Synthesis and Pharmacological Evaluation of Quinazoline based Inhibitors of Phosphodiesterase 4

A Thesis Submitted to
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In Chemistry

By

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Dedicated to

My Parents

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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.

Raju Adepu

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
September 2014

DR. REDDY'S
INSTITUTE OF LIFE SCIENCES

CERTIFICATE

This is to certify that the thesis entitled ***"Synthesis and Pharmacological Evaluation of Quinazoline based Inhibitors of Phosphodiesterase 4"*** being submitted by ***Mr. Raju Adepu*** 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 with that the thesis has reached to the standard fulfilling the requirements of the regulations relating to the nature of the degree.

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September 2014

Biography

Mr. Raju Adepu was born in Kamalapur, Karimnagar Dist., Andhra Pradesh, India, on 15th December, 1987. He received his B.Sc. Degree in Botany, Zoology and Chemistry from Bhargavi Degree College, Kakatiya University, Warangal, Andhra Pradesh, India in 2007. In 2009 he received M.Sc. degree in Chemistry with specialization in Organic Chemistry from Andhra Vidyalaya PG centre, Osmania University, Hyderabad, Andhra Pradesh, India. He was awarded "Gold Medal" for securing highest marks in M.Sc. Examinations, 2009 in AV PG Centre. Then, he qualified in CSIR-UGC NET and awarded a Junior Research Fellowship (JRF) from the CSIR, Government of India in June 2009. He also secured 17th rank in national wide and attended the interview for "Shyama Prasad Mukherjee Fellowship". In 2010 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

This thesis entitled "**Synthesis and Pharmacological Evaluation of Quinazoline based Inhibitors of Phosphodiesterase 4**" comprises five chapters.

Chapter 1

An overview of Phosphodiesterase 4 inhibitors: Targeting inflammatory diseases

In this chapter, we describe introduction, classification and functions of phosphodiesterases (PDEs) and importance of PDE4. Uses of PDE4 inhibitors in drug discovery especially in inflammatory diseases like asthma and COPD (Chronic Obstructive Pulmonary Disease) along with known literature examples.

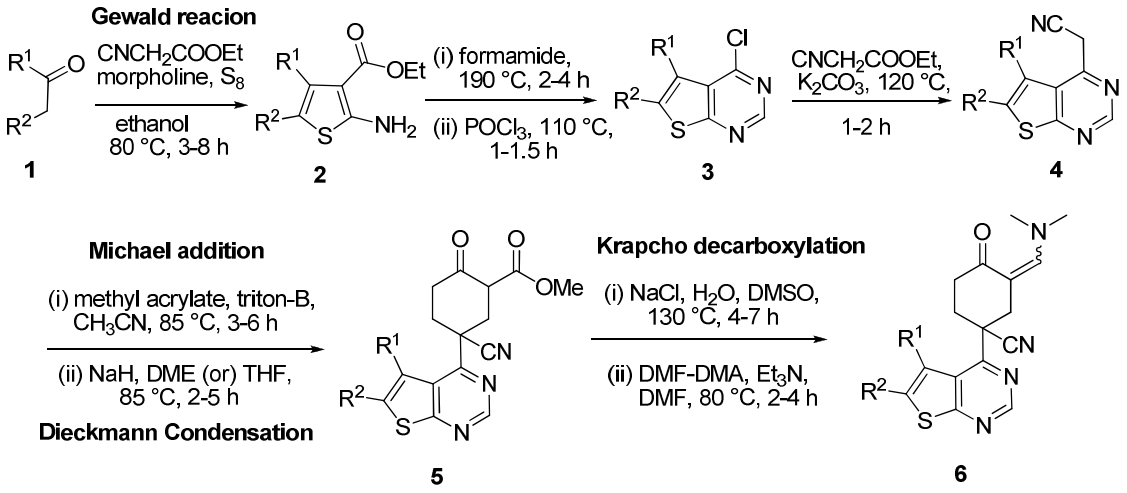
Chapter 2

Synthesis of novel thieno[2,3-*d*]pyrimidine based quinazolines

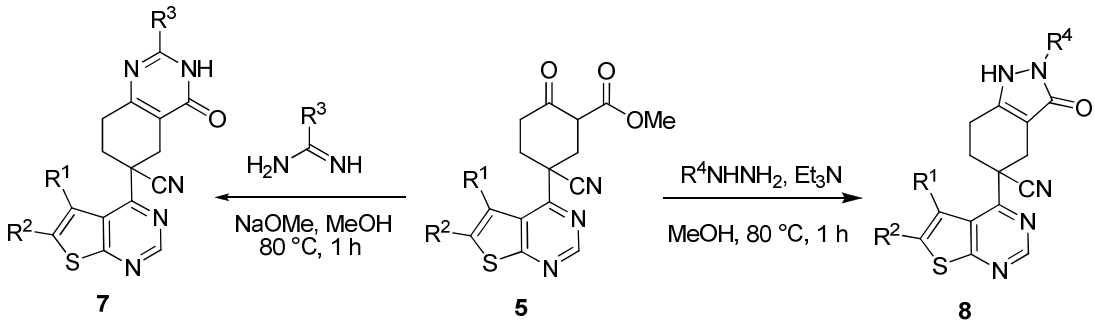
(*Org. Biomol. Chem.* **2012**, *10*, 5554-5569)

In this chapter, we have demonstrated design and synthesis of novel thieno[2,3-*d*]pyrimidine based library of small molecules containing cyclohexane ring fused with a six- or five-membered heterocyclic moiety along with a benzylic nitrile as potential inhibitors of PDE4. These molecules were prepared conveniently *via* a multi-step sequence consisting of few key steps such as Gewald reaction, Dieckmann type cyclization and Krapcho decarboxylation (Scheme 1, 2 & 3). A number of thieno[2,3-*d*]pyrimidine based derivatives were synthesized and the molecular structure of a representative compound was established unambiguously by single crystal X-ray diffraction along with the molecular arrangement and hydrogen bonding patterns.

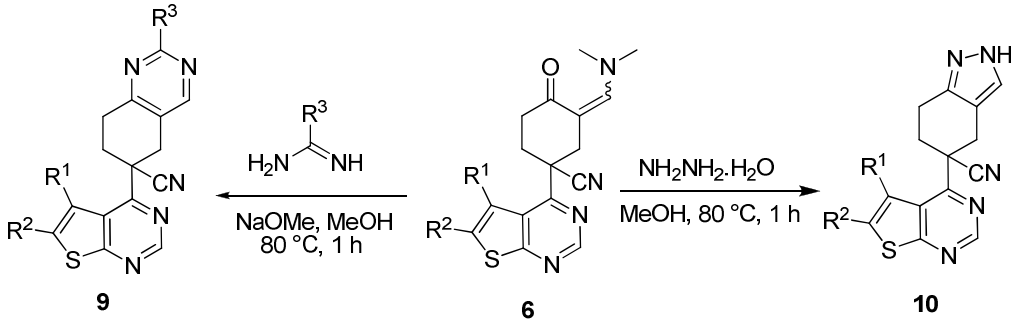
Many of these compounds were evaluated for their PDE4B inhibitory potential *in vitro*. Some of these compounds showed promising inhibition of PDE4B initially at a single dose and then subsequently in a dose dependent manner. One compound showed IC_{50} ~2 μM in PDE4B inhibition. It was also tested for PDE4D inhibition *in vitro* and dose dependent inhibition of TNF-α. The docking results showed good overall correlations to the observed PDE4B inhibitory properties *in vitro*.



Scheme 1: Synthesis of key intermediates **5** and **6**.



Scheme 2: Preparation of 4-oxo-3,4,5,6,7,8-hexahydroquinazoline (**7**) and 3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole (**8**) derivatives.



Scheme 3: Preparation of 5,6,7,8-tetrahydroquinazoline (**9**) and 4,5,6,7-tetrahydro-1*H*-indazole (**10**) derivatives.

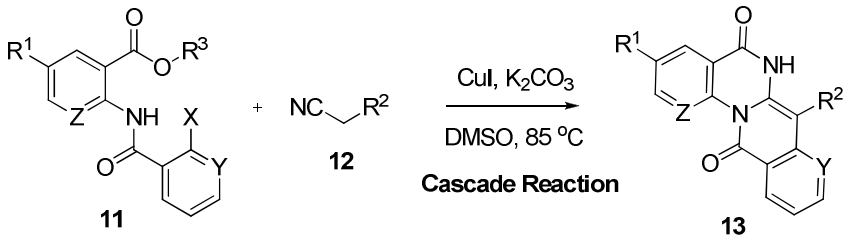
Chapter 3

Copper catalyzed one pot synthesis of isoquinolino[2,3-*a*]quinazolines

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

Over the past years, metal catalyzed cascade/domino reactions, 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.

In this chapter, we have presented design and synthesis of novel isoquinolino[2,3-*a*]quinazoline based small molecules as potential PDE4 inhibitors. 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 in one pot (Scheme 4). The cascade reaction proceeds *via* copper catalyzed Ullmann type C-C bond formation, which subsequently undergo intramolecular nucleophilic addition of NH to CN followed by intramolecular nucleophilic attack by amine to ester group, allow the formation of a fused ring leading to isoquinolino[2,3-*a*]quinazoline frame work. Some of the synthesized compounds showed promising inhibition of PDE4B when tested *in vitro* at 30 μM.



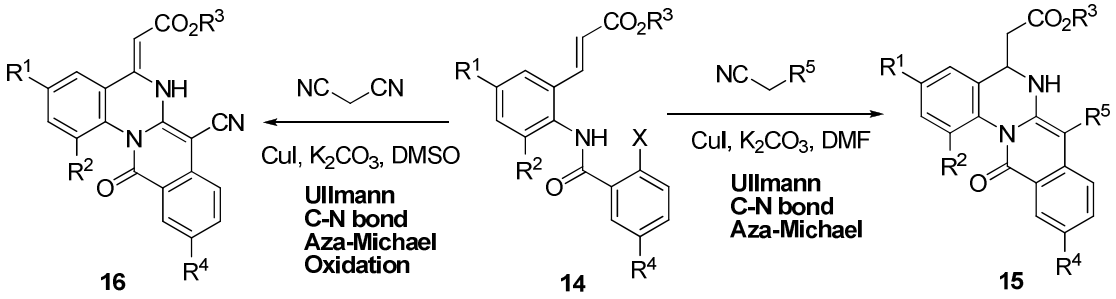
Scheme 4: one pot synthesis of isoquinolino[2,3-*a*]quinazolines.

Chapter 4

Synthesis of *N*-heterocyclic acetic acid derivatives *via* copper catalyzed cascade reaction

(*Org. Biomol. Chem.* **2014**, *12*, 2514-2518)

In continuation to the work presented in chapter 3, we extended the copper catalyzed cascade reaction for the synthesis of isoquinolino[2,3-*a*]quinazoline acetic acid framework (Scheme 5). The reaction proceeds *via* a Cu-catalyzed domino reaction involving (i) an Ullmann type intermolecular C–C followed by (ii) an intramolecular C–N bond formation and then (iii) an intramolecular aza-Michael type addition (and subsequent aerial oxidation). The reaction conditions were quiet simple as it does not require the use of any co-catalyst, ligand or additive and inert atmosphere. Several of these compounds showed promising PDE4B inhibition *in vitro* when tested using a reporter assay that was supported by *in silico* studies. Two of these compounds showed a dose dependent inhibition of PDE4B with an IC_{50} (the half maximal inhibitory concentration) ~1.06 µM and ~2.25 µM, respectively.

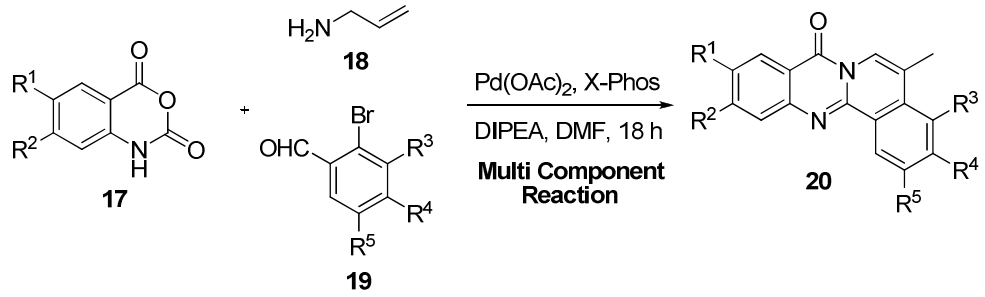


Scheme 5: Copper catalyzed synthesis of *N*-heterocyclic acetic acid derivatives.

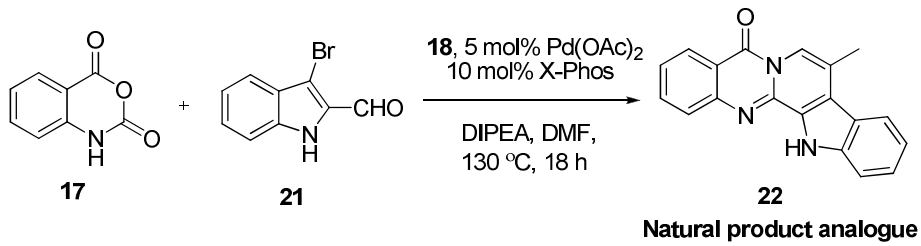
Chapter 5

Palladium catalyzed synthesis of isoquinolino[1,2-*b*]quinazolinones and a methyl analogue of 7,8-dehydrorutaecarpine

Multi-component reactions (MCRs) allow simple and flexible assembly of three or more building blocks in user friendly one pot operations with high atom economy and bond-forming efficiency by avoiding isolation and purification of any intermediates. In this chapter, we demonstrated a new multi component reaction for the synthesis of novel isoquinolino[1,2-*b*]quinazolinones *via* intramolecular palladium catalyzed Heck reaction using isatoic anhydride, allyl amine and bromo aryl aldehydes as starting materials (Scheme 6). This straightforward and operationally simple methodology does not require the monitoring or completion of the initial step before adding the Pd-catalyst. This strategy extended successfully towards the synthesis of a methyl analog of 7,8-dehydrorutaecarpine avoiding multi step procedures (Scheme 7).



Scheme 6: Palladium catalyzed synthesis of isoquinolino[1,2-*b*]quinazolinones.



Scheme 7: Synthesis of 8-methyl 7,8-dehydrorutaecarpine.

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

An overview of Phosphodiesterase 4 inhibitors: Targeting inflammatory diseases

1.1. Introduction: Asthma and chronic obstructive pulmonary disease (COPD):

Inflammation is a part of the complex biological response of the immune system to harmful stimuli such as damaged cells or irritants *via* a cascade of biochemical events that propagates and matures the inflammatory response.[1] It is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process. However, if uncontrolled, inflammation can lead to a diverse array of acute, chronic and systemic inflammatory disorders.[1, 2]

Some of the diseases related to chronic inflammation include cardiovascular disease, autoimmune disease, periodontal disease and Alzheimer's disease, along with asthma, diabetes, COPD etc. Among these, bronchial asthma, a chronic lung disorder, is characterized by hyperactivity of the respiratory tract to external stimuli such as cold or warm or moist air, exercise, exertion, and emotional stress, resulting in shortness of breath, coughing, wheezing and feelings of tightness in the chest. Under this condition the airway occasionally constricts, becomes inflamed, and filled with excessive amounts of mucus which lead to a number of severe lung diseases including asthma and chronic obstructive pulmonary diseases (COPD). COPD is associated with multiple comorbidities, including heart disease,[3] cancer,[4] depression,[5] and decreased bone mineral density,[6] among others. It is currently the fourth leading cause of death worldwide and the WHO predicts that it will rise to third leading cause by 2030.[7]

Currently, there is no definitive cure for COPD. However, the major goals involve preventing disease progression, reduction and treatment of symptoms, increased exercise tolerance, improved overall health status, prevention of exacerbations, prevention of COPD related complications, and, of course, reduction in mortality.[8] Bronchodilatory drugs are currently the preferred choice for the symptomatic management of COPD, as lung volumes improve with bronchodilator therapy and reduction of hyperinflation of the lungs can improve exercise tolerance and reduce dyspnea. However, these agents do not address the underlying chronic inflammation or the changes in airway structure. While the introduction of more effective treatments and the use of nonpharmacological interventions, such as pulmonary rehabilitation and noninvasive ventilation (NIV), have improved the management of COPD considerably,[9] no existing therapies have been shown to reduce the disease

progression. Notably, among the new anti-inflammatory agents currently being developed, phosphodiesterase 4 (PDE4) inhibitors[10,11] proved to be very effective in attenuating the responses of various inflammatory cells through their ability to elevate cyclic 3′,5′-adenosine monophosphate (cAMP) levels.

1.2. Phosphodiesterases classification and function:

Phosphodiesterases (PDE) are enzymes that hydrolyze intracellular cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) into inactive linear 5′ monophosphate (Figure 1.1).

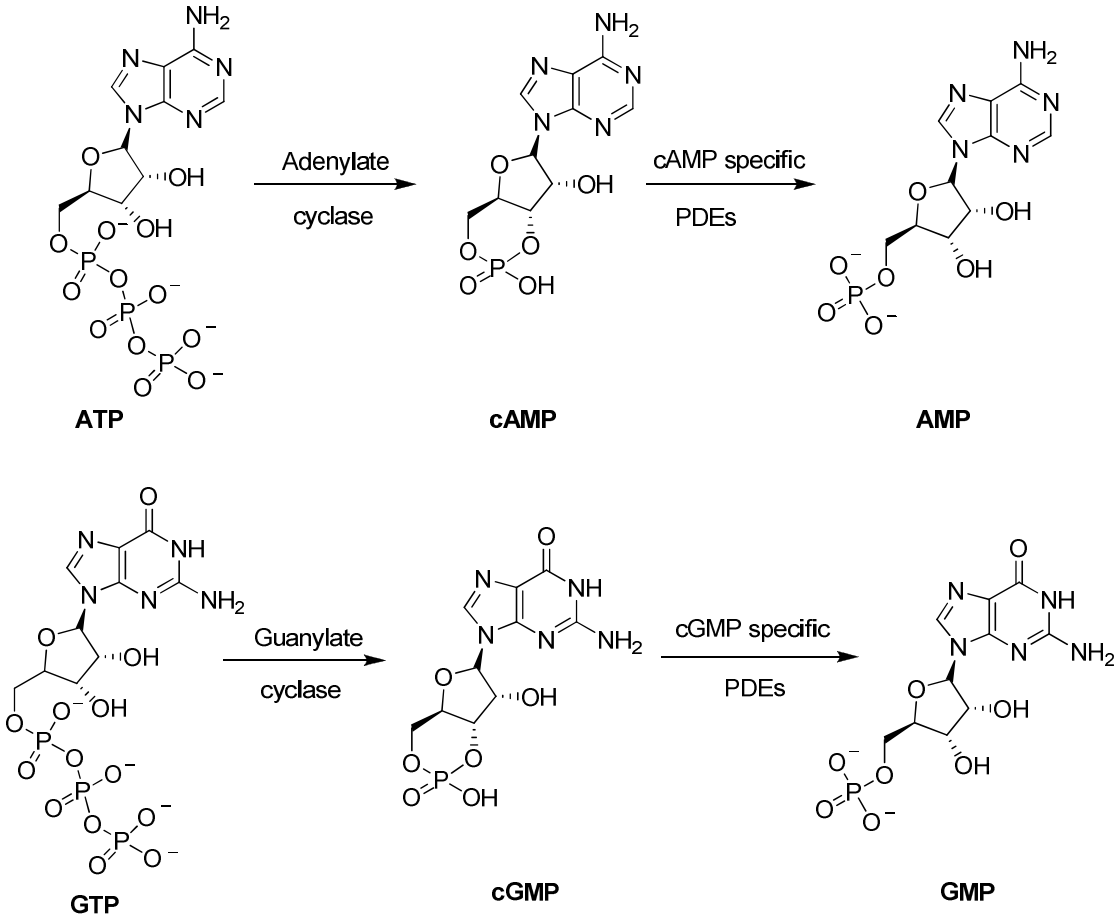


Fig. 1.1: Enzymatic conversions of cyclic and linear nucleotides.

In cyclic nucleotide signaling pathway cAMP and cGMP plays important role in mediating cellular signals in response to hormones, neurotransmitters, chemokines and cytokines.[12] These signals usually begin through binding of hormone neurotransmitter to a G-protein coupled receptor with subsequent triggering of

adenylyl cyclase and guanylyl cyclase. The newly synthesized nucleotides bind and activate effectors such as protein kinase A and protein kinase G. These protein kinases phosphorylate the variety of substrates including ion channels and transcription factors. The resulting signals can lead to a change in the gene expression, cell metabolism and ultimately regulate the wide variety of cellular functions such as immune response, cardiac smooth muscle contraction, visual response, cell growth control and apoptosis.

PDEs hydrolyze the cyclic nucleotides to their inactive linear 5'-AMP and 5'-GMP and are considered as negative regulators of cyclic nucleotide signaling pathways. The PDEs are classified into 11 families namely PDE1-PDE11. The classification is based on amino acid sequences, substrate specificities, regulatory properties, pharmacological properties and tissue distribution. Different PDE of the same family are functionally related but can have different substrate specificities, some are cAMP selective hydrolyses (PDE4, 7 and 8), some are cGMP selective (PDE5, 6 and 9) and other are selective towards both cAMP and cGMP (PDE1, 2, 3, 10 and 11) (Figure 1.2).

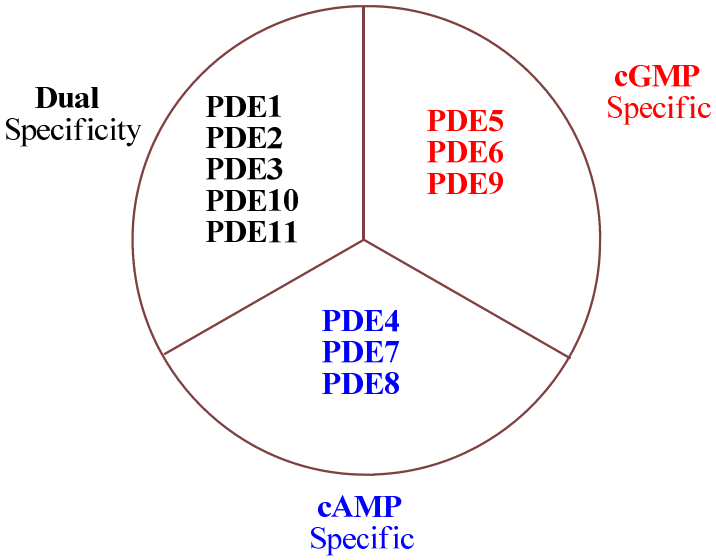


Fig. 1.2: Classification of PDEs based on specificity toward cAMP and cGMP.

Among these, PDE4 enzymes are cAMP specific which specifically hydrolyses cAMP. The family of PDE4 enzymes is encoded by 4 distinct genes (A-D) that gives rise to four isoforms *i.e.,* PDE4A, PDE4B, PDE4C and PDE4D.[13] PDE4 is encoded by four separate genes (PDE4A–D), each with multiple splice variants that can

encode more than 15 different isozymes that vary among cell type. These isoforms are distributed at various tissues and differ in their sensitivity to inhibitor. They are mainly found in the brain, inflammatory cells, smooth muscle and cardiovascular tissues. They are nearly absent in platelets and are expressed in inflammatory cells such as T cells, B cells, eosinophils, neutrophils, airway epithelial cells and endothelial cells.[14]

In the inflammatory cells, cAMP plays the role of a negative regulator by activating the primary pathways such as cytokine release by T-cells. As cAMP levels are regulated by cAMP-specific PDE isozymes, PDE4 isozyme regulation plays a key role in the case of mediators of inflammatory response because it is predominantly expressed in inflammatory and immune cells.

1.3. Effect of PDE4 inhibition:

Inhibition of the PDE4 in inflammatory cells effectively elevates the intracellular cAMP levels, thereby subsequent PKA activity leads to specific protein phosphorylation that elicit a variety of functional responses. This in turn inhibits the release of inflammatory mediators such as cytokines e.g. tumor necrosis factor-R (TNF-R), interleukin-2 (IL-2), interleukin-12 (IL-12), leukotriene B4 (LTB4), interferon-γ (IFN-γ a), as well as activation of inflammatory cells and thereby inactivation of the anti-inflammatory response. Since the cellular mediators play a key role in the inflammatory diseases such as asthma and COPD, the development of PDE4 inhibitors as therapeutic agents has been a major pharmaceutical focus (Figure 1.3 & 1.4).[10a] In addition, elevation of intracellular cAMP levels *via* inhibition of PDE4 activity led to smooth muscle relaxation and thereby bronchodilation which is beneficial for the management of respiratory diseases like asthma or COPD.

Elevation of cAMP levels has several other beneficial effects that include inhibition of mast cell mediator release, suppression of neutrophils degranulation, inhibition of basophil degranulation, and inhibition of monocyte and macrophage activation. However, the common side effects of PDE4 inhibitors include nausea, emesis and diarrhea. These side effects are explained on the basis of PDE4 subtypes. Among the PDE4 subtypes, the PDE4B is linked to inflammatory cell regulation[15] whereas the

PDE4D is implied in the emetic response.[16] However, none of the PDE4 inhibitors under development are PDE4B selective.

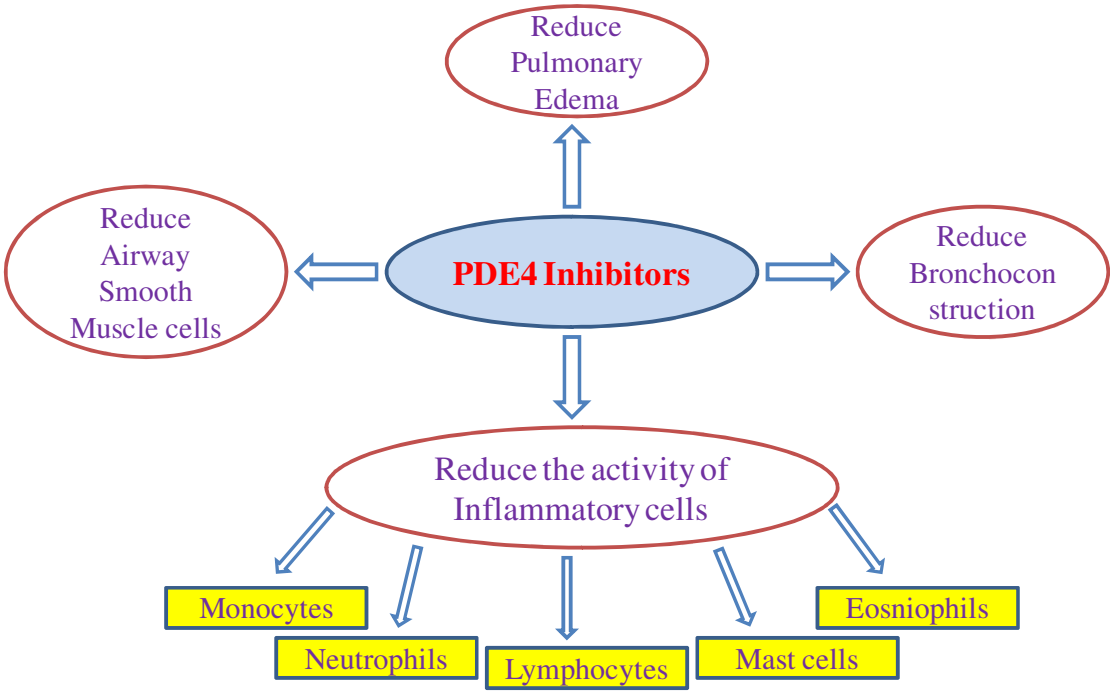


Fig. 1.3: Therapeutic actions of PDE4 inhibitors (Asthma).

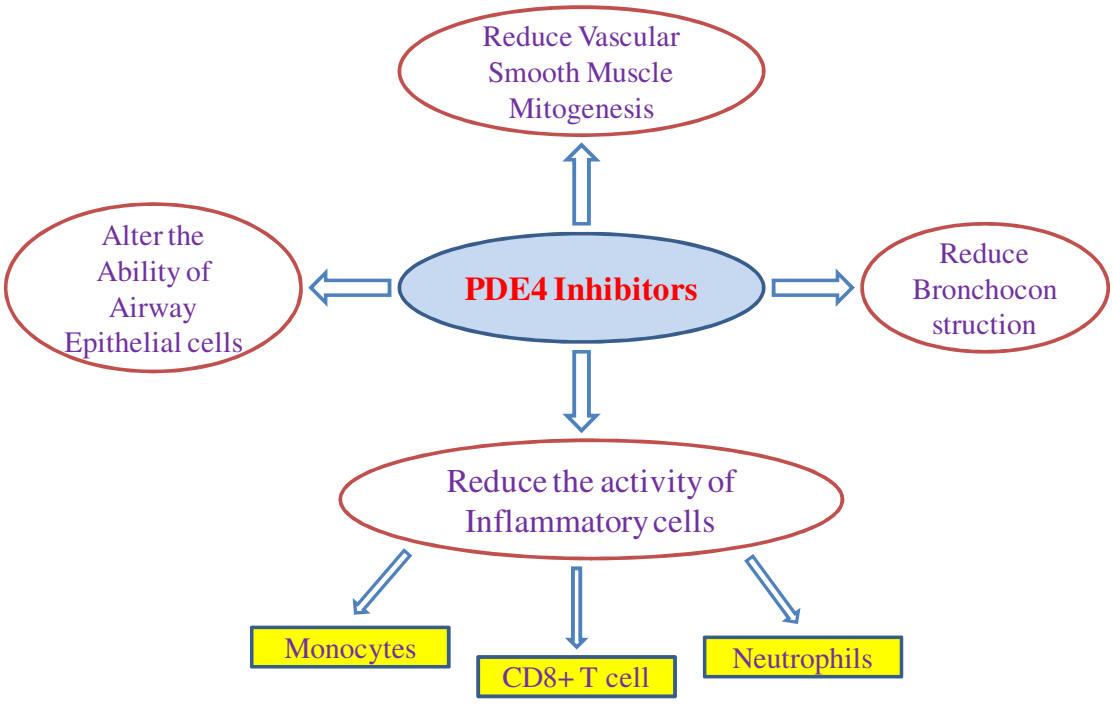


Fig. 1.4: Therapeutic actions of PDE4 inhibitors (COPD).

Recent studies have indicated that the development of PDE4B selective inhibitors as potential therapeutics is beneficial for allergic inflammation and asthma,[17] and the development of PDE4D selective allosteric modulators is beneficial to reduce inflammation and improve cognition.[18] Recent studies showed that PDE inhibitors, especially PDE4 inhibitors, may be useful for treating certain metabolic diseases, including obesity, type 2 diabetes and metabolic syndrome.[19] Given the current understanding of the crucial role of inflammation in these diseases,[20] the anti-inflammatory actions of PDE4 inhibitors may provide considerable therapeutic benefit.

1.4. PDE4 inhibitors:

Identification of the specific PDE isoform that mediates inflammatory lung disease and developing orally available small molecule inhibitors are important concepts and goals in targeting PDEs for the treatment of lung diseases. A wide variety of PDE4 inhibitors have been reported so far. Theophylline (**1**, Figure 1.5) could be considered the first PDE inhibitor marketed for the treatment of lung diseases over 60 years. However, it is a nonspecific inhibitor of all PDEs and is least effective against PDE4.[21]

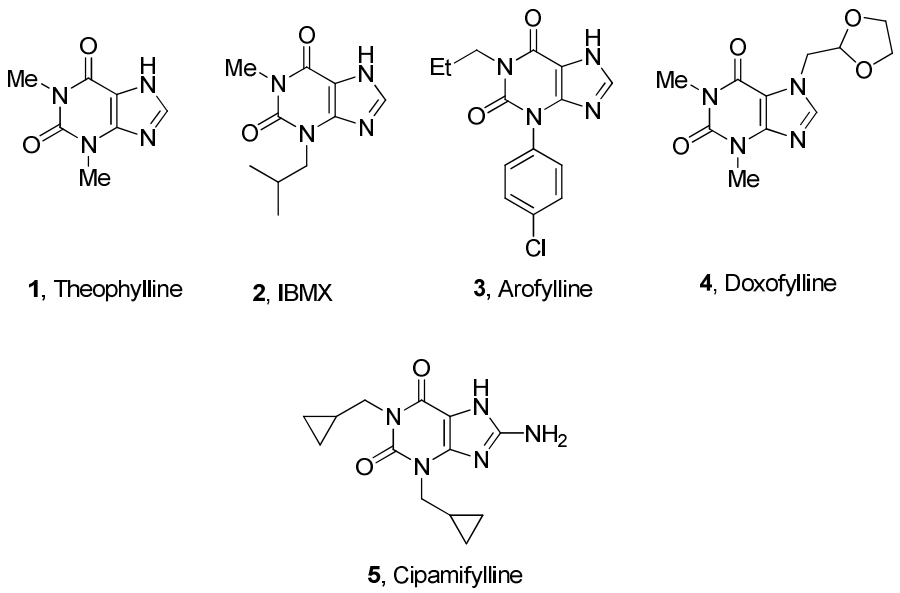
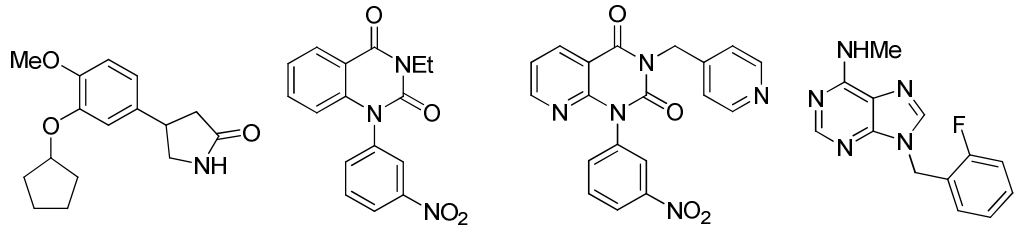


Fig. 1.5: Theophylline and related compounds.

Traditionally, the effect of theophylline was thought to be bronchodilation. Theophylline has good oral bioavailability but has a very narrow therapeutic index.

However, it has fallen out of favor for the treatment of lung disease and has been replaced with inhaled bronchodilators and anti-inflammatory drugs that are more specific in effect and delivery and have markedly less potential for adverse effects. Other members of theophylline analogues resulted in identification of a novel series of xanthenes (**2-5**, Figure 1.5) with acceptable PDE4 activity and improved selectivity.[22]

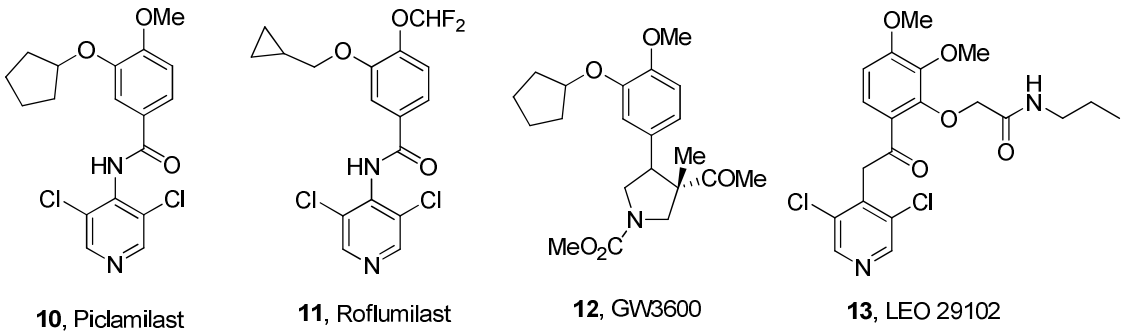
The first generation of PDE4-selective inhibitors improved selectivity over theophylline in which one of the earliest one was rolipram (**6**, Figure 1.6).[23, 24] Rolipram is a highly selective first generation PDE4 inhibitor that has been used for many years as a research tool to investigate the role of PDE4. Rolipram possesses anti-inflammatory and anti-immunomodulatory effects with a PDE4/PDE (all forms) selectivity of more than 100 fold. However, its development as a therapeutic has been halted mainly because of undesired side effects, including nausea and vomiting.[25] Analogs of rolipram and other PDE4 inhibitors like nitraquazone (**7**, Figure 1.6) exhibited rolipram binding site affinity in the nanomolar range with anti-inflammatory and analgesic pharmacological profile.[26] Structural analogs of nitraquazone were also identified as potent PDE4 inhibitors (**8-9**, Figure 1.6).[27]



6, Rolipram **7**, Nitraquazone **8**, RS-25344 **9**, BWA78U

Fig. 1.6: Structures of rolipram (**6**), nitraquazone (**7**) and its analogues.

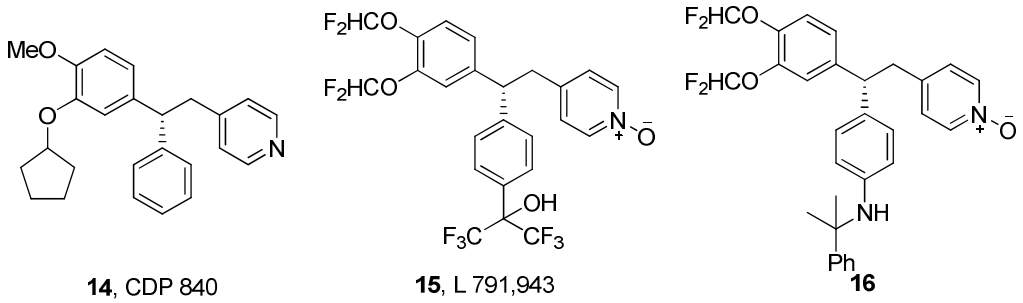
Later, the potent analogues piclamilast (**10**, Figure 1.7),[28] roflumilast (**11**, Figure 1.7)[29] and GW3600 (**12**, Figure 1.7)[30] were achieved by changing pyrrolidinone ring in rolipram by an appropriate pharmacophore. Indeed, 3,5-dichloropyridyl-4-carboxamide group identified as an effective pharmacophore after a detailed SAR work study. However, the clinical development of **10** (Sanofi-aventis) and **12** discontinued and Roflumilast was approved in the EU (Daxas) in June 2010 and approval in US (Daliresp) in Mar 2011.[31] Recently, LEO 29102 (**13**, Figure 1.7) was developed as PDE4 inhibitor using the pharmacophore of roflumilast.[32]



10, Piclamilast **11**, Roflumilast **12**, GW3600 **13**, LEO 29102

Fig. 1.7: Structures of Piclamilast (**10**) and related compounds.

Another potent PDE4 inhibitor **14** (PDE4 IC_{50} 4.5 nM),[33] derived from rolipram significantly reduced antigen-induced bronchoconstriction in animal models and in asthmatic patients. However, it discontinued due to extensive metabolism *in vitro* and thereby a short half-life *in vivo*. Later highly potent metabolically stable PDE4 inhibitor **15** (GST-PDE4A IC_{50} 4.2 nM)[34] was developed by introducing a stable bisdifluoromethoxy catechol and a pendent bistrifluoromethylcarbinol groups and showed long half life in animal studies. To decrease the half life of compound **15**, compound **16** was chosen and it exhibited a good PDE4 inhibitor activity (GST-PDE4A IC_{50} 0.8 nM)[35] as well as an improved pharmacokinetic profile over **15**.



14, CDP 840 **15**, L 791,943 **16**

Fig. 1.8: Tri aryl ethane based inhibitors.

The indole or benzimidazole was identified as novel and potent PDE4 inhibitors by replacing catechol moiety in rolipram with 3,4-substituted indole derivative, **17** (PDE4 IC_{50} 12 nM).[36] Later a new series **18**,[37] was developed as PDE4 inhibitors by introducing a 2-substituted-7-methoxybenzimidazole moiety. Even, benzofuran was also found to be an isoster for catechol in rolipram as exemplified by potent PDE4 inhibitors **19, 20** and **21** (Figure 1.9).[38]

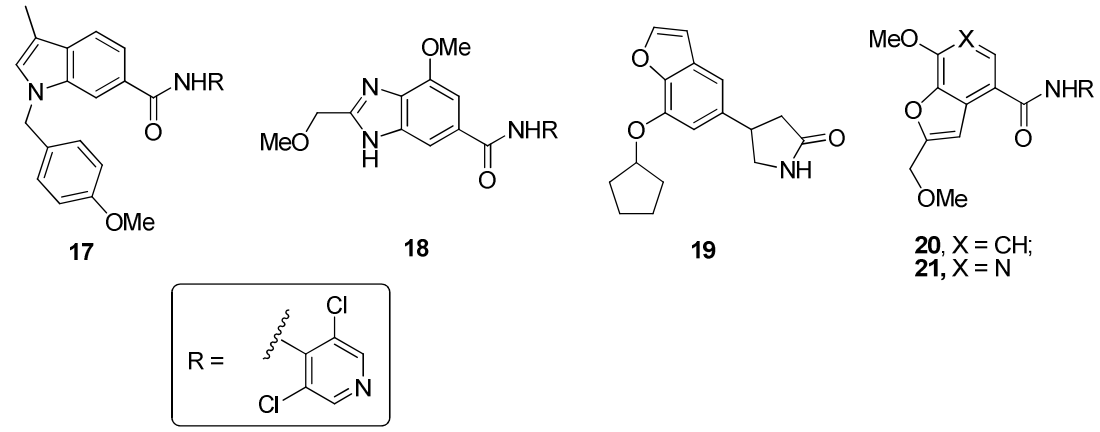


Fig. 1.9: Indole, benzimidazole and furan based PDE4 inhibitors.

Later, an orally active second generation PDE4 inhibitors, *cis*-4-cyano-4-[3-cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid, cilomilast (**22**, Figure 1.10)[39] was designed and explored after an extensive SAR study. These molecules showed significant improvement in reducing the side effects. By introducing a nitrogen atom into the six membered ring (**23**-**24**, Figure 1.10)[40] the potency was improved compared to compound **22**. In another study, introduction of rigid ring by employing bicycle[3.3.0.]octane template (**25**, Figure 1.10)[41] instead of six membered ring showed improved therapeutic potential with fewer side effects.

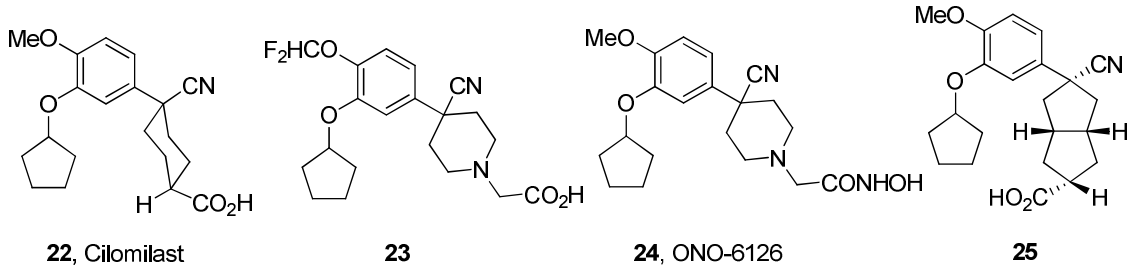


Fig. 1.10: Cilomilast (**22**) and its related derivatives.

Later, many cyclohexane derivatives (**27**-**30**, Figure 1.11)[42] having benzylic nitrile as one of the pharmacophore along with cyclohexane ring were reported as PDE4 inhibitors. In these cases, the cyclohexane ring was fused with a heterocyclic ring (R = NH_2, **27** & **30**, Figure 1.11)[42a, 42d] and the corresponding compounds showed improved potency over others.

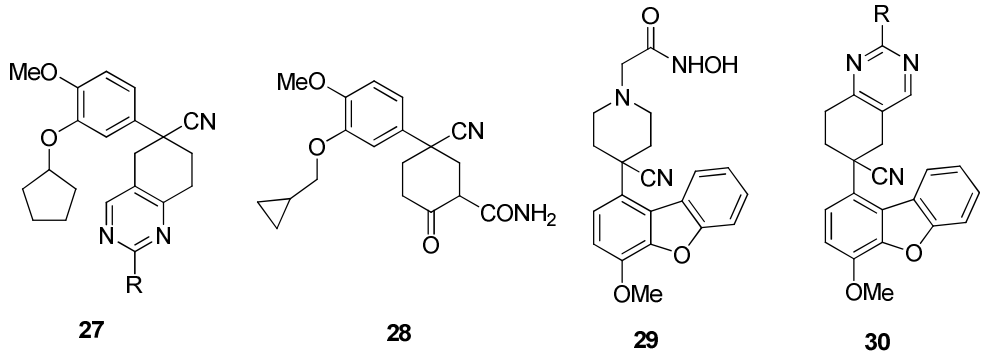


Fig. 1.11: Cyclohexane based PDE4 inhibitors.

With the use of PDE4 inactive thalidomide (**31**, Figure 1.12)[43], which is known to be a selective TNF-α inhibitor, its analogues **32**[44] and aprimilast, (**33**, Figure 1.12)[44] were developed as TNF- α as well as PDE4 inhibitors (Figure 1.12). Recently, aprimilast has been approved for the treatment of adults with active psoriatic arthritis.[45] Another molecule, GSK 256066 (**34**, Figure 1.13)[46] showed an exceptionally high affinity and selectivity to the PDE4 inhibition which showed IC_{50} value 0.01 nM.

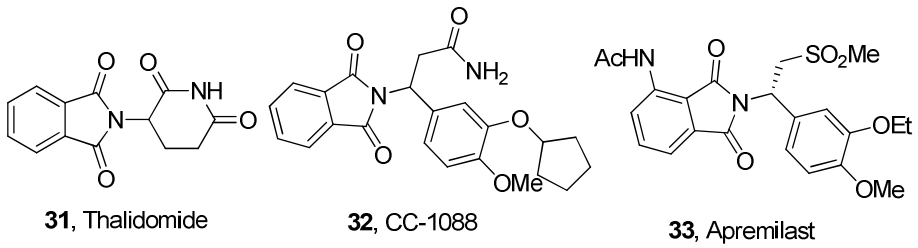


Fig. 1.12: PDE4 inactive thalidomide (**31**) and its PDE4 active derivatives.

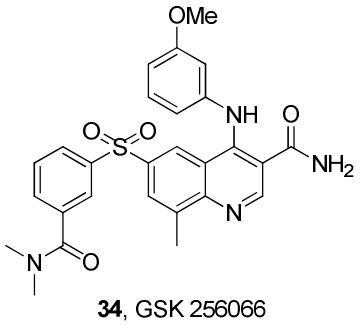


Fig. 1.13: Structure of GSK 256066.

1.5. Conclusions:

A major focus has been devoted on targeting PDE4 in the development of novel therapeutics for pulmonary inflammatory diseases for almost two decades. It is evident that as a result of intense research towards the development of PDE4 inhibitors, numerous candidates have been placed into the clinical trials for asthma and/or COPD. The recent approval of roflumilast as a drug for COPD therapy provides proof that the PDE4 isozyme family can be a therapeutic target, even though this second-generation PDE4 inhibitor is still not free from the side effects. Several strategies have been proposed to minimize this problem, such as designing inhibitors as inhaled drugs or topically applied agents, as well as improving subtype selectivity. The development of PDE4 inhibitors with PDE4B selectivity has been considered as a promising approach because inhibition of PDE4B produces a broad spectrum of anti-inflammatory effects by minimizing unwanted side effects. Nevertheless, the impact of PDE4B selective inhibitors on inflammatory diseases awaits further clinical trials. Several PDE4B and PDE4D selective inhibitors have been designed and synthesized, and their effects on inflammation are under investigation. Overall, owing to their unique anti inflammatory and immunomodulatory properties coupled with their potential for disease modification, PDE4 inhibitors would continue to be preferred and the potential option for the treatment of severe asthma and COPD.

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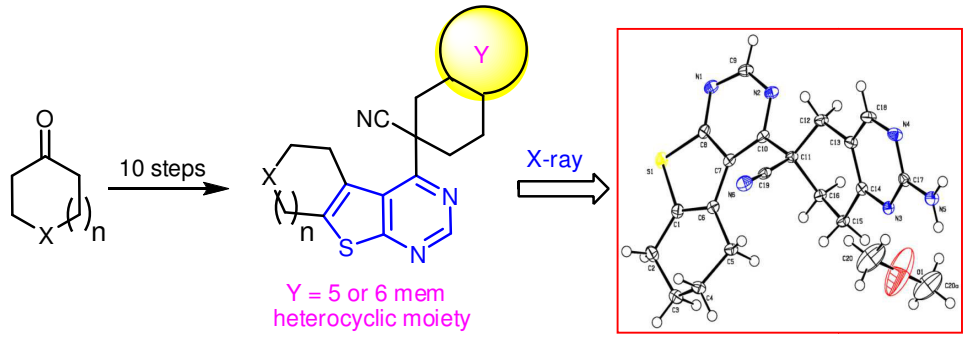
CHAPTER 2

Synthesis of novel thieno[2,3-d]pyrimidine based quinazolines



2.1. Introduction:

Phosphodiesterases (PDEs) are enzymes that regulate the cellular levels of the second messengers like cAMP and cGMP, by controlling their rates of degradation.[1] Among these phosphodiesterases, PDE4 is a cAMP specific PDE present in majority of inflammatory cells.[2] Elevated levels of cAMP trigger a variety of functional responses such as suppression of inflammatory mediators which is beneficial in treating inflammatory diseases especially pulmonary diseases. Thus inhibition of PDE4 is beneficial for the treatment of respiratory diseases including asthma and COPD.[2] A number of PDE4 inhibitors are under development with a wide variety of scaffolds. Some of the new chemical entities containing pyrimidine rings such as **1** and **2** showed good inhibition of PDE4 (Figure 2.1).[3] As pyrimidine nucleus fused with another heterocycle has found wide applications in the design and discovery of novel bioactive molecules and drugs,[4] we focused on thieno[2,3-*d*]pyrimidine scaffold to explore the potential towards the inhibition of PDE4.

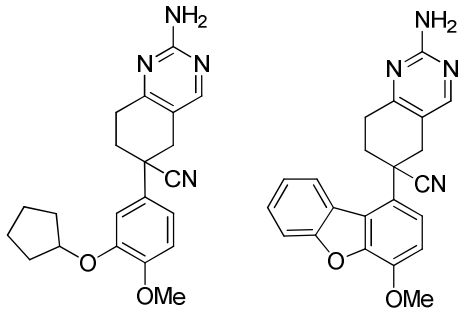


Fig. 2.1: Examples of pyrimidine ring containing PDE4 inhibitors.

A wide range of biological activities are known for compounds containing thieno[2,3-*d*]pyrimidine scaffold such as selective 5-HT3 and 5-HT4 receptor ligands (**3** and **4**, Figure 2.2),[5,6] tyrosine kinase inhibitors (**5**, Figure 2.2),[7] TGase 2 inhibitors (**6**, Figure 2.2),[8] TNF-α inhibitors (**7**, Figure 2.2),[9] potential dual thymidylate synthase (TS) and dihydrofolate reductase (DHFR) inhibitors (**8**, Figure 2.2),[10] agents that cause apoptosis through inhibition of tubulin polymerization (**9**, Figure 2.2).[11] All these reports on biological activities prompted us to explore various functionalizations around the thieno[2,3-*d*]pyrimidine moiety. The earlier reports for the synthesis of thieno[2,3-*d*]pyrimidines are discussed below.

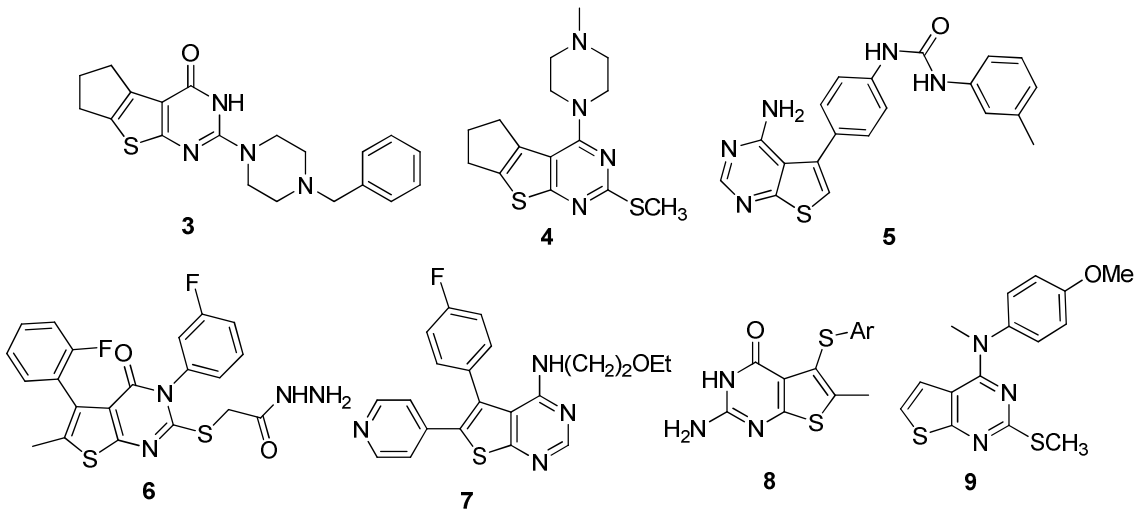
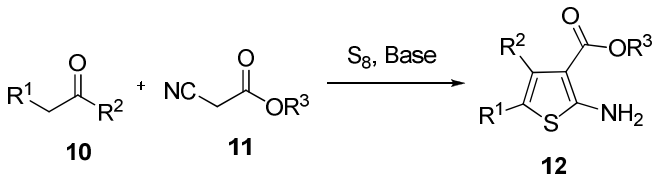


Fig. 2.2: Examples of biologically active thieno[2,3-*d*]pyrimidine derivatives.

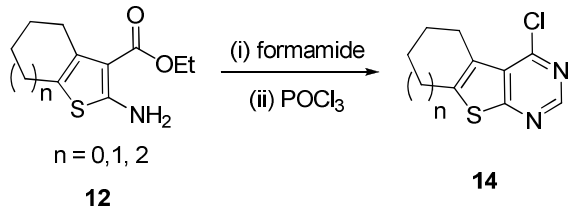
2.2. Previous work on thieno[2,3-*d*]pyrimidine core:

Synthesis of thieno[2,3-*d*]pyrimidine core was achieved by starting with classical Gewald reaction reported by Gewald[12] in 1966 which produced 2-amino thiophene carboxylates from the condensation of a ketone (or aldehyde when R^2 = H) with a α-cyanoester in the presence of elemental sulfur as shown in Scheme 2.1.



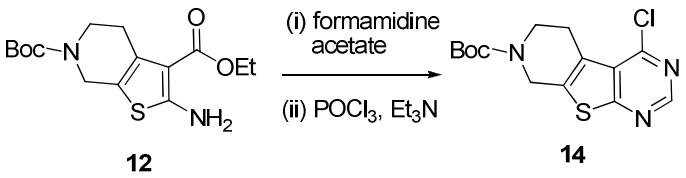
Scheme 2.1: Synthesis of 2-amino thiophene carboxylate *via* a multi component reaction.

In 2010, Pal and coworkers reported 2-step protocol for the synthesis of 4-chloro thieno[2,3-*d*]pyrimidine derivatives from 2-amino thiophene carboxylate as shown in Scheme 2.2.[13, 14]



Scheme 2.2: Synthesis of 4-chloro thieno[2,3-*d*]pyrimidine from 2-amino thiophene carboxylate.

In 2010, Hsieh and coworkers reported synthesis of 4-chloro tetrahydropyridothieno[2,3-*d*]pyrimidine scaffold from 2-amino tetrahydrothieno[2,3-*c*]pyridine carboxylate as a starting material in two step process shown in Scheme 2.3.[15]



12 **14**

Scheme 2.3: Synthesis of 4-chloro tetrahydropyridothieno[2,3-*d*]pyrimidine.

2.3. Present work:

A wide variety of PDE4 inhibitors have been reported so far and several of them entered into the clinical trials. A highly selective first generation PDE4 inhibitor, Rolipram was associated with undesired side effects such as nausea and vomiting. While these side effects were reduced in second generation inhibitors like cilomilast[16] (Ariflo), it therapeutic index has delayed market launch so far. These side effects were thought to be due to the inhibition of PDE4D subtype that was responsible for the emetic response[17] whereas inhibition of PDE4B subtype was linked to the inflammatory cell regulation.[18] However, none of the PDE4 inhibitors under development are PDE4B selective.[19]

To identify novel PDE4 inhibitors, we became interested in the synthesis of 4-cyano cyclohexane-1-carboxylic acid derivatives as this type of compounds was reported to cause significant improvement in reducing the side effects of rolipram (**A**, Figure 2.3). For example, cilomilast (**B**, Figure 2.3) that belongs to this family entered into phase-3 clinical trials as it did not show the side effects of rolipram in the earlier stages. Then, the new cyclohexane derivatives (**C**, Figure 2.3) were designed with the replacement of CO_2H group in cyclohexane ring by a six- or five-membered heterocyclic moiety and maintaining the benzylic nitrile as one of the pharmacophore.[3a] Recently, cyclohexane derivatives (**D**, Figure 2.3) containing a tricyclic fused aryl ring have been reported as potential PDE4 inhibitors.[3b] Based on these observations, we designed novel cyclohexane derivatives (**E**, Figure 2.3) having a benzylic nitrile group as a pharmacophore and thieno[2.3-*d*]pyrimidine ring as a fused aryl ring. The cyclic ring 'X' and 'Y' were chosen to introduce diversity into

basic scaffold for the generation of library of compounds. To best of our knowledge template **E** has not been previously explored for the discovery of PDE4 inhibitors.

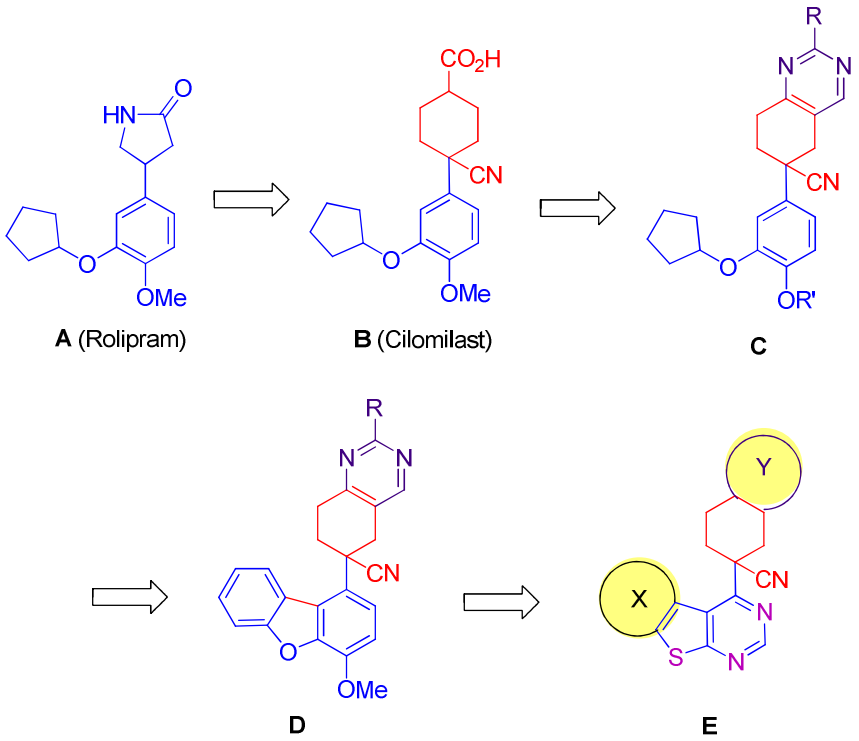


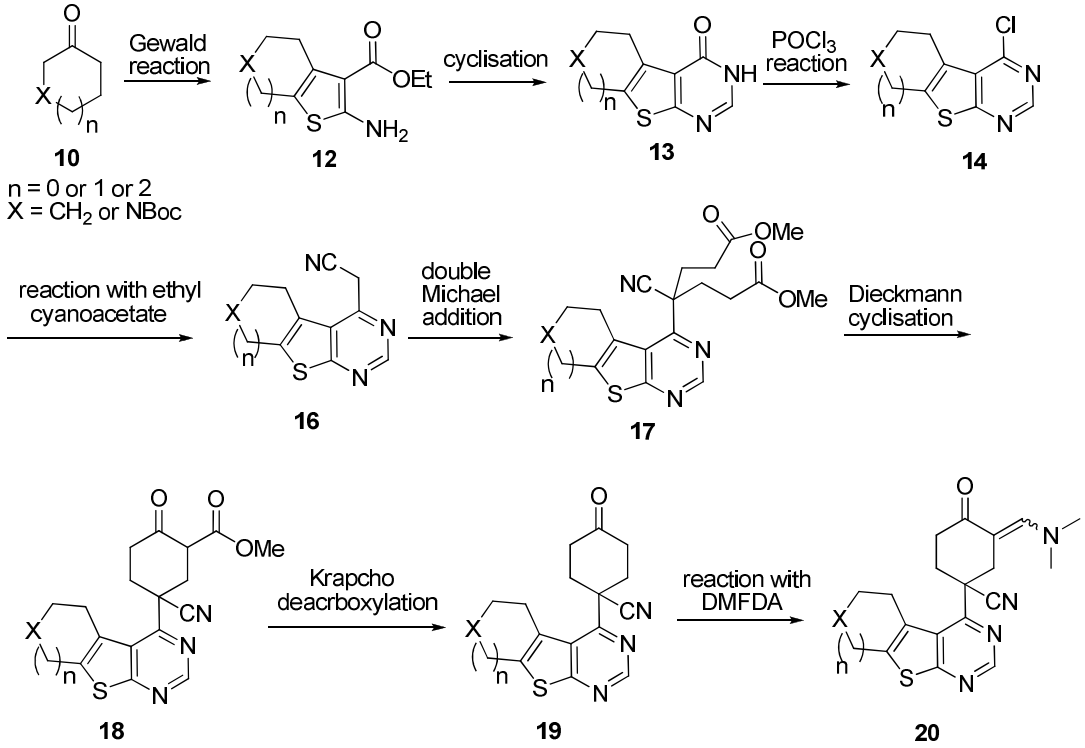
Fig. 2.**3**: Design of novel inhibitors (**E**) of PDE4 based on known inhibitors (**A-D**).

2.4. Results and discussion:

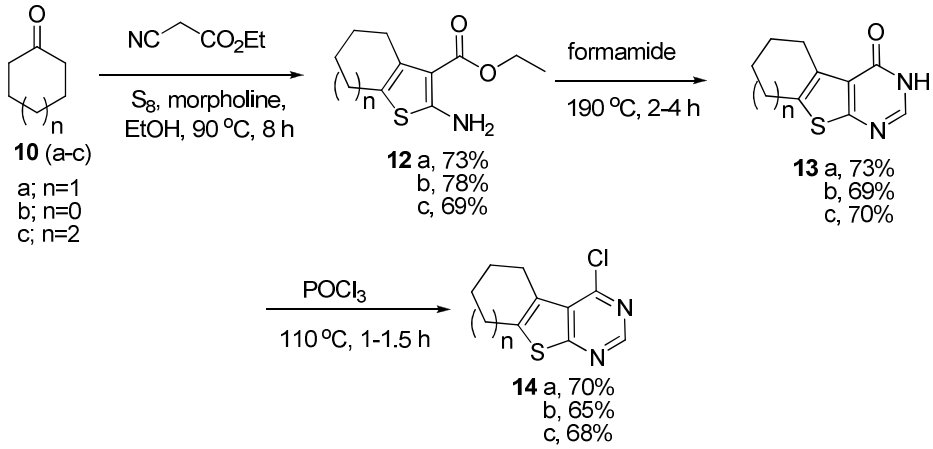
The construction of a cyclohexane ring at C-4 of the thieno-[2,3-*d*]pyrimidine moiety is the key step to achieve the target compound **E**. The synthesis of target compound is divided into three parts *i.e.,* construction of thieno pyrimidine ring (step 10-14, Scheme 2.4) followed by construction of six membered ring (step 16-20, Scheme 2.4), subsequently introduction of five or six membered heterocyclic ring. The key reactions involved in this scheme are Gewald reaction,[12] Krapcho decarboxylation,[20] Michael addition and Dieckmann type condensation.[21]

The starting material ethyl 2-aminothiophene carboxylate **12** was prepared using Gewald reaction conditions *i.e.,* condensation of corresponding ketone **10**, ethyl cyanoacetate and sulphur in the presence of a base. Condensation with formamide provided thieno[2,3-*d*]pyrimidone **13a-c** followed by treatment with phosphorous oxychloride gave required compound **14a-c** (Scheme 2.5).[13] For the synthesis of **13d**,

formamidine hydrochloride was used instead of formamide which on treatment with 1:1 $POCl_3$ and Et_3N provided **14d** (Scheme 2.6).[15]



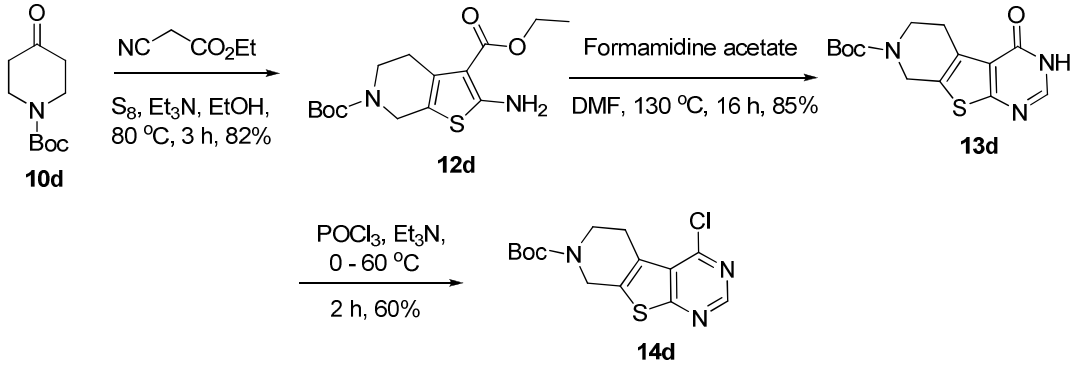
Scheme 2.4: Synthetic route for synthesis of key intermediates **18** and **20**.



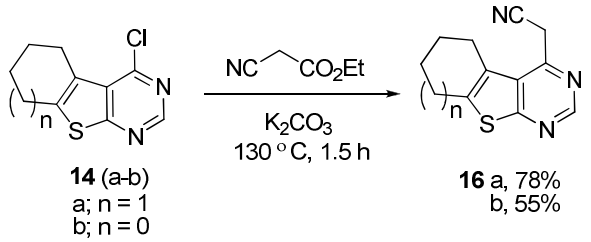
Scheme 2.5: Synthesis of 4-chloro thieno[2,3-*d*]pyrimidine from cyclo alkanones.

Then, the key step was the introduction of acetonitrile group. The reaction of ethyl cyanoacetate with chloro derivative **14a-b** followed by *in situ* decarboxylation of the resulting ester afforded the cyano derivative **16a-b** in one pot (Scheme 2.7). For the synthesis of **16c-d**, a two step protocol was used *i.e.,* the reaction with ethyl

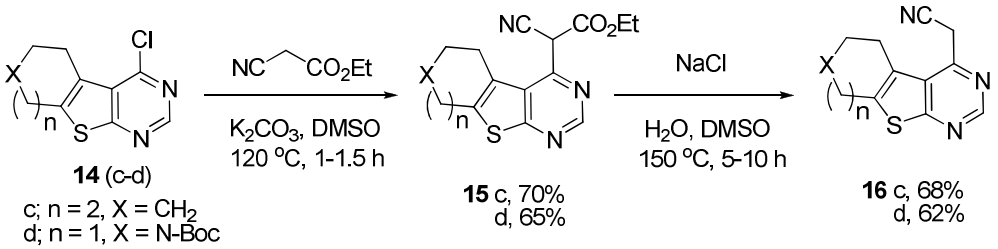
cyanoacetate followed by Krapcho decarboxylation using sodium chloride in DMSO-H_2O furnished the required compound (Scheme 2.8).



Scheme 2.6: Synthesis of 4-chloro tetrahydropyridothieno[2,3-*d*]pyrimidine from *N*-Boc-4-piperidone.

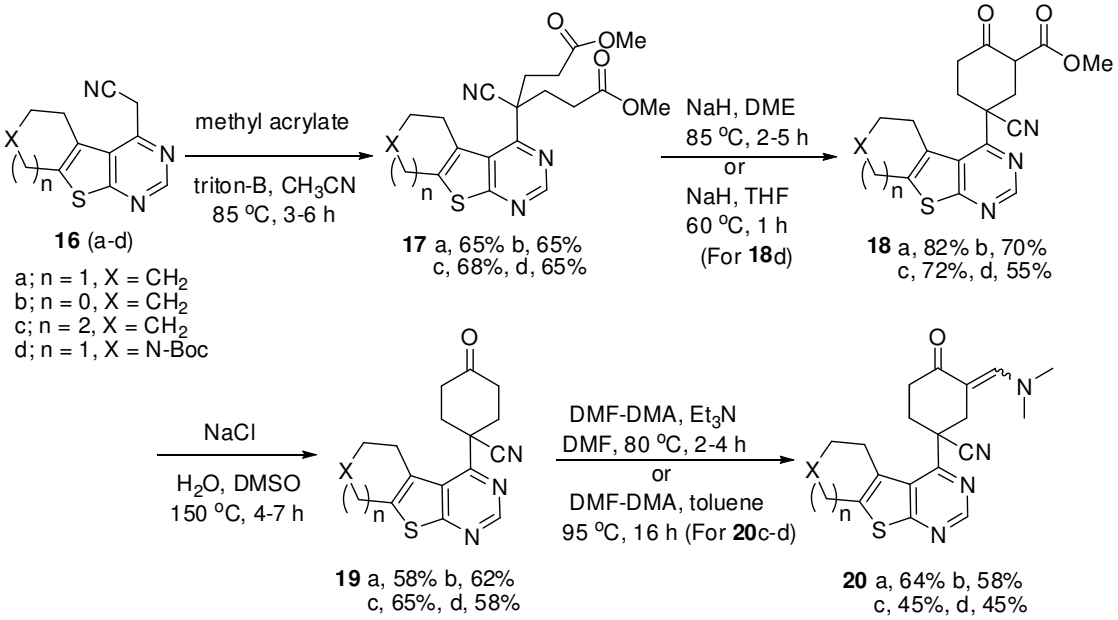


Scheme 2.7: Synthesis of 2-(thieno[2,3-*d*]pyrimidin-4-yl)acetonitrile in one pot.



Scheme 2.8: Synthesis of 2-(thieno[2,3-*d*]pyrimidin-4-yl)acetonitrile.

Double Michael addition of cyano compound **16** with methyl acrylate using triton-B as a base gave the diester **17**, which on Dieckmann condensation with sodium hydride resulted in beta keto ester **18**. Krapcho decarboxylation of compound **18** with sodium chloride in DMSO and H_2O provided the cyclohexanone derivative **19**. The reaction of *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) with the cyclohexanone derivative **19** furnished the required 2-((dimethylamino)methylene)cyclohexanone derivative **20**. The intermediates **18** and **20** were used for the preparation of target compounds (Scheme 2.9).



Scheme 2.9: Synthesis of key intermediates **18** and **20**.

All the intermediates synthesized were well characterized by spectral (NMR, MS and IR) data. Additionally, the molecular structure of the intermediate **18a** (methyl-5-cyano-5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-2-oxocyclohexane carboxylate) was established unambiguously by single crystal X-ray diffraction (Figure 2.4).

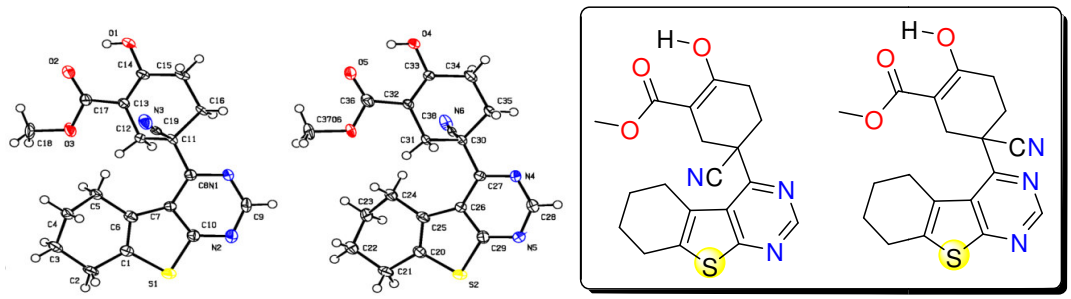
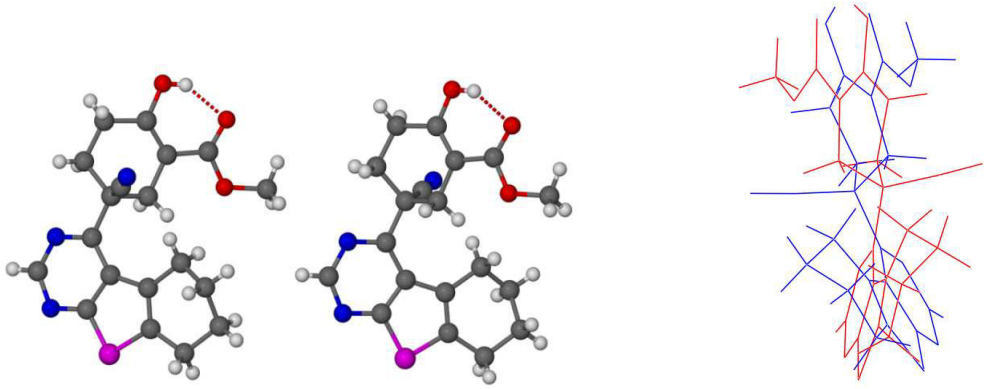


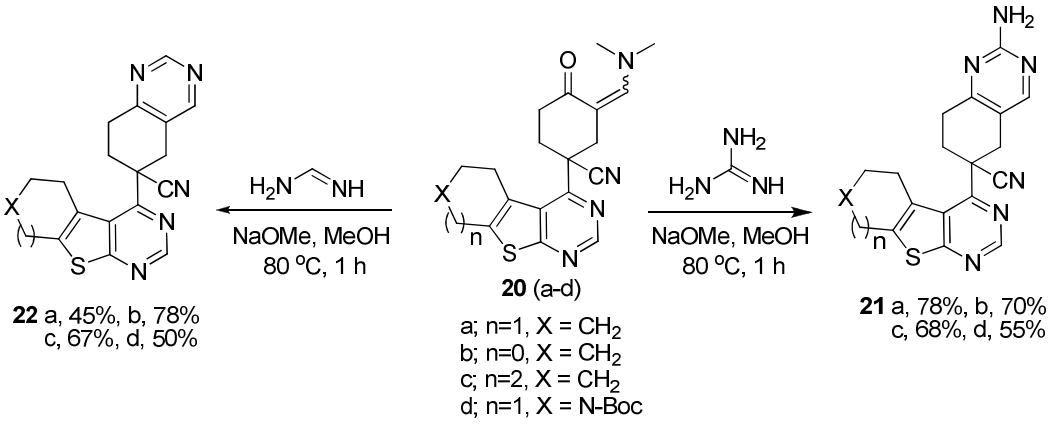
Fig. 2.4: ORTEP representation of the **18a** (Thermal ellipsoids are drawn at 50% probability level).

The X-ray diffraction study indicated that **18a** existed in enol tautomeric form predominantly stabilized by the 6-membered ring formed due to an intramolecular H-bond (Figure 2.5). The existence of enol form in solution was also supported by ^{1}H NMR data as the enolic hydroxyl group appeared at ~ 12.0-12.3 δ.



(a) **(b)**

Fig. 2.5: (a) showing the intra molecular hydrogen bonding present in the molecule **18a** *via* O-H···O synthon; (b) showing the conformations present in the asymmetric unit (i) conformer-**i** in blue colour, (ii) conformer-**ii** in red colour.



Scheme 2.10: Preparation of 5,6,7,8-tetrahydroquinazoline derivatives.

The reaction of **20** with guanidine hydrochloride afforded the 2-amino tetra hydro quinazoline ring **21** and formamidine acetate provided tetrahydroquinazoline ring **22** (Scheme 2.10). The molecular structure of a representative compound **21a** was established unambiguously by single crystal X-ray diffraction (Figure 2.6).

Compound **21a** crystallizes in the triclinic P-1 space group with one molecule and one solvent molecule in the asymmetric unit (Z = 6, Z' = 2) (Figure 2.6). The molecule in the asymmetric unit contains a pyrimidine ring substituted with a free amine and has capability to form supra molecular synthon *via* inter molecular hydrogen bonding (Figure 2.7).

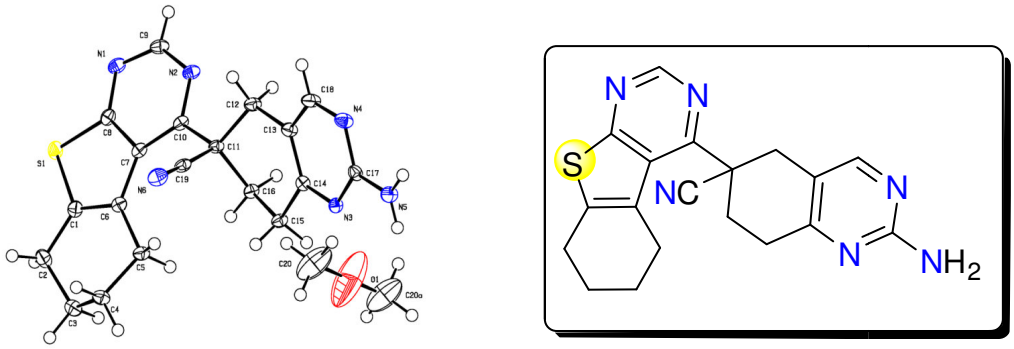


Fig. 2.6: ORTEP representation of the **21a** (thermal ellipsoids are drawn at 50% probability level).

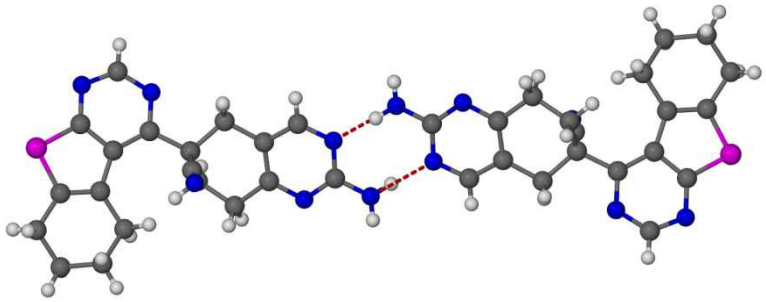
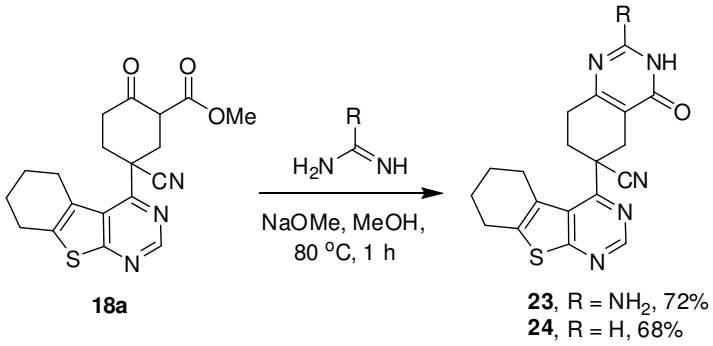


Fig. 2.7: Showing the intra molecular hydrogen bonding present in the molecule **21a** *via* N-H···N synthon.

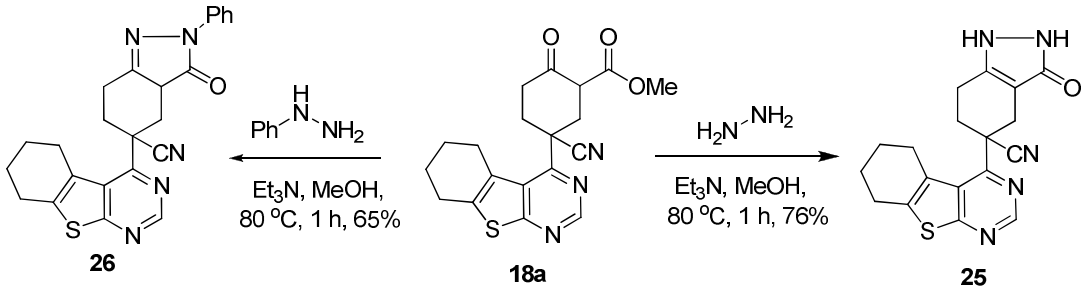
The reaction of **18a** with guanidine hydrochloride provided 2-amino hexahydroquinazolin-4-one derivative **23** whereas the reaction with formamidine acetate afforded hexahydroquinazolin-4-one derivative **24** (Scheme 2.11). The keto form of compound **23** and **24** was characterized by the IR absorption at 1650 and 1655 cm^{-1} respectively, due to the C=O moiety.



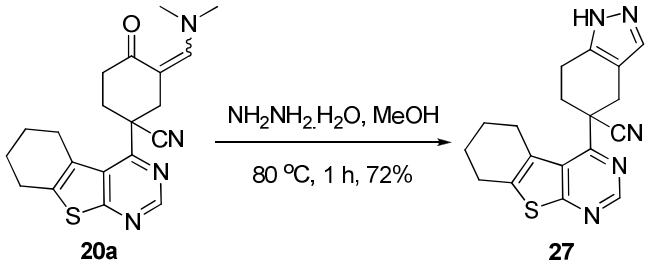
Scheme 2.11: Preparation of 4-oxo-3,4,5,6,7,8-hexahydroquinazoline derivatives.

Hydrazine hydrate reacted with compound **18a** provided 3-oxo hexahydro-1*H*-indazole derivative **25** whereas phenyl hydrazine afforded 2-phenyl hexahydro-2*H*-

indazole derivative **26** (Scheme 2.12). The structure of compound **25** was assigned based on two broad ^{1}H NMR signals at 11.3 and 9.6 δ due to the two NH groups and IR absorption at 1734 cm^{-1} due to the amide C=O moiety. The keto form of compound **26** was also indicated by IR absorption at 1730 cm^{-1} due to the C=O group and the absence of any NH IR absorption beyond 3000 cm^{-1}. The reaction of **20a** with hydrazine provided the tetra hydro indazole **27** (Scheme 2.13).



Scheme 2.12: Preparation of 3-oxo-2,3,4,5,6,7-hexahydroindazole derivatives.



Scheme 2.13: Preparation of 4,5,6,7-tetrahydro-1*H*-indazole derivative.

The stereo centre of the compounds **21-27** was generated during the conversion of intermediate **17** to **18** and **19** to **20**. This conversion was expected to afford a mixture of steroisomers as the methodology used was not an enantiospecific one. Thus, a chiral HPLC method was used to determine the enantiomeric purity of some representative compounds e.g. **21a**, **24**, **25** and **27**. All these compounds were found to be a ~1:1 mixture of both the antipodes. All the target compounds (**21–27**) synthesized were evaluated for their PDE4 inhibitory properties *in vitro*.

2.5. Pharmacology:

2.5.1. *In vitro* data

Many of these novel compounds synthesized were evaluated for their PDE4 inhibitory properties *in vitro*.[22] The compounds were tested at 30 μM and the results are summarized in Table 2.1.

Table 2.1: *In vitro* data of compounds of **21-27** for inhibition of PDE4B enzyme.

Compound No	%PDE4B inhibition (30μM)	IC_{50}
21a	80.0	4.48 ± 0.91
21b	66.0	nd
21c	70.4	5.64 ± 1.26
21d	78.9	3.24 ± 0.73
22a	80.0	4.51 ± 0.89
22b	65.5	nd
22c	74.8	6.19 ± 1.30
22d	71.0	5.01 ± 0.56
23	68.0	nd
24	70.7	nd
25	51.4	nd
26	41.9	nd
27	83.6	2.0 ± 0.41

A number of compounds showed significant inhibition (>70%) of PDE4. Change in ring size (**21a** *vs* **21b** and **21c**) or functionalization of the saturated cycloalkyl ring (**21a** *vs* **21d**) fused with the thiophene moiety or removal of NH_2 group from the pyrimidine ring (**21a** *vs* **22a**) did not show significant effect on inhibitory activities (Table 2.1).

The functionalization of the pyrimidine ring of 5,6,7,8-tetrahydroquinazoline-6-carbonitrile moiety with pyrimidone or 2-amino pyrimidone was tolerated (**22a** *vs* **23** and **24**). Replacement of 5,6,7,8-tetrahydroquinazoline-6-carbonitrile moiety by 3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole-5-carbonitrile group decreased the activity significantly (**22a** *vs* **25** and **26**). With the introduction of 4,5,6,7-tetrahydro-1*H*-

indazole-5-carbonitrile moiety restored the activity (**22a** *vs* **27**). Rolipram, a well known inhibitor of PDE4 was used as a reference compound in all these assays which showed 100% inhibition of PDE4B at 30 μM. Based on their initial PDE4B inhibitory properties, compounds **21a**, **21c, 21d**, **22a**, **22c**, **22d** and **27** were evaluated for dose dependent inhibitions (Figure 2.8) and the corresponding IC_{50} values are presented in Table 2.1. Few of these compounds were also tested their TNF-α inhibitory activity in lipopolysaccharide (LPS) stimulated RAW 264.7 cells,[22] values presented in Table 2.2.

Table 2.2: *In vitro* data of compounds of **10-16** for inhibition of TNF-α.

Compound No	TNF-α inhibition (30μM)
21a	66.0
21d	48.5
22a	35.7
22d	32.6
24	50.0
27	87.4

Among all the compounds, compound **27** appeared as a promising inhibitor tested its dose dependent inhibition of TNF-α.was also determined (Figure 2.9). Further the PDE4D inhibitory potential of compound **27** was evaluated using the PDE4D enzyme assay (Figure 2.9). Based on the PDE4B and PDE4D inhibitory data it is evident that compound **27** has 1.5 fold or balanced selectivity towards PDE4B. Notably potent inhibitor cilomilast (**D**, Figure 2.3) showed reverse selectivity *i.e.* ~10 fold selectivity towards PDE4D over PDE4B and induced emesis at the first and /or second doses though this effect apparently disappeared with continued treatment.[23]

Overall, with respect to the *in vitro* data (PDE4 & TNF-α) the compound **27** seemed to be comparable with phase 2 clinical candidate CC-1088 (PDE4 IC_{50} = 1.1 μM, TNF-α IC_{50} = 2.5 μM) of Celgene[24] and was identified as a PDE4 inhibitor of further interest.

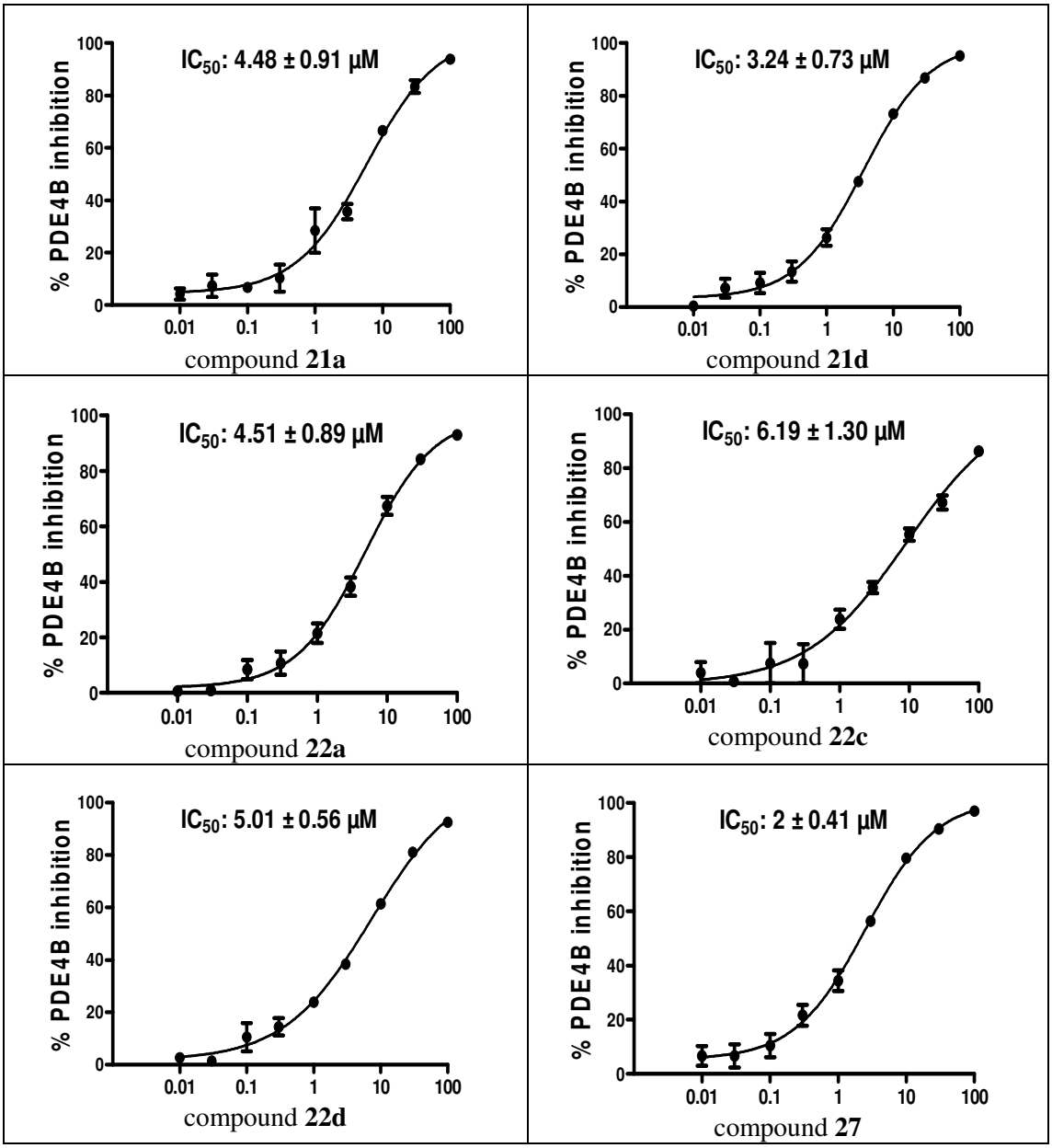


Fig. 2.8: Dose dependent inhibition of PDE4B by compounds **21a**, **21d**, **22a**, **22c**, **22d** and **27**.

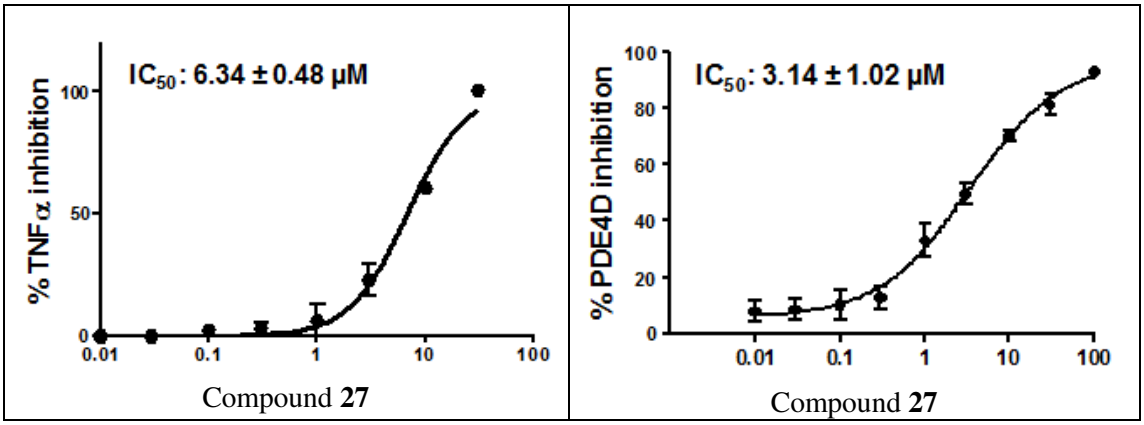


Fig. 2.9: Dose dependent inhibition of TNF-α and PDE4D by compound **27**.

2.5.2. Docking studies

To understand the nature of interactions of this class of molecules with PDE4B docking study was performed using compound **27** and PDB ID 3O0J.[25] This molecule was docked using Schrodinger 2011 software and results are summarized in Table 2.3. The compound **27** can exists in two tautomeric forms and hence both the forms e.g. **27(A)** and **27(B)** were considered for docking studies.

Table 2.3: Docking scores and other parameters of compounds after docking with PDE4B

Compound	Dock Score	ligand efficiency (ln)	XP PhobEn	XP LowMW	XP Lipophilic EvdW	XP Electro
(*R*)-**27(A)**	-6.09	-1.45	-0.65	-0.38	-2.78	-1.98
(*R*)-**27(B)**	-6.76	-1.61	-1.63	-0.38	-3.96	-0.49
(*S*)-**27(A)**	-7.18	-1.71	-0.97	-0.38	-3.23	-0.63
(*S*)-**27(B)**	-6.08	-1.45	-0.67	-0.38	-3.16	-1.19

ligand efficiency (ln) = GlideScore/[1 + ln(number of heavy atoms)]

XP PhobEn = Hydrophobic enclosure reward hydrophobic atoms on the protein that enclose hydrophobic groups on the ligand

XP LowMW = Reward for low molecular weight

XP LipophilicEvdW = Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy, XP Electro = electrostatic reward

Table 2.4: The binding energy of compounds after docking with PDE4B.

Compound	E.Complex (Kcal/mol)	E.Receptor (Kcal/mol)	E.Ligand in Complex (Kcal/mol)	dG.binding, (Kcal/mol)
(*R*)-**27(A)**	-15503.10	-15424.16	-8.77	-70.16
(*R*)-**27(B)**	-15526.80	-15424.16	-12.18	-90.45
(*S*)-**27(A)**	-15506.01	-15424.16	-8.07	-73.78
(*S*)-**27(B)**	-15491.86	-15424.16	-9.24	-58.45

The docking results of compound (*R*)-**27(A)** showed hydrogen bond between NH of pyarazole and backbone of threonine 345 (Figure 2.10) whereas that of (*R*)-**27(B)** showed (i) π-π stacking interaction between pyrimidine and thiophene rings and phenylalanine 414 and 446 (ii) hydrogen bonding between NH of pyrazole and aspartate 392 (Figure 2.11). As evident from the dock score that the tautomeric form (*R*)-**27(B)** of compound (*R*)-**27** showed better binding with PDE4B compared to its other form (*R*)-**27(A)**. The binding energy of (*R*)-**27(B)** was also found to be higher than (*R*)-**27(A)** (Table 2.4).

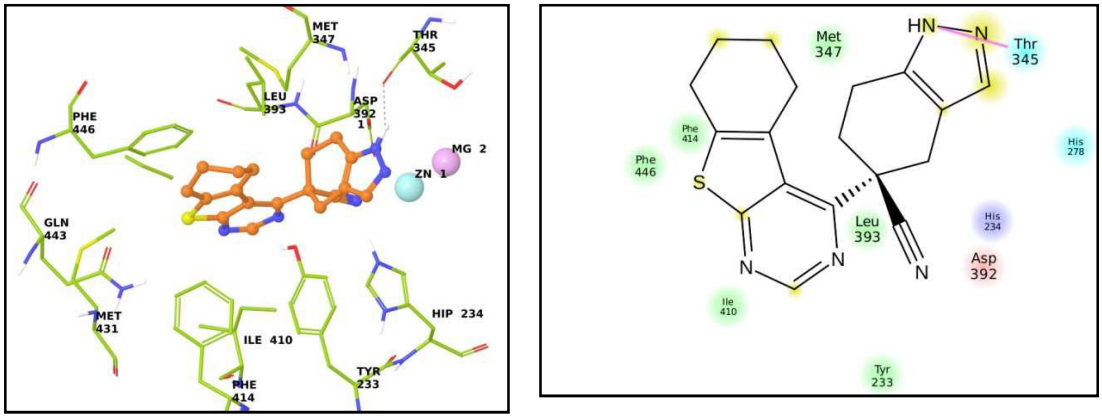


Fig. 2.10: Docking of tautomeric form (*R*)-**27(A)** at the active site of PDE4B.

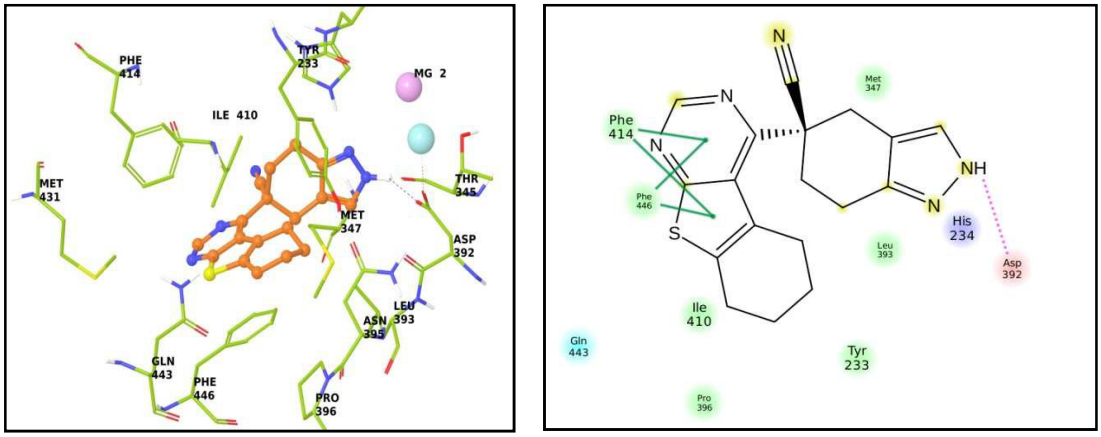


Fig. 2.11: Docking of tautomeric form (*R*)-**27(B)** at the active site of PDE4B.

The docking results of compound (*S*)-**27(A)** showed (i) Hydrogen bond between Pyrimidine nitrogen and glutamine 443; (ii) Hydrogen bond between Pyrazole ring nitrogen with threonine 345 (Figure 2.12) whereas that of (*S*)-**27(B)** showed (i) Hydrogen bond between pyrazole and threonine 345; (ii) Pi-Pi stacking interaction between pyrazole ring and histidine 234; (iii) Pi-Pi stacking interaction between pyrimidine ring and tyrosine 233 (Figure 2.13). Both tautomeric forms showed Pi-Pi

stacking interaction of thiophene ring with phenylalanine 414 and 446. Two tautomers could be generated for the compound (*S*)-**27** out of which tautomer (*S*)-**27(A)** showed slightly more score than that of (*S*)-**27(B)** due to more contribution by hydrophobic enclosure (Table 2.3).

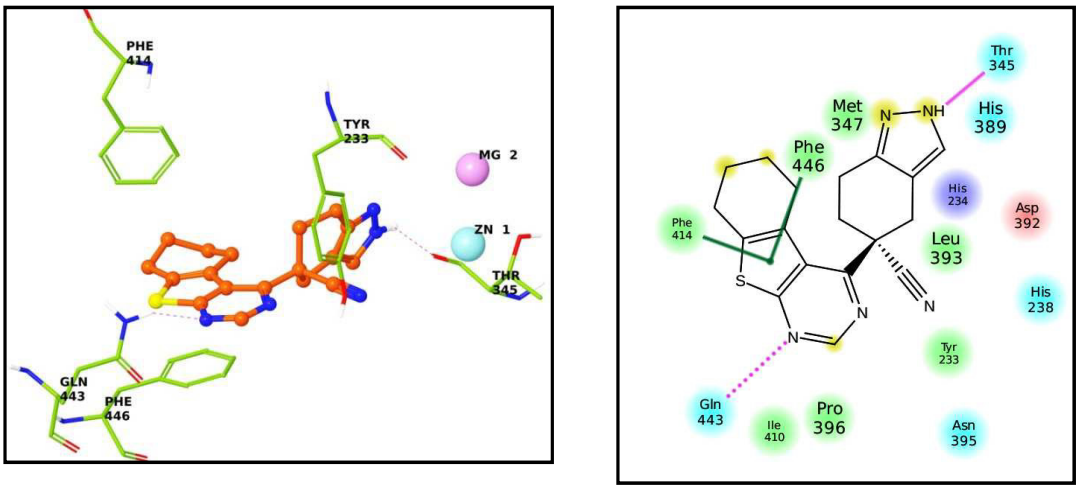


Fig. 2.12: Docking of tautomeric form (*S*)-**27(A)** at the active site of PDE4B.

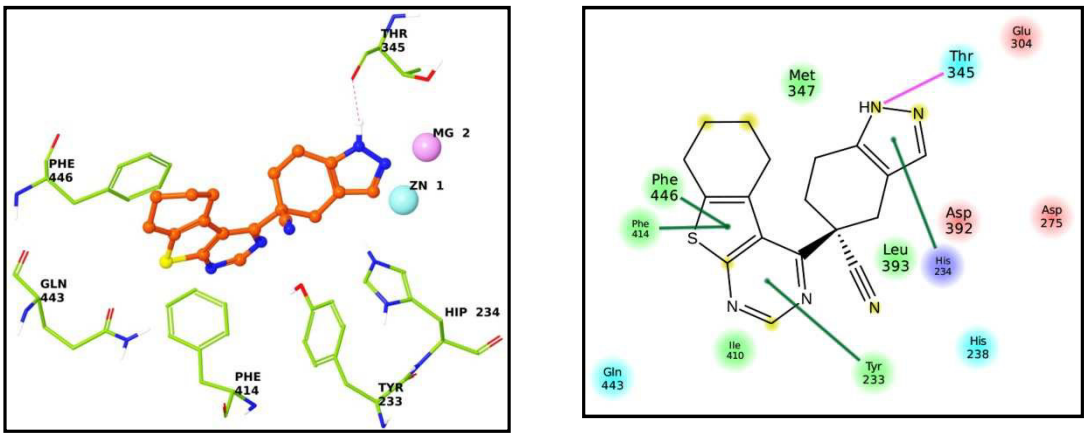


Fig. 2.13: Docking of tautomeric form (*S*)-**27(B)** at the active site of PDE4B.

When compared to R isomers, S isomers of compound **27(A)** showed better scores. Nevertheless, docking results of active molecule (including tautomers) showed good overall correlations to their PDE4B inhibitory properties *in vitro*.

2.6. Conclusion:

The thieno[2,3-*d*]pyrimidine based library of small molecules containing cyclohexane ring fused with a six- or five-membered heterocyclic moiety along with a benzylic nitrile was designed as potential inhibitors of PDE4. These molecules were prepared conveniently *via* a multi-step sequence consisting of few key steps such as Gewald

reaction, Dieckmann type cyclization and Krapcho decarboxylation. A number of thieno[2,3-*d*]pyrimidine based derivatives were synthesized and the molecular structure of a representative compound was established unambiguously by single crystal X-ray diffraction. The crystal structure analysis of this compound provided an insight on the hydrogen bonding patterns and molecular arrangement present within the molecule. Many of these compounds were evaluated for their PDE4B inhibitory potential *in vitro*.

Some of these compounds showed promising inhibition of PDE4B initially at a single dose and then subsequently in a dose dependent manner. One of them *i.e.* 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4,5,6,7-tetrahydro-1*H*-indazole-5-carbonitrile was tested for PDE4D inhibition *in vitro* and dose dependent inhibition of TNF-α. The docking results of active molecule showed good overall correlations to observed PDE4B inhibitory properties *in vitro*. Overall, the strategy involving the sequential construction of thieno pyrimidine ring followed by the cyclohexanone moiety and subsequently the fused heterocyclic ring provided a new framework that appeared to be a promising template for the discovery of novel inhibitors of PDE4.

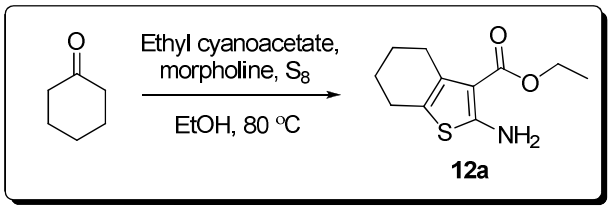
2.7. Experimental section

2.7.1. 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, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were recorded 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), t (triplet) q (quartet) and m (multiplet) as well as bs (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. 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 time. The enantiomeric purity of some representative compounds e.g. **21a**, **24**, **25** and **27** was determined by using a chiral HPLC method.

2.7.1.1. Typical procedure for the synthesis of ethyl 2-amino-4,5,6,7tetrahydro benzo[*b*]thiophene-3-carboxylate (12a)

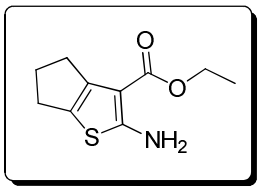


12a

A mixture of cyclo hexanone (5.3 mL, 50 mmol), ethyl cyanoacetate (5.7 mL, 50 mmol), morpholine (4.5 mL, 50 mmol), and sulphur (1.6 g, 50 mmol) in ethanol (50 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 to give the desired product **12a** (9.1 g**,** 73%) as a brown solid.

mp: 114-116 °C (lit[26] 115-117 °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).

2.7.1.2. Ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (12b)

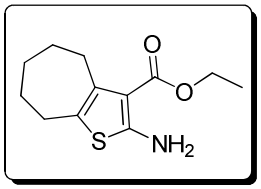


The compound **12b** was synthesized in 78% yield from cyclo pentanone following a procedure similar to that of compound **12a**. Purification done by column chromatography using ethyl acetate-hexane.

Brown solid; mp: 180-182 °C (lit[26] 182-184 °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).

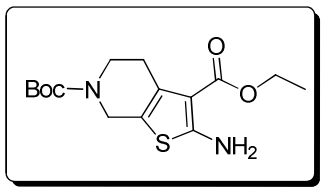
2.7.1.3. Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (12c)



The compound **12c** was synthesized in 69% yield from cyclo heptanone following a procedure similar to that of compound **12a**. Purification done by column chromatography using ethyl acetate-hexane.

Light yellow solid; mp: 87-89 °C (lit[26] 89-90 °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).

2.7.1.4. 6-*tert*-Butyl 3-ethyl 2-amino-4, 5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (12d)

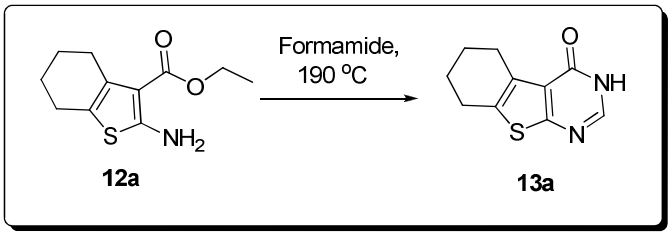


The compound **12d** was synthesized in 82% yield from *N*-Boc-4-piperidone and Et_3N as a base following a procedure similar to that of compound **12a**.

Light yellow solid; mp: 156-158 °C (lit[26] 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).

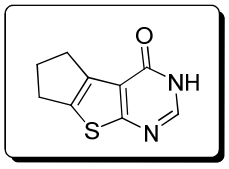
2.7.1.5. Typical procedure for preparation of 5,6,7,8-tetrahydrobenzo[*b*]thieno [2,3-*d*]pyrimidin-4-one (13a)

A mixture of compound **12a** (8 g, 35.5 mmol) and formamide (16 mL) was heated to 190 °C for 2 h and cooled to room temperature. The mixture was then poured into crushed ice. The precipitate appeared was filtered off, washed with cold water and dried to give the desired product **13a** (5.3 g**,** 73%) as a brown solid.



mp: 244-246 °C (lit[13] 245-247 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (s, 1H), 2.99-3.02 (m, 2H), 2.76-2.79 (m, 2H),1.83-2.02 (m, 4H).

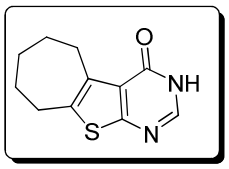
2.7.1.6. 6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (13b)



The compound **13b** was synthesized in 69% yield from compound **12b** following a procedure similar to that of compound **12a**.

Light yellow solid; mp 262-264 °C (lit[13] 258-260 °C); ^{1}H NMR (300 MHz, CD_3OD): δ 7.80 (s, 1H), 2.93 (t, *J* = 5.6 Hz, 2H), 2.71 (t, *J* = 6.0 Hz, 2H), 1.83 - 1.77 (m, 2H).

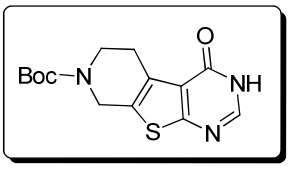
2.7.1.7. 6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (13c)



The compound **13c** was synthesized in 70% yield from compound **12c** following a procedure similar to that of compound **13a**.

Brown solid; mp: 252-254 °C; ^{1}H NMR (400 MHz, CDCl3): 12.21 (s, 1H), 7.96 (s, 1H), 3.29-3.18 (m, 2H), 2.83-2.77 (m, 2H), 1.89-1.77 (m, 2H), 1.63-1.46 (m, 4H).

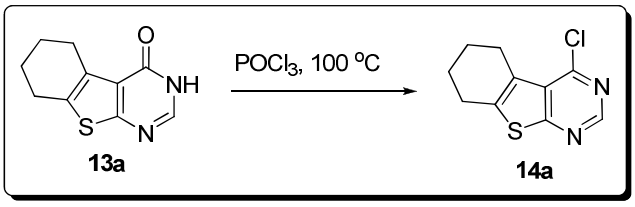
2.7.1.8. Typical procedure for preparation of *tert*-butyl-4-oxo-3,4,5,6,7,8-hexahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7-carboxylate (13d)



A mixture of **12d** (3 g, 9.2 mmol) and formamidine acetate (1.43 g, 13.8 mmol) in DMF (30 mL) were heated at 100 °C for 16 h. The reaction mixture was cooled, DMF removed under vacuum, and the solid obtained was washed thoroughly with water to give the desired product **13d** (2.4 g, 85%) as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.33 (bs, 1NH), 7.99 (s, 1H), 4.66 (s, 2H), 3.70-3.76 (bs, 2H), 3.10-3.16 (bs, 2H), 1.50 (s, 9H); MS (ES mass): m/z 308.1 (M+1).

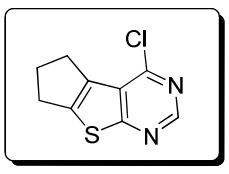
2.7.1.9. Typical procedure for preparation of 4-chloro-5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidine (14a)



A mixture of compound **13a** (5 g, 24.2 mmol), $POCl_3$ (15.0 mL) was refluxed at 100 °C for 1 hour. Then, the reaction mixture was cooled to room temperature, poured into crushed ice, neutralized (pH 7.0) with $NaHCO_3$ and extracted with ethyl acetate (2 x 50 mL). The organic layers were collected, combined, washed with brine solution (40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:6) to give the desired product **14a** (3.8 g, 70%) as a white solid.

mp: 107-109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71 (s, 1H), 3.11 (t, *J* = 4.2 Hz, 2H), 2.90 (t, *J* = 4.2 Hz, 2H), 1.95-1.92 (m, 4H).

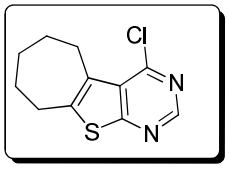
2.7.1.10. 4-chloro-6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidine (14b)



The compound **14b** was synthesized in 65% yield from compound **13b** following a procedure similar to that of compound **14a**.

Light yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, 1H), 3.11 (t, *J* = 4.0, 2H), 2.89 (t, *J* = 4.4 Hz, 2H), 1.97-1.90 (m, 2H).

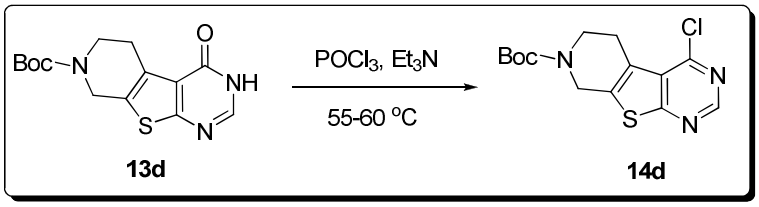
2.7.1.11. 4-chloro-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidine (14c)



The compound **14c** was synthesized in 68% yield from compound **13c** following a procedure similar to that of compound **14a**.

White solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 3.30-3.21 (m, 2H), 2.86-2.77 (m, 2H), 1.89-1.75 (m, 2H), 1.65-1.49 (m, 4H).

2.7.1.12. Typical procedure for preparation of *tert*-butyl-4-chloro-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7-carboxylate (14d)



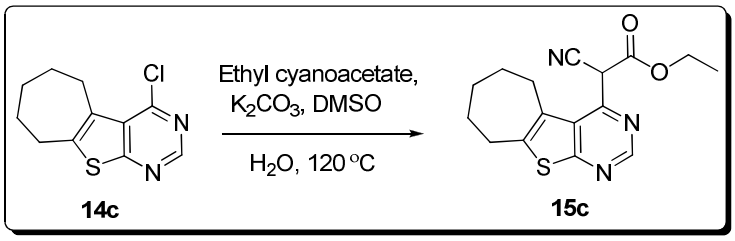
To a mixture of $POCl_3$ (5 mL) and triethylamine (5 mL) at 0 °C was added **13d** (2 g, 6.5 mmol). The reaction mixture was heated at 55-60 °C for 3 h, then the reaction mixture was cooled to rt, excess of $POCl_3$ was co evaporated with toluene (2 x 30 mL) under vacuum, and the remaining residue neutralized carefully by adding saturated aqueous sodium bicarbonate solution. The resulting mixture was extracted with dichloromethane (3 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:5) to give the desired product **14d** (1.3 g**,** 60%) as a light yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.77 (s, 1H), 4.75 (s, 2H), 3.79 (t, *J* = 5.2Hz, 2H), 3.20-3.21 (bs, 2H), 1.51 (s, 9H); MS (ES mass): m/z 326.0 (M+1).

2.7.1.13. Typical procedure for preparation of ethyl-2-cyano-2-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)acetate (15c)

A mixture of **14c** (1 g, 0.42 mmol), ethyl cyanoacetate (0.04 mL, 0.42 mmol) and K_2CO_3 (86 mg, 0.63 mmol) in DMSO (10 mL) and water (1 mL) was heated at 120 °C for 1.5 h under anhydrous conditions. After completion of the reaction the

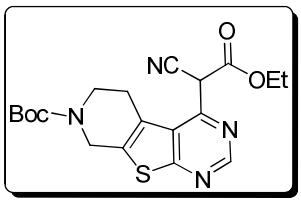
mixture was cooled to room temp, diluted with water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:6) to give the desired product **15c** (0.9 g, 70%) as a white solid.



14c **15c**

mp: 139-141°C; R_f = 0.5 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2927, 2856, 2200, 1656; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.83 (bs, 1H), 8.41 (s, 1H), 4.23 (q, *J* = 4.5 Hz, 2H), 3.05-3.02 (m, 2H), 2.95-2.92 (m, 2H), 1.88-1.83 (m, 2H), 1.68-1.54 (m, 4H), 1.28 (t, *J* = 4.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.5, 162.1, 152.8, 141.9, 139.1, 136.7, 121.9, 119.4, 66.7, 61.3, 32.0, 30.6, 27.9, 27.0, 14.4, 14.3; MS (ES mass): 315.5 (M+1).

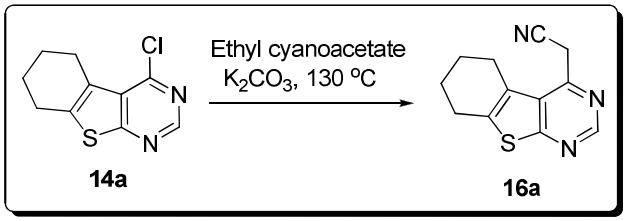
2.7.1.14. Ethyl-2-cyano-2-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)acetate (15d)



The compound **15d** was synthesized in 65% yield from **14d** following a procedure similar to that of compound **15c**.

Light brown solid; mp: 128-130 °C; R_f = 0.3 (30% EtOAc/n-hexane); IR (KBr, cm^{-1}): 2975, 2202, 1710, 1661; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 14.75 (s, 1H), 8.08 (d, *J* = 3.2 Hz, 1H), 4.72 (s, 2H), 4.33 (q, *J* = 7.2 Hz, 2H), 3.60-3.61 (bs, 2H), 3.29-3.31 (bs, 2H), 1.51 (s, 9H), 1.39 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.3, 170.7, 152.5 (3C), 140.1 (2C), 134.0, 120.9, 80.6, 61.6, 43.4, 41.9, 31.0, 28.4 (3C), 25.9, 14.3; MS (ES mass): 401.1 (M+1).

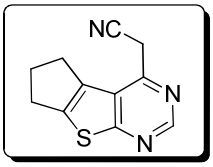
2.7.1.15. Typical procedure for preparation of 2-(5,6,7,8-tetrahydrobenzo[*b*] thieno[2,3-*d*]pyrimidin-4-yl)acetonitrile (16a)



To a mixture of ethyl cyanoacetate (4.2 mL, 40.05 mmol) and K_2CO_3 (3.7 g, 26.70 mmol) was added the compound **14a** (3 g, 13.35 mmol). The mixture was initially heated to 60 °C for 30 minutes and then at 130 °C for 1 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temperature and diluted with EtOAc (60 mL). The organic layer was collected, washed with water (2 x 30 mL) followed by brine solution (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:6) to give the desired product **16a** (2.4 g**,** 78%) as a white solid.

mp 164-166 °C; R_f = 0.45 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2931, 2853, 2256, 1539; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 4.27 (s, 2H), 2.99-2.92 (m, 4H), 1.97-1.96 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.8, 152.0, 151.0, 140.1, 129.0, 125.5, 115.7, 26.3, 26.0, 25.9, 22.4, 22.3; MS (ES mass): m/z 229.9 (M+1); HPLC: 99.3%, column: ZORBAX XDB C-18 150 x 4.6 mm5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.34 min.

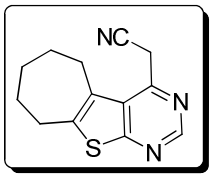
2.7.1.16. 2-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)aceto nitrile (16b)



The compound **16b** was synthesized in 55% yield from **14b** following a procedure similar to that of compound **16a**.

White solid; mp: 205-207 °C; R_f = 0.6 (30% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2911, 2861, 2261, 1552; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 4.18 (s, 2H), 3.18-3.09 (m, 4H), 2.64-2.56 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.2, 152.1, 150.7, 145.6, 134.6, 125.9, 115.5, 30.0, 29.4, 27.6, 24.9; MS (ES mass): m/z 216.1 (M+1).

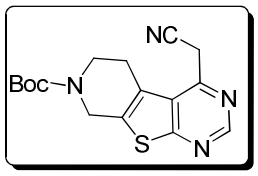
2.7.1.17. Typical procedure for preparation of 2-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)acetonitrile (16c)



A mixture of compound **15c** (1 g, 0.32 mmol) and NaCl (1.47 g, 2.53 mmol) in DMSO (10 mL) and water (1 mL) was heated at 150 °C for 4.5 h under anhydrous conditions. After completion of the reaction the mixture was cooled to room temp, diluted with water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:6) to give desired product **16c** (524 mg, 68%) as a white solid.

mp: 133-135 °C; R_f = 0.5 (10% EtOAc/DCM); IR (KBr, cm^{-1}): 2925, 2853, 2254, 1533;^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.93 (s, 1H), 4.31 (s, 2H), 3.10-3.08 (m, 2H), 3.01-2.99 (m, 2H), 2.00-1.99 (m, 2H), 1.85-1.78 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.6, 151.5, 150.9, 144.1, 131.0, 129.7, 115.6, 31.4, 29.9, 29.3, 26.9, 26.8, 26.5; MS (ES mass): 243.5 (M+1).

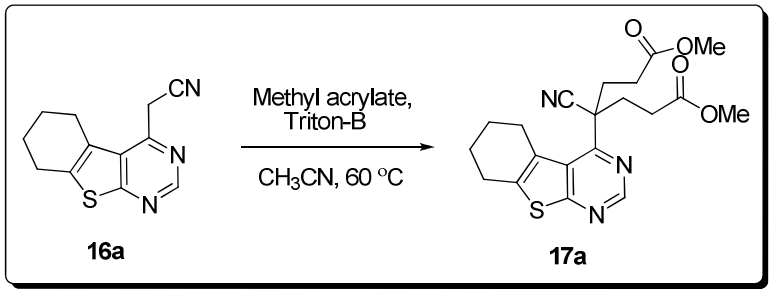
2.7.1.18. *tert*-Butyl-4-acetonitrilo-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidine-7-carboxylate (16d)



Compound **16d** was synthesized in 62% yield from **15d** following a procedure similar to that of compound **16c**.

White solid; mp: 161-163°C; R_f = 0.4 (40% EtOAc/ n-hexane); IR (KBr, cm^{-1}): 2976, 2257, 2190, 1683; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.99 (s, 1H), 4.77 (s, 2H), 4.26 (s, 2H), 3.84 (t, *J* = 5.6 Hz, 2H), 3.08-3.09 (bs, 2H), 1.51 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ**:** 169.0, 154.2, 152.6 (3C), 128.3 (2C), 115.4, 80.9, 43.9, 40.0, 28.4 (3C), 25.9 (2C); MS (ES mass): 331.1 (M+1).

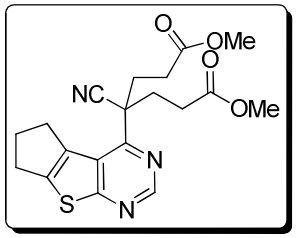
2.7.1.19. Typical procedure for preparation of 4-cyano-4-(5,6,7,8-tetrahydro benzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-heptane dioic acid dimethylester (17a)



To a solution of **16a** (2 g, 8.72 mmol) in acetonitrile (12.5 mL) was added 40% solution of triton-B (1mL) and the mixture was heated to reflux under anhydrous conditions. To this was added methyl acrylate (7.9 mL, 87.22 mmol) in acetonitrile (12.5 mL) under refluxing condition. The mixture was refluxed for 3 h. After completion of the reaction the mixture was cooled to room temperature and solvent as well as excess of methyl acrylate was evaporated under reduced pressure. The residue was dissolved in EtOAc (40 mL). The organic layer was washed with water (2 x 20 mL) followed by brine solution (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate-hexane (1:8) to give desired product **17a** (2.3 g**,** 65%) as a light yellow liquid.

R_f = 0.5 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2946, 2861, 2236, 1738; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 3.67 (s, 6H), 3.22 (bs, 2H), 2.98 (bs, 2H), 2.86-2.79 (m, 2H), 2.69-2.61 (m, 2H), 2.53-2.45 (m, 2H), 2.37-2.29 (m, 2H), 1.96 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.4 (2C), 170.2, 156.4, 150.2, 140.1, 129.3, 126.3, 121.7, 51.9 (2C), 32.9 (2C), 29.8 (3C), 29.6, 26.6, 23.2, 22.2; MS (ES mass): 401.9 (M+1).

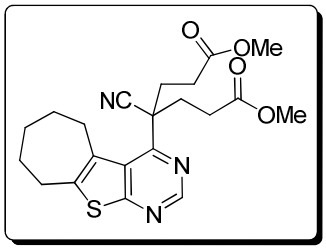
2.7.1.20. 4-Cyano-4-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-heptane dioic acid dimethyl ester (17b)



Compound **17b** was synthesized in 65% yield from **16b** following a procedure similar to that of compound **17a**.

Light yellow liquid; R_f = 0.7 (30% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2954, 2859, 2240, 1735; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 3.65 (s, 6H), 3.39-3.35 (m, 2H), 3.10 (t, *J* = 7.2 Hz, 2H), 2.81-2.74 (m, 2H), 2.62-2.45 (m, 6H), 2.41-2.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.3, 172.4 (2C), 156.3, 150.3, 146.4, 134.6, 125.7, 121.3, 51.9 (2C), 47.3, 33.5, 32.5 (2C), 30.1, 29.8 (2C), 28.1; MS (ES mass): 387.5 (M+1).

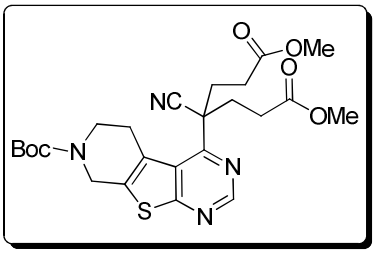
2.7.1.21. 4-Cyano-4-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*] pyrimidin-4-yl)-heptane dioic acid dimethyl ester (17c)



Compound **17c** was synthesized in 68% yield from **16c** following a procedure similar to that of compound **17a**.

Light yellow liquid; R_f = 0.55 (10% EtOAc/DCM); IR (KBr, cm^{-1}): 2929, 2853, 2233, 1738; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 3.68 (s, 6H), 3.32-3.29 (m, 2H), 3.03-3.00 (m, 2H), 2.88-2.80 (m, 2H), 2.73-2.64 (m, 2H), 2.60-2.48 (m, 2H), 2.39-2.32 (m, 2H), 2.03-1.95 (m, 2H), 1.78-1.72 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.5 (2C), 168.9, 156.3, 149.9, 144.8, 132.1, 129.1, 120.8, 51.9 (2C), 32.4 (2C), 32.3, 31.0, 30.7, 29.8 (3C), 26.8, 26.7; MS (ES mass): 415.5 (M+1).

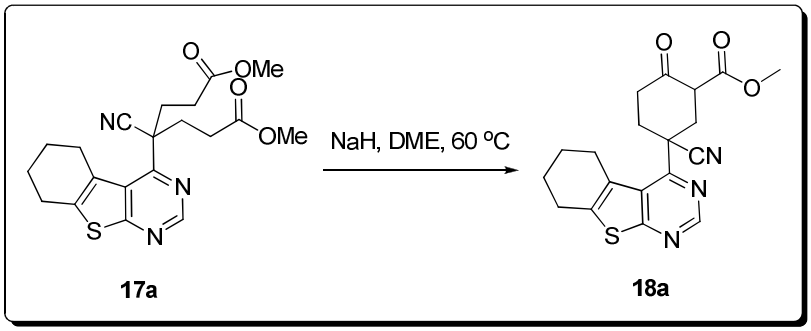
2.7.1.22. 4-Cyano-4-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)-heptane dioic acid dimethyl ester (17d)



Compound **17d** was synthesized in 65% yield from **16d** following a procedure similar to that of compound **17a**.

White solid; mp: 133-135 °C; R_f = 0.5 (35% EtOAc/ n-hexane); IR (KBr, cm^{-1}): 2976, 2236, 1736, 1686; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 4.79 (s, 2H), 3.80-3.78 (bs, 2H), 3.67 (s, 6H), 3.35-3.33 (bs, 2H), 2.85-2.78 (m, 2H), 2.69-2.62 (m, 2H), 2.54-2.46 (m, 2H), 2.38-2.31 (m, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.3 (2C), 170.4, 152.9, 150.7 (3C), 128.4 (2C), 121.5, 80.8, 51.9 (2C), 46.4, 45.1, 32.7 (2C), 32.3, 29.7 (2C), 28.4 (3C), 28.0. MS (ES mass): 503.2 (M+1).

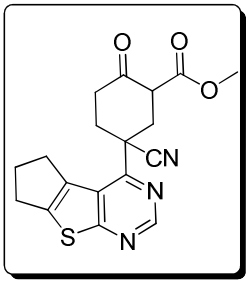
2.7.1.23. Typical procedure for preparation of methyl-5-cyano-5-(5,6,7,8-tetra hydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxo cyclohexane carboxylate (18a)



A cold solution of compound **17a** (2 g, 4.98 mmol) in dry DME (15 mL) was added slowly to a mixture of 60% NaH (359 mg, 14.96 mmol) in dry DME (15 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was heated at 60-70 °C for 2.5 h. After completion of the reaction, the mixture was quenched with ice cold 1N hydrochloric acid (20 mL) and extracted with ethyl acetate (2 x 30 mL). The organic layers were collected, combined, washed with water (2 x 30 mL) followed by brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate-hexane (1:9) to give the desired product **18a** (1.5 g, 82%) as a white solid.

mp: 171-173 °C; R_f = 0.6 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3267, 2951, 2230, 1657; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.25 (s, OH), 8.89 (s, 1H), 3.81 (s, 3H), 3.30-3.26 (m, 3H), 3.05-3.00 (m, 3H), 2.93-2.85 (m, 1H), 2.63-2.58 (m, 2H), 2.44-2.37 (m, 1H), 1.98 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 170.6, 169.7, 157.7, 150.4, 140.0, 129.1, 126.2, 121.7, 94.5, 51.8, 42.2, 32.8, 31.3, 29.2, 26.8, 26.6, 23.2, 22.3; MS (ES mass): 369.9 (M+1); HPLC: 98.9%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/80, 2/80, 9/98, 12/98, 15/80, 18/80; flow rate: 1.0 mL/min; UV 245 nm, retention time 4.37 min.

2.7.1.24. Methyl-5-cyano-5-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*] pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (18b)

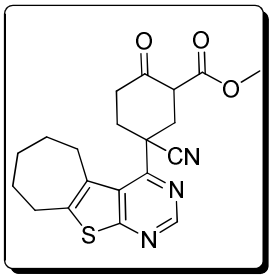


Compound **18b** was synthesized in 70% yield from **17b** following a procedure similar to that of compound **18a**.

White solid; mp: 153-155 °C; R_f = 0.8 (30% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3535, 2955, 2235, 1656; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.26 (bs, 1H), 8.90 (s, 1H), 3.81 (s, 3H), 3.41-3.36 (m, 2H), 3.25 (d, *J* = 16.0 Hz, 1H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.97 (d, *J* = 16.5 Hz, 1H), 2.92-2.84 (m, 1H), 2.64-2.53 (m, 4H), 2.44-2.36 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.9, 171.8, 170.6, 157.4, 150.6, 146.2, 134.8, 125.8, 121.6, 94.4, 51.8, 41.9, 33.2, 32.3, 30.4, 30.1, 28.2, 26.6; MS (ES mass): 355.4 (M+1).

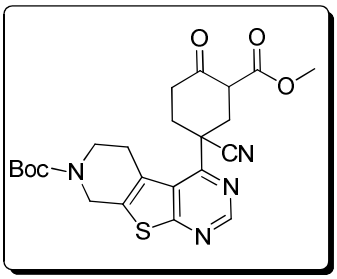
2.7.1.25. Methyl-5-cyano-5-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (18c)

Compound **18c** was synthesized in 72% yield from **17c** following a procedure similar to that of compound **18a**.



White solid; mp: 166-168 °C; R_f = 0.7 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3482, 2931, 2232, 1656; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.26 (bs, 1H), 8.90 (s, 1H), 3.80 (s, 3H), 3.39-3.26 (m, 3H), 3.04-2.98 (m, 3H), 2.94-2.85 (m, 1H), 2.66-2.60 (m, 2H), 2.46-2.38 (m, 1H), 2.01-1.98 (m, 2H), 1.78-1.70 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 170.7, 168.5, 157.6, 150.3, 144.7, 132.1, 128.9, 121.0, 94.5, 51.8, 42.0, 32.5, 32.4, 30.9(2C), 30.6, 26.9, 26.8, 26.7; MS (ES mass): 384.2 (M+1).

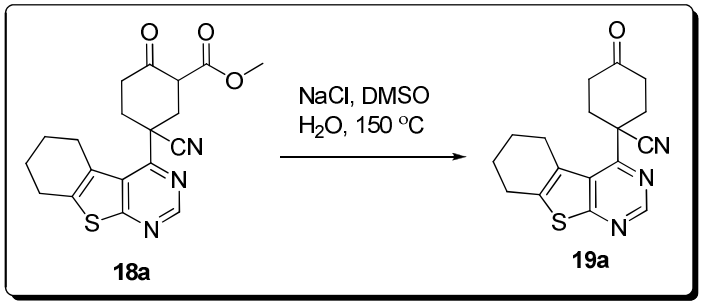
2.7.1.26. Methyl-5-cyano-5-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido [4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (18d)



Compound **18d** was synthesized in 55% yield from **17d** using dry THF as a solvent following a procedure similar to that of compound **18a**.

White solid; mp: 189-191°C; R_f = 0.5 (40% EtOAc/ n-hexane); IR (KBr, cm^{-1}): 2975, 2232, 1727, 1695, 1664 ; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.25 (bs, 1OH), 8.94 (s, 1H), 4.81 (s, 2H), 3.81 (s, 5H), 3.42-3.35 (m, 2H), 3.26 (d, *J* = 16.0 Hz, 1H), 2.98 (d, *J* = 16.0 Hz, 1H), 2.92-2.85 (m, 1H), 2.65-2.58 (m, 2H), 2.47-2.39 (m, 1H), 1.52 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.7, 170.6, 169.9, 158.3, 154.2, 151.0 (2C), 128.2 (2C), 121.5, 94.3, 80.7, 51.9, 42.1 (2C), 37.0, 32.7, 30.9, 29.6, 28.4 (3C), 26.7. MS (ES mass): 471.2 (M+1). HPLC: 95.7%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 5 mM Ammonium Acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/95, 13/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.29 min.

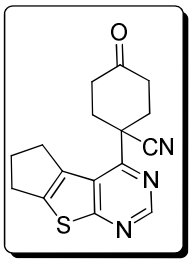
2.7.1.27. Typical procedure for preparation of 1-(5,6,7,8-tetrahydro benzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (19a)



A mixture of **18a** (0.5 g, 1.35 mmol) and NaCl (628 mg, 10.84 mmol) in DMSO (5 mL) and water (0.5 mL) was heated at 150 °C for 5 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temp, diluted with water (25 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were collected, combined, washed with brine solution (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The isolated residue was purified by column chromatography using ethyl acetate-hexane (1:6) to give desired product **19a** (240 mg, 58%) as a white solid.

mp: 167-169 °C; R_f = 0.5 (30% EtOAc/Hexane); IR (KBr, cm^{-1}): 2947, 2883, 2233, 1713; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s,1H), 3.28(m, 2H), 3.01 (m, 2H), 2.96-2.87 (m, 2H), 2.87-2.76 (m, 2H), 2.67-2.56 (m, 4H), 1.99 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 206.8, 169.8, 157.2, 150.4, 140.4, 129.1, 125.9, 121.5, 43.3, 37.8 (2C), 35.5 (2C), 29.3, 26.6, 23.3, 22.3; MS (ES mass): 311.9 (M+1); HPLC: 98.4%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/90, 14/90, 16/50, 20/50; flow rate: 0.8 mL/min; UV 245 nm, retention time 7.74 min.

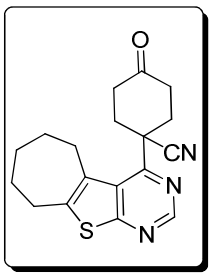
2.7.1.28. 1-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxo cyclohexanecarbonitrile (19b)



Compound **19b** was synthesized in 62% yield from **18b** following a procedure similar to that of compound **18a**.

White solid; mp: 152-154 °C; R_f = 0.5 (30% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2960, 2909, 2234, 1710; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 3.42 (t, *J* = 7.2 Hz, 2H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.95-2.87 (m, 2H), 2.78-2.72 (m, 2H), 2.69-2.63 (m, 2H), 2.61-2.53 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 206.8, 174.9, 156.8, 150.6, 146.5, 134.6, 125.8, 121.3, 42.9, 37.8 (2C), 34.9 (2C), 33.2, 30.1, 28.2; MS (ES mass): 297.5 (M+1).

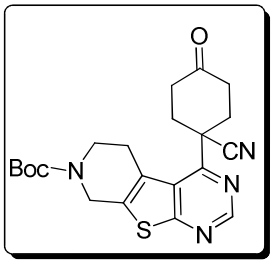
2.7.1.29. 1-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl) -4-oxocyclohexanecarbonitrile (19c)



Compound **19c** was synthesized in 65% yield from **18c** following a procedure similar to that of compound **19a**.

White solid; mp: 144-146 °C; R_f = 0.5 (25% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 2916, 2856, 2232, 1720; ^1H NMR (400 Hz, $CDCl_3$) δ: 8.89 (s, 1H), 3.37-3.34 (m, 2H), 3.05-3.02 (m, 2H), 2.96-2.87 (m, 2H), 2.80-2.76 (m, 2H), 2.67-2.55 (m, 4H), 2.04-1.98 (m, 2H), 1.81-1.74 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 206.8, 168.6, 157.0, 150.2, 144.9, 131.8, 128.9, 120.7, 43.1, 37.8 (2C), 35.1 (2C), 32.3, 30.9, 30.6, 26.9, 26.7; MS (ES mass): 326.2 (M+1).

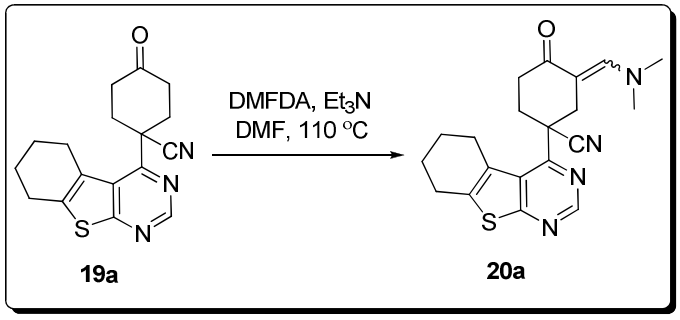
2.7.1.30. 1-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno [2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (19d)



Compound **19d** was synthesized in 58% yield from **18d** following a procedure similar to that of compound **19a**.

White solid; mp: 259-261°C; R_f = 0.4 (40% EtOAc/ n-hexane); IR (KBr, cm^{-1}): 2973, 2935, 2235, 1699; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 4.75 (s, 2H), 3.76 (t, *J* = 4.8 Hz, 2H), 3.33 (bs, 2H), 2.88-2.80 (m, 2H), 2.71-2.67 (m, 2H), 2.61-2.49 (m, 4H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 206.6, 170.1, 150.9, 150.1, 132.1, 129.8, 128.7, 126.9, 121.2, 80.9, 43.7, 43.2, 37.7 (2C), 35.2 (2C), 29.6, 28.4 (3C), 28.3. MS (ES mass): 413.2 (M+1); HPLC: 97.6%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 5 mM Ammonium Acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 13/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.72 min.

2.7.1.31. Typical procedure for preparation of 3-[(dimethylamino)methylene]-1-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbo nitrile (20a)



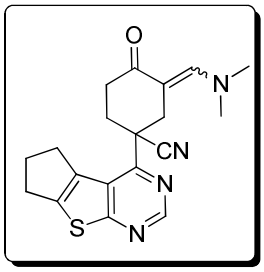
19a **20a**

A mixture of **19a** (0.5 g, 1.60 mmol), DMFDA (0.8 mL, 6.43 mmol) and Et_3N (0.7 mL, 4.82 mmol) in dry DMF (5 mL) was heated to 110 °C for 4 h under a nitrogen atmosphere. After completion of the reaction the mixture was cooled to room temp, diluted with water (25 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were collected, combined, washed with brine solution (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate-hexane (4:1) to give desired product **20a** (0.4 g, 64%) as a brown solid.

mp: 214-216 °C; R_f = 0.2 (100% EtOAc); IR (KBr, cm^{-1}): 2947, 2230, 1735, 1647; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 7.69 (s, 1H), 3.61 (s, 2H), 3.41-3.37 (m, 1H), 3.17 (s, 6H), 3.13-3.08 (m, 1H), 2.99 (s, 2H), 2.87-2.77 (m, 1H), 2.72-2.62 (m, 1H), 2.60-2.53 (m, 1H), 2.36-2.28 (m, 1H), 1.97 (bs, 4H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ: 193.7, 169.7, 158.4, 152.4, 150.4, 139.9, 129.1, 126.2, 122.2, 98.9, 43.7, 43.7, 43.2, 35.6, 34.6, 32.9, 29.1, 26.5, 23.2, 22.3; MS (ES mass): 367.0 (M+1).

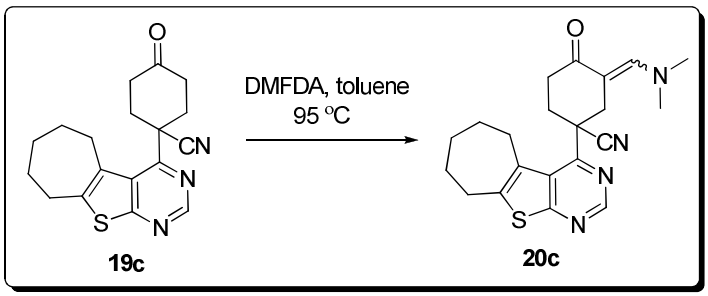
2.7.1.32. 3-[(dimethylamino)methylene]-1-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno [2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (20b)



Compound **20b** was synthesized in 58% yield from **19b** following a procedure similar to that of compound **20a**.

Light brown solid; mp: 227-229 °C; R_f = 0.1 (100% EtOAc); IR (KBr, cm^{-1}): 2947, 2232, 1646, 1541; ^{1}H NMR (400 Hz, $CDCl_3$) δ: 8.89 (s, 1H), 7.70 (s, 1H), 3.57 (s, 2H), 3.51-3.43 (m, 1H), 3.34-3.25 (m, 1H), 3.17 (s, 6H), 3.11 (t, *J* = 7.2 Hz, 2H), 2.88-2.80 (m, 1H), 2.67-2.52 (m, 4H), 2.38-2.30 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 193.6, 174.8, 158.1, 152.7, 150.6, 146.1, 134.9, 125.8, 122.0, 98.8, 43.8, 42.9, 34.8, 34.5, 33.1, 32.4, 30.1 (2C), 28.2; MS (ES mass): 353.2 (M+1).

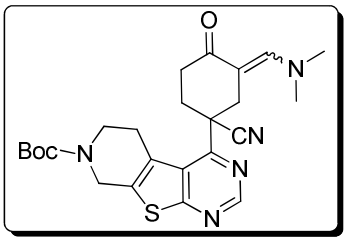
2.7.1.33. 3-[(dimethylamino)methylene]-1-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5] thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (20c)



A mixture of **19c** (0.2 g, 0.615 mmol) and DMFDA (0.16 mL, 1.23 mmol) in toluene (5 mL) was heated to 95 °C for 16 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temp and the solvent was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under

reduced pressure. The residue was purified by column chromatography using ethyl acetate-hexane (4:1) to give desired product **20c** (105 mg, 45%) as a white solid. mp: 185-187 °C; R_f = 0.1 (100% EtOAc); IR (KBr, cm^{-1}): 2920, 2851, 2228, 1646; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 7.71 (s, 1H), 3.61 (s, 2H), 3.33 (t, *J* = 5.6Hz, 2H), 3.17 (s, 6H), 3.03-3.01 (m, 2H), 2.88-2.78 (m, 1H), 2.68-2.56 (m, 2H), 2.39-2.30 (m, 1H), 2.04-1.96 (m, 2H), 1.78-1.74 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 193.7, 168.4, 158.3, 152.7, 150.2, 144.5, 132.2, 128.9, 121.5, 99.0, 43.8, 43.2, 35.6, 34.6, 32.5 (2C), 32.4, 30.8, 30.6, 27.1, 26.7; MS (ES mass): 381.2 (M+1).

2.7.1.34. 3-[(dimethylamino)methylene]-1-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetra hydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbo nitrile (20d)



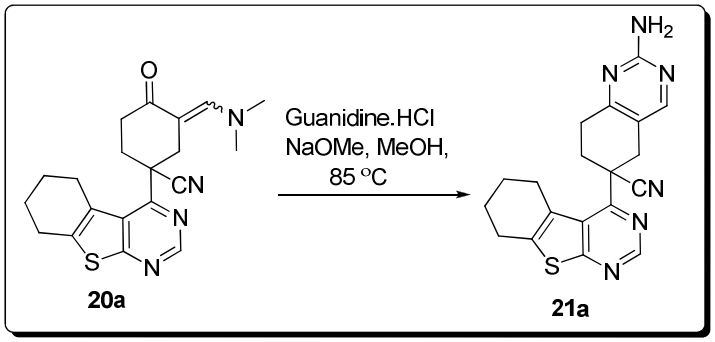
Compound **20d** was synthesized in 45% yield from **19d** following a procedure similar to that of compound **20c**.

Brown solid; mp: 113-115 °C; R_f = 0.1 (100 % EtOAc); IR (KBr, cm^{-1}): 2976, 2237, 1729, 1696; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.67 (s, 1H), 4.80 (s, 2H), 3.83 (bs, 2H), 3.60 (bs, 3H), 3.39-3.29 (m, 2H), 3.08 (s, 3H), 3.03 (s, 3H), 2.71-2.63 (m, 3H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 193.5, 169.9, 159.0, 152.7 (2C), 150.9 (2C), 128.2, 122.1, 121.2, 98.7, 80.8, 43.8, 43.2, 42.3, 35.4, 34.6, 32.8, 31.0, 29.2, 28.4 (3C), 28.3; MS (ES mass): 468.2 (M+1).

2.7.1.35. Typical procedure for preparation of 2-amino-6-(5,6,7,8-tetrahydro benzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbo nitrile (21a)

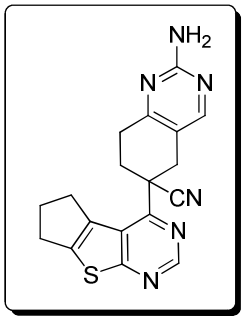
A mixture of **20a** (0.1 g, 0.27 mmol), guanidine HCl (24 mg, 0.41 mmol) and NaOMe (22 mg, 0.41 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction the excess of sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was

diluted with water (25 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate-hexane (4:1) to give desired product **21a** (80 mg, 78%) as a light brown solid.



mp: 153-155 °C; R_f = 0.35 (100% EtOAc); IR (KBr, cm^{-1}): 3320, 3172, 2937, 2235; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.17 (s, 1H), 5.15 (s, 2H), 3.67 (d, *J* = 16.1 Hz, 1H), 3.43-3.36 (m, 2H), 3.26-3.16 (m, 2H), 3.01-2.91 (m, 3H), 2.79-2.75 (m, 1H), 2.45-2.37 (m, 1H), 1.99 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 163.9, 161.9, 158.6, 157.5, 150.4, 140.3, 129.1, 126.1, 121.5, 115.7, 42.3, 35.9, 32.5, 29.3, 29.2, 26.6, 23.3, 22.3; MS (ES mass): 362.9 (M+1); HPLC: 99.3%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN (Isocratic) (A : B) 40 : 60 ; flow rate: 0.8 mL/min; UV 245 nm, retention time 2.9 min; Chiral HPLC: column: chiral pak IC (250 x 4.6 mm) 5 µm, mobile phase: A: MeOH: B: 0.1% DEA, flow : 1.0 mL/min, wave Length : 245 nm, retention time (area %): 16.9 min (50%) and 19.4 min (50%).

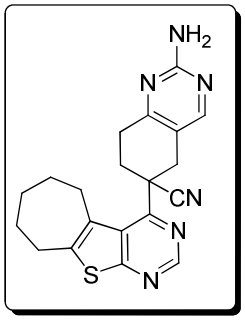
2.7.1.36. 2-Amino-6-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (21b)



Compound **21b** was synthesized in 70% yield from **20b** ollowing a procedure similar to that of compound **21a**.

White solid; mp: 200-202 °C; R_f = 0.3 (100% EtOAc); IR (KBr, cm^{-1}): 3318, 3161, 2950, 2242; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.16 (s, 1H), 5.10 (bs, 2H), 3.62 (d, *J* = 16.0 Hz, 1H), 3.52-3.45 (m, 1H), 3.40-3.36 (m, 1H), 3.34-3.17 (m, 2H), 3.15-3.11 (m, 2H), 2.96-2.89 (m, 1H), 2.78-2.72 (m, 1H), 2.62-2.54 (m, 2H), 2.46-2.38 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.3, 163.9, 161.7, 158.6, 157.0, 150.6, 146.5, 134.7, 125.8, 121.4, 115.5, 41.9, 34.7, 33.2, 31.9, 30.1, 29.7, 28.9; MS (ES mass): 349.1 (M+1); HPLC: 90.7%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 9/95, 12/95, 15/30, 18/30; flow rate: 0.8 mL/min; UV 241 nm, retention time 7.0 min.

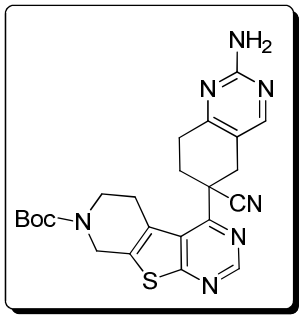
2.7.1.37. 2-Amino-6-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*] pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (21c)



Compound **21c** was synthesized in 68% yield from **20c** following a procedure similar to that of compound **21a**.

White solid; mp: 234-236 °C; R_f = 0.2 (100% EtOAc); IR (KBr, cm^{-1}): 3456, 3314, 2930, 2232; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.15 (s, 1H), 5.17 (s, 2H), 3.64 (d, *J* = 16.4 Hz, 1H), 3.44-3.17 (m, 4H), 3.04 (t, *J* = 5.4 Hz, 2H), 2.99-2.92 (m, 1H), 2.81-2.76 (m, 1H), 2.45-2.37 (m, 1H), 2.05-1.97 (m, 2H), 1.79-1.76 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 164.1, 161.9, 158.6, 157.3, 150.3, 144.9, 131.9, 128.9, 120.8, 115.7, 42.1, 35.7, 32.4, 32.1, 30.9, 30.6, 29.1, 26.9, 26.7; MS (ES mass): 377.1 (M+1); HPLC: 99.1%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL/min; UV 245 nm, retention time 4.5 min.

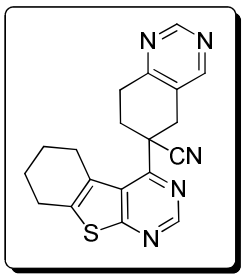
2.7.1.38. 2-Amino-6-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4',3':4,5] thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (21d)



Compound **21d** was synthesized in 55% yield from **20d** following a procedure similar to that of compound **21a**.

Light yellow solid; mp: 141-143 °C; R_f = 0.3 (100% EtOAc); IR (KBr, cm^{-1}): 3327, 3194, 2971, 2235, 1696; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 8.21 (s, 1H), 5.82 (s, 2H), 4.82 (s, 2H), 3.89-3.77 (m, 2H), 3.72 (d, *J* = 16.1 Hz, 1H), 3.57-3.46 (m, 1H), 3.40 (d, *J* = 16.3 Hz, 1H), 3.31-3.19 (m, 3H), 3.03-2.91 (m, 1H), 2.81-2.71 (m, 1H), 2.49-2.39 (m, 1H), 2.09 (s, 1H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.3, 170.1, 163.8, 161.2 (2C), 158.3, 154.2, 151.0 (2C), 128.2, 121.3, 115.4, 80.9, 42.1, 35.4, 32.1, 31.5, 28.7, 28.4 (3C), 28.3, 26.9. MS (ES mass): 464.2 (M+1). HPLC: 97.9%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 5 mM Ammonium Acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 13/95, 15/20, 18/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 8.6 min.

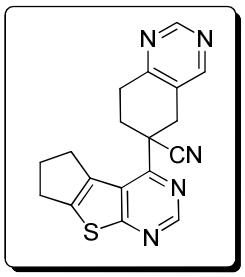
2.7.1.39. 6-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetra hydroquinazoline-6-carbonitrile (22a)



Compound **22a** was synthesized in 45% yield from **20a** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **21a**.

Brown solid; mp: 182-184 °C; R_f = 0.45 (100% EtOAc); IR (KBr, cm^{-1}): 2941, 2868, 2234, 1557; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.86 (s, 1H), 8.61 (s, 1H), 3.99 (d, *J* = 16.9 Hz, 1H), 3.54 (d, *J* = 16.9 Hz, 1H), 3.46-3.34 (m, 2H), 3.15-3.02 (m, 4H), 2.86-2.82 (m, 1H), 2.51-2.43 (m, 1H), 2.05-1.99 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9, 162.9, 157.1, 157.0, 156.7, 150.4, 140.6, 129.0, 126.7, 125.9, 121.4, 41.7, 36.3, 32.3, 29.3, 29.1, 26.6, 23.2, 22.2; MS (ES mass): 347.9 (M+1); HPLC: 98.5%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN (Isocratic) (A : B) 40 : 60 ; flow rate: 0.8 mL/min; UV 245 nm, retention time 4.2 min.

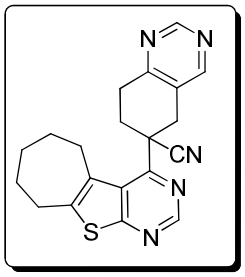
2.7.1.40. 6-(6,7-dihydro-5*H*–cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (22b)



Compound **22b** was synthesized in 78% yield from **20b** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **21a**.

White solid; mp: 248-250°C; R_f = 0.5 (100% EtOAc); IR (KBr, cm^{-1}): 2954, 2863, 2243, 1535; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.86 (s, 1H), 8.60 (s, 1H), 3.91 (d, *J* = 16.8 Hz, 1H), 3.57-3.49 (m, 2H), 3.41-3.25 (m, 2H), 3.13 (t, *J* = 7.2 Hz, 2H), 3.08-3.01 (m, 1H), 2.84-2.77 (m, 1H), 2.65-2.54 (m, 2H), 2.51-2.44 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.0, 162.9, 157.2, 157.1, 156.3, 150.5, 146.8, 134.6, 126.5, 125.7, 121.2, 41.3, 34.9, 33.2, 31.9, 30.2, 28.9, 28.2; MS (ES mass): 334.1 (M+1); HPLC: 97.9%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 9/95, 12/95, 15/30, 18/30; flow rate: 0.8 mL/min; UV 241 nm, retention time 7.9 min.

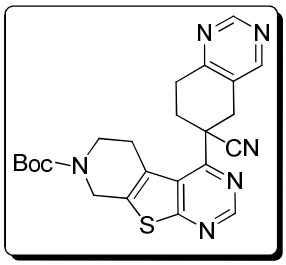
2.7.1.41. 6-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (22c)



Compound **22c** was synthesized in 67% yield from **20c** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **21a**.

White solid; mp: 157-159 °C; R_f = 0.4 (100% EtOAc); IR (KBr, cm^{-1}): 2934, 2853, 2230, 1551; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.06 (s, 1H), 8.88 (s, 1H), 8.61 (s, 1H), 3.97 (d, *J* = 16.8 Hz, 1H), 3.55 (d, *J* = 16.8 Hz, 1H), 3.43-3.28 (m, 3H), 3.13-3.00 (m, 3H), 2.87-2.84 (m, 1H), 2.50-2.42 (m, 1H), 2.10-1.95 (m, 2H), 1.80-1.77 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.7, 163.0, 157.2, 157.1, 156.6, 150.2, 145.2, 131.8, 128.9, 126.7, 120.6, 41.6, 36.1, 32.4, 31.9, 30.9, 30.6, 29.1, 26.9, 26.7; MS (ES mass): 362.1 (M+1); HPLC: 97.3%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL/min; UV 245 nm, retention time 6.4 min.

2.7.1.42. 6-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (22d)

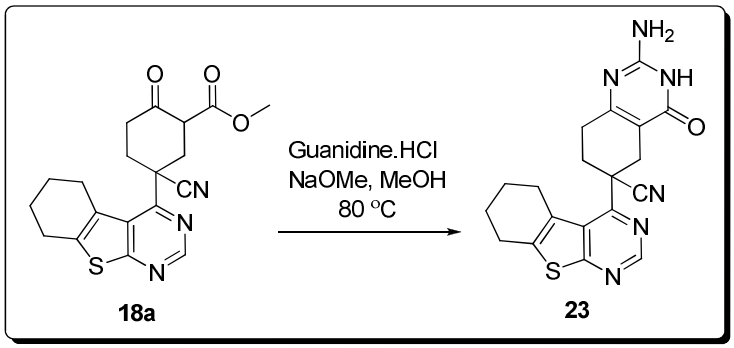


Compound **22d** was synthesized in 50% yield from **20d** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **21a**.

Brown solid; mp: 191-193°C; R_f = 0.5 (100% EtOAc); IR (KBr, cm^{-1}): 2975, 2930, 2228, 1698 ; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.11 (s, 1H), 8.89 (s, 1H), 8.72 (m, 1H), 4.84 (s, 2H), 4.08 (d, *J* = 16.0Hz, 1H), 3.83 (s, 2H), 3.66-3.51 (m, 2H), 3.51-3.37 (m, 2H), 3.35-3.03 (m, 3H), 2.89-2.77 (m, 1H), 2.60-2.48 (m, 1H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3 (2C), 163.3, 156.8, 155.9, 154.2, 150.9 (3C), 128.1,

121.1, 109.9, 80.9, 41.5, 35.9, 32.0 (2C), 28.8 (2C), 28.4 (3C), 28.3; MS (ES mass): 449.1 (M+1); HPLC: 98.2%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 13/95, 15/50, 18/50; flow rate: 0.8 mL/min; UV 240 nm, retention time 6.6 min.

2.7.1.43. Typical procedure for preparation of 2-amino-6-(5,6,7,8-tetrahydro benzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxo-3,4,5,6,7,8-hexahydro quinazoline-6-carbonitrile (23)

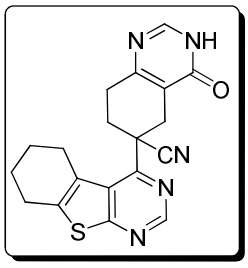


A mixture of **18a** (0.1 g, 0.27 mmol), guanidine HCl (48 mg, 0.81 mmol) and NaOMe (73 mg, 1.35 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction the excess sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using methanol-DCM (1:19) to give desired product **23** (73 mg, 72%) as a white solid.

mp: 279-281°C; R_f = 0.5 (10% MeOH/DCM); IR (KBr, cm^{-1}): 3448, 3314, 3125, 2940, 2229, 1650; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.8 (bs, 1H), 9.04 (s, 1H), 6.46 (bs, 2H), 3.28 (m, 3H), 3.23 (m, 3H), 2.81-2.63 (m, 3H), 2.39-2.36 (m, 1H), 1.94 (bs, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.8, 162.9, 158.3, 154.1, 150.6, 145.1, 139.7, 128.4, 126.1, 122.1, 104.4, 42.2, 32.0, 31.6, 28.9, 28.5, 25.9, 22.7, 21.8; MS (ES mass): 378.9 (M+1); HPLC: 97.4%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient

(T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 1.0 mL/min; UV 246 nm, retention time 6.2 min.

2.7.1.44. 6-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxo-3,4,5,6,7,8-hexahydroquinazoline-6-carbonitrile (24)



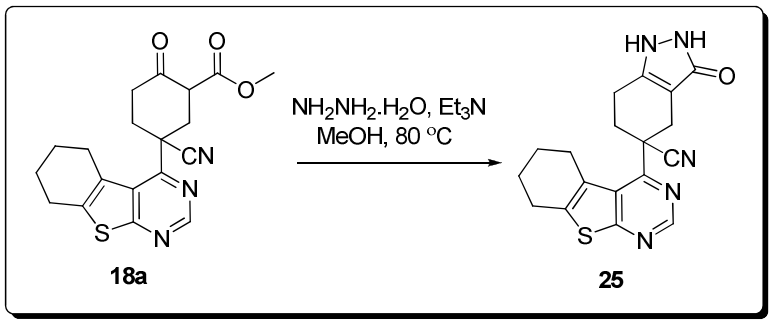
Compound **24** was synthesized in 68% yield from **18a** and formimidine acetate (3 mmol) following a procedure manner similar to that of compound **23**.

White solid; mp: 202-204 °C; R_f = 0.6 (10% MeOH/DCM); IR (KBr, cm^{-1}): 3153, 2943, 2233, 1655; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.12 (s, 1H), 3.58 (d, *J* = 17.6 Hz, 1H), 3.46-3.34 (m, 2H), 3.26-3.18 (m, 2H), 3.01 (s, 2H), 2.95-2.89 (m, 1H), 2.78-2.74 (m, 1H), 2.55-2.47 (m, 1H), 1.99-1.98 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 163.5, 160.6, 157.2, 150.4, 145.9, 140.2, 129.1, 126.1, 121.7, 119.1, 41.5, 32.4, 31.8, 29.2, 29.1, 26.6, 23.2, 22.2; MS (ES mass): 363.9 (M+1); HPLC: 98.6%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 0.8 mL/min; UV 244 nm, retention time 8.1 min; Chiral HPLC: column: chiral pak AD (250 x 4.6 mm) 3 µm, mobile phase: A: n-hexane: B: 0.1% IPA, flow: 0.8 mL/min, wave length: 245 nm, retention time (area %): 12.6 min (49.5%) and 15.8 min (50.5%).

2.7.1.45. Typical procedure for preparation of 5-(5,6,7,8-tetrahydrobenzo [*b*]thieno[2,3-*d*]pyrimidin-4-yl)-3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole-5-carbo nitrile (25)

A mixture of **18a** (0.1 g, 0.27 mmol), hydrazine (0.03 mL, 0.54 mmol) and Et_3N (0.09 mL, 0.81 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL),

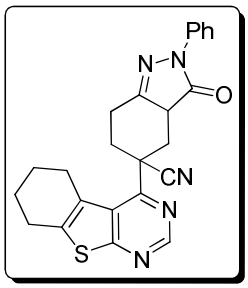
dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The isolated residue was purified by column chromatography using methanol-DCM (1:49) to give desired product **25** (75 mg, 76%) as a white solid.



mp: 271-273 °C; R_f = 0.5 (5% MeOH/DCM); IR (KBr, cm^{-1}): 3231, 2944, 2234, 1734; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.31 (bs, 1H), 9.61 (bs, 1H), 9.00 (s, 1H), 3.22-3.15 (m, 4H), 3.02 (s, 2H), 2.87 (s, 2H), 2.66-2.62 (m, 1H), 2.38-2.28 (m, 1H), 1.91 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.7, 158.5, 150.5, 150.3, 139.6, 137.9, 128.3, 126.1, 122.1, 109.5, 43.6, 32.3, 30.5, 28.5, 25.9, 22.7, 21.8, 19.4; MS (ES mass): 351.9 (M+1); HPLC: 97.7%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 0.8 mL/min; UV 245 nm, retention time 7.9 min; Chiral HPLC: column: Lux Cellulose-2 (250 x 4.6 mm) 3 µm, mobile phase: A: n-hexane: D: 0.1% TFA in EtOH, flow : 0.8 mL/min, wave length : 245 nm, retention time (area %): 16.9 min (45.7%) and 20.9 min (49.8%).

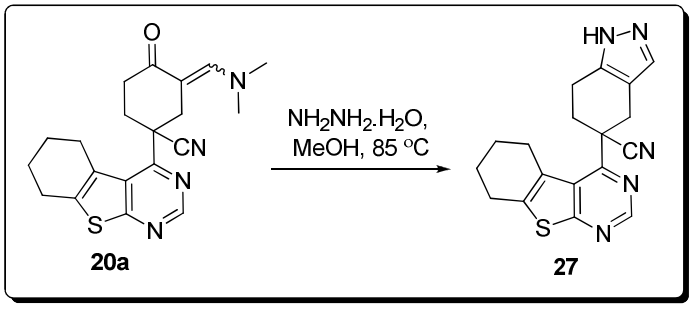
2.7.1.46. 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-3-oxo-2-phenyl-3,3a,4,5,6,7-hexahydro-2*H*-indazole-5-carbonitrile (26)

Compound **26** was prepared in 65% yield from **18a** and phenyl hydrazine (2 mmol) following a procedure similar to compound **25**.



White solid; mp: 218-220 °C; R_f = 0.3 (70% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3062, 2861, 2237, 1730; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.22-7.13 (m, 1H), 3.41-3.33 (m, 2H), 3.27-3.17 (m, 2H), 3.09-2.84 (m, 5H), 2.76-2.65 (m, 1H), 2.01-1.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 159.2, 157.9, 150.4, 148.1, 140.1, 129.1, 128.9, 128.9(2C), 126.3, 125.7, 121.7, 120.3, 118.9, 42.8, 37.3, 32.1, 30.5, 29.2, 26.6, 23.2, 22.2, 20.4; MS (ES mass): 427.9 (M+1); HPLC: 97.9%, column: ZORBAX XDB C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL/min; UV 245 nm, retention time 5.9 min.

2.7.1.47. Typical procedure for preparation of 5-(5, 6, 7, 8-tetrahydrobenzo[*b*] thieno[2,3-*d*]pyrimidin-4-yl)-4,5,6,7-tetrahydro-1*H*-indazole-5-carbonitrile (27)



A mixture of **20a** (0.1 g, 0.27 mmol) and hydrazine (0.02 mL, 0.41 mmol) in methanol (5 mL) was stirred at 80 °C for 1 h under nitrogen. Then, methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate-*n*-hexane (3:2) to give desired product **27** (65 mg, 72%) as a light brown solid.

mp: 109-111°C; R_f = 0.3 (70% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3647, 3248, 2230, 1513; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 7.54 (s, 1H), 6.45 (bs, 1H), 3.61 (d, *J* = 16.0 Hz, 1H), 3.48 (d, *J* = 16.0 Hz, 1H), 3.38-3.09 (m, 4H), 3.00 (bs, 2H), 2.83-2.78 (m, 1H), 1.98 (bs, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.5, 158.2, 150.2 (2C), 139.8 (2C), 129.0, 126.1 (2C), 121.8, 43.5, 33.4, 31.9, 29.1, 26.4, 23.1, 22.1, 20.1; MS (ES mass): 336.2 (M+1); HPLC: 99.1%, column: ZORBAX XDB C-18 150

x 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, 9/95, 13/95, 15/20, 18/20; flow rate: 1.0 mL/min; UV 245 nm, retention time 8.5 min; Chiral HPLC: column: chiral pak IC (250 x 4.6 mm) 5 µm, mobile phase: A: MeOH: B: 0.1% DEA, flow : 1.0 mL/min, wave Length: 295 nm, retention time (area %): 8.8 min (50%) and 10.9 min (50%).

2.7.2. Single crystal X-ray data

Single crystals suitable for X-ray diffraction of **18a** and **21a** were grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα 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. [27] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[28]

Crystal data of 18a: Molecular formula = $C_{19}H_{19}N_3O_3S$, Formula weight = 369.44, Crystal system = Triclinic, space group = P-1, a = 11.092 (5) Å, b = 11.448 (5) Å, c = 15.672 (7) Å, V = 1761.7 (13)Å^3, T = 296 K, Z = 4, D_c = 1.401 Mg m^{-3}, µ(Mo-Kα) = 0.21 mm^{-1}, 20533 reflections measured, 7395 independent reflections, 5076 observed reflections [I > 2.0 σ (I)], R_1_obs = 0.081, Goodness of fit =1.003. Crystallographic data (excluding structure factors) for 7a have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 864130.

Crystal data of 21a: Molecular formula = $C_{19}H_{18}N_6S$, Formula weight = 362.13, Crystal system = Triclinic, space group = P-1, a = 7.625 (4) Å, b = 10.1757 (5) Å, c = 12.243 (6) Å, V = 903.66 (8)Å^3, T = 296 K, Z = 6, Dc = 1.387 Mg m^{-3}, µ(Mo-Kα) = 0.21 mm^{-1}, 15612 reflections measured, 3949 independent reflections, 3342 observed reflections [I > 2.0 σ (I)], R1_obs = 0.029, Goodness of fit = 0.876. Crystallographic data (excluding structure factors) for 10a have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 864129.

2.7.3. Pharmacology

2.7.3.1. Cells and Reagents

HEK 293 and Sf9 cells were obtained from ATCC (Washington D.C., USA). HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC and routinely cultured in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc.). cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDE4B1 clone was from OriGene Technologies (Rockville, MD, USA). PDE4D2 enzyme was purchased from BPS Bioscience (San Diego, CA, USA). Lipopolysaccharide (LPS) was from *Escherichia coli* strain 0127:B8 obtained from Sigma (St. Louis, MO, USA). Mouse TNF-α ELISA kit was procured from R&D Systems (Minneapolis, MN, USA).

2.7.3.2. 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.[22] 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.

2.7.3.3. 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.

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

2.7.3.4. TNF-α production assay

RAW 264.7 cells were pre-incubated either with DMSO (vehicle control) or compound for 30 minutes and then stimulated with 1 µg/mL of LPS overnight. Dose response studies were carried out at eight different concentrations (30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 µM). Post-stimulation, cell supernatants were harvested, centrifuged to clear cell debris and the amount of TNF-α in the supernatants was measured using mouse TNF-α DuoSet ELISA kit from R&D Systems according to manufacturer's recommendations. The percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 - \left[\frac{(\text{ LPS stimulated}_{\text{compound}} - \text{unstimulated})}{(\text{LPS stimulated}_{\text{DMSO}} - \text{unstimulated})} \text{ X } 100 \right]$$

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 expressed as mean ± SD.

2.7.3.5. Docking Method:

We docked all the molecules by using Schrodinger 2011 software. The PDB ID 3O0J was used for the docking study. 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. The search grid was generated by picking the cocrystal ligand upto 20 Å search area. The hydroxyl groups of search area were allowed to move.

All the molecules were minimized by using macromodule application. 1000 iteration were used for minimization using OPLS 2005 force field and charges were added from force field only. The PRCG (Polak-Ribier conjugate gradient) method was used for minimization. All the molecules were docked by using glide XP (extra precision) dock application by rigid docking method. The compound 16 was docked in both the tautomeric forms separately. All the free energy and binding energy calculations were carried out by using MMGBSA module of Prime application. The MMGBSA approach employs molecular mechanics, the generalized born model and solvent accessibility method to elicit free energies from input structural information. The output results of xp docking (pose viewer file) were used to run MMGBSA energy calculations.

2.8. References:

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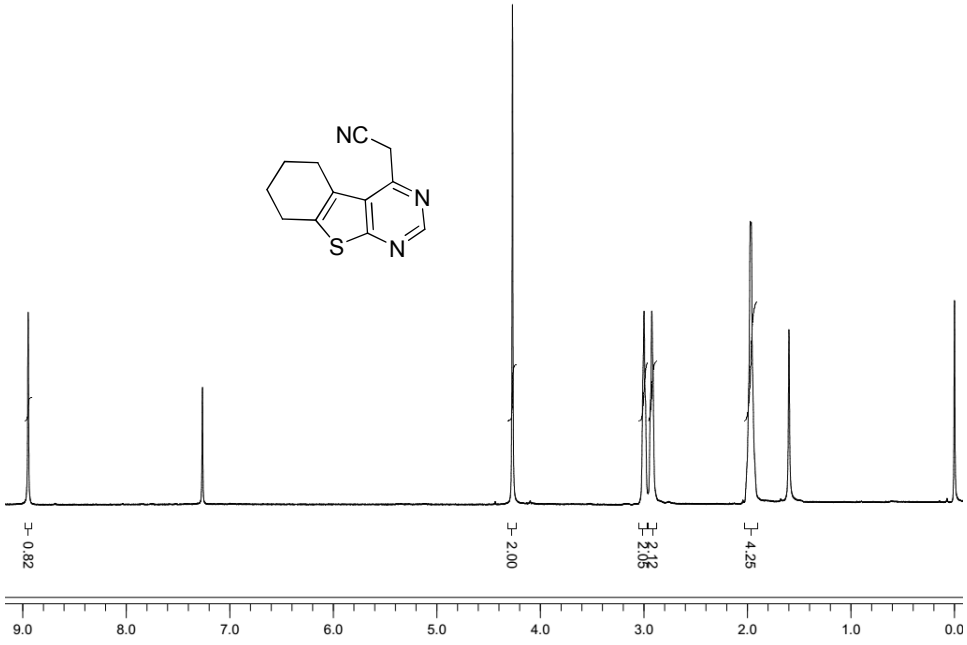
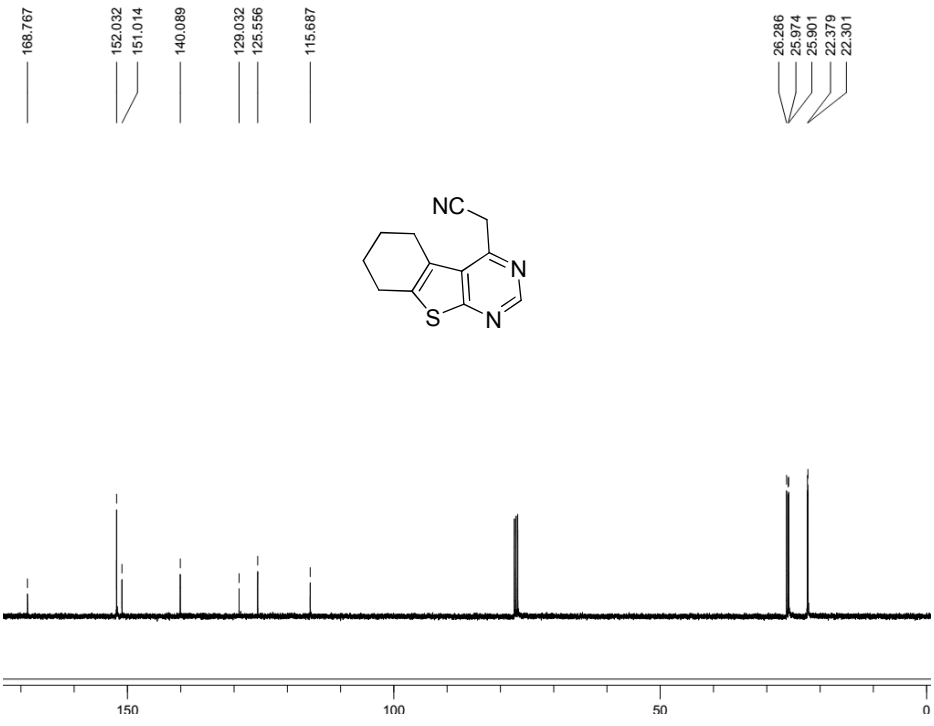
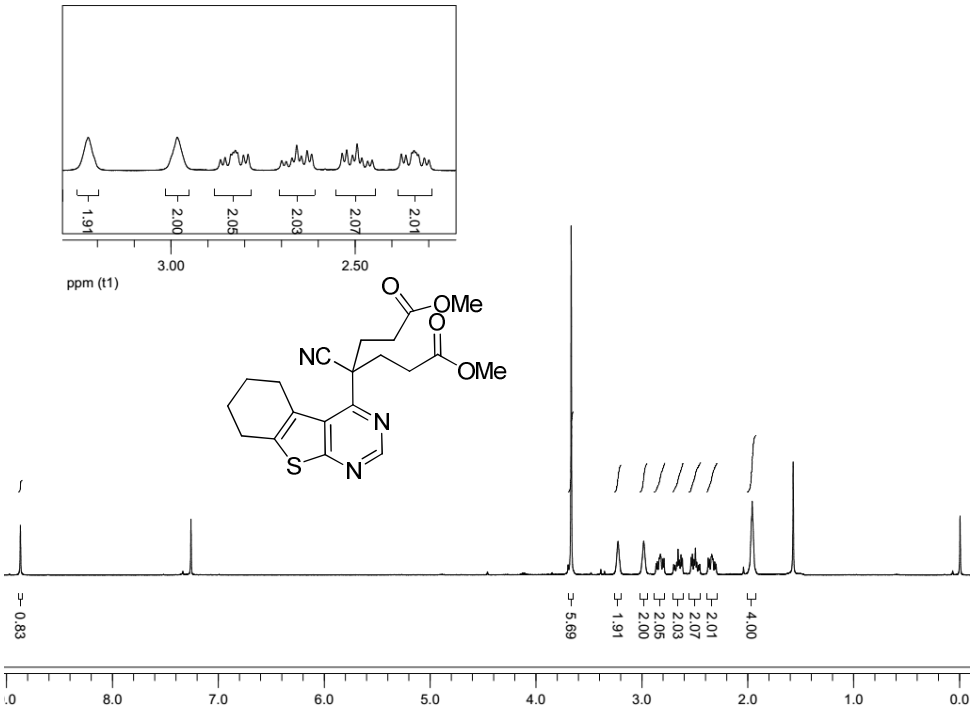
Appendix



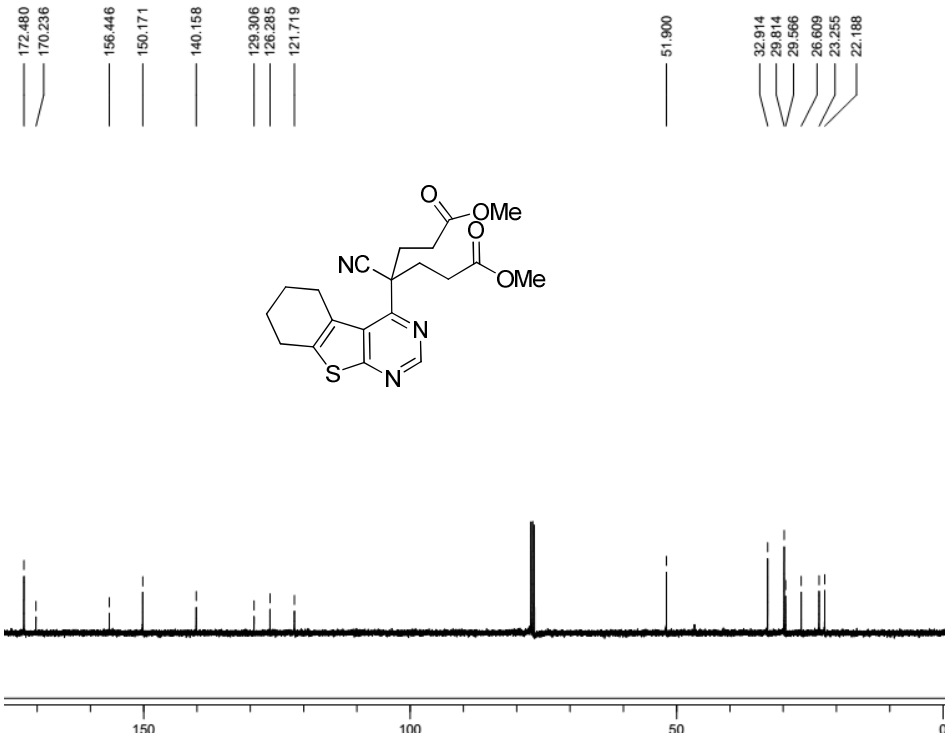
^{1}H NMR spectra of **16a** (400 MHz, $CDCl_3$)



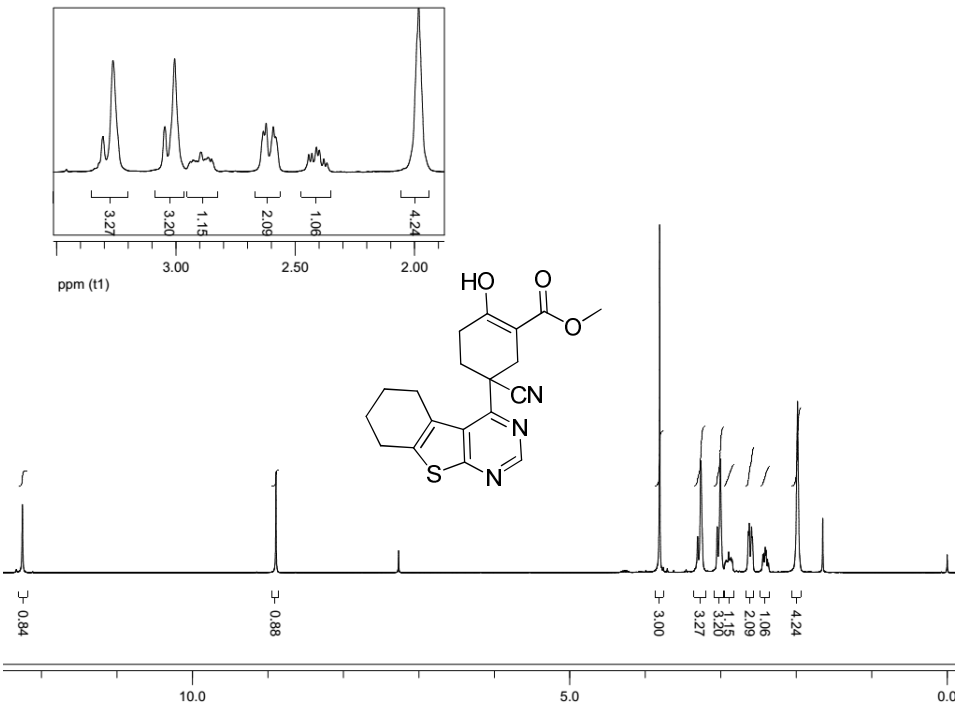
^{13}C NMR spectra of **16a** (100 MHz, $CDCl_3$)



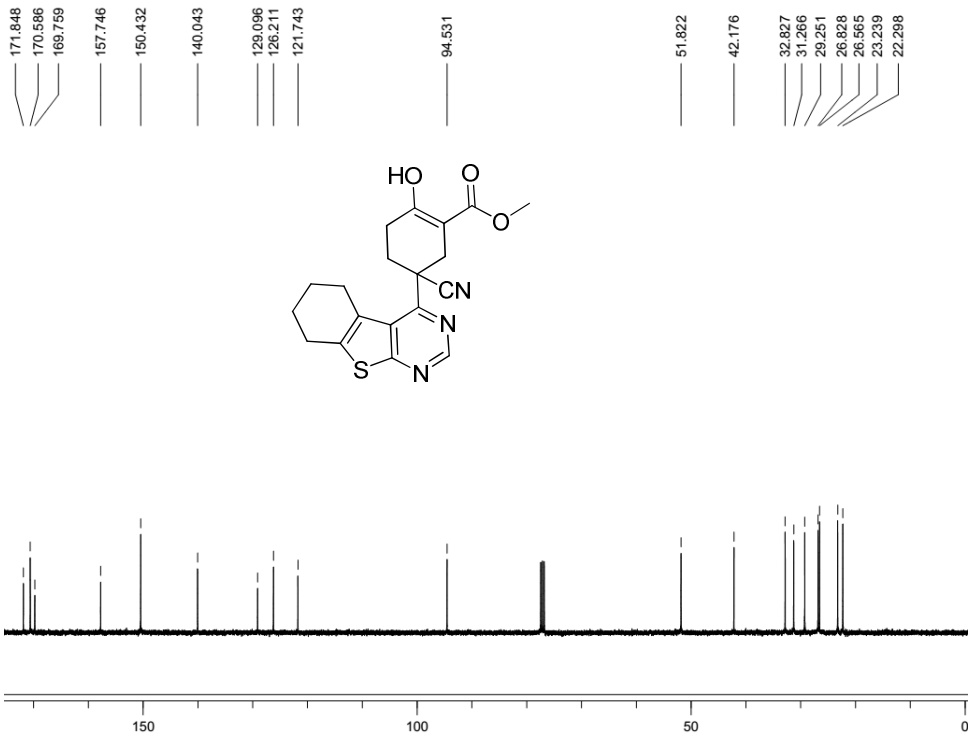
^{1}H NMR spectra of **17a** (400 MHz, $CDCl_3$)



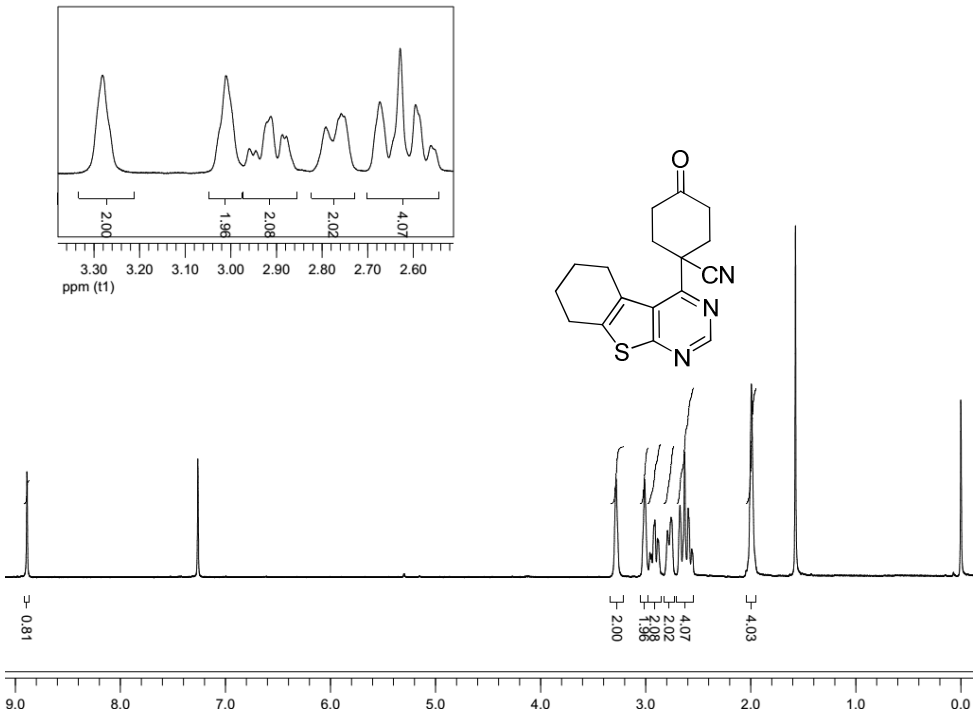
^{13}C NMR spectra of **17a** (100 MHz, $CDCl_3$)



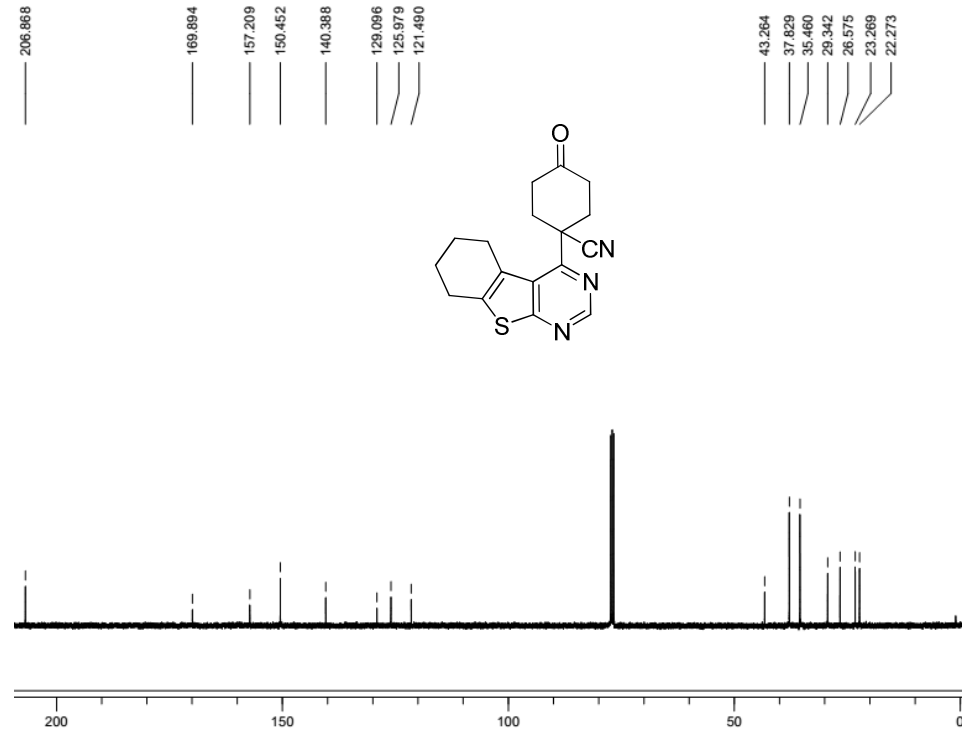
^{1}H NMR spectra of **18a** (400 MHz, $CDCl_3$)



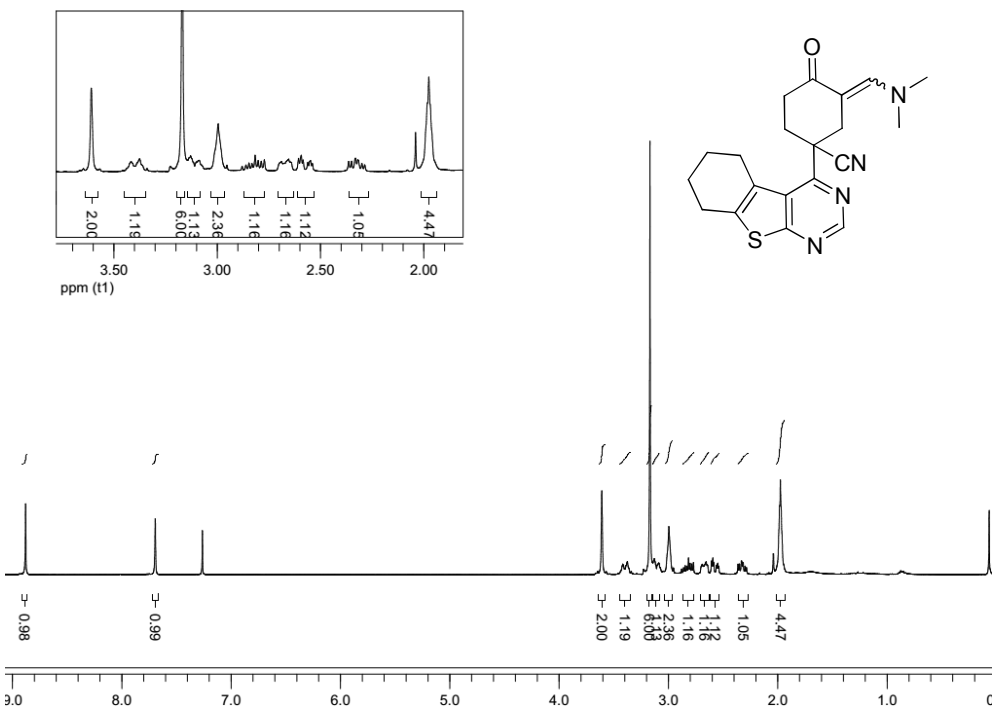
^{13}C NMR spectra of **18a** (100 MHz, $CDCl_3$)



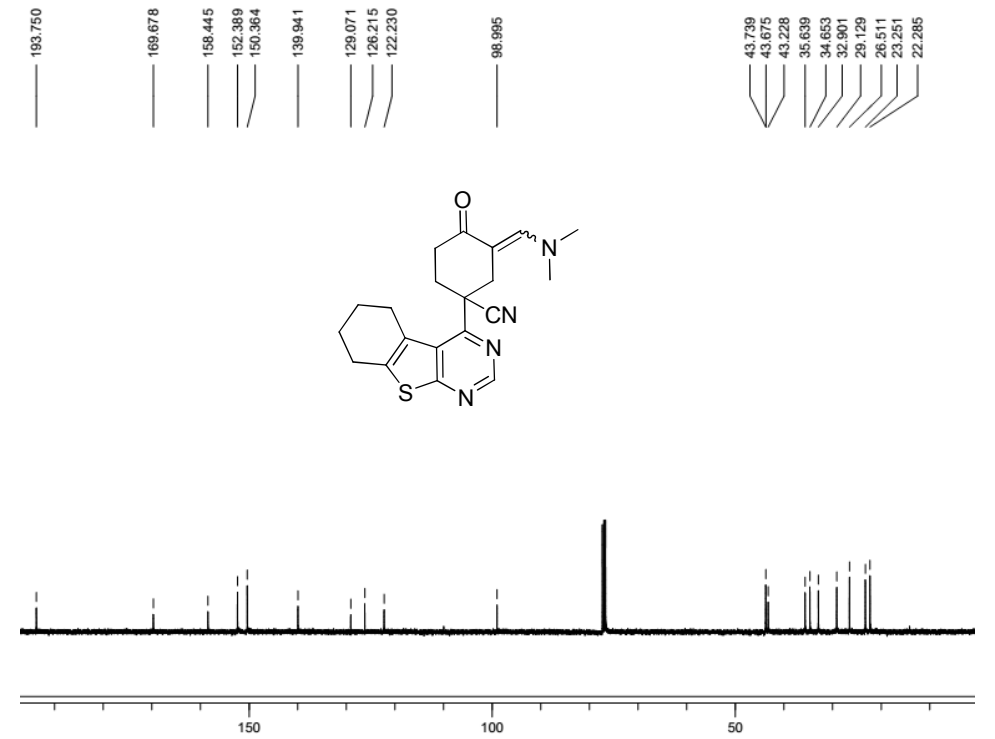
^{1}H NMR spectra of **19a** (400 MHz, $CDCl_3$)



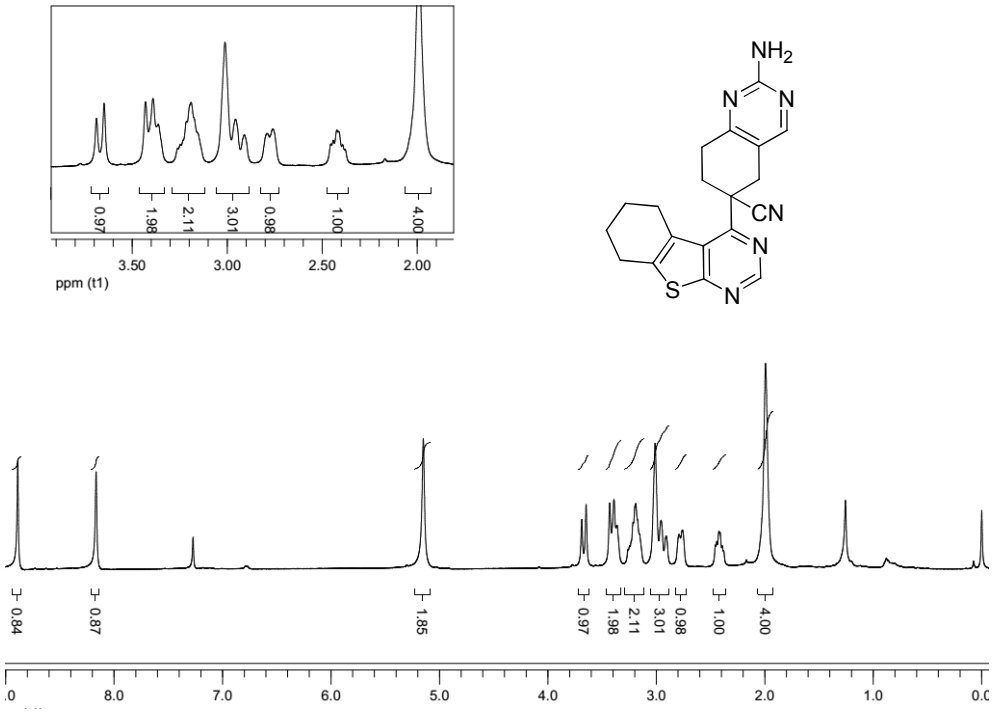
^{13}C NMR spectra of **19a** (100 MHz, $CDCl_3$)



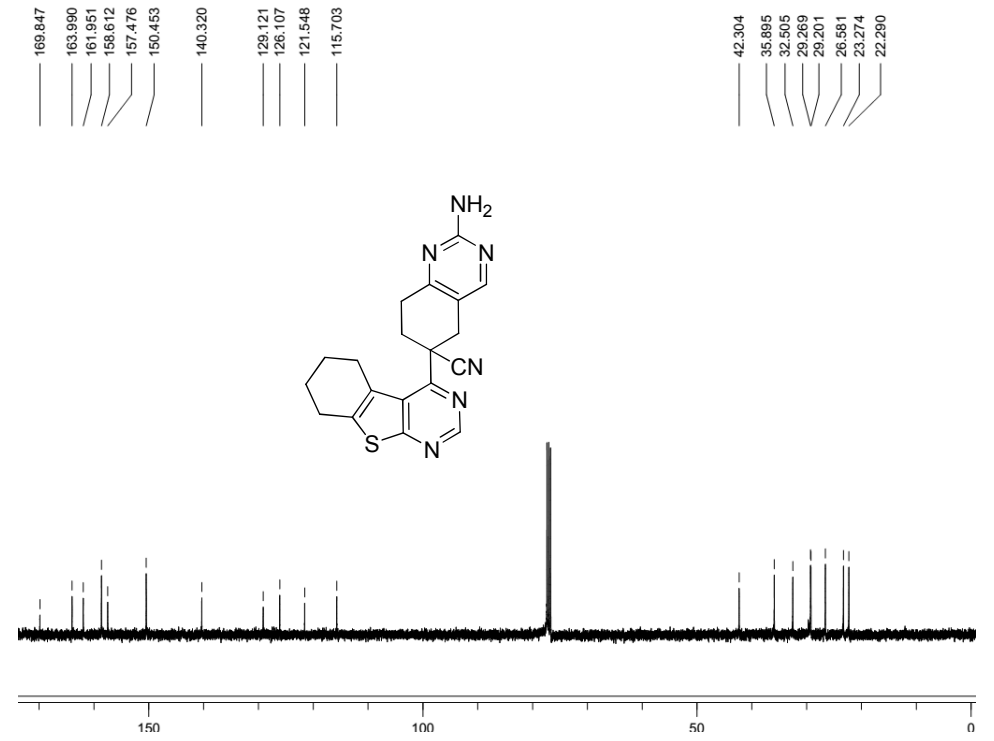
^{1}H NMR spectra of **20a** (400 MHz, $CDCl_3$)



^{13}C NMR spectra of **20a** (100 MHz, $CDCl_3$)



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



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

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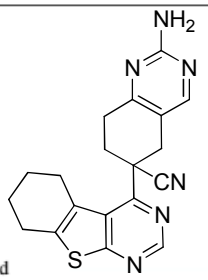
Sample Name	:ILS-ARJ-10A
Tray#	: 1
Vail #	: 53
Injection Volume	: 10 uL
Data File Name	: 1_0512048.lcd
Method File Name	: Method_2.lcm
Batch File Name	: 1_May_164.lcb
Data Acquired	: 5/4/2012 3:25:43 AM
Data Processed	: 5/4/2012 12:39:03 PM

Chromatogram conditions:
Column ID:CHIRALPAK IC(4.6 x 250 mm) 5µ
M.P:MeOH:DEA(100:0.1)
Flow:1.0 ml/min,Temp:25°C
Sample Conc:0.5mg/mL
Diluent:M.P



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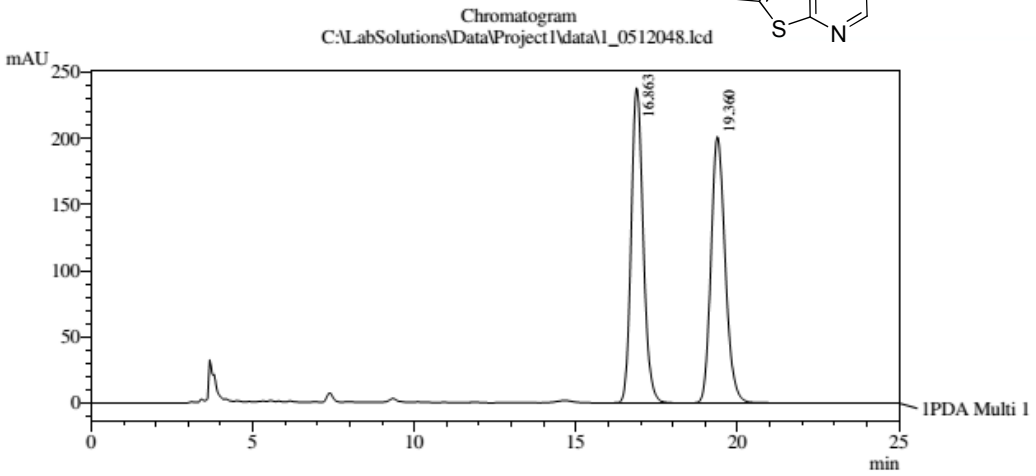

1 PDA Multi 1 / 245nm 4nm

PeakTable

PDA Ch1 245nm 4nm

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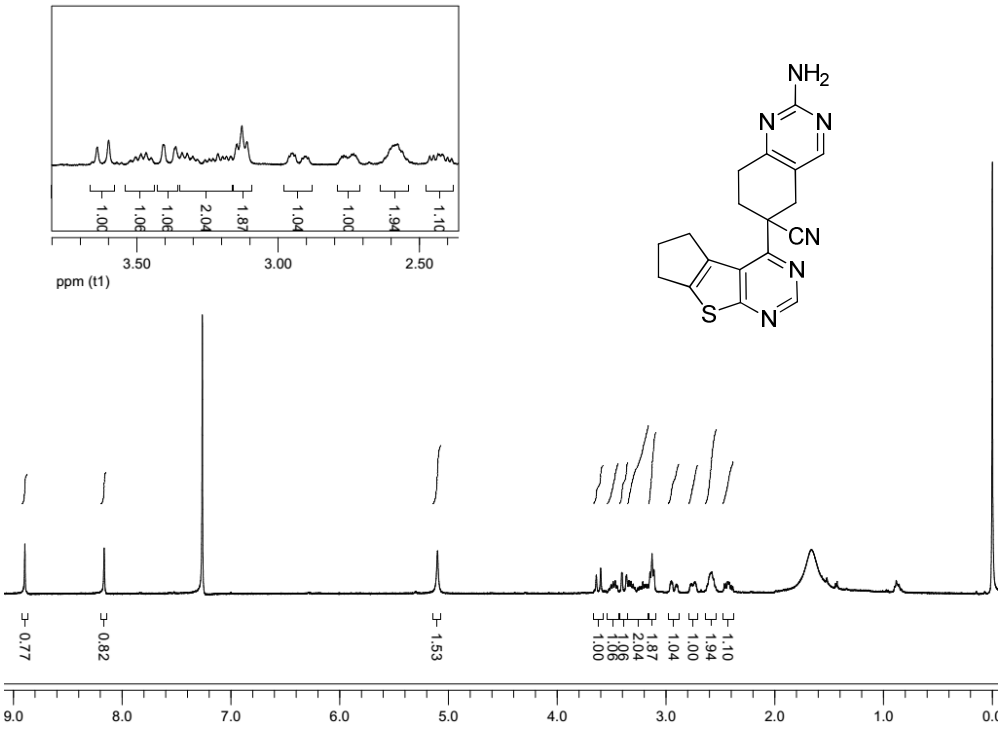
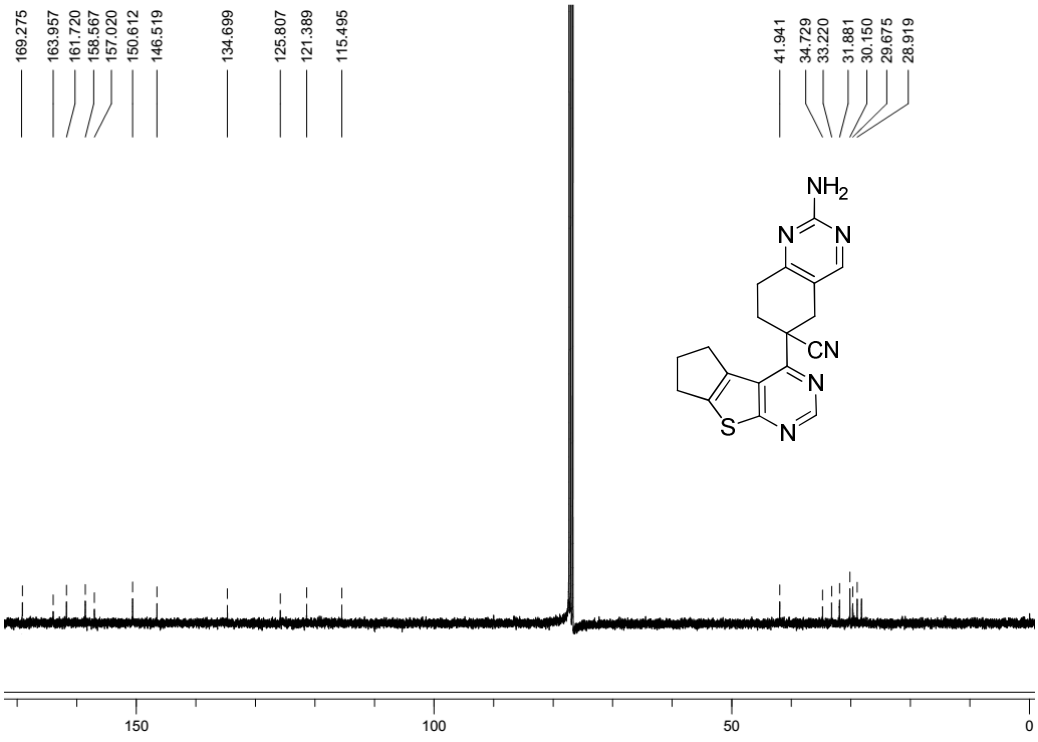
Total		12350599		100.000			

Chiral HPLC of **21a**



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



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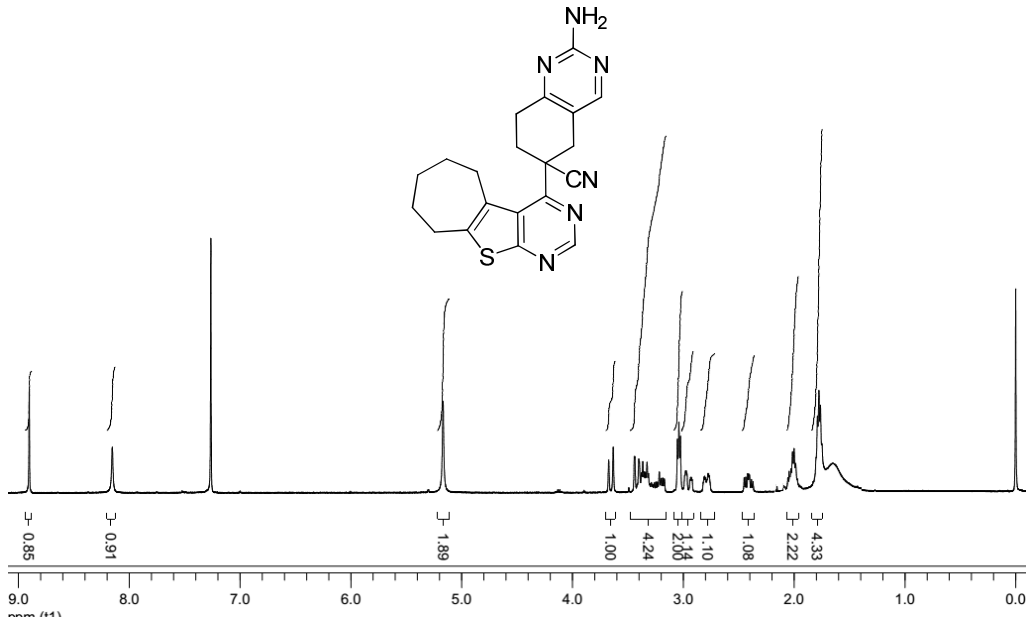

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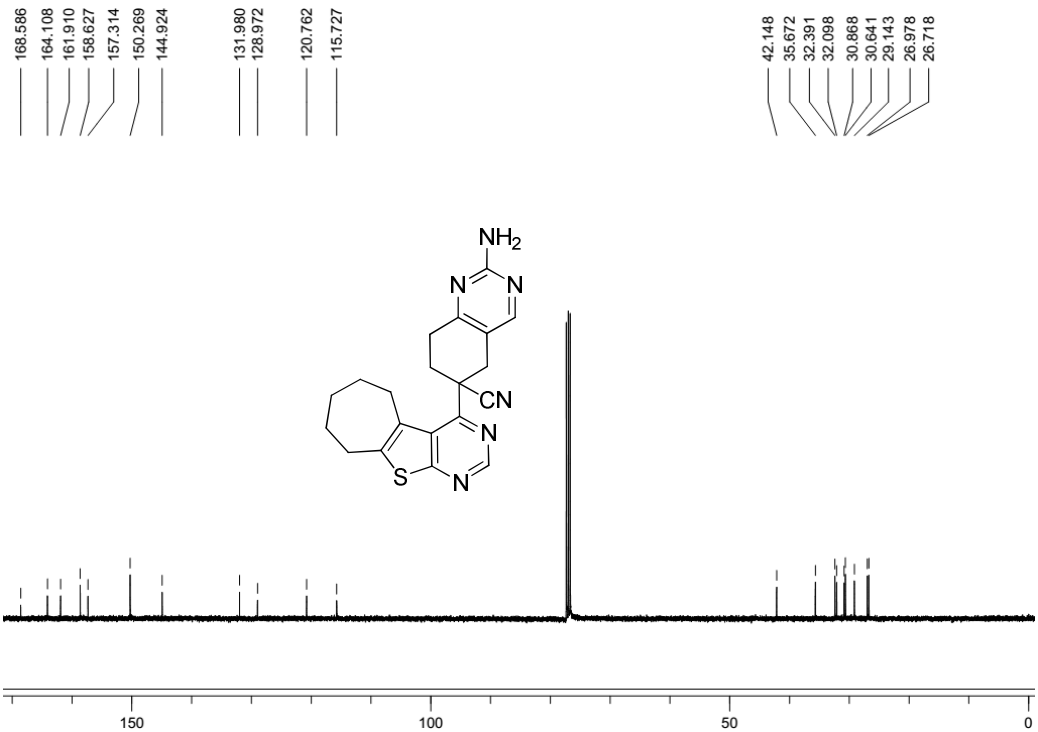
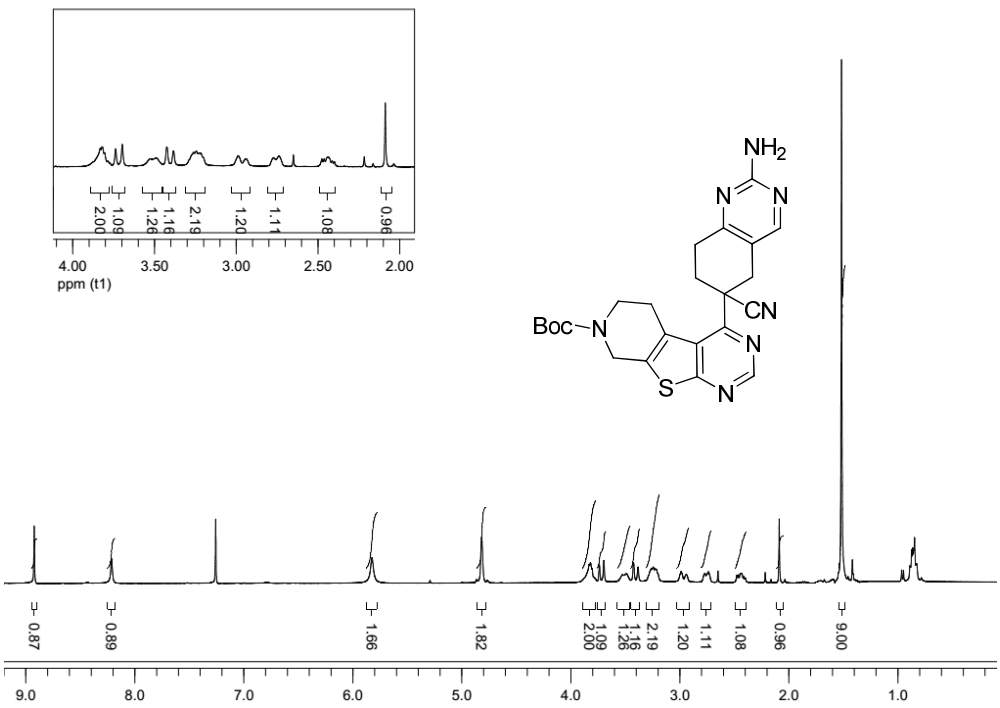


^{13}C NMR spectra of **21c** (100 MHz, $CDCl_3$)



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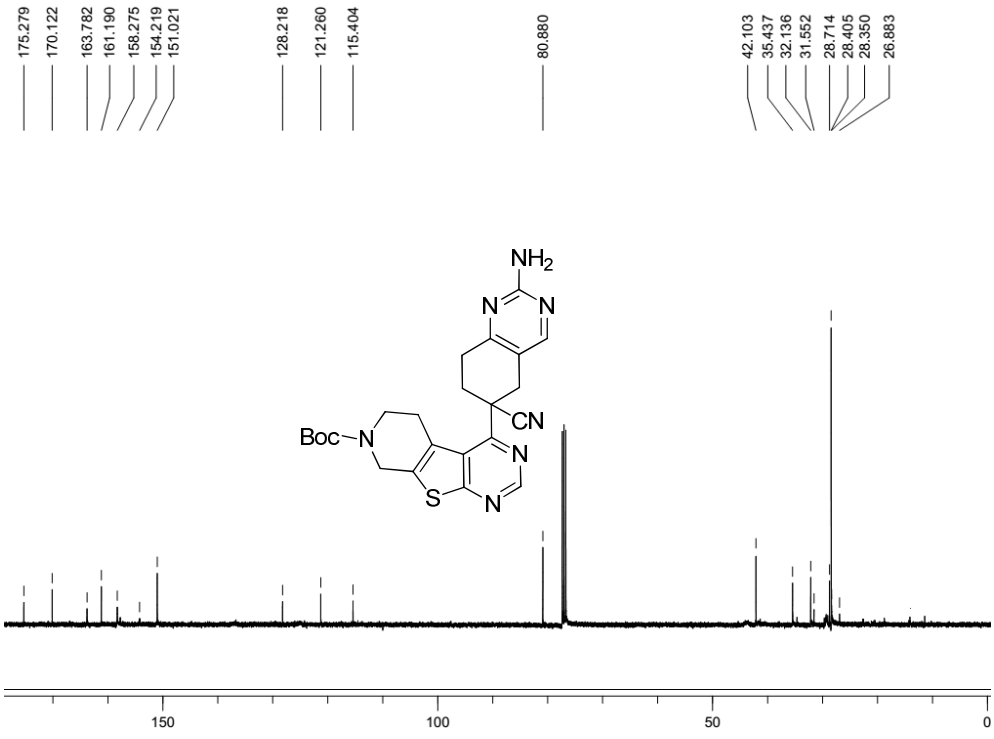

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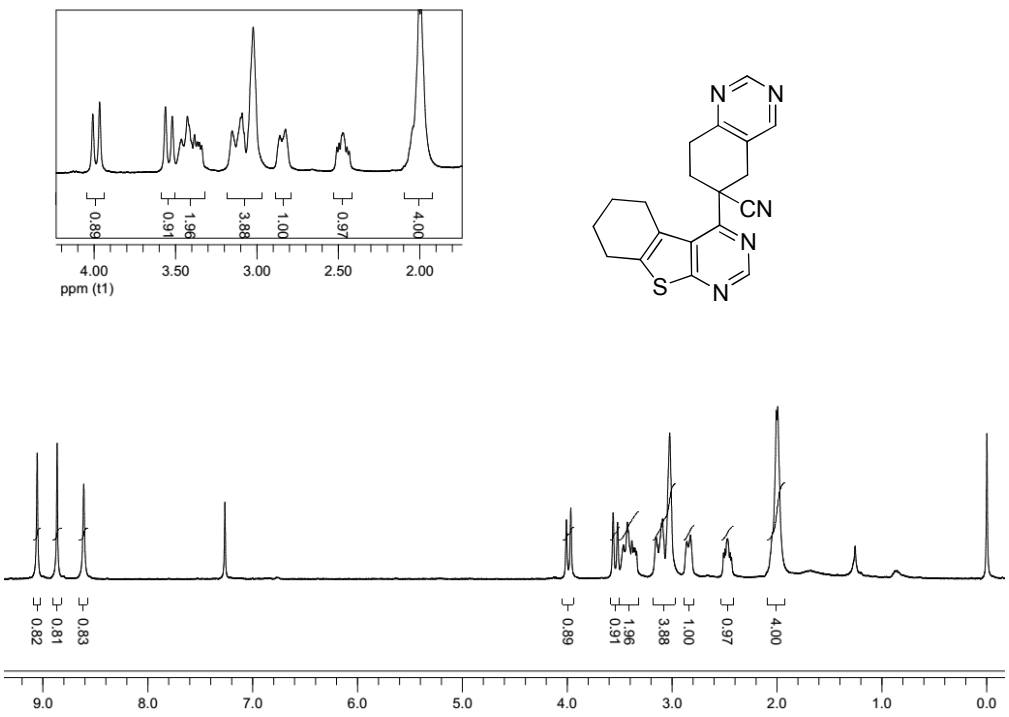
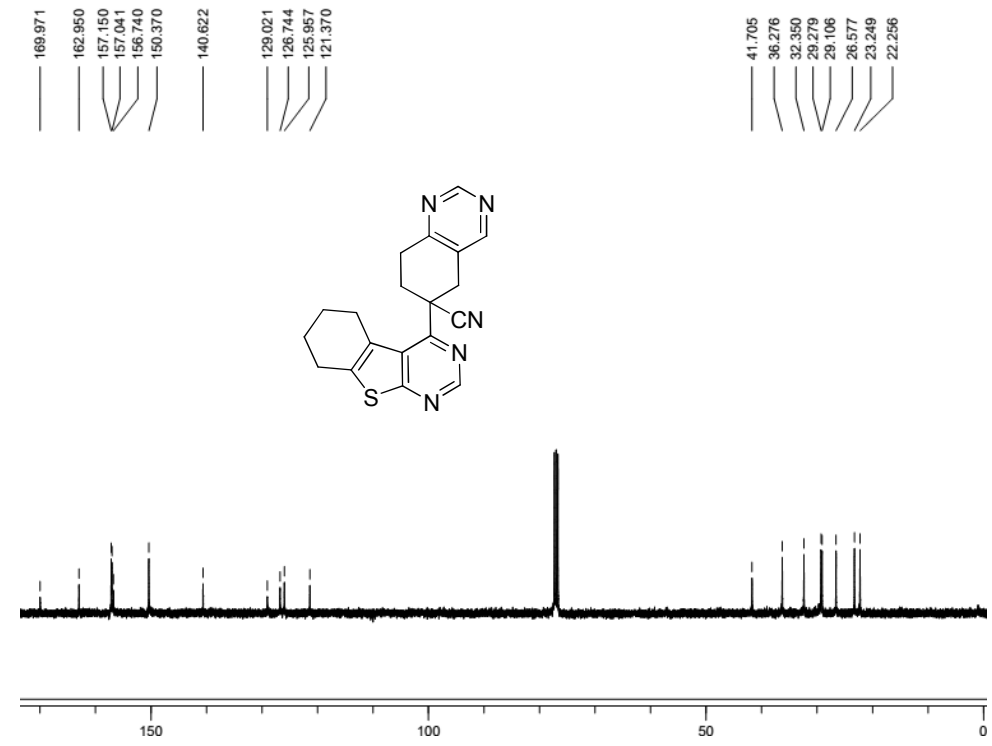
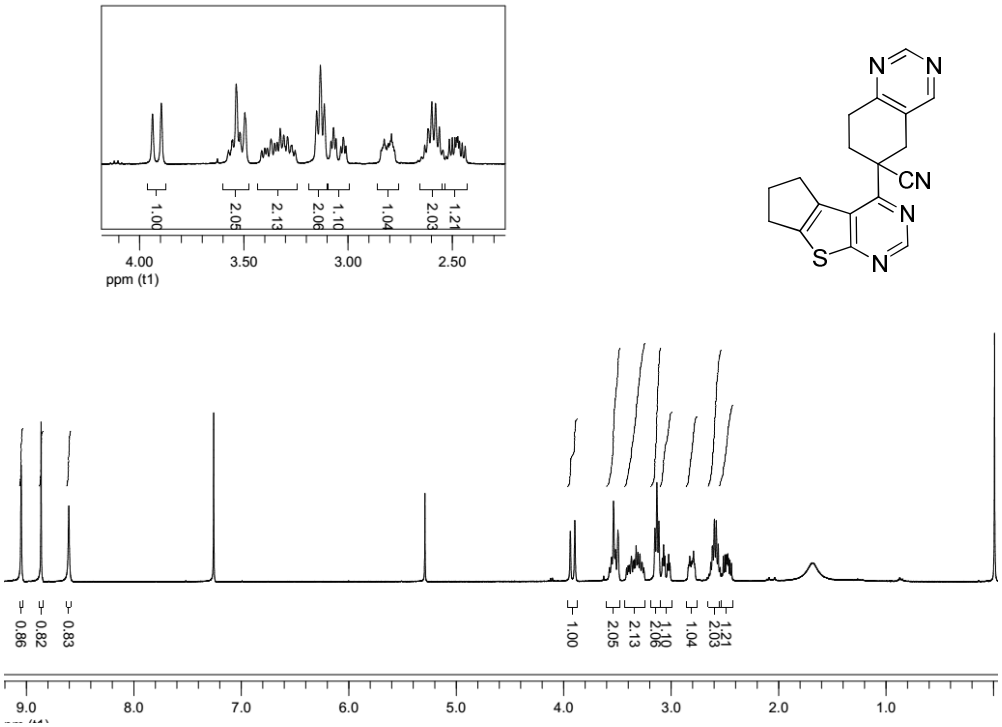


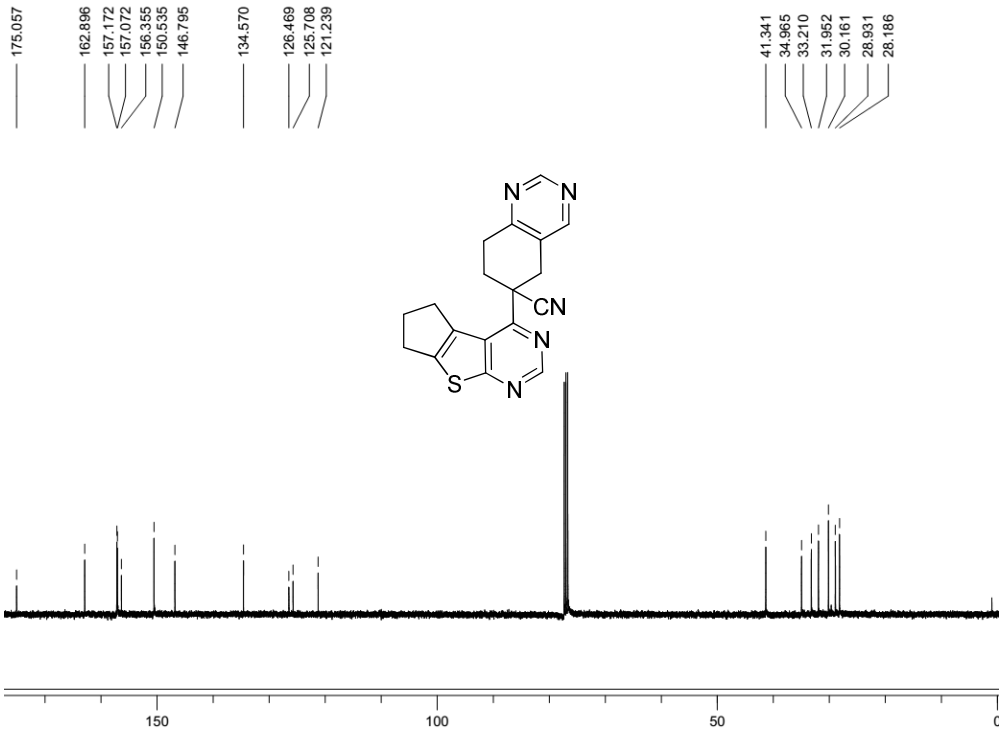
^{1}H NMR spectra of **22a** (400 MHz, $CDCl_3$)



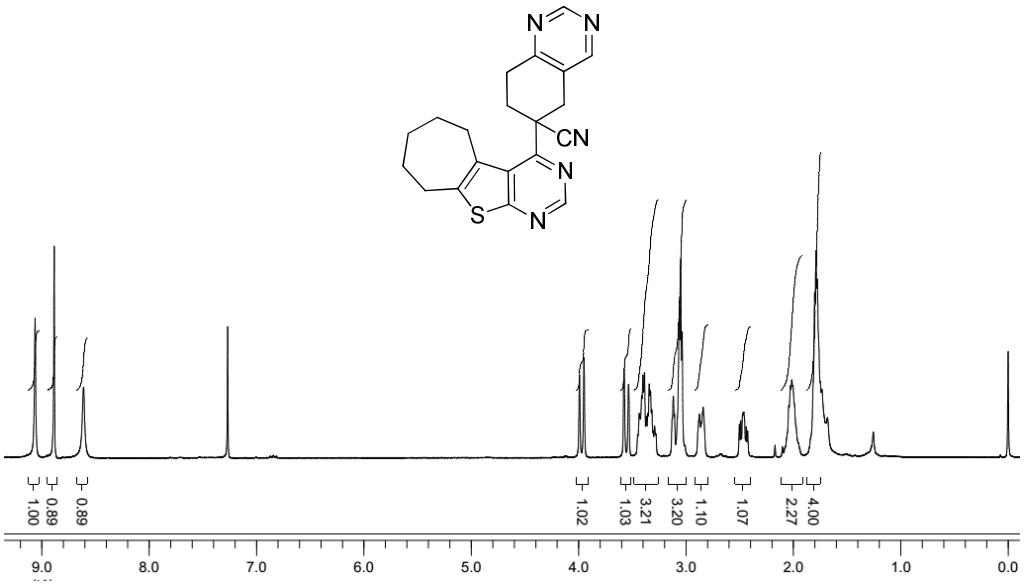
^{13}C NMR spectra of **22a** (100 MHz, $CDCl_3$)



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



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



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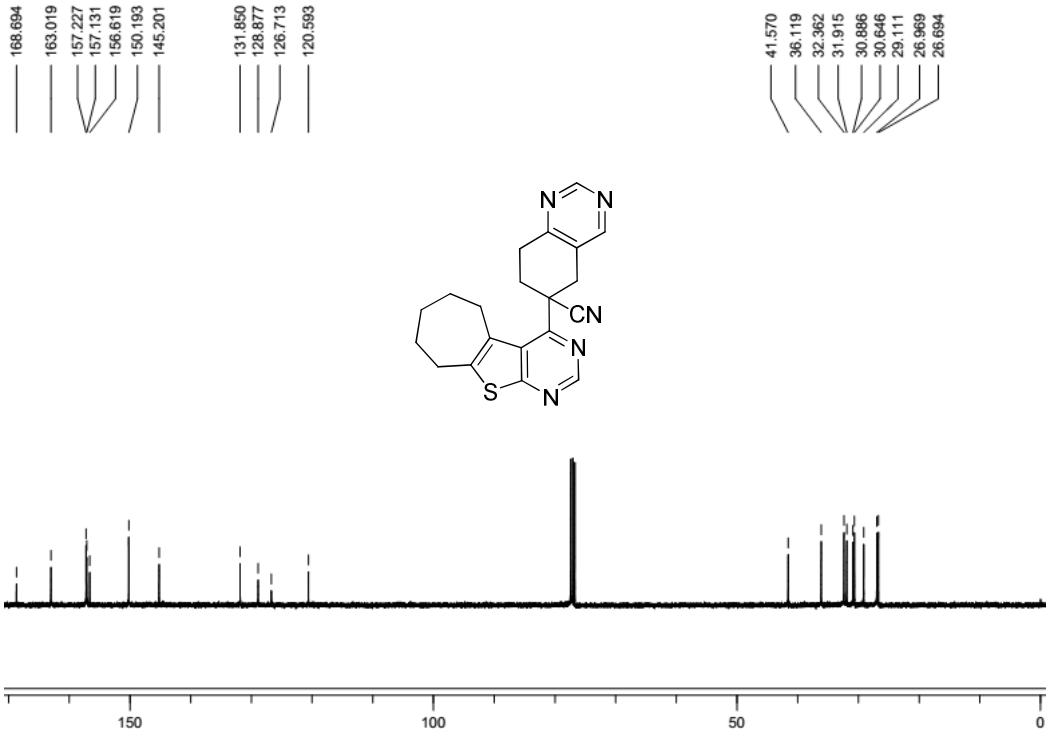

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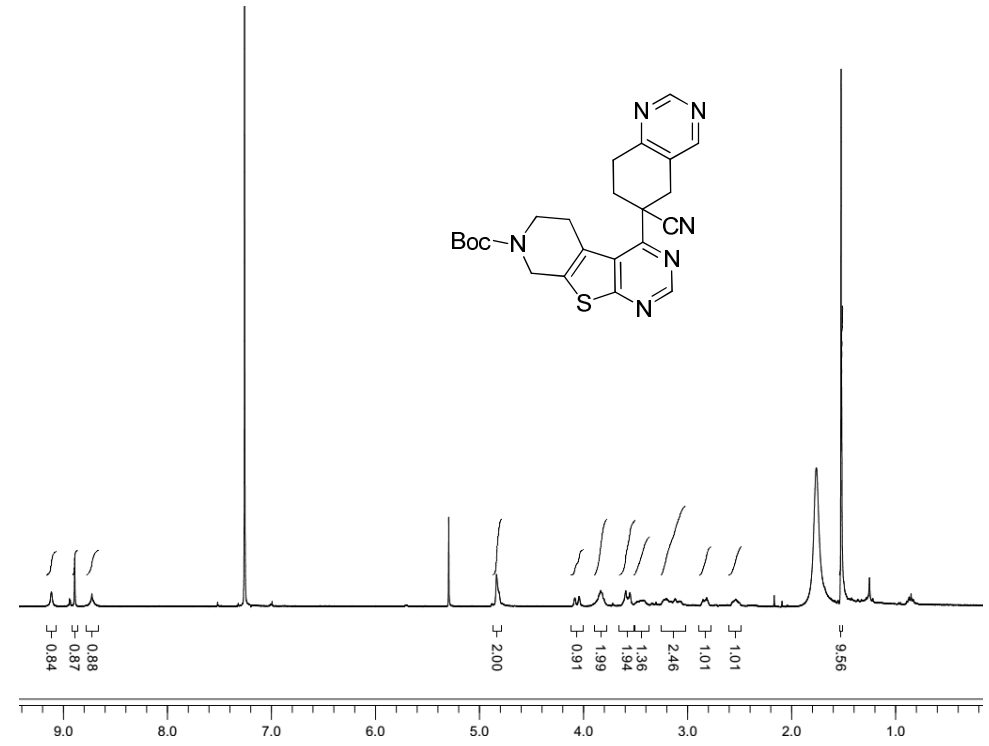

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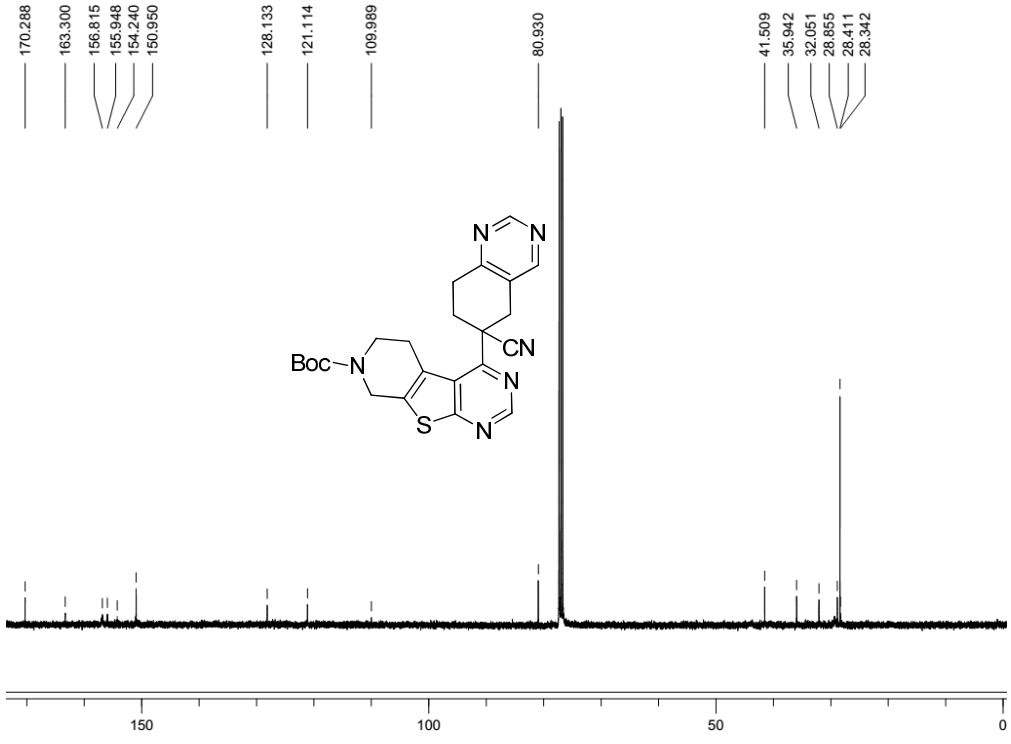

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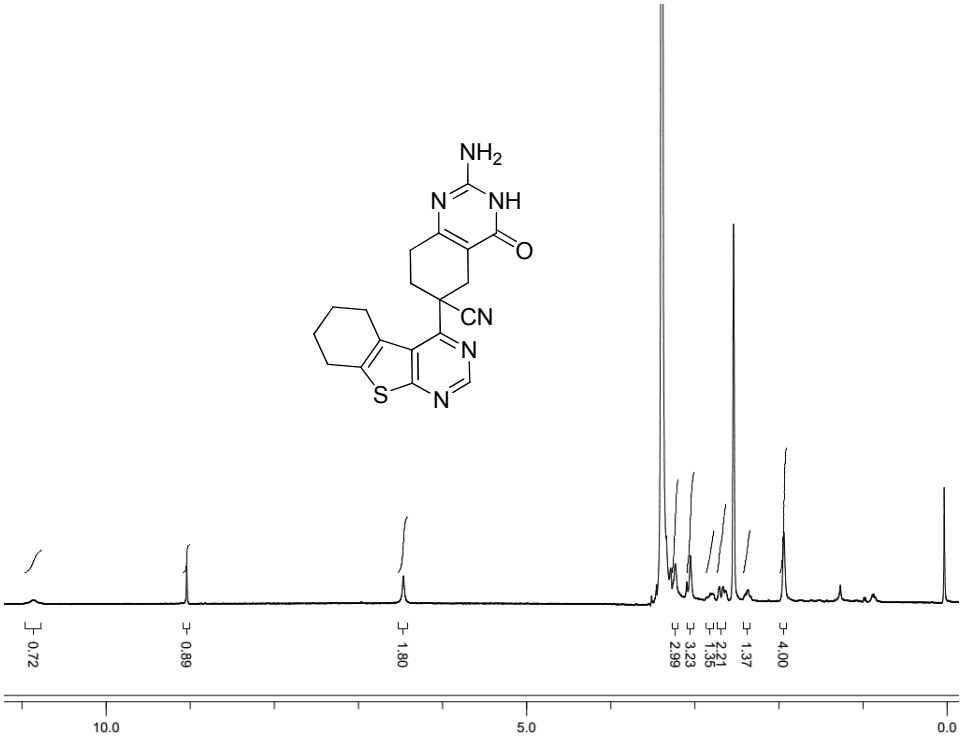
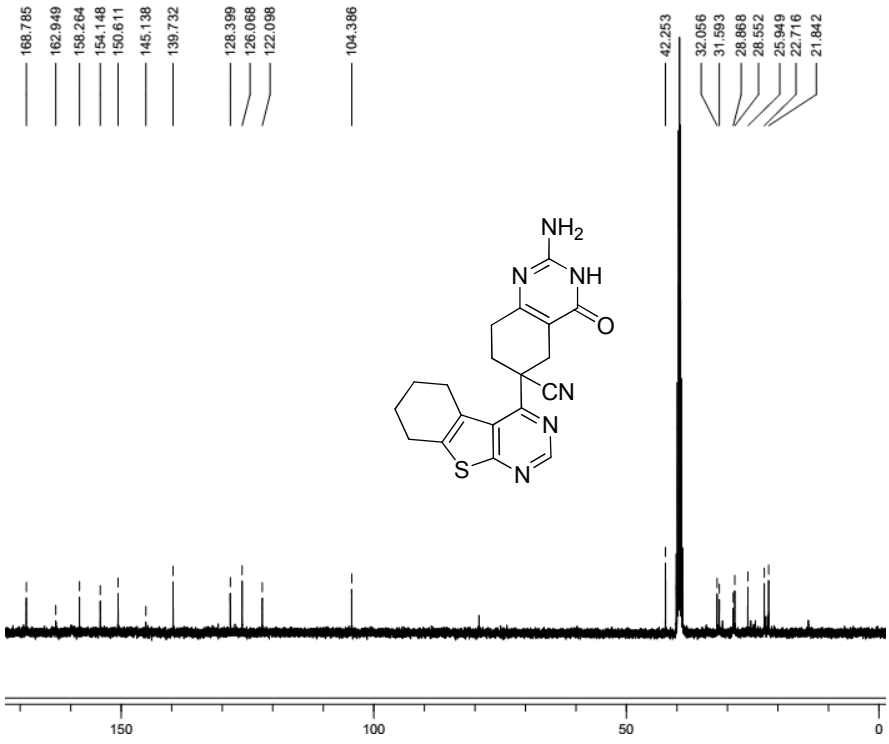
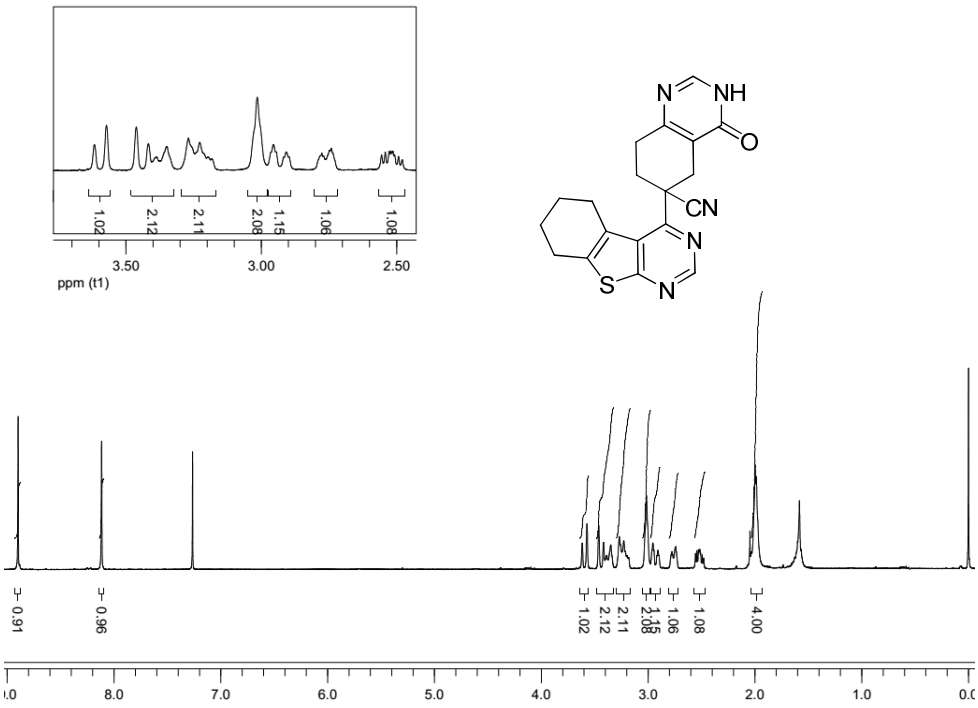


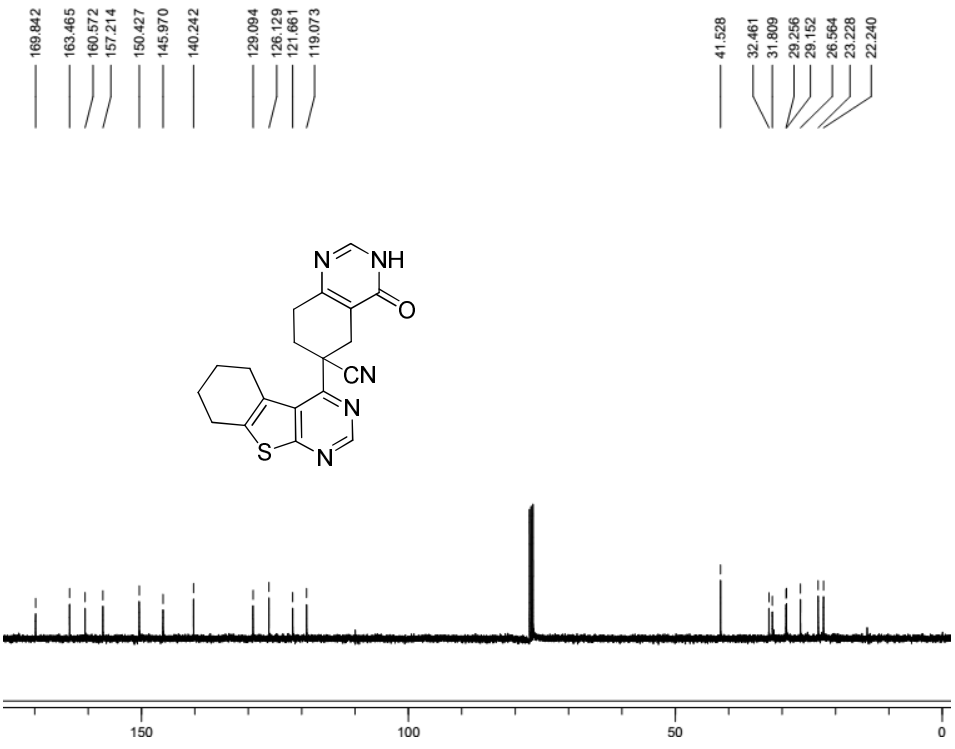
^{1}H NMR spectra of **23** (400 MHz, DMSO-d_6)



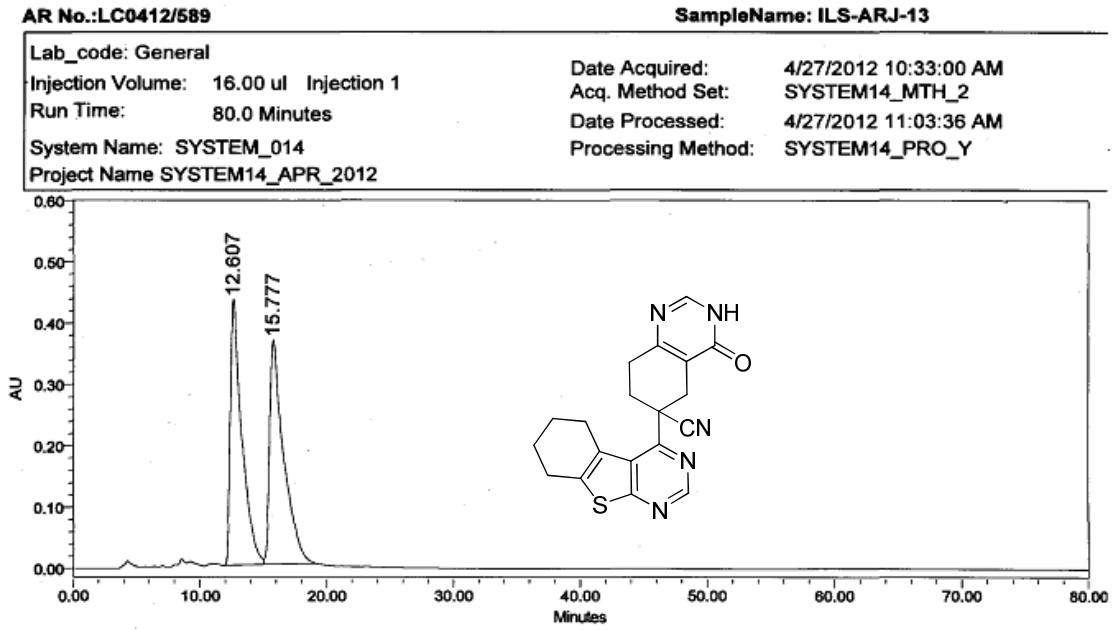
^{13}C NMR spectra of **23** (100 MHz, DMSO-d_6)



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



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



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Diluent: MeOH+MP (sonicated)
Wavelength: PDA 245.0 nm
Concn_mg_pr_mL NA

Isocratic information
A: B : 75 : 25

Flow rate: 0.8 mL/min

Peak results

	RT	Area	% Area
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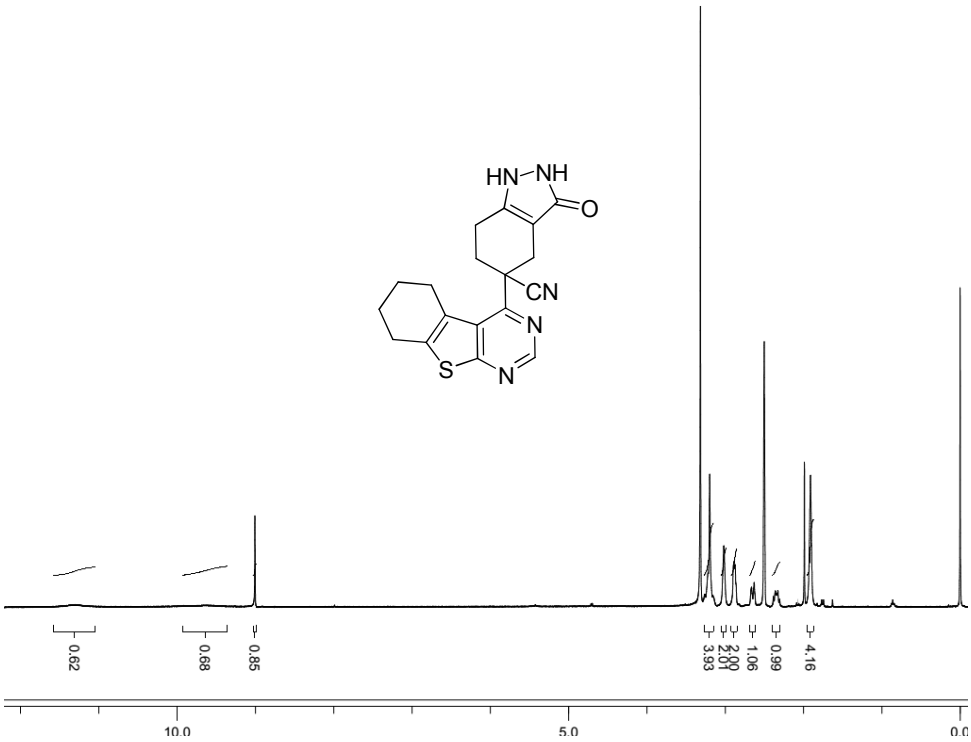
2	15.78	28251307	50.53

Chiral HPLC of **24**



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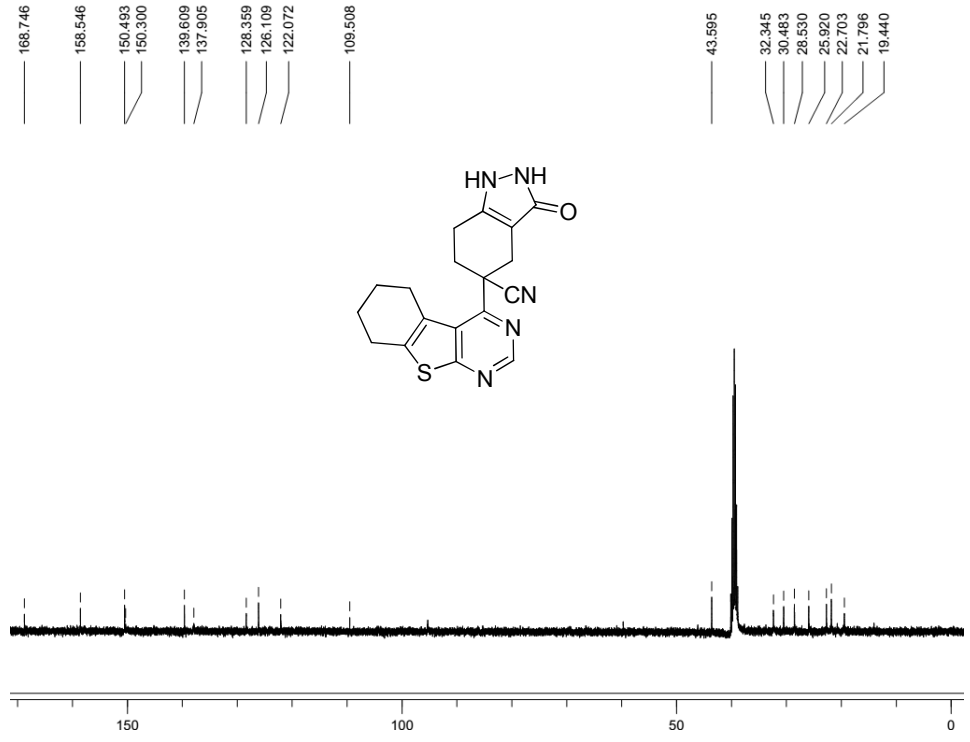
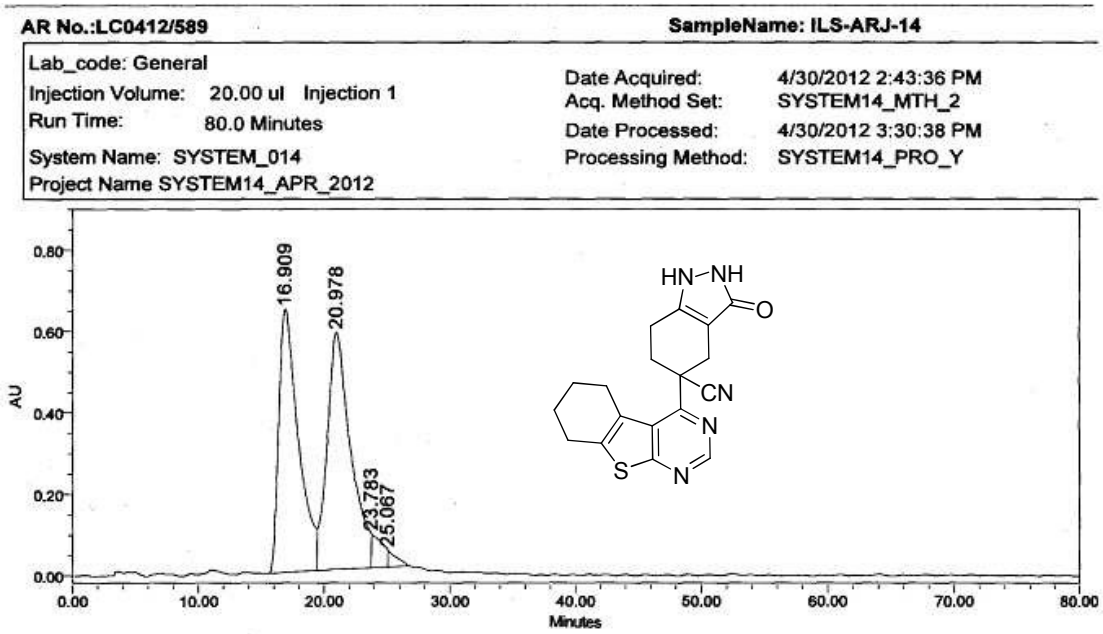


^{13}C NMR spectra of **25** (100 MHz, DMSO-d_6)



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MobilePhase_used: A:n-Hexane,D:EtOH(0.1%TFA)
Diluent: MeOH+MP (sonicated)
Wavelength: PDA 245.0 nm
Concn_mg_pr_mL NA

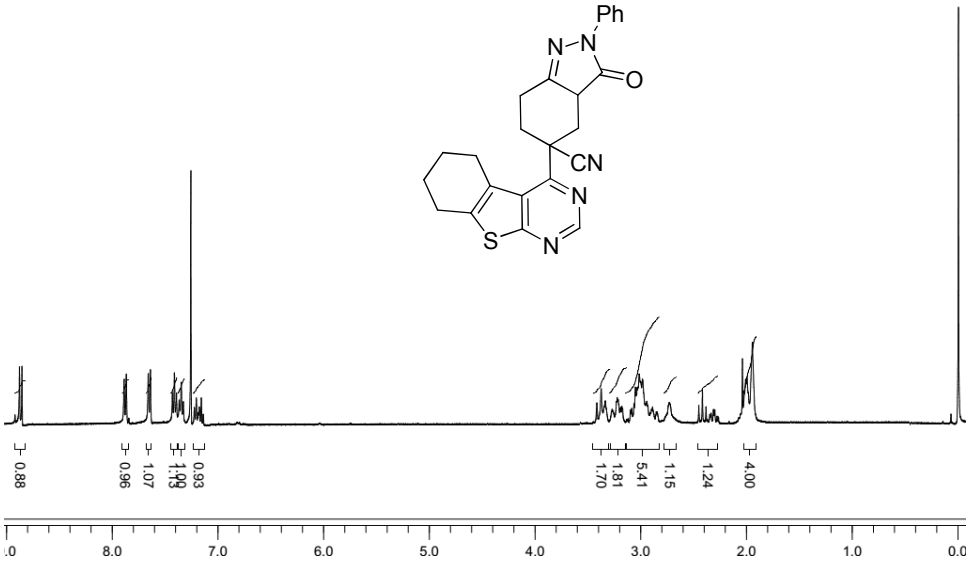
Isocratic information
A: D : 70 : 30

Flow rate: 0.8 mL/min

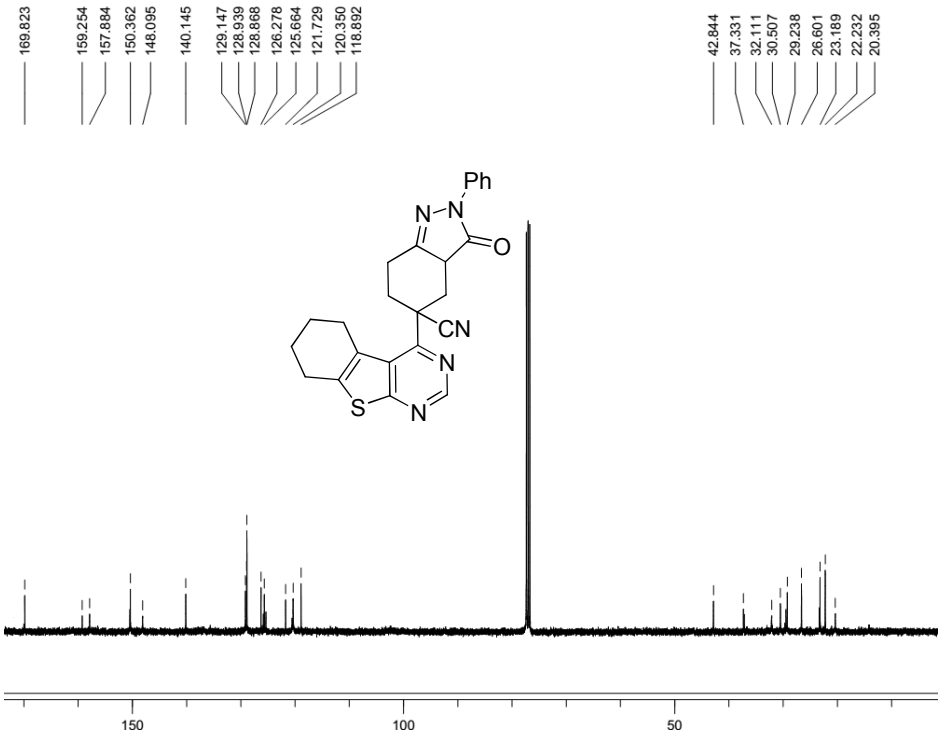
Peak results

	RT	Area	% Area
1	16.91	69901269	45.72
2	20.98	76156973	49.81
3	23.78	4905898	3.21
4	25.07	1937331	1.27

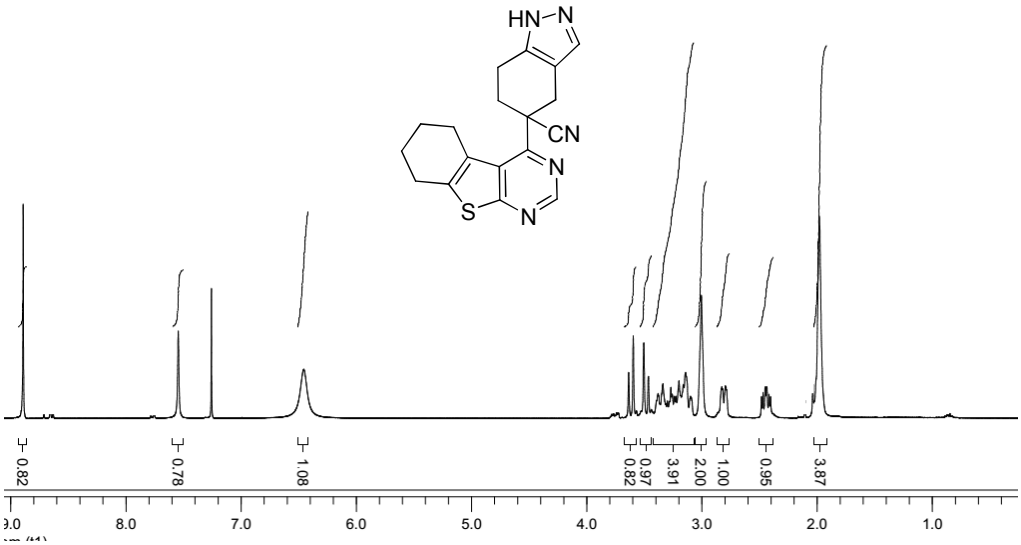
Chiral HPLC of **25**



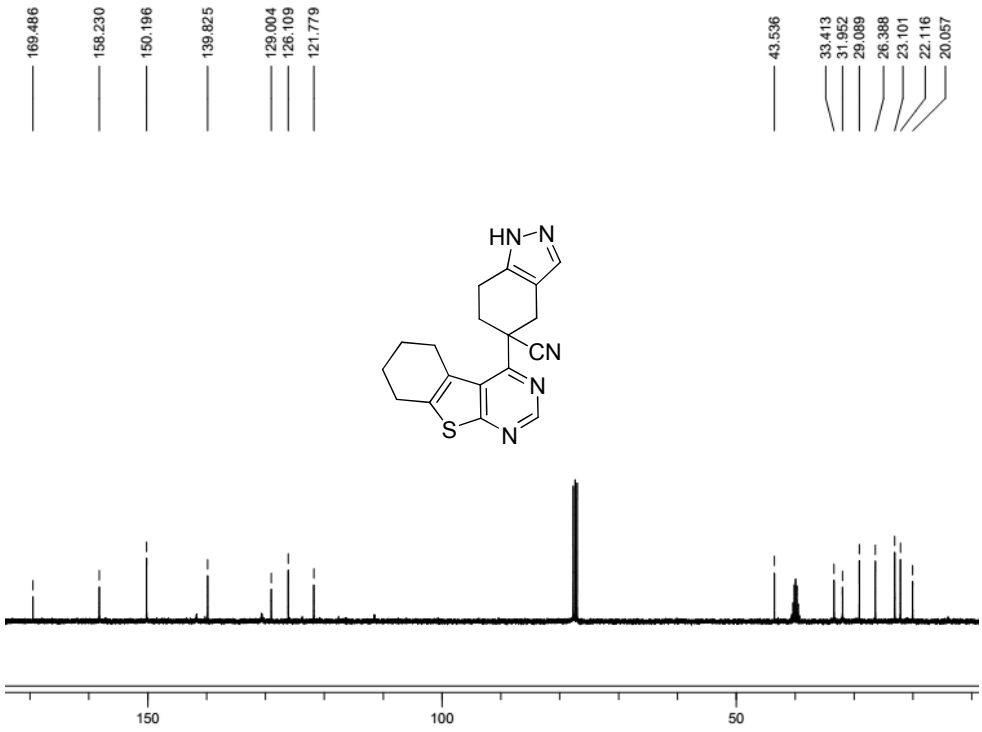
^{1}H NMR spectra of **26** (400 MHz, $CDCl_3$)



^{13}C NMR spectra of **26** (100 MHz, $CDCl_3$)



^{1}H NMR spectra of **27** (400 MHz, $CDCl_3$)

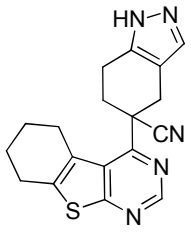


^{13}C NMR spectra of **27** (100 MHz, $CDCl_3$ + DMSO-d_6)

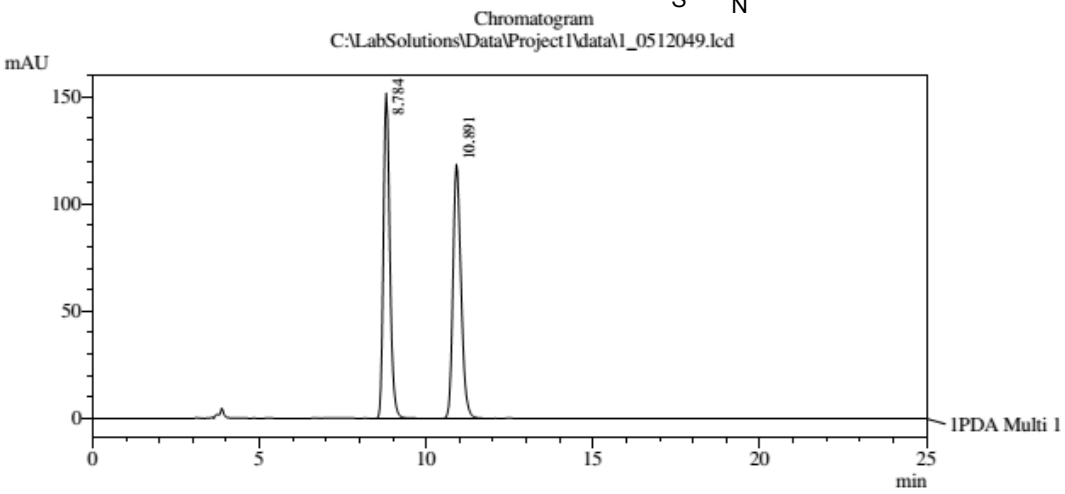
C:\LabSolutions\Data\Project1\data\1_0512049.lcd

Acquired by	: Chandrasekhar
Sample Name	:ILS-ARJ-16
Tray#	: 1
Vail #	: 54
Injection Volume	: 10 uL
Data File Name	: 1_0512049.lcd
Method File Name	: Method_2.lcm
Batch File Name	: 1_May_164.lcb
Data Acquired	: 5/4/2012 3:51:13 AM
Data Processed	: 5/4/2012 9:33:52 AM

Chromatogram conditions:
Column ID:CHIRALPAK IC(4.6 x 250 mm) 5µ
M.P:MeOH:DEA(100:0.1)
Flow:1.0 ml/min,Temp:25°C
Sample Conc:0.5mg/mL
Diluent:M.P



<Chromatogram>



1 PDA Multi 1 / 295nm 4nm

PeakTable

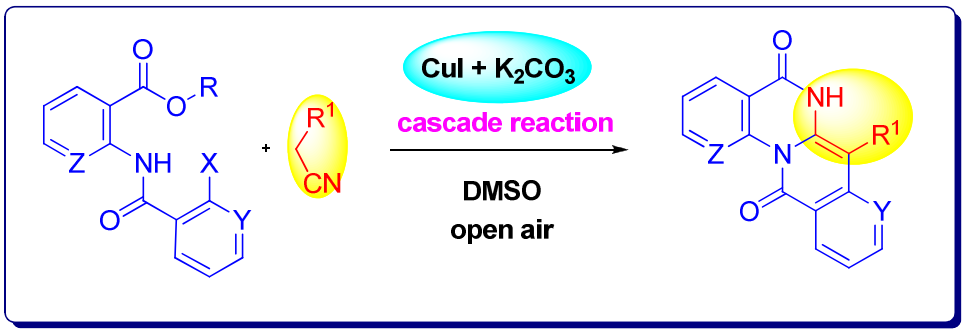
PDA Ch1 295nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Theoretical Plate#	Resolution	Tailing Factor
1	8.784	2004284	151763	50.060	7862.573	0.000	1.352
2	10.891	1999476	118523	49.940	7366.006	4.662	1.315
Total		4003760		100.000			

Chiral HPLC of **27**

CHAPTER 3

Copper catalyzed one pot synthesis of isoquinolino[2,3-a]quinazolines



3.1. Introduction:

Nitrogen heterocycles are widespread in many natural products and also found in biologically active molecules.[1] Synthesis of these heterocycles is an important goal in organic chemistry, as they are considered as privileged structures in drug discovery and development.[2] Among them, quinazoline derivatives found in natural products[3] (**1-10**, Figure 3.1) show various biological and pharmacological activities such as psychotropic, hypnotic, cardiotonic and antihistaminic properties. They also exhibit CNS (central nervous system) related effects as well as cardiovascular and anti-inflammatory activities.[4] They are used as potent antibacterial, antifungal, antiviral, anti mycobacterial and antimalarial agents.[5] Quinazoline derivatives are also known to inhibit various enzymes such as monoamine oxidase, aldose reductase, tumor necrosis factor α, and thymidylate synthase.[6]

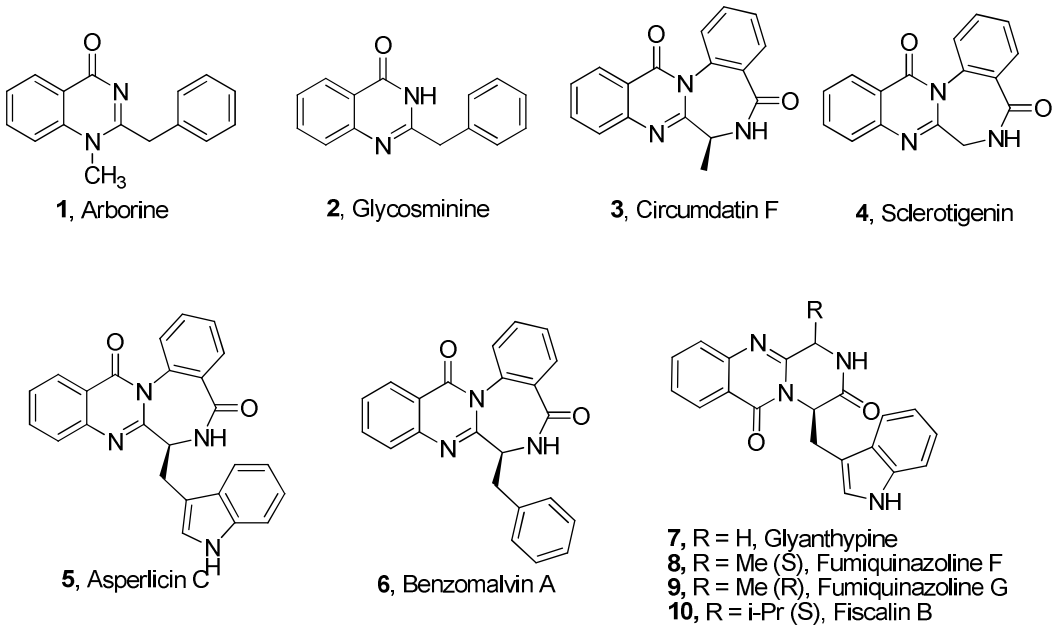


Fig. 3.1: Biologically active natural products containing quinazolinone scaffold.

On the other hand, isoquinolinone moiety is present in many natural products,[7] (**11-16**, Figure 3.2), and isoquinolinone derivatives are versatile building blocks for the total synthesis of several natural alkaloids.[8] Isoquinolinones also exhibit various pharmacological properties such as antihypertensive activity[9] and NK3 antagonism,[10] melatonin MT1 and MT2 receptor agonism,[11] Rho-kinase inhibition,[12] and JNK inhibition.[7c] They are also known as orally active 5-HT3 antagonists,[13] thymidylate synthase (TS) inhibitors,[14] and use for the treatment of stomach tumors and diseases

of human brain cells.[15] These features e.g. diverse biological properties including anti inflammatory and anti cancer activities have attracted the attention of medicinal / organic chemists towards the synthesis of isoquinolinone derivatives.

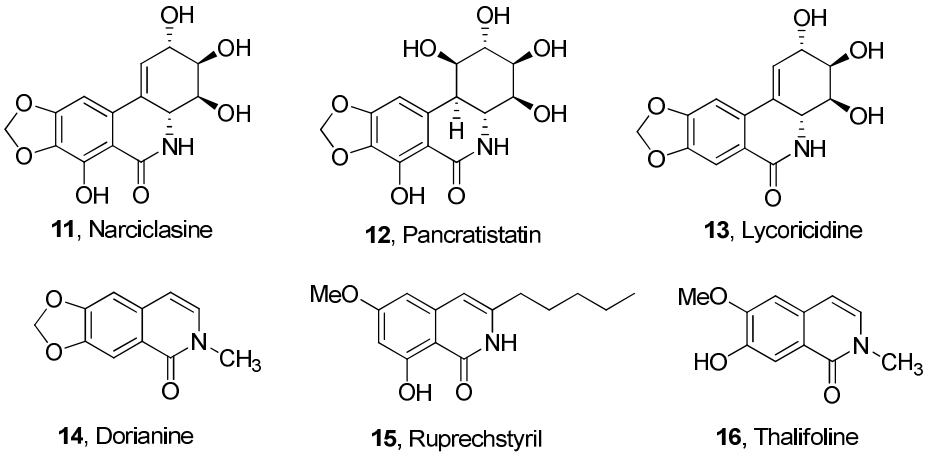


Fig. 3.2: Biologically active natural products containing isoquinolinone scaffold.

However, synthesis of the hybrid structure of isoquinoline and quinazoline motifs, namely isoquinolino[2,3-*a*]quinazolinone derivatives (Figure 3.3), are uncommon in literature.[16] Therefore, the development of a convenient and efficient synthetic approach towards isoquinolino[2,3-*a*]quinazolinone derivatives will be valuable for their screening against various biological targets.

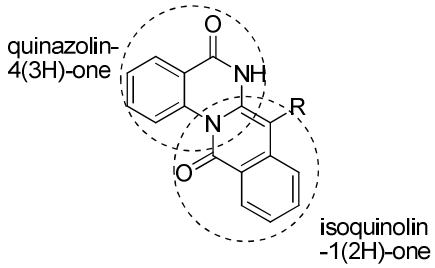


Fig. 3.3: Isoquinolino[2,3-*a*]quinazolinone as a hybrid structure of isoquinoline and quinazolinone motifs.

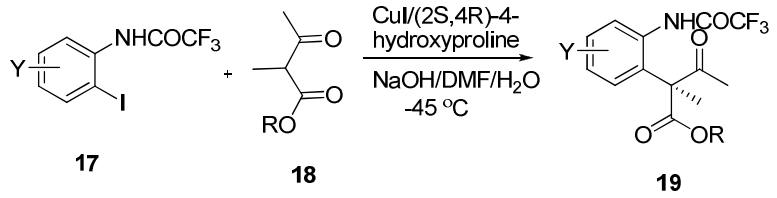
Over the past years, metal catalyzed cascade/domino reactions,[17] have occupied the center stage due to their ability to provide an array of diverse and novel compounds especially for medicinal/pharmaceutical uses or early drug discovery effort. Among them, a copper-catalyzed coupling reaction in C-C, C-heteroatom bond formation is an important transformation and has been developed to a wide range of substrates.[18] Recently, great advances have been achieved on the conceptual evolution of copper

catalyzed Ullmann-type reactions by using activated methylene groups to construct tricyclic and tetra heterocyclic ring systems. The earlier reports for copper catalyzed Ullmann type C-C bond formation, synthesis of *N*-heterocycles *via* copper catalyzed domino process and synthesis of substituted isoquinolino[2,3-*a*]quinazolines are discussed below.

3.2. Previous work:

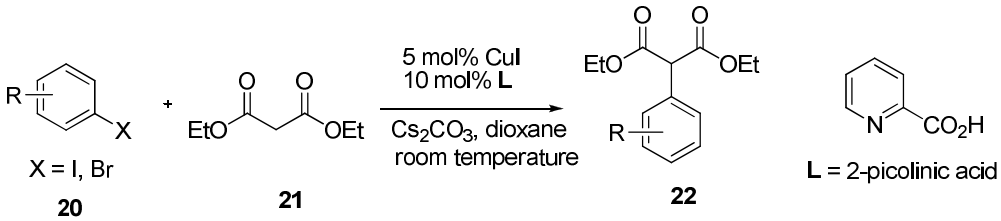
3.2.1. Some examples for copper catalyzed Ullmann type C-C bond formation.

In 2006, Ma and coworkers reported enantioselective synthesis of compound **19** from 2-iodo trifluoroacetanilides with 2-methylacetoacetates at -45 ºC using catalytic CuI and *trans*-4-hydroxy-L-proline as a ligand as shown in Scheme 3.1.[19] Up to 93% ee was achieved when *tert*-butyl ester was used. This is the first time Ullmann type coupling reaction was performed at low temperature.



Scheme 3.1: Copper catalyzed enantioselective synthesis of compound **19**.

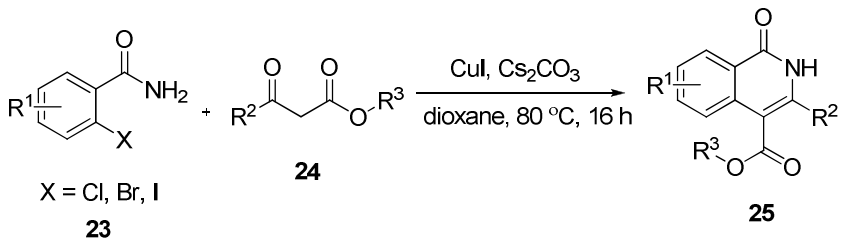
In 2007, Kwong and coworkers developed the synthesis of α-aryl malonates by the reaction of aryl iodides with diethyl malonate in the presence of a catalytic amount of 2-picolinic acid and CuI at room temperature as shown in Scheme 3.2.[20]



Scheme 3.2: Synthesis of α-aryl malonates from aryl halides and diethyl malonate.

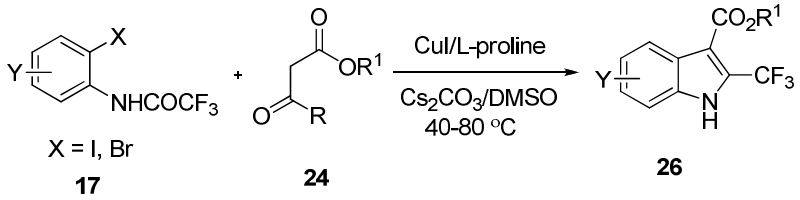
3.2.2. Recent examples for *N*-heterocycles construction *via* copper catalyzed domino process:

In 2009, Zhao and coworkers reported the synthesis of 3,4-disubstituted isoquinolin-1(2*H*)-one derivatives *via* cascade reactions of substituted 2-halobenzamides with β-keto esters under mild conditions using CuI as a catalyst without using any co catalyst or ligand as shown in Scheme 3.3.[21]



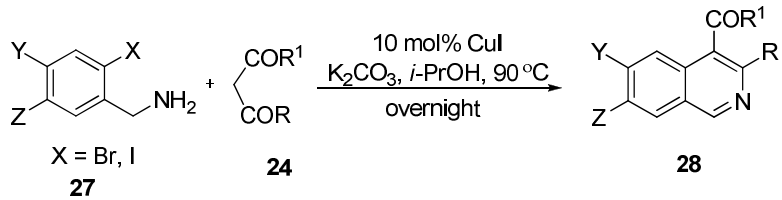
Scheme 3.3: Synthesis of 3,4-disubstituted isoquinolin-1(2*H*)-one from 2-halo benzamide.

In 2008, Ma and coworkers disclosed the CuI/L-proline-catalyzed coupling of 2-halotrifluoro acetanilides with β-keto esters in anhydrous DMSO under the action of Cs_2CO_3 at 40–80°C that produced poly substituted 2-(trifluoromethyl) indoles *via* coupling/condensation/deacylation mechanism as shown in Scheme 3.4.[22]



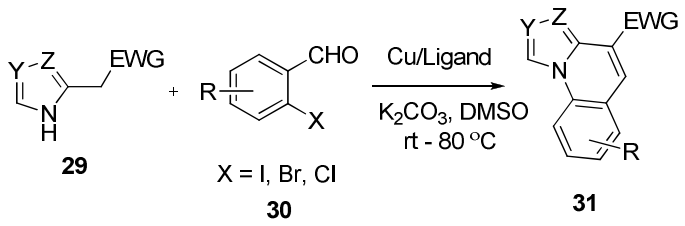
Scheme 3.4: Synthesis of 2-(triflouromethyl)indoles from 2-halotrifluoro acetanilides.

In 2008, the same group reported CuI-catalyzed coupling of 2-halobenzylamines with β-keto esters or 1,3-diketones in *i*-PrOH that produced 1,2-dihydroisoquinolines as the coupling/condensative cyclization products, which underwent smooth dehydrogenation under air to afford substituted isoquinolines as shown in Scheme 3.5.[23]



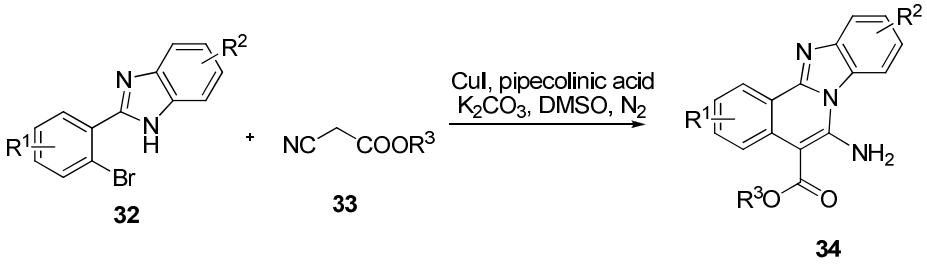
Scheme 3.5: Synthesis of 1,2-dihydroisoquinolines from 2-halo benzylamines.

In 2010, Ding and coworkers developed an efficient method for the synthesis of aza-fused polycyclic quinolines (e.g., benzimidazo[1,2-*a*]quinolines) as shown in Scheme 3.6.[24] This reaction proceeds *via* an intermolecular condensation followed by a copper-catalyzed intramolecular C-N coupling reaction.



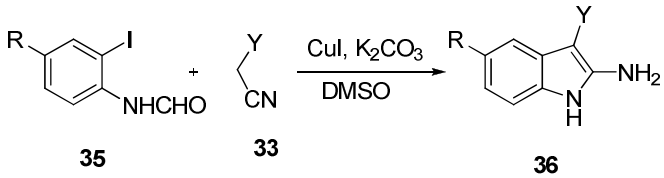
Scheme 3.6: Synthesis of aza fused quinolines from 2-halo benzaldehydes.

In 2011, Fu and coworkers developed a convenient and efficient copper-catalyzed cascade method for synthesis of benzimidazoisoquinoline derivatives *via* the reaction of readily available substituted 2-(2-halophenyl)benzoimidazoles with alkyl cyanoacetates under mild conditions as shown in Scheme 3.7.[21]



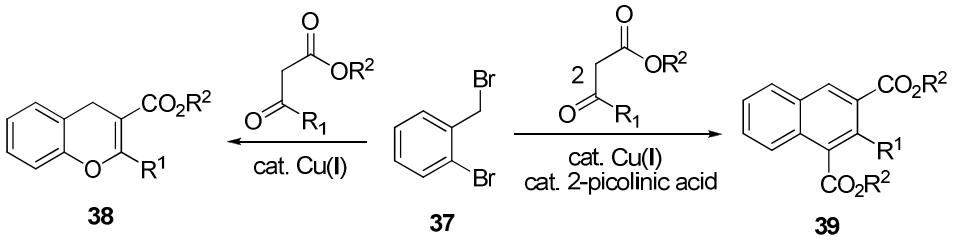
Scheme 3.7: Synthesis of benzimidazoisoquinoline from 2-(2-halophenyl)benzoimidazoles

In 2011, Konishi and coworkers reported the synthesis of 2-aminoindole-3-carbonitriles and 2-aminoindole-3-carboxylates by the reaction of *N*-(2-iodophenyl)formamides with malononitrile and cyanoacetates, respectively, in the presence of a catalytic amount of copper(I) iodide using potassium carbonate as a base as shown in Scheme 3.8.[25]



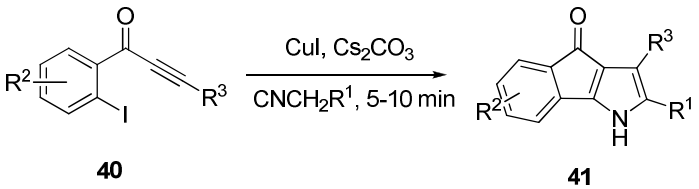
Scheme 3.8: Synthesis of 2-aminoindole-3-carbonitriles/carboxylates from *N*-(2-iodophenyl)formamides

In 2011, Beifuss and coworkers developed a methodology for the synthesis of 4*H*-chromenes and napthalenes by maintaining the ratio of the substrates and reaction conditions using Cu catalyzed domino reaction between bromobenzyl bromides and β-ketoesters as shown in Scheme 3.9.[26]



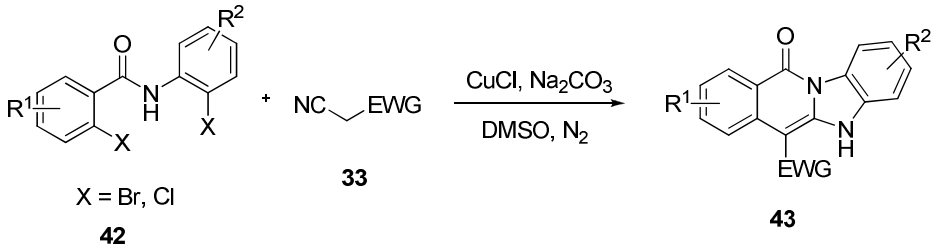
Scheme 3.9: Synthesis of 4*H*-chromenes (**38**) and napthalenes (**39**) from 2-bromobenzylbromide

In 2011, Ding and coworkers disclosed a novel copper-catalyzed tandem reaction of 1-(2-iodoaryl)-2-yn-1-ones with isocyanides for the synthesis of 4-oxo-indeno[1,2-*b*]pyrroles as shown in Scheme 3.10.[27] The reaction proceeded through a formal [3+2] cycloaddition/coupling tandem process.



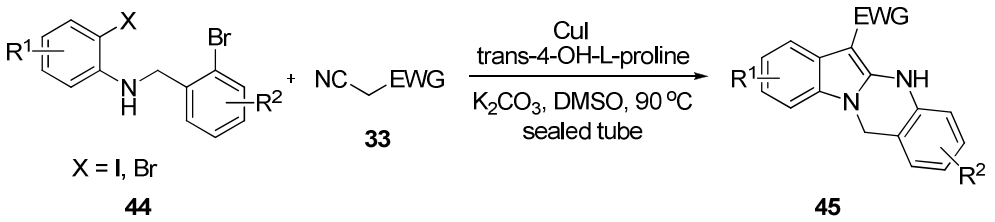
Scheme 3.10: Synthesis of 4-oxo-indeno[1,2-*b*]pyrroles from 1-(2-iodoaryl)-2-yn-1-ones.

In 2010, Fu and coworkers reported a copper-catalyzed one-pot tandem method for synthesis of benzimidazo[1,2-*b*]isoquinolin-11-one derivatives *via* the reaction of substituted 2-halo-*N*-(2-halophenyl)benzamides with alkyl 2-cyanoacetates or malononitrile under mild conditions as shown in Scheme 3.11.[28]



Scheme 3.11: Synthesis of benzimidazo[1,2-*b*]isoquinolin-11-one from 2-halo-*N*-(2-halophenyl)benzamide.

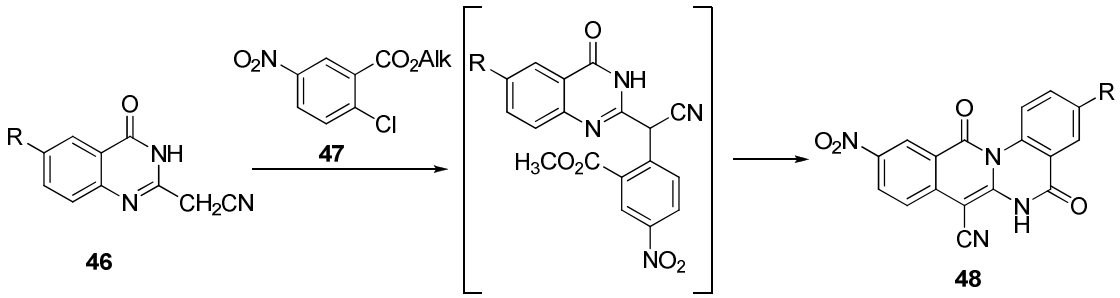
In 2012, Zhao and coworkers developed a domino synthesis of 5,12-dihydroindolo[2,1-*b*]quinazoline derivatives *via* the copper-catalyzed Ullmann-type intermolecular C-C and intramolecular C-N couplings as shown in Scheme 3.12.[29]



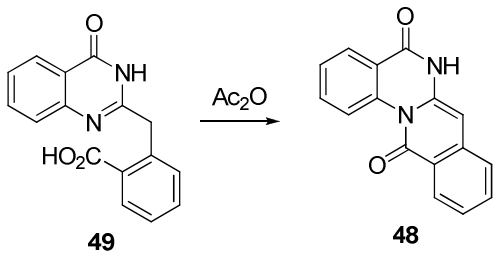
Scheme 3.12: Synthesis of 5,12-dihydroindolo[2,1-*b*]quinazoline from *N*-(2-bromobenzyl)-2-haloaniline.

3.2.3. Earlier reports for the synthesis of isoquinolino[2,3-*a*]quinazolinone:

In 1997, Volovenko reported the synthesis of isoquino[2,3-a]quinazolines by the reaction of alkyl 2-chloro-5-nitrobenzoates with 2-cyanomethylquinazolones in boiling dimethylformamide in the presence of an equimolar amount of potash as shown in Scheme 3.13.[16a]



Scheme 3.13: Synthesis of substituted isoquino[2,3-*a*]quinazolines from 2-cyano methyl quinazolones.

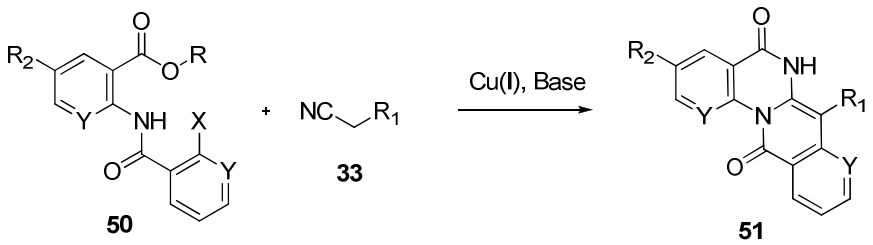


Scheme 3.14: Synthesis of 5*H*-Isoquino[2,3-*a*]quinazoline-5,12-(6*H*)-dione from 4-oxo quinazoline acid.

In 2008, Kucherenko and coworkers reported the synthesis of 5*H*-isoquino[2,3-*a*]quinazoline-5,12-(6*H*)-dione from 4-oxoquinazoline acid by treatment with acetic anhydride as shown in Scheme 3.14.[16b]

3.3. Present work:

Earlier from our group, we identified fused *N*-heterocycle containing isoquinolinone and quinazolinone scaffold as PDE4-4/ TNF-α inhibitors.[30] In continuation of this work we became interested in the synthesis of isoquino[2,3-*a*]quinazoline scaffold (Figure 3.3). While chemistry of quinazolin-4(3*H*)-ones and isoquinolin-1(2*H*)-ones is well documented their combined form as shown in Figure 3.3 remained unexplored. To synthesize our target molecule, we envisioned that Cu-mediated C-arylation of substituted nitriles on compound **50**, which subsequently undergo intramolecular nucleophilic addition of NH to CN followed by intramolecular nucleophilic attack by amine to ester group, may allow the formation of a fused ring leading to our target compound **51**. Accordingly, we have developed a new and versatile Cu-mediated domino reaction leading to one-pot synthesis of **51** under mild conditions without using any co-catalyst, ligand or additive. Herein we report the results of our study (Scheme 3.15).



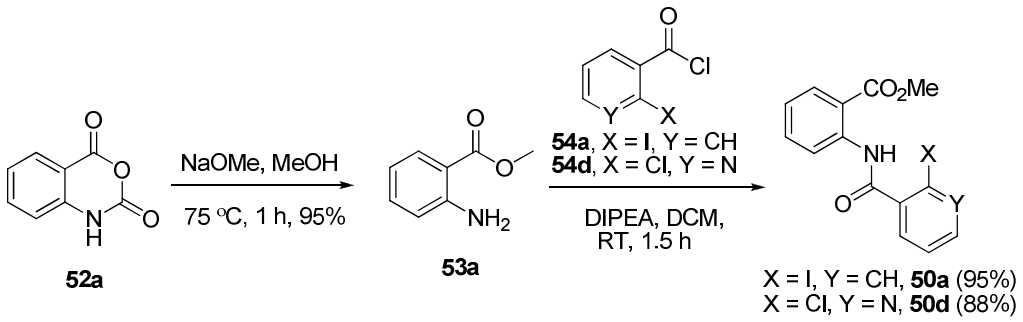
Scheme 3.15: Synthesis of isoquino[2,3-*a*]quinazoline scaffold from alkyl 2-(2-halobenzamido)benzoate.

3.4. Results and discussion:

3.4.1. Preparation of starting materials:

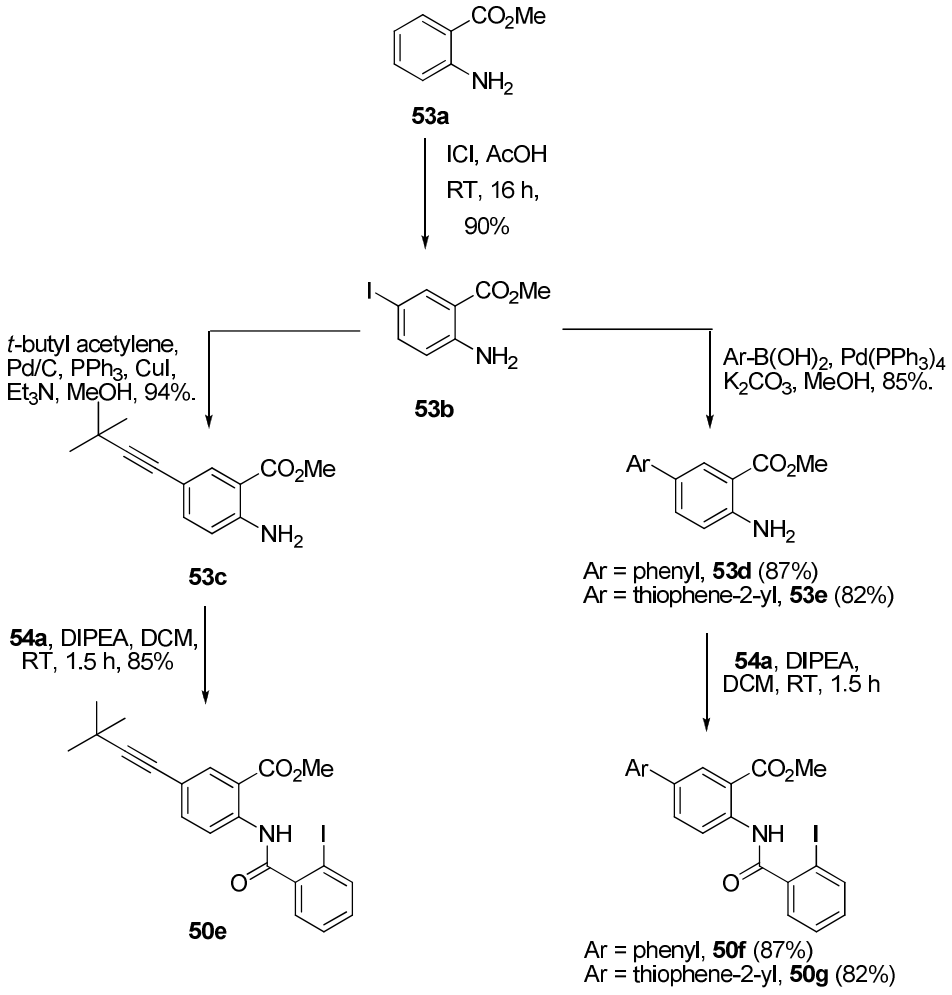
The required starting material alkyl 2-(2-halobenzamido)benzoate, **50** was synthesized from the isatoic anhydride **52a**. Opening of isatoic anhydride with sodium methoxide in methanol provided methyl 2-amino benzoate, **53a** which on coupling

with corresponding acid chloride, **54** which was synthesized from the corresponding acid provided **50a** and **50d** as shown in Scheme 3.16.



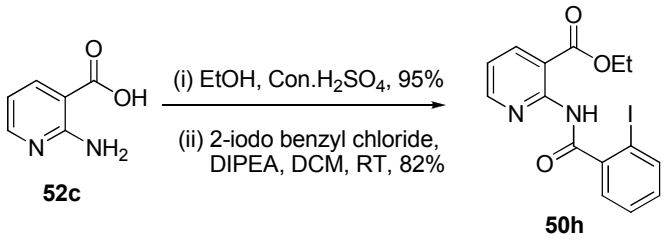
Scheme 3.16: Synthesis of methyl 2-(2-halobenzamido)benzoate.

Iodination on methyl 2-amino benzoate,[31] followed by Sonogashira coupling / Suzuki coupling provided **53c** and **53d-e**, respectively. Then coupling with acid chloride, **54a** afforded compound **50e** and **50f-g**, respectively (Scheme 3.17).

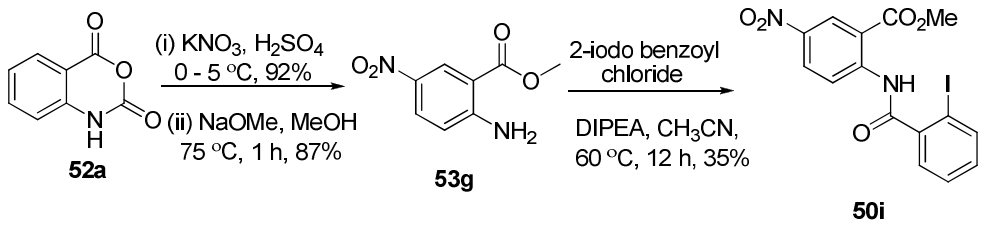


Scheme 3.17: Synthesis of methyl 2-(2-iodobenzamido)-5-(alkynyl/aryl)benzoate.

Ethyl 2-(2-iodobenzamido)nicotinate, **50h** was synthesized from the 2 aminonicotinic acid in 2-steps *i.e.,* esterification and coupling reaction as shown in Scheme 3.18. Nitration of isatoic anhydride provided 4-nitro isatoic anhydride[32] which on esterification and coupling reaction as mentioned above afforded the required starting material **50i** (Scheme 3.19).



Scheme 3.18: Synthesis of ethyl 2-(2-iodobenzamido)nicotinate.

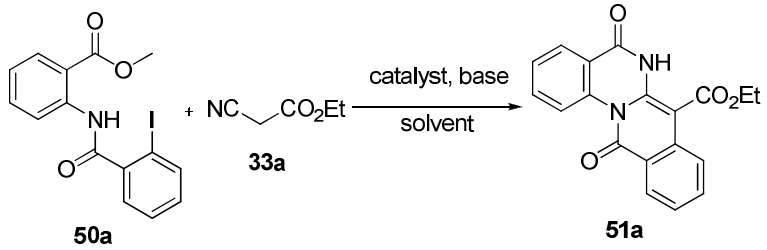


Scheme 3.19: Synthesis of methyl 2-(2-iodobenzamido)-5-nitrobenzoate.

3.4.2. Reaction optimization:

Initially the coupling of methyl 2-(2-iodobenzamido)benzoate (**50a**) with ethyl cyanoacetate (**33a**) was used to establish the optimized reaction conditions including catalysts, base and solvents. The reaction was screened by using 0.1equiv. of CuI and 3.0 equiv. of K_2CO_3 in DMSO that provided the highest yield of desired product (entry 1, Table 3.1). By replacing K_2CO_3 with Na_2CO_3, decreased the product yield (entry 2, Table 3.1) whereas the effect of Cs_2CO_3 was found to be the same as K_2CO_3 (entry 3, Table 3.1). Among solvent selection DMSO was found to be much better than DMF and 1,4-dioxane (entry 5-6, Table 3.1) whereas toluene was not suitable for this reaction (entry 7, Table 3.1). Other copper source like CuBr was found to be similar with CuI (entry 8, Table 3.1) whereas CuCl provided less yield of product (entry 9, Table 3.1). No target compound was formed in the absence of catalyst (entry 4, Table 3.1).

Table 3.1: Reaction conditions and optimization.



Entry[a]	Catalyst	Base	Solvent	Yield[b] (%)
1	CuI	K_2CO_3	DMSO	87
2	CuI	Na_2CO_3	DMSO	71
3	CuI	Cs_2CO_3	DMSO	86
4	-	K_2CO_3	DMSO	0[c]
5	CuI	K_2CO_3	DMF	74
6	CuI	K_2CO_3	1,4-Dioxane	46
7	CuI	K_2CO_3	Toluene	0
8	CuBr	K_2CO_3	DMSO	81
9	CuCl	K_2CO_3	DMSO	69

[a]Reactions were carried out using **50a** (1 mmol), **33a** (1.2 mmol), catalyst (0.1 mmol) and base (3 mmol) in solvent (2 mL) at 85 ºC for 3 h under anhydrous conditions.

[b]Isolated yield.

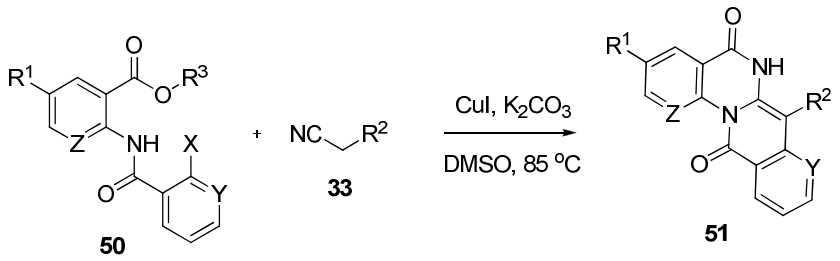
[c]No addition of catalyst.

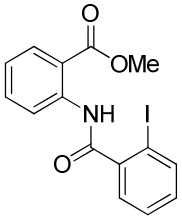
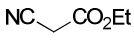
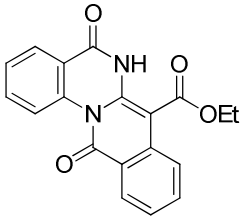
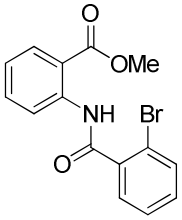
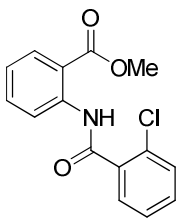
3.4.3. Scope of the reaction:

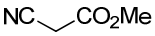
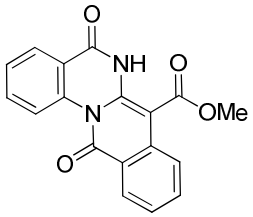
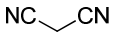
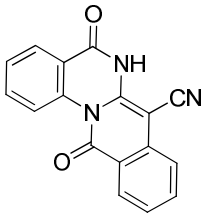
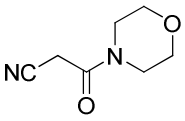
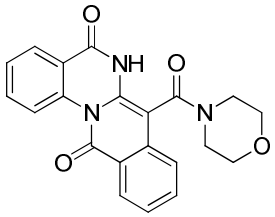
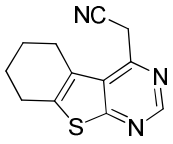
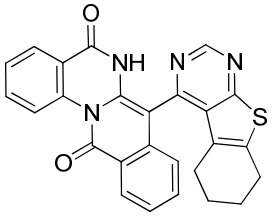
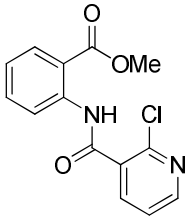
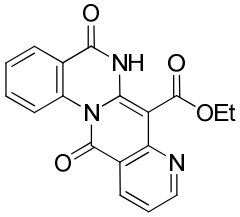
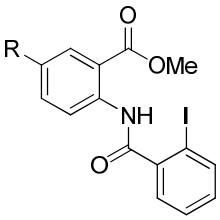
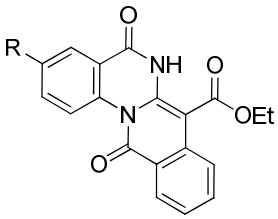
The reaction scope was then examined under the optimized conditions, and the results are summarized in Table 3.2. For the substitutions on methyl anthranilate moiety such as 3,3-dimethyl-but-1-ynyl (entry 9-11, Table 3.2), phenyl (entry 12-14, Table 3.2), thiophen-2-yl (entry 15, Table 3.2), and an electron-withdrawing nitro group (entry 17, Table 3.2) afforded good to high yields of the desired products and also ethyl 2-amino nicotinate (entry 16, Table 3.2) gave good yield. On the other side, reactivity of the iodo and bromo derivative (compare entry 1-2, Table 3.2) was found to be same

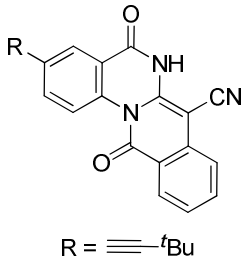
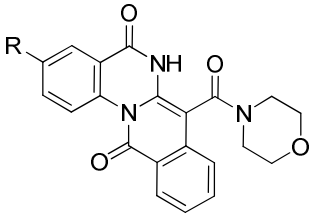
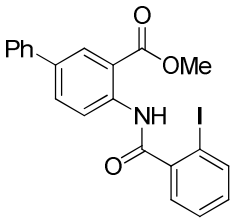
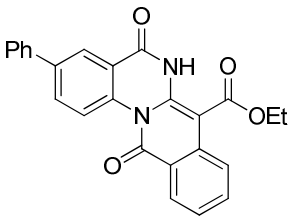
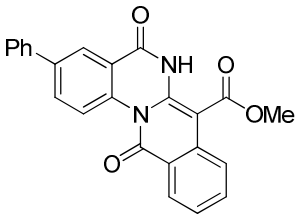
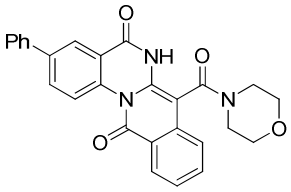
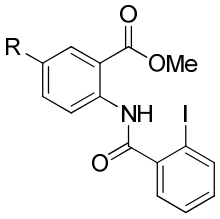
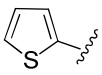
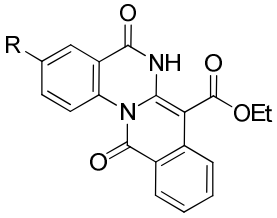
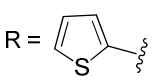
whereas the chloro derivative (entry 3, Table 3.2) was found to be inactive. However, the chloro attached at the ortho position of the pyridine ring (entry 8, Table 3.2) found to be highly reactive in this copper catalyzed domino reaction. Furthermore, other types of acetonitriles, such as ethyl cyano acetate, methyl cyano acetate, malano nitrile provided high yields, where as 3-morpholino-3-oxopropane nitrile, **33d**[33] and 2-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)acetonitrile, **33e**[34] afforded good yields.

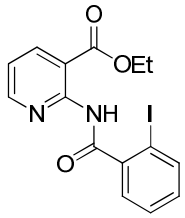
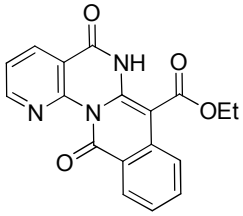
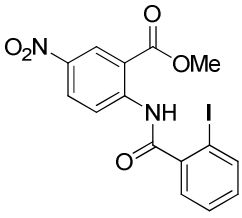
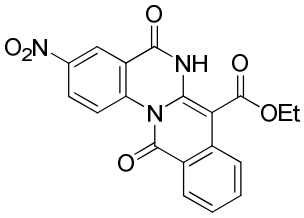
Table 3.2: Copper catalyzed synthesis of 5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (**51**).[a]



S.No	Halide (**50**)	Nitrile (**33**)	Time (h)	Product (**51**)	Yield (%)[b]
1	 **50a**	 **33a**	3.0	 **51a**	87
2	 **50b**	**33a**	3.5	**51a**	84
3	 **50c**	**33a**	6.0	**51a**	-

4	**50a**	 **33b**	3.0	 **51b**	86
5	**50a**	 **33c**	4.0	 **51c**	76
6	**50a**	 **33d**	3.5	 **51d**	77
7	**50a**	 **33e**	3.0	 **51e**	63
8	 **50d**	**33a**	4.0	 **51f**	73
9	 R = **50e**	**33a**	3.0	 R = **51g**	85

10	**50e**	**33c**	3.5	 R = **51h**	72
11	**50e**	**33d**	3.5	 R =  **51i**	72
12	 **50f**	**33a**	3.0	 **51j**	74
13	**50f**	**33b**	3.0	 **51k**	69
14	**50f**	**33d**	3.5	 **51l**	68
15	 R =  **50g**	**33a**	3.0	 R =  **51m**	72

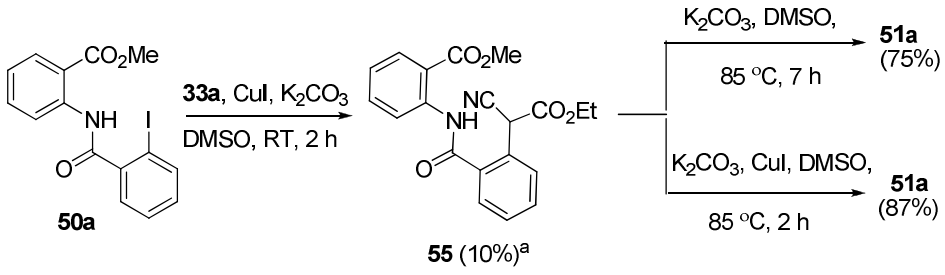
16	 **50h**	**33a**	3.0	 **51n**	63
17	 **50i**	**33a**	3.5	 **51o**	65

[a]Reactions were carried out using **50** (1 mmol), **33** (1.2 mmol), CuI (0.1 mmol) and K_2CO_3 (3 mmol) in DMSO (2 mL) under anhydrous conditions.

[b]Isolated yield.

3.4.4. Proposed mechanism:

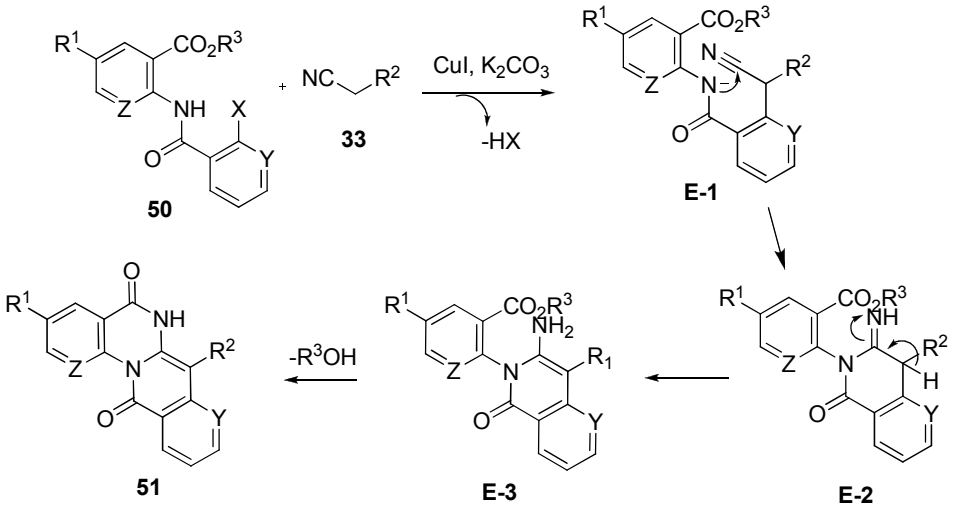
In order to understand the reaction mechanism, the following control experiments were performed under standard reaction conditions as shown in Scheme 3.20. Reaction of methyl 2-(2-iodobenzamido)benzoate (**50a**) with ethyl cyanoacetate (**33a**) at room temperature for 2 hours provided the intermediate **54** which showed copper mediated Ullmann type coupling reaction as the key step in this cascade process. Then treatment of **55** with potassium carbonate in DMSO gave **51a** after 7 hours, whereas in the presence of CuI the reaction was completed within 2.5 hours. This observation clearly showed that CuI accelerate the reaction towards the product.



[a] Unreacted **50a** was recovered.

Scheme 3.20: Reaction control experiments.

Based on the above results, a reaction mechanism is proposed as shown in Scheme 3.21. Copper catalyzed Ullmann type intermolecular C-C bond formation provided **E-1.** Then CuI/base promoted intramolecular nucleophilic attack of nitrogen of amide bond of **E-1** to CN moiety afforded **E-2** which then tautomerized to **E-3**. Finally, intramolecular amide bond formation within **E-3** afforded the target compound **51**.



Scheme 3.21: Proposed reaction mechanism.

3.5. Pharmacology:

3.5.1. *In vitro* data:

All the compounds synthesized were evaluated for their PDE4 inhibitory properties *in vitro*.[35] These compounds were tested at 30 μM and the results are summarized in Table 3.3. Among all the compounds, compound **51n** showed maximum inhibition *i.e.,* 56%.

Table 3.3: *In vitro* data of compounds of **51** for inhibition of PDE4B enzyme.

Entry	Compound No	% of PDE4B inhibition @ 30 μM
1	**51a**	38.88
2	**51c**	41.07
3	**51d**	14.93
4	**51f**	50.74
5	**51g**	15.14

6	**51h**	27.96
7	**51i**	9.51
8	**51l**	39.58
9	**51m**	15.87
10	**51n**	56.69

3.5.2. Docking studies:

The molecular docking was performed using most potent molecule against PDE4. Docking studies predicted good binding interaction with the PDE4B enzyme.[36]

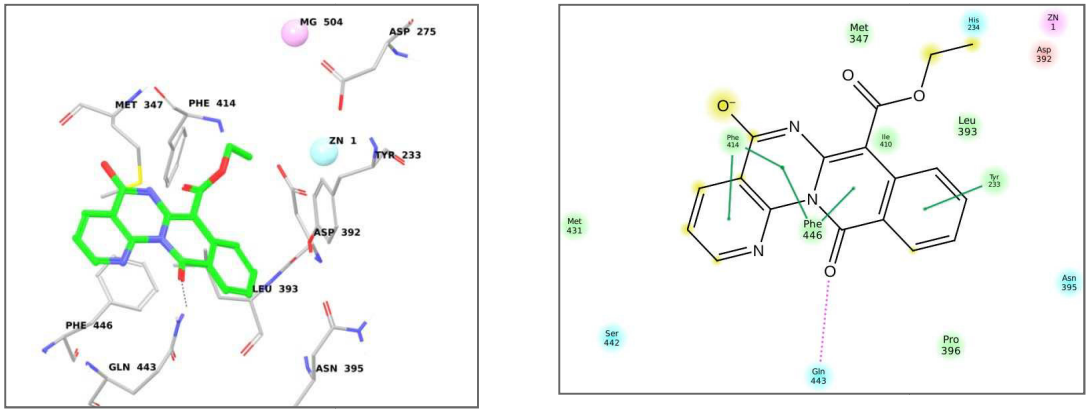


Fig. 3.4: Binding mode and interactions of molecule **51n** with PDE4B.

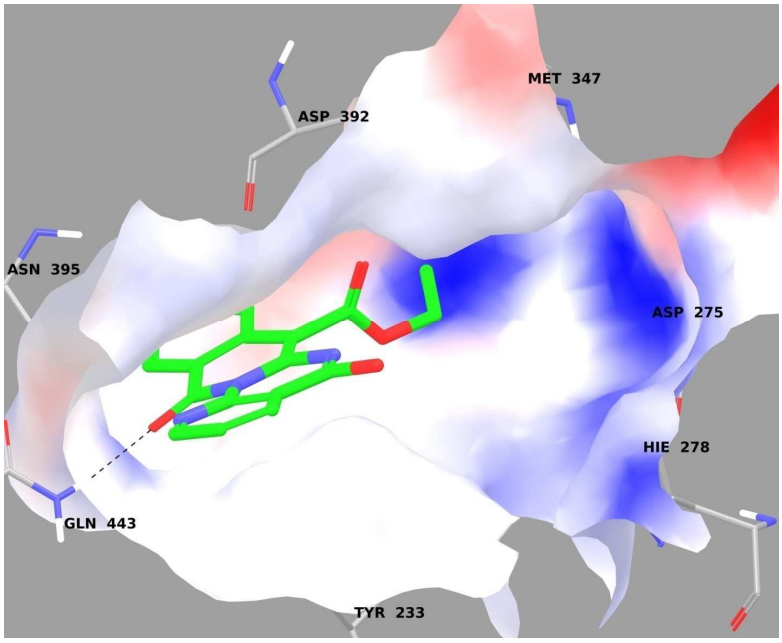


Fig. 3.5: Binding orientation of molecule **51n** at the active site t of PDE4B.

In molecule **51n**, hydrophobic interactions were found to be the primary binding interactions with glide score -7.3. Aromatic rings of molecule **51n** participated in good pi-pi stacking with active site residues (Tyr-233, Phe-414, and Phe-446) of the protein. In addition a hydrogen bond interaction between molecule **51n** and amino group of Gln-443 was also observed (Figure 3.4 and 3.5).

3.6. Conclusion:

A new, one pot and versatile Cu-mediated domino reaction has been developed for the synthesis of isoquinolino[2,3-*a*]quinazolinones. The protocol uses cheap and readily available CuI as the catalyst and substituted methyl 2-(2-halobenzamido)benzoates and nitriles as the starting materials, and the corresponding isoquinolino[2,3-*a*]quinazolinones were obtained in moderate to good yields under mild conditions. This inexpensive, convenient and efficient copper-catalyzed method should provide a new and useful strategy for the construction of nitrogen-containing heterocycles.

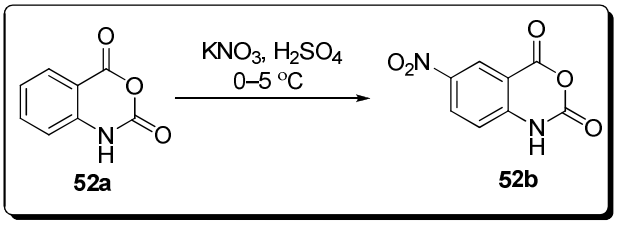
3.7. Experimental section:

3.7.1. 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 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.

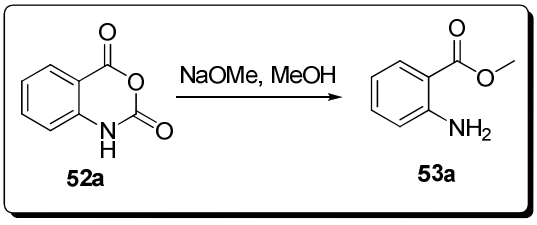
3.7.1.1. Typical procedure for preparation of 6-nitro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione[32] (52b)



To a solution of isatoic anhydride (2 g, 12.27 mmol) in con.H_2SO_4 (3 mL) at 0 ºC was added potassium nitrate (1.24 g, 12.27 mmol) in small batches over a period of 30min and the stirring was continued for an additional 15 min. Then, the reaction mixture was poured into crushed ice with stirring and the precipitate formed was filtered. The solid obtained was washed with water and dried under vacuum to afford the nitro compound **52b**.

Yield: 92% (2.4 g); light brown solid; mp: 259-261 °C (lit[37] 260 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.33 (bs, 1H), 8.56 (d, J = 2.4 Hz, 1H), 8.51 (dd, J = 8.9, 2.5 Hz, 1H), 7.30 (d, J = 9.0 Hz, 1H).

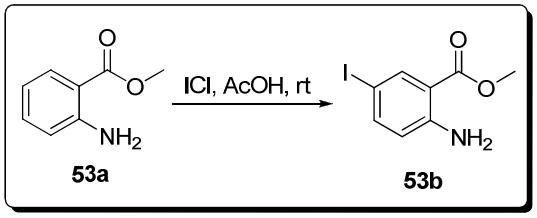
3.7.1.2. Typical procedure for preparation of methyl 2-amino benzoate (53a)



To the solution of sodium methoxide (1.36 g, 24.52 mmol) in methanol (20 mL), S-1a (2 g, 12.26 mmol) was slowly added at 0 ºC and stirred at 75 ºC for 1 h. After completion of the reaction, the excess of sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was diluted with water (50 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give desired compound **53a**.

Yield: 95% (1.76 g); color less liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.85 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.31-7.23 (m, 1H), 6.66-6.62 (m, 2H), 5.70 (bs, 2H), 3.87 (s, 3H).

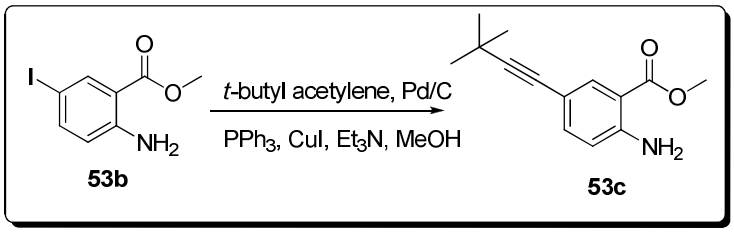
3.7.1.3. Typical procedure for preparation of methyl 2-amino-5-iodobenzoate (53b)[31]



To a solution of methyl 2-aminobenzoate (1 g, 6.62 mmol) in glacial acetic acid (10 mL), iodine monochloride (1 g, 6.62 mmol) in glacial acetic acid (10 mL) was added over 10 min. The resulting mixture was stirred at room temperature for 24 h. The ensuing precipitate was filtered, washed with glacial acetic acid followed by diethyl ether, and dried to provide the title compound.

Yield: 90% (1.65 g); white solid; mp: 188-190 °C (lit[31] 188-192 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.91 (d, *J* = 2.1 Hz, 1H), 7.47 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 3.77 (s, 3H).

3.7.1.4. Typical procedure for preparation of methyl 2-amino-5-(3,3-dimethyl but-1-ynyl)benzoate (53c)

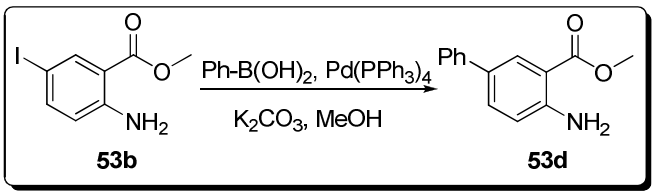


To a solution of methyl 2-amino-5-iodobenzoate (300 mg, 1.08 mmol) in methanol (5.0 mL), 5% Pd/C (1.0 mg, 0.01 mmol), CuI (1.9 mg, 0.01 mmol), PPh_3 (5.2 mg, 0.02 mmol) and triethyl amine (0.18 mL, 2.71 mmol) was added under nitrogen atmosphere. The reaction mixture was allowed to stir at room temperature for 15 min, and then added *t*-butyl acetylene (0.16 mL, 1.29 mmol). The mixture was refluxed for 3 hours. Upon completion, the reaction mixture was diluted with saturated NH_4Cl solution (15 ml) and the product was extracted with ethyl acetate (3 x 15 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 **53c**.

Yield: 94% (234 mg); light red solid; mp: 141-143 °C; R_f = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3336, 3172, 3053, 2219, 1713; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 1.8 Hz, 1H), 7.28-7.26 (m, 1H), 6.56 (d, *J* = 8.5 Hz, 1H), 5.79 (bs, 2H), 3.87 (s, 3H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0, 149.6, 137.0, 134.6, 116.5, 111.7, 110.4, 95.9, 78.4, 51.6, 31.1 (3C), 27.8; MS (ES mass): 231.9 (M+1); HPLC: 92.6%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 225 nm, retention time 10.34 min.

3.7.1.5. Typical procedure for preparation of methyl 4-aminobiphenyl-3-carboxylate (53d)

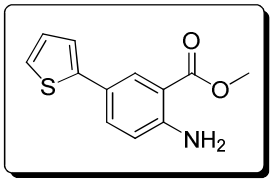


To a solution of methyl 2-amino-5-iodobenzoate (300 mg, 1.08 mmol) in methanol (5.0 ml), $Pd(PPh_3)_4$ (37 mg, 0.03 mmol), K_2CO_3 (298 mg, 2.16 mmol) and phenyl boronic acid (157 mg, 1.29 mmol) was added under nitrogen atmosphere. The reaction mixture was allowed to stir at 70 ºC for 8 h. Upon completion of the reaction, solvent was removed under reduced pressure. The residue 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, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **53d**.

Yield: 85% (254 mg); white solid; mp: 75-77 °C (lit[38] 78.1-80.3 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (d, *J* = 2.1 Hz, 1H), 7.58-7.52 (m, 3H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.29-7.27 (m, 1H), 6.75 (d, *J* = 7.9 Hz, 1H), 6.12 (bs, 2H), 3.90 (s, 3H).

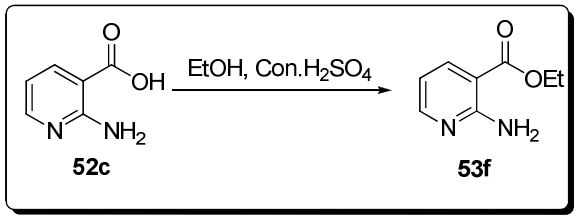
3.7.1.6. Methyl 2-amino-5-(thiophen-2-yl)benzoate (53e)

Compound **53e** was synthesized from the reaction of **53b** and thiophen-2-ylboronic acid following a procedure similar to that of compound **53d**.



Yield: 88% (221 mg); light green solid; mp: 159-161 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3421, 3327, 3100, 2948, 1702; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 2.1 Hz, 1H), 7.53 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.19-7.17 (m, 2H), 7.03 (d, *J* = 4.6 Hz, 1H), 6.69 (d, *J* = 8.5 Hz, 1H), 5.70 (bs, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.3, 149.7, 144.1, 131.9, 128.4, 127.8, 123.2, 123.1, 121.5, 117.1, 110.7, 51.6; MS (ES mass): 233.9 (M+1); HPLC: 96.3%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 220 nm, retention time 10.02 min.

3.7.1.7. Typical procedure for preparation of ethyl 2-aminonicotinate (53f)

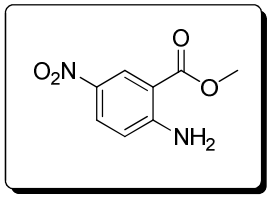


To a solution of 2-aminonicotinic acid (1g, 7.24 mmol) in ethanol (20 mL) was added con. Sulfuric acid (3 mL). The reaction mixture was heated at reflux for 16 hours, and then cooled to room temperature. The solvent was removed under reduced pressure. Then, water was added and the crude basified to pH 8.0 with 1N NaOH solution. The product was extracted into ethyl acetate (50 mL), dried over anhydrous sodium sulphate, filtered and concentrated to afford compound **53f**.

Yield: 95% (1.14 g); light brown solid; mp: 90-92 °C (lit[39] 97-99 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.14 (dd, *J* = 7.8, 1.7 Hz, 1H), 6.62 (dd, *J* = 7.8, 4.7 Hz, 1H), 6.34 (bs, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 1.38 (t, *J* = 7.2 Hz, 3H).

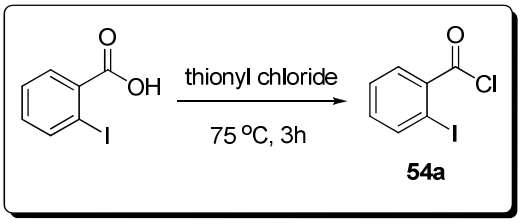
3.7.1.8. Methyl 2-amino-5-nitrobenzoate (53g)

Compound **53g** was synthesized from **52b** following a procedure similar to that of compound **53a**.



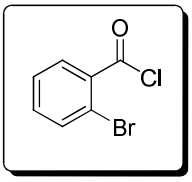
Yield: 87% (1.64 g); yellow solid; mp: 165-167 °C (lit[40] 166-168 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.84 (d, *J* = 2.6 Hz, 1H), 8.13 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.66 (d, *J* = 9.1 Hz, 1H), 3.93 (s, 3H).

3.7.1.9. Typical procedure for preparation of 2-iodo benzoyl chloride (54a)



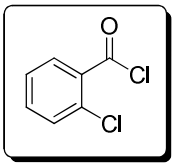
Thionyl chloride (10 equiv.) was slowly added to the 2-iodo benzoic acid (1 equiv.) at 0 ºC and the reaction mixture was heated to 75 ºC for 3 h. Then, the reaction mixture cooled to room temperature and excess of thionyl chloride removed under reduced pressure to give desired product which was used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

3.7.1.10. 2-bromo benzoyl chloride (54b)



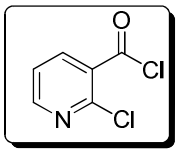
Compound **54b** was synthesized from 2-bromo benzoic acid following a procedure similar to that of compound **54a**.

3.7.1.11. 2-chloro benzoyl chloride (54c)



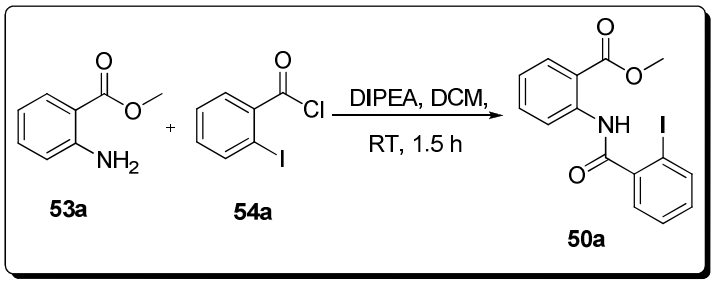
Compound **54c** was synthesized from 2-chloro benzoic acid following a procedure similar to that of compound **54a**.

3.7.1.12. 2-chloro nicotinoyl chloride (54d)



Compound **54d** was synthesized from 2-chloro nicotinic acid following a procedure similar to that of compound **54a**.

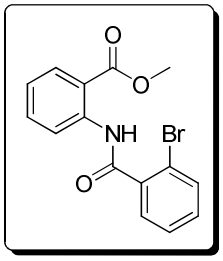
3.7.1.13. Typical procedure for preparation of methyl 2-(2-iodobenzamido) benzoate (50a)



To a solution of compound **53a** (100 mg, 0.66 mmol) in dry DCM (5 mL), DIPEA (0.23 mL, 1.32 mmol) was added at 0 ºC under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.11 mL, 0.79 mmol) was slowly added and the reaction mixture stirred at room temperature for 1.5 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, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **50a**.

Yield: 95% (239 mg); white solid; mp: 102-104 °C (lit[41] 102-103 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.41 (bs, 1H), 8.89 (d, *J* = 8.2 Hz, 1H), 8.10-8.07 (m, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.66-7.59 (m, 1H), 7.56 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.49-7.42 (m, 1H), 7.18-7.13 (m, 2H), 3.91 (s, 3H).

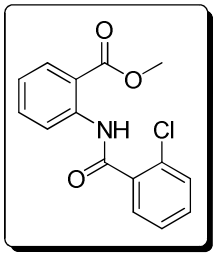
3.7.1.14. Methyl 2-(2-bromobenzamido)benzoate (50b)



Compound **50b** was synthesized from the reaction of **53a** and **54b** following a procedure similar to that of compound **50a**.

Yield: 95% (209 mg); white solid; mp: 80-82 °C (lit[41] 80-81 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.49 (bs, 1H), 8.90 (d, *J* = 8.0 Hz, 1H), 8.08 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.70-7.58 (m, 3H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.34-7.30 (m, 1H), 7.18-7.11 (m, 1H), 3.91 (s, 3H).

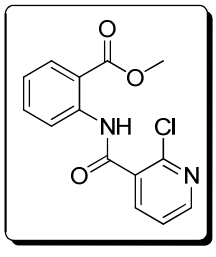
3.7.1.15. Methyl 2-(2-chlorobenzamido)benzoate (50c)



Compound **50c** was synthesized from the reaction of **53a** and **54c** following a procedure similar to that of compound **50a**.

Yield: 92% (176 mg); light red solid; mp: 75-77 °C (lit[41] 81-82 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.52 (bs, 1H), 8.91 (d, *J* = 8.5 Hz, 1H), 8.07 (d, *J* = 7.3 Hz, 1H), 7.69-7.58 (m, 2H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.45-7.34 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 3.90 (s, 3H).

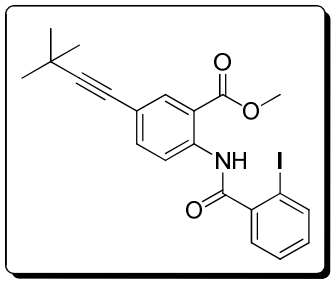
3.7.1.16. Methyl 2-(2-chloronicotinamido)benzoate (50d)



Compound **50d** was synthesized from the reaction of **53a** and **54d** following a procedure similar to that of compound **50a**.

Yield: 88% (168 mg); light brown solid; mp: 144-146 °C; R_f = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3276, 2956, 1704, 1657; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.67 (bs, 1H), 8.85 (d, *J* = 8.3 Hz, 1H), 8.53 (d, *J* = 1.2 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 8.01 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.38 (dd, *J* = 7.4, 4.9 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 163.8, 150.9, 140.7, 138.4, 134.8, 132.6, 130.9, 129.4, 123.5, 122.6, 120.7, 115.6, 52.5; MS (ES mass): 290.8 (M+1); HPLC: 93.2%, 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, 1.0/50, 9/98, 16/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.43 min.

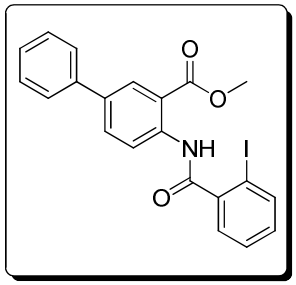
3.7.1.17. Methyl 5-(3,3-dimethylbut-1-ynyl)-2-(2-iodobenzamido)benzoate (50e)



Compound **50e** was synthesized from the reaction of **53c** and **54a** following a procedure similar to that of compound **50a**.

Yield: 85% (199 mg); white solid; mp: 135-137 °C; R_f = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3294, 2964, 2217, 1683, 1654; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.39 (bs, 1H), 8.82 (d, *J* = 8.4 Hz, 1H), 8.13-8.07 (m, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.61 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.58-7.53 (m, 1H), 7.47-7.43 (m, 1H), 7.20-7.11 (m, 1H), 3.91 (s, 3H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 167.5, 161.2, 142.1, 140.5, 137.5, 134.1, 132.2, 128.2, 120.3, 119.1, 115.2, 98.9, 95.3, 92.7, 77.8, 52.5, 30.1 (3C), 27.9; MS (ES mass): 461.7 (M+1); HPLC: 93.6%, column: X-Bridge C-18 150 x 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 230 nm, retention time 11.86 min.

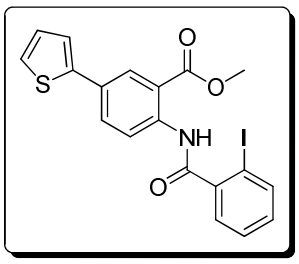
3.7.1.18. Methyl 4-(2-iodobenzamido)biphenyl-3-carboxylate (50f)



Compound **50f** was synthesized from the reaction of **53d** and **54a** following a procedure similar to that of compound **50a**.

Yield: 88% (177 mg); white solid; mp: 134-136 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3275, 2987, 1697, 1654; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.42 (bs, 1H), 8.32 (d, *J* = 2.1 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.87 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.65-7.56 (m, 3H), 7.50-7.43 (m, 4H), 7.37 (t, *J* = 7.2 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 167.3, 141.8, 140.1, 139.1, 135.7, 133.7, 131.9, 131.2, 128.9, 128.5 (2C), 128.0, 127.8, 127.7, 126.4 (2C), 120.7, 115.4, 92.3, 52.2; MS (ES mass): 457.7 (M+1); HPLC: 93.2%, 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, 1.0/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.89 min.

3.7.1.19. Methyl 2-(2-iodobenzamido)-5-(thiophen-2-yl)benzoate (50g)

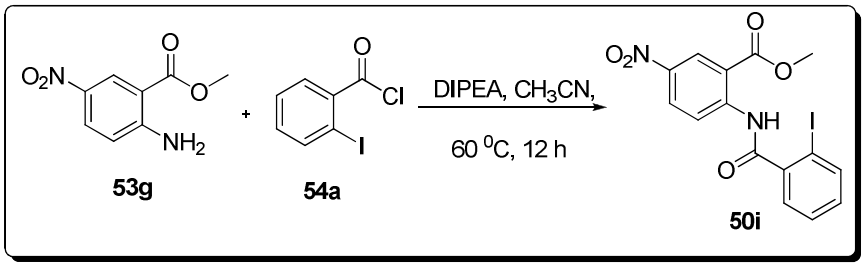


Compound **50g** was synthesized from the reaction of **53e** and **54a** following a procedure similar to that of compound **50a**.

Yield: 85% (168 mg); light green solid; mp: 131-133 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3267, 2976, 1698, 1657; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.39 (bs, 1H), 8.31 (d, *J* = 2.0 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.3, 2.0 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.50-7.44 (m, 3H), 7.34 (d, *J* = 3.6 Hz, 1H), 7.30 (d, *J* = 3.6 Hz, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.9, 167.2, 141.8, 140.1, 133.7, 131.9, 131.5, 131.2, 128.0, 127.8 (2C),

127.7, 127.6, 124.7, 122.9, 120.7, 115.4, 92.3, 52.3; MS (ES mass): 463.7 (M+1); HPLC: 95.3%, 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): 0/50, 1.0/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 8.34 min.

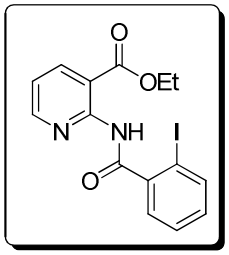
3.7.1.20. Typical procedure for preparation of methyl 2-(2-iodobenzamido)-5-nitrobenzoate (50i)



To a solution of compound **53g** (100 mg, 0.51 mmol) in CH_3CN (5 mL), DIPEA (0.18 mL, 1.02 mmol) was added at 0 ºC under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.09 mL, 0.61 mmol) was slowly added and the reaction mixture heated 60 ºC for 12 h. Then, solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (15 mL). The organic layer was washed with water (15 mL), saturated $NaHCO_3$ solution (15 mL), followed by brine solution (10 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 **50i**.

Yield: 35% (75 mg); white floppy solid; mp: 159-161 °C; R_f = 0.4 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3170, 2960, 1696, 1611; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.74 (bs, 1H), 9.12 (d, *J* = 9.3 Hz, 1H), 8.99 (d, *J* = 2.3 Hz, 1H), 8.47 (dd, *J* = 9.3, 2.2 Hz, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 167.2, 146.2, 142.2, 140.8, 140.7, 132.1, 129.5, 128.5, 128.2, 126.9, 120.7, 115.3, 92.6, 53.2; MS (ES mass): 424.7 (M-1); HPLC: 99.0 %, column: X-Bridge 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): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.16 min.

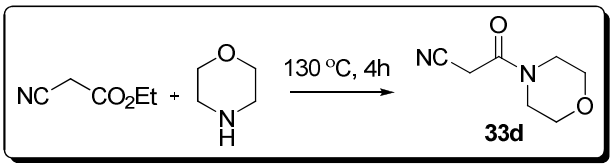
3.7.1.21. Ethyl 2-(2-iodobenzamido)nicotinate (50h)



Compound **50h** was synthesized from the reaction of **53f** and **54a** following a procedure similar to that of compound **50a**.

Yield: 82% (195 mg); brown liquid; R_f = 0.3 (40% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3169, 3071, 2983, 1703, 1651; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.16 (bs, 1H), 8.69 (d, *J* = 3.7 Hz, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.19-7.10 (m, 2H), 4.39 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.6, 153.1, 152.2, 142.0, 140.2, 139.9, 131.4 (2C), 128.3, 128.2, 118.8, 112.1, 92.3, 62.1, 14.1; MS (ES mass): 396.7 (M+1); HPLC: 92.8%, 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): 0/30, 1/30, 3/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.01 min.

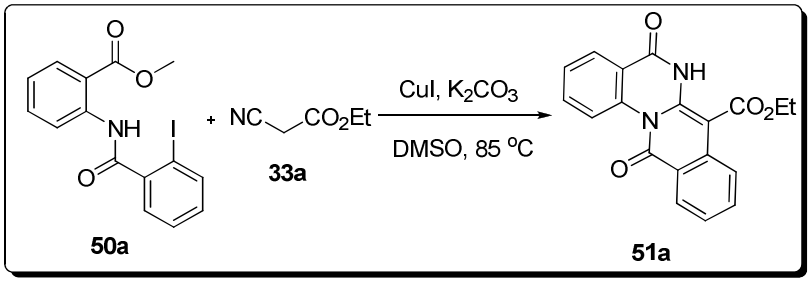
3.7.1.22. Typical procedure for preparation of 3-morpholino-3-oxopropane nitrile[33] (33d)



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 2d.

Yield: 75% (1.02 g); brown solid; mp: 81-83 °C; (lit[42] 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).

3.7.1.23. Typical procedure for preparation of Ethyl 5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (51a)

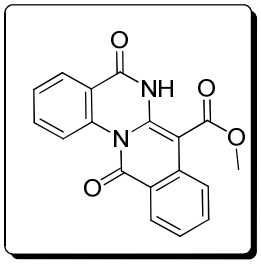


A mixture of compound **50a** (100 mg, 0.26 mmol), K_2CO_3 (107 mg, 0.78 mmol), ethyl cyano acetate (**33a**) (0.04 mL, 0.31 mmol) and CuI (4.9 mg, 0.026 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions ($CaCl_2$ filled guard tube) for 3 h. After completion of the reaction, reaction 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, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **51a**.

White solid; mp: 153-155 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2956, 1704, 1634, 1587; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.12 (bs, 1H), 8.77 (d, *J* = 8.8 Hz, 1H), 8.42-8.38 (m, 2H), 8.30 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.76-7.67 (m, 2H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 4.52 (q, *J* = 7.1 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 161.8, 157.8, 144.5, 137.1, 133.8, 133.6, 133.4, 128.5, 127.3, 126.9, 125.4, 125.2, 122.4, 121.9, 119.4, 86.8, 61.9, 14.2; MS (ES mass): 332.9 (M-1); HPLC: 99.2%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 9.12 min. HRMS (ESI): calcd for $C_{19}H_{15}N_2O_4$ $(M+H)^+$ 335.1032, found 335.1016.

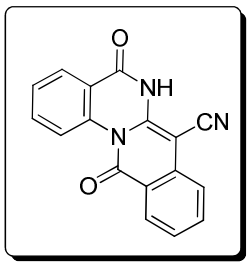
3.7.1.24. Methyl 5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (51b)

Compound **51b** was synthesized from the reaction of **50a** and methyl cyano acetate (**33b**) following a procedure similar to that of compound **51a**.



White solid; mp: 189-191 °C; R_f = 0.5 (15% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2965, 1712, 1634, 1590; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.06 (bs, 1H), 8.76 (d, *J* = 8.4 Hz, 1H), 8.39 (d, *J* = 7.9 Hz, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.29 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.77-7.66 (m, 2H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 1H), 4.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:169.1, 161.9, 157.8, 144.7, 137.2, 133.9, 133.7, 133.3, 128.6, 127.4, 127.1, 125.5, 125.4, 122.4, 122.0, 119.4, 86.8, 52.4; MS (ES mass): 320.8 (M+1); HPLC: 99.6%, 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): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.83 min.

3.7.1.25. 5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carbo nitrile (51c)

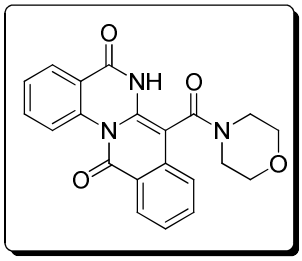


Compound **51c** was synthesized from the reaction of **50a** and malano nitrile (**33c**) following a procedure similar to that of compound **51a**.

Brown solid; mp: 292-294 °C; R_f = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3145, 2209, 1691, 1606; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.05 (bs, 1H), 8.86 (d, *J* = 8.8 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.13 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.87-7.79 (m, 2H), 7.80-7.55 (m, 2H), 7.49 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.7, 159.2, 146.5, 137.6, 133.9, 128.9, 126.9, 126.8, 126.7, 121.9, 121.8 (2C), 121.3, 121.2, 119.8, 115.7, 72.3; MS (ES mass): 285.9 (M-1); HPLC: 99.2%, column: X-Bridge C-18 150 x 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 230 nm, retention time 8.48 min; HRMS (ESI): calcd for $C_{17}H_{10}N_3O_2$ $(M+H)^+$ 288.0845, found 288.0854.

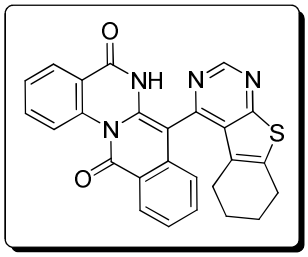
3.7.1.26. 7-(morpholine-4-carbonyl)-5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (51d)



Compound **51d** was synthesized from the reaction of **50a** and **33d** following a procedure similar to that of compound **51a**.

White solid; mp: 236-238 °C; R_f = 0.4 (40% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3206, 3087, 1682, 1628, 1562; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.75 (bs, 1H), 9.15 (d, *J* = 8.8 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.28 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.77-7.69 (m, 2H), 7.50-7.39 (m, 3H), 3.98-3.18 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.7, 161.7, 157.9, 137.8, 136.5, 134.2, 134.0, 132.7, 129.3, 127.7, 126.7, 125.6, 122.1, 121.9, 121.5, 118.7, 92.7, 66.7 (2C), 44.4, 42.9; MS (ES mass): 374.0 (M-1); HPLC: 96.2%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 230 nm, retention time 8.15 min. HRMS (ESI): calcd for $C_{21}H_{18}N_3O_4$ $(M+H)^+$ 376.1297, found 376.1280.

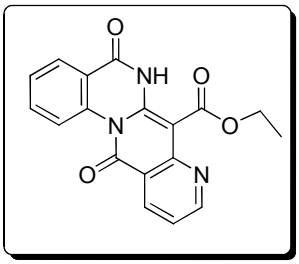
3.7.1.27. 7-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (51e)



Compound **51e** was synthesized from the reaction of **50a** and **33e** following a procedure similar to that of compound **51a**.

Light brown solid; mp: 329-331 °C; R_f = 0.4 (25% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3224, 2940, 1697, 1678, 1619; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.27 (d, *J* = 8.8 Hz, 1H), 9.18 (s, 1H), 8.53-8.50 (m, 2H), 8.21 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.78 (t, *J* = 8.6 Hz, 1H), 7.53-7.41 (m, 3H), 6.73 (d, *J* = 7.8 Hz, 1H), 2.89-2.86 (m, 2H), 2.29-2.22 (m, 1H), 1.87-1.80 (m, 2H), 1.75-1.66 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 162.2, 158.0, 153.1, 152.7, 140.7, 138.2, 135.5, 134.5, 134.1, 133.9, 131.9, 129.2, 127.9, 126.7, 126.6, 125.8, 122.6, 122.2, 121.8, 118.7, 96.8, 26.0, 24.8, 22.3, 22.1; MS (ES mass): 449.0 (M-1); HRMS (ESI): calcd for $C_{26}H_{19}N_4O_2S$ $(M+H)^+$ 451.1229, found 451.1225.

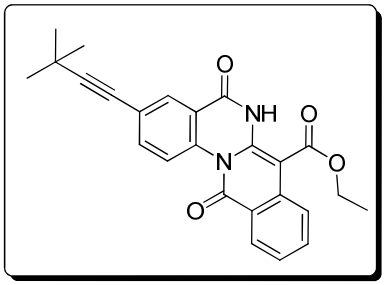
3.7.1.28. Ethyl 5,12-dioxo-6,12-dihydro-5*H*-[1,6]naphthyridino[6,7-*a*] quinazoline -7-carboxylate (51f)



Compound **51f** was synthesized from the reaction of **50d** and **33a** following a procedure similar to that of compound **51a**.

Light brown solid; mp: 134-136 °C; R_f = 0.4 (40% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3078, 2923, 1701, 1646, 1590; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.65 (bs, 1H), 8.98-8.97 (m, 1H), 8.89 (d, *J* = 8.4 Hz, 1H), 8.64 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.33 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.38-7.35 (m, 1H), 4.55 (q, *J* = 7.1 Hz, 2H), 1.46 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 161.9, 157.6, 154.9, 150.5, 145.6, 137.1, 136.9, 134.3, 127.8, 127.5, 121.8, 120.4, 119.3, 117.9, 89.9, 62.2, 14.2; MS (ES mass): 335.8 (M+1); HPLC: 92.5%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/10, 3/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.43 min. HRMS (ESI): calcd for $C_{18}H_{14}N_3O_4$ $(M+H)^+$ 336.0984, found 336.0988

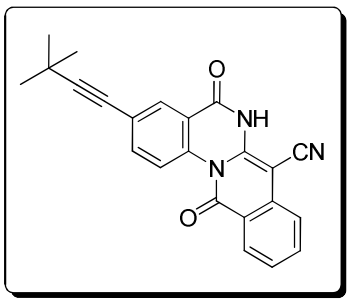
3.7.1.29. Ethyl 3-(3,3-dimethylbut-1-ynyl)-5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (51g)



Compound **51g** was synthesized from the reaction of **50e** and **33a** following a procedure similar to that of compound **51a**.

White solid; mp: 183-185 °C; R_f = 0.5 (15% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2968, 2218, 1700, 1654, 1599; ^1H NMR (400 MHz, $CDCl_3$) δ: 13.12 (bs, 1H), 8.72 (d, *J* = 8.8 Hz, 1H), 8.40-8.37 (m, 2H), 8.28 (d, *J* = 2.0 Hz, 1H), 7.71-7.67 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 4.52 (q, *J* = 7.2 Hz, 2H), 1.49 (t, *J* = 7.2 Hz, 3H), 1.33 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 161.7, 157.3, 144.3, 136.4, 135.8, 133.7, 133.4, 130.2, 128.6, 125.5, 125.3, 123.4, 122.3, 121.9, 119.3, 101.3, 87.1, 77.3, 61.9, 30.8 (3C), 28.0, 14.3; MS (ES mass): 414.9 (M+1); HPLC: 99.3%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 11.2 min. HRMS (ESI): calcd for $C_{25}H_{23}N_2O_4$ $(M+H)^+$ 415.1658, found 415.1657.

3.7.1.30. 3-(3,3-dimethylbut-1-ynyl)-5,12-dioxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazoline-7-carbonitrile (51h)

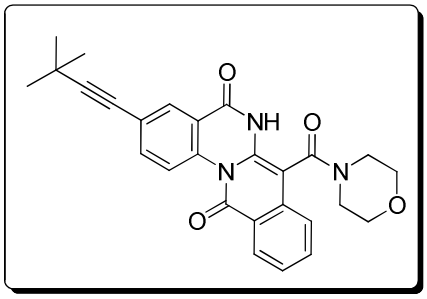


Compound **51h** was synthesized from the reaction of **50e** and **33c** following a procedure similar to that of compound **51a**.

Brown solid; mp: 318-320 °C; R_f = 0.2 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3194, 2964, 2223, 1703, 1617, 1559; ^1H NMR (400 MHz, DMSO d_6) δ: 8.88 (d, *J* = 8.8 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 1.8 Hz, 1H), 7.86 (t, *J* = 8.0 Hz,

1H), 7.76 (dd, *J* = 8.8, 1.8 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 161.8, 160.2, 136.9, 135.3, 135.0, 129.9, 129.2, 128.8 (2C), 122.1 (2C), 121.9, 121.7, 120.7, 119.4, 109.8, 100.8, 79.5, 77.7, 30.9 (3C), 29.3; MS (ES mass): 366.0 (M-1); HPLC: 98.8%, column: X-Bridge C-18 150 x 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 240 nm, retention time 11.03 min.

3.7.1.31. 3-(3,3-dimethylbut-1-ynyl)-7-(morpholine-4-carbonyl)-5*H*-isoquinolino [2,3-*a*]quinazoline-5,12(6*H*)-dione (51i)

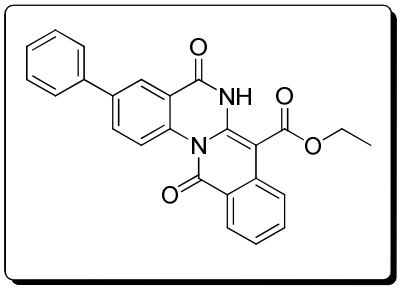


Compound **51i** was synthesized from the reaction of **50e** and **33d** following a procedure similar to that of compound **51a**.

Light brown solid; mp: 233-235 °C; R_f = 0.3 (45% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3198, 2970, 2215, 1697, 1623, 1572; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.83 (bs, 1H), 9.12 (d, *J* = 8.4 Hz, 1H), 8.44 (d, *J* = 7.6 Hz, 1H), 8.28 (d, *J* = 2.0 Hz, 1H), 7.76-7.68 (m, 2H), 7.48-7.37 (m, 2H), 3.94-3.41 (m, 8H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.8, 161.7, 157.5, 136.9, 136.6, 136.5, 134.2, 132.8, 130.6, 129.4, 125.9, 125.8, 123.1, 122.2, 121.9, 121.6, 118.7, 101.2, 93.0, 66.8 (2C), 45.6, 43.8, 30.8 (3C), 29.7; MS (ES mass): 454.0 (M-1); HPLC: 93.6%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 9.18 min. HRMS (ESI): calcd for $C_{27}H_{26}N_3O_4$ $(M+H)^+$ 456.1923, found 456.1909.

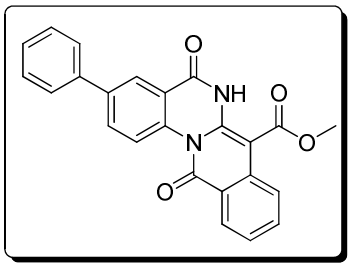
3.7.1.32. Ethyl-5,12-dioxo-3-phenyl-6,12-dihydro-5*H*-isoquinolino[2,3-*a*] quinazoline-7-carboxylate (51j)

Compound **51j** was synthesized from the reaction of **50f** and **33a** following a procedure similar to that of compound **51a**.



White solid; mp: 209-211 °C; R_f = 0.5 (15% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2995, 1694, 1643, 1595; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.21 (bs, 1H), 8.90 (d, *J* = 8.9 Hz, 1H), 8.55 (d, *J* = 2.1 Hz, 1H), 8.44 (t, *J* = 9.0 Hz, 2H), 8.00 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.75-7.69 (m, 3H), 7.55-7.41 (m, 4H), 4.56 (q, *J* = 7.1 Hz, 2H), 1.52 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.7, 161.9, 157.9, 144.4, 139.8, 138.3, 136.3, 133.8, 133.6, 132.3, 129.1 (2C), 128.7, 128.3, 126.9 (2C), 125.6, 125.4, 125.2, 122.6, 122.5, 119.8, 87.1, 61.9, 14.3; MS (ES mass): 408.9 (M-1). HPLC: 92.8%, 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, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.75 min. HRMS (ESI): calcd for $C_{25}H_{19}N_2O_4$ $(M+H)^+$ 411.1345, found 411.1337.

3.7.1.33. Methyl-5,12-dioxo-3-phenyl-6,12-dihydro-5*H*-isoquinolino[2,3-*a*] quinazoline-7-carboxylate (51k)

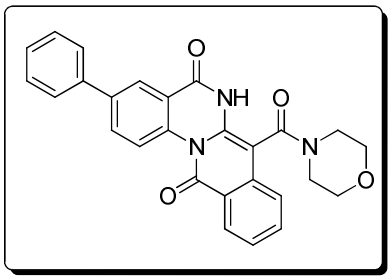


Compound **51k** was synthesized from the reaction of **50f** and **33b** following a procedure similar to that of compound **51a**.

Light yellow floppy solid; mp: 209-211 °C; R_f = 0.6 (15% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3010, 1699, 1642, 1589; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.13 (bs, 1H), 8.89 (d, *J* = 8.8 Hz, 1H), 8.54 (d, *J* = 2.4 Hz, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.37 (d, *J* = 8.2 Hz, 1H), 7.99 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.73-7.71 (m, 3H), 7.52-7.48 (m, 2H),

7.48-7.41 (m, 2H), 4.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.1, 161.9, 157.9, 144.6, 139.9, 138.3, 136.2, 133.8, 133.4, 132.3, 129.1 (2C), 128.7, 128.3, 127.1, 126.9 (2C), 125.7, 125.5, 125.2, 122.6, 119.9, 87.0, 52.5; MS (ES mass): 396.7 (M+1); HPLC: 98.6%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.43 min.

3.7.1.35. 7-(morpholine-4-carbonyl)-3-phenyl-5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (51l)

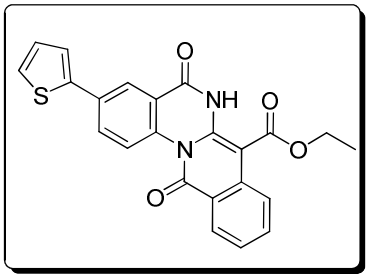


Compound **51l** was synthesized from the reaction of **50f** and **33d** following a procedure similar to that of compound **51a**.

Light brown solid; mp: 366-368 °C; R_f = 0.4 (45% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3208, 2889, 1689, 1620, 1570; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.98 (bs, 1H), 9.27 (d, *J* = 9.0 Hz, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.99 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.74-7.71 (m, 3H), 7.51-7.38 (m, 5H), 4.01-3.24 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.5, 165.8, 163.4, 139.2, 138.3, 134.6, 134.1, 132.9, 132.5, 129.4, 129.0 (2C), 128.6, 128.2, 127.1, 126.9 (2C), 126.6, 125.7, 122.2, 121.9, 114.0, 95.2, 66.8 (2C), 43.8, 41.5; MS (ES mass): 449.9 (M-1); HPLC: 92.8%, 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, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.36 min. HRMS (ESI): calcd for $C_{27}H_{22}N_3O_4$ $(M+H)^+$ 452.1610, found 452.1627

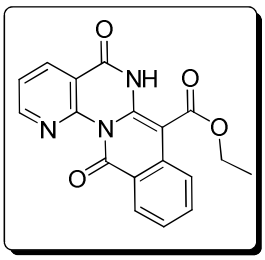
3.7.1.36. Ethyl-5,12-dioxo-3-(thiophen-2-yl)-6,12-dihydro-5*H*-isoquinolino[2,3-*a*] quinazoline-7-carboxylate (51m)

Compound **51m** was synthesized from the reaction of **50g** and **33a** following a procedure similar to that of compound **51a**.



White solid; mp: 184-186 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3023, 2975, 1712, 1691, 1643, 1599; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.18 (s, 1H), 8.83 (d, *J* = 9.0 Hz, 1H), 8.49 (d, *J* = 2.3 Hz, 1H), 8.41 (t, *J* = 8.6 Hz, 2H), 7.94 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.73-7.66 (m, 1H), 7.48 (d, *J* = 2.6 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 5.6 Hz, 1H), 7.14-7.12 (m, 1H), 4.53 (q, *J* = 7.2 Hz, 2H), 1.50 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 161.7, 157.6, 144.2, 141.5, 135.8, 133.6, 133.3, 133.2, 130.7, 128.6, 128.3, 126.1, 125.5, 125.3, 124.4, 123.4, 122.6, 122.3, 119.7, 87.0, 61.9, 14.2; MS (ES mass): 414.9 (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, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.19 min.

3.7.1.37. Ethyl-5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]pyrido[2',3'-*d*] pyrimidine-7-carboxylate (51n)

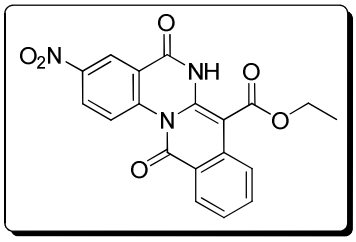


Compound **51n** was synthesized from the reaction of **50h** and **33a** following a procedure similar to that of compound **51a**.

White solid; mp: 214-216 °C; R_f = 0.3 (40% EtOAc/*n*-hexane); IR (KBr, cm^{-1}): 3071, 2983, 1703, 1651, 1606; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.16 (bs, 1H), 8.87 (dd, *J* = 4.8, 1.9 Hz, 1H), 8.56 (dd, *J* = 7.7, 1.9 Hz, 1H), 8.40 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 7.73-7.65 (m, 1H), 7.49 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.43 (t, *J* = 7.3 Hz, 1H), 4.52 (q, *J* = 7.1 Hz, 2H), 1.49 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.6, 161.1, 157.8, 153.1, 149.9, 144.3, 136.6, 133.7, 133.3, 128.7, 125.8, 125.7, 124.1, 122.8, 115.3, 87.3, 61.9, 14.3; MS (ES mass): 335.8 (M+1); HPLC:

97.4%, 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): 0/30, 1/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 245 nm, retention time 4.42 min.

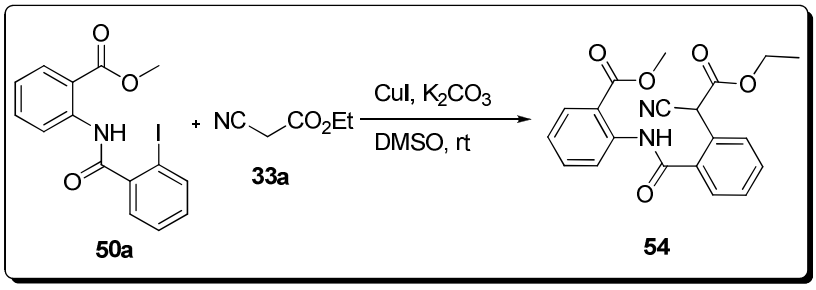
3.7.1.38. Ethyl-3-nitro-5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*] quinazoline-7-carboxylate (51o)



Compound **51o** was synthesized from the reaction of **50i** and **33a** following a procedure similar to that of compound **51a**.

Light yellow solid; mp: 179-181 °C; R_f = 0.4 (15% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2990, 1696, 1644, 1598; ^1H NMR (400 MHz, $CDCl_3$) δ: 13.25 (bs, 1H), 9.12 (d, *J* = 2.5 Hz, 1H), 8.98 (d, *J* = 9.5 Hz, 1H), 8.53 (dd, *J* = 9.5, 2.5 Hz, 1H), 8.40 (t, *J* = 8.7 Hz, 2H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 7.2 Hz, 1H), 4.55 (q, *J* = 7.2 Hz, 2H), 1.51 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.5, 167.2, 161.8, 156.2, 155.0, 143.7, 141.4, 133.9, 133.2, 128.6, 127.4, 126.1, 123.6, 123.0, 120.4, 116.3, 92.2, 62.4, 14.2; MS (ES mass): 377.9 (M-1); HPLC: 98.6%, column: X-Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 9.07 min.

3.7.1.39. Methyl 2-(2-(1-cyano-2-ethoxy-2-oxoethyl)benzamido)benzoate (54)



A mixture of compound **50a** (100 mg, 0.26 mmol), K_2CO_3 (36 mg, 0.26 mmol), ethyl cyano acetate (**33a**) (0.04 mL, 0.31 mmol) and CuI (4.9 mg, 0.026 mmol) in DMSO

(2 mL) was stirred at room temperature under anhydrous conditions for 2 h. Then the reaction mixture was 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 **54**.

Light brown liquid; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3265, 2949, 2252, 1748, 1682, 1593; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.84 (bs, 1H), 8.80 (d, *J* = 8.0 Hz, 1H), 8.09 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.87 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.65-7.57 (m, 3H), 7.16 (t, *J* = 7.1 Hz, 1H), 6.09 (s, 1H), 4.27-4.19 (m, 2H), 3.94 (s, 3H), 1.24 (t, *J* = 7.15 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.9, 166.7, 165.0, 141.2, 134.8, 134.5, 131.8, 130.9, 130.4, 130.2, 129.7, 127.7, 123.2, 120.4, 116.1, 115.5, 63.1, 52.5, 40.3, 13.8; MS (ES mass): 366.9 (M+1); HPLC: 94.5 %, 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): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.36 min.

3.8. References:

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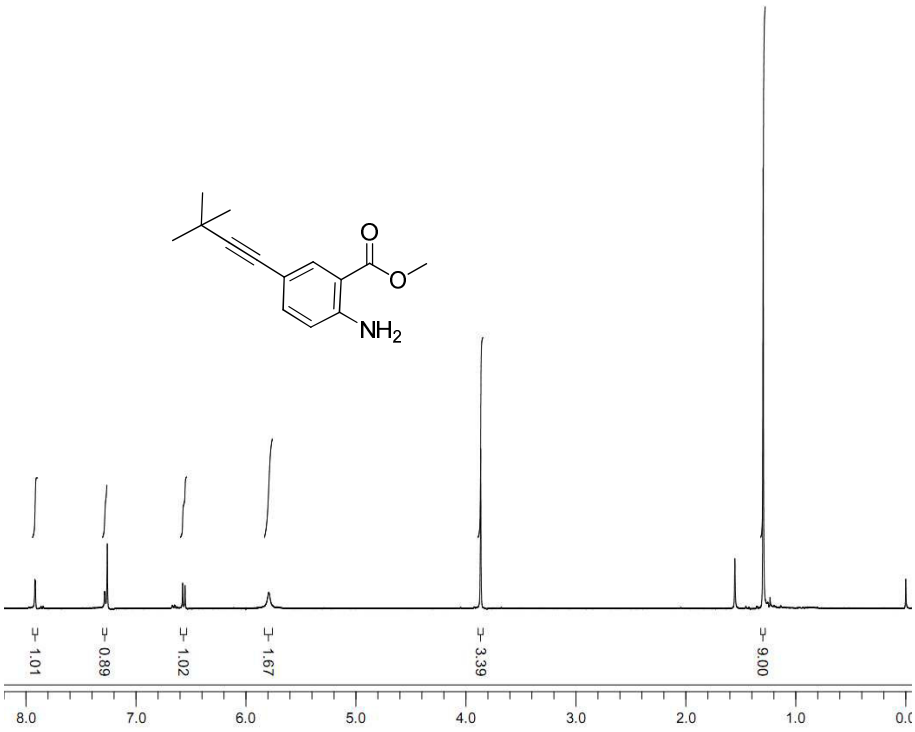
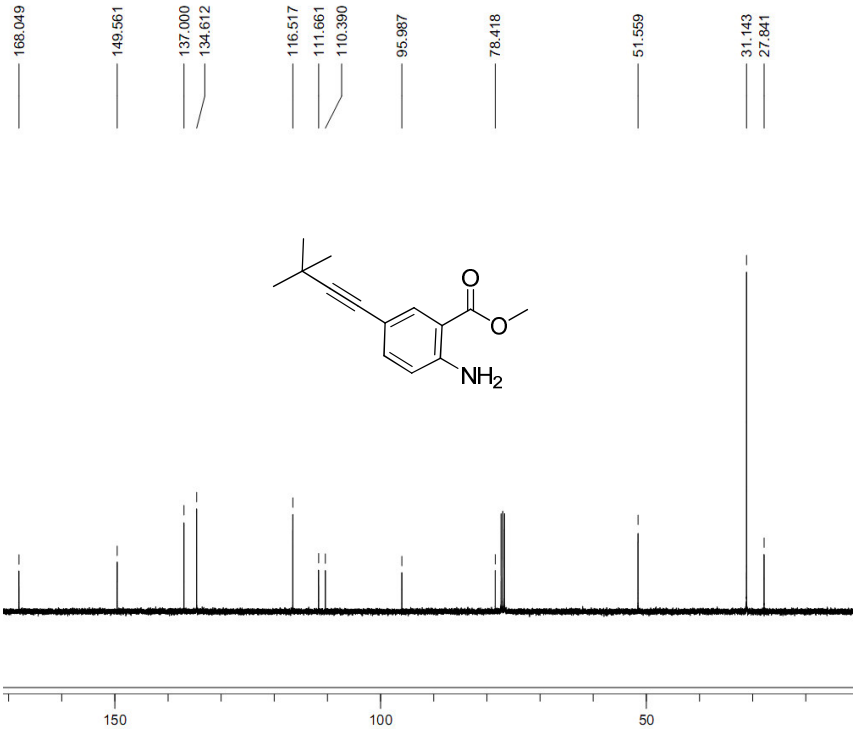
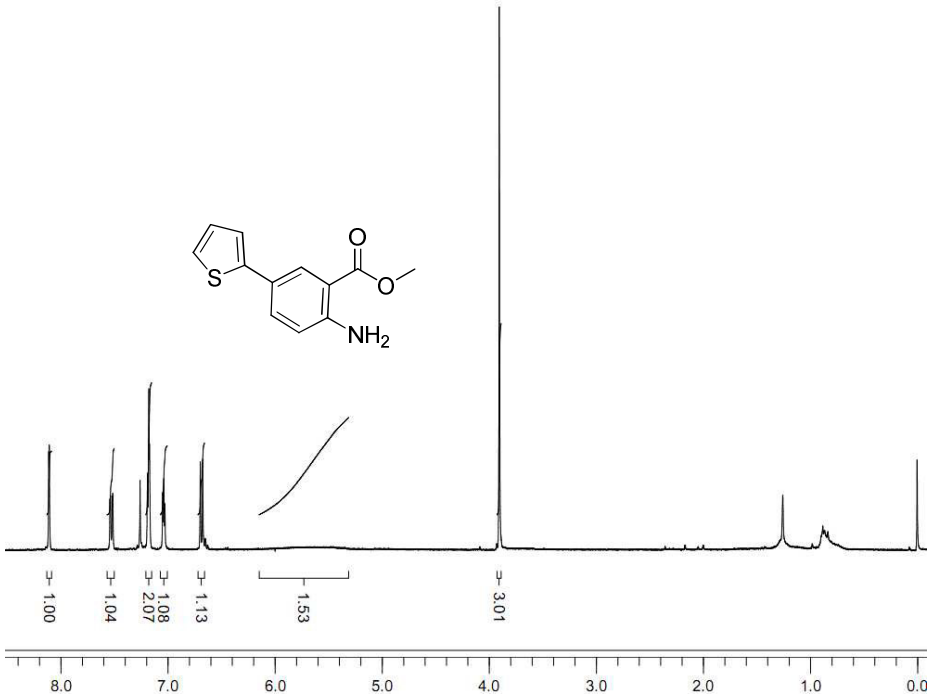
Appendix



^{1}H NMR spectra of compound **53c** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **53c** ($CDCl_3$, 100 MHz)



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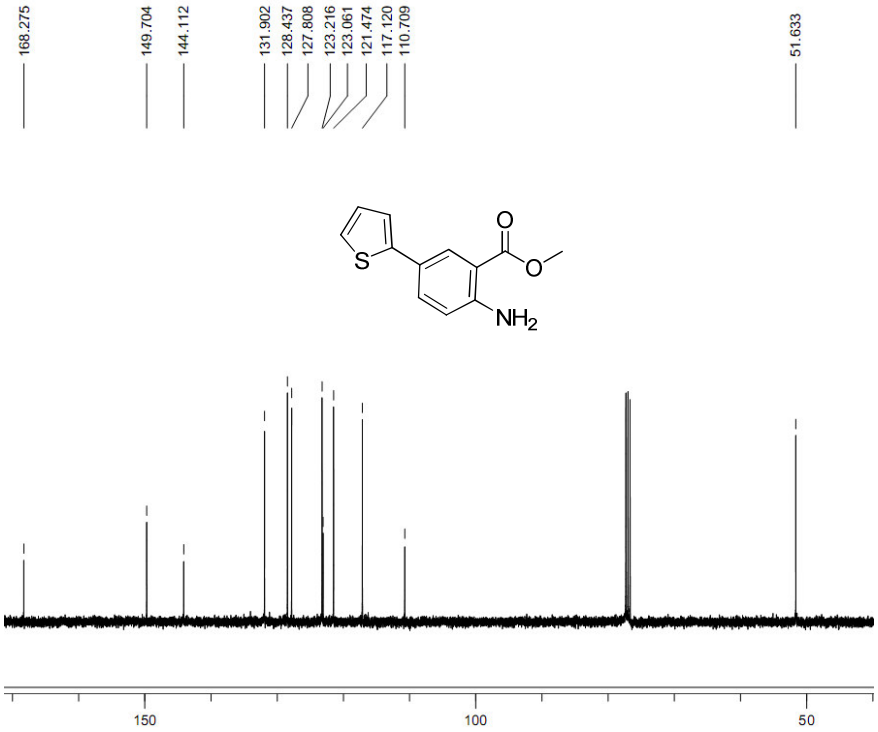

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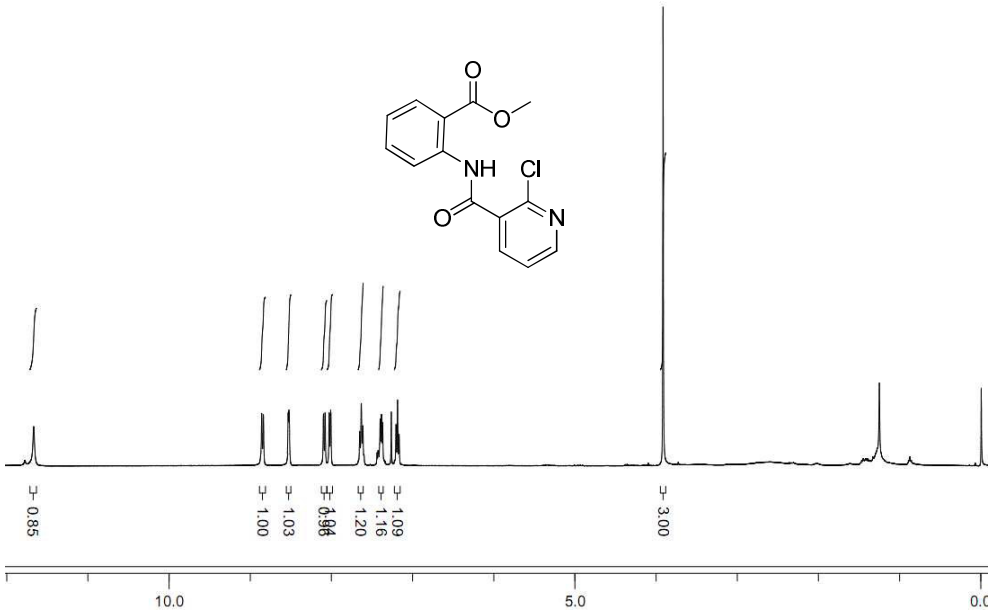

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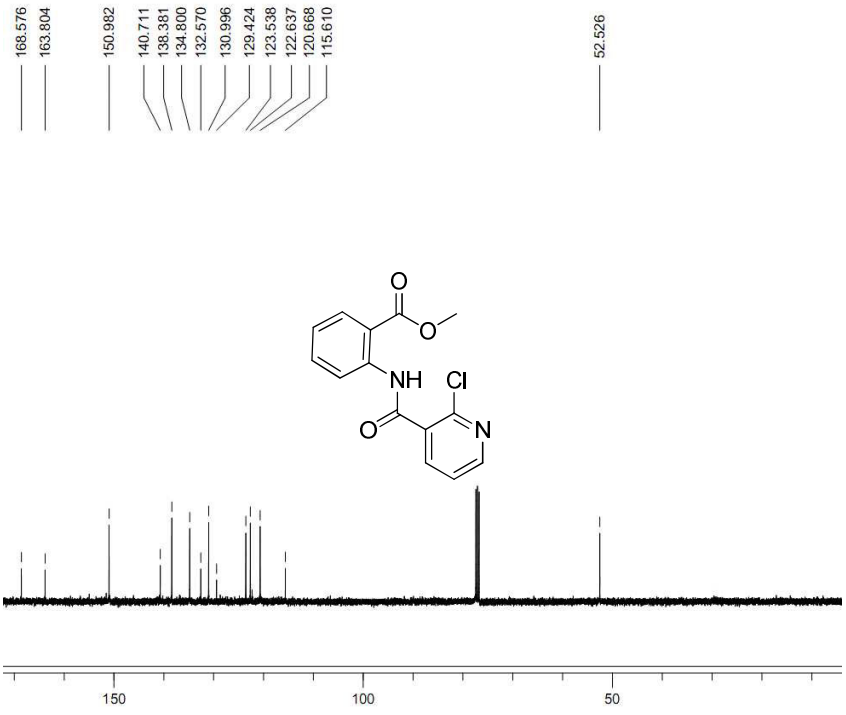

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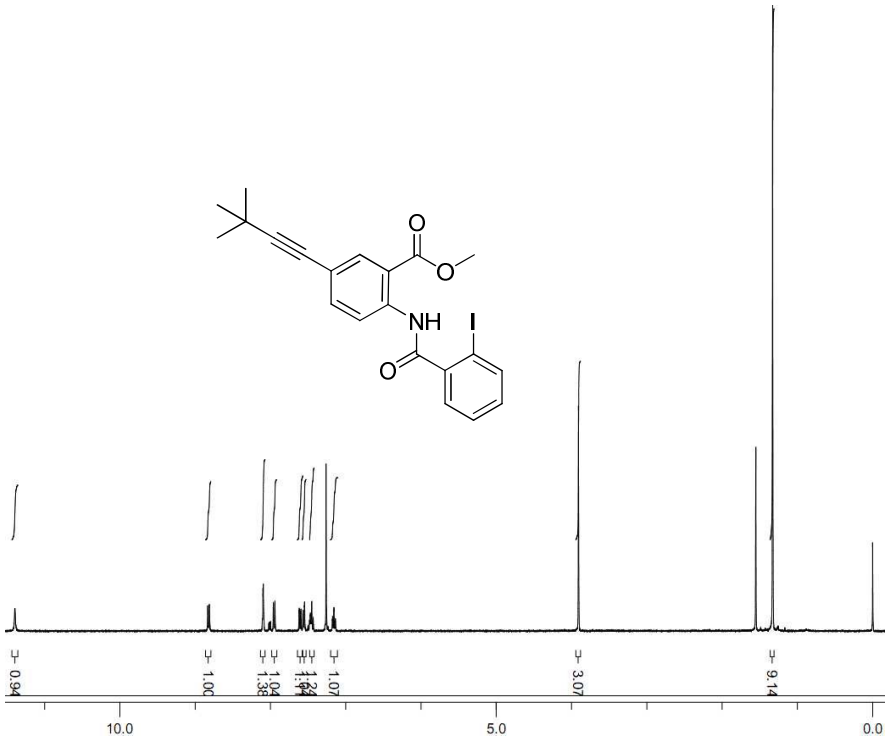
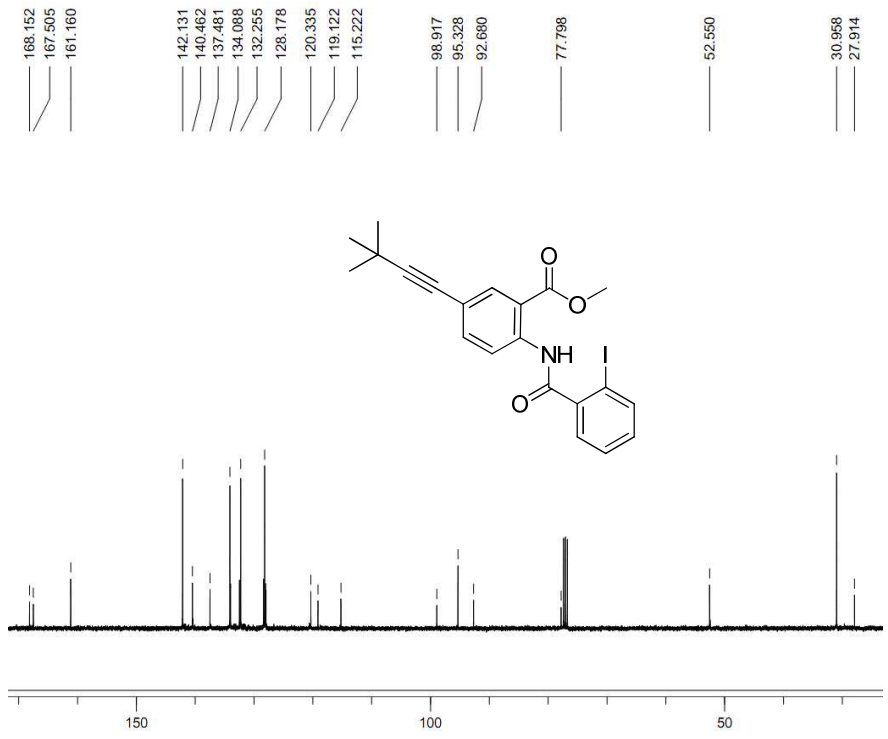


^{1}H NMR spectra of compound **50e** ($CDCl_3$, 400 MHz)



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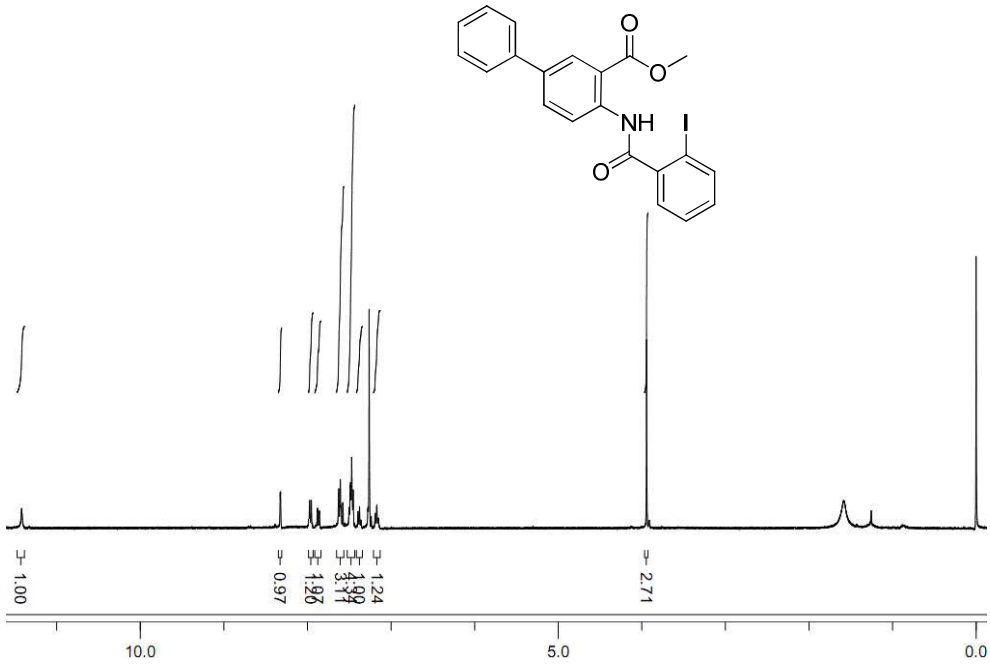

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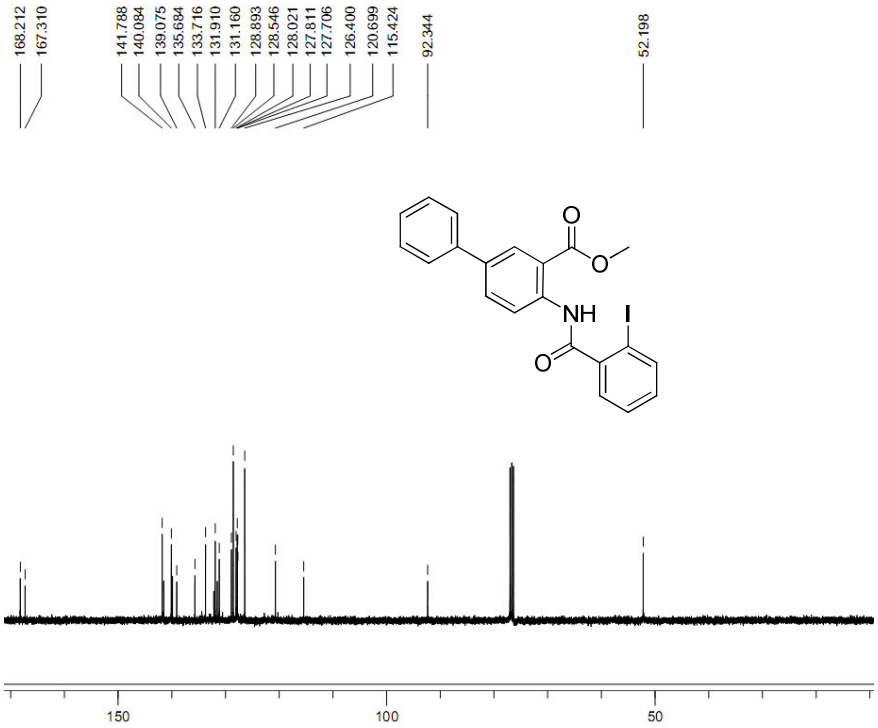

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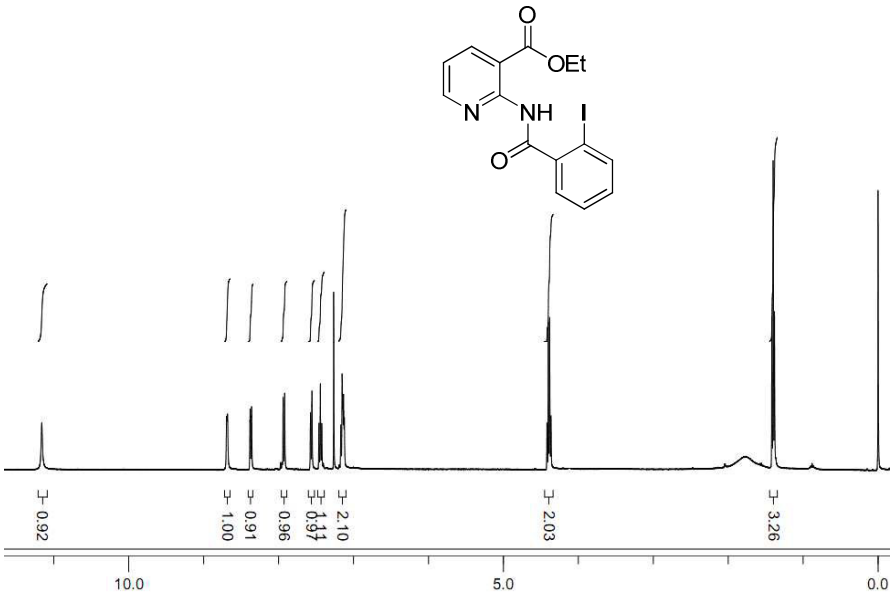

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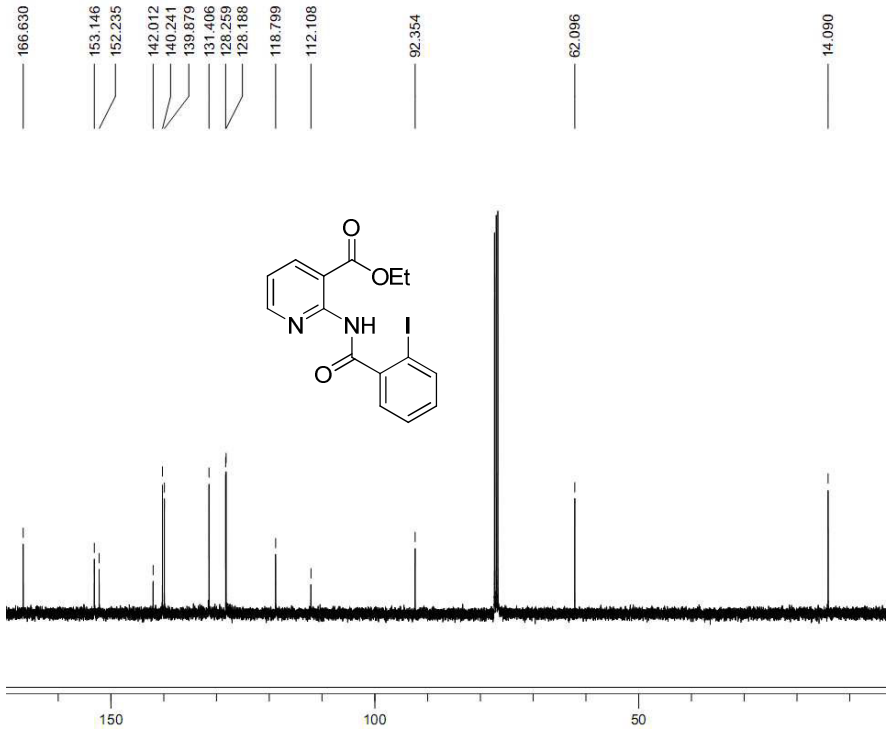

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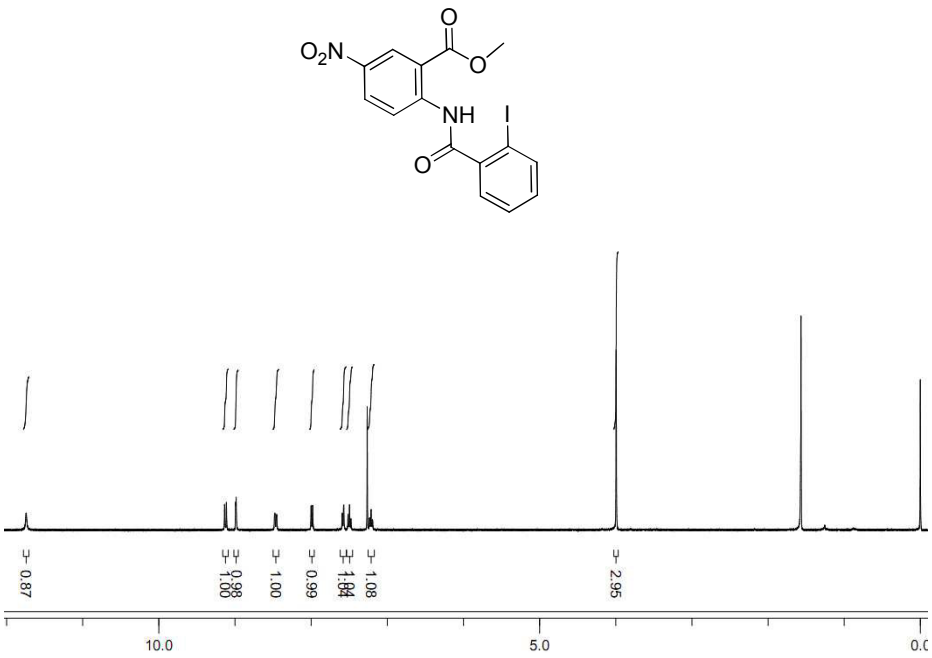

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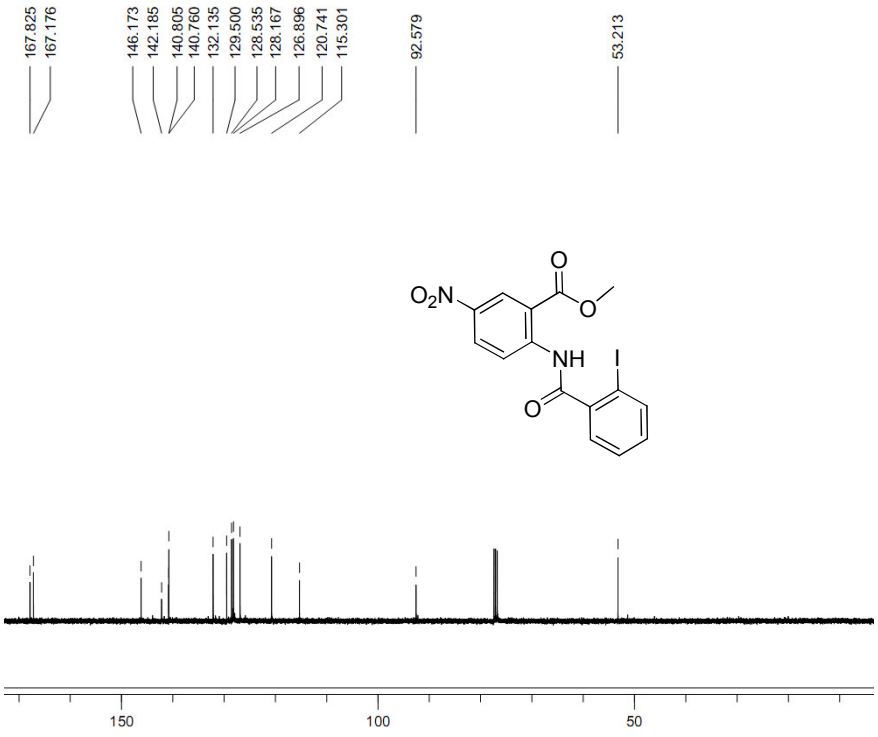

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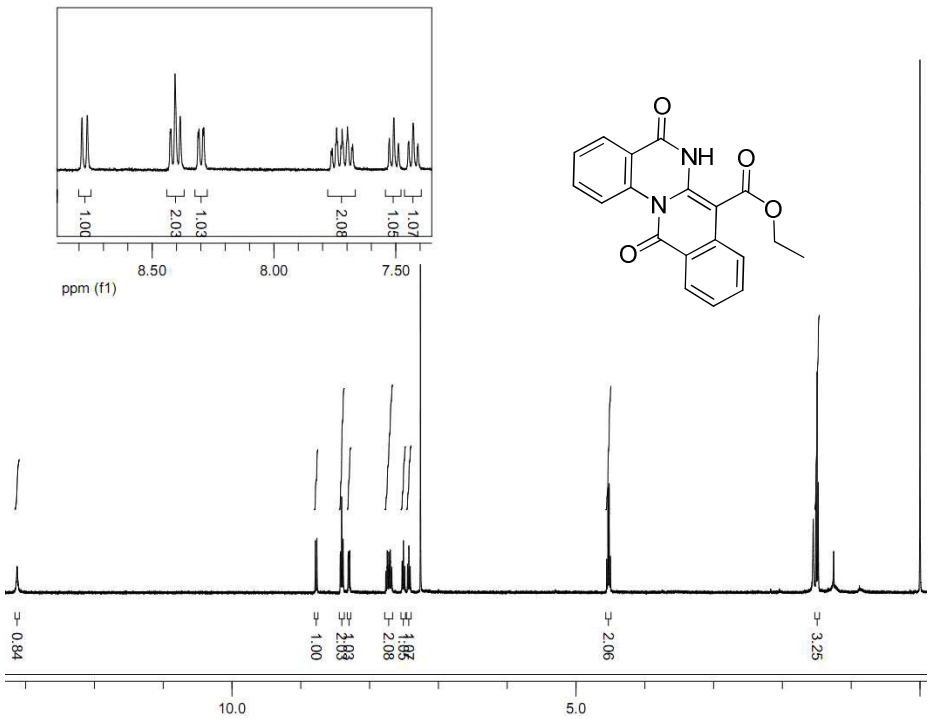
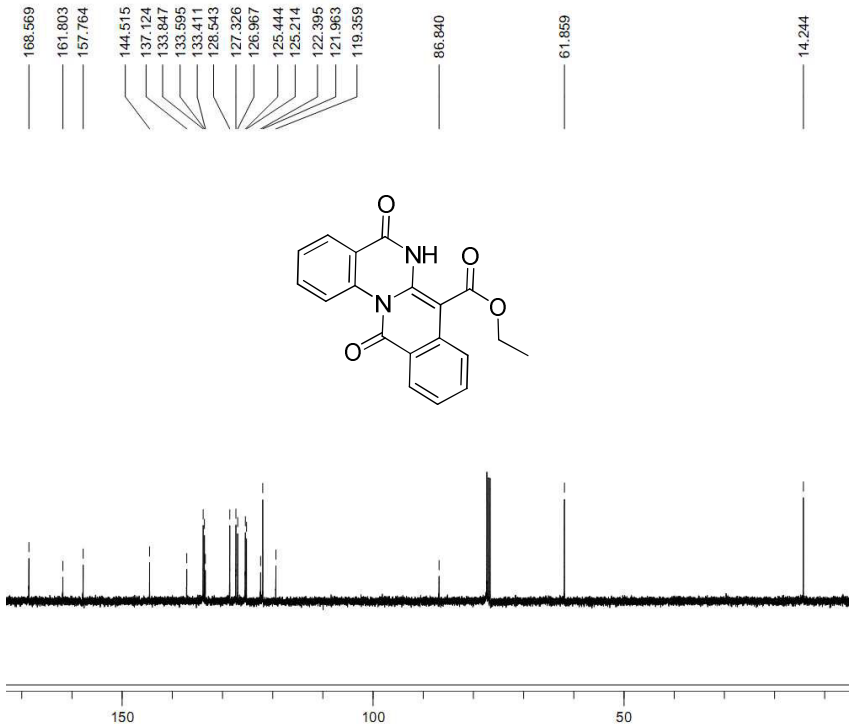


^{1}H NMR spectra of compound **51a** ($CDCl_3$, 400 MHz)



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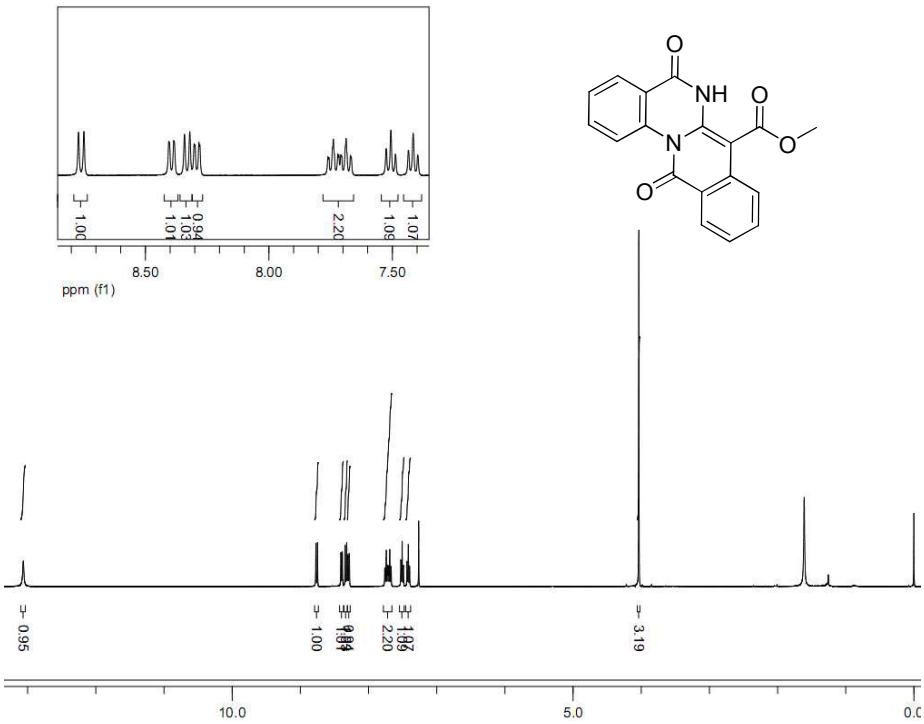

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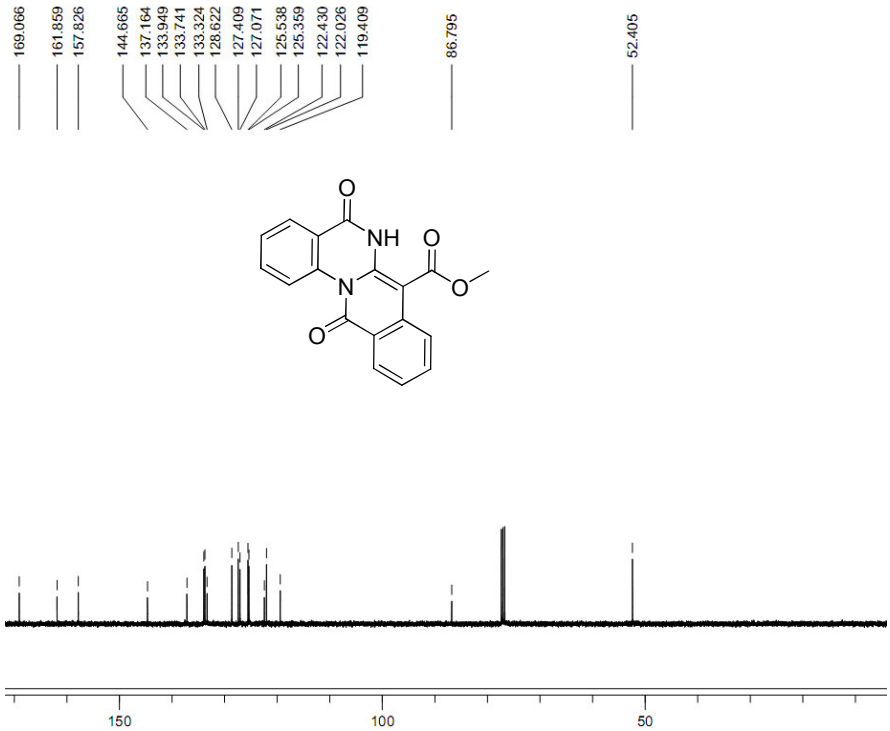

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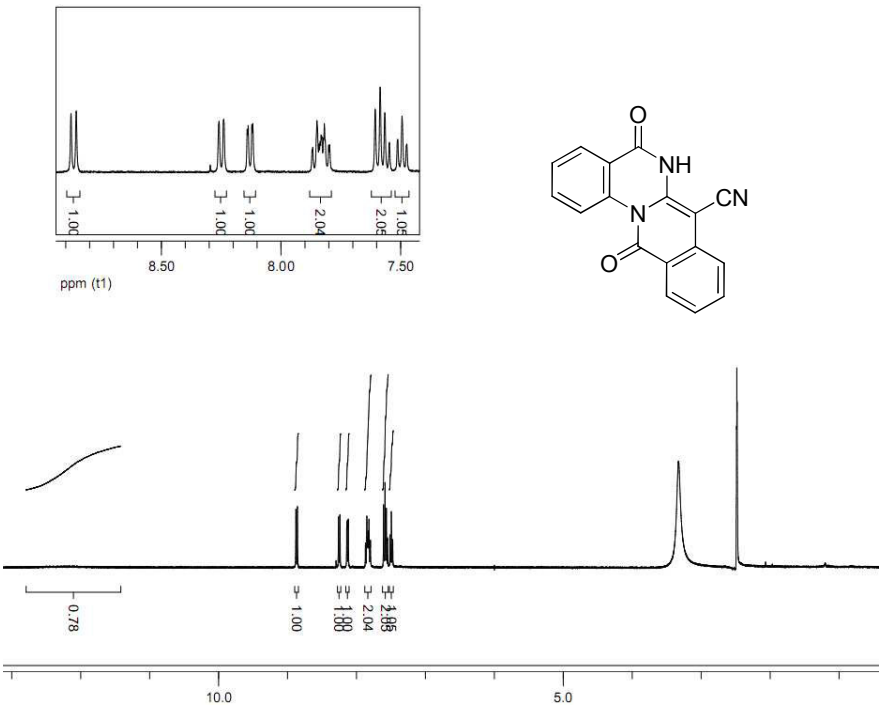

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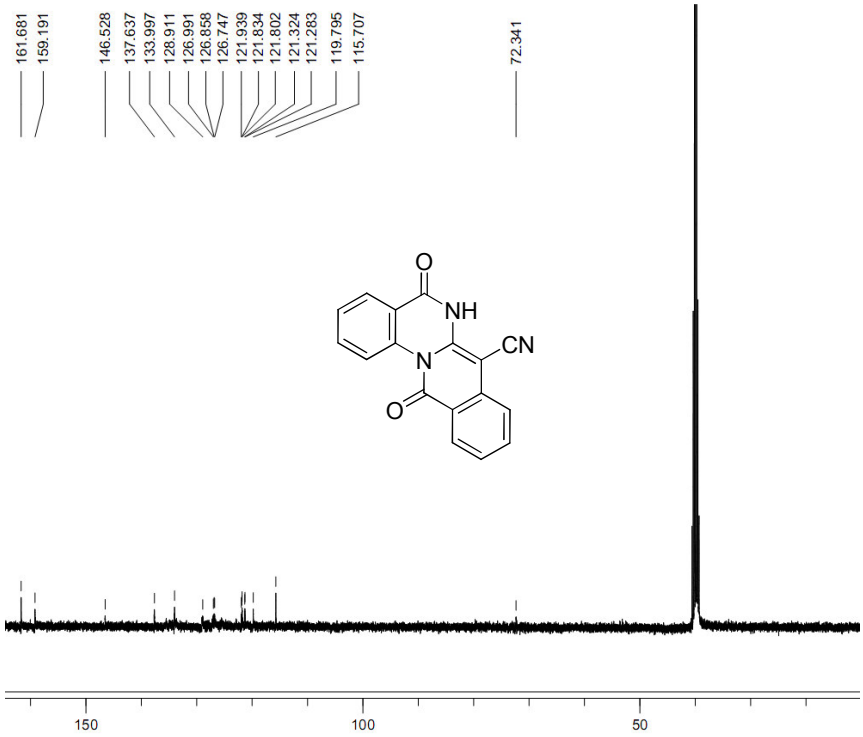
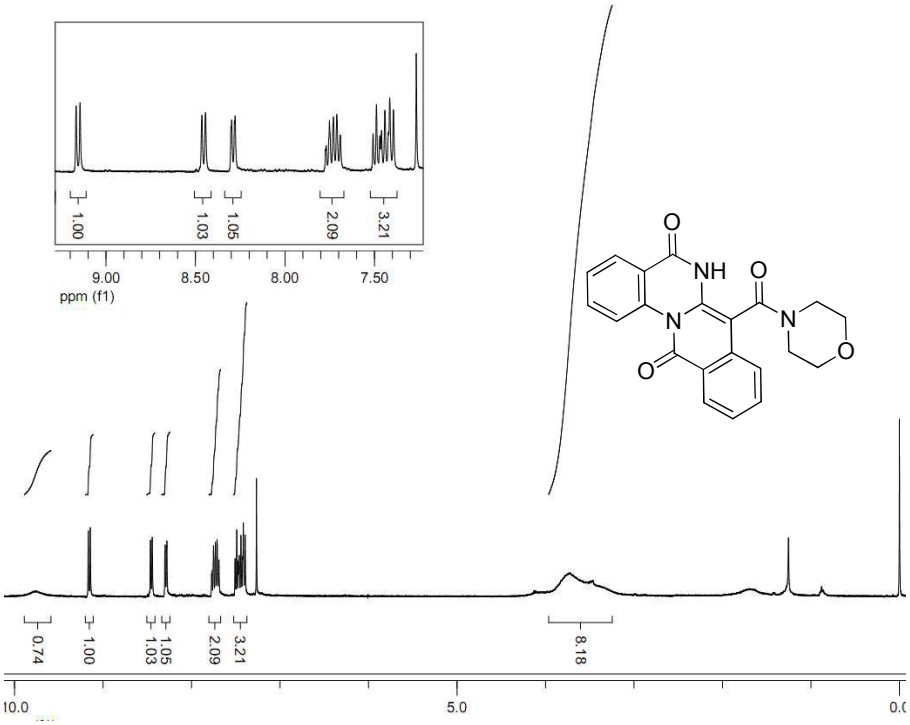


^{13}C NMR spectra of compound **51c** (DMSO-d_6, 100 MHz)



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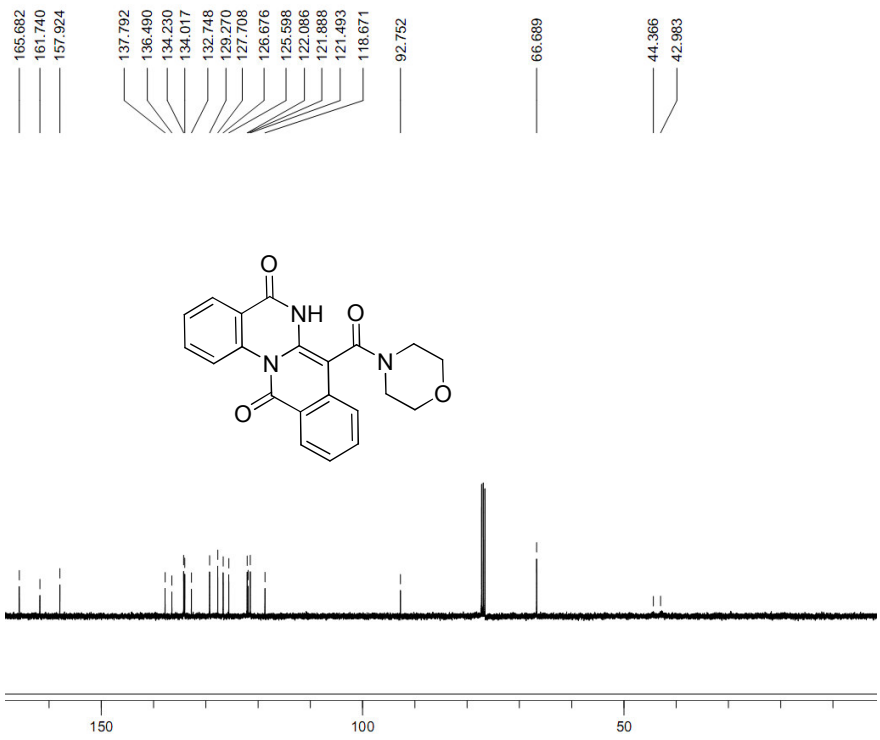

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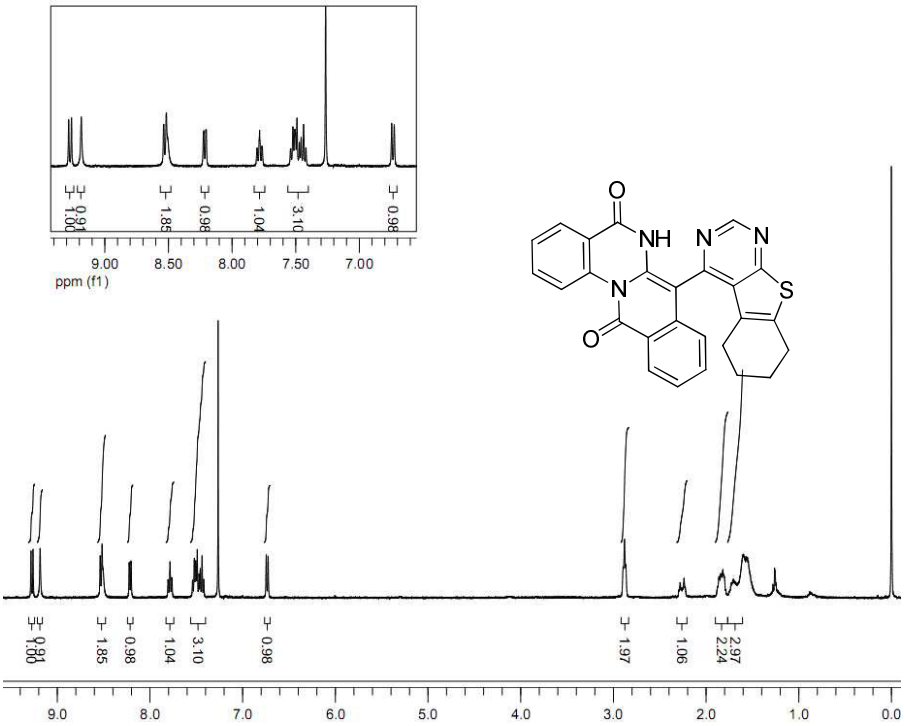

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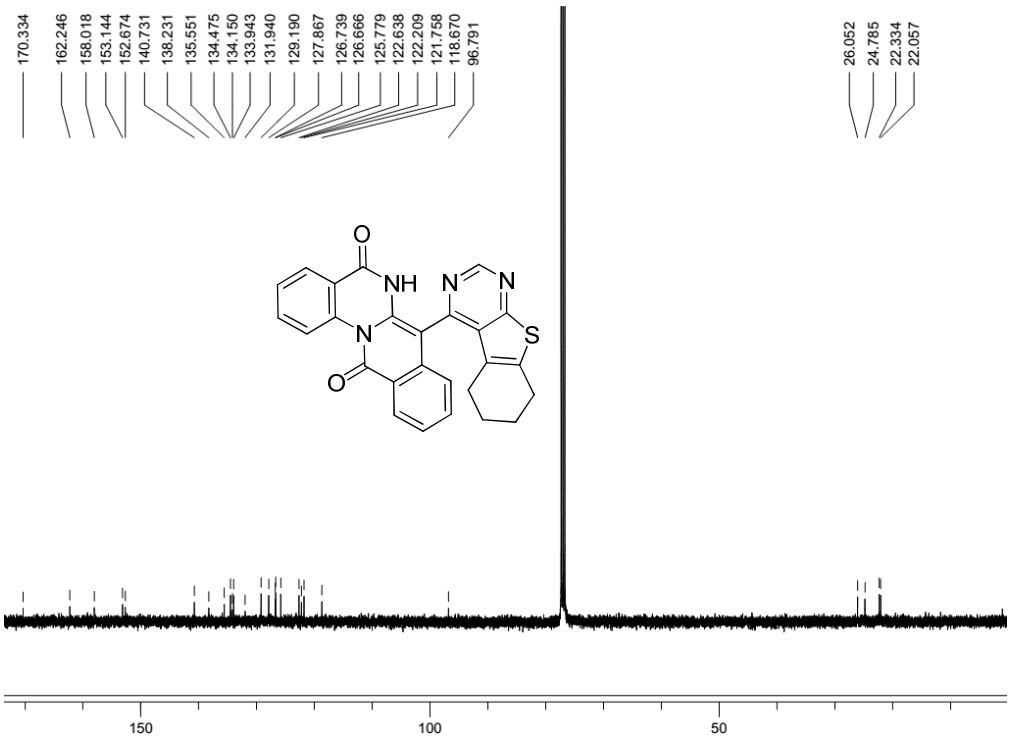

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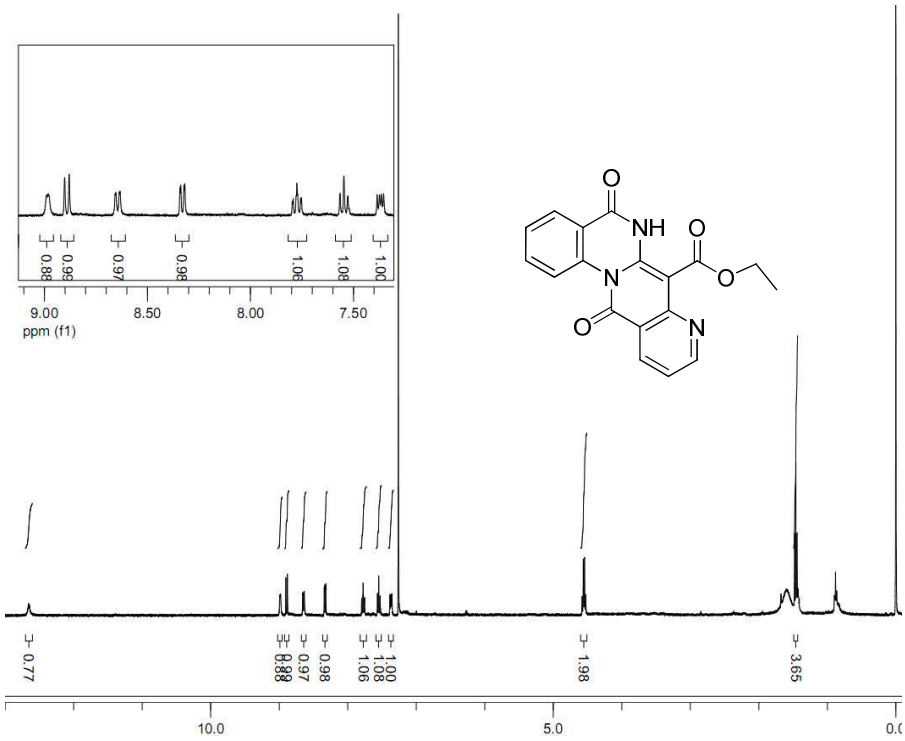

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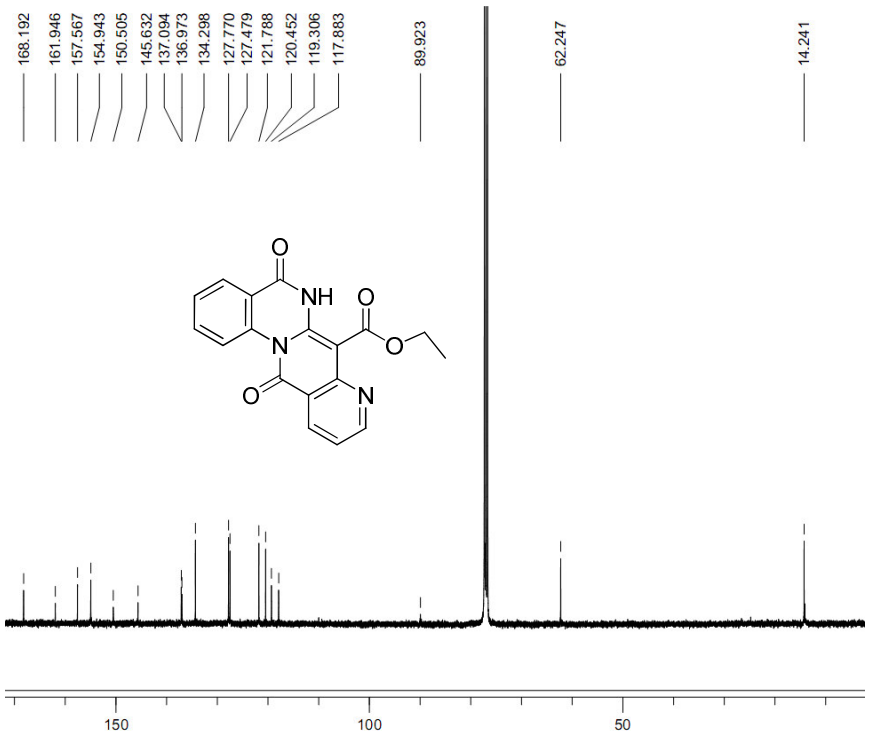

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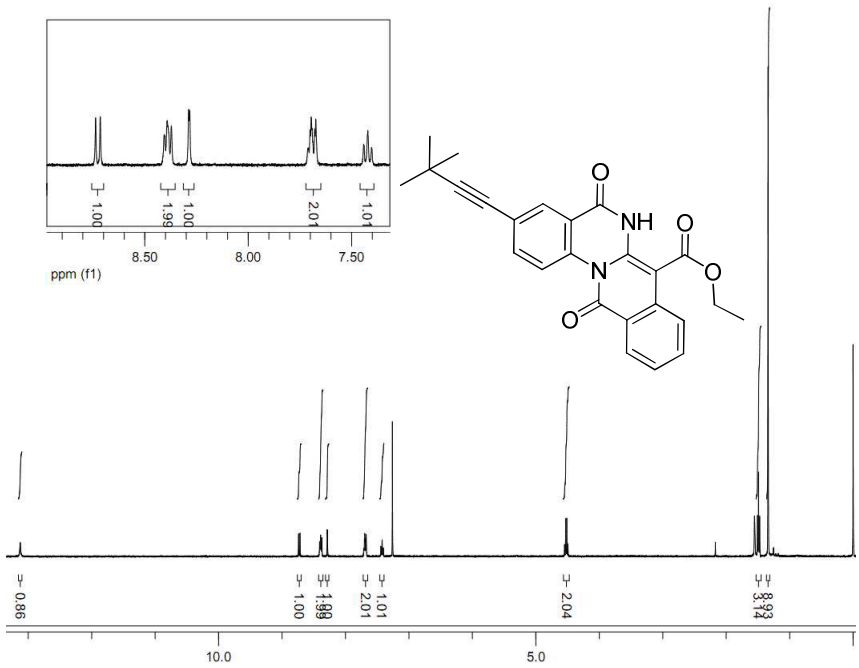

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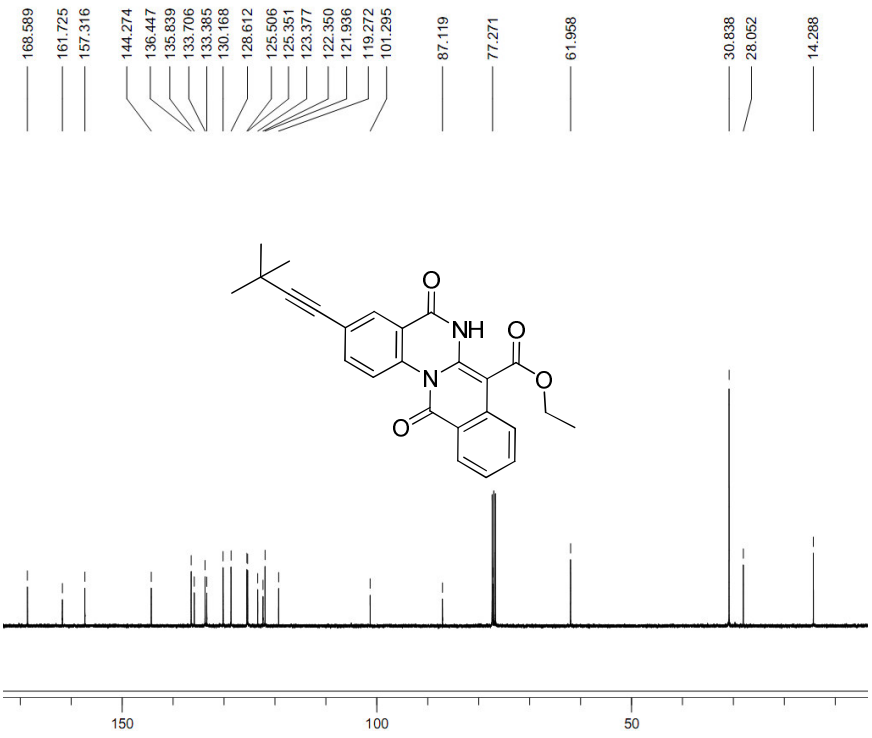

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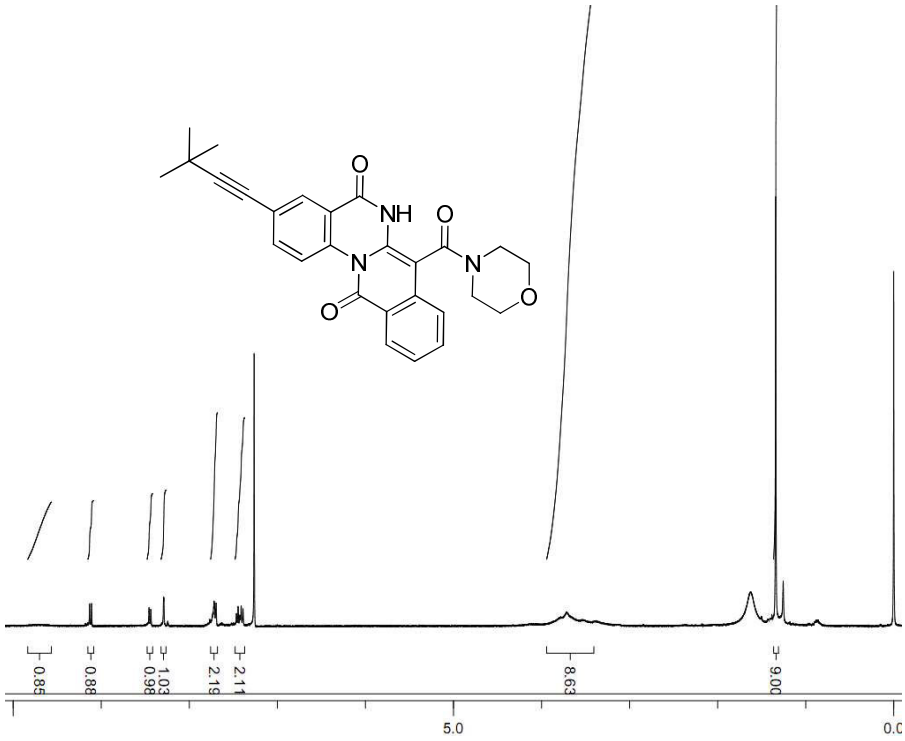

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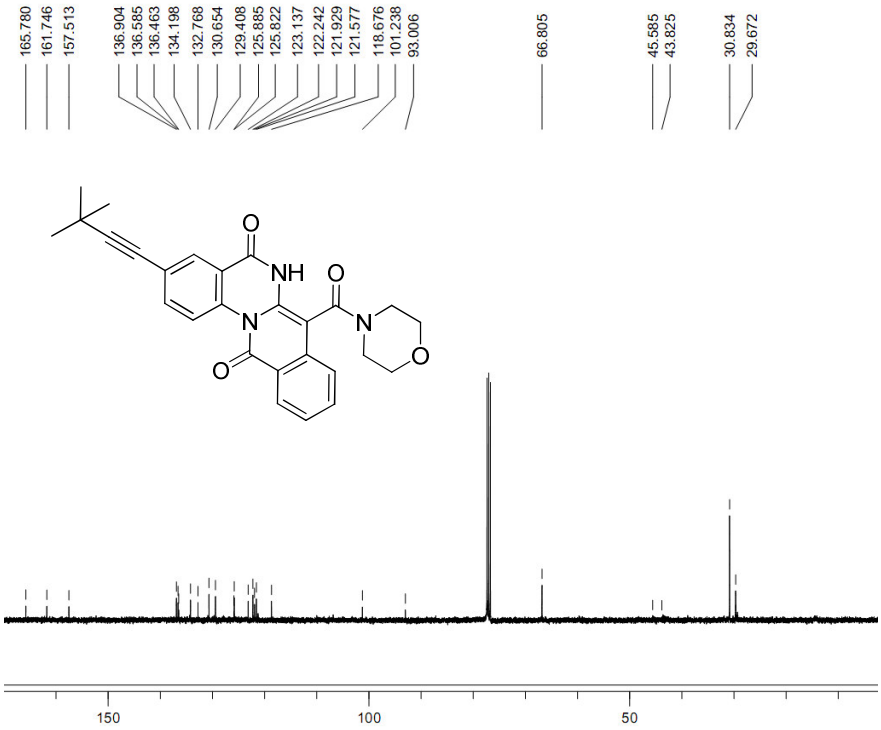

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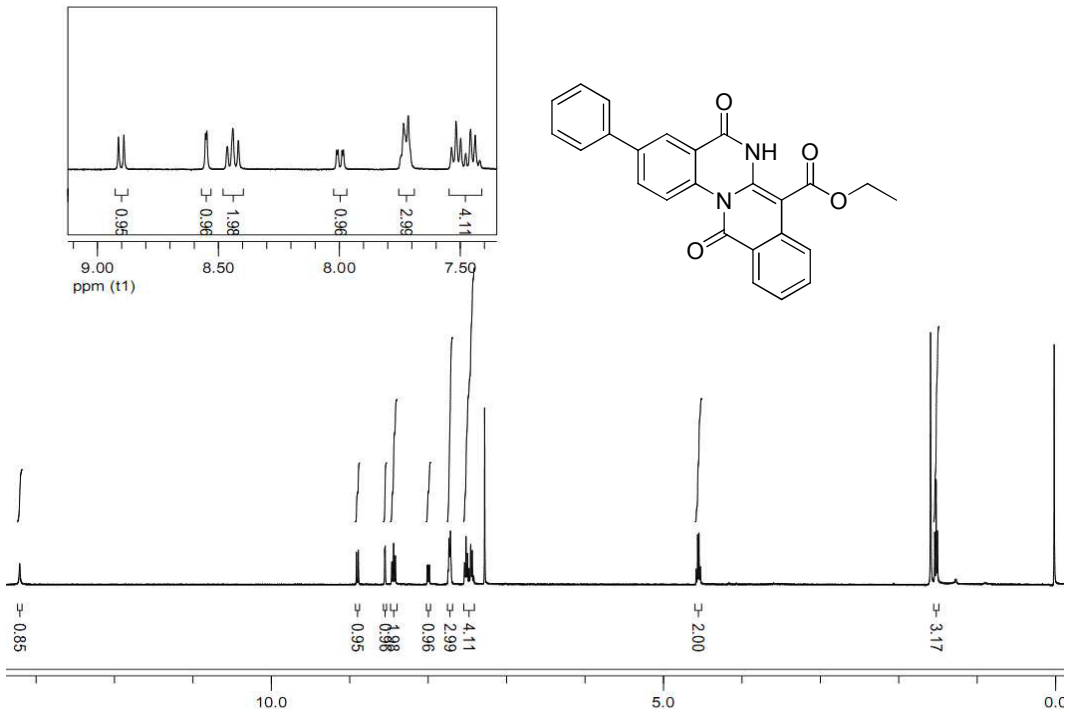

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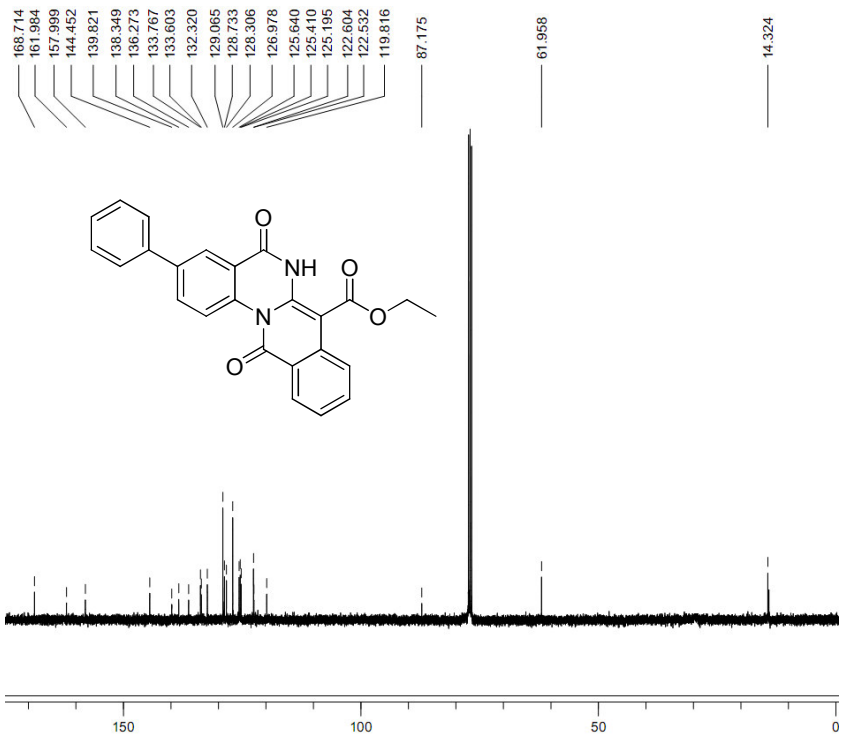

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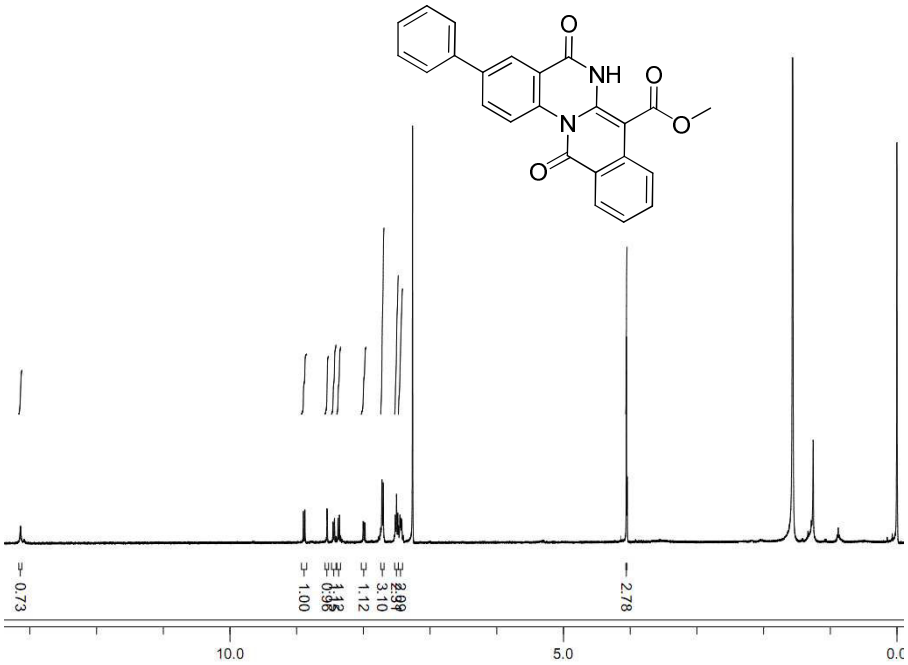
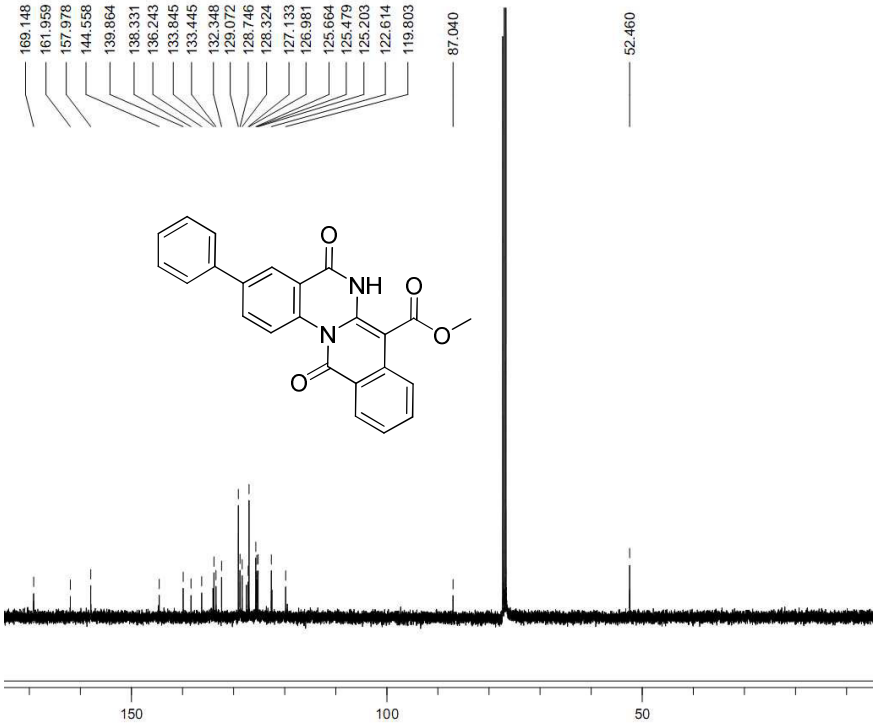
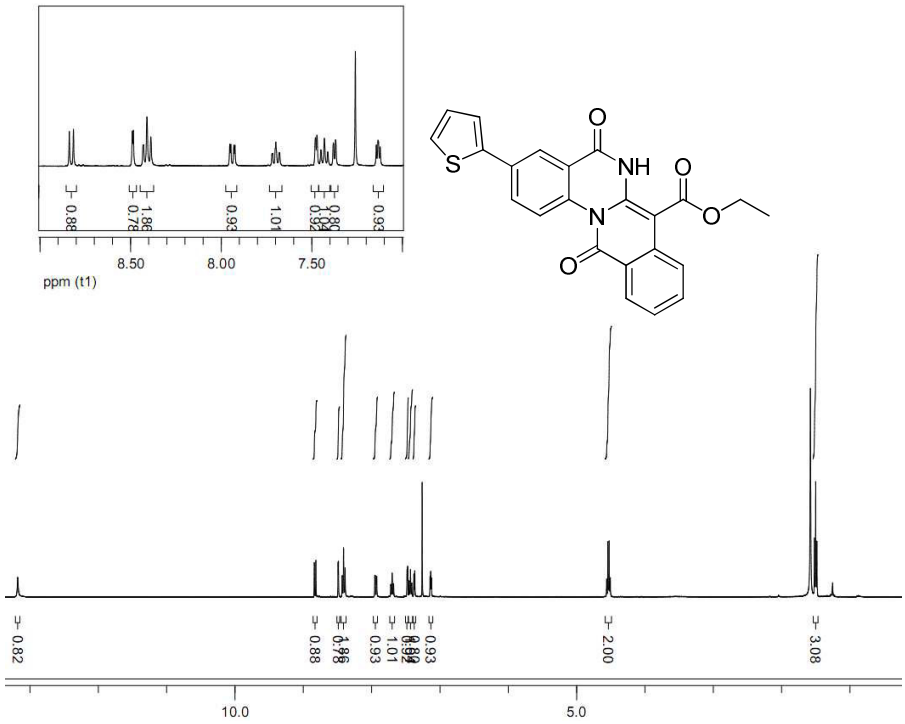


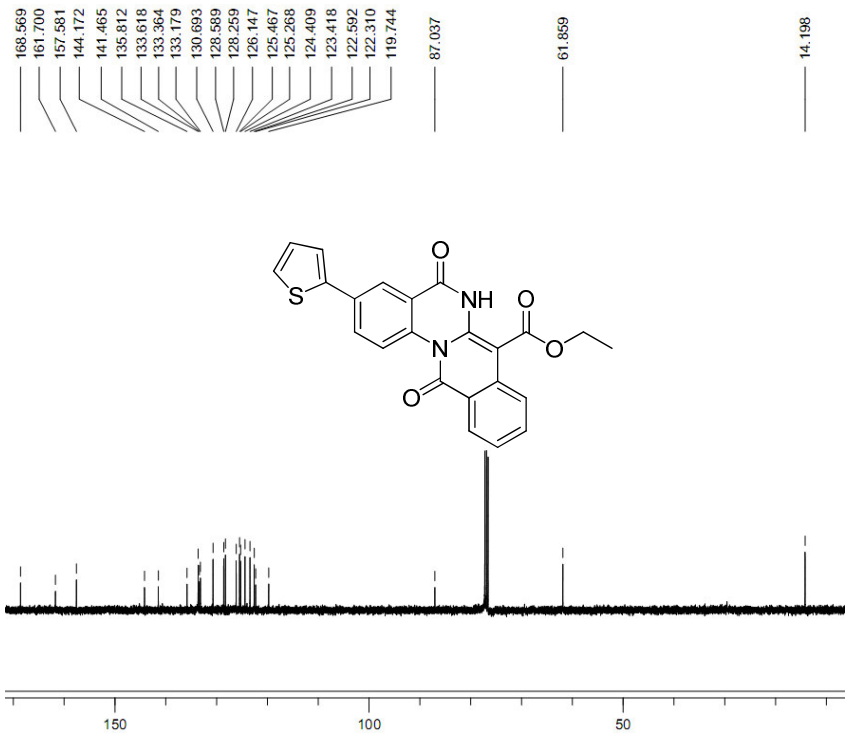
^{1}H NMR spectra of compound **51k** ($CDCl_3$, 400 MHz)



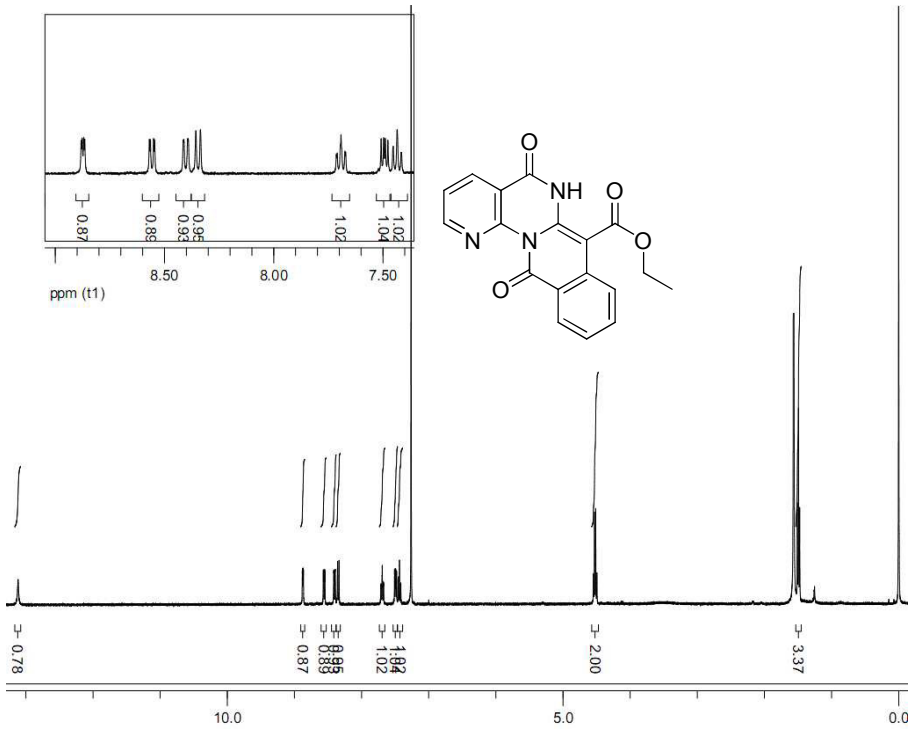
^{13}C NMR spectra of compound **51k** ($CDCl_3$, 100 MHz)



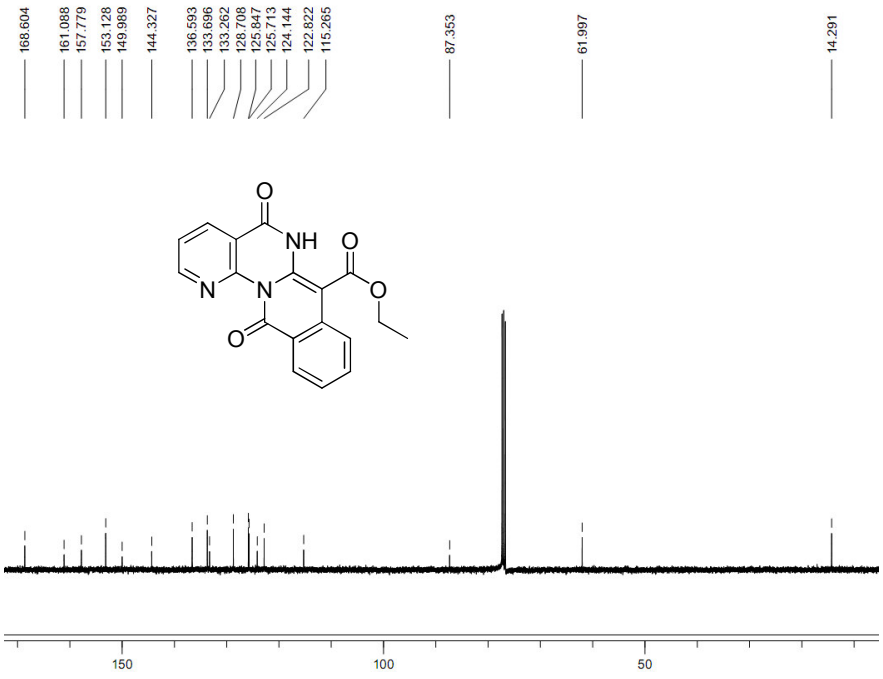
^{1}H NMR spectra of compound **51m** ($CDCl_3$, 400 MHz)



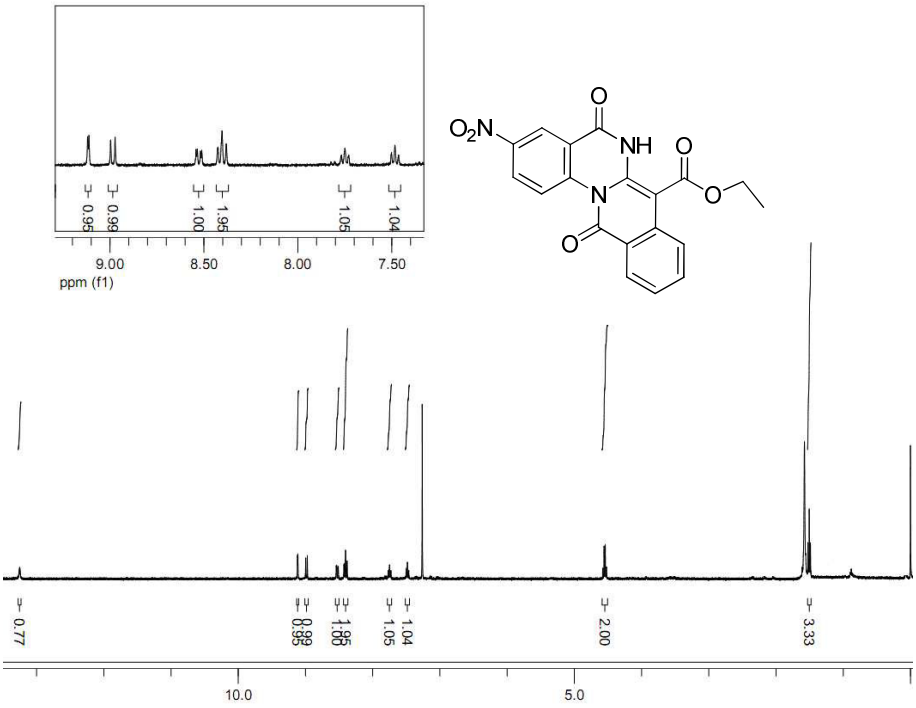
^{13}C NMR spectra of compound **51m** ($CDCl_3$, 100 MHz)



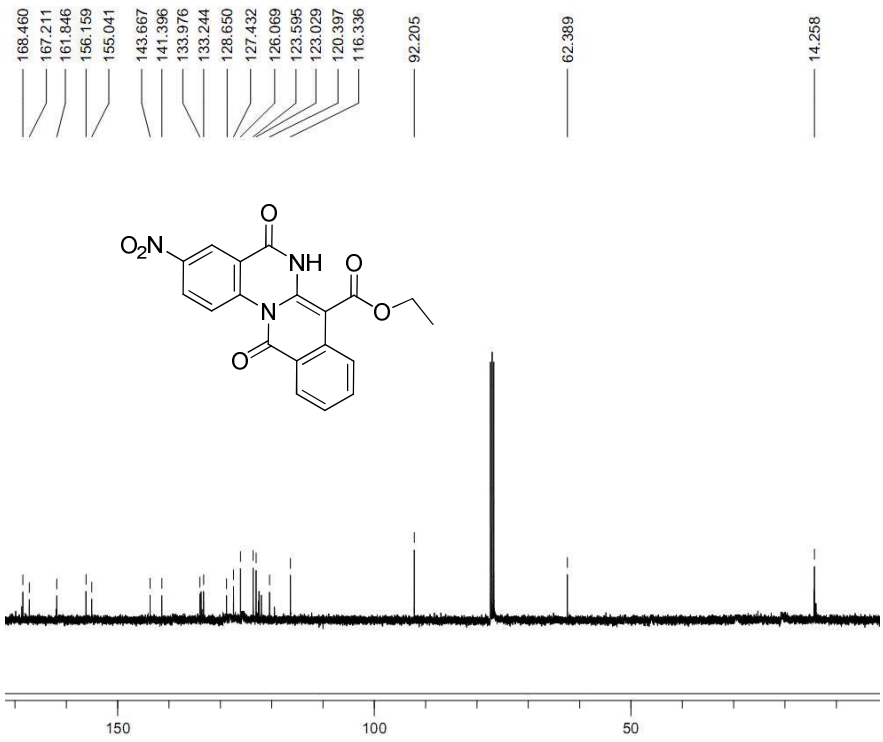
^{1}H NMR spectra of compound **51n** ($CDCl_3$, 400 MHz)



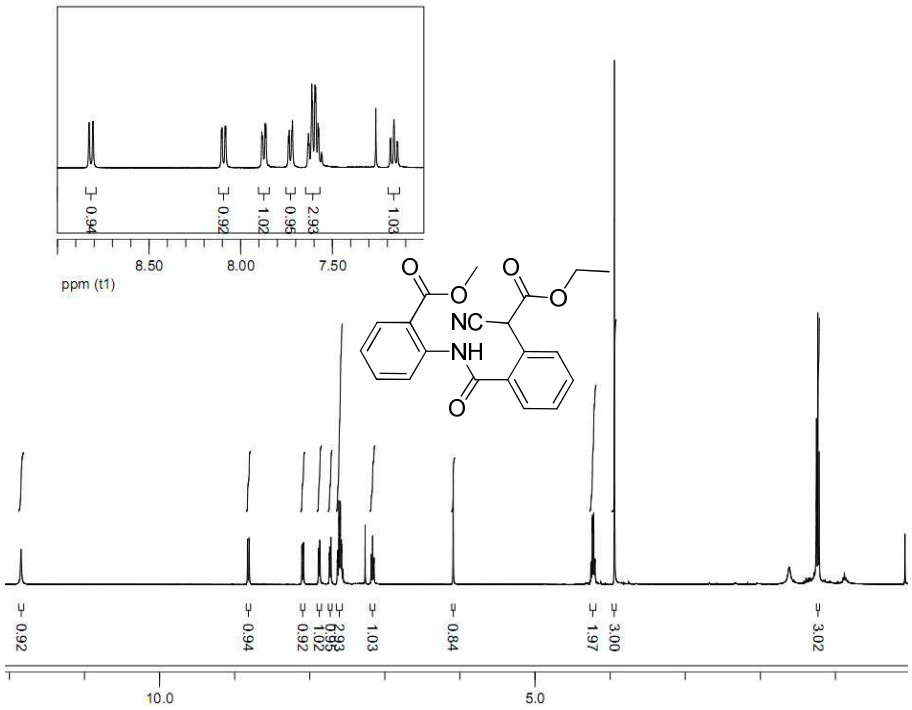
^{13}C NMR spectra of compound **51n** ($CDCl_3$, 100 MHz)



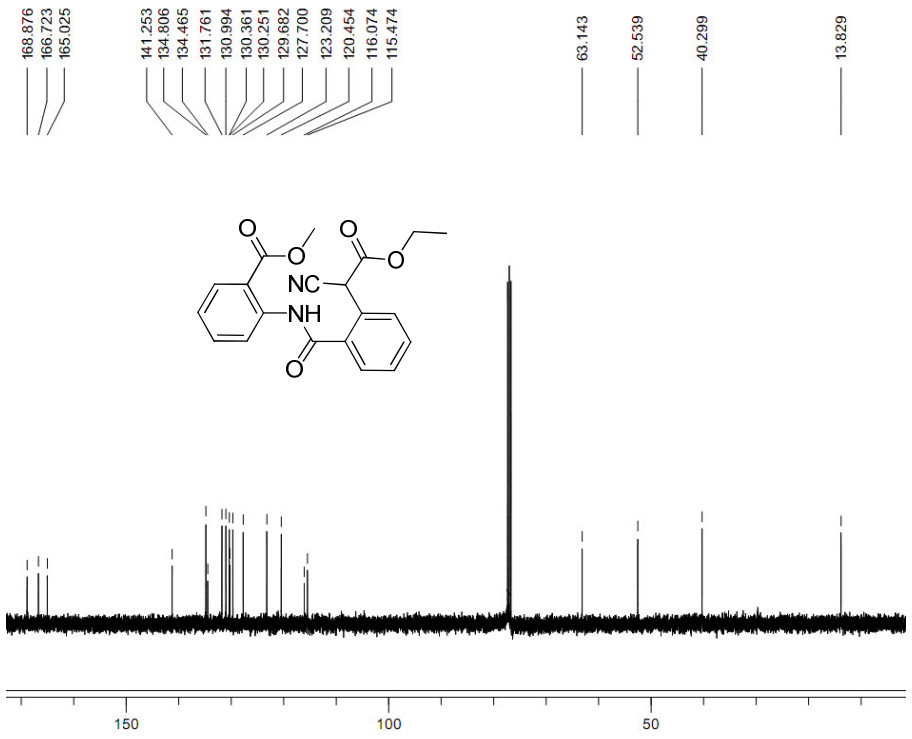
^{1}H NMR spectra of compound **51o** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **51o** ($CDCl_3$, 100 MHz)



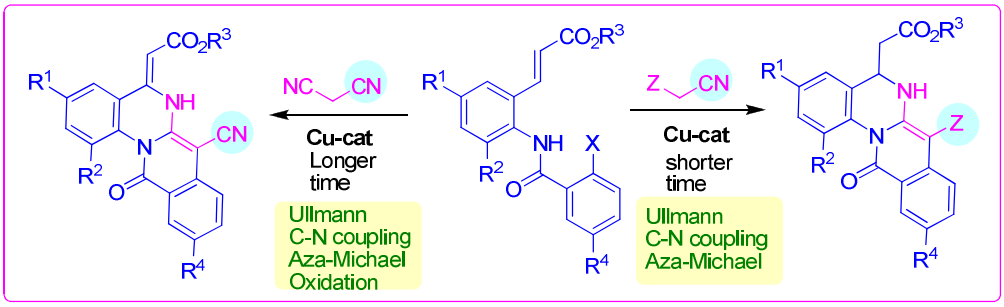
^{1}H NMR spectra of compound **54** ($CDCl_3$, 400 MHz)



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

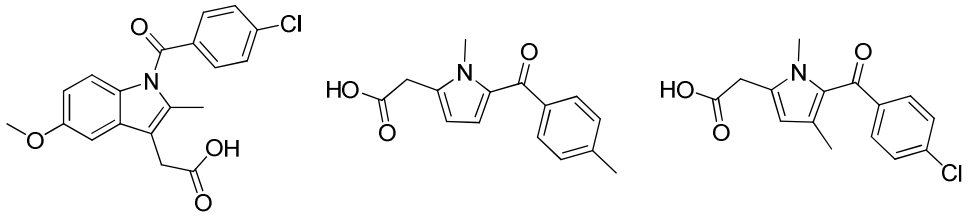
CHAPTER 4

Synthesis of N-heterocyclic acetic acid derivatives via copper catalyzed cascade reaction



4.1. Introduction:

Nitrogen containing heteroaryl acetic acid framework is found in many naturally occurring bioactive compounds possessing anti bacterial and anti fungal activities.[1] Penicillin belongs to this class is derived from the *Penicillium* fungi, and is used for the treatment of bacterial infections caused by susceptible gram positive organisms.[1-2] Some non-steroidal anti inflammatory drugs such as indomethacin, tolmetin, zomepirac also possess *N*-heterocyclic acetic acid framework (Figure 4.1).[3]



1, Indometacin **2**, Tolmetin **3**, zomepirac

Fig. 4.1: Structures of non-steroidal anti inflammatory drugs.

Isoquinoline and quinazoline frameworks are present in several natural products and possess various biological activities as discussed in chapter 3. We hypothesized that the combination of these two frameworks into a single molecule may be useful for the identification of new chemical entities (NCEs) against various biological targets. Since compounds containing isoquinolino[2,3-*a*]quinazoline framework showed impressive anti inflammatory activities (chapter 3), hence the introduction of acetic acid group into this scaffold may afford NCEs possessing potential anti inflammatory activities. In view of the fact that PDE4 inhibitors are effective anti-inflammatory agents, the isoquinolino[2,3-*a*]quinazoline acetic acid framework therefore could be a basis for designing NCEs as potential inhibitors of PDE4[4] (Figure 4.2). Notably, PDE4 inhibitors are reported to be useful for the treatment of various inflammatory diseases including COPD and asthma.

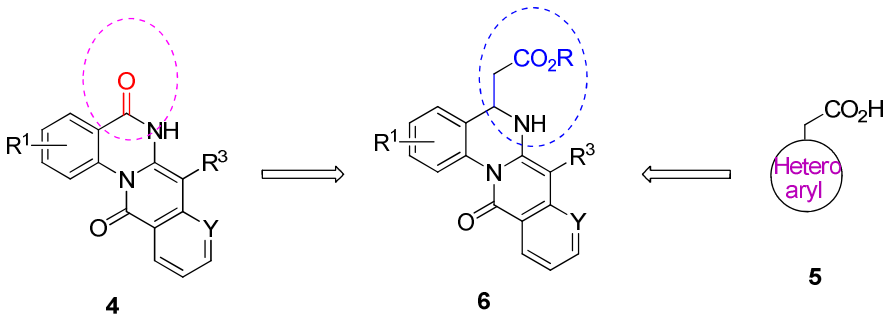
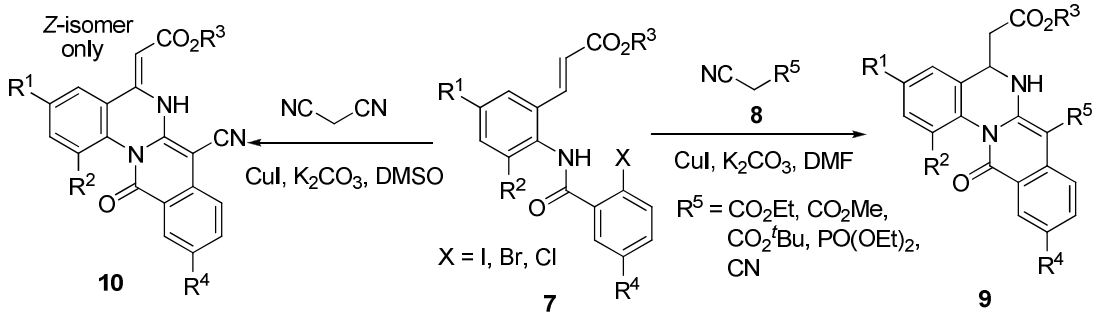


Fig. 4.2: Isoquinolino[2,3-*a*]quinazoline acetatic acid frame work (**6**)

A literature search revealed that the heterocyclic compounds represented by **6** can potentially be synthesized by using metal catalyzed cascade reactions.[5] Among various cascade reactions, copper catalyzed domino reactions are useful in the formation of C-C and C-N bond. Especially, copper catalyzed Ullmann type coupling reactions of active methylene groups with aryl halides are well known in the literature and found to be useful in the construction of tri and tetra cyclic heterocyclic motifs (discussed in chapter 3).

4.2. Present work:

While simpler heterocyclic acid derivatives have been prepared *via* a number of efficient methods,[6] none of them appeared to be useful for the synthesis of our target molecules represented by **6**. In view of our recent success on copper catalyzed domino reactions[7] to construct tri- and tetracyclic ring systems, we envisioned that a Cu-mediated Ullmann type intermolecular C–C coupling, followed by intramolecular C-N bond formation, then intramolecular aza Michael addition[8] and oxidation would furnish our target molecule. Accordingly, we developed a new copper catalyzed one pot domino reaction for the synthesis of **9** and **10** using **7** as a starting material under mild conditions without using any ligand, co catalyst or additive (Scheme 4.1).



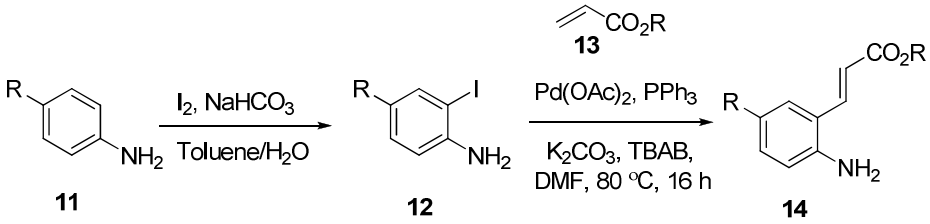
Scheme 4.1: copper catalyzed synthesis of **9** and **10**.

4.3. Results and discussion:

4.3.1. Preparation of starting materials:

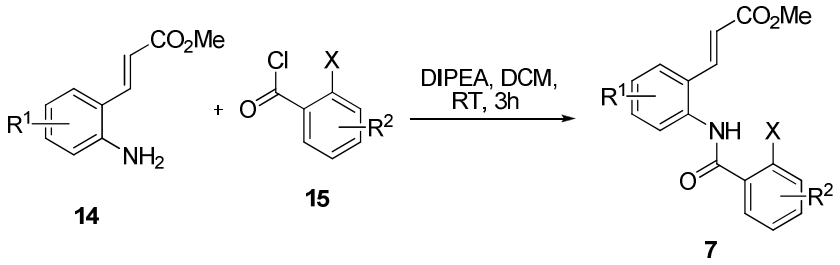
The required starting material (*E*)-alkyl 3-(2-(2-halobenzamido)-5-substitutedphenyl)acrylate, **7** was synthesized from substituted anilines. Iodination on anilines provided 2-iodo substituted anilines (**12**) which on Heck reaction with various acrylates (**13**) afforded (*E*)-alkyl 3-(2-amino-5-substitutedphenyl)acrylate, **14**

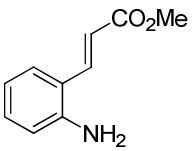
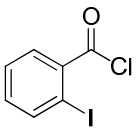
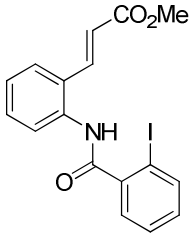
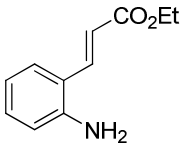
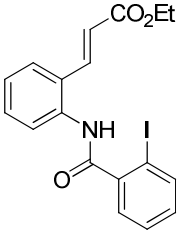
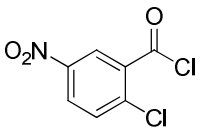
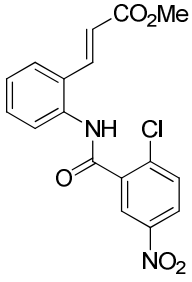
as shown in Scheme 4.2. Reaction of **14** with various 2-halo acid chlorides (**15**) gave the desired starting material **7** as shown in Table 4.1.

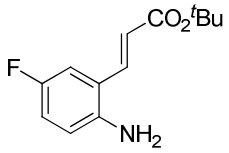
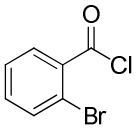
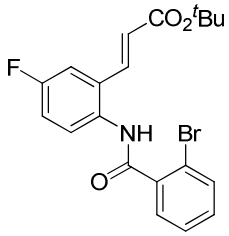
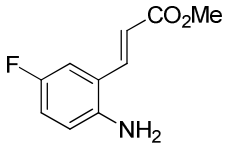
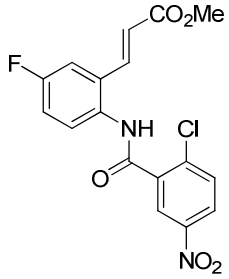
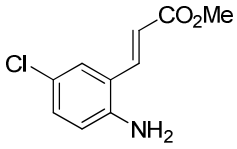
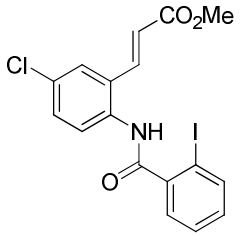
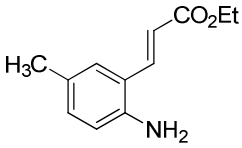
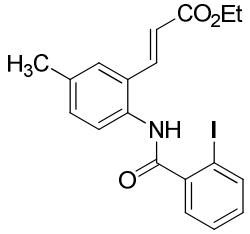
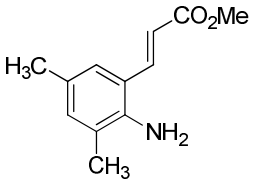
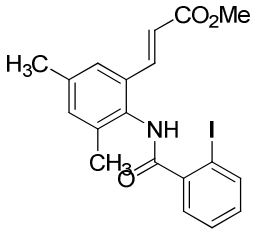
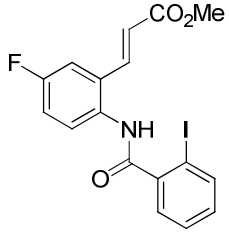


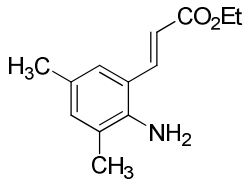
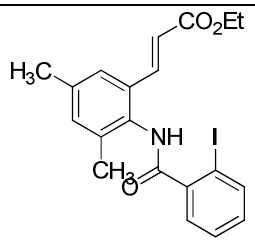
Scheme 4.2: Synthesis of (*E*)-alkyl 3-(2-amino-5-substitutedphenyl)acrylate.

Table 4.1: Synthesis of (*E*)-alkyl 3-(2-(2-halobenzamido)-5-substitutedphenyl) acrylate.[a]



S.No	Acrylate (**13**)	Acid chloride (**14**)	Product (**7**)	Yield[b]
1	 **14a**	 **15a**	 **7a**	52
2	 **14b**	**15a**	 **7b**	58
3	**14a**	 **15b**	 **7c**	56

4	 **14c**	 **15c**	 **7d**	62
5	 **14d**	**15b**	 **7e**	56
6	 **14e**	**15a**	 **7f**	74
7	 **14f**	**15a**	 **7g**	66
8	 **14g**	**15a**	 **7h**	64
9	**14d**	**15a**	 **7i**	67

10	 **14h**	**15a**	 **7j**	63

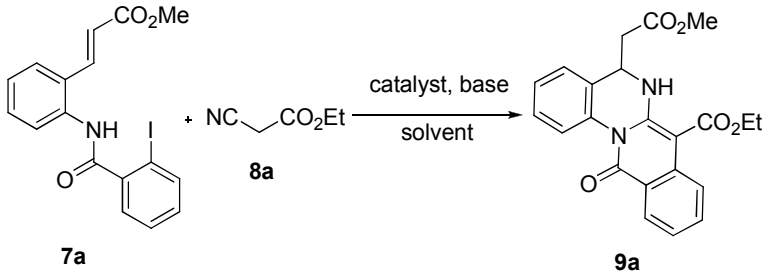
[a]Reactions were carried out using **14** (1 mmol), **15** (1.2 mmol) and DIPEA (1.5 mmol) in DCM under nitrogen atmosphere for 3 hours.

[b]Isolated yield.

4.3.2. Reaction optimization:

Initially the coupling of (*E*)-Methyl-3-(2-(2-iodobenzamido)phenyl)acrylate (**7a**) with ethyl cyano acetate (**8a**) was used to establish the optimized reaction conditions by changing various factors including catalysts, base and solvents. The reaction carried out using 0.1 equiv. of CuI and 3.0 equiv. of K_2CO_3 in DMF provided the highest yield of desired product within 0.5 h (entry 1, Table 4.2). By replacing K_2CO_3 with Na_2CO_3, the yield was decreased (entry 2, Table 4.2) whereas Cs_2CO_3 was found to be same as K_2CO_3 (entry 3, Table 4.2). Among the solvents used, DMF and DMSO was found to be similar and better than 1,4-dioxane (entry 1, 4-5, Table 4.2). The use of other copper salts like CuBr, and CuCl also provided good yields (entry 6-7, Table 4.2). The Cu (II) salts such as $Cu(OAc)_2$ and $Cu(OTf)_2$ also gave good yields of product (entry 8-9, Table 4.2). However, among all the copper salts examined the copper iodide provided superior yield. The reaction did not proceed at room temperature (entry 10, Table 4.2).

Table 4.2: Optimization of reaction conditions.



Entry[a]	Catalyst	Base	Solvent	Yield[b] (%)
1	CuI	K_2CO_3	DMF	89
2	CuI	Na_2CO_3	DMF	79
3	CuI	Cs_2CO_3	DMF	88
4	CuI	K_2CO_3	DMSO	88
5	CuI	K_2CO_3	1.4-dioxane	53
6	CuBr	K_2CO_3	DMF	80
7	CuCl	K_2CO_3	DMF	71
8	$Cu(OAc)_2$	K_2CO_3	DMF	78
9	$Cu(OTf)_2$	K_2CO_3	DMF	80
10	CuI	K_2CO_3	DMF	0[c]

[a]Reactions were carried out using **7a** (1 mmol), **8a** (1.2 mmol), catalyst (0.1 mmol) and base (3 mmol) in solvent (2 mL) at 80 ºC for 0.5 h under anhydrous conditions (no inert atmosphere).

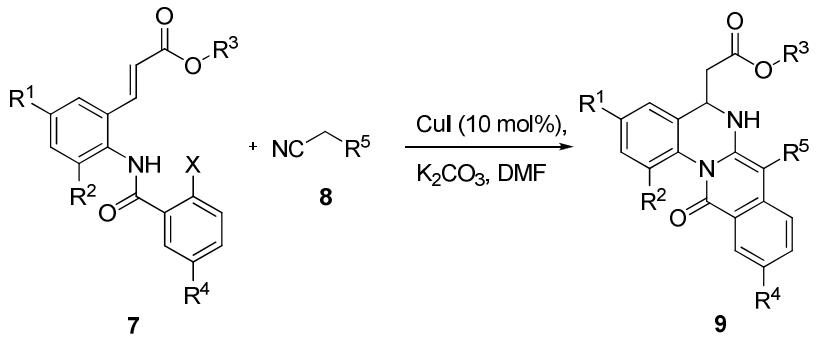
[b]Isolated yield.

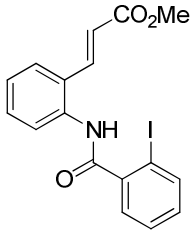
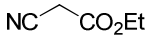
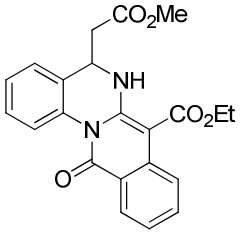
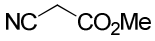
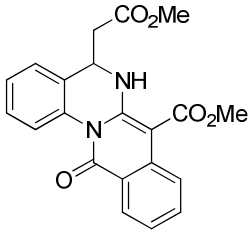
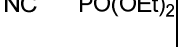
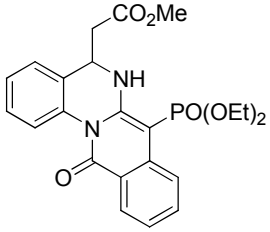
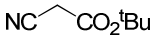
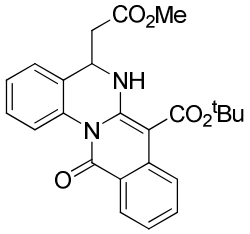
[c]Reaction performed at room temperature.

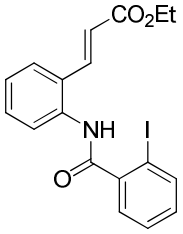
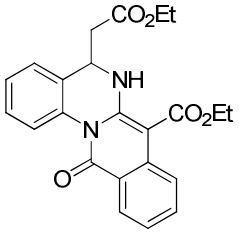
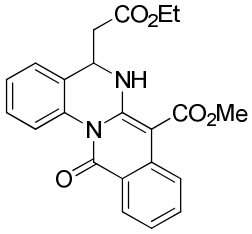
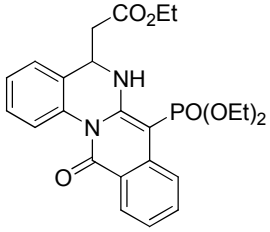
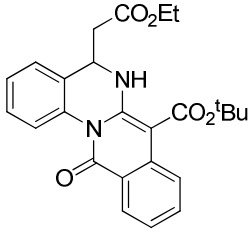
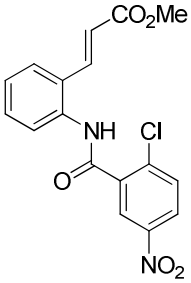
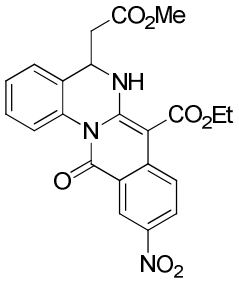
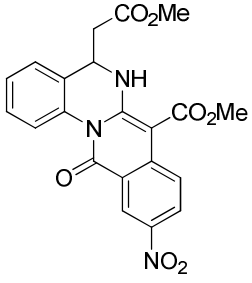
4.3.3. Scope of the reaction:

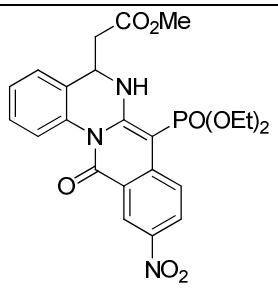
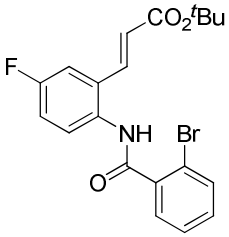
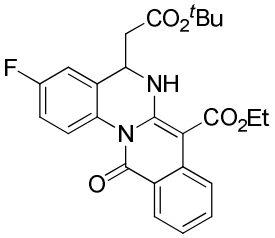
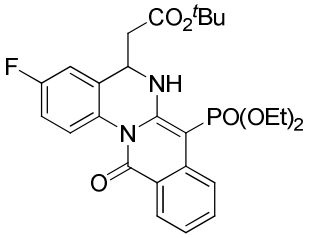
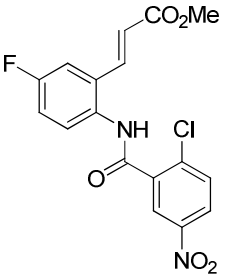
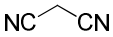
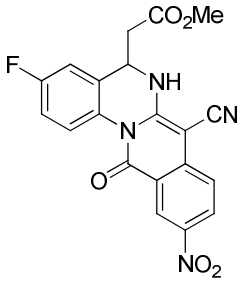
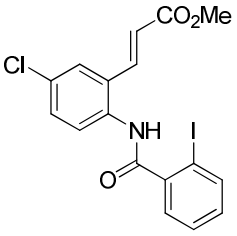
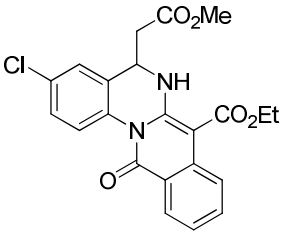
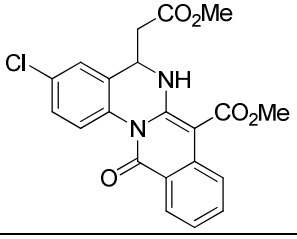
The reaction scope was then examined under the optimized reaction conditions, and the results are summarized in Table 4.2. The substitutions on acrylate group like fluorine, chlorine, methyl and di methyl groups afforded good yields of desired products (entry 12-25, Table 4.3). On the other side, reactivity of bromo derivative was found to be inferior compared to the iodo compound (entry 1 *vs.* 12, Table 4.3) and chlorine also participated well in the reaction when an electron withdrawing group was present (entry 9-11 & 14, Table 4.3). Furthermore, other types of acetonitriles, such as ethyl cyano acetate, methyl cyano acetate, *t*-butyl cyano acetate, diethyl cyano methyl phosphonate and malano nitrile provided high yields. Interestingly, continuation of the reaction with malononitrile provided compound containing exocyclic double bond with *Z*-geometry exclusively (Table 4.4). This was further explored using various moiety having F, Cl, CH_3 and 2,4-di methyl functional groups with good to moderate yields of desired products (entry 1-8, Table 4.4).

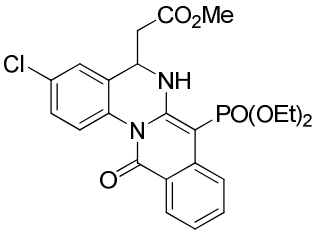
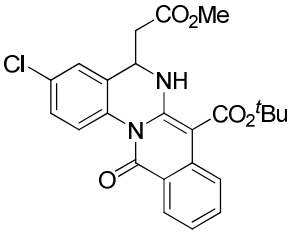
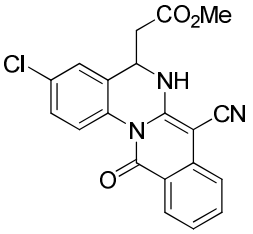
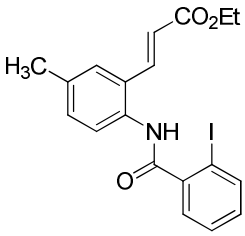
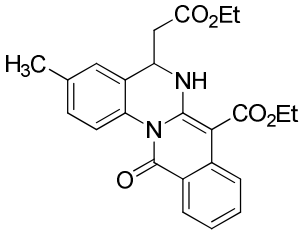
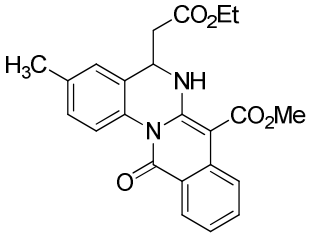
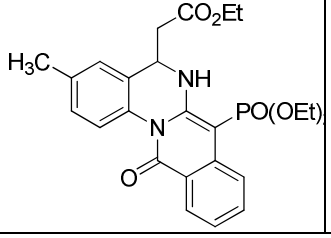
Table 4.3: Cu catalyzed synthesis of 2-(12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (**9**)[a]

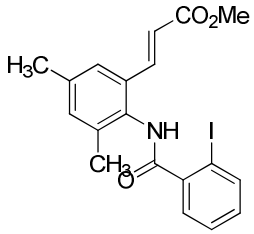
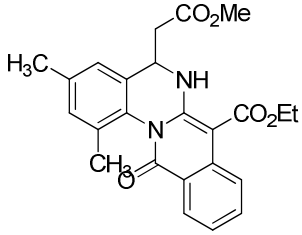
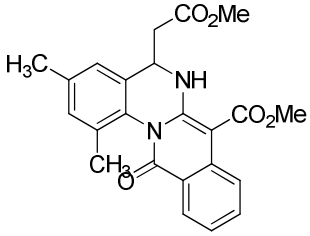
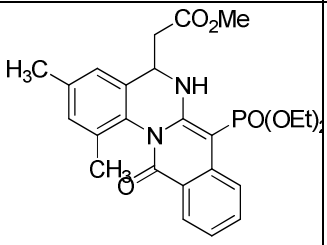


Entry	Halide (**7**)	Nitrile (**8**)	Time/h	Product (**9**)	Yield (%)[b]
1	 **7a**	 **8a**	0.5	 **9a**	89
2	**7a**	 **8b**	0.5	 **9b**	84
3	**7a**	 **8c**	2.0	 **9c**	81
4	**7a**	 **8d**	0.5	 **9d**	81

5	 **7b**	**8a**	0.5	 **9e**	87
6	**7b**	**8b**	0.5	 **9f**	83
7	**7b**	**8c**	1.5	 **9g**	79
8	**7b**	**8d**	0.5	 **9h**	81
9	 **7c**	**8a**	1.5	 **9i**	76
10	**7c**	**8b**	1.5	 **9j**	73

11	**7c**	**8c**	2.5	 **9k**	73
12	 **7d**	**8a**	2.0	 **9l**	80
13	**7d**	**8c**	2.5	 **9m**	75
14	 **7e**	 **8e**	2.5	 **9n**	68
15	 **7f**	**8a**	0.5	 **9o**	85
16	**7f**	**8b**	0.5		81

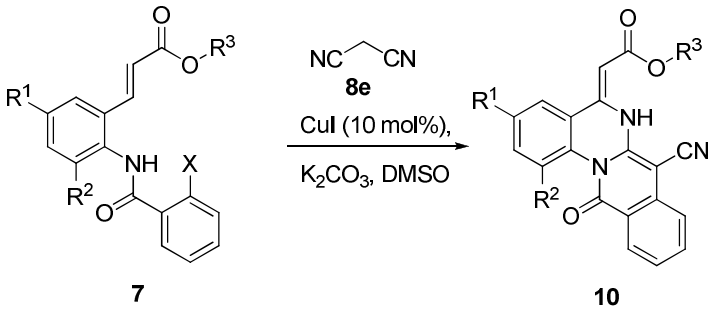
				9p	
17	**7f**	**8c**	1.5	 **9q**	78
18	**7f**	**8d**	1.0	 **9r**	77
19	**7f**	**8e**	1.0	 **9s**	72
20	 **7g**	**8a**	0.5	 **9t**	87
21	**7g**	**8b**	0.5	 **9u**	84
22	**7g**	**8c**	2.0		82

				9v	
23	 **7h**	**8a**	0.5	 **9w**	86
24	**7h**	**8b**	0.5	 **9x**	81
25	**7h**	**8c**	1.5	 **9y**	78

[a]Reactions were carried out using **7** (1 mmol), **8** (1.2 mmol), CuI (0.1 mmol) and K_2CO_3 (3 mmol) in DMF (2 mL) at 80 ºC under anhydrous conditions (no inert atmosphere).

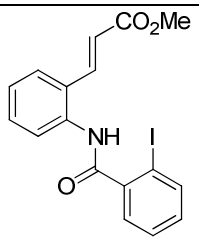
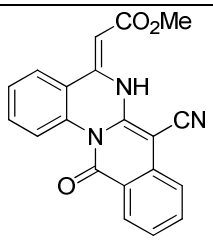
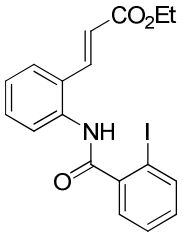
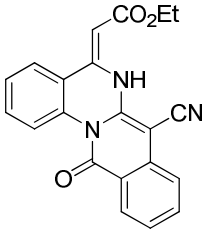
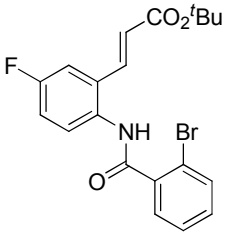
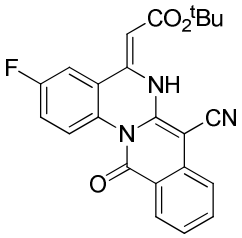
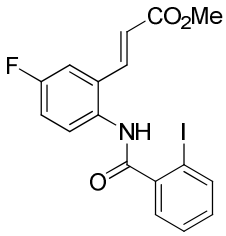
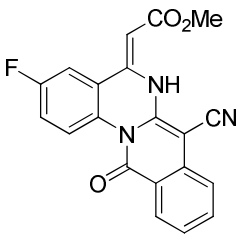
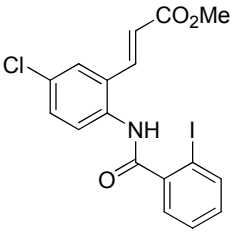
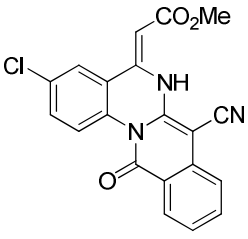
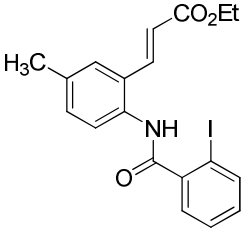
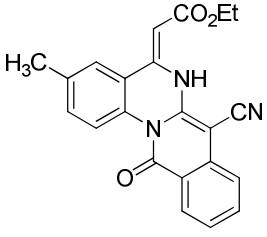
[b]Isolated yield.

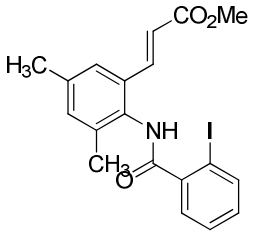
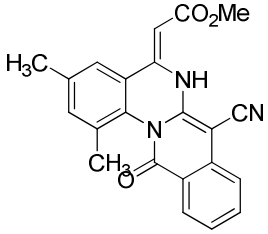
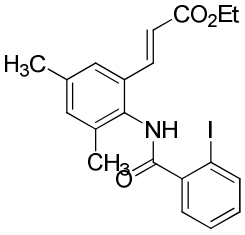
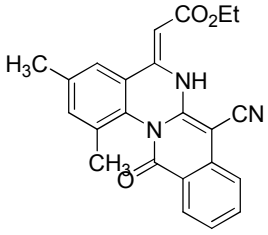
Table 4.4: Cu catalyzed synthesis of (*Z*)-alkyl-2-(7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-ylidene)acetate (**10**)[a]



7 **10**

Entry	Halide (**7**)	Time/h	Product (**10**)	Yield (%)[b]

1	 **7a**	4.0	 **10a**	72
2	 **7b**	4.0	 **10b**	71
3	 **7d**	5.0	 **10c**	49
4	 **7i**	6.0	 **10d**	56
5	 **7f**	5.0	 **10e**	48
6	 **7g**	4.0		65

			10f	
7	 **7h**	4.0	 **10g**	73
8	 **7j**	6.0	 **10h**	72

[a]Reactions were carried out using **7** (1 mmol), malononitrile (1.2 mmol), CuI (0.1 mmol) and K_2CO_3 (3 mmol) in DMSO (2 mL) at 80 ºC under anhydrous conditions (no inert atmosphere). [b]Isolated yield.

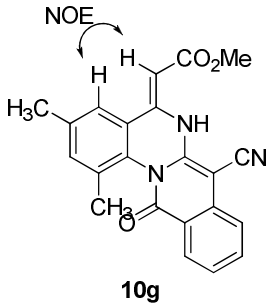


Fig. 4.3: NOE correlation of exocyclic vinyl proton and aromatic proton.

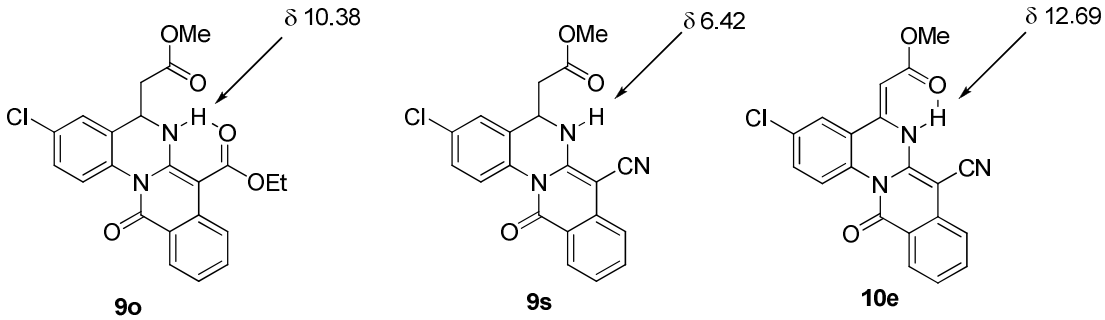


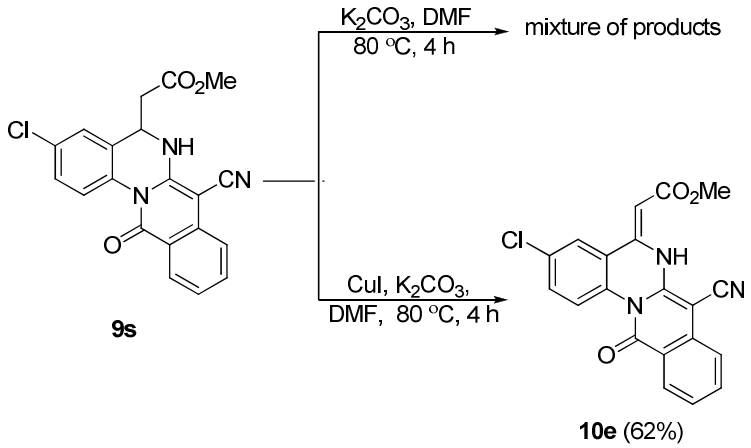
Fig. 4.4: Various chemical shift values of NH proton and hydrogen bonding pattern.

The geometry of the exocyclic double bond conformed by the NOE study of **10g** (Figure 4.3). From the H^1 NMR data, intramolecular hydrogen bond observed between NH proton and ester carbonyl group in compounds **9o** and **10e** (chemical

shift values of NH proton δ 10.38 and 12.69 ppm respectively) and no hydrogen bond observed in compound **9s** because of absence of carbonyl group (chemical shift value δ 6.42 ppm) (Figure 4.4).

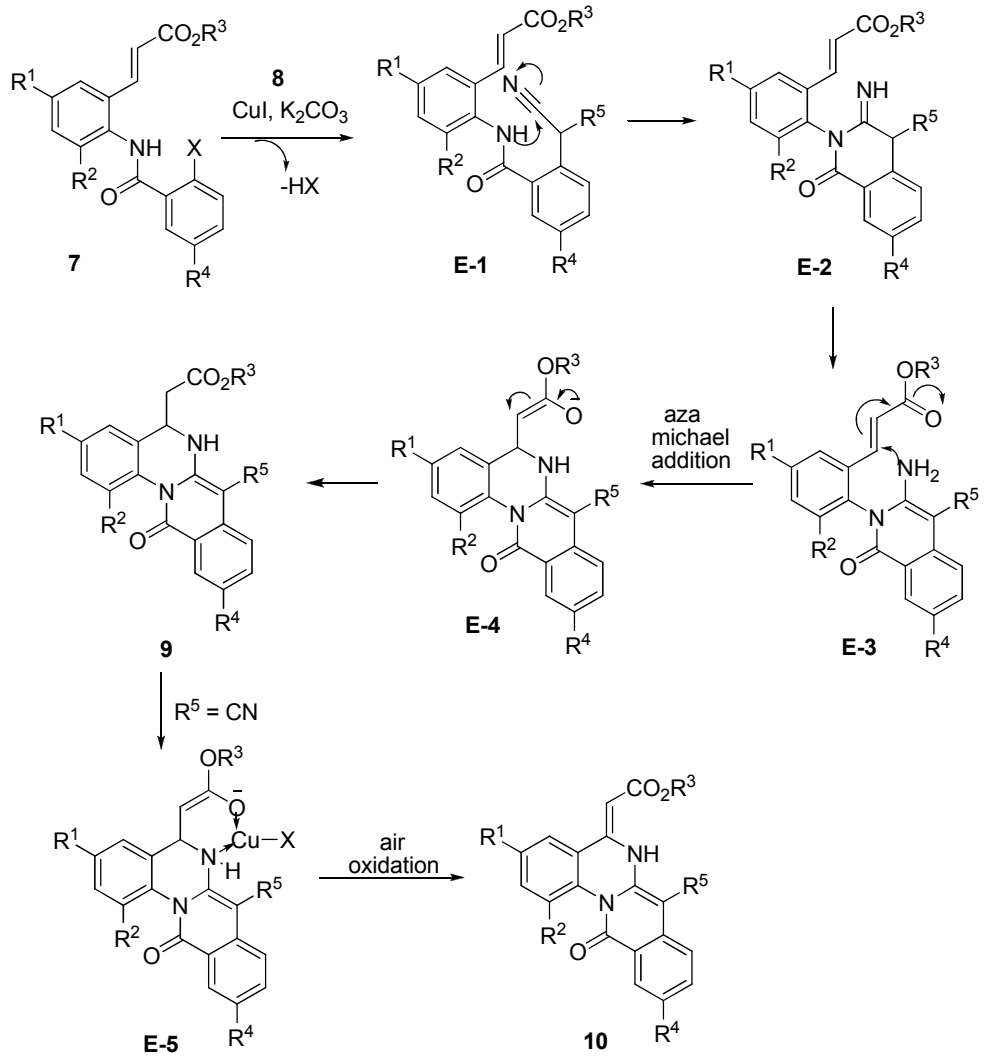
4.3.4. Proposed mechanism:

In order to explain the reaction mechanism, the following control experiments are preformed as shown in Scheme 4.3. The reaction of **9s** with K_2CO_3 provided mixture of products whereas in presence of copper iodide, the formation of desired product (**10e**) was observed in 62% yield. This observation clearly showed the key role of copper in the formation of exocyclic double bond.



Scheme 4.3: Reaction control experiments.

Based on these results, a reaction mechanism is proposed in Scheme 4.4. Copper catalyzed Ullmann type intermolecular C-C bond formation provided **E-1** which on CuI/base promoted intramolecular nucleophilic attack by the nitrogen of amide bond to –CN moiety gave **E-2**. The **E-2** then tautomerized to **E-3**. Finally, intramolecular aza-Michael addition afforded the target compound **9**. Formation of **10** was explained based on the formation of **E-5** intermediate, where free NH availability (**9s**, Figure 4.4) provided the 6-membered complex with the copper, and then air oxidation provided exocyclic double bond as well as *Z*-isomer exclusively.



Scheme 4.4: Proposed reaction mechanism.

4.4. Pharmacology:

4.4.1. *In vitro* data:

Some of the compounds synthesized were tested against PDE4B using an *in vitro* enzyme assay[9] and the results were summarized in Table 4.5.

Table 4.5: *In vitro* PDE4B inhibition by isoquinolino[2,3-*a*]quinazoline (**9**).

S.No	Compound	% inhibition of PDE4B @ 30 µM
1	**9b**	70.61 ± 0.75
2	**9e**	92.72 ± 3.22
3	**9f**	86.45 ± 2.19

4	**9i**	65.52 ± 1.18
5	**9j**	66.20 ± 3.18
6	**9k**	77.97 ± 1.27
7	**9l**	42.47 ± 3.13
8	**9o**	61.65 ± 3.93
9	**9p**	62.30 ± 0.53
10	**9r**	32.28 ± 2.84
11	**9s**	59.16 ± 0.68
12	**9t**	83.31 ± 5.81
13	**9u**	92.90 ± 3.54
14	**9w**	70.79 ± 0.65 (16 µM)
15	**9x**	87.62 ± 0.06

All the compounds showed excellent inhibition at 30 µM (Table 4.5). The compounds **9e** and **9u** showed maximum inhibition *i.e.*, 92% at 30 µM and IC_{50} values are 1.0 and 2.2 µM for PDE4B and 1.9 and 5.8 µM for PDE4D (Figure 4.5) respectively.

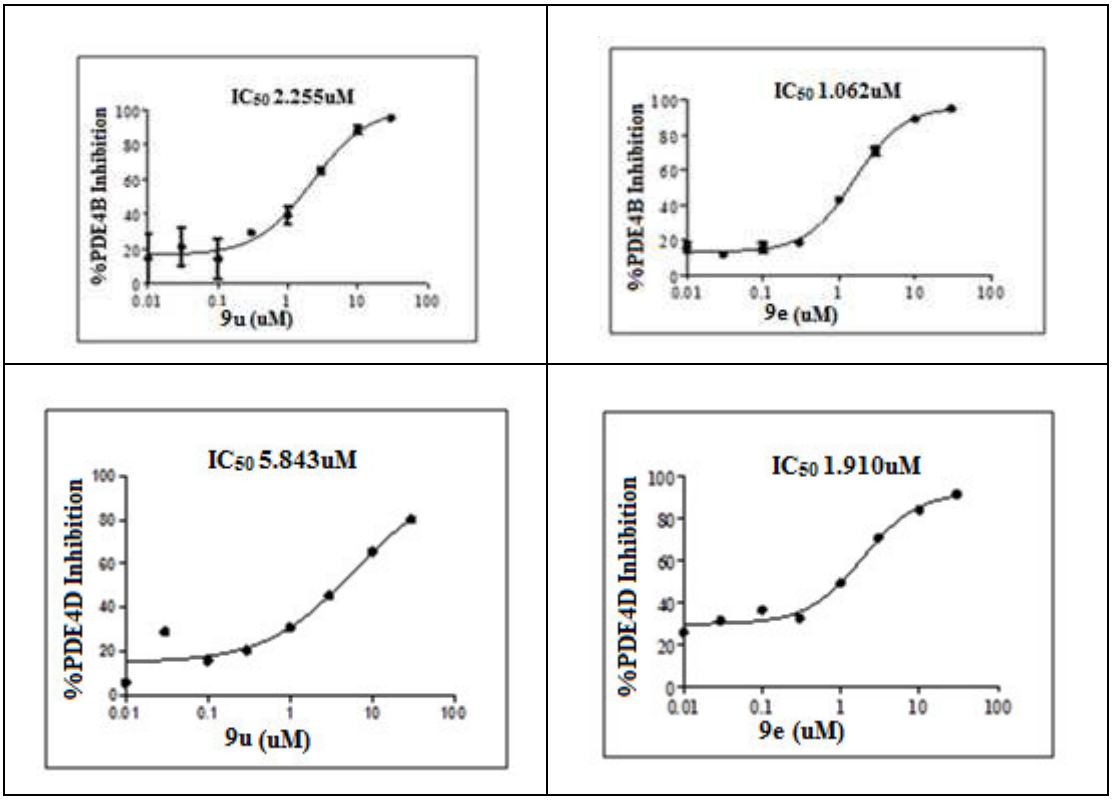


Fig. 4.5: Dose dependent inhibition of PDE4B & PDE4D by compounds **9u** and **9e**.

4.4.2. Docking studies:

The following molecular docking Simulation was done with Chemical Computing Group's Molecular Operating Environment (MOE) software 2008.10 Version, "DOCK" application Module.[10] The compound **9e** and **9u** were docked into the PDE4B protein and their respective Docking scores and interactions were observed. In molecule **9e**, hydrogen bond interactions with gly 443 and hydrophibic interactions with phe 446 were observed with glide score -23.05. In **9u**, hydrogen bond observed with his 234 and hydrophibic interactions with phe 446 were observed with glide score -22.05 (Figure 4.6).

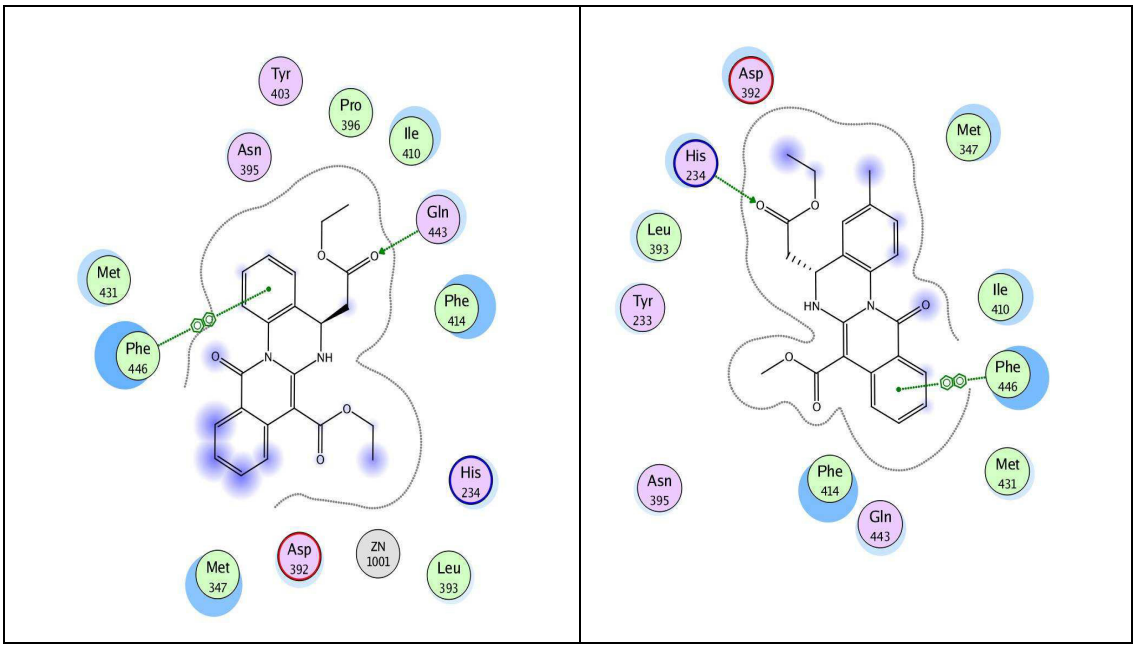


Fig. 4.6: Binding mode and interactions of molecule **9e** and **9u** with PDE4B.

4.5. Conclusion:

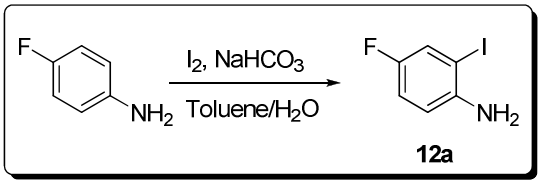
In conclusion, a robust, mild and ligand/additive-free copper catalyzed onepot domino reaction has been developed that allowed a rapid access to the novel fused *N*-heterocyclic acetic acid derivatives. The reaction proceeds *via* a copper catalyzed domino reaction involving (i) Ullmann type intermolecular C-C followed by (ii) an intramolecular C-N coupling and then (iii) intramolecular Aza-Michael type addition (and subsequent aerial oxidation). Several of these compounds showed promising PDE4B inhibition *in vitro* and seemed to have potential for the related medicinal applications. Overall, the one-pot methodology presented here may find wide usage in constructing diversity based library of small molecules for chemical and medicinal applications.

4.6. Experimental section:

4.6.1. 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 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 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.

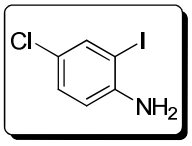
4.6.1.1. Typical procedure for preparation of 4-Fluoro-2-iodoaniline (12a)[11]



A mixture of 4-fluoro aniline (1.0 g, 9.0 mmol), iodine (2.28 g, 9.0 mmol) and sodium bicarbonate (1.13 g, 13.5 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 ethyl acetate – hexane to give the desired compound **12a**;

Yield: 85% (1.8 g); dark brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38 (dd, *J* = 7.8, 2.2 Hz, 1H), 6.90 (tb, *J* = 8.1, 2.4 Hz, 1H), 6.69 (dd, *J* = 8.6, 4.9 Hz, 1H), 3.92 (bs, 2H).

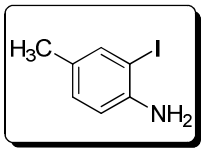
4.6.1.2. 4-Chloro-2-iodoaniline (12b)[12]



Compound **12b** was synthesized from 4-chloro aniline following a procedure similar to that of compound **12a**.

Yield: 88% (1.7 g); dark brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 2.3 Hz, 1H), 7.11 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 1H), 4.15 (bs, 2H).

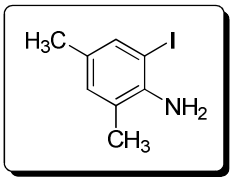
4.6.1.3. 2-Iodo-4-methyl aniline (12c)[11]



Compound **12c** was synthesized from 4-methyl aniline following a procedure similar to that of compound **12a**.

Yield: 89% (1.9 g); dark brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48 (s, 1H), 6.96 (d, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 3.23 (bs, 2H), 2.22 (s, 3H).

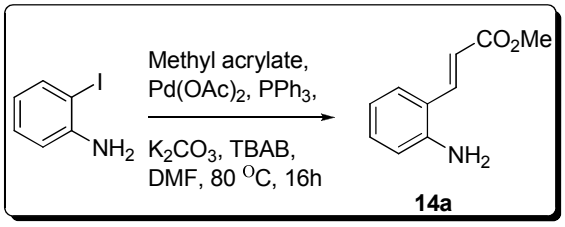
4.6.1.4. 2-Iodo-4,6-dimethylaniline (12d)



Compound **12d** was synthesized from 2,4-dimethyl aniline following a procedure similar to that of compound **12a**.

Yield: 89% (1.8 g); light brown solid; mp: 62-64 °C (lit[13] 64-65 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37 (s, 1H), 6.85 (s, 1H), 3.98 (bs, 2H), 2.22 (s, 3H), 2.21 (s, 3H).

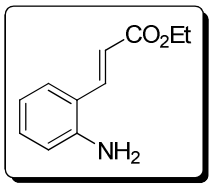
4.6.1.5. Typical procedure for preparation of (*E*)-Methyl 3-(2-aminophenyl) acrylate (14a)



The reaction vessel was charged with 2-iodo aniline (1.0 g, 4.58 mmol), methyl acrylate (0.83 mL, 9.17 mmol), K_2CO_3 (1.26 g, 9.17 mmol), palladium acetate (10 mg, 0.04 mmol), triphenyl phoshine (17 mg, 0.09 mmol) and tetra butyl ammonium bromide (74 mg, 0.23 mmol) in *N*,*N*-dimethylformamide (8 mL). The reaction mixture was stirred at 80 °C for 16 hours under nitrogen. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc (30 mL), 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 **14a**.

Yield: 78% (630 mg); light yellow solid; mp: 70-72 °C (lit[14] 65-67 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 15.8 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 15.8 Hz, 1H), 3.97 (bs, 2H), 3.80 (s, 3H).

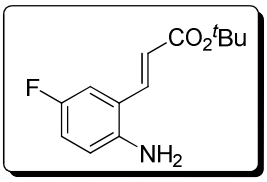
4.6.1.6. (*E*)-Ethyl-3-(2-aminophenyl)acrylate (14b)



Compound **14b** was synthesized from the reaction of 2-iodo aniline and ethyl acrylate following a procedure similar to that of compound **14a**.

Yield: 80% (700 mg); light yellow solid; mp: 67-69 °C (lit[15] 68-69 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 15.8 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.23-7.12 (m, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.36 (d, *J* = 15.8 Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.97 (bs, 2H), 1.34 (t, *J* = 7.1 Hz, 3H).

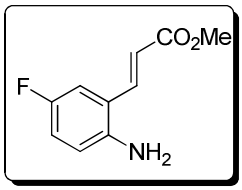
4.6.1.6. (*E*)-^{t}Butyl-3-(2-amino-5-fluorophenyl)acrylate (14c)



Compound **14c** was synthesized from the reaction of **12a** and *tert*-butyl acrylate following a procedure similar to that of compound **14a**.

Yield: 89% (895 mg); brown solid; mp: 94-96 °C; R_f = 0.2 (10% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3361, 3332, 2983, 1699, 1631; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (dd, *J* = 15.7, 1.1 Hz, 1H), 7.08 (dd, *J* = 8.5, 2.8 Hz, 1H), 6.89 (tb, *J* = 8.4, 3.2 Hz, 1H), 6.65 (dd, *J* = 8.5, 4.6 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 3.80 (bs, 2H), 1.54 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.2, 157.3 (C-F *J* = 235.6 Hz), 141.5 (C-F *J* = 1.7 Hz), 137.8 (C-F *J* = 2.3 Hz), 121.3, 121.1 (C-F *J* = 7.2 Hz), 117.9 (C-F *J* = 18.6 Hz), 117.6 (C-F *J* = 2.4 Hz), 113.4 (C-F *J* = 25.4 Hz), 80.7, 28.1 (3C); MS (ES mass): 238.1 (M+1);

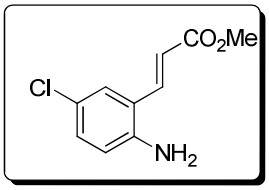
4.6.1.7. (*E*)-Methyl-3-(2-amino-5-fluorophenyl)acrylate (14d)[16]



Compound **14d** was synthesized from the reaction of **12a** and methyl acrylate following a procedure similar to that of compound **14a**.

Yield: 72% (590 mg); yellow solid; mp: 93-95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, *J* = 15.7 Hz, 1H), 7.08 (dd, *J* = 9.2, 2.8 Hz, 1H), 6.91 (tb, *J* = 8.4, 2.9 Hz, 1H), 6.66 (dd, *J* = 8.8, 4.7 Hz, 1H), 6.33 (d, *J* = 15.7 Hz, 1H), 3.84 (bs, 2H), 3.82 (s, 3H).

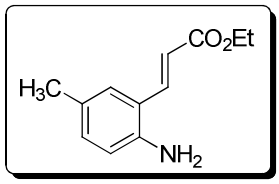
4.6.1.8. (*E*)-Methyl-3-(2-amino-5-chlorophenyl)acrylate (14e)[17]



Compound **14e** was synthesized from the reaction of **12b** and methyl acrylate following a procedure similar to that of compound **14a**.

Yield: 62% (520 mg); yellow solid; mp: 67-69 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 15.7 Hz, 1H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.12 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 6.35 (d, *J* = 15.7 Hz, 1H), 3.92 (bs, 2H), 3.81 (s, 3H).

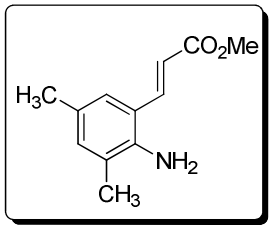
4.6.1.9. (*E*)-Ethyl-3-(2-amino-5-methylphenyl)acrylate (14f)



Compound **14f** was synthesized from the reaction of **12c** and ethyl acrylate following a procedure similar to that of compound **14a**.

Yield: 72% (635 mg); yellow solid; mp: 72-74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 15.8 Hz, 1H), 7.20 (s, 1H), 6.99 (dd, *J* = 8.2, 1.1 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.35 (d, *J* = 15.8 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.85 (bs, 2H), 2.24 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 3H).

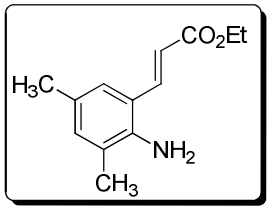
4.6.1.10. (*E*)-Methyl-3-(2-amino-3,5-dimethylphenyl)acrylate (14g)



Compound **14g** was synthesized from the reaction of **12d** and methyl acrylate following a procedure similar to that of compound **14a**.

Yield: 72% (600 mg); brown solid; mp: 92-94 °C; R_f = 0.7 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3391, 3336, 2953, 1715, 1621; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (d, *J* = 15.7 Hz, 1H), 7.09 (s, 1H), 6.93 (s, 1H), 6.34 (d, *J* = 15.7 Hz, 1H), 3.84 (bs, 2H), 3.80 (s, 3H), 2.22 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 141.6, 140.7, 133.5, 127.3, 125.9, 123.3, 119.3, 117.4, 51.6, 20.3, 17.6; MS (ES mass): 206.2 (M+1);

4.6.1.11. (*E*)-Methyl-3-(2-amino-3,5-dimethylphenyl)acrylate (14h)

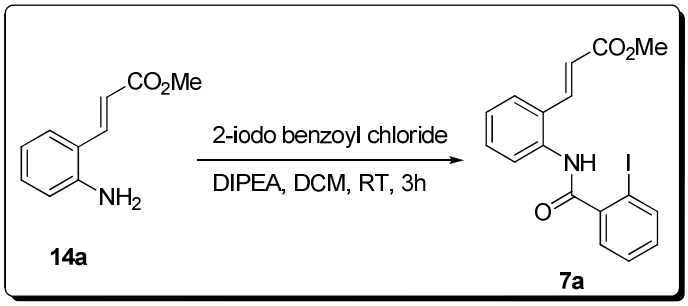


Compound **14h** was synthesized from the reaction of **12d** and ethyl acrylate following a procedure similar to that of compound **14a**.

Yield: 69% (610 mg); yellow solid; mp: 68-70 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3364, 2965, 1705, 1625; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 15.7 Hz, 1H), 7.09 (s, 1H), 6.93 (s, 1H), 6.34 (d, *J* = 15.7 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.84 (s, 2H), 2.22 (s, 3H), 2.16 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.4, 141.5, 140.5, 133.4, 127.3, 125.9, 123.3, 119.4, 117.9, 60.4, 20.3, 17.6, 14.4; MS (ES mass): 219.7 (M+1).

4.6.1.12. Typical procedure for preparation of (*E*)-Methyl-3-(2-(2-iodobenzamido)phenyl)acrylate (7a)

To a solution of compound **14a** (500 mg, 2.82 mmol) in dry DCM (20 mL) was added DIPEA (0.73 mL, 4.23 mmol) at 0 ºC under a nitrogen atmosphere. To this 2-iodo benzoyl chloride [7] (0.48 mL, 3.38 mmol) was slowly added and the reaction mixture was stirred at room temperature for 3 hours. After completion of the reaction, the mixture was diluted with DCM (25 mL), washed with saturated $NaHCO_3$ solution (2 x 30 mL) and water (30 mL) followed by brine solution (30 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 the desired compound **7a**.



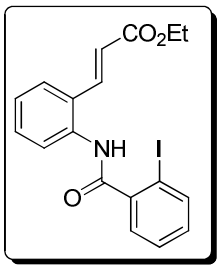
14a **7a**

Yield: 52% (590 mg); white solid; mp: 155-157 °C; R_f = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3204, 2948, 1711, 1655; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, *J* = 15.9 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.66-7.51 (m, 3H), 7.52-7.39 (m, 2H), 7.33-

7.24 (m, 1H), 7.16 (t, *J* = 7.5 Hz, 1H), 6.41 (d, *J* = 15.8 Hz, 1H), 3.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.0, 167.1, 141.8, 139.9, 139.6, 135.4, 131.5, 130.9, 128.5, 128.3, 128.2, 127.1, 126.4, 125.4, 120.0, 92.3, 51.8; MS (ES mass): 408.1 (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 210 nm, retention time 4.44 min.

4.6.1.13. (*E*)-Ethyl-3-(2-(2-iodobenzamido)phenyl)acrylate (7b)

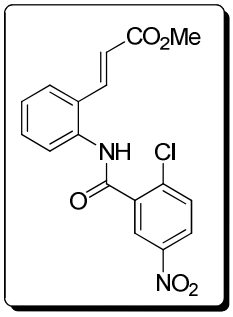
Compound **7b** was synthesized from the reaction of **14b** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.



Yield: 58% (640 mg); white solid; mp: 136-138 °C; R_f = 0.5 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3246, 2982, 1711, 1656; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03-7.90 (m, 3H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.60-7.52 (m, 2H), 7.51-7.38 (m, 2H), 7.30-7.27 (m, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.44 (d, *J* = 15.9 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.9, 166.7, 141.9, 139.9, 139.3, 135.3, 131.5, 130.8, 128.5, 128.3, 128.2, 127.1, 126.4, 125.3, 120.8, 92.2, 60.7, 14.3; MS (ES mass): 422.1 (M+1); HPLC: 96.5%, 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 4.68 min.

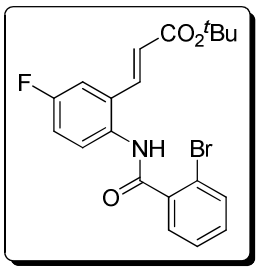
4.6.1.14. (*E*)-Methyl-3-(2-(2-chloro-5-nitrobenzamido)phenyl)acrylate (7c)

Compound **7c** was synthesized from the reaction of **14a** and 2-chloro-5-nitrobenzoyl chloride [18] following a procedure similar to that of compound **7a**.



Yield: 56% (570 mg); white solid; mp: 191-193 °C; R_f = 0.6 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3183, 2949, 1716, 1655; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 2.5 Hz, 2H), 8.19 (dd, *J* = 8.8, 2.6 Hz, 2H), 8.07 (d, *J* = 15.9 Hz, 1H), 7.69-7.74 (m, 1H), 7.57 (d, *J* = 8.8 Hz, 2H), 7.48-7.44 (m, 1H), 7.42-7.39 (m, 2H), 6.50 (d, *J* = 15.9 Hz, 1H), 3.91 (s, 3H) (extra protons due to rotamers);; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.5, 166.2, 145.9, 137.9, 137.2, 136.1, 135.2, 133.3, 131.2, 130.9, 130.6, 129.3, 127.6, 125.8, 123.2, 122.8, 52.1; MS (ES mass): 358.4 (M-1); HPLC: 93.2%, 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 270 nm, retention time 5.14 min.

4.6.1.15. (*E*)-^{t}Butyl-3-(2-(2-bromobenzamido)-5-fluorophenyl)acrylate (7d)

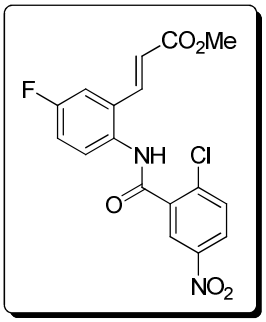


Compound **7d** was synthesized from the reaction of **14c** and 2-bromo benzoyl chloride[8] following a procedure similar to that of compound **7a**.

Yield: 62% (550 mg); light brown solid; mp: 142-144 °C; R_f = 0.3 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3212, 2982, 1742, 1675; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.87 (dd, *J* = 8.9, 5.2 Hz, 1H), 7.81 (d, *J* = 15.7 Hz, 1H), 7.71 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.67-7.64 (m, 2H), 7.44 (t, *J* = 7.1 Hz, 1H), 7.36 (tb, *J* = 7.7, 1.6 Hz, 1H), 7.30 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.15 (tb, *J* = 8.7, 2.8 Hz, 1H), 6.35 (d, *J* = 15.7 Hz, 1H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.3, 165.5, 161.8 (C-F *J* = 245.1 Hz), 137.2, 136.9 (C-F *J* = 2.1 Hz), 133.4, 131.8, 131.2 (C-F *J* = 2.8 Hz), 130.8 (C-F *J* = 8.0 Hz), 129.9, 127.7, 127.6 (C-F *J* = 8.4 Hz), 123.9, 119.2, 117.5 (C-F *J* = 22.6 Hz),

113.3 (C-F *J* = 23.3 Hz), 81.1, 28.1 (3C); MS (ES mass): 419.4 (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 210 nm, retention time 5.08 min.

4.6.1.16. (*E*)-Methyl-3-(2-(2-chloro-5-nitrobenzamido)-5-fluorophenyl)acrylate (7e)

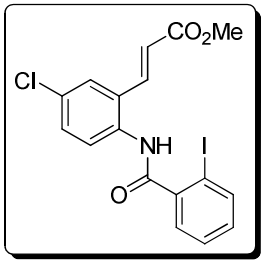


Compound **7e** was synthesized from the reaction of **14d** and 2-chloro-5-nitrobenzoyl chloride following a procedure similar to that of compound **7a**.

Yield: 56% (540 mg); light yellow solid; mp: 154-156 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3212, 2998, 1704, 1648; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.27 (d, *J* = 2.2 Hz, 2H), 8.18 (dd, *J* = 8.7, 2.3 Hz, 2H), 7.98 (d, *J* = 15.9 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.45 (dd, *J* = 8.7, 5.0 Hz, 1H), 7.30 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.10 (tb, *J* = 8.7, 2.6 Hz, 1H), 6.44 (d, *J* = 15.8 Hz, 1H), 3.90 (s, 3H) (extra protons due to rotamers); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.5, 165.9, 164.2 (C-F *J* = 251.3 Hz), 146.0, 137.2, 136.9 (C-F *J* = 2.0 Hz), 136.0, 135.7 (C-F *J* = 8.4 Hz), 131.4 (C-F *J* = 9.1 Hz), 131.2, 131.1, 125.9, 123.9, 123.2, 118.5 (C-F *J* = 23.1 Hz), 114.5 (C-F *J* = 23.5 Hz), 52.3; MS (ES mass): 376.7 (M-1); HPLC: 93.4%, 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): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 270 nm, retention time 5.20 min.

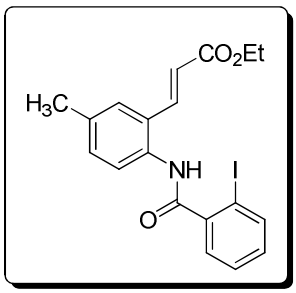
4.6.1.17. (*E*)-Methyl-3-(5-chloro-2-(2-iodobenzamido)phenyl)acrylate (7f)

Compound **7f** was synthesized from the reaction of **14e** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.



Yield: 74% (770 mg); white solid; mp: 141-143 °C; R_f = 0.6 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3212, 3011, 1721, 1658; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97-7.90 (m, 3H), 7.63-7.55 (m, 2H), 7.54-7.41 (m, 3H), 7.20 (t, *J* = 7.1 Hz, 1H), 6.45 (d, *J* = 15.7 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 166.8, 141.8, 140.0, 138.2, 133.8, 131.8, 131.7, 130.7, 128.6, 128.5, 127.9, 126.9, 126.4, 121.8, 92.1, 52.0; MS (ES mass): 439.5 (M-1); HPLC: 94.8%, 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 4.82 min.

4.6.1.18. (*E*)-Ethyl-3-(2-(2-iodobenzamido)-5-methylphenyl)acrylate (7g)

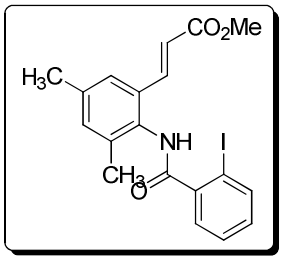


Compound **7g** was synthesized from the reaction of **14f** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.

Yield: 66% (700 mg); white solid; mp: 166-168 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3196, 2988, 1708, 1647; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.87 (d, *J* = 15.8 Hz, 1H), 7.83 (d, *J* = 7.9 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.47 (d, *J* = 6.6 Hz, 1H), 7.43 (bs, 1H), 7.37-7.34 (m, 2H), 7.20-7.19 (m, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.33 (d, *J* = 15.8 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 2.30 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.1, 166.8, 141.9, 139.8, 139.5, 136.3, 132.8, 131.6, 131.4, 128.5, 128.4, 128.3, 127.3, 125.6, 120.2, 92.3, 60.6, 21.0, 14.3; MS (ES mass): 436.1 (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): 0/20, 0.5/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.86 min.

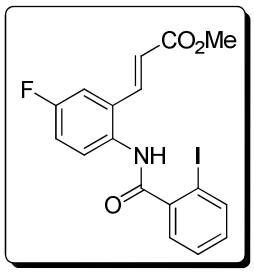
4.6.1.19. (*E*)-Methyl-3-(2-(2-iodobenzamido)-3,5-dimethylphenyl)acrylate (7h)



Compound **7h** was synthesized from the reaction of **14g** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.

Yield: 64% (680 mg); white solid; mp: 171-173 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3222, 2948, 1718, 1655; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 15.9 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 7.34 (s, 1H), 7.23-7.13 (m, 3H), 6.41 (d, *J* = 15.9 Hz, 1H), 3.78 (s, 3H), 2.39 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 167.2, 141.9, 141.0, 140.1, 137.8, 136.5, 133.5, 132.2, 131.4, 130.9, 128.5, 128.2, 125.1, 119.6, 92.1, 51.7, 21.0, 18.9; MS (ES mass): 436.2 (M+1); HPLC: 98.9%, 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 4.73 min.

4.6.1.20. (*E*)-Methyl-3-(5-fluoro-2-(2-iodobenzamido)phenyl)acrylate (7i)

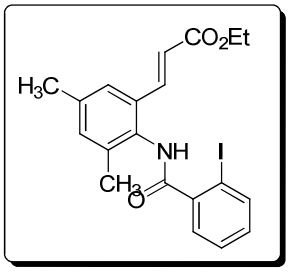


Compound **7i** was synthesized from the reaction of **14c** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.

Yield: 67% (730 mg); light brown solid; mp: 118-120 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3173, 2987, 1721, 1657; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.94 (d, *J* = 5.4 Hz, 1H), 7.91 (bs, 1H), 7.86 (dd, *J* = 8.4, 5.4 Hz, 1H), 7.57 (d, *J* = 7.3

Hz, 1H), 7.48-7.42 (m, 2H), 7.31 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.23-7.14 (m, 2H), 6.42 (d, *J* = 15.8 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 166.8, 161.9 (C-F *J* = 245.4 Hz), 141.6, 139.9, 138.6, 131.6, 131.4 (C-F *J* = 2.4 Hz), 130.8 (C-F *J* = 8.0 Hz), 128.5, 128.3, 127.9 (C-F *J* = 8.3 Hz), 121.1, 117.9 (C-F *J* = 22.5 Hz), 113.3 (C-F *J* = 23.2 Hz), 92.3, 51.9; MS (ES mass): 425.5 (M+1); HPLC: 98.9%, 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 4.54 min.

4.6.1.21. (*E*)-Ethyl-3-(2-(2-iodobenzamido)-3,5-dimethylphenyl)acrylate (7j)



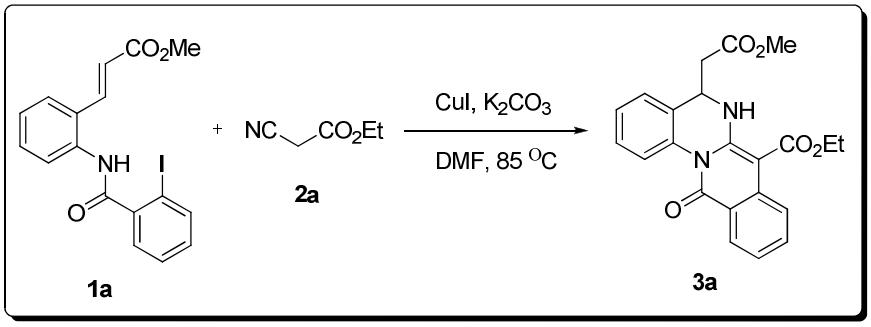
Compound **7j** was synthesized from the reaction of **14h** and 2-iodo benzoyl chloride following a procedure similar to that of compound **7a**.

Yield: 63% (645 mg); light brown solid; mp: 171-173 °C; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3215, 2948, 1708, 1645; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 15.9 Hz, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.37 (s, 1H), 7.24-7.13 (m, 3H), 6.44 (d, *J* = 15.9 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 2.42 (s, 3H), 2.37 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.2, 166.8, 142.0, 140.6, 140.1, 137.8, 136.5, 133.5, 132.3, 131.4, 130.9, 128.5, 128.2, 125.1, 120.1, 92.2, 60.5, 21.1, 18.9, 14.4; MS (ES mass): 449.5 (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 210 nm, retention time 4.93 min.

4.6.1.22. Typical procedure for preparation of Ethyl-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9a)

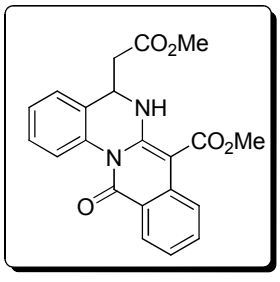
A mixture of compound **7a** (50 mg, 0.12 mmol), K_2CO_3 (50 mg, 0.36 mmol), ethyl cyano acetate (**8a**) (0.016 mL, 0.14 mmol) and CuI (2.3 mg, 0.012 mmol) in DMF (2 mL) was heated to 85 °C under anhydrous conditions ($CaCl_2$ filled guard tube) for 0.5

h. After completion of the reaction, the mixture was cooled to room temp, 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 **9a**.



Yield: 89% (44 mg); brown solid; mp: 131-133 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3227, 2983, 1725, 1684, 1632; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.43 (d, *J* = 2.4 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.58 (tb, *J* = 7.6, 1.4 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.30-7.22 (m, 3H), 4.95-4.86 (m, 1H), 4.49-4.34 (m, 2H), 3.71 (s, 3H), 2.86-2.71 (m, 2H), 1.45 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6, 169.1, 162.0, 151.6, 135.8, 133.2, 132.3, 129.5, 128.5, 127.9, 126.9, 125.3, 125.1, 123.4, 122.9, 121.8, 85.5, 60.6, 52.0, 48.9, 40.4, 14.4; MS (ES mass): 392.5 (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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.14 min. Elemental analysis found C, 67.55; H, 5.17; N, 7.01; $C_{22}H_{20}N_2O_5$ requires C, 67.34; H, 5.14; N, 7.14.

4.6.1.23. Methyl-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazoline-7-carboxylate (9b)

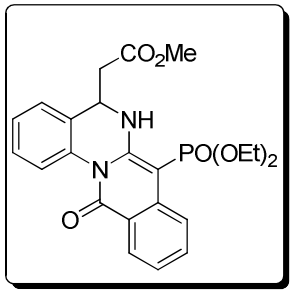


Compound **9b** was synthesized from the reaction of **7a** and methyl cyano acetate (**8b**) following a procedure similar to that of compound **9a**.

Yield: 84% (39 mg); brown semi solid; R_f = 0.6 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3212, 2983, 1728, 1674, 1631; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.41 (d, *J* = 2.4 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.27 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.59 (tb, *J* = 8.0, 1.2 Hz, 1H), 7.37 (tb, *J* = 8.4, 1.5 Hz, 1H), 7.30-7.23 (m, 3H), 4.96-4.86 (m, 1H), 3.94 (s, 3H), 3.70 (s, 3H), 2.82-2.74 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6, 169.5, 162.0, 151.7, 135.7, 133.3, 132.3, 129.4, 128.5, 127.9, 126.9, 125.3, 125.1, 123.5, 122.9, 121.7, 85.3, 52.0, 51.5, 48.9, 40.4; MS (ES mass): 379.3 (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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.50 min.

4.6.1.24. Methyl-2-(7-(diethoxyphosphoryl)-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-yl)acetate (9c)

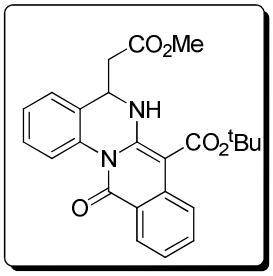
Compound **9c** was synthesized from the reaction of **7a** and diethyl cyano methyl phosphonate (**8c**) following a procedure similar to that of compound **9a**.



Yield: 81% (45 mg); brown liquid; R_f = 0.3 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2951, 2925, 1738, 1679, 1590; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.80 (d, *J* = 2.7 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.55 (d, *J* = 8.3, 1.2 Hz, 1H), 7.37 (d, *J* = 8.4, 1.5 Hz, 1H), 7.32-7.21 (m, 3H), 4.86-4.82 (m, 1H), 4.22-3.99 (m, 3H), 3.96-3.84 (m, 1H), 3.75 (s, 3H), 2.84-2.71 (m, 2H), 1.32 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 162.3, 152.9 (C-P *J* = 22.0 Hz), 136.7 (C-P *J* = 7.1 Hz), 133.3, 132.5, 130.2, 128.7, 127.8, 126.8, 125.3, 123.5 (C-P *J* = 3.2 Hz), 123.3, 122.9, 121.4 (C-P *J* = 12.1 Hz), 74.7, 61.8, 61.7, 52.1, 49.3, 40.3, 16.3 (C-P *J* = 7.0 Hz), 16.2 (C-P *J* = 7.2 Hz); MS (ES mass): 457.3 (M+1); HPLC: 94.2%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.33 min.

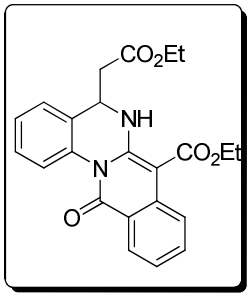
4.6.1.25. ^{t}Butyl-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazoline-7-carboxylate (9d)



Compound **9d** was synthesized from the reaction of **7a** and *tert*-butyl cyano acetate (**8d**) following a procedure similar to that of compound **9a**.

Yield: 81% (42 mg); brown solid; mp: 119-121 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2955, 2923, 1738, 1682, 1644; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.31 (d, *J* = 2.8 Hz, 1H), 8.35 (dd, *J* = 7.9, 1.2 Hz, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 8.3 Hz, 1H), 7.57 (td, *J* = 7.9, 1.6 Hz, 1H), 7.37 (td, *J* = 8.3, 2.0 Hz, 1H), 7.30-7.28 (m, 1H), 7.27-7.22 (m, 2H), 4.92-4.87 (m, 1H), 3.71 (s, 3H), 2.85-2.71 (m, 2H), 1.66 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.5, 168.4, 162.1, 151.1, 136.1, 132.9, 132.4, 129.8, 128.4, 127.9, 126.8, 125.2, 125.1, 123.3, 123.0, 121.8, 86.9, 81.7, 52.0, 48.9, 40.4, 28.6 (3C); MS (ES mass): 421.3 (M+1); HPLC: 93.1%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.21 min.

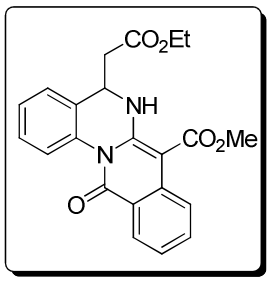
4.6.1.26. Ethyl-5-(2-ethoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9e)



Compound **9e** was synthesized from the reaction of **7b** and **8a** following a procedure similar to that of compound **9a**.

Yield: 87% (42 mg); brown solid; mp: 125-127 °C; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2969, 2931, 1720, 1676, 1632; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.43 (d, *J* = 2.5 Hz, 1H), 8.35 (dd, *J* = 8.3, 1.0 Hz, 1H), 8.32 (d, *J* = 8.3 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.58 (tb, *J* = 8.4, 1.2 Hz, 1H), 7.37 (tb, *J* = 8.4, 1.1 Hz, 1H), 7.32-7.22 (m, 3H), 4.95-4.87 (m, 1H), 4.51-4.32 (m, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.84-2.70 (m, 2H), 1.44 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.1, 162.1, 151.7, 135.9, 133.3, 132.4, 129.6, 128.5, 127.9, 126.9, 125.3, 125.1, 123.4, 122.9, 121.8, 85.4, 61.2, 60.6, 48.9, 40.6, 14.5, 14.1; MS (ES mass): 407.2 (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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.65 min. Elemental analysis found C, 67.79; H, 5.45; N, 6.99; $C_{23}H_{22}N_2O_5$ requires C, 67.97; H, 5.46; N, 6.89.

4.6.1.27. Methyl-5-(2-ethoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazoline-7-carboxylate (9f)

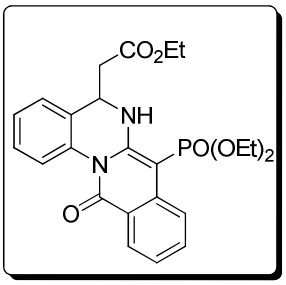


Compound **9f** was synthesized from the reaction of **7b** and **8b** following a procedure similar to that of compound **9a**.

Yield: 83% (38 mg); light brown solid; mp: 105-107 °C; R_f = 0.3 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2968, 2942, 1721, 1682, 1631; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.43 (s, 1H), 8.36 (d, *J* = 7.9 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.7 Hz, 1H), 7.32-7.25 (m, 3H), 4.94-4.90 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.93 (s, 3H), 2.82-2.73 (m, 2H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.5, 162.1, 151.8, 135.7, 133.3, 132.4, 129.5, 128.6, 127.9, 126.9, 125.3, 125.1, 123.5, 122.9, 121.7, 85.3, 61.2, 51.5, 48.9, 40.7, 14.1; MS (ES mass): 392.6 (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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.05 min.

4.6.1.28. Ethyl-2-(7-(diethoxyphosphoryl)-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-yl)acetate (9g)

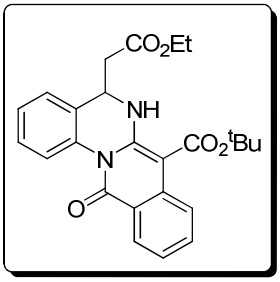


Compound **9g** was synthesized from the reaction of **7b** and **8c** following a procedure similar to that of compound **9a**.

Yield: 79% (44 mg); brown liquid; R_f = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2971, 2923, 1731, 1685, 1642; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.80 (d, *J* = 2.3 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.37 (tb, *J* = 8.3, 2.7 Hz, 1H), 7.32-7.22 (m, 3H), 4.89-4.81 (m, 1H), 4.26-4.13 (m, 3H), 4.12-4.00 (m, 2H), 3.95-3.84 (m, 1H), 2.84-2.70 (m, 2H), 1.33-1.26 (m, 6H), 1.25-1.23 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 162.3, 153.0 (C-P *J* = 28.9 Hz), 136.7 (C-P *J* = 7.3 Hz), 133.3, 132.5, 130.3, 128.7, 127.8, 126.8, 125.4, 123.5 (C-P *J* = 3.2 Hz), 123.3, 122.9, 121.4 (C-P *J* = 11.2 Hz), 74.6, 61.8, 61.7, 61.1, 49.2, 40.5, 16.3 (C-P *J* = 7.1 Hz), 16.2 (C-P *J* = 7.2 Hz), 14.1; MS (ES mass): 470.6 (M+1); HPLC: 93.1%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.87 min.

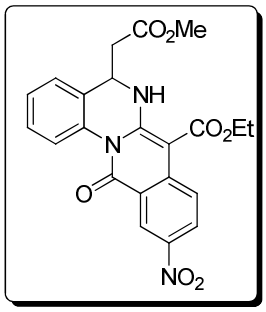
4.6.1.29. ^{t}Butyl-5-(2-ethoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9h)

Compound **9h** was synthesized from the reaction of **7b** and **8d** following a procedure similar to that of compound **9a**.



Yield: 81% (41 mg); brown liquid; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2968, 2931, 1721, 1678, 1633; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.32 (d, *J* = 2.6 Hz, 1H), 8.34 (dd, *J* = 7.9, 1.1 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 7.56 (tb, *J* = 8.2, 1.2 Hz, 1H), 7.36 (tb, *J* = 8.4, 1.2 Hz, 1H), 7.29-7.27 (m, 1H), 7.26-7.20 (m, 2H), 4.93-4.85 (m, 1H), 4.20-4.11 (m, 2H), 2.81-2.69 (m, 2H), 1.64 (s, 9H), 1.24 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1, 168.4, 162.1, 151.2, 136.2, 132.9, 132.5, 129.8, 128.4, 127.9, 126.8, 125.3, 125.1, 123.3, 123.0, 121.8, 86.8, 81.7, 61.1, 48.9, 40.6, 28.6 (3C), 14.1; MS (ES mass): 434.7 (M+1); HPLC: 95.1%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.65 min.

4.6.1.30. Ethyl-5-(2-methoxy-2-oxoethyl)-10-nitro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9i)

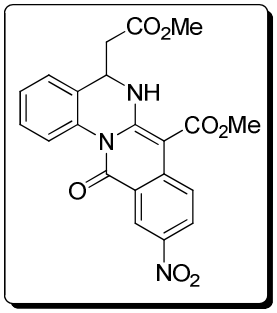


Compound **9i** was synthesized from the reaction of **7c** and **8a** following a procedure similar to that of compound **9a**.

Yield: 76% (46 mg); yellow solid; mp: 142-144 °C; R_f = 0.3 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2926, 2854, 1736, 1687, 1649; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.90 (d, *J* = 2.9 Hz, 1H), 9.22 (d, *J* = 2.5 Hz, 1H), 8.48 (d, *J* = 9.4 Hz, 1H), 8.35 (dd, *J* = 9.4, 2.6 Hz, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.44 (tb, *J* = 8.4, 1.4 Hz, 1H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.30-7.28 (m, 1H), 5.03-4.99 (m, 1H), 4.51-4.42 (m, 2H), 3.75 (s, 3H), 2.89-2.80 (m, 2H), 1.48 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3,

168.5, 160.8, 153.3, 142.8, 141.2, 131.7, 128.4, 128.3, 127.7, 127.0, 125.9, 125.5, 125.1, 122.5, 120.7, 85.7, 61.2, 52.2, 48.9, 40.7, 14.4; MS (ES mass): 435.7 (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): 0/50, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.62 min.

4.6.1.31. Methyl-5-(2-methoxy-2-oxoethyl)-10-nitro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9j)

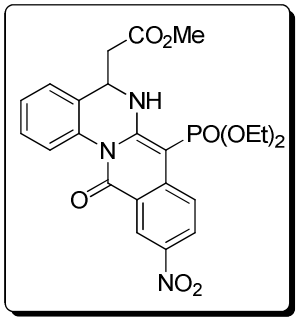


Compound **9j** was synthesized from the reaction of **7c** and **8b** following a procedure similar to that of compound **9a**.

Yield: 73% (42 mg); brown solid; mp: 170-172 °C; R_f = 0.3 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2925, 2799, 1722, 1681, 1639; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.89 (d, *J* = 2.3 Hz, 1H), 9.22 (d, *J* = 2.5 Hz, 1H), 8.44 (d, *J* = 9.3 Hz, 1H), 8.35 (dd, *J* = 9.4, 2.6 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.44 (tb, *J* = 8.3, 1.2 Hz, 1H), 7.37 (t, *J* = 7.2 Hz, 1H), 7.29-7.27 (m, 1H), 5.04-5.00 (m, 1H), 3.99 (s, 3H), 3.75 (s, 3H), 2.88-2.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 168.9, 160.8, 153.3, 142.8, 141.1, 131.7, 128.4, 128.3, 127.7, 127.1, 125.9, 125.5, 125.1, 122.5, 120.7, 85.6, 52.2, 51.9, 48.9, 40.7; MS (ES mass): 423.6 (M+1); HPLC: 92.5%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.31 min.

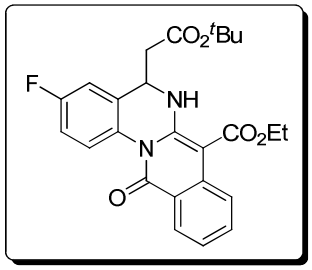
4.6.1.32. Methyl-2-(7-(diethoxyphosphoryl)-10-nitro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9k)

Compound **9k** was synthesized from the reaction of **7c** and **8c** following a procedure similar to that of compound **9a**.



Yield: 73% (50 mg); brown solid; mp: 166-168 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2931, 2892, 1739, 1642; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.27 (d, *J* = 3.0 Hz, 1H), 9.19 (d, *J* = 2.0 Hz, 1H), 8.30 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.42 (tb, *J* = 8.4, 1.4 Hz, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.28-7.27 (m, 1H), 4.96-4.89 (m, 1H), 4.28-4.01 (m, 3H), 4.02-3.90 (m, 1H), 3.75 (s, 3H), 2.83-2.75 (m, 2H), 1.34 (t, *J* = 7.0 Hz, 3H), 1.30 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9, 161.1, 154.5 (C-P *J* = 21.1 Hz), 142.7, 142.4 (C-P *J* = 7.0 Hz), 131.8, 129.0, 128.2, 127.5, 127.1, 125.5, 125.4, 124.2 (C-P *J* = 3.2 Hz), 122.5, 120.4 (C-P *J* = 2.3 Hz), 75.7, 62.3, 62.2 (C-P *J* = 3.4 Hz), 52.2, 49.2, 40.8, 16.1 (C-P *J* = 7.0 Hz, 2C); MS (ES mass): 501.6 (M+1); HPLC: 95.7%, 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, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 225 nm, retention time 5.01 min. Elemental analysis found C, 55.19; H, 4.85; N, 8.23; $C_{23}H_{24}N_3O_8P$ requires C, 55.09; H, 4.82; N, 8.38.

4.6.1.34. Ethyl-5-(2-tert-butoxy-2-oxoethyl)-3-fluoro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9l)

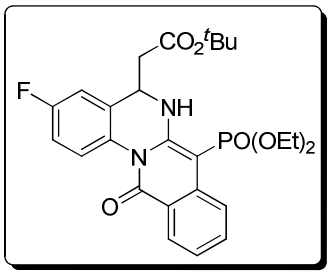


Compound **9l** was synthesized from the reaction of **7d** and **8a** following a procedure similar to that of compound **9a**.

Yield: 80% (38 mg); light brown solid; mp: 120-122 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2980, 2920, 1731, 1671, 1627; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.37 (d, *J* = 2.8 Hz, 1H), 8.34 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.31 (d, *J* = 8.3 Hz, 1H),

8.08 (dd, *J* = 8.8, 4.9 Hz, 1H), 7.59 (tb, *J* = 8.4, 1.6 Hz, 1H), 7.29-7.27 (m, 1H), 7.07 (tb, *J* = 8.4, 2.9 Hz, 1H), 6.97 (dd, *J* = 7.9, 2.8 Hz, 1H), 4.89-4.80 (m, 1H), 4.50-4.30 (m, 2H), 2.72-2.64 (m, 2H), 1.48-1.41 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.0, 168.9, 161.9, 161.7 (C-F *J* = 247.2 Hz), 151.3, 135.8, 133.3, 132.0 (C-F *J* = 7.8 Hz), 128.5, 128.4 (C-F *J* = 3.2 Hz), 125.1, 125.1 (C-F *J* = 8.4 Hz), 123.5, 121.7, 115.0 (C-F *J* = 22.7 Hz), 112.0 (C-F *J* = 23.3 Hz), 85.6, 82.0, 60.6, 48.9, 41.3, 27.9 (3C), 14.4; MS (ES mass): 453.3 (M+1); HPLC: 99.7%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.93 min. Elemental analysis found C, 66.20; H, 5.56; N, 6.33; $C_{25}H_{25}FN_2O_5$ requires C, 66.36; H, 5.57; N, 6.19.

4.6.1.35. ^{t}Butyl-2-(7-(diethoxyphosphoryl)-3-fluoro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9m)

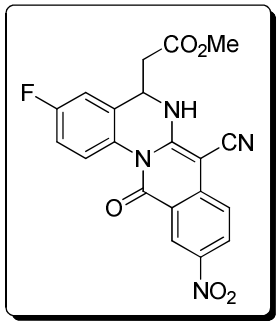


Compound **9m** was synthesized from the reaction of **7d** and **8c** following a procedure similar to that of compound **9a**.

Yield: 75% (41 mg); light brown semi solid; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2982, 2914, 1735, 1611; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.77 (d, *J* = 3.0 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.12 (dd, *J* = 8.8, 4.9 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.55 (td, *J* = 8.6, 1.6 Hz, 1H), 7.26-7.22 (m, 1H), 7.06 (tb, *J* = 8.6, 2.7 Hz, 1H), 6.99 (dd, *J* = 8.0, 2.8 Hz, 1H), 4.79-4.74 (m, 1H), 4.25-3.99 (m, 3H), 3.97-3.83 (m, 1H), 2.73-2.61 (m, 2H), 1.47 (s, 9H), 1.31 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.8, 162.2, 161.6 (C-F *J* = 247.0 Hz), 152.8 (C-P *J* = 21.9 Hz), 136.6 (C-P *J* = 7.0 Hz), 133.4, 132.8 (C-F *J* = 7.7 Hz), 128.7, 128.5 (C-F *J* = 3.4 Hz), 124.9 (C-P *J* = 8.3 Hz), 123.5 (C-F *J* = 3.1 Hz), 123.4, 121.3 (C-P *J* = 12.1 Hz), 114.8 (C-F *J* = 22.6 Hz), 112.2 (C=F *J* = 23.2 Hz), 81.8, 74.9, 61.8, 61.7, 49.1, 41.1, 28.0 (3C), 16.2 (C-P *J* = 7.0 Hz), 16.1 (C-P *J* = 7.3 Hz); MS (ES mass): 517.3 (M+1); HPLC: 91.3%, 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): 0/50, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.27 min.

4.6.1.36. Methyl-2-(7-cyano-3-fluoro-10-nitro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9n)

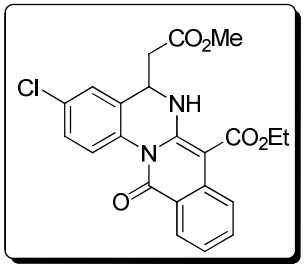


Compound **9n** was synthesized from the reaction of **7e** and malononitrile (**8e**) following a procedure similar to that of compound **9a**.

Yield: 68% (36 mg); brown solid; mp: 115-117 °C; R_f = 0.1 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2978, 2923, 2221, 1731, 1671; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.16 (d, *J* = 2.0 Hz, 1H), 8.45 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.34 (dd, *J* = 9.1, 4.8 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.18 (tb, *J* = 7.6, 2.8 Hz, 1H), 6.99 (dd, *J* = 7.6, 2.6 Hz, 1H), 6.88 (d, *J* = 2.0 Hz, 1H), 4.93 (tb, *J* = 6.9, 3.0 Hz, 1H), 3.81 (s, 3H), 2.87 (d, *J* = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9, 166.3 (C-F *J* = 259.1 Hz), 160.0, 152.4, 143.4, 140.7, 130.6 (C-F *J* = 8.0 Hz), 128.1, 127.3, 125.5, 124.1 (C-F *J* = 8.2 Hz), 122.5, 119.3 (C-F *J* = 2.2 Hz), 115.7, 115.5 (C-F *J* = 25.1 Hz), 113.0 (C-F *J* = 22.7 Hz), 68.6, 52.1, 49.1, 40.3; MS (ES mass): 406.7 (M-1); HPLC: 95.8%, 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): 0/50, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 225 nm, retention time 3.93 min. Elemental analysis found C, 58.79; H, 3.25; N, 13.93; $C_{20}H_{13}FN_4O_5$ requires C, 58.83; H, 3.21; N, 13.72.

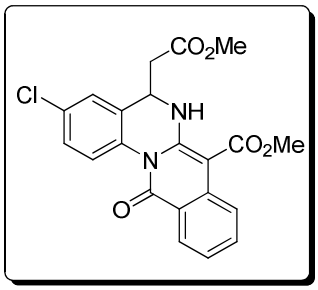
4.6.1.37. Ethyl-3-chloro-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9o)

Compound **9o** was synthesized from the reaction of **7f** and **8a** following a procedure similar to that of compound **9a**.



Yield: 85% (41 mg); light yellow solid; mp: 151-153 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2947, 2843, 1731, 1676, 1654; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.39 (d, *J* = 3.0 Hz, 1H), 8.32 (t, *J* = 8.3 Hz, 2H), 8.05 (d, *J* = 8.8 Hz, 1H), 7.60 (tb, *J* = 8.2, 1.6 Hz, 1H), 7.34 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.30-7.27 (m, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 4.90-4.83 (m, 1H), 4.50-4.34 (m, 2H), 3.71 (s, 3H), 2.83-2.71 (m, 2H), 1.45 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.0, 161.9, 151.2, 135.7, 133.4, 132.2, 131.2, 130.9, 128.5, 128.1, 125.1 (2C), 124.5, 123.7, 121.6, 85.9, 60.8, 52.1, 48.7, 40.1, 14.4; MS (ES mass): 426.6 (M+1); HPLC: 98.8%, 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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.22 min. Elemental analysis found C, 61.79; H, 4.45; N, 6.83; $C_{22}H_{19}ClN_2O_5$ requires C, 61.90; H, 4.49; N, 6.56.

4.6.1.38. Methyl-3-chloro-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9p)

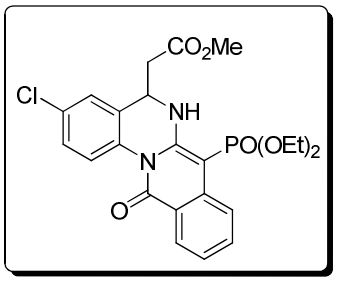


Compound **9p** was synthesized from the reaction of **7f** and **8b** following a procedure similar to that of compound **9a**.

Yield: 81% (37 mg); light brown solid; mp: 175-177 °C; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2950, 2835, 1726, 1672, 1655; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.37 (d, *J* = 2.9 Hz, 1H), 8.34 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.60 (tb, *J* = 8.5, 1.5 Hz, 1H), 7.34 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.29-7.24 (m, 2H), 4.91-4.83 (m, 1H), 3.94 (s, 3H), 3.72 (s, 3H), 2.83-2.71 (m, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.4, 161.8, 151.3, 135.6, 133.5, 132.3, 131.2, 130.9, 128.6, 128.1, 125.2, 125.1, 124.5, 123.7, 121.6, 85.7, 52.1, 51.6, 48.7, 40.2; MS (ES mass): 412.6 (M+1); HPLC: 95.8%, 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): 0/50, 0.5/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.92 min.

4.6.1.39. Methyl-2-(3-chloro-7-(diethoxyphosphoryl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9q)

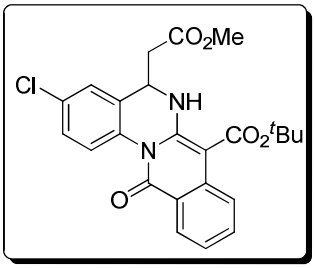


Compound **9q** was synthesized from the reaction of **7f** and **8c** following a procedure similar to that of compound **9a**.

Yield: 78% (43 mg); brown liquid; mp: 121-123 °C; R_f = 0.2 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2953, 2834, 1732, 1686; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.79 (d, *J* = 2.3 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.37-7.32 (m, 1H), 7.30-7.23 (m, 2H), 4.85-4.77 (m, 1H), 4.22-4.01 (m, 3H), 3.96-3.87 (m, 1H), 3.76 (s, 3H), 2.82-2.71 (m, 2H), 1.35-1.30 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.9, 162.1, 152.5 (C-P *J* = 22.0 Hz), 139.2, 136.5 (C-P *J* = 6.3 Hz), 133.5, 132.1, 131.9, 128.7, 127.9, 125.2, 124.5, 123.6 (C-P *J* = 3.2 Hz), 123.5, 121.4 (C-P *J* = 12.0 Hz), 80.1, 61.9, 61.8, 52.2, 49.1, 40.0, 16.3 (C-P *J* = 6.9 Hz), 16.2 (C-P *J* = 7.1 Hz); MS (ES mass): 490.6 (M+1); HPLC: 89.9%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.59 min.

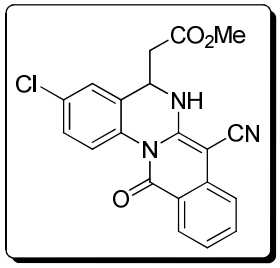
4.6.1.40. ^{t}Butyl-3-chloro-5-(2-methoxy-2-oxoethyl)-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9r)

Compound **9r** was synthesized from the reaction of **7f** and **8d** following a procedure similar to that of compound **9a**.



Yield: 77% (39 mg); brown solid; mp: 150-152 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2953, 2797, 1730, 1673, 1624; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.26 (d, *J* = 2.9 Hz, 1H), 8.31 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.56 (tb, *J* = 8.2, 1.2 Hz, 1H), 7.32 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.28-7.21 (m, 2H), 4.87-4.80 (m, 1H), 3.70 (s, 3H), 2.81-2.67 (m, 2H), 1.64 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 168.3, 161.9, 150.6, 135.9, 133.1, 132.2, 131.5, 130.9, 128.5, 128.1, 125.2, 125.1, 124.6, 123.5, 121.7, 87.3, 81.9, 52.2, 48.8, 40.1, 28.6 (3C); MS (ES mass): 454.6 (M+1); HPLC: 97.3%, 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): 0/50, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 7.09 min.

4.6.1.41. Methyl-2-(3-chloro-7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9s)

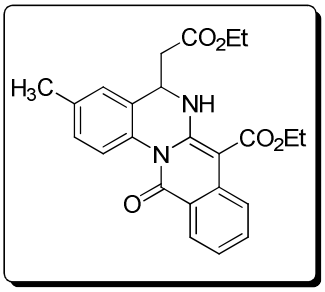


Compound **9s** was synthesized from the reaction of **7f** and **8e** following a procedure similar to that of compound **9a**.

Yield: 72% (31 mg); light yellow solid; mp: 214-216 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2959, 2878, 2217, 1723, 1686; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.32-8.29 (m, 2H), 7.70 (tb, *J* = 7.9, 1.2 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.41 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.27-7.24 (m, 1H), 6.43 (d, *J* = 2.3 Hz, 1H), 4.85-4.80 (m, 1H), 3.81 (s, 3H), 2.86-2.79 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.5, 161.0, 150.3, 134.6, 134.4, 132.7, 130.4, 130.3, 129.0, 128.6, 125.5, 125.0, 124.2, 122.1, 120.8, 116.3, 70.5, 52.6, 49.4, 39.3; MS (ES mass): 377.6 (M-1);

HPLC: 98.2%, column: X TERRA C-18 250 x 4.6 mm 5µ, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 3/20, 15/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.36 min.

4.6.1.42. Ethyl-5-(2-ethoxy-2-oxoethyl)-3-methyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9t)

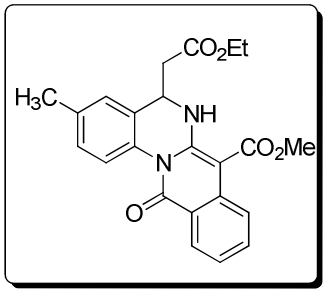


Compound **9t** was synthesized from the reaction of **7g** and **8a** following a procedure similar to that of compound **9a**.

Yield: 87% (42 mg); light brown solid; mp: 114-116 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3235, 2968, 1726, 1681, 1627; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.35 (d, *J* = 2.9 Hz, 1H), 8.30-8.23 (m, 2H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.50 (tb, *J* = 7.0, 1.2 Hz, 1H), 7.21-7.16 (m, 1H), 7.10 (dd, *J* = 8.4, 1.4 Hz, 1H), 6.97 (s, 1H), 4.81-4.77 (m, 1H), 4.42-4.25 (m, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.72-2.61 (m, 2H), 2.30 (s, 3H), 1.37 (t, *J* = 7.2 Hz, 3H), 1.18-1.15 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.1, 162.0, 151.7, 136.9, 135.8, 133.1, 129.8, 129.4, 128.6, 128.5, 125.6, 125.0, 123.3, 122.7, 121.7, 85.3, 61.1, 60.6, 48.9, 40.7, 20.9, 14.4, 14.1; MS (ES mass): 421.3 (M+1); HPLC: 97.9%, 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 5.19 min. Elemental analysis found C, 68.79; H, 5.74; N, 6.43; $C_{24}H_{24}N_2O_5$ requires C, 68.56; H, 5.75; N, 6.66.

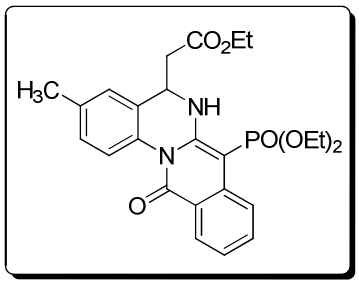
4.6.1.43. Methyl-5-(2-ethoxy-2-oxoethyl)-3-methyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9u)

Compound **9u** was synthesized from the reaction of **7g** and **8b** following a procedure similar to that of compound **9a**.



Yield: 84% (39 mg); light brown solid; mp: 112-114 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 3185, 2952, 1730, 1680, 1641; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.33 (d, *J* = 2.9 Hz, 1H), 8.27 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.50 (tb, *J* = 7.8, 1.2 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.10 (dd, *J* = 8.3, 1.4 Hz, 1H), 6.96 (s, 1H), 4.83-4.76 (m, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 2.73-2.61 (m, 2H), 2.30 (s, 3H), 1.18 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 169.5, 161.9, 151.7, 136.9, 135.7, 133.2, 129.8, 129.3, 128.6, 128.5, 125.6, 125.0, 123.4, 122.7, 121.7, 85.2, 61.1, 51.4, 48.9, 40.7, 20.9, 14.1; MS (ES mass): 407.2 (M+1); HPLC: 97.9%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.66 min.

4.6.1.44. Ethyl-2-(7-(diethoxyphosphoryl)-3-methyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9v)

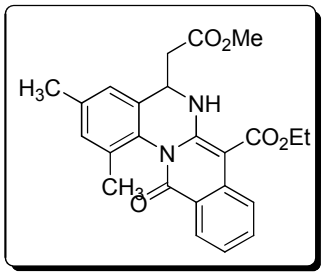


Compound **9v** was synthesized from the reaction of **7g** and **8c** following a procedure similar to that of compound **9a**.

Yield: 82% (45 mg); brown liquid; R_f = 0.1 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2978, 2926, 1732, 1679, 1599; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.78 (d, *J* = 2.7 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.54 (tb, *J* = 8.4, 1.5 Hz, 1H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.17 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.06

(s, 1H), 4.84-4.77 (m, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.14-3.98 (m, 3H), 3.95-3.82 (m, 1H), 2.78-2.69 (m, 2H), 2.37 (s, 3H), 1.33-1.27 (m, 6H), 1.25-1.21 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.8, 162.3, 153.0 (C-P *J* = 28.9 Hz), 136.7, 136.6 (C-P *J* = 7.1 Hz), 133.2, 130.1, 129.9, 128.7, 128.5, 125.7, 123.4 (C-P *J* = 3.2 Hz), 123.2, 122.7, 121.4 (C-P *J* = 12.2 Hz), 74.5, 61.7, 61.8, 61.1, 49.2, 40.6, 20.9, 16.2 (C-P *J* = 7.3 Hz), 16.1 (C-P *J* = 7.3 Hz), 14.1; MS (ES mass): 485.3 (M+1); HPLC: 89.9%, 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, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.40 min.

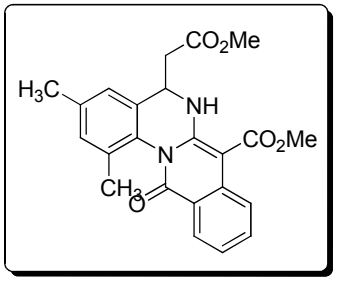
4.6.1.45. Ethyl-5-(2-methoxy-2-oxoethyl)-1,3-dimethyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9w)



Compound **9w** was synthesized from the reaction of **7h** and **8a** following a procedure similar to that of compound **9a**.

Yield: 86% (41 mg); light brown solid; mp: 176-178 °C; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2943, 2834, 1732, 1688, 1642; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.03 (d, *J* = 3.1 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 7.60 (tb, *J* = 8.4, 1.2 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.07 (s, 1H), 6.93 (s, 1H), 4.88-4.80 (m, 1H), 4.50-4.41 (m, 1H), 4.38-4.30 (m, 1H), 3.67 (s, 3H), 2.80-2.69 (m, 2H), 2.36 (s, 3H), 2.05 (s, 3H), 1.45 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7, 168.8, 161.3, 153.0, 136.9, 135.6, 132.9, 132.5, 132.4, 132.0, 129.1, 127.8, 125.5, 123.6, 123.5, 122.8, 86.5, 60.5, 51.9, 50.2, 40.1, 20.8, 20.5, 14.5; MS (ES mass): 420.6 (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): 0/50, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.29 min.

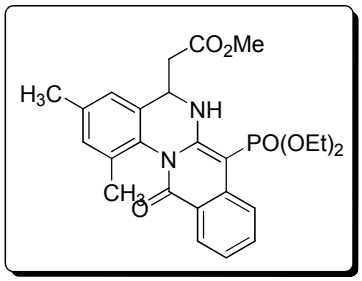
4.6.1.46. Methyl-5-(2-methoxy-2-oxoethyl)-1,3-dimethyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (9x)



Compound **9x** was synthesized from the reaction of **7h** and **8b** following a procedure similar to that of compound **9a**.

Yield: 81% (37 mg); light yellow solid; mp: 135-137 °C; R_f = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2941, 2823, 1731, 1685, 1638; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.02 (d, *J* = 2.9 Hz, 1H), 8.27-8.24 (m, 2H), 7.60 (t, *J* = 7.8 Hz, 1H), 7.27 (t, *J* = 7.5 Hz, 1H), 7.07 (s, 1H), 6.93 (s, 1H), 4.89-4.79 (m, 1H), 3.93 (s, 3H), 3.67 (s, 3H), 2.79-2.69 (m, 2H), 2.36 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7, 169.2, 161.3, 153.1, 136.9, 135.5, 132.9, 132.5, 132.4, 132.0, 129.0, 127.8, 125.4, 123.6, 123.5, 122.8, 86.3, 51.9, 51.4, 50.2, 40.1, 20.8, 20.5; MS (ES mass): 406.6 (M+1); HPLC: 97.1%, 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, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.67 min.

4.6.1.47. Methyl-2-(7-(diethoxyphosphoryl)-1,3-dimethyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (9y)

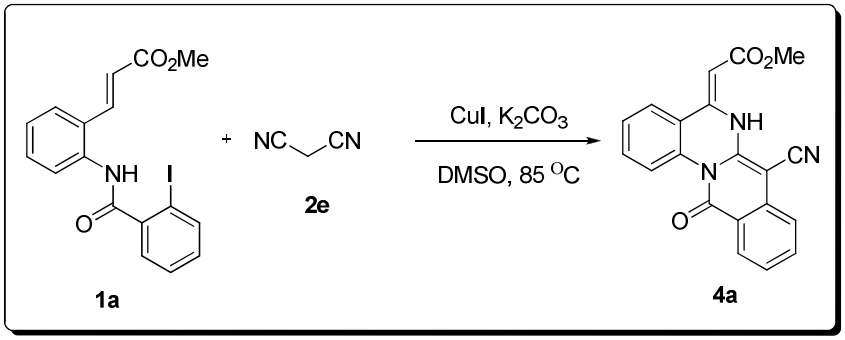


Compound **9y** was synthesized from the reaction of **7h** and **8c** following a procedure similar to that of compound **9a**.

Yield: 78% (43 mg); light brown semi solid; R_f = 0.4 (30% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2921, 2834, 1731, 1682, 1639; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.45 (d, *J* = 2.6 Hz, 1H), 8.28 (d, *J* = 7.9 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.26-7.21 (m, 1H), 7.08 (s, 1H), 6.94 (s, 1H), 4.80-4.76 (m, 1H), 4.25-4.14 (m,

1H), 4.14-3.99 (m, 2H), 3.84-3.76 (m, 1H), 3.73 (s, 3H), 2.80-2.69 (m, 2H), 2.36 (s, 3H), 2.07 (s, 3H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 161.4, 154.2 (C-P *J* = 21.4 Hz), 136.8, 136.5 (C-P *J* = 7.0 Hz), 133.0, 132.9, 132.4, 131.9, 129.2, 128.1, 123.7 (C-P *J* = 3.1 Hz), 123.5, 123.4, 122.3 (C-P *J* = 11.7 Hz), 74.8, 61.8, 61.7, 52.1, 50.4, 39.9, 20.8, 20.6, 16.3 (C-P *J* = 6.9 Hz), 16.1 (C-P *J* = 7.2 Hz); MS (ES mass): 484.7 (M+1); HPLC: 97.8%, 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): 0/50, 1.0/50, 6/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.44 min. Elemental analysis found C, 61.69; H, 6.05; N, 5.93; $C_{25}H_{29}N_2O_6P$ requires C, 61.98; H, 6.03; N, 5.78.

4.6.1.48. Typical procedure for preparation of (*Z*)-Methyl-2-(7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-ylidene)acetate (10a)

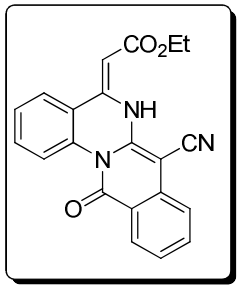


A mixture of compound **7a** (50 mg, 0.12 mmol), K_2CO_3 (50 mg, 0.36 mmol), malononitrile (**2e**) (9.4 mg, 0.14 mmol) and CuI (2.3 mg, 0.012 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions ($CaCl_2$ filled guard tube) for 4 h. After completion of the reaction, reaction mixture was 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, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **10a**.

Yield: 72% (30 mg); yellow solid; mp: 205-207 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2969, 2853, 2221, 1681, 1642; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.70 (s, 1H), 8.87 (d, *J* = 8.7 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.80-7.66 (m, 3H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.43-7.38 (m, 2H), 5.77 (s, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.4, 161.3, 144.0, 143.8, 134.7, 133.6, 132.9, 132.4, 129.1, 127.4, 125.7,

124.1, 122.5, 122.2, 121.2, 119.0, 115.4, 85.8, 71.3, 51.9; MS (ES mass): 341.5 (M-1); HPLC: 95.1%, 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, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.72 min.

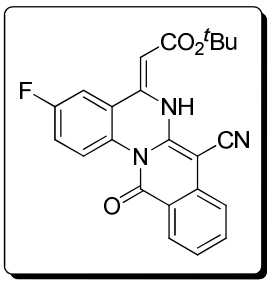
4.6.1.49. (*Z*)-Ethyl-2-(7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*] quinazolin-5-ylidene)acetate (10b)



Compound **10b** was synthesized from the reaction of **7b** and **8e** following a procedure similar to that of compound **10a**.

Yield: 71% (30 mg); yellow solid; mp: 214-216 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2972, 2855, 2214, 1676, 1638; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.75 (s, 1H), 8.86 (d, *J* = 8.7 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.73-7.68 (m, 2H), 7.61 (tb, *J* = 8.6, 1.4 Hz, 1H), 7.43-7.38 (m, 2H), 5.77 (s, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1, 161.3, 144.1, 143.7, 134.7, 133.6, 132.9, 132.3, 129.1, 127.4, 125.7, 124.1, 122.5, 122.2, 121.2, 119.1, 115.4, 86.2, 71.2, 60.7, 14.4; MS (ES mass): 357.6 (M+1); HPLC: 93.2%, 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, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.24 min.

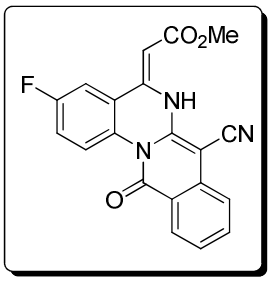
4.6.1.50. (*Z*)-^{t}Butyl-2-(7-cyano-3-fluoro-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-ylidene)acetate (10c)



Compound **10c** was synthesized from the reaction of **7d** and **8e** following a procedure similar to that of compound **10a**.

Yield: 49% (23 mg); brown solid; mp: 175-177 °C; R_f = 0.7 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2958, 2854, 2221, 1698, 1643; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.75 (s, 1H), 8.89 (dd, *J* = 9.3, 4.9 Hz, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 7.75-7.68 (m, 2H), 7.41-7.38 (m, 2H), 7.34-7.27 (m, 1H), 5.63 (s, 1H), 1.57 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3, 169.5, 161.7 (C-F *J* = 248.4 Hz), 161.3, 143.9, 142.0 (C-F *J* = 2.7 Hz), 134.8, 133.8, 129.0, 125.7, 124.8 (C-F *J* = 8.1 Hz), 122.5, 121.8 (C-F *J* = 8.1 Hz), 121.0, 119.3 (C-F *J* = 22.3 Hz), 115.2, 110.3 (C-F *J* = 14.2 Hz), 89.1, 81.7, 71.2, 28.3 (3C); MS (ES mass): 401.7 (M-1); HPLC: 90.9%, 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, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.79 min.

4.6.1.51. (*Z*)-Methyl-2-(7-cyano-3-fluoro-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-ylidene)acetate (10d)

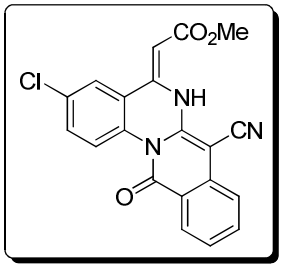


Compound **10d** was synthesized from the reaction of **7i** and **8e** following a procedure similar to that of compound **10a**.

Yield: 56% (23 mg); light yellow solid; mp: 172-174 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2963, 2845, 2216, 1692, 1645; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.69 (s, 1H), 8.96 (dd, *J* = 9.3, 5.0 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.80-7.65 (m, 2H), 7.47-7.39 (m, 2H), 7.35-7.33 (m, 1H), 5.70 (s, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 168.2, 161.7 (C-F *J* = 258.9 Hz), 161.2, 143.6, 142.9 (C-F *J* = 2.7 Hz), 134.8, 133.4, 129.0, 125.8, 124.8 (C-F *J* = 8.0 Hz), 122.6, 121.2 (C-F *J* = 8.4 Hz), 121.1, 119.7 (C-F *J* = 22.2 Hz), 115.3, 110.4 (C-F *J* = 24.6 Hz), 86.6, 71.5, 52.1; MS (ES mass): 359.6 (M-1); HPLC: 92.3%, 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): 0/50, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.42 min.

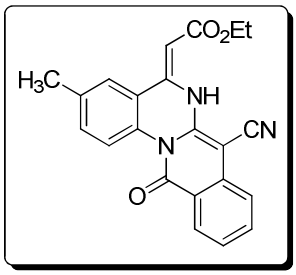
4.6.1.52. (*Z*)-Methyl-2-(3-chloro-7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-ylidene)acetate (10e)



Compound **10e** was synthesized from the reaction of **7f** and **8e** following a procedure similar to that of compound **10a**.

Yield: 48% (20 mg); yellow solid; mp: 205-207 °C; R_f = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2943, 2851, 2220, 1682, 1645; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.69 (s, 1H), 8.89 (d, *J* = 9.0 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.76-7.69 (m, 3H), 7.56 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.42 (t, *J* = 8.0 Hz, 1H), 5.74 (s, 1H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 161.2, 143.6, 142.6, 134.9, 133.4, 133.2, 132.2, 131.5, 129.1, 125.9, 123.8, 123.7, 122.6, 121.1, 120.7, 115.2, 86.6, 71.7, 52.1; MS (ES mass): 375.4 (M-1); HPLC: 94.2%, 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, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.72 min. Elemental analysis found C, 63.39; H, 3.25; N, 11.33; $C_{20}H_{12}ClN_3O_3$ requires C, 63.59; H, 3.20; N, 11.12.

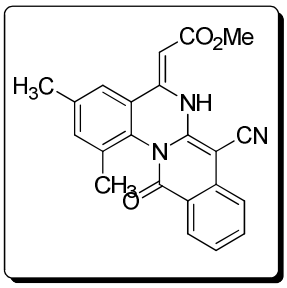
4.6.1.53. (*Z*)-Ethyl-2-(7-cyano-3-methyl-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-ylidene)acetate (10f)



Compound **10f** was synthesized from the reaction of **7g** and **8e** following a procedure similar to that of compound **10a**.

Yield: 65% (27 mg); brown solid; mp: 189-191 °C; R_f = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2920, 2853, 2208, 1682, 1643; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.74 (s, 1H), 8.79 (d, *J* = 8.6 Hz, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 7.73-7.68 (m, 2H), 7.56 (s, 1H), 7.42-7.37 (m, 2H), 5.75 (s, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 2.43 (s, 3H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1, 161.3, 143.9, 143.8, 137.5, 134.5, 133.6, 133.2, 128.9 (2C), 125.5, 124.1, 122.5, 122.0, 121.1, 118.8, 109.9, 85.9, 71.0, 60.6, 20.9, 14.4; MS (ES mass): 372.2 (M+1); HPLC: 91.5%, 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, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.65 min.

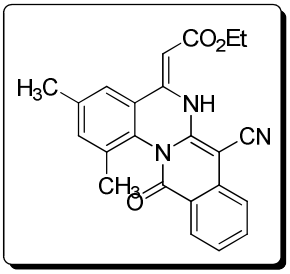
4.6.1.54. (*Z*)-Methyl-2-(7-cyano-1,3-dimethyl-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-ylidene)acetate (10g)



Compound **10g** was synthesized from the reaction of **7h** and **8e** following a procedure similar to that of compound **10a**.

Yield: 73% (31 mg); brown solid; mp: 178-180 °C; R_f = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2931, 2855, 2212, 1679, 1645; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.37 (s, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 7.74-7.68 (m, 2H), 7.43-7.36 (m, 2H), 7.29 (s, 1H), 5.81 (s, 1H), 3.86 (s, 3H), 2.42 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 160.2, 145.7, 144.9, 137.3, 136.1, 134.4, 133.9, 132.7, 128.8, 128.4, 125.5, 122.8, 122.4, 122.2, 121.8, 115.5, 87.3, 70.5, 51.9, 21.5, 20.9; MS (ES mass): 371.7 (M+1); HPLC: 98.3%, 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): 0/50, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.31 min.

4.6.1.55. (*Z*)-Ethyl-2-(7-cyano-1,3-dimethyl-12-oxo-6,12-dihydro-5*H*-isoquinolino [2,3-*a*]quinazolin-5-ylidene)acetate (10h)



Compound **10h** was synthesized from the reaction of **7j** and **8e** following a procedure similar to that of compound **10a**.

Yield: 72% (31 mg); light brown solid; mp: 188-190 °C; R_f = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr, cm^{-1}): 2935, 2843, 2209, 1672, 1644; ^1H NMR (400 MHz, $CDCl_3$) δ: 12.41 (s, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 7.74-7.68 (m, 2H), 7.42-7.36 (m, 2H), 7.29 (s, 1H), 5.81 (s, 1H), 4.33 (m, *J* = 7.0, 2.3 Hz, 2H), 2.41 (s, 3H), 2.15 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.9, 160.2, 145.8, 144.8, 137.3, 136.1, 134.3, 134.0, 132.7, 128.7, 128.4, 125.5, 122.8, 122.5, 122.2, 121.8, 115.5, 87.8, 70.5, 60.7, 21.5, 20.9, 14.4; MS (ES mass): 385.7 (M+1); HPLC: 98.3%, 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): 0/50, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.61 min. Elemental analysis found C, 71.39; H, 5.05; N, 11.01; $C_{23}H_{19}N_3O_3$ requires C, 71.67; H, 4.97; N, 10.90.

4.7. References:

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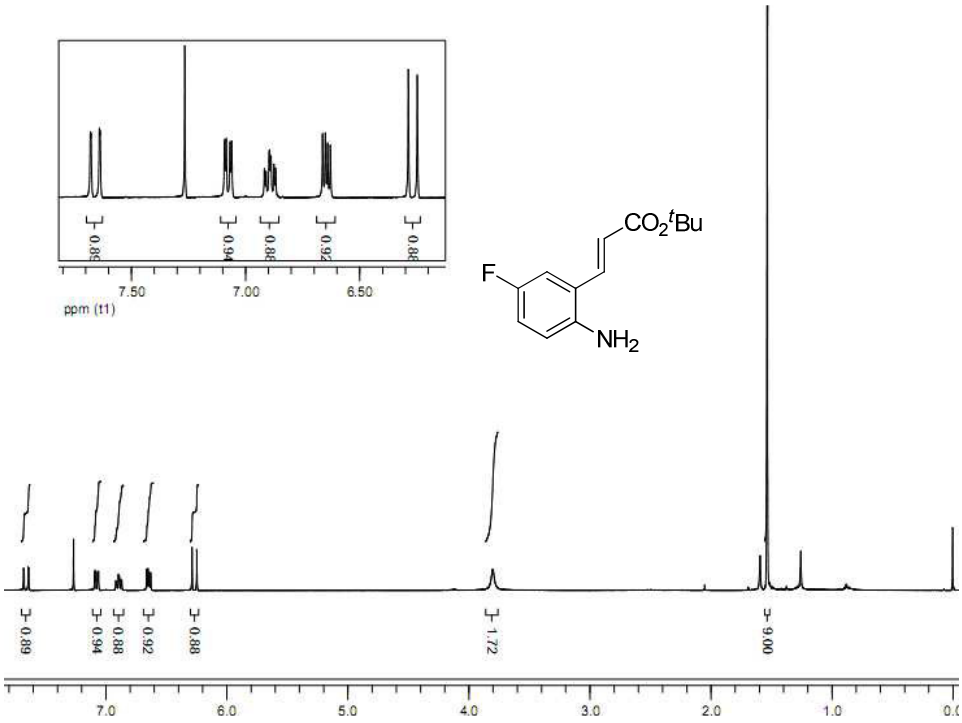
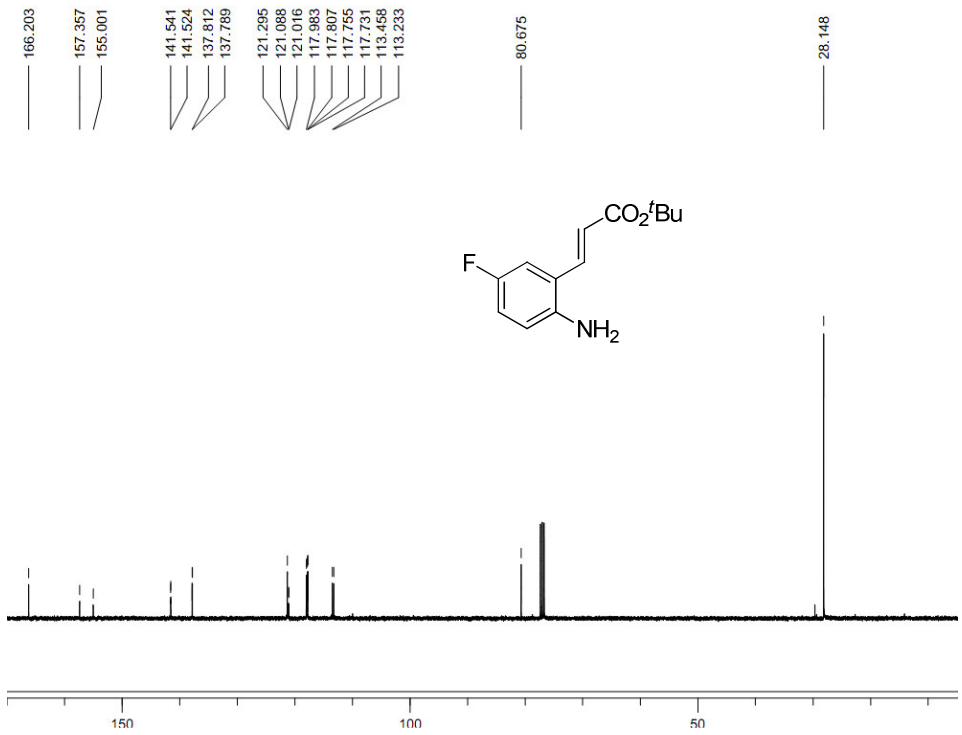
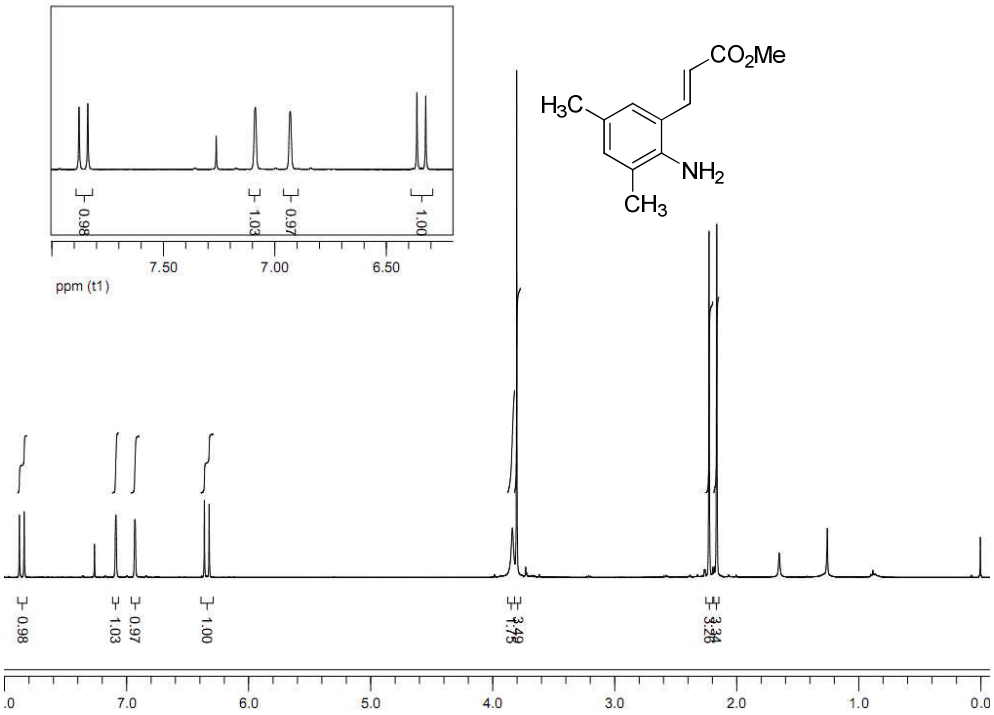
Appendix



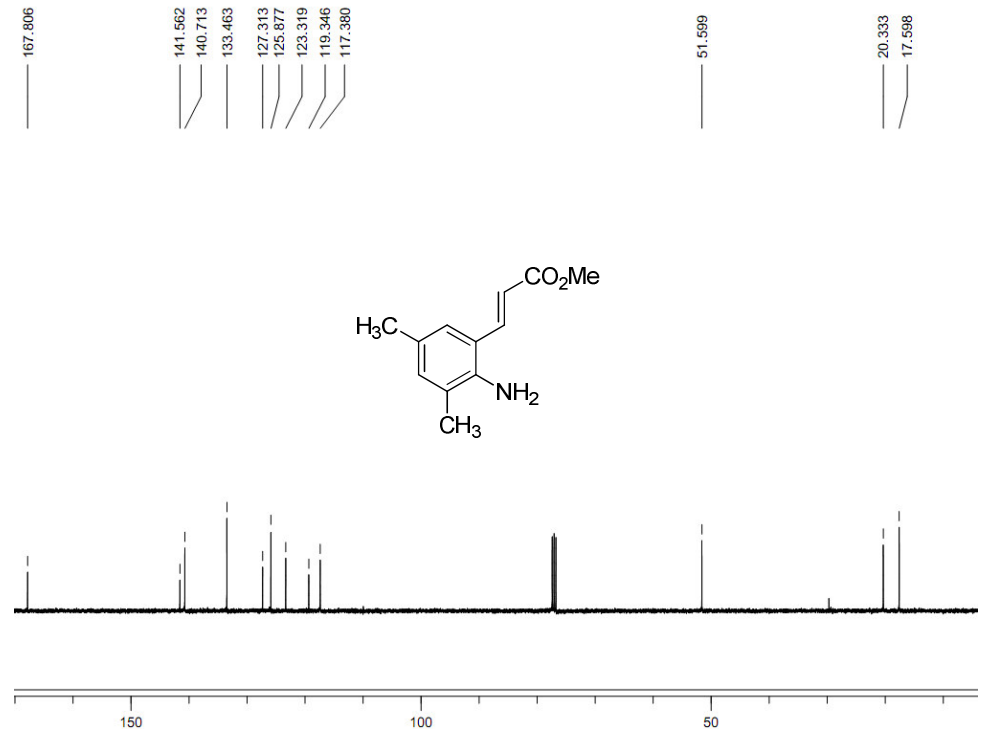
^{1}H NMR spectra of compound **14c** ($CDCl_3$, 400 MHz)



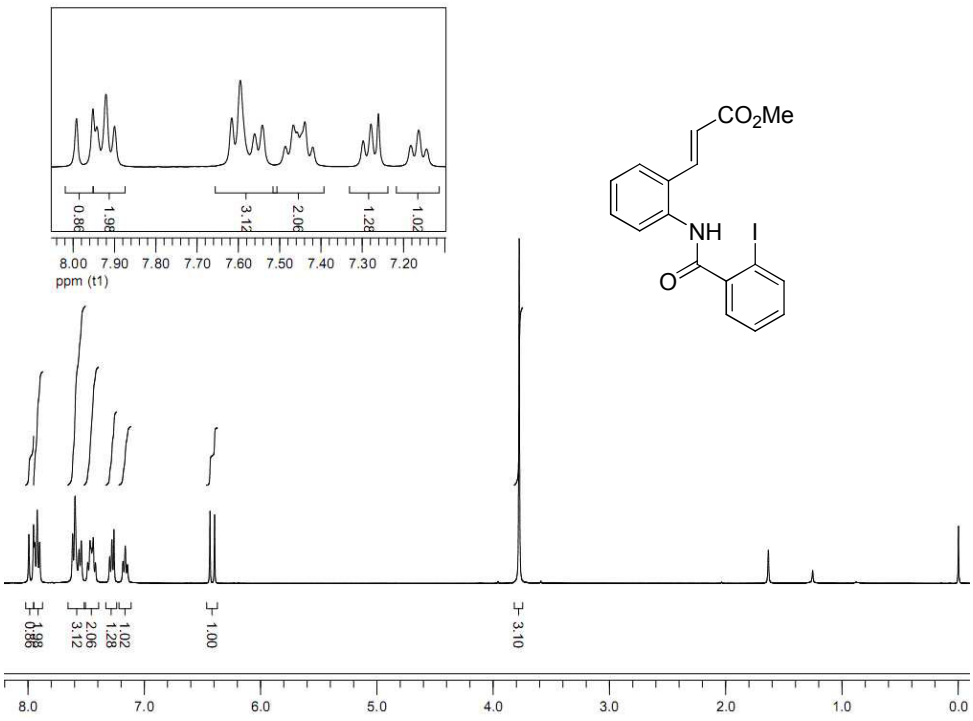
^{13}C NMR spectra of compound **14c** ($CDCl_3$, 100 MHz)



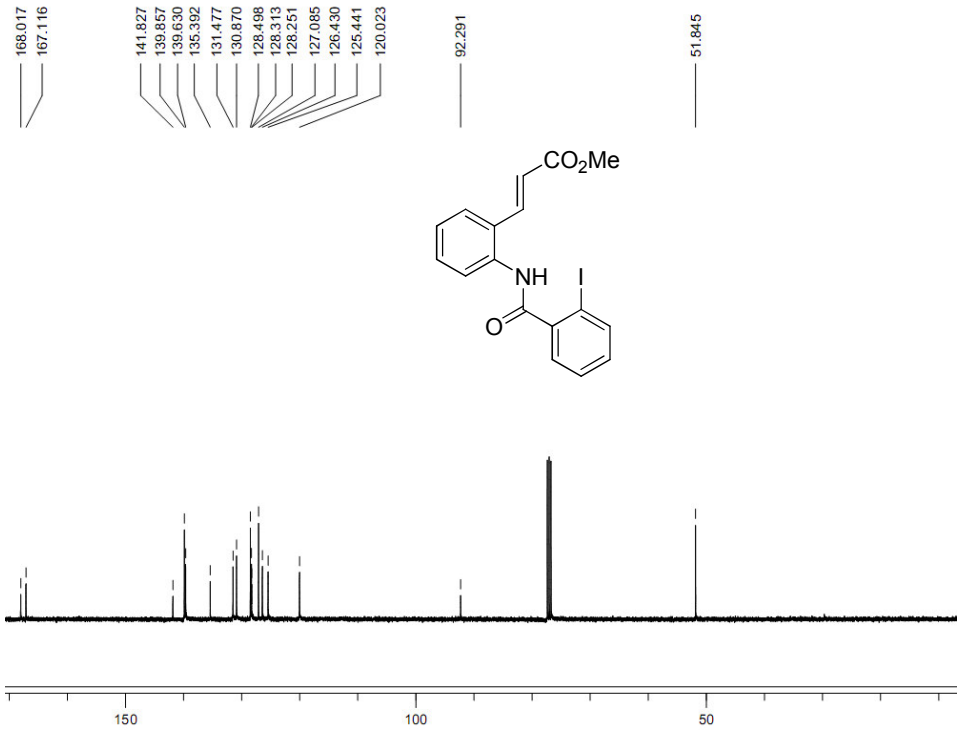
^{1}H NMR spectra of compound **14g** ($CDCl_3$, 400 MHz)



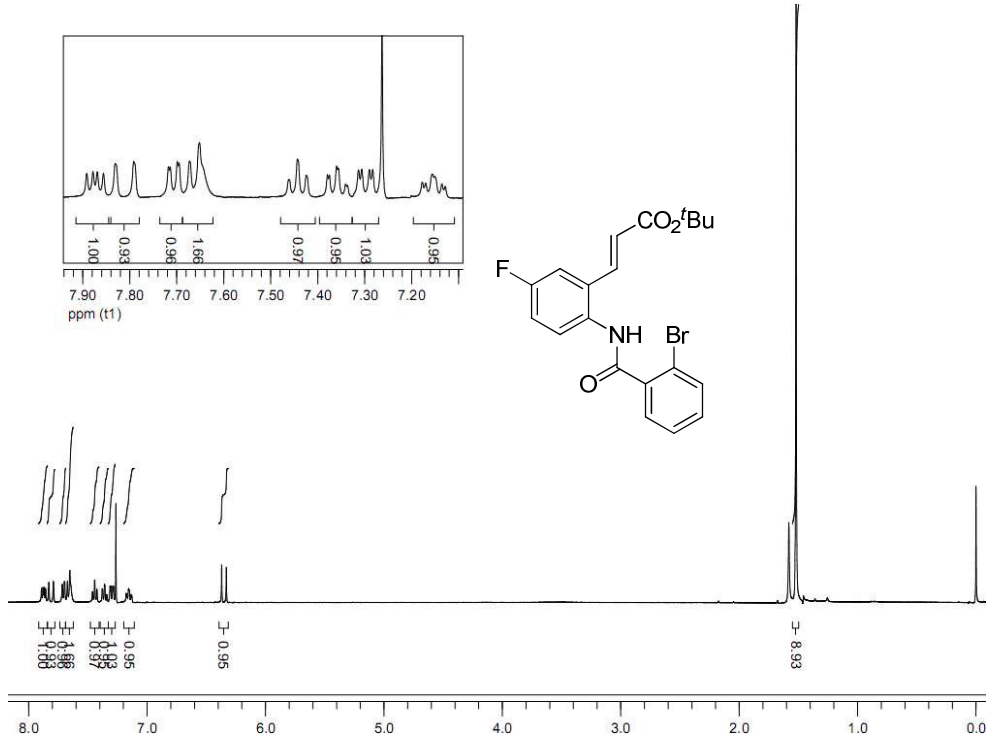
^{13}C NMR spectra of compound **14g** ($CDCl_3$, 100 MHz)



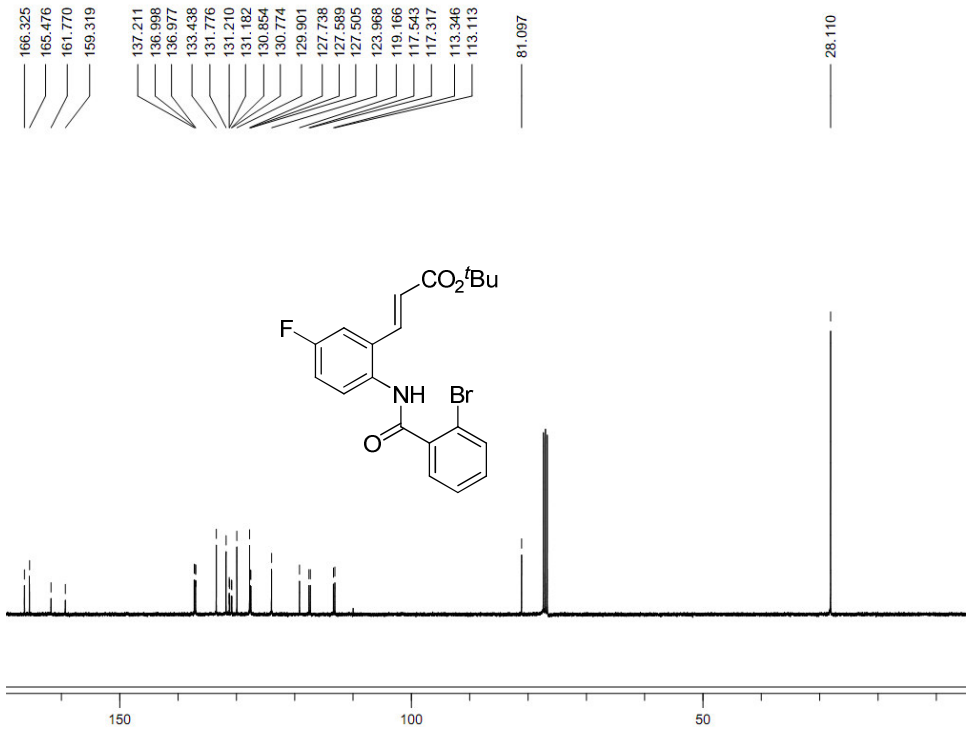
^{1}H NMR spectra of compound **7a** ($CDCl_3$, 400 MHz)



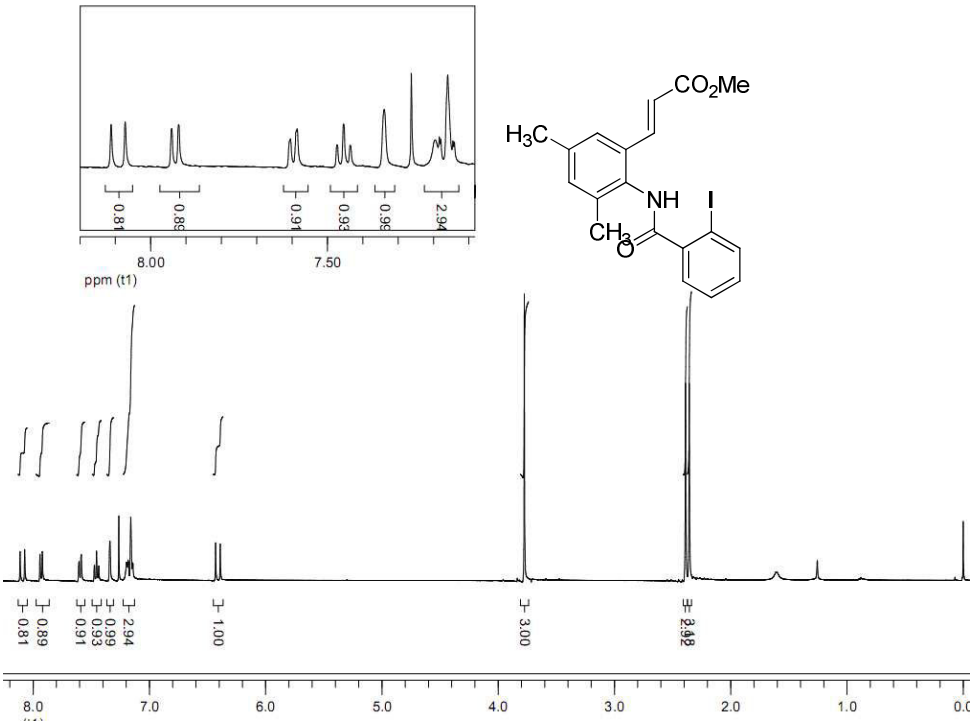
^{13}C NMR spectra of compound **7a** ($CDCl_3$, 100 MHz)



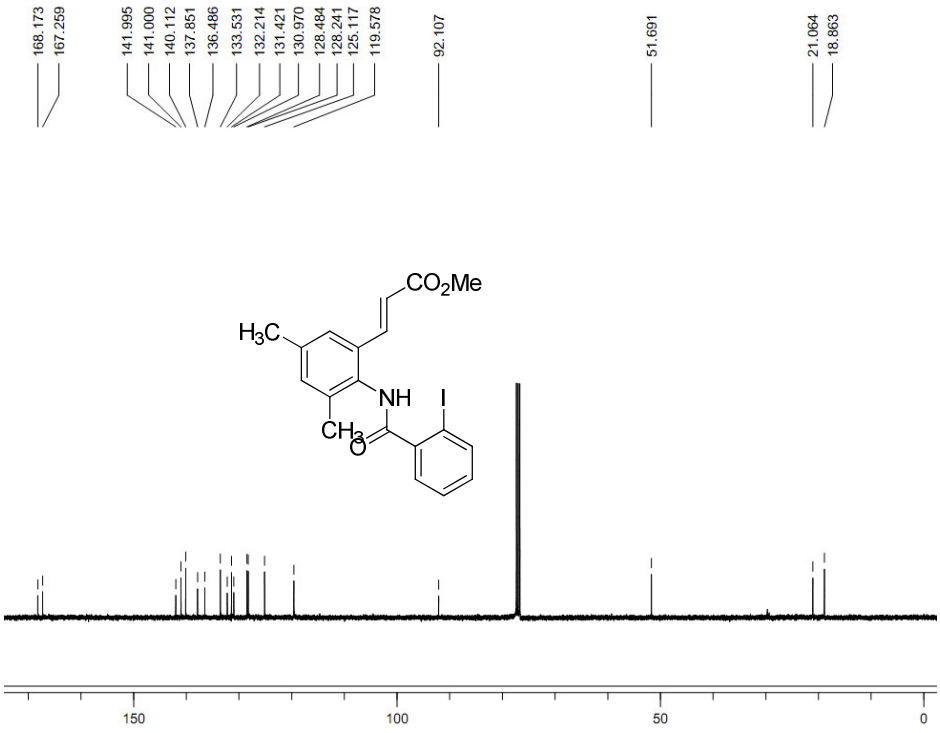
^{1}H NMR spectra of compound **7d** ($CDCl_3$, 400 MHz)



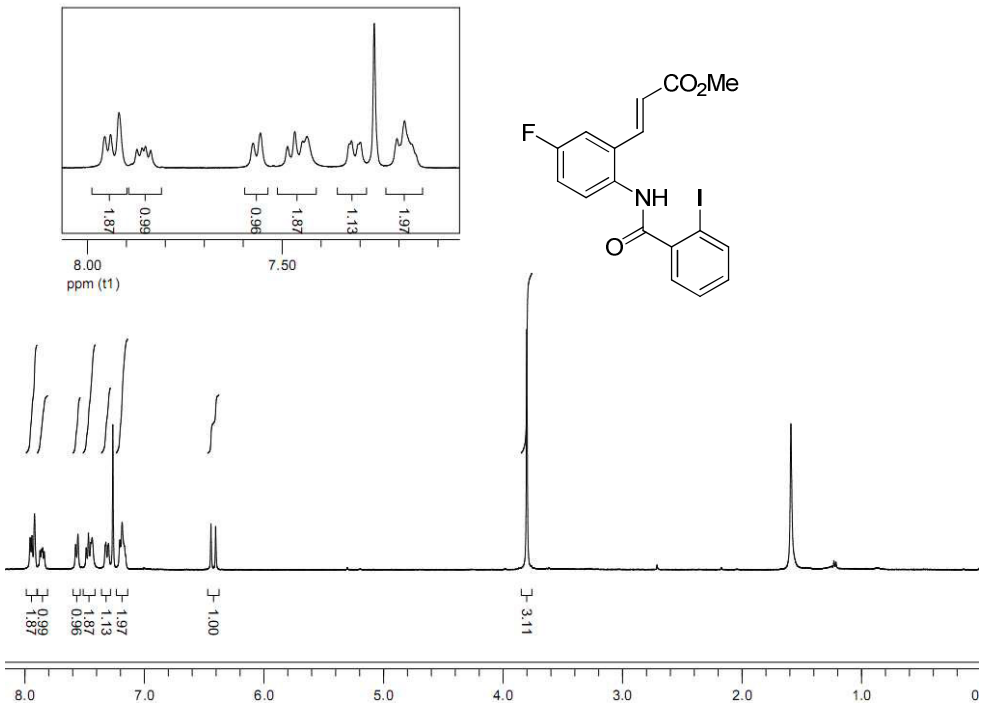
^{13}C NMR spectra of compound **7d** ($CDCl_3$, 100 MHz)



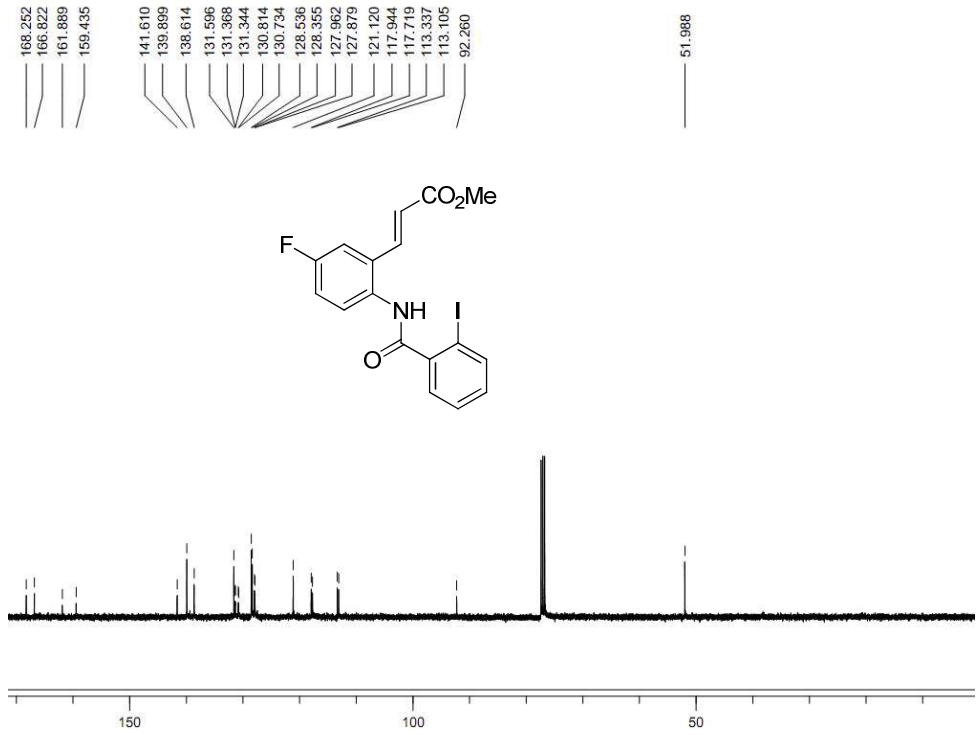
^{1}H NMR spectra of compound **7h** ($CDCl_3$, 400 MHz)



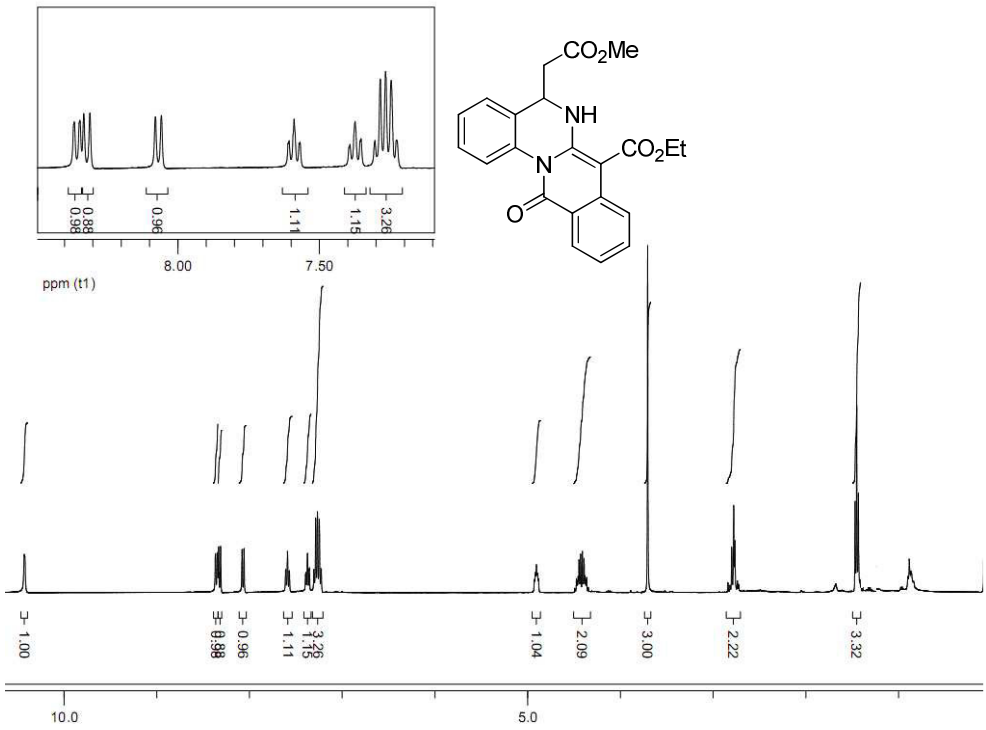
^{13}C NMR spectra of compound **7h** ($CDCl_3$, 100 MHz)



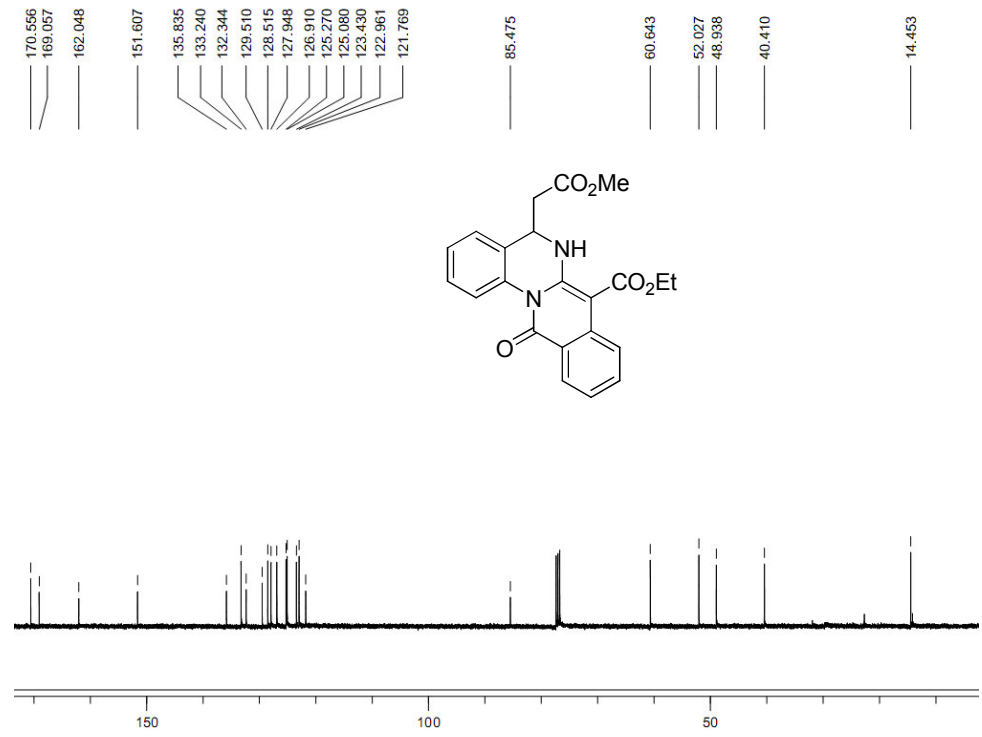
^{1}H NMR spectra of compound **7i** ($CDCl_3$, 400 MHz)



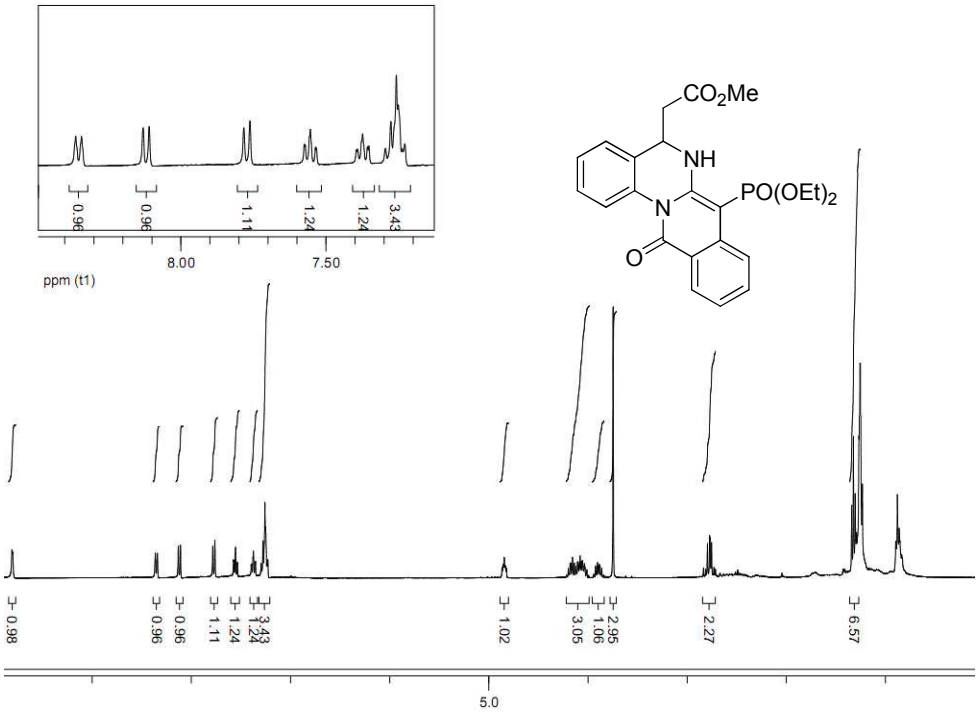
^{13}C NMR spectra of compound **7i** ($CDCl_3$, 100 MHz)



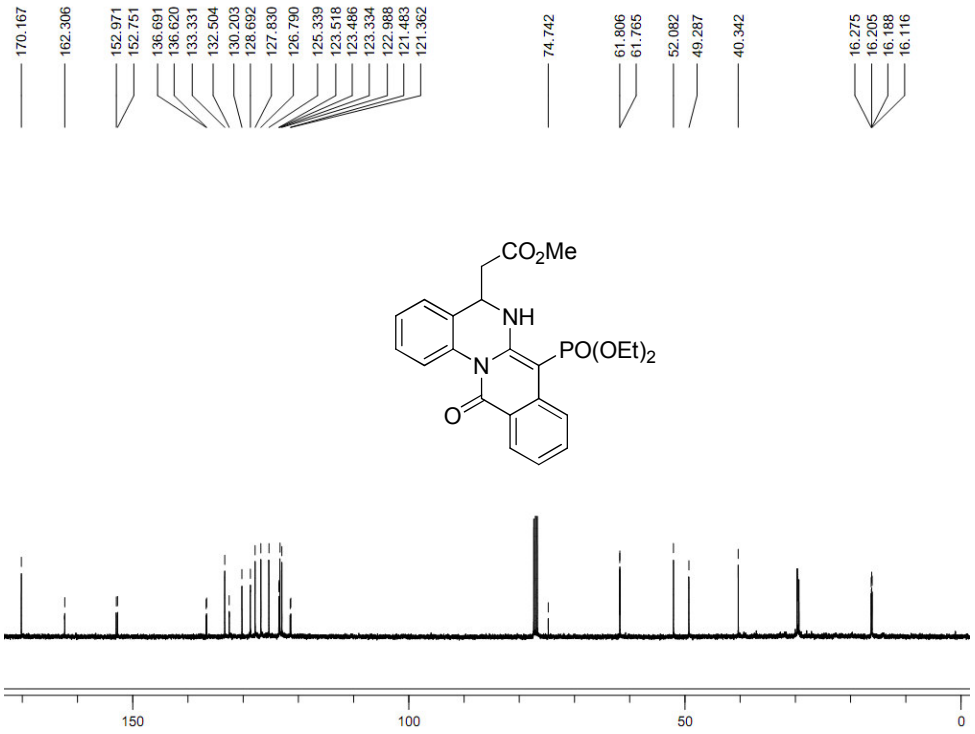
^{1}H NMR spectra of compound **9a** ($CDCl_3$, 400 MHz)



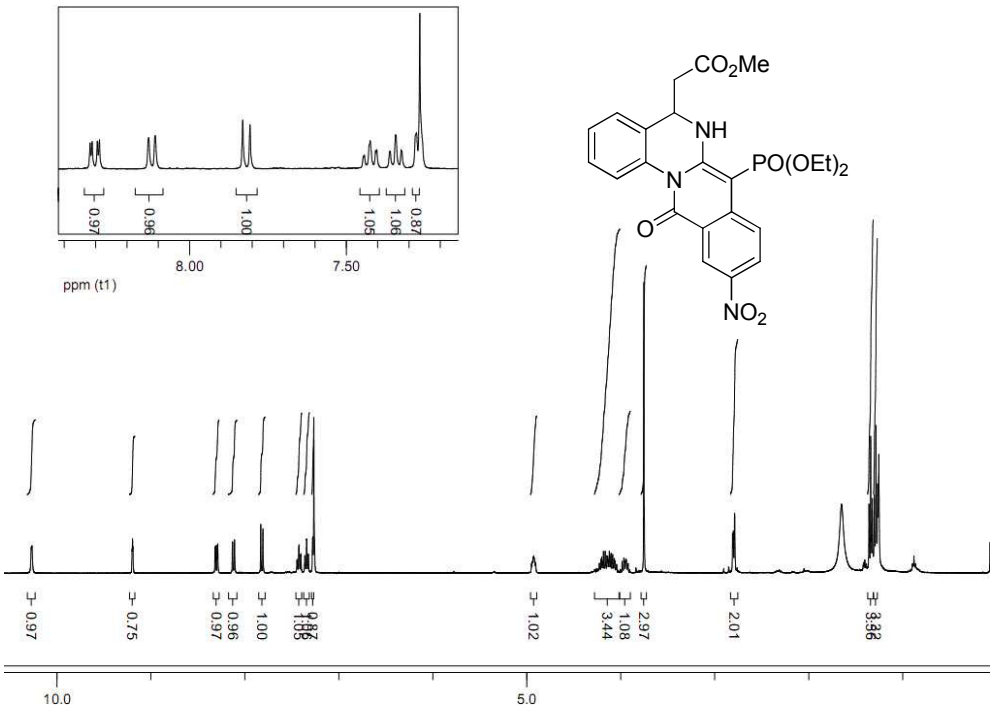
^{13}C NMR spectra of compound **9a** ($CDCl_3$, 100 MHz)



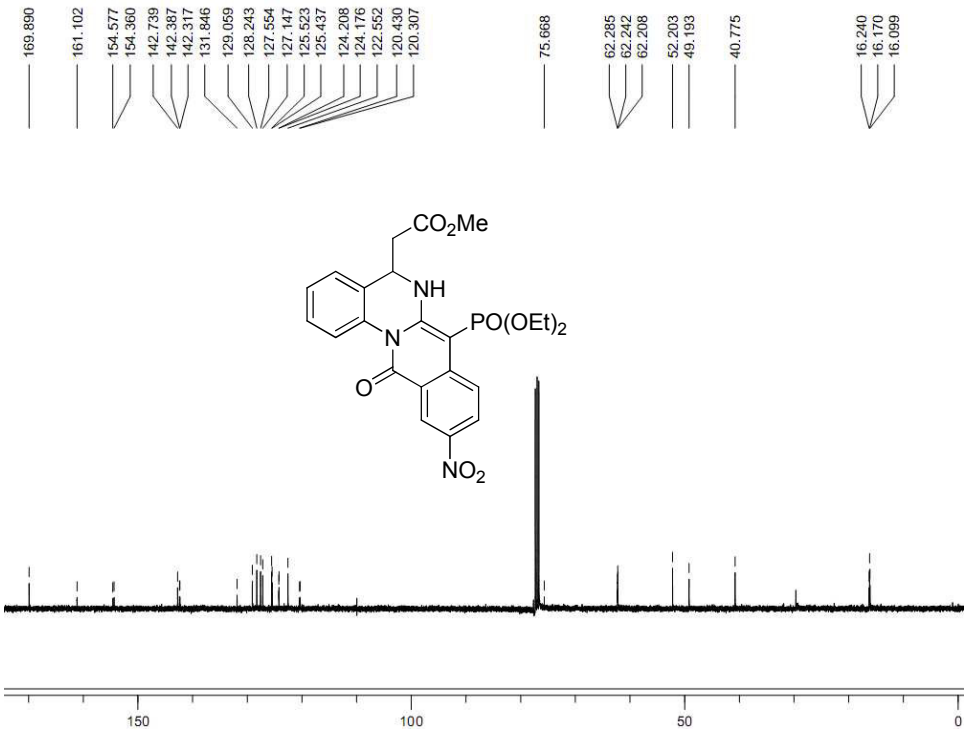
^{1}H NMR spectra of compound **9c** ($CDCl_3$, 400 MHz)



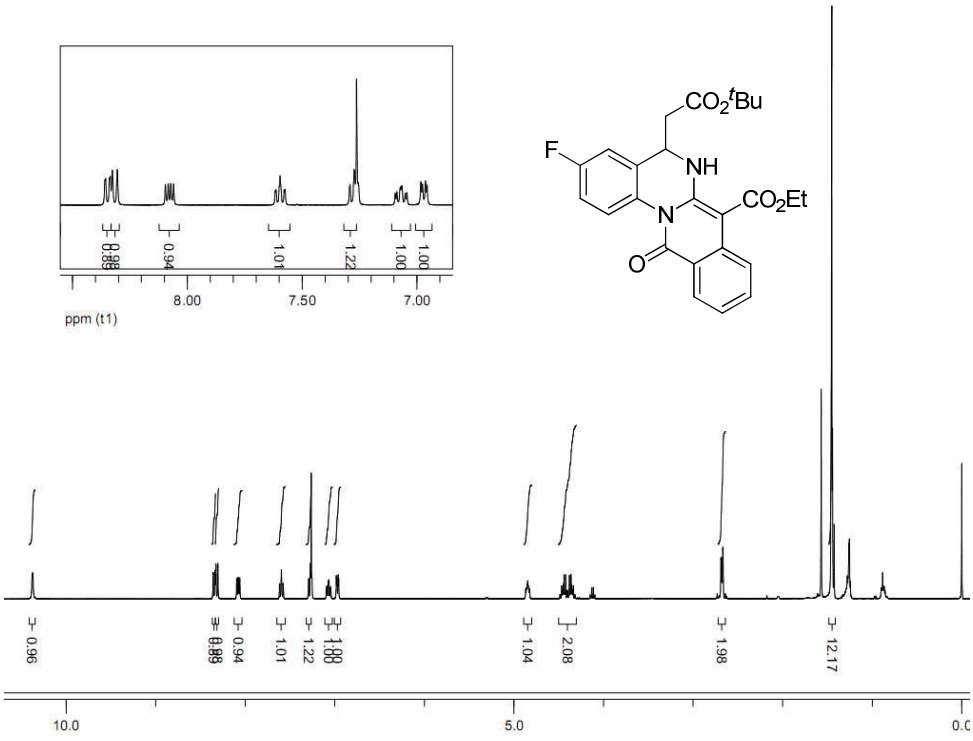
^{13}C NMR spectra of compound **9c** ($CDCl_3$, 100 MHz)



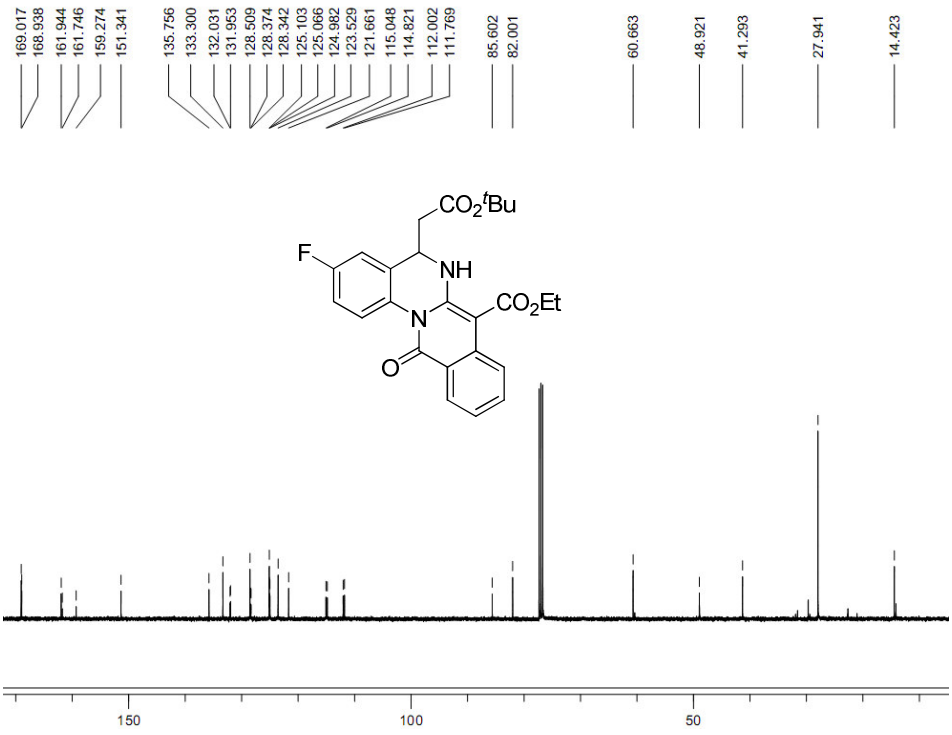
^{1}H NMR spectra of compound **9k** ($CDCl_3$, 400 MHz)



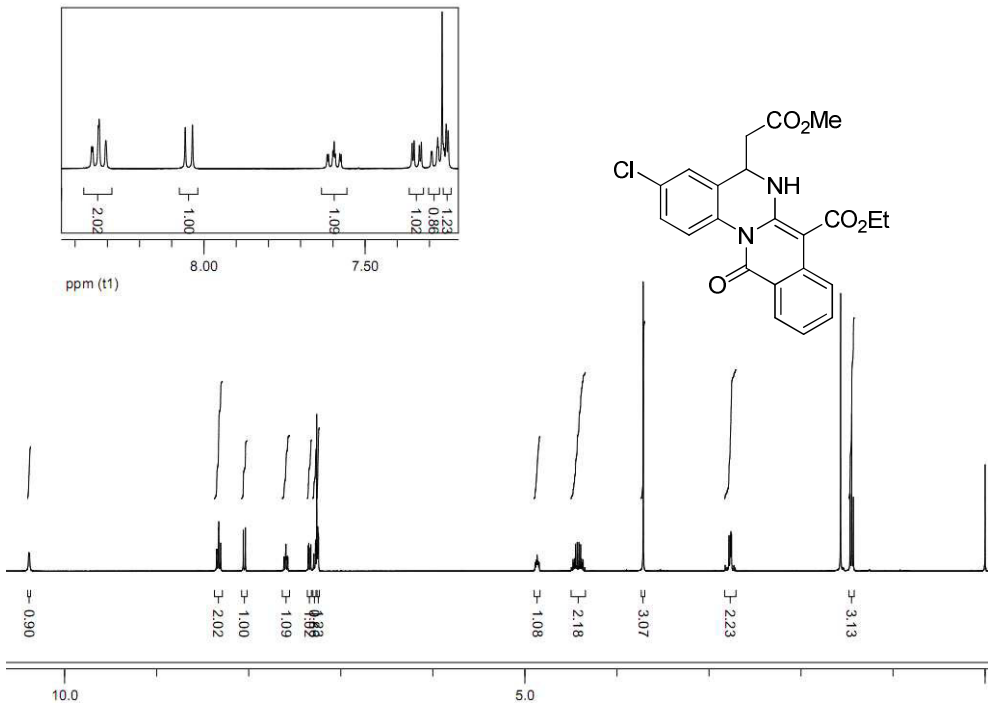
^{13}C NMR spectra of compound **9k** ($CDCl_3$, 100 MHz)



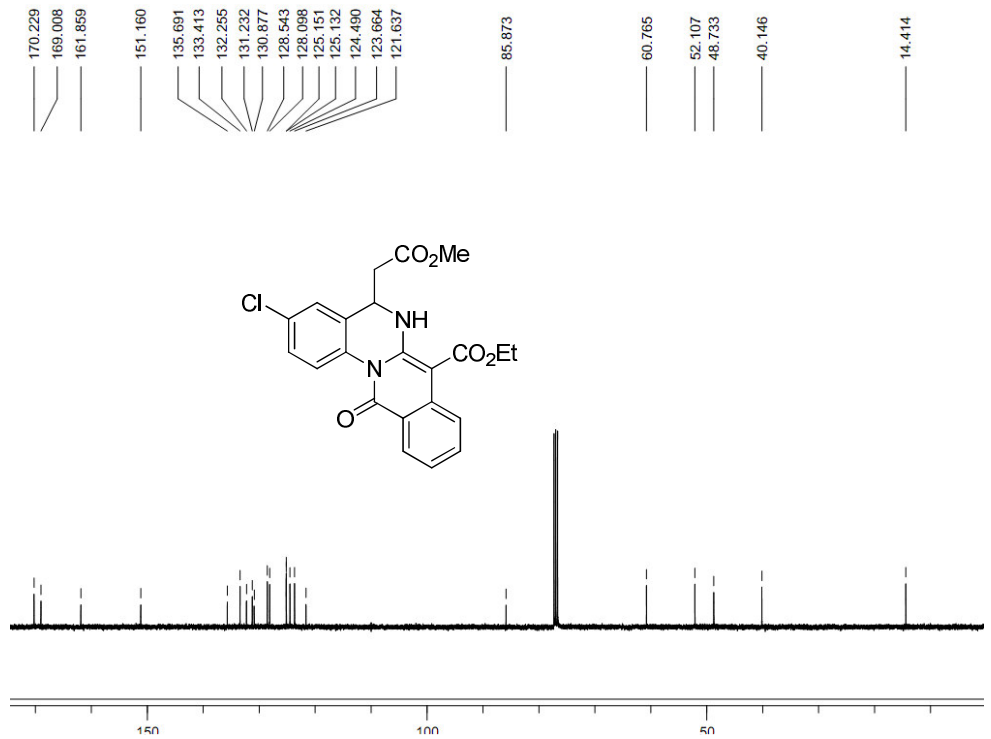
^{1}H NMR spectra of compound **9l** ($CDCl_3$, 400 MHz)



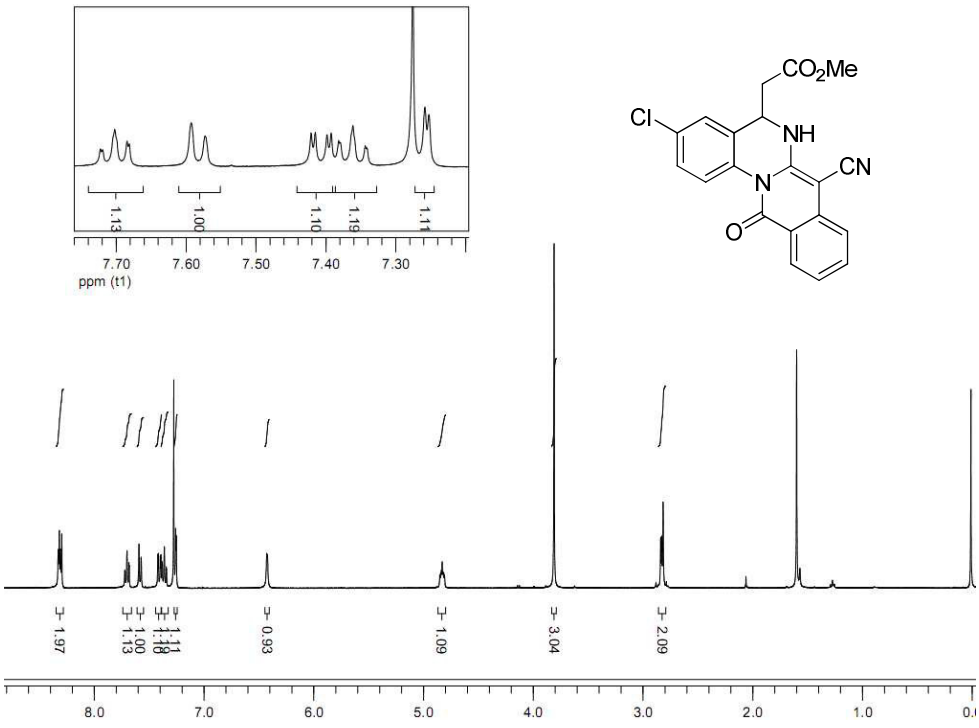
^{13}C NMR spectra of compound **9l** ($CDCl_3$, 100 MHz)



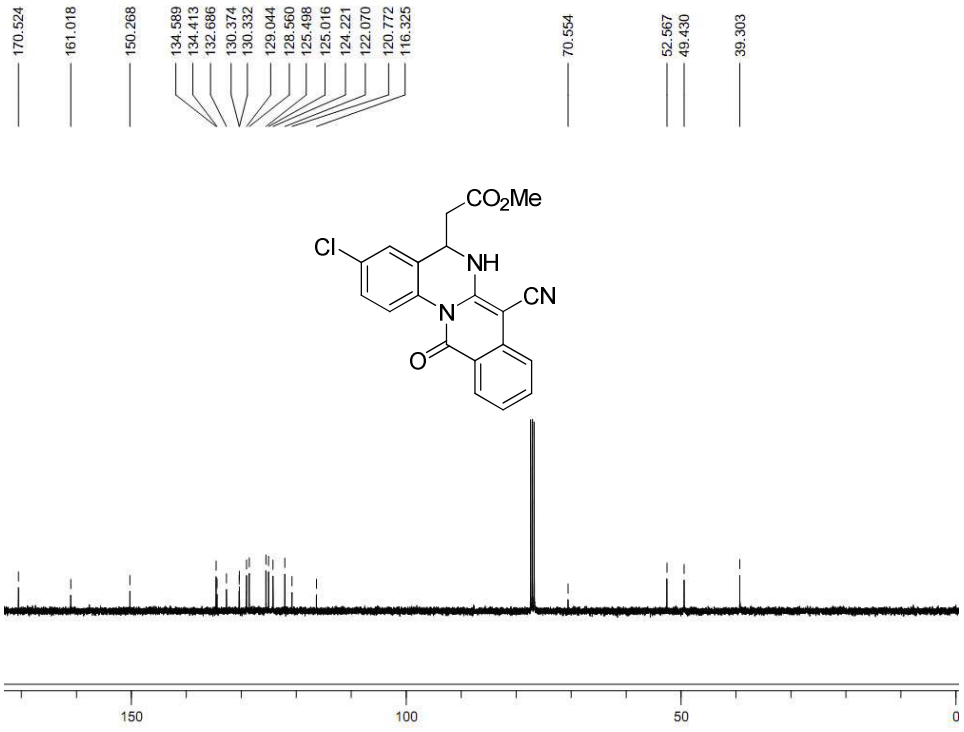
^{1}H NMR spectra of compound **9o** ($CDCl_3$, 400 MHz)



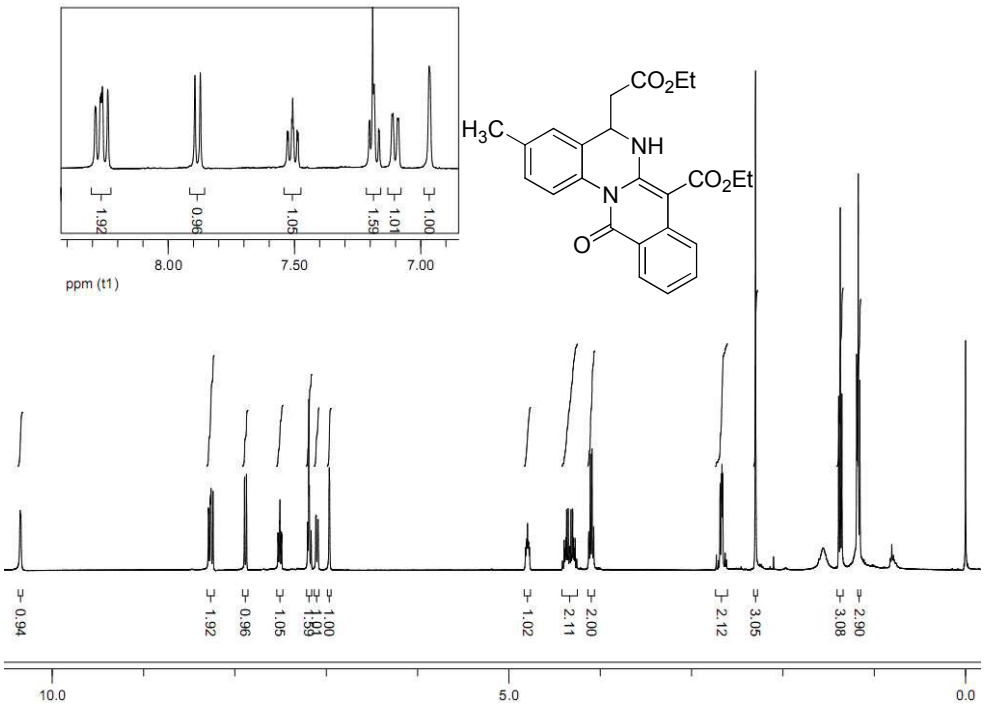
^{13}C NMR spectra of compound **9o** ($CDCl_3$, 100 MHz)



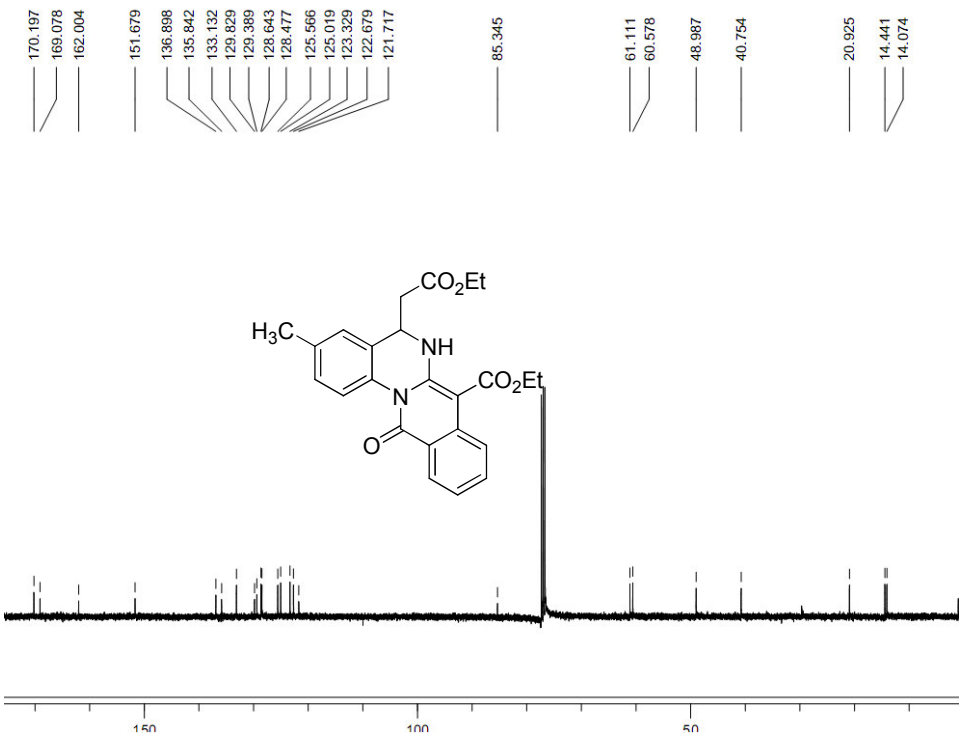
^{1}H NMR spectra of compound **9s** ($CDCl_3$, 400 MHz)



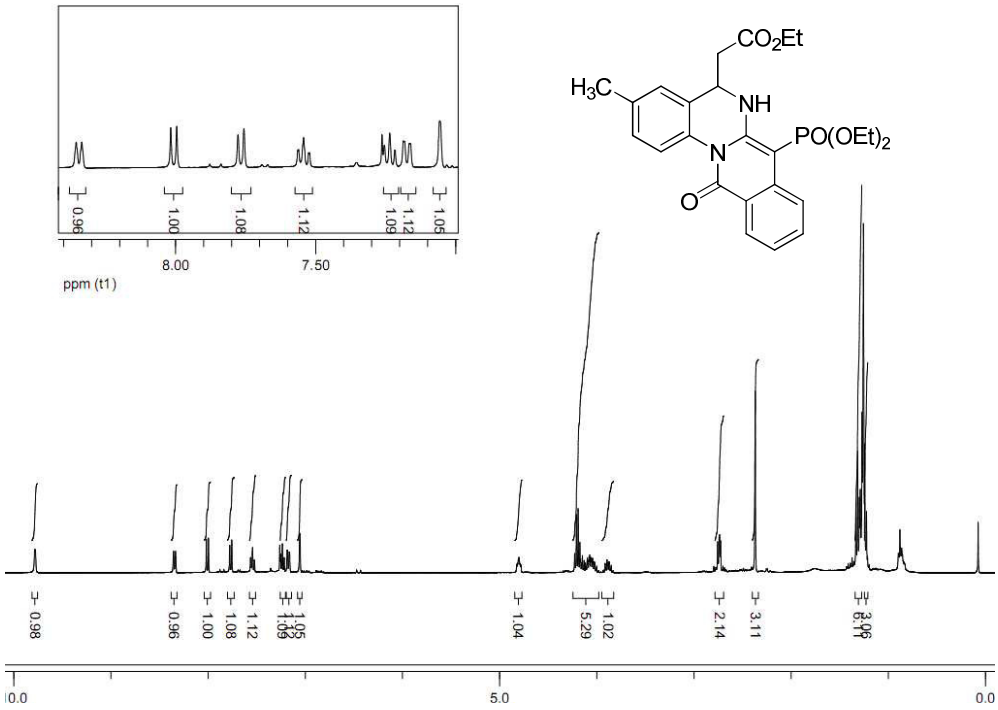
^{13}C NMR spectra of compound **9s** ($CDCl_3$, 100 MHz)



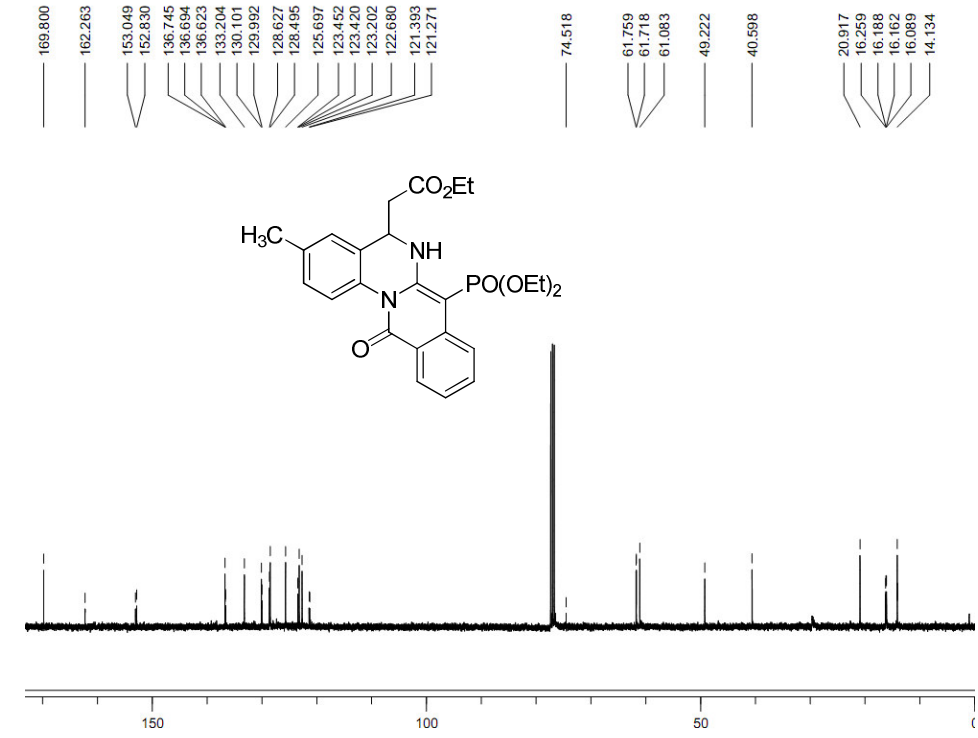
^{1}H NMR spectra of compound **9t** ($CDCl_3$, 400 MHz)



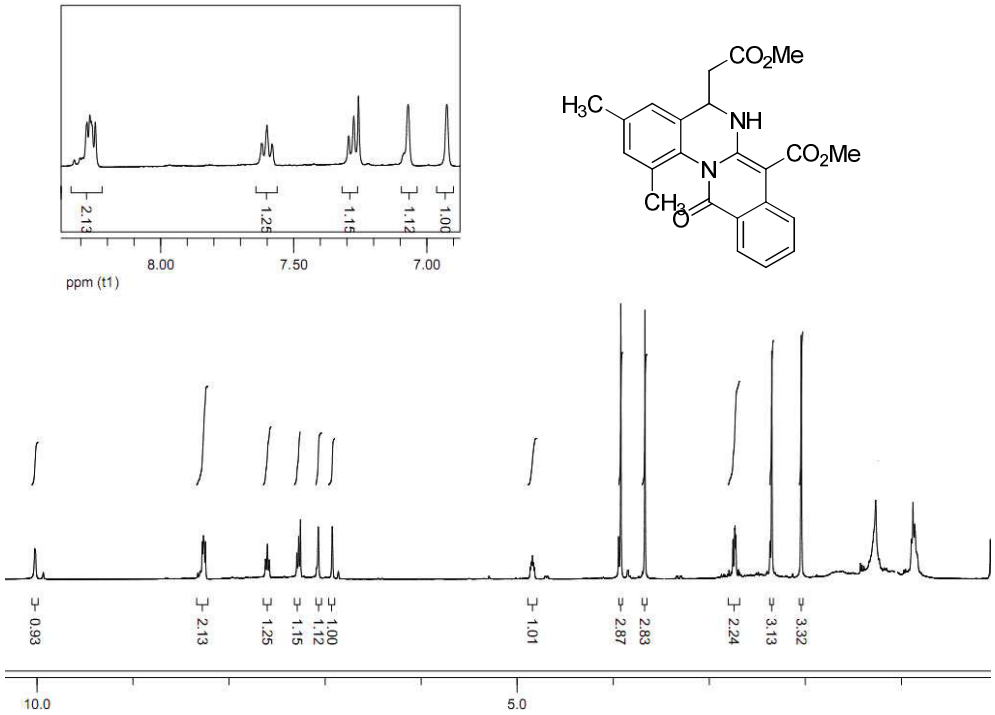
^{13}C NMR spectra of compound **9t** ($CDCl_3$, 100 MHz)



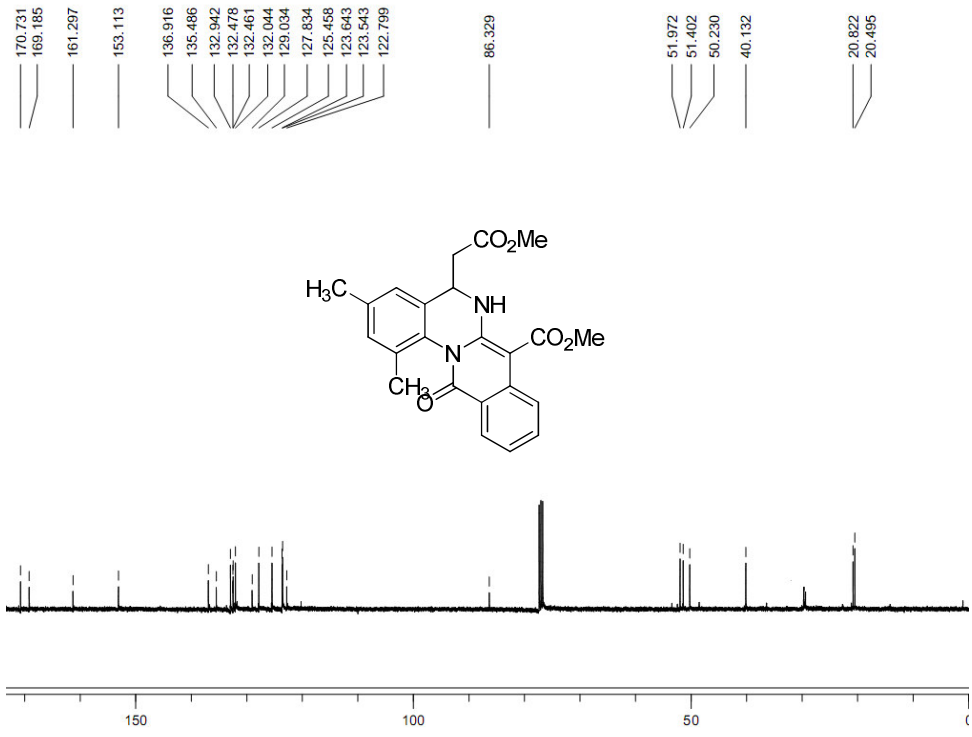
^{1}H NMR spectra of compound **9v** ($CDCl_3$, 400 MHz)



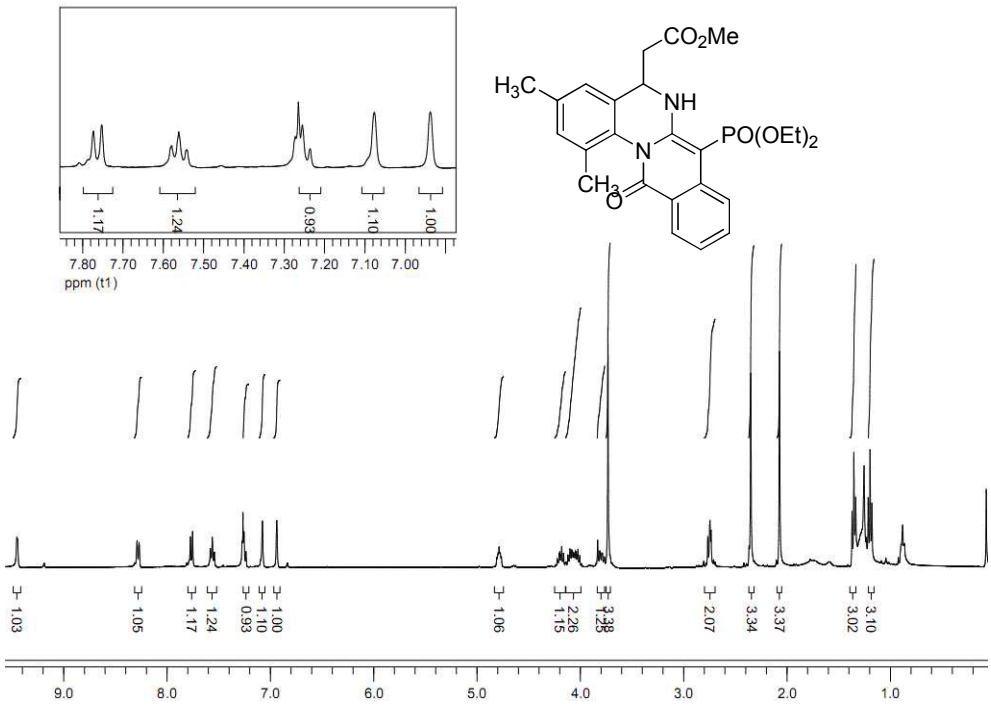
^{13}C NMR spectra of compound **9v** ($CDCl_3$, 100 MHz)



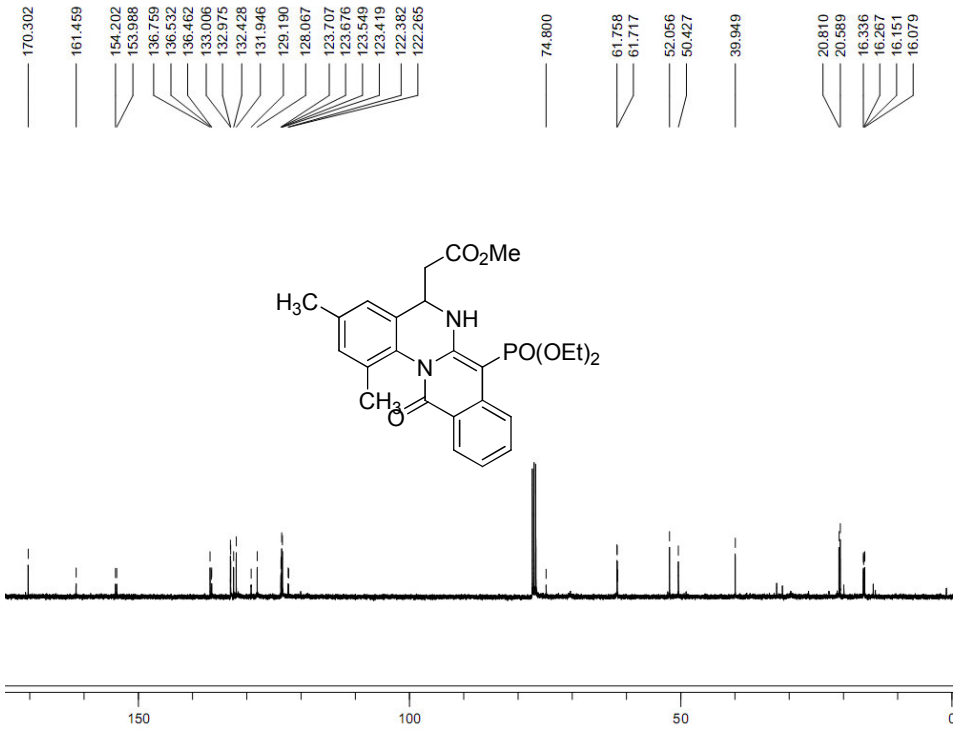
^{1}H NMR spectra of compound **9x** ($CDCl_3$, 400 MHz)



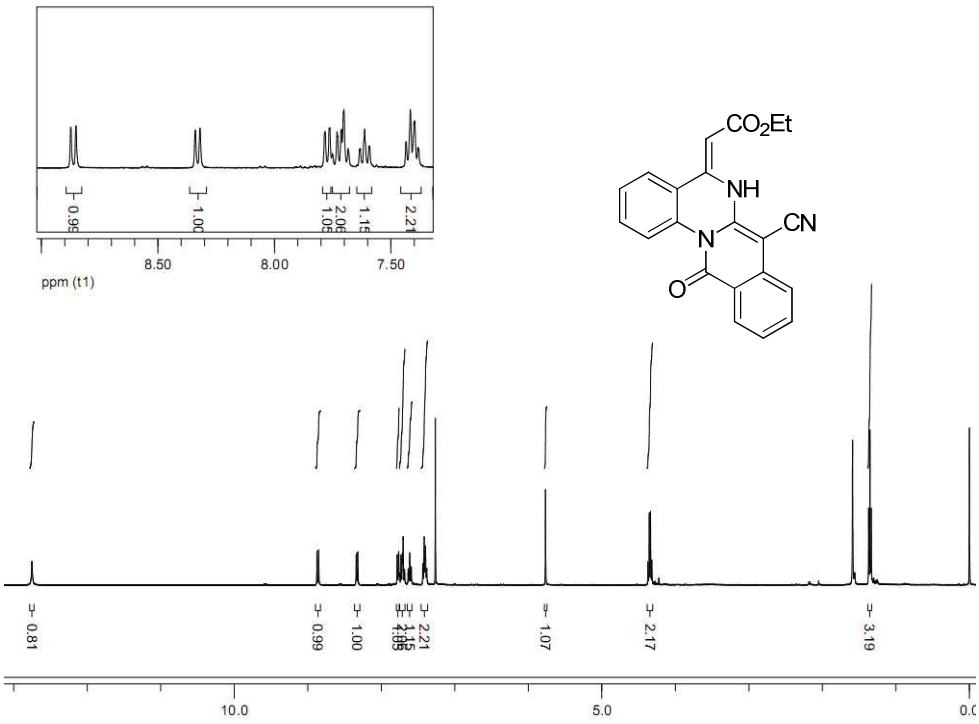
^{13}C NMR spectra of compound **9x** ($CDCl_3$, 100 MHz)



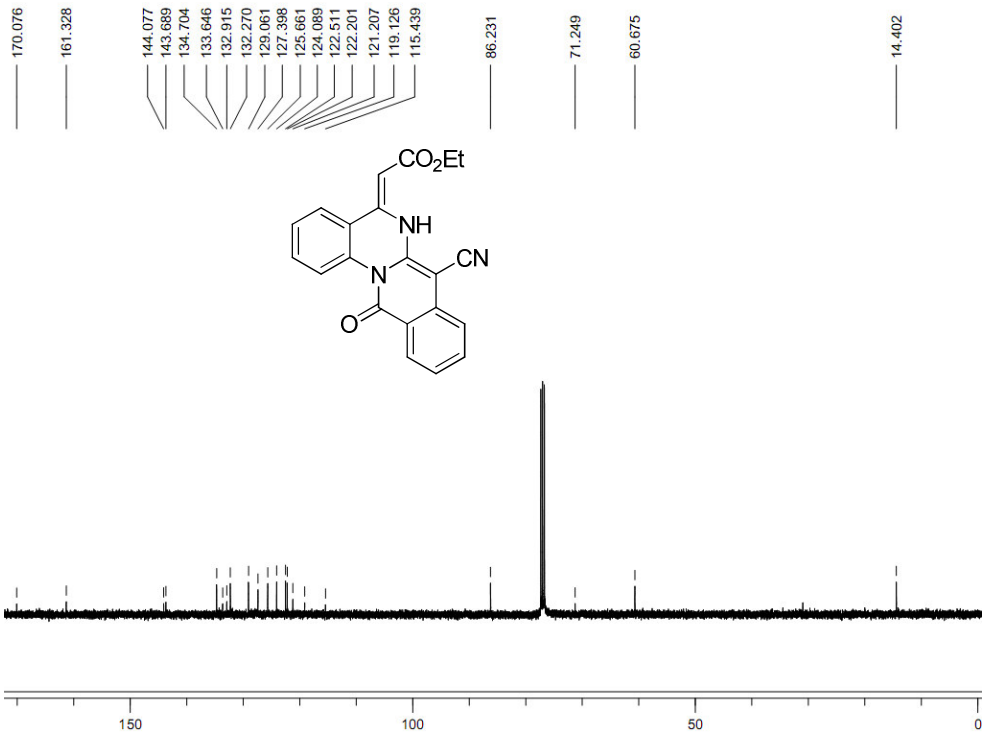
^{1}H NMR spectra of compound **9y** ($CDCl_3$, 400 MHz)



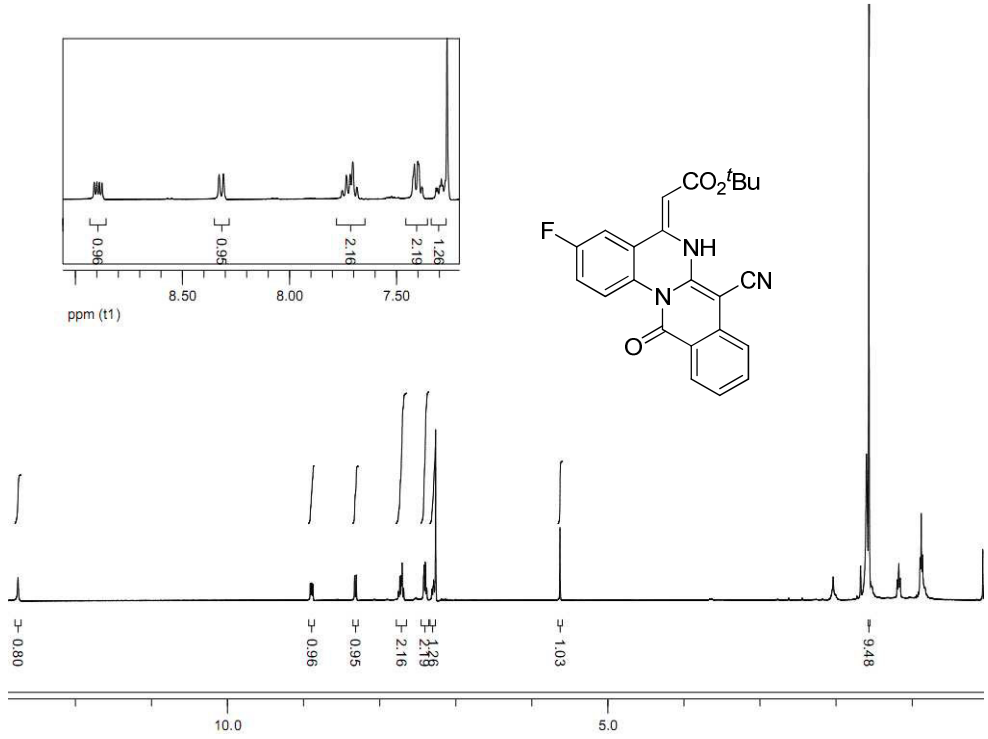
^{13}C NMR spectra of compound **9y** ($CDCl_3$, 100 MHz)



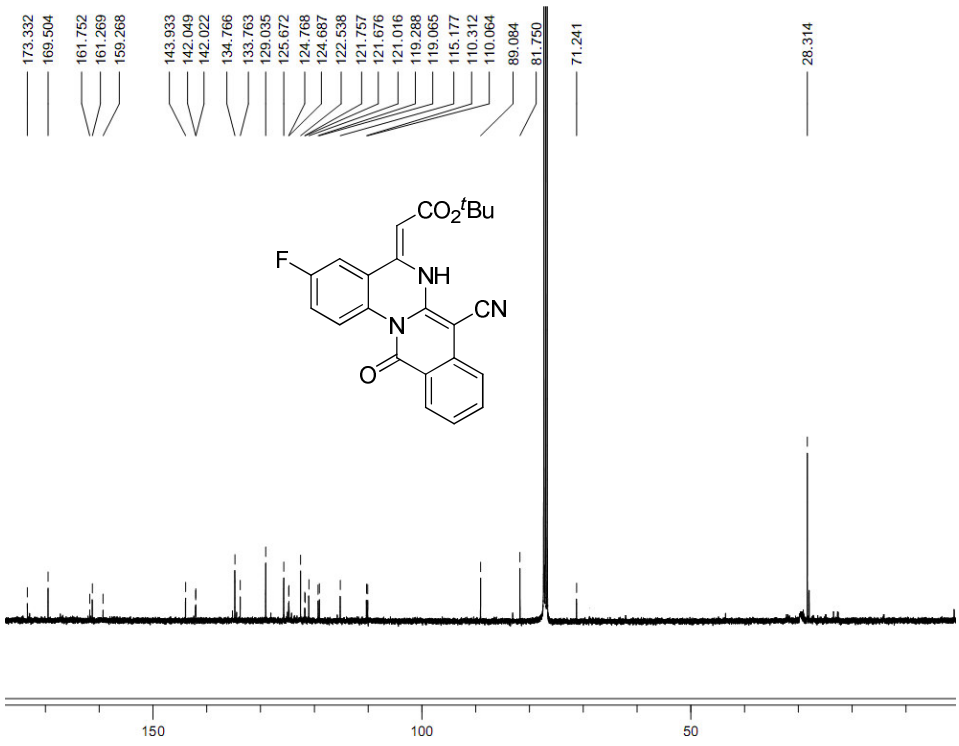
^{1}H NMR spectra of compound **10b** ($CDCl_3$, 400 MHz)



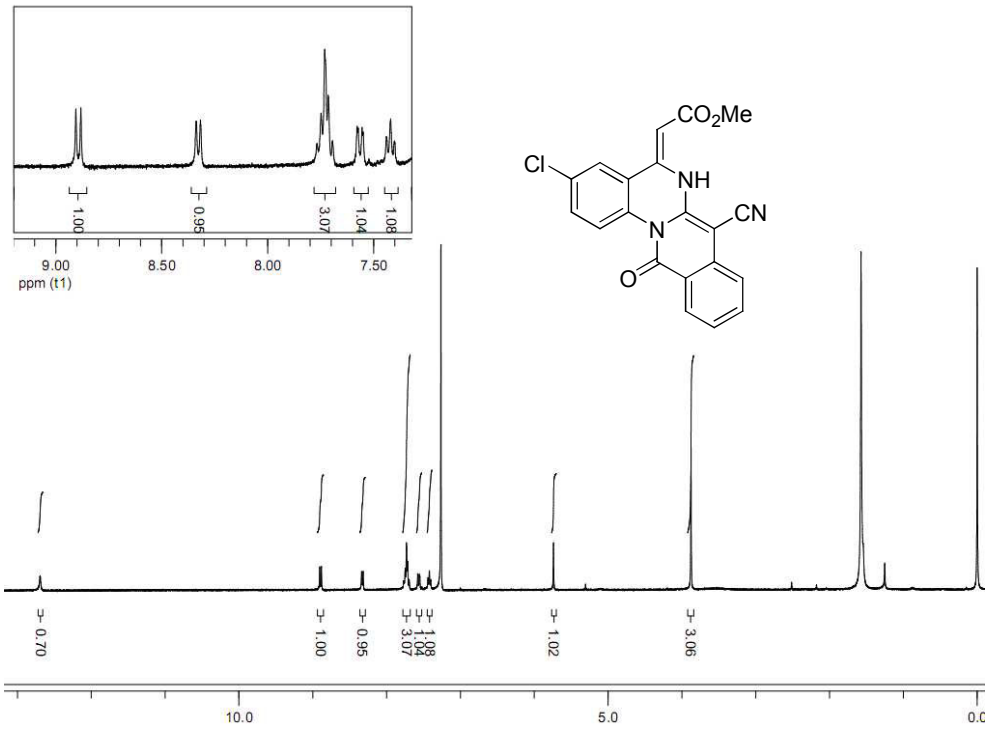
^{13}C NMR spectra of compound **10b** ($CDCl_3$, 100 MHz)



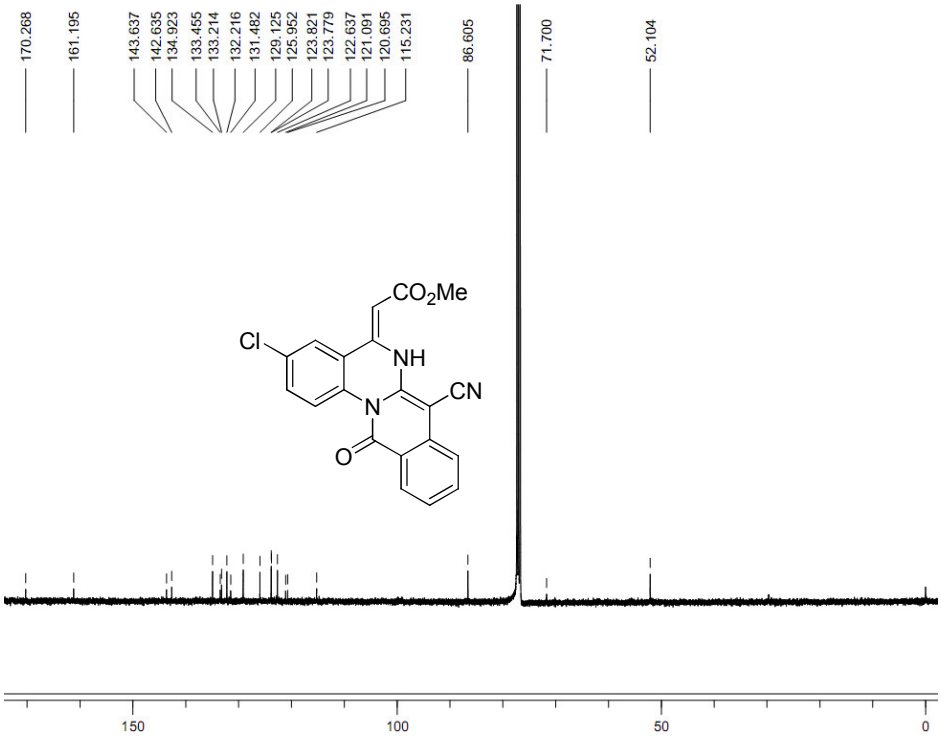
^{1}H NMR spectra of compound **10c** ($CDCl_3$, 400 MHz)



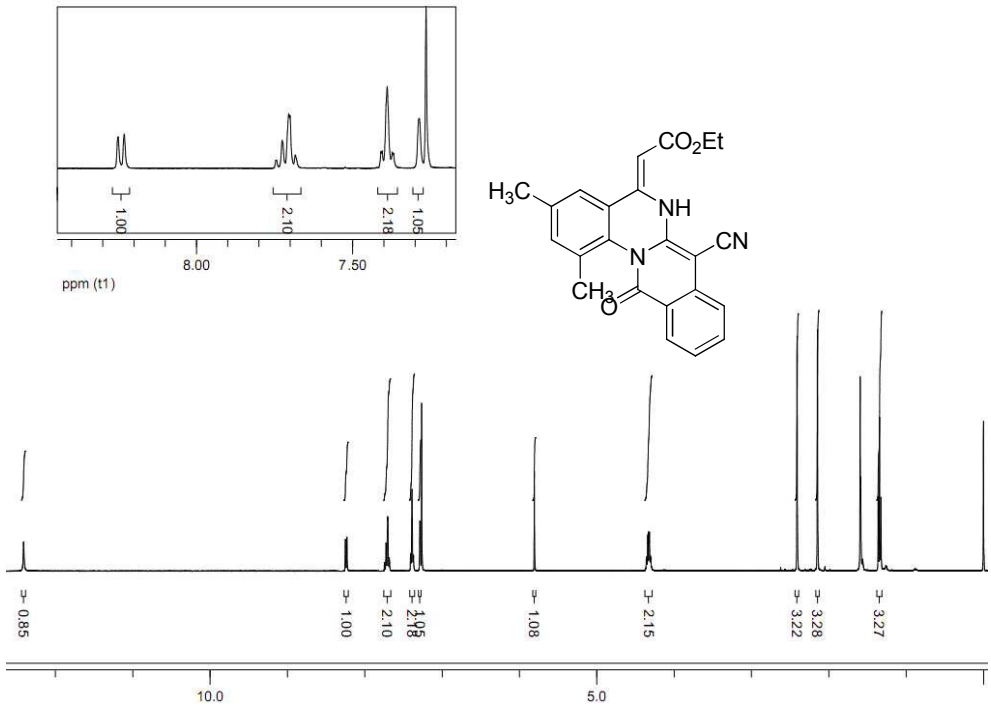
^{13}C NMR spectra of compound **10c** ($CDCl_3$, 100 MHz)



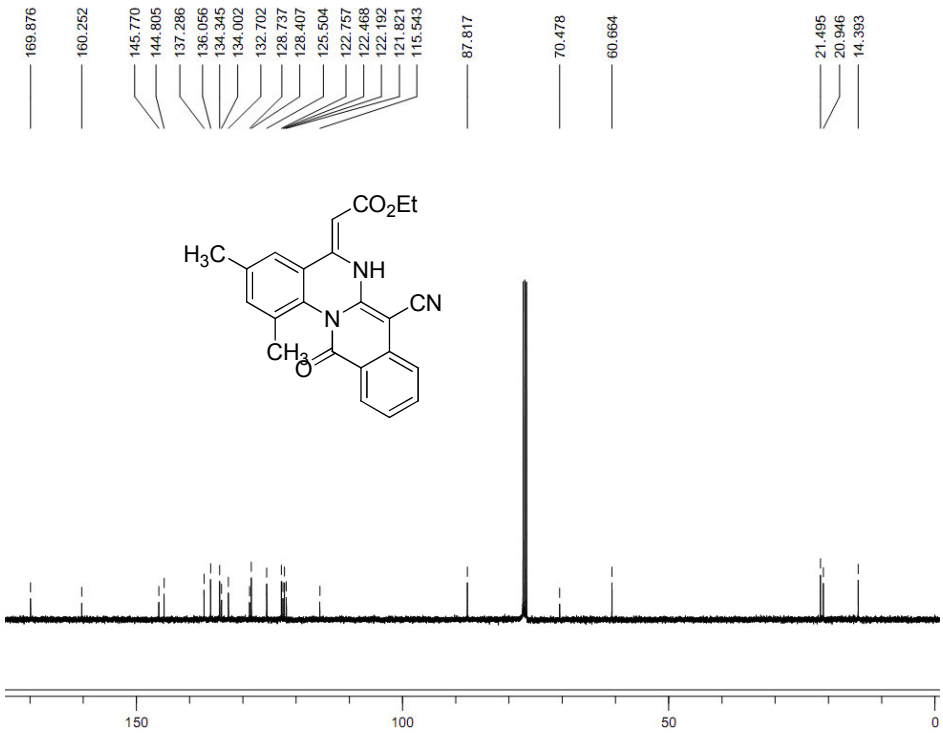
^{1}H NMR spectra of compound **10e** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **10e** ($CDCl_3$, 100 MHz)



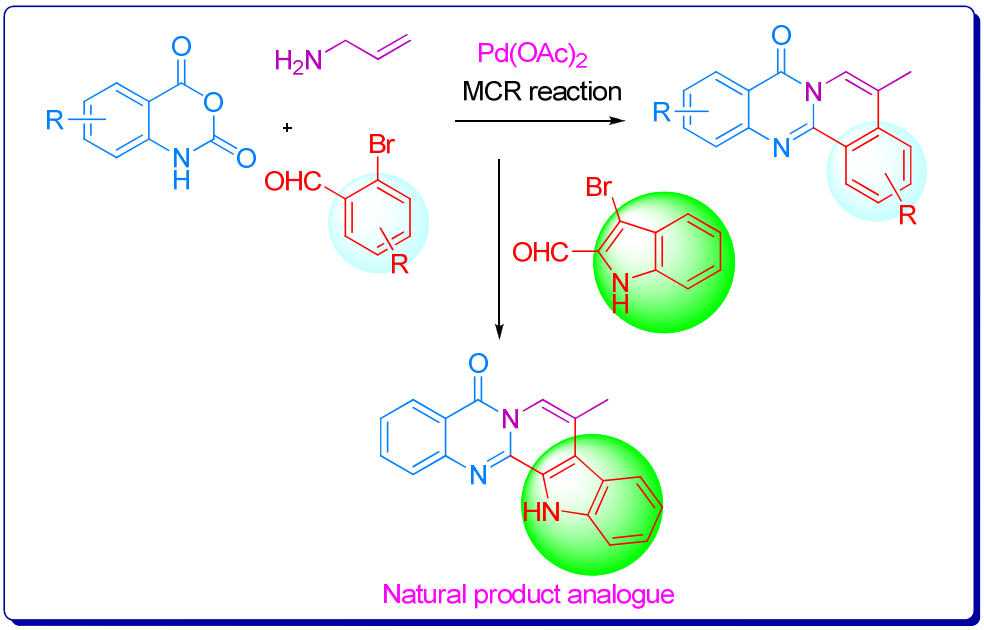
^{1}H NMR spectra of compound **10h** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **10h** ($CDCl_3$, 100 MHz)

CHAPTER 5

Palladium catalyzed synthesis of isoquinolino[1,2-b]quinazolinones and a methyl analogue of 7,8-dehydrorutaecarpine

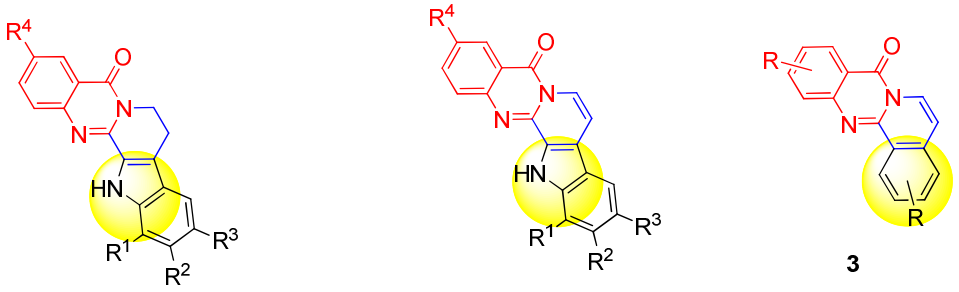


5.1. Introduction:

Multicomponent reactions (MCRs)[1] are defined as processes involving sequential reactions among three or more reactants in the same reaction mixture. MCRs allow simple and flexible assembly of three or more building blocks in user friendly one pot operations to form a product containing substantial elements of all the reactants. MCRs not only allow union of three or more starting materials in a single synthetic operation with high atom economy and bond-forming efficiency, but also avoid isolation and purification of any intermediates thereby minimizing waste, labor, and cost.[2] MCRs have become an extremely powerful tool in combinatorial chemistry and drug discovery, since it offers significant advantages over the conventional linear step syntheses. MCRs generally allow to promote new reactions and develop straightforward synthetic routes for bioactive heterocycles[3] and natural products.[1d] Transition metal-catalyzed MCRs[4] have attracted considerable attention due to the fact that complex organic molecules and drugs can be easily prepared from simple compounds in one reaction sequence. Pd-catalyzed reactions on the other hand allow creation of new C-Z (Z = C or N or O) bonds under milder conditions.[5] Thus development of new MCRs based on Pd catalyzed reactions[5b] is of high demand as these methodologies can easily construct pre-designed complex frameworks relevant to natural products access of which may be difficult or cumbersome *via* conventional multi-step methods.

Natural products containing *N*-heterocycles are attractive scaffolds in medicinal/pharmaceutical chemistry as well as in the early stage of drug discovery due to their wide range of remarkable pharmacological properties. For example, indolopyridoquinazolinone alkaloid rutaecarpine and its natural analogs (**1**-**2**, Figure 5.1) isolated from rutaceae (a tropical family of trees and shrubs) has been explored and evaluated as a potential agent in various therapeutic areas.[6] The reported synthesis of these natural products and its analogues are few and involved a lengthy and multistep process. Here we became interested in the structurally similar another framework *i.e.* isoquinolino[1,2-*b*]quinazolinone (**3**, Figure 5.1) as combination of isoquinoline and quinazoline framework proved potential anti inflammatory agents[7] as discussed in chapter 3 and 4. Notably, the reported synthesis of isoquinolino[1,2-*b*]quinazolinones are also few. We therefore decided to develop a new and general

route to construct the framework **3** extendable to the framework of **2**. The earlier reports for palladium catalyzed multicomponent reaction and synthesis of substituted isoquinolino[1,2-*b*]quinazolines are discussed below.



1a, $R^1 = R^2 = R^3 = R^4 = H$
Rutaecarpine
1b, $R^1 = R^2 = R^3 = H$, $R^4 = OCH_3$
Hortiacine
1c, $R^1 = R^2 = R^3 = OCH_3$, $R^4 = H$
Euxylophoricine A
1d, $R^2 = R^3 = OCH_2O$, $R^4 = R^1 = H$
Euxylophoricine C
1e, $R^2 = R^3 = R^4 = OCH_3$, $R^1 = H$
Euxylophoricine D

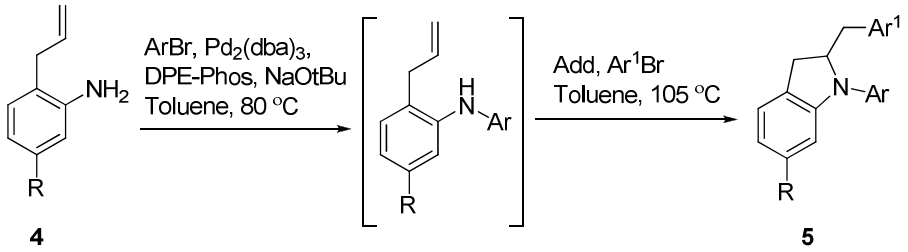
2a, $R^1 = R^2 = R^3 = R^4 = H$
7,8-dehydrorutaecarpine
2b, $R^2 = R^3 = OCH_3$, $R^4 = R^1 = H$
Euxylophoricine B
2c, $R^2 = R^3 = R^4 = OCH_3$, $R^1 = H$
Euxylophoricine E
2d, $R^2 = OH$, $R^3 = OCH^3$, $R^4 = R^1 = H$
Euxylophoricine F
2e, $R^2 = R^3 = R^4 = H$, $R^1 = OCH_3$
1-methoxy-7,8-dehydrorutaecarpine

Fig. 5.1: Biologically active rutaceae family alkaloids (**1** & **2**) and designed molecule (**3**).

5.2. Previous work:

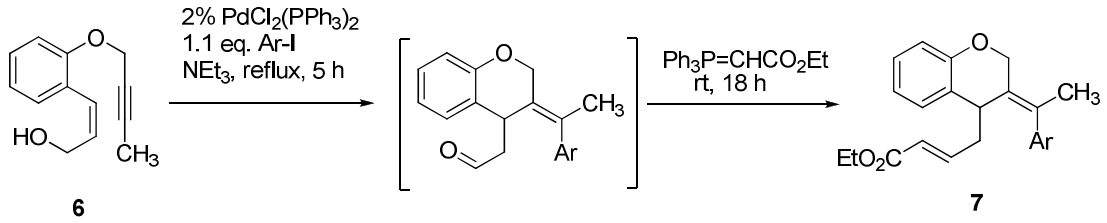
5.2.1. Palladium catalyzed multicomponent reactions *via* Heck type coupling:

In 2006, Wolfe and coworkers have invented a 3-CR with sequential *N*-arylation and carbopalladation starting with 2-allylanilines and two different aryl bromides. The *N*-arylation proceeds with formation of an alkene-coordinating aryl palladium complex, then, insertion of the olefin fragment followed by a reductive elimination to afford the indoline as shown in Scheme 5.1.[8]



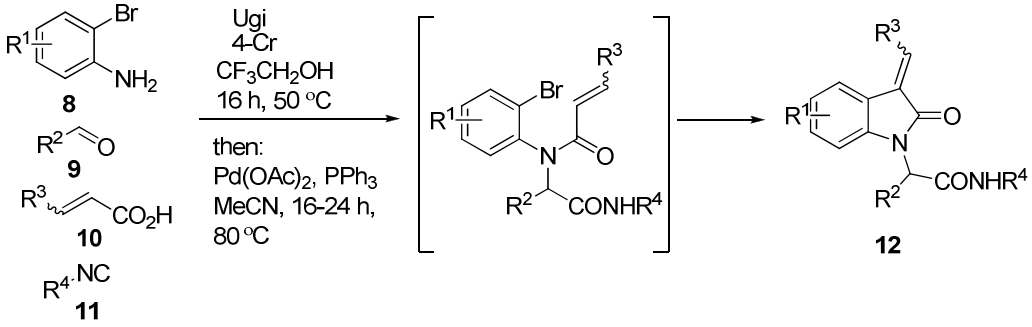
Scheme 5.1: Synthesis of *N*-Aryl-2-benzylindolines *via* tandem arylation of 2-allylaniline.

In 2004, Mullar and coworkers reported an intramolecular approach to an exo-methylene tetra hydro furan skeleton. The incorporation of yne allyl alcohol derivatives in a Heck type insertion/ cyclization cascade furnishes enols as elusive intermediates which rapidly undergo a keto–enol tautomerism yielding aliphatic aldehydes. These aldehydes were trapped by a subsequent Wittig olefination to provide a chromane derivative with an α,β-unsaturated ester as a side chain as shown in Scheme 5.2.[9]



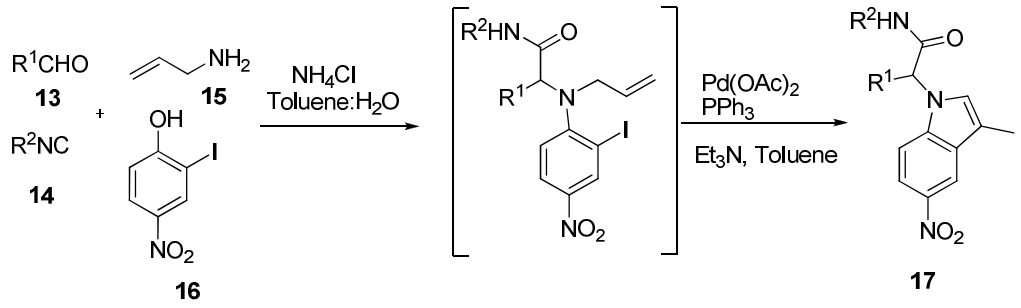
Scheme 5.2: Synthesis of tetra hydro furan skeleton *via* intramolecular Heck type cyclization followed by Wittig reaction.

In 2006, Umkehrer and coworkers reported a novel one-pot synthesis of highly substituted indol-2-ones using a combination of Ugi and Heck reaction (U-4-CR-Heck) as shown in Scheme 5.3. In this reaction, addition of palladium catalyst was at the end of Ugi reaction which was monitored by TLC.[10]



Scheme 5.3: Synthesis of indol-2-ones *via* Ugi-four-component-Heck reaction.

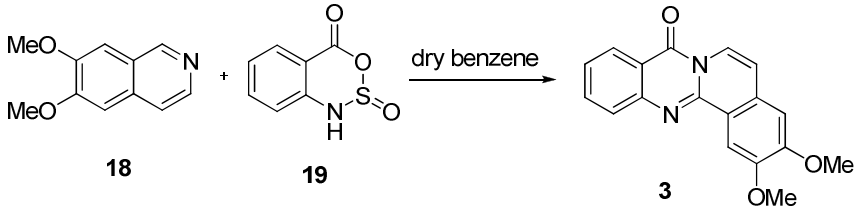
In 2008, Grimaud and coworkers reported the synthesis of indole scaffolds by using Ugi-Smiles reaction followed by Heck cyclization as shown in Scheme 5.4. The sequence can be performed in a one-pot reaction if the residual isocyanide is neutralized prior to the addition of the palladium catalyst.[11]



Scheme 5.4: Synthesis of substituted indole scaffold Heck cyclization.

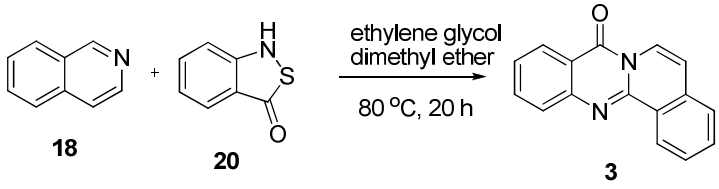
5.2.2. Earlier reports of isoquinolino[1,2-*b*]quinazoline:

In 1976, Kametani and coworkers reported the synthesis of 7,8-dihydro-2,3-dimethoxy-8-oxoisoquinolo[1,2-*b*]quinazoline from the cycloaddition between the iminoketene, derived from anhydride (**19**), and 6,7-dimethoxyisoquinoline followed by *in situ* dehydrogenation as shown in Scheme 5.5.[12]



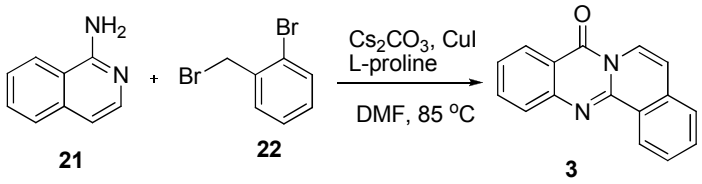
Scheme 5.5: Cycloaddition of isoquinoline and anhydride.

In 2000, Prager and coworkers described the synthesis of isoquinolo[1,2-*b*]quinazoline from the pyrolysis between isoquinoline and benzisothiazolinone as shown in Scheme 5.6.[13]



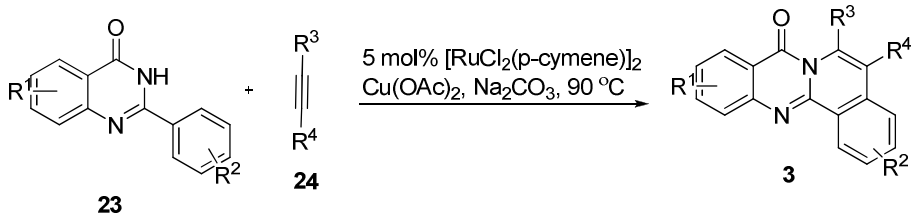
Scheme 5.6: Pyrolysis of isoquinoline and benisothiazolinone.

In 2011, Mondal and coworkers developed the synthesis isoquinolo[1,2-*b*]quinazoline from the reaction between isoquinolin-1-amine and *o*-bromo benzyl bromides using CuI/L-proline system which proceeds *via* nucleophilic aromatic substitution of the *N*-heteroaromatic cationic intermediate followed by *in situ* aerial oxidation at the benzylic position to the quinazolinone scaffold as shown in Scheme 5.7.[14]



Scheme 5.7: Copper catalyzed synthesis of isoquinolo[1,2-*b*]quinazoline.

In 2014, Peng and coworkers developed Ruthenium-catalyzed regioselective oxidative cross-coupling/annulations of quinazolones with alkynes for a direct access to the fused polycyclic heteroarenes as shown in Scheme 5.8.[15]



Scheme 5.8: Ruthenium catalyzed cross-coupling/annulations of quinazolones with alkynes.

5.3. Present work:

Synthesis of isoquinolino[1,2-*b*]quinazolinones are uncommon in the literature. We envisaged that isatoic anhydride and allyl amine could provide the precursor of quinazolin-4-one moiety *in situ* that on reaction with *o*-halo benzaldehyde could complete the construction of the fused isoquinolinone ring *via* an intramolecular heck reaction in the presence of palladium catalyst (Figure 5.2).

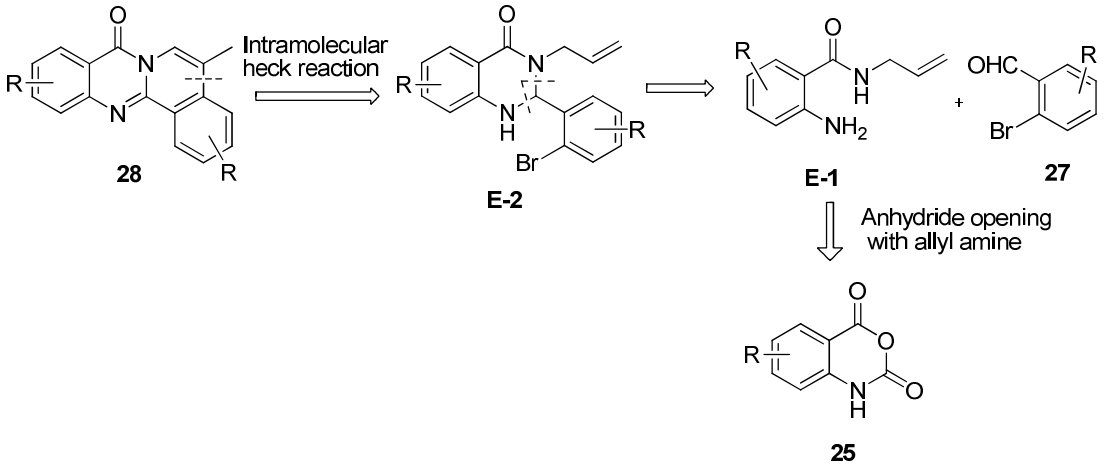
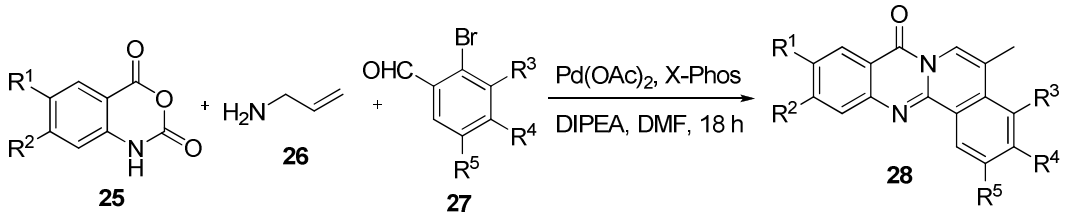


Fig. 5.2: Retro synthetic approach for the synthesis of isoquinolino[1,2-*b*]quinazolinone.

Indeed, this strategy worked well as the MCR of isatoic anhydrides (**25**), allyl amine (**26**) and *o*-bromo aryl aldehydes (**27**) in the presence of $Pd(OAc)_2$, ligand X-Phos to afford the desired isoquinolino[1,2-*b*]quinazolinones (**28**) smoothly (Scheme 5.9).



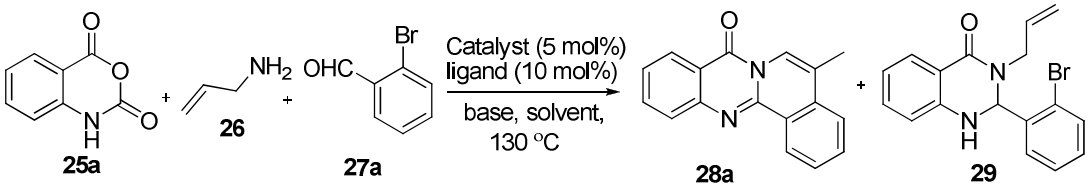
Scheme 5.9: Synthesis of **28** *via* palladium catalyzed intramolecular Heck reaction.

5.4. Results and discussion:

5.4.1. Reaction optimization:

Initially, the reaction of isatoic anhydride (**25a**), allyl amine (**26**) and 2-bromo benzaldehyde (**27a**) was examined under various conditions as shown in Table 5.1. Initially the use of catalyst $Pd(OAc)_2$ and DIPEA as a base in DMF provided desired product **28a** in poor yield along with intermediate **29** as a major product (entry 1, Table 5.1). Combination of $Pd(OAc)_2$ with ligands such as triphenyl phosphine, X-phos and bipyridine improved the yield of required product, although combination with X-phos provided the good yield of product compared to others (compare entry 3 with entry 2 & 4, Table 5.1). Replacing $Pd(OAc)_2$ with $Pd_2(dba)_3$, $Pd(PPh_3)_2Cl_2$ and $Pd(PPh_3)_4$ did not improve the yield of product (entry 5-7, Table 5.1). The use of additive also did not improve the yield of product (entry 8, Table 5.1). Replacing the base with triethyl amine decreased the yield (entry 9, Table 5.1) and inorganic bases like potassium carbonate and cesium carbonate also didn't improve the yield of product (entry 10-11, Table 5.1). Solvents like DMA, 1,4-dioxane and acetonitrile afforded the product in low yield compare to DMF (compare entry 3 with entry 12-14). The use of a lower quantity of Pd-catalyst decreased the product yield (entry 15, Table 5.1) and the MCR did not provide **28a** in the absence of $Pd(OAc)_2$ confirming the key role played by the catalyst (entry 16, Table 5.1). Overall, the combination of $Pd(OAc)_2$, X-phos and DIPEA in DMF was found to be optimum for this MCR reaction.

Table 5.1: Optimization of reaction conditions.[a]



25a 26 27a 28a 29

				Yield[b]	
Entry	Catalyst	Ligand/Additive	Base/Solvent	**28a**	**29**
1	$Pd(OAc)_2$	-	DIPEA/DMF	30	57
2	$Pd(OAc)_2$	PPh_3	DIPEA/DMF	55	21
3	$Pd(OAc)_2$	X-Phos	DIPEA/DMF	78	-
4	$Pd(OAc)_2$	2,2'-bipyridine	DIPEA/DMF	64	12
5	$Pd(dba)_3$	-	DIPEA/DMF	34	51
6	$Pd(PPh_3)_2Cl_2$	-	DIPEA/DMF	34	49
7	$Pd(PPh_3)_4$	-	DIPEA/DMF	31	49
8[c]	$Pd(OAc)_2$	$Cu(OAc)_2$	DIPEA/DMF	42	33
9	$Pd(OAc)_2$	X-Phos	Et_3N/DMF	45	29
10	$Pd(OAc)_2$	X-Phos	K_2CO_3/DMF	32	45
11	$Pd(OAc)_2$	X-Phos	Cs_2CO_3/DMF	35	44
12	$Pd(OAc)_2$	X-Phos	DIPEA/DMA	73	-
13[d]	$Pd(OAc)_2$	X-Phos	DIPEA/1,4-dioxane	45	34
14[e]	$Pd(OAc)_2$	X-Phos	DIPEA/CH_3CN	22	25
15[f]	$Pd(OAc)_2$	X-Phos	DIPEA/DMF	52	30
16[g]	-	-	DIPEA/DMF	-	20

[a]Reactions were carried out using **25a** (1 mmol), **26** (1 mmol), **27a** (1 mmol), 5 mol% catalyst, 10 mol% ligand and base (3 mmol) in solvent (2 mL) at 130 ºC for 18 h under anhydrous conditions. [b]Isolated yield. [c]1 equivalent additive used. [d]Reaction at 100 ºC. [e]Reaction at 80 ºC. [f]2.5 mol% catalyst and 5 mol% of ligand used. [g]Reaction performed without catalyst.

The compound **28a** was characterized by the use of methyl protons at δ 2.53 and allylic coupling (J = 1.2 Hz) was observed between methyl protons and aromatic C-H

(**A**, Figure 5.3). The NOE study indicated the proximity of methyl group with aromatic protons (**B**, Figure 5.3).

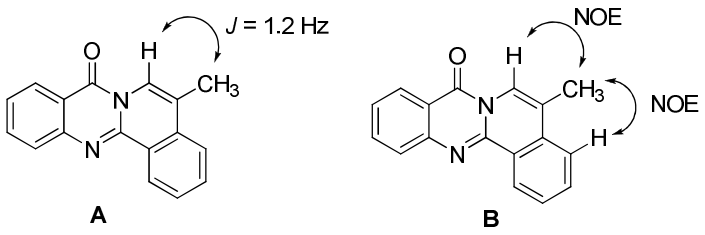
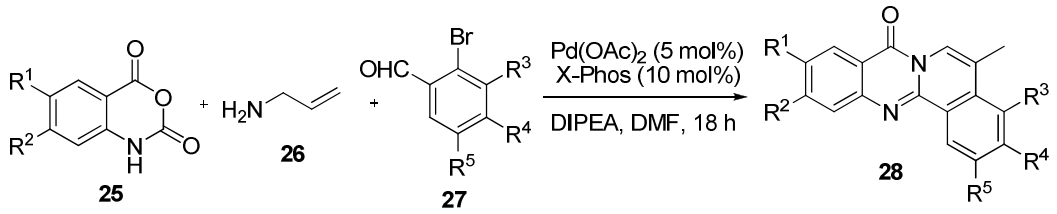


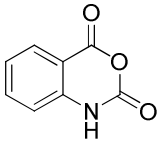
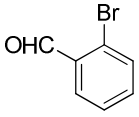
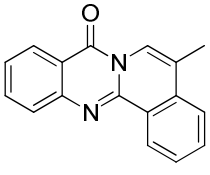
Fig. 5.3: Allylic coupling (A) and NOE correlations (B).

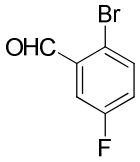
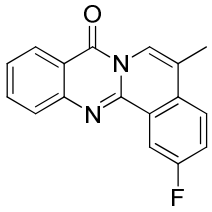
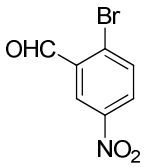
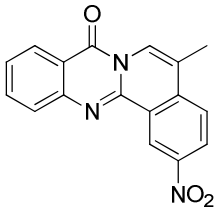
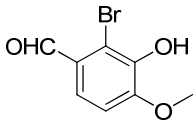
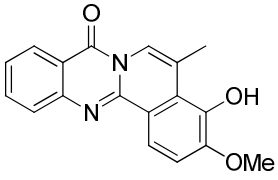
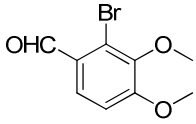
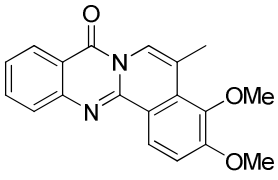
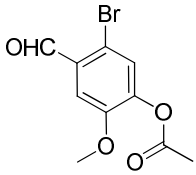
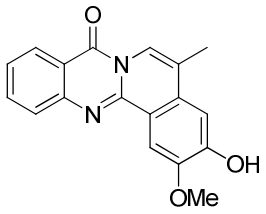
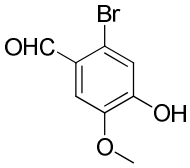
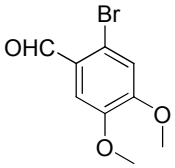
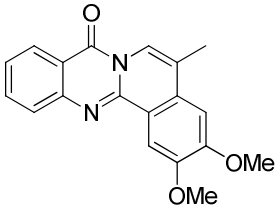
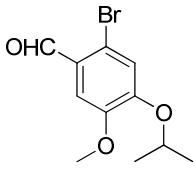
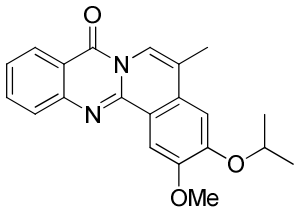
5.4.2. Scope of the reaction:

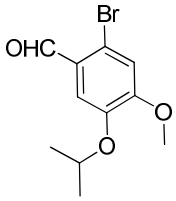
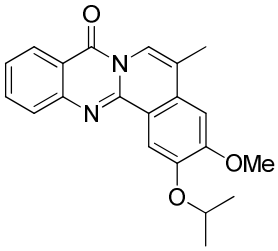
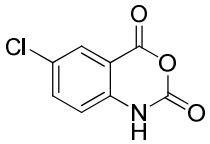
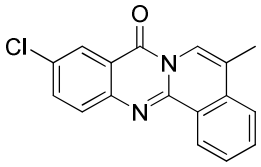
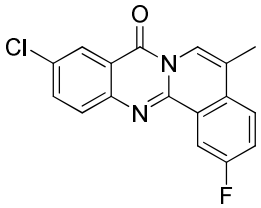
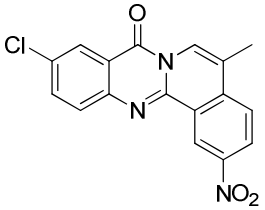
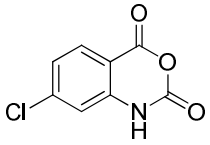
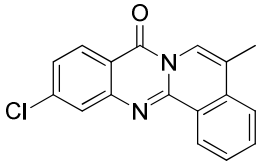
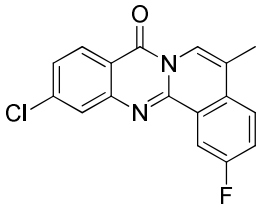
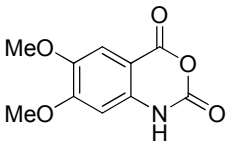
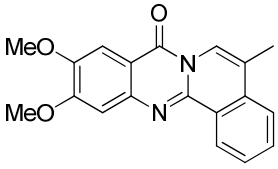
The reaction scope was then examined under the optimized conditions. A number of isatoic anhydrides (**25a-e**) and a range of 2-bromo aryl aldehydes (**27a-j**) were employed in the present MCR and results are summarized in Table 5.2. Electron donating groups such as OH, OMe, O^{i}Pr present on aldehyde provided good yields of products (entry 4-5 & 7-10, Table 5.2). The electron withdrawing group like NO_2 also participated well in this MCR reaction (entry 3 & 13, Table 5.2). Deprotection of *O*-acetyl group was observed (entry 6, Table 5.2) due to the presence of base. On the other hand, substituents on isatoic anhydride like chloro, dimethoxy provided good yields with all types of aldehydes irrespective of position of substituent (entry 11-17, Table 5.2). An electron withdrawing group *i.e.,* NO_2, present on isatoic anhydride was not favorable as yield of the product was only 45% in this case (entry 18, Table 5.2).

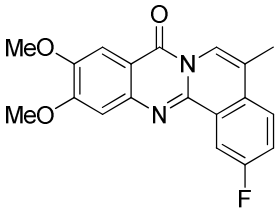
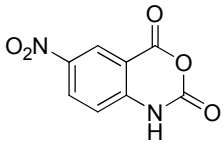
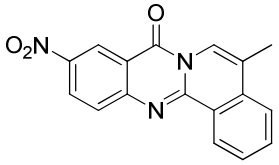
Table 5.2: Pd catalyzed synthesis of 5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (**28**)[a]



S. No	Isatoic anhydride (**1**)	Aldehyde (**3**)	Product (**4**)	Yield[b]
1	 **25a**	 **27a**	 **28a**	78

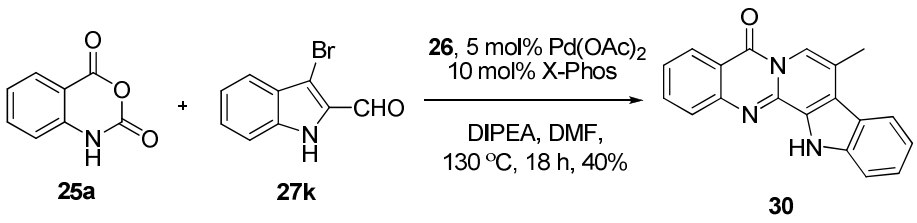
2	**25a**	 **27b**	 **28b**	69
3	**25a**	 **27c**	 **28c**	65
4	**25a**	 **27d**	 **28d**	70
5	**25a**	 **27e**	 **28e**	71
6	**25a**	 **27f**	 **28f**	62[c]
7	**25a**	 **27g**	**28f**	65
8	**25a**	 **27h**	 **28g**	70
9	**25a**	 **27i**		73

			28h	
10	**25a**	 **27j**	 **28i**	72
11	 **25b**	**27a**	 **28j**	79
12	**25b**	**27b**	 **28k**	64
13	**25b**	**27c**	 **28l**	64
14	 **25c**	**27a**	 **28m**	75
15	**25c**	**27b**	 **28n**	62
16	 **25d**	**27a**	 **28o**	66

17	**25d**	**27b**	 **28p**	60
18	 **25e**	**27a**	 **28q**	45

[a]All the reactions were carried out using **25** (1 mmol), **26** (1 mmol), **27** (1 mmol), 5 mol% $Pd(OAc)_2$, 10 mol% X-Phos and DIPEA (3 mmol) in DMF (2 mL) at 130 ºC for 18 h under anhydrous conditions. [b]Isolated yield. [c]Deacetylated product observed.

To expand the scope of this methodology further we extended this strategy successfully towards the synthesis of a natural product analogue *i.e.,* 8-methyl-7,8-dehydrorutaecarpine (**30**). We successfully achieved the direct synthesis of 8-methyl 7,8-dehydrorutaecarpine (**30**) by the reaction of isatoic anhydride (**25a**), allyl amine (**26**) and 3-bromo-1*H*-indole-2-carbaldehyde (**27k**) in a single synthetic operation (Scheme 5.10) indicating the potential of this methodology over the conventional multi-step sequences.

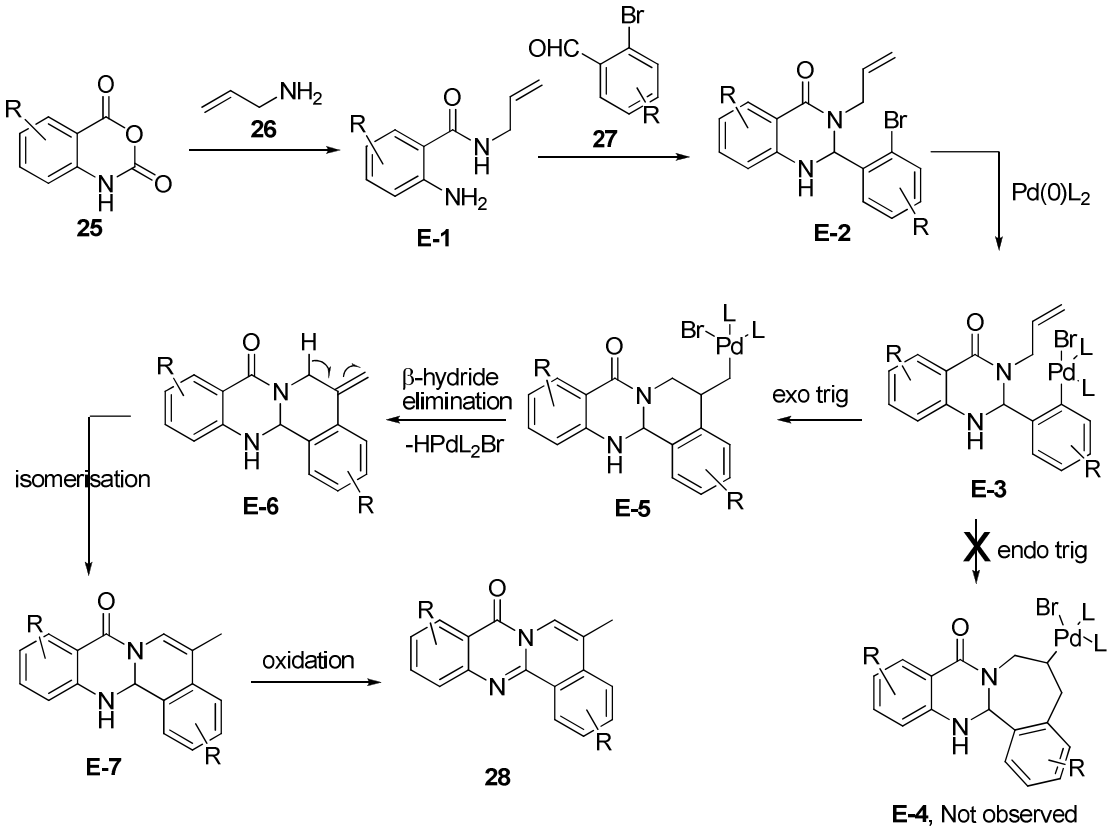


Scheme 5.10: Synthesis of 8-methyl 7,8-dehydrorutaecarpine (**30**)

5.4.3. Proposed mechanism:

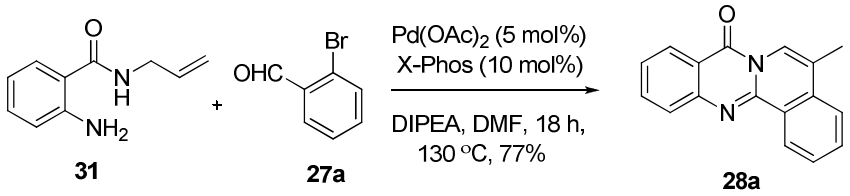
A plausible mechanism is proposed based on our observations (Scheme 5.11) for the present MCR. Mechanistically, the MCR reaction seems to proceed *via in situ* generation of *N*-allyl-2-aminobenzamide intermediate **E-1** from **25** and **26** followed by condensation with aldehyde to provide 2,3-dihydroquinazolin-4(1*H*)-one intermediate **E-3** (*cf* compound **29**, Table 5.1). The Pd(II) complex **E-3** formed *via* the insertion of Pd(0) to the bromoarene moiety, undergoes intramolecular Heck reaction

via an exo trig fashion to give **E-5**. The **E-5** intermediate formed contains a 6-membered ring. The alternative intermediate **E-4** was not formed *via* an endo trig fashion perhaps due to the unfavourable ring strain encountered by the 7-membered ring that would formed in the second case. Intermediate **E-5** undergoes β-hydride elimination to afford **E-6** followed by base mediated hydrogen isomerisation to give **E-7** which on aerial oxidation provided the desired product **28**.

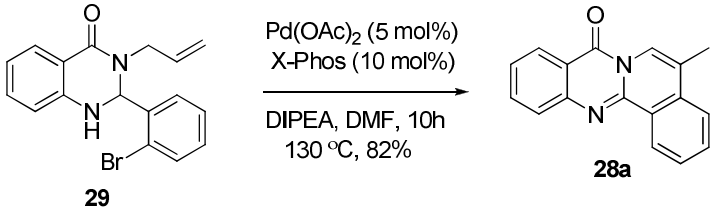


Scheme 5.11: Proposed mechanism.

To gain further evidence on the intermediacy of **E-1** and **E-2** we performed two individual reactions separately. The *N*-allyl-2-aminobenzamide **31**, prepared *via* the reaction of **25a** and **26** under heating condition, was treated with aldehyde **27a** under the condition of entry 3 of Table 1 for 18h when the desired product **28a** was isolated in 77% yield (Scheme 5.12). Similarly, the compound **29** afforded **28a** in 82% yield when treated with the Pd catalyst maintaining the condition of entry 3 of Table 1 for 10h (Scheme 5.13). These observations suggested that the reaction proceeded *via* **E-1** followed by **E-2**.



Scheme 5.12: The reaction of *N*-allyl-2-aminobenzamide **31** with aldehyde **27a**.



Scheme 5.13: Conversion of **29** to **28a** *via* intramolecular Heck reaction.

5.5. Conclusion:

In conclusion, a new multi component reaction has been developed for the synthesis of novel isoquinolino[1,2-*b*]quinazolinones *via* intramolecular palladium catalyzed heck reaction. This straightforward and operationally simple methodology does not require the monitoring or completion of the initial step before adding the Pd-catalyst. This strategy was extended successfully towards the synthesis of a methyl analog of 7,8-dehydrorutaecarpine avoiding multi step procedures. The methodology would find application in constructing library of molecules based on complex and fused *N*-heterocycles useful for medicinal/pharmaceutical chemistry.

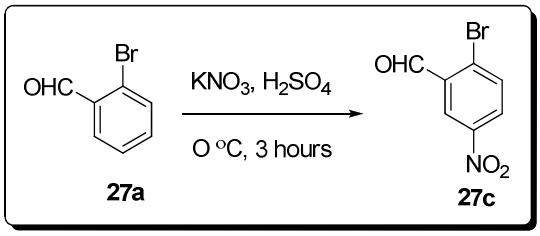
5.6. Experimental section:

5.6.1. 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 recorded 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 an 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 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.

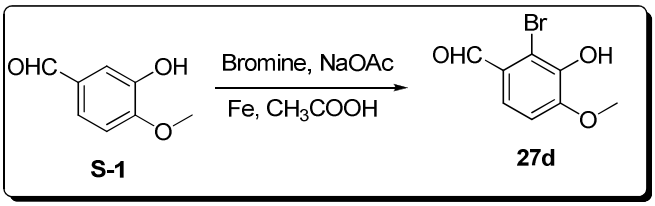
5.6.1.1. Typical procedure for preparation of 2-bromo-5-nitrobenzaldehyde (27c)[16]



Potassium nitrate (330 mg, 3.27 mmol) was slowly added to a stirred and chilled (ice bath) solution of 2-bromobenzaldehyde (0.5 g, 2.73 mmol) in sulfuric acid (6 mL) over 10 minutes. And the reaction mixture was stirred at 0 °C for 3 hours. Then the reaction mixture was poured into ice water, and the solid separated was filtered, washed with water, and dried to give the desired product **27c**.

Yield: 52% (325 mg); light yellow solid; mp: 102-104 (lit[17] 105-107 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.41 (s, 1H), 8.73 (d, *J* = 2.1 Hz, 1H), 8.34 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H).

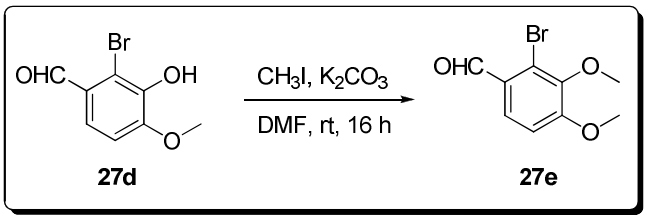
5.6.1.2. Typical procedure for the synthesis of 2-bromo-3-hydroxy-4-methoxybenzaldehyde (27d)[18]



Isovanillin (**S-1**) (2 g, 13.15 mmol) was dissolved in glacial acetic acid (10 ml). To this, was added anhydrous sodium acetate (2.15 g, 26.23 mmol), followed by powdered iron (0.05 g). Then, a solution of bromine (0.7 mL, 14.46 mmol) in glacial acetic acid (10 ml) was added over a period of 15 min under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 45 min. The reaction mixture was poured into an aqueous solution of 5% sodium bisulfite (50 mL) and stirred for 10 min. The precipitate was filtered washed with water (50 ml), and dried to give the desired product **27d**.

Yield: 60% (1.8 g); white solid; mp: 195-197 °C (lit[18] 200-202 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.26 (s, 1H), 7.58 (d, *J* = 8.55 Hz, 1H), 6.93 (d, *J* = 8.56 Hz, 1H), 6.10 (s, 1H), 4.01 (s, 3H).

5.6.1.3. Typical procedure for the synthesis of 2-bromo-3,4-dimethoxybenzaldehyde (27e)

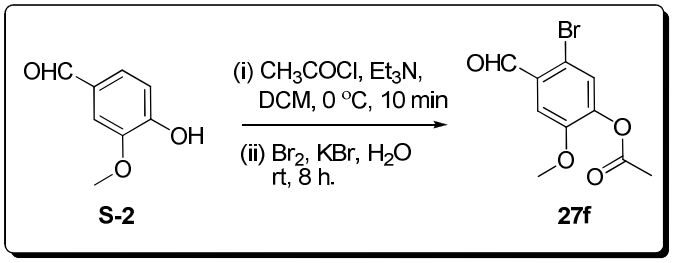


A mixture of compound **27d** (200 mg, 0.87 mmol), methyl iodide (0.06 mL, 1.04 mmol) and potassium carbonate (180 mg, 1.30 mmol) in DMF (5 mL) was stirred at room temperature for 16 hours. Upon completion of the reaction, the mixture was diluted with water (15 mL) and extracted with ethyl acetate (2 x 10 mL). The combined organic layer was washed with brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **27e.**

Yield: 78% (165 mg); white solid; mp: 81-83 °C (lit[19] 83-84 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.26 (s, 1H), 7.75 (d, *J* = 8.67 Hz, 1H), 6.96 (d, *J* = 8.69 Hz, 1H), 3.99 (s, 4H), 3.89 (s, 3H).

5.6.1.4. Typical procedure for the synthesis of 5-bromo-4-formyl-2-methoxyphenyl acetate (27f)[20]

To a mixture of vanillin (3 g, 19.73 mmol) and triethylamine (3.5 mL, 25.65 mmol) in DCM (50 mL), was added acetyl chloride (1.8 mL, 25.65 mmol) drop wise at 0 °C. The reaction mixture was stirred at 0 °C for 20 minutes, and then filtered. The solid separated was washed with methylene chloride (2 x 20 mL). The combined filtrate was washed with water (50 mL) followed by brine (50 mL), dried over anhydrous Na_2SO_4 and filtered. The organic layer was concentrated under reduced pressure to give vanillin acetate as a yellow solid.

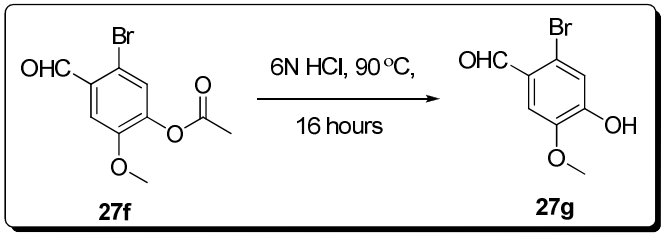


Yield: quantitative; mp: 72 – 74 °C (lit[21] 74-76 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.94 (s, 1H), 7.52-7.45 (m, 2H), 7.23 (d, *J* = 7.8 Hz, 1H), 3.92 (s, 3H), 2.36 (s, 3H).

To a solution of potassium bromide (5.5 g, 46.38 mmol) in water was added vanillin acetate (3 g, 15.46 mmol) and bromine (0.8 mL, 15.46 mmol), and the mixture was stirred at room temperature for 8 h. The precipitate was collected by filtration, washed with water, and dried to give desired compound **27f**.

Yield: 72% (3 g); white solid; mp 162-164 °C (lit[22] 164-165 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.28 (s, 1H), 7.50 (s, 1H), 7.36 (s, 1H), 3.90 (s, 3H), 2.35 (s, 3H).

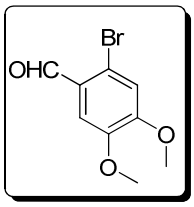
5.6.1.5. Typical procedure for the synthesis of 2-bromo-4-hydroxy-5-methoxybenzaldehyde (27g)[23]



To the compound **27f** (2.7 g, 10 mmol), was added 6 N hydrochloric acid (50 mL), and the mixture was stirred at 90 °C for 16 h. The precipitate was collected by filtration, and washed with saturated aqueous sodium hydrogen carbonate (50 mL) and water (50 mL). The obtained solid was dried to give the title compound **27g**.

Yield: 65% (1.4 g); white solid; mp: 176-178 °C (lit[24] 174-175 °C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.02 (s, 1H), 7.35 (s, 1H), 7.11 (s, 1H), 3.83 (s, 3H).

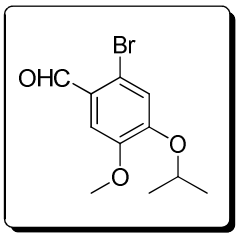
5.6.1.6. 2-bromo-4,5-dimethoxybenzaldehyde (27h)



Compound **27h** was synthesized from the reaction of **27g** and methyl iodide following a procedure similar to that of compound **27e**.

Yield: 65% (136 mg); white solid; mp: 145-147 °C (lit[25] 144-145 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.18 (s, 1H), 7.40 (s, 1H), 7.05 (s, 1H), 3.92 (s, 3H), 3.91 (s, 3H).

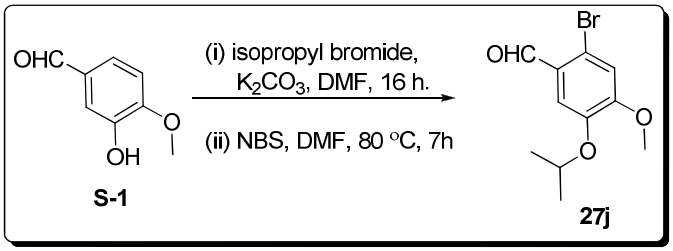
5.6.1.7. 2-bromo-4-isopropoxy-5-methoxybenzaldehyde (27i)



Compound **27i** was synthesized from the reaction of **27g** and isopropyl bromide following a procedure similar to that of compound **27e**.

Yield: 71% (167 mg); white solid; mp: 102-104 °C (lit[26] 105-107 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.17 (s, 1H), 7.41 (s, 1H), 7.04 (s, 1H), 4.68-4.62 (m, 1H), 3.93 (s, 3H), 1.42 (d, J = 6.0 Hz, 6H).

5.6.1.8. Typical procedure for the synthesis of 2-bromo-5-isopropoxy-4-methoxybenzaldehyde (27j)[27]



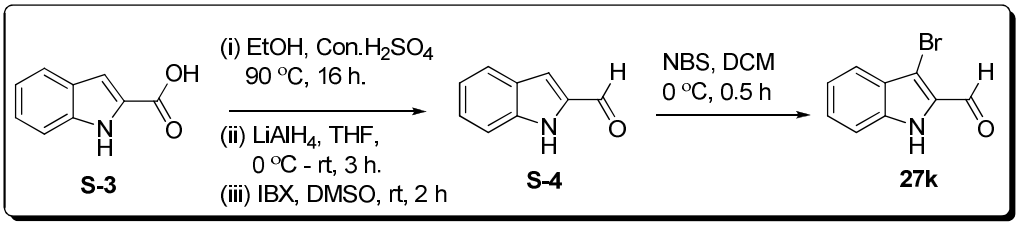
Step 1: A solution of isovanillin (500 mg, 3.28 mmol) in DMF (10 mL) was treated with K_2CO_3 (678 mg, 4.92 mmol) and isopropyl bromide (0.37 mL, 3.93 mmol). The resulting mixture was stirred at room temperature under a nitrogen atmosphere for 16 h. Then poured into H_2O (20 mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give 3-isopropoxy-4-methoxy benzaldehyde.

Yield: 82% (520 mg); light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.83 (s, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.43 (s, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 4.66-4.62 (m, 1H), 3.93 (s, 3H), 1.40 (d, *J* = 6.0 Hz, 6H).

Step 2: NBS (330 mg, 1.85 mmol) was added to a solution of 3-isopropoxy-4-methoxybenzaldehyde (300 mg, 1.54 mmol) in DMF (10 mL) at room temperature, and heated to 80 °C. After 7 h, the solution was cooled to room temperature, diluted with ethyl acetate (25 mL), washed with 10% $Na_2S_2O_5$ solution (30 mL), water (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **27j.**

Yield: 62% (260 mg); white solid; mp: 79-81 °C (lit[28] 78-79 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.12 (s, 1H), 7.42 (s, 1H), 7.04 (s, 1H), 4.63-4.60 (m, 1H), 3.93 (s, 3H), 1.38 (d, *J* = 6.0 Hz, 6H).

5.6.1.9. Typical procedure for the synthesis of 3-bromo-1H-indole-2-carbaldehyde (27k)[29]



Step 1: To the solution of indole 2-carboxylic acid (**S-3**) (500 mg, 3.10 mmol) in EtOH (5 mL), was added con. H_2SO_4 (0.5 mL), and the reaction mixture stirred at 90 °C for 16 hours. Then the reaction mixture was cooled to room temperature and the

solid separated was filtered and washed with H_2O to give ethyl indole 2-caboxylate as a light brown solid (550 mg, 95%).

Step 2: In a dry THF (10 mL), $LiAlH_4$ (200 mg, 5.29 mmol) was added slowly at 0 °C. To this was added a solution of ethyl indole-2-carboxylate (500 mg, 2.65 mmol) in THF (10 mL) drop wise. The reaction mixture was stirred at the same temperature for 1 h. After that the reaction mixture was quenched with H_2O (2 mL), 10% NaOH solution (2 mL), H_2O (3 mL) successively. Then the reaction mixture was extracted with EtOAc (2 x 20 mL). The combined organic layer was washed with water (20 mL) followed by brine solution (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the (1*H*-indol-2-yl)methanol as a light brown solid (342 mg, 88%).

Step 3: The obtained alcohol (340 mg, 2.31 mmol) was dissolved in DMSO (4 mL), and then IBX (971 mg, 3.46 mmol) was added. The reaction mixture was stirred for 2 hours at room temperature, and then diluted with cold water (20 mL). The mixture was extracted with ethyl acetate (2 x 15 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **S-4.**

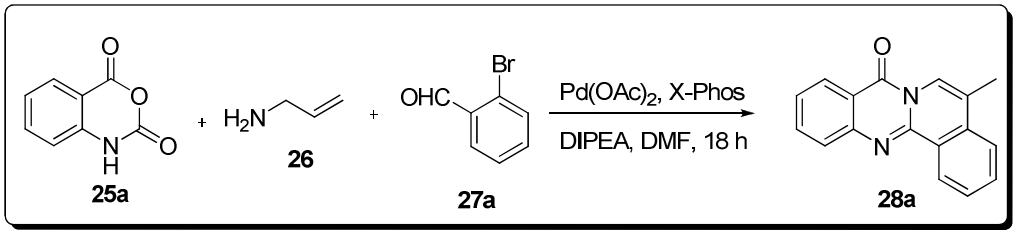
Yield: 62% (207 mg); white solid; mp: 136-138 °C (lit[29b] 138-140 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.89 (s, 1H), 9.68 (bs, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.49-7.50 (m, 1H), 7.41 (t, *J* = 8.1 Hz, 1H), 7.29 (s, 1H), 7.16-7.19 (m, 1H).

Step 4: To the solution of 1*H*-indole-2-carbaldehyde (**S-4**) (200 mg, 1.37 mmol) in DCM (10 mL), was added *N*-bromo succinimide (294 mg, 1.65 mmol) portion wise for about 10 minutes at 0 °C and the reaction mixture was stirred for 1 hour at the same temperature. Then the reaction mixture was diluted with water (20 mL) and extracted with DCM (2 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **27k**.

Yield: 55% (165 mg); white solid; mp: 172-174 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.00 (s, 1H), 9.30 (bs, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.49-7.42 (m, 2H), 7.30-7.21 (m, 1H).

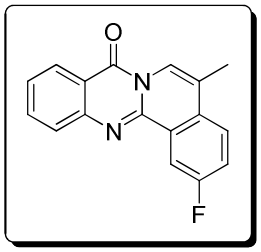
5.6.1.10. Typical procedure for preparation of 5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28a)

A mixture of isatoic anhydride (**25a**) (30 mg, 0.18 mmol), allyl amine (**26**) (10 mg, 0.18 mmol), 2-bromo benzaldehyde (**27a**) (33 mg, 0.18 mmol), DIPEA (0.94 mL, 0.54 mmol), $Pd(OAc)_2$ (2 mg, 0.009 mmol) and X-Phos (8 mg, 0.018 mmol) in DMF (2 mL) was heated to 130 °C under anhydrous conditions for 18 hours. After completion of the reaction, reaction mixture was cooled to room temperature, diluted with ethyl acetate (15 mL) and passed through the celite. The resulting solution was washed with water (2 x 15 mL) followed by brine solution (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **28a**.



Yield: 78% (36 mg); white solid; mp: 203-205 °C; R_f = 0.4 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.19 (d, *J* = 7.9 Hz, 1H), 8.53 (d, *J* = 1.2 Hz, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.86 (t, *J* = 7.6 Hz, 1H), 7.82-7.74 (m, 2H), 7.70 (t, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 2.53 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.1, 146.1, 134.7, 133.9, 132.2, 128.2, 127.5, 127.2, 127.2 (2C), 125.6, 123.3 (2C), 119.7, 119.3, 117.6, 16.4; MS (ES mass): 260.6 (M+1); HPLC: 98.2%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 280 nm, retention time 14.2 min.

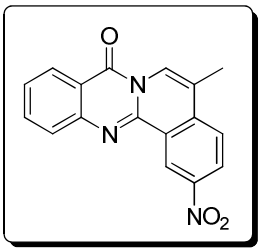
5.6.1.11. 2-fluoro-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28b)



Compound **28b** was synthesized from the reaction of **25a** and 2-bromo-5-fluorobenzaldehyde (**27b**) following a procedure similar to that of compound **28a**.

Yield: 69% (34 mg); light brown solid; mp: 196-198 °C; R_f = 0.4 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.81 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.48-8.46 (m, 2H), 7.94-7.83 (m, 2H), 7.76 (dd, *J* = 8.4, 5.2 Hz, 1H), 7.56-7.49 (m, 2H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.3 (C-F *J* = 235.6 Hz), 159.1, 147.2, 134.7, 130.4, 129.4, 127.5 (C-F *J* = 39.0 Hz), 125.9, 125.6 (C-F *J* = 8.3 Hz), 120.5 (C-F *J* = 23.2 Hz), 118.9, 118.8 (2C), 117.8, 113.2 (C-F *J* = 24.2 Hz), 109.9, 16.4; MS (ES mass): 279.0 (M+1); HPLC: 95.1%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 235 nm, retention time 15.2 min.

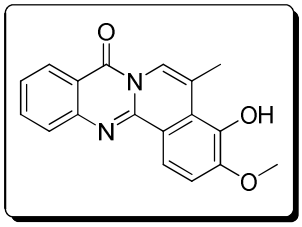
5.6.1.12. 5-methyl-2-nitro-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28c)



Compound **28c** was synthesized from the reaction of **25a** and 2-bromo-5-nitrobenzaldehyde (**27c**) following a procedure similar to that of compound **28a**.

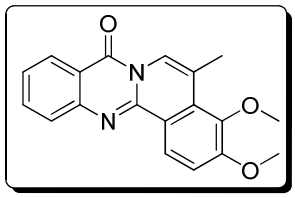
Yield: 65% (35 mg); brown solid; mp: 224-226 °C; R_f = 0.3 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.96 (d, *J* = 2.2 Hz, 1H), 8.63 (s, 1H), 8.56 (dd, *J* = 8.6, 2.2 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 7.98-7.88 (m, 3H), 7.58 (t, *J* = 7.6 Hz, 1H), 2.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.8, 146.9, 144.7, 138.4, 137.2, 135.2, 128.3, 127.8, 127.3, 126.6, 126.0, 124.8, 123.3, 122.9, 118.2, 118.0, 16.4; MS (ES mass): 305.9 (M+1); HPLC: 91.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.65 min.

5.6.1.13. 4-hydroxy-3-methoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28d)



Compound **28d** was synthesized from the reaction of **25a** and 2-bromo-3-hydroxy-4-methoxybenzaldehyde (**27d**) following a procedure similar to that of compound **28a**. Yield: 70% (38 mg); white solid; mp: 251-253 °C; R_f = 0.4 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78 (d, *J* = 8.5 Hz, 1H), 8.43 (d, *J* = 8.1 Hz, 1H), 8.30 (s, 1H), 7.84-7.78 (m, 2H), 7.45 (tb, *J* = 8.3, 2.0 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 6.19 (s, 1H), 4.07 (s, 3H), 2.74 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.1, 153.8, 148.7, 147.8, 146.0, 142.2, 139.7, 134.4, 127.5, 127.2, 125.1, 121.7, 119.9 (2C), 118.9, 110.9, 56.5, 20.9; MS (ES mass): 307.0 (M+1); HPLC: 97.9%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 315 nm, retention time 4.97 min.

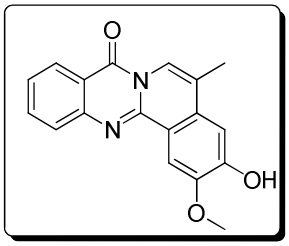
5.6.1.14. 3,4-dimethoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28e)



Compound **28e** was synthesized from the reaction of **25a** and 2-bromo-3,4-dimethoxybenzaldehyde (**27e**) following a procedure similar to that of compound **28a**. Yield: 71% (40 mg); light brown solid; mp: 176-178 °C; R_f = 0.1 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.00 (d, *J* = 8.5 Hz, 1H), 8.44 (d, *J* = 7.9 Hz, 1H), 8.36 (s, 1H), 7.88-7.79 (m, 2H), 7.51-7.45 (m, 1H), 7.30 (d, *J* = 8.6 Hz, 1H), 4.06 (s, 3H), 3.94 (s, 3H), 2.72 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.0, 156.1, 147.8, 145.9, 145.0, 134.5, 128.4, 127.2, 125.1, 124.6, 121.5, 120.4, 119.1, 117.4, 112.9, 109.9, 61.4, 56.1, 20.5; MS (ES mass): 321.0 (M+1); HPLC: 99.3%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 290 nm, retention time 14.0 min.

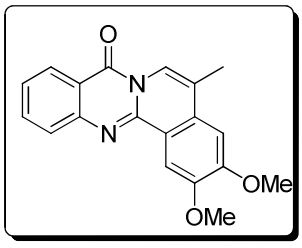
5.6.1.15. 3-hydroxy-2-methoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28f)

Compound **28f** was synthesized from the reaction of **25a** and 2-bromo-4-hydroxy-5-methoxybenzaldehyde (**27g**) following a procedure similar to that of compound **28a**.



Yield: 65% (35 mg); white solid; mp: 205-207 °C; R_f = 0.4 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 8.49 (d, *J* = 1.1 Hz, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 7.89-7.80 (m, 2H), 7.46 (td, *J* = 8.0, 1.1 Hz, 1H), 7.26 (s, 1H), 6.27 (s, 1H), 4.19 (s, 3H), 2.47 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.8, 152.2, 149.3, 147.6, 145.6, 135.1, 129.4, 127.2, 127.0, 125.1, 119.6, 119.3, 117.7, 116.6, 108.8, 108.0, 56.1, 16.4; MS (ES mass): 307.0 (M+1); HPLC: 96.3%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 280 nm, retention time 11.0 min.

5.6.1.16. 2,3-dimethoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28g)

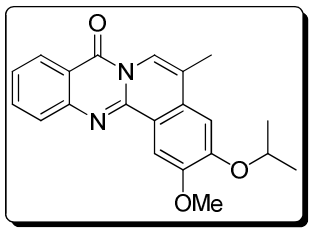


Compound **28g** was synthesized from the reaction of **25a** and 2-bromo-4,5-dimethoxybenzaldehyde (**27h**) following a procedure similar to that of compound **28a**.

Yield: 70% (40 mg); light yellow solid; mp: 246-248 °C; R_f = 0.1 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 8.52 (s, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.84 (t, *J* = 7.1 Hz, 1H), 7.47 (t, *J* = 7.0 Hz, 1H), 7.10 (s, 1H), 4.17 (s, 3H), 4.08 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:

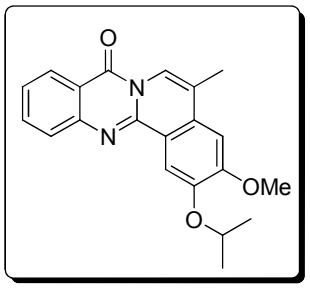
159.3, 153.1, 149.7, 147.7, 145.6, 134.4, 129.2, 127.2, 127.0, 124.8, 121.0, 119.2, 118.3, 116.9, 107.8, 103.8, 56.3, 56.1, 16.6; MS (ES mass): 321.0 (M+1); HPLC: 95.3%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 275 nm, retention time 12.0 min.

5.6.1.17. 3-isopropoxy-2-methoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28h)



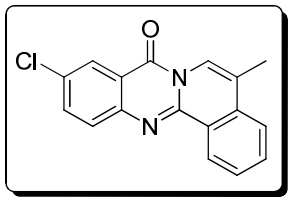
Compound **28h** was synthesized from the reaction of **25a** and 2-bromo-4-isopropoxy-5-methoxybenzaldehyde (**27i**) following a procedure similar to that of compound **28a**. Yield: 73% (45 mg); light brown solid; mp: 196-198 °C; R_f = 0.5 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (s, 1H), 8.49 (s, 1H), 8.45 (d, *J* = 7.90 Hz, 1H), 7.88 (t, *J* = 8.08 Hz, 1H), 7.86-7.79 (m, 1H), 7.45 (tb, *J* = 8.0, 1.2 Hz, 1H), 7.12 (s, 1H), 4.85-4.79 (m, 1H), 4.14 (s, 3H), 2.49 (s, 3H), 1.50 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.4, 151.6, 150.7, 147.8, 145.7, 134.4, 129.1, 127.2, 127.0, 124.8, 120.8, 119.2, 118.2, 116.9, 108.2, 106.7, 71.4, 56.3, 21.9 (2C), 16.6; MS (ES mass): 349.0 (M+1); HPLC: 97.6%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 280 nm, retention time 12.9 min.

5.6.1.18. 2-isopropoxy-3-methoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28i)



Compound **28i** was synthesized from the reaction of **25a** and 2-bromo-5-isopropoxy-4-methoxybenzaldehyde (**27j**) following a procedure similar to that of compound **28a**. Yield: 72% (45 mg); light yellow solid; mp: 177-179 °C; R_f = 0.5 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.50 (s, 1H), 8.45 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.82 (td, *J* = 7.4, 1.8 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.10 (s, 1H), 5.01-4.95 (m, 1H), 4.06 (s, 3H), 2.52 (s, 3H), 1.52 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.4, 154.2, 148.1, 147.8, 145.7, 134.4, 128.9, 127.2, 127.1, 124.8, 121.0, 119.2, 118.2, 116.9, 110.7, 104.3, 71.4, 56.1, 21.9 (2C), 16.6; MS (ES mass): 349.0 (M+1); HPLC: 94.3%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 280 nm, retention time 13.1 min.

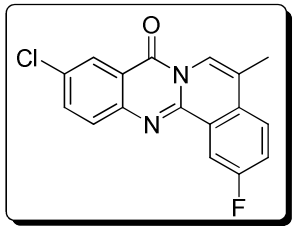
5.6.1.19. 10-chloro-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28j)



Compound **28j** was synthesized from the reaction of 5-chloro isatoic anhydride (**25b**) and **27a** following a procedure similar to that of compound **28a**.

Yield: 79% (35 mg); light yellow solid; mp: 217-219 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.13 (d, *J* = 8.2 Hz, 1H), 8.51 (s, 1H), 8.43 (d, *J* = 2.3 Hz, 1H), 7.87-7.75 (m, 4H), 7.70 (tb, *J* = 8.4, 1.8 Hz, 1H), 2.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.3, 148.3, 145.9, 135.1, 133.8, 132.3, 131.1, 129.1, 128.3, 127.5, 127.0, 126.3, 123.4, 120.2, 119.2, 118.5, 16.4; MS (ES mass): 294.9 (M+1); HPLC: 91.3%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 285 nm, retention time 6.73 min.

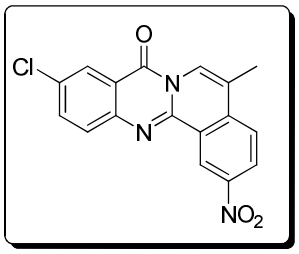
5.6.1.20. 10-chloro-2-fluoro-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28k)



Compound **28k** was synthesized from the reaction of **25b** and **27b** following a procedure similar to that of compound **28a**.

Yield: 64% (30 mg); white solid; mp: 224-226 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78 (dd, *J* = 8.8, 2.5 Hz, 1H), 8.47 (d, *J* = 0.9 Hz, 1H), 8.43 (d, *J* = 2.4 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.81-7.75 (m, 2H), 7.53 (tb, *J* = 8.1, 2.5 Hz, 1H), 2.53 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.5 (C-F *J* = 247.9 Hz), 158.1, 145.7, 135.2, 131.5, 130.4, 129.2, 126.3, 125.8 (C-F *J* = 8.2 Hz), 123.4, 120.7 (C-F *J* = 23.1 Hz), 119.5, 118.7 (C-F *J* = 13.5 Hz), 118.7, 113.3 (C-F *J* = 24.3 Hz), 111.0, 16.5; MS (ES mass): 312.9 (M+1); HPLC: 92.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 285 nm, retention time 6.86 min.

5.6.1.21. 10-chloro-5-methyl-2-nitro-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28l)

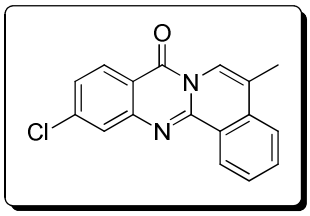


Compound **28l** was synthesized from the reaction of **25b** and **27c** following a procedure similar to that of compound **28a**.

Yield: 64% (32 mg); yellow solid; mp: 279-281 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.93 (d, *J* = 2.3 Hz, 1H), 8.62 (s, 1H), 8.59 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.44 (d, *J* = 2.2 Hz, 1H), 7.92 (dd, *J* = 8.0, 1.8 Hz, 2H), 7.85 (dd, *J* = 8.4, 2.2 Hz, 1H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.9, 147.2, 145.5, 139.3, 138.3, 135.7, 132.3, 129.5, 128.1, 126.5, 126.3, 124.9, 123.3, 122.8, 118.9, 118.8, 16.4; MS (ES mass): 339.9 (M+1); HPLC: 92.2%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN,

gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.16 min.

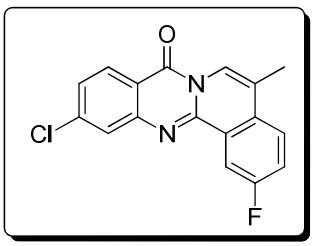
5.6.1.22. 11-chloro-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28m)



Compound **28m** was synthesized from the reaction of 4-chloro isatoic anhydride (**25c**) and **27a** following a procedure similar to that of compound **28a**.

Yield: 75% (33 mg); white solid; mp: 186-188 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.09 (d, *J* = 8.1 Hz, 1H), 8.48 (s, 1H), 8.36 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 1.7 Hz, 1H), 7.83-7.74 (m, 2H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.42 (dd, *J* = 8.4, 1.8 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.7, 148.3, 147.0, 140.8, 133.9, 132.5, 128.7, 128.3, 127.6, 126.9, 126.7, 126.2, 123.3, 120.1, 119.2, 116.0, 16.4; MS (ES mass): 294.9 (M+1); HPLC: 95.6%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 275 nm, retention time 15.6 min.

5.6.1.23. 11-chloro-2-fluoro-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28n)

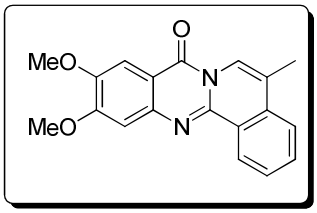


Compound **28n** was synthesized from the reaction of **25c** and **27b** following a procedure similar to that of compound **28a**.

Yield: 62% (29 mg); white solid; mp: 221-223 °C; R_f = 0.5 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (dd, *J* = 9.2, 2.1 Hz, 1H), 8.40 (s, 1H), 8.34 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 1.2 Hz, 1H), 7.73 (dd, *J* = 8.4, 5.1 Hz, 1H), 7.50 (td, *J* = 8.4, 2.2 Hz, 1H), 7.42 (dd, *J* = 8.4, 1.4 Hz, 1H), 2.49 (s, 3H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ: 163.4 (C-F J = 246.9 Hz), 158.5, 148.0, 146.2, 140.9, 130.6, 129.1 (C-F J = 5.8 Hz), 128.7, 126.8 (C-F J = 27.0 Hz), 125.7, 125.6, 120.9 (C-F J = 23.3 Hz), 119.4, 118.7, 116.1, 113.3 (C-F J = 23.7 Hz), 16.5; MS (ES mass): 312.9 (M+1); HPLC: 99.5%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 285 nm, retention time 15.8 min.

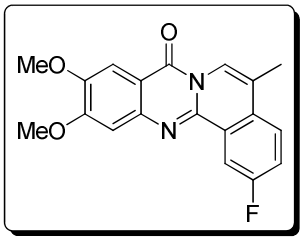
5.6.1.24. 10,11-dimethoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28o)



Compound **28o** was synthesized from the reaction of 4,5-dimethoxy isatoic anhydride (**25d**) and **27a** following a procedure similar to that of compound **28a**.

Yield: 66% (28 mg); light brown solid; mp: 184-186 °C; R_f = 0.1 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.11 (d, J = 7.8 Hz, 1H), 8.56 (s, 1H), 7.80-7.78 (m, 2H), 7.76 (s, 1H), 7.72-7.65 (m, 1H), 7.30 (s, 1H), 4.09 (s, 3H), 4.06 (s, 3H), 2.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.3, 155.7, 148.7, 145.2, 144.2, 133.5, 131.7, 128.1, 127.2, 126.9, 123.3, 119.5, 119.4, 111.2, 107.3, 105.5, 56.4 (2C), 16.4; MS (ES mass): 321.0 (M+1); HPLC: 99.1%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 285 nm, retention time 13.4 min.

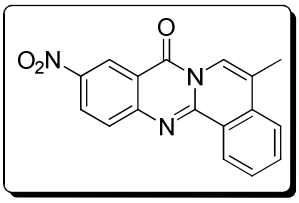
5.6.1.25. 2-fluoro-10,11-dimethoxy-5-methyl-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28p)



Compound **28p** was synthesized from the reaction of **25d** and **27b** following a procedure similar to that of compound **28a**.

Yield: 60% (27 mg); light brown solid; mp: 209-211 °C; R_f = 0.3 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.73 (dd, *J* = 8.6, 2.5 Hz, 1H), 8.50 (s, 1H), 7.77-7.74 (m, 1H), 7.73 (s, 1H), 7.48 (tb, *J* = 8.0, 2.4 Hz, 1H), 7.27 (s, 1H), 4.09 (s, 3H), 4.06 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.4 (C-F *J* = 246.7 Hz), 158.0, 155.7, 148.9, 143.9, 130.1, 130.0, 129.3, 125.6 (C-F *J* = 8.1 Hz), 120.0 (C-F *J* = 23.2 Hz), 118.9 (C-F *J* = 27.0 Hz), 118.9, 112.7 (C-F *J* = 24.1 Hz), 111.3, 107.3, 105.4, 56.4, 56.3, 16.5; MS (ES mass): 339.0 (M+1); HPLC: 97.9%, column: X-Terra RP18 250 x 4.6 mm 5.0µm, mobile phase A: 0.1 % TFA 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 280 nm, retention time 14.1 min.

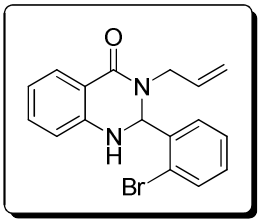
5.6.1.26. 5-methyl-10-nitro-8*H*-isoquinolino[1,2-*b*]quinazolin-8-one (28q)



Compound **28q** was synthesized from the reaction of 5-nitro isatoic anhydride (**25e**) and **27a** following a procedure similar to that of compound **28a**.

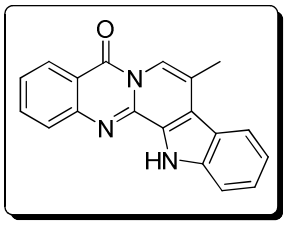
Yield: 45% (20 mg); brown solid; mp: 253-255 °C; R_f = 0.4 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.35 (d, *J* = 2.3 Hz, 1H), 9.20 (d, *J* = 8.4 Hz, 1H), 8.59 (dd, *J* = 8.6, 2.1 Hz, 1H), 8.56 (d, *J* = 0.9 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 1H), 7.90-7.84 (m, 2H), 7.76 (td, *J* = 8.1, 1.5 Hz, 1H), 2.58 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.5, 151.3, 144.2, 134.6, 133.4, 128.9, 128.7, 128.4, 128.1, 126.7, 124.6, 123.6, 121.4, 119.1, 116.9, 115.4, 16.4; MS (ES mass): 306.0 (M+1); HPLC: 94.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 4/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.82 min.

5.6.1.27. 3-allyl-2-(2-bromophenyl)-2,3-dihydroquinazolin-4(1*H*)-one (29)



Colorless semi solid; R_f = 0.1 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 7.8 Hz, 1H), 7.59 (dd, *J* = 7.4, 1.0 Hz, 1H), 7.26-7.16 (m, 4H), 6.83 (t, *J* = 7.5 Hz, 1H), 6.51 (d, *J* = 7.8 Hz, 1H), 6.04 (d, *J* = 2.2 Hz, 1H), 5.91-5.81 (m, 1H), 5.32-5.22 (m, 2H), 5.12 (s, 1H), 5.03-4.95 (m, 1H), 3.21 (dd, *J* = 15.2, 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.2, 144.3, 137.6, 133.7, 133.5, 132.3, 130.4, 128.5, 127.9, 127.4, 121.9, 119.3, 118.2, 115.7, 114.6, 69.2, 46.7; MS (ES mass): 343.0 (M+1).

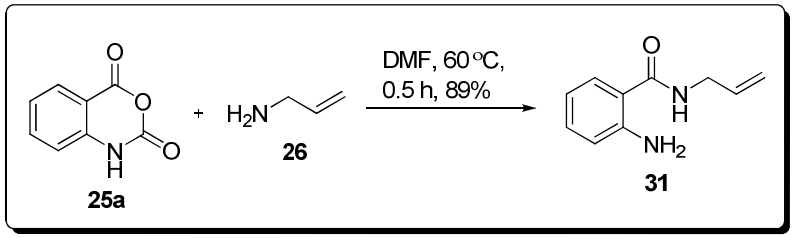
5.6.1.28. 8-methyl 7,8-dehydrorutaecarpine (30)



Compound **30** was synthesized from the reaction of **25a** and 3-bromo-1*H*-indole-2-carbaldehyde (**27k**) following a procedure similar to that of compound **28a**.

Yield: 40% (21 mg); light brown solid; mp: 285-287 °C; R_f = 0.2 (15% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.07 (bs, 1H), 8.56-8.48 (m, 2H), 8.17 (d, *J* = 8.2 Hz, 1H), 7.84-7.79 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.51 (t, *J* = 7.8 Hz,1H), 7.49-7.43 (m, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 2.85 (d, *J* = 1.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.0, 141.2, 134.6, 134.5, 132.9, 129.0, 127.6, 126.9, 124.5, 122.3, 121.7, 121.2, 120.0, 119.9, 119.6, 115.9, 112.2, 105.3, 17.8; MS (ES mass): 300.0 (M+1); HPLC: 94.1%, column: Symmetry C-18 75 x 4.6 mm 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 1/20, 6/98, 10/98, 12/20, 15/20; flow rate: 1.0 mL/min; UV 250 nm, retention time 6.08 min.

5.6.1.29. Typical procedure for the synthesis of *N*-allyl-2-aminobenzamide (31)[30]



A mixture of isatoic anhydride, **25a** (100 mg, 0.61 mmol) and allyl amine, **26** (34 mg, 0.61 mmol) in DMF (2 mL) was heated to 60 °C for 30 minutes. Then the reaction mixture was cooled to room temperature, diluted with water (10 mL), and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine solution (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate-hexane to give the desired product **31**.

Yield: 89% (95 mg); white solid; mp: 84-86 °C (lit[17] 83-84 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (d, *J* = 7.8 Hz, 1H), 7.24 (td, *J* = 8.0, 1.0 Hz, 1H), 6.70-6.64 (m, 2H), 6.12 (bs, 1H), 5.99-5.90 (m, 1H), 5.40 (bs, 2H), 5.28 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.20 (dd, *J* = 10.2, 1.2 Hz, 1H), 4.11-4.02 (m, 2H).

5.7. References:

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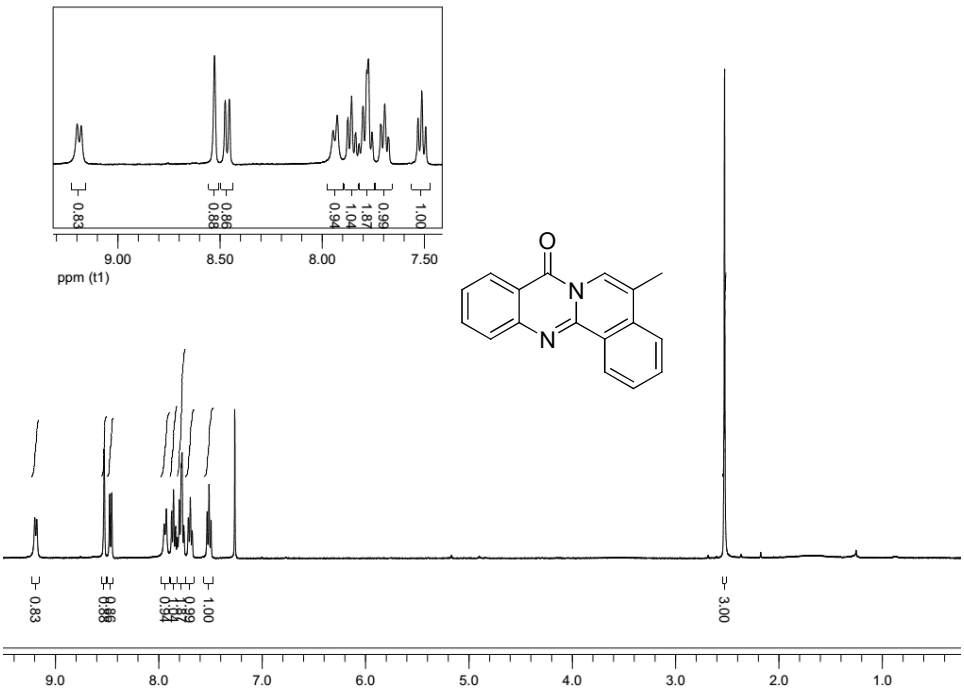
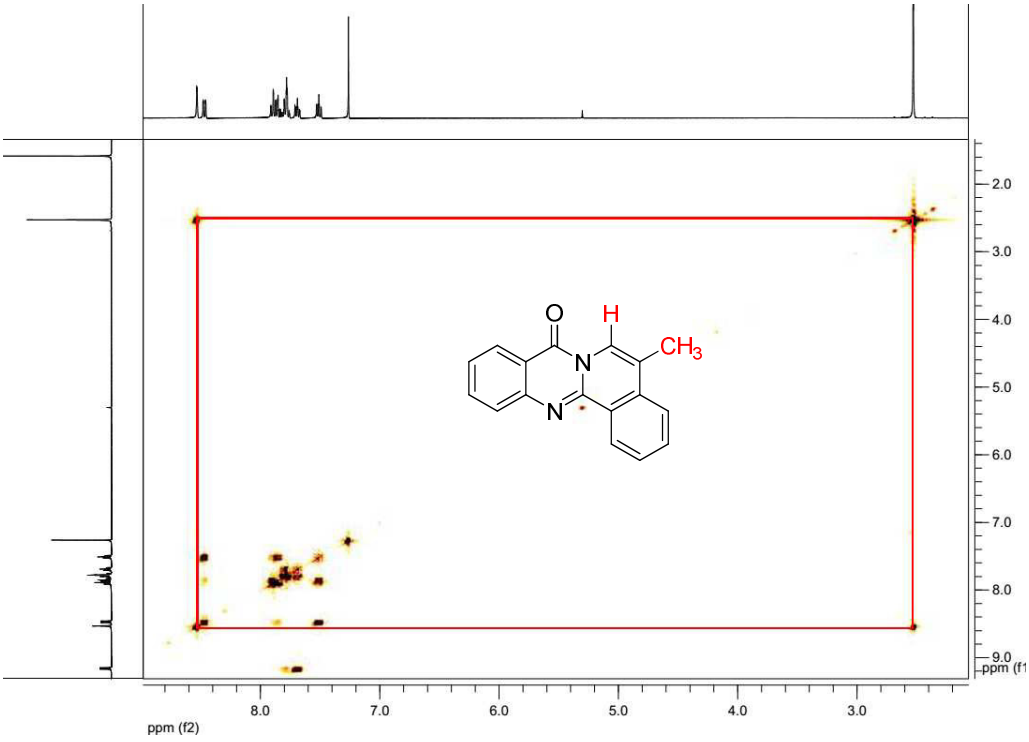
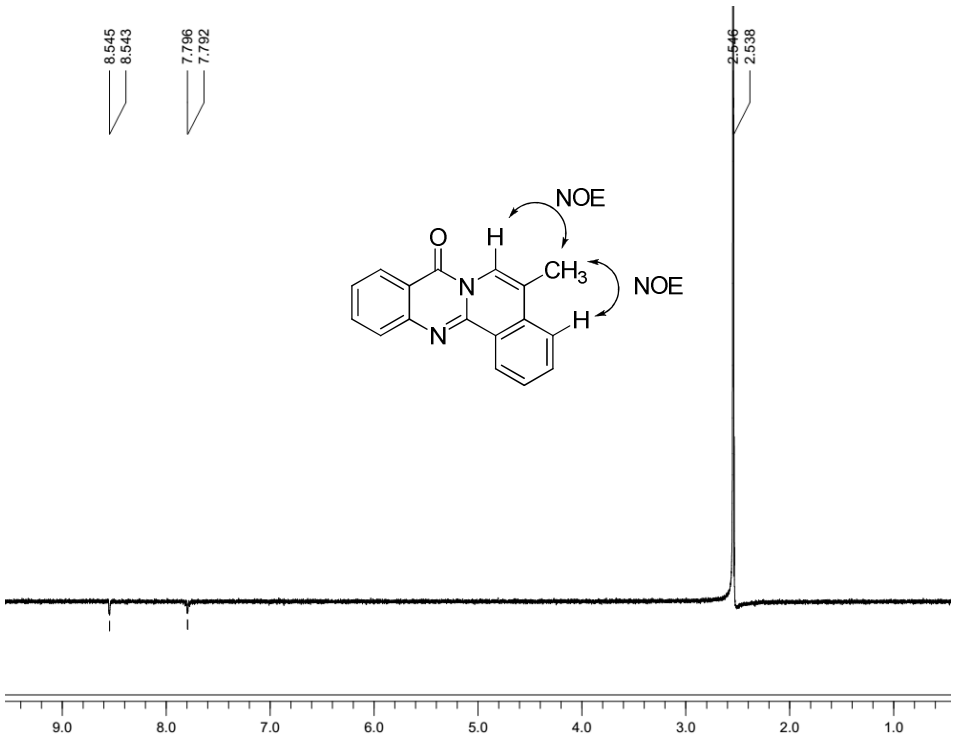
Appendix



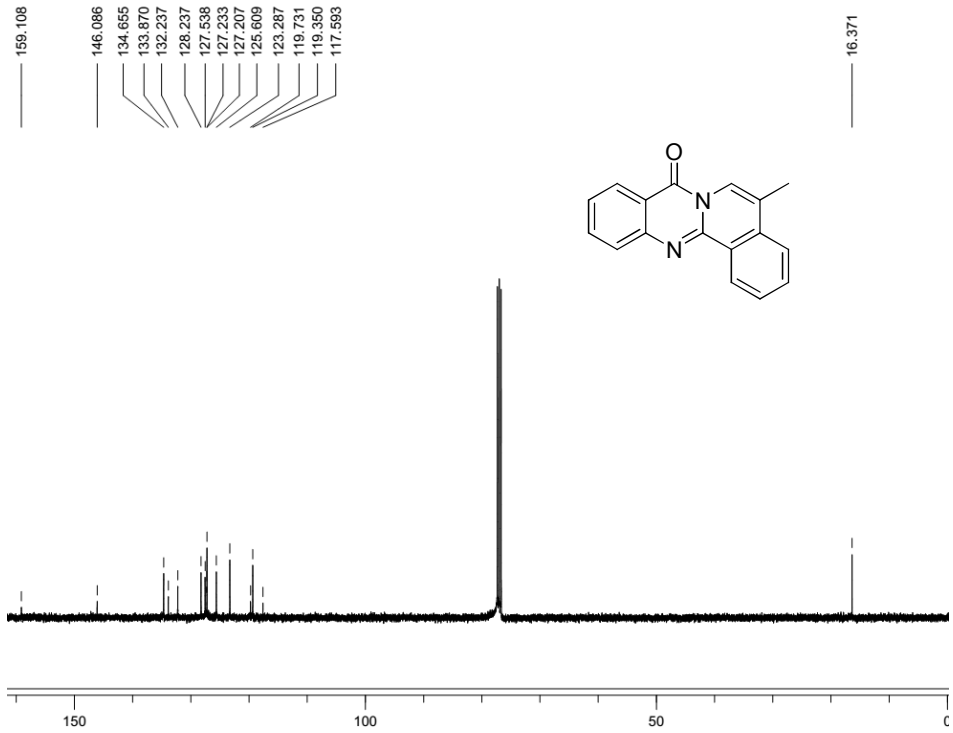
^{1}H NMR spectra of compound **28a** ($CDCl_3$, 400 MHz)



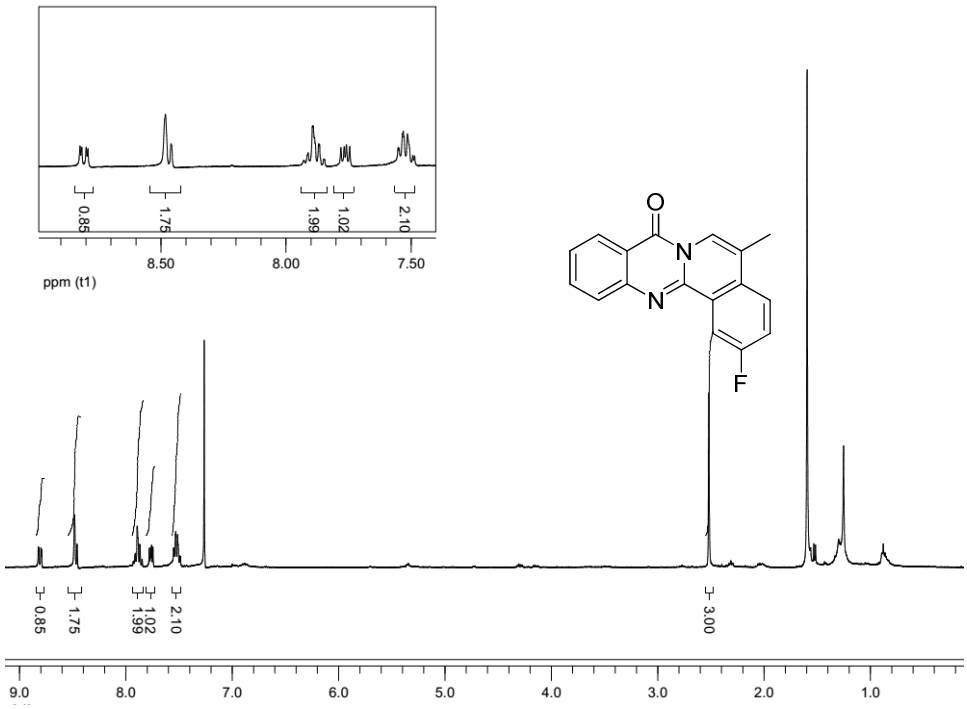
^{1}H-^{1}H COSY spectra of compound **28a** ($CDCl_3$, 400 MHz)



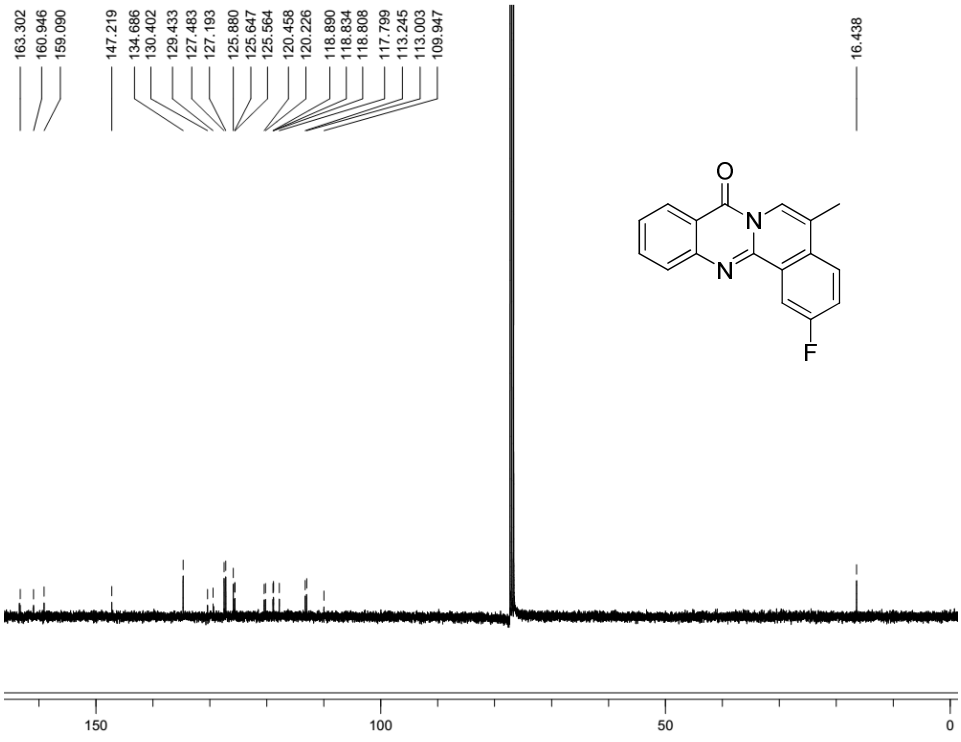
1D-NOE spectra of compound **28a** ($CDCl_3$, 400 MHz)



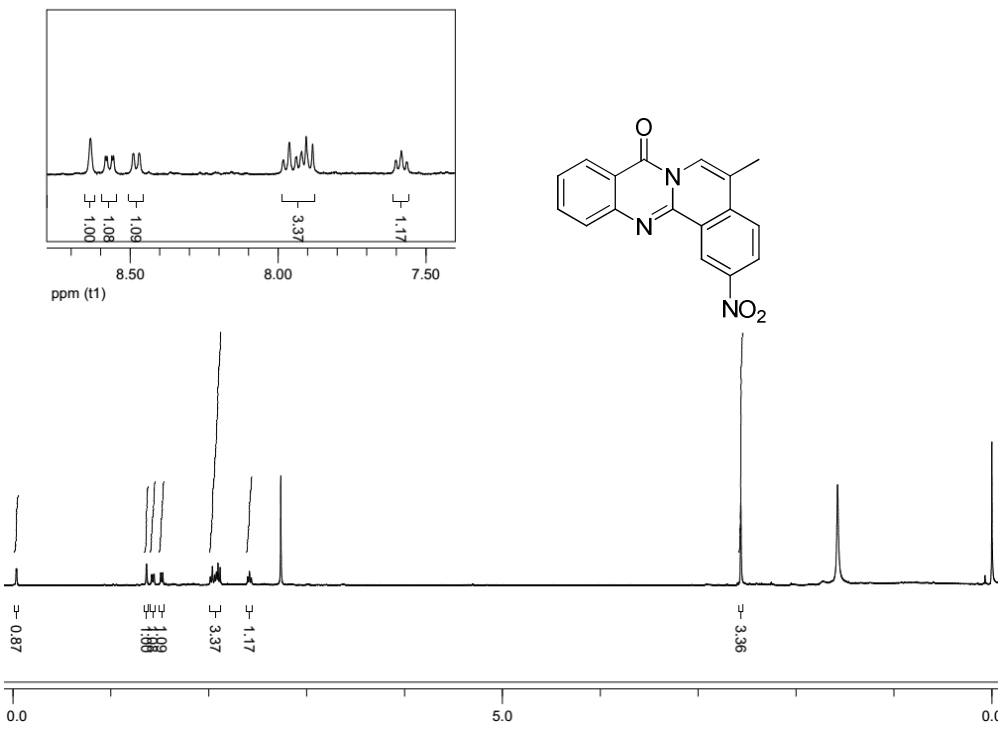
^{13}C NMR spectra of compound **28a** ($CDCl_3$, 100 MHz)



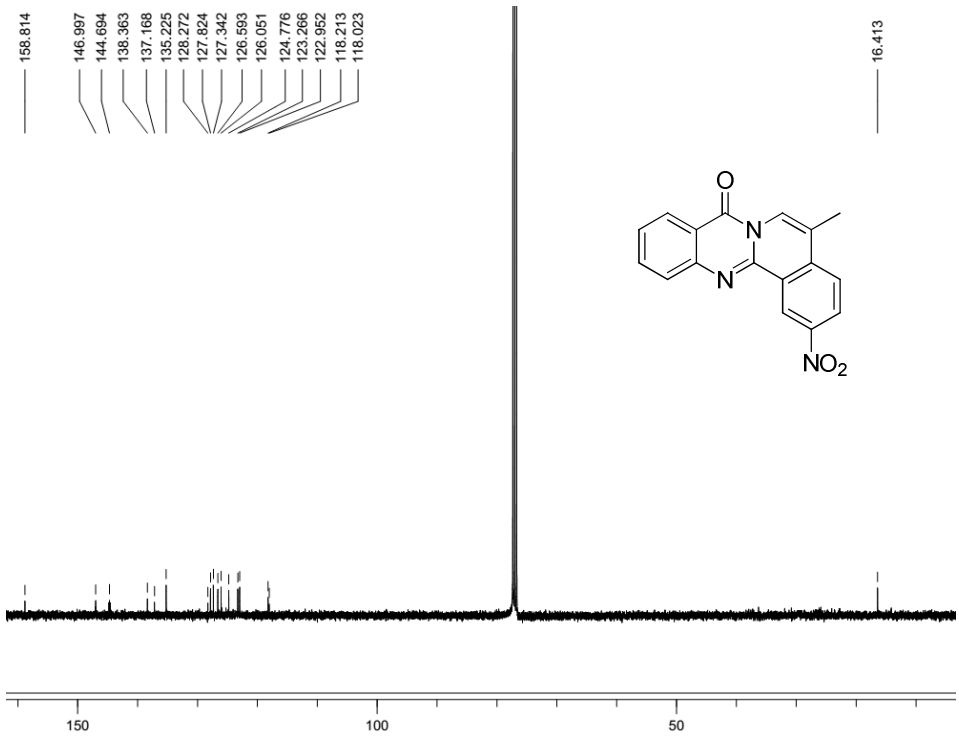
^{1}H NMR spectra of compound **28b** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **28b** ($CDCl_3$, 100 MHz)



^{1}H NMR spectra of compound **28c** ($CDCl_3$, 400 MHz)



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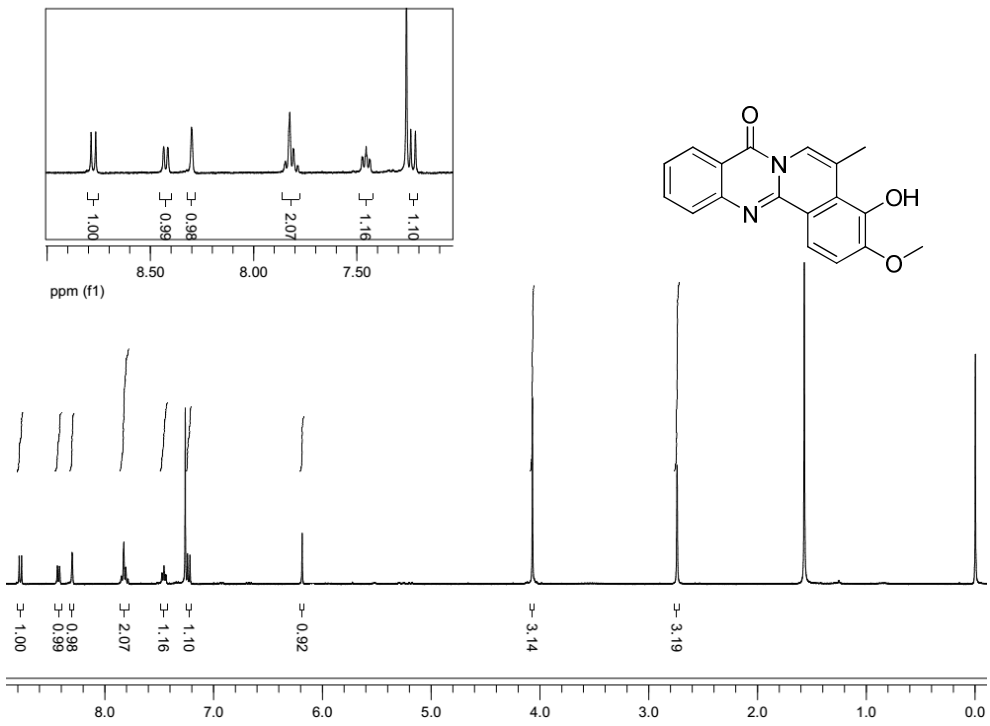

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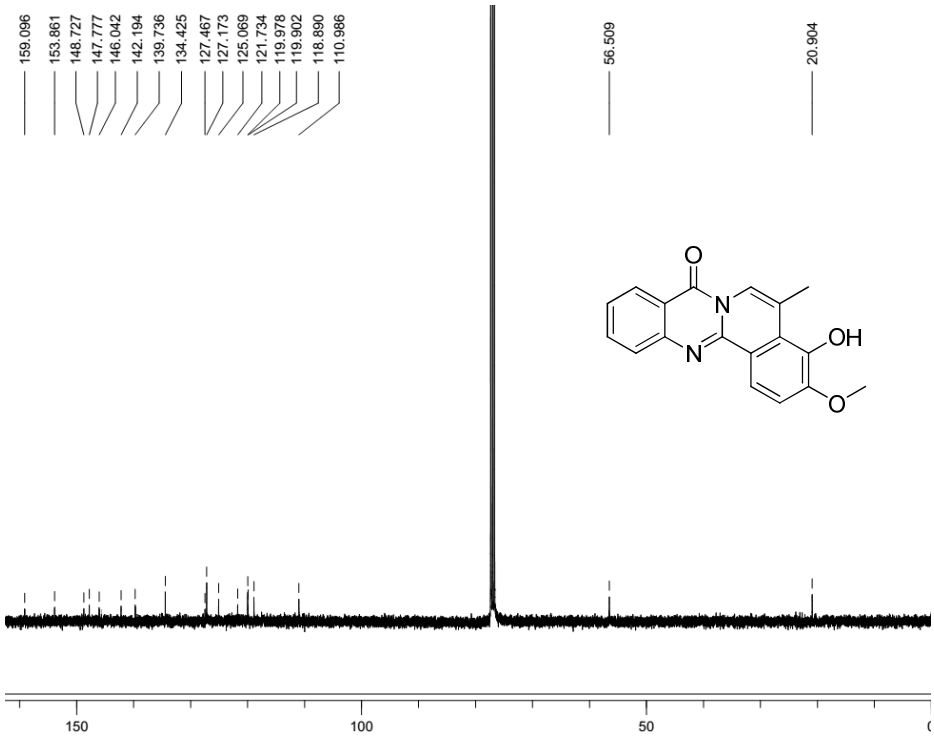

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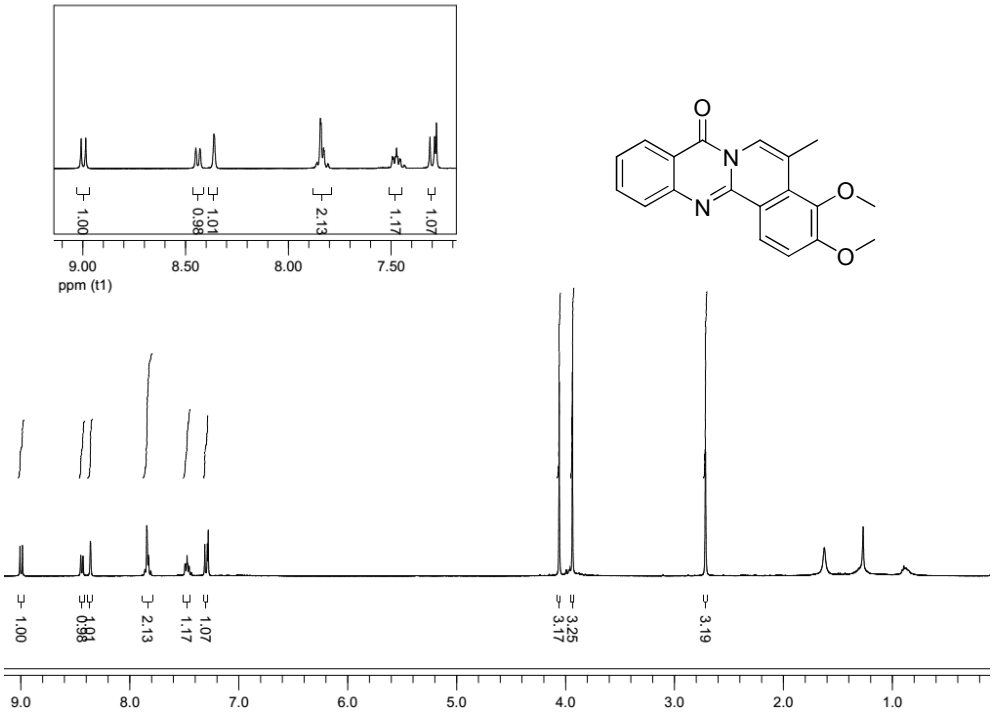
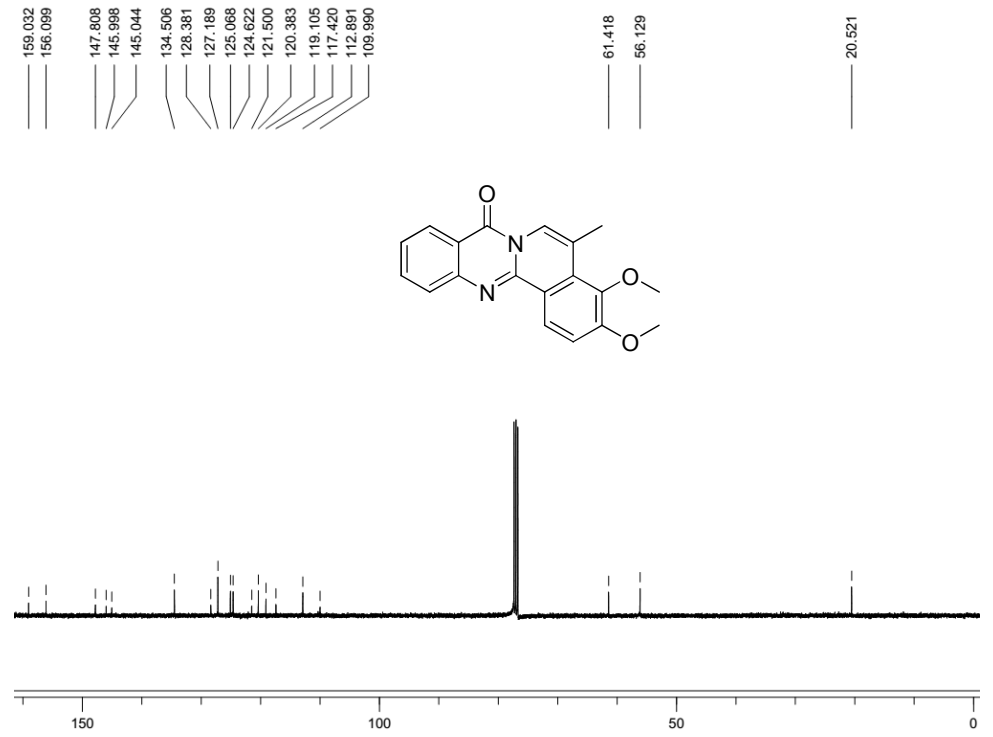


^{1}H NMR spectra of compound **28e** ($CDCl_3$, 400 MHz)



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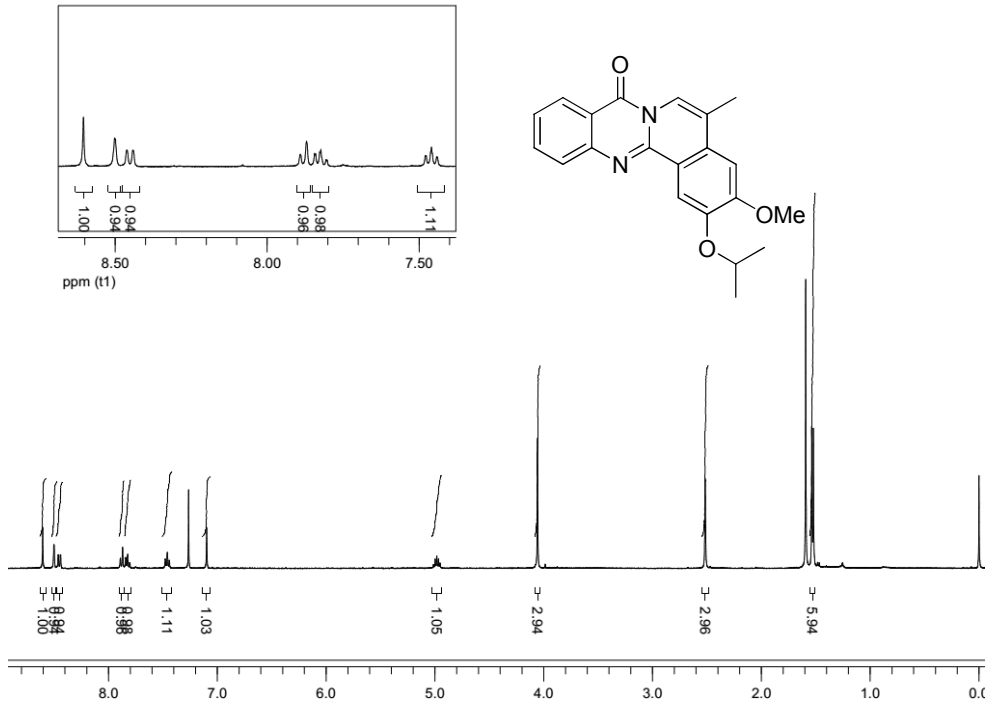
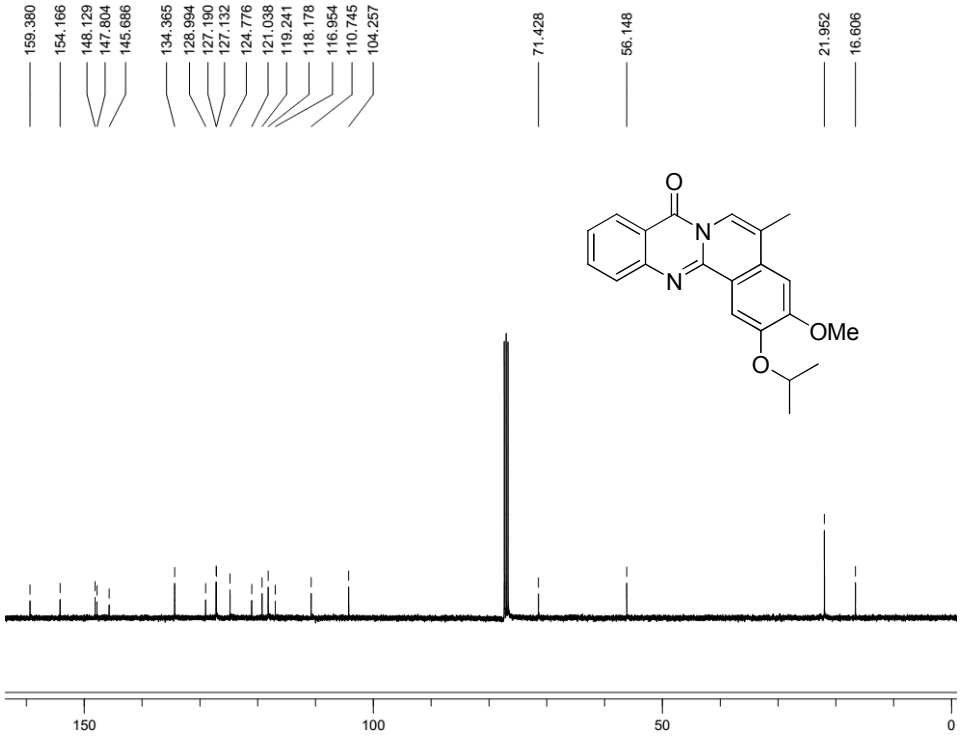


^{1}H NMR spectra of compound **28i** ($CDCl_3$, 400 MHz)



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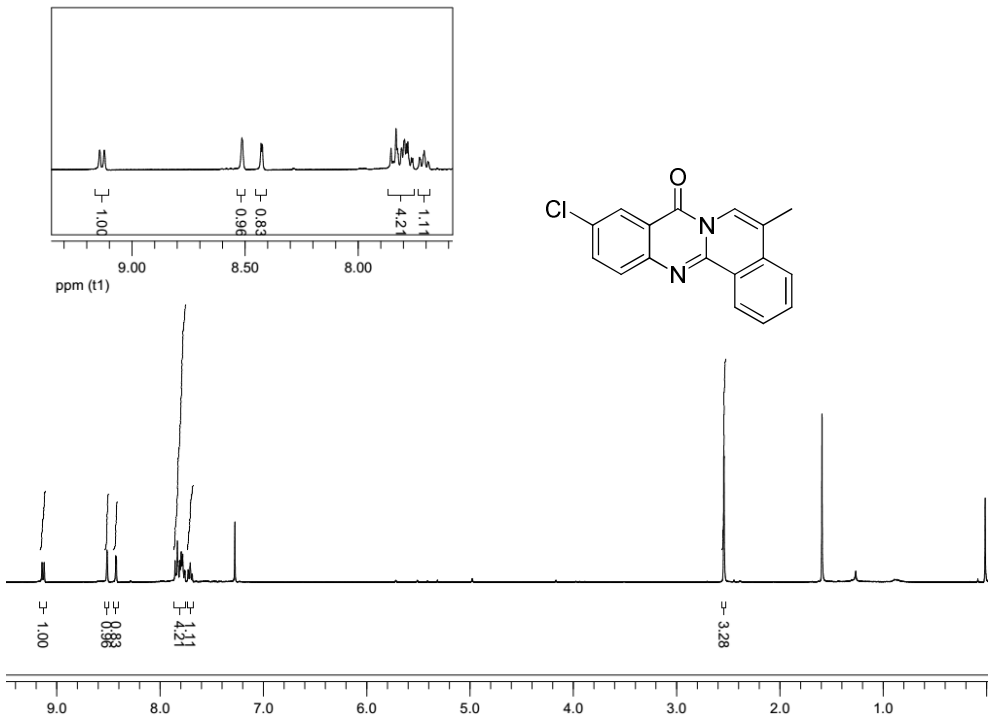

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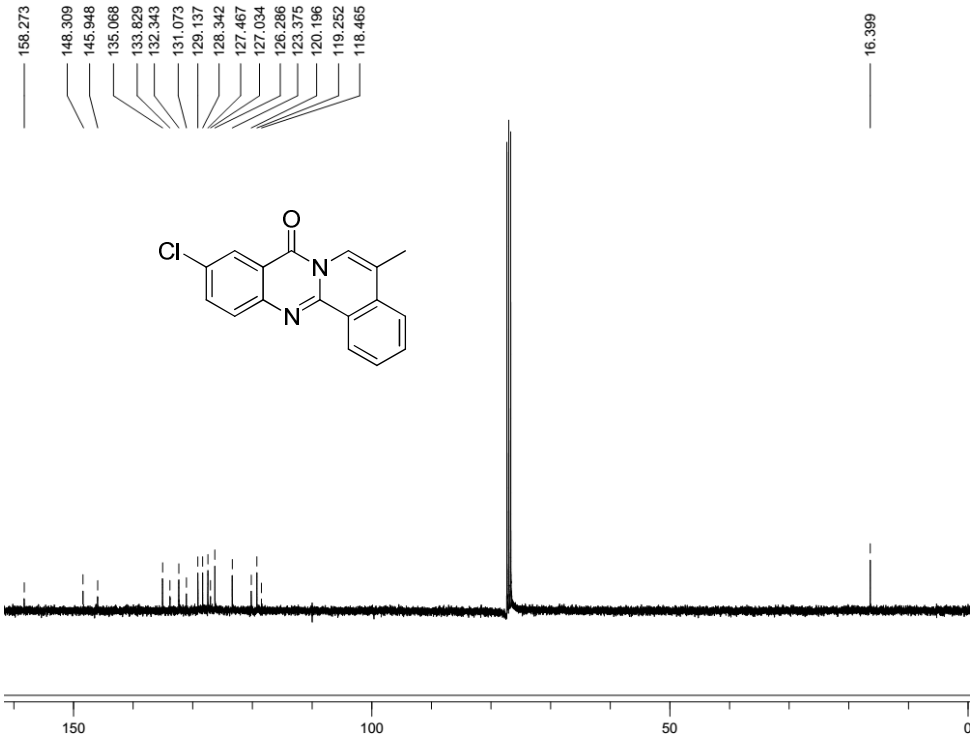
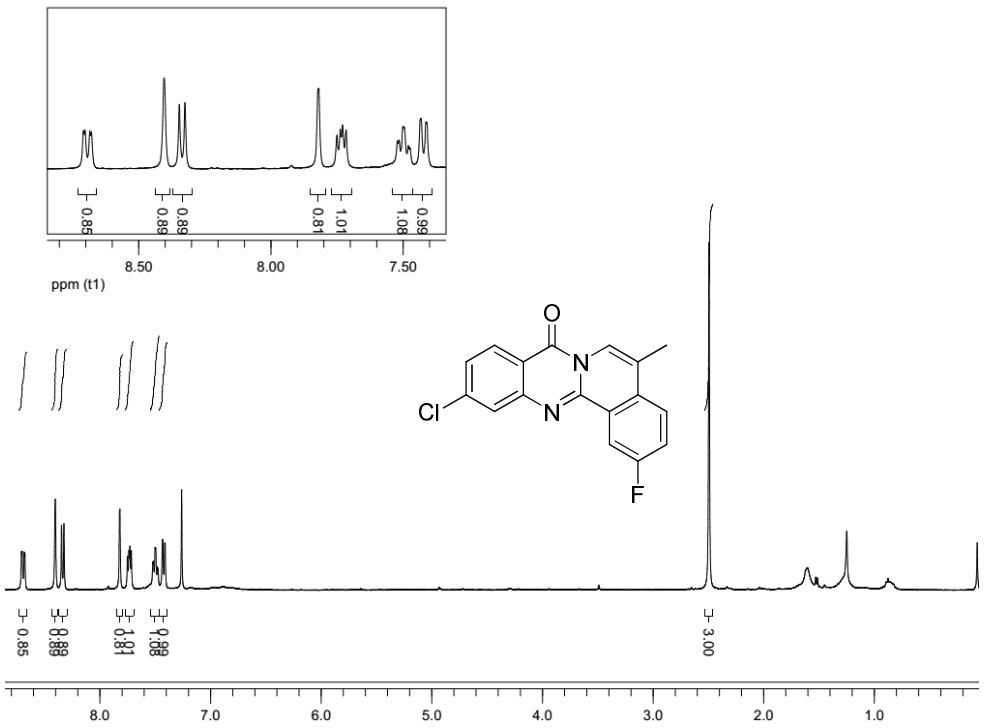
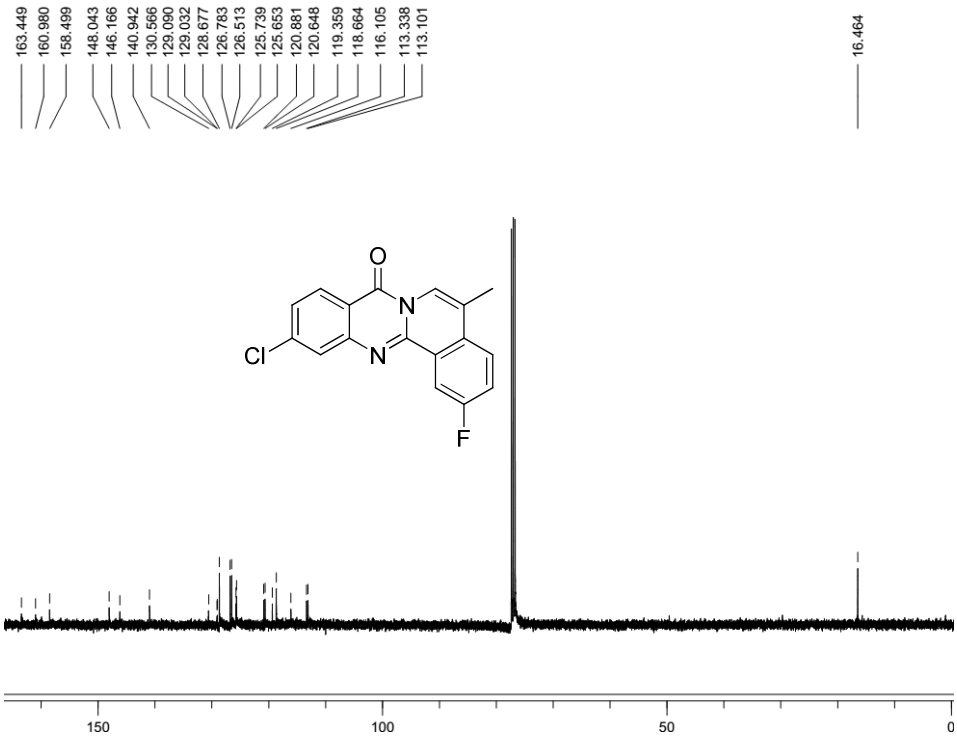


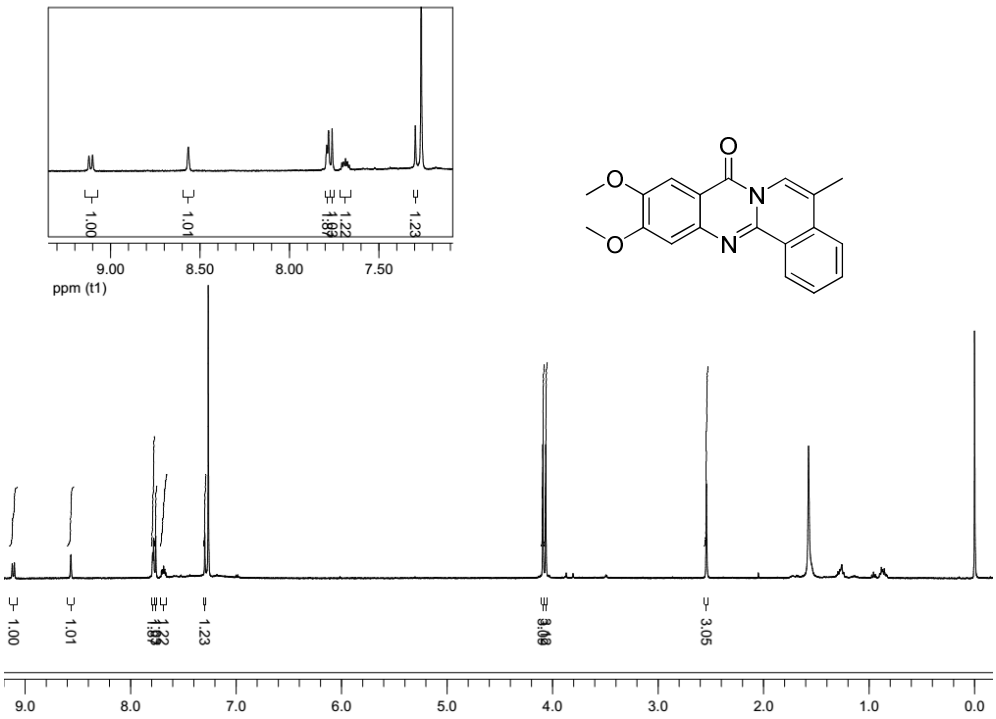
^{13}C NMR spectra of compound **28j** ($CDCl_3$, 100 MHz)



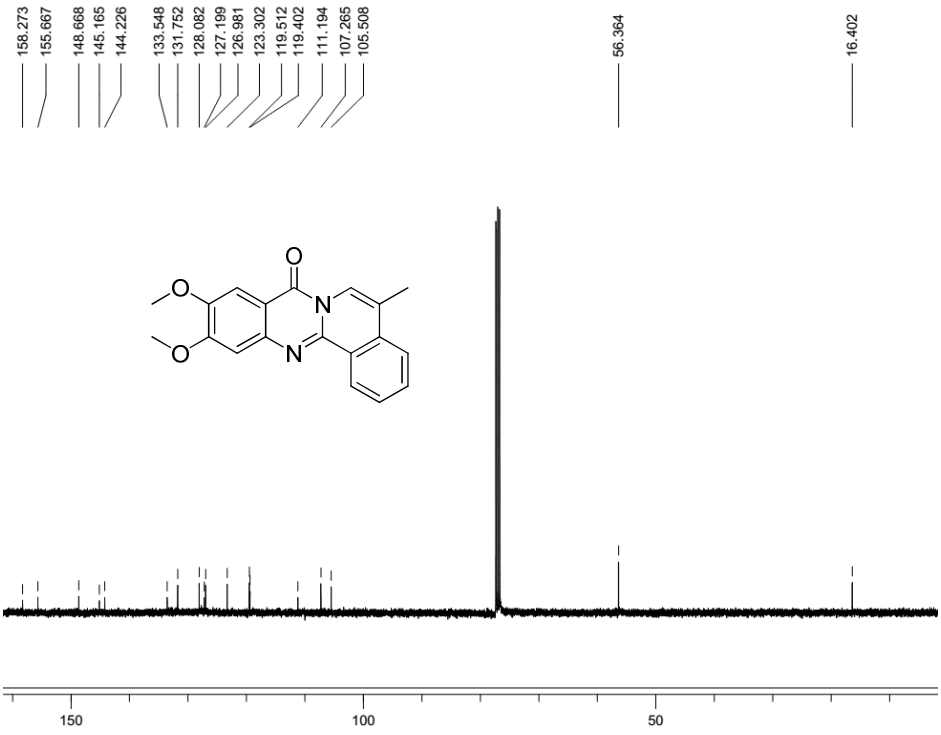
^{1}H NMR spectra of compound **28n** ($CDCl_3$, 400 MHz)



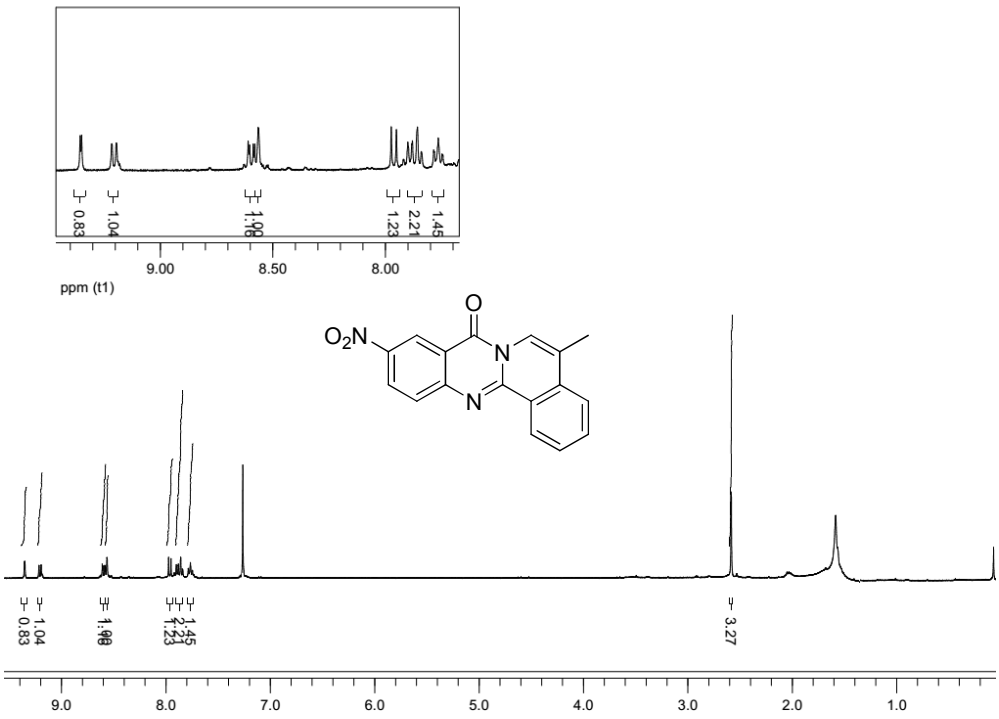
^{13}C NMR spectra of compound **28n** ($CDCl_3$, 100 MHz)



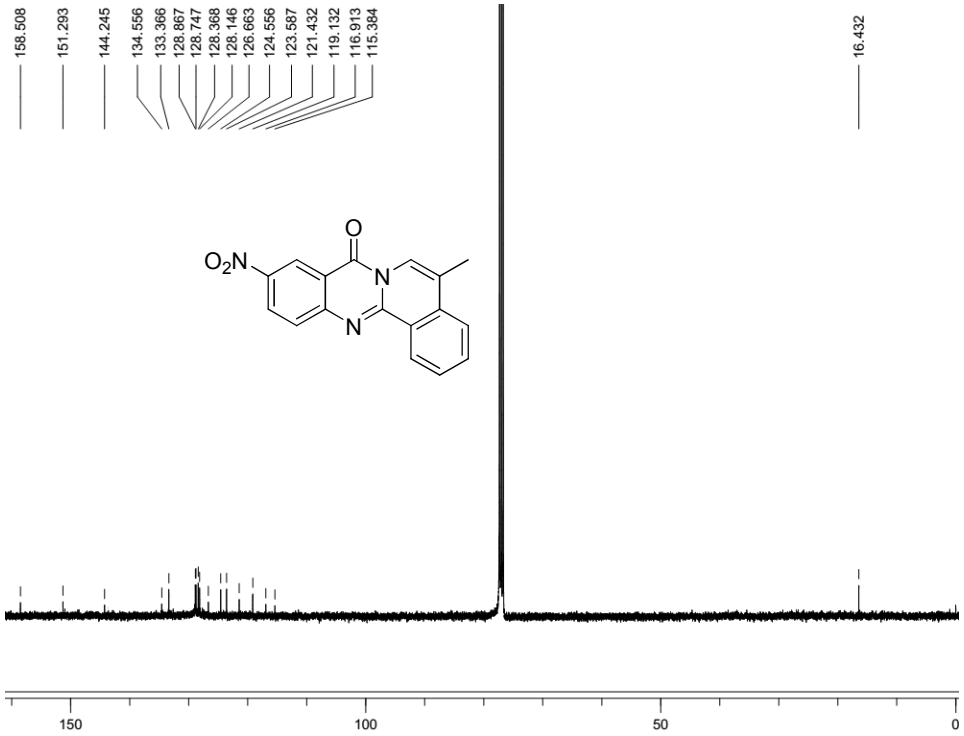
^{1}H NMR spectra of compound **28o** ($CDCl_3$, 400 MHz)



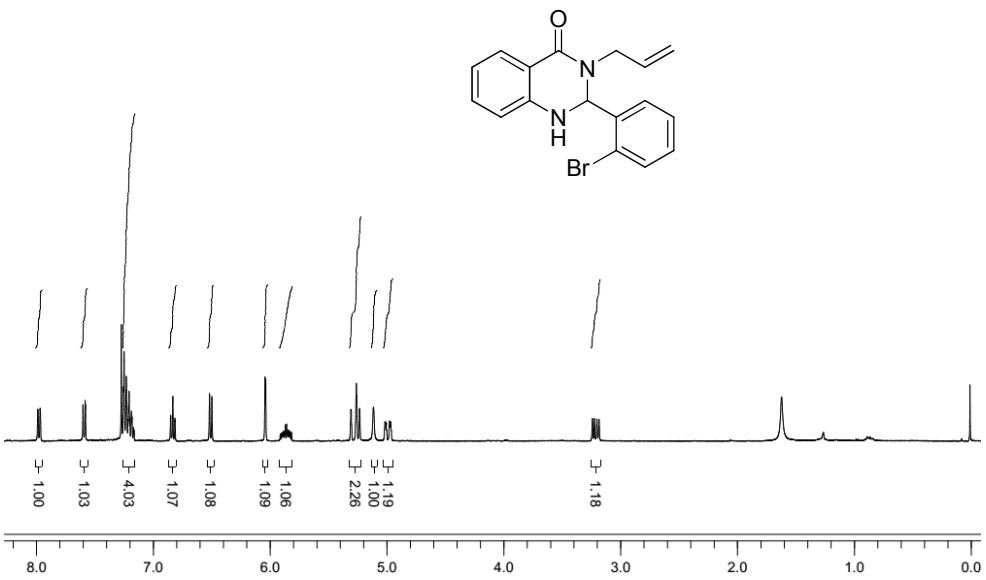
^{13}C NMR spectra of compound **28o** ($CDCl_3$, 100 MHz)



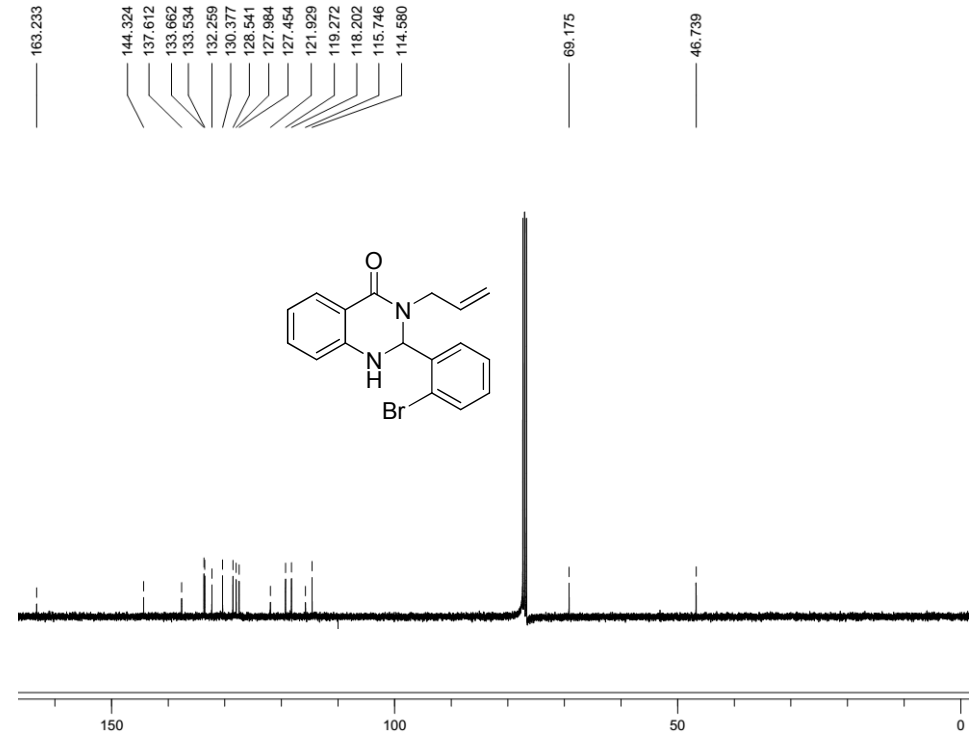
^{1}H NMR spectra of compound **28q** ($CDCl_3$, 400 MHz)



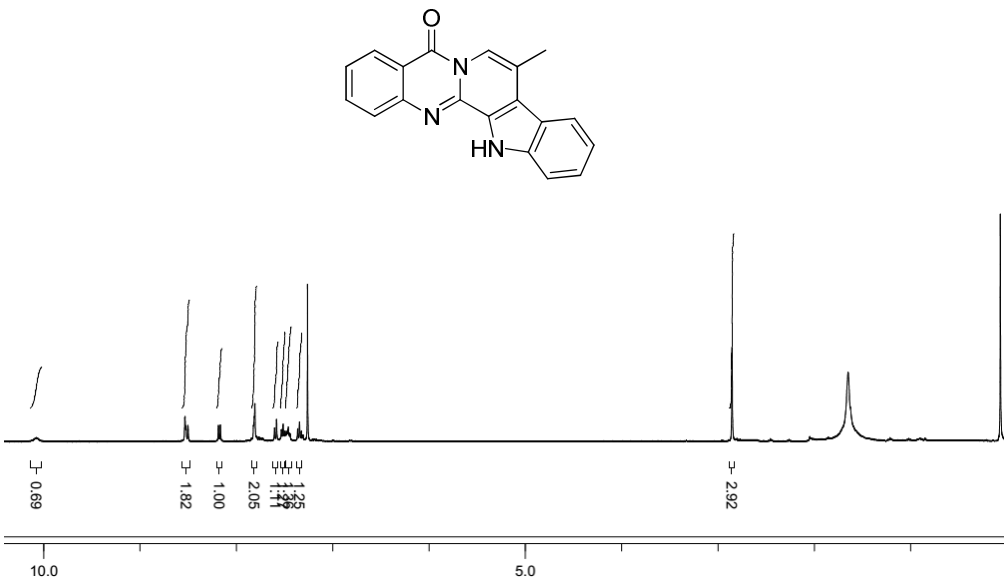
^{13}C NMR spectra of compound **28q** ($CDCl_3$, 100 MHz)



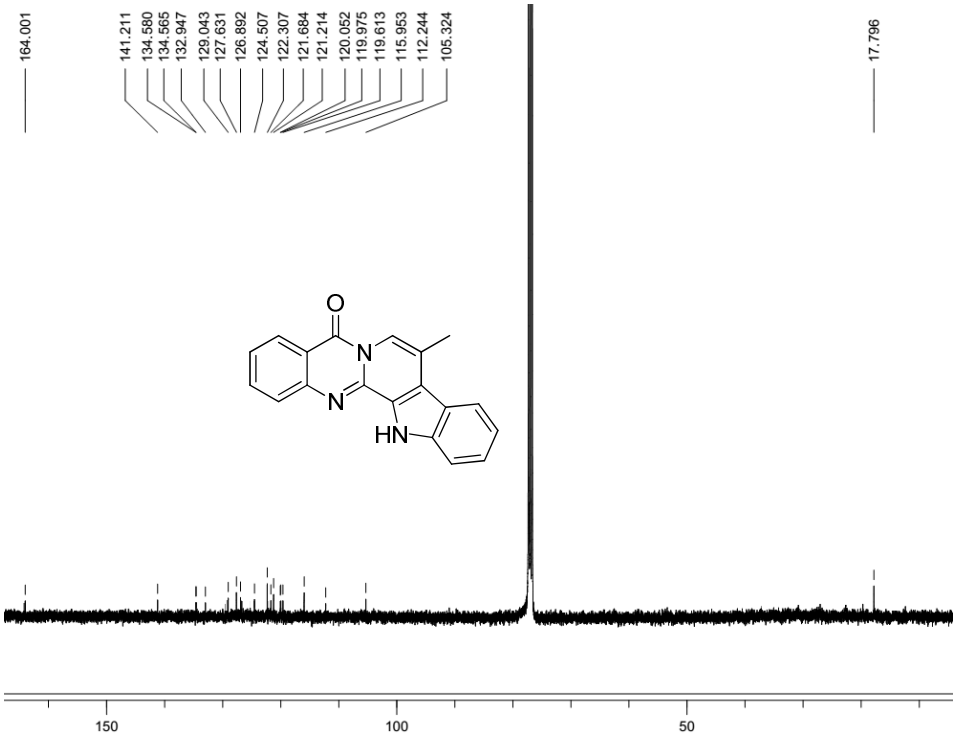
^{1}H NMR spectra of compound **29** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **29** ($CDCl_3$, 100 MHz)



^{1}H NMR spectra of compound **30** ($CDCl_3$, 400 MHz)



^{13}C NMR spectra of compound **30** ($CDCl_3$, 100 MHz)

List of publications

Publications included in the thesis:

1. Novel thieno[2,3-*d*]pyrimidines: their design, synthesis, crystal structure analysis and pharmacological evaluation. **Adepu, R.**; Rambabu, D.; Prasad, B.; Meda, C. L. T.; Kandale, A.; Krishna, G. R.; Reddy, C. M.; Chennuru, L. N.; Parsa, K. V. L.*; Pal, M.* *Org. Biomol. Chem.* **2012**, *10*, 5554-5569.
2. Facile assembly of two 6-membered fused *N*-heterocyclic rings: a rapid access to novel small molecules *via* Cu-mediated reaction. **Adepu, R.**; Sunke, R.; Meda, C. L. T.; Rambabu, D.; Krishna, G. R.; Reddy, C. M.; Deora, G. S.; Parsa, K. V. L.; Pal, M.* *Chem. Comm.* **2013**, *49*, 190-192.
3. A direct access to bioactive fused *N*-heterocyclic acetic acid derivatives. **Adepu, R.**; Rajitha, A.; Ahuja, D.; Sharma, A. K.; Ramudu, B.; Kapavarapu, R.; Parsa, K. V. L.; Pal, M.* *Org. Biomol. Chem.* **2014**, *12*, 2514-2518.
4. Palladium catalyzed Multi Component Reaction: A direct access to isoquinolino[1,2-*b*]quinazolinones and a methyl analogue of 7,8-dehydro rutaecarpine. **Adepu, R.**; Pal, M.* *Manuscript under preparation.*

Publication not included in the thesis

5. C–N bond formation under Cu-catalysis: Synthesis and in vitro evaluation of *N*-aryl substituted thieno[2,3-*d*]pyrimidin-4(3*H*)-ones against chorismate mutase. **Adepu, R.**; Kumar, K. S.; Sandra, S.; Rambabu, D.; Krishna, G. R.; Reddy, C. M.; Kandale, A.; Misra, P.; Pal, M.* *Bioorg. Med. Chem.* **2012**, *20*, 5127-5138.
6. Cu-mediated *N*-arylation of 1,2,3-triazin-4-ones: Synthesis of fused triazinone derivatives as potential inhibitors of chorismate mutase. Kumar, K. S.; **Adepu, R.**; Sandra, S.; Rambabu, D.;

Krishna, G. R.; Reddy, C. M.; Misra, P.; Pal, M.* *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1146-1150.

7. Thieno[3,2-*c*]pyran-4-one based novel small molecules: Their synthesis, crystal structure analysis and in vitro evaluation as potential anticancer agents. Nakhi, A.; **Adepu, R.**; Rambabu, D.; Kishore, R.; Vanaja, G. R.; Kalle A. M.; Pal, M.* *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4418-4427.
8. $AlCl_3$ induced C-arylation/cyclization in a single pot: a new route to benzofuran fused *N*-heterocycles of pharmacological interest. Kumar, K. S.; **Adepu, R.**; Kapavarapu, R. K.; Rambabu, D.; Krishna, G. R.; Reddy, C. M.; Priya, K. K.; Parsa, K. V. L.; Pal, M.* *Tet. Lett.* **2012**, *53*, 1134-1138.
9. $AlCl_3$ mediated unexpected migration of sulfonyl group: regioselective synthesis of 7-sulfonyl indoles of potential pharmacological interest. Prasad, B.; **Adepu, R.**; Sandra, S.; Rambabu, D.; Krishna, G. R.; Reddy, C. M.; Deora, G. S.; Misra, P.; Pal, M.* *Chem. Commun.* **2012**, *48*, 10434-10436.
10. Catalysis by molecular iodine: A rapid synthesis of 1,8-dioxo-octahydroxanthenes and their evaluation as potential anticancer agents. Mulakayala, N.; Murthy, P. V. N. S.; Rambabu, D.; Aeluri, M.; **Adepu, R**.; Krishna, G. R.; Reddy, C. M.; Prasad, K. R. S.; Chaitanya, M.; Kumar, C. S.; Rao, M. V. B.; Pal, M.* *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 2186-2191.
11. Vinylic amino group activation: a new and general strategy leading to functionalized fused heteroaromatics. Sunke, R.; **Adepu, R.**; Kapavarapu, R.; Chintala, S.; Meda, C. L. T.; Parsa, K. V. L.; Pal, M.* *Chem. Commun.* **2013**, *49*, 3570-3572.

Poster Presentations in Conferences

- Poster presentation on "Design, synthesis and pharmacological evaluation of novel thieno[2,3-*d*]pyrimidines as PDE4 inhibitors", **Raju Adepu** and Manojit Pal.
  8th National Organic Symposium Trust (J-NOST)
  IIT Guwahati, Assam, India, Dec 15th-17th, 2012.

- Poster presentation on "Synthesis of fused *N*-heterocyclic compounds *via* copper catalyzed one-pot domino reaction", **Raju Adepu** and Manojit Pal.
  9th National Organic Symposium Trust (J-NOST)
  IISER Bhopal, Madhya Pradesh, India, Dec 4th-6th, 2013.

- Poster presentation on "Synthesis of bioactive *N*-heterocyclic acetic acid derivatives", **Raju Adepu** and Manojit Pal.
  ***International Symposium on Nature Inspired Initiatives in Chemical Trends* (NIICT)**
  IICT Hyderabad, India, Mar 2nd-4th, 2014.

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PAPER

Novel thieno[2,3-*d*]pyrimidines: their design, synthesis, crystal structure analysis and pharmacological evaluation†

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Novel thieno[2,3-*d*]pyrimidines containing a cyclohexane ring fused with a six- or five-membered heterocyclic moiety along with a benzylic nitrile were designed as potential inhibitors of PDE4. Expeditious synthesis of these compounds was carried out *via* a multi-step sequence consisting of a few key steps such as Gewald reaction, Dieckmann type cyclisation and Krapcho decarboxylation. This newly developed strategy involved construction of the thienopyrimidine ring followed by the cyclohexanone moiety and subsequently the fused heterocyclic ring. A number of thieno[2,3-*d*]pyrimidine based derivatives were synthesized using this method some of which showed promising PDE4B inhibitory properties. One of them was tested for PDE4D inhibition *in vitro* and dose dependent inhibition of TNF-α. A few selected molecules were docked into the PE4B protein the results of which showed good overall correlations to their observed PDE4B inhibitory properties *in vitro*. The crystal structure analysis of representative compounds along with hydrogen bonding patterns and molecular arrangement present within the molecule is described.

Introduction

A pyrimidine nucleus fused with another heterocycle has found wide applications in the design and discovery of novel bioactive molecules and drugs.[1] For example, thieno[2,3-*d*]pyrimidine derivatives such as **A** and **B** (Fig. 1) exhibited remarkable affinity and selectivity for the 5-HT3 receptor.[2,3] In continuation of our research under the new drug discovery program, we became interested in evaluating a library of small-molecules based on thieno[2,3-*d*]pyrimidine that were designed as potential inhibitors of PDE4 (phosphodiesterase 4). PDEs are a diverse family of enzymes that hydrolyse cyclic nucleotides and thus play a key role in regulating intracellular levels of the second messenger cAMP and cGMP, and hence cell function.[4] PDE4 is a cAMP-specific PDE and predominant isoenzyme in the majority of inflammatory cells, with the exception of platelets, implicated in inflammatory airways disease. Elevated levels of cAMP play a major role in relaxation of vascular smooth muscle, which is beneficial in treating inflammatory diseases especially pulmonary diseases. Thus, inhibition of PDE4 is beneficial for the treatment of respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD).[5] The use of first-generation PDE4 inhibitor rolipram[6] (**C**, Fig. 2) however was associated with dose-limiting side effects *e.g.* nausea and vomiting. While these side effects were reduced by second-generation inhibitors like cilomilast[7] (Ariflo) and roflumilast (Daxas®, Nycomed), their therapeutic index has delayed market launch so far. Recent studies have indicated that the PDE4B subtype is linked to inflammatory cell regulation[8] whereas the PDE4D subtype is implied in the emetic response.[9] However, none of the PDE4 inhibitors under development are PDE4B selective.[10a] Recently it has been demonstrated that inhibition of PDE4D by allosteric inhibitors (maximum inhibition,

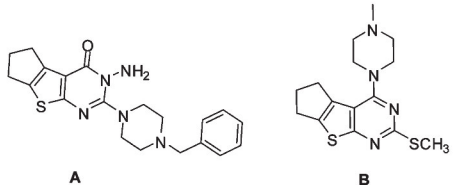

Fig. 1 Examples of biologically active thieno[2,3-*d*]pyrimidine derivatives.

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†Electronic supplementary information (ESI) available: Docking studies, copies of NMR spectra for all new compounds. CCDC 864129 and 864130. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25420d

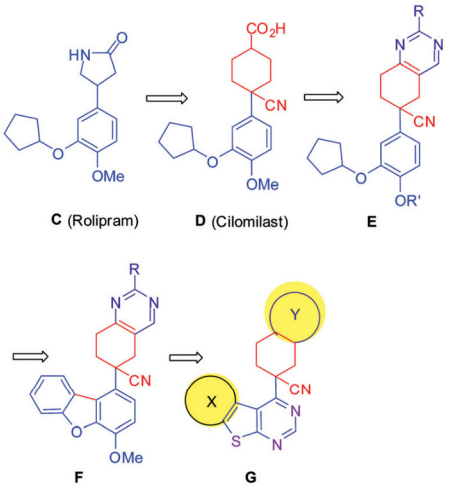


Fig. 2 Design of novel inhibitors (**G**) of PDE4 based on known inhibitors (**C**–**F**).

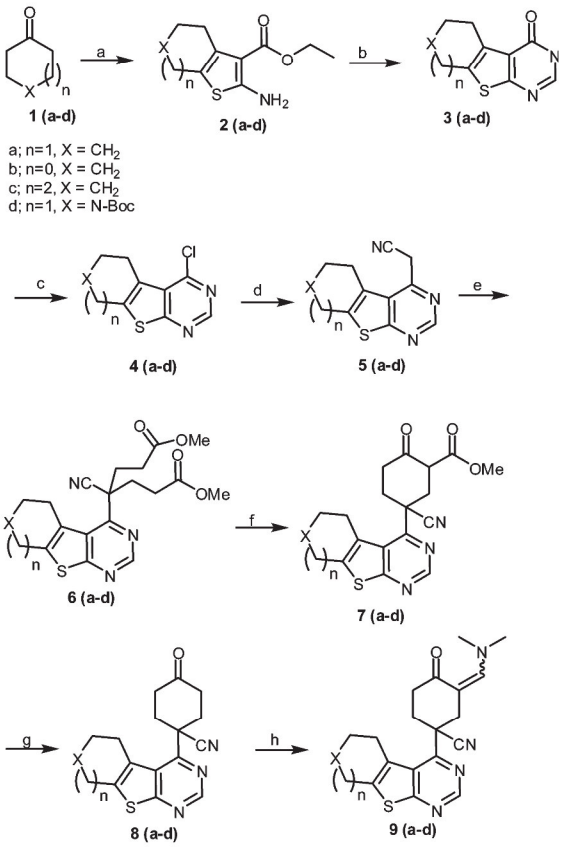
I_{max} 80–90%) did not cause emetic side effects raising a possibility that PDE4B inhibitors with partial but not complete inhibition of PDE4D (I_{max} of ~60–80%) could be developed to treat COPD and asthma without causing emetic side effects.[10b]

To identify novel and orally active PDE4 inhibitors with decreased potential for side effects the design and synthesis of 4-cyano cyclohexane-1-carboxylic acid derivatives was undertaken which resulted in significant improvement in reducing the side effects of **C**.[11] Thus, cilomilast (**D**, Fig. 2) that belongs to this class was discovered and finally entered into phase 3 clinical trials. However, to address the issue of configurational isomerism of **D** around the CO_2H group new cyclohexane derivatives (**E**, Fig. 2) were designed maintaining the benzylic nitrile as one of the pharmacophores[12] and the cyclohexane ring fused with a six- or five-membered heterocyclic moiety. Recently, cyclohexane derivatives (**F**, Fig. 2) containing a tricyclic fused aryl ring have been reported to possess PDE4 inhibitory properties.[13] Based on these observations we designed novel cyclohexane derivatives (**F**, Fig. 2) containing the thieno[2,3-*d*]pyrimidine moiety along with benzylic nitrile as potential and new inhibitors of PDE4. The cyclic rings 'X' and 'Y' were chosen to introduce diversity into the basic scaffold for the generation of library of small molecules. To the best of our knowledge template **G** has not been previously explored for the discovery of PDE4 inhibitors.

Results and discussions

Chemistry

The retro-synthetic analysis of the target compound **G** revealed that construction of a cyclohexane ring at C-4 of the thieno-[2,3-*d*]pyrimidine moiety could be a key step. Overall, we envisioned that sequential construction of (i) the thienopyrimidine ring followed by (ii) the cyclohexanone moiety and subsequently (iii) the fused heterocyclic ring could provide us with the target compounds based on **G**. While introduction of an aryl or heteroaryl or alkyne moiety[14,15] at C-4 of the thieno[2,3-*d*]-pyrimidine moiety is known a similar method to introduce a cyclohexyl moiety at the same position was unprecedented. Nevertheless, the 4-chloro-thieno[2,3-*d*]pyrimidines (**4**) appeared to be appropriate starting materials for our synthesis and were prepared following a 3-step method (step a–c, Scheme 1) as reported earlier.[14,15] The use of **4** for the preparation of subsequent intermediates is shown in Scheme 1. Thus, the reaction[16] of ethyl cyanoacetate with chloro derivative **4** followed by *in situ* decarboxylation of the resulting ester afforded the cyano derivative **5** which on double Michael reactions with methyl acrylate furnished the diester **6**. A Dieckmann type cyclisation[17] of **6** followed by Krapcho decarboxylation[18] of the resulting β-ketoester **7** provided the cyclohexanone derivative **8** which on reaction with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) furnished the required 2-((dimethylamino)methylene)cyclohexanone derivative **9**. The intermediates **7** and **9** were used for the



Scheme 1 Reagents and conditions: (a) ethyl cyanoacetate, morpholine, sulphur, ethanol, 90 °C, 3–8 h; (b) for **5a–c**: formamide, 190 °C, 2–4 h; for **5d**: formimidine acetate, DMF, 130 °C, 16 h; (c) for **5a–c**: $POCl_3$, 110 °C, 1–1.5 h; for **5d**: $POCl_3$, Et_3N, 60 °C, 2 h; (d) for **5a–b**: ethyl cyanoacetate, K_2CO_3, 130 °C, 1–2 h; for **5c–d**: (i) ethyl cyanoacetate, K_2CO_3, DMSO, 120 °C, 1–1.5 h; (ii) NaCl, H_2O, DMSO, 150 °C, 5–10 h; (e) methyl acrylate, triton-B, CH_3CN, 85 °C, 3–6 h; (f) for **5a–c**: NaH, DME, 85 °C, 2–5 h; for **5d**: NaH, THF, 60 °C, 1 h; (g) NaCl, H_2O, DMSO, 150 °C, 4–7 h; (h) for **9a–b**: DMF-DMA, Et_3N, DMF, 80 °C, 2–4 h; for **9c–d**: DMF-DMA, toluene, 95 °C, 16 h.

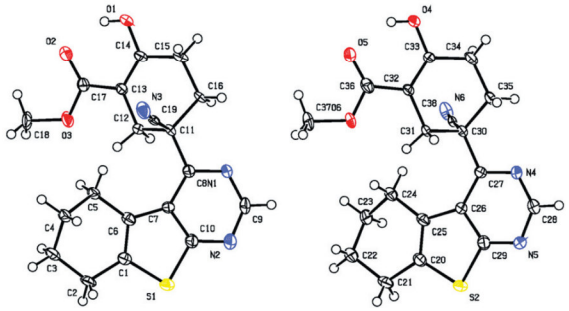


Fig. 3 ORTEP representation of the **7a** (thermal ellipsoids are drawn at 50% probability level).

preparation of the target compounds. All the intermediates synthesized were well characterized by spectral (NMR, MS and IR) data. Additionally, the molecular structure of intermediate **7a** (methyl-5-cyano-5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-2-oxocyclohexanecarboxylate) was established unambiguously by single crystal X-ray diffraction (Fig. 3) the details of which are presented in the following section. The X-ray diffraction study indicated that **7a** existed in the enol tautomeric form predominantly stabilized by the 6-membered ring formed due to an intramolecular H-bond. The existence of the enol form in solution was also supported by ^{1}H NMR data as the enolic hydroxyl group appeared at ~ 12.0–12.3 δ.

Compound **7a** crystallizes in the triclinic $P\bar{1}$ space group with two symmetry molecules in the asymmetric unit ($Z = 4$, $Z' = 2$) (Fig. 3). The two molecules in the asymmetric unit contain free hydroxyl and ester functional groups. These molecules have the capability to form supramolecular synthons and are conformationally different (Fig. 4). The inversion related molecules of conformer-i, and conformer-ii are both forming the same type of intramolecular O–H···O synthon in between the substituted hydroxyl group with the *ortho* oriented methyl ester group and the C–H···O, C–H···S intermolecular hydrogen bonding. Consequently, the two groups OH and ester come closer and interact with the O–H···O synthon. These interactions propagate 3D network packing along the ac axis (see ESI†).

The reaction of **9** with guanidine or formamidine afforded the compounds **10** or **11** (Scheme 2). Similarly, the reaction of **7a** with guanidine or formamidine afforded the compounds **12** or **13** whereas treating **7a** with hydrazines afforded compounds **14** or **15** (Scheme 3). The reaction of **9a** with hydrazine provided the compound **16** (Scheme 4).[12] All the target compounds synthesized were characterized by spectral (NMR, MS and IR) data. For example, the presence of a –CN group was confirmed by an IR absorption in the region 2240–2230 cm^{-1}. The keto form of compound **12** and **13** was characterized by the IR absorption at 1650 and 1655 cm^{-1} respectively, due to the C═O moiety. The structure of compound **14** was assigned based on two broad ^{1}H NMR signals at 11.3 and 9.6 δ due to the two NH groups and an IR absorption at 1734 cm^{-1} due to the amide C═O moiety. The keto form of compound **15** was also indicated by IR absorption at 1730 cm^{-1} due to the C═O group and the absence of any NH IR absorption beyond 3000 cm^{-1}. Nevertheless, the molecular structure of a representative compound **10a**

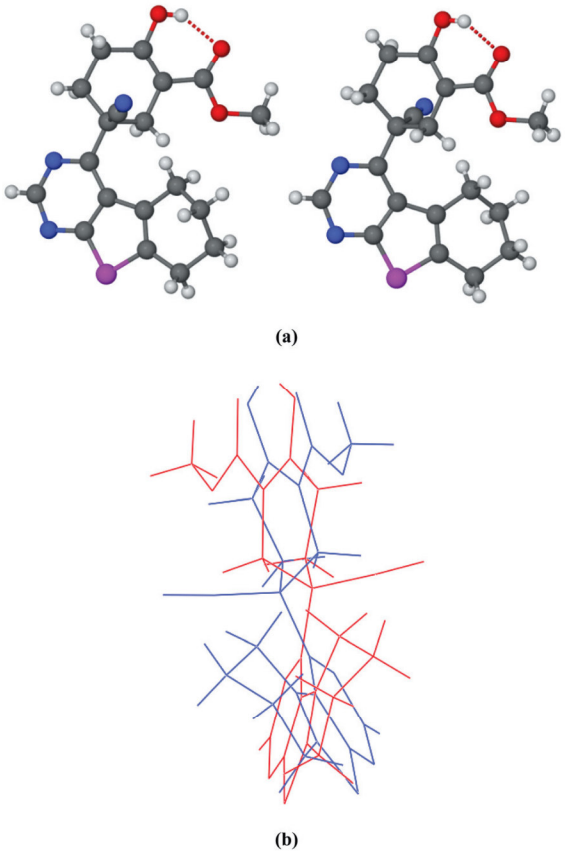
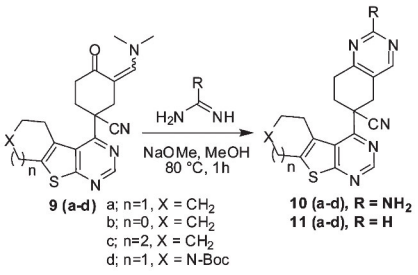


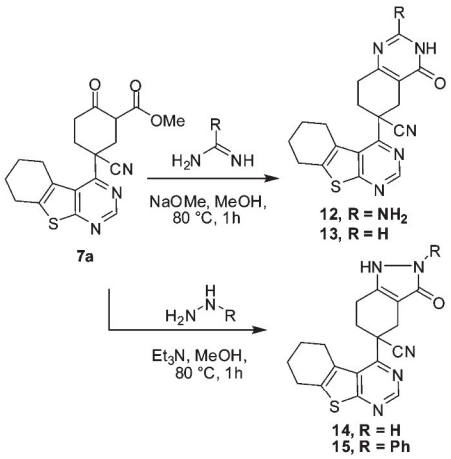
Fig. 4 (a) Showing the intramolecular hydrogen bonding present in the molecule **7a** *via* O–H···O synthon. (b) Showing the conformations present in the asymmetric unit (i) conformer-**i** in blue, (ii) conformer-**ii** in red.



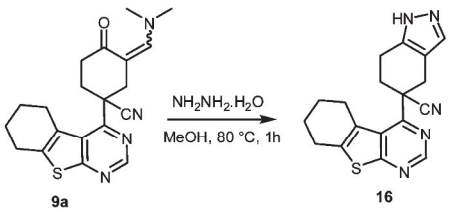
Scheme 2 Preparation of 5,6,7,8-tetrahydroquinazoline derivatives.

was established unambiguously by single crystal X-ray diffraction (Fig. 5). The X-ray diffraction study also indicated the *R*-isomer of compound **10a**.

Compound **10a** crystallizes in the triclinic $P\bar{1}$ space group with one molecule and one solvent molecule in the asymmetric unit ($Z = 6$, $Z' = 2$) (Fig. 5). The molecule in the asymmetric unit contains pyrimidine substituted with a free amine and has capability to form a supramolecular synthon *via* intermolecular



Scheme 3 Preparation of 4-oxo-3,4,5,6,7,8-hexahydroquinazoline and 3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole derivatives.



Scheme 4 Preparation of 4,5,6,7-tetrahydro-1*H*-indazole derivative.

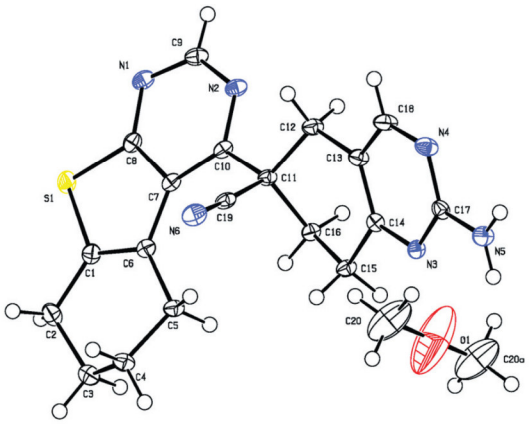


Fig. 5 ORTEP representation of the **10a** (thermal ellipsoids are drawn at 50% probability level).

hydrogen bonding. The inversion related molecule in the asymmetric unit formed the dimer synthon through pyrimidine amine like N–H···N interactions (Fig. 6) and is stabilised by C–H···C, C–H···N and C–H···S interactions with dimethoxy solvent molecules. These interactions propagate in a 3D network packing along bc axis (see ESI†).

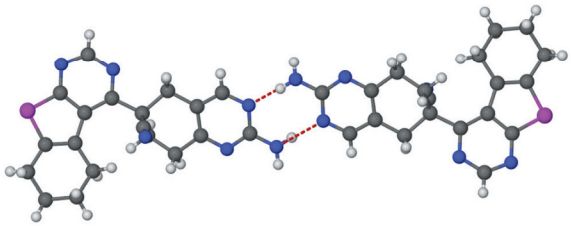


Fig. 6 Showing the intermolecular hydrogen bonding present in the molecule **10a** *via* N–H···N synthon.

While only the *R*-isomer of compound **10a** was isolated during generation of the single crystal *via* crystallization the possibility of the presence of the *S*-isomer in the solution could not be ruled out. It was therefore necessary to assess the chiral purity of **10a** and other target compounds synthesized. It is worthy to mention that the stereocentre in compounds **10–16** was generated during the conversion of intermediate **6** to **7** and **8** to **9**. This conversion was expected to afford a mixture of stereoisomers as the methodology used was not an enantiospecific one. Thus, a chiral HPLC method was used to determine the enantiomeric purity of some representative compounds *e.g.* **10a**, **13**, **14** and **16**. All these compounds were found to be a ~1 : 1 mixture of both the antipodes.

In vitro pharmacology

All the target compounds (**10–16**) synthesized were evaluated for their PDE4 inhibitory properties *in vitro*. Initially, PDE4B inhibitory potential was assessed by using PDE4B enzyme isolated from Sf9 cells.[19] Some of the compounds showed significant inhibition of PDE4B at 30 μM and the data generated for most of the compounds are summarized in Table 1. As is evident from Table 1 the change in ring size (**10a** *vs.* **10b** and **10c**) or functionalization of the saturated cycloalkyl ring (**10a** *vs.* **10d**) fused with the thiophene moiety or removal of NH_2 group from the pyrimidine ring (**10a** *vs.* **11a**) did not show significant effect on inhibitory activities. A similar trend regarding the effect of fused cycloalkyl ring was observed for compounds **11**. The functionalization of the pyrimidine ring of 5,6,7,8-tetrahydroquinazoline-6-carbonitrile moiety was tolerated (**11a** *vs.* **12** and **13**). While replacing the 5,6,7,8-tetrahydroquinazoline-6-carbonitrile moiety by 3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole-5-carbonitrile group decreased the activity significantly (**11a** *vs.* **14** and **15**) the 4,5,6,7-tetrahydro-1*H*-indazole-5-carbonitrile moiety restored the activity (**11a** *vs.* **16**). Based on their initial PDE4B inhibitory properties compounds **10a**, **10c**, **10d**, **11a**, **11c**, **11d** and **16** were evaluated for dose dependent inhibitions (Fig. 7, see also ESI†) and the corresponding IC_{50} values are presented in Table 1. A few of these compounds were also tested in a cell based cAMP reporter assay in HEK 293 cells and their TNF-α inhibitory activity was measured in lipopolysaccharide (LPS) stimulated RAW 264.7 cells.[19] Rolipram, a well known inhibitor of PDE4 was used as a reference compound in all these assays which showed 100% inhibition of PDE4B at 30 μM. Since compound **16** appeared as the promising inhibitor among all the compounds tested its dose dependent inhibition of TNF-α was

Table 1 *In vitro* data of compounds **10–16**

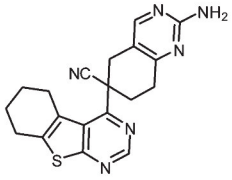
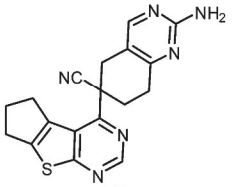
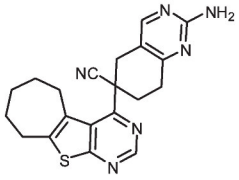
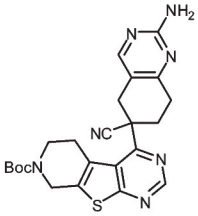
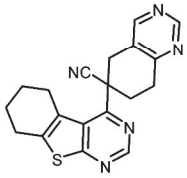
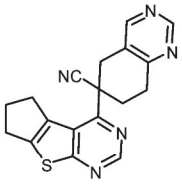
Compound	%PDE4B inhibition (30 μM)	IC_{50} (μM)	TNF-α inhibition (30 μM)	Fold elevation of cAMP[a]
 **10a**	80.0	4.48 ± 0.91	66.0	3.70
 **10b**	66.0	nd	nd	nd
 **10c**	70.4	5.64 ± 1.26	nd	3.09
 **10d**	78.9	3.24 ± 0.73	48.5	nd
 **11a**	80.0	4.51 ± 0.89	35.7	2.34
 **11b**	65.5	nd	nd	nd

Table 1 *(Contd.)*

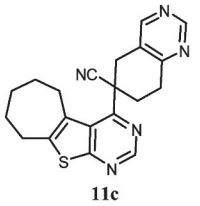
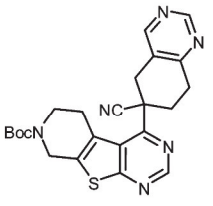
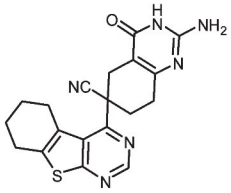
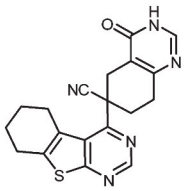
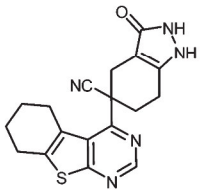
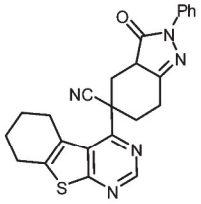
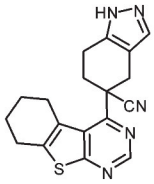
Compound	%PDE4B inhibition (30 μM)	IC_{50} (μM)	TNF-α inhibition (30 μM)	Fold elevation of cAMP[a]
 **11c**	74.8	6.19 ± 1.30	nd	nd
 **11d**	71.0	5.01 ± 0.56	32.6	nd
 **12**	68.0	nd	nd	nd
 **13**	70.7	nd	50.0	3.17
 **14**	51.4	nd	nd	nd
 **15**	41.9	nd	nd	nd

Table 1 *(Contd.)*

Compound	%PDE4B inhibition (30 μM)	IC_{50} (μM)	TNF-α inhibition (30 μM)	Fold elevation of cAMP[a]
 **16**	83.6	2.0 ± 0.41	87.4	3.90

[a] In a cell based reporter assay.

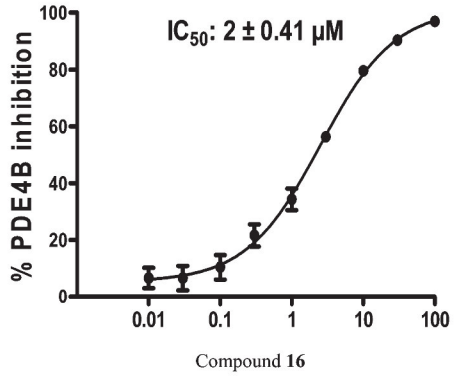


Fig. 7 Dose dependent inhibition of PDE4B by compound **16**.

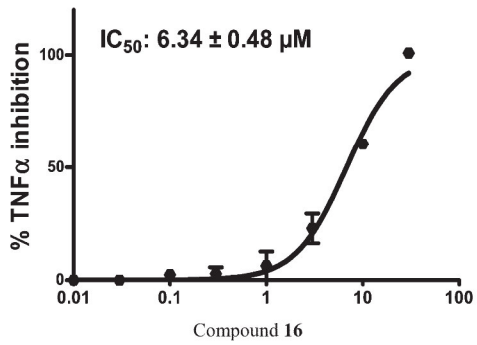


Fig. 8 Dose dependent inhibition of TNF-α by compound **16**.

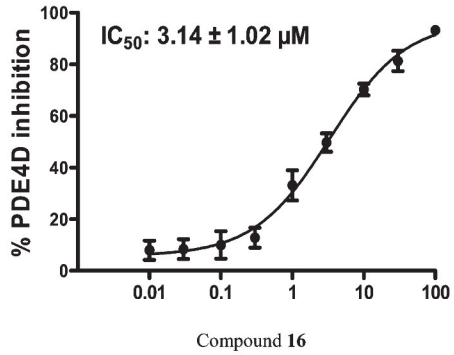


Fig. 9 Dose dependent inhibition of PDE4D by compound **16**.

also determined (Fig. 8). Further the PDE4D inhibitory potential of compound **16** was evaluated using the PDE4D enzyme assay (Fig. 9). Based on the PDE4B and D inhibitory data it is evident that compound **16** has 1.5 fold or balanced selectivity towards PDE4B. Notably potent inhibitor cilomilast (**D**, Fig. 2) showed reverse selectivity *i.e.* ~10 fold selectivity towards PDE4D over PDE4B and induced emesis at the first and/or second doses though this effect apparently disappeared with continued treatment.[19c] Overall, with respect to the *in vitro* data (PDE4 & TNF-α) compound **16** seemed to be comparable with phase 2 clinical candidate CC-1088 (PDE4 IC_{50} = 1.1 μM, TNF-α IC_{50} = 2.5 μM)[19b] of Celgene and was identified as a PDE4 inhibitor of further interest.

To understand the nature of interactions of this class of molecules with PDE4B a few selected molecules (only *R*-isomer) were docked[20] into the PDE4B protein (see ESI†). The results of this study showed good overall correlations to their observed PDE4B inhibitory properties *in vitro*. For example, due to the absence of an amine moiety on the pyrimidine ring though **11a** and **11b** showed different orientation of binding at the active site of PDE4B protein their overall interactions however were not better than **10a** or **10b** (compounds **11a** and **11d** showed better dock score but not better binding energy compared to **10a** and **10d** respectively). This is supported by the observed inhibition of PDE4B shown by compounds **10a**, **10d**, **11a** and **11d**.

Conclusions

The thieno[2,3-*d*]pyrimidine based library of small molecules containing a cyclohexane ring fused with a six- or five-membered heterocyclic moiety along with a benzylic nitrile was designed as potential inhibitors of PDE4. These molecules were prepared conveniently *via* a multi-step sequence consisting of a few key steps such as Gewald reaction, Dieckmann type cyclisation and Krapcho decarboxylation. A number of thieno[2,3-*d*]-pyrimidine based derivatives were synthesized and the molecular structure of a representative compound was established

unambiguously by single crystal X-ray diffraction. The crystal structure analysis of this compound provided an insight on the hydrogen bonding patterns and molecular arrangement present within the molecule. Many of these compounds were evaluated for their PDE4B inhibitory potential *in vitro*. Some of these compounds showed promising inhibition of PDE4B initially at a single dose and then subsequently in a dose dependent manner. One of them *i.e.* 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-4,5,6,7-tetrahydro-1*H*-indazole-5-carbonitrile was tested for PDE4D inhibition *in vitro* and dose dependent inhibition of TNF-α. The docking results of a few selected molecules showed good overall correlations to their observed PDE4B inhibitory properties *in vitro*. Overall, the strategy involving the sequential construction of the thienopyrimidine ring followed by the cyclohexanone moiety and subsequently the fused heterocyclic ring provided a new framework that appeared to be a promising template for the discovery of novel inhibitors of PDE4.

Experimental section

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, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were determined 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), t (triplet) q (quartet) and m (multiplet) as well as bs (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the conditions specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention time. The enantiomeric purity of some representative compounds *e.g.* **10a**, **13**, **14** and **16** was determined by using a chiral HPLC method.

Preparation of 2-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)acetonitrile (5a)

To a mixture of ethyl cyanoacetate (4.2 mL, 40.05 mmol) and K_2CO_3 (3.7 g, 26.70 mmol) was added compound **4a** (3 g, 13.35 mmol). The mixture was initially heated to 60 °C for 30 min and then at 140 °C for 1 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temperature and diluted with EtOAc (60 mL). The organic layer was collected, washed with water (2 × 30 mL) followed by brine solution (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate–hexane (1 : 6) to give the desired product **5a** (2.4 g, 78%) as a white solid; mp 164–166 °C; R_f = 0.45 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2931, 2853, 2256, 1539; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 4.27 (s, 2H), 2.99–2.92 (m, 4H), 1.97–1.96 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.8, 152.0, 151.0, 140.1, 129.0, 125.5, 115.7, 26.3, 26.0, 25.9, 22.4, 22.3; MS (ES mass): *m*/*z* 229.9 (M + 1); HPLC: 99.3%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 1.0 mL min^{-1}; UV 240 nm, retention time 5.34 min.

Preparation of 2-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]-pyrimidin-4-yl)acetonitrile (5b)

Compound **5b** was synthesized in 55% yield from **4b** following a similar procedure as presented above; white solid; mp: 205–207 °C; R_f = 0.6 (30% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2911, 2861, 2261, 1552; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.95 (s, 1H), 4.18 (s, 2H), 3.18–3.09 (m, 4H), 2.64–2.56 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 174.2, 152.1, 150.7, 145.6, 134.6, 125.9, 115.5, 30.0, 29.4, 27.6, 24.9; MS (ES mass): *m*/*z* 216.1 (M + 1).

Preparation of ethyl 2-cyano-2-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)acetate (5cc)

A mixture of **4c** (1 g, 0.42 mmol), ethyl cyanoacetate (0.04 mL, 0.42 mmol) and K_2CO_3 (86 mg, 0.63 mmol) in DMSO (10 mL) and water (1 mL) was heated at 120 °C for 1.5 h under anhydrous conditions. After completion of the reaction the mixture was cooled to room temp, diluted with water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate–hexane (1 : 6) to give the desired product (0.9 g, 70%) as a white solid; mp: 139–141 °C; R_f = 0.5 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2927, 2856, 2200, 1656; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.83 (bs, 1H), 8.41 (s, 1H), 4.23 (q, J = 4.5 Hz, 2H), 3.05–3.02 (m, 2H), 2.95–2.92 (m, 2H), 1.88–1.83 (m, 2H), 1.68–1.54 (m, 4H), 1.28 (t, J = 4.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.5, 162.1, 152.8, 141.9, 139.1, 136.7, 121.9, 119.4, 66.7, 61.3, 32.0, 30.6, 27.9, 27.0, 14.4, 14.3; MS (ES mass): 315.5 (M + 1).

Preparation of 2-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno-[2,3-*d*]pyrimidin-4-yl)acetonitrile (5c)

A mixture of compound **5cc** (1 g, 0.32 mmol) and NaCl (1.47 g, 2.53 mmol) in DMSO (10 mL) and water (1 mL) was heated at 150 °C for 4.5 h under anhydrous conditions. After completion of the reaction the mixture was cooled to room temp, diluted with water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue isolated was

purified by column chromatography using ethyl acetate–hexane (1 : 6) to give desired product (524 mg, 68%) as a white solid; mp: 133–135 °C; R_f = 0.5 (10% EtOAc–DCM); IR (KBr, cm^{-1}): 2925, 2853, 2254, 1533; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.93 (s, 1H), 4.31 (s, 2H), 3.10–3.08 (m, 2H), 3.01–2.99 (m, 2H), 2.00–1.99 (m, 2H), 1.85–1.78 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 167.6, 151.5, 150.9, 144.1, 131.0, 129.7, 115.6, 31.4, 29.9, 29.3, 26.9, 26.8, 26.5; MS (ES mass): 243.5 (M + 1).

Preparation of ethyl 2-cyano-2-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-yl)acetate (5dd)

The compound **5dd** was synthesized in 65% yield from **4d** following a similar procedure as presented above; light brown solid; mp: 128–130 °C; R_f = 0.3 (30% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2975, 2202, 1710, 1661; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 14.75 (s, 1H), 8.08 (d, J = 3.2 Hz, 1H), 4.72 (s, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.60–3.61 (m, 2H), 3.29–3.31 (m, 2H), 1.51 (s, 9H), 1.39 (t, J = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 179.3, 170.7, 152.5 (3C), 140.1 (2C), 134.0, 120.9, 80.6, 61.6, 43.4, 41.9, 31.0, 28.4 (3C), 25.9, 14.3. MS (ES mass): 401.1 (M + 1).

Preparation of *tert*-butyl 4-acetonitrilo-5,6,7,8-tetrahydropyrido-[4′,3′:4,5]thieno[2,3-*d*]pyrimidine-7-carboxylate (5d)

Compound **5d** was synthesized in 62% yield from **5dd** following a similar procedure as presented above; white solid; mp: 161–163 °C; R_f = 0.4 (40% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2976, 2257, 2190, 1683; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.99 (s, 1H), 4.77 (s, 2H), 4.26 (s, 2H), 3.84 (t, J = 5.6 Hz, 2H), 3.08–3.09 (m, 2H), 1.51 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.0, 154.2, 152.6 (3C), 128.3 (2C), 115.4, 80.9, 43.9, 40.0, 28.4 (3C), 25.9 (2C); MS (ES mass): 331.1 (M + 1).

Preparation of 4-cyano-4-(5,6,7,8-tetrahydrobenzo[*b*]thieno-[2,3-*d*]pyrimidin-4-yl)-heptanedioic acid dimethyl ester (6a)

To a solution of **5a** (2 g, 8.72 mmol) in acetonitrile (12.5 mL) was added 40% solution of triton-B (1 mL) and the mixture was heated to reflux under anhydrous conditions. To this was added methyl acrylate (7.9 mL, 87.22 mmol) in acetonitrile (12.5 mL) under refluxing conditions. The mixture was refluxed for 3 h. After completion of the reaction the mixture was cooled to room temperature and solvent as well as excess of methyl acrylate was evaporated under reduced pressure. The residue was dissolved in EtOAc (40 mL). The organic layer was washed with water (2 × 20 mL) followed by brine solution (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue isolated was purified by column chromatography using ethyl acetate–hexane (1 : 8) to give desired product **6a** (2.3 g, 65%) as a light yellow liquid; R_f = 0.5 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2946, 2861, 2236, 1738; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 3.67 (s, 6H), 3.22 (bs, 2H), 2.98 (bs, 2H), 2.86–2.79 (m, 2H), 2.69–2.61 (m, 2H), 2.53–2.45 (m, 2H), 2.37–2.29 (m, 2H), 1.96 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.4 (2C), 170.2, 156.4, 150.2, 140.1, 129.3, 126.3, 121.7, 51.9 (2C), 32.9 (2C), 29.8 (3C), 29.6, 26.6, 23.2, 22.2; MS (ES mass): 401.9 (M + 1).

Preparation of 4-cyano-4-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno-[2,3-*d*]pyrimidin-4-yl)-heptanedioic acid dimethyl ester (6b)

Compound **6b** was synthesized in 65% yield from **5b** following a similar procedure as described above; light yellow liquid; R_f = 0.7 (30% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2954, 2859, 2240, 1735; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 3.65 (s, 6H), 3.39–3.35 (m, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.81–2.74 (m, 2H), 2.62–2.45 (m, 6H), 2.41–2.33 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 175.3, 172.4 (2C), 156.3, 150.3, 146.4, 134.6, 125.7, 121.3, 51.9 (2C), 47.3, 33.5, 32.5 (2C), 30.1, 29.8 (2C), 28.1; MS (ES mass): 387.5 (M + 1).

Preparation of 4-cyano-4-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]-thieno[2,3-*d*]pyrimidin-4-yl)-heptanedioic acid dimethyl ester (6c)

Compound **6c** was synthesized in 68% yield from **5c** following a similar procedure as described above; light yellow liquid; R_f = 0.55 (10% EtOAc–DCM); IR (KBr, cm^{-1}): 2929, 2853, 2233, 1738; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 3.68 (s, 6H), 3.32–3.29 (m, 2H), 3.03–3.00 (m, 2H), 2.88–2.80 (m, 2H), 2.73–2.64 (m, 2H), 2.60–2.48 (m, 2H), 2.39–2.32 (m, 2H), 2.03–1.95 (m, 2H), 1.78–1.72 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.5 (2C), 168.9, 156.3, 149.9, 144.8, 132.1, 129.1, 120.8, 51.9 (2C), 32.4 (2C), 32.3, 31.0, 30.7, 29.8 (3C), 26.8, 26.7; MS (ES mass): 415.5 (M + 1).

Preparation of 4-cyano-4-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′: 4,5]thieno[2,3-*d*]pyrimidin-4-yl)-heptanedioic acid dimethyl ester (6d)

Compound **6d** was synthesized in 65% yield from **5d** following a similar procedure as described above; white solid; mp: 133–135 °C; R_f = 0.5 (35% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2976, 2236, 1736, 1686; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 4.79 (s, 2H), 3.80–3.78 (m, 2H), 3.67 (s, 6H), 3.35–3.33 (m, 2H), 2.85–2.78 (m, 2H), 2.69–2.62 (m, 2H), 2.54–2.46 (m, 2H), 2.38–2.31 (m, 2H), 1.52 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.3 (2C), 170.4, 152.9, 150.7 (3C), 128.4 (2C), 121.5, 80.8, 51.9 (2C), 46.4, 45.1, 32.7 (2C), 32.3, 29.7 (2C), 28.4 (3C), 28.0. MS (ES mass): 503.2 (M + 1).

Preparation of methyl 5-cyano-5-(5,6,7,8-tetrahydrobenzo[*b*]-thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (7a)

A cold solution of compound **6a** (2 g, 4.98 mmol) in dry DME (15 mL) was added slowly to a mixture of 60% NaH (359 mg, 14.96 mmol) in dry DME (15 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was heated at 60–70 °C for 2.5 h. After completion of the reaction, the mixture was quenched with ice cold 1 N hydrochloric acid (20 mL) and extracted with ethyl acetate (2 × 30 mL). The organic layers were collected, combined, washed with water (2 × 30 mL) followed by brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column

chromatography using ethyl acetate–hexane (1 : 9) to give the desired product **7a** (1.5 g, 82%) as a white solid; mp: 171–173 °C; R_f = 0.6 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 3267, 2951, 2230, 1657; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.25 (s, OH), 8.89 (s, 1H), 3.81 (s, 3H), 3.30–3.26 (m, 3H), 3.05–3.00 (m, 3H), 2.93–2.85 (m, 1H), 2.63–2.58 (m, 2H), 2.44–2.37 (m, 1H), 1.98 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.8, 170.6, 169.7, 157.7, 150.4, 140.0, 129.1, 126.2, 121.7, 94.5, 51.8, 42.2, 32.8, 31.3, 29.2, 26.8, 26.6, 23.2, 22.3; MS (ES mass): 369.9 (M + 1); HPLC: 98.9%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/80, 2/80, 9/98, 12/98, 15/80, 18/80; flow rate: 1.0 mL min^{-1}; UV 245 nm, retention time 4.37 min.

Preparation of methyl 5-cyano-5-(6,7-dihydro-5*H*-cyclopenta-[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (7b)

Compound **7b** was synthesized in 70% yield from **6b** following a procedure similar to that of compound **7a**; white solid; mp: 153–155 °C; R_f = 0.8 (30% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 3535, 2955, 2235, 1656; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.26 (bs, 1H), 8.90 (s, 1H), 3.81 (s, 3H), 3.41–3.36 (m, 2H), 3.25 (d, J = 16.0 Hz, 1H), 3.12 (t, J = 7.2 Hz, 2H), 2.97 (d, J = 16.5 Hz, 1H), 2.92–2.84 (m, 1H), 2.64–2.53 (m, 4H), 2.44–2.36 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 174.9, 171.8, 170.6, 157.4, 150.6, 146.2, 134.8, 125.8, 121.6, 94.4, 51.8, 41.9, 33.2, 32.3, 30.4, 30.1, 28.2, 26.6; MS (ES mass): 355.4 (M + 1).

Preparation of methyl 5-cyano-5-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclo-hexanecarboxylate (7c)

Compound **7c** was synthesized in 72% yield from **6c** following a procedure similar to that of compound **7a**; white solid; mp: 166–168 °C; R_f = 0.7 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 3482, 2931, 2232, 1656; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.26 (bs, 1H), 8.90 (s, 1H), 3.80 (s, 3H), 3.39–3.26 (m, 3H), 3.04–2.98 (m, 3H), 2.94–2.85 (m, 1H), 2.66–2.60 (m, 2H), 2.46–2.38 (m, 1H), 2.01–1.98 (m, 2H), 1.78–1.70 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.8, 170.7, 168.5, 157.6, 150.3, 144.7, 132.1, 128.9, 121.0, 94.5, 51.8, 42.0, 32.5, 32.4, 30.9(2C), 30.6, 26.9, 26.8, 26.7; MS (ES mass): 384.2 (M + 1).

Preparation of methyl 5-cyano-5-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-yl)-2-oxocyclohexanecarboxylate (7d)

Compound **7d** was synthesized in 55% yield from **6d** using dry THF as a solvent following a procedure similar to that of compound **7a**; white solid; mp: 189–191 °C; R_f = 0.5 (40% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2975, 2232, 1727, 1695, 1664; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.25 (bs, 1OH), 8.94 (s, 1H), 4.81 (s, 2H), 3.81 (s, 5H), 3.42–3.35 (m, 2H), 3.26 (d, J = 16.0 Hz, 1H), 2.98 (d, J = 16.0 Hz, 1H), 2.92–2.85 (m, 1H), 2.65–2.58 (m, 2H), 2.47–2.39 (m, 1H), 1.52 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.7, 170.6, 169.9, 158.3, 154.2, 151.0 (2C), 128.2 (2C), 121.5, 94.3, 80.7, 51.9, 42.1 (2C), 37.0, 32.7, 30.9, 29.6, 28.4 (3C), 26.7. MS (ES mass): 471.2 (M + 1). HPLC: 95.7%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 5 mM ammonium acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/95, 13/95, 15/70, 18/70; flow rate: 1.0 mL min^{-1}; UV 240 nm, retention time 5.29 min.

Preparation of 1-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (8a)

A mixture of **7a** (0.5 g, 1.35 mmol) and NaCl (628 mg, 10.84 mmol) in DMSO (5 mL) and water (0.5 mL) was heated at 150 °C for 5 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temp, diluted with water (25 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were collected, combined, washed with brine solution (15 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The isolated residue was purified by column chromatography using ethyl acetate–hexane (1 : 6) to give desired product **8a** (240 mg, 58%) as a white solid; mp: 167–169 °C; R_f = 0.5 (30% EtOAc–hexane); IR (KBr, cm^{-1}): 2947, 2883, 2233, 1713; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s,1H), 3.28–3.26 (m, 2H), 3.01–2.99 (m, 2H), 2.96–2.87 (m, 2H), 2.87–2.76 (m, 2H), 2.67–2.56 (m, 4H), 1.99 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 206.8, 169.8, 157.2, 150.4, 140.4, 129.1, 125.9, 121.5, 43.3, 37.8 (2C), 35.5 (2C), 29.3, 26.6, 23.3, 22.3; MS (ES mass): 311.9 (M + 1); HPLC: 98.4%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/90, 14/90, 16/50, 20/50; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 7.74 min.

Preparation of 1-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]-pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (8b)

Compound **8b** was synthesized in 62% yield from **7b** following a procedure similar to that of compound **8a**; white solid; mp: 152–154 °C; R_f = 0.5 (30% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2960, 2909, 2234, 1710; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 3.42 (t, J = 7.2 Hz, 2H), 3.12 (t, J = 7.2 Hz, 2H), 2.95–2.87 (m, 2H), 2.78–2.72 (m, 2H), 2.69–2.63 (m, 2H), 2.61–2.53 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 206.8, 174.9, 156.8, 150.6, 146.5, 134.6, 125.8, 121.3, 42.9, 37.8 (2C), 34.9 (2C), 33.2, 30.1, 28.2; MS (ES mass): 297.5 (M + 1).

Preparation of 1-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno-[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (8c)

Compound **8c** was synthesized in 65% yield from **7c** following a procedure similar to that of compound **8a**; white solid; mp: 144–146 °C; R_f = 0.5 (25% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2916, 2856, 2232, 1720; ^{1}H NMR (400 Hz, $CDCl_3$) δ: 8.89 (s, 1H), 3.37–3.34 (m, 2H), 3.05–3.02 (m, 2H), 2.96–2.87 (m, 2H), 2.80–2.76 (m, 2H), 2.67–2.55 (m, 4H), 2.04–1.98 (m, 2H), 1.81–1.74 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 206.8, 168.6, 157.0, 150.2, 144.9, 131.8, 128.9, 120.7, 43.1,

37.8 (2C), 35.1 (2C), 32.3, 30.9, 30.6, 26.9, 26.7; MS (ES mass): 326.2 (M + 1).

Preparation of 1-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (8d)

Compound **8d** was synthesized in 58% yield from **7d** following a procedure similar to that of compound **8a**; white solid; mp: 259–261 °C; R_f = 0.4 (40% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 2973, 2935, 2235, 1699; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 4.75 (s, 2H), 3.76 (t, J = 4.8 Hz, 2H), 3.33 (bs, 2H), 2.88–2.80 (m, 2H), 2.71–2.67 (m, 2H), 2.61–2.49 (m, 4H), 1.45 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 206.6, 170.1, 150.9, 150.1, 132.1, 129.8, 128.7, 126.9, 121.2, 80.9, 43.7, 43.2, 37.7 (2C), 35.2 (2C), 29.6, 28.4 (3C), 28.3. MS (ES mass): 413.2 (M + 1). HPLC: 97.6%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 5 mM ammonium acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 13/95, 15/50, 18/50; flow rate: 1.0 mL min^{-1}; UV 240 nm, retention time 6.72 min.

Preparation of 3-[(dimethylamino)methylene]-1-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (9a)

A mixture of **8a** (0.5 g, 1.60 mmol), DMF-DMA (0.8 mL, 6.43 mmol) and Et_3N (0.7 mL, 4.82 mmol) in dry DMF (5 mL) was heated to 110 °C for 4 h under a nitrogen atmosphere. After completion of the reaction the mixture was cooled to room temp, diluted with water (25 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were collected, combined, washed with brine solution (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane (4 : 1) to give desired product **9a** (0.4 g, 64%) as a brown solid; mp: 214–216 °C; R_f = 0.2 (100% EtOAc); IR (KBr, cm^{-1}): 2947, 2230, 1735, 1647; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1H), 7.69 (s, 1H), 3.61 (s, 2H), 3.41–3.37 (m, 1H), 3.17 (s, 6H), 3.13–3.08 (m, 1H), 2.99 (s, 2H), 2.87–2.77 (m, 1H), 2.72–2.62 (m, 1H), 2.60–2.53 (m, 1H), 2.36–2.28 (m, 1H), 1.97 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 193.7, 169.7, 158.4, 152.4, 150.4, 139.9, 129.1, 126.2, 122.2, 98.9, 43.7, 43.7, 43.2, 35.6, 34.6, 32.9, 29.1, 26.5, 23.2, 22.3; MS (ES mass): 367.0 (M + 1).

Preparation of 3-[(dimethylamino)methylene]-1-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (9b)

Compound **9b** was synthesized in 58% yield from **8b** following a procedure similar to that of compound **9a**; light brown solid; mp: 227–229 °C; R_f = 0.1 (100% EtOAc); IR (KBr, cm^{-1}): 2947, 2232, 1646, 1541; ^{1}H NMR (400 Hz, $CDCl_3$) δ: 8.89 (s, 1H), 7.70 (s, 1H), 3.57 (s, 2H), 3.51–3.43 (m, 1H), 3.34–3.25 (m, 1H), 3.17 (s, 6H), 3.11 (t, J = 7.2 Hz, 2H), 2.88–2.80 (m, 1H), 2.67–2.52 (m, 4H), 2.38–2.30 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 193.6, 174.8, 158.1, 152.7, 150.6, 146.1, 134.9, 125.8, 122.0, 98.8, 43.8, 42.9, 34.8, 34.5, 33.1, 32.4, 30.1 (2C), 28.2; MS (ES mass): 353.2 (M + 1).

Preparation of 3-[(dimethylamino)methylene]-1-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (9c)

A mixture of **8c** (0.2 g, 0.615 mmol) and DMF-DMA (0.16 mL, 1.23 mmol) in toluene (5 mL) was heated to 95 °C for 16 h under anhydrous conditions. After completion of the reaction, the mixture was cooled to room temp and the solvent was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, combined, 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 (4 : 1) to give desired product **9c** (105 mg, 45%) as a white solid; mp: 185–187 °C; R_f = 0.1 (100% EtOAc); IR (KBr, cm^{-1}): 2920, 2851, 2228, 1646; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 7.71 (s, 1H), 3.61 (s, 2H), 3.33 (t, J = 5.6 Hz, 2H), 3.17 (s, 6H), 3.03–3.01 (m, 2H), 2.88–2.78 (m, 1H), 2.68–2.56 (m, 2H), 2.39–2.30 (m, 1H), 2.04–1.96 (m, 2H), 1.78–1.74 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 193.7, 168.4, 158.3, 152.7, 150.2, 144.5, 132.2, 128.9, 121.5, 99.0, 43.8, 43.2, 35.6, 34.6, 32.5 (2C), 32.4, 30.8, 30.6, 27.1, 26.7; MS (ES mass): 381.2 (M + 1).

Preparation of 3-[(dimethylamino)methylene]-1-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno-[2,3-*d*]pyrimidin-4-yl)-4-oxocyclohexanecarbonitrile (9d)

Compound **9d** was synthesized in 45% yield from **8d** following a procedure similar to that of compound **9c**; brown solid; mp: 113–115 °C; R_f = 0.1 (100% EtOAc); IR (KBr, cm^{-1}): 2976, 2237, 1729, 1696; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.67 (s, 1H), 4.80 (s, 2H), 3.83 (bs, 2H), 3.60 (bs, 3H), 3.39–3.29 (m, 2H), 3.08 (s, 3H), 3.03 (s, 3H), 2.71–2.63 (m, 3H), 1.52 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 193.5, 169.9, 159.0, 152.7 (2C), 150.9 (2C), 128.2, 122.1, 121.2, 98.7, 80.8, 43.8, 43.2, 42.3, 35.4, 34.6, 32.8, 31.0, 29.2, 28.4 (3C), 28.3. MS (ES mass): 468.2 (M + 1).

Preparation of 2-amino-6-(5,6,7,8-tetrahydrobenzo[*b*]thieno-[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (10a)

A mixture of **9a** (0.1 g, 0.27 mmol), guanidine HCl (24.1 mg, 0.41 mmol) and NaOMe (22 mg, 0.41 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction the excess of sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, combined, 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 (4 : 1) to give desired product **10a** (80 mg, 78%) as a light brown solid; mp: 153–155 °C; R_f = 0.35 (100% EtOAc); IR (KBr, cm^{-1}): 3320, 3172, 2937, 2235; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.17 (s, 1H), 5.15 (s, 2H), 3.67 (d, J = 16.1 Hz, 1H), 3.43–3.36 (m, 2H), 3.26–3.16 (m, 2H), 3.01–2.91 (m, 3H), 2.79–2.75 (m, 1H), 2.45–2.37 (m, 1H), 1.99 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.8, 163.9, 161.9, 158.6, 157.5, 150.4, 140.3, 129.1, 126.1, 121.5, 115.7, 42.3, 35.9, 32.5, 29.3, 29.2, 26.6, 23.3, 22.3; MS (ES mass): 362.9 (M + 1); HPLC: 99.3%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN (isocratic) (A : B) 40 : 60; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 2.9 min; chiral HPLC: column: chiral pak IC (250 × 4.6 mm) 5 µm, mobile phase: A: MeOH: B: 0.1% DEA, flow: 1.0 mL min^{-1}, wavelength: 245 nm, retention time (area %): 16.9 min (50%) and 19.4 min (50%).

Preparation of 2-amino-6-(6,7-dihydro-5*H*-cyclopenta-[4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (10b)

Compound **10b** was synthesized in 70% yield from **9b** following a procedure similar to that of compound **10a**; white solid; mp: 200–202 °C; R_f = 0.3 (100% EtOAc); IR (KBr, cm^{-1}): 3318, 3161, 2950, 2242; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.16 (s, 1H), 5.10 (bs, 2H), 3.62 (d, J = 16.0 Hz, 1H), 3.52–3.45 (m, 1H), 3.40–3.36 (m, 1H), 3.34–3.17 (m, 2H), 3.15–3.11 (m, 2H), 2.96–2.89 (m, 1H), 2.78–2.72 (m, 1H), 2.62–2.54 (m, 2H), 2.46–2.38 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.3, 163.9, 161.7, 158.6, 157.0, 150.6, 146.5, 134.7, 125.8, 121.4, 115.5, 41.9, 34.7, 33.2, 31.9, 30.1, 29.7, 28.9; MS (ES mass): 349.1 (M + 1); HPLC: 90.7%, column: X Bridge C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/30, 2/30, 9/95, 12/95, 15/30, 18/30; flow rate: 0.8 mL min^{-1}; UV 241 nm, retention time 7.0 min.

Preparation of 2-amino-6-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]-thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (10c)

Compound **10c** was synthesized in 68% yield from **9c** following a procedure similar to that of compound **10a**; white solid; mp: 234–236 °C; R_f = 0.2 (100% EtOAc); IR (KBr, cm^{-1}): 3456, 3314, 2930, 2232; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.90 (s, 1H), 8.15 (s, 1H), 5.17 (s, 2H), 3.64 (d, J = 16.4 Hz, 1H), 3.44–3.17 (m, 4H), 3.04 (t, J = 5.4 Hz, 2H), 2.99–2.92 (m, 1H), 2.81–2.76 (m, 1H), 2.45–2.37 (m, 1H), 2.05–1.97 (m, 2H), 1.79–1.76 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.6, 164.1, 161.9, 158.6, 157.3, 150.3, 144.9, 131.9, 128.9, 120.8, 115.7, 42.1, 35.7, 32.4, 32.1, 30.9, 30.6, 29.1, 26.9, 26.7; MS (ES mass): 377.1 (M + 1); HPLC: 99.1%, column: X Bridge C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.1% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 4.5 min.

Preparation of 2-amino-6-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (10d)

Compound **10d** was synthesized in 55% yield from **9d** following a procedure similar to that of compound **10a**; light yellow solid; mp: 141–143 °C; R_f = 0.3 (100% EtOAc); IR (KBr, cm^{-1}): 3327, 3194, 2971, 2235, 1696; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.92 (s, 1H), 8.21 (s, 1H), 5.82 (s, 2H), 4.82 (s, 2H), 3.89–3.77 (m, 2H), 3.72 (d, J = 16.1 Hz, 1H), 3.57–3.46 (m, 1H), 3.40 (d, J = 16.3 Hz, 1H), 3.31–3.19 (m, 2H), 3.03–2.91 (m, 1H), 2.81–2.71 (m, 1H), 2.49–2.39 (m, 1H), 1.52 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 175.3, 170.1, 163.8, 161.2 (2C), 158.3, 154.2, 151.0 (2C), 128.2, 121.3, 115.4, 80.9, 42.1, 35.4, 32.1, 31.5, 28.7, 28.4 (3C), 28.3, 26.9. MS (ES mass): 464.2 (M + 1). HPLC: 97.9%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 5 mM ammonium acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 13/95, 15/20, 18/20; flow rate: 1.0 mL min^{-1}; UV 240 nm, retention time 8.6 min.

Preparation of 6-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (11a)

Compound **11a** was synthesized in 45% yield from **9a** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **10a**; brown solid; mp: 182–184 °C; R_f = 0.45 (100% EtOAc); IR (KBr, cm^{-1}): 2941, 2868, 2234, 1557; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.86 (s, 1H), 8.61 (s, 1H), 3.99 (d, J = 16.9 Hz, 1H), 3.54 (d, J = 16.9 Hz, 1H), 3.46–3.34 (m, 2H), 3.15–3.02 (m, 4H), 2.86–2.82 (m, 1H), 2.51–2.43 (m, 1H), 2.05–1.99 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.9, 162.9, 157.1, 157.0, 156.7, 150.4, 140.6, 129.0, 126.7, 125.9, 121.4, 41.7, 36.3, 32.3, 29.3, 29.1, 26.6, 23.2, 22.2; MS (ES mass): 347.9 (M + 1); HPLC: 98.5%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN (Isocratic) (A:B) 40 : 60; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 4.2 min.

Preparation of 6-(6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]-pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (11b)

Compound **11b** was synthesized in 78% yield from **9b** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **10a**; white solid; mp: 248–250 °C; R_f = 0.5 (100% EtOAc); IR (KBr, cm^{-1}): 2954, 2863, 2243, 1535; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.05 (s, 1H), 8.86 (s, 1H), 8.60 (s, 1H), 3.91 (d, J = 16.8 Hz, 1H), 3.57–3.49 (m, 2H), 3.41–3.25 (m, 2H), 3.13 (t, J = 7.2 Hz, 2H), 3.08–3.01 (m, 1H), 2.84–2.77 (m, 1H), 2.65–2.54 (m, 2H), 2.51–2.44 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 175.0, 162.9, 157.2, 157.1, 156.3, 150.5, 146.8, 134.6, 126.5, 125.7, 121.2, 41.3, 34.9, 33.2, 31.9, 30.2, 28.9, 28.2; MS (ES mass): 334.1 (M + 1); HPLC: 97.9%. column: X Bridge C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient

(T/%B): 0/30, 2/30, 9/95, 12/95, 15/30, 18/30; flow rate: 0.8 mL min^{-1}; UV 241 nm, retention time 7.9 min.

Preparation of 6-(6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno-[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydroquinazoline-6-carbonitrile (11c)

Compound **11c** was synthesized in 67% yield from **9c** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **10a**; white solid; mp: 157–159 °C; R_f = 0.4 (100% EtOAc); IR (KBr, cm^{-1}): 2934, 2853, 2230, 1551; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.06 (s, 1H), 8.88 (s, 1H), 8.61 (s, 1H), 3.97 (d, J = 16.8 Hz, 1H), 3.55 (d, J = 16.8 Hz, 1H), 3.43–3.28 (m, 3H), 3.13–3.00 (m, 3H), 2.87–2.84 (m, 1H), 2.50–2.42 (m, 1H), 2.10–1.95 (m, 2H), 1.80–1.77 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 168.7, 163.0, 157.2, 157.1, 156.6, 150.2, 145.2, 131.8, 128.9, 126.7, 120.6, 41.6, 36.1, 32.4, 31.9, 30.9, 30.6, 29.1, 26.9, 26.7; MS (ES mass): 362.1 (M + 1); HPLC: 97.3%. column: X Bridge C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.1% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 6.4 min.

Preparation of 6-(7-(*tert*-butoxycarbonyl)-5,6,7,8-tetrahydro-pyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-yl)-5,6,7,8-tetrahydro-quinazoline-6-carbonitrile (11d)

Compound **11d** was synthesized in 50% yield from **9d** and formimidine acetate (1.5 mmol) following a procedure similar to that of compound **10a**: brown solid; mp: 191–193 °C; R_f = 0.5 (100% EtOAc); IR (KBr, cm^{-1}): 2975, 2930, 2228, 1698; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.11 (s, 1H), 8.89 (s, 1H), 8.72 (m, 1H), 4.84 (s, 2H), 4.08 (d, J = 16.0 Hz, 1H), 3.83 (s, 2H), 3.66–3.51 (m, 2H), 3.51–3.37 (m, 1H), 3.35–3.03 (m, 2H), 2.89–2.77 (m, 1H), 2.60–2.48 (m, 1H), 1.52 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.3 (2C), 163.3, 156.8, 155.9, 154.2, 150.9 (3C), 128.1, 121.1, 109.9, 80.9, 41.5, 35.9, 32.0 (2C), 28.8 (2C), 28.4 (3C), 28.3; MS (ES mass): 449.1 (M + 1). HPLC: 98.2%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 5 mM ammonium acetate in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 13/95, 15/50, 18/50; flow rate: 0.8 mL min^{-1}; UV 240 nm, retention time 6.6 min.

Preparation of 2-amino-6-(5,6,7,8-tetrahydrobenzo[*b*]thieno-[2,3-*d*]pyrimidin-4-yl)-4-oxo-3,4,5,6,7,8-hexahydroquinazoline-6-carbonitrile (12)

A mixture of **7a** (0.1 g, 0.27 mmol), guanidine HCl (48 mg, 0.81 mmol) and NaOMe (73 mg, 1.35 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction the excess sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, combined, 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 methanol–DCM (1 : 19) to give desired product **12** (73 mg, 72%) as a white solid; mp: 279–281 °C; R_f = 0.5 (10% MeOH–DCM); IR (KBr, cm^{-1}): 3448, 3314, 3125, 2940, 2229, 1650; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.8 (bs, 1H), 9.04 (s, 1H), 6.46 (bs, 2H), 3.28–3.26 (m, 3H), 3.23–3.21 (m, 3H), 2.81–2.63 (m, 3H), 2.39–2.36 (m, 1H), 1.94 (bs, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 168.8, 162.9, 158.3, 154.1, 150.6, 145.1, 139.7, 128.4, 126.1, 122.1, 104.4, 42.2, 32.0, 31.6, 28.9, 28.5, 25.9, 22.7, 21.8; MS (ES mass): 378.9 (M + 1); HPLC: 97.4%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 1.0 mL min^{-1}; UV 246 nm, retention time 6.2 min.

Preparation of 6-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-4-oxo-3,4,5,6,7,8-hexahydroquinazoline-6-carbonitrile (13)

Compound **13** was synthesized in 68% yield from **7a** and formimidine acetate (3 mmol) following a procedure manner similar to that of compound **12**; white solid; mp: 202–204 °C; R_f = 0.6 (10% MeOH–DCM); IR (KBr, cm^{-1}): 3153, 2943, 2233, 1655; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 8.12 (s, 1H), 3.58 (d, J = 17.6 Hz, 1H), 3.46–3.34 (m, 2H), 3.26–3.18 (m, 2H), 3.01 (s, 2H), 2.95–2.89 (m, 1H), 2.78–2.74 (m, 1H), 2.55–2.47 (m, 1H), 1.99–1.98 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.8, 163.5, 160.6, 157.2, 150.4, 145.9, 140.2, 129.1, 126.1, 121.7, 119.1, 41.5, 32.4, 31.8, 29.2, 29.1, 26.6, 23.2, 22.2; MS (ES mass): 363.9 (M + 1); HPLC: 98.6%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 0.8 mL min^{-1}; UV 244 nm, retention time 8.1 min; chiral HPLC: column: chiral pak AD (250 × 4.6 mm) 3 µm, mobile phase: A: *n*-hexane: B: 0.1% IPA, flow: 0.8 mL min^{-1}, wave length: 245 nm, retention time (area %): 12.6 min (49.5%) and 15.8 min (50.5%).

Preparation of 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-3-oxo-2,3,4,5,6,7-hexahydro-1*H*-indazole-5-carbonitrile (14)

A mixture of **7a** (0.1 g, 0.27 mmol), hydrazine (0.03 mL, 0.54 mmol) and Et_3N (0.09 mL, 0.81 mmol) in methanol (8 mL) was stirred at 80 °C for 1 h under nitrogen. After completion of the reaction methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The isolated residue was purified by column chromatography using methanol–DCM (1 : 49) to give desired product **14** (75 mg, 76%) as a white solid; mp: 271–273 °C; R_f = 0.5 (5% MeOH–DCM); IR (KBr, cm^{-1}): 3231, 2944, 2234, 1734; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.31 (bs, 1H), 9.61 (bs, 1H), 9.00 (s, 1H), 3.22–3.15 (m, 4H), 3.02 (s, 2H), 2.87 (s, 2H), 2.66–2.62 (m, 1H), 2.38–2.28 (m, 1H), 1.91 (s, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 168.7, 158.5, 150.5, 150.3, 139.6, 137.9, 128.3, 126.1, 122.1, 109.5, 43.6, 32.3, 30.5, 28.5, 25.9,

22.7, 21.8, 19.4; MS (ES mass): 351.9 (M + 1); HPLC: 97.7%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 7.9 min; chiral HPLC: column: Lux Cellulose-2 (250 × 4.6 mm) 3 µm, mobile phase: A: *n*-hexane: D: 0.1% TFA in EtOH, flow: 0.8 mL min^{-1}, wavelength: 245 nm, retention time (area %): 16.9 min (45.7%) and 20.9 min (49.8%).

Preparation of 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-3-oxo-2-phenyl-3,3a,4,5,6,7-hexahydro-2*H*-indazole-5-carbonitrile (15)

Compound **15** was prepared in 65% yield from **7a** and phenyl hydrazine (2 mmol) following a procedure similar to compound **14**; white solid; mp: 218–220 °C; R_f = 0.3 (70% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 3062, 2861, 2237, 1730; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.22–7.13 (m, 1H), 3.41–3.33 (m, 2H), 3.27–3.17 (m, 2H), 3.09–2.84 (m, 5H), 2.76–2.65 (m, 1H), 2.40–2.33 (m, 1H), 2.01–1.94 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.8, 159.2, 157.9, 150.4, 148.1, 140.1, 129.1, 128.9, 128.9 (2C), 126.3, 125.7, 121.7, 120.3, 118.9, 42.8, 37.3, 32.1, 30.5, 29.2, 26.6, 23.2, 22.2, 20.4; MS (ES mass): 427.9 (M + 1); HPLC: 97.9%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.05% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL min^{-1}; UV 245 nm, retention time 5.9 min.

Preparation of 5-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]-pyrimidin-4-yl)-4,5,6,7-tetrahydro-1*H*-indazole-5-carbonitrile (16)

A mixture of **9a** (0.1 g, 0.27 mmol) and hydrazine (0.02 mL, 0.41 mmol) in methanol (5 mL) was stirred at 80 °C for 1 h under nitrogen. Then, methanol was removed under reduced pressure. The residue was diluted with water (25 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were collected, combined, washed with brine solution (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–*n*-hexane (3 : 2) to give desired product **16** (65 mg, 72%) as a light brown solid; mp: 109–111 °C; R_f = 0.3 (70% EtOAc–*n*-hexane); IR (KBr, cm^{-1}): 3647, 3248, 2230, 1513; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H), 7.54 (s, 1H), 6.45 (bs, 1H), 3.61 (d, J = 16.0 Hz, 1H), 3.48 (d, J = 16.0 Hz, 1H), 3.38–3.09 (m, 4H), 3.00 (bs, 2H), 2.83–2.78 (m, 1H), 2.48–2.43 (m, 1H), 1.98 (bs, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.5, 158.2, 150.2 (2C), 139.8 (2C), 129.0, 126.1 (2C), 121.8, 43.5, 33.4, 31.9, 29.1, 26.4, 23.1, 22.1, 20.1. MS (ES mass): 336.2 (M + 1); HPLC: 99.1%, column: ZORBAX XDB C-18 150 × 4.6 mm 5 µ, mobile phase A: 0.1% formic acid in water, mobile phase B: CH_3CN, gradient (T/%B): 0/20, 2/20, 9/95, 13/95, 15/20, 18/20; flow rate: 1.0 mL min^{-1}; UV 245 nm, retention time 8.5 min. Chiral HPLC: column: chiral pak IC (250 × 4.6 mm) 5 µm, mobile phase: A: MeOH: B: 0.1% DEA, flow : 1.0 mL min^{-1}, wave length: 295 nm, retention time (area %): 8.8 min (50%) and 10.9 min (50%).

Single crystal X-ray data for compound 7a and 10a

Single crystals suitable for X-ray diffraction of **7a** and **10a** were grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα 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.[21] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[22]

Crystal data of **7a**: Molecular formula = $C_{19}H_{19}N_3O_3S$, formula weight = 369.44, crystal system = triclinic, space group = $P\bar{1}$, a = 11.092 (5) Å, b = 11.448 (5) Å, c = 15.672 (7) Å, V = 1761.7 (13) $Å^3$, T = 296 K, Z = 4, D_c = 1.401 Mg m^{-3}, μ(Mo-Kα) = 0.21 mm^{-1}, 20 533 reflections measured, 7395 independent reflections, 5076 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.081, goodness of fit = 1.003. CCDC 864130.

Crystal data of **10a**: Molecular formula = $C_{19}H_{18}N_6S$, formula weight = 362.13, crystal system = triclinic, space group = $P\bar{1}$, a = 7.625 (4) Å, b = 10.1757 (5) Å, c = 12.243 (6) Å, V = 903.66 (8)$Å^3$, T = 296 K, Z = 6, D_c = 1.387 Mg m^{-3}, μ(Mo-Kα) = 0.21 mm^{-1}, 15 612 reflections measured, 3949 independent reflections, 3342 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.029, goodness of fit = 0.876. CCDC 864129.

Pharmacology

Materials and methods

Cells and reagents. HEK 293 and Sf9 cells were obtained from ATCC (Washington, DC, USA). HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC and routinely cultured in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc.). cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDE4B1 clone was from OriGene Technologies (Rockville, MD, USA). PDE4D2 enzyme was purchased from BPS Bioscience (San Diego, CA, USA). Lipopolysaccharide (LPS) was from *Escherichia coli* strain 0127:B8 obtained from Sigma (St. Louis, MO, USA). Mouse TNF-α ELISA kit was procured from R&D Systems (Minneapolis, MN, USA).

Evaluation of PDE4 inhibitory potential by cell based cAMP reporter assay. One day prior to transfection, HEK 293 cells were seeded in p60 cell culture dish (Tarsons Inc.). These were

transfected using Lipofectamine 2000 (as per the manufacturer's instructions) with 2.4 μg of PDE4B1 expression plasmid and 4.0 μg of pCRELuc plasmid. After 5 h of transfection, medium was aspirated, cells were trypsinized and seeded in 96 well plates at a density of 60 000 cells per well. Plates were incubated overnight in a CO_2 incubator set to 37 °C and 5% CO_2. Twenty four hours post transfection, cells were pre-treated with various concentrations (0.001 to 30 μM) of compounds for 30 min, followed by stimulation with 5 μM forskolin for 4 h. Subsequently medium was removed and cells were lysed in reporter lysis buffer (Promega Inc) for 15 min with gentle rocking at RT. Luciferase activity in the lysates was measured by a Multilabel Plate Reader (Perkin Elmer 1420 Multilabel Counter). Fold elevation of cAMP is calculated using the following formula.

$$\text{Fold activation} = \frac{(\text{RLU of compound} - \text{Rlu of vehicle control})}{(\text{Rlu of forskolin} - \text{RLU of vehicle control})}$$

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 °C 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 °C. After incubation, lysate–Ni-NTA mixture was centrifuged at 4500 rpm for 5 min at 4 °C 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 °C in 50% glycerol until further use.

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 min 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, USA). IC_{50} values are presented as mean ± SD.

$$\%\,\text{inhibition} = \frac{(\text{RLU of vehicle control} - \text{Rlu of inhibitor})}{\text{RLU of vehicle control}} \times 100$$

TNF-α production assay. RAW 264.7 cells were pre-incubated either with DMSO (vehicle control) or compound for 30 min and then stimulated with 1 μg mL^{-1} of LPS overnight. Dose response studies were carried out at eight different concentrations (30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 μM). Post-stimulation, cell supernatants were harvested, centrifuged to clear cell debris and the amount of TNF-α in the supernatants was measured using mouse TNF-α DuoSet ELISA kit from R&D Systems according to manufacturer's recommendations. The percentage of inhibition was calculated using the following formula:

$$\%\,\text{inhibition} = 100 - \left[\frac{(\text{LPS stimulated}_{\text{compound}} - \text{unstimulated})}{(\text{LPS stimulated}_{\text{DMSO}} - \text{unstimulated})}\right] \times 100$$

The IC_{50} values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, USA). IC_{50} values are expressed as mean ± SD.

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Facile assembly of two 6-membered fused *N*-heterocyclic rings: a rapid access to novel small molecules *via* Cu-mediated reaction†

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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.

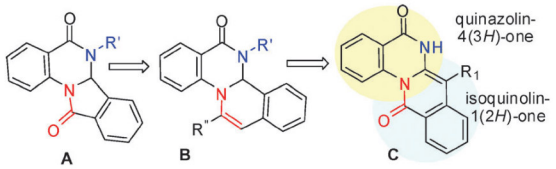
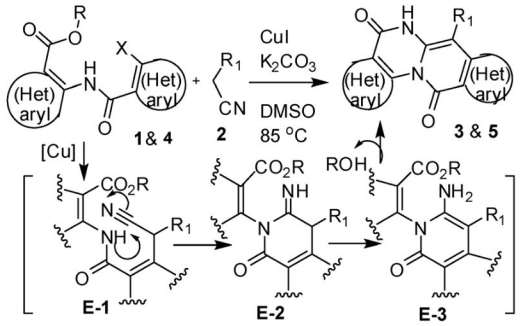


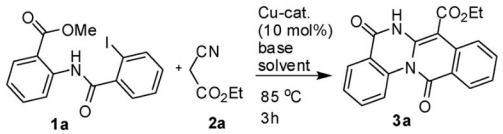
Fig. 1 Design of novel PDE-4/TNF-α inhibitors (**C**) derived from **A/B**.[3a,b]



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.

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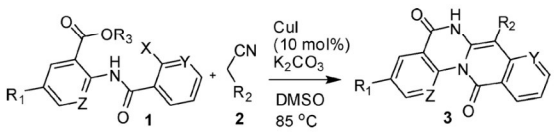
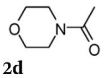
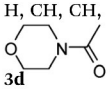
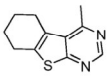
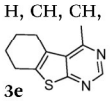
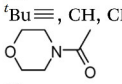
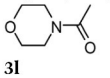
† Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, and results of *in vitro* and docking study. CCDC 902761. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc37070k

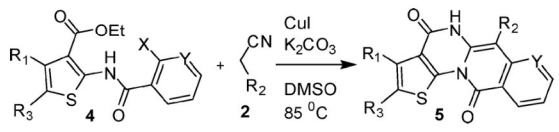
Table 2 Cu-catalyzed synthesis of 5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (**3**)[a]

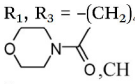
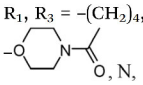
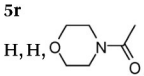


Entry	Halide (**1**) R_1, R_3, X, Y, Z	Nitrile (**2**) R_2	*T*/h	Product (**3**) R_1, Y, Z, R_2	Yield[b] (%)
1	H, CH_3, I, CH, CH **1a**	CO_2Et **2a**	3.0	H, CH, CH, CO_2Et **3a**	87
2	H, CH_3, Br, CH, CH **1b**	**2a**	3.5	**3a**	84
3	H, CH_3, Cl, CH, CH **1c**	**2a**	6.0	**3a**	0
4	**1a**	CO_2Me **2b**	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	H, H,  **5s**	59

[a] For reaction conditions, see footnote of Table 2. [b] Isolated yield.

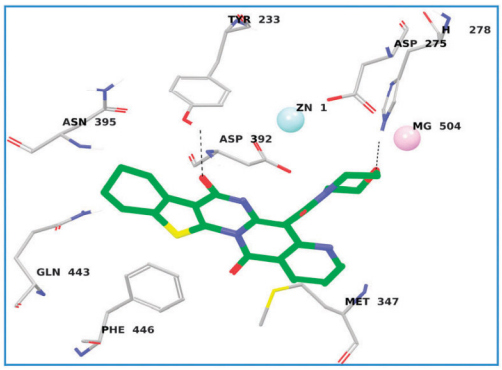


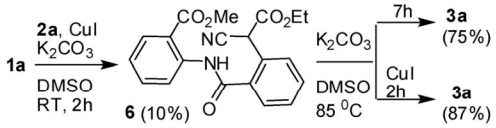
Fig. 2 Binding mode and interactions of **5e** with PDE4B active sites.

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–c**) 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 **μ**M (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.

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Notes and references

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A direct access to bioactive fused N-heterocyclic acetic acid derivatives†

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A Cu-catalyzed new sequence involving the Ullmann type intermolecular C–C followed by an intramolecular C–N coupling and then intramolecular aza-Michael type addition (and oxidation) in a single pot afforded various fused N-heterocyclic acetic acid derivatives as inhibitors of PDE4.

The development of direct, simple and efficient strategies that offer ample flexibilities is of high demand in modern organic synthesis. The use of metal catalyzed domino reactions[1] has attracted considerable interest for this purpose, because of their ability to produce diverse and novel classes of compounds in a single synthetic operation.

The N-heterocyclic acetic acid framework **A** (Fig. 1) is prevalent in many bioactive compounds, including non-steroidal anti-inflammatory drugs[2] (NSAIDs) *e.g.* indomethacin, tolmetin, zomepirac *etc.* The impressive and proven anti-inflammatory activities of these drugs prompted us to explore fused N-heterocyclic acetic acids as a novel class of potential inhibitors[3] of phosphodiesterase 4 (PDE4). The development of PDE4 inhibitors[4] is beneficial, as in addition to chronic obstructive pulmonary

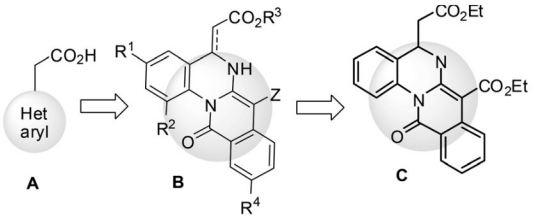

Fig. 1 Design of novel bioactive molecules **B/C** (as potential PDE4 inhibitors) derived from **A**.

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†Electronic supplementary information (ESI) available: Experimental procedures, spectral data for all new compounds, copies of spectra, results of *in vitro* and docking studies. See DOI: 10.1039/c3ob42535e

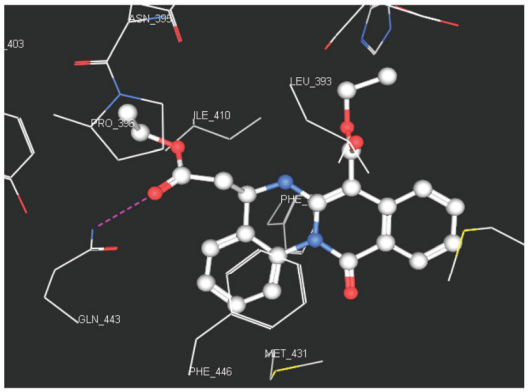
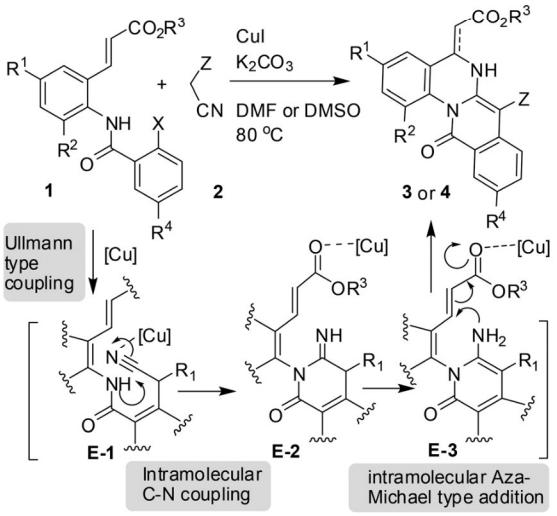
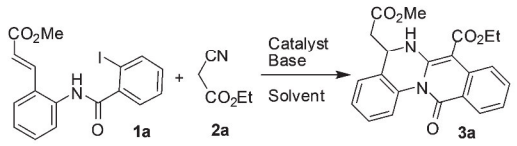


Fig. 2 Docking of **C** into the active site of PDE4B (PDB code 1XMY).



Scheme 1 Synthesis of **3** *via* Cu-catalyzed domino reactions.

Table 1 Effect of conditions on domino reaction of **1a** with **2a**[a]



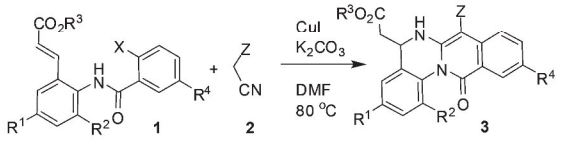
Entry	Catalyst	Base	Solvent	Yield[b] (%)
1	CuI	K_2CO_3	DMF	89
2	CuI	Na_2CO_3	DMF	79
3	CuI	Cs_2CO_3	DMF	88
4	CuI	K_2CO_3	DMSO	88
5	CuI	K_2CO_3	1,4-Dioxane	53
6	CuBr	K_2CO_3	DMF	80
7	CuCl	K_2CO_3	DMF	71
8	$Cu(OAc)_2$	K_2CO_3	DMF	78
9	$Cu(OTf)_2$	K_2CO_3	DMF	80
10	CuI	K_2CO_3	DMF	0[c]
11	—	K_2CO_3	DMF	0

[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) at 80 °C for 0.5 h under anhydrous conditions (no inert atmosphere). [b] Isolated yield. [c] Reaction performed at room temperature.

disease (COPD) and asthma, PDE4 has been reported as a potential therapeutic target for neurodegenerative diseases, cancer and memory loss. Most of the leading PDE4 inhibitors, including the marketed drug roflumilast (Daxas®, Nycomed), suffer from side effects including nausea and emesis.[4] It is therefore desirable to identify novel PDE4 inhibitors having fewer side effects. In our effort, initial *in silico* docking studies of a representative compound **C**, in the active site of PDE4B, aided us in the design of the target molecules **B** (Fig. 1 and 2). To obtain **B** we have developed a direct, Cu-mediated domino reaction, leading to the one-pot synthesis of **B** (or **3** and **4**, Scheme 1) under mild conditions without using any co-catalyst, ligand or additive. Herein, we report our preliminary results.

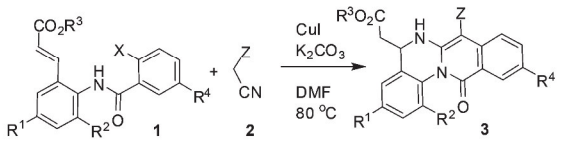
While more simple heterocyclic acid derivatives have been prepared *via* a number of efficient methods,[5] none of them appeared to be useful for the synthesis of **3** or **4**. In view of the recent success of Cu-catalyzed domino reactions[6] to construct tri- and tetracyclic ring systems, we envisioned[3f] that a Cu-mediated Ullmann type intermolecular C–C coupling of **1** with **2** (*e.g.* **E-1**), followed by an intramolecular nucleophilic addition of the amidic NH to CN, would afford the initial

Table 2 Cu-catalyzed synthesis of 2-(12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-yl)acetate (**3**)[a]



Entry	Halide (**1**) R^1, R^2, R^3, R^4, X	Nitrile (**2**) Z	Time (h)	Product (**3**) R^1, R^2, R^3, R^4, Z	Yield[b] (%)
1	H, H, Me, H, I **1a**	CO_2Et **2a**	0.5	H, H, Me, H, CO_2Et **3a**	89
2	**1a**	CO_2Me **2b**	0.5	H, H, Me, H, CO_2Me **3b**	84
3	**1a**	$PO(OEt)_2$ **2c**	2.0	H, H, Me, H, $PO(OEt)_2$ **3c**	81
4	**1a**	$CO_2{}^tBu$ **2d**	0.5	H, H, Me, H, $CO_2{}^tBu$ **3d**	81
5	H, H, Et, H, I **1b**	**2a**	0.5	H, H, Et, H, CO_2Et **3e**	87
6	**1b**	**2b**	0.5	H, H, Et, H, CO_2Me **3f**	83
7	**1b**	**2c**	1.5	H, H, Et, H, $PO(OEt)_2$ **3g**	79
8	**1b**	**2d**	0.5	H, H, Et, H, $CO_2{}^tBu$ **3h**	81
9	H, H, Me, NO_2, Cl **1c**	**2a**	1.5	H, H, Me, NO_2, CO_2Et **3i**	76
10	**1c**	**2b**	1.5	H, H, Me, NO_2, CO_2Me **3j**	73
11	**1c**	**2c**	2.5	H, H, Me, NO_2, $PO(OEt)_2$ **3k**	73
12	F, H, ^{t}Bu, H, Br **1d**	**2a**	2.0	F, H, ^{t}Bu, H, CO_2Et **3l**	80
13	**1d**	**2c**	2.5	F, H, ^{t}Bu, H, $PO(OEt)_2$ **3m**	75
14	F, H, Me, NO_2, Cl **1e**	CN **2e**	2.5	F, H, Me, NO_2, CN **3n**	68
15	Cl, H, Me, H, I **1f**	**2a**	0.5	Cl, H, Me, H, CO_2Et **3o**	85

Table 2 *(Contd.)*



Entry	Halide (**1**) R^1, R^2, R^3, R^4, X	Nitrile (**2**) Z	Time (h)	Product (**3**) R^1, R^2, R^3, R^4, Z	Yield[b] (%)
16	**1f**	**2b**	0.5	Cl, H, Me, H, CO_2Me **3p**	81
17	**1f**	**2c**	1.5	Cl, H, Me, H, $PO(OEt)_2$ **3q**	78
18	**1f**	**2d**	1.0	Cl, H, Me, H, $CO_2{}^tBu$ **3r**	77
19	**1f**	**2e**	1.0	Cl, H, Me, H, CN **3s**	72
20	Me, H, Et, H, I **1g**	**2a**	0.5	Me, H, Et, H, CO_2Et **3t**	87
21	**1g**	**2b**	0.5	Me, H, Et, H, CO_2Me **3u**	84
22	**1g**	**2c**	2.0	Me, H, Et, H, $PO(OEt)_2$ **3v**	82
23	Me, Me, Me, H, I **1h**	**2a**	0.5	Me, Me, Me, H, CO_2Et **3w**	86
24	**1h**	**2b**	0.5	Me, Me, Me, H, CO_2Me **3x**	81
25	**1h**	**2c**	1.5	Me, Me, Me, H, $PO(OEt)_2$ **3y**	78

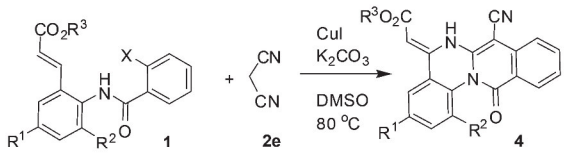
[a] All the reactions were carried out using **1** (1 mmol), **2** (1.2 mmol), CuI (0.1 mmol) and K_2CO_3 (3 mmol) in DMF (2 mL) at 80 °C under anhydrous conditions (no inert atmosphere). [b] Isolated yield.

6-membered ring *in situ* (**E-3** *via* **E-2**, Scheme 1). A subsequent intramolecular aza-Michael type addition[7] of **E-3** would furnish **3** (or **4** after aerial oxidation).[8]

The required starting material, **1**, was prepared *via* an amide bond formation between a 2-haloaryl carboxylic acid chloride and 3-(2-aminoaryl)acrylate ester, produced *via* a Heck reaction (see the ESI†). Initially, the coupling of iodo compound **1a** with ethyl cyanoacetate (**2a**) was examined (Table 1) using a range of bases (*e.g.* K_2CO_3, Na_2CO_3 and Cs_2CO_3), solvents (*e.g.* DMSO, DMF and 1,4-dioxane) and catalysts [*e.g.* CuI, CuBr, CuCl, $Cu(OAc)_2$ and $Cu(OTf)_2$]. While good results were obtained in several cases (entries 1, 3, 4, 6 and 9), the combination of CuI and K_2CO_3 in DMF (entry 1, Table 1) was chosen for further studies. All these reactions were performed at 80 °C. The reaction did not proceed at room temperature or in the absence of a catalyst (entries 10 and 11, Table 1).

To expand the scope of the present Cu-catalyzed domino reaction, compound **3** was prepared with a variety of substitution patterns (Table 2). The reaction proceeded well with various substituents on **1** including F, Cl, Me, Et, ^{t}Bu, or NO_2, irrespective of X being either I, or Br, or Cl. The use of various nitriles (**2a–e**) was also successful. Notably, the reaction of **1** with malononitrile **2e** in DMSO for a longer period afforded the compound **4** containing an exocyclic double bond with a *Z*-stereochemistry (Table 3). Moreover, the formation of the *Z*-isomer was found to be exclusive and was supported by a

Table 3 Cu-catalyzed synthesis of (*Z*)-alkyl 2-(7-cyano-12-oxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazolin-5-ylidene)acetate (**4**)[a]



Entry	Halide (**1**) R_1, R_2, R_3, X	Time/h	Product (**4**) R_1, R_2, R_3	Yield[b] (%)
1	H, H, Me, I **1a**	4.0	H, H, Me **4a**	72
2	H, H, Et, I **1b**	4.0	H, H, Et **4b**	71
3	F, H, ^{t}Bu, Br **1d**	5.0	F, H, ^{t}Bu **4c**	49
4	F, H, Me, I **1i**	6.0	F, H, Me **4d**	56
5	Cl, H, Me, I **1f**	5.0	Cl, H, Me **4e**	48
6	Me, H, Et, I **1g**	4.0	Me, H, Et **4f**	65
7	Me, Me, Me, I **1h**	4.0	Me, Me, Me **4g**	73
8	Me, Me, Et, I **1j**	6.0	Me, Me, Et **4h**	72

[a] Reactions were carried out using **1** (1 mmol), **2e** (1.2 mmol), CuI (0.1 mmol) and K_2CO_3 (3 mmol) in DMSO (2 mL) at 80 °C under anhydrous conditions (no inert atmosphere). [b] Isolated yield.

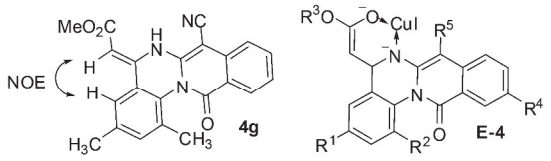
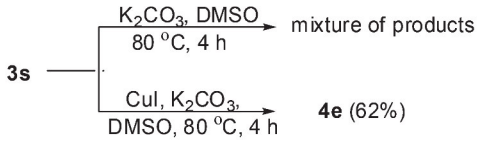


Fig. 3 (A) NOE study of **4g** and (B) complexation of **3** with Cu-catalyst.



Scheme 2 Role of CuI in the generation of compound **4**.

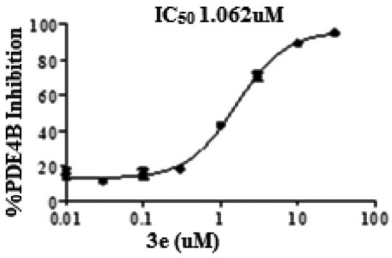


Fig. 4 Dose dependent inhibition of PDE4B by compound **3e**.

NOE study of **4g** (Fig. 3A). Mechanistically, the *Z*-isomer of **4** seemed to have been formed *in situ via* the generation of **3** (Scheme 1) and then a Cu-complex **E-4** (Fig. 3B) which on aerial oxidation afforded the olefin **4**. To gain further evidence, **3s** was treated with K_2CO_3 and CuI + K_2CO_3, separately, whereby a mixture of products was obtained in the first case and the desired **4e** (62% yield) in the second case (Scheme 2).

Several of the synthesized compounds showed promising inhibition of PDE4B [*e.g.* **3b** (71%), **3e** (93%), **3f** (86%), **3i** (66%), **3j** (66%), **3k** (78%), **3o** (62%), **3p** (62%), **3t** (83%), **3u** (93%), **3w** (71%) and **3x** (88%)] when tested *in vitro*[9] at 30 μM (see the ESI†). This result was further supported by the results of the docking of **3e** (Fig. 2) and **3u** (see the ESI†) into the PDE4B protein (Glide score −23.05 and −22.05 *vs.* rolipram's −24.61). The ester carbonyl group participated in H-bonding with the Gln443 of the Q pocket in the case of **3e** and the His234 of the metal binding pocket in case of **3u**, respectively. Additionally, both **3e** and **3u** showed a common Ar–Ar interaction with the Phe446 of PDE4B (see the ESI†). The compound **3e** showed a dose dependent inhibition of PDE4B with an IC_{50} (the half maximal inhibitory concentration) ~ 1.06 μM comparable to rolipram's IC_{50} ~ 1.0 μM (Fig. 4).

In conclusion, a robust, mild and ligand/additive-free Cu-mediated domino reaction has been developed, that allows a rapid access to novel, fused N-heterocyclic acetic acid derivatives. The reaction proceeds *via* a Cu-catalyzed domino reaction involving (i) an Ullmann type intermolecular C–C followed by (ii) an intramolecular C–N coupling and then (iii) an intramolecular aza-Michael type addition (and subsequent aerial oxidation). Several of these compounds showed promising PDE4B inhibition *in vitro* and seem to have potential for related medical applications. Overall, the one-pot methodology, presented here, may find wide use in constructing a diversity based library of small molecules for chemical and medicinal applications.

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