.1; MS (ES mass): 425.1 (M+1); HPLC: 99.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 285.0 nm, retention time 5.7 min.

### 4.5.1.63. Ethyl 2-(9-methoxy-3,6-dimethyl-6*H*-indolo[2,3-*b*]quinolin-11-yl)acetate (38u)

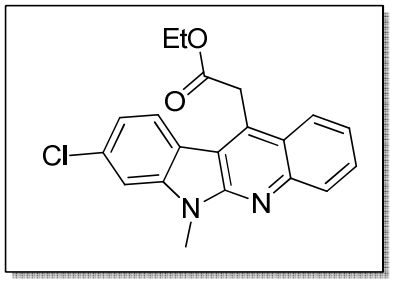



Compound (**38u)** was synthesized from (**37u)** following a procedure similar to that of compound (**38a)**

Yield: 84%; white solid; mp: 180-182 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8.8 Hz, 1H), 7.91-7.89 (m, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.21 (dd, *J* = 8.8, 2.8 Hz, 1H), 4.64 (s, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 6H), 2.60 (s, 3H), 1.18 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 169.8, 154.1, 152.6, 147.0, 139.0, 137.5, 133.8, 127.1, 125.2, 123.7, 121.4, 120.9, 116.5, 115.1, 108.8, 108.2, 61.4, 56.2, 35.1, 27.6, 21.7, 14.1; MS (ES mass): 363.2 (M+1);

HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 3.6 min.

**4.5.1.64. Ethyl 2-(8-chloro-6-methyl-6H-indolo[2,3-*b*]quinolin-11-yl)acetate (38v)**




Compound (**38v)** was synthesized from (**37v)** following a procedure similar to that of compound (**38a)**

Yield: 81%; off white solid; mp: 173-175 °C; $R_f$ = 0.2 (20% EtOAc/ *n*-hexane); $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.27 (d, *J* = 8.4 Hz, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.76-7.72 (m, 1H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.42 (s, 1H), 7.29 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.64 (s, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 3H), 1.17 (t, *J* = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): 169.5, 152.2, 146.6, 143.5, 133.8, 133.7, 128.9, 128.1, 124.1, 123.9, 123.4, 120.2, 118.8, 116.4, 109.9, 108.8, 61.4, 35.0, 27.6, 14.1; MS (ES mass): 353.1 (M+1); HPLC: 98.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 270.0 nm, retention time 3.9 min.

**4.5.2. Pharmacology**

**4.5.2.1. Sulphorhodamine B (SRB) Assay:**

**The principle:** The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on cancer cells was evaluated using the SRB (Sulforhodamine B) cell proliferation assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as to vehicle-treated cells. Further, this assay is the standardized assay of choice for screening of anticancer

compounds at the National Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number.

**The methodology:** Cancer cells (around 5000 in number) were seeded in 96-well plates and incubated overnight. The optimum cell number to be seeded was determined by a growth curve analysis for the cell line. Compounds (dissolved in 100% DMSO to a stock concentration of 200mM) were added to the adhered cells at a final concentration of 10µM. After 72h of treatment, the cells were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid was added to the cells to precipitate the proteins. It was incubated for 1h at 4 $^{0}$C. The cells were then washed with water and air-dried. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 30 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye was solubilized with 10mM Tris solution (pH 10.5). Absorbance of solubilized dye was measured at a wavelength of 590 nm. Percentage growth was determined by the formula

[(At-A0/Ac-A0)] X 100
where At=absorbance after 72h of test compound treatment,
A0=Absorbance at time 0,
Ac=Absorbance after 72h without treatment.

The known cytotoxic agent, gemcitabine was used as a positive control in the assay.

**4.5.2.2. Zebrafish embryo studies:**

**Materials and Methods:**

**<u>Husbandry:</u>**

Zebrafish obtained from a local vendor were maintained in in-house built re-circulatory system under 14-10 h light dark cycle and 28 ˚C temperature as described earlier (Banote et al., 2013). Breeding was carried out using females and males in ratio of 2:3 and the embryos obtained were collected in petridishes and maintained at 28$^{0}$C. (Westerfield et al., 2000, Nakhi et al.,2013).

**Apoptosis Assay:**

24hpf embryos were de-chorinated manually. 6 embryos were distributed as two sets in each well of 24 well plates with 250 µl of 0.1% DMSO. The working stock solutions were prepared by serial dilution as described earlier. Each well was added with 250µl of respective concentration to obtain final working concentration. Embryos were incubated at 28˚C for 24 hrs and 48hrs. Check apoptotic effect at 24 h and 48 h by washing drug exposed embryos thrice with E3 medium. Acridine orange (2µg/ml) solution of dye in E3 medium was added and incubated for 30 min. The embryos were rinsed thoroughly twice in fresh E3 medium to wash the acridine orange solution. Stained embryos were anesthetized with tricaine and photographed under UV illumination using Zeiss AxioCamMR camera attached to a Zeiss florescence microscope (GFP filter set : excitation 473,emission 520 ) under 5X magnification. The Images were taken and analyzed using Image J software.

**Teratogenicity assay:**

In this assay, 1dpf embryos at same developmental stage were sorted out and dechorionated using protease K. Test compounds stock solutions were prepared by dissolving in 100% DMSO. By serial dilution from stock solutions various concentrations were prepared and the final concentration of DMSO becomes 0.1%. The embryos were distributed in 24 well plate (3/well) and concentrations of test compounds starting from 1µM to 30µM compound was added to each well accordingly where n=6.The plate was incubated at 28˚C until 5dpf. The embryos were washed with PBS and anesthetized using tricaine (0.008%). Morphological scoring was done based on the procedure previously described (Panzica-Kelly et al, 2010).

## 4.6. References:


1. (a) Gellert, E.; Schlittler, E., *Helv. Chim. Acta.* **1951,** *34* (2), 642-651; (b) Tackie, A. N.; Sharaf, M. H.; Schiff, P. L.; Boye, G. L.; Crouch, R. C.; Martin, G. E., *J. Hterocycl. Chem* **1991,** *28* (5), 1429-1435; (c) Spitzer, T. D.; Crouch, R. C.; Martin, G. E.; Sharaf, M. H.; Schiff, P. L.; Tackie, A. N.; Boye, G. L., *J. Heterocycl. Chem.* **1991,** *28* (8), 2065-2070; (d) Tackie, A. N.; Boye, G. L.; Sharaf, M. H.; Schiff Jr, P. L.; Crouch, R. C.; Spitzer, T. D.; Johnson, R. L.; Dunn, J.; Minick, D.; Martin, G. E., *J. Nat. Prod.* **1993,** *56* (5), 653-670; (e) Crouch, R. C.; Davis, A. O.; Spitzer, T. D.; Martin, G. E.; Sharaf, M. M.; Schiff, P. L.; Phoebe, C. H.; Tackie, A. N., *J. Heterocycl. Chem.* **1995,** *32* (3), 1077-1080; (f) Paulo, A.; Gomes, E. T.; Houghton, P. J., *J. Nat. Prod.* **1995,** *58* (10), 1485-1491; (g) Hadden, C. E.; Sharaf, M. H.; Guido, J. E.; Robins, R. H.; Tackie, A. N.; Phoebe, C. H.; Schiff, P. L.; Martin, G. E., *J. Nat. Prod.* **1999,** *62* (2), 238-240.
2. Subbaraju, G. V.; Kavitha, J.; Rajasekhar, D.; Jimenez, J. I., *J. Nat. Prod.* **2004,** *67* (3), 461-462.
3. (a) Sawer, I.; Berry, M.; Brown, M.; Ford, J., *J. Appl. Bacteriol.* **1995,** *79* (3), 314-321; (b) Kirby, G.; Paine, A.; Warhurst, D.; Noamese, B.; Phillipson, J., *Phytother. Res.* **1995,** *9* (5), 359-363; (c) Cimanga, K.; De Bruyne, T.; Lasure, A.; Van Poel, B.; Pieters, L.; Claeys, M.; Berghe, D. V.; Kambu, K.; Tona, L.; Vlietinck, A. J., *Planta Med.* **1996,** *62* (1), 22-27; (d) Cimanga, K.; De Bruyne, T.; Pieters, L.; Vlietinck, A. J.; Turger, C. A., *J. Nat. Prod.* **1997,** *60* (7), 688-691; (e) Boakye-Yiadom, K.; Heman-Ackah, S. M., *J. Pharm. Sci.* **1979,** *68* (12), 1510-1514.
4. (a) Lu, Y.-J.; Ou, T.-M.; Tan, J.-H.; Hou, J.-Q.; Shao, W.-Y.; Peng, D.; Sun, N.; Wang, X.-D.; Wu, W.-B.; Bu, X.-Z., *J. Med. Chem.* **2008,** *51* (20), 6381-6392; (b) El Sayed, I.; Van der Veken, P.; Steert, K.; Dhooghe, L.; Hostyn, S.; Van Baelen, G.; Lemiere, G.; Maes, B. U.; Cos, P.; Maes, L., *J. Med. Chem.* **2009,** *52* (9), 2979-2988.
5. Chang, M.-R.; Takeuchi, Y.; Hashigaki, K.; Yamato, M., *Heterocycles.* **1992,** *33* (1), 147-152.
6. Görlitzer, K.; Stockmann, R.; Walter, R., *Pharmazie.* **1995,** *50* (2), 105-111.
7. Görlitzer, K.; Stockmann, R.; Walter, R., *Pharmazie.* **1994,** *49* (4), 231-235.

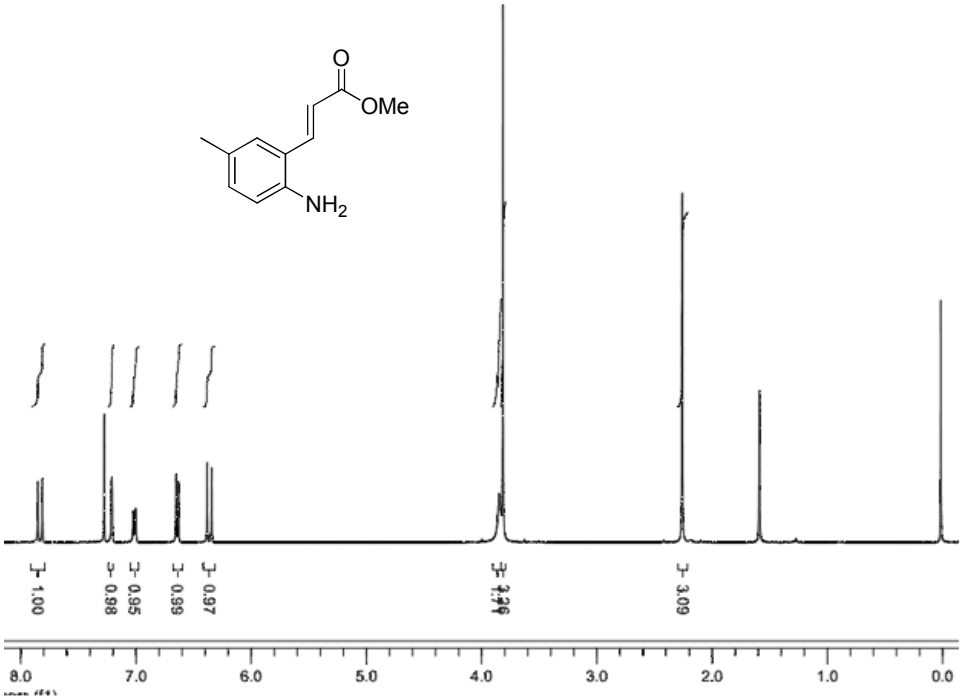
8. Görlitzer, K.; Stockmann, R.; Walter, R., *Die Pharmazie.* **1994,** *49* (4), 231-235.
9. Lavrado, J.; Moreira, R.; Paulo, A., *Curr. Med. Chem.* **2010,** *17* (22), 2348-2370.
10. Cimanga, K.; De Bruyne, T.; Lasure, A.; Van Poel, B.; Pieters, L.; Claeys, M.; Berghe, D. V.; Kambu, K.; Tona, L.; Vlietinck, A. J., *Planta medica.* **1996,** *62* (1), 22-27.
11. Garbett, N. C.; Graves, D. E., *Curr. Med. Chem.* **2004,** *4* (2), 149-172.
12. Kaczmarek, L.; Luniewski, W.; Zagrodzki, B.; Godlewska, J.; Osiadacz, J.; Wietrzyk, J.; Opolski, A.; Peczyńska-Czoch, W., *Acta Pol. Pharm.* **2001,** *59* (3), 199-207.
13. (a) Gamage, S.; Rewcastle, G.; Atwell, G.; Baguley, B.; Denny, W., *Anti-cancer Drug Des.* **1992,** *7* (5), 403-414; (b) Futami, H.; Eader, L.; Back, T. T.; Gruys, E.; Young, H. A.; Wiltrout, R. H.; Baguley, B. C., *J. Immunother.* **1992,** *12* (4), 247-255.
14. Lu, W.-J.; Świtalska, M.; Wang, L.; Yonezawa, M.; El-Sayed, I. E.-T.; Wietrzyk, J.; Inokuchi, T., *Med. Chem. Res.* **2013,** *22* (9), 4492-4504.
15. Takeda, H.; Ishida, T.; Takemoto, Y., *Chem. Lett.* **2009,** *38* (8), 772-773.
16. Tokimizu, Y.; Oishi, S.; Fujii, N.; Ohno, H., *Org. Lett.* **2014,** *16* (11), 3138-3141.
17. Nguyen, H. H.; Fettinger, J. C.; Haddadin, M. J.; Kurth, M. J., *Tetrahedron Lett.* **2015,** *56* (40), 5429-5433.
18. Zhou, Y.; Zhang, M.; Yin, M.; Wang, X., *Chin. J. Org. Chem.* **2013,** *31* (2), 237-242.
19. Ghorbani-Vaghei, R.; Malaekehpoor, S. M., *Tetrahedron Lett.* **2012,** *53* (35), 4751-4753.
20. Parvatkar, P. T.; Parameswaran, P. S.; Tilve, S. G., *J. Org. Chem.* **2009,** *74* (21), 8369-8372.
21. Vecchione, M. K.; Sun, A. X.; Seidel, D., *Chem. Sci.* **2011,** *2* (11), 2178-2181.
22. Ali, S.; Li, Y.-X.; Anwar, S.; Yang, F.; Chen, Z.-S.; Liang, Y.-M., *J. Org. Chem.* **2011,** *77* (1), 424-431.
23. Kandukuri, S. R.; Jiao, L. Y.; Machotta, A. B.; Oestreich, M., *Adv. Synth. Catal.* **2014,** *356* (7), 1597-1609.

24. (a) Jia, C.; Piao, D.; Oyamada, J.; Lu, W.; Kitamura, T.; Fujiwara, Y., *Science. . Science* **2000,** *287* (5460), 1992-1995; (b) Murai, S.; Kakiuchi, F.; Sekine, S.; Tanaka, Y.; Kamatani, A.; Sonoda, M.; Chatani, N., *Nature.* **1993**.
25. (a) Alberico, D.; Scott, M. E.; Lautens, M., *Chem. Rev.* **2007,** *107* (1), 174-238; (b) Campeau, L.-C.; Stuart, D. R.; Fagnou, K., *Aldrichimica Acta.* **2007,** *40* (2), 35-41; (c) Seregin, I. V.; Gevorgyan, V., *Chem. Soc. Rev.* **2007,** *36* (7), 1173-1193; (d) Lewis, J. C.; Bergman, R. G.; Ellman, J. A., *Acc. Chem. Res.* **2008,** *41* (8), 1013-1025; (e) Colby, D. A.; Bergman, R. G.; Ellman, J. A., *Chem. Rev.* **2009,** *110* (2), 624-655.
26. Merlic, C. A.; You, Y.; McInnes, D. M.; Zechman, A. L.; Miller, M. M.; Deng, Q., *Tetrahedron.* **2001,** *57* (24), 5199-5212.
27. (a) Li, Y.-X.; Wang, H.-X.; Ali, S.; Xia, X.-F.; Liang, Y.-M., *Chem. Commun.* **2012,** *48* (17), 2343-2345; (b) YogiáSreenivas, B.; RamaáKrishna, G.; áMalla Reddy, C.; LalitháKumar, K., *Chem. Commun.* **2013,** *49* (38), 3970-3972.
28. Ali, S.; Li, Y.-X.; Anwar, S.; Yang, F.; Chen, Z.-S.; Liang, Y.-M., *J. Org. Chem.* **2011,** *77* (1), 424-431.
29. (a) Ge, H.; Niphakis, M. J.; Georg, G. I., *J. Am. Chem. Soc.* **2008,** *130* (12), 3708-3709; (b) Liu, Y.; Li, D.; Park, C. M., *Angew. Chem., Int. Ed.* **2011,** *123* (32), 7471-7474; (c) Pankajakshan, S.; Xu, Y. H.; Cheng, J. K.; Low, M. T.; Loh, T. P., *Angew. Chem., Int. Ed.* **2012,** *51* (23), 5701-5705; (d) Lafrance, M.; Fagnou, K., *J. Am. Chem. Soc.* **2006,** *128* (51), 16496-16497; (e) Liégault, B.; Lee, D.; Huestis, M. P.; Stuart, D. R.; Fagnou, K., *J. Org. Chem.* **2008,** *73* (13), 5022-5028; (f) Jia, C.; Lu, W.; Oyamada, J.; Kitamura, T.; Matsuda, K.; Irie, M.; Fujiwara, Y., *J. Am. Chem. Soc.* **2000,** *122* (30), 7252-7263; (g) Giri, R.; Lam, J. K.; Yu, J.-Q., *J. Am. Chem. Soc.* **2009,** *132* (2), 686-693; (h) Ji, X.; Huang, H.; Wu, W.; Li, X.; Jiang, H., *J. Org. Chem.* **2013,** *78* (22), 11155-11162.
30. (a) Ramsay, J. M.; Feist, G. W.; Varga, Z. M.; Westerfield, M.; Kent, M. L.; Schreck, C. B., *Aquaculture* **2006,** *258* (1), 565-574; (b) Nakhi, A.; Archana, S.; Seerapu, G. P. K.; Chennubhotla, K. S.; Kumar, K. L.; Kulkarni, P.; Haldar, D.; Pal, M., *Chem. Commun.* **2013,** *49* (56), 6268-6270; (c) Panzica-Kelly, J. M.; Zhang, C. X.; Danberry, T. L.; Flood, A.; DeLan, J. W.;

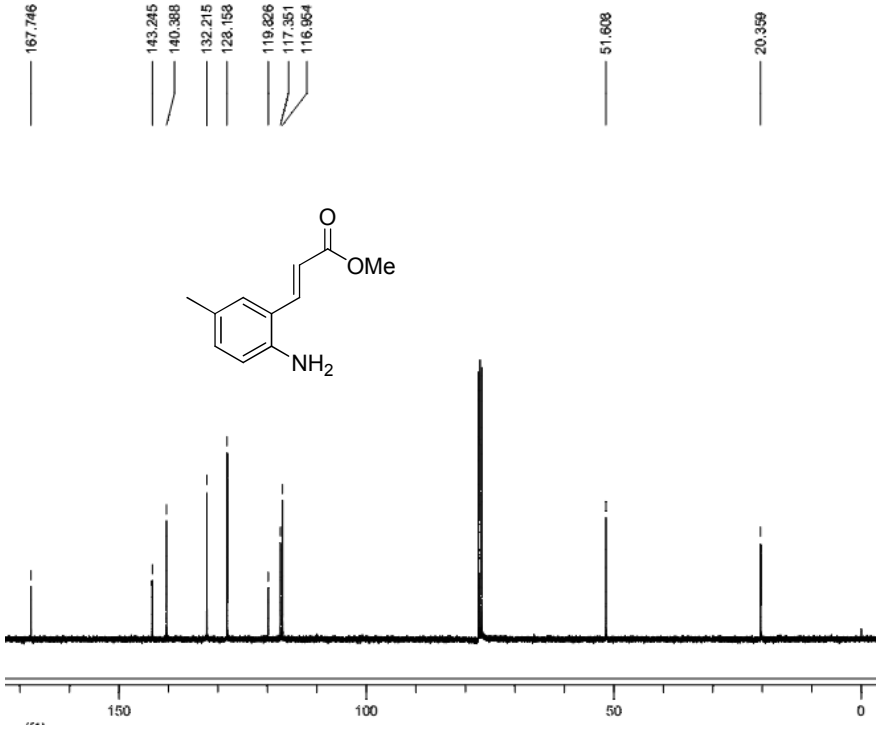
Brannen, K. C.; Augustine-Rauch, K. A., *Birth Defects Research Part B: Developmental and Reproductive Toxicology* **2010,** *89* (5), 382-395.

31. (a) Rubinstein, L.; Shoemaker, R.; Paull, K.; Simon, R.; Tosini, S.; Skehan, P.; Scudiero, D.; Monks, A.; Boyd, M., *J. Natl. Cancer Inst.* **1990,** *82* (13), 1113-1117; (b) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R., *J. Natl. Cancer Inst.* **1990,** *82* (13), 1107-1112.
32. Adepu, R.; Rajitha, A.; Ahuja, D.; Sharma, A. K.; Ramudu, B.; Kapavarapu, R.; Parsa, K. V.; Pal, M., *Org. Biomol. Chem.* **2014,** *12* (16), 2514-2518.
33. Hayashi, S.; Ueno, N.; Murase, A.; Nakagawa, Y.; Takada, J., *Eur. J. Med. Chem.* **2012,** *50*, 179-195.

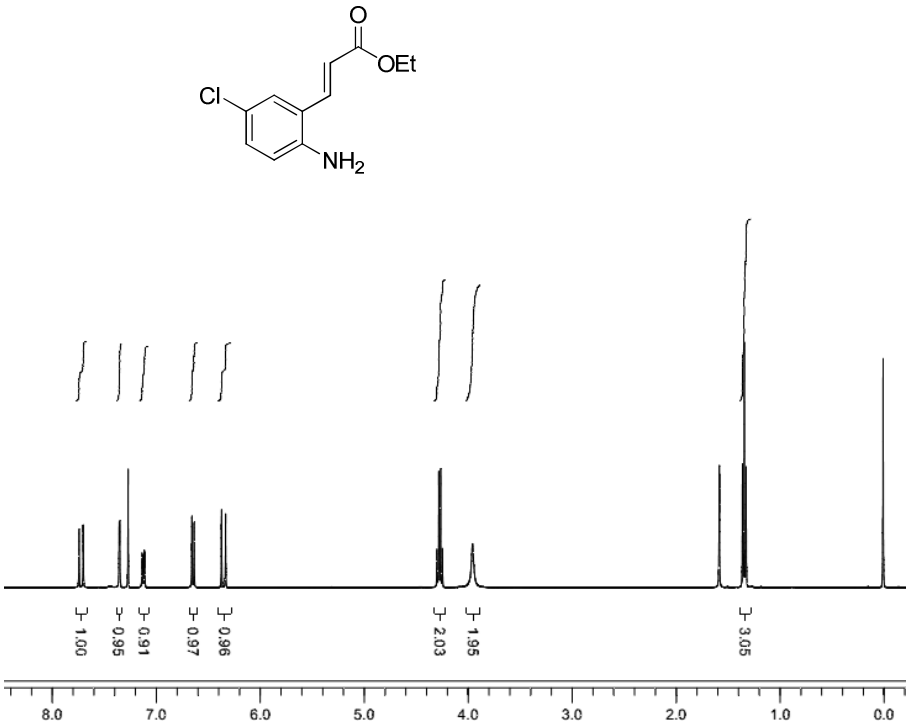
# Appendix



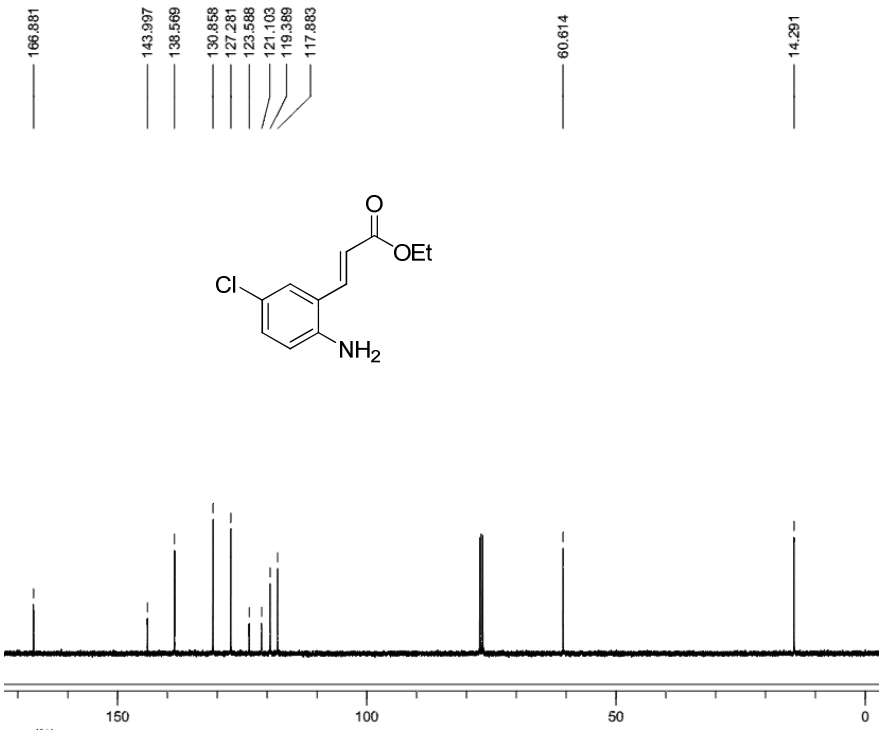

$^{1}$H NMR spectra of compound **32a** ($CDCl_3$, 400 MHz)

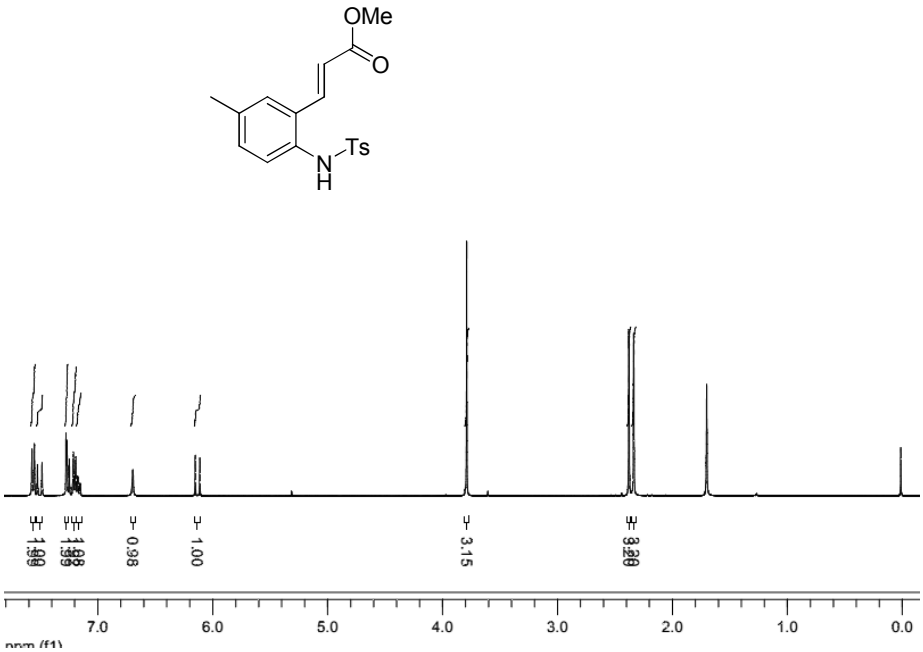



$^{13}$C NMR spectra of compound **32a** ($CDCl_3$, 100 MHz)

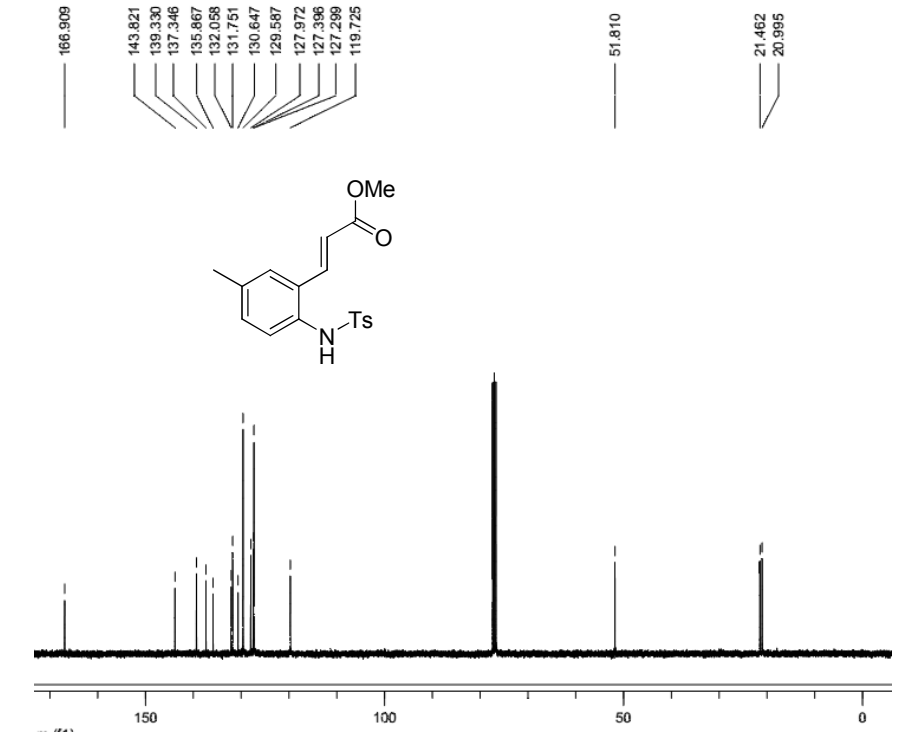



$^{1}$H NMR spectra of compound **32b** ($CDCl_3$, 400 MHz)

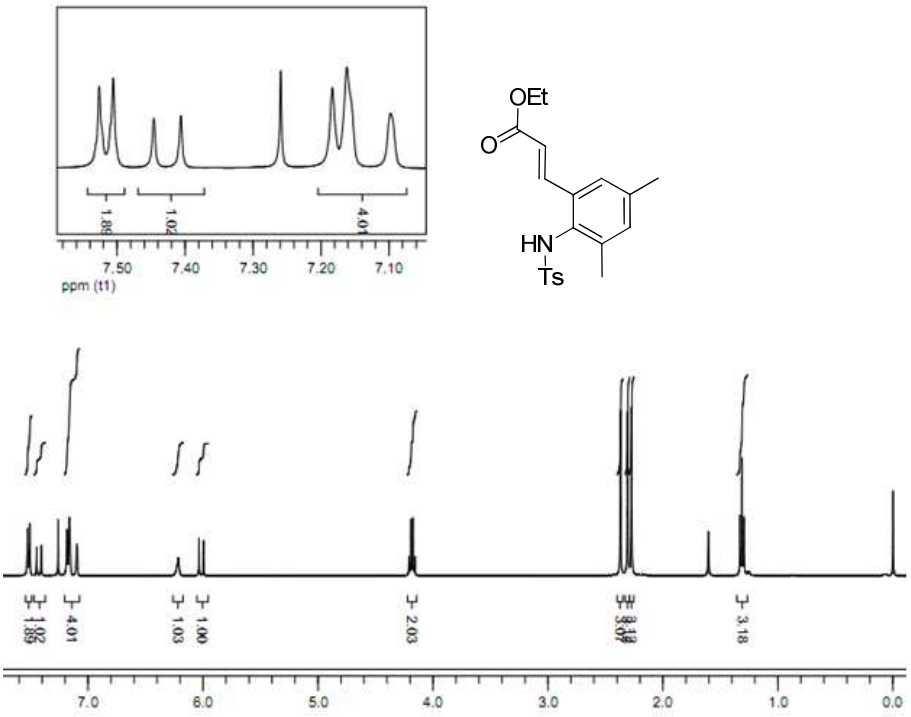




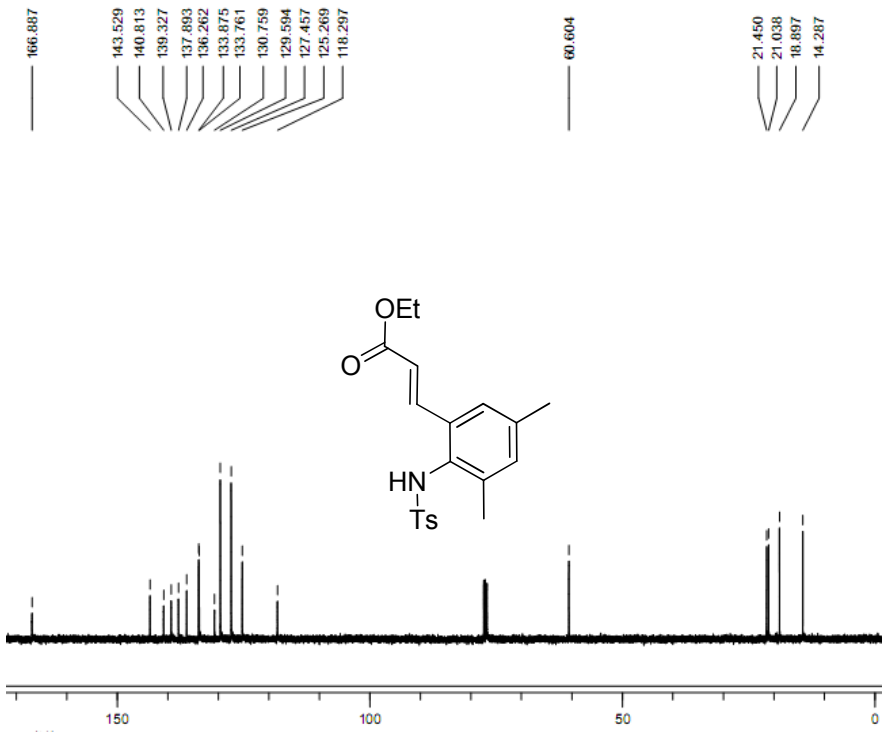

$^{1}$H NMR spectra of compound **33a** ($CDCl_3$, 400 MHz)



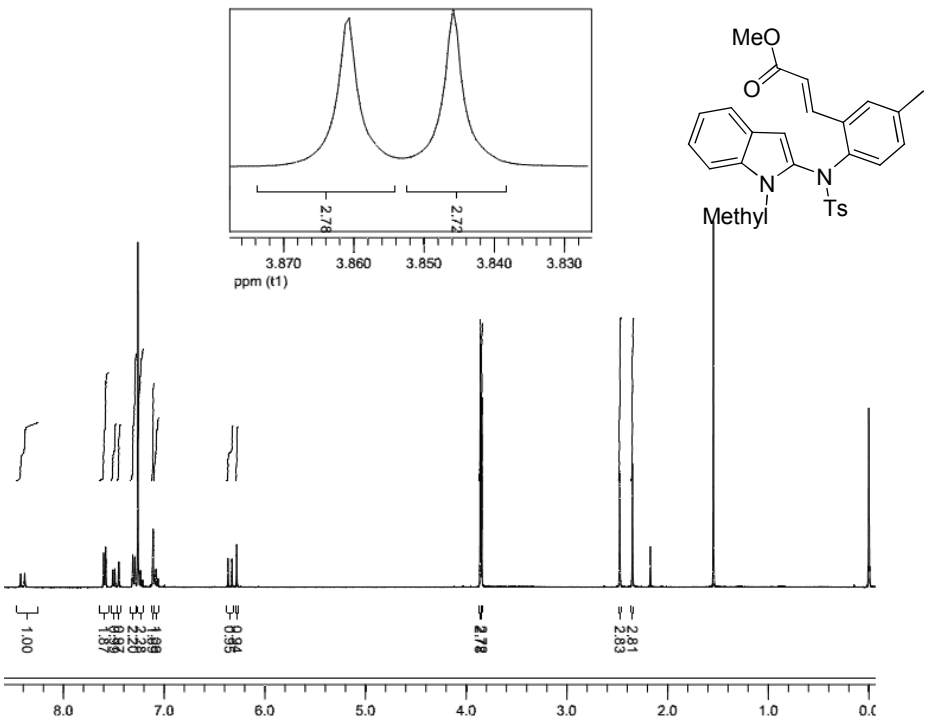

$^{13}$C NMR spectra of compound **33a** ($CDCl_3$, 100 MHz)



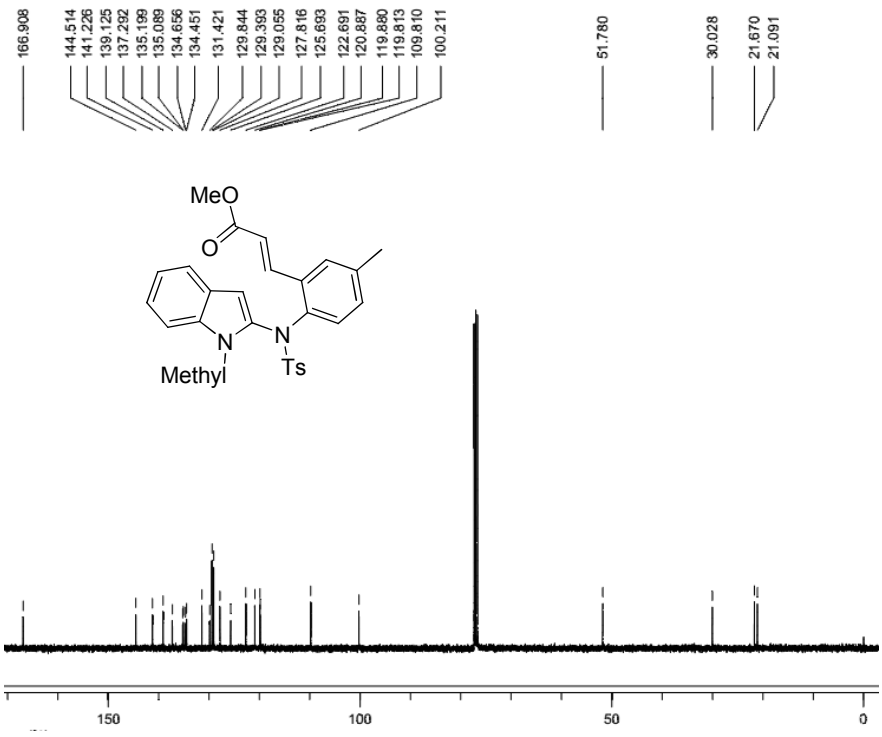

$^{1}$H NMR spectra of compound **33k** ($CDCl_3$, 400 MHz)



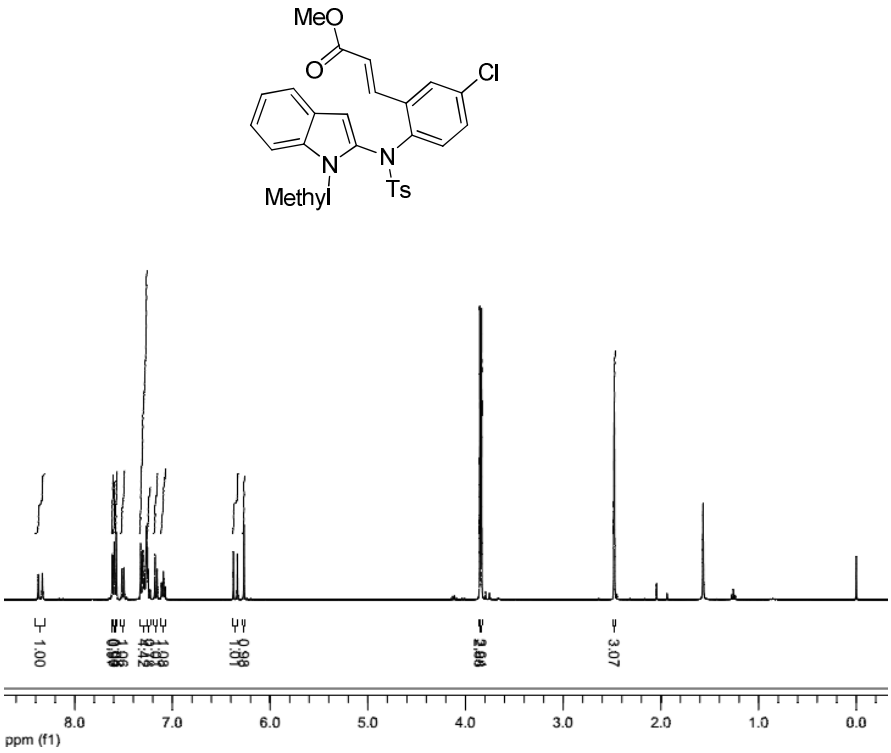

$^{13}$C NMR spectra of compound **33g** ($CDCl_3$, 100 MHz)



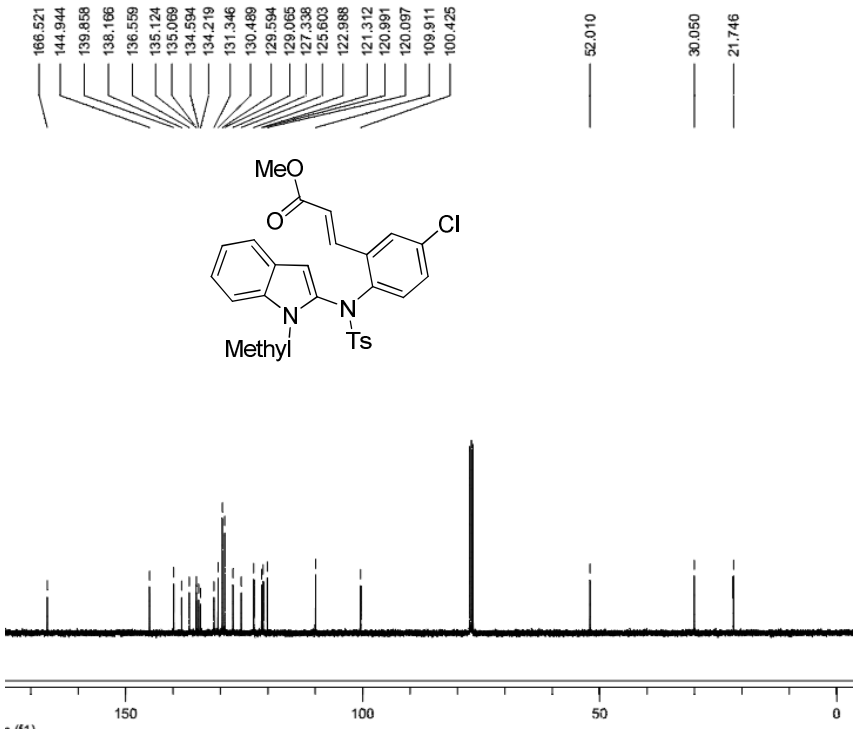

$^{1}$H NMR spectra of compound **37g** ($CDCl_3$, 400 MHz)



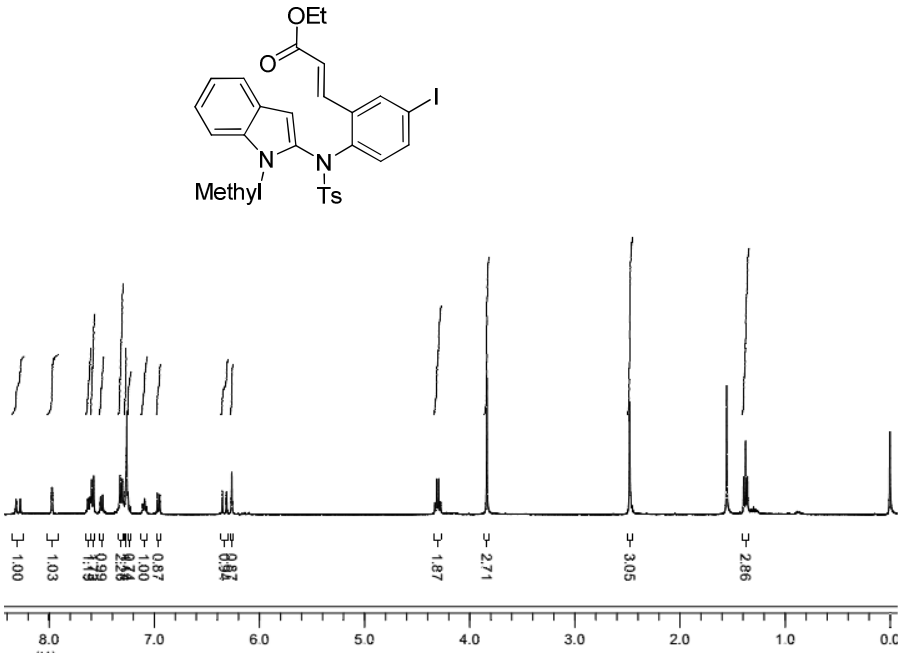

$^{13}$C NMR spectra of compound **37g** ($CDCl_3$, 100 MHz)



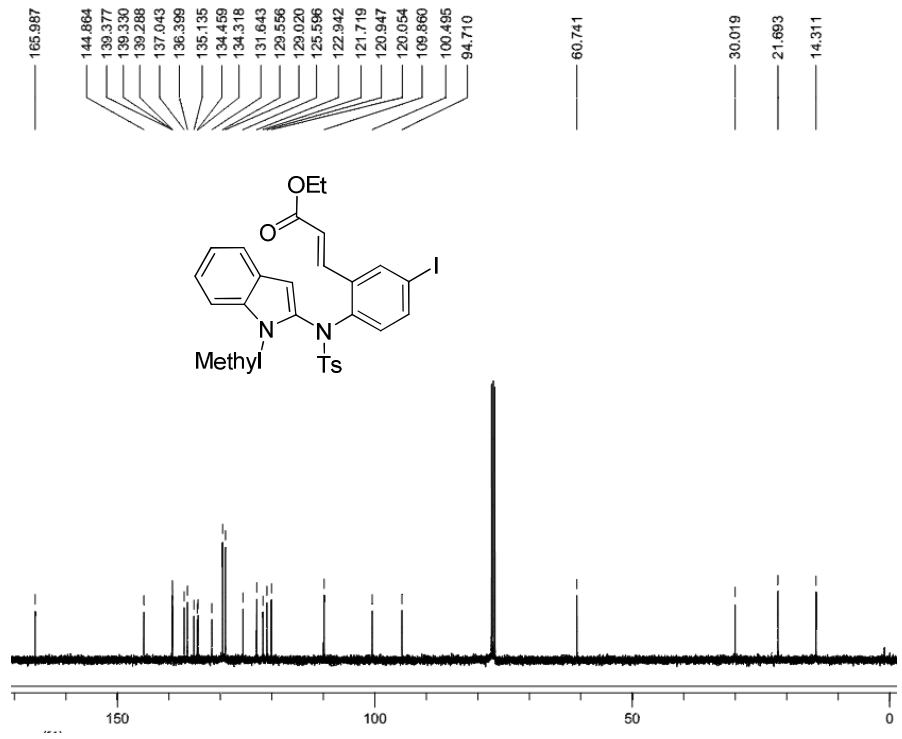

$^{1}$H NMR spectra of compound **37i** ($CDCl_3$, 400 MHz)



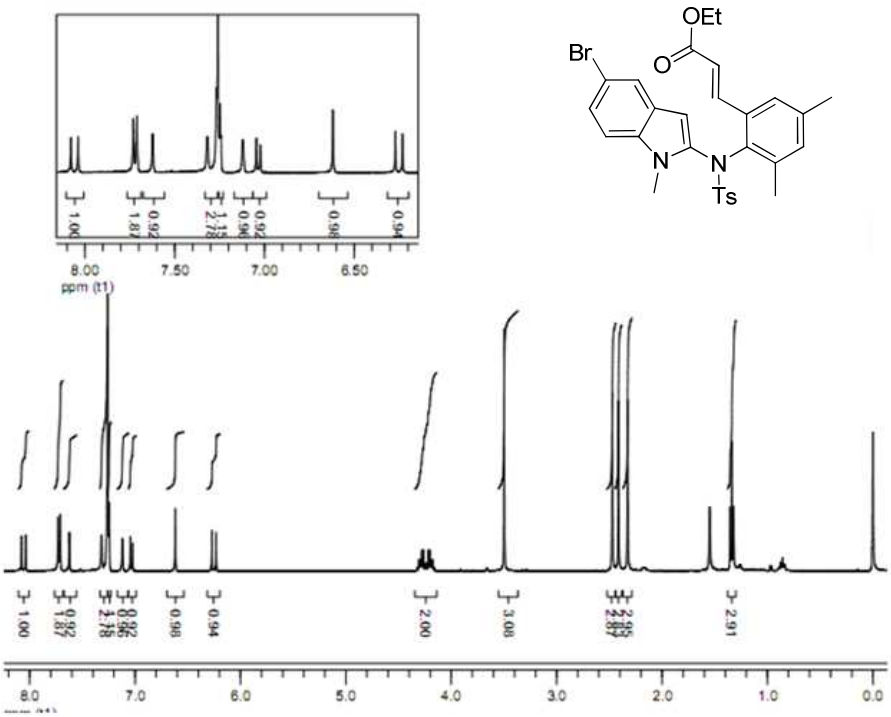

$^{13}$C NMR spectra of compound **37o** ($CDCl_3$, 100 MHz)



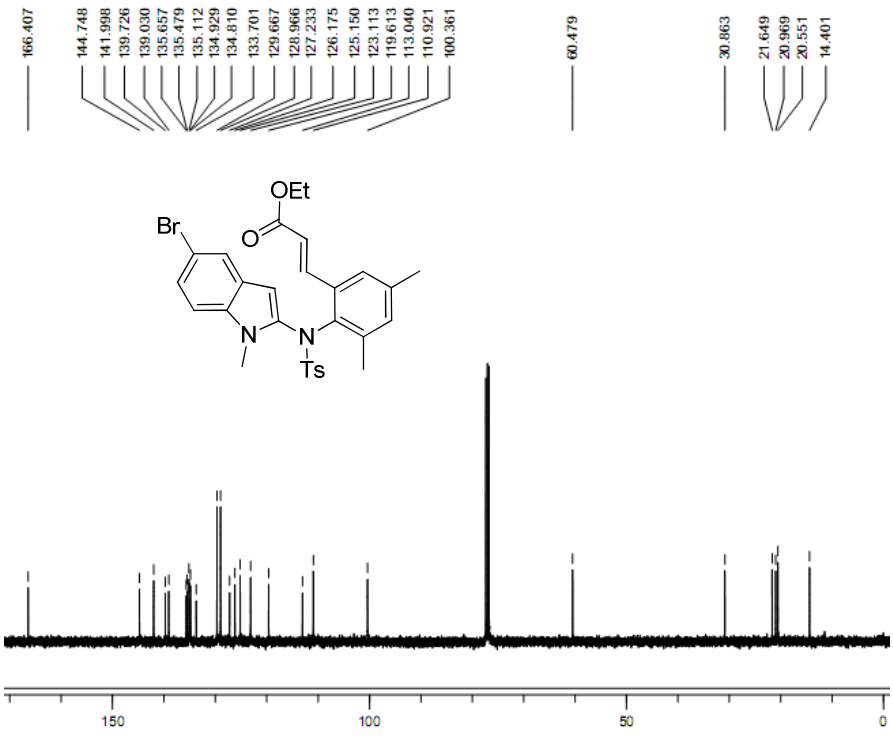

$^{1}$H NMR spectra of compound **37s** ($CDCl_3$, 400 MHz)



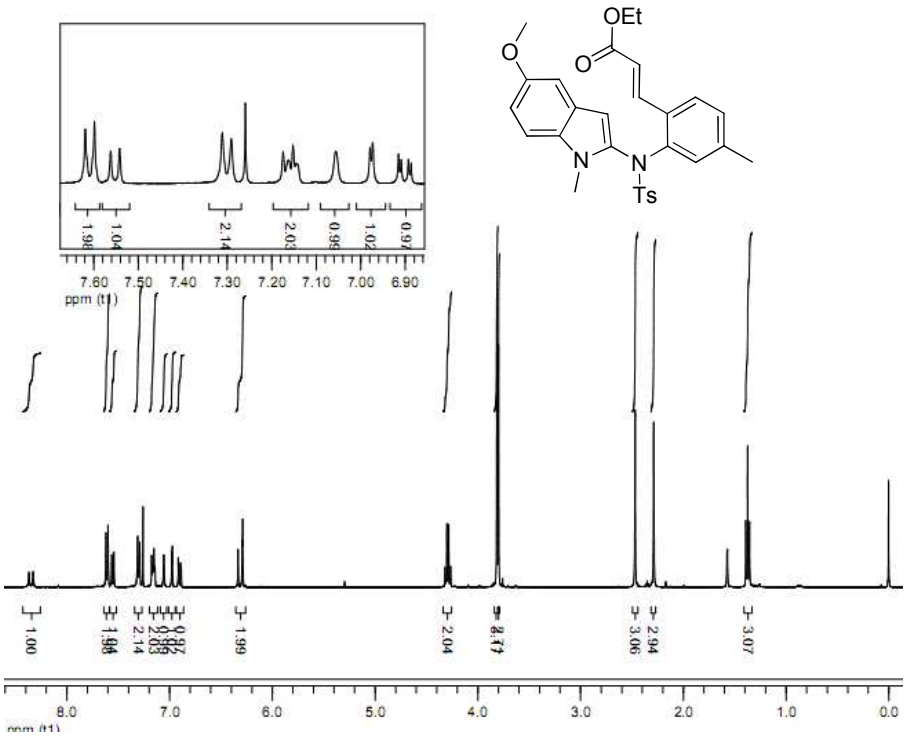

$^{13}$C NMR spectra of compound **37s** ($CDCl_3$, 100 MHz)



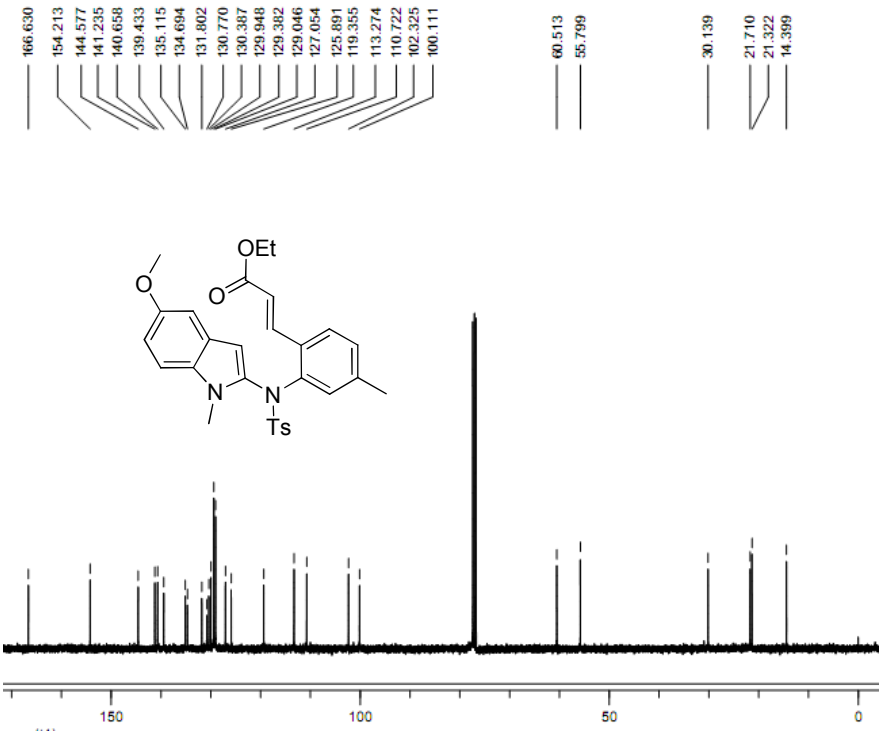

$^{1}$H NMR spectra of compound **37t** ($CDCl_3$, 400 MHz)



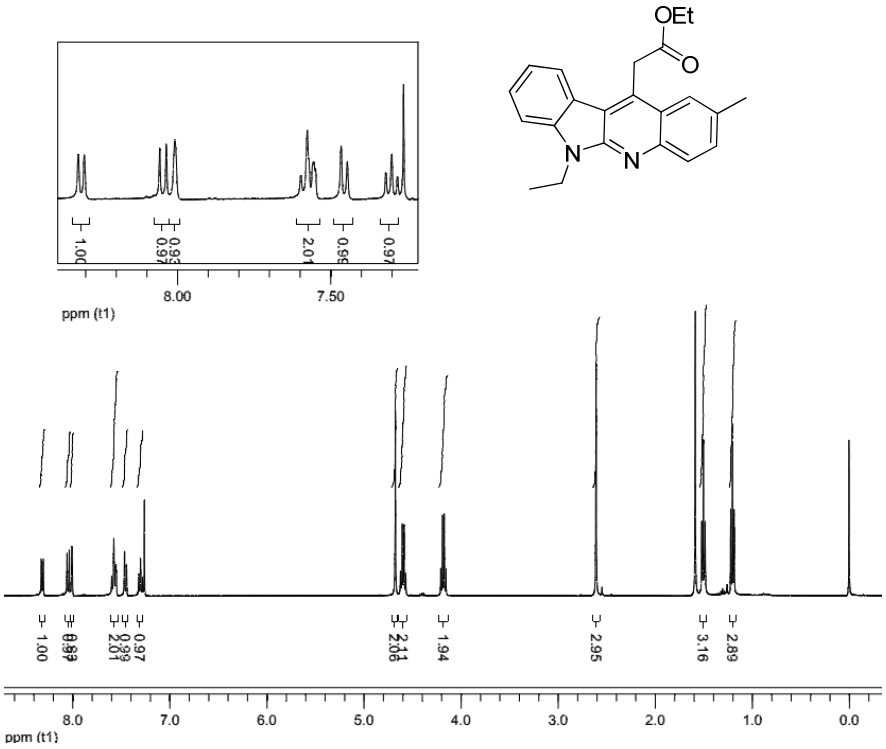

$^{13}$C NMR spectra of compound **37t** ($CDCl_3$, 100 MHz)



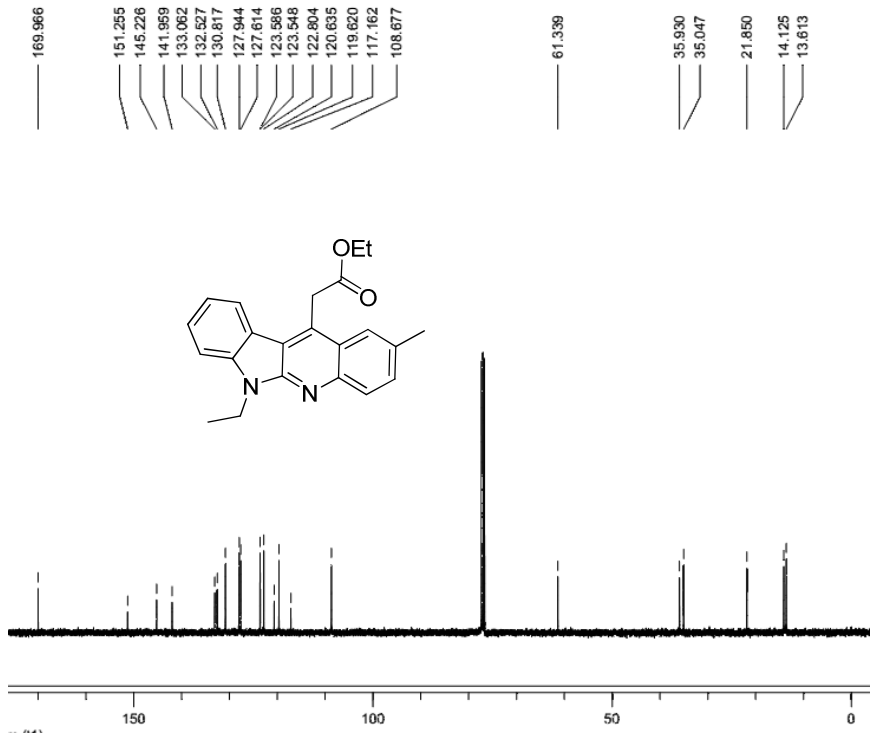

$^{1}$H NMR spectra of compound **37u** ($CDCl_3$, 400 MHz)



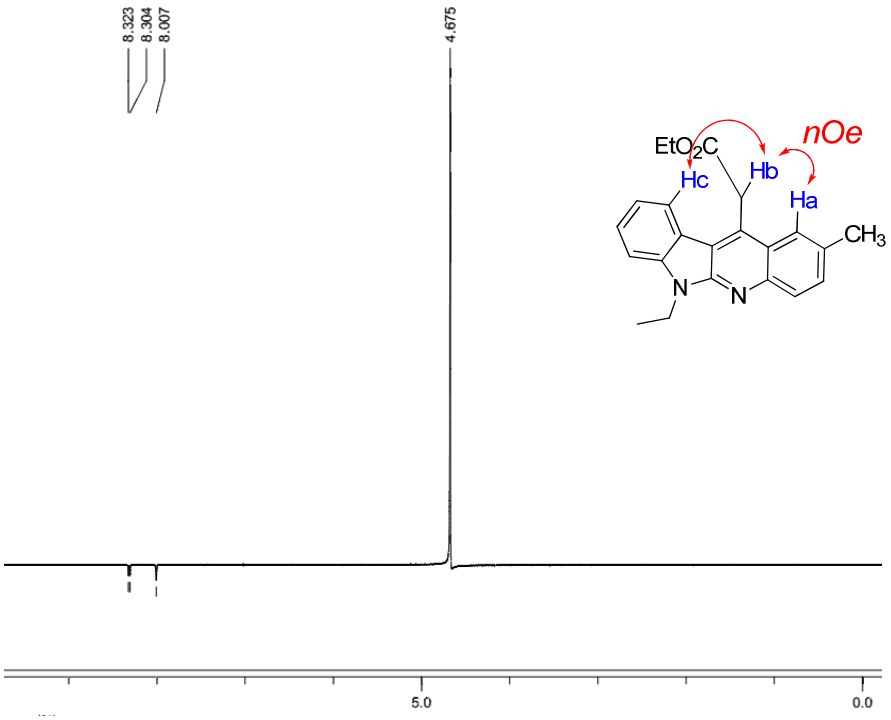

$^{13}$C NMR spectra of compound **37u** ($CDCl_3$, 100 MHz)



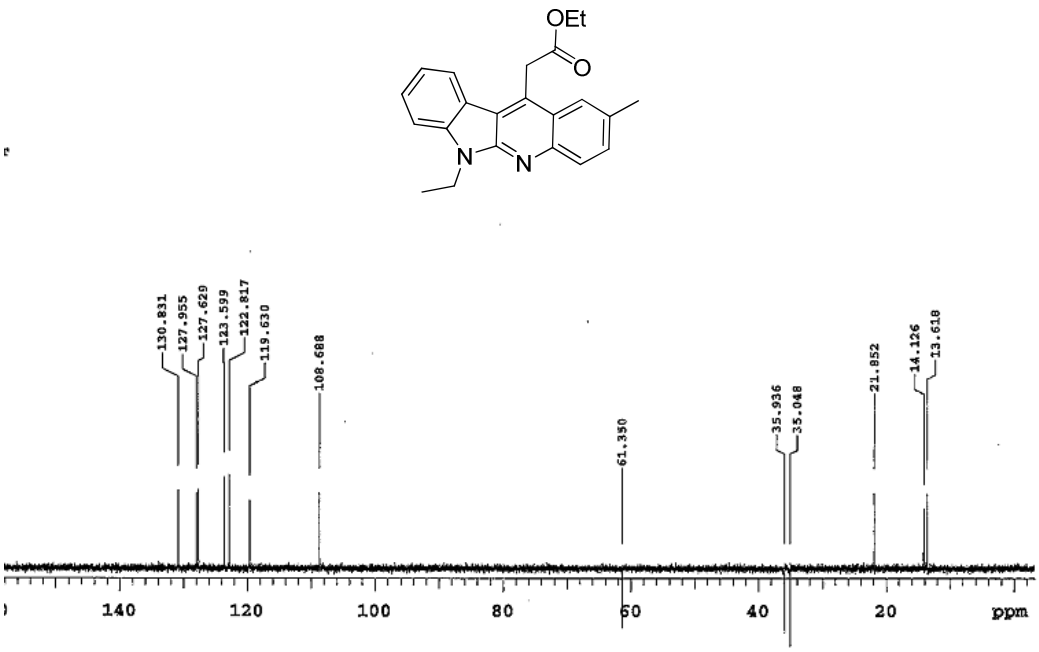

$^{1}$H NMR spectra of compound **38b** ($CDCl_3$, 400 MHz)



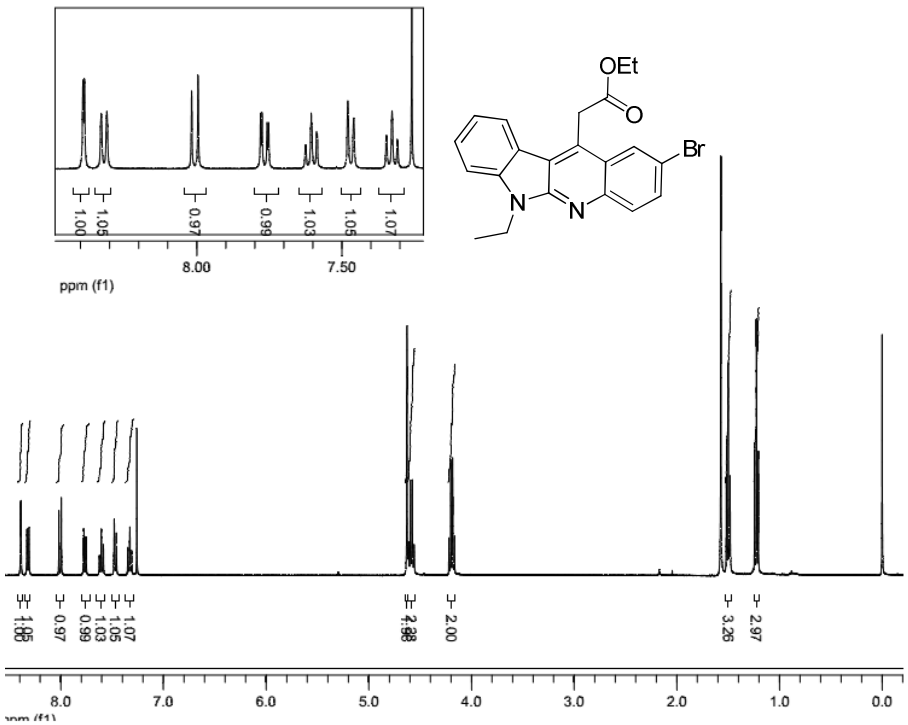

$^{13}$C NMR spectra of compound **38b** ($CDCl_3$, 100 MHz)



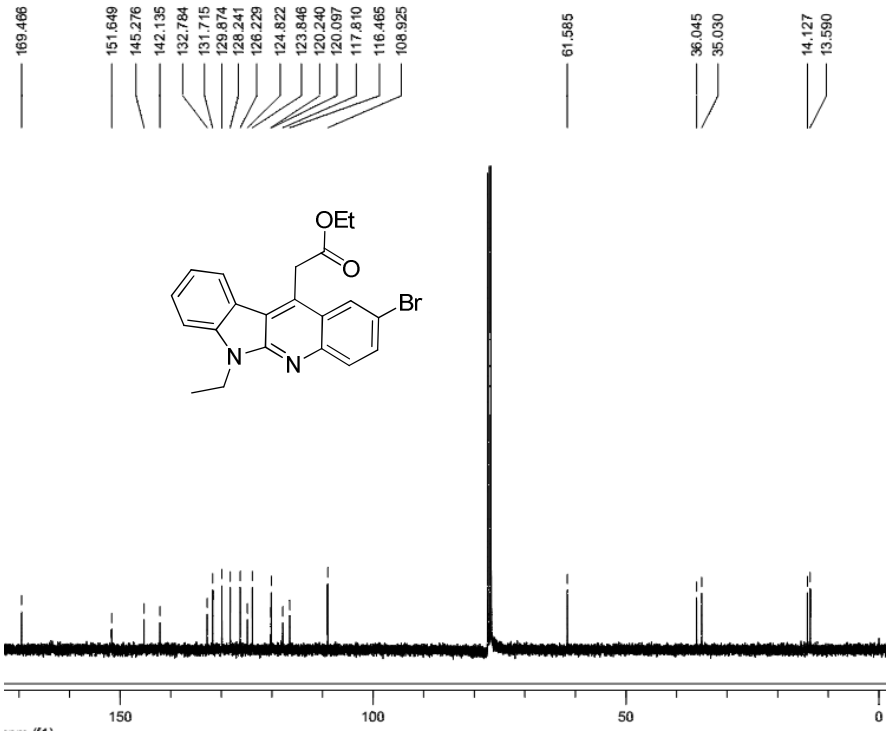

1D-NOE spectra of compound **38b**



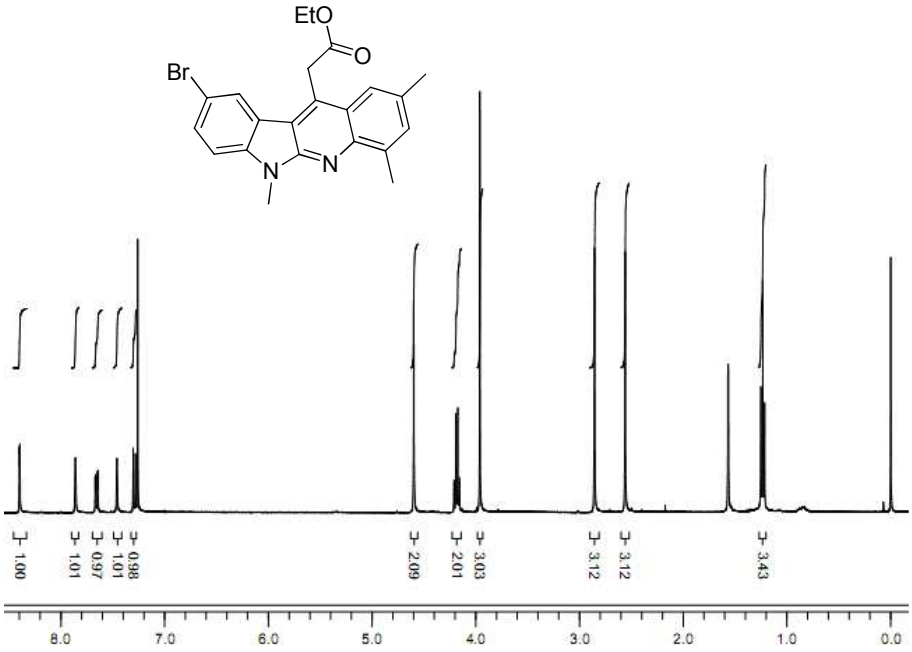

DEPT spectra of compound **38b**



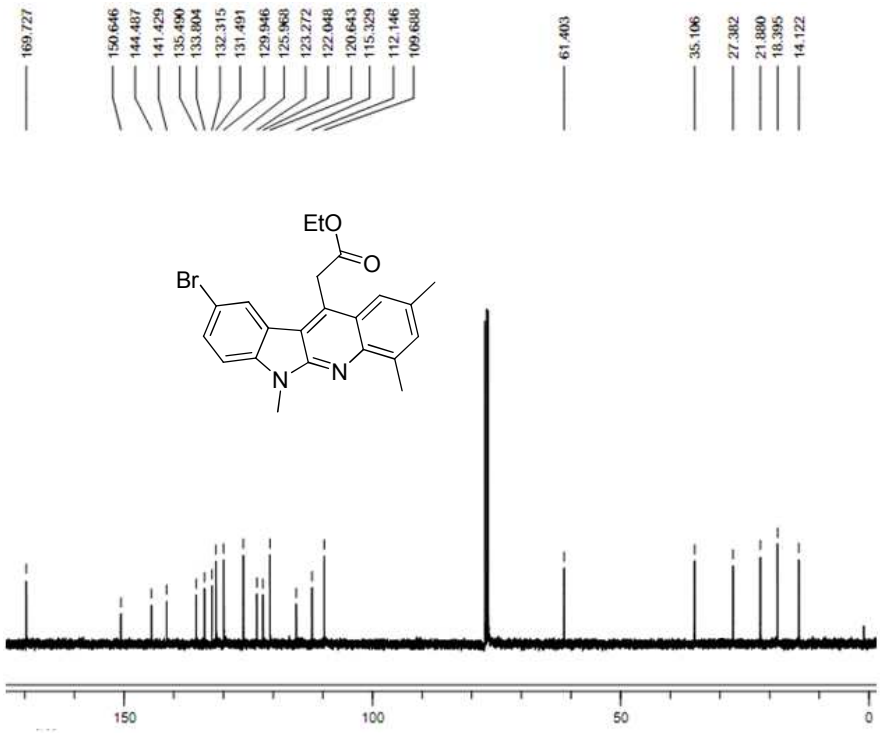

$^{1}$H NMR spectra of compound **38f** ($CDCl_3$, 400 MHz)



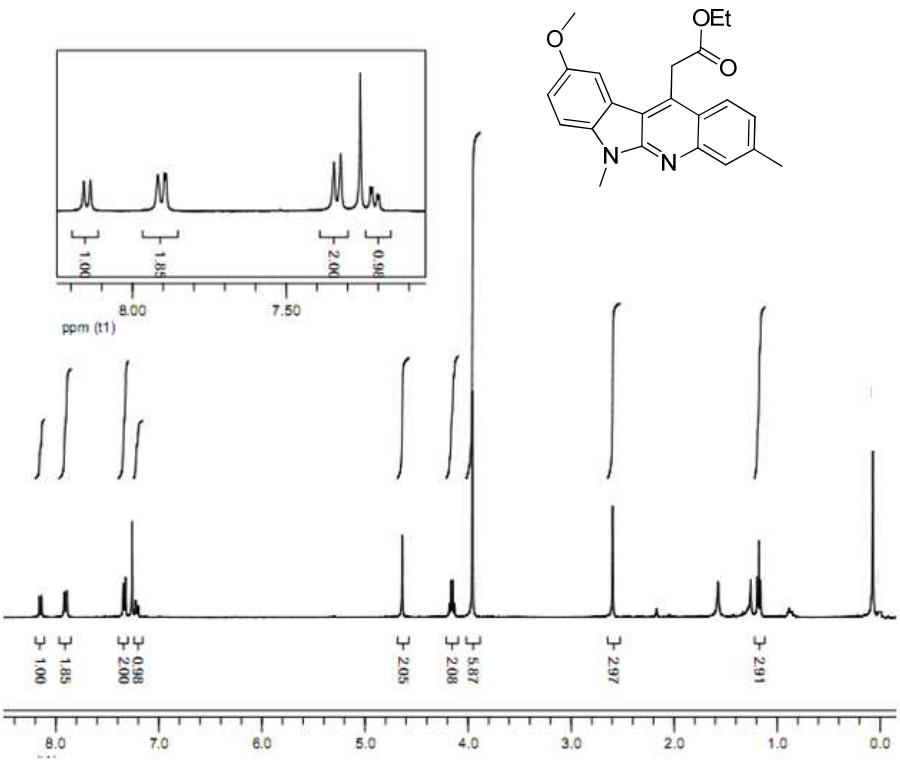

$^{13}$C NMR spectra of compound **38f** ($CDCl_3$, 100 MHz)



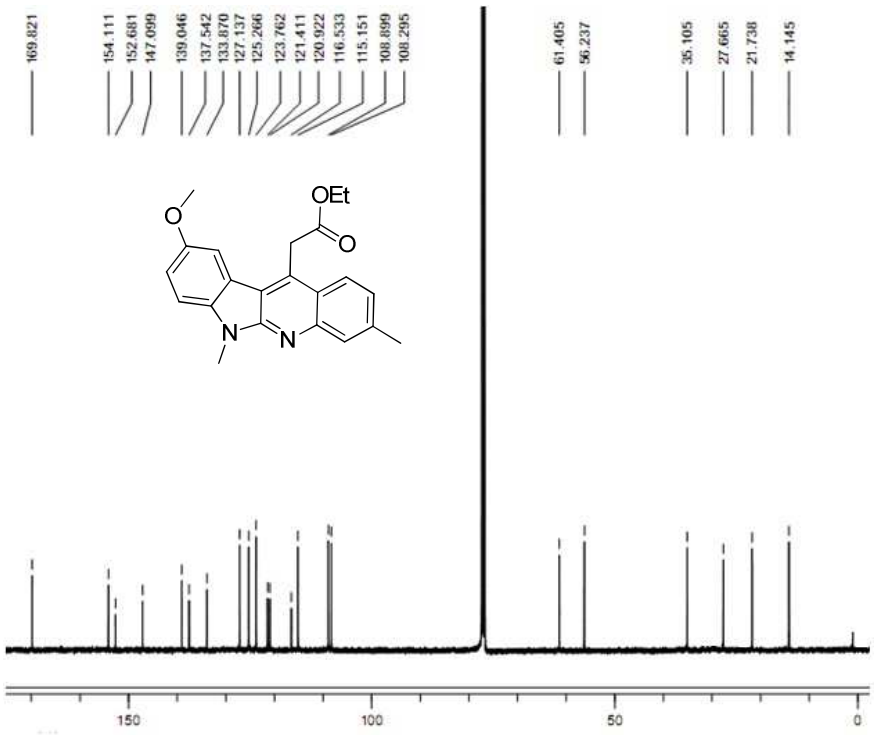

$^{1}$H NMR spectra of compound **38t** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **38t** ($CDCl_3$, 100 MHz)



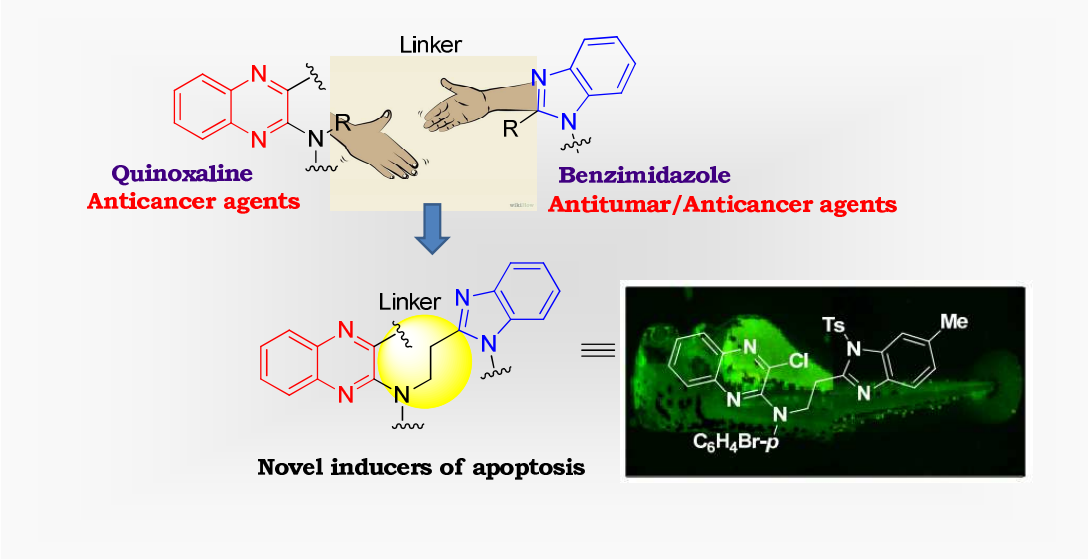

$^{1}$H NMR spectra of compound **38u** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **38u** ($CDCl_3$, 100 MHz)

# *CHAPTER 5*

# Copper-catalyzed one-pot synthesis of hybrid benzo[*d*]imidazoloquinoxalines as potential inducers of apoptosis



## 5.1. Introduction:

Hybrid molecules are chemical entities with two (or more) structural domains having different biological functions that show dual activity (e.g. **A**, Figure 5.1), and can act as two distinct pharmacophores.[1] Both entities of the hybrid molecule are not necessarily acting on the same biological target. Nevertheless, the strategy of hybrid molecule is also used to enhance the pharmacological activities of the individual pharmacophore (e.g. **B**, Figure 5.1). For example, weak cytotoxicity of distamycin A (a naturally occurring antibiotic agent isolated from *Streptomyces distallicus* active against some viruses) has been enhanced by tethering it with the known antitumor compounds or simple active moieties of known antitumor agents (Figure 5.2).[2]

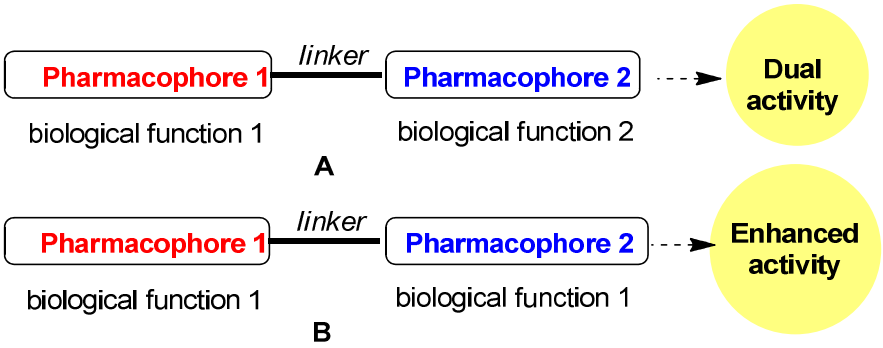



**Fig. 5.1**. Hybrid molecule **A** and **B.**

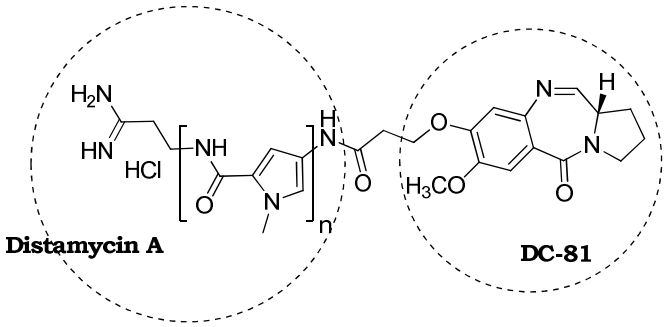



**Fig. 5.2**. Hybrid molecule of distamycin A.

Bleomycin (Figure 5.3, glycopeptide) is an excellent example of a hybrid molecule-based drug which was isolated from *Streptomyces Verticillus* by Umezawa and coworkers, and is an efficient anticancer agent.[3] This drug has three distinct structural domains: one for DNA binding, a second for metal binding, and a third containing carbohydrates which is essential for the permeability to the cell

membrane.

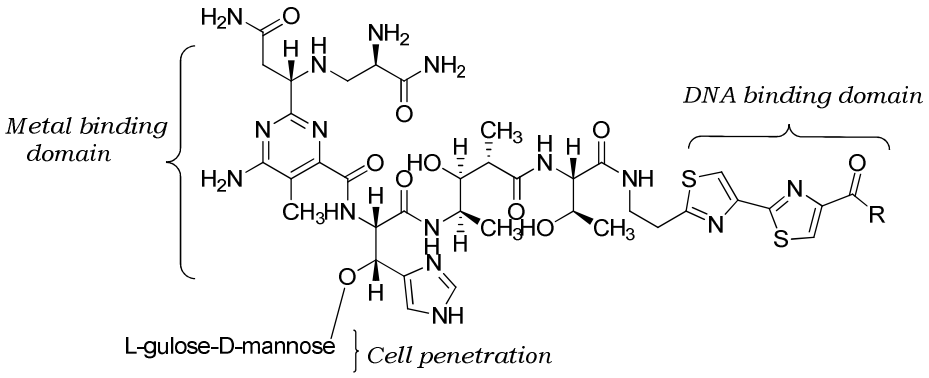



**Fig. 5.3**. Three distinct structural domains of bleomycin.

Hybrid molecules with a dual mode of action can be classified into three different categories. The category **A** (Figure 5.4) relates to both entities of the hybrid molecule that are able to interact with the single target. For example in the case of antimalarial drugs trioxaquines (**1,** Figure 5.4) a "double-edged sword" hybrid molecule was developed and the two pharmacophores of trioxaquines[4] were able to interact with the heme (a single target). These molecules are highly active campared to their corresponding two separate precursors.

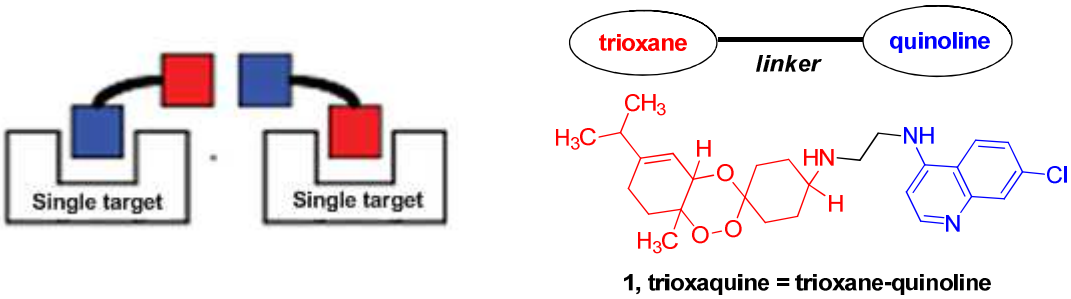



**Fig. 5.4**: Type **A** hybrid molecules with single target.

In the second category **B** (Figure 5.5), the two entities of the hybrid molecule act independently on two different and non related targets. This case has been recently demonstrated by developing of both acetylcholinesterase and serotonin transporters inhibitors for the treatment of Alzheimer's disease.[5]

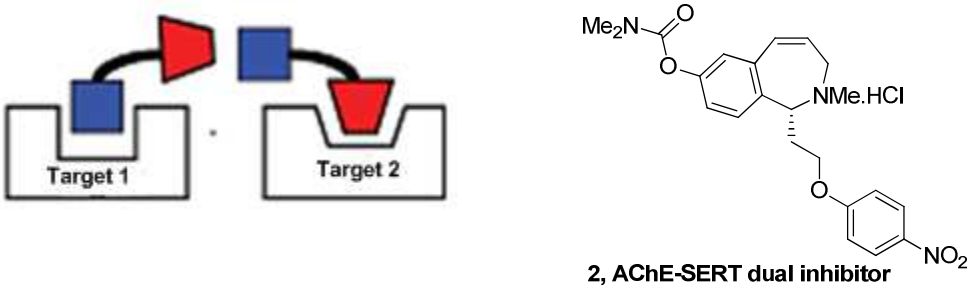



**Fig. 5.5**: Type **B** hybrid molecules with two independent targets.

In case of third category C (Figure 5.6), both entities of the hybrid molecule acts on the related targets. For example in the case of antiprion drugs a hybrid molecule (**3,** Figure 5.6) was developed by linking acridines to iminodibenzyl entities and the two pharmacophores were able to interact with two related targets. These hybrid molecules was the most active antiprion compounds with an $EC_{50}$ value of 20 nM determined using a cell model of prion disease.[6]

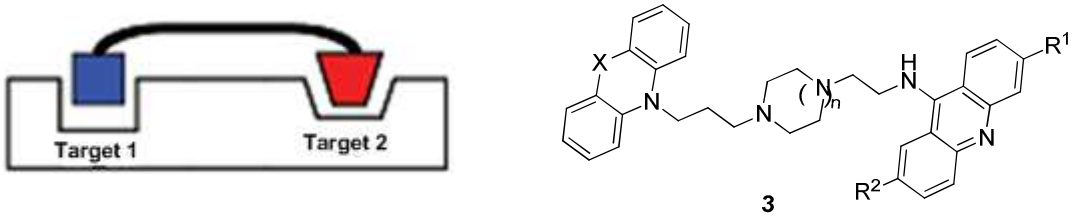



**Fig. 5.6**: Type **C** hybrid molecules with two related targets.

### 5.1.1. Literature reports on hybrid molecules:

In 2007, Walsh and coworkers demonstrated a novel artemisinin–quinine hybrid molecule (**4**, Figure 5.7) with potent antimalarial activity.

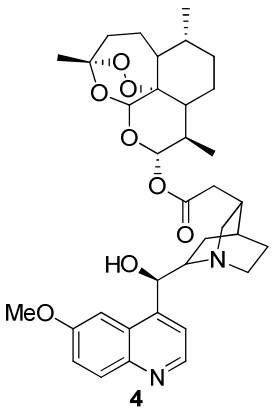
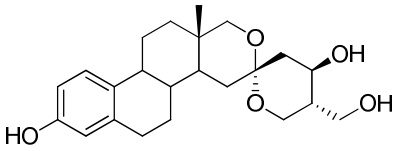



**Fig. 5.7**: Artemisinin–quinine based hybrid molecule

The hybrid was more effective against drug-sensitive and drug-resistant malaria than the individual drugs alone suggested that the two molecules joined together were more active than the same two molecules administered separately.[7]

In 2000, Tietze and coworkers illustrated the structural features of a highly active mycotoxin(-)-talaromycin **B** with the hormone estrone to design a novel hybridmolecule (**5,** Figure 5.8) that exhibited anticancer activity.[8]




**5**

**Fig. 5.8**: Estron-talaromycin based hybrid molecule.

Guantai and coworkers reported a series of analogs, based on the chalcone chloroquinoline hybrid (**6,** Figure 5.9) as the promising antimalarial agents. Thus, aiming to identify new analogs with improved solubility and retained antimalarial potency they again devised a novel hybrid framework (**7**) *via in silico* studies as shown in Figure 5.9.[9]

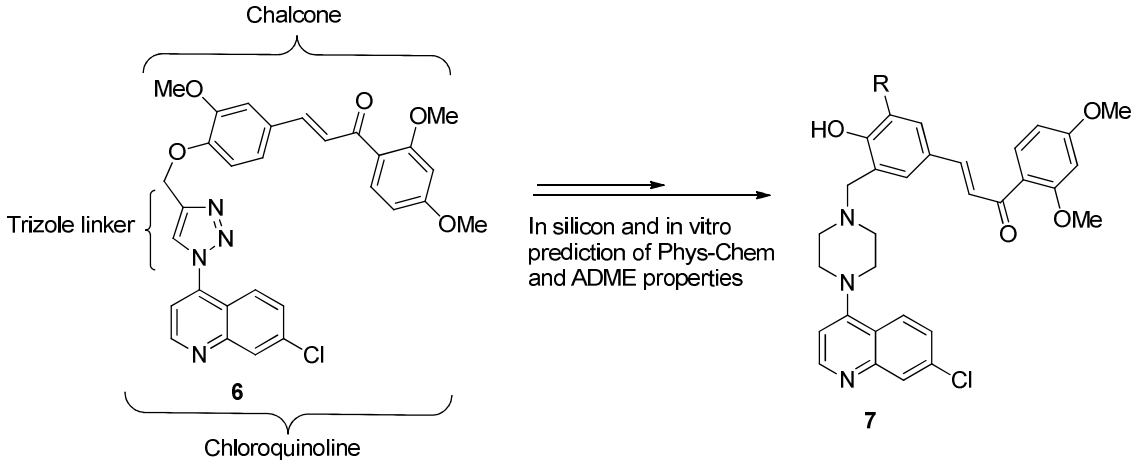
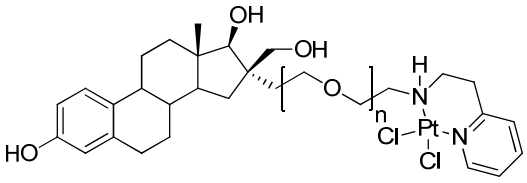



**Fig. 5.9**: Chalcone-chloroquinoline based hybrid molecule.

In 2000, Berube and coworkers synthesized a series of novel 17$\beta$-estradiol-platinum(II) hybrid molecules (**8,** Figure 5.10) made of a polyethylene glycol (PEG) linking chain of various length and 2-(2´-aminoethyl)pyridine ligand. The hybrid

molecule possesses cytotoxic activity on breast cancer cell lines and the derivatives with the longest PEG chain showed the best activity.[10]




**8**

**Fig. 5.10**: 17$\beta$-estradiol-platinum(II) hybrid molecule.

### 5.1.2. Design of our target hybrid molecule C:

We discussed in Chapter 4 that the quinoxaline framework has been reported to be integral part of several anticancer agents.[11] The benzimidazole nucleus on the other hand has also found to be present in various antitumor/anticancer agents.[12] Thus, we anticipated that the combination of these two moieties connected through an appropriate linker in a single molecular entity may provide a new hybrid molecule (**C,** Figure 5.11) for the design and identification of novel inducers of apoptosis. To the best of our knowledge pharmacological evaluation of this class of compounds especially as apoptotic agents has not been explored earlier.

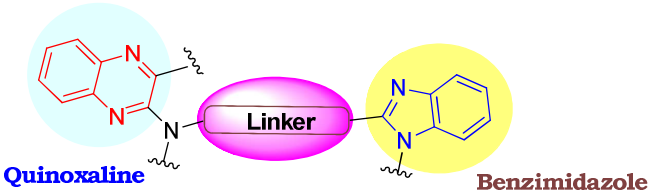
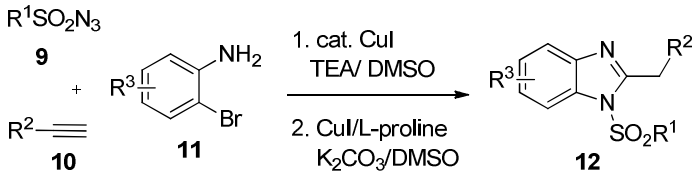



**Fig. 5.11:** New framework (**C**) for the design and identification of novel inducers of apoptosis

## 5.2. Results and discussion:

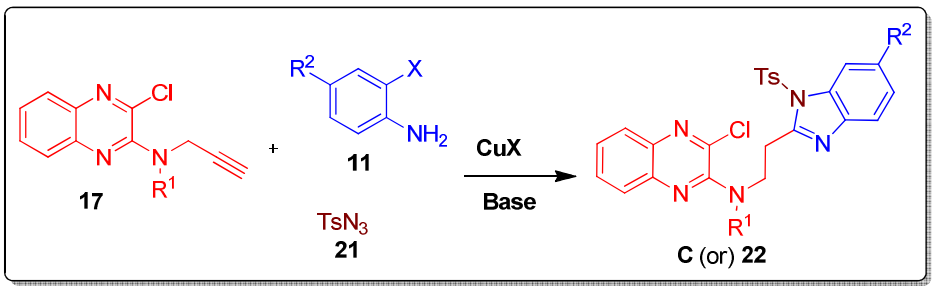
Synthesis of type **C** hybrid molecules (Figure 5.11) with quinoxaline and benzimidazole as pharmacophores, we required a direct and straightforward synthetic method. A literature studies disclosed that the synthesis of 2-substituted benzimidazole can be performed *via* the reaction of 1,2-phenylenediamines with the corresponding carboxylic acids or with aldehydes followed by oxidation[13] and which can be functionalized further such as sulfonylation using an alkyl or aryl sulfonyl

chloride.[14] This method however appeared to be less effective for the synthesis of molecules based on **C** as the introduction of quinoxaline moiety seemed to be difficult. This problem was found to be common with the other methods of constructing 1,2-disubstituted benzimidazole moiety possessing the required quinoxaline substituent. However, during the literature search a recent report on Cu-catalyzed 3-component cascade reaction of sulfonyl azides, alkynes and 2-bromoaniline (Scheme 5.1) leading to the functionalized benzimidazoles[15] attracted our attention which is shown in Scheme 5.1.




**Scheme 5.1**: Three-component synthesis of benzimidazoles (**12)**.

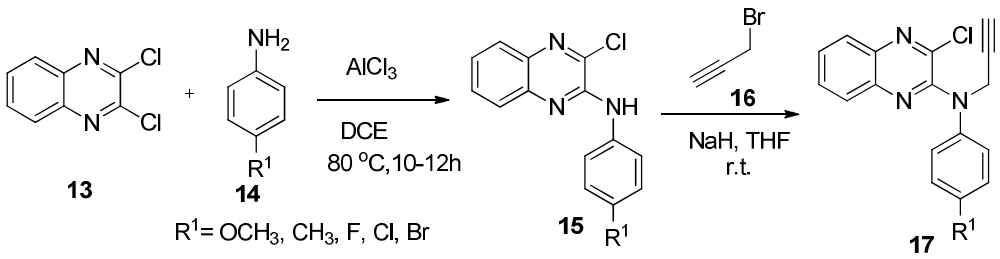
The substitution pattern on the benzimidazole ring was very similar to that we were aiming for. Indeed, we envisioned that the incorporation of quinoxaline moiety into the alkyne reactant might afford our desired product in a single step.




**Scheme 5.2**: Synthesis of hybrid molecules (**22)** based on **C.**

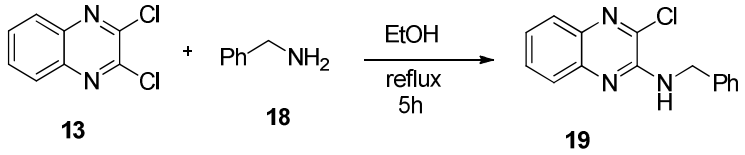
### 5.2.1. Preparation of starting materials:

The key starting material *i.e*. 3-chloro-*N*-aryl-*N*-(prop-2-ynyl)quinoxalin-2-amine (**17**), required for our study was obtained *via* $AlCl_3$ induced C-N bond formation reaction between 2,3 dichloroquinoxaline **(13)** and substituted anilines (**14)** in dichloroethane at 80 °C to afforded compound (**15**). Further on propargylation of compound (**15**) with propargyl bromide in the presence of sodium hydride in THF gives the product (**17**) as shown in Scheme 5.3 (mechanism for $AlCl_3$ induced C-N bond formation reaction is explained in Chapter 4).[16]



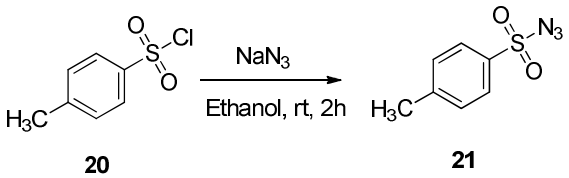

**Scheme 5.3**: Synthesis of key starting material (**17)**.

The requisite other starting material *N*-benzyl-3-chloroquinoxalin-2-amine (**19**) was prepared *via* the reaction of 2,3-dichloroquinoxaline (**13**) with benzyl amine (**18**) under reflux in ethanol as shown in Scheme 5.4.[17]




**Scheme 5.4**: Synthesis of *N*-benzyl-3-chloroquinoxalin-2-amine (**19**).

The requisite 4-methylbenzenesulfonyl azide (**21**) was prepared by the reaction of tosyl chloride (**20)** with sodium azide in ethanol at room temperature as shown in Scheme 5.5.[18]



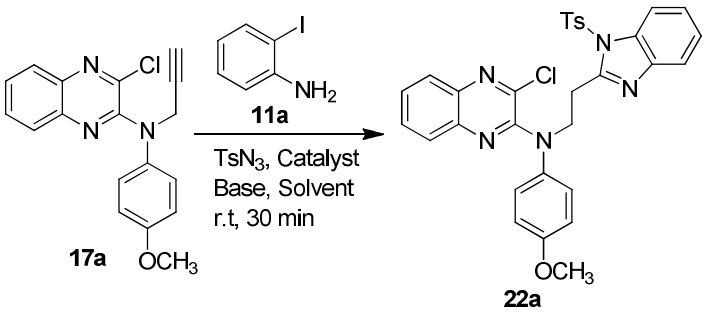

**Scheme 5.5**: Synthesis of 4-methylbenzenesulfonyl azide (**21**).

### 5.2.2. Reaction optimization:

Having prepared the required starting materials according to the reported methods we commenced our studies by the reaction of 3-chloro-*N*-(4-methoxyphenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (**17a**) with 2-iodoaniline (**11a**) and tosyl azide (**21**) to establish the optimized reaction conditions. The reaction was initially performed in the presence of CuI (10 mol%) and $Et_3N$ at room temperature in the absence of any ligand in a number of solvents to investigate the effect of solvent. This analysis revealed that the reaction medium significantly affects the yields of

product shown in Table 5.1. Solvents like THF, MeCN, DCM (dichloromethane), toluene, DMSO and DMF (entries 1-7, Table 5.1) were examined and found that the reaction proceeded well in all these solvents affording the desired product (**22a)** in variable yields. The best result was obtained when the reaction was performed in DMSO (entry 6, Table 5.1) affording (**22a)** in 94% yield. When we changed the catalyst to $Cu(OTf)_2$ and base to $K_2CO_3$ (entry 10, Table 5.1) the product yield was decreased indicating that they were not effective for this cascade reaction.

**Table 5.1**: Reaction conditions and optimization.




| Entry | Catalyst | Base | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | CuI | $Et_3N$ | THF | 32 |
| 2 | CuI | $Et_3N$ | $CH_3CN$ | 57 |
| 3 | CuI | $Et_3N$ | DCM | 65 |
| 4 | CuI | $Et_3N$ | DCE | 66 |
| 5 | CuI | $Et_3N$ | Toluene | 48 |
| 6 | CuI | $Et_3N$ | DMSO | 94 |
| 7 | CuI | $Et_3N$ | DMF | 88 |
| 8 | CuI | $Et_3N$ | DMSO | 94[c] |
| 9 | $Cu(OTf)_2$ | $Et_3N$ | DMSO | 10 |
| 10 | CuI | $K_2CO_3$ | DMSO | 70 |

[a]All the reactions are carried out using compound **17a** (0.30 mmol), **11a** (0.34 mmol), $TsN_3$ (0.36 mmol), in the presence of a Cu catalyst (0.03 mmol) and base (0.36 mmol) in a solvent (2 mL) at room temp for 30 min under nitrogen. [b]Isolated yield. [c]The reaction was carried using 30 mol% CuI and completed within 10 min.

We were delighted with this observation as the reaction proceeded in the absence of any ligand and was completed within 30 min. Moreover, the use of 10 mol% of CuI was found to be enough to catalyze this MCR though the use of higher quantity of catalyst e.g. 30 mol% of CuI completed the reaction within 10 min (entry 8, Table 5.1). Since the yield of (**22a)** was not improved further in this case hence the condition of entry 6 was identified as the best reaction conditions for further studies. Nevertheless, being truly an MCR the present method seemed to have advantages over the earlier method that was more of a cascade reaction and required the use of additional quantity of Cu catalyst as well as a different base to facilitate the second step.

### 5.2.3. Scope of the reaction:

Having identified the optimum condition (entry 6, Table 5.1) we then examined the substrate scope and generality of this method. Thus, a range of 3-chloro-*N*-(4-methoxyphenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (**17a**) with a number of 2-iodoaniline derivatives were employed in the present MCR and the results are summarized in Table 5.2. The reaction proceeded well in all these cases affording a variety of *N*-substituted 3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amines (**22**) in good to excellent yield (73-95%). All the compounds synthesized were well characterized by spectral (NMR and MS) data and the representative compound (**22a)** was confirmed by $^{1}$H and $^{13}$C NMR spectral data. Some of the characteristic signals appeared in $^{1}$H and $^{13}$C NMR spectra are shown in the following figure (Figure 5.12).

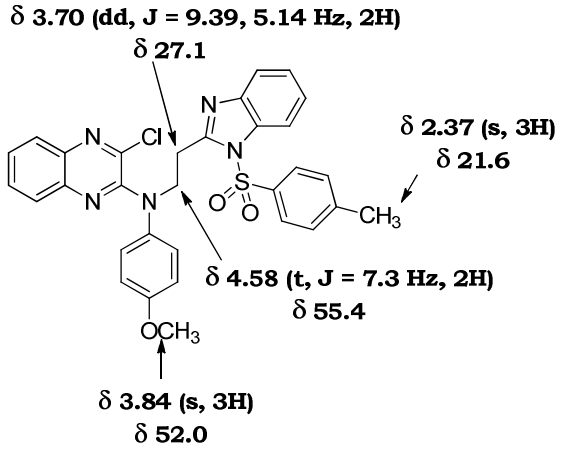
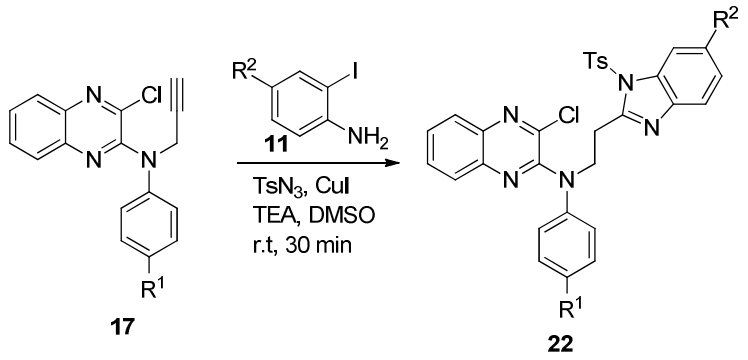
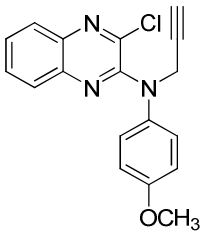
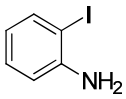
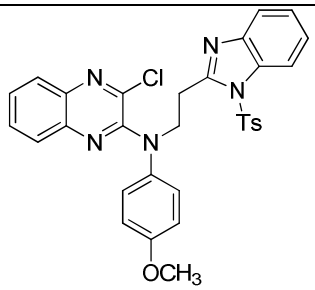
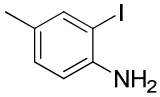
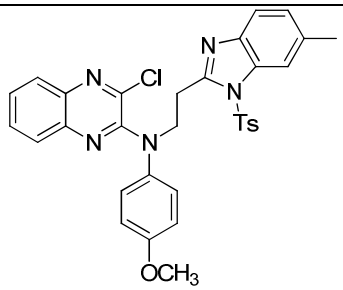
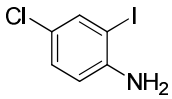
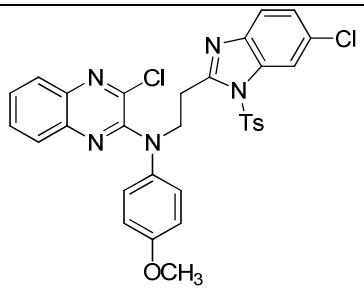


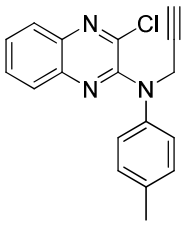
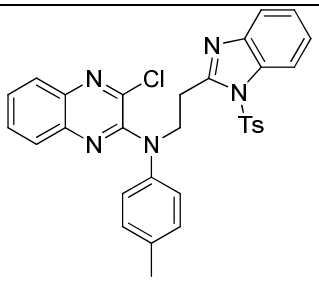
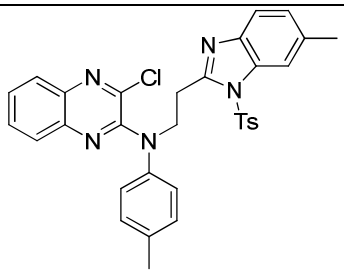
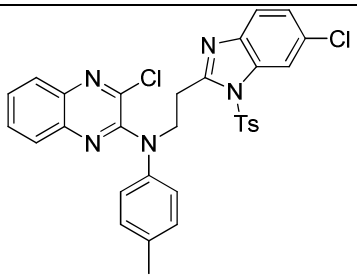
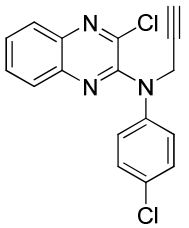
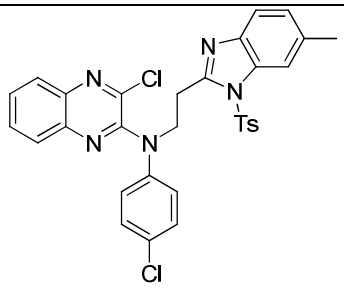
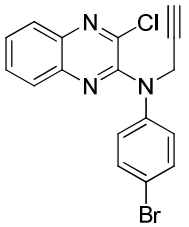
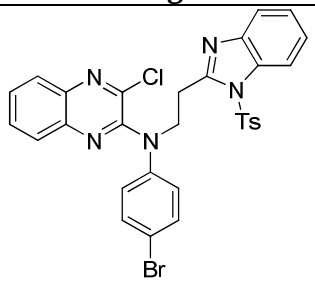

**Fig. 5.12**: Characteristic $^{1}$H and $^{13}$C NMR signals of (**22a)**

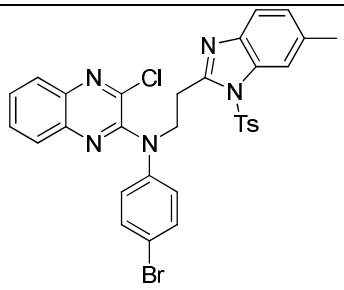
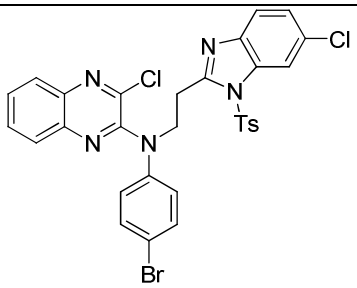
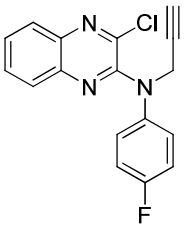
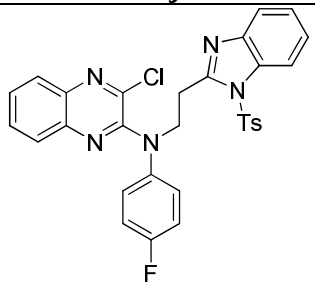
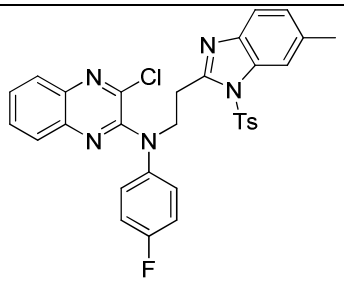
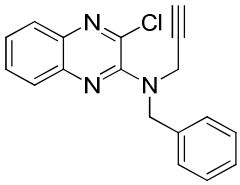
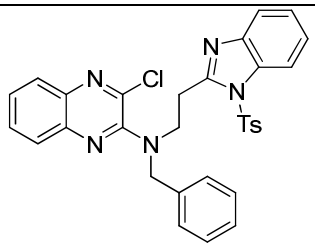
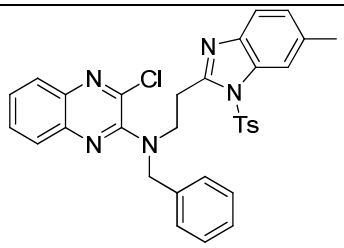
The appearance of singlets at δ 3.84 ppm and δ 2.37 ppm in $^{1}$HNMR spectra of (**22a)** were due to methoxy group present on the aromatic ring and methyl protons corresponding to tosyl group. The corresponding $^{13}$C signals appeared at δ 52.0, and δ 21.6 respectively. The two methylene protons present in (**22a**) appeared at δ 4.58, δ 3.70 in $^{1}$H NMR spectra and δ 55.4, δ 27.1 ppm in $^{13}$CNMR spectra respectively.

**Table 5.2:** Copper catalyzed synthesis of compound (**22**).[a]




| Entry | Alkyne (**17**) | Iodo Aniline (**11**) | Product **(7)** | Yield[b] (%) |
|---|---|---|---|---|
| 1 |  **17a** |  **11a** |  **22a** | 94 |
| 2 | **17a** |  **11b** |  **22b** | 89 |
| 3 | **17a** |  **11c** |  | 90 |

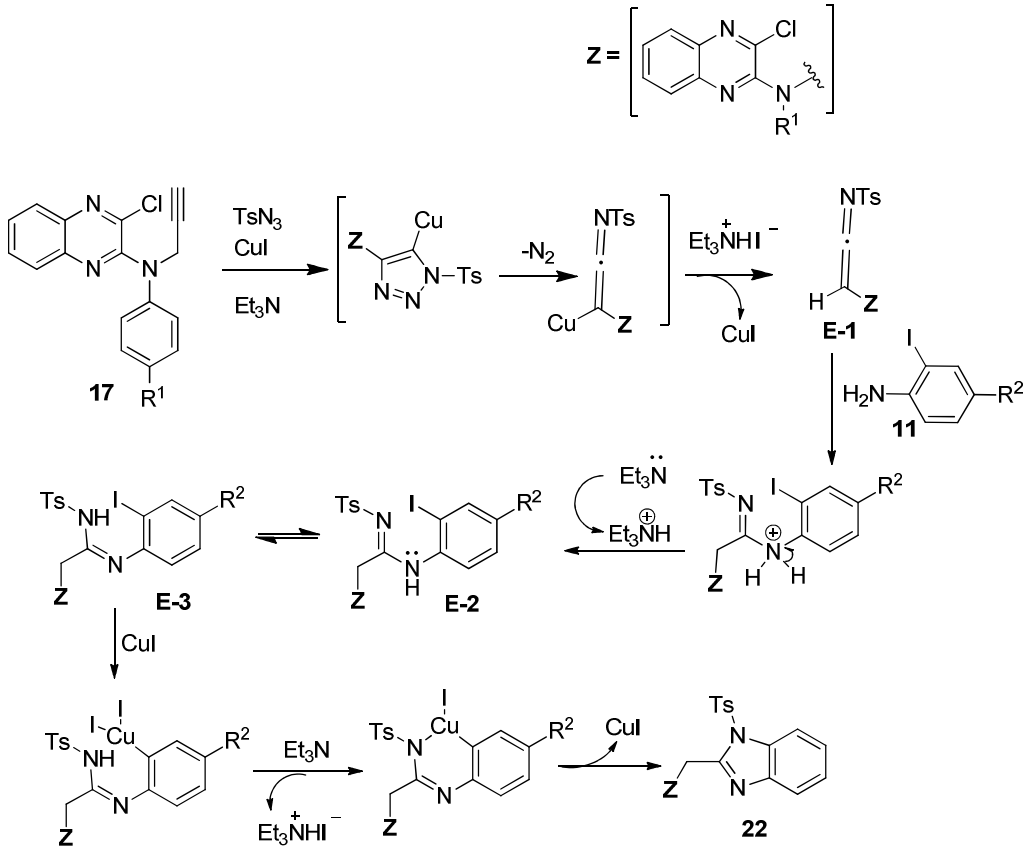
| | | | | |
|---|---|---|---|---|
| | | | **22c** | |
| 4 |  **17b** | **11a** |  **22d** | 92 |
| 5 | **17b** | **11b** |  **22e** | 86 |
| 6 | **17b** | **11c** |  **22f** | 90 |
| 7 |  **17c** | **11b** |  **22g** | 89 |
| 8 |  **17d** | **11a** |  **22h** | 95 |

| | | | | |
|---|---|---|---|---|
| 9 | **17d** | **11b** |  **22i** | 91 |
| 10 | **17d** | **11c** |  **22j** | 89 |
| 11 |  **17e** | **11a** |  **22k** | 93 |
| 12 | **17e** | **11b** |  **22l** | 86 |
| 13 |  **17f** | **11a** |  **22m** | 73 |
| 14 | **17f** | **11b** |  **22n** | 81 |

[a]All the reactions are carried out using compound **17** (0.30 mmol), **11** (0.34 mmol), $TsN_3$ (0.36 mmol), TEA (0.36 mmol) and CuI (0.03 mmol) in DMSO (2 mL), rt, $N_2$, 30 min.
[b]Isolated yield.

### 5.2.4. Proposed mechanism:

A plausible mechanism for the present MCR to synthesise compound (**22**) is depicted in Scheme 5.6. The MCR seems to proceed *via* a number of steps including the formation of (i) ketenimine, (ii) tosylamide and finally (iii) intramolecular C-N bond formation as shown in Scheme 5.6.




**Scheme 5.6**: Proposed reaction mechanism.

Initially, the terminal alkyne (**17)** and sulfonyl azide undergoes a copper-catalyzed azide-alkyne cycloaddition (CuAAC) in the presence of CuI and $Et_3N$ to form ketenimine species (**E-1)** *via*[19] formation of triazolo-Cu intermediate followed by the release of nitrogen gas. Then nucleophilic attack of 2-iodoanilines (**11**) at the sp-carbon of **E-1** leads to *N*-tosylamidine intermediate **E-2** which on tautomerization leads to tosylamide **E-3**. Finally the Cu-catalyzed intramolecular C-N bond formation of **E-3** affords the desired product (**22**).

The possible reason for (i) low catalyst loading, (ii) rapid reaction, (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products in the present case in compared to the earlier protocol was perhaps due to use of 2-iodoaniline derivative in place of 2-bromo analog. It is well known that reactivity order of halogen substituent towards the transition metal catalyst is I > Br >> Cl and therefore 10 mol% Cu catalyst alone in the presence of $Et_3N$ was enough to facilitate the overall transformation efficiently. The other reason could be the nature of terminal alkynes used. The alkynes (**17**) used in the present MCR contain a tertiary amino group which because of its bulkiness could force the orientation of tosylamide moiety of **E-3** to a position favorable for intramolecular cyclization thereby accelerating the Cu-catalyzed C-N bond forming step

## 5.3. Pharmacology:

In order to assess their potential to induce apoptosis the synthesized compounds were tested in Zebrafish embryos[20] along with a known drug methotrexate at 30 µM.

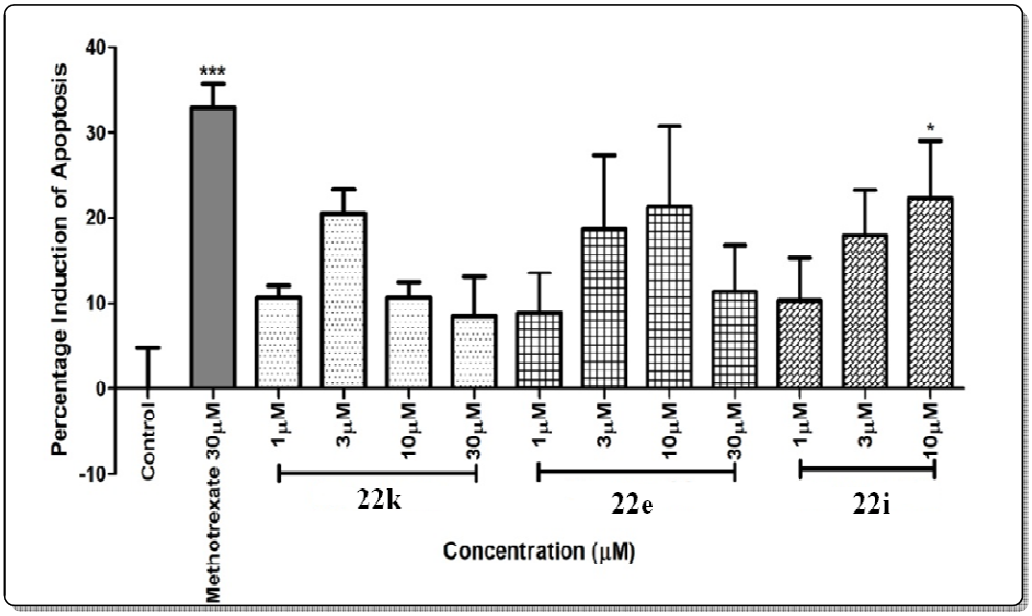



**Fig. 5.13**: Results of apoptosis assay: The percentage induction of apoptosis caused by compounds **22k**, **22e** and **22i** at different concentrations along with Methotrexate. All the statistical analysis was performed using GraphPad Prism® software.

Based on their considerable effects in the present assay of apoptosis compounds (**22k)**, (**22e)** and (**22i)** were further tested at 1, 3, 10 and 30 µM along with methotrexate (Figure 5.13 & 5.14). While the compound (**22k)** showed an increase in its apoptotic activities up to 3 µM a decrease in activity was observed at 10 and 30 µM. The embryos were found to be safe at all concentrations. In the case of compound (**22e)** the increase in apoptotic activities was observed with the increase of concentration from 1 to 10 µM, but the activity was decreased at 30 µM. Compound (**22i)** showed significant apoptotic activity at 10 µM but embryos were dead when the concentration was increased to 30 µM.

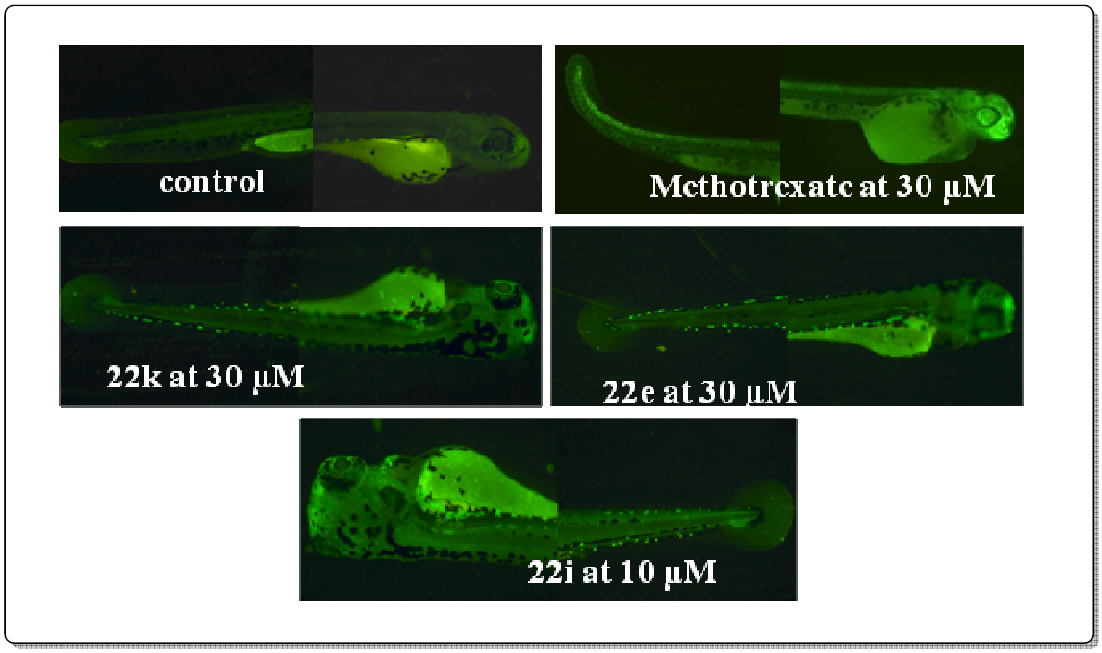



**Fig. 5.14**: Representative images of the embryos treated with compounds assayed for apoptosis.

These compounds were also evaluated for their potential toxicities[14] e.g. teratogenicity in Zebrafish embryo at a range of 1.0-30 µM. The toxicological evaluation was carried out in a blinded fashion. All the embryos in control group were found normal. Phenobarbital (3 mM) was used as a positive control in this assay (Figure 5.15 & 5.16). The compound (**22k)** was found to be non toxic in all the tested concentrations. While the compound (**22e)** showed mild toxicity at 30 µM, it was found to be safe at lower concentrations e.g. 1, 3 and 10 µM. Compound (**22i)** was found to be safe at 1 and 3 µM but showed toxicity at 10 and 30 µM.

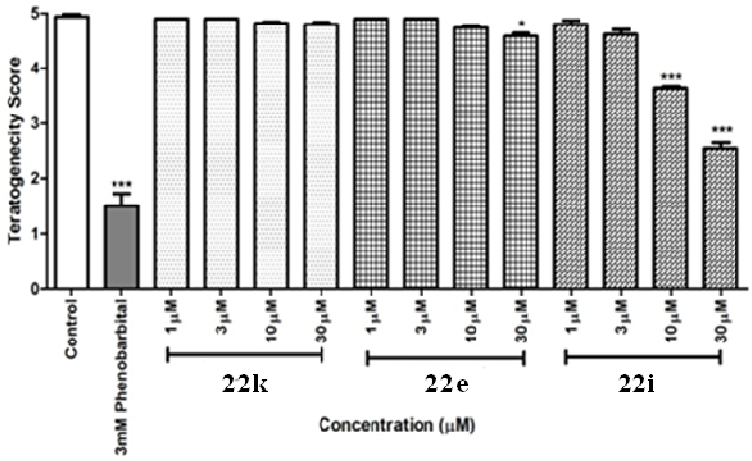



**Fig. 5.15**: Results of teratogenicity assay: Each embryo was scored based on their level of toxicity from 5 being non toxic and 0.5 being highly toxic. Statistical analysis for scoring was done using GraphPad Prism® software using two-way ANOVA. The graph represents the teratogenic scoring given compared to the positive control Phenobarbital.

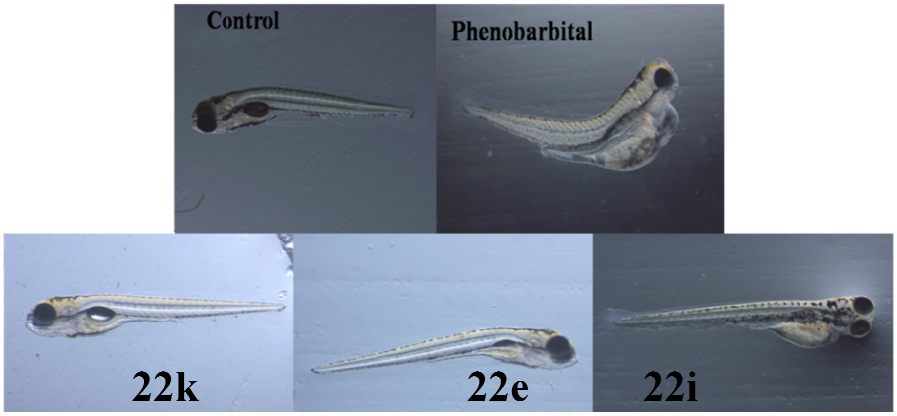



**Fig. 5.16**: Representative zebrafish images of teratogenicity assay of compounds tested at 30 µM.

Based on the summery of $EC_{50}$ values (apoptosis), NOAEL (No Observed Adverse Effect Level) and the overall therapeutic index (Fig. 5.16 and Table 5.3) the safety order of the tested compounds appeared as **22k** > **22e** > **22i**. Overall, the compound **22k** was found to be safest whereas **22i** was identified as the most potent inducer of apoptosis in zebrafish indicating the present class of compounds are of further interest.

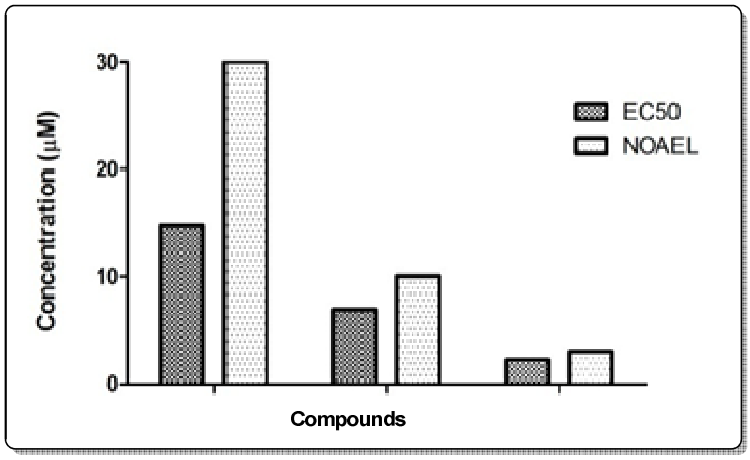



**Fig. 5.16**: The $EC_{50}$ (apoptosis) and NOAEL of test compound **22k** ($EC_{50}$ = 14.76 µM & NOAEL = 30 µM), **22e** ($EC_{50}$ = 6.94 µM & NOAEL = 10 µM), and **22i** ($EC_{50}$ = 2.23 µM & NOAEL = 3 µM), The overall therapeutic index (ratio of NOAEL/$EC_{50}$) of **22k** is 2.032, **22e** is 1.44, and **22i** is 1.34.

**Table 5.3:** Summary of pharmacological evaluations of compounds **22k, 22e,** and **22i**.

| Pharmacological evaluations | | | | Test compounds data | | |
|---|---|---|---|---|---|---|
| Tests | Endpoint | Positive Control | Parameters | **22k** | **22e** | **22i** |
| Apoptosis | Acridine Orange staining of apoptotic cells | Methotrexate | $EC_{50}$ | 14.76 | 6.94 | 2.23 |
| Teratogenecity | Morphological assessment of phenotypic changes | Phenobarbital | NOAEL | 30 µM | 10 µM | 3 µM |
| Overall Therapeutic Index | Ratio of NOAEL/$EC_{50}$ | --- | Therapeutic Index | 2.032 | 1.44 | 1.34 |

## 5.4. Conclusion:

In conclusion, an efficient MCR has been developed involving the reaction of *N*-(prop-2-ynyl)quinoxalin-2-amine derivative with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and $Et_3N$ in DMSO to afford the pre-designed target compounds containing quinoxaline framework linked with benzimidazole nucleus. In contrast to the previously reported cascade reaction for the synthesis of similar class of compounds the present MCR seemed to have following favorable features e.g. (i) rapid reaction (30 min), (ii) low catalyst loading (10 mol%), (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products (73-95%). A range of novel hybrid molecules originally designed as potential inducers of apoptosis were prepared using this methodology and tested for apoptosis, and teratogenicity in zebrafish embryos. Some of these compounds showed encouraging apoptosis inducing properties and therefore seemed to have potential medicinal value. The MCR presented here could be useful in building library of hybrid molecules useful for medicinal / pharmaceutical chemistry and drug discovery efforts.

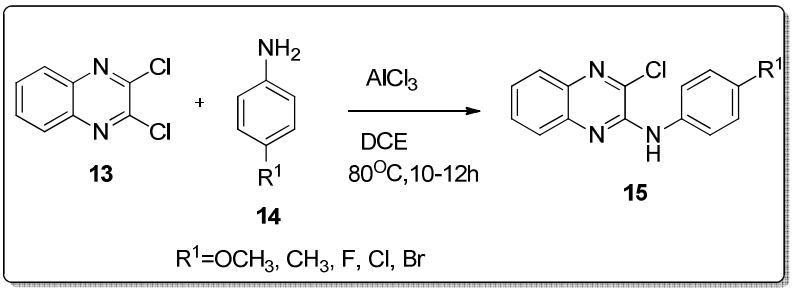
## 5.5. Experimental section:

### 5.5.1. Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. $^1H$ and $^{13}C$ NMR spectra were recodred in $CDCl_3$ or DMSO-$d_6$ solution by using a 400 MHz spectrometers. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT- IR spectrometer. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Melting points (mp) were determined by using Buchi B-540 melting point appratus and are

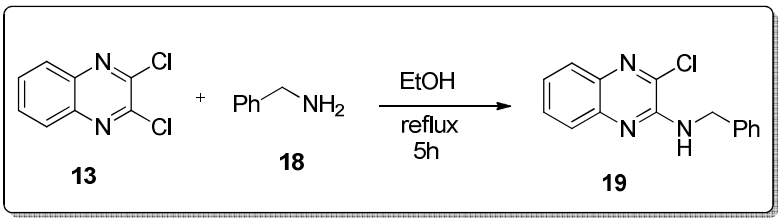
uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

### 5.5.1.1. General Procedure for the preparation of 3-Chloro-*N*-aryl quinoxalin-2-amine (15)




A mixture of 2,3-dichloroquinoxaline (**13)** (1.0 mmol), an appropriate amine (**14)** (1.0 mmol) and $AlCl_3$ (1.25 mmol) in 1,2-dichloroethane (5mL) was stirred at 80°C for 10-12 h under a nitrogen atmosphere. After completion of the reaction, the mixture was cooled to room temperature, poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 × 10 mL). The combined organic layers were washed with cold water (2 × 10 mL), brine (4mL) and dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The residue obtained was purified by column chromatography on silica gel (230-400 mesh) using ethylacetate/hexane to give the desired product (**15)**.

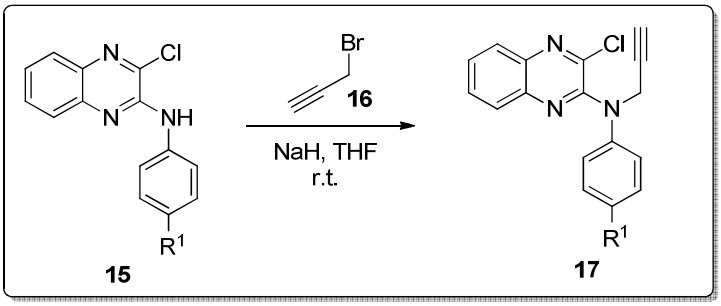
### 5.5.1.2. Preparation of *N*-benzyl-3-chloroquinoxalin-2-amine (19)




A mixture of 2,3-dichloroquinoxaline (**13)** (0.01 mmol) and benzylamine (**18)** (0.015 mmol) in EtOH (5 mL) was heated under reflux for 5 h. After completion of the reaction, the reaction mass was cooled to room temperature and ethanol was removed under reduced pressure. The resulting solid was washed with water and

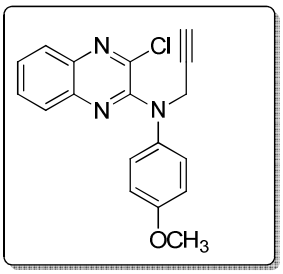
dried to afford the desired products (**19)**.

### 5.5.1.3. General procedure for the preparation of 3-chloro-*N*-aryl-N-(prop-2-ynyl)quinoxalin-2-amine (17)




Proparyl bromide (**16**, 3 mmol) was added to a solution of *N*-substituted quinoxaline-2-amine derivatives (**15,** 1 mmol) and sodium hydride in THF (10 mL) under nitrogen atmosphere. The reaction mixture was then stirred for 2h at room temperature. After completion of the reaction (confirmed by TLC), the mixture was diluted with ice water (3 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using ethylacetate/ hexane as eluent to afford the N-propallylated quinoxaline-2-amine derivatives (**17)**.

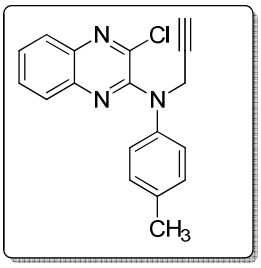
### 5.5.1.4. 3-chloro-*N*-(4-methoxyphenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (17a)




Yield: 92%; Light yellow; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3265.7, 3057.5, 3008.2, 2964.3, 2926.0, 2849.3, 1512.3, 1435.6, 1402.7, 1221.9, 1084.9; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.95-7.90 (m, 1H), 7.90-7.85 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.71-7.64 (m, 1H), 7.59-7.51 (m, 1H), 7.09 (m, 2H), 6.93-6.85 (m, 2H), 4.69 (d, *J* = 2.3 Hz, 2H), 3.82 (s, 3H), 2.19 (t, *J* = 2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): 158.0, 149.4, 141.0, 139.8, 138.3, 138.2, 130.1 (2C), 127.6, 127.2, 127.1, 127.0, 114.5

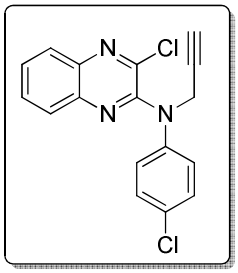
(2C), 79.8, 72.2, 55.4, 43.7; MS (ES mass): 323.8 (M+1); HPLC: 85.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 2/50, 10/98, 15/98, 18/50, 20/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 215.5 nm, retention time 7.0 min.

**5.5.1.5. 3-chloro-*N*-*(*prop-2-ynyl)-*N*- tolylquinoxalin-2-amine (17b)**




Yield: 94%; Yellow solid; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3265.7, 1545.2, 1512.3, 1457.5, 1397.2, 1227.4, 1084.9; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H), 7.72-7.66 (m, 1H), 7.58-7.54 (m, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 4.73 (d, *J* = 2.4 Hz, 2H), 2.37 (s, 3H), 2.19 (t, *J* = 2.4 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 149.3, 142.9, 141.3, 139.9, 138.3, 136.0, 130.1, 130.0, 129.4, 127.6, 127.4, 127.1, 125.1, 109.9, 79.8, 72.1, 43.5, 21.1; MS (ES mass): 307.9 (M+1); HPLC: 92.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 2/50, 10/98, 15/98, 18/50, 20/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.9 min.

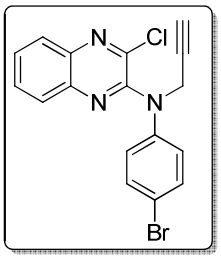
**5.5.1.6. 3-chloro-*N*-(4-chlorophenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (17c)**




Yield: 90%; White solid; $R_f$ = 0.2 (5% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3275.3, 1541.6, 1490.3, 1362.3, 1225.9, 1073.2; $^1H$ NMR (400 MHz, $CDCl_3$) δ: 7.94 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.91 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.73-7.68 (m, 1H), 7.62-7.57 (m, 1H), 7.36-7.31 (m, 2H), 7.09-7.04 (m, 2H), 4.74 (d, *J* = 2.4 Hz, 2H), 2.21 (t, *J* = 2.4

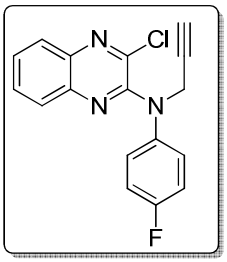
Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 148.9, 144.0, 141.2, 139.8, 138.6, 131.4, 130.3, 129.5(2C), 127.9, 127.7, 127.2, 126.1(2C), 79.4, 72.6, 43.3; MS (ES mass): 328.0 (M+1); HPLC: 99.1%, Column: Symmetry C-18 250 * 4.6 mm, 5µm, mobile phase A: 5mm Ammonium Acetate in water, mobile phase B: $CH_3CN$ (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20,30/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (50:50); UV 210.4 nm, retention time 14.4 min.

**5.5.1.7. *N*-(4-bromophenyl)-3-chloro-*N*-(prop-2-ynyl)quinoxalin-2-amine (17d)**




Yield: 89%; Light yellow solid; $R_f$ = 0.2 (5% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3276.7, 1545.2, 1495.8, 1364.3, 1221.9, 1073.9; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.95 (d, *J* = 8.4 Hz, 1H), 7.92 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.74-7.69 (m, 1H), 7.64-7.58 (m, 1H), 7.50-7.47 (m, 2H), 7.02-7.00 (m, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 2.22 (t, *J* = 2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 148.9, 144.5, 141.3, 139.8, 138.6, 132.5, 131.8, 130.3, 128.0, 127.7, 127.2, 126.3 (2C), 119.2, 79.3, 72.6, 43.3; MS (ES mass): 372.5 (M+1); HPLC: 98.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 6.2 min.

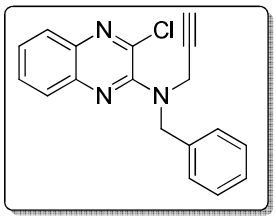
**5.5.1.7. 3-chloro-*N*-(4-fluorophenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (17e)**




Yield: 90%; Yellow solid; $R_f$ = 0.2 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3274.4, 1535.1, 1486.8, 1334.3, 1218.9, 1072.9; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* =

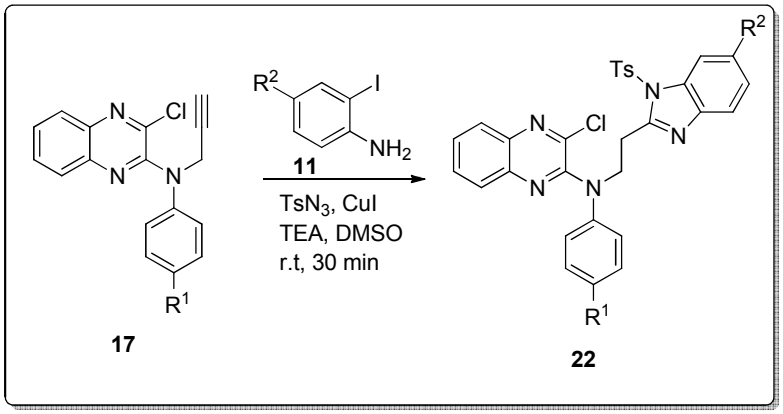
8.4 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.71-7.67 (m, 1H), 7.60-7.56 (m, 1H), 7.14-7.11 (m, 2H), 7.08-7.04 (m, 2H), 4.71 (d, *J* = 2.3 Hz, 2H), 2.21 (t, *J* = 2.3 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 162.1 (C-F *J* = 244.8 Hz), 159.6, 149.2, 141.4 (2C), 140.9, 139.8, 138.4, 130.2, 127.6, 127.2 (2 C), 127.1 (C-F *J* = 8.5 Hz ), 116.4 (2C), 116.1 (C-F *J* = 22.5 Hz), 109.9, 79.4, 72.5, 43.6; MS (ES mass): 311.8 (M+1); HPLC: 98.9%, Column: X-Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: Formic acid in water B: $CH_3CN$ (T/%B): 0/40, 2/40, 9/98, 14/98, 16/40, 18/40; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 215.4 nm, retention time 9.5 min.

**5.5.1.8. *N*-benzyl-3-chloro-*N*-(prop-2-ynyl)quinoxalin-2-amine (17f)**



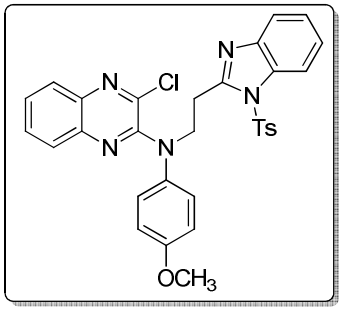

Yield: 91%; Yellow solid; $R_f$ = 0.2 (5% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3274.7, 1535.3, 1497.3, 1367.2, 1231.9, 1063.7; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.92-7.88 (m, 2H), 7.69-7.65 (m, 1H), 7.59-7.57 (m, 1H), 7.48 (d, *J* = 7.4 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.31 (d, *J* = 7.2 Hz, 1H), 4.86 (s, 2H), 4.24 (d, *J* = 2.3 Hz, 2H), 2.25 (t, *J* = 2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 151.5, 141.0, 139.9, 138.4, 136.8, 130.2 (2C), 128.5 (2C), 128.4, 127.6 (2), 127.5, 127.2, 79.1, 72.8, 53.2, 39.1; MS (ES mass): 307.9 (M+1); HPLC: 88.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: $NH_4OAc$ in water B: $CH_3CN$ (T/%B): 0/20, 2/20, 7/98, 11/98, 12/20, 15/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210.4 nm, retention time 8.3 min.

**5.5.1.9. General procedure for preparation of 3-chloro-*N*-(4-aryl)-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22)**




To a solution of sulfonyl azide (0.36 mmol), alkynes (**17**, 0.30 mmol), 2-iodoanline (**11**, 0.34 mmol), and CuI (0.03 mmol) in DMSO (2mL) was added TEA (0.36 mmol) slowly via syringe. The reaction solution was stirred at room temperature under $N_2$ for 30min. After completion of the reaction, the reaction mixture was partitioned between ethyl acetate and saturated $NH_4Cl$, the organic layer was washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound (**22)**.

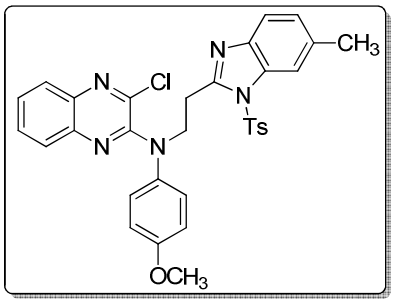
### 5.5.1.10. 3-chloro-*N*-(4-methoxyphenyl)-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22a)




Yield: 94%; Yellow solid; mp: 164-166 °C ; $R_f$ = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3409.3, 2936.6, 2832.5, 2719.7, 1604.9, 1539.1, 1369.2, 1303.4; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.08-8.05 (m, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.71-7.63 (m, 4H), 7.56-7.52 (m, 1H), 7.41-7.31 (m, 2H), 7.11 (dd, *J* = 14.5, 8.5 Hz, 4H), 6.90 (d, *J* = 8.8 Hz, 2H), 4.58 (t, *J* = 7.3 Hz, 2H), 3.84 (s, 3H), 3.70 (dd, *J* = 9.39, 5.14 Hz, 2H), 2.37 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 157.5, 152.8, 149.6, 145.7, 141.9, 141.2, 140.9, 140.0, 138.7, 137.8, 135.2, 132.9, 130.0

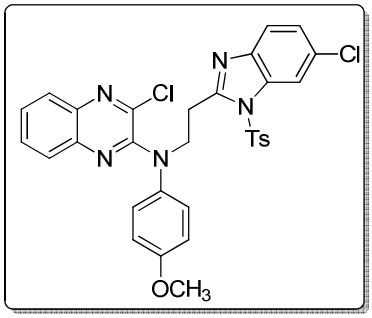
(2C), 129.9, 127.5, 126.7 (2C), 126.6 (2C), 126.5, 124.7, 124.5, 119.7, 114.6 (2C), 113.4, 55.4, 52.0, 27.1, 21.6; MS (ES mass): 584.1 (M+1); HPLC: 99.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/40, 1/40, 6/98, 10/98, 12/40, 15/40; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210 nm, retention time 7.6 min.

**5.5.1.11. 3-chloro-*N*-(4-methoxyphenyl)-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22b)**



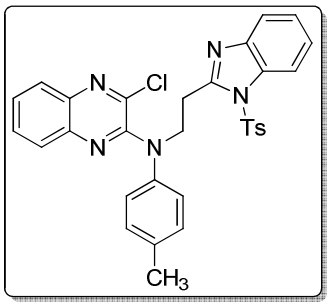

Yield: 89%; Yellow solid; m.p.170-175 $^0$C; $R_f$ = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3381.3, 3302.2, 2928.0, 2831.6, 2718.7, 1626.0, 1597.1, 1353.1, 1307.7; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.86 (d, *J* = 8.3 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.68-7.59 (m, 3H), 7.52 (m, 2H), 7.18-7.03 (m, 5H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.54 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 3.63 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 157.4, 152.0, 149.5, 145.5, 141.1, 139.9, 139.8, 138.7, 137.7, 135.3, 134.9, 133.1, 129.9 (2C), 129.8, 127.4, 126.7, 126.6 (2C), 126.5, 126.4 (2C), 125.8, 119.0, 114.5 (2C), 113.4, 55.3, 51.9, 27.0, 21.8, 21.5; MS (ES mass): 598 (M+1); HPLC: 97.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.5 min.

**5.5.1.12. 3-chloro-*N*-(2-(6-chloro-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-*N*-(4-methoxyphenyl)quinoxalin-2-amine (22c)**




Yield: 90%; Yellow solid; m.p.174-175 $^{0}$C; $R_f$ = 0.4 (10% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3409.3, 2955.0, 2848.0, 2719.7, 1626.0, 1596.1, 1383.0, 1352.1; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 8.07 (s, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.70-7.60 (m, 3H), 7.52 (t, *J* = 6.92 Hz, 2H), 7.30 (d, *J* = 1.89 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 4.54 (t, *J* = 6.8Hz, 2H), 3.82 (s, 3H), 3.63 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 157.5, 153.4, 149.5, 146.0, 141.1, 140.4, 139.8, 138.5, 137.7, 134.8, 133.4, 130.5, 130.1 (2C), 129.8, 127.4, 126.7, 126.6 (2C), 126.6 (2C), 126.5, 125.1, 120.3, 114.5 (2C), 113.6, 55.3, 51.8, 27.0, 21.5; MS (ES mass): 617.9 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.9 min.

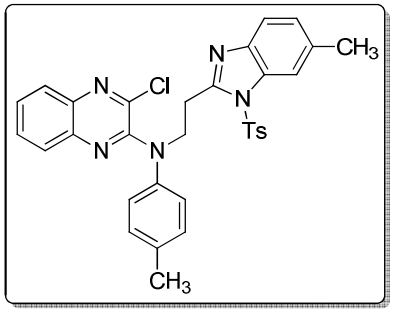
**5.5.1.13. 3-chloro-*N*-p-tolyl-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2 amine (22d)**




Yield: 92%; Yellow solid; m.p.162-167 $^{0}$C; $R_f$ = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 2958.5, 2925.6, 2848.9, 1741.9, 1593.9, 1544.6, 1511.2, 1374.7, 1265.1; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 8.07-8.01 (m, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.67-6.63 (m, 4H), 7.56-7.50 (m, 1H), 7.36-7.29 (m, 2H), 7.13 (dd, *J* =

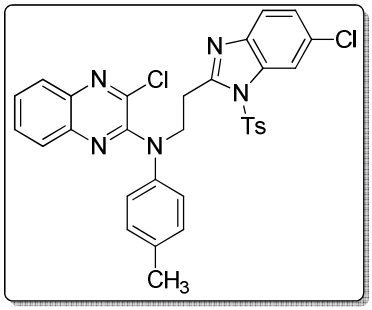
8.4, 8.0 Hz, 4H), 7.01 (d, *J* = 8.4 Hz, 2H), 4.59 (t, *J* = 7.2 Hz, 2H), 3.69-3.65 (m, 2H), 2.35 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 152.7, 149.6, 145.7, 143.1, 141.8, 141.5, 140.0, 138.0, 135.3, 135.2, 132.9, 130.1, 130.0 (2C), 129.8, 127.5, 126.9, 126.8, 126.6 (2C), 124.9 (2C), 124.7, 124.5, 119.7, 113.4, 109.9, 51.8, 27.2, 21.6, 20.9; MS (ES mass): 568 (M+1); HPLC: 97.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.7 min.

### 5.5.1.14. 3-chloro-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-*N*-p-tolylquinoxalin-2-amine (22e)



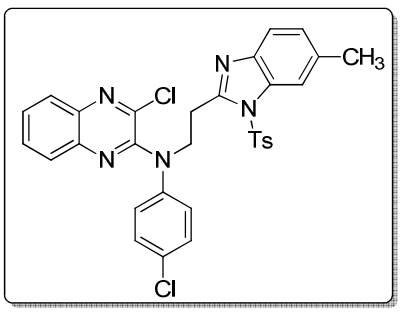

Yield: 86%; Yellow solid; m.p.160-162 $^0$C; $R_f$ = 0.2 (40% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3024.3, 2914.7, 2859.9, 1741.9, 1604.9, 1544.6, 1511.7, 1380.2, 1265.1; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.91-7.83 (m, 2H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.66-7.61 (m, 3H), 7.55-7.49 (m, 2H), 7.15-7.09 (m, 5H), 7.01 (d, *J* = 8.2 Hz, 2H), 4.57 (t, *J* = 7.3 Hz, 2H), 3.63 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.35 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 152.1, 149.6, 145.6, 143.2, 141.6, 140.0, 139.9, 138.0, 135.4, 135.3, 134.9, 133.1, 130.0 (2C), 129.8, 127.5, 126.8 (2C), 126.5 (2C), 125.9 (2C), 124.8 (2C), 119.1, 113.4, 109.9, 51.8, 27.2, 21.9, 21.6, 20.9; MS (ES mass): 582 (M+1); HPLC: 99.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 8.0 min.

### 5.5.1.15. 3-chloro-*N*-(2-(6-chloro-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)-*N*-p-tolylquinoxalin-2-amine (22f)




Yield: 90%; Yellow solid; m.p.170-173 $^0$C; $R_f$ = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 2953.0, 2926.6, 2854.4, 1747.3, 1599.4, 1544.6, 1506.2, 1380.2, 1265.1; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.06 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.70-7.61 (m, 3H), 7.54 (t, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 1.8 Hz, 1H), 7.14 (dd, *J* = 7.6, 5.4 Hz, 4H), 6.99 (d, *J* = 8.3 Hz, 2H), 4.57 (t, *J* = 7.2 Hz, 2H), 3.63 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 153.4, 149.6, 146.1, 143.1, 141.5, 140.4, 139.9, 138.0, 135.4, 134.9, 133.5, 130.6, 130.2 (2C), 130.0, 129.9, 127.5, 127.0, 126.8, 126.7 (2C), 126.7, 125.1, 124.8 (2C), 120.4, 113.7, 51.7, 27.3, 21.6, 21.0; MS (ES mass): 601.9 (M+1); HPLC: 95.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 230.5 nm, retention time 8.4 min.

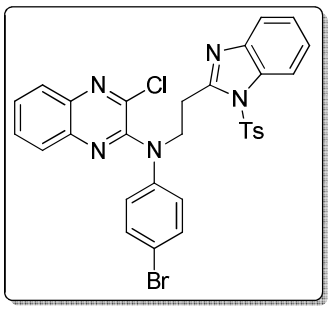
**5.5.1.16. 3-chloro-*N*-(4-chlorophenyl)-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22g)**




Yield: 89%; Yellow solid; m.p.162-166 $^0$C; $R_f$ = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 2947.6, 2920.2, 1599.4, 1539.1, 1495.2, 1374.7, 1265.1; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.07 Hz, 1H), 7.86-7.79 (m, 2H), 7.71-7.55 (m, 4H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.12 (t, *J* = 9.3 Hz, 3H), 7.04 (d, *J* = 8.6 Hz, 2H), 4.59 (t, *J* = 6.9 Hz, 2H), 3.61 (t, *J* = 7.1 Hz, 2H), 2.50 (s, 3H), 2.35 (s,

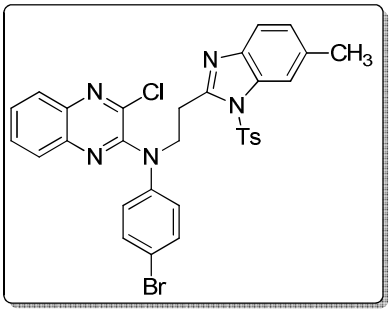
3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 151.6, 149.1, 145.7, 144.4, 141.4, 139.8, 139.7, 138.2, 135.2, 135.0, 133.1, 130.5, 130.0 (2C), 129.9, 129.4 (2C), 127.5, 127.4 (2C), 126.9, 126.4, 125.9, 125.6 (2C), 119.0, 113.4, 51.7, 27.5, 21.8, 21.5; MS (ES mass): 601.9 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.9 min.

**5.5.1.17. Preparation of *N*-(4-bromophenyl)-3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[d]imidazol-2-yl)ethyl)quinoxalin-2-amine (22h)**



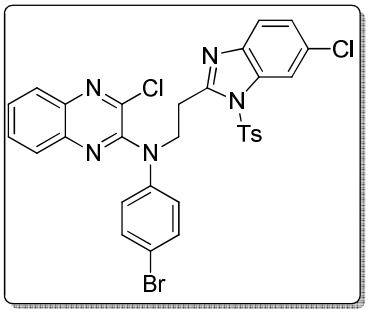

Yield: 95%; Light yellow solid; m.p.145-150 $^0$C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3035.2, 2931.1, 2859.9, 1621.3, 1599.4, 1544.6, 1374.7, 1193.8; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.07-8.00 (m, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.71-7.55 (m, 5H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.37-7.30 (m, 2H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 4.62 (dd, *J* = 9.4, 4.9 Hz, 2H), 3.65 (t, *J* = 7.0 Hz, 2H), 2.35 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 152.9, 152.4, 149.2, 145.9, 144.9, 141.8, 141.5, 139.9, 138.3, 135.2, 132.9, 132.5 (2C), 130.1 (2C), 127.6, 127.0, 126.5 (2C), 126.0 (2C), 125.9, 124.9, 124.6, 119.7, 118.4, 113.5, 51.7, 27.5, 21.6; MS (ES mass): 633.9 (M+1); HPLC: 99.2%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 7.9 min.

**5.5.1.18. *N*-(4-bromophenyl)-3-chloro-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22i)**




Yield: 91%; Brown solid; m.p.165-169 $^{0}$C; $R_f$ = 0.4 (10% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3783.5, 3433.4, 3407.4, 2926.1, 2828.7, 1595.2, 1492.2, 1437.9, 1354.0; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 8.0 Hz, 1H), 7.86-7.80 (m, 2H), 7.71-7.56 (m, 4H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 2H), 7.12 (t, *J* = 8.3 Hz, 3H), 6.98 (d, *J* = 8.7 Hz, 2H), 4.60 (t, *J* = 7.10 Hz, 2H), 3.62 (t, *J* = 7.1 Hz, 2H), 2.51 (s, 3H), 2.35 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ:151.7, 149.2, 145.8, 145.0, 141.6 (2C), 139.9, 139.8, 138.3,135.3, 135.1, 132.4, 130.1, 130.0, 127.6, 127.5, 127.1, 126.5, 126.0, 125.9 (2C), 125.8, 125.7, 119.1, 118.3, 113.5 (2C), 51.7, 27.6, 21.9, 21.6; MS (ES mass): 647.8 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.5 nm, retention time 8.0 min.

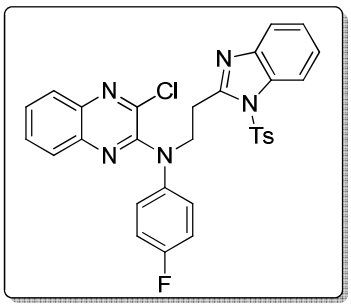
**5.5.1.19. *N*-(4-bromophenyl)-3-chloro-*N*-(2-(6-chloro-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22j)**




Yield: 89%; Brown solid; m.p.190-193 $^{0}$C; $R_f$ = 0.4 (10% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3786.4, 3432.4, 3383.2, 2958.9, 2830.6, 2719.7, 1595.2 , 1486.2, 1443.7, 1380.1, 1353.; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 7.91-7.88 (m, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.69-7.56 (m, 4H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.45 (t, *J* = 8.8 Hz, 2H), 7.28 (dd, *J* = 8.6, 1.2 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 2H), 6.98 (t, *J* = 8.8 Hz, 2H),

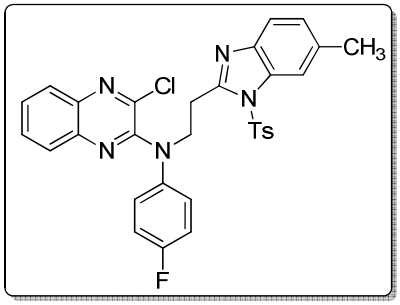
4.59 (t, *J* = 7.1 Hz, 2H), 3.61 (t, *J* = 7.0 Hz, 2H), 2.36 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 153.1, 149.1, 146.3, 144.9, 141.5, 140.4, 139.9, 138.3, 134.9, 133.5, 132.5 (2C), 130.7, 130.2 (2C), 130.1, 127.6, 127.0, 126.6, 126.5, 126.0, 125.9, 125.3, 120.4, 118.4, 113.7, 109.9, 51.6, 27.6, 21.6; MS (ES mass): 667.8 (M+1); HPLC: 88.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 2/50, 10/98, 15/98, 18/50, 20/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.5 nm, retention time 7.9 min.

**5.5.1.20. 3-chloro-*N*-(4-fluorophenyl)-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22k)**



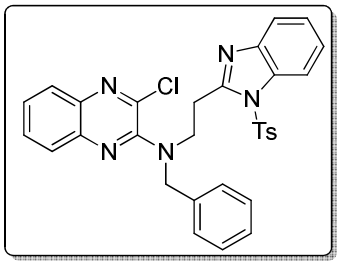

Yield: 93%; Brown solid; m.p.141-142.9 $^0$C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3057.2, 2953.0, 2920.2, 1599.4, 1539.7, 1473.3, 1418.5, 1369.2, 1226; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.64-7.61 (m, 4H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.36-7.28 (m, 2H), 7.10 (t, *J* = 6.9 Hz, 4H), 7.02 (t, *J* = 8.4 Hz, 2H), 4.58 (t, *J* = 7.2 Hz, 2H), 3.65 (t, *J* = 7.2 Hz, 2H), 2.33 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 161.6 (C-F *J* = 244.1 Hz), 159.1, 152.5, 149.4, 145.8, 142.0, 141.9, 141.8, 141.2, 139.9, 138.0, 135.2, 132.9 (2C), 130.0, 129.2, 127.5, 127.2, 126.9, 126.7 (C-F *J* = 8.3 Hz), 126.7, 126.5, 124.8, 124.6, 119.7, 116.3 (C-F *J* = 22.6 Hz), 116.1, 113.4 ( 2C), 109.9, 52.0, 27.3, 21.5; MS (ES mass): 572 (M+1); HPLC: 98.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210 nm, retention time 7.4 min.

**5.5.1.21. 3-chloro-*N*-(4-fluorophenyl)-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22l)**




Yield: 86%; White solid; m.p.141-142.9 $^{0}$C; $R_f$ = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3799.0, 3714.0, 3461.4, 3293.5, 2927.1, 2833.5, 2719.7, 1597.1, 1486.2, 1358.9; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 7.91-7.83 (m, 2H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.70-7.61 (m, 3H), 7.60-7.53 (m, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.17-7.07 (m, 5H), 7.03 (t, *J* = 8.5 Hz, 2H), 4.57 (t, *J* = 7.1 Hz, 2H), 3.62 (t, *J* = 7.2 Hz, 2H), 2.51 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 161.6 (C-F *J* = 244.3 Hz), 159.1, 152.5, 149.4, 145.8, 142.0, 141.9, 141.8, 141.2, 139.9, 138.0, 135.3, 135.0, 133.1, 129.9 (2C) , 127.5, 127.1, 126.8, 126.8, 126.6 (C-F *J* = 8.4 Hz), 126.5 (2C), 126.4 (2C), 125.9, 119.0, 116.2 (C-F *J* = 22.7 Hz), 116.0 (2C), 113.4, 52.0, 27.3, 21.8, 21.5; MS (ES mass): 586 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210 nm, retention time 7.6 min.

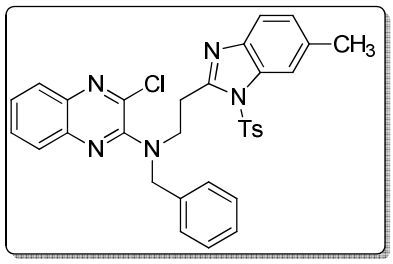
**5.5.1.22. *N*-benzyl-3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22m)**




Yield: 73%; White solid; m.p.125-130 $^{0}$C; $R_f$ = 0.3 (10% EtOAc/ *n*-hexane); IR (KBr, cm$^{-1}$): 3431.5, 3412.2, 2966.6, 2827.7, 2720.7, 1594.2, 1380.1, 1353.1; $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 8.04-7.96 (m, 1H), 7.91-7.84 (m, 1H), 7.71-7.62 (m, 3H), 7.60-1,56 (m, 2H), 7.55-7.47 (m, 1H), 7.46-7.37 (m, 2H), 7.29 (d, *J* = 7.18 Hz, 5H), 7.13 (d, *J* = 7.6 Hz, 2H), 4.87 (s, 2H), 4.13 (s, 2H), 3.57 (d, *J* = 0.49 Hz, 2H), 2.34 (s, 3H); $^{13}$C

NMR (100 MHz, $CDCl_3$) δ: 152.5, 151.5, 145.8, 141.8, 140.7, 139.8, 137.8, 137.6 (2C), 135.2, 132.9, 130.2, 130.1, 130.0, 128.4, 127.6, 127.2, 126.9, 126.8, 126.6, 124.8, 124.6, 121.9, 119.7, 113.5 (2C), 109.9, 54.9, 47.9, 27.9, 21.6; MS (ES mass): 568 (M+1); HPLC: 98.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 250 nm, retention time 7.5 min

### 5.5.1.23. *N*-benzyl-3-chloro-*N*-(2-(6-methyl-1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amine (22n)




Yield: 81%; Light green solid; m.p.174-175 $^0$C; $R_f$ = 0.3 (10% EtOAc/ *n*-hexane); IR (KBr, $cm^{-1}$): 3433.4, 3407.4, 3383.2, 2927.1, 2829.6, 2720.7, 1594.2, 1590.3, 1492.9, 1443.7, 1381.0, 1354.0; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.86 (d, *J* = 8.4 Hz, 1H), 7.80 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.65-7.58 (m, 3H), 7.52 (t, *J* = 7.2 Hz, 1H), 7.44-7.40 (m, 3H), 7.30 (t, *J* = 7.08 Hz, 3H), 7.15-7.08 (m, 3H), 4.86 (s, 2H), 4.12 (t, *J* = 7.3 Hz, 2H), 3.53 (t, *J* = 7.3 Hz, 2H), 2.49 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 151.8, 151.5, 145.7, 140.7, 139.8, 137.8, 135.4, 135.0, 133.1, 130.1 (2C), 129.9, 128.4 (2C), 127.8 (2C), 127.5, 127.2, 126.9, 126.8, 126.5 (2C), 125.9, 119.1 (2C), 113.5 (2C), 54.8, 48.0, 27.9, 21.9, 21.6; MS (ES mass): 582 (M+1); HPLC: 99%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: $CH_3CN$ (T/%B): 0/50, 1/50, 6/98, 12/98, 13/50, 15/50; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 250 nm, retention time 7.8 min.

## 5.6. References:

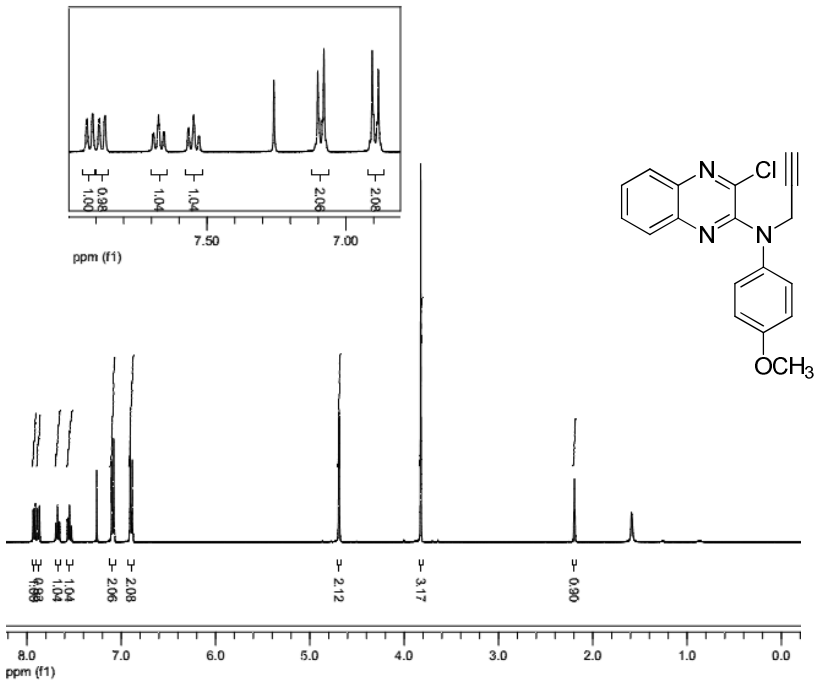

1. Meunier, B., *Acc. Chem. Res.* **2007,** *41* (1), 69-77.
2. (a) Tietze, L. F.; Bell, H. P.; Chandrasekhar, S., *Angew. Chem. Int. Ed.* **2003,** *42* (34), 3996-4028; (b) Baraldi, P. G.; Zaid, A. N.; Preti, D.; Fruttarolo, F.; Tabrizi, M. A.; Iaconinoto, A.; Pavani, M. G.; Carrion, M. D.; Cara, C. L.; Romagnoli, R., *Arkivoc* **2006,** *7*, 20-34.
3. Sausville, E. A.; Peisach, J.; Horwitz, S. B., *Biochemistry* **1978,** *17* (14), 2740-2746.
4. (a) Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B., *ChemBioChem.* **2000,** *1* (4), 281-283; (b) Dechy-Cabaret, O.; Benoit-Vical, F.; Loup, C.; Robert, A.; Gornitzka, H.; Bonhoure, A.; Vial, H.; Magnaval, J. F.; Séguéla, J. P.; Meunier, B., *Chem. Eur. J.* **2004,** *10* (7), 1625-1636.
5. Kogen, H.; Toda, N.; Tago, K.; Marumoto, S.; Takami, K.; Ori, M.; Yamada, N.; Koyama, K.; Naruto, S.; Abe, K., *Org. Lett.* **2002,** *4* (20), 3359-3362.
6. Dollinger, S.; Löber, S.; Klingenstein, R.; Korth, C.; Gmeiner, P., *J. Med. Chem.* **2006,** *49* (22), 6591-6595.
7. Walsh, J. J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A., *Bioorg. Med. Chem. Lett.* **2007,** *17* (13), 3599-3602.
8. Tietze, L. F.; Schneider, G.; Wölfling, J.; Fecher, A.; Nöbel, T.; Petersen, S.; Schuberth, I.; Wulff, C., *Chem. Eur. J.* **2000,** *6* (20), 3755-3760.
9. Guantai, E. M.; Ncokazi, K.; Egan, T. J.; Gut, J.; Rosenthal, P. J.; Bhampidipati, R.; Kopinathan, A.; Smith, P. J.; Chibale, K., *J. Med. Chem.* **2011,** *54* (10), 3637-3649.
10. Provencher-Mandeville, J.; Descôteaux, C.; Mandal, S. K.; Leblanc, V.; Asselin, E.; Bérubé, G., *Biorg. Med. Chem. Lett.* **2008,** *18* (7), 2282-2287.
11. (a) Sprague, E.; Undevia, S.; Innocenti, F.; Ramirez, J.; House, L.; Kindler, H.; Singh, D.; Desai, A.; Schilsky, R.; Ratain, M. ASCO Annual Meeting Proceedings, 2004; p 2021; (b) Kakodkar, N. C.; Peddinti, R.; Kletzel, M.; Tian, Y.; Guerrero, L. J.; Undevia, S. D.; Geary, D.; Chlenski, A.; Yang, Q.; Salwen, H. R., *Pediatr Blood Cancer.* **2011,** *56* (1), 164-167.
12. (a) Xiang, P.; Zhou, T.; Wang, L.; Sun, C.-Y.; Hu, J.; Zhao, Y.-L.; Yang, L., *Molecules* **2012,** *17* (1), 873-883; (b) Ramla, M. M.; Omar, M. A.; El-

Khamry, A.-M. M.; El-Diwani, H. I., *Bioorg. Med. Chem. Lett.* **2006,** *14* (21), 7324-7332.

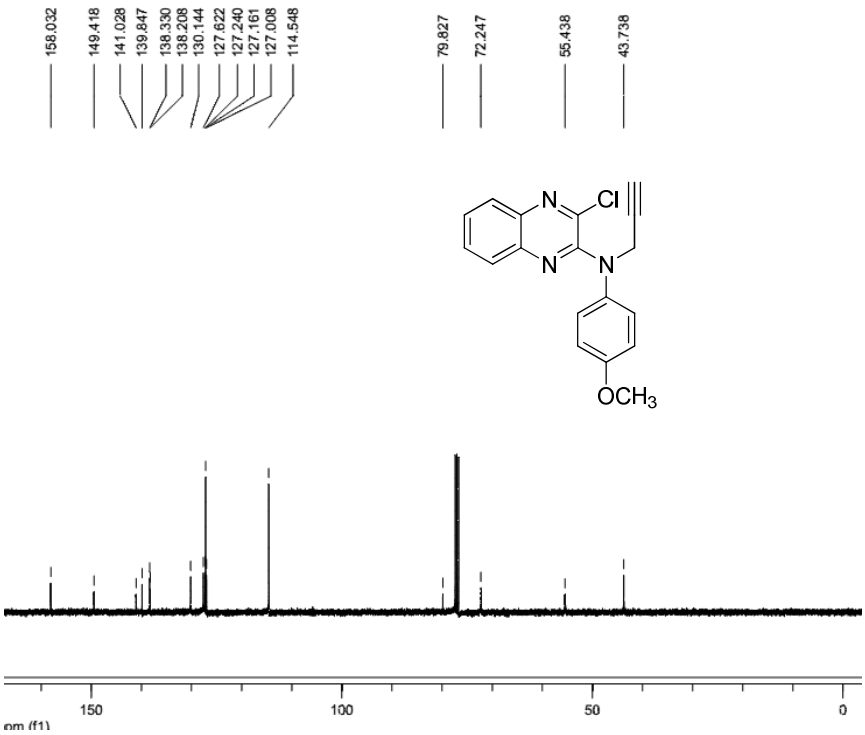
13. (a) Raban, M.; Chang, H.; Craine, L.; Hortelano, E., *J. Org. Chem.* **1985,** *50* (13), 2205-2210; (b) Garuti, L.; Roberti, M.; Cermelli, C., *Bioorg. Med. Chem. Lett.* **1999,** *9* (17), 2525-2530; (c) Dudd, L. M.; Venardou, E.; Garcia-Verdugo, E.; Licence, P.; Blake, A. J.; Wilson, C.; Poliakoff, M., *Green Chem.* **2003,** *5* (2), 187-192; (d) Charton, J.; Girault-Mizzi, S.; Sergheraert, C., *Chem. Pharm. Bull.* **2005,** *53* (5), 492-497; (e) Lin, S.-Y.; Isome, Y.; Stewart, E.; Liu, J.-F.; Yohannes, D.; Yu, L., *Tetrahedron Lett.* **2006,** *47* (17), 2883-2886; (f) Das, B.; Holla, H.; Srinivas, Y., *Tetrahedron Lett.* **2007,** *48* (1), 61-64.
14. (a) Puratchikody, A.; Sivajothi, V.; Jaswanth, A.; Ruckmani, K.; Nallu, M., *Indian J. Heterocycl. Chem.* **2002,** *11* (3), 241-242; (b) Garuti, L.; Roberti, M.; De Clercq, E., *Bioorg. Med. Chem. Lett.* **2002,** *12* (19), 2707-2710; (c) Foks, H.; Pancechowska-Ksepko, D.; Kuzmierkiewicz, W.; Zwolska, Z.; Augustynowicz-Kopec, E.; Janowiec, M., *Chem. Heterocycl. Compd.* **2006,** *42* (5), 611-614.
15. Jin, H.; Xu, X.; Gao, J.; Zhong, J.; Wang, Y., *Adv. Synth. Catal.* **2010,** *352* (2-3), 347-350.
16. Prasad, B.; Kumar, K. S.; Babu, P. V.; Anusha, K.; Rambabu, D.; Kandale, A.; Vanaja, G.; Kalle, A. M.; Pal, M., *Tetrahedron Lett.* **2012,** *53* (45), 6059-6066.
17. Babu, P. V.; Mukherjee, S.; Deora, G. S.; Chennubhotla, K. S.; Medisetti, R.; Yellanki, S.; Kulkarni, P.; Sripelly, S.; Parsa, K. V.; Chatti, K., *Org. Biomol. Chem.* **2013,** *11* (39), 6680-6685.
18. Bélanger, D.; Tong, X.; Soumaré, S.; Dory, Y. L.; Zhao, Y., *Chem. Eur. J.* **2009,** *15* (17), 4428-4436.
19. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B., *Angew. Chem. Int. Ed.* **2002,** *114* (14), 2708-2711; (b) Tornøe, C. W.; Christensen, C.; Meldal, M., *J. Org. Chem.* **2002,** *67* (9), 3057-3064; (c) Yoo, E. J.; Ahlquist, M. r.; Bae, I.; Sharpless, K. B.; Fokin, V. V.; Chang, S., *J. Org. Chem.* **2008,** *73* (14), 5520-5528.
20. (a) Ramsay, J. M.; Feist, G. W.; Varga, Z. M.; Westerfield, M.; Kent, M. L.; Schreck, C. B., *Aquaculture.* **2006,** *258* (1), 565-574; (b) KumaráSeerapu, G.

P.; SarmaáChennubhotla, K.; LalithaKumar, K., *Chem. Commun.* **2013,** *49* (56), 6268-6270; (c) Panzica-Kelly, J. M.; Zhang, C. X.; Danberry, T. L.; Flood, A.; DeLan, J. W.; Brannen, K. C.; Augustine-Rauch, K. A., *Birth Defects Res. B Dev. Reprod Toxicol.* **2010,** *89* (5), 382-395.

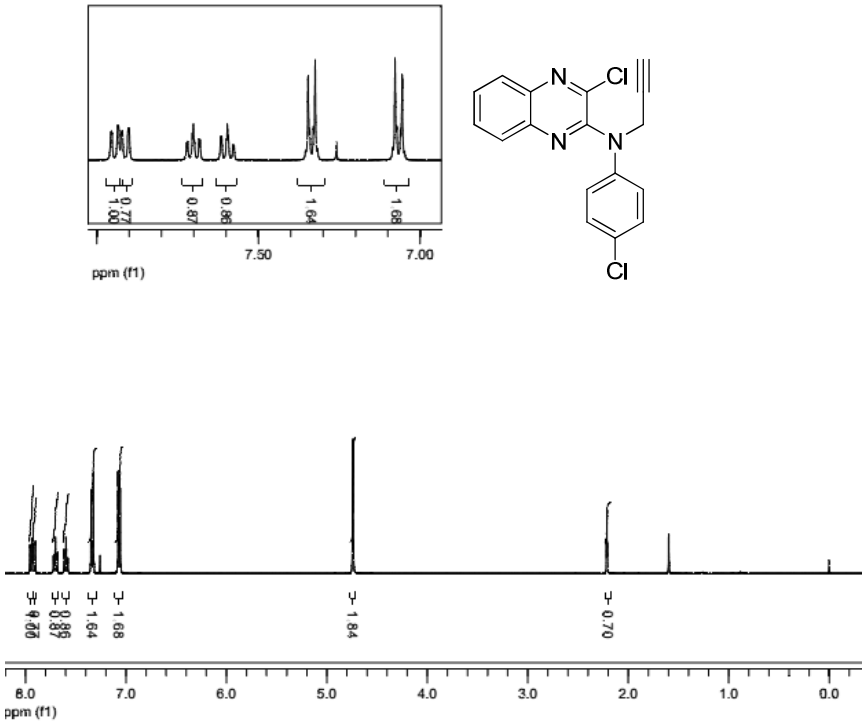
# Appendix



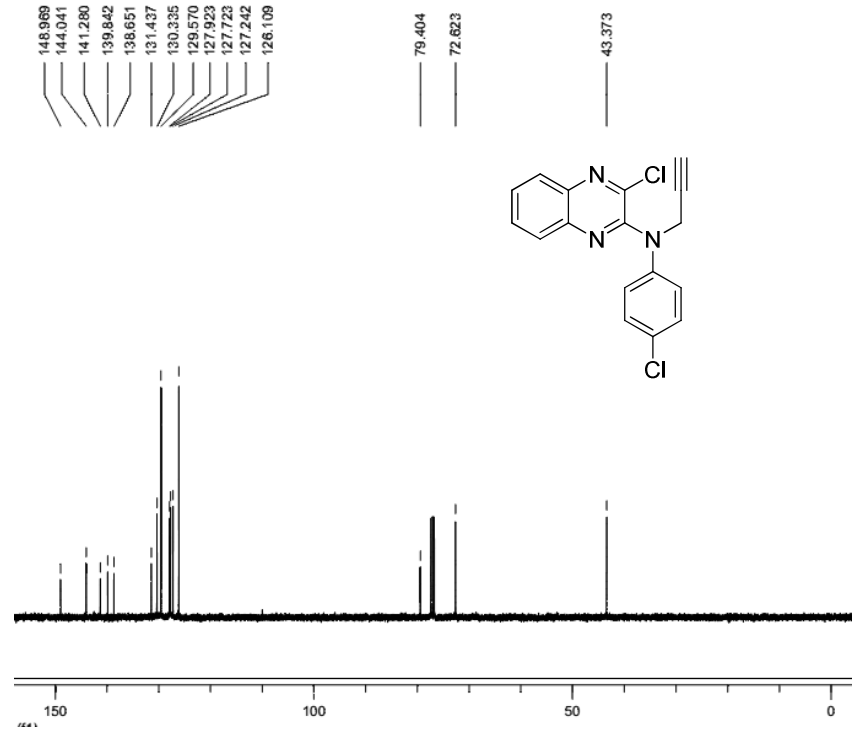

$^{1}$H NMR spectra of compound **17a** ($CDCl_3$, 400 MHz)



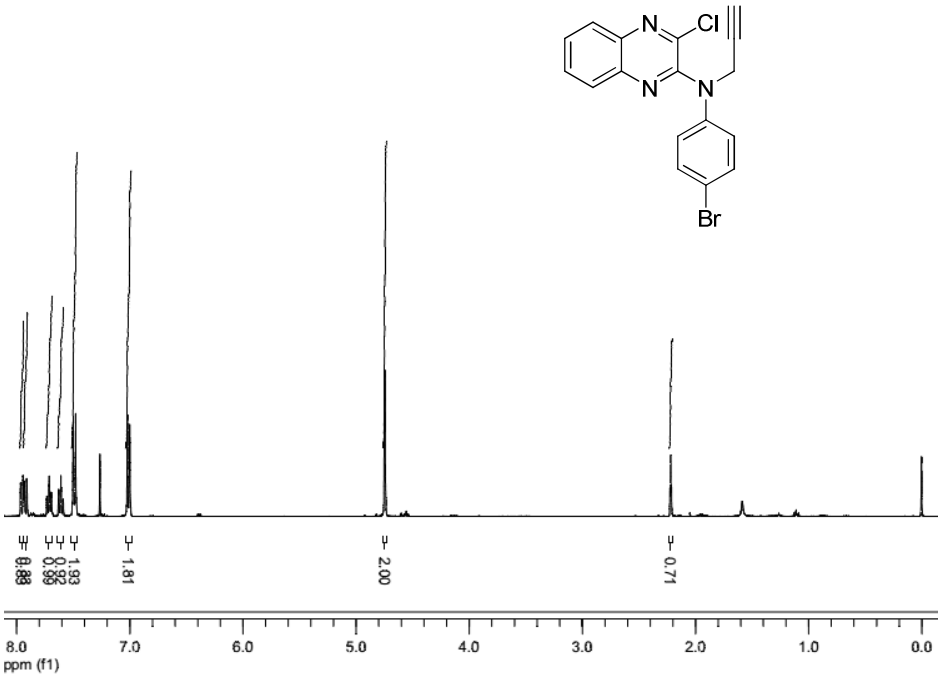

$^{13}$C NMR spectra of compound **17a** ($CDCl_3$, 100 MHz)



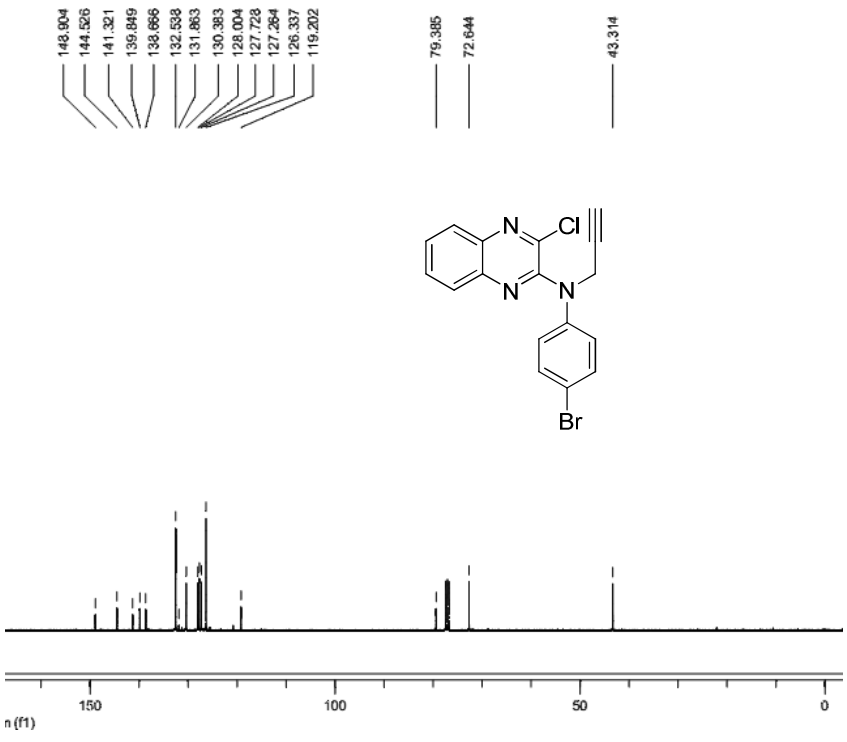

$^{1}$H NMR spectra of compound **17c** ($CDCl_3$, 400 MHz)



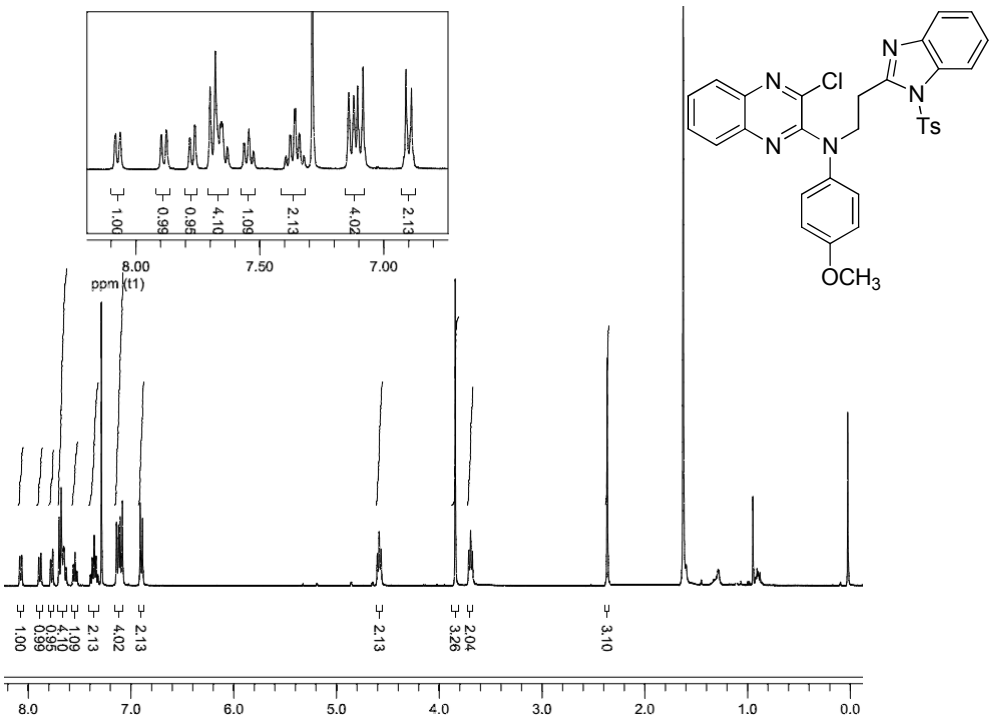

$^{13}$C NMR spectra of compound **17c** ($CDCl_3$, 100 MHz)



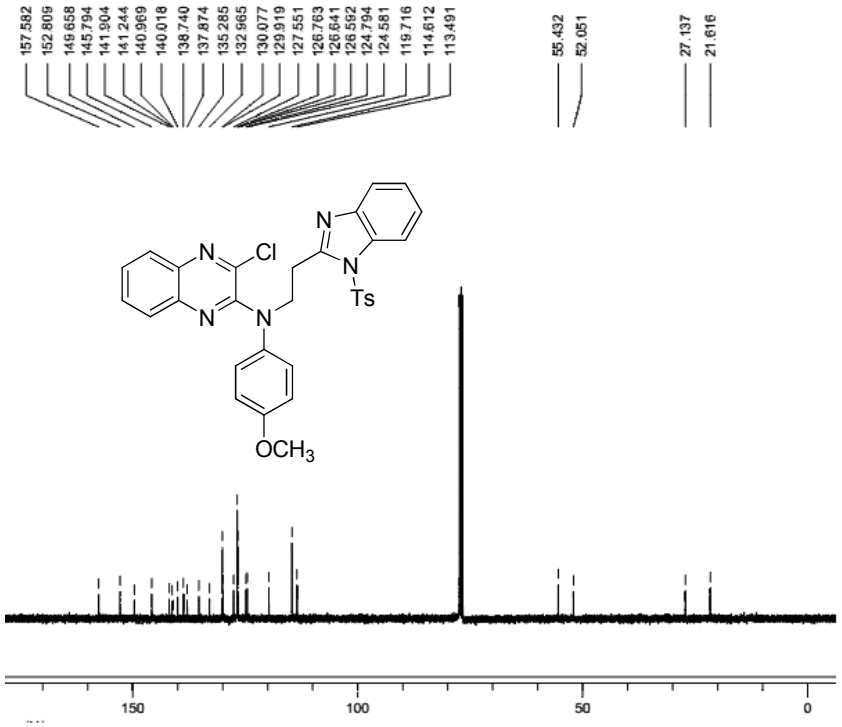

$^{1}$H NMR spectra of compound **17d** ($CDCl_3$, 400 MHz)



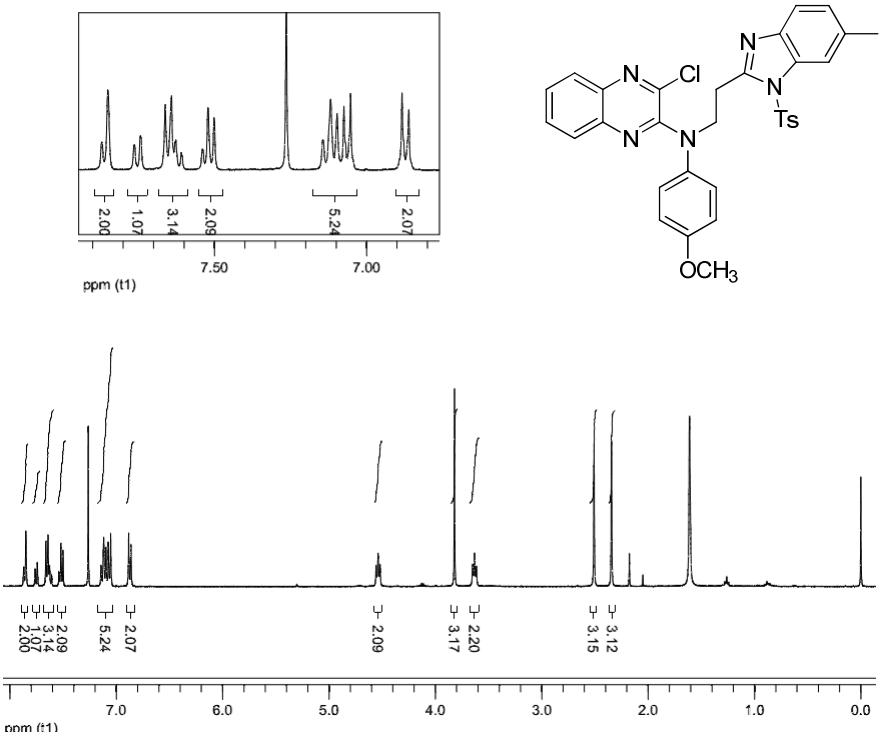

$^{13}$C NMR spectra of compound **17d** ($CDCl_3$, 100 MHz)



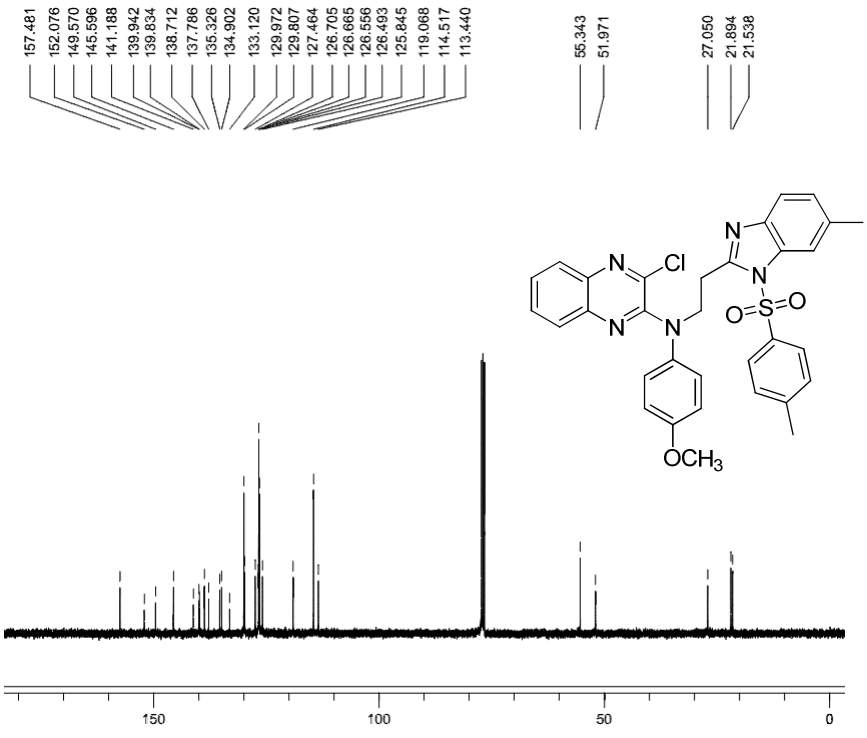

$^{1}$H NMR spectra of compound **22a** ($CDCl_3$, 400 MHz)



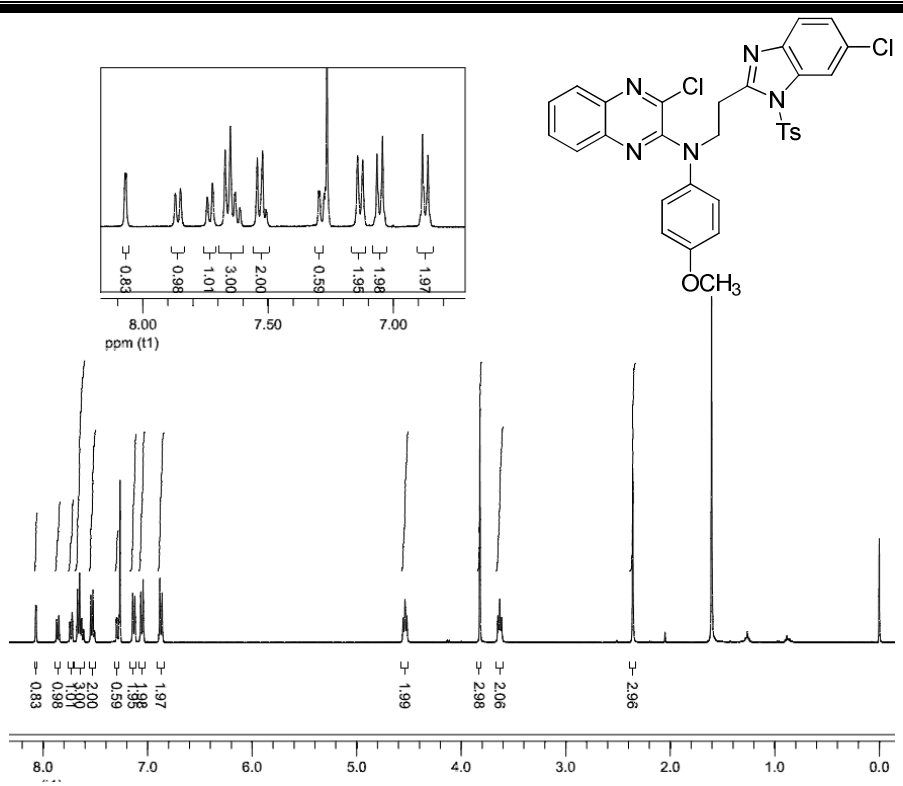

$^{13}$C NMR spectra of compound **22a** ($CDCl_3$, 100 MHz)



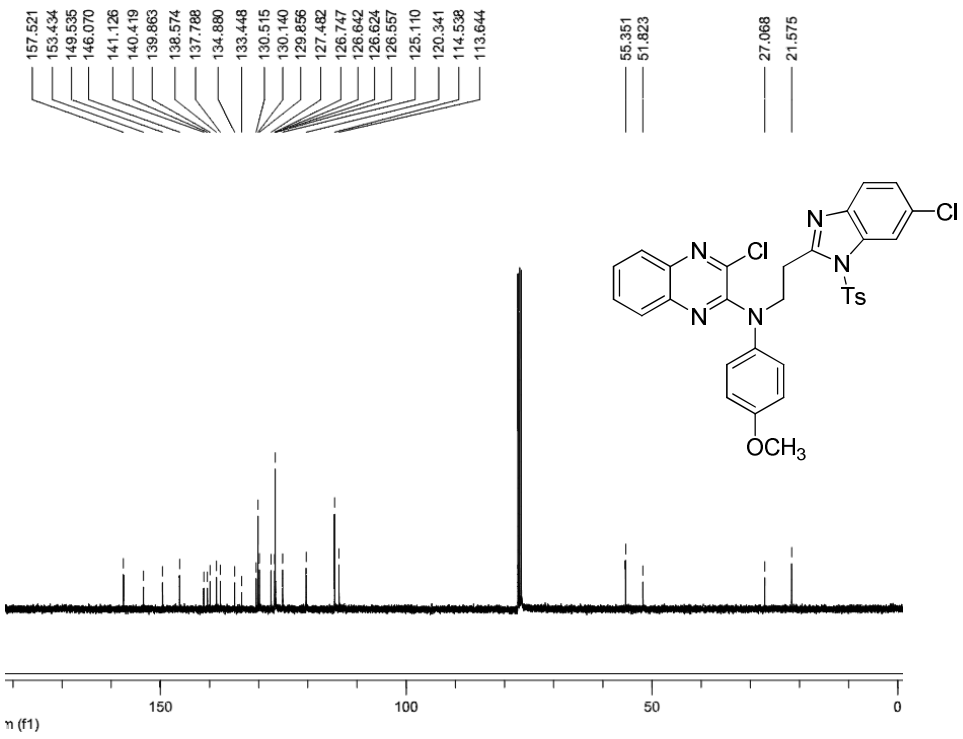

$^{1}$H NMR spectra of compound **22b** ($CDCl_3$, 400 MHz)



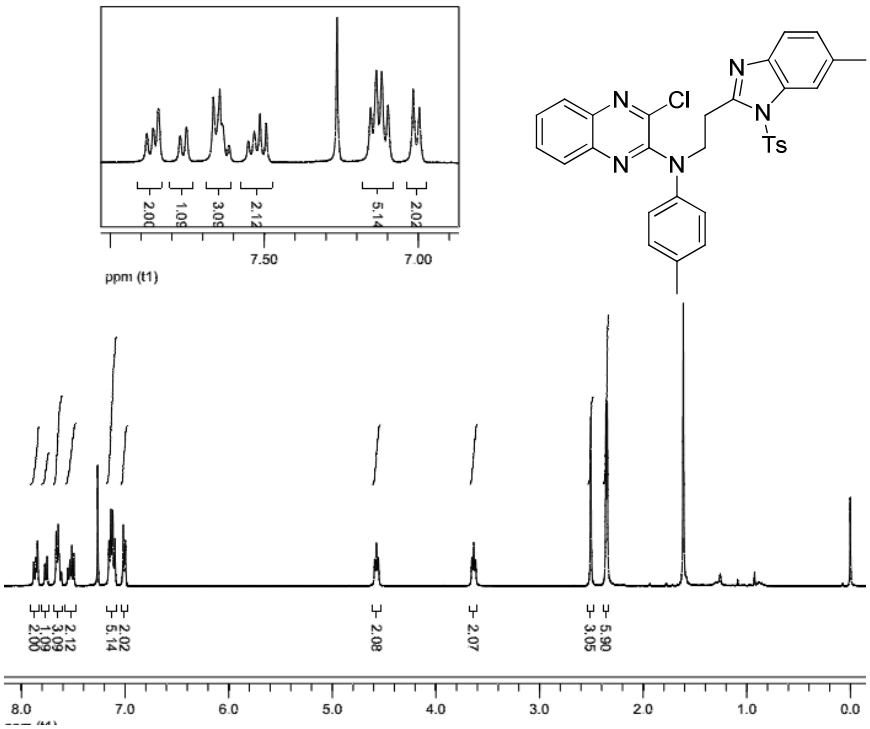

$^{13}$C NMR spectra of compound **22b** ($CDCl_3$, 100 MHz)



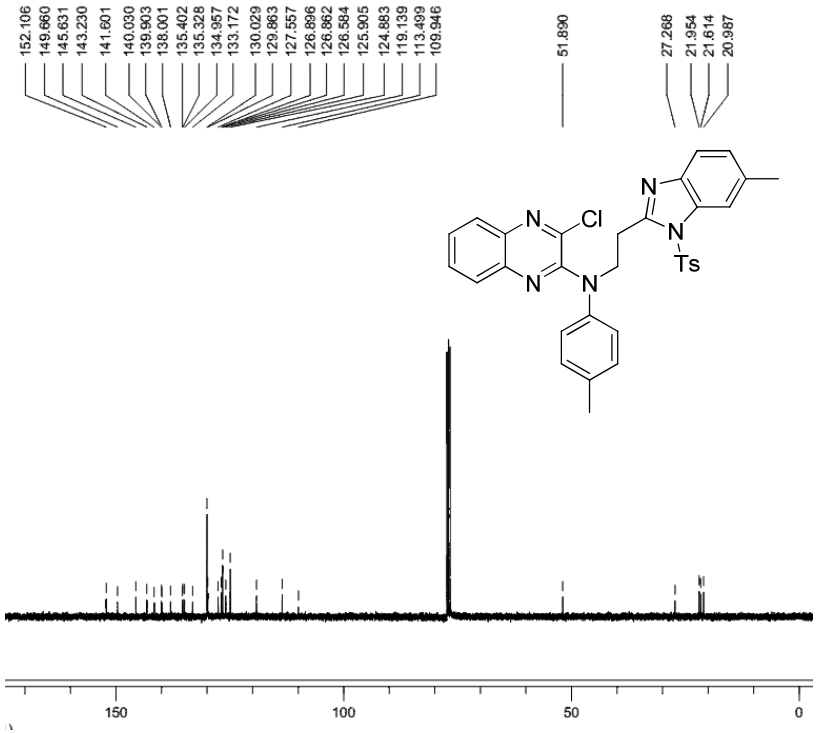

$^{1}$H NMR spectra of compound **22c** ($CDCl_3$, 400 MHz)



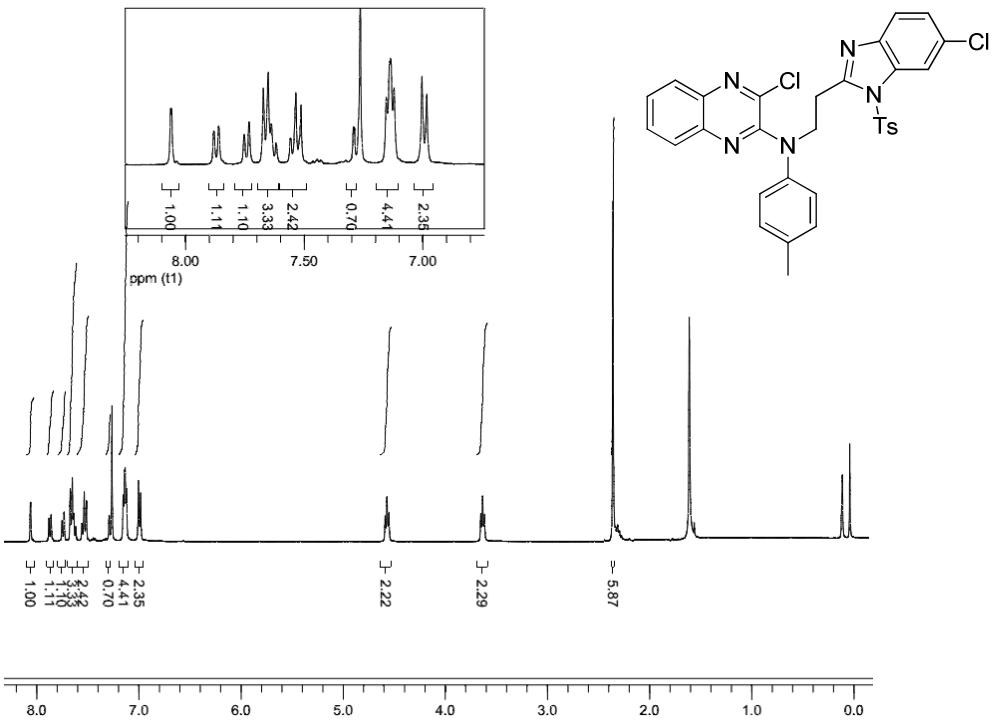

$^{13}$C NMR spectra of compound **22c** ($CDCl_3$, 100 MHz)



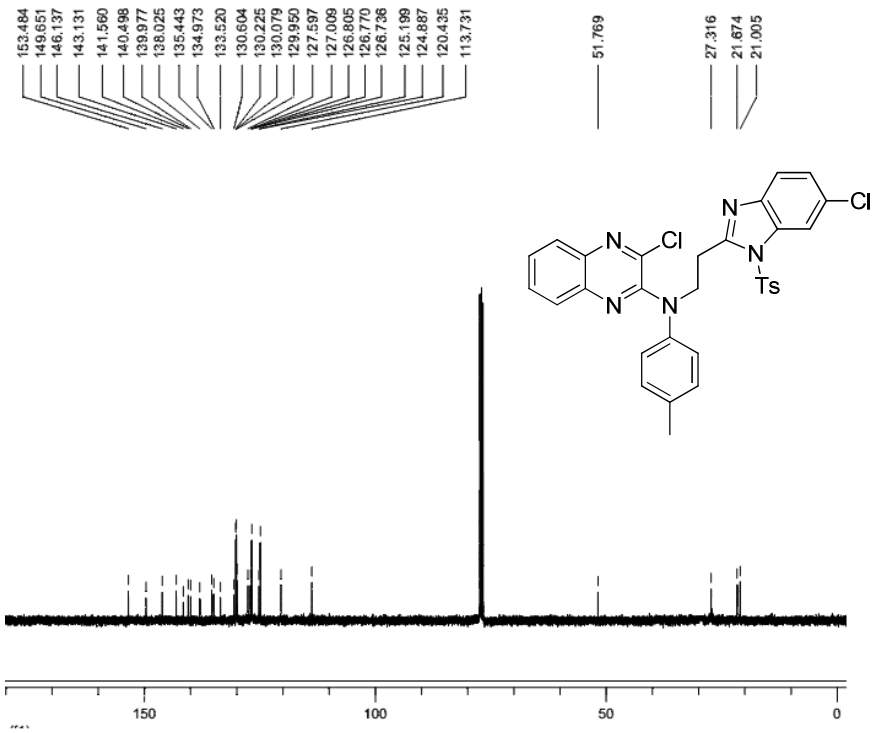

$^{1}$H NMR spectra of compound **22e** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **22e** ($CDCl_3$, 100 MHz)




$^{1}$H NMR spectra of compound **22f** ($CDCl_3$, 400 MHz)




$^{13}$C NMR spectra of compound **22f** ($CDCl_3$, 100 MHz)

# *List of Publications*

1. "Vinylic amino group activation: a new and general strategy leading to functionalized fused heteroaromatics", **Rajnikanth Sunke**, R. Adepu**,** R. Kapavarapu, S. Chintala, C. L. T. Meda, K. V. L. Parsa and M. Pal, *Chem. Comm., 2013*, **49**, 3570.

2. "Ligand-free MCR for linking quinoxaline framework with a benzimidazole nucleus: a new strategy for the identification of novel hybrid molecules as potential inducers of apoptosis", **Rajnikanth Sunke**, P. V. Babu, S. Yellanki, R. Medisetti, P. Kulkarni, and M. Pal, *Org.Biomol.Chem., 2014*, **12**, 6800.

3. "A Pd(II)-catalyzed C–H activation approach to densely functionalized N-heteroaromatics related to neocryptolepine and their evaluation as potential inducers of apoptosis", **Rajnikanth Sunke**, V. Kumar, M. A. Ashfaq, S. Yellanki, R. Medisetti, P. Kulkarni, E. V. V. S. Ramarao, N. Z. Ehtesham and M. Pal, *RSC Adv., 2015*, **5**, 44722.

4. "Quinoxaline: a new directing group for ortho C–H alkenylation/intramolecular ortho C–H cycloamination under open air leading to bioactive polynuclear *N*-heteroarenes", **Rajnikanth Sunke**, V. Kumar, E. V. V. S. Ramarao, R.Bankala, K. V. L. Parsa and M. Pal, *RSC Adv., 2015*, **5**, 70604.

5. "Facile assembly of two 6-membered fused N-heterocyclic rings: a rapid access to novel small molecules *via* Cu-mediated reaction", R. Adepu**,** **Rajnikanth Sunke,** C. L. T. Meda, D. Rambabu, G. R. Krishna, C. M. Reddy, G. S. Deora, K. V. L. Parsa and M. Pal, *Chem.Comm., 2013*, **49**, 190.

## *Oral/Poster Presentations*

1. **Best Oral Award on** "Synthesis of bioactive polynuclear *N*-heteroaromatics by C-H alkenylation / intramolecular ortho C–H cycloamination", **Rajnikanth Sunke** and Manojit Pal. International Conference On "***Trend setting innovations in chemical sciences & technology applications in pharma industry-2015***", *held on16th -18th December,* ***2015,*** Center for chemical sciences & technology, JNTU Hyderabad, India.

2. Poster presentation on "Synthesis of Indoloquinolines related to neocryptolepine via Pd-catalyzed C–H activation", **Rajnikanth Sunke** and Manojit Pal. ***Drils Science Cafe-2015,*** *held on10th August,* ***2015,*** Dr. Reddy's Institute of Life Sciences, University of Hyderabad, India.

3. Poster presentation on "Copper-catalyzed one-pot Synthesis of hybrid benzo[*d*]imidazolo quinoxalines scaffolds as potential inducers of apoptosis", **Rajnikanth Sunke** and Manojit Pal. ***10th National Organic Symposium Trust (J-NOST),*** *held on 4h - 6th December,* ***2014,*** IIT Madras, Chennai, India.

4. Poster presentation on "Amberlyst-15 mediated activation of a vinylic amino group towards synthesis of functionalized heteroaromatics**",** **Rajnikanth Sunke** and Manojit Pal. ***9th National Organic Symposium Trust (J-NOST),*** *held on 4h - 6th December,* ***2013,*** IISER Bhopal, Madhya Pradesh, India.

5. Poster presentation on "Cu-mediated synthesis of fused N-heterocyclic compounds", **Rajnikanth Sunke** and Manojit Pal. ***Catalyst 2013 Dr. Reddy's chemistry conclave,*** *held on 9th and 10th January.*

ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue



Cite this: *Chem. Commun.*, 2013, **49**, 190

Received 28th September 2012,
Accepted 5th November 2012

DOI: 10.1039/c2cc37070k

www.rsc.org/chemcomm

# Facile assembly of two 6-membered fused *N*-heterocyclic rings: a rapid access to novel small molecules *via* Cu-mediated reaction†

Raju Adepu,[a] Rajnikanth Sunke,[a] Chandana L. T. Meda,[a] D. Rambabu,[a] G. Rama Krishna,[b] C. Malla Reddy,[b] Girdhar Singh Deora,[a] Kishore V. L. Parsa[a] and Manojit Pal*[a]

**A rapid, versatile and one-pot Cu-mediated domino reaction has been developed for facile assembly of two six membered fused *N*-heterocyclic rings leading to novel small molecules as potential inhibitors of PDE4.**

The development of elegant, versatile and new synthetic methodologies leading to the diversity based *N*-heterocycles is of enormous importance. Metal catalyzed cascade/domino reactions[1] have occupied the center stage due to their ability to provide an array of diverse and novel compounds especially for medicinal/pharmaceutical uses or early drug discovery effort.

Evaluation of phosphodiesterase 4 (PDE4) inhibition for the potential treatment of CNS related diseases in addition to COPD and asthma has underlined the importance of development of PDE4 inhibitors.[2] Only one drug *i.e.* roflumilast (Daxas®, Nycomed) has been launched so far and side effects including nausea and emesis[2] have delayed the market launch of cilomilast. Thus, discovery of novel PDE4 inhibitors having fewer side effects is desirable. In pursuance of our research on identification of fused *N*-heterocycle based PDE-4/TNF-α inhibitors[3] we required new routes to access our target compound **C** that was derived from our earlier inhibitors[3a,b] **A/B** (Fig. 1). Accordingly, we have developed a new and versatile Cu-mediated domino reaction[4] leading to one-pot synthesis of **C** (or **3** and **5**, Scheme 1) under mild conditions without using any co-catalyst, ligand or additive. Herein we report our preliminary results.

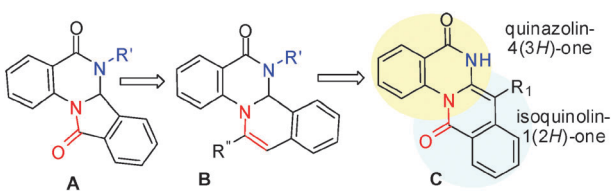
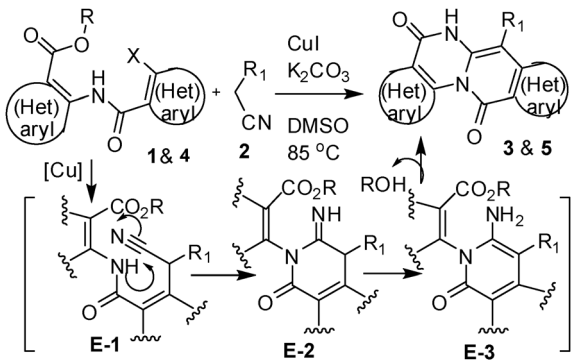


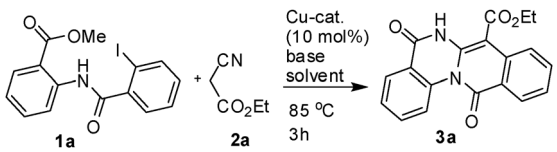

**Fig. 1** Design of novel PDE-4/TNF-α inhibitors (**C**) derived from **A/B**.[3a,b]




**Scheme 1** Synthesis of **3** *via* Cu-catalyzed domino reactions.

While chemistry of quinazolin-4(3*H*)-ones and isoquinolin-1(2*H*)-ones is well documented their combined form **C** (**3** and **5**) remained unexplored.[5] Thus, in addition to evaluating their

**Table 1** Effect of conditions on domino reaction of **1a** with **2a**[a]



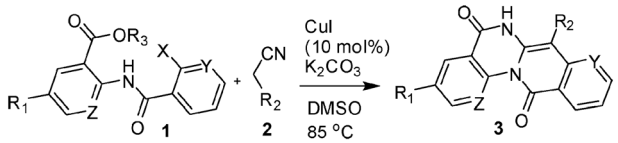

| Entry | Catalyst | Base | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | CuI | $K_2CO_3$ | DMSO | 87 (60, 32)[c] |
| 2 | CuI | $Na_2CO_3$ | DMSO | 71 |
| 3 | CuI | $Cs_2CO_3$ | DMSO | 86 |
| 4 | CuI | $K_2CO_3$ | DMF | 74 |
| 5 | CuI | $K_2CO_3$ | 1,4-Dioxane | 46 |
| 6 | CuI | $K_2CO_3$ | Toluene | 0 |
| 7 | CuBr | $K_2CO_3$ | DMSO | 81 |
| 8 | CuCl | $K_2CO_3$ | DMSO | 69 |
| 9 | No cat. | $K_2CO_3$ | DMSO | 0 |

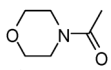
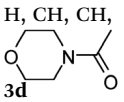
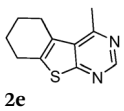
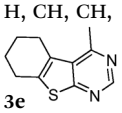
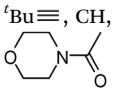
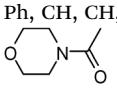
[a] Reactions were carried out using **1a** (1 mmol), **2a** (1.2 mmol), catalyst (0.1 mmol) and base (3 mmol) in a solvent (2 mL) under anhydrous conditions. [b] Isolated yield. [c] 0.05 and 0.02 mmol of CuI used.

[a] *Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India. E-mail: manojitpal@rediffmail.com; Tel: +91 40 6657 1500*
[b] *Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, West Bengal 741252, India*

† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, and results of *in vitro* and docking study. CCDC 902761. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc37070k

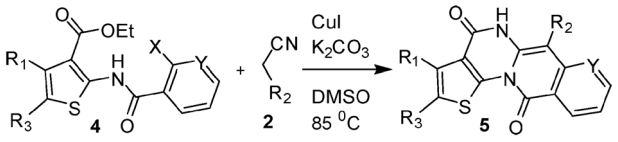
**Table 2** Cu-catalyzed synthesis of 5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (**3**)[a]

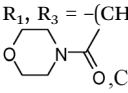
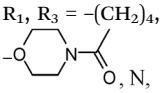
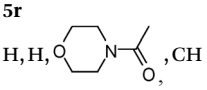



| Entry | Halide (**1**) $R_1$, $R_3$, X, Y, Z | Nitrile (**2**) $R_2$ | *T*/h | Product (**3**) $R_1$, Y, Z, $R_2$ | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | H, $CH_3$, I, CH, CH **1a** | $CO_2Et$ **2a** | 3.0 | H, CH, CH, $CO_2Et$ **3a** | 87 |
| 2 | H, $CH_3$, Br, CH, CH **1b** | **2a** | 3.5 | **3a** | 84 |
| 3 | H, $CH_3$, Cl, CH, CH **1c** | **2a** | 6.0 | **3a** | 0 |
| 4 | **1a** | $CO_2Me$ **2b** | 3.0 | H, CH, CH, $CO_2Me$ **3b** | 86 |
| 5 | **1a** | CN **2c** | 4.0 | H, CH, CH, CN **3c** | 76 |
| 6 | **1a** |  **2d** | 3.5 | H, CH, CH,  **3d** | 77 |
| 7 | **1a** |  **2e** | 3.0 | H, CH, CH,  **3e** | 63 |
| 8 | H, $CH_3$, Cl, N, CH **1d** | **2a** | 4.0 | H, N, CH, $CO_2Et$ **3f** | 73 |
| 9 | $^t$Bu≡, $CH_3$, I, CH, CH **1e** | **2a** | 3.0 | $^t$Bu≡, CH, CH, $CO_2Et$ **3g** | 85 |
| 10 | **1e** | **2c** | 3.5 | $^t$Bu≡, CH, CH, CN **3h** | 72 |
| 11 | **1e** | **2d** | 3.5 | $^t$Bu≡, CH, CH,  **3i** | 72 |
| 12 | Ph, $CH_3$, I, CH, CH **1f** | **2a** | 3.0 | Ph, CH, CH, $CO_2Et$ **3j** | 74 |
| 13 | **1f** | **2b** | 3.0 | Ph, CH, CH, $CO_2Me$ **3k** | 69 |
| 14 | **1f** | **2d** | 3.5 | Ph, CH, CH,  **3l** | 68 |
| 15 | 2-Thienyl, $CH_3$, I, CH, CH **1g** | **2a** | 3.0 | 2-Thienyl, CH, CH, $CO_2Et$ **3m** | 72 |
| 16 | H, $C_2H_5$, I, CH, N **1h** | **2a** | 3.0 | H, CH, N, $CO_2Et$ **3n** | 63 |
| 17 | $NO_2$, $CH_3$, I, CH, CH **1i** | **2a** | 3.5 | $NO_2$, CH, CH, $CO_2Et$ **3o** | 65 |

[a] All the reactions were carried out using **1** (1 mmol), **2** (1.2 mmol), CuI (0.1 mmol) and $K_2CO_3$ (3 mmol) in DMSO (2 mL) under anhydrous conditions (no inert atmosphere). [b] Isolated yield.

**Table 3** Cu-catalyzed synthesis of 4*H*-pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine-4,9(5*H*)-dione (**5**)[a]




| Entry | Halide (**4**) $R_1$, $R_3$, X, Y | Nitrile (**2**) $R_2$ | *T*/h | Product (**5**) $R_1$, $R_3$, $R_2$, Y | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | $R_1$, $R_3$ = $-(CH_2)_4-$, I, CH **4a** | $CO_2Et$ **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_4-$, $CO_2Et$, CH **5a** | 75 |
| 2 | **4a** | CN **2c** | 7.0 | $R_1$, $R_3$ = $-(CH_2)_4-$, CN, CH **5b** | 64 |
| 3 | **4a** | **2d** | 6.5 | $R_1$, $R_3$ = $-(CH_2)_4-$,  **5c** | 69 |
| 4 | $R_1$, $R_3$ = $-(CH_2)_4-$, Cl, N **4b** | **2a** | 8.0 | $R_1$, $R_3$ = $-(CH_2)_4-$, $CO_2Et$, N **5d** | 61 |
| 5 | **4b** | **2d** | 6.5 | $R_1$, $R_3$ = $-(CH_2)_4$,  **5e** | 60 |
| 6 | $R_1$, $R_3$ = $-(CH_2)_2-$ $N(Boc)-CH_2-$, I, CH **4c** | **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_2-$ $N(Boc)-CH_2-$, $CO_2Et$, CH **5f** | 70 |
| 7 | **4c** | **2c** | 7.0 | $R_1$, $R_3$ = $-(CH_2)_2-$ $N(Boc)-CH_2-$, CN, CH **5g** | 61 |
| 8 | $R_1$, $R_3$ = $-(CH_2)_2-$ $N(CO_2Et)-CH_2-$, I, CH **4d** | **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_2-$ $N(CO_2Et)-CH_2-$, $CO_2Et$, CH **5h** | 71 |
| 9 | $R_1$, $R_3$ = $-(CH_2)_3-$, I, CH **4e** | **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_3-$, $CO_2Et$, CH **5i** | 68 |
| 10 | $R_1$, $R_3$ = $-(CH_2)_5-$, I, CH **4f** | **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_5-$, $CO_2Et$, CH **5j** | 70 |
| 11 | **4f** | $CO_2Me$ **2b** | 6.5 | $R_1$, $R_3$ = $-(CH_2)_5-$, $CO_2Me$, CH **5k** | 72 |
| 12 | **4f** | **2c** | 7.0 | $R_1$, $R_3$ = $-(CH_2)_5-$, CN, CH **5l** | 61 |
| 13 | $R_1$, $R_3$ = $-(CH_2)_5-$, Cl, N **4g** | **2a** | 8.5 | $R_1$, $R_3$ = $-(CH_2)_5-$, $CO_2Et$, N **5m** | 63 |
| 14 | $R_1$, $R_3$ = $-(CH_2)_6-$, I, CH **4h** | **2a** | 6.0 | $R_1$, $R_3$ = $-(CH_2)_6-$, $CO_2Et$, CH **5n** | 68 |
| 15 | **4h** | **2b** | 6.5 | $R_1$, $R_3$ = $-(CH_2)_6-$, $CO_2Me$, CH **5o** | 69 |
| 16 | Ph, H, I, CH **4i** | **2a** | 6.0 | Ph, H, $CO_2Et$, CH **5p** | 70 |
| 17 | Ph, H, Cl, N **4j** | **2a** | 8.0 | Ph, H, $CO_2Et$, N **5q** | 62 |
| 18 | H, H, I, CH **4k** | **2a** | 6.5 | H, H, $CO_2Et$, CH **5r** | 72 |
| 19 | **4k** | **2d** | 7.5 |  **5s** | 59 |

[a] For reaction conditions, see footnote of Table 2. [b] Isolated yield.

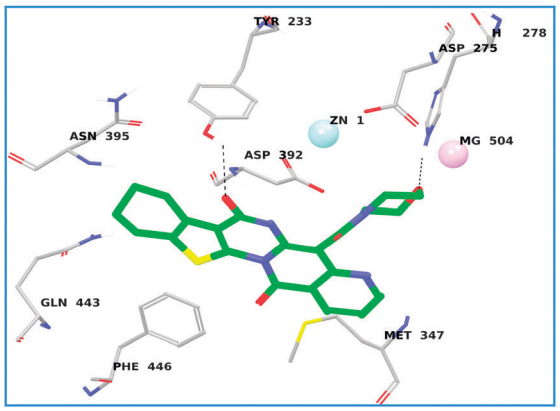


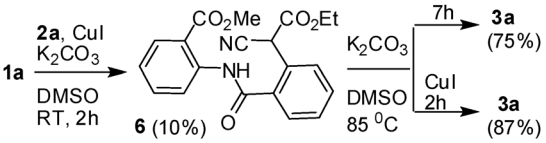

**Fig. 2** Binding mode and interactions of **5e** with PDE4B active sites.

PDE4 inhibiting properties the development of a suitable methodology leading to **C** was a major challenge. We envisioned that Cu-mediated C-arylation of nitriles (*e.g.* **E-1**) followed by an intramolecular nucleophilic addition of NH to CN would afford the initial 6-membered ring *in situ* (**E-3** *via* **E-2**, Scheme 1). A subsequent intramolecular nucleophilic attack by the 3-amino moiety of **E-3** on its ester group would allow the formation of a fused pyrimidone ring leading to **3** or **5**.

The key starting material **1** or **4** required for our synthesis was prepared *via* amide bond formation between 2-halo (het)aryl carboxylic acid chloride and 2-amino (het)aryl carboxylate ester (see ESI†). We then examined the coupling of iodo compound **1a** with ethyl cyanoacetate (**2a**) under various conditions. After assessing a range of bases *e.g.* $K_2CO_3$, $Na_2CO_3$ and $Cs_2CO_3$ (entries 1–3, Table 1), solvents *e.g.* DMSO, DMF, 1,4-dioxane and toluene (entries 1 and 4–6, Table 1) and catalysts *e.g.* CuI, CuBr and CuCl (entries 1, 7 and 8, Table 1) a combination of CuI and $K_2CO_3$ in DMSO was found to be optimum. A decrease in CuI loading decreased the product yield and no reaction in the absence of CuI (entries 1 and 9, Table 1) indicated the key role played by the catalyst.

We then examined the scope of the present Cu-catalyzed domino reaction which afforded compound **3** with a variety of substitution patterns (Table 2). The reaction proceeded well with various substituents on **1** *e.g.* $R_1$ = alkynyl (entries 9–11, Table 2), phenyl (entries 12–14, Table 2), 2-thienyl (entry 15, Table 2), or $NO_2$ (entry 17, Table 2) group irrespective of X being I or Br (entry 1 *vs.* 2, Table 2) except Cl (entry 3, Table 2) unless it is attached to an azomethine carbon (entry 8, Table 2). In addition to the use of various nitriles (**2a–e**) the reaction was also successful for thiophene analogues of **1** (Table 3) *i.e.* **4** containing various substituents *e.g.* $R_1$, $R_3$ representing a fused alicyclic (entries 1–5 and 9–15, Table 3) or azaalicyclic (entries 6–8, Table 3) ring or hydrogens (entries 18 and 19, Table 3) or $R_1$ = Ph and $R_3$ = H (entries 16 and 17, Table 3). All the compounds synthesized were well characterized by spectral (NMR, IR and MS) data and the molecular structure of **5k** was confirmed unambiguously by single crystal X-ray diffraction study (see ESI†).[6]

Mechanistically, the intermediacy of **E-1** (Scheme 1) was confirmed by isolation of **6** from the reaction of **1a** with **2a** at room temperature (Scheme 2). The shorter reaction time (2 h) for the conversion of **6** to **3a** in the presence of CuI indicated the




**Scheme 2** Preparation of intermediate **6** and its conversion to **3a**.

favorable role played by the catalyst in the cycloaddition step (perhaps *via* coordination with CN) in addition to C-arylation.

Some of the synthesized compounds showed promising inhibition of PDE4B [*e.g.* **3f** (51%), **3n** (57%) and **5e** (62%)] when tested *in vitro*[7] at 30 **μM** (see ESI†). This was further supported by the docking results of **5e** (Fig. 2) (and **3n**, see ESI†) with PDE4B protein (Glide score −7.4). The oxygen atom of the morpholine ring of **5e** along with the CO group participated in H-bonding with NH of His-278 and OH of Tyr 233 respectively. A pi–pi stacking between **5e** and Phe-446 was also observed (Fig. 2). The morpholine ring of **5e** was found to be well occupied in the partially charged pocket of active sites (see ESI†).

In conclusion, a new, one pot and versatile Cu-mediated domino reaction has been developed that allowed rapid access to a library of small molecules based on novel structural motifs. Three of these compounds showed inhibition of PDE4B *in vitro* and may have potential for therapeutic applications.

RA and RS thank CSIR for research fellowships. The authors thank Prof. J. Iqbal and DST (Grant SR/S1/OC-53/2009) for support.

## Notes and references

1 For excellent reviews, see: (*a*) V. Michelet, P. Y. Toullec and J.-P. Genêt, *Angew. Chem., Int. Ed.*, 2008, **47**, 4268; (*b*) E. Jiménez-Núñez and A. M. Echavarren, *Chem. Rev.*, 2008, **108**, 3326; (*c*) N. T. Patil and Y. Yamamoto, *Chem. Rev.*, 2008, **108**, 3395; (*d*) D. J. Gorin, B. D. Sherry and F. D. Toste, *Chem. Rev.*, 2008, **108**, 3351; (*e*) S. F. Kirsch, *Synthesis*, 2008, 3183; (*f*) D. M. D'Souza and T. J. J. Muller, *Chem. Soc. Rev.*, 2007, **36**, 1095; (*g*) K. C. Nicolaou, D. J. Edmonds and P. G. Bulger, *Angew. Chem., Int. Ed.*, 2006, **45**, 7134; (*h*) H. Pellissier, *Tetrahedron*, 2006, **62**, 2143; (*i*) L. F. Tietze, *Chem. Rev.*, 1996, **96**, 115.

2 For reviews, see: (*a*) A. Kodimuthali, S. L. Jabaris and M. Pal, *J. Med. Chem.*, 2008, **51**, 5471; (*b*) M. D. Houslay, P. Schafer and K. Y. Zhang, *Drug Discovery Today*, 2005, **10**, 1503.

3 (*a*) K. S. Kumar, P. M. Kumar, K. A. Kumar, M. Sreenivasulu, A. A. Jafar, D. Rambabu, G. R. Krishna, C. M. Reddy, R. Kapavarapu, K. S. Kumar, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 5010; (*b*) K. S. Kumar, P. M. Kumar, M. A. Reddy, Md. Ferozuddin, M. Sreenivasulu, A. A. Jafar, G. R. Krishna, C. M. Reddy, D. Rambabu, K. S. Kumar, S. Pal and M. Pal, *Chem. Commun.*, 2011, **47**, 10263; (*c*) G. R. Reddy, T. R. Reddy, S. C. Joseph, K. S. Reddy, L. S. Reddy, P. M. Kumar, G. R. Krishna, C. M. Reddy, D. Rambabu, R. Kapavarapu, C. Lakshmi, T. Meda, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 7779; (*d*) P. M. Kumar, K. S. Kumar, P. K. Mohakhud, K. Mukkanti, R. Kapavarapu, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2012, **48**, 431.

4 For selected Cu-mediated domino reaction, see: (*a*) M. Jiang, J. Li, F. Wang, Y. Zhao, X. Dong and W. Zhao, *Org. Lett.*, 2012, **14**, 1420; (*b*) T. Liu, C. Zhu, H. Yang and H. Fu, *Adv. Synth. Catal.*, 2012, **354**, 1579; (*c*) J. Lu, X. Gong, H. Yang and H. Fu, *Chem. Commun.*, 2010, **46**, 4172; (*d*) V. A. Vaillard, R. A. Rosi and S. E. Martin, *Org. Biomol. Chem.*, 2011, **9**, 4927; (*e*) B. Zou, Q. Yuan and D. Ma, *Angew. Chem., Int. Ed.*, 2007, **46**, 2598.

5 Only two synthesis of isoquino[2,3-*a*]quinazolines have been reported, see: (*a*) Y. M. Volovenko, *Chem. Heterocycl. Compd.*, 1997, **33**, 997; (*b*) V. M. Kisel', V. A. Kovtunenko, A. V. Turov, A. K. Tyltin and F. S. Babichev, *Dokl. Akad. Nauk SSSR*, 1989, **306**, 628.

6 CCDC 902761 (**5k**)†.

7 P. Wang, J. G. Myers, P. Wu, B. Cheewatrakoolpong, R. W. Egan and M. M. Billah, *Biochem. Biophys. Res. Commun.*, 1997, **234**, 320.

ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

# Vinylic amino group activation: a new and general strategy leading to functionalized fused heteroaromatics†

Cite this: *Chem. Commun.*, 2013, **49**, 3570

Received 22nd February 2013,
Accepted 11th March 2013

DOI: 10.1039/c3cc41337c

www.rsc.org/chemcomm

Rajnikanth Sunke,[a] Raju Adepu,[a] Ravikumar Kapavarapu,[b] Swetha Chintala,[a] Chandana Lakshmi Teja Meda,[a] Kishore V. L. Parsa[a] and Manojit Pal*[a]



**A conceptually new and general strategy has been developed for the construction of a benzimidazole or a benzoxazole ring fused with isoquinolinone affording a diverse and unique class of small molecules as potential and novel inhibitors of PDE4.**

The development of new and powerful chemical methodologies leading to fused heteroaromatics is of immense value as it allows access to the diversity based novel chemical space useful for pharmaceutical/drug discovery efforts. Through the generation of a combinatorial library of small molecules these methodologies often provide crucial breakthroughs in the discovery of new chemical entities (NCEs) required by pharmaceutical/agrochemical industries.

While benzimidazole or benzoxazole and isoquinolinone are well known structural motifs in drug discovery/medicinal chemistry their combined form *i.e.* (benzimidazo or benzoxazolo)isoquinolinones largely remained unexplored perhaps due to the limited or no accessibility of this class of compounds.[1] This prompted us to explore[2] a new and general method of accessing benzimidazo[1,2-*b*]isoquinolin-11-one/benzoxazolo[3,2-*b*]isoquinolin-11-one derivatives as potential inhibitors of phosphodiesterase 4 (PDE4). PDE4 inhibitors are known to be useful anti-inflammatory agents for the potential treatment of chronic obstructive pulmonary disease (COPD) and asthma.[3] Our target molecules **B** derived from a known anti-inflammatory agent[4] CP-77059 (Fig. 1) were designed based on the *in silico* docking studies of a representative compound **A** (Fig. 1) into the active site of PDE4B (Fig. 2). The study showed binding of **A** deep into the active site (docking score −22.07) along with an H-bond interaction of carbonyl oxygen of ester with the side chain amino group of Gln 443 (see ESI†).

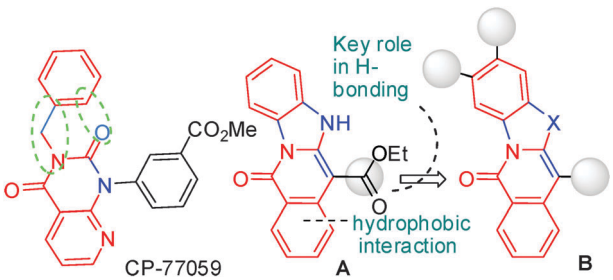



**Fig. 1** Design of **A**/**B** as novel inhibitors of PDE4.

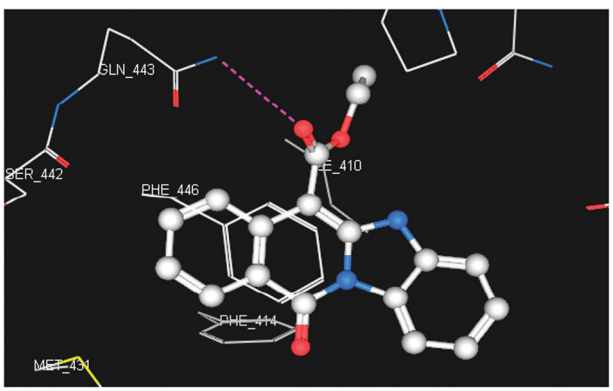


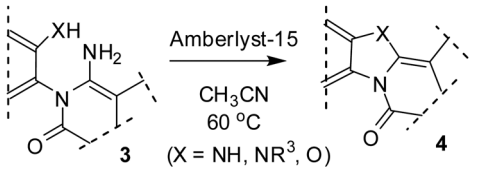

**Fig. 2** Binding mode of **A** in PDE4B (PDB code-1XMY).

In view of the central role played by heterogeneous catalysts in various organic transformations,[5] inexpensive and commercially available Amberlyst-15 attracted our attention due to its non-hazardous nature and easy removal from the reaction mixture *e.g. via* simple filtration. We anticipated that Amberlyst-15 mediated activation of the vinylic amino group of 3-amino-2-(2-hydroxy/aminophenyl)isoquinolin-1(2*H*)-one could trigger its intramolecular cyclization leading to our target compounds **A**/**B**. Herein we report our preliminary results on intramolecular cyclization of 3-amino-2-(2-amino/hydroxyphenyl)isoquinolin-1(2*H*)-one derivative **3** leading to benzimidazo[1,2-*b*]isoquinolin-11-ones/benzoxazolo[3,2-*b*]isoquinolin-11-ones **4** (or **B**, Scheme 1).

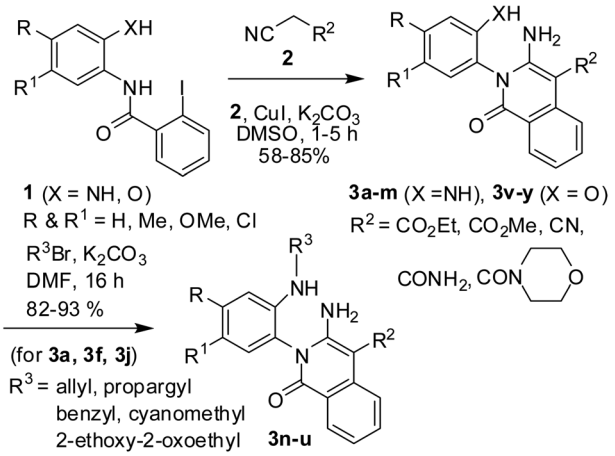
[a] *Dr Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India. E-mail: manojitpal@rediffmail.com; Tel: +91 40 6657 1500*

[b] *Doctoral Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal*

† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, results of *in vitro* and docking studies. See DOI: 10.1039/c3cc41337c




**Scheme 1** Amberlyst-15 mediated activation of a vinylic amino group.



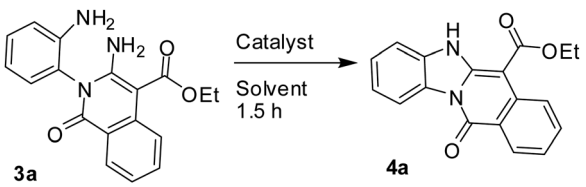

**Scheme 2** Preparation of key starting material **3**.

To the best of our knowledge the use of this strategy leading to **4** is unprecedented.[1,6] The key starting material **3** (**3a–m;** X = NH and **3v–y;** X = O) required was prepared *via* Cu-mediated[2f,7] coupling–cyclization of 2-iodobenzamides **1** with appropriate cyano derivatives **2** in the same pot (followed by selective *N*-alkylation to prepare **3n–u**) (Scheme 2).

The Amberlyst-15 mediated intramolecular cyclization of **3a** was examined initially in a variety of solvents (Table 1). The reaction proceeded well in MeCN, PEG and MeOH (entries 1, 2 and 4, Table 1) but not in DMF (entry 3, Table 1). Notably, the reaction proceeded in water affording product **4a** albeit in lower yield (entry 5, Table 1). The reaction, however, did not proceed in the absence of Amberlyst-15 (entry 6, Table 1) or in the presence of another catalyst *i.e.* Amberlite (entry 7, Table 1) indicating the key role played by Amberlyst-15 in the present reaction. To test the recyclability of the catalyst, Amberlyst-15 was recovered by simple filtration and reused additional three times when **4a** was isolated without significant loss of its yield (entry 1, Table 1). Notably, all these reactions were performed without using any inert atmosphere. Overall, Amberlyst-15 in MeCN was found to be optimum for the preparation of **4a**.

We then examined the generality and substrate scope of the present reaction. A range of substituents *e.g.* Me, OMe, Cl on the *N*-aryl ring and ester, CN, amide on the isoquinolinone ring of **3** were well tolerated (Table 2). Moreover, the reaction proceeded well irrespective of the nature of participating amino groups (*e.g.* primary or secondary) on the *N*-aryl ring of **3**. Secondary amines possessing various $R^3$ groups like allyl, propargyl, benzyl, cyanomethyl or 2-ethoxy-2-oxoethyl participated well in the reaction. The generality of this methodology was demonstrated further by synthesizing benzoxazolo[3,2-*b*]isoquinolin-11-ones (**4v–y**) where the phenolic hydroxyl group of the *N*-aryl ring of **3** participated in the reaction. All the desired products were synthesized in good to excellent yields[8] and well characterized by spectral (NMR, IR and MS) data (see ESI†).

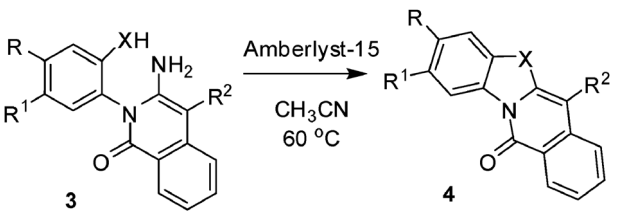
**Table 1** Effect of conditions on Amberlyst-15 mediated synthesis of **4a**[a]




| Entry | Catalyst | Solvent | Yield[b] (%) |
|---|---|---|---|
| 1 | Amberlyst-15 | MeCN | 98 (95, 90, 88)[c] |
| 2 | Amberlyst-15 | PEG-800 | 92 |
| 3 | Amberlyst-15 | DMF | 47 |
| 4 | Amberlyst-15 | MeOH | 90 |
| 5 | Amberlyst-15 | $H_2O$ | 75[d] |
| 6 | No cat. | MeCN | No reaction |
| 7 | Amberlite | MeCN | No reaction |

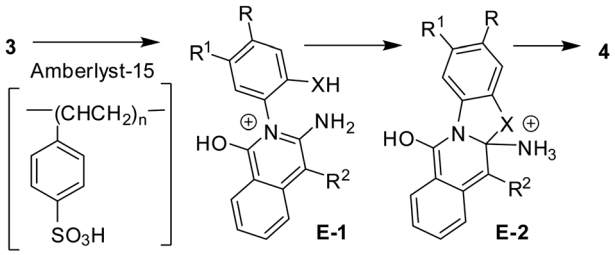
[a] Reaction was carried out using **3a** (1.0 mmol), catalyst (10%, w/w) in solvent (5 mL) at 60 °C. [b] Isolated yield. [c] Catalyst was reused for additional three runs and figures within parentheses indicate the corresponding yields for each run. [d] The reaction was carried out at 75 °C.

**Table 2** Amberlyst-15 mediated synthesis of **4**[a]




| Entry | X, R, $R^1$, $R^2$ substrate (**3**) | *t*/h | Product (**4**) | Yield[b] (%) |
|---|---|---|---|---|
| 1 | NH, H, H, $CO_2Et$ **3a** | 1.5 | **4a** | 98 |
| 2 | NH, H, H, $CO_2Me$ **3b** | 2.0 | **4b** | 87 |
| 3 | NH, H, H, CN **3c** | 7.0 | **4c** | 71 |
| 4 | NH, H, H, mor[c] **3d** | 11.5 | **4d** | 63 |
| 5 | NH, H, H, $CONH_2$ **3e** | 8.5 | **4e** | 65 |
| 6 | NH, $CH_3$, H, $CO_2Et$ **3f** | 1.5 | **4f** | 91 |
| 7 | NH, $CH_3$, H, $CO_2Me$ **3g** | 2.0 | **4g** | 83 |
| 8 | NH, $CH_3$, H, CN **3h** | 7.0 | **4h** | 75 |
| 9 | NH, $CH_3$, H, mor **3i** | 12.0 | **4i** | 61 |
| 10 | NH, $OCH_3$, H, $CO_2Et$ **3j** | 1.5 | **4j** | 92 |
| 11 | NH, $OCH_3$, H, $CO_2Me$ **3k** | 2.0 | **4k** | 91 |
| 12 | NH, Cl, Cl, $CO_2Et$ **3l** | 2.0 | **4l** | 93 |
| 13 | NH, Cl, Cl, $CO_2Me$ **3m** | 2.0 | **4m** | 90 |
| 14 | *N*-Allyl, H, H, $CO_2Et$ **3n** | 3.5 | **4n** | 89 |
| 15 | *N*-Allyl, $CH_3$, H, $CO_2Et$ **3o** | 3.5 | **4o** | 91 |
| 16 | *N*-Propargyl, H, H, $CO_2Et$ **3p** | 3.0 | **4p** | 89 |
| 17 | *N*-Propargyl, $CH_3$, H, $CO_2Et$ **3q** | 3.0 | **4q** | 86 |
| 18 | NBn, H, H, $CO_2Et$ **3r** | 4.5 | **4r** | 95 |
| 19 | NBn, $OCH_3$, H, $CO_2Et$ **3s** | 4.0 | **4s** | 95 |
| 20 | $NCH_2CN$, $CH_3$, H, $CO_2Et$ **3t** | 5.0 | **4t** | 96 |
| 21 | $NCH_2CO_2Et$, $CH_3$, H, $CO_2Et$ **3u** | 6.0 | **4u** | 91 |
| 22 | O, $OCH_3$, H, $CO_2Et$ **3v** | 5.0 | **4v** | 90 |
| 23 | O, $OCH_3$, H, $CONH_2$ **3w** | 8.0 | **4w** | 85 |
| 24 | O, $OCH_3$, H, mor **3x** | 8.0 | **4x** | 72 |
| 25 | O, $CH_3$, H, $CO_2Et$ **3y** | 5.0 | **4y** | 93 |

[a] All the reactions were carried out using compound **3** (1 mmol) and Amberlyst-15 (10%, w/w) in $CH_3CN$ (5 mL) at 60 °C. [b] Isolated yield. [c] mor = morpholine-4-carbonyl.




**Scheme 3** The proposed reaction mechanism.

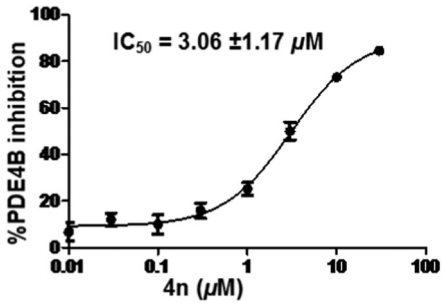



**Fig. 3** Dose dependent inhibition of PDE4B by **4n**.

Amberlyst-15 (Scheme 3), a macro-reticular polystyrene-based ion exchange resin, possesses strongly acidic sulfonic groups. Thus, mechanistically (Scheme 3), the intramolecular cyclization of **3** seemed to proceed *via* a two-step process involving (i) a nucleophilic attack by the –XH moiety[9a] of **E-1** on its activated and nearby –C═N– affording the intermediate **E-2** followed by (ii) elimination of ammonia to give the desired compound **4**.[9b] An attempt to isolate the intermediate **E-1** or **E-2** from the reaction of **3a** under the conditions employed however failed, perhaps due to its rapid participation in the next step leading to **4a**.

Some of the compounds synthesized were tested against PDE4B using an *in vitro* enzyme assay.[10] The compounds **4a** (**A** in Fig. 1 and 2), **4f**, **4g**, **4d** and **4n** showed 62, 53, 57, 48 and 85% inhibition, respectively, at 30 μM and **4n** ($IC_{50}$ ~ 3 μM, Fig. 3) was comparable with rolipram ($IC_{50}$ ~ 1 μM). Since COPD and asthma are the major health burden worldwide, the present class of compounds is of further interest.

In conclusion, a facile assembly of a benzimidazole or a benzoxazole ring with isoquinolinone has been achieved *via* a conceptually new and general strategy involving Amberlyst-15 mediated activation of a vinylic amino group leading to a diverse and unique class of small molecules as potential inhibitors of PDE4.

RS and RA thank CSIR, for research fellowships. The authors thank Prof. J. Iqbal and DST (Grant SR/S1/OC-53/2009) for support.

## Notes and references

1 For the only report on biological activities of benzimidazoisoquinolinone, see: M. Bollini, J. J. Casal, D. E. Alvarez, L. Boiani, M. González, H. Cerecetto and A. M. Bruno, *Bioorg. Med. Chem.*, 2009, **17**, 1437.
2 For our earlier effort on the synthesis of functionalized heteroaromatics, see: (*a*) K. S. Kumar, P. M. Kumar, K. A. Kumar, M. Sreenivasulu, A. A. Jafar, D. Rambabu, G. R. Krishna, C. M. Reddy, R. Kapavarapu, K. S. Kumar, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 5010; (*b*) K. S. Kumar, P. M. Kumar, M. A. Reddy, M. Ferozuddin, M. Sreenivasulu, A. A. Jafar, G. R. Krishna, C. M. Reddy, D. Rambabu, K. S. Kumar, S. Pal and M. Pal, *Chem. Commun.*, 2011, **47**, 10263; (*c*) G. R. Reddy, T. R. Reddy, S. C. Joseph, K. S. Reddy, L. S. Reddy, P. M. Kumar, G. R. Krishna, C. M. Reddy, D. Rambabu, R. Kapavarapu, C. Lakshmi, T. Meda, K. K. Priya, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2011, **47**, 7779; (*d*) P. M. Kumar, K. S. Kumar, P. K. Mohakhud, K. Mukkanti, R. Kapavarapu, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2012, **48**, 431; (*e*) B. Prasad, R. Adepu, S. Sandra, D. Rambabu, G. R. Krishna, C. M. Reddy, G. S. Deora, P. Misra and M. Pal, *Chem. Commun.*, 2012, **48**, 10434; (*f*) R. Adepu, R. Sunke, C. L. T. Meda, D. Rambabu, G. R. Krishna, C. M. Reddy, G. S. Deora, K. V. L. Parsa and M. Pal, *Chem. Commun.*, 2013, **49**, 190.
3 A. Kodimuthali, S. S. L. Jabaris and M. Pal, *J. Med. Chem.*, 2008, **51**, 5471.
4 J. A. Lowe, III, R. L. Archer, D. S. Chapin, J. B. Cheng, D. Helweg, J. L. Johnson, B. K. Koe, L. A. Lebel, P. F. Moore, J. A. Nielsen, L. L. Russo and J. T. Shirley, *J. Med. Chem.*, 1991, **34**, 624.
5 (*a*) S. H. Lee, D. R. Park, H. Kim, J. Lee, J. C. Jung, S. Y. Woo, W. S. Song, M. S. Kwon and I. K. Song, *Catal. Commun.*, 2008, **9**, 1920; (*b*) S. Park, K. M. Cho, M. H. Youn, J. G. Seo, S. H. Baeck, T. J. Kim, Y. M. Chung, S. H. Oh and I. K. Song, *Catal. Lett.*, 2008, **122**, 349; (*c*) I. K. Song and W. Y. Lee, *Appl. Catal., A*, 2003, **256**, 77.
6 For earlier methods, see: (*a*) J. Lu, X. Gong, H. Yang and H. Fu, *Chem. Commun.*, 2010, **46**, 4172; (*b*) A. Senthilvelan, D. Thirumalai and V. T. Ramakrishnan, *Tetrahedron*, 2005, **61**, 4213; (*c*) K.-Q. Ling, X.-Y. Chen, H.-K. Fun, X.-Y. Huang and J.-H. Xu, *J. Chem. Soc., Perkin Trans. 1*, 1998, 4147. See also ref. 1.
7 For a similar Cu-mediated coupling, see: T. Liu, C. Zhu, H. Yang and H. Fu, *Adv. Synth. Catal.*, 2012, **354**, 1579; see also ref. 6*a*.
8 Except **4c–e**, **4h**, **4i**, **4w** and **4x** other compounds do not require any chromatographic purification after isolation.
9 (*a*) The possibility of the –XH moiety to play the role of a leaving group (*cf.* ref. 6*a* where Br was used as a leaving group) was ruled out as the corresponding benzimidazo[1,2-*b*]isoquinolin-11-ones was not isolated in the case of **3v–y** instead of benzoxazolo[3,2-*b*]isoquinolin-11-ones **4v–y** (see Table 2). Indeed, the present role of –XH allowed us to introduce further diversity *i.e.* the $R^3$ group into the product **4**; (*b*) Alternatively, protonation of $R^2$ (*i.e.* ester, CN or amide) of **3** followed by intramolecular nucleophilic attack of XH on the –C═N– moiety could also lead to the formation of product **4**.
10 P. Wang, J. G. Myers, P. Wu, B. Cheewatrakoolpong, R. W. Egan and M. M. Billah, *Biochem. Biophys. Res. Commun.*, 1997, **234**, 320.



RSC Advances

COMMUNICATION

View Article Online
View Journal | View Issue



Cite this: *RSC Adv.*, 2015, **5**, 70604

Received 24th July 2015
Accepted 11th August 2015

DOI: 10.1039/c5ra14671b

www.rsc.org/advances

# Quinoxaline: a new directing group for *ortho* C–H alkenylation / intramolecular *ortho* C–H cycloamination under open air leading to bioactive polynuclear *N*-heteroarenes†

Rajnikanth Sunke,[a] Vimal Kumar,[b] E. V. Venkat Shivaji Ramarao,[a] Ramudu Bankala,[a] Kishore V. L. Parsa[a] and Manojit Pal*[a]

Quinoxaline has been identified as a new directing group for the Pd (or Ru)-catalyzed *ortho* C–H alkenylation of aniline derivatives and subsequent hypervalent iodine promoted intramolecular *ortho* C–H cycloamination of the resulting *N*-arylquinoxalin-2-amine derivatives. This two-step strategy afforded alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines as inhibitors of PDE4. The Pd-catalyzed *ortho* C–H alkenylation of phenol derivatives was also performed successfully when quinoline was found to be an effective directing group.

The strategy consisting of directed *ortho* C–H functionalization followed by converting the directing group into an integral part of the target molecule is of fundamental interest as this may allow easy and quick access to functionalized heteroarenes for their potential applications in organic/medicinal/pharmaceutical chemistry.

While functionalized alkenes[1] have been explored in the discovery of new drugs[2] *e.g.* tamoxifen[2a] in the past, assembly of polynuclear heteroarene and an alkenyl moiety in the same molecule largely remain underexplored. Their potential applications in medicinal and pharmaceutical chemistry and our interest in bioactive alkenyl substituted heteroarenes[3] prompted us to focus on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]-quinoxalines (**A**, Fig. 1) and their pharmacological evaluation *in vitro*. Indeed the selection of benzo[4,5]imidazo[1,2-*a*]quinoxaline ring was inspired by the promising pharmacological properties of structurally related imidazo[1,2-*a*]quinoxaline based molecules[4,5] *e.g.* EAPB0203 (**B**, Fig. 1). The core structure of **A** *i.e.* the central polynuclear heterocyclic ring can be realized simply by moving and fusing the benzene ring of 3-methoxy phenyl group with the imidazole ring of **B**.

Alkenylation is one of the most powerful methods for accessing substituted alkenes[6] that involved Pd-catalyzed coupling of aryl halides with alkenes (the Mizoroki–Heck reaction).[7] However, because of drawbacks such as limited availability of expensive aryl halide component, or their cumbersome preparation, direct C–H bond olefination (the Fujiwara–Moritani reaction)[8] has attracted huge attention as a greener alternative during past several years. While transition metals have been used extensively for the C–H functionalization leading to C–C and C-heteroatom bond formation,[9] the Pd-catalyzed chelation-directed $sp^2$ C–H activation has been found to be a highly effective strategy for this purpose.[10] This approach involves the use of σ-chelating directing groups with a metal center that leads to *o*-selectivity *via in situ* generation of conformationally rigid rings. A range of directing groups has been reported to aid C(Ar)–H activation until recently *e.g.* pyridine,[10a–c] imidazoline,[10d] pyrazole,[10e] oxazoline,[10f,g] amide,[10h,i] oxime ether,[10j] ketones,[10k] hydroxyl,[10l,m] carboxylic acids,[10n,o] 2-pyridylsulfinyl,[10p] quinoline[10q] *etc.* However, several of these groups are non-removable and are considered as serious limitations for practical applications of these processes. In our strategy we wondered if any of these groups or a new one could be considered as a pre-installed precursor required for further chemical transformation instead of attempting their removal after the C–H activation step. This strategy appeared to be attractive and economical as it could allow salvaging of directing groups. A retro synthetic analysis of **A** (Fig. 2) revealed that a quinoxaline moiety could serve the purpose and it was therefore necessary for the quinoxaline moiety to play the role of a directing group in the present case.

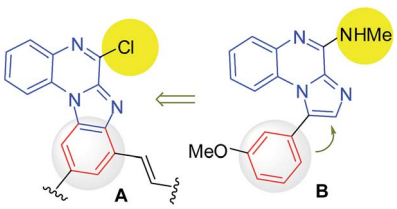



Fig. 1 New alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines (**A**) and a known imidazo[1,2-*a*]quinoxaline derivative EAPB0203 (**B**).

[a]*Dr Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India. E-mail: manojitpal@rediffmail.com*

[b]*University of Delhi, Delhi, India*

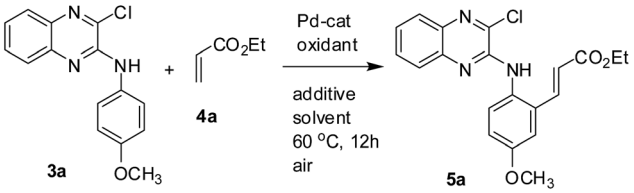
† Electronic supplementary information (ESI) available: Experimental procedures, copies of the $^1$H and $^{13}$C NMR spectra. See DOI: 10.1039/c5ra14671b

This prompted us to test quinoxaline as a new directing group for the C–H functionalization with *o*-selectivity.[11] Herein we report our preliminary results on the Pd (or Ru)-catalyzed *o*-alkenylation of aniline derivatives *via* C–H activation assisted by quinoxaline (Scheme 1). We also report subsequent conversion of the resulting *N*-arylquinoxalin-2-amine derivatives (**5**) to **A** (or **6**) *via* hypervalent iodine promoted intra-molecular C–H cycloamination reaction[12] under mild and environmental friendly conditions (Scheme 1). To our knowledge the use of this two-step strategy consisting of *ortho* C–H functionalization followed by intramolecular C–H cycloamination involving quinoxaline moiety leading to alkenyl substituted polynuclear *N*-heteroarenes *e.g.* benzo[4,5]imidazo[1,2-*a*]quinoxalines[13] is not common in the literature. Moreover, though synthesis of this class of *N*-heteroarenes has been reported earlier their alkenyl analogues are not known. The effectiveness of quinoline in the *o*-alkenylation of phenol derivatives *via* C–H activation under the conditions studied was also examined.

Having prepared the required starting materials (**3a–g**) according to the reported methods[14] (see ESI†) we began our study with the coupling of **3a** with ethyl acrylate (**4a**) under various conditions (Table 1). The reaction was initially performed in the presence of a Pd-catalyst, $Cu(OAc)_2$, trifluoroacetic acid (TFA), in $CH_3CN$ at 60 °C for 12 h under open air. The use of $Pd(PPh_3)_4$, $Pd(dba)_3$, and $Pd(PPh_3)_2Cl_2$ did not afford good yields of **5a** (entries 1–3, Table 1). However, a dramatic increase in yield of **5a** was observed when $Pd(OAc)_2$ was used (entry 4, Table 1). The change of oxidants *e.g.* the use of $K_2S_2O_8$ and $CuCl_2$ in place of $Cu(OAc)_2$ was discouraging (entries 5 & 6, Table 1) and $CuCl_2$ acted as an inhibitor. While some progress of reaction was observed when no oxidant was used (perhaps assisted by aerial oxygen) the yield of **5a** was low (entry 7, Table 1). The use of other solvents such as 1,4-dioxane, DMF, DCE (1,2-dichloroethane), EtOH and toluene in place of $CH_3CN$ was also ineffective (entries 8–12, Table 1) except 1,4-dioxane. The role of $Pd(OAc)_2$ and TFA was confirmed by performing the reaction in the absence of these reagents where no or poor yield of **5a** was observed (entries 13 & 14, Table 1). Indeed, TFA was better compared to other additives *e.g.* PivOH and $CH_3COOH$ (entries 15 & 16, Table 1). The decrease of reaction temperature from 60 °C decreased the product yield (entry 17, Table 1) whereas increase of temperature (*e.g.* to 80 °C) resulted quick evaporation of TFA (bp 72.4 °C). Moreover, a longer reaction time was also found to be less effective (entry 18, Table 1). Notably, the present quinoxaline directed *ortho* C–H alkenylation proceeded well in the presence of a Ru(II) catalyst (*vide infra*). However, requirement of sealed tube in this case prompted us to proceed with Pd(II)-catalyzed method and the conditions of entry 4 appeared to be optimal.

To expand the generality and scope of this methodology a range of substrates *e.g.* **3a–f** carrying substituents such as MeO, Me, F, Cl and Br on the *N*-phenyl ring were employed (Table 2).

Table 1 Effect of conditions on the reaction of **3a** with **4a**[a]




| Entry | Catalyst | Additive/oxidant | Solvent | % Yield[b] |
|---|---|---|---|---|
| 1 | $Pd(PPh_3)_4$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 26 |
| 2 | $Pd(dba)_3$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 35 |
| 3 | $Pd(PPh_3)_2Cl_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 52 |
| 4 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 84 |
| 5 | $Pd(OAc)_2$ | $TFA/K_2S_2O_8$ | $CH_3CN$ | 22 |
| 6 | $Pd(OAc)_2$ | $TFA/CuCl_2$ | $CH_3CN$ | 0 |
| 7 | $Pd(OAc)_2$ | TFA/— | $CH_3CN$ | 30[c] |
| 8 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | 1,4-Dioxane | 82 |
| 9 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | DMF | 10 |
| 10 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | DCE | 48 |
| 11 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | EtOH | 0 |
| 12 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | Toluene | 72 |
| 13 | — | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 0[d] |
| 14 | $Pd(OAc)_2$ | —/$Cu(OAc)_2$ | $CH_3CN$ | 28[e] |
| 15 | $Pd(OAc)_2$ | $PivOH/Cu(OAc)_2$ | $CH_3CN$ | Trace |
| 16 | $Pd(OAc)_2$ | $AcOH/Cu(OAc)_2$ | $CH_3CN$ | 19 |
| 17 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 45[f] |
| 18 | $Pd(OAc)_2$ | $TFA/Cu(OAc)_2$ | $CH_3CN$ | 80[g] |

[a] All the reactions are carried out using **3a** (1 mmol), alkene **4a** (1.5 mmol), a Pd-catalyst (5 mol%), an oxidant (1.5 mmol) and an additive (1.2 mmol) in a solvent (2.5 mL) at 60 °C for 12 h under open air. [b] Isolated yield. [c] No oxidant. [d] No catalyst. [e] No additive. [f] Performed at 40 °C. [g] Reaction time was 24 h.

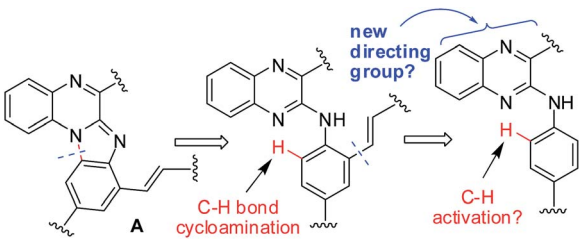
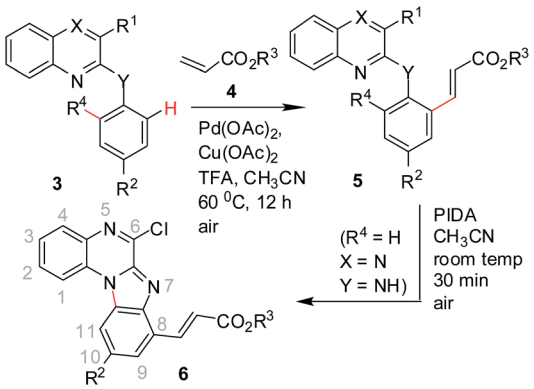
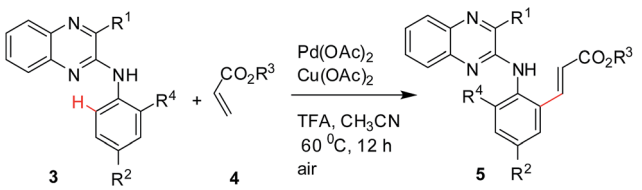



Fig. 2 Retrosynthetic analysis of compound **A**.




Scheme 1 Pd-catalyzed *ortho* C–H functionalization followed by intramolecular C–H cycloamination under open air leading to alkenyl substituted *N*-heteroarenes (**6**).

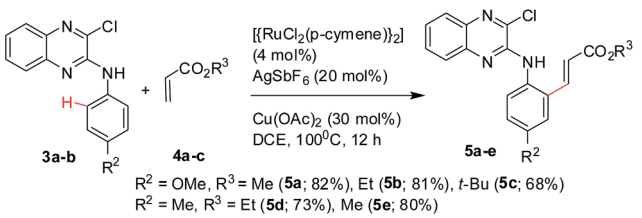
Table 2 Synthesis of alkenyl substituted *N*-arylquinoxalin-2-amine derivatives (**5**)[a]



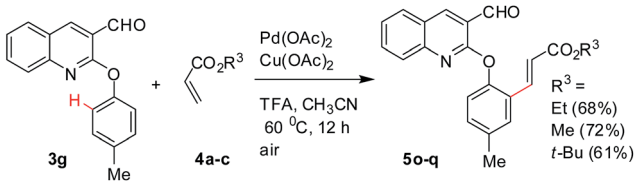

| | Substrate (**3**) | Alkene (**4**) | Product (**5**) | |
|---|---|---|---|---|
| Entry | $R^1$, $R^2$, $R^4$= | $R^3$= | $R^1$, $R^2$, $R^4$, $R^3$= | Yield[b] (%) |
| 1 | **3a** Cl, $OCH_3$, H | **4a** Et | **5a** Cl, $OCH_3$, H, Et | 84 |
| 2 | **3a** | **4b** Me | **5b** Cl, $OCH_3$, H, Me | 82 |
| 3 | **3a** | **4c** *t*-Bu | **5c** Cl, $OCH_3$, H, *t*-Bu | 67 |
| 4 | **3b** Cl, $CH_3$, H | **4a** | **5d** Cl, $CH_3$, H, Et | 75 |
| 5 | **3b** | **4b** | **5e** Cl, $CH_3$, H, Me | 80 |
| 6 | **3b** | **4c** | **5f** Cl, $CH_3$, H, *t*-Bu | 62 |
| 7 | **3c** Cl, Cl, H | **4a** | **5g** Cl, Cl, H, Et | 77 |
| 8 | **3c** | **4b** | **5h** Cl, Cl, H, Me | 71 |
| 9 | **3c** | **4c** | **5i** Cl, Cl, H, *t*-Bu | 59 |
| 10 | **3d** Cl, Br, H | **4a** | **5j** Cl, Br, H, Et | 78 |
| 11 | **3d** | **4b** | **5k** Cl, Br, H, Me | 74 |
| 12 | **3e** Cl, F, H | **4a** | **5l** Cl, F, H, Et | 79 |
| 13 | **3e** | **4c** | **5m** Cl, F, H, *t*-Bu | 74 |
| 14 | **3f** Cl, H, $OCH_3$ | **4b** | **5n** Cl, H, $OCH_3$, Me | 55 |
| 15 | **3a** Cl, $OCH_3$, H | Et **4a** | **5a** Cl, $OCH_3$, H, Et | 84 |

[a] All the reactions are carried out using compound **3** (1 mmol), alkene **4** (1.5 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1.5 mmol) and TFA (1.2 mmol) in $CH_3CN$ (2.5 mL) at 60 °C, under air. [b] Isolated yield.

The other coupling partner *e.g.* **4a–c** generally included Me, Et or *t*-Bu ester of acrylic acid. The reaction proceeded well in all these cases affording the corresponding desired product **5a–n** in good to acceptable yield. To test the effectiveness of




Scheme 2 Ru-catalyzed direct *ortho* C–H alkenylation of **3a–b**.

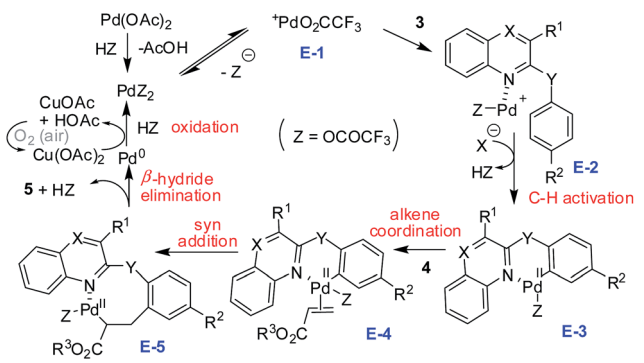



Scheme 3 Pd-catalyzed *ortho* C–H alkenylation of a phenol derivative **3g**.

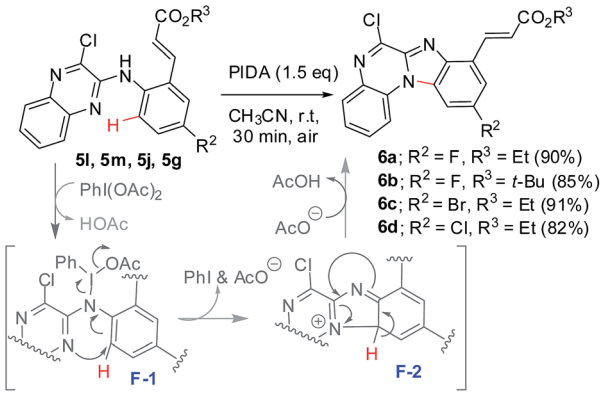
quinoxaline moiety towards Ru(II)-catalyzed *o*-alkenylation of **3** the coupling of **3a–b** with **4a–c** was performed under reported conditions[15] when the desired product **5a–e** was obtained almost in the same yields (Scheme 2) as observed in case of Pd-catalyzed reaction. Notably, the Ru(II)-catalyzed *o*-alkenylation of **3** was carried out in a sealed tube as the reaction did not proceed well when performed in an open reaction vessel.

We then focused on the Pd-catalyzed *ortho* C–H alkenylation of a phenol derivative **3g** that was coupled with the alkene **4a–c** under the conditions of entry 4 of Table 1. The reaction proceeded smoothly affording the corresponding alkenyl substituted analogs **5o–q** (Scheme 3). Notably, quinoline was found to be an effective directing group in these cases. While alkenylation of phenol derivatives are known in the literature[16] the use of quinoline moiety as a directing group for this purpose has not been explored earlier. Thus the present strategy of *ortho* C–H alkenylation of phenol is of further interest.

According to the proposed mechanism (Scheme 4), the reaction appeared to proceed[17] *via* (i) *in situ* generation of highly electrophilic Pd(II) cationic species **E-1** in TFA, (ii) stabilization of **E-1** by the quinoxaline/quinoline nitrogen aided by the C-2 arylamine/aryloxy moiety (*via* + M effect) in **E-2**, (iii) generation of **E-3** *via* σ-bond formation between the "Pd" center and the proximate *ortho* C-aryl following a C(aryl)–H activation, (iv) alkene coordination with **E-3** to give **E-4**, (v) *syn* addition *via* 1,2-migratory insertion to afford **E-5**, that undergoes (vi) β-hydride elimination to give **5** and the $Pd^0$ species, and (vii) finally, oxidation of $Pd^0$ to $Pd^{II}$ by $Cu(OAc)_2$ to complete the catalytic cycle. The $Cu(OAc)_2$ is regenerated from the reduced copper species *i.e.* CuOAc by the aerial oxygen. A similar 6-membered ruthenacycle (like **E-3**, Scheme 4) generated *in situ* from $[Ru(OAc)L]^+[SbF_6]^-$ (L = *p*-cymene) species and **3** can be




Scheme 4 The plausible reaction mechanism.




Scheme 5 Synthesis of **6a–d** *via* intramolecular C–H cycloamination under open air.

proposed[15] for the Ru(II)-catalyzed alkenylation of **3** that on coordinative insertion of alkene **4** followed by β-hydride elimination could afford the product **5** (see ESI†).

The intramolecular C–H cycloamination of **5** was performed by using a hypervalent iodine(III) reagent[18] such as PIDA [phenyliodine diacetate or $PhI(OAc)_2$] at room temperature (Scheme 5) to afford alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines[19a] (**6**) in good to excellent yields. The Br, Cl, F and alkenyl ester[19b] substituents remained intact during this mild and selective oxidative cyclization. The reaction seemed to involve an initial activation of the aniline nitrogen of **5** by PIDA that facilitated a nucleophilic attack by the proximate quinoxaline nitrogen atom on the aniline ring of **F-1** affording the cyclic intermediate **F-2** (Scheme 5). Deprotonation followed by aromatization of **F-2** afforded product **6**.

Due to the reported PDE4 (phosphodiesterase type 4) inhibitory activities of related imidazo[1,2-*a*]quinoxalines[5] the compounds **6a–d** were tested for their PDE4 inhibition. Notably, inhibitors of PDE4 are known to be generally useful for the treatment of chronic obstructive pulmonary disease (COPD) and asthma.[20] All these compounds showed promising inhibition of PDE4B [*e.g.* **6a** (69.77 ± 9.69%), **6b** (42.15 ± 1.23%) **6c** (60.66 ± 3.93%), **6d** (79.50 ± 1.12%)] when tested *in vitro*[21] at 30 μM. In a dose response study the compound **6d** showed dose

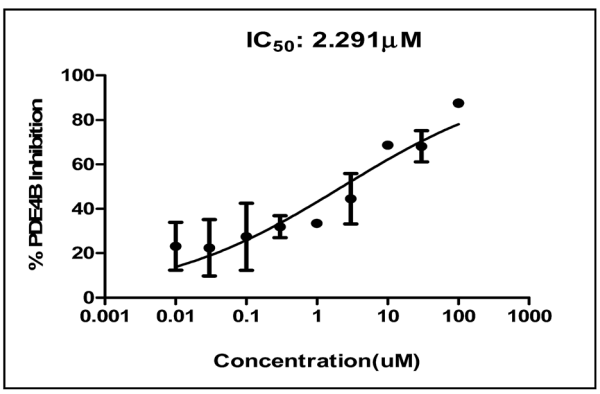



Fig. 3 Dose dependent inhibition of PDE4B by compound **6d**.

depended inhibition of PDE4B with $IC_{50} \sim 2.3$ μM (comparable to rolipram's $IC_{50} \sim 1.0$ μM) (Fig. 3) indicating potential medicinal value of this class of heterocycles.

In conclusion, we have developed a two-step strategy for accessing new chemical entities based on alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxaline framework *via* C–H activation methods. The strategy involved use of a quinoxaline moiety as a new directing group for the Pd (or Ru)-catalyzed *ortho* C–H alkenylation of aniline derivatives and subsequent hypervalent iodine(III)-promoted intramolecular *ortho* C–H cycloamination of the resulting *N*-arylquinoxalin-2-amine derivatives. Both the steps were performed under open air and mild conditions. All these alkenyl substituted benzo[4,5]-imidazo[1,2-*a*]quinoxalines were identified as inhibitors of PDE4 indicating their potential medicinal importance. The Pd-catalyzed *ortho* C–H alkenylation of phenol derivatives was also performed successfully when quinoline was found to be an effective directing group. Overall, our efforts on exploration of new C–H activation strategies for Med Chem purpose would be of further interest.

## Acknowledgements

RS thank CSIR, New Delhi, India for a research fellowship. The authors thank management of DRILS and CSIR [Grant 02(0127)/13/EMR-II] for support.

## Notes and references

1 For excellent reviews, see: (*a*) R. R. Schrock, *Angew. Chem., Int. Ed. Engl.*, 2006, **45**, 3748; (*b*) R. H. Grubbs, *Angew. Chem., Int. Ed. Engl.*, 2006, **45**, 3760.

2 (*a*) For an anticancer drug, see: V. C. Jordan, *Nat. Rev. Drug Discovery*, 2003, **2**, 205; (*b*) For 1,1-diarylalkenes as dual inhibitors of tubulin polymerization and phosphodiesterase 4, see: A. L. Ruchelman, H. W. Man, R. Chen, W. Liu, L. Lu, D. Cedzik, L. Zhang, J. Leisten, A. Collette, R. K. Narla, H. K. Raymon and G. W. Muller, *Bioorg. Med. Chem.*, 2011, **19**, 6356.

3 (*a*) A. Nakhi, S. Archana, G. P. K. Seerapu, K. S. Chennubhotla, K. L. Kumar, P. Kulkarni, D. Haldar and M. Pal, *Chem. Commun.*, 2013, **49**, 6268; (*b*) T. B. Kumar, C. Sumanth, S. Vaishaly, M. S. Rao, K. B. C. Sekhar, C. L. T. Meda, A. Kandale, D. Rambabu, G. R. Krishna, C. M. Reddy, K. S. Kumar, K. V. L. Parsa and M. Pal, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5639.

4 (*a*) S. Khier, C. Deleuze-Masquéfa, G. Moarbess, F. Gattacceca, D. Margout, I. Solassol, J. F. Cooper, F. Pinguet, P. A. Bonnet and F. M. Bressolle, *Eur. J. Pharm. Sci.*, 2010, **39**, 23; (*b*) C. Deleuze-Masquefa, G. Moarbess, S. Khier, N. David, S. Gayraud-Paniagua, F. Bressolle, F. Pinguet and P.-A. Bonnet, *Eur. J. Med. Chem.*, 2009, **44**, 3406.

5 (*a*) S. Parra, F. Laurent, G. Subra, C. Deleuze-Masquefa, V. Benezech, J. Fabreguettes, J. Vidal, T. Pocock, K. Elliott, R. Small, R. Escale, A. Michel, J. Chapat and P. Bonnet, *Eur. J. Med. Chem.*, 2001, **36**, 255; (*b*) C. Deleuze-Masquéfa,

G. Gerebtzoff, G. Subra, J.-R. Fabreguettes, A. Ovens, M. Carraz, M.-P. Strub, J. Bompart, P. George and P.-A. Bonnet, *Bioorg. Med. Chem.*, 2004, **12**, 1129.

6 For an excellent review, see: I. P. Beletskaya and A. V. Cheprakov, *Chem. Rev.*, 2000, **100**, 3009.

7 (*a*) T. Mizoroki, K. Mori and A. Ozaki, *Bull. Chem. Soc. Jpn.*, 1971, **44**, 581; (*b*) R. F. Heck, *Acc. Chem. Res.*, 1979, **12**, 146.

8 For selected references, see: (*a*) I. Moritani and Y. Fujiwara, *Tetrahedron Lett.*, 1967, 1119; (*b*) C. G. Jia, T. Kitamura and Y. Fujiwara, *Acc. Chem. Res.*, 2001, **34**, 844; (*c*) L. Zhou and W. Lu, *Chem.–Eur. J.*, 2014, **20**, 634.

9 For selected reviews, see: (*a*) J. F. Hartwig, *Nature*, 2008, **455**, 314; (*b*) L. Ackermann, R. Vicente and A. R. Kapdi, *Angew. Chem., Int. Ed.*, 2009, **48**, 9792; (*c*) O. Daugulis, H.-Q. Do and D. Shabashov, *Acc. Chem. Res.*, 2009, **42**, 1074; (*d*) D. A. Colby, R. G. Bergman and J. A. Ellman, *Chem. Rev.*, 2009, **110**, 624; (*e*) X. Chen, K. M. Engle, D.-H. Wang and J.-Q. Yu, *Angew. Chem., Int. Ed.*, 2009, **48**, 5094; (*f*) T. W. Lyons and M. S. Sanford, *Chem. Rev.*, 2010, **110**, 1147; (*g*) C.-L. Sun, B.-J. Li and Z.-J. Shi, *Chem. Rev.*, 2010, **111**, 1293; (*h*) R. Jazzar, J. Hitce, A. Renaudat, J. Sofack-Kreutzer and O. Baudoin, *Chem.–Eur. J.*, 2010, **16**, 2654; (*i*) L. McMurray, F. O'Hara and M. J. Gaunt, *Chem. Soc. Rev.*, 2011, **40**, 1885; (*j*) G. Song, F. Wang and X. Li, *Chem. Soc. Rev.*, 2012, **41**, 3651; (*k*) P. B. Arockiam, C. Bruneau and P. H. Dixneuf, *Chem. Rev.*, 2012, **112**, 5879.

10 (*a*) G. Deng, L. Zhao and C.-J. Li, *Angew. Chem., Int. Ed.*, 2008, **47**, 6278; (*b*) D. Kalyani, N. R. Deprez, L. V. Desai and M. S. Sanford, *J. Am. Chem. Soc.*, 2005, **127**, 7330; (*c*) K. L. Hull, E. L. Lanni and M. S. Sanford, *J. Am. Chem. Soc.*, 2006, **128**, 14047; (*d*) S. Oi, E. Aizawa, Y. Ogino and Y. Inoue, *J. Org. Chem.*, 2005, **70**, 3113; (*e*) D. Shabashov and O. Daugulis, *Org. Lett.*, 2005, **7**, 3657; (*f*) K. M. Gericke, D. I. Chai, N. Bieler and M. Lautens, *Angew. Chem., Int. Ed.*, 2009, **48**, 1447; (*g*) B. Saito and G. C. Fu, *J. Am. Chem. Soc.*, 2007, **129**, 9602; (*h*) B.-J. Li, S.-L. Tian, Z. Fang and Z.-J. Shi, *Angew. Chem., Int. Ed.*, 2008, **47**, 1115; (*i*) O. Daugulis and V. G. Zaitsev, *Angew. Chem., Int. Ed.*, 2005, **44**, 4046; (*j*) D.-H. Wang, M. Wasa, R. Giri and J.-Q. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 7190; (*k*) Y. Terao, Y. Kametani, H. Wakui, T. Satoh, M. Miura and M. Nomura, *Tetrahedron*, 2001, **57**, 5967; (*l*) T. Satoh, Y. Kawamura, M. Miura and M. Nomura, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1740; (*m*) T. Satoh, J.-I. Inoh, Y. Kawamura, M. Miura and M. Nomura, *Bull. Chem. Soc. Jpn.*, 1998, **71**, 2239; (*n*) R. Giri and J.-Q. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 14082; (*o*) D.-H. Wang, T.-S. Mei and J.-Q. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 17676; (*p*) M. Yu, Z. Liang, Y. Wang and Y. Zhang, *J. Org. Chem.*, 2011, **76**, 4987; (*q*) A. Deb, S. Bag, R. Kancherla and D. Maiti, *J. Am. Chem. Soc.*, 2014, **136**, 13602.

11 While selective *ortho* monofluorination directed by the quinoxaline moiety has been reported earlier (see: S.-J. Lou, D.-Q. Xu, Y.-F. Wang, Y.-K. Liu, X.-H. Du, and Z.-Y. Xu, *Chem. Commun.* 2013, **49**, 6218) its use for selective *ortho* alkenylation is not known.

12 For similar examples using *N*-aryl-2-aminopyrimidines, see: G. Qian, B. Liu, Q. Tan, S. Zhang and B. Xu, *Eur. J. Org. Chem.*, 2014, 4837.

13 For a Cu-mediated synthesis, see: A. Huang, Y. Chen, Y. Zhou, W. Guo, X. Wu and C. Ma, *Org. Lett.*, 2013, **15**, 5480.

14 (*a*) B. Prasad, K. S. Kumar, P. V. Babu, K. Anusha, D. Rambabu, A. Kandale, G. R. Vanaja, A. M. Kalle and M. Pal, *Tetrahedron Lett.*, 2012, **53**, 6059; (*b*) D. C. Mungra, M. P. Patel, D. P. Rajani and R. G. Patel, *Eur. J. Med. Chem.*, 2011, **46**, 4192.

15 (*a*) K. Graczyk, W. Ma and L. Ackermann, *Org. Lett.*, 2012, **14**, 4110; (*b*) K. Padala and M. Jeganmohan, *Org. Lett.*, 2012, **14**, 1134.

16 For recent examples, see: (*a*) H.-X. Dai, G. Li, X.-G. Zhang, A. F. Stepan and J.-Q. Yu, *J. Am. Chem. Soc.*, 2013, **135**, 7567; (*b*) C. Feng and T.-P. Loh, *Chem. Commun.*, 2011, **47**, 10458; (*c*) B. Li, J. Ma, Y. Liang, N. Wang, S. Xu, H. Song and B. Wang, *Eur. J. Org. Chem.*, 2013, 1950; (*d*) C. Martínez and K. Muñiz, *ChemCatChem*, 2013, **5**, 3502.

17 (*a*) H. Ge, M. J. Niphakis and G. I. Georg, *J. Am. Chem. Soc.*, 2008, **130**, 3708; (*b*) Y. Liu, D. Li and C.-M. Park, *Angew. Chem., Int. Ed.*, 2011, **50**, 7333; (*c*) S. Pankajakshan, Y.-H. Xu, J. K. Cheng, M. T. Low and T.-P. Loh, *Angew. Chem., Int. Ed.*, 2012, **51**, 5701.

18 For an in depth review, see: V. V. Zhdankin, *ARKIVOC*, 2009, (i), 1–62.

19 (*a*) All the synthesized compounds *i.e.* **6a–d** were characterized by spectral data. The NOE experiment performed using **6c** indicated close proximity of C(1)–H with C(11)–H as they interacted with each other (see ESI†); (*b*) The presence of alkenyl moiety with *E*-geometry in compound **6** was confirmed by the appearance of a pair of doublets in the region $\delta$ 8.2–8.1 and $\delta$ 7.5–7.3 with $J = 16$ Hz.

20 A. Kodimuthali, S. S. L. Jabaris and M. Pal, *J. Med. Chem.*, 2008, **51**, 5471.

21 P. Wang, J. G. Myers, P. Wu, B. Cheewatrakoolpong, R. W. Egan and M. M. Billah, *Biochem. Biophys. Res. Commun.*, 1997, **234**, 320.

RSC Advances

COMMUNICATION

Cite this: *RSC Adv.*, 2015, **5**, 44722

Received 15th April 2015
Accepted 1st May 2015

DOI: 10.1039/c5ra06764b

www.rsc.org/advances

# A Pd(II)-catalyzed C–H activation approach to densely functionalized *N*-heteroaromatics related to neocryptolepine and their evaluation as potential inducers of apoptosis†

Rajnikanth Sunke,[a] Vimal Kumar,[a] Mohd Ashraf Ashfaq,[a] Swapna Yellanki,[ab] Raghavender Medisetti,[ab] Pushkar Kulkarni,[ab] E. V. Venkat Shivaji Ramarao,[a] Nasreen Z. Ehtesham[c] and Manojit Pal*[a]

11-Carboxymethyl substituted 6*H*-indolo[2,3-*b*]quinolines, which are potential inducers of apoptosis, were obtained by one-pot Pd(II)-catalyzed intramolecular oxidative C3–H alkenylation of the indole ring of (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives followed by desulfonylation.

The development of one-pot direct access to densely functionalized heteroaromatics is of great interest for exploring diverse regions of pharmaceutical and medicinal chemical space, and for obtaining rare analogues of complex heteroarene-based natural products. Recently, cross-coupling *via* transition metal-catalyzed C–H activation[1] has been a focus of much research[2] and has found many applications in forming C–C and C-heteroatom bonds, particularly using Pd. However, this method for the straightforward synthesis of densely functionalized heteroaromatics is not commonly used and needs further investigation.

Indoloquinolines are commonly found in nature and display a range of pharmacological properties; therefore, they are attractive templates for drug discovery,[3] particularly for anticancer compounds. For example, the plant alkaloid, neocryptolepine[4] **A** (5-methyl-5*H*-indolo[2,3-*b*]quinoline, Fig. 1), and its synthetic analogue, 5,11-dimethyl-5*H*-indolo-[2,3-*b*]quinoline[5a] (DIMIQ), showed promising cytotoxic and anticancer activities. Similar cytotoxic effects were also observed in 6-substituted 6*H*-indolo[2,3-*b*]quinolines.[5b] In view of the close link between cancer and apoptosis[6] and our longstanding interest in developing apoptotic agents,[7] we became interested in using the 6*H*-indolo[2,3-*b*]quinoline framework **B** (Fig. 1) for discovering novel apoptotic agents. The $-CH_2CO_2R$ moiety at C-11 of **B** was introduced because some aryl acetic acids[8] (**C**) (Fig. 1), such as acridone-4-acetic acids, show potent activity against solid tumors[8a] *via* induction of cytokines, including tumor necrosis factor (which affects tumor blood flow) and other host-mediated cytotoxicity mechanisms. To obtain **B**, we developed a new one-pot strategy involving sequential Pd-catalyzed C–H activation and intramolecular alkenylation followed by desulfonation (Scheme 1). The products were evaluated for their cytotoxicity and apoptosis-inducing properties. Here, we present the preliminary results of this study.

Although a number of interesting and elegant methods[9,10] have been reported for the construction of indoloquinoline rings, none of them were suitable for preparing our target compounds, 11-substituted 6*H*-indolo[2,3-*b*]quinoline derivatives (**4**). A recent one-pot approach[11] that afforded this class of

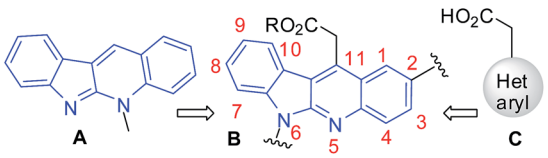
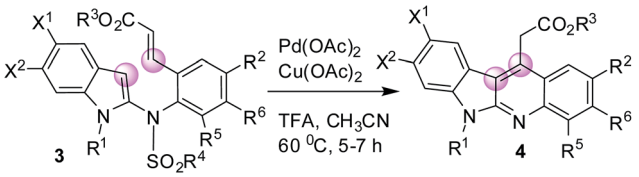



Fig. 1 Design of novel bioactive molecules **B** based on known anticancer alkaloid neocryptolepine **A** and reported antitumor agent **C**.




$R^1$ = Me, Et, Hex, Bn; $R^2$ = Cl, Br, F, Me, H (or I); $R^3$ = Me, Et
$R^4$ = *p*-tolyl, 2-thienyl, $R^5$ & $R^6$ = H, Me; $X^1$ = Br, OMe; $X^2$ =H, Cl

Scheme 1 Pd-mediated synthesis of 11-carboxymethyl-substituted 6*H*-indolo[2,3-*b*]quinoline derivatives.

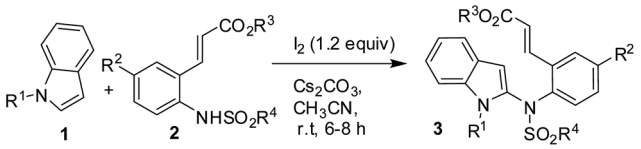
[a]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India. E-mail: manojitpal@rediffmail.com; Tel: +91 40 6657 1500*

[b]*Zephase Therapeutics (an incubated company at the DRILS), University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India*

[c]*National Institute of Pathology, Safdarjang Hospital Campus, New Delhi-110029, India*

† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, copies of spectra and results of zebrafish embryo study. See DOI: 10.1039/c5ra06764b

Table 1 Iodine-mediated synthesis of compound 3[a]

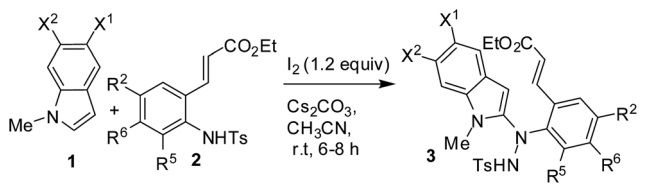



| Entry | Indole 1; $R^1$ = | Sulfonamide 2; $R^2$, $R^3$, $R^4$ = | Time (h) | Product 3 | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | **1a**; Et | **2a**; Me, Me, *p*-tolyl | 6 | **3a** | 85 |
| 2 | **1a** | **2b**; Me, Et, *p*-tolyl | 6 | **3b** | 87 |
| 3 | **1a** | **2c**; F, Et, *p*-tolyl | 6.5 | **3c** | 83 |
| 4 | **1a** | **2d**; F, Me, 2-thienyl | 8 | **3d** | 68 |
| 5 | **1a** | **2e**; Br, Me, *p*-tolyl | 7 | **3e** | 75 |
| 6 | **1a** | **2f**; Br, Et, *p*-tolyl | 7 | **3f** | 80 |
| 7 | **1b**; Me | **2a** | 6 | **3g** | 71 |
| 8 | **1b** | **2b** | 6 | **3h** | 83 |
| 9 | **1b** | **2g**; Cl, Me, *p*-tolyl | 6 | **3i** | 68 |
| 10 | **1b** | **2e** | 7.5 | **3j** | 60 |
| 11 | **1b** | **2h**; H, Et, *p*-tolyl | 5 | **3k** | 73 |
| 12 | **1c**; Bn | **2a** | 7 | **3l** | 84 |
| 13 | **1c** | **2i**; Cl, Et, *p*-tolyl | 6 | **3m** | 83 |
| 14 | **1c** | **2j**; F, Me, *p*-tolyl | 6.5 | **3n** | 82 |
| 15 | **1c** | **2c** | 6 | **3o** | 79 |
| 16 | **1c** | **2e** | 7 | **3p** | 76 |
| 17 | **1d**; hexyl | **2h** | 8 | **3q** | 55 |
| 18 | **1a** | **2h** | 12 | **3r**; $R^2$ = I | 73[c] |
| 19 | **1b** | **2h** | 12 | **3s**; $R^2$ = I | 68[c] |

[a] Reactions were performed using compound **1** (1.2 mmol), **2** (1.0 mmol), $I_2$ (1.2 mmol) and $Cs_2CO_3$ (1.5 mmol) in $CH_3CN$ (5.0 mL) at room temperature under nitrogen. [b] Isolated yield. [c] 3.0 equiv. of $I_2$ was used for 12 h.

compounds with alkyl or aryl substituents at C-11 was also inconvenient for direct access to **4** (or **B**, Fig. 1). However, based on a recent report[12] we anticipated that an intramolecular C3–H alkenylation of the indole ring of **3** under oxidative Pd(II) catalysis could afford target compound **4** in a straightforward manner. Starting material **3** was prepared *via* $I_2$-mediated addition of sulfonamide derivative[13a–c] **2** to indole **1** (Tables 1

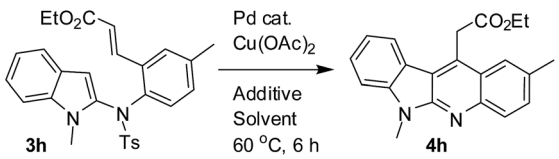
Table 2 Iodine-mediated synthesis of compound 3[a]




| Entry | Indole 1; $X^1$, $X^2$ = | Sulfonamide 2; $R^2$, $R^5$, $R^6$ = | Time (h) | Product 3 | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | **1e**; Br, H | **2k**; Me, H, Me | 6 | **3t** | 84 |
| 2 | **1f**; OMe, H | **2l**; H, Me, H | 6 | **3u** | 87 |
| 3 | **1g**; H, Cl | **2h**; H, H, H | 6.5 | **3v** | 82 |

[a] Reactions were performed using compound **1** (1.2 mmol), **2** (1.0 mmol), $I_2$ (1.2 mmol) and $Cs_2CO_3$ (1.5 mmol) in $CH_3CN$ (5.0 mL) at room temperature under nitrogen. [b] Isolated yield.

Table 3 Effect of reaction conditions on intramolecular C–H alkenylation of 3h[a]




| Entry | Catalyst | Additive | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | $Pd(OAc)_2$ | — | $CH_3CN$ | 22 |
| 2 | $Pd(OAc)_2$ | TFA | $CH_3CN$ | 82 |
| 3 | $PdCl_2$ | TFA | $CH_3CN$ | 67 |
| 4 | $Pd(PPh_3)_2Cl_2$ | TFA | $CH_3CN$ | 9 |
| 5 | $Pd(PPh_3)_4$ | TFA | $CH_3CN$ | 40 |
| 6 | $Pd(OAc)_2$ | Amberlyst | $CH_3CN$ | 0 |
| 7 | $Pd(OAc)_2$ | PTSA | $CH_3CN$ | Trace |
| 8 | $Pd(OAc)_2$ | TFA | DMSO | 30 |
| 9 | $Pd(OAc)_2$ | TFA | DMF | 27 |
| 10 | $Pd(OAc)_2$ | TFA | Toluene | 52 |
| 11 | $Pd(OAc)_2$ | TFA | DCE | 11 |
| 12 | $Pd(OAc)_2$ | TFA | $CH_3CN$ | 81[c] |
| 13 | — | TFA | $CH_3CN$ | 0 |
| 14 | — | TFA | $CH_3CN$ | 0[d] |

[a] Reactions were performed using compound **3h** (0.20 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (0.30 mmol) and TFA (0.24 mmol) in $CH_3CN$ (2.5 mL) at 60 °C for 6 h under air. [b] Isolated yield. [c] Reaction was performed under nitrogen. [d] Reaction was performed without $Pd(OAc)_2$ and $Cu(OAc)_2$.

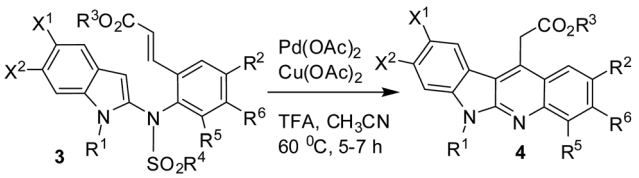
and 2, also see ESI†).[13d,e] Although the reaction proceeded well in the presence of 1.2 equiv.[13g] of $I_2$ (entries 1–17, Table 1, and entries 1–3, Table 2), 3.0 equiv. of $I_2$ afforded desired products **3r** and **3s** with an iodo group on the *N*-phenyl ring (entries 18 and 19, Table 1).

The Pd(II)-mediated intramolecular C–H alkenylation was then examined under various conditions by using compound **3h** (Table 3). Although the $Pd(OAc)_2$-catalyzed reaction afforded a low yield of desired product **4h** (entry 1, Table 3) the yield was increased dramatically when trifluoroacetic acid (TFA) was used as an additive (entry 2, Table 3). To improve the yield further we changed the Pd catalysts (entries 3–5, Table 3) and the additive (entries 6 and 7, Table 3). However, **4h** was obtained in low or moderate yields or not formed at all. $CH_3CN$ was used as a solvent in these reactions; other solvents, such as DMSO, DMF, toluene and 1,2-dichloroethane (DCE), were less effective (entries 8–11, Table 3). Notably, the present synthesis of **4h** does not require an inert atmosphere, as the yield was not affected by whether the reaction was performed under air or nitrogen (entry 2 *vs.* 12, Table 3). The reaction did not proceed in the absence of $Pd(OAc)_2$ or $Pd(OAc)_2/Cu(OAc)_2$, indicating the key role played by the catalyst and the oxidant in the present reaction. However, the reaction proceeded slowly in the absence of $Cu(OAc)_2$ when performed under air affording **4h** in 70% yield after 12 h. The oxygen in the air functioned as the oxidant. Notably, **4h** was not formed when **3h** was treated with 12 M HCl under previously reported conditions[11] even after 12 h, indicating that the earlier method was inappropriate in the present case.

Having identified the optimum conditions (entry 2, Table 3), we examined the substrate scope and generality of the method. A range of (*E*)-alkyl-3-(2-(1*H*-indol-2-ylamino)phenyl)acrylate derivatives (**3**) were used to give the desired 6*H*-indolo[2,3-*b*]-quinoline derivatives[13f] (**4**) in acceptable to good yield (Table 4, also see ESI†). In addition to various $R^1$, $R^2$, $R^3$, $R^5$, $R^6$, $X^1$ and $X^2$ substituents (Table 4), the Pd(0) labile iodo group was also tolerated in this reaction (entries 18 and 19, Table 4), which is amenable for further functionalization *via* Pd(0) chemistry.

As shown in Scheme 2, the reaction proceeded[14,15] *via* (i) *in situ* generation of highly electrophilic Pd(II) cationic species **D-1** in TFA, (ii) formation of σ-indole-Pd complexes **D-2** through metalation of the indolyl C3–H bond[15g] in the presence of **D-1**, (iii) intramolecular 6-*exo*-trig cyclization (*via cis*-arylpalladation of a C–C double bond) of **D-2** leading to intermediate **D-3**, which (iv) undergoes β-hydride elimination to give **D-4** and the $Pd^0$

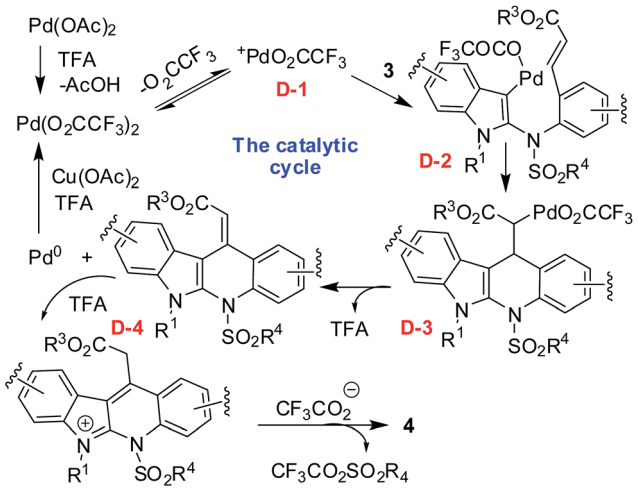
Table 4 Pd-mediated synthesis of 6*H*-indolo[2,3-*b*]quinoline derivatives[a]




| Entry | Indole **3** | Product **4**; $R^1$, $R^2$, $R^3$, $R^5$, $R^6$, $X^1$, $X^2$ | Time (h) | Yield[b] (%) |
|---|---|---|---|---|
| 1 | **3a** | **4a**; Et, Me, Me, H, H, H, H | 5 | 86 |
| 2 | **3b** | **4b**; Et, Me, Et, H, H, H, H | 5 | 89 |
| 3 | **3c** | **4c**; Et, F, Et, H, H, H, H | 6 | 78 |
| 4 | **3d** | **4d**; Et, F, Me, H, H, H, H | 7 | 52 |
| 5 | **3e** | **4e**; Et, Br, Me, H, H, H, H | 7 | 80 |
| 6 | **3f** | **4f**; Et, Br, Et, H, H, H, H | 7 | 88 |
| 7 | **3g** | **4g**; Me, Me, Me, H, H, H, H | 6 | 75 |
| 8 | **3h** | **4h**; Me, Me, Et, H, H, H, H | 6 | 82 |
| 9 | **3i** | **4i**; Me, Cl, Me, H, H, H, H | 6 | 85 |
| 10 | **3j** | **4j**; Me, Br, Me, H, H, H, H | 7 | 77 |
| 11 | **3k** | **4k**; Me, H, Et, H, H, H, H | 6.5 | 84 |
| 12 | **3l** | **4l**; Bn, Me, Me, H, H, H, H | 5 | 89 |
| 13 | **3m** | **4m**; Bn, Cl, Et, H, H, H, H | 6 | 75 |
| 14 | **3n** | **4n**; Bn, F, Me, H, H, H, H | 5 | 81 |
| 15 | **3o** | **4o**; Bn, F, Et, H, H, H, H | 5 | 83 |
| 16 | **3p** | **4p**; Bn, Br, Et, H, H, H, H | 7 | 77 |
| 17 | **3q** | **4q**; hexyl, H, Et, H, H, H, H | 6 | 67 |
| 18 | **3r** | **4r**; Et, I, Et, H, H, H, H | 7 | 72 |
| 19 | **3s** | **4s**; Me, I, Et, H, H, H, H | 7 | 69 |
| 20 | **3t** | **4t**; Me, Me, Et, H, Me, Br, H | 5 | 87 |
| 21 | **3u** | **4u**; Me, H, Et, Me, H, OMe, H | 5 | 84 |
| 22 | **3v** | **4v**; Me, H, Et, H, H, H, Cl | 7 | 81 |

[a] Reactions were performed by using compound **3** (1 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1.5 mmol) and TFA (1.2 mmol) in $CH_3CN$ (2.5 mL) at 60 °C under air. [b] Isolated yield.




Scheme 2 Proposed reaction mechanism.

species, (v) cleavage of the *N*-(het)arylsulfonyl group in the presence of TFA gives product **4**, and (vi) oxidation of $Pd^0$ to $Pd^{II}$ completes the catalytic cycle.

Most of the synthesized compounds were tested at 10 μM initially for their ability to inhibit the growth of A549 (lung), Cal27 (oral) MCF-7 (breast) and TZM-BL (cervical) cancer cells using the sulforhodamine B (SRB) assay[16a,b] with gemcitabine[16c] as a reference compound. Among the active compounds, **4a**, **4b**, **4d–k** and **4q** (>90% inhibition, comparable to 90% inhibition by gemcitabine) against lung cancer cells, **4b** (40%) and **4j** (46%) against oral cancer cells (gemcitabine 92%), **4d** (62%), **4f** (55%), **4i** (57%), **4j** (42%) and **4q** (63%) against breast cancer cells (gemcitabine 49%), **4a** (53%), **4j** (63%), and **4q** (76%) against cervical cancer cells (gemcitabine 90%) were of particular interest. These cytotoxic agents were then tested for their apoptotic activities in zebrafish embryos[17a–c] (1, 3, 10 and 30 μM, positive control of methotrexate[17d] at 30 μM). Compounds **4k**, **4j** and **4a** showed considerable effects (Fig. 2 and 3) with an $EC_{50}$ of 1.59, 4.18 and 2.20 μM, respectively. These compounds showed a no observed adverse effect level (NOAEL) of 10 (**4k**), 3

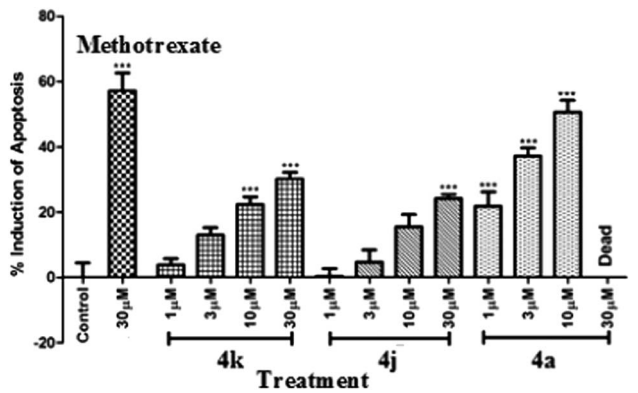



Fig. 2 The percentage induction of apoptosis caused by compounds **4k**, **4j** and **4a** at different concentrations along with methotrexate. All the statistical analysis was performed using GraphPad Prism software.

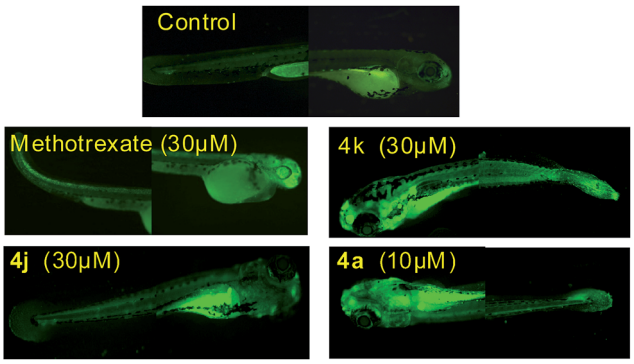



Fig. 3 Representative images of the embryos treated with compounds assayed for apoptosis. Only the selected parts of embryos are shown.

(**4j**) and 1 μM (**4a**), respectively, when evaluated for potential toxicities, such as teratogenicity, in zebrafish embryos at 1.0–30 μM with phenobarbital (3 mM) as a positive control.[18] Based on the overall therapeutic index (TI = NOEL/$EC_{50}$), compound **4k** (TI = 6.26) was found to be promising and is of further interest.

In conclusion, a sequential method has been developed for synthesizing novel indolo[2,3-*b*]quinolines related to neocryptolepine. The one-pot strategy involved Pd(II)-catalyzed intramolecular oxidative C3–H alkenylation of an indole ring followed by desulfonylation. This straightforward, facile methodology afforded an array of 11-carboxymethyl-substituted 6*H*-indolo[2,3-*b*]quinoline derivatives. Several of these compounds showed promising cytotoxicities against cancer cells and apoptosis inducing properties in zebrafish embryos, indicating the potential of these compounds for treating cancer. Overall, these findings could be a new and useful addition to the C–H activation/cascade reaction as well as indoloquinoline chemistry.

## Acknowledgements

RS thanks CSIR, New Delhi, India for a research fellowship. The authors thank the management of DRILS and CSIR [Grant 02(0127)/13/EMR-II] for support.

## Notes and references

1 (*a*) S. Murai, F. Kakiuchi, S. Sekine, Y. Tanaka, A. Kamatani, M. Sonoda and N. Chatani, *Nature*, 1993, **366**, 529; (*b*) C. Jia, D. Piao, J. Oyamada, W. Lu, T. Kitamura and Y. Fujiwara, *Science*, 2000, **287**, 1992.

2 For excellent reviews, see: (*a*) D. Alberico, M. E. Scott and M. Lautens, *Chem. Rev.*, 2007, **107**, 174; (*b*) L.-C. Campeau, D. R. Stuart and K. Fagnou, *Aldrichimica Acta*, 2007, **40**, 35; (*c*) I. V. Seregin and V. Gevorgyan, *Chem. Soc. Rev.*, 2007, **36**, 1173; (*d*) J. C. Lewis, R. G. Bergman and J. A. Ellman, *Acc. Chem. Res.*, 2008, **41**, 1013; (*e*) D. A. Colby, R. G. Bergman and J. A. Ellman, *Chem. Rev.*, 2009, **110**, 624.

3 L. Lavrado, R. Moreira and A. Paulo, *Curr. Med. Chem.*, 2010, **17**, 2348.

4 K. Cimanga, T. de Bruyne, A. Lasure, B. V. Poel, L. Pieters, M. Claeys, D. V. Berghe, K. Kambu, L. Tona and A. J. Vlietinck, *Planta Med.*, 1996, **62**, 22.

5 (*a*) N. C. Garbett and D. E. Graves, *Curr. Med. Chem.: Anti-Cancer Agents*, 2004, **4**, 149; (*b*) L. Kaczmarek, W. Luniewski, B. Zagrodzki, J. Godlewska, J. Osiadacz, J. Wietrzyk, A. Opolski and W. Peczyńska-Czoch, *Acta Pol. Pharm.*, 2002, **59**, 199.

6 (*a*) C. B. Thompson, *Science*, 1995, **267**, 1456; (*b*) Cytotoxic anticancer agents are known to induce apoptosis, as cancer is associated with lower levels of apoptosis.

7 (*a*) P. V. Babu, S. Mukherjee, D. R. Gorja, S. Yellanki, R. Medisetti, P. Kulkarni, K. Mukkanti and M. Pal, *RSC Adv.*, 2014, **4**, 4878; (*b*) B. Prasad, B. Y. Sreenivas, A. Sushma, S. Yellanki, R. Medisetti, P. Kulkarni and M. Pal, *Org. Biomol. Chem.*, 2014, **12**, 2864; (*c*) R. Sunke, P. V. Babu, S. Yellanki, R. Medishetti, P. Kulkarni and M. Pal, *Org. Biomol. Chem.*, 2014, **12**, 6800; (*d*) S. K. Kolli, A. Nakhi, R. Medishetti, S. Yellanki, P. Kulkarni, R. R. Raju and M. Pal, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 4460; (*e*) S. K. Kolli, A. Nakhi, S. Archana, M. Saridena, G. S. Deora, S. Yellanki, R. Medisetti, P. Kulkarni, R. R. Raju and M. Pal, *Eur. J. Med. Chem.*, 2014, **63**, 270.

8 (*a*) S. A. Gamage, G. W. Rewcastle, G. J. Atwell, B. C. Baguley and W. A. Denny, *Anti-Cancer Drug Des.*, 1992, **7**, 403; (*b*) H. Futami, L. Eader, T. T. Back, E. Gruys, H. A. Young, R. H. Wiltrout and B. C. Baguley, *J. Immunother.*, 1992, **12**, 247.

9 (*a*) P. Molina, M. Alajarin and A. Vidal, *J. Chem. Soc., Chem. Commun.*, 1990, 1277; (*b*) P. Molina, M. Alajarin, A. Vidal and P. Sanchez-Andrada, *J. Org. Chem.*, 1992, **57**, 929; (*c*) C. Shi, Q. Zhang and K. K. Wang, *J. Org. Chem.*, 1999, **64**, 925; (*d*) T. Saito, H. Ohmori, E. Furuno and S. Motoki, *J. Chem. Soc., Chem. Commun.*, 1992, 22; (*e*) T. Saito, H. Ohmori, T. Ohkubo and S. Motoki, *J. Chem. Soc., Chem. Commun.*, 1993, 1802; (*f*) Q. Zhang, C. Shi, H.-R. Zhang and K. K. Wang, *J. Org. Chem.*, 2000, **65**, 7977; (*g*) P. Molina, P. M. Fresneda and S. Delgado, *Synthesis*, 1999, 326; (*h*) P. M. Fresneda, P. Molina and S. Delgado, *Tetrahedron Lett.*, 1999, **40**, 7275; (*i*) M. Alajarin, P. Molina and A. Vidal, *J. Nat. Prod.*, 1997, **60**, 747; (*j*) G. Timari, T. Soos and G. Hajos, *Synlett*, 1997, 1067; (*k*) P. T. Parvatkar, P. S. Parameswaran and S. G. Tilve, *J. Org. Chem.*, 2009, **74**, 8369; (*l*) P. T. Parvatkar and S. G. Tilve, *Tetrahedron Lett.*, 2011, **52**, 6594.

10 M. K. Vecchione, A. X. Sun and D. Seidel, *Chem. Sci.*, 2011, **2**, 2178.

11 S. Ali, Y.-X. Li, S. Anwar, F. Yang, Z.-S. Chen and Y.-M. Liang, *J. Org. Chem.*, 2012, **77**, 424.

12 For intramolecular C–H alkenylation of indoles at the C-2 position, see: S. R. Kandukuri, L.-Y. Jiao, A. B. Machotta and M. Oestreich, *Adv. Synth. Catal.*, 2014, **356**, 1597.

13 (*a*) Compound 2 was prepared via iodination of appropriate aniline derivatives followed by Heck coupling with alkenyl esters and mono (het)aryl sulfonation of the -$NH_2$ group as reported previoulsy, see for example: C. Ma, X. Liu, X. Li, J. F. Anderson, S. Yu and J. M. Cook, *J. Org. Chem.*, 2001, **66**, 4525; (*b*) R. Adepu, A. Rajitha, D. Ahuja, A. K. Sharma, B. Ramudu, R. Kapavarapu, K. V. L. Parsa and M. Pal, *Org. Biomol. Chem.*, 2014, **12**, 2514; (*c*) N. Ueno, A. Murase, Y. Nakagawa, J. Takada and S. Hayashi, *Eur. J. Med. Chem.*, 2012, **50**, 179; (*d*) Y. X. Li, H. X. Wang, S. Ali, X. F. Xia and Y. M. Liang, *Chem. Commun.*, 2012, **48**, 2343; (*e*) B. Prasad, B. Y. Sreenivas, D. Rambabu, G. R. Krishna, C. M. Reddy, K. L. Kumar and M. Pal, *Chem. Commun.*, 2013, **49**, 3970; (*f*) The requirement of 1.2 equiv. (excess) of $I_2$ can be rationalized by the fact that $I_2$ was not regenerated after formation of the product and was converted to HI, which does not catalyze the reaction; (*g*) All the compounds were characterized by $^1$H and $^{13}$C NMR, MS and HPLC. The disappearance of certain $^1$HNMR signals of 3, namely the indole C3–H singlet near $\delta$ 6.2–6.5 and two trans olefinic proton doublets near $\delta$ 8.3–8.5 and 6.3–6.4 ppm ($J \sim 16$ Hz), and the appearance of a 11-carboxymethylene (–$CH_2$COO–) singlet near $\delta$ 4.6–4.7 ppm and a $^{13}$C NMR signal near $\delta$ 35 ppm indicated the formation of product 4. Representative compound 4b was further characterized by $^1$H-$^1$H COSY, 2D NOESY, 1D-NOE and DEPT analysis (see ESI†).

14 (*a*) H. Ge, M. J. Niphakis and G. I. Georg, *J. Am. Chem. Soc.*, 2008, **130**, 3708; (*b*) Y. Liu, D. Li and C.-M. Park, *Angew. Chem., Int. Ed.*, 2011, **50**, 7333; (*c*) S. Pankajakshan, Y.-H. Xu, J. K. Cheng, M. T. Low and T.-P. Loh, *Angew. Chem., Int. Ed.*, 2012, **51**, 5701.

15 (*a*) M. Lafrance and K. Fagnou, *J. Am. Chem. Soc.*, 2006, **128**, 16496; (*b*) B. Liégault, D. Lee, M. P. Huestis, D. R. Stuart and K. Fagnou, *J. Org. Chem.*, 2008, **73**, 5022; (*c*) C. Jia, W. Lu, J. Oyamada, T. Kitamura, K. Matsuda, M. Irie and Y. Fujiwara, *J. Am. Chem. Soc.*, 2000, **122**, 7252; (*d*) J. Oyamada and T. Kitamura, *Chem. Commun.*, 2008, 4992; (*e*) R. Giri, J. K. Lam and J.-Q. Yu, *J. Am. Chem. Soc.*, 2010, **132**, 686; (*f*) X. Ji, H. Huang, W. Wu, X. Li and H. Jiang, *J. Org. Chem.*, 2013, **78**, 11155; (*g*) This is favored because the C-3 of the indole ring is a more electrophilic (electron-rich) position than the double bond; (*h*) An alternative intramolecular cyclization of E-2 proceeding via the electrophilic metalation of the proximate olefinic C–H bond is unlikely as this would afford an unfavorable 7-membered cyclic intermediate containing Pd in the ring.

16 (*a*) L. V. Rubinstein, R. H. Shoemaker, K. D. Paull, R. M. Simon, S. Tosini, P. Skehan, D. A. Scudiero, A. Monks and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1113; (*b*) P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107; (*c*) E. Chu and V. T. DeVita, *Physicians' Cancer Chemotherapy Drug Manual*, Jones & Bartlett, 2007.

17 Embryos of zebrafish Danio rerio are excellent animal models for studying the effects of small molecules in early development, thus allowing effective *in vivo* evaluation of potential drugs before embarking on expensive studies in mice and humans (*a*) M. Westerfield, *The Zebrafish Book. a Guide for the Laboratory Use of Zebrafish (Danio rerio)*, University of Oregon Press, Eugene, OR, 4th edn, 2000; (*b*)

A. Nakhi, S. Archana, G. P. Seerapu, K. S. Chennubhotla, K. L. Kumar, P. Kulkarni, D. Haldar and M. Pal, *Chem. Commun.*, 2013, **49**, 6268; (*c*) J. M. Panzica-Kelly, C. X. Zhang, T. L. Danberry, A. Flood, J. W. DeLan, K. C. Brannen and K. A. Augustine-Rauch, *Birth Defects Res., Part B*, 2010, **89**, 382, DOI: 10.1002/bdrb.20260; (*d*) Y.-X. Chen, W.-G. Lv, H.-Z. Chen, F. Ye and X. Xie, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2009, **142**, 107.

18 See ESI for (a) detailed results of the apoptosis (Graph S-1, S-2) and teratogenicity (Graph S-3, Fig. S-1) assays in zebrafish embryos, including results of the toxicity study with toxicological indices and major organs/systems affected in embryos treated with test compounds at the maximum test concentration (MTC) (Table S-4) and (b) a summary of the results (Table S-5).†

Organic & Biomolecular Chemistry

COMMUNICATION

**Cite this:** *Org. Biomol. Chem.*, 2014, **12**, 6800

Received 18th June 2014,
Accepted 10th July 2014

DOI: 10.1039/c4ob01268b

www.rsc.org/obc

# Ligand-free MCR for linking quinoxaline framework with a benzimidazole nucleus: a new strategy for the identification of novel hybrid molecules as potential inducers of apoptosis†

Rajnikanth Sunke,[a] P. Vijaya Babu,[a,b] Swapna Yellanki,[a,c] Raghavender Medishetti,[a,c] Pushkar Kulkarni[c] and Manojit Pal*[a]

**We report a true MCR involving the reaction of *N*-(prop-2-ynyl)-quinoxalin-2-amine derivatives with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and $Et_3N$ in DMSO to afford the pre-designed hybrid molecules containing quinoxaline framework linked with a benzimidazole nucleus. The MCR proceeds in the absence of any ligand and/or lateral addition of the catalyst/base affording products within 30 min in good yields, some of which showed encouraging apoptosis inducing properties in zebrafish.**

Apoptosis, also termed as programmed cell death, is a series of genetically controlled events that result in the elimination of damaged or abnormal cells. Being an important method of cellular control, any disruption of apoptosis leads to abnormal growth of cells *e.g.* cancer. Thus, the induction of apoptosis in tumor cells is considered as an effective approach in the management and therapy of cancer as well as its prevention.[1] Indeed, many of the known anticancer agents and drugs work by inducing apoptosis in cancer cells. While various natural products and small molecules have been explored as inducers of apoptosis earlier, there is still a continued need for a new framework or scaffold for the design and discovery of potential novel apoptotic agents.

The hybrid molecules are generally defined as agents with two (or more) structural frameworks having different biological functions and dual activity (*e.g.*, **A**, Fig. 1), and can act as two distinct pharmacophores.[2a] However, the strategy of a hybrid molecule is also used to enhance the pharmacological activities of the individual pharmacophore (*e.g.* **B**, Fig. 1). For example, the weak cytotoxicity of distamycin A (Fig. 1) has been enhanced by tethering it with the known antitumor compounds or simple active moieties of known antitumor agents.[2b,c] Prompted by this idea we adopted a similar strategy to design our target hybrid molecules as potential apoptotic agents.

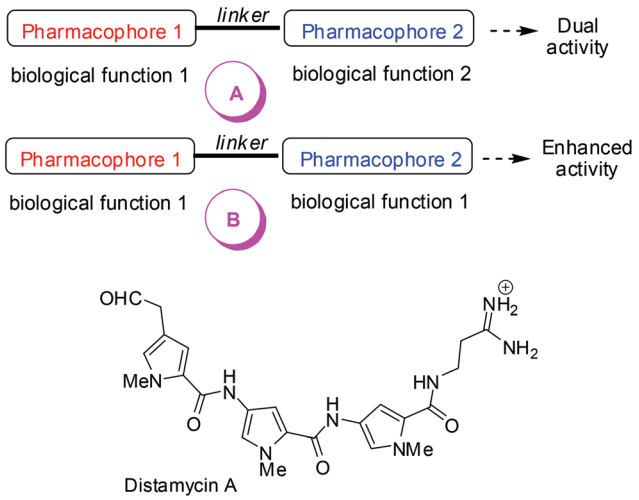



**Fig. 1** The strategy of hybrid molecule **A** and **B** in the design and discovery of potential new drugs.

The quinoxaline framework has been reported to be an integral part of several anticancer agents.[3] The benzimidazole nucleus on the other hand has also found to be present in various antitumor/anticancer agents.[4] Thus we anticipated

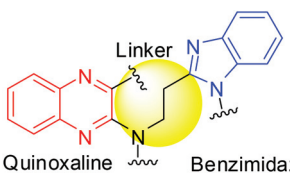
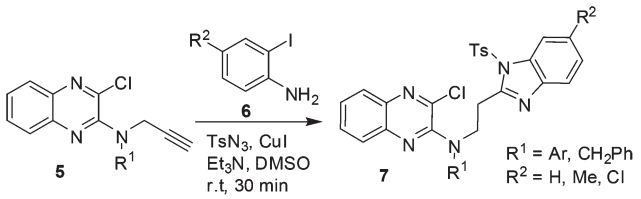


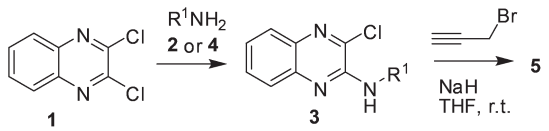

**Fig. 2** New hybrid framework (**C**) for the design and identification of novel inducers of apoptosis.

[a]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India. E-mail: manojitpal@rediffmail.com; Tel: +91 40 6657 1500*
[b]*Chemistry Division, Institute of Science and Technology, JNT University, Kukatpally, Hyderabad 500072, India*
[c]*Zephase Therapeutics (an incubated company at the DRILS), University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India*

†Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds. See DOI: 10.1039/c4ob01268b

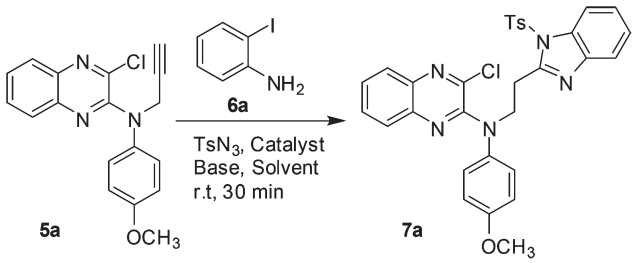



**Scheme 1** Synthesis of hybrid molecules **7** based on **C** *via* a ligand-free MCR.




**Scheme 2** Synthesis of compound **5**.

**Table 1** Optimization of reaction conditions[a]



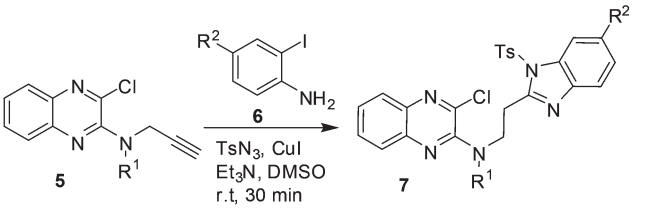

| Entry | Catalyst | Base | Solvent | Yield[b] (%) |
|---|---|---|---|---|
| 1 | CuI | $Et_3N$ | THF | 32 |
| 2 | CuI | $Et_3N$ | $CH_3CN$ | 57 |
| 3 | CuI | $Et_3N$ | DCM | 65 |
| 4 | CuI | $Et_3N$ | DCE | 66 |
| 5 | CuI | $Et_3N$ | Toluene | 48 |
| 6 | CuI | $Et_3N$ | DMSO | 94 |
| 7 | CuI | $Et_3N$ | DMF | 88 |
| 8 | CuI | $Et_3N$ | DMSO | 94[c] |
| 9 | $Cu(OTf)_2$ | $Et_3N$ | DMSO | 10 |
| 10 | CuI | $K_2CO_3$ | DMSO | 70 |

[a] All the reactions are carried out using compound **5a** (0.30 mmol), **6a** (0.34 mmol), $TsN_3$ (0.36 mmol), in the presence of a Cu catalyst (0.03 mmol) and base (0.36 mmol) in a solvent (2 mL) at room temp for 30 min under nitrogen. [b] Isolated yield. [c] The reaction was carried using 30 mol% CuI and completed within 10 min.

that the combination of these two moieties connected through an appropriate linker in a single molecule may provide a new framework (**C**, Fig. 2) suitable for the design and identification of novel inducers of apoptosis. Indeed, the strategy was found to be operative in our case. Herein, we report our preliminary findings on the synthesis and pharmacological evaluation of compounds based on **C** as potential inducers of apoptosis. To the best of our knowledge, pharmacological evaluation of this class of compounds, especially as apoptotic agents, has not been explored earlier.

To achieve our project goal, we required a direct and straightforward synthetic method for the rapid supply of our

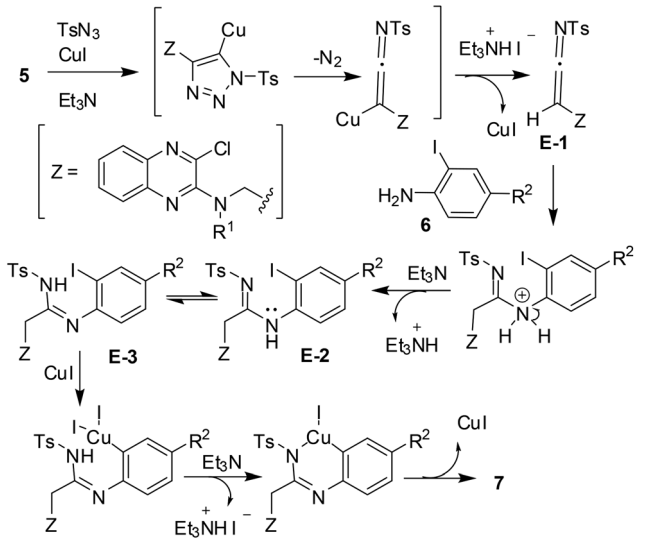
**Table 2** Synthesis of *N*-substituted 3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]-imidazol-2-yl)ethyl)quinoxalin-2-amines (**7**)[a]




| Entry | Alkyne (**5**); $R^1$ = | Aniline (**6**); $R^2$ = | Product (**7**); $R^1$, $R^2$ = | Yield[b] (%) |
|---|---|---|---|---|
| 1 | **5a**-$C_6H_4OMe$-*p* | **6a** H | **7a**-$C_6H_4OMe$-*p*, H | 94 |
| 2 | **5a** | **6b** Me | **7b**-$C_6H_4OMe$-*p*, Me | 89 |
| 3 | **5a** | **6c** Cl | **7c**-$C_6H_4OMe$-*p*, Cl | 90 |
| 4 | **5b**-$C_6H_4Me$-*p* | **6a** | **7d**-$C_6H_4Me$-*p*, H | 92 |
| 5 | **5b** | **6b** | **7e**-$C_6H_4Me$-*p*, Me | 86 |
| 6 | **5b** | **6c** | **7f**-$C_6H_4Me$-*p*, Cl | 90 |
| 7 | **5c**-$C_6H_4Cl$-*p* | **6b** | **7g**-$C_6H_4Cl$-*p*, Me | 89 |
| 8 | **5d**-$C_6H_4Br$-*p* | **6a** | **7h**-$C_6H_4Br$-*p*, H | 95 |
| 9 | **5d** | **6b** | **7i**-$C_6H_4Br$-*p*, Me | 91 |
| 10 | **5d** | **6c** | **7j**-$C_6H_4Br$-*p*, Cl | 89 |
| 11 | **5e**-$C_6H_4F$-*p* | **6a** | **7k**-$C_6H_4F$-*p*, H | 93 |
| 12 | **5e** | **6b** | **7l**-$C_6H_4F$-*p*, Me | 86 |
| 13 | **5f**-$CH_2Ph$ | **6a** | **7m**-$CH_2Ph$, H | 73 |
| 14 | **5f**-$CH_2Ph$ | **6b** | **7n**-$CH_2Ph$, Me | 81 |

[a] All the reactions are carried out using compound **5** (0.30 mmol), **6** (0.34 mmol), $TsN_3$ (0.36 mmol), TEA (0.36 mmol) and CuI (0.03 mmol) in DMSO (2 mL), rt, $N_2$, 30 min. [b] Isolated yield.

target molecules based on **C**. A literature search revealed that the 2-substituted benzimidazole synthesized *via* the reaction of 1,2-phenylenediamines with the corresponding carboxylic acids or with aldehydes followed by oxidation[5] can be functionalized further, for example, by sulfonylation using an alkyl or aryl sulfonyl chloride.[6] However, this method appeared to be less attractive for the synthesis of molecules based on **C** as the introduction of quinoxaline moiety seemed to be difficult.




**Scheme 3** Proposed reaction mechanism.

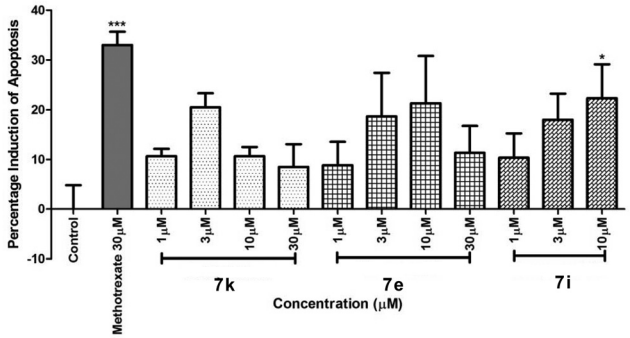



Fig. 3 Results of apoptosis assay: the percentage induction of apoptosis caused by compounds **7k**, **7e** and **7i** at different concentrations along with methotrexate (*$p$ < 0.05, ***$p$ < 0.001). All the statistical analysis was performed using GraphPad Prism® software.

This problem was found to be common with the other methods of constructing 1,2-disubstituted benzimidazole[7] moieties possessing the required quinoxaline[8] substituent.[9] Nevertheless, during the literature search a recent report on Cu-catalyzed 3-component cascade reaction of sulfonyl azides, alkynes and 2-bromoaniline leading to the functionalized benzimidazoles[10] attracted our attention. The substitution pattern on the benzimidazole ring was very similar to that we were looking for. Indeed, we envisioned that the incorporation of quinoxaline moiety into the alkyne reactant may afford our desired product in a single step. However, a closer look at the reported reaction[10] revealed that the methodology required the use of a ligand and lateral addition of an extra quantity of Cu catalyst as well as a base to facilitate the second step of the cascade sequence making it not truly an MCR (multi-component reaction[11]). Moreover, the methodology involved the overall use of 20 mol% of Cu catalyst and required a total reaction time of 6 h to complete the reaction. The yields of the products obtained were also not particularly high being in the range of 43%–78%. In contrast, we have observed that the reaction of *N*-(prop-2-ynyl)quinoxalin-2-amine derivative (**5**) (prepared *via* selective amination[12] of 2,3-dichloroquinoxaline **1** followed by propargylation, Scheme 2) with 2-iodoanilines (**6**) and tosyl azide in the presence of 10 mol% of CuI and $Et_3N$ in DMSO afforded the desired target compound **7** (Scheme 1) within 30 min in the absence of any ligand and/or lateral addition of the catalyst/base. The methodology was used to prepare a range of target compounds in good to excellent yields, the details of which are presented here.

The reaction of 3-chloro-*N*-(4-methoxyphenyl)-*N*-(prop-2-ynyl)quinoxalin-2-amine (**5a**) with 2-iodoaniline (**6a**) and tosyl azide was used to establish the optimized reaction conditions. The reaction was initially performed in the presence of CuI (10 mol%) and $Et_3N$ at room temperature in the absence of any ligand in a number of solvents, such as THF, MeCN, DCM, toluene, DMSO and DMF (entries 1–7, Table 1). While the reaction proceeded well in all these solvents affording the desired product **7a** in variable yields, the best result was obtained when the reaction was performed in DMSO (entry 6, Table 1) affording **7a** in 94% yield. We were delighted with this observation as the reaction proceeded in the absence of any ligand and was completed within 30 min. Moreover, the use of 10 mol% of CuI was found to be enough to catalyze this MCR though the use of higher quantity of catalyst *e.g.*, 30 mol% of CuI completed the reaction within 10 min (entry 8, Table 1). Since the yield of **7a** was not improved further in this case, the conditions in entry 6 were identified as the best reaction

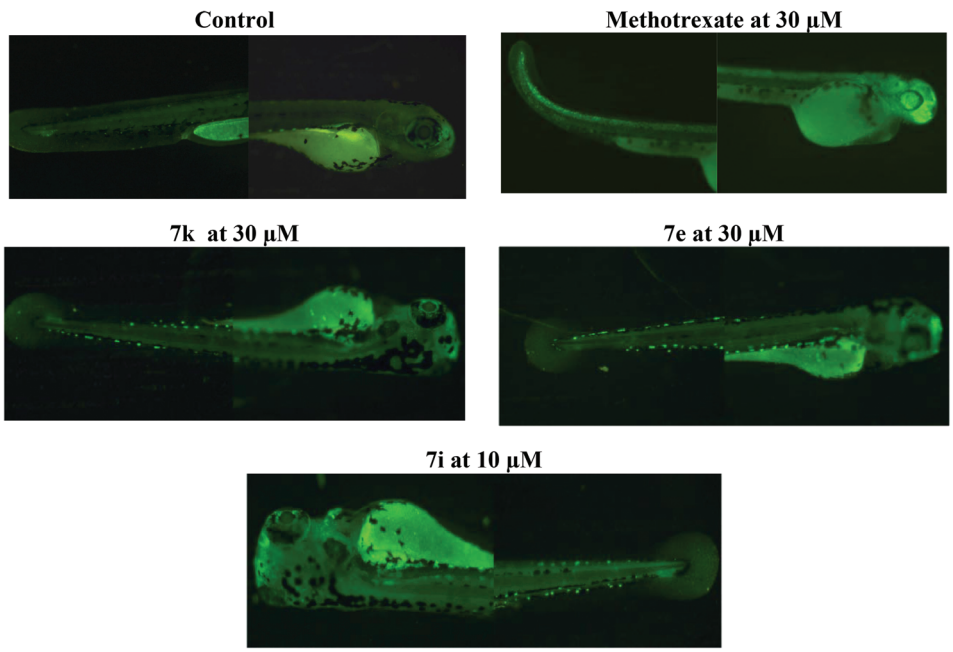



Fig. 4 Representative images of the embryos treated with compounds assayed for apoptosis.

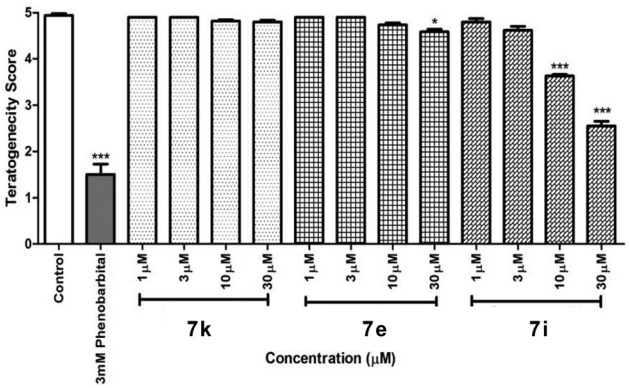



Fig. 5 Results of teratogenicity assay: each embryo was scored based on their level of toxicity from 5 being non toxic and 0.5 being highly toxic (*$p < 0.05$, ***$p < 0.001$). Statistical analysis for scoring was done using GraphPad Prism® software using two-way ANOVA. The graph represents the teratogenic scoring given compared to the positive control Phenobarbital.

conditions for further studies. We also examined the use of another catalyst $Cu(OTf)_2$ (entry 9, Table 1) and base $K_2CO_3$ (entry 10, Table 1), but these were found to be less efficient in terms of product yield. Nevertheless, being truly an MCR, the present method seemed to have advantages over the earlier method that was more of a cascade reaction.

The ligand-free MCR was further explored to expand its scope and generality. Thus a range of alkynes (**5**) along with a number of 2-iodoaniline derivatives were employed in the present MCR, and the results are summarized in Table 2 (see also Table S1 in ESI†). The reaction proceeded well in all these cases affording a variety of *N*-substituted 3-chloro-*N*-(2-(1-tosyl-1*H*-benzo[*d*]imidazol-2-yl)ethyl)quinoxalin-2-amines (**7**) in good to excellent yield (73%–95%).

From the view point of the reaction mechanism, the present MCR seems to proceed *via* a number of steps, including the formation of (i) ketenimine, (ii) tosylamide and finally (iii) intramolecular C–N bond as shown in Scheme 3. The terminal alkyne **5** reacts with the tosyl azide in the presence of CuI and $Et_3N$ to form the ketenimine species **E-1** *via* a copper-catalyzed azide–alkyne cycloaddition (CuAAC) process[13] followed by the release of nitrogen gas from the resulting triazolo-Cu intermediate. **E-1** then undergoes nucleophilic attack at the sp-carbon by the 2-iodoaniline derivative (**6**), which triggers several changes including the tautomerism of *N*-tosylamidine intermediate **E-2** to the tosylamide **E-3**. The Cu-catalyzed intramolecular C–N bond formation of **E-3** then shifts the tautomerism equilibrium from towards **E-3** and affords product **7**. Thus, the organo-copper(III) species generated from **E-3** and CuI undergoes intramolecular cyclization, involving the initial formation of N–Cu(III) bond followed by the reductive elimination of CuI to give **7**.

While the reason for the (i) rapid reaction, (ii) low catalyst loading, (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products in the present case in compared to the earlier protocol[10] is not clear if the use of the 2-iodoaniline derivative in place of 2-bromo analog could be a possible reason. It is well known

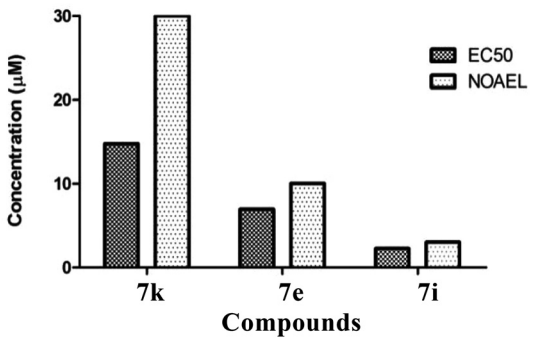



Fig. 7 The $EC_{50}$ (apoptosis) and NOAEL of test compound **7k** ($EC_{50}$ = 14.76 μM & NOAEL = 30 μM), **7e** ($EC_{50}$ = 6.94 μM & NOAEL = 10 μM), and **7i** ($EC_{50}$ = 2.23 μM & NOAEL = 3 μM) (*$p < 0.05$). The overall therapeutic index (ratio of NOAEL/$EC_{50}$) of **7k** is 2.032, **7e** is 1.44, and **7i** is 1.34.

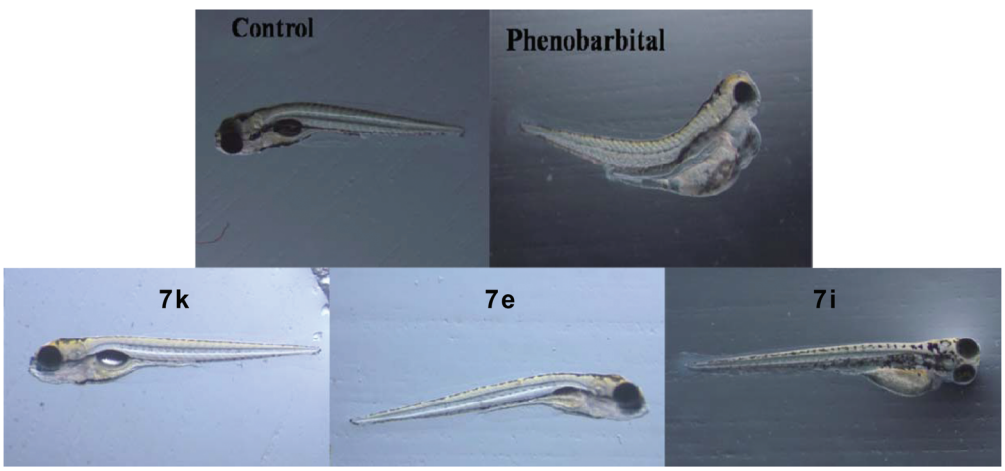



Fig. 6 Representative zebrafish images from the teratogenicity assay of compounds tested at 30 μM.

Table 3 Summary of the pharmacological evaluations of compounds **7k**, **7e**, and **7i**

| Pharmacological evaluations | | | | Test compounds data | | |
|---|---|---|---|---|---|---|
| Tests | Endpoint | Positive control | Parameters | **7k** | **7e** | **7i** |
| Apoptosis | Acridine Orange staining of apoptotic cells | Methotrexate | $EC_{50}$ | 14.76 | 6.94 | 2.23 |
| Teratogenicity | Morphological assessment of phenotypic changes | Phenobarbital | NOAEL | 30 | 10 | 3 |
| Overall therapeutic index | Ratio of NOAEL/$EC_{50}$ | — | Therapeutic index | 2.032 | 1.44 | 1.34 |

that the reactivity order of the halogen substituent towards the transition metal catalyst is I > Br > Cl and therefore 10 mol% Cu catalyst alone in the presence of $Et_3N$ was found to be enough to facilitate the overall transformation efficiently. The other reason could be the nature of terminal alkynes used. The alkynes (**5**) used in the present MCR contain a tertiary amino group, which because of its bulkiness, could force the orientation of the tosylamide moiety of **E-3** to a position favorable for intramolecular cyclization thereby accelerating the Cu-catalyzed C–N bond forming step.

In order to assess their potential to induce apoptosis, the synthesized compounds were tested in zebrafish embryos[14] along with a known drug methotrexate[15] at 30 μM. Based on their considerable effects in the present assay of apoptosis compounds **7k**, **7e** and **7i** were further tested at 1, 3, 10 and 30 μM along with methotrexate (Fig. 3 and 4). While the compound **7k** showed an increase in its apoptotic activities up to 3 μM, a decrease in activity was observed at 10 and 30 μM. The embryos were found to be safe at all concentrations. In the case of compound **7e**, the increase in apoptotic activities was observed with the increase of concentration from 1 to 10 μM, but the activity was decreased at 30 μM. Compound **7i** showed significant apoptotic activity at 10 μM; however, the embryos were dead when the concentration was increased to 30 μM.

These compounds were also evaluated for their potential toxicities[16] *e.g.* teratogenicity in the zebrafish embryo at a range of 1.0–30 μM. The toxicological evaluation was carried out in a blind fashion. All the embryos in the control group were found to be normal. Phenobarbital (3 mM) was used as a positive control in this assay (Fig. 5 and 6). The compound **7k** was found to be non-toxic in all the tested concentrations. While the compound **7e** showed mild toxicity at 30 μM, it was found to be safe at lower concentrations *e.g.*, 1, 3 and 10 μM. Compound **7i** was found to be safe at 1 and 3 μM but showed toxicity at 10 and 30 μM.

Based on the summary of $EC_{50}$ values (apoptosis), NOAEL (no observed adverse effect level) and the overall therapeutic index (Fig. 7 and Table 3), the safety order of the tested compounds appears to be **7k** > **7e** > **7i**. Overall, the compound **7k** was found to be safest whereas **7i** was identified as the most potent inducer of apoptosis in zebrafish,[17] indicating the present class of compounds are of further interest. To assess their anti-proliferative properties *in vitro*, compound **7i** and **7k** were tested against cancer cell line derived from tongue tissue *e.g.* CAL 27 at 10 μM using the sulphorhodamine B (SRB) assay.[18] Two compounds *e.g.*, 3-chloro-*N*-(4-fluorophenyl)quinoxalin-2-amine[12] (**8**) and 1-tosyl-1*H*-benzo[*d*]imidazole[19] (**9**) in addition to the reference compound gemcitabine[20] were also included in this assay to test the concept presented in Fig. 1 (*e.g.* **B**). While **7i** and **7k** showed 61% and 46% growth inhibition of CAL 27, respectively, the compound **8** and **9** showed only 20%–25% inhibition, indicating the usefulness of this concept.

In conclusion, an efficient MCR has been developed involving the reactions of *N*-(prop-2-ynyl)quinoxalin-2-amine derivatives with 2-iodoanilines and tosyl azide in the presence of 10 mol% of CuI and $Et_3N$ in DMSO to afford the pre-designed target compounds containing the quinoxaline framework linked with a benzimidazole nucleus. In contrast to the previously reported cascade reaction for the synthesis of a similar class of compounds, the present MCR seemed to have the following favorable features *e.g.*, (i) rapid reaction (30 min), (ii) low catalyst loading (10 mol%), (iii) non-requirement of any ligand and/or lateral addition of the catalyst/base and (iv) good to high yields of products (73%–95%). A range of novel hybrid molecules originally designed as potential inducers of apoptosis were prepared using this methodology and tested for apoptosis, and teratogenicity in zebrafish embryos. Furthermore, some of these compounds showed encouraging apoptosis inducing properties and therefore seem to have potential medicinal value. MCR presented here could be useful in building a library of hybrid molecules that are helpful for medicinal/pharmaceutical chemistry and drug discovery efforts.

RS thank CSIR for a research fellowship. The authors thank the management of DRILS and CSIR [Grant 02(0127)/13/EMR-II] for support.

## Notes and references

1 P. Workman, in *Apoptosis and Cell Cycle Control in Cancer*, ed. N. S. B. Thomas, BIOS Scientific Publishers Ltd, Oxford, 1996, pp. 205–232.

2 (*a*) For an account, see: B. Meunier, *Acc. Chem. Res.*, 2008, **41**, 69; (*b*) L. F. Tietze, H. P. Bell and S. Chandrasekhar, *Angew. Chem., Int. Ed.*, 2003, **42**, 3996; (*c*) P. G. Baraldi, A. N. Zaid, D. Preti, F. Fruttarolo, M. A. Tabrizi, A. Iaconinoto, M. G. Pavani, M. D. Carrion, C. L. Cara and R. Romagnoli, *ARKIVOC*, 2006, **vii**, 20.

3 (*a*) E. Sprague, S. D. Undevia, F. Innocenti, J. Ramirez, L. K. House, H. L. Kindler, D. A. Singh, A. A. Desai, R. L. Schilsky and M. J. Ratain, *J. Clin. Oncol*, (Meeting Abstracts) July 2004, **22**(14_suppl), 2021; (*b*) N. C. Kakodkar, R. Peddinti, M. Kletzel, Y. Tian,

L. J. Guerrero, S. D. Undevia, D. Geary, A. Chlenski, Q. Yang, H. R. Salwen and S. L. Cohn, *Pediatr. Blood Cancer*, 2011, **56**, 164, DOI: 10.1002/pbc.22639; (*c*) R. R. Becklin, C. L. Chepanoske, J. M. Peltier, L. Qi, P. B. Robbins, S. R. Sahasrabudhe, R. Selliah, K. Simmon, B. R. Stockwell, R. G. Venkat, M. von Rechenberg and E. Y. Zhen, *US Patent application number* US20090214465 A1, Aug 27, 2009.

4 (*a*) P. Xiang, T. Zhou, L. Wang, C.-Y. Sun, J. Hu, Y.-L. Zhao and L. Yang, *Molecules*, 2012, **17**, 873; (*b*) M. M. Ramla, M. A. Omar, A.-M. M. El-Khamry and H. I. El-Diwani, *Bioorg. Med. Chem. Lett.*, 2006, **14**, 7324.

5 (*a*) M. Raban, H. Chang, L. Craine and E. Hortelano, *J. Org. Chem.*, 1985, **50**, 2205; (*b*) L. Garuti, M. Roberti and C. Cermelli, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2525; (*c*) L. M. Dudd, E. Venardou, E. Garcia-Verdugo, P. Licence, A. J. Blake, C. Wilson and M. Poliakoff, *Green Chem.*, 2003, **5**, 187; (*d*) J. Charton, S. Girault-Mizzi and C. Sergheraert, *Chem. Pharm. Bull.*, 2005, **53**, 492; (*e*) S. Y. Lin, Y. Isome, E. Stewart, J. F. Liu, D. Yohannes and L. Yu, *Tetrahedron Lett.*, 2006, **47**, 2883; (*f*) B. Das, H. Holla and Y. Srinivas, *Tetrahedron Lett.*, 2007, **48**, 61.

6 (*a*) A. Puratchikody, V. Sivajothi, A. Jaswanth, K. Ruckmani and M. Nallu, *Indian J. Heterocycl. Chem.*, 2002, **11**, 241; (*b*) L. Garuti, M. Roberti and E. D. Clercq, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2707; (*c*) H. Foks, D. Pancechowska-Ksepko, W. Kuzmierkiewicz, Z. Zwolska, E. Augustynowicz-Kopec and M. Janowiec, *Chem. Heterocycl. Compd.*, 2006, **42**, 611.

7 For a recent reviews on benzimidazole synthesis, see: S. S. Panda, R. Malik and S. C. Jain, *Curr. Org. Chem.*, 2012, **16**, 1905.

8 For a recent review on quinoxaline synthesis, see: Y. V. D. Nageswar, K. H. V. Reddy, K. Ramesh and S. N. Murthy, *Org. Prep. Proced. Int.*, 2013, **45**, 1.

9 For the synthesis of benzimidazole linked quinoxaline derivatives, see: L.-H. Chen, C.-M. Chang, D. B. Salunke and C.-M. Sun, *ACS Comb. Sci.*, 2011, **13**, 391.

10 H. Jin, X. Xu, J. Gao, J. Zhong and Y. Wang, *Adv. Synth. Catal.*, 2010, **352**, 347.

11 (*a*) R. C. Cioc, E. Ruijter and R. V. A. Orru, *Green Chem.*, 2014, **16**, 2958; (*b*) A. Dömling, W. Wang and K. Wang, *Chem. Rev.*, 2012, **112**, 3083.

12 B. Prasad, K. S. Kumar, P. V. Babu, K. Anusha, D. Rambabu, A. Kandale, G. R. Vanaja, A. M. Kalle and M. Pal, *Tetrahedron Lett.*, 2012, **53**, 6059.

13 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; (*b*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057; (*c*) E. J. Yoo, M. Ahlquist, I. Bae, K. B. Sharpless, V. V. Fokin and S. Chang, *J. Org. Chem.*, 2008, **73**, 5520.

14 (*a*) M. Westerfield, *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)*, University of Oregon Press, Eugene, OR, 4th edn, 2000; (*b*) A. Nakhi, S. Archana, G. P. Seerapu, K. S. Chennubhotla, K. L. Kumar, P. Kulkarni, D. Haldar and M. Pal, *Chem. Commun.*, 2013, **49**, 6268; (*c*) J. M. Panzica-Kelly, C. X. Zhang, T. L. Danberry, A. Flood, J. W. DeLan, K. C. Brannen and K. A. Augustine-Rauch, *Birth Defects Res., Part B*, 2010, **89**, 382, DOI: 10.1002/bdrb.20260.

15 Y.-X. Chen, W.-G. Lv, H.-Z. Chen, F. Ye and X. Xie, *Eur J. Obstet. Gynecol. Reprod. Biol.*, 2009, **142**, 107.

16 For results of zebrafish embryo toxicity study with toxicological indices and major organs/systems affected in positive control and at MTC (maximum tolerable concentration) of test compounds, see Table S2 in the ESI.†

17 (*a*) While, the observed order of $EC_{50}$ (apoptosis) of compounds *e.g.* **7i** > **7e**> **7k** (low to high) is not clear at this stage the possibility of different rate of metabolism under *in vivo* conditions employed could play an important role. For example, a possible metabolic site *i.e.* C-5 position of the benzimidazole ring was blocked by the Me group in case of **7i** and **7e** but not in case of **7k**. Secondly, a medium sized group like Br or Me at C-4 of the N–Ph ring (*e.g.* **7i** and **7e**) seemed to be beneficial rather than a smaller group like F (*e.g.* **7k**); (*b*) Although the compound **7k** showed slightly better "therapeutic index", the compound **7i** however appeared to be a better candidate in terms of drug like properties. We thank one of the reviewers for pointing out this.

18 (*a*) L. V. Rubinstein, R. H. Shoemaker, K. D. Paull, R. M. Simon, S. Tosini, P. Skehan, D. A. Scudiero, A. Monks and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1113; (*b*) P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107.

19 Z. Peng, J. W. Wong, E. C. Hansen, A. L. A. Puchlopek-Dermenci and H. J. Clarke, *Org. Lett.*, 2014, **16**, 860.

20 E. Chu and V. T. DeVita, *Physicians' Cancer Chemotherapy Drug Manual, 2007*, Jones & Bartlett, 2007.

# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL ISOQUINOLINE

*by* Rajanikanth S

# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL ISOQUINOLINE

ORIGINALITY REPORT

| 24% | 21% | 9% | 17% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | Submitted to Jawaharlal Nehru Technological University<br>Student Paper | 7% |
| 2 | www.rsc.org<br>Internet Source | 5% |
| 3 | www.freshpatents.com<br>Internet Source | 4% |
| 4 | www.faqs.org<br>Internet Source | 2% |
| 5 | www.beilstein-journals.org<br>Internet Source | 1% |
| 6 | beilstein-journals.org<br>Internet Source | 1% |
| 7 | www.ncbi.nlm.nih.gov<br>Internet Source | 1% |
| 8 | www.heterocycles.jp<br>Internet Source | 1% |
| 9 | www.plosone.org<br>Internet Source | 1% |

| | | |
|---|---|---|
| 10 | daecr1.harvard.edu<br>Internet Source | 1% |
| 11 | Submitted to Yonsei University<br>Student Paper | <1% |
| 12 | www.chm.ulaval.ca<br>Internet Source | <1% |
| 13 | Raheem, Mohammed Abdul Edmunds, Michael . "Regioselective palladium-catalyzed ring-opening reactions of [C.sub.1]-substituted oxabenzonorbornad", Canadian Journal of Chemistry, Sept 2014 Issue<br>Publication | <1% |
| 14 | Submitted to University of Strathclyde<br>Student Paper | <1% |
| 15 | steacie.nrc-cnrc.gc.ca<br>Internet Source | <1% |
| 16 | Saleh, Moussa. "Triphenylene-based polymers for organic electronics", 09: Chemie, Pharmazie und Geowissenschaft. 09: Chemie, Pharmazie und Geowissenschaft, 2010.<br>Publication | <1% |
| 17 | www.wiley-vch.de<br>Internet Source | <1% |
| 18 | BORGNE, MARC LE, PASCAL MARCHAND, MARIE-RENEE NOURRISSON, DENIS LOQUET, MARTINA PALZER, GUILLAUME | <1% |

LE BAUT, and ROLF W. HARTMANN. "Synthesis and biological evaluation of 3-(azolylmethyl)-1H-indoles and 3-(α-azolylbenzyl)-1H-indoles as selective aromatase inhibitors", Journal of Enzyme Inhibition and Medicinal Chemistry, 2007.
Publication

| | | |
|---|---|---|
| 19 | www.google.com<br>Internet Source | <1% |
| 20 | www.chembuyersguide.com<br>Internet Source | <1% |
| 21 | www.google.com.tr<br>Internet Source | <1% |
| 22 | Enzenberg, Anne; Laschewsky, André; Boeffel, Christine and Wischerhoff, Erik. "Influence of the Near Molecular Vicinity on the Temperature Regulated Fluorescence Response of Poly(N-vinylcaprolactam)", Polymers (20734360), 2016.<br>Publication | <1% |
| 23 | Pigment & Resin Technology, Volume 45, Issue 4 (2016)<br>Publication | <1% |

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON
EXCLUDE MATCHES < 5 WORDS