Synthetic Studies on N, O and S containing Heterocycles of Medicinal Interest

A Thesis Submitted to
University of Hyderabad
For the Degree of

Doctor of Philosophy
In Chemistry

By

Alinakhi

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
Gachibowli, Hyderabad 500046, India

December 2013

Dedicated to

My Parents
&
Shohda-e-Karbala

Contents

Statement

Certificate

Acknowledgement

Biography

Synopsis

Page

CHAPTER 1 **Pharmacological activities of N, O and S containing heterocycles and Sirtuin modulators**

1.1. Introduction 1

1.2. Brief introduction to cancer and diabetes 3

1.3. SIRTUINS (Silent Information Regulator) 5

1.3.1. Histones 6

1.3.2. Sirtuin family 6

1.3.3. Overview of the mechanism of deacetylation catalyzed by Sirtuins 9

1.3.4. SIRT1- A multifaceted protein of sirtuins family 11

1.4. SIRT1 modulation 12

1.4.1. SIRT1 activators 13

1.4.2. SIRT1 inhibitors 14

1.5. Yeast cell based *in vitro* URA3 reporter silencing assay 15

1.6. Zebrafish as *in vivo* model 16

1.7. Previous work from our group 17

1.7.1. Design of JGB1741 17

1.7.2. Synthesis of JGB1741 18

1.7.3. Conclusion 19

1.8. Experimental section 19

References 23

Appendix 27

CHAPTER 2 2A **Synthesis of thieno[3,2-*c*]pyran-4-one derivatives as potential anticancer agents**

2A.1. Introduction 30
2A.2. Previous work 31
2A.3. Present work 34
2A.4. Results and discussion 36
2A.5. Pharmacology 50
2A.6. Conclusion 55
2A.7. Experimental section 56
Reference 81
Appendix 84

CHAPTER 2 2B **Design and synthesis of novel pyrano[4,3-*b*]pyran-5(4*H*)-ones as potential inhibitors of sirtuins**

2B.1. Introduction 92
2B.2. Previous work 93
2B.3. Present work 95
2B.4. Results and discussion 97
2B.5. Pharmacology 108
2B.6. Conclusion 113
2B.7. Experimental section 114
Reference 131
Appendix 133

CHAPTER 3 **$AlCl_3$-mediated synthesis of functionalized olefins as potential inhibitors of sirtuins**

3.1. Introduction 145
3.2. Previous work 146
3.2.1. Hydroarylation reactions 146

3.2.2. Heteroarylation reactions 147
3.3. Present work 148
3.4. Results and discussion 149
3.5. Pharmacology 159
3.5.1. Yeast cell based *in vitro* URA3 reporter silencing assay 159
3.5.2. Dose response study on mammalian SIRT1 161
3.5.3. Treatment of HepG2 cancerous cells with inhibitor 161
3.5.4. Docking studies with yeast Sir2 (*in silico*) 162
3.5.5. Toxicity studies in Zebrafish embryo (*in vivo*) 163
3.6. Conclusion 164
3.7. Experimental section 165
3.7.1. Analytical data of intermediate compounds 165
3.7.2. General procedure for the synthesis of 2-(2,2-diarylvinyl)-3-arylquinoxaline (4) 166
Reference 187
Appendix 190

CHAPTER 4 **$AlCl_3$-mediated synthesis of functionalized olefins as potential inhibitors of sirtuins**

4.1 Introduction 196
4.2. Previous work 197
4.3. Present work 198
4.4. Results and discussion 199
4.5. Pharmacology 210
4.5.1. Yeast cell based *in vitro* URA3 reporter silencing assay 210
4.5.2. Docking studies with yeast Sir2 (*in silico*) 212
4.5.3. Toxicity studies in Zebrafish embryo (*in vivo*) 214
4.6. Conclusion 216
4.7. Experimental section 216
Reference 235
Appendix 238

CHAPTER 5
5A

Synthesis of 2-(hetero)aryl indoles *via* coupling/desilylation–coupling/cyclization under Pd/C-Cu catalysis

5A.1.	Introduction	247
5A.2.	Previous work	248
5A.3.	Present work	250
5A.4.	Results and discussion	252
5A.5.	Pharmacology	260
5A.6.	Conclusion	262
5A.7.	Experimental	262
	Reference	275
	Appendix	279

CHAPTER 5
5B

Pd/C-Cu catalyzed one pot synthesis of 2-substituted indoles

5B.1.	Introduction	285
5B.2.	Present work	286
5B.3.	Results and discussion	287
5B.4.	Scope of the reaction	294
5B.5.	Conclusion	296
5B.6.	Experimental section	297
	Reference	309
	Appendix	311

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 esteemed 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.

Alinakhi

Dr. Reddy's Institute of Life Sciences
University of Hyderabad
December 2013

DR. REDDY'S
INSTITUTE OF LIFE SCIENCES

CERTIFICATE

This is to certify that the thesis entitled "***Synthetic Studies on N, O and S Containing Heterocycles of Medicinal Interest***" being submitted by ***Mr. Alinakhi*** to University of Hyderabad for the award of the Degree of Doctor of Philosophy in Chemistry has been carried out absolutely by him under my supervision and the same has not been submitted elsewhere for a degree. I am satisfied that the thesis has reached the standard fulfilling the requirements of the regulations relating to the nature of the degree.

Prof. Manojit Pal
(Supervisor)
****
Manojit Pal, PhD
Professor-Organic and Medicinal Chemistry
Dean of Academic, Research and Development (DOARD)
Dr Reddy's Institute of Life Sciences
University of Hyderabad Campus
Gachibowli, Hyderabad 500 046
Andhra Pradesh, India
Tel: +91 40 6657 1500
Fax: +91 40 6657 1581
e-mail: manojitpal@rediffmail.com
manojitp@drils.org
http://www.drils.org/
Former Sr. Director, Discovery Research, Dr. Rreddy's Laboratories Ltd, Hyderabad, India
Former Sr. GM and Head, New Drug Discovery, Matrix / Mylan Lab Ltd, Hyderabad, India

Acknowledgement

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises to Allah for the strengths and his blessing in completing this thesis.

For any research scholar, the most important factor is to get a right person as a supervisor. The person to whom I am gratefully indebted is my Ph.D. supervising guide Prof. Manojit Pal. I sincerely thank him for his constructive guidance, valuable suggestions, inspiration and motivation has helped me profoundly during course my research. It would have been impossible to accomplish my research work, without his esteemed guidance, unfailing attention and constant encouragement. He has paved me a platform in medicinal chemistry research. His simplicity, moral ethics, patience, behavior towards the students inspired me a lot, which will be useful in different stages of my personal and professional life. Dear Sir, I consider it as a God's blessing to work with you.

With respect and admiration, I express my sincere gratitude to Prof. Javed Iqbal for his constant motivation and valuable suggestions at different stages of my research work.

I would like sincerely thank to Prof. F.A. Khan for his continuous assessment of my work during my Ph.D. His suggestions really helped me in completing my thesis work.

I am very much grateful to CSIR (Council of Scientific and Industrial Research) for providing Senior Research Fellowship, DST (Department of Science and Technology) and DBT (Department of Biotechnology) for funding during my thesis work.

I take this opportunity to thank Dr. Pinaki Talukdar for his kind introduction to laboratory techniques and giving a way to start my research. It is an immense pleasure to thank Dr. Rajamohan Reddy Poondra, Dr. Devyani Halder, Dr. Kishore Parsa, Dr. Sacchidanad, Dr. Arvind Shanmugam, Prof. Parimal Mishra, and Prof. Prabhat Arya for their encouragement and support. I would like to thank all non

teaching staff in Dr. Reddy's Institute of Life Sciences, especially Mrs. Deepa didi, Mr. Buchi Prasad for their help and encouragement. I also thank Mr. Ramanamurthy and Mr. Vijaybhaskar for their support.

I feel fortunate to have an unstinting support from my lab mates. My special thanks to Mr. Raja Mohan Rao, Mr. Bagineni Prasad, Mr. Adepu Raju, Dr. Soumita Mukherjee and Dr. Shiva Kumar for their great support, constant encouragement and for creating joyful environment during my thesis work. I am grateful to Mr. Bagineni Prasad and Mr. Adepu Raju for their support in subject discussions. I am thankful to Dr. Dhilli Rao and Mr. Vijay Babu for their support. Finally I would like to thank Mr. Rajnikanth, the budding researcher of our lab for accompanying me in my course work. I sincerely thank to my juniors who worked with me for their dissertation. My best wishes to Mr. Md. Shafiqur Rehman, Ms. Archana Shivakumar, Ms. Manisha Saridena, Ms. Snigdha Gupta, Mr. Yogi Srinivas and Mr. Sunder. I would like to thank group members of Lab I & Lab III for their friendly affection. Special thanks to my friend Mr. Narendar Reddy for his support and productive chemistry conversations during my thesis work. I would also extend my thanks to Mr. Ratnam, Mr. Srinivas, Mr. Madhu, Mr. Bhanudas, Mr. Shivakrishna Reddy, and Mr. Ravi kumar.

Some friends really make difference in our life. I really feel proud and happy to have friends Mr. Sayyad Arshad Rizvi, Ms. Amrita Sen Gupta, Mrs. Nandita Jain, Ms. Iqra Basheer Nehvi, Mrs. Samra Akram, Mr. Ronald Benjamine, Ms. Atoshi Benerjee and Mr. Gowrav Sing. I will surely miss all our crazy, memorable and joyful moments together, but I know that someday our paths will cross again.

I am truly grateful to school of chemistry faculty Dr. D. B. Ramachari and Dr. Ramu Sridhar for their immense encouragement. And also I thank my friends Dr. Kishore Ravada and Dr. Seshadri for their help during my thesis work.

I would like to thank all my School teachers, Undergraduate teacher Mr. Chalapati Rao, Graduate teachers Prof. Saraswati and Mr. Satyadev. I thank Mr. SrinuBabu, Mr. Raju Babu, Mr. Kishore Addanki, Mr. Naresh, Mr. Koteswra Rao, Mrs. Vijaya Lakshmi and Mr. Suresh for being my friends.

I invariably feel short of words to express my love and affection to my family members Mohammad Ali Baig (Daddy), Razia Shahin (Ammy), Jafar Saheb (Babji), Zainab Faizy (Bhabi), Amatul Zainab (Wife), my little champ Danish Abbas Baig (Nephew) and Wajjatunnisa (Dadjan) for their adorable support which has driven me to this stage. I really feel great to have my parents and brother for educating me, with all the opportunities they could provide to explore my potentials and pursue my dreams ever since I was a child.

A special thanks and love to my dearest Wife Amatul Zainab (Amat) who has given a wonderful support and constant encouragement during my thesis writing. Her prayers really helped me to achieve my goal.

It gives me an immense pleasure to show my respect to Janab Barkat Ali (Nanhe Dadsahab) for his encouragement throughout my education. I thank my aunt Farida (Maami) for her great support. I would like to extend my thanks to Hyder Ali (Chacha), Tahera Ashraf (Chahi), Burhan Askari (Brother) and Wajjatunnisa (Sister).

I would like to express my gratitude to my dearest uncle Dr. Mohammad Takhi (Dadbhai Mama), who has been a continuous motivation for me. It would have been impossible for me to accomplish my goal without his guidance, unfailing attention and wise counsel. I would also like to acknowledge Dr. Abbas Raza (Dudbhai Mama) for his valuable suggestions and continuous support. I thank my late grandparents Mazhar Hussain (Nansahab), Jawadul Qhatun (Nani Ammy) and Jafar Saheb (Dadsahab) for their blessings. Their memories are always with me.

At the end, with sincere gratitude, I want to pay homage to Ahlulbaith and Imam Mehdi-ul-hadi (peace be upon them). A special homage to Hazrat Abul Fazlil Abbas (a.s) and Imam Ali Reza (a.s).

December 2013

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

Alinakhi

Biography

Mr. Alinakhi was born in Amalapuram, East Godavari Dist., Andhra Pradesh, India, on July 19, 1984. He received his B.Sc. Degree in Botany, Zoology and Chemistry from Arts College (Rajahmundry), Andhra University, Andhra Pradesh, India in the year 2004. He was awarded Topper in Chemistry for securing highest marks in B.Sc. In 2006 he received his M.Sc degree in Organic Chemistry from Acharya Nagarjuna University, Guntur, Andhra Pradesh. Then in 2007 he joined in Dr. Reddy's Institute of Life Sciences as a Research Trainee in Medicinal Chemistry Department at University of Hyderabad, Andhra Pradesh, India. After completion of 2 years in 2009 he joined as Project Fellow under Department of Science and Technology (DST), Government of India, sponsored project. In 2011 he qualified Council of Scientific and Industrial Research (CSIR) and awarded Senior Research Fellowship from CSIR, Government of India. In the same year he registered in University of Hyderabad for Ph.D. During his doctoral programme at Dr. Reddy's Institute of Life Sciences, under the guidance of Prof. Manojit Pal he has published number of papers in International Journals especially in the area of Medicinal Chemistry. He has given oral presentation at 6th Junior National Organic Symposium (J-NOST) in 2011. And in 2012 he was awarded cash prize for his first best poster at Royal Society of Chemistry (RSC- London) - DS, National Poster Symposium on Organic/Medicinal Chemistry for Ph.D. Students. In addition he presented poster at different National and International Conferences.

His areas of research interest include Synthetic Organic Chemistry, Organometallic Chemistry, Heterocyclic Chemistry and Medicinal Chemistry.

This Thesis entitled "**Synthetic Studies on N, O and S containing Heterocycles of Medicinal Interest**" consists of five chapters.

CHAPTER 1

Pharmacological activities of N, O and S containing heterocycles and Sirtuin modulators

(*Biochem. Biophys. Res. Commun.* **2010**, *401*,13)

The nitrogen, oxygen and sulfur containing heterocycles are of immense importance because of their interesting biological and pharmaceutical properties. In the current scenario of drug discovery, medicinal chemistry has become an indispensible division to ameliorate the deleterious consequences of increasing population with different diseases. Understanding of the biology and pharmacology that relates to the target protein often shed light on the development of new drug candidates.

In this chapter, we discuss about the medicinal values of nitrogen, oxygen and sulfur containing heterocycles followed by the brief introduction to cancer and diabetes. Here we have given a major focus on Sirtuins (Fig. 1, A) and its small molecule modulators. Sirtuins are class III histone deacetylases (HDACS) that require NAD+ for their catalytic action. Hence, identification of compounds that can either activate or inhibit specific sirtuins is expected to give insight in the development of human therapeutics. SIRT1 is a mammalian homolog of the yeast Sir2 (*Saccharomyces cerevisiae*). The *in vitro* assay of compounds against Sir2 generally provides information about SIRT1 modulatory properties of test compounds. Finally, we discussed the use of Zebrafish as an *in vitro* model. We also presented our preliminary work on the synthesis of a novel small molecule as SIRT1 inhibitor (**5**) - JGB1741 (Fig. 1, B). Based on encouraging results we became interested in the synthesis of other potential modulators of Sirtuins.

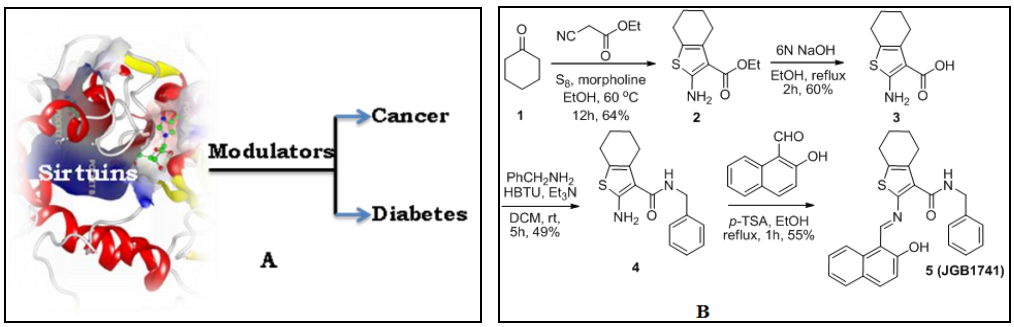


Figure 1. (A) Sirtuin modulation for cancer and diabetes. (B) Synthesis of SIRT1 inhibitor.

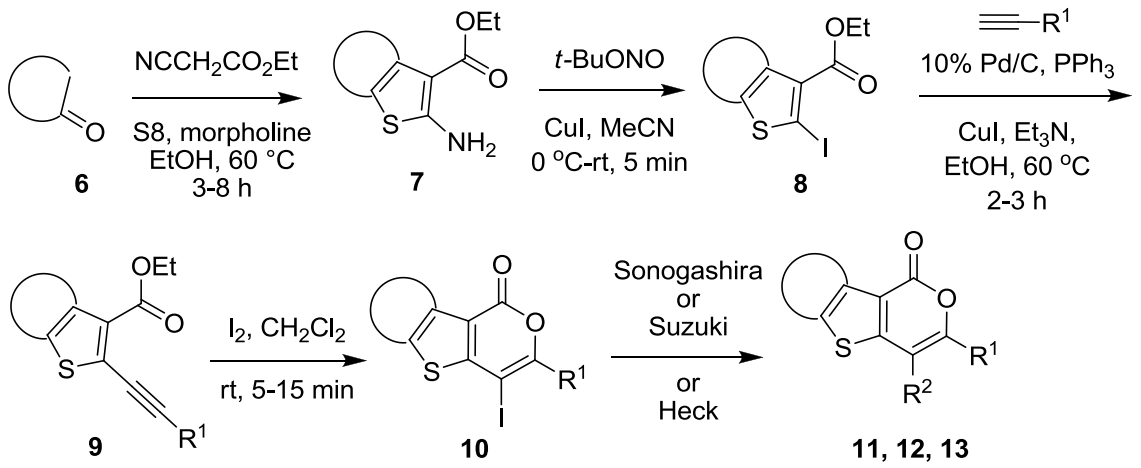
CHAPTER 2

Chapter 2A

Synthesis of thieno[3,2-*c*]pyran-4-one derivatives as potential anticancer agents

(*Bioorg. Med. Chem. Lett*. **2012**, *22*, 4418)

In this chapter, we have demonstrated a design and synthesis of novel thieno[3,2-*c*]pyran-4-one (**10**) based small molecules as potential anticancer agents. The synthesis (Scheme 1) was carried out *via* a multistep sequence involving the use of Pd/C-catalyzed coupling-iodocyclization-coupling as a key strategy. The utility of this strategy has been demonstrated by synthesizing a wide variety of thienopyranone derivatives (**11**, **12** and **13**) *via* Pd catalyzed C-C bond forming reactions such as Sonogashira, Suzuki and Heck coupling reactions. Some of these compounds were evaluated *in vitro* against K562, HEPG2 and MDA-MB231 cancer cell lines along with a normal cell line HEK293. Two compounds showed IC_{50} values in the range of 2.0-2.5 μM. The crystal structure analysis of an active compound has been described. Finally the anticancer activity of an active compound was supported by *in vitro* yeast Sir2 reporter gene based assay, which showed the anticancer property may be possibly due to the inhibition of Sirtuin. Overall, the thienopyranone framework presented here could be an attractive template for the identification of novel anticancer agents and the corresponding synthetic strategy described could be useful for generating diversity based library of small molecules related to thieno[3,2-*c*]pyran-4-one.



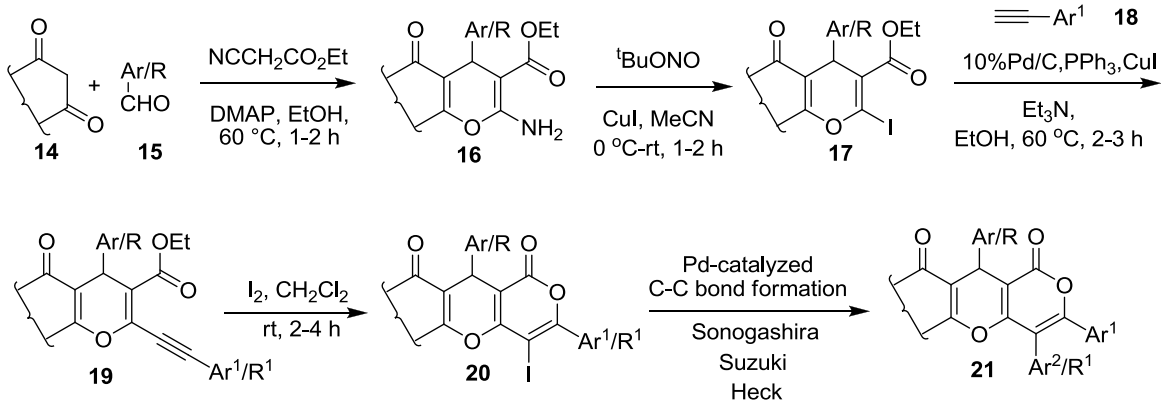
Scheme 1. Synthesis of thieno[3,2-*c*]pyran-4-one based novel compounds.

Chapter 2B

Design and synthesis of novel pyrano[4,3-*b*]pyran-5(4*H*)-ones as potential inhibitors of sirtuins

(*Bioorg. Med. Chem. Lett*. **2013**, *23,* 4195)

In continuation to the work presented in chapter 2A, we designed and extentended the Pd/C-catalyzed coupling-iodocyclization-coupling strategy to synthesize novel pyrano[4,3-*b*]pyran-5(4*H*)-one (**20**) based small molecules as potential inhibitors of sirtuins (i.e. yeast sir2, a homolog of human SIRT1). The synthesis of these compounds was performed *via* a multi-step sequence consisting of a multi component reaction, Sandmeyer type iodination, Sonogashira type coupling followed by iodocyclization and then Pd-mediated various C-C bond forming reactions (Scheme 2). The overall strategy involved the construction of a pyran ring followed by the fused pyranone moiety and subsequent functionalization at C-8 position of the resultant core pyrano[4,3-*b*]pyran-5(4*H*)-one (**21**) framework *via* Pd catalyzed C-C bond forming reactions such as Sonogashira, Suzuki and Heck coupling reactions. The crystal structure analysis of a representative iodolactonized compound has been presented. Some of the synthesized compounds showed promising inhibitory activities when tested against yeast sir2 *in vitro*. The active compound showed dose dependent inhibition with IC_{50} ~ 78.05 µM in yeast sir2 and good binding interactions with this protein *in silico*.



Scheme 2. Synthesis of novel pyrano[4,3-*b*]chromendione and pyrano[4,3-*b*]pyran based small molecules

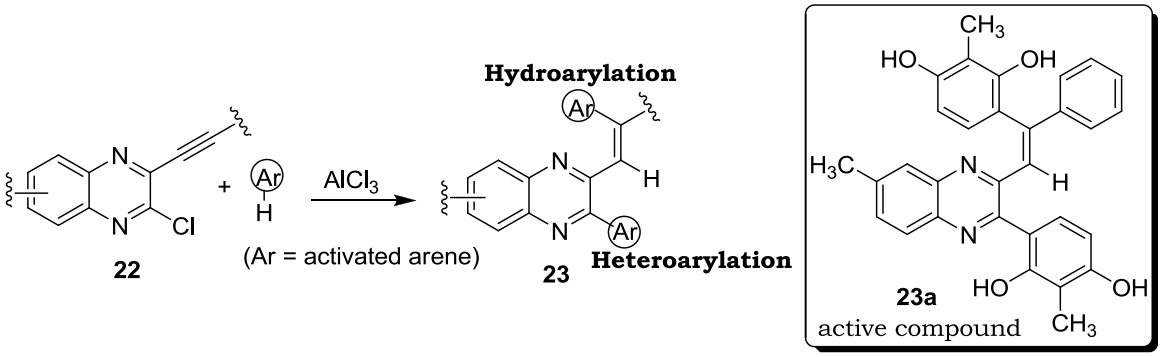
CHAPTER 3

$AlCl_3$-mediated synthesis of functionalized olefins as potential inhibitors of sirtuins

(*Chem. Commun.* **2013**, *49*, 6268).

Synthesis of densely functionalized olefins with highly substituted heteroaromatics is of fundamental interest as it enables the easy access to compounds for potential applications in chemical/pharmaceutical industries. On the other hand, Sirtuins are known to be up-regulated in various types of cancer and hence are considered as promising targets for cancer therapeutics.

In this chapter, we demonstrate a new route for the synthesis of 2-(2,2-diarylvinyl)-3-arylquinoxaline (**23**) *via* $AlCl_3$ mediated hydroarylation/heteroarylation of 2-chloro-3-(arylethynyl)quinoxalines (**22**) (Scheme 3). These compounds have shown good inhibition of yeast Sir2 *in vitro* when tested using a reporter assay that was supported by *in silico* studies. A representative compound (**23a**) showed IC_{50}~13.5 µM in a dose response study (Fig. 1, A). It also showed IC_{50}~32.9 µM when tested against mammalian SIRT1. In an MTT assay this compound showed inhibition of cell growth at 50 µM when tested against human hepatocellular liver carcinoma (HepG2) cells. Further we carried out safety studies of this compound using Zebrafish as an *in vivo* model and no adverse effects were observed when tested at 10 µM for toxicity in zebrafish embryo (Fig. 1, B).



Scheme 3. Synthesis of 2-(2,2-diarylvinyl)-3 aryl quinoxaline.

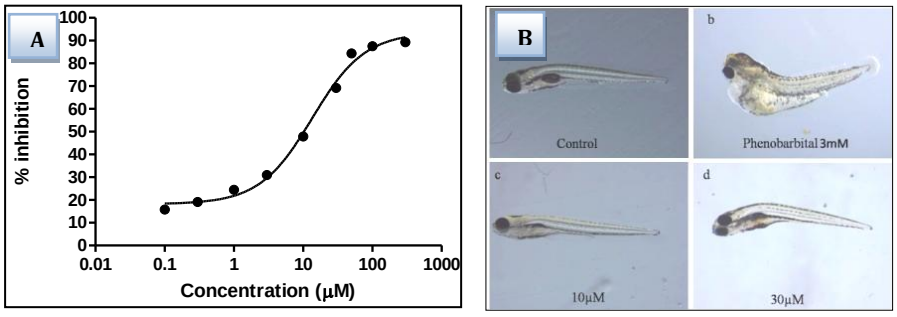


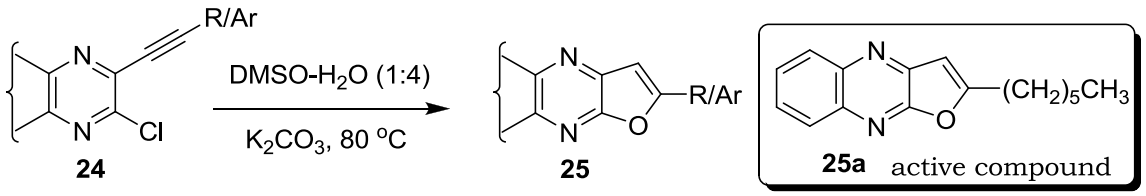
Figure 1. (A) Dose dependent inhibition of Sir2 by **23a**. (B) *In vivo* toxicity study of **23a** in Zebrafish embryo.

CHAPTER 4

Synthesis of fused furan *N*-heterocycles as potential inhibitors of sirtuins

(*Org. Biomol. Chem.* **2013**, *11*, 4930)

The use of aqueous media in organic synthesis has gain high popularity as water is naturally abundant, safe and inexpensive which can be recycled. The functionalized fused *N*-heteroaromatics have played key roles in the early stage of drug discovery and many of them have been marketed as successful drugs. In this chapter, we describes the synthesis of a novel and unique class of fused furo *N*-heterocycles **25** *via* a tandem two-step strategy which involves hydrolysis of 2-chloro-3-alkynyl quinoxalines / pyrazines **24** followed by *in situ* cyclization of the corresponding 2-hydroxy-3-alkynyl intermediates in a single pot (Scheme 4). One of the representative compound **25a** showed significant inhibition when tested *in vitro* on yeast Sir2 and showed IC_{50}~15.02 µM in a dose response study (Fig. 1, A) and also showed IC_{50}~23.5 µM when tested against mammalian SIRT1 (Fig. 1, B). In a preliminary MTT assay compound **25a** strongly inhibited the cell growth when tested against human hepatocellular liver carcinoma (HepG2) and Cervical Cancer (Hela) cells at 50 µM. Further, no adverse effects were observed till 30 µM when tested for toxicity in zebrafish embryo at a concentration range of 1.0-50 µM.



Scheme 4. Synthesis of furo[2,3-*b*]quinoxalines / pyrazines.

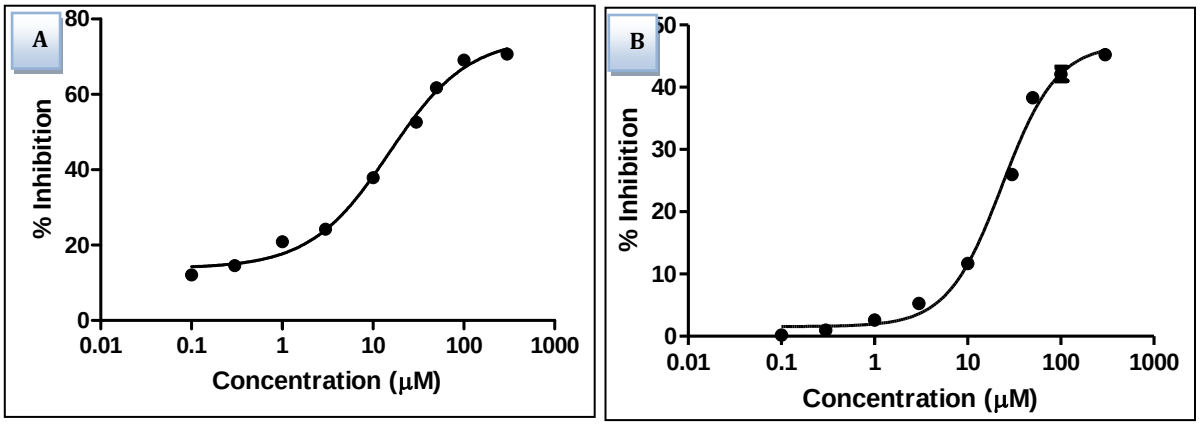


Figure 1. Dose dependent assay of **25a** against (A) Sir2 and (B) mammalian SIRT1.

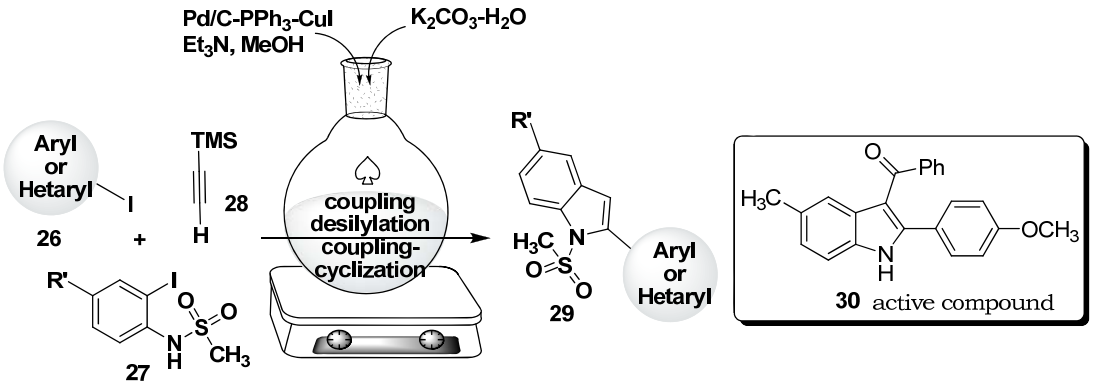
CHAPTER 5

Chapter 5A

Synthesis of 2-(hetero)aryl indoles *via* coupling/desilylation–coupling/cyclization under Pd/C-Cu catalysis

(*Org. Biomol. Chem.* **2011**, *9*, 3808)

2-substituted indole is one of the key structural frameworks found in many naturally occurring alkaloids, bioactive compounds and drugs. In this chapter, we have demonstrated a Pd/C-catalyzed new one-pot synthesis of 2-(hetero)aryl indoles (**29**) *via* sequential C-C coupling followed by C-Si bond cleavage and subsequent tandem C-C / C-N bond forming reaction in the same pot (Scheme 5). A variety of indole derivatives were prepared by using this methodology. Its application has been demonstrated in preparing compound (**30**) with promising mammalian SIRT1 activating properties (Fig. 4, A) that was supported by the *in silico* docking studies (Fig. 4, B).



Scheme 5. One pot synthesis of 2-aryl indoles.

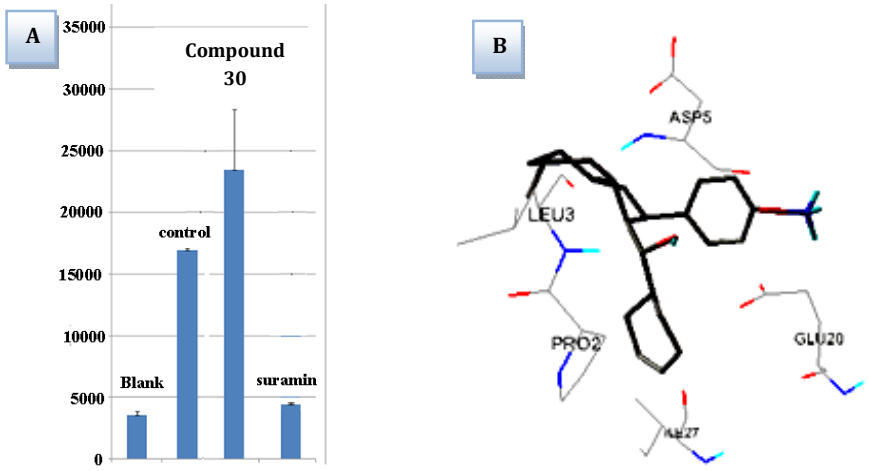


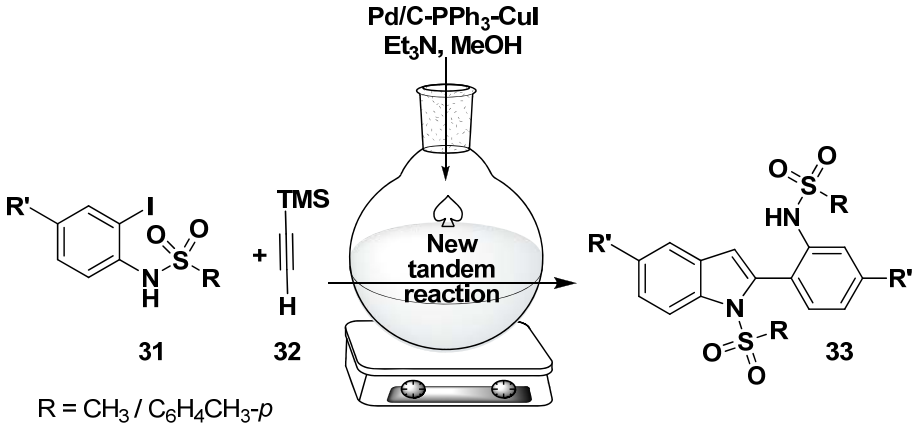
Figure 4. (A) SITR1 activation by compound **30**. (B) Docking study of compound **30**.

Chapter 5B

Pd/C-Cu catalyzed one pot synthesis of 2-substituted indoles

(*Med. Chem. Commun*. **2011**, *2*, 1006)

In continuation of the work that has been described in chapter 5A, we further developed a new, direct and general method for the construction of indole derivatives (**33**) containing an *o*-($MeSO_2NH)C_6H_4$ group at C-2 position in a single pot (Scheme 6).



Scheme 6. Synthesis of indole containing a *o*-($RSO_2NH)C_6H_4$ group at C-2.

List of Publications

1. **Ali Nakhi**, S. Archana, S. Manisha, K. Lalit Kumar, Manojit Pal. Palladium catalyzed one pot synthesis of *N*-thiophenyl substituted pyrrole[2,3-*b*]quinoxalines of pharmacological interest via Buchwald coupling strategy.
   (***2013*** *- Manuscript under preparation*).

2. **Ali Nakhi**, Md. Shafiqur Rahman, G. P. K. Seerapu, B. Rakesh Kumar, K. Lalith Kumar, Pushkar Kulkarni, Devyani Haldar, Manojit Pal. Transition metal free hydrolysis / cyclization strategy in a single pot: Synthesis of fused furo *N*-heterocycles of pharmacological interest.
   (*Org. Biomol. Chem.* **2013**, *11*, 4930). Most Read Article

3. **Ali Nakhi**, S. Archana, G. P. K. Seerapu, Ch. Keerthana Sarma, K. Lalith Kumar, Pushkar Kulkarni, Devyani Haldar, Manojit Pal. $AlCl_3$ -mediated hydroarylation–heteroarylation in a single pot: a direct access to densely functionalized olefins of pharmacological interest.
   (*Chem. Commun.* **2013**, *49*, 6268).

4. **Ali Nakhi,** Md. Shafiqur Rahman, S. Archana, Ravada Kishore, G. P. K. Seerapu, K. Lalith Kumar, Devyani Haldar, Manojit Pal. Construction and functionalization of pyranone ring fused with pyran moiety: Design and synthesis of novel pyrano[4,3- b]pyran-5(4 H)-ones as potential inhibitors of sirtuins.
   (*Bioorg. Med. Chem. Lett*. **2013**, *23*, 4195).

5. **Ali Nakhi**, P. T. V. A. Srinivas, Md. Shafiqur Rahman, Ravada Kishore, G. P. K. Seerapu, K. Lalith Kumar, Devyani Haldar, M. V. Basaveswara Rao, Manojit Pal. Amberlite IR-120H catalyzed MCR: Design, synthesis and crystal structure analysis of 1,8-dioxodecahydroacridines as potential inhibitors of sirtuins.
   (*Bioorg. Med. Chem. Lett*. **2013**, *23*, 1828).

6. **Ali Nakhi**, Md. Shafiqur Rahman, Ravada Kishore, Chandana L.T. Meda, Girdhar Singh Deora, Kishore V. L. Parsa, Manojit Pal. Pyrrole[2,3-*b*]quinoxalines as inhibitors of firefly luciferase: Their Cu-mediated synthesis and evaluation as false positives in a reporter Gene Assay.
   (*Bioorg. Med. Chem. Lett*. **2012**, *22*, 6433).

7. **Ali Nakhi**, Raju Adepu, D. Rambabu, Ravada Kishore, G. R. Vanaja, Arunasree M. Kalle, Manojit Pal**.** Thieno[3,2-*c*]pyran-4-one based novel small molecules: their synthesis, crystal structure analysis and in vitro evaluation as potential anticancer agents.
   (*Bioorg. Med. Chem. Lett.* **2012**, *22*, 4418).

8. **Ali Nakhi**, Bagineni Prasad, Raja Mohan Rao, Uppender Reddy, Sandhya Sandra, Ravada Kishore, Javed Iqbal, Manojit Pal. A new route to indoles via Sila-Sonogashira strategy: Identification of novel small molecules as potential anti tuberculosis agents.
   (*Med. Chem. Commun.* **2011**, *2* , 1006).

9. R.M. Rao, Upendar Reddy, **Ali Nakhi**, M. K. Arunasree, Rajamohan Reddy.P, Javed Iqbal, Manojit Pal. Sequential coupling/desilylation-coupling/cyclization in a single pot under Pd/C-Cu catalysis: Synthesis of 2-(hetero)aryl indoles.
   (*Org. Biomol. Chem.* **2011**, *9*, 3808).

10. Arunasree M. Kalle, A. Mallika, **Ali Nakhi**, Jayasree Badiger, Pinaki Talukdar, Sachchidanand. Inhibition of SIRT1 by a small molecule induces apoptosis in breast cancer cells.
    (*Biochem. Biophys. Res. Commun*. **2010**, *401*, 13).

Oral and Poster Presentation

Jan 9-10, 2013

1. Pd/C-CuI catalyzed one pot synthesis of 2-substituted indoles as potential anti tuburculosis agents.
   Catalyst 2013 - Dr. Reddy' Chemistry Conclave,
   Dr.Reddy's Research Laboratory – Hyderabad-India.

Dec 15, 2012

2. (Best Poster Award).
   Synthesis of thieno[3,2-c]pyran-4-one based novel small molecules: Crystal structure analysis and their in vitro evaluation as potential anticancer agents.
   Royal Society of Chemistry (RSC- London)-DS,
   National Poster Symposium on Organic/Medicinal Chemistry for Ph.D students,
   Indian Institute of Chemical Technology – Hyderabad-India.

Dec 16-17, 2011

3. A one pot synthesis of 2-(hetero)aryl indoles through coupling/desilylation–coupling/cyclization under Pd/C-Cu catalysis.
   Catalyst 2011- Dr. Reddy' Chemistry Conclave (International year of Chemistry),
   Dr.Reddy's Research Laboratory – Hyderabad-India.

Jan 28-31, 2011

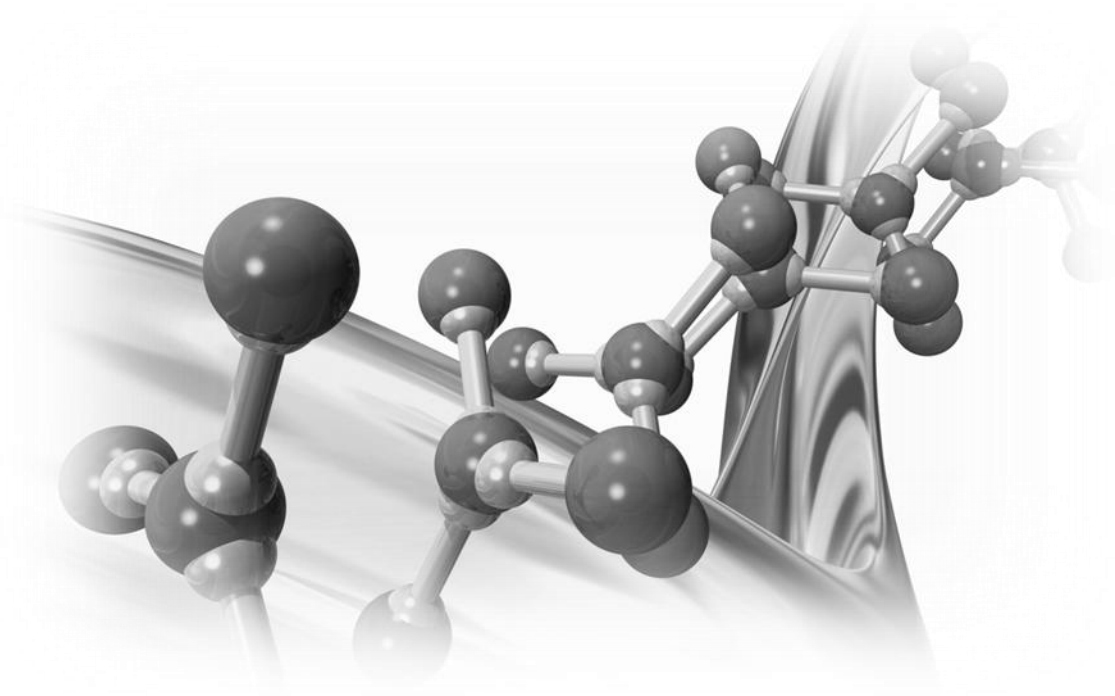
4. Oral Presentation:
   A one pot synthesis of 2-(hetero)aryl indoles under Pd/C-Cu catalysis.
   6th National Organic Symposium Trust (J-NOST),
   University of Hyderabad-India.

Dec 21, 2013

5. (Best Poster Award).
   AlCl3 mediated synthesis of functionalized olefins as potential inhibitors of sirtuins.
   Royal Society of Chemistry (RSC- London)-DS,
   National Poster Symposium on Organic/Medicinal Chemistry for Ph.D students,

CHAPTER 1

Pharmacological activities of N, O and S containing heterocycles and sirtuin modulators



1.1. Introduction

Chemistry is a key to all fascinating facts of life. The life starts with genes. The basic skeleton of the genetic material DNA is made up of heterocycles like adenine, guanine, cytosine and thymine. Extension of human life span through direct intervention has been an important objective of medicinal chemistry.[1] Synthetic organic chemistry coupled with understanding of the basic biology of cellular target proteins for the human diseases *via* medicinal chemistry approach has become a key milestone to ameliorate the deleterious consequences of increasing population. This is possible by the study of different chemical entities like natural products, heterocycles and their pharmacological properties. Heterocycles forms largest divisions of organic chemistry and are of immense importance biologically and pharmaceutically.[2] Heterocyclic systems containing mainly nitrogen, oxygen and sulfur atom constitutes a large class of compounds of biological and medicinal interest. Many natural and synthetic drugs are heterocycles.[3] Some of the drug properties that can be modulated by a strategic manipulation of heterocyclic moiety to maintain or improve the activity of the ligand and selectivity through bioisosteric replacements while improving its absorption, brain penetration and metabolic stability characteristics.

Any attempt to discuss the medicinal properties of heterocyclic compounds is confined to a limited aspect of the subject, since otherwise we would be wading in a stupendous data available in literature. Therefore, some of the marketed drugs containing nitrogen, oxygen and sulfur heterocycles along with their medicinal values are presented in Figure 1. Lapatinib (**1**) is an orally active drug for breast cancer and other solid tumours.[4] Rosiglitazone (**2**) is an antidiabetic drug[5] that belongs to the thiazolidinedione class of compounds. It works as an insulin sensitizer, by binding to the PPAR receptors in fat cells and making the cells more responsive to insulin. Ciprofloxacin (**3**) is one of the broad-spectrum fluoroquinolone antibiotics with low side effects.[6] Warfarin (**4**) has been used extensively as an oral anticoagulant[7] and is often being referred to as the 'gold standard' for oral anticoagulants. Vilazodone (**5**) an antidepressant,[8] combines the effects of a selective serotonin reuptake inhibitor with 5-HT(1A) receptor partial agonist activity. Duloxetine (**6**) an approved antidepressant[9] inhibits both serotonin 5-HT and norepinephrine reuptake.

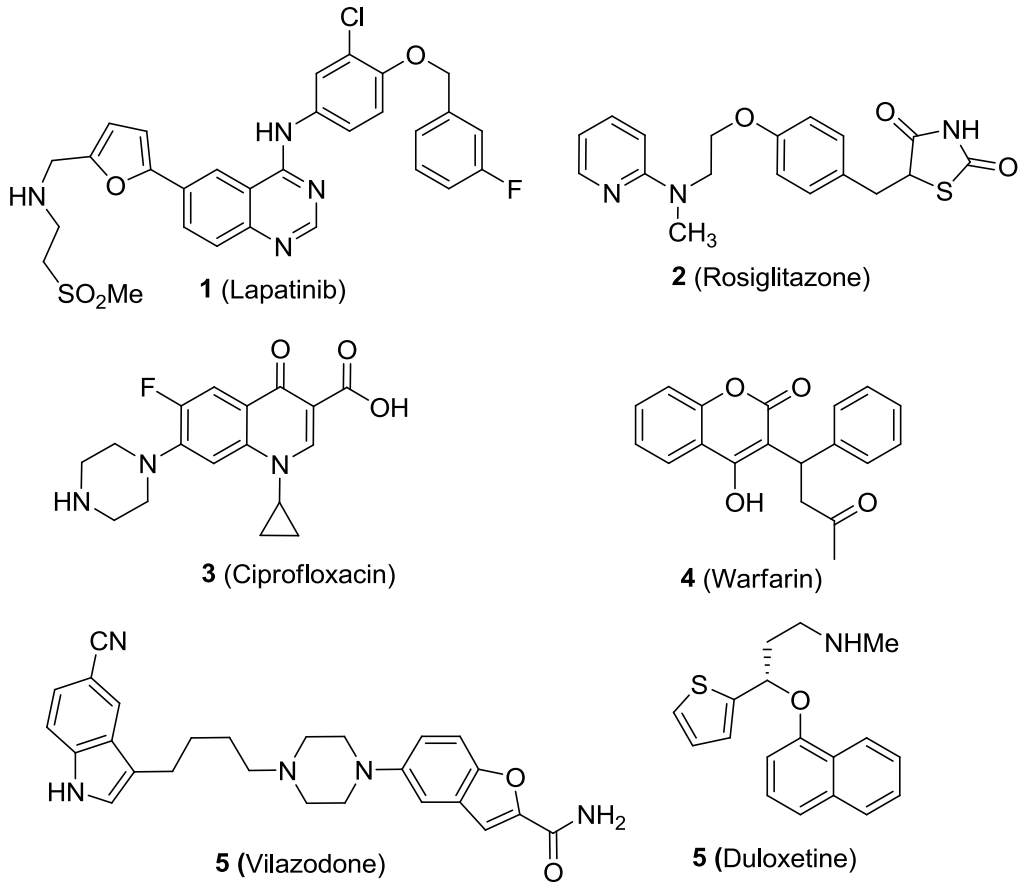


Figure 1. Drugs containing N, O and S heterocycles

The need for the pharmaceutical industry to produce a constant stream of new chemical entities (NCE) has never been more paramount but with constantly changing research and development paradigms in drug discovery where medicinal chemistry plays a key role in modern drug discovery. In drug discovery approaches although high throughput screening (HTS) *in vitro* studies will have beneficial results, some of leads obtained often fail in humans in spite of passing through several prior expensive *in vivo* studies. To avoid this problem the use of Zebrafish as an *in vivo* model to get an early reading has attracted considerable attention in drug discovery.[10a] Zebrafish (or *Danio rerio*), a small pet-shop fish, provides an inexpensive, reliable and efficient first-level screening model for testing toxicity, efficacy, and tissue-targeting for a large number of new chemical entities (NCEs). The use of Zebrafish embryos are also being considered as a one-step strategy to perform developmental screens for identifying compounds with bioactivities relevant to vertebrates.[10b]

1.2. Brief introduction to cancer and diabetes

This section mainly focuses on the diseases such as Cancer and Diabetes with emphasis on Sirtuin modulation as a possible strategy for the potential treatment of these diseases. Advances in our understanding of biology of these diseases have led to the discovery of a spectrum of new therapeutic targets. However, despite the remarkable progress made in the identification and characterization of novel mechanisms of the oncogenic processes and diabetes the success rate for approval of drugs remains low relative to other therapeutic areas. The understanding of key roles of different targeted proteins in signaling pathways leading to the development of cancer and diabetes has helped medicinal chemists to focus on targeted therapies in a more specific and effective way. Recent findings of life span extension in yeast raise the expectation that molecular mechanisms mediating life span extension may also shares commonality between species. Pharmacological modulation of these mechanisms could lead to effective results. In *Saccharomyces cerevisiae*, Sir2 (Sirtuin) protein has been proposed to have a link with metabolism, epigenetic silencing, genome stability and lifespan control.[11] Sirtuin modulation has become one of the active areas of cancer and diabetes research. Before describing the Sirtuin protein in detail, a short introduction to cancer and diabetes are presented below.

Cancer

Cancer is a general term used to refer to a condition where the body's cells begin to grow and reproduce in an uncontrollable way. These cells can then invade and destroy healthy tissue, including organs. Cancer sometimes begins in one part of the body before spreading to other parts. Cancer is one of the greatest health challenges faced by modern medicine in today's world accounting for 7.6 million deaths (around 13% of all deaths) in 2008 and the number is expected to rise to 21 Million by 2030. In India, around 5, 55,000 people died of cancer in 2010 according to the International Agency for Research on Cancer (IARC).[12] These figures pose serious challenges for researchers worldwide who are exploring different approaches to tackle the menace. Of all types of cancers breast, blood, cervical and colon cancer have been studied well. The deregulation of signaling pathways in tumors can lead to enhanced cancer cell growth, proliferation, survival, invasion, and metastasis. Such pathways has become the focus of the development of

targeted cancer therapies during the last decades.[13] There are different types of cellular targets like Tyrosine kinase inhibitors, Histone Deacetylase (HDAC) inhibitors, Proteasome inhibitor, Bcl-2 inhibitors, PARP inhibitors, PI3K inhibitors, and CDK inhibitors etc., Some of the anticancer drugs with different cellular targets are shown in figure 2.

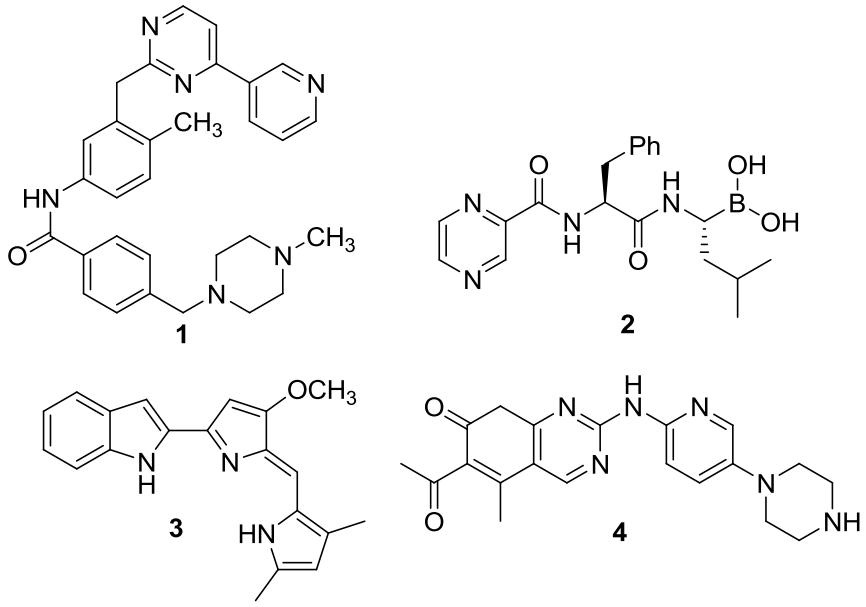


Figure 2. Some of the available anticancer drugs in the market with different cellular targets. 1) Imatinib mesylate (Tyrosine kinase inhibitor). 2) Bortizomib (Proteasome inhibitor). 3) Obatoclax (Bcl2 inhibitor- Phase II clinical trials). 4) Palbociclib (CDK inhibitor)

Diabetes

Diabetes is a chronic disease characterized by high blood glucose (blood sugar) that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin produced in the body. The insulin is a hormone which acts in anabolic pathway that regulates glycogen from glucose, amino acids from TCA cycle intermediates, gluconeogenesis, fatty acid synthesis and protein synthesis. The hallmark of diabetes mellitus is an inability to control blood glucose.

There are two major clinical syndromes namely characterized by (i) Early age of onset with weight loss and ketonuria which is called as Type 1 (Insulin dependent) diabetes and (ii) Relatively later onset, insensitivity to insulin and partial insulin deficiency which is called as Type 2 (Insulin independent) diabetes.

According to International Diabetes Federation, 366 million people have diabetes in 2011 which is expected to increase to 552 million by 2030.[14] Most active research areas of diabetes research are related to various signaling pathways, such as insulin signaling pathway,[15] carbohydrate metabolism pathway,[16,17] ER Stress related pathway,[18] the pathways involving insulin secretion[19] and PPARα receptor agonists,[20] Along with these there are some important cellular drug targets such as Sirtuins (HDACs),[21] AMP Kinases,[22] GLP-1 analogues, dipeptidyl peptidase 4 (DPP4)[23] and Protein tyrosine phosphatase 1B (PTP1B) inhibitors[24] which become the major source of the promising novel drug targets to treat diabetes.

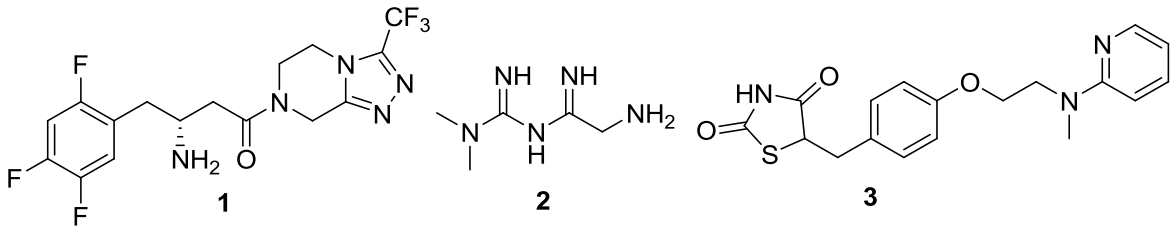


Figure 3. Some available drugs. 1) Sitagliptin - DPP4 inhibitor 2) Metformin - AMPK activator 3) Rosiglitazone – PPAR agonist

1.3. SIRTUINS (Silent Information Regulator)

Sirtuins are class III histone deacetylase enzymes that require nicotinamide adenine dinucleotide (NAD+) to catalyze their reactions.[25] Sirtuins have been found in bacteria to eukaryotes. Caloric restriction promotes the lifespan of many species. SIRT1, an NAD+ - dependent deacetylase, was identified as a molecule involved in calorie restriction related anti aging strategies.[26] SIRT1 functions as an intracellular energy sensor to detect the concentration of NAD+, and controls *in vivo* metabolic changes under calorie restriction and starvation through its deacetylase activity to many targets including histones. Before understanding the biology of sirtuins it is most important to understand Histone proteins.

1.3.1. Histones

Histones are a family[27] of basic proteins their positive charges allow them to associate with negatively charged DNA which is in the nucleus and condense it into chromatin. Nuclear DNA does not appear in free linear strands, it is highly condensed and wrapped around histones in order to fit inside the nucleus and take part in the formation of chromosomes. Under the microscope in its extended form, chromatin looks like beads on a string. The beads are called nucleosomes. Each nucleosome is made of DNA wrapped around eight Histone proteins that function like a spool and are called a Histone octamer (Fig.4). Each histone octamer is composed of two copies each of the histone proteins H2A, H2B, H3, and H4. The chain of nucleosomes is then wrapped into a 30 nm spiral called a solenoid, where additional H1 histone proteins are associated with each nucleosome to maintain the chromosome structure. The packaging of DNA into chromatin profoundly influences DNA-dependent processes such as transcription, replication, repair and recombination.

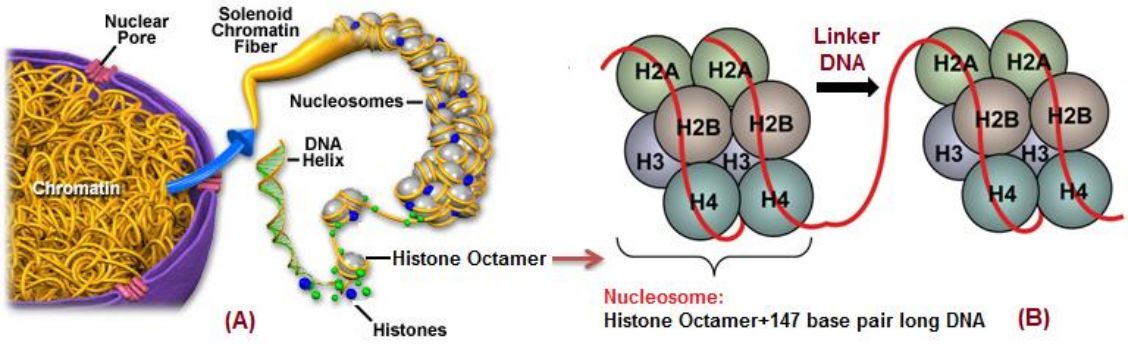


Figure 4. (A) Winding of DNA around Histones to form chromatin. (B) Histone octamere.

1.3.2. Sirtuin family

From their humble origins as silencing factors in yeast, members of the sirtuin family have emerged as broad regulators of cellular fate and mammalian physiology.[28] Yeast silent information regulator 2 (Sir2) protein and its homologues in other prokaryotes and eukaryotes, also known as sirtuins, are highly conserved NAD+-dependant protein deacetylases and/or ADP-ribosyltransferases. The history of sirtuins began almost 3

decades ago, with the identification of *Saccharomyces cerevisiae* an yeast Sir2 (silent information regulator 2, a homolog of human SIRT1), a protein forming part of a complex that enabled gene silencing at selected regions of the yeast genome and another major turning point in the history of Sir2 came from the discovery that Sir2 was involved in the yeast replicative aging process.[29] The mammalian sirtuin family (Table 1) consists of seven members, SIRT1-SIRT7.[30]

Table 1. Mammalian sirtuin family in a cell.

Sirtuin	Localization	Enzyme activity	Function
SIRT1	Nucleus	NAD-dependent Deacetylase	Metabolism/Aging/Cancer/ rRNA Synthesis
SIRT2	Cytoplasm	NAD-dependent Deacetylase	Cell cycle/Adiposgenesis/ Neurodegeneration
SIRT3	Mitochondria	NAD-dependent Deacetylase	Mitochondrial deacetylation
SIRT4	Mitochondria	ADP-ribosyltransferase	Mitochondrial deacetylation/ Insulin metabolism
SIRT5	Mitochondria	NAD-dependent Deacetylase	Mitochondrial deacetylation
SIRT6	Nucleus	NAD-dependent Deacetylase ADP-ribosyltransferase	Genome instability/ Telomeric chromatin
SIRT7	Nucleus	RNA-polymerase	Stress resistance (heart)/ RNA pol1 Transcription

Moreover it is intriguing to investigate whether each member of the sirtuins family exerts its effect on cell cycle individually or in combination with other sirtuin family members or other cell cycle regulators.[31] Sirtuins perform myriad functions which respond in an epigenetic fashion to variety of environmental factors.[32] It plays important role in organism's response to certain types of stress and toxicity which have drawn interest in situations like lifespan extension, cancer, obesity, neurological functions, and age related disorders like diabetes etc., Some of the important aspects of SIRT1 are discussed below.

- **Cancer:** The effects of SIRT1 on tumorigenesis is not clearly understood but is actively being investigated as multiple mediators of cell survival and apoptosis are known substrates (p53 tumor suppressor gene, the FOXO transcription factors and Ku70. These substrates indicate that SIRT1 might be involved in apoptosis, cell cycle regulation, transcription, and many other cellular and organismal regulatory pathways) of SIRT1 deacetylation. It determines changes in gene transcription and most of its effects in oncogenesis is by deacetylating and thus regulating the function of a large number of tumor suppressors or oncogenes such as p53,[33] FOXO transcription factors Ku70, and NF-κB [34, 35] etc. p53 is a critical factor for cell cycle checkpoint regulation, apoptosis, and tumor suppression. Most of the human cancers are related to p53 mutations. SIRT1 regulates p53 via deacetylation, which induce inactivation of p53 and associates with inhibition of p53-dependent apoptosis. SIRT1 activity may elevate cancer risk in mammals by inhibiting p53-induced apoptosis. Regulation of SIRT1 expression in cancer is perhaps the most interesting case, as it involves multiple factors that are highly relevant to cancer.

- **Diabetes:** Diabetes mellitus is characterized by insufficient or inefficient insulin secretary response and elevated blood glucose level. SIRT1 plays a major role in insulin sensitivity. Pancreatic β cells are systemic metabolic sensors that release insulin in response to blood glucose levels. Dysfunction of these cells causes type 1 diabetes mellitus and partially contributes to the pathogenesis of type 2 diabetes.[36] SIRT1 has also been suggested to be involved in the processes of glucose homeostasis and insulin secretion. SIRT1 shown to be a major positive regulator for pancreatic insulin secretion, which in turn triggers glucose uptake and utilization. For example, SIRT1 controls hepatic glucose metabolism by interacting with and deacetylating PGC-1α,[37] a key transcriptional coactivator that controls glucose metabolism in the liver at the level of gene transcription.

Histone acetylation and deacetylation are very specific phenomenon with various isoforms playing distinct roles.[38] It is a dynamic phenomenon with the steady state mediated by the opposing activities of histone acetyltransferases (HATs) and deacetylases (HDACs).

These activities involve large regulatory complexes that are capable of responding to specific DNA sequences and can contain transcription factors, regulatory ligands, and signal transduction and cell cycle proteins. As sirtuins are class III histone deacetylases a brief discussion is presented below about the deacetylation by sirtuins.

1.3.3. Overview of the mechanism of deacetylation catalyzed by Sirtuins

Major families of enzymes that function to deacetylate lysine residues of proteins are the sirtuin enzymes. Deacetylation reaction catalyzed by class III deacetylases requires the consumption of NAD+ and links chromatin epigenetic changes and transcriptional regulation with energy metabolism. Another important difference between sirtuins and the other classes of deacetylases is that a part from deacetylase, some class III family members possesses ADP-ribosyl transferase activity.[39] The mitochondrial localization of these enzymes and their involvement in acetylation/deacetylation processes of mitochondrial proteins is an additional indication of the coupling between metabolic networks and acetylation. These characteristics of the sirtuin family members implicate them in a wide range of diverse cellular processes ranging from glucose homeostasis, to cellular growth, senescence, stress resistance, and metabolism.[40] This in combination with the fact that there are seven members of the sirtuins family raise the theoretical proposal that intervention in each one of the different cellular processes modulated by distinct members of the sirtuins family could increase the specificity of therapeutics by selectively targeting different sirtuins mediated pathways. Consequently inhibitors specific for each member of the sirtuins family could provide a valuable tool towards understanding the link of individual sirtuin member specific biological functions to alter native pathological situations.

Post-translational modification of proteins at lysine residues by reversible acetylation is catalyzed by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs),[38] which act on both histone and non-histone substrates. Post-translational modifications of histones, such as acetylation, methylation, phosphorylation, ubiquitination, etc., are now known to be some of the mechanisms that regulate chromatin structure and function. Among all these acetylation and deacetylation of nucleosomal

histones play an important role in the modulation of chromatin structure, chromatin function and in the regulation of gene expression. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are two opposing classes of enzymes, which tightly control the equilibrium of histone acetylation. An imbalance in the equilibrium of histone acetylation has been associated with carcinogenesis and cancer progression.

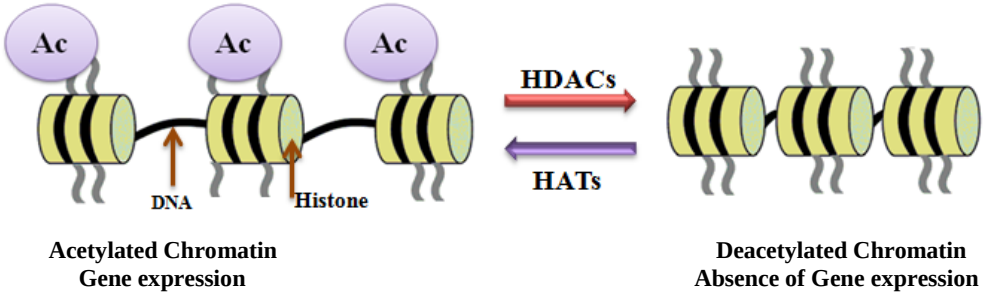


Figure 5. Histone acetylation and deacetylation showing gene regulation

The NAD+ binding pocket, present in the large domain between the interface of the large and small domains, can be divided into three spatially distinct regions: Pocket A, where the adenine-ribose moiety of NAD+ is bound. Pocket B, where the nicotinamide-ribose moiety is bound, and Pocket C, located deep in the NAD+ binding pocket (Fig. 6).[41] The B and C pockets are thought to be directly involved in catalysis. In the presence of an acetyl-lysine, NAD+ bound to the pocket B undergo a conformational change, bringing the nicotinamide group in proximity to the pocket C, where it can be cleaved. The ADP-ribose product of this reaction may then return to the B site, where deacetylation of the acetyl-lysine occurs.

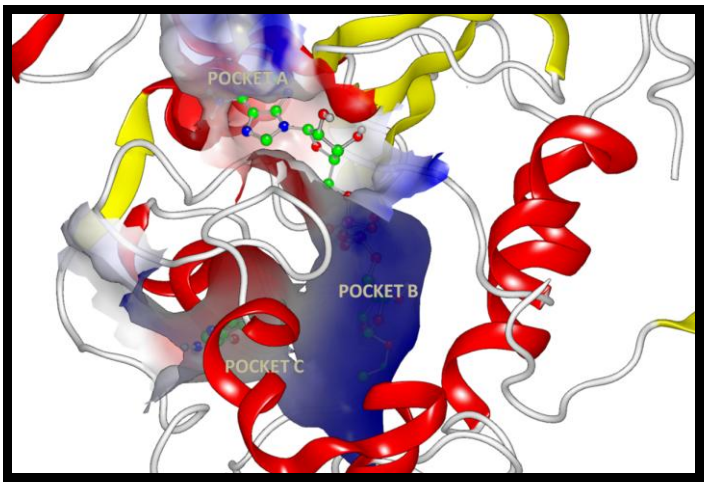


Figure 6. Catalytic core of yeast Sir2 along with Pockets A,B and C showing NAD+ dependent deacetylation and Nicotinamide regulation.

Sirtuins deacetylate lysine residues in an unusual chemical reaction that allows them to be tightly regulated in the cell. The deacetylation reaction catalyzed by these enzymes is coupled to the cleavage of NAD+, yielding nicotinamide and *O*-acetyl ADP-ribose (OAADPr) along with the deacetylated lysine *via* formation of a positively charged O-alkylamidate intermediate.[42] The mechanism of deacetylation is presented in figure 7.

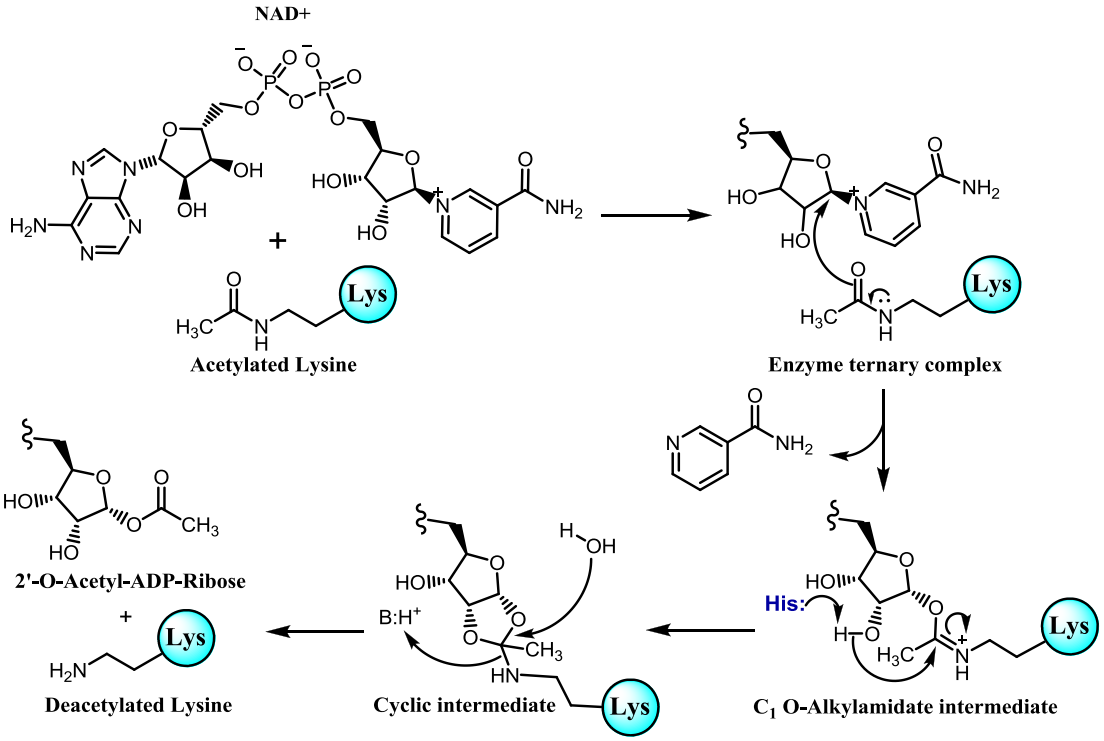


Figure 7. Mechanism of the Sirtuin-catalyzed NAD+-Dependent Deacetylation and Nicotinamide Regulation.

1.3.4. SIRT1- A multifaceted protein of sirtuins family

The uniqueness of sirtuins is that their function as transcriptional regulators is directly linked to intracellular energetics (Fig. 8). Some cellular substrates of SIRT1 include p53 (a tumor suppressor and apoptosis-linked factor),[33] the transcription factor NF-ƙB, and the FOXO[35] family of transcription factors. These molecules are related to the transcriptional control of cell proliferation- and survival-involved genes. SIRT1 can regulate other targets linked to cell death, including Ku70, E2F1 signalling.[34, 43] The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is a known target of Sirtuin dependent

deacetylation[44] and this coactivator also plays a fundamental part in regulating gluconeogenesis and fatty acid oxidation pathways within the liver. SIRT1 mobilizes fat from white adipose tissue by blocking the activity of peroxisome proliferator-activated receptor-gamma (PPAR-γ) through its interaction with nuclear receptor co-repressor (NCOR).

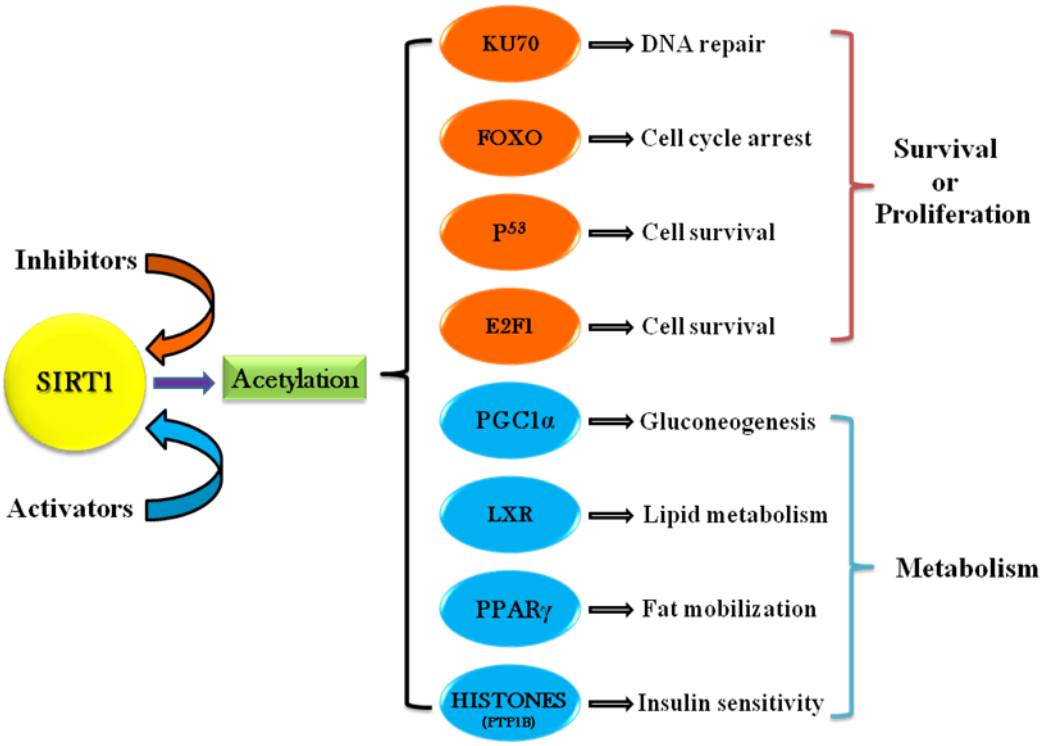


Figure 8. SIRT1 regulate the activity of numerous transcriptional regulators indirectly affecting the outcome of several cellular functions.

1.4. SIRT1 modulation

A thorough understanding of sirtuins is not only of fundamental importance, but also of medicinal importance, since there is enormous current interest in identification and development of small chemical modulators.[45] Based on this concept either activation or inhibition of sirtuins may be desirable for ameliorating disease depending on the pathological condition and the target tissue. In this section the development of small molecule activators and inhibitors of the SIRT1 enzyme is discussed. SIRT1 is more closely related to yeast Sir2 (Sir2 ortholog, SIRT1). In the upcoming chapters we carried out a yeast based reporter assay to check the activity (inhibitory) of synthesized compounds.

1.4.1. SIRT1 activators

Till date, several SIRT1 activators have been identified (Figure 9). They can be mainly categorized into two classes: natural plant polyphenols and synthesized small molecule based activators. Small molecule activators of SIRT1 have been identified that exhibited efficacy in animal models of diseases typically associated with aging including type 2 diabetes. The first activator of SIRT1 identified was resveratrol (**1**) (Fig. 5), a polyphenol commonly found in red wine. In the initial report resveratrol was also shown to increase yeast replicative life span in a Sir2 dependent fashion. This work resulted in tremendous excitement and led to many studies that evaluated resveratrol as an antiaging drug which is a calorie restriction mimetic for the treatment of type 2 diabetic mice. Meanwhile, small molecules with structures unrelated to resveratrol had been identified to activate SIRT1 *in vitro* by using the fluorophore labeled substrates, which include compounds **2**,[46] **3**,[47] **4** (SRT1460),[48] **5** (SRT1720),[49] and **6**.[50] These compounds were shown to activate SIRT1 at submicromolar concentrations and increase SIRT1 catalytic activity several hundred fold. This prompted investigators to look into sirtuins for the discovery and development of small molecule based activators of sirtuins.

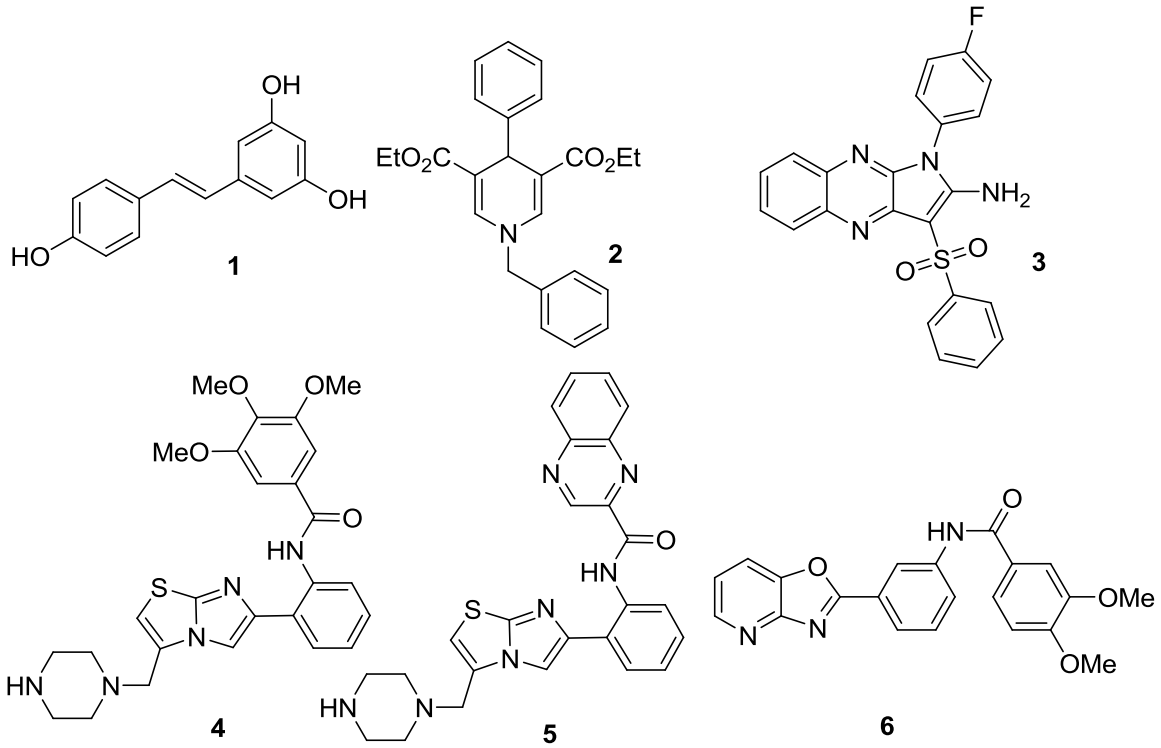


Figure 9. Reported Sirtuin activators.

1.4.2. SIRT1 inhibitors

Since the discovery of sirtuin's enzymatic activity 10 years ago several compound that inhibit this class of enzymes have been described. Both whole cells and biochemical screens have been employed for identification of these inhibitors.[51a] Sirtuins require nicotinamide adenine dinucleotide (NAD+) as an essential cofactor. Nicotinamide **7** is a potent product inhibitor of the reaction because it can rebind to the *o*-alkylamidate intermediate form of the enzyme and attack the intermediate. Sirtuin inhibitors may be potentially used as cancer therapeutic agents because up-regulation of SIRT1 has been described in cancer cell lines.[52] This helps in raising the possibility that SIRT1 inhibition to suppress cancer cell proliferation. Splitomicin (**8**) inhibits Sir2 with an IC_{50} of 60 μM.[51b] However, Splitomicin showed rather weak inhibition of human SIRT1. Another sirtuin inhibitor, Sirtinol (**9**) inhibited yeast Sir2 activity *in vitro*. The 2-hydroxyl-1-napthol moiety played a key role in the inhibition.[53] Two analogues, *m*- and *p*-sirtinol, were 2- to 10-fold more potent than sirtinol against human SIRT1.[54] SIRT1 expression may play an important role in promoting cell growth. Down regulation of p53 activity by deacetylation may result in reduced apoptosis thus, inhibitors of SIRT1 such as sirtinol may have anticancer potential. Salermide (**10**) with a strong *in vitro* inhibitory effect on SIRT1 induced p53-independent apoptosis in cancer but not in normal cells.[55] Cambinol (**11)** a β-naphthol derivative with a substituted thiouracil ring represents the most promising sirtuin inhibitor and shares a β-naphthol pharmacophore with sirtinol and splitomicin. Cambinol inhibits SIRT1 *in vitro* with IC_{50} values of 56 μM.[56] Suramin is also a potent inhibitor of SIRT1 with IC_{50} ~ 0.29 μM.[57] Indole based SIRT1 inhibitor EX-527 i.e. compound (**12)** showed IC_{50} ~ 0.098 μM.[58] This compound was found to be useful in understanding the role of SIRT1 in cell survival and its interaction with p53. Currently, EX-527 is in phase 1 clinical trials for the treatment of Huntington's disease. Tenovins, a family of small molecule inhibitors, are able to inhibit SIRT1 at single digit micromolar concentration and prevent tumor growth *in vivo* in a p53-dependent manner.[59]

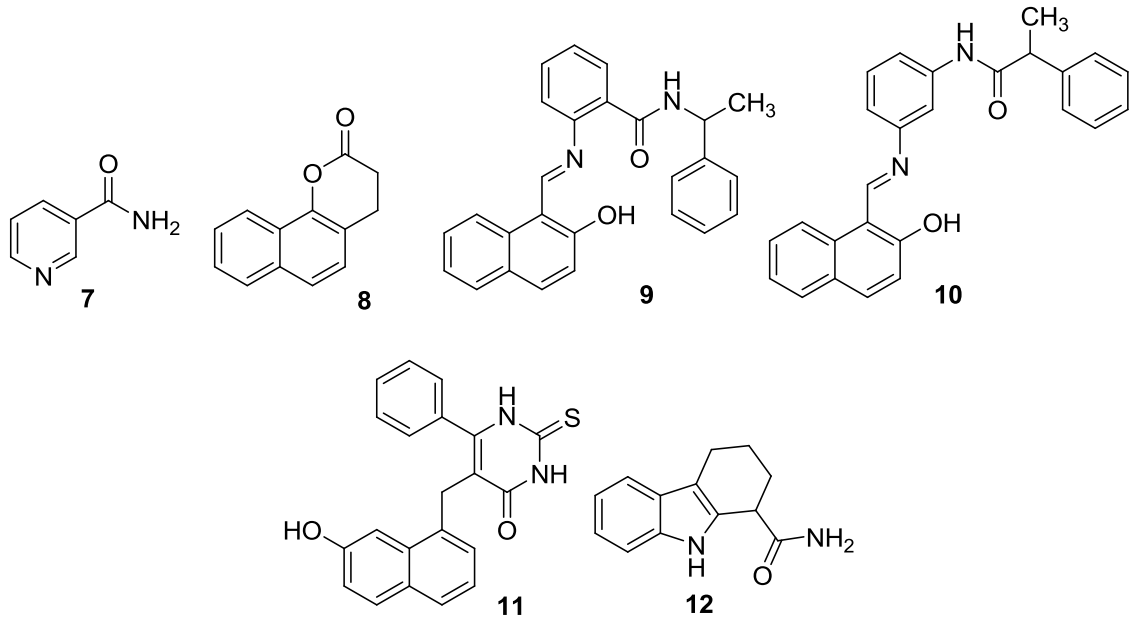


Figure 10. Reported Sirtuin inhibitors. 1) Nicotinamide (**7**). 2) Splitomicin (**8**). 3) Sirtinol (**9**). 4) Selrimide (**10**). 5) Cambinol (**11**). 6) EX-527 (**12**)

1.5. Yeast cell based *in vitro* URA3 reporter silencing assay

The identification of novel inhibitors of sirtuin being the goal of the present work and due to our continuing interest in this area we carried out *in vitro* by using yeast cell based reporter silencing assay (Fig. 11). Compounds were tested at the concentration of 50 μM for their ability to inhibit yeast sirtuin family NAD-dependent histone deacetylase (HDAC) Sir 2 protein (a yeast homologue of mammalian SIRT1). Splitomicin,[13] a known inhibitor of sirtuin, was used as a reference compound. In this assay a yeast strain (TEL::URA3 strain MATα ura3-52 lys2-801 ade2-101 trpΔ63 his3Δ200 leu3Δ200 leu2-Δ1 TEL adh4::URA) was used in which, a reporter gene URA3 was ectopically inserted in the silenced telomeric region where it is silenced by yeast Sir2 protein. Inhibition of Sir2 protein by an inhibitor would allow the URA3 gene to be expressed thereby resulting in death of the yeast cell in presence of 5-fluorooratic acid (5-FOA) through the formation of toxic 5-fluorouracil (5-FUR) which is toxic to the cell.

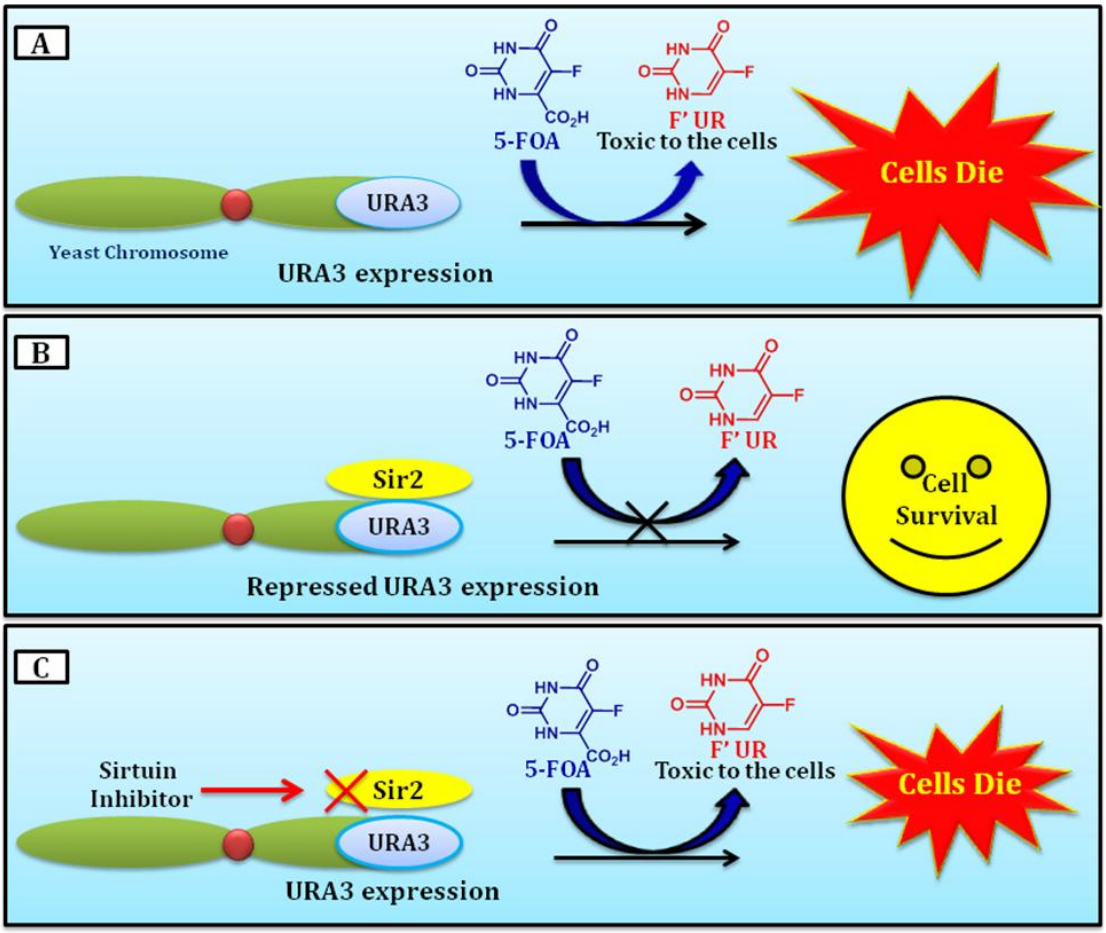


Figure 11. (A) Cell death in presence of 5-FOA. **(B)** Growth in presence of 5-FOA (Sir2 mediated silencing of URA3 gene permits growth in 5-FOA). **(C)** Cell death in presence of 5-FOA: Inhibition of Sir2 results in expression of URA3 gene and cell death in presence of 5-FOA.

1.6. Zebrafish as *in vivo* model

As mentioned in the section 1.1, many of the lead compounds fail in human studies in drug discovery process because of metabolic inactivation, failure to reach target tissues, and off-target toxicity.[60] Recently, the emergence of Zebrafish as an *in vivo* model has provided some initial solution to these problems.[61] The rapid development and transparency of zebrafish embryos has made them an ideal model for the study of vertebrate-specific developmental processes. Since zebrafish are also amenable to closer genetic, morphological, and physiological relationship to humans, it is becoming the model of choice to discover and assess new potential leads. Some important stages of zebrafish development are presented below.

Figure 12. 1.Transparent developing zebrafish embryos. 2. Five day old zebrafish embryo. 3. Adult zebrafish.

In the upcoming chapters we will discuss about safety studies and No Observed Adverse Effect Level (NOAEL) obtained for some of our synthesized compounds.

1.7. Previous work from our group

In the year 2010, we reported inhibition of SIRT1 by a small molecule i.e. compound **5** (JGB1741) induces apoptosis in breast cancer.[62] This molecule showed inhibitory effects on the proliferation of human metastatic breast cancer cells, MDA-MB 231 and significant inhibition of SIRT1 activity compared to sirtinol. Studies on the antitumor effects of JGB1741 on three different cancer cell lines, K562, HepG2 and MDA-MB 231 showed an IC_{50} of 1, 10 and 0.5 μM respectively. Further studies on MDA-MB 231 cells showed a dose dependent increase in K9 and K382 acetylation of H3 and p53 respectively. In conclusion, our study showed the potent apoptotic effects of JGB1741 in MDA-MB 231 cells *via* SIRT1 inhibition. (For complete work see *Biochemical and Biophysical Research Communications* **2010**, *401*, 13)

1.7.1. Design of Compound 5 (JGB1741)

In this study we have designed and developed a small molecule inhibitor of SIRT1, JGB1741, based on sirtinol structure which is having promising antitumor activities. The bioisosteric equivalence between benzene and thiophene prompted us to replace the benzene of 3-amino benzamide of sirtinol with thiophene ring which we thought might give a molecule with better or similar activity to sirtinol. Since the crystal structure of SIRT1 is not available, the best model of the catalytic core of SIRT1 was developed.[63] A series of thiophene derivatives were docked with modeled SIRT1 catalytic domain.

Docking studies using Auto Dock program, showed that compound **5** has given a better binding score (-7.53 kCal/mol) compared to sirtinol (-6.9 kCal/mol). Therefore we synthesized the compound **5** as presented in the following section.

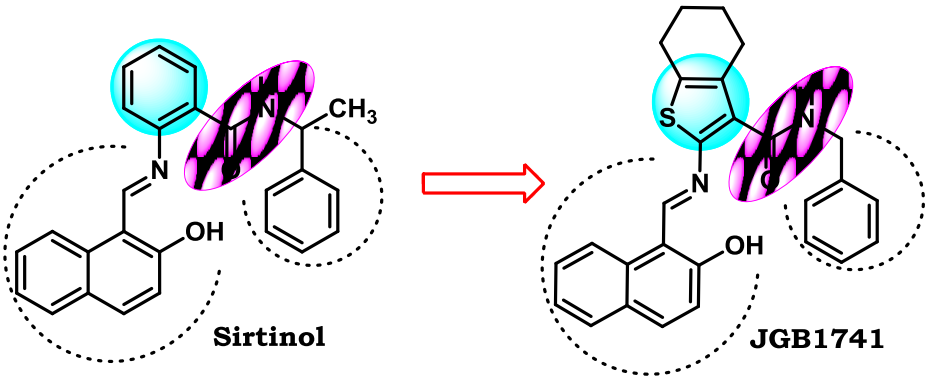
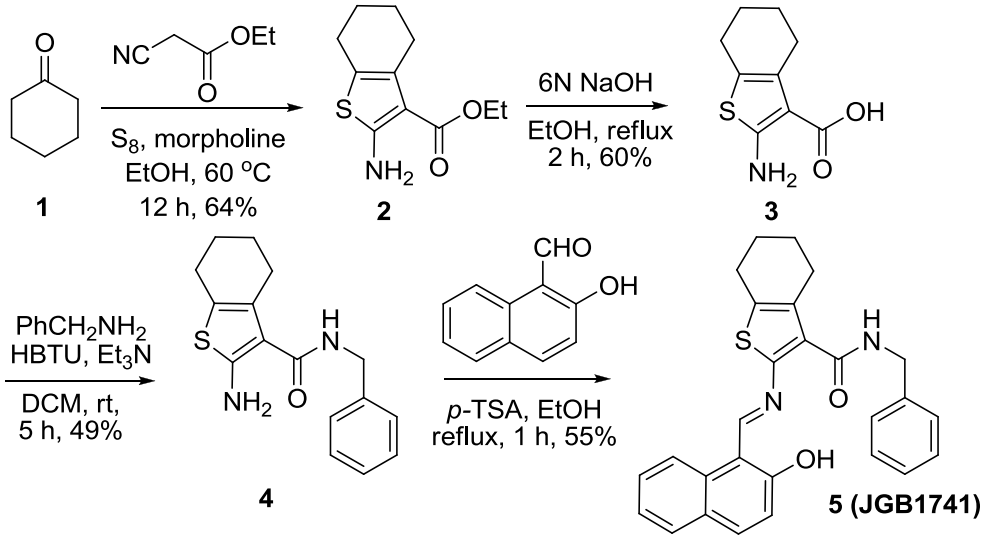


Figure 12. Design of Compound **5** (JGB1741) from known SIRT1 inhibitor

1.7.2. Synthesis of compound 5 (JGB1741)

Synthesis of the inhibitor JGB1741 was carried out from cyclohexanone in four steps (Scheme 6). In the first step, multi-component Gewald reaction[64] of cyclohexanone **1**was carried out in the presence of ethylcyanoacetate and elemental sulfur that afforded thiophene ester **2**. The compound **2** on base-catalyzed hydrolysis produced thiophene acid **3** that was subsequently converted to amide **4** by reacting with benzylamine in the presence of HBTU. Finally, the compound **4** on treatment with 2-hydroxy-1-naphthaldehyde in presence of the catalyst, *p*-toluenesulfonic acid, furnished the inhibitor molecule **5** (JGB1741). The structure of JGB1741 was confirmed by spectroscopic data (see experimental section).



Scheme 6. Synthesis of sirtinol based compound **5** (JGB1741).

1.7.3. Conclusion

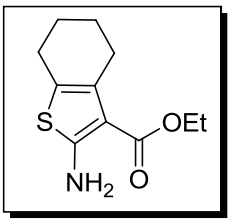
In conclusion we have designed and synthesized an inhibitor of SIRT1. The *in vitro* cell based data clearly indicates that it is more potent in inhibiting cancer cell proliferation, specifically metastatic breast cancer cells MDA-MB 231, with an IC_{50} ~ 512 nM. The study also demonstrates that compound **5** is a specific inhibitor of SIRT1 and thereby increases the acetylated p53 levels leading to p53-mediated apoptosis with modulation of Bax/Bcl2 ratio, cytochrome c release and PARP cleavage. Compound **5** showed 95% inhibition of SIRT1 at100 µM with IC_{50} ~ 15 µM, better inhibition of SIRT1 compared to sirtinol and Splitomicin at 10 µM. However, compound **5** was found to be less potent compared to another known SIRT1 inhibitor Suramin. Overall, the compound **5** could become a potential therapeutic hit candidate towards the treatment of breast cancer.

1.8. Experimental section

Melting points (mp) were measured on a Buchi B-540. 1H and 13C spectra were recorded (as indicated) either on a Varian (Mercury Plus) 400MHz or on a Jeol NMR -ECS400 or on a Gemini (Varian) 200 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (*J*) given in Hz, or

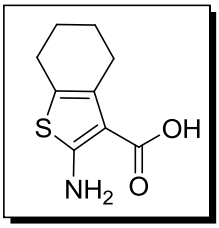
multiplet (m). Broad peaks are marked as br. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. UV-Vis spectra were measured on a Perkin-Elmer-Lambda 25/35/45 UV/Visible spectrophotometer. Low resolution mass spectra (LRMS) for compounds were measured on API 3000TM LC/MS/MS system. High resolution mass spectra (HRMS) were measured on a Waters LCT Premier XE instrument. PXRD data was recorded on a RIGAKU Dmax 2200 instrument and analyzed using RINT2000 software.

Procedure for the Preparation of compound [ethyl 2-amino-4,5,6,7 tetrahydrobenzo [*b*]thiophene-3-carboxylate (2)



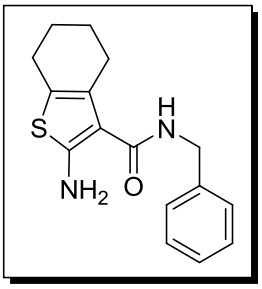
A mixture of cyclohexanone 1 (1.0 g, 10 mmol), ethylcyanoacetate (1.15 g, 10 mmol), morpholine (0.89 g, 10 mmol), sulphur (0.32 g, 10 mmol) in ethanol (10 ml) was stirred and refluxed for overnight. After completion of the reaction, the reaction mixture was cooled to room temperature and solvent was removed under vacuum. Crude solid was washed with cold ethanol and filtered though sintered funnel, dried in vacuo. Crude product was dissolved in dichloromethane and washed with brine followed by evaporation of the solvent to give 1.469 g of the compound 2 (64%) as light yellow solid. mp: 111.0-112.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.92 (s, 2H), 4.25 (q, *J* = 7.5 Hz, 2H), 2.70 (t, *J* = 5.6 Hz, 2H), 2.50 (t, *J* = 5.6 Hz, 2H), 1.70-1.80 (m, 2H), 1.33 (t, *J* = 7.5 Hz, 3H). IR (KBr, cm^{-1}): 3404, 2935, 1599, 1647. EI-MS: m/z 226.3 [M+1]. The spectroscopic data were consistent with reported literature values.

Procedure for the Preparation of compound [2-amino-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxylic acid (3)



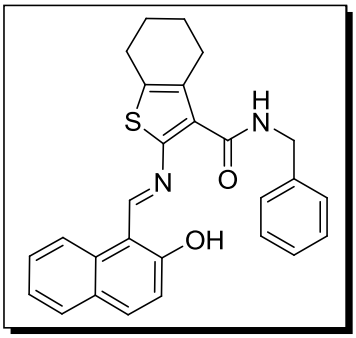
To a solution of ester 2 (1.0 g, 5.07 mmol) in ethanol (10 ml), was added NaOH (6 N, 8 ml) and the solution was refluxed for 2 h at 60-70 oC. After completion of the reaction, ethanol was evaporated in vacuo. The reaction mixture was poured on cold water (15 ml) and washed with diethylether (2 × 10 ml). The aqueous layer was acidified to pH 3 - 4 with 6 N HCl solution till product was precipitated. The solid was separated by filtration, dried and purified by recrystallization from dichloromethane /hexane (1:1) to yield 0.52 g of 2 (60 %). mp: 155-156 °C; cm-1. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.69 (br s, 1H) 7.15 (br s, 2H), 2.58 (t, J = 5.8 Hz, 2H), 2.41 (t, J = 5.8 Hz, 2H), 1.62-1.70 (m, 4H). ^{13}C NMR (CD_3OD, 50 MHz): δ 167.8, 162.6, 132.6, 117.2, 104.9, 26.5, 24.2, 23.1, 22.5. IR (KBr, cm^{-1}): 3324, 3367, 3324, 2935, and 1635. EI-MS: m/z 196.2 [M-H]. The spectroscopic data were consistent with reported literature values.

Procedure for the Preparation of compound [2-amino-N-benzyl-4,5,6,7 tetrahydro benzo[*b*]thiophene-3-carboxamide (4)



To a solution of the of the compound 3 (0.5 g, 2.53 mmol) in dry dichloromethane (15 ml) was added benzyl amine (0.35 g, 3.289 mmol) and cooled to 0 ^{o}C. To the solution was added HBTU (1.9 g, 5.06 mmol), triethylamine (1.7 ml, 12.65 mmol) and stirred for 5 h at room temperature. The reaction mixture was diluted with dichloromethane (50 ml), successively washed with saturated $NaHSO_4$ solution (5 ml), $NaHCO_3$ solution (5 ml), brine solution (10 ml) and concentrated in vacuo. The crude mass was purified by column chromatography over silical gel (hexane/ethylacetate 8:2) to yield 0.352 g of the compound **4** (49%). mp: 184-186 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.34-7. 26 (m, 5H), 4.8 (d, J = 5.6 Hz, 2H), 2.59-2.53 (m, 4H), 1.79-1.76 (m, 4H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 166.3, 161.8, 138.5, 132.2, 128.7 (2 C), 127.5 (2 C), 127.3, 104.4, 43.3, 26.9, 24.5, 23.2, 22.8 18.5; IR (KBr, cm^{-1}): 3564, 2938, 1697, 1134 cm-1. EI-MS: m/z 287.2 [M+1]. The spectroscopic data were consistent with reported literature values.

Procedure for the Preparation of compound [(*E*)-*N*-benzyl-2-(((2-hydroxynaphthalen-1 -yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (5)



To a solution of compound 3 (0.5 g, 1.84 mmol) in ethanol (8 ml) was added 2-hydroxy-1-naphthaldehyde (0.315 g, 1.84 mmol), catalytic amount of p-toluenesulfonic acid (16 mg, 0.09 mmol) and heated to reflux at 70oC for 1 h. The reaction mixture was cooled to room temperature and the separated solid was filtered, washed with cold ethanol (15 ml) and purified by column chromatography over silica gel (hexane/ethylacetate 9:1) to yield 0.4 g of the compound **5** (55.2 %). mp: 242-243 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.74 (s, 1H), 9.34 (s, 1H), 8.93 (t, *J* = 6.0 Hz, 1H), 8.53 (d, *J* = 8.7 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 7.3 Hz), 7.58-7.53 (m, 1H), 7.44-7.39 (m, 1H), 7.37-7.27 (m, 4H), 7.26-7.20 (m, 2H), 4.49 (d, *J* = 6.0 Hz, 2H), 2.78-2.69 (m, 2H), 2.59-2.50 (m, 2H), 1.89-1.68 (m, 4H); ^{13}C NMR (DMSO-d_6, 50 MHz): δ 207.0, 163.9, 161.2, 154.3, 147.0, 139.3, 135.1, 133.8, 131.9, 131.8, 129.1, 128.3 (2 C), 128.2, 127.7, 127.2 (2 C), 126.7, 123.9, 121.2, 119.0, 110.0, 42.6, 24.9, 24.5, 22.7, 22.0; IR (KBr, cm^{-1}): 3297, 2937, 1632, 1534, 819. EI-MS: m/z 441.3 [M+1]. HR-ES MS calculated for $C_{27}H_{25}N_2O_2S$, [M+1] 441.1637, found 441.1653.

Reference:

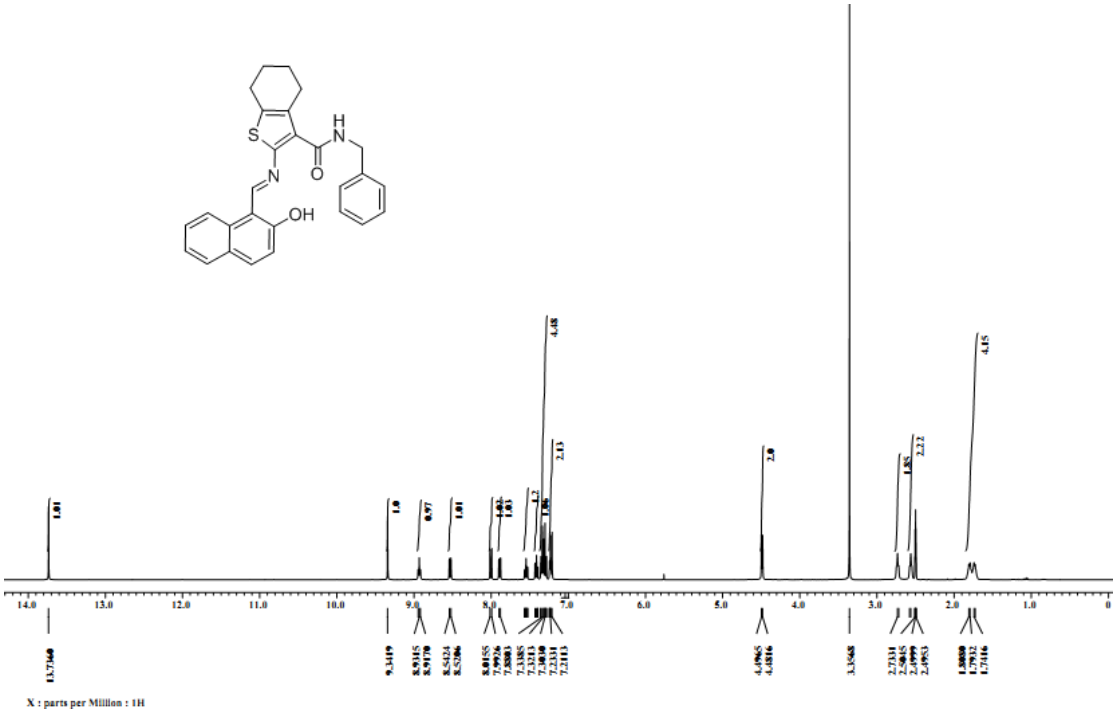
1. Lombardino, J. G.; Lowe III, J. A. *Nat. Rev. Drug. Discov.* **2004**, *3*, 853.
2. Pozharskii, A. F.; Soldatenkov, A.; ; Katritzky, A. R. Heterocycles in Life and Society. Second Edition, *Wiley*, **2011**
3. (a) Chin, Y. W.; Balunas, M. J.; Chai, H. B.; Kinghorn, A. D. *Amer. Asso. Pharm. Sci. J.* **2006**, *8*(2), 239. (b) Cordell, G. A.; Quinn-Beattie, M. L.and Farnsworth, N. R. *Phytother. Res.*, **2001**, *15* (3), 183.
4. Burris, H. A. *The Oncolo.* **2004**, *9*, 3, 10.
5. Werner, A. L.; Travaglini, M. T. *Pharmacotherapy.* **2001**, *21*(9), 1082.
6. Azema, J.; Guidetti, B.; Dewelle, J.; Le Calve, B.; Mijatovic, T.; Korolyov, A.; Vaysse, J.; Martino, M. M.; Martino, R.; Kiss, R. *Bioorg. Med. Chem.* **2009**, *17*, 5396.
7. Holbrook, A. M.; Pereira, J. A.; Labiris, R.; McDonald, H.; Douketis, J. D.; Crowther, M.; Wells, P.S. *Arch. Intern. Med.* **2005**, *165* (10), 1095.
8. Khan A. *Expert. Opin. Investig. Drugs.* **2009**, *18*(11), 1753.
9. Bymaster, F. P.; Ahmad, L. J.; Threlkeld, P. G.; Shaw, J. L.; Thompson, L.; Nelson, D.L.; Luecke, M. S. K.; Wong, D. T. *Neuropsychopharm*. **2001**, *25*, 871.
10. (a) Kari, G.; Rodeck, U.; Dicker, A.P. *Clin*. *Pharmacol*. *Ther*. **2007**, *82*, 70. (b) Basu, S.; Sachidanandan, C. Chem. Rev., **2013**, *113* (10), 7952.
11. Lin, S. J.; Ford, E.; Haigis, M.; Liszt, G.; Guarente, L. *Genes. Dev*. **2004**, *18*, 12.
12. http://www.who.int/gho/ncd/mortality_morbidity/cancer/en/index.html (accessed on 10 october 2013)
13. a) Hanahan, D.; Weinberg, R.A. *Cell.* **2000**, *100* (1), 57. b) Widakowich, C. *Int J Biochem Cell Biol.* **2007**, *39* (7-8), 1375.
14. (a) http://www.idf.org/diabetesatlas/5e/the-global-burden (b) Wild, S.; Roglic, G.; Gree n, A.; Sicree, R.; King, H. *Diabetes Care.* **2004**, *27*, 1047.
15. Huang, X.F.; Chen, J.Z.; *Obes. Rev*. **2009**, *10*, 610.
16. Henke, B.R.; Sparks, S.M. *Mini Rev. Med. Chem.* **2006**, 6, 845.
17. Hardie, D.G. *Annu. Rev. Pharmacol*. *Toxicol*. **2007**, *47*, 185.
18. Ozcan, U.; Cao, Q.; Yilmaz, E.; Lee, A.H.; Iwakoshi, N.N.; Ozdelen, E.; Tuncman, G.; Gorgun, C.; Glimcher, L.H.; Hotamisligil, G.S.; *Science*, **2004**, 306, 457.

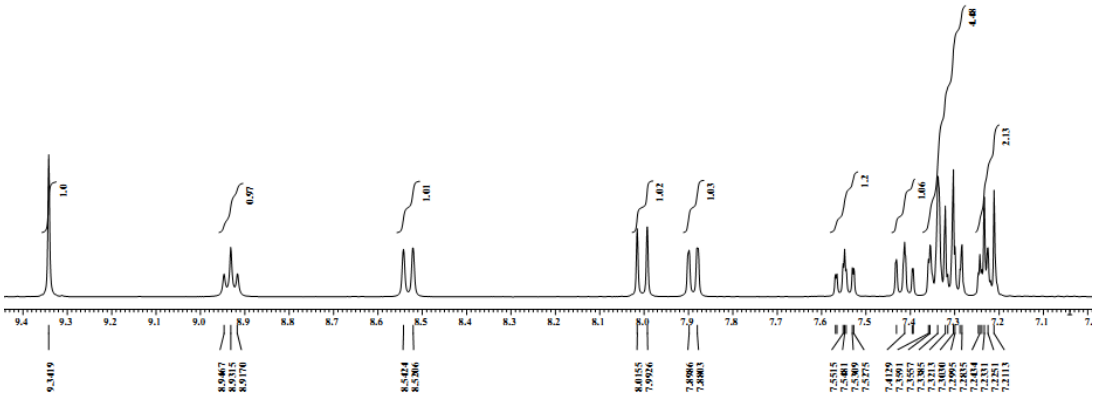
19. K.N. Frayn, B.A. Fielding, F. Karpe, Adipose tissue fatty acid metabolism and cardiovascular disease, Curr. Opin. Lipidol. 16 (2005) 409 –415.
20. B.L. Balint, L. Nagy, Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases, Endocr. Metab. Immune Disord. Drug Targets 6 (2006) 33–43.
21. Kitada M, Kume S, Kanasaki K, Takeda-Watanabe A, Koya D. Curr Drug Targets. 2013 Jun 1;14(6):622-36.
22. Zhang BB, Zhou G, Li C. Cell Metab. 2009 May; 9(5):407-16
23. Rajesh Gupta, Sameer S. Walunj, Ranjeet K. Tokala, Kishore V.L. Parsa, Santosh Kumar Singh and Manojit Pal. Current Drug Targets, 2009, 10, 71-87.
24. Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug. Discov.* **2002**, *1*, 696.
25. (a) Michan, S.; Sinclair, D. *Biochem. J.* 2007, *404*, 1. (b) Frye, R. A. Biochem. Biophys. Res. Commun. **1999**, *260*, 273.
26. Finkel, T.; Deng, C. X.; Mostoslavsky, R. *Nature*. **2009**, *460*, 587.
27. Bartova, E.; Krejci, J.; Harnicarova, A.; Galiova, G.; Kozubek, S. *J. Histochem. Cytochem.* **2008**, *56*(8), 711.
28. Blander, G.; G uarente, L. *Annu. Rev. Biochem*. **2004**, *73*, 417.
29. (a) Kaeberlein, M.; McVey, M.; Guarente, L. *Genes. Dev.* **1999**, *13*, 2570. (b) Lin, S. J.; Defossez, P. A.; Guarente, L. *Science*, **2000**, 289, 2126.
30. (a) Haigis, M. C.; Guarente, L. P. *Genes. Dev*, **2006**, 20, 21, 2913. (b) Haigis, M. C.; Sinclair, D. A. *Biochem. J.*, **2007**, *404*, 1, 1.
31. Houtkooper, R. H.; Pirinen, E.; Auwerx, J. *Nat. Rev. Mol. Cell Biol*, **2012**, 13, 225.
32. Moazed, D. *Mol. Cell*, **2001**, *8*, 489.
33. Vazir i, H.; Dessain, S. K.; Ng Eaton, E.; Imai, S.I.; Fr ye, R. A.; Pandita, T. K.; Guarente, L.; Weinberg , R. A. Cell, **2001**, *107*, 149.
34. Cohen, H. Y.; Miller, C.; Bitter man, K. J.; Wall, N. R.; Hekking , B.; Kessler, B.; Howitz, K. T.; Gorospe, M.; Cabo, R.; Sinclair, D. A. *Science*, **2004**, *305*, 390.
35. Westphal, C. H.; Dipp, M. A.; Guarente, L. *Trends in Biochem. Sciences*, **2007**, 32, 12, 555.
36. American Diabetes Association. *Diabetes Care*. **2010**, *33*(Suppl 1), S62.

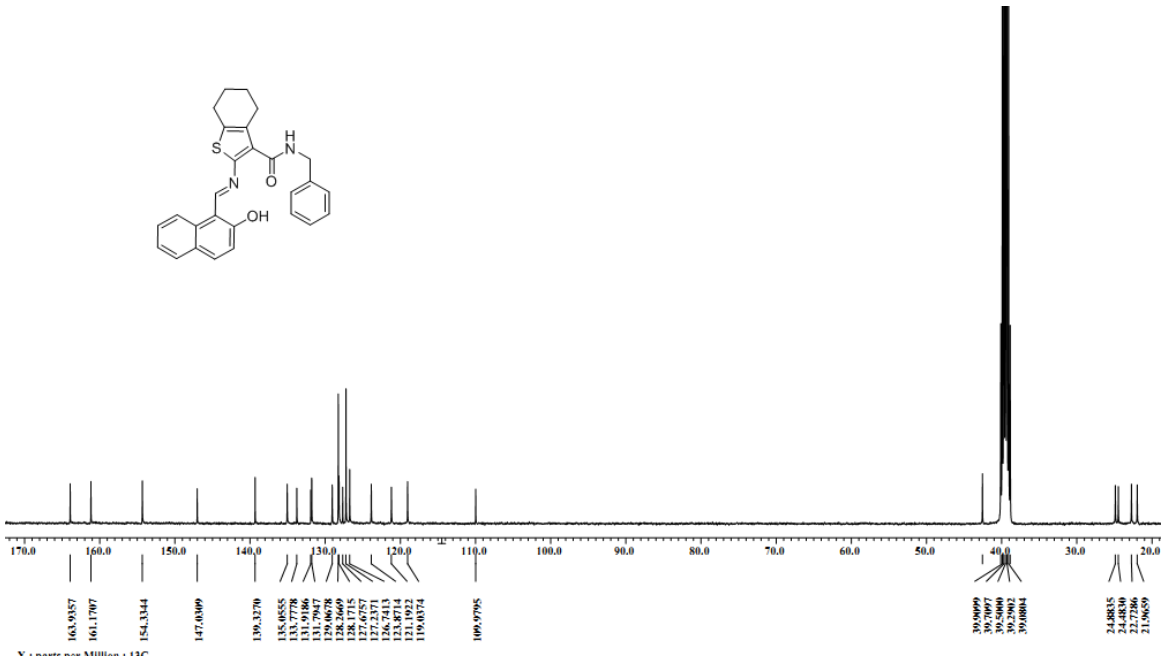
37. Rodgers, J.T.; Lerin, C.; Haas, W.; Gygi, S.P.; Spiegelman, B.M.; Puigserver, P. *Nature*, 2005, *434*, 113.
38. (a) Cheung, P.; Allis, C. D.; Sassone Corsi, P. *Cell*, **2000**,103, 2, 263. (b) Hildmann, C.; Riester, D.; Schwienhorst, A. *Appl. Microbio. Biotech*, **2007**, 75, 3, 487.
39. Tanny, J.C.; Dowd, G.J.; Huang, J.; Hilz, H.; Moazed, D. *Cell*, **1999**, 99, 735.
40. Rajendran, R.; Garva, R.; Demonacos, M.K.; Demonacos, C. *J. Biomed. Biotech.* **2011**, Article ID 368276.
41. Avalos, J. L.; Bever, K. M.; Wolberger, C. *Mol. Cell*, **2005**, *17*, 855.
42. (a) Chang, J. H.; Kim, H. C.; Hwang, K. Y.; Lee, J. W.; Jackson, S. P.; Bell, S. D.; Cho, Y. *J. Biol. Chem.* **2002**, *277*, 34489. (b) Sauve, A. A.; Celic, I.; Avalos, J.; Deng, H.; Boeke, J. D.; Schramm, V. L. *Biochemistry*. 2001, *40*, 15456.
43. Revollo, J. R.; Grimm, A. A.; Imai, S.I. *J. Biol. Chem.* 2004, *279*, 50754.
44. Dimova, D.K.; Dyson, N.J. *Oncogene*. **2005**, *24*, 2810.
45. Villalba, J.M.; Alcain F.J. (International Union of Biochemistry and Molecular biology) *Bio Fact*. **2012**, *38*, 5, 349.
46. Mai, A.; Valente, S.; Meade, S.; Carafa, V.; Tardugno, M.; Nebbioso, A.; Galmozzi, A.; Mitro, N.; De Fabiani, E.; Altucci, L.; Kazant sev, A. *J. Med. Chem.* **2009** , *52* , 5496.
47. Nayagam, V. M.; Wang, X.; Tan, Y. C.; Poulsen, A.; Goh, K. C.; Ng, T.; Wang, H. Song, H. Y.; Ni, B.; Entzeroth, M.; Stunkel, W. *J. Biomol. Screening*. **2006**, *11*, 959.
48. Milne, J. C.; Lambert, P. D.; Schenk, S.; Carney, D. P.; Smith, J. J.; Gagne, D. J.; Jin, L.; Boss, O.; Perni, R. B.; Vu, C. B.; Bemis, J. E.; Xie, R.; Disch, J. S.; Ng, P. Y.; Nunes, J. J.; Lynch, A. V.; Yang, H.; Galonek, H.; Israelian, K.; Choy, W.; Iffland, A.; Lavu, S.; Medvedik, O.; Sinclair, D. A.; Olefsky, J. M.; Jirousek, M. R.; Elliott, P. J.; Westphal, C. H. *Nature*. **2007**, *450*, 712.
49. Vu, C. B.; Bemis, J. E.; Disch, J. S.; Ng, P. Y.; Nunes, J. J.; Milne, J. C.; Carney, D. P.; Lynch, A. V.; Smith, J. J.; Lavu, S.; Lambert, P. D.; Gagne, D. J.; Jirousek, M. R.; Schenk, S.; Olefsky, J. M.; Perni, R. B. *J. Med. Chem.* **2009**, *52*, 1275.
50. Bemis, J. E.; Vu, C. B.; Xie, R.; Nunes, J. J.; Ng, P. Y.; Disch, J. S.; Milne, J. C.; Carney, D. P.; Lynch, A. V.; Jin, L.; Smith, J. J.; Lavu, S.; Iffland, A.; Jirousek, M. R.; Perni, R. B. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 2350.

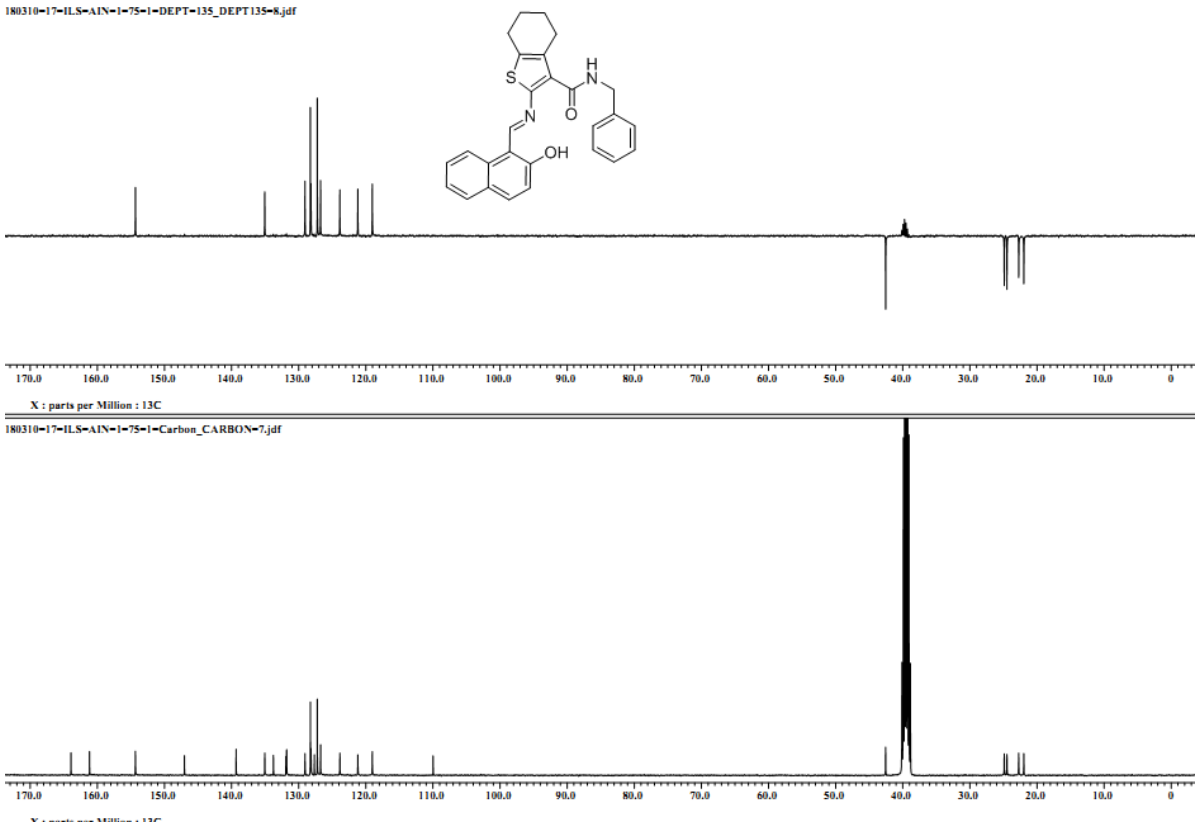
51. (a) Mahajan, V. L. S. S.; Simon, J. A.; Bedalov, A. *The Biology and Clinical Implication*. **2001**. p. 241-255. (b) Bedalov, A.; Gatbonton, T.; Irvine, W. P.; Gottschling, D. E. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 15113.
52. (a) Alcain, F. J.; Villalba, J. M.; *Expert Opin Ther Pat.* **2009**, *19*, 283. (b) Audrito, V.; Vaisitti, T.; Rossi, D.; Gottardi, D. *Cancer Res*. **2011**, *71*, 4473.
53. Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D. *J. Biol. Chem*. **2001**, *276*, 38837.
54. Mai, A.; Massa, S.; Lavu, S.; Pezzi, R.; Simeoni, S.; Ragno, R.; Mariotti, F. R.; Chiani, F.; Camilloni, G.; Sinclair, D. A. *J. Med. Chem.* **2005**, *48*, 7789.
55. Lara, E.; Mai, A.; Calvanese, V.; Altucci, L.; Lopez-Nieva, P.; Martinez-Chantar, M. L.;
56. Varela-Rey, M.; Rotili, D.; Nebbioso, A.; Ropero, S.; Montoya, G.; Oyarzabal, J.; Velasco, S.; Serrano, M.; Witt, M.; Villar-Garea, A.; Inhof, A.; Mato, J. M.; Esteller, M.; Fraga, M. F. *Oncogene*. **2009**, *28*, 781.
57. Medda, F.; Russell, R. J. M.; Higgins, M.; McCarthy, A. R.; Campbell, J.; Slawin, A. M. Z.; Lane, D. P.; Lain, S.; Westwood, N. J. *J. Med. Chem*. **2009**, *52*, 2673.
58. Trapp, J.; Meier, R.; Hongwiset, D.; Kassack, M.U.; Sippl, W.; Jung, M. *Chem. Med. Chem*. **2007**, *2*, 10, 1419.
59. Napper, A. D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J. F.; Barker, J.; Yau, W. T.; Amouzegh, P.; Flegg, A.; Hamelin, E.; Thomas, R. J.; Kates, M.; Jones, S.; Navia, M. A.; Saunders, J. O.; DiStefano, P. S.; Curtis, R. Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. *J. Med. Chem*. **2005**, *48*, 8045.
60. Keseru, G.M.; Makara G.M. *Nat. Rev. Drug. Discov*. **2009**, *8*, 203.
61. Basu, S.; Sachidanandan, C. *Chem. Rev.*, **2013**, *113* (10), 7952.
62. Kalle, A.M.; Mallika, A.; Badiger, J.; Alinakhi.; Talukdar,P.; Sachchidanand. *Biochem. Biophy. Res. Comm.* **2010**, *401*, 13
63. Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. PROCHECK. *J. Appl. Crystallogr*. **1993**, 26, 283.
64. Puterova, Z.; Krutosikova, A.; Vegh, D. *Arkivoc*. **2010**, 209.

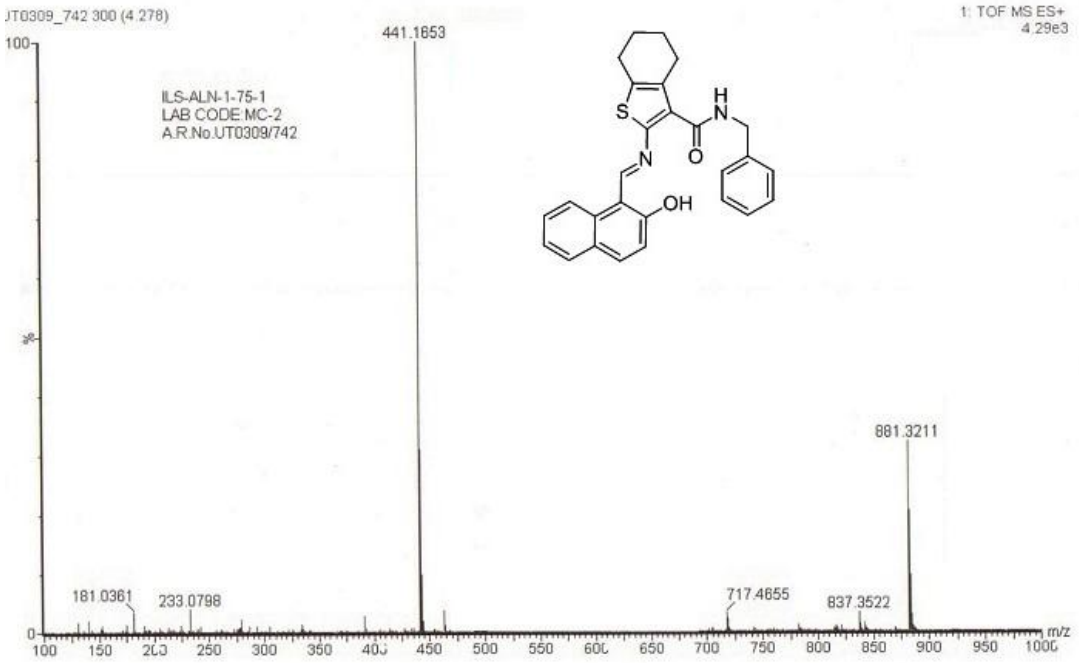
Appendix

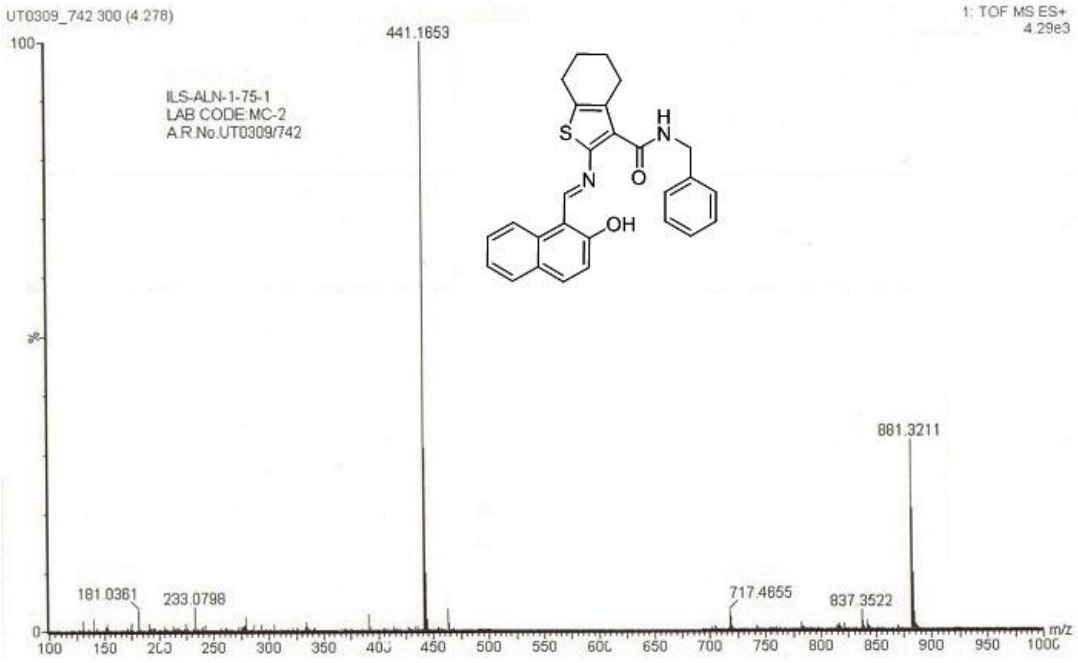





S
N
H
O
OH
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
163.9357
161.1707
154.3344
147.0309
139.3270
135.0555
133.1778
131.9186
131.7947
129.0678
128.2669
128.1715
127.6577
127.2371
126.7413
123.8714
121.1922
119.0374
109.9795
39.9899
39.7097
39.5000
39.2902
39.0804
24.8835
24.4830
22.7286
21.9669


180310-17-ILS-AIN-1-75-1-DEPT-135_DEPT135-8.jdf
S
N
H
O
OH
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
X : parts per Million : 13C
180310-17-ILS-AIN-1-75-1-Carbon_CARBON-7.jdf
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0





75-1

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -5.0, max = 60.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

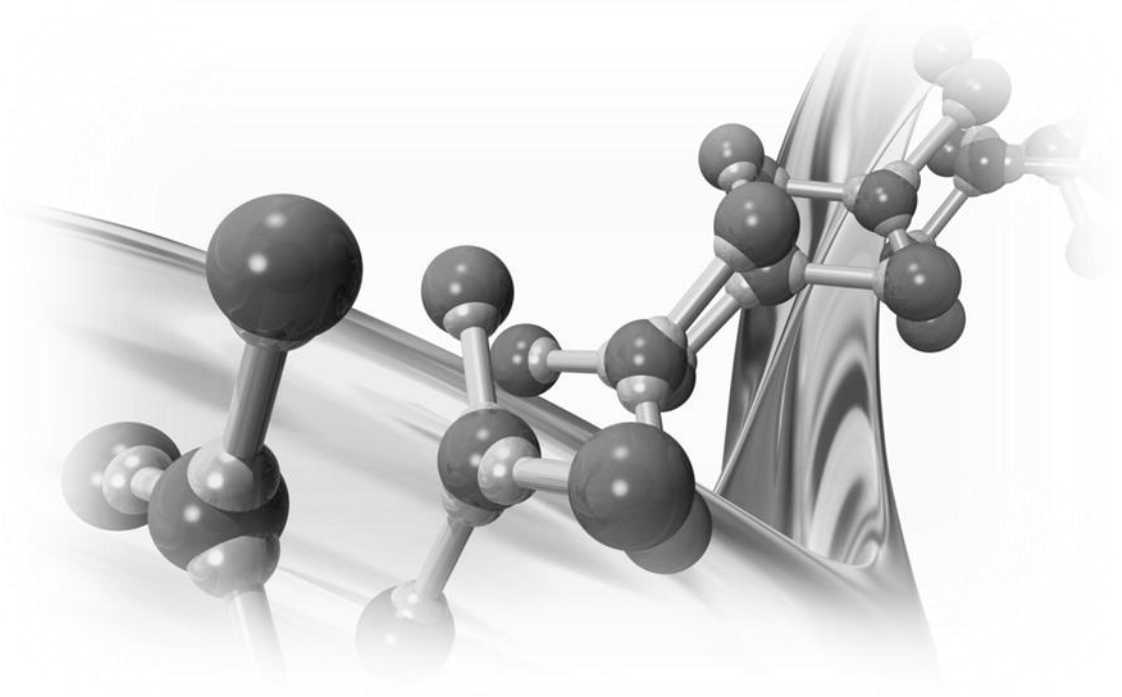
Monoisotopic Mass, Even Electron Ions
312 formula(e) evaluated with 1 results within limits (up to 4 best isotopic matches for each mass)
Elements Used:
C: 0-35 H: 0-50 N: 1-6 O: 0-5 S: 0-1

Minimum:				-5.0		
Maximum:		5.0	5.0	60.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
441.1653	441.1637	1.6	3.6	16.5	0.8	C27 H25 N2 O2 S

CHAPTER 2

CHAPTER 2A

Synthesis of thieno[3,2-*c*]pyran-4-one derivatives as potential anticancer agents



2A.1. Introduction

The pyranone motif often shows different biological activities. It is also found in biosynthetic precursors/intermediates and can be easily metabolized. This particular facet of the pyranone ring system makes them very interesting to study as potential pharmaceutical agents. Pyranones can exist in one of two major isomeric forms, 2-pyranone and 4- pyranone (also known as α and γ Pyranones, respectively) which are assigned on the position of the carbonyl relative to the oxygen atom within the ring system.

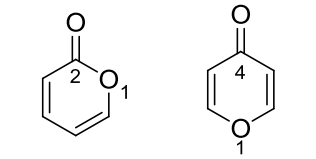


Figure 1. The two variations of the pyranone motif.

2-pyrones have gained much interest over the past few decades. There have been a number of reports in the literature. More attention has been payed towards 2-pyranone motif as it possesses broad spectrum biological activity. Functionalized 2-pyrones (2*H*-pyran-2-ones) have attracted considerable interest as medicinal agents, natural products, fluorescent derivatives, and synthetic intermediates. Such compounds are validated as being capable of binding to specific protein domains and able to exert a remarkable range of biological effects. 2-pyrones is a six-membered cyclic unsaturated ester, which is highly abundant in bacteria, microbial, plant, insect and animal systems and takes part in many different types of biological processes such as defence against other organisms, as key biosynthetic intermediates, and as metabolites.[1]A wide range of bioactivities such as antitumor, [2, 3] antimicrobial and antifungal activities[4, 5] have demonstrated the medicinal importance of 2-pyrones. Historically, simple 2-pyrones such as triacetic acid lactone **1b** and tetraacetic acid lactone **1c**, are used as precursors for the synthesis of biologically important compounds such as pheromones, solanopyrones, elastase, coumarins and analogues.

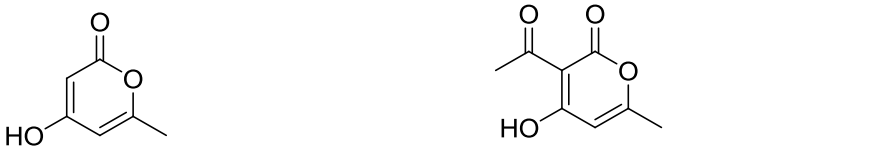


Figure 2. Simple 2-pyranones found in nature.

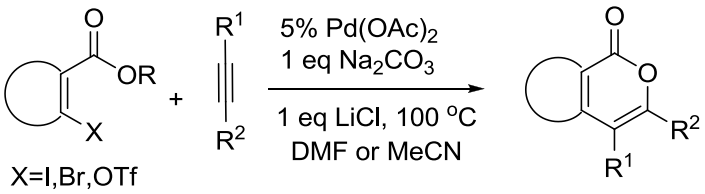
2A.2. Previous work

Methods for synthesis of 2-pyranone fused heterocycles using transition metal catalyst

Many synthetic routes to fused 2-pyrones have utilized novel methodology involving both organic and organometallic pathways. Transition metals such as Pd, Ru, Au, Cu, Zn and Cu etc., have been shown to be highly useful for the formation of fused 2-pyranones. Here some synthetic routes for the formation of fused 2-pyranones using transition metal catalysts are described.

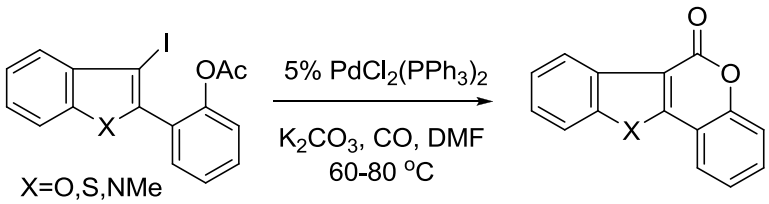
Palladium (Pd) Catalysts

Organometallic approaches utilizing palladium (Pd) for the synthesis of fused 2-pyrones have been a major focus since last few years. In 1999 Larock and his group developed a new route for the synthesis of 3,4-disubstituted isocoumarins and a variety of 3,4,6-tri- and 3,4,5,6- tetrasubstituted α-pyrones using the palladium-catalyzed annulation of internal alkynes via appropriate halogen and/or triflate-substituted esters (Scheme1).[6]



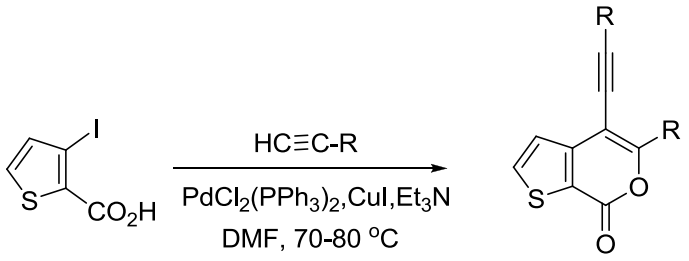
Scheme1. Synthesis of substituted isocoumarins and a variety of substituted α-pyrones.

In the year 2005 the Larock and his co workers disclosed an efficient synthetic route to coumestans *via* palladium-catalyzed carbonylative lactonization. The nucleophile employed in our lactonization process is an acetoxy group, which serves as a latent hydroxyl group (Scheme 2).[7]



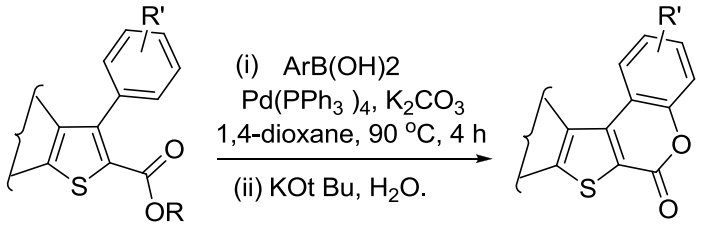
Scheme 2. Synthesis of coumestans and analogues *via* Pd catalyzed carbonylative lactonization.

Later on, in 2006 Pal and his co workers developed the first palladium-mediated tandem C–C bond forming reaction between 3-iodothiophene-2-carbox-ylic acid and terminal alkynes to afford the 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones (Scheme 3).[8]



Scheme 3. Pd mediated synthesis of 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones.

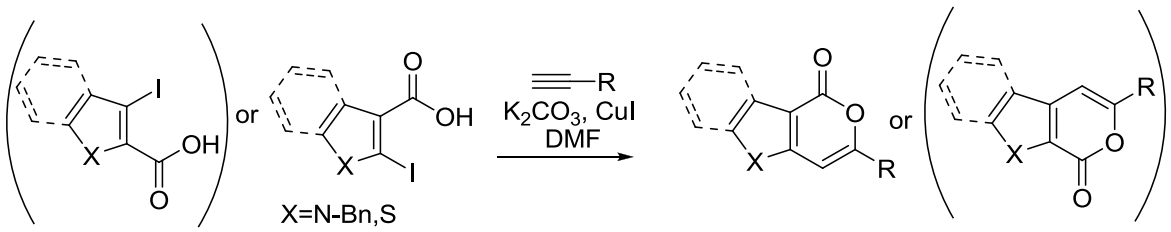
In 2012, Iaroshenko and his group developed a concise approach to a series of chromen-4-ones with fused thiophene ring four step synthetic strategy starting from readily available amino thiophenes *via* diazotization–bromination/Suzuki–Miyaura reaction/KOtBu induced lactonization.[9]



Scheme 4. Synthesis of chromen-4-ones with fused thiophene *via* Suzuki–Miyaura reaction

Copper (Cu) Catalyst

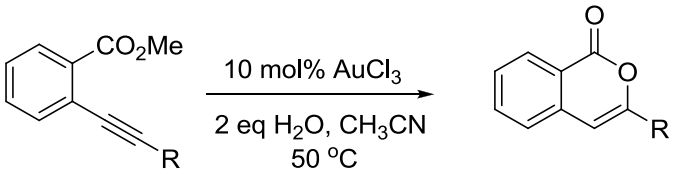
Thibonnet *et al*. in 2011 described a copper mediated regioselective 6-*endo-dig* cyclization via a tandem coupling oxacyclization route to thieno[2,3-*c*]pyrane-7-one, indolo[2,3-*c*] pyrane-1-ones, and indolo[3,2-*c*] pyrane-1-ones (Scheme 5).[10]



Scheme 5. Selective 6-*endo-dig* cyclization *via* a tandem coupling oxacyclization reaction.

Gold (Au) catalyst

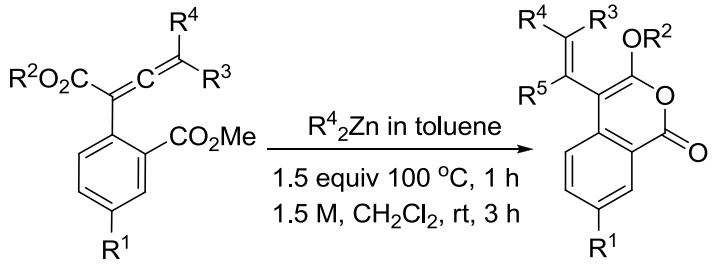
Pierre van de Weghe *et al*. in 2007 developed a gold(I)-catalyzed intramolecular cyclization of γ-alkyne acids in mild conditions yielding various alkylidene lactones (Scheme 6).[11]



Scheme 6. Synthesis of alkylidene lactones

Zinc (Zn) catalyst

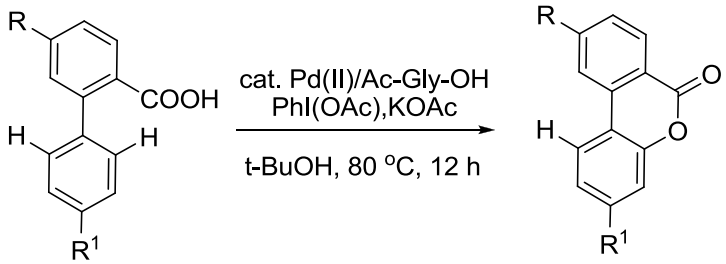
Recently in 2013 Ma *et al*. developed an efficient synthesis 2-(*o* (methoxy carbonyl) phenyl)-2,3-alenoates and organozincs at room temperature (for dialkylzinc) or 100 °C (for Ph_2Zn).[12] This method allows the introduction of an alkoxy group in the heterocyclic portion of the isocoumarin ring.



Scheme 7. Synthesis of synthesis 2-(*o*(methoxycarbonyl)phenyl)-2,3-alenoates

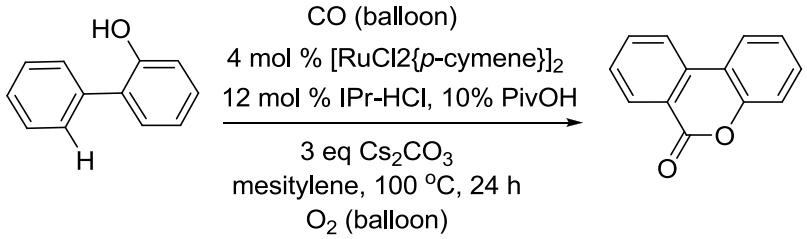
Using C-H/C-S bond activation

In 2013 Xi-Sheng Wang *et al*. developed a practical Pd(II)/Pd(IV)-catalyzed carboxyl-directed C–H activation/C–O cyclization to construct biaryl lactones. And also utility of this new reaction was demonstrated in total synthesis of the natural product cannabinol (Scheme 8).[13]



Scheme 8. Pd-catalyzed C–H lactonization.

Later on in 2013 Kondo and co workers developed a new Ru based catalytic system that successfully effects carbonylative C-H cyclization of 2-arylphenols to produce 6H-dibenzo[*b,d*]pyran-6-one (scheme 9).[14]



Scheme 9. Synthesis of 6 *H*-dibenzo[*b,d*]pyran-6-one *via* C-H activation

2A.3. Present work

In drug development and drug discovery process, methodology development and combinatorial synthesis of natural product like compounds with privileged scaffolds have attracted much attention. In the current scenario of diseases, cancer has become one of the most devastating diseases worldwide. According to WHO, the number of new cases is expected to grow the next 20 years to reach 15 million by year 2020. Among various types of cancers hepatocellular carcinoma (HCC), chronic myelogenous leukemia (CML), and breast cancer have emerged as most common cancers worldwide.[15] It is therefore necessary to identify new and more effective anticancer agents for the potential treatment of HCC, CML and breast cancer. As described in chapter 1, in 2010, our group identified an anticancer agents *via* sirtuin inhibition,[16] where we designed a novel small molecule (JGB1741) based on a known inhibitor of SIRT1, sirtinol, by replacing thiophene ring in place benzene of sirtinol using bioinformatic tools. We envisaged that replacement of benzene ring of in NM3 and benzofuran ring in **A3** with thiophene moiety could give good biological activity. All these reasons and our continued interest prompted us to design novel scaffold based on a known anticancer agent i.e. 8,9-dihydroxy-6*H*-benzofuro[3,2-*c*]chromen-6-one[17] or **A3** (Fig 1.3). While the compound **A3** possess flexible structural features its structural modification for the identification of new anticancer agent has not been explored well earlier. The structures **D** of target molecules were arrived *via* **B** by (i) replacing the furan moiety of **A3** by a thiophene ring and (ii) incising the benzene ring fused with the pyranone moiety. The substituents R^1 and R^2 were chosen to introduce diversity into the basic structure of **D**. Our design was essentially based on the fact that the central thienopyranone core of **D** would mimic an isocoumarins moiety that is known to

be integral part of several pharmacologically active agents and drugs including a clinical candidate[18] NM-3 (**C**, Fig. 3).

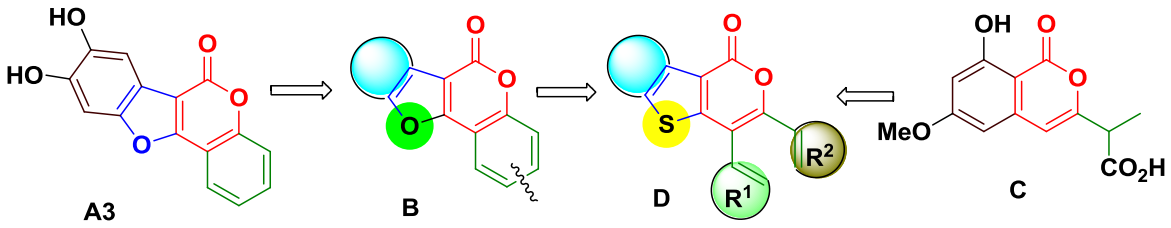
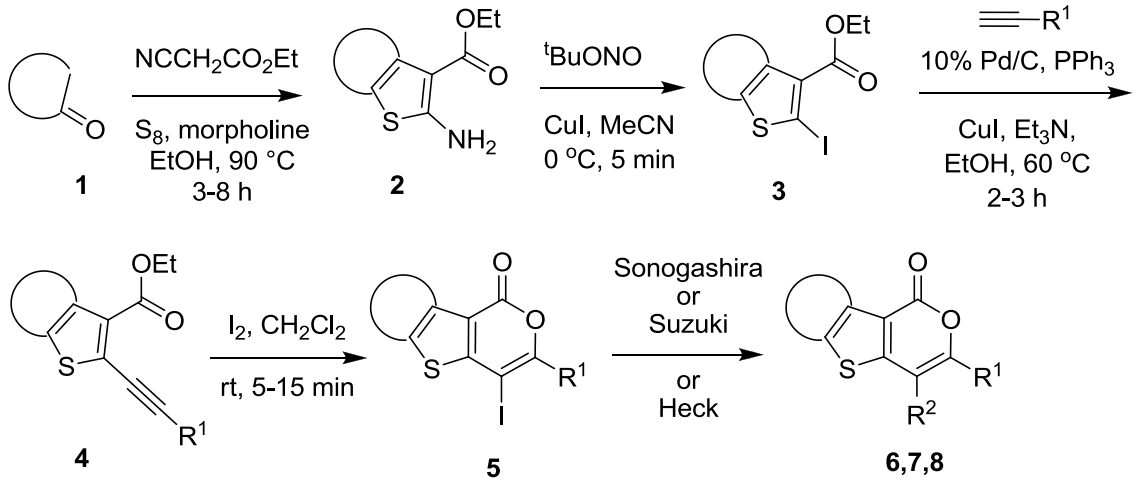


Figure 3. Design of thieno[3,2-*c*] pyran-4-one based novel and potential anticancer agents (**D**) from a known coumestan derivative **A3** and a isocoumarin derivative NM-3 (**C**).

Thiophene moiety is common in many bioactive agents and drugs[19] as a class of compounds thienopyranones however are rather unusual. Only a few number of thieno[2,3-*c*]pyran-4-ones were synthesized and evaluated for their antileishmanial and antifungal activities[20]. In 2006, Pal and co workers reported regioselective synthesis of various mono and disubstituted thieno[2,3-c]pyran-7-one, and thieno[3,2-c]pyran-4-ones some of which showed anticancer properties *in vitro*.[21] In further continuation of this work we undertook the synthesis of thieno[3,2-*c*]pyran-4-one based target molecules represented by **D** (or **6**, Scheme 10). To the best of our knowledge, most of these compounds were unknown in the literature earlier and consequently their synthesis remained unexplored. Herein we report the use of Pd/C-mediated coupling-iodocyclization-coupling as a key strategy to generate a library of compounds based on **D** leading to the identification of novel anticancer agents.

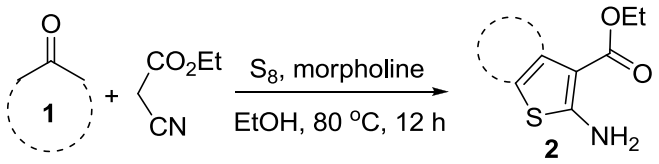


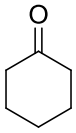
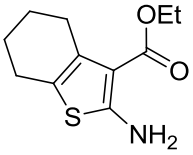
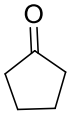
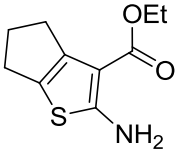
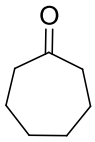
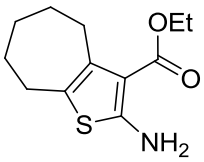
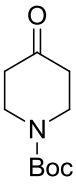
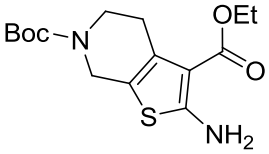
Scheme 10. Synthesis of thieno[3,2-*c*]pyran-4-one based novel compounds.

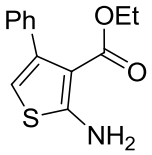
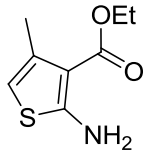
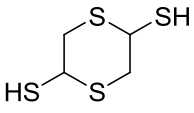
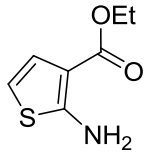
2A.4. Results and discussion

The key starting material **2** (Scheme 10) was prepared from the corresponding ketone **1** by using a Gewald type of reaction[22] (a multi-component condensation between sulfur, an α-methylene carbonyl compound and an α-cyanoester, which was first described in 1960s by Gewald and co-workers) to give the required 2-aminothiophene derivative **2**. The Gewald reaction with cyclohexanone gave product with good crystal without any column chromatography. The yields of 2-amino thiophene derivatives **2** decreased based on the alicyclic ring size.

Table 1. Synthesis of 2-amino substituted thiophenes *via* Gewald reaction.



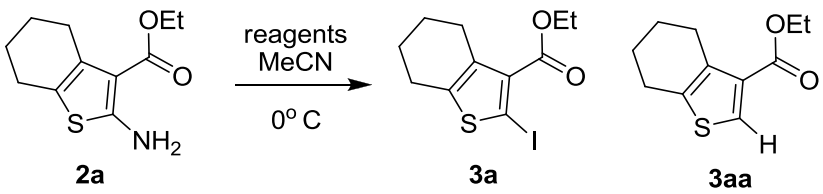
Entry	Compound (**1**)	Time	Product (**2**)	%Yield
1	 **1a**	12	 **2a**	65
2	 **1b**	12	 **2b**	60
3	 **1c**	12	 **2c**	70
4	 **1d**	8	 **2d**[b]	62

5	 **1e**	12	 **2e**	58
6	 **1f**	12	 **2f**	60
7	 **1g**	8	 **2g**[c]	64

[a] All the 2-aminothiophene derivatives (**2**) were prepared by using the corresponding ketone with an ethyl cyanoacetate (1.0 equiv) in the presence of elemental sulfur (1.0 equiv), morpholine (1.0 equiv) in EtOH under Gewald reaction conditions. [b] Et_3N (1.0 equiv) was used as a base instead of morpholine. [c] The compound **2g** was prepared by using 1,4-dithiane-2,5-dithiol (1.0 equiv), ethyl cyanoacetate (1.0 equiv), Et_3N (1.0 equiv) in DMF. [d] Isolated yield.

Then 2-aminothiophene derivatives **2a-g** were converted to the 2-iodo derivative **3** under a modified Sandmeyer conditions.[23] Our initial attempt to prepare **3** under a standard Sandmeyer conditions was not successful and therefore the conversion of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**2a**) to the corresponding iodo derivative (**3a**) was examined under a number of reaction conditions (Table 2). The compound **2a** did not provide **3a** when treated with HCl, $NaNO_2$, CuI (entry 1, Table 1) whereas the use of combination of *p*-toluene sulfonic acid (*p*-TSA)-$NaNO_2$-KI (entry 2, Table 2; condition A) or *p*-TSA-$NaNO_2$-CuI (entry 3, Table 1.2) provided **3a** as a major product along with a side product **3aa**. The formation of **3aa** however, was suppressed when ^{t}BuONO and CuI was used (entry 4, Table 2; condition B). Nevertheless, the condition **A** or **B** was used to prepare the other iodo derivatives **3**.

Table 2. Different reaction conditions for iodination on **2a**.

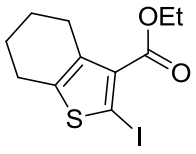
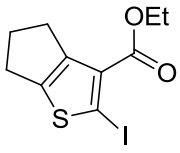
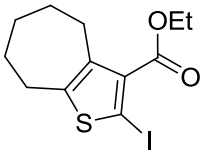
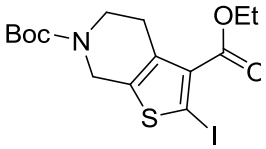
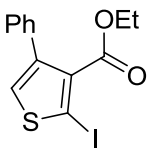


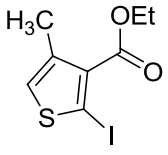
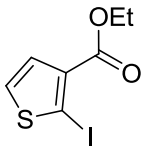
2a **3a** **3aa**

Entry	Reagents	Time (min)	Yield %[a]	
			3a	**3aa**
1	HCl, $NaNO_2$, CuI	30	0	0
2	*p*-TSA, $NaNO_2$, KI	05	54	8
3	*p*-TSA, $NaNO_2$, CuI	15	50	10
4	$(CH_3)_3CONO$, CuI	05	52	0

[a] isolated yields.

Table 3. Preparation of 2-iodothiophene derivatives **3**.[a]

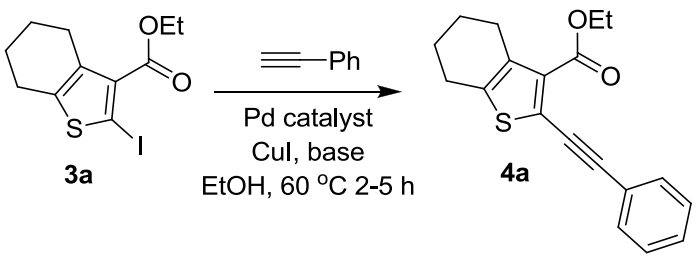
Entry	2-aminothiophene derivative (**2**)	Condition; Time(min)	Product (**3**)	%Yield
1	**2a**	A; 5	 **3a**	54
2	**2b**	A; 5	 **3b**	52
3	**2c**	A; 5	 **3c**	54
4	**2d**	B; 10	 **3d**	52
5	**2e**	B; 10	 **3e**	50

6	**2f**	B; 10	 **3f**	53
7	**2g**	B; 15	 **3g**	51

[a]All the reactions were carried out using the amine **2** either under Condition A (*p*-TSA)-$NaNO_2$-KI) or Condition B (^{t}BuONO-CuI) in MeCN at 0 °C.

We then examined the alkynylation of **3a** using a terminal alkyne under various conditions and the results are summarized in Table 4. Recently, the use of Pd/C as an inexpensive, easily separable and recyclable catalyst has been explored for the alkynylation of aryl and heteroaryl halides.[24] Accordingly, the initial coupling reaction of **3a** with phenylacetylene was carried out in the presence of 10%Pd/C, PPh_3, CuI and Et_3N in ethanol when the desired product **4a** was isolated in 72% yield (entry 1, Table 4). Replacing Et_3N by piperidine provided the product **4a** but required longer reaction time (entry 2, Table 4). The use of other Pd catalysts e.g. $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$ in the presence or absence of CuI was also examined but decreased the product yield (entries 3-4, Table 4). Thus, combination of 10% Pd/C-CuI-PPh_3 and Et_3N in EtOH was found to be optimum in the present alkynylation reaction and was used to prepare other 2-alkynyl thiophene esters represented by **4** (Table 5).

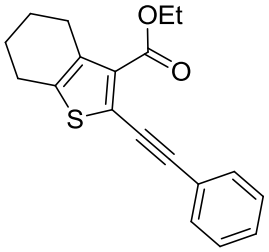
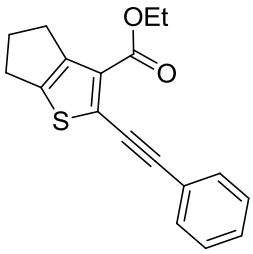
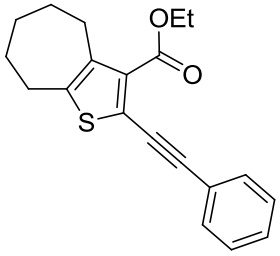
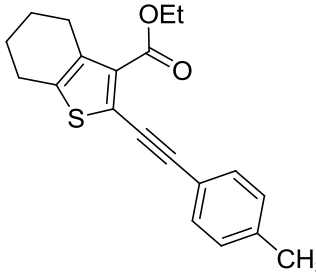
Table 4. Effect of reaction conditions on Sonogashira coupling of **3a** with phenylacetylene[a]

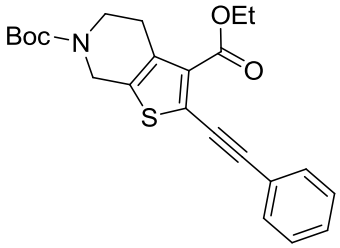
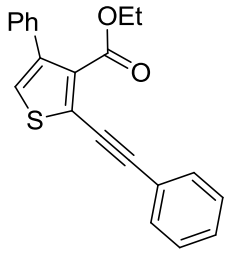
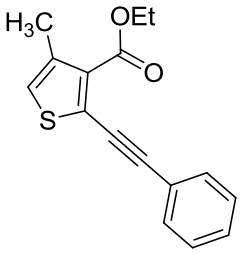
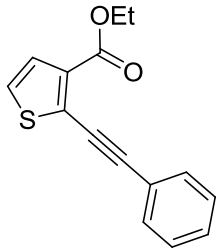


Entry	Pd-catalysts	Base	Time[b] (h)	%Yield[c]
1	10%Pd/C-PPh_3	Et_3N	2	72
2	10%Pd/C-PPh_3	Piperidine	5	61
3	$Pd(PPh_3)_4$	Et_3N	4	45
4	$Pd(PPh_3)_2Cl_2$[d]	Et_3N	5	58
5	$Pd(PPh_3)_2Cl_2$[d]	Piperidine	5	55

[a]All the reactions were carried out using ethyl 2-iodo-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate **3a** (0.5952 mmol), phenylacetylene (0.8928 mmol), 10% Pd/C (0.0059 mmol), PPh_3 (0.0238 mmol), CuI (0.0059 mmol), and base (1.1904 mmol) in EtOH (5.0 mL) at 60 ºC;
[b]After adding **3a**. [c]Isolated yield. [d]The reaction was carried out without CuI.

Table 5. Pd/C-mediated preparation of 2-alkynyl thiophene ester derivatives (**4**)[a]

Entry	2-Iodothiophene derivative (**3**)	Time (h)	Alkynyl ester (**4**)	%Yield[b]
1	**3a**	2	 **4a**	72
2	**3b**	2	 **4b**	64
3	**3c**	4	 **4c**	67
4	**3a**	2	 **4d**	61

5	**3d**	5	 **4e**	62
6	**3e**	3	 **4f**	60
7	**3f**	3	 **4g**	65
8	**3g**	4	 **4h**	62

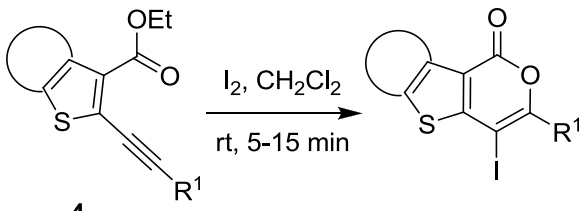
[a]All the reactions were carried out using 2-Iodothiophene derivative **3** (0.5952 mmol), a terminal alkyne (0.8928 mmol), 10% Pd/C (0.0059 mmol), PPh_3 (0.0238 mmol), CuI (0.0059 mmol), and Et_3N (1.1904 mmol) in EtOH (5.0 mL) at 60 ºC. [b]Isolated yields

In recent years, molecular iodine as an inexpensive, nontoxic, readily available and mild catalyst has found numerous applications in organic synthesis[25] and intramolecular regioselective iodocyclization[26, 27] of alkynes leading to various heterocyclic structures. Iodine has high tolerance to air as well as moisture and can be easily removed from the reaction mixture by washing with reducing agents. The development of safe, atom efficient acid-catalyzed organic process is one of the most important challenges for green chemistry. While acid catalysis remains the most widely used type of catalysis, the commonly used acid catalysts continue to pose serious health and safety problems.

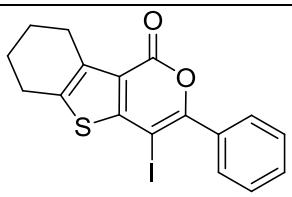
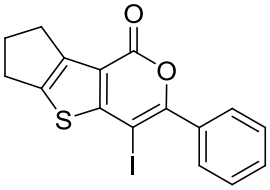
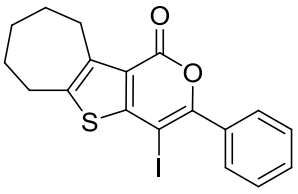
Moreover, the mild Lewis acidity of iodine has enhanced its utility for several organic transformations starting from minor catalytic amounts to higher stoichiometric levels.

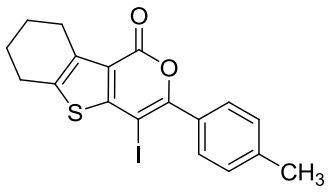
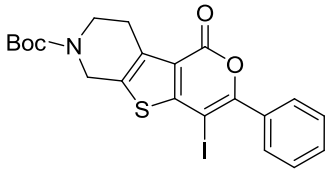
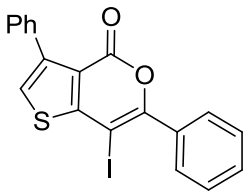
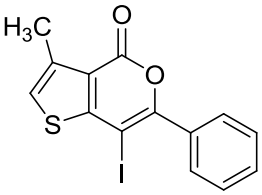
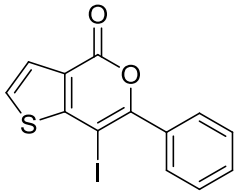
We therefore used this strategy to construct the desired pyranone ring possessing an iodo group for further functionalization. Initially, the iodocyclization of **4a** was examined using molecular I_2 in dichloromethane (DCM) at room temperature when the desired regioisomeric product **5a** was isolated in 80% yield (Table 6, entry 1). The use of other agent e.g. ICl however decreased the product yield when employed under the same reaction conditions. The I_2-mediated cyclization strategy therefore was chosen to prepare a variety of 6-aryl substituted 7-iodo-4*H*-thieno[3,2-*c*]pyran-4-one derivatives (**5**) in good yields (Table 6).

Table 6. I_2 mediated synthesis of 6-aryl substituted 7-iodo-4*H*-thieno[3, 2-*c*]pyran-4-one derivatives (**5**)[a]



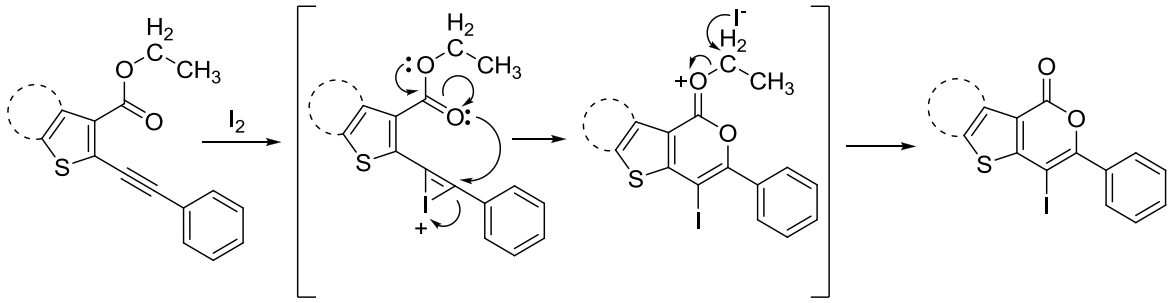
4 **5**

Entry	Alkynyl ester (**4**)	Time (min)	Product (**5**)	%Yield[b]
1	**4a**	5	 **5a**	80
2	**4b**	15	 **5b**	64
3	**4c**	10	 **5c**	67

4	**4d**	15	 **5d**	61
5	**4e**	15	 **5e**	62
6	**4f**	10	 **5f**	60
7	**4g**	15	 **5g**	65
8	**4h**	15	 **5h**	62

[a]All reactions were carried out by using alkynes **4** (0.9677 mmol) and I_2 (0.9677 mmol) in DCM (3 mL). [b]Isolated yields.

The mechanism of iodolactonization should involve the electrophilic attack of iodine on the triple bond, regioselectively forming a vinyl cation. A subsequent intramolecular nucleophilic attack of the methoxycarbonyl group, followed by elimination of methyl iodide, yields the iodo cyclized products.



Scheme 1.11. Mechanism of I_2 mediated electrolactonization.

All the iodo compounds (**5**) prepared were characterized by spectral data. The regioselective formation has been established by NMR, IR showing six-membered lactone *via 6 endo-dig* fashion rather than five-membered lactone and the molecular structure of a representative compound **5d** was determined unambiguously by X-ray crystallographic analysis (Fig. 4). Single crystals suitable for X-ray diffraction of compound **5d** were grown from ethyl acetate and hexane mixture (1:1). Single crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at liquid nitrogen temperature (100 K) on a Bruker SMART APEX CCD single crystal diffractometer using graphite monochromated Mo-Kα radiation (0.71073 Å). Absorption corrections using multi ψ-scans were applied. Structure was solved using SHELXS-97, and refined by full-matrix least squares against F^2 using SHELXL-97 software. All non-hydrogen atoms were refined anisotropically. With this weak Van der Waals interactions[28] compound **5d** formed 2D network in its crystal packing as shown in figure 1.8. Hydrogen atoms on the C atoms of compound **5d** were introduced on calculated positions and were included in the refinement riding on their respective parent atoms.

Crystal data of **5d** showed the Molecular formula = $C_{18}H_{15}IO_2S$, Formula weight = 422.27, Monoclinic, *P2(1)/c*, *a* = 9.137 (2) Å, *b* = 23.917 (6) Å, *c* = 7.2826 (19) Å, *V* = 1565.9 (7) $Å^3$, *T* = 100 K, *Z* = 4, D_c = 1.791 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1}, 13961 reflections were measured with 2748 unique reflections (R_{int} = 0.0439), of which 2748 ($I > 2\sigma(I)$) were used for the structure solution. Final R_1 (w R_2) = 0.0330 (0.0769), 200 parameters. The final Fourier difference synthesis showed minimum and maximum peaks of -0.361 and +1.584 e.$Å^{-3}$ respectively. Goodness of fit was 1.060.

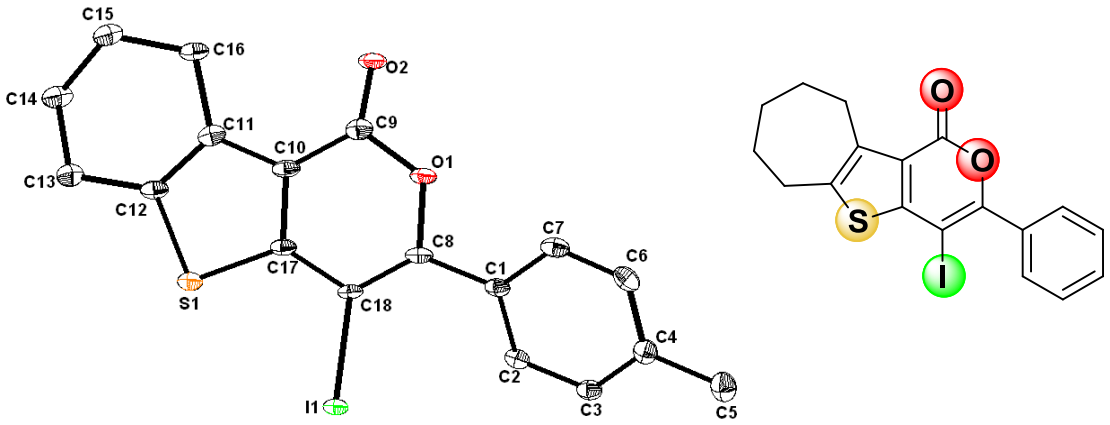


Figure 4. X-ray crystal structure of **5d** (ORTEP diagram). Thermal ellipsoidal diagram is drawn at 30% probability (hydrogen atoms are omitted for clarity).

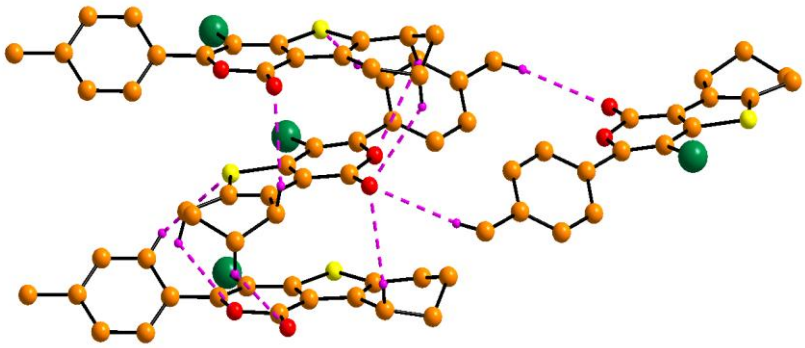


Figure 5. Showing the intermolecular interaction of compound **5d**.

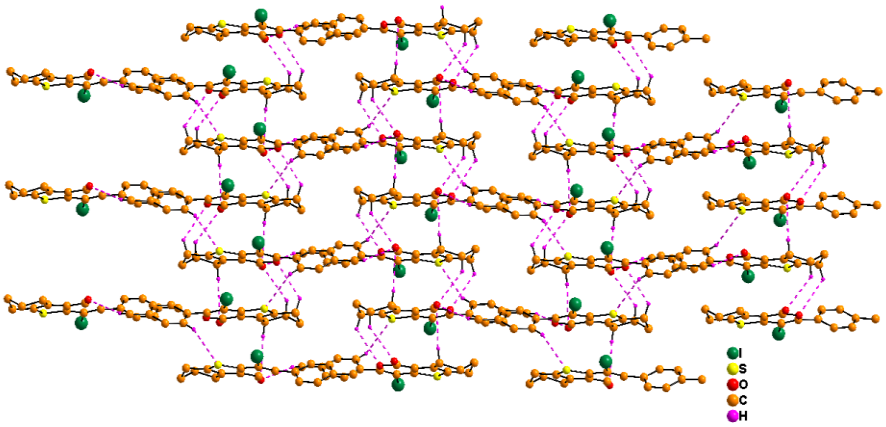


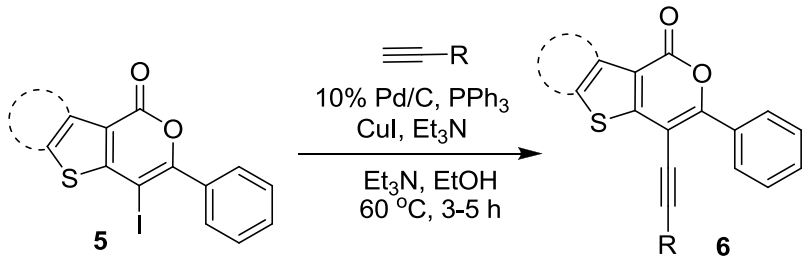
Figure 6. Showing the formation of 2D network in compound **5d**.

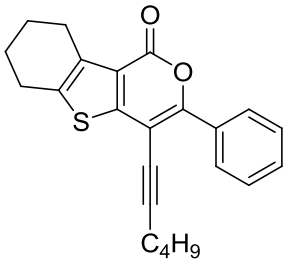
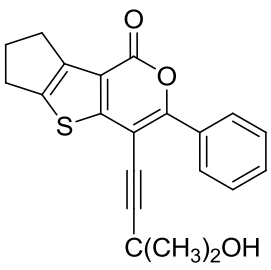
Functionalization of 6-aryl substituted 7-iodo-4*H*-thieno[3, 2-*c*]pyran-4-one *via* Pd catalyzed cross coupling reactions: Sonogashira, Suzuki and Heck reactions

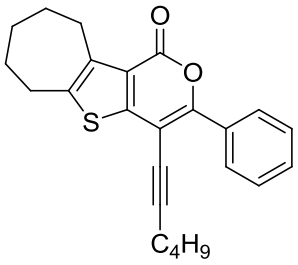
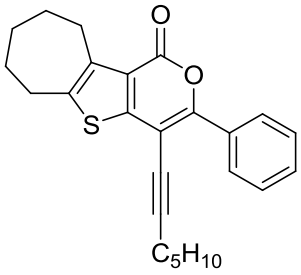
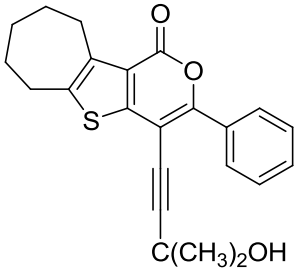
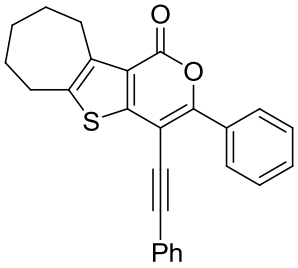
Thus, with the prepared iodo derivatives **5**, we then focused on further structure elaboration of these compounds by using various Pd-catalyzed C-C bond forming reactions such as Sonogashira, Suzuki, and Heck coupling reactions.

Thus, the compound **5** was reacted with various terminal alkynes in the presence of 10%Pd/C-PPh_3-CuI as catalysts and Et_3N as a base in EtOH to afford a variety of 7-alkynyl substituted-6-phenyl-4*H*-thieno [3,2-*c*]pyran-4-one derivatives in good yields (Table 7).

Table 7. Synthesis of 7-alkynyl substituted-6-phenyl-4*H* -thieno [3,2- *c*]pyran-4-one derivatives (**6**).[a]



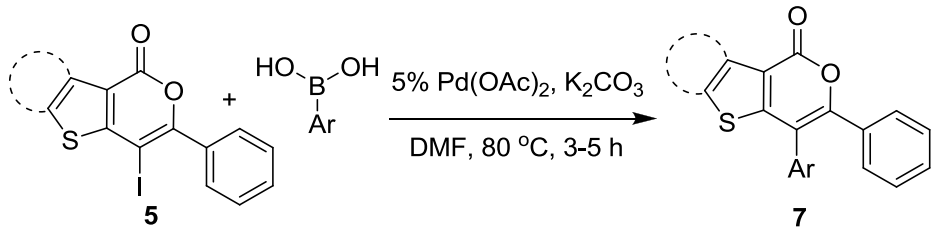
Entry	Compound (**5**)	Alkynes; R =	Time (h)	Product		% yield[b]
1	**5a**	*n*-C_4H_9	4		**6a**	60
2	**5b**	$C(CH_3)_2OH$	4		**6b**	72

3	**5c**	*n*-C_4H_9	4		**6c**	63
4	**5c**	*n*-C_5H_{10}	5		**6d**	60
5	**5c**	$C(CH_3)_2OH$	3		**6e**	68
6	**5c**	Ph	3		**6f**	65

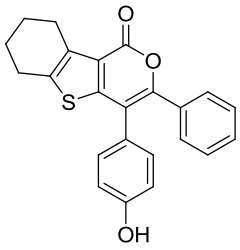
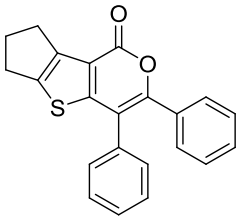
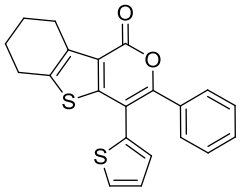
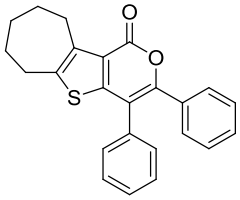
[a]All reactions were carried out using 10% Pd/C (0.0048 mmol), PPh_3 (0.0195), CuI (0.0048), Et_3N (0.9798 mmol), an appropriate terminal alkyne (0.7348 mmol) in EtOH. [b]Isolated yields.

Similarly, Suzuki reaction of iodo compound **5** was carried out using a number of boronic acids in the presence of 5% $Pd(OAc)_2$, K_2CO_3 in DMF at 80 ºC for 3-5 h to give the corresponding 7-(hetero)aryl substituted products (**7**) in good yields (Table 8).

Table 8. Synthesis of 7-aryl substituted-6-phenyl-4*H*-thieno[3,2-*c*]pyaran-4-one derivatives (**7**).[a]



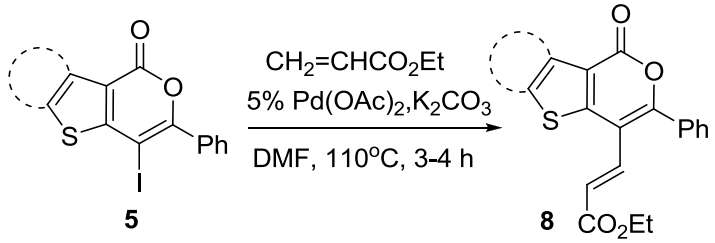
5 **7**

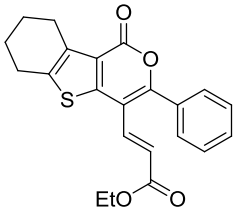
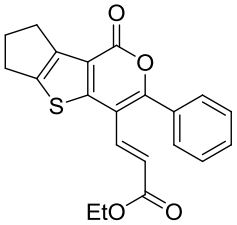
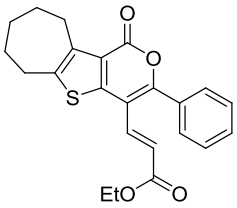
Entry	Compound (**5**)	Time (h)	Products (**7**)		%yield[b]
1	**5a**	3		**7a**	52
2	**5b**	5		**7b**	57
3	**5a**	3		**7c**	63
4	**5c**	4		**7d**	53

[a]All reactions were carried out by using $Pd(OAc)_2$ (0.0050 mmol), K_2CO_3 (1.0152 mmol) an appropriate boronic acid (0.7614 mmol) in DMF. [b]Isolated yields.

Heck coupling of compound **5** was also carried out using ethyl acrylate as an alkene in the presence of 5% $Pd(OAc)_2$, K_2CO_3 in DMF at 110 ºC for 3-4 h (Table 9).

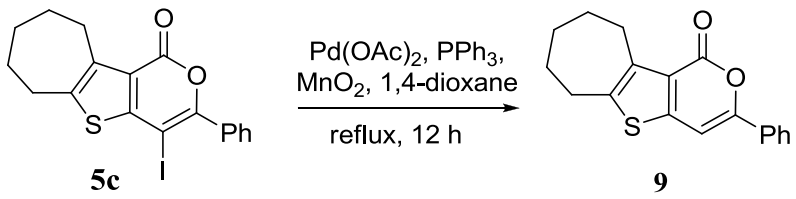
Table 9. Synthesis of 7-alkenyl substituted-6-phenyl-4*H*-thieno[3,2-*c*]pyaran-4-one derivatives (**8**) [a]



Entry	Compound (**5**)	Time (h)	Products (**8**)	%Yield[b]
1	**5a**	4	 **8a**	63
2	**5b**	4	 **8b**	55
3	**5c**	3	 **8c**	60

[a]All reactions were carried out by using $Pd(OAc)_2$ (0.0029 mmol), K_2CO_3 (0.5878 mmol), ethyl acrylate (0.9798 mmol) in DMF. [b]Isolated yields.

One of the iodo compounds i.e. **5c** was converted to the corresponding deiodinated product (**9**) (Scheme 12) showing the flexibility of the strategy presented i.e. the iodo group could either be functionalized (as shown earlier) or removed providing options in generating library of diversity based compounds.



Scheme 12. Pd-mediated deiodination of compound **5c**.

The synthesized compounds were then carried out for *in vitro* studies. And also we performed sirtuin protein inhibition studies which are one of the responsible proteins for cancer.

2.5. Pharmacology

Many of these novel compounds synthesized were evaluated for their anti proliferative properties against human chronic myeloid leukemia cells (K562), human metastatic breast cancer cells (MDA-MB 231), hepatocellular carcinoma cells (HepG2) and non-cancerous human embryonic kidney cells (HEK293). All the compounds were tested initially at the concentration of 10 µM in a MTT assay, in which Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 10 µM concentration (five different concentrations i.e. 10, 5, 1, 0.5, 0.1 and 0.01 µM for dose response study) in duplicates for 24 h in a final volume of 200 µl. After treatment, the medium was removed and 20 µL of MTT (5 mg/mL in PBS) was added to the fresh medium. After 3h incubation at 37 °C, 100 µL of DMSO was added to each well and plates were agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader (Victor3, Perkin Emler). Percent inhibition of proliferation was calculated as a fraction of control (without compound). The results are summarized in Table 10 (see also Fig 7-9). The compound **5d** showed good activity against both K562 as well as HEPG2 cell lines (entry 3, Table 10). The compound **6c** also showed significant activity against K562 cells (Entry 7, Table 10). Among other compounds **5c, 6d, 8c** showed moderate activities against MDA-MB 231 cells (entries 2,6,12. Table 10). Notably, these three compounds possess common structural features except the substituent present at C-7 position. All these compounds were then tested against non cancerous HEK293 cells. None of these compounds had any effect on HEK293 cells indicating their selectivity

towards the growth inhibition of cancer cells (Table 10). Based on their promising *in vitro* data IC_{50} values were determined for compounds **5d** and **6c** and were compared with known anticancer agents e.g. imatinib (a drug used to treat chronic myelogenous leukemia) and doxorubicin (commonly used for the treatment of a wide range of cancers) (Table 11). While both **5d** and **6c** were 20 fold less potent than imatinib in leukemia (K562) cells the compound **5d** however showed more than 2-fold potency over doxorubicin when tested against hepatocellular carcinoma (HepG2) cells.

Table 10. *In vitro* antiproliferative properties of compounds against various cells

		% Inhibition of the growth@10μM[a]			
Entry	Compounds	K562	MDA-MB 231	HepG2	HEK293[b]
1.	**5a**	38.06	22.68	0.00	0.6
2.	**5c**	30.29	40.11	12.00	0.23
3.	**5d**	64.23	32.55	68.23	0
4.	**5e**	8.34	27.53	15.49	9.03
5.	**6c**	66.44	25.21	24.13	3.2
6.	**6d**	12.33	41.87	24.84	0.44
7.	**6e**	9.32	21.59	25.03	4.2
8.	**6f**	13.89	34.66	20.05	1.7
9.	**7a**	9.85	22.85	23.00	0.5
10.	**7b**	26.74	12.39	13.44	4
11.	**7d**	24.01	7.42	35.89	0.01
12.	**8c**	27.43	42.59	12.00	1.11

[a]Data represent the mean values of three independent determinations. [b]Non-cancerous cell line.

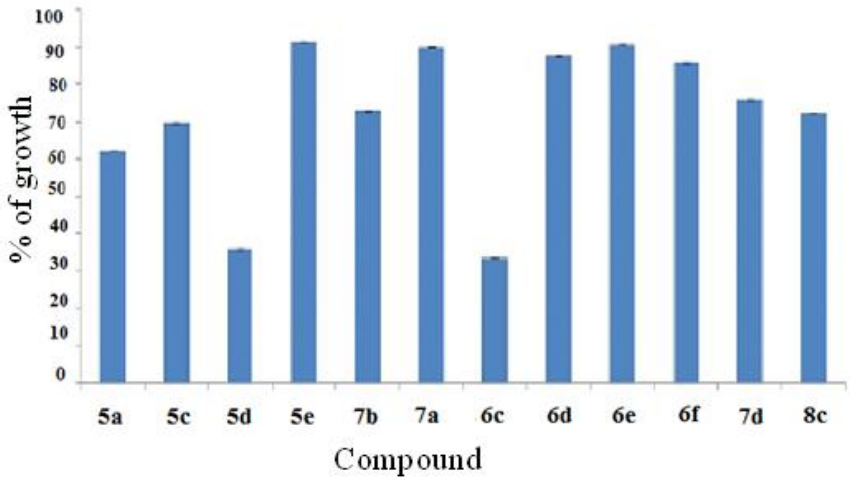


Figure 7. Effect of compounds on the growth of K562 cell line at 10 µM.

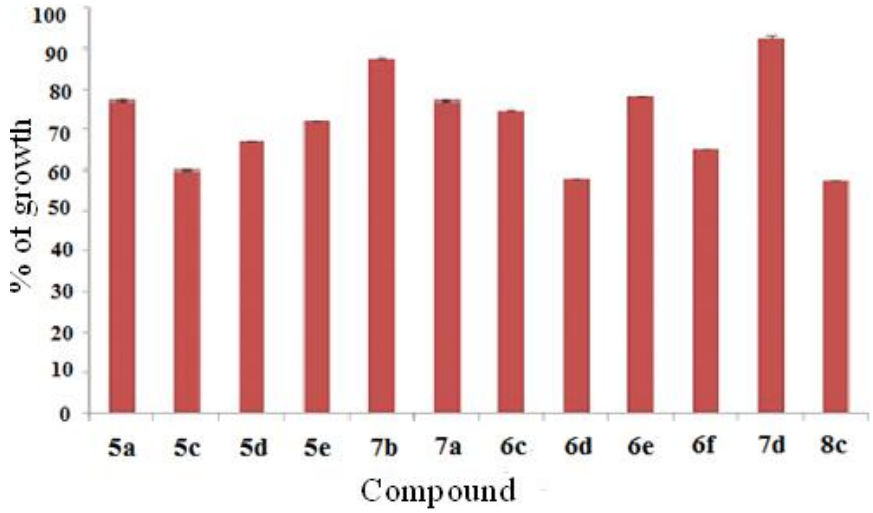


Figure 8. Effect of compounds on the growth of MDA MB 231 cell line at 10 µM.

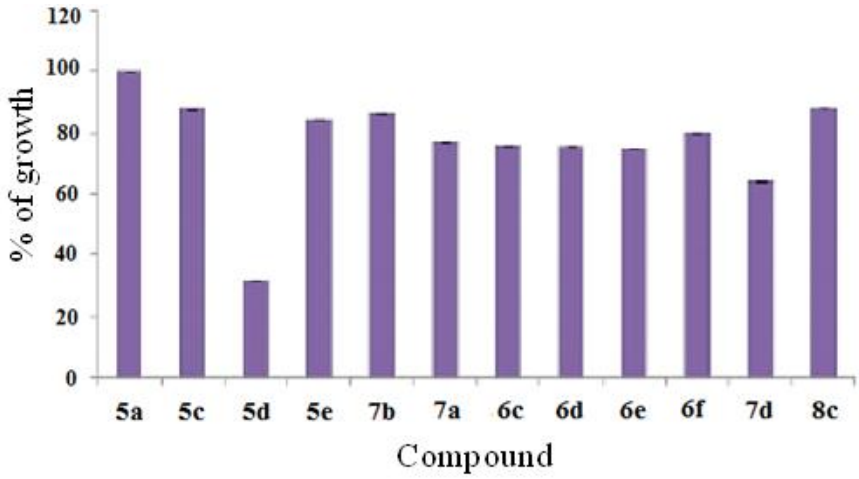


Figure 9. Effect of compounds on the growth of HepG2 cell line at 10 µM.

Table 11. IC_{50} values of compound **5d** and **6c**

Compound	IC_{50} (μM)[a]		s.d.[b]
	K562	HepG2	
5d	2.33	2.38	0.00167
6c	2.28	-	0.0043
Imatinib	0.10	-	0.0041
Doxorubicin	-	5.00	0.0018

[a] IC_{50} represent the concentration of compound that causes a 50% growth inhibition to untreated cells using an MTT assay. [b]s.d. = standard deviation

In 2010, our group reported that the inhibition of SIRT1 by a small molecule which induces apoptosis in breast cancer cells (MDA MB 231) and identified a sirtuin inhibitor (JGB-1741) a Splitomycin (standard sirtuin inhibitor) analogue (JGB-1741 having thiophene ring in place of benzene ring of Sirtinol). This and our continuous interest in synthesis of sirtuin inhibitors[29] we selected some of the compounds, to study inhibition property of sirtuins. To understand their mechanism of action compounds **5c**, **5d** and **6c** were tested for their inhibitory potential against sirtuins *in vitro*. Sirtuins (class III NAD-dependent deacetylases) are shown to up-regulated in various types of cancer[30] and inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. Compounds **5d** and **6c** were tested at 50 μM for their ability to inhibit yeast sirtuin family NAD-dependent histone deacetylase (HDAC) Sir 2 protein (by estimating inhibition of growth of yeast strain containing Ura3 gene at telomeric locus) in presence of 5-fluoroorotic acid (5-FAO) as reported previously.[22] Inhibition of Sir2 in presence of 5-FAO in yeast cell results in de-repressing of URA3 gene thereby causing the death of yeast cell. Among these compound **5c** (entry 3, Table 6) which was showing yeast Sir2 inhibition 58.13% (Fig. 10). Compounds **5d** and **6c** showed 30-35 % inhibition in the presence of 5-FAO indicating that the anticancer properties of these molecules are possibly due to their sirtuin inhibiting properties at 50μM concentration. Further we carried out the dose response study of **5c** on yeast Sir2p showed MIC of 18.77 μM and IC_{50} at 21.09 μM. Nevertheless, the compounds **5c, 5d** and **6c** were identified as promising anticancer agents of further interest. Among these compound **5c** (entry 3, Table 6) which was showing yeast Sir2 inhibition 58.13% at 50μM.

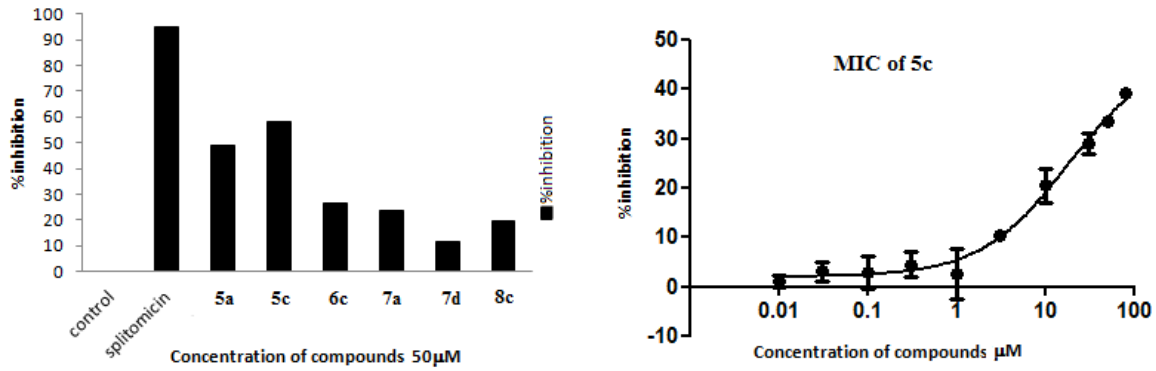
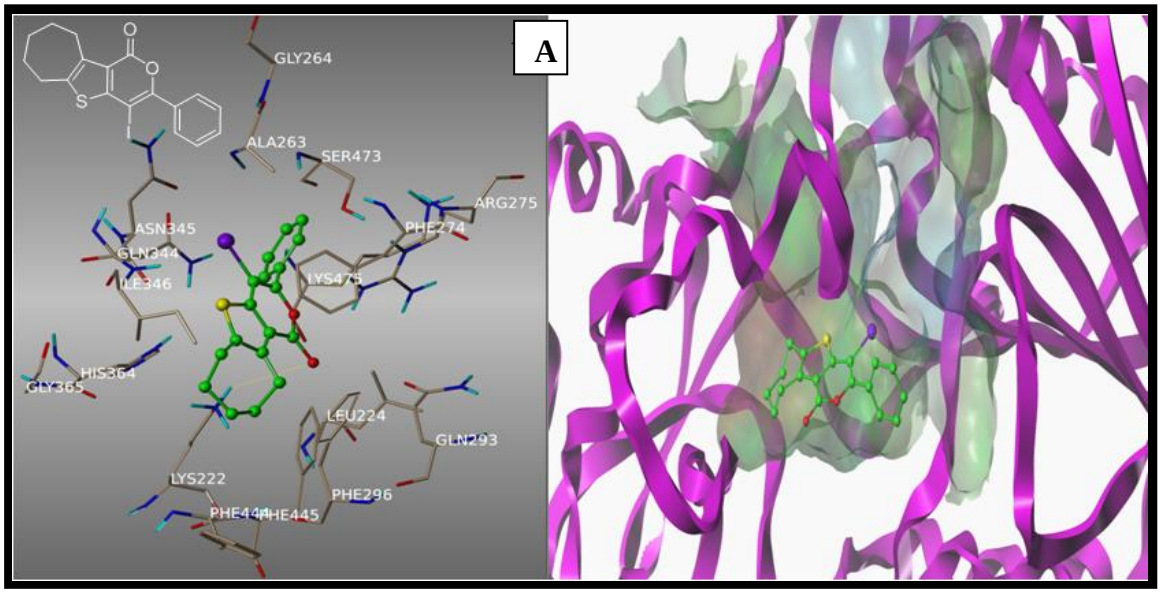


Figure 10. Screening of compounds on yeast Sir2 and MIC study of compound **5c.**

The protein yeast sir2 (PDB ID – 2HJH) crystal structure was retrieved from the protein data bank and it is refined with the Protein Preparation Wizard application in which the hydrogens were added and missing side chains and loops were filled with PRIME application. Water molecules were observed within the 5A° distance and were deleted beyond 5A° from heteroatom groups. Finally the protein is then optimized and minimized using OPLS_2005 force filed. GRID based docking were done in the present study. The following Glide score -4.54 K.cal/mol was obtained for **5c** when docked with yeast sir2.



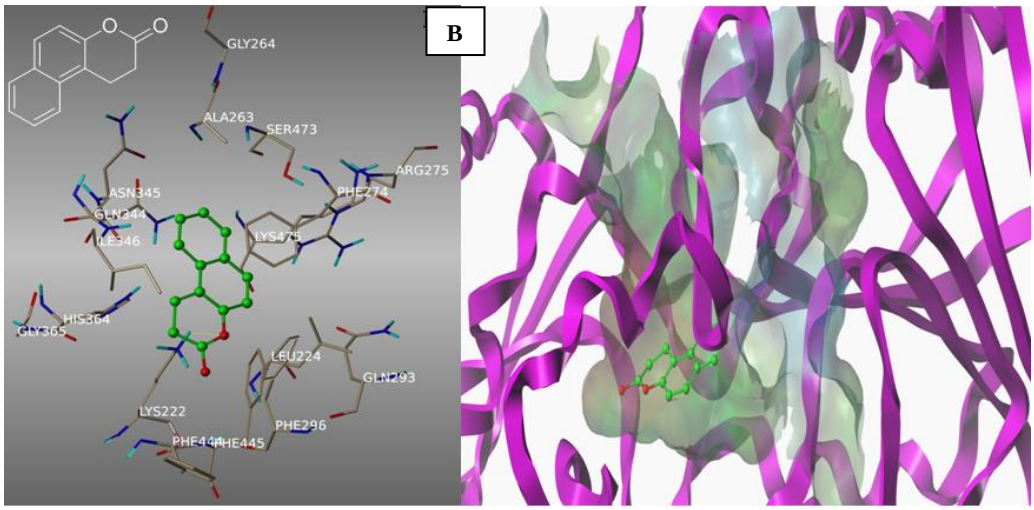


Figure 11. Binding mode of compound **5c** and Splitomicin with catalytic site of Sir2.

2A.6. Conclusion

In conclusion, novel small molecules based on thieno[3,2-*c*]pyran-4-one framework were designed as potential anticancer agents. These molecules were synthesized *via* a multi-step method consisting of several steps such as Gewald reaction, Sandmeyer type iodination, Sonogashira type coupling followed by iodocyclization and then Pd-mediated various C-C bond forming reactions. The overall strategy involved the construction of thiophene ring followed by the fused pyranone moiety and then functionalization at C-7 position of the resultant thieno[3,2-*c*]pyran-4-one framework. The utility of this strategy has been demonstrated by synthesizing a wide variety of thienopyranone derivatives some of which were evaluated for their anti-proliferative properties *in vitro* against three cancer cell lines e.g. K562, MDA-MB 231 and HepG2 as well as noncancerous cell line e.g. HEK293. All these compounds showed selective growth inhibition of cancer cells and two of them e.g. 5d and 6c were found to be promising with IC_{50} values in the range of 2.0-2.5 µM indicating their potential as novel anticancer agents. Further *in vitro* studies indicated that inhibition of sirtuins (yeast Sir2 MIC of 18.77µM) could be the possible mechanism of action of these molecules. The crystal structure analysis of an active compound provided an insight on hydrogen bonding patterns and molecular arrangement present within the molecule. Overall, the thienopyranone framework presented here could be an attractive template for the identification of novel anticancer agents and the corresponding synthetic strategy described could be useful for generating diversity based library of small molecules related to thienopyranone.

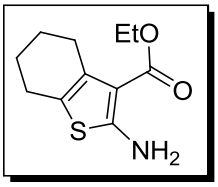
2A.7. Experimental Section

Chemistry

General methods: Unless stated otherwise, reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Column chromatography was performed on silica gel (230–400 mesh) using distilled petroleum ether and ethyl acetate. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solutions using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, d = 0.0) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FTIR spectrometer. Melting points were determined by using a Buchi melting point B-540 apparatus. MS spectra were obtained on a mass spectrometer. All the ketones used are commercially available.

Preparation of amino ester 2

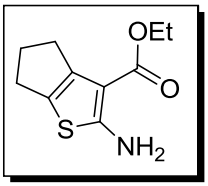
Typical procedure for the synthesis of ethyl 2-amino-4,5,6,7 tetrahydrobenzo [*b*] thiophene-3-carboxylate (2a) [1a]:



A mixture of cyclohexanone (1.06 mL, 10 mmol), ethyl cyanoacetate (1.15 mL, 10 mmol), morpholine (0.90 mL, 10 mmol), elemental sulfur (0.32 g, 10 mmol) in ethanol (10 mL) was stirred and refluxed for overnight. After completion of the reaction, the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The crude solid was washed with cold ethanol and filtered though sintered funnel, dried under vacuum. The crude product was dissolved in dichloromethane and washed with brine. The organic layer was collected and concentrated under low vacuum to give the compound **2a**; yield: 73% (1.83 g); brown solid; mp: 116.2-117.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (t, *J* = 7.3 Hz, 3H), 1.74-1.80 (m, 4H), 2.47-2.51 (m, 2H), 2.68-2.71(m, 2H), 4.25 (q, *J* = 7.3 Hz, 2H), 5.93 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.4, 22.8, 23.2, 24.5, 26.9,

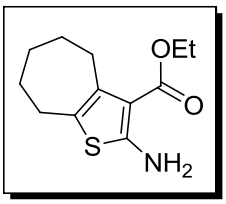
59.3, 105.7, 117.6, 132.4, 162.6, 166.1; IR (KBr) ν_{max} (cm^{-1}): 3408, 3300, 2938, 1646, 639; MS (ESI) m/z : 226.2 [M + H].

Ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (2b)[1a]:



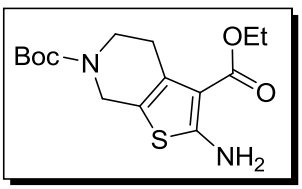
This compound was prepared by using cyclopentanone (1.05 mL, 11.90 mmol) according to a procedure similar to that of **2a**; yield: 78% (1.79 g); brown solid; mp: 182.5-183.5 °C; ^{1}H NMR(400 MHz, $CDCl_3$) δ: 1.40 (t, *J* = 7.3 Hz, 3H), 2.26-2.30 (m, 2H), 2.68-2.70 (m, 2H), 2.81-2.83 (m, 2H), 4.23 (q, *J* = 7.3 Hz, 2H), 5.89 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.4, 27.2, 28.8, 30.7, 59.4, 102.8, 121.8, 142.6, 165.8, 166.4; IR (KBr) ν_{max} (cm^{-1}) : 3443, 3315, 2920, 1635, 649; MS (ESI) m/z: 212.2 [M + H]$^+$.

Ethyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (2c)[1b]:



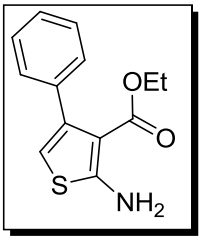
This compound was prepared by using cycloheptanone (1.05 mL, 8.92 mmol) according to a procedure similar to that of **2a**; yield: 69% (1.47 g); light yellow solid; mp: 89.5-90.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.34 (t, *J* = 6.9 Hz, 3H), 1.58-1.66 (m, 4H), 1.77-1.83 (m, 2H), 2.57 (t, *J* = 5.6 Hz, 2H), 2.97 (t, *J* = 5.5 Hz, 2H), 4.27 (q, *J* = 6.9 Hz, 2H), 5.77 (s, 2H); IR (KBr) ν_{max} (cm^{-1}): 3399, 2921, 1650, 1482; MS (ESI) m/z: 240.2 [M + H].

6-*tert*-Butyl 3-ethyl 2-amino-4, 5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)dicarboxylate (2d)[1b]:



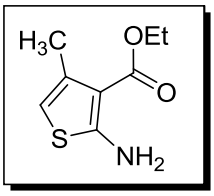
This compound was prepared by using N-Boc-4-piperidone (1.0 g, 5.02 mmol) according to a procedure similar to that of **2a** using Et_3N instead of morpholine; yield: 80% (1.31 g); light yellow solid; mp: 157-158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (t, *J* = 7.2 Hz, 3H), 1.48 (s, 9H), 2.78 (bs, 2H), 3.62 (t, *J* = 4.8 Hz, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 4.35 (bs, 2H), 6.05 (s, 2H); IR (KBr) ν_{max} (cm^{-1}): 3341, 2952, 1633, 1621, 1568; MS (ESI) m/z: 327.0 [M+H];

Ethyl 2-amino-4-phenylthiophene-3-carboxylate (2e) [1c]**:**



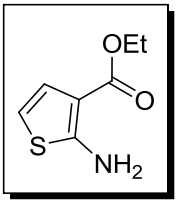
This compound was prepared by using acetophenone (0.97 mL, 8.33 mmol) according to a procedure similar to that of **2a**; yield: 68% (1.15g); light green solid; mp: 97.5-98.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, *J* = 6.9 Hz, 3H), 4.10 (q, *J* = 6.9 Hz, 2H), 6.06 (s, 1H), 6.08 (s, 2H), 7.28-7.31 (m, 5H); IR (KBr) ν_{max} (cm^{-1}): 3264, 2951, 1613, 1587; MS (ES mass) m/z: 248.1 [M + H];

Ethyl 2-amino-4-methylthiophene-3-carboxylate (2f)[1a]**:**



This compound was prepared by using acetone (1.26 mL, 17.24 mmol) according to a procedure similar to that of **2a**; Yield: 76% (2.42 g); white solid; mp: 76-78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (t, *J* = 7.1 Hz, 3H), 2.29 (s, 3H), 4.30 (q, *J* = 7.1 Hz, 2H), 5.84 (s, 1H), 6.02 (s, 2H); IR (KBr) ν_{max} (cm^{-1}): 3352, 2963, 1628, 1542; MS (ESI) m/z: 185.7 [M+H];

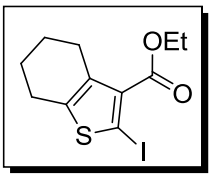
Ethyl 2-aminothiophene-3-carboxylate (2g)[1d]**:**



The compound **2g** was prepared by using 1,4-dithiane-2,5-dithiol (1.0 g, 6.45 mmol), ethyl cyanoacetate (0.72 mL, 6.45 mmol), triethylamine (0.90 mL, 6.45 mmol) in DMF under a Gewald reaction condition; yield: 71% (0.78 g); off white solid; mp: 47-48 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (t, *J* = 6.3 Hz, 3H), 4.30 (q, *J* = 6.9 Hz, 2H), 6.06 (bs, 2H), 6.24 (d, *J* = 5.7 Hz, 1H), 6.97 (d, *J* = 5.7 Hz, 1H); IR (KBr) ν_{max} (cm^{-1}): 3314, 2892, 1622, 1566; MS (ES mass) m/z : 171.9 [M + H].

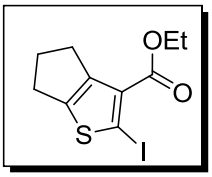
Preparation of iodo compound 3

Typical procedure for the synthesis of ethyl 2-iodo-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxylate (3a):



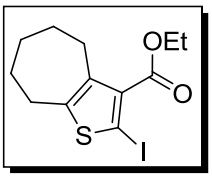
2A.6.2.1. Condition A: To the solution of *p*-TSA (1.14 g, 6.66 mmol) in MeCN (5 mL) was added the compound **2a** (0.5 g, 2.22 mmol) at 0 °C. To this suspension was added a mixture of $NaNO_2$ (0.3 g, 4.44 mmol) and KI (0.9 g, 5.55 mmol) in H_2O (5 mL). Then, the mixture was stirred for 5 min at room temperature and poured into cold water (50 mL). To this was added saturated solution of K_2CO_3 carefully until the pH become ~ 9. The mixture was extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and evaporated under low vacuum. The crude product was purified by column chromatography using 9:1 hexane/ethyl acetate to give of the desired compound **3a**; yield: 54% (0.4 g); brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.37 (t, *J* = 6.9 Hz, 3H), 1.24-1.27 (m, 4H), 2.84-2.86 (m, 2H), 2.88-2.91 (m, 2H), 4.32 (q, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 22.2 (2C), 24.7, 25.1, 60.2, 78.1, 129.8, 141.3, 144.0, 155.0; IR (KBr) ν_{max} (cm^{-1}): 2920,1714, 1445,741; MS (ESI) m/z: 337.2 [M + H].

Ethyl 2-iodo-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (3b):



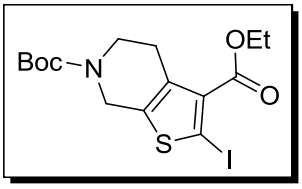
This compound was prepared by using compound **2b** (0.5 g, 2.36 mmol) according to a procedure similar to that of **3a**; Yield: 52% (0.39 g); brown liquid: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (t, *J* = 7.6 Hz, 3H), 2.48-2.38 (m, 2H), 2.87 (t, *J* = 6.6 Hz, 2H), 2.94 (t, *J* = 6.3 Hz, 2H), 4.36 (q, *J* = 7.4 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.2, 28.4, 29.7, 30.5, 61.5, 82.1, 135.1, 143.1, 147.2, 162.5; IR (KBr) ν_{max} (cm^{-1}): 2930, 1730, 1465, 710; MS (ESI) m/z: 323.2 [M + H];

Ethyl 2-iodo-5,6,7,8-tetrahydro-4H-cyclohepta[*b*]thiophene-3-carboxylate (3c):



This compound was prepared by using compound **2c** (0.5 g, 2.09 mmol) according to a procedure similar to that of **3a**; Yield: 54% (0.39 g); brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (t, *J* = 7.4 Hz, 3H), 1.58-1.69 (m, 4H), 1.81-1.88 (m, 2H), 2.74-2.77 (m, 2H), 2.82-2.88 (m, 2H), 4.36 (q, *J* = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 27.7, 28.6 (2C), 29.7, 29.8, 61.0, 72.7, 131.9, 141.5, 147.1, 164.6; IR (KBr) ν_{max} (cm^{-1}): 2953, 1720, 1425, 723; MS (ESI) m/z: 351.2 [M+H].

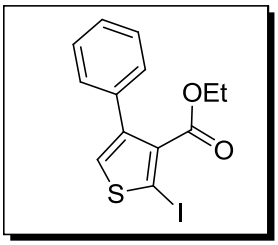
Synthesis of 6-tert-butyl 3-ethyl 2-iodo-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (3d):



2A.7.2.2. Condition B: To a solution of tert-butyl nitrite (0.27 mL, 2.30 mmol) and CuI (0.29 g, 1.53 mmol) in MeCN (5 mL) was added compound **2d** (0.5 g, 1.53 mmol). The reaction mixture was stirred for 10 min and then solvent was removed under low vacuum. The residue was treated with water (15 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and evaporated under low vacuum. The crude product was purified by column chromatography using 9:1 hexane/ethyl acetate to give of compound **3d**; yield: 52% (0.34g); light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.23 (t, *J* = 4.2 Hz, 3H), 1.52 (s, 9H), 2.98 (bs, 2H), 3.61 (bs, 2H), 4.31 (q, *J* = 6.8 Hz, 2H), 4.60 (bs, 2H); ^{13}C NMR (100

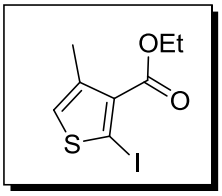
MHz, $CDCl_3$) δ: 14.3 (2C), 28.4 (3C), 40.6, 42.3 (2C), 60.3, 79.9, 131.2, 140.0, 144.0, 158.8, 162.8; IR (KBr) ν_{max} (cm^{-1}): 2867, 1713, 1620, 1564, 1105, 797; MS (ESI) m/z: 438.2 [M + H];

Ethyl 2-iodo-4-phenylthiophene-3-carboxylate (3e):



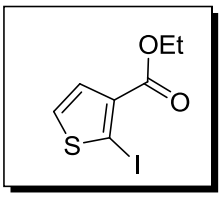
This compound was prepared by using compound **2e** (0.5 g, 2.02 mmol) according to a procedure similar to that of **3d**; yield: 50% (0.36 g); brown liquid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, *J* = 6.2 Hz, 3H), 4.18 (q, *J* = 7.3 Hz, 2H), 7.28 (s, 1H), 7.37-7.39 (m, 3H), 7.42-7.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 60.7, 84.9, 122.9, 129.9 (2C), 130.5 (2C), 131.6, 138.5, 141.7, 145.3, 156.7; IR (KBr) ν_{max} (cm^{-1}): 2934, 1698, 1574; MS (ESI) m/z: 358.8 [M + H];

Ethyl 2-iodo-4-methylthiophene-3-carboxylate (3f):



This compound was prepared by using compound **2f** (0.5g, 2.70 mmol) according to a procedure similar to that of **3d**; yield: 53% (0.42g); light brown liquid; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.22 (t, *J* = 5.6 Hz, 3H), 2.2 (s, 3H), 4.18 (q, *J* = 6.2 Hz, 2H), 6.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 15.0, 60.3, 81.3, 128.3, 131.3, 137.9, 150.4; IR (KBr) ν_{max} (cm^{-1}): 2854, 2937, 1704; MS (ESI) m/z: 296.8 [M + H];

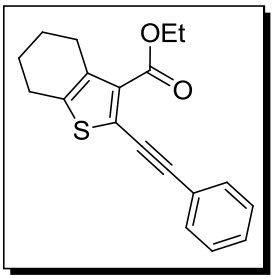
Ethyl 2-iodothiophene-3-carboxylate (3g):



This compound was prepared by using compound **2g** (0.5g, 2.92 mmol) according to a procedure similar to that of **3d**; yield: 51% (0.42 g); light green liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32 (t, *J* = 5.7 Hz, 3H), 4.18 (q, *J* = 6.1 Hz, 2H), 6.25 (d, *J* = 5.3 Hz, 1H), 6.89 (d, *J* = 5.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.5, 63.8, 80.7, 128.0, 133.9, 144.5, 155.5; IR (KBr) ν_{max} (cm^{-1}): 2872, 1698, 1549; MS (ES mass) m/z : 282.8 [M + H].

Preparation of alkynes (4)

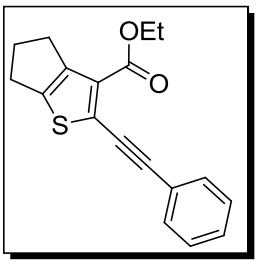
Typical procedure for the synthesis of ethyl 2-(phenylethynyl)-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxylate (4a):



To a solution of compound **3a** (0.2 g, 0.59 mmol) in ethanol (3mL) was added 10% Pd/C (0.0006 g, 0.0059 mmol), PPh_3 (0.006 g, 0.02 mmol), CuI (0.001 g, 0.0059 mmol) and Et_3N (0.16 mL, 1.18 mmol) and the mixture was stirred for 15 min under nitrogen. Then, phenyl acetylene (0.1 mL, 0.89 mmol) was added and the mixture was stirred at 60 °C for 2h. After completion of the reaction, the mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford the compound **4a;** yield 72% (0.13 g); off white solid; mp: 88.5-89.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (t, *J* = 7.2 Hz, 3H), 1.78-1.84(m, 4H), 2.72 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H), 4.36 (q, *J* = 6.2 Hz, 2H), 7.28-7.31 (m, 3H), 7.33-7.35 (m, 2H); ^{13}C NMR (100 MHz,

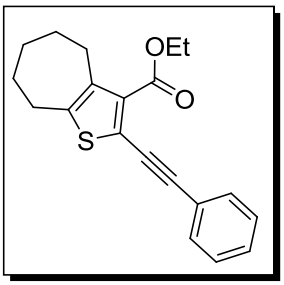
$CDCl_3$) δ: 14.1, 21.0, 22.3, 22.7, 26.1, 60.3, 82.8, 97.5, 107.3, 114.1, 114.3 (2C), 116.5, 128.3, 131.3, 136.2, 137.9, 150.4, 171.1; HPLC: 97.5%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water mobile phase B: CH_3CN, gradient (T/%B): 0/50, 2/50, 9/98, 12/98, 15/50, 18/50, 15/90; flow rate: 1.0 mL/min; UV 368 nm, retention time 8.6 min; IR (KBr) ν_{max} (cm^{-1}): 2946,1698, 1228, 756; MS (ESI) m/z: 311.4 [M + H];

Ethyl-2-(phenylethynyl)-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (4b):



This compound was prepared by using compound **3b** (0.2 g, 0.62 mmol) according to a procedure similar to that of **4a**; yield 64% (0.12 g); light brown solid; mp: 105-106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (t, *J* = 6.9 Hz, 3H), 2.40-2.48 (m, 2H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 4.34 (q, *J* = 6.9 Hz, 2H), 7.36-7.37 (m, 3H), 7.55-7.57 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 28.5, 28.9, 29.9, 60.4, 83.2, 97.5, 123.0, 128.3 (2C), 128.5, 129.3, 131.3, 131.4 (2C), 143.0, 147.3, 162.7; HPLC: 98.0%, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 248 nm, retention time 7.2 min; IR (KBr) ν_{max} (cm^{-1}): 2980, 1705, 1535, 755; MS (ES mass) m/z : 297.3[M + H];

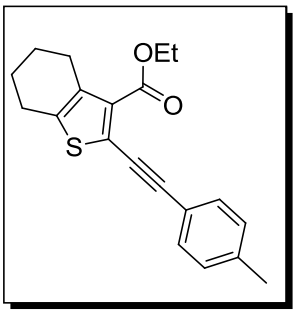
Ethyl-2-(phenylethynyl)-5,6,7,8-tetrahydrobenzo[*b*]thiophene-3-carboxylate (4c):



This compound was prepared by using compound **3c** (0.2g, 0.57 mmol) according to a procedure similar to that of **4a**; yield 67% (0.124 g); light yellow liquid; ^{1}H NMR (400

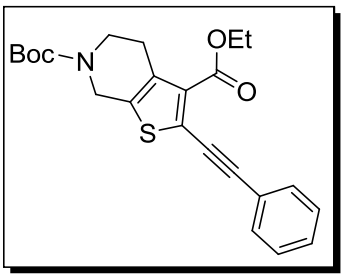
MHz, $CDCl_3$) δ: 1.39 (t, *J* = 7.3 Hz, 3H), 1.61-1.69 (m, 4H), 1.84-1.88 (m, 2H), 2.96 (t, *J* = 5.2 Hz, 2H), 2.96 (t, *J* = 5.6 Hz, 2H), 4.37 (q, *J* = 7.3 Hz, 2H), 7.47-7.49 (m, 3H), 7.52-7.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 22.6, 27.0, 27.2, 28.1, 29.7, 31.9, 32.4, 60.7, 82.4, 95.9, 123.1, 128.3 (2C), 131.3 (2C), 135.0, 141.4, 142.6, 163.9; IR (KBr) ν_{max} (cm^{-1}): 3020, 2126, 1625, 636; MS (ESI) m/z 325.4 [M + H]; HRMS: calcd for $C_{20}H_{21}O_2S$ (M + H): 325.1262, found 325.1247.

Ethyl 2-(*p*-tolylethynyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (4d):



This compound was prepared by using compound **3a** (0.2g, 0.59 mmol) and *p*-tolyl acetylene (0.1 mL, 0.89 mmol) according to a procedure similar to that of **4a**; Yield: 61% (0.11g); brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.25 (t, *J* = 4.6 Hz, 3H), 1.81-1.82 (m, 4H), 2.38 (s, 3H), 2.77-2.79 (m, 2H), 2.98-2.99 (m, 2H), 4.24 (q, *J* = 6.6 Hz, 2H), 7.24-7.25 (m, 2H), 7.59-7.60 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.8, 21.8, 22.3, 25.8, 25.9, 30.9, 64.8, 82.4, 97.7, 109.9, 120.6, 128.1, 129.6, 130.8, 136.4, 137.1, 140.2, 154.8, 155.7, 157.5; IR (KBr) ν_{max} (cm^{-1}) : 2897, 2917, 1625, 874 ; MS (ES mass) m/z : 325.3 [M + H].

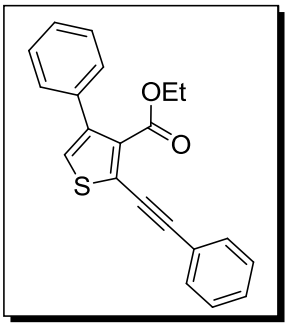
6-*tert*-Butyl 3-ethyl 2-(phenylethynyl)-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (4e):



This compound was prepared by using compound **3d** (0.2g, 0.45 mmol) according to a procedure similar to that of **4a**; yield 62% (0.1 g); light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (t, *J* = 7.3 Hz, 3H), 1.49 (s, 9H), 2.92-3.01 (m, 2H), 3.67-3.69 (m, 2H),

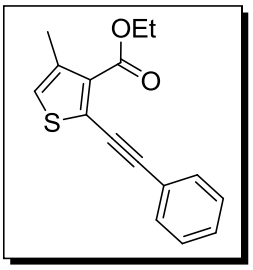
4.25-4.31 (m, 2H), 4.60 (s, 2H), 7.35-7.38 (m, 3H), 7.53-7.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 15.2, 18.4, 28.4 (3C), 29.6, 60.3, 70.8, 81.5, 94.2, 121.7, 128.4, 128.5 (2C), 129.1 (2C), 131.4, 132.1, 132.3, 132.4, 154.5, 162.8; IR (KBr) ν_{max} (cm^{-1}): 2913, 2210, 1682, 1594, 1207, 865; MS (ESI) m/z: 412.7 [M + H];

Ethyl 4-phenyl-2-(phenylethynyl)thiophene-3-carboxylate (4f):



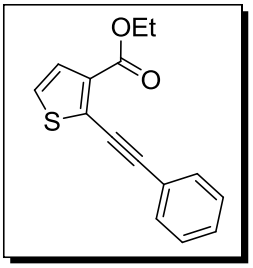
This compound was prepared by using compound **3e** (0.2g, 0.56 mmol) according to a procedure similar to that of **4a**; Yield: 60% (0.11 g); brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, *J* = 6.2 Hz, 3H), 4.18 (q, *J* = 7.3 Hz, 2H), 7.28 (s, 1H), 7.37-7.40 (m, 3H), 7.43-7.47 (m, 3H), 7.49 (dd, *J* = 7.4, 1.8 Hz, 2H), 7.90-7.94 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9, 61.2, 85.4, 91.0, 114.1, 115.7 (2C), 120.6, 125.0 (2C), 124.5, 127.0 (2C), 128.2 (2C), 129.4 (2C), 131.6, 136.2, 145.4, 159.4; IR (KBr) ν_{max} (cm^{-1}) : 2951, 2230, 1698, 1572; MS (ES mass) m/z : 333.2 [M + H].

Ethyl 4-methyl-2-(phenylethynyl)thiophene-3-carboxylate (4g):



This compound was prepared by using compound **3f** (0.2 g, 0.67 mmol) according to a procedure similar to that of **4a**; Yield: 65% (0.11g); light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.17 (t, *J* = 7.1 Hz, 3H), 2.17 (s, 3H), 4.19 (q, *J* = 7.5 Hz, 2H), 7.46 (m, 3H), 7.52 (s, 1H), 7.80-7.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.4, 15.8, 60.6, 85.3, 94.0, 122.9, 123.1, 126.2, 128.6 (2C), 132.1 (2C), 134.3, 137.5, 139.4, 158.0; IR (KBr) ν_{max} (cm^{-1}) : 2862, 2906, 2213, 1717, 1547; MS (ES mass) m/z : 270.8 [M + H].

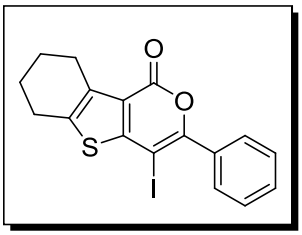
Ethyl 2-(phenylethynyl)thiophene-3-carboxylate (4h):



This compound was prepared by using compound **3g** (0.2g, 0.70 mmol) according to a procedure similar to that of **4a**; Yield: 62% (0.11 g); light green liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (t, *J* = 6.2 Hz, 3H), 4.25 (q, *J* = 6.9 Hz, 2H), 6.60 (d, *J* = 5.6 Hz, 1H), 6.98 (d, *J* = 5.6 Hz, 1H), 7.56 (m, 3H), 7.74 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 64.0, 90.4, 122.9, 123.7, 128.1 (2C), 128.4, 129.7 (2C), 130.4, 131.4, 133.3, 136.7, 156.8; IR (KBr) ν_{max} (cm^{-1}): 2931, 1718, 1522; MS (ES mass) m/z : 257.2 [M + H].

Preparation of 7-iodo-4*H*-thieno[3, 2-*c*]pyran-4-one derivatives (5)

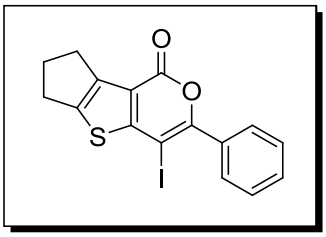
Typical procedure for the synthesis of 1-iodo-2-phenyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-one (5a): 5a:



A solution of compound **4a** (0.2 g, 0.64 mmol) and I_2 (0.16 g, 0.64 mmol) in dichloromethane (3 mL) was placed in a round bottom flask and stirred for 5 min under a nitrogen atmosphere. The mixture was then diluted with ether (25 mL) and washed with aq $Na_2S_2O_3$ (20 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford the compound **5a**; **y**ield: 80% (0.21 g); light yellow solid; mp: 213.7-214.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.81-1.93 (m, 4H), 2.82 (t, *J* = 5.6 Hz, 2H), 2.99 (t, *J* = 6.0 Hz, 2H), 7.44-7.45 (m, 3H), 7.73-7.75 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.0, 27.6, 28.3, 29.9, 64.7, 121.1, 128.0 (2C), 129.7 (2C), 130.1, 133.6, 141.8, 142.0, 153.9, 154.4, 158.1; HPLC: 98.7%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water

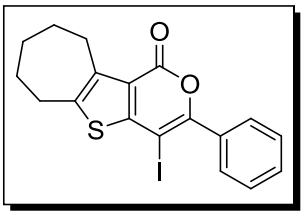
mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 5.9 min; IR (KBr) ν_{max} (cm^{-1}): 2920, 1654, 1138, 786; MS (ESI) m/z: 409.0 [M + H]; HRMS: calcd for $C_{17}H_{14}IO_2S$ (M + H): 408.9833, found 408.9830.

1-Iodo-2-phenyl-4*H*-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[3,2-*c*]pyran-4-one (5b):



This compound was prepared by using compound **4b** (0.2 g, 0.67 mmol) according to a procedure similar to that of **5a**; Yield: 72% (0.19 g); light yellow solid; mp: 208.2-209.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.48-2.55 (m, 2H), 2.98 (t, *J* = 7.4 Hz, 2H), 3.07 (t, *J* = 7.3 Hz, 2H), 7.46-7.73 (m, 3H), 7.74-7.76 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.2, 29.9, 32.4, 64.7, 125.1, 128.0 (2C), 128.8 (2C), 129.7, 130.1, 141.8, 142.0, 153.9, 154.4, 158.1; HPLC: 99.1%, 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): 0/50, 2/50, 9/98, 12/98, 15/50, 18/50; flow rate: 1.0 mL/min; UV 367 nm, retention time 7.3 min; IR (KBr) ν_{max} (cm^{-1}): 2964, 2854, 1717, 1060; MS (ESI) m/z: 395.1 [M + H]; HRMS: calcd for $C_{16}H_{11}IO_2S$ (M + H): 394.9603, found 394.9594.

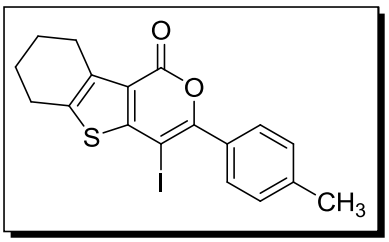
1-Iodo-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (5c):



This compound was prepared by using compound **4c** (0.2 g, 0.61 mmol) according to a procedure similar to that of **5a**; Yield: 75% (0.19 g); brown solid; mp: 180.2-182.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.67-1.69 (m, 4H), 1.90-1.93 (m, 2H), 2.88-2.91 (m, 2H), 3.26-3.28 (m, 2H), 7.44-7.46 (m, 3H), 7.72-7.74 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.0, 27.6, 28.3, 29.9, 32.5, 64.7, 121.1, 128.0 (2C), 129.7 (2C), 130.1, 133.6, 141.8,

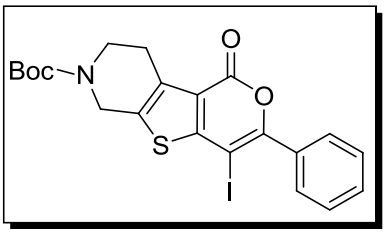
142.0, 153.9, 154.4, 158.1; HPLC: 98.7%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 5.9 min; IR (KBr) ν_{max} (cm^{-1}): 2920, 1713, 1556, 1066, 693; MS (ESI) m/z: 423.2[M + H]; HRMS: calcd for $C_{18}H_{16}IO_2S$ (M + H): 422.9916, found 422.9911.

1-Iodo-2-*p*-tolyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-one (**5d)**:



This compound was prepared by using compound **4d** (0.2 g, 0.61 mmol) according to a procedure similar to that of **5a**; Yield: 66% (0.17 g); brown solid; mp: 182-183 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72-1.87 (m, 4H), 2.35 (s, 3H), 2.74 (t, *J* = 5.5 Hz, 2H), 2.92 (t, *J* = 5.4 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.8, 22.7, 25.2, 25.9, 30.9, 64.3, 109.9, 120.6, 128.7, 129.6 (2C), 130.8, 136.4, 137.1, 140.4, 154.8, 155.7, 157.8; HPLC: 98.5%, 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): 0/90, 2/90, 9/98, 12/98, 15/90, 18/90; flow rate: 7.40 mL/min; UV 258 nm, retention time 20.0 min; IR (KBr) ν_{max} (cm^{-1}): 2856, 1643, 1201, 842; MS (ESI) m/z: 422.8 [M + H].

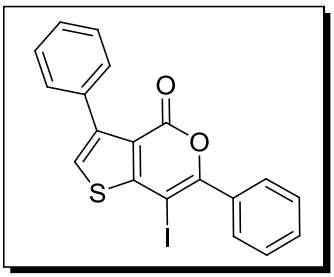
1-Iodo-2-phenyl-4*H*-7-(tert-butoxycarbonyl)-5,6,7,8 tetrahydropyrido [4',3':4,5] thieno[3,2-*c*]pyran-4-one (5e):



This compound was prepared by using compound **4c** (0.2 g, 0.48 mmol) according to a procedure similar to that of **5a**; yield: 58% (0.14 g); light brown solid; mp: 174-175 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (s, 9H), 3.09 (s, 2H), 3.71 (t, *J* = 5.2 Hz, 2H), 4.67 (s, 2H), 7.43-7.49 (m, 3H), 7.71-7.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.6, 28.2

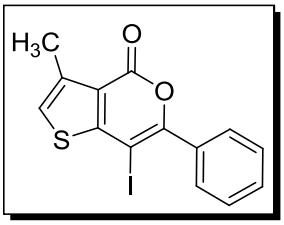
(3C), 40.3, 42.7, 56.8, 80.4, 128.0 (2C), 129.6 (2C), 130.2, 131.6, 133.6, 139.0, 141.6, 144.8, 146.9, 152.8, 156.9; HPLC: 98.8%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 6.4 min; IR (KBr) ν_{max} (cm^{-1}): 2902, 1678, 1584, 846; MS (ESI) m/z: 510.3 [M+H].

7-Iodo-3,6-diphenyl-4*H*-thieno[3,2-*c*]pyran-4-one (5f):



This compound was prepared by using compound **4f** (0.2g, 0.60 mmol) according to a procedure similar to that of **5a**; yield: 70% (0.18 g); off white solid; mp: 136-137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30 (s, 1H), 7.41-7.43 (m, 3H), 7.45-7.50 (m, 3H), 7.53 (dd, *J* = 7.4, 1.8 Hz, 2H), 7.73-7.79 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 59.5, 127.0, 127.3 (2C), 127.5 (2C), 127.7, 128.0, 128.2 (2C), 128.8 (2C), 129.3 (2C), 143.7, 144.3, 145.5, 147.0, 156.3; HPLC: 98.7%, 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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 5.9 min; IR (KBr) ν_{max} (cm^{-1}): 1588, 1610, 1743, 867; MS (ESI) m/z: 431.5 [M+H].

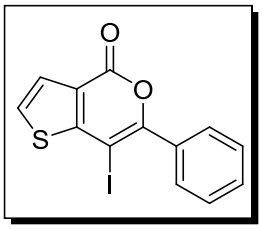
7-Iodo-3-methyl-6-phenyl-4*H*-thieno[3,2-*c*]pyran-4-one (5g):



This compound was prepared by using compound **4g** (0.2g, 0.74 mmol) according to a procedure similar to that of **5a**; yield: 68% (0.185 g); light yellow solid; mp: 123-124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (s, 3H), 7.08 (s, 1H), 7.48-7.50 (m, 3H), 7.76-7.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.8, 60.7, 120.9, 123.7, 128.1 (2C), 129.7 (2C), 133.3, 138.8, 139.7, 141.6, 147.8, 157.7; HPLC: 95.2 %, 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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 4.5 min; IR (KBr) ν_{max} (cm^{-1}): 2935, 1649, 1572, 879; MS (ESI) m/z: 369.3 [M+H].

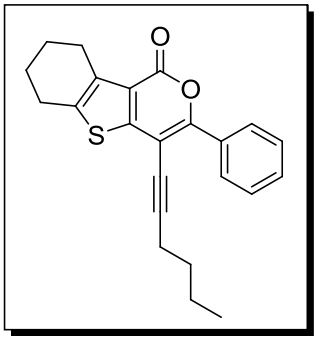
7-Iodo-6-phenyl-4*H*-thieno[3,2-*c*]pyran-4-one (5h):



This compound was prepared by using compound **4h** (0.2g, 0.78 mmol) according to a procedure similar to that of **5a**; yield: 63% (0.17 g); light yellow solid; mp: 135-136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, *J* = 5.4 Hz, 1H) 7.48-7.50 (m, 3H), 7.72-7.79 (m, 2H), 7.89 (d, *J* = 5.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 63.8, 122.5, 125.9, 127.2, 128.1, 129.7 (2C), 130.4 (2C), 133.6, 149.2, 155.5, 157.8; HPLC: 92.8 %, 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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 6.2 min; IR (KBr) ν_{max} (cm^{-1}): 2931, 1618, 1524, 863; MS (ESI) m/z: 354.9 [M+H].

Preparation of 7-alkynyl substituted-6-phenyl-4*H* -thieno[3,2- *c*]pyaran-4-one derivatives (6)

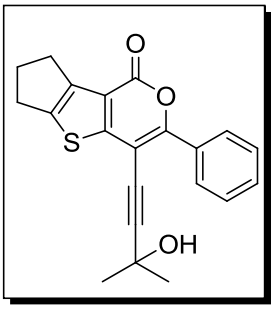
Typical procedure for the synthesis of 1-(hex-1-ynyl)-2-phenyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-one (6a):



A mixture of compound **5a** (0.2 g, 0.49 mmol), 10% Pd/C (0.0005 g, 0.0049 mmol), PPh_3 (0.005 g, 0.02mmol), CuI (0.0009 g, 0.0049 mmol) and Et_3N (0.1 mL, 0.98 mmol) in

ethanol/methanol (3.0 mL) was stirred for 15 min under nitrogen. Then, 1-hexyne (0.08 mL, 0.73 mmol) was added and the mixture was stirred at 80 °C for 4 h. After completion, the reaction mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford the compound **6a**; yield: 60% (0.135 g); brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, *J* = 6.9 Hz, 3H), 1.41-1.49 (m, 2H), 1.61-1.66 (m, 2H), 1.83-1.89 (m, 4H), 2.49 (t, *J* = 6.9 Hz, 2H), 2.80 (t, *J* = 5.8 Hz, 2H), 3.00 (t, *J* = 6.1 Hz, 2H), 7.41-7.45 (m, 3H), 8.17-8.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 19.0, 21.3, 21.9, 22.6, 24.7, 29.6, 31.4, 73.5, 96.2, 98.2, 125.0, 127.8 (2C), 129.3 (2C), 131.2, 131.9, 141.3, 141.7, 144.0, 147.1, 150.9; HPLC: 97.3%, 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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 8.1 min; IR (KBr) ν_{max} (cm^{-1}): 2928, 2224, 1732, 1548, 1066, 690. MS (ESI) m/z: 363.3 [M+H]; HRMS: calcd for $C_{23}H_{23}O_2S$ (M + H): 362.9452, found 362.9451.

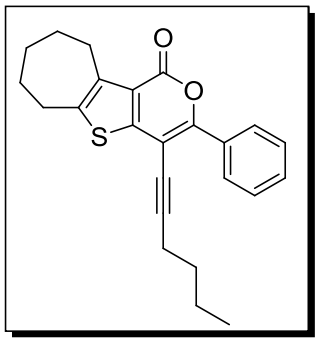
1-(3-Hydroxy-3-methylbut-1-ynyl)-2-phenyl-4*H*-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[3,2-*c*]pyran-4-one (**6b)**:



This compound was prepared by using compound **5b** (0.2 g, 0.51 mmol) and 2-methyl but-3-yn-2-ol (0.07 mL, 0.76 mmol) according to a procedure similar to that of **6a**; Yield: 72% (0.13 g); brown color semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (s, 6H), 2.23-2.25 (m, 2H), 2.61-2.63 (m, 2H), 2.79-2.81 (m, 2H), 7.41-7.43 (m, 3H), 7.81-7.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.0, 26.2, 31.4 (2C), 31.5, 64.1, 85.4, 98.4, 103.0, 121.9, 125.8, 127.0, 128.6, 129.2, 131.6, 137.1, 141.9, 142.1, 149.3, 156.9, 157.7; HPLC: 87.6

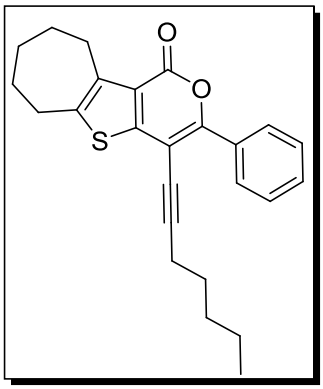
%, column: X Bridge C-18 150 x 4.6 mm 5µ, mobile phase A: 0.05 % Formic Acid in water mobile phase B: CH_3CN, gradient (T/%B): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 6.8 min; IR (KBr) ν_{max} (cm^{-1}): 3356, 2930, 1730, 1465; MS (ESI) m/z: 350.9 [M+H];

1-(Hex-1-ynyl)-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (**6c)**:



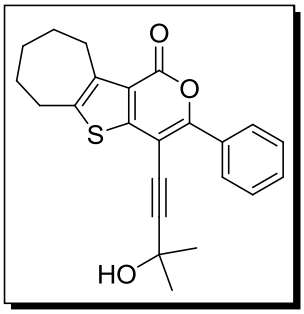
This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) and hex-1-yne (0.08 mL, 0.70 mmol) according to a procedure similar to that of **6a**; yield: 63% (0.11 g); light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (t, *J* = 7.3 Hz, 3H), 1.48-1.53 (m, 2H), 1.59-1.73 (m, 6H), 1.88-1.91 (m, 2H), 2.49 (t, *J* = 6.9 Hz, 2H), 2.89 (t, *J* = 5.5 Hz, 2H), 3.29 (t, *J* = 5.1 Hz, 2H), 7.41-7.45 (m, 3H), 8.16-8.19 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8, 14.1, 19.0, 21.3, 21.9, 25.1, 27.6, 29.6 (2C), 32.5, 73.5, 96.3, 99.1, 113.2, 125.1, 127.8, 128.2, 128.8 (2C), 129.8, 140.7, 141.3, 147.1, 157.6; HPLC: 99.6%, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 6.6 min IR (KBr) ν_{max} (cm^{-1}): 2924, 2228, 1730, 1068, 690; MS (ESI) m/z: 377.4 [M+H]; HRMS: calcd for $C_{24}H_{25}O_2S$ (M + H): 377.1575, found 377.1576.

1-(Hept-1-ynyl)-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (**6d)**:



This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) and hept-1-yne (0.09 mL, 0.70 mmol) according to a procedure similar to that of **6a**; yield: 60% (0.26 g); brown color semi solid; ^{1}H NMR(400 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 6.2 Hz, 3H), 1.42-1.61 (m, 6H), 1.65-1.68 (m, 4H), 1.81-1.84 (m, 2H), 2.25-2.29 (m, 2H), 2.82-2.85 (m, 2H), 3.35-3.38 (m, 2H), 7.36-7.42 (m, 3H), 7.77-7.80 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9, 19.2, 22.2, 27.0, 27.6, 27.7, 29.6, 29.9, 32.4, 37.2, 62.8, 96.2, 98.2, 125.1, 127.8 (2C), 128.0, 128.4, 128.5 (2C), 128.8, 129.7, 132.1, 141.8, 158.1; IR (KBr) ν_{max} (cm^{-1}): 2915, 1732, 1448, 810. MS (ESI) m/z: 390.8 [M + H];

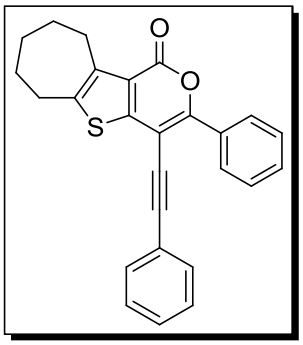
1-(3-Hydroxy-3-methylbut-1-ynyl)-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (**6e)**:



This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) and 2-methylbut-3-yn-2-ol (0.07 mL, 0.70 mmol) according to a procedure similar to that of **6a**; yield: 68% (0.15 g); colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.65 (s, 6H), 1.68-1.77 (m, 4H), 1.92 (m, 2H), 2.85-2.92 (m, 2H), 3.25-3.33 (m, 2H), 7.45-7.47 (m, 3H), 8.11-8.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.0, 27.6, 30.0, 31.0, 31.1 (2C), 32.3, 65.5, 83.9, 96.9, 102.0, 121.2, 127.8, 128.1 (2C), 130.2 (2C), 131.6, 140.8, 141.9, 150.1, 155.8, 157.4; HPLC: 88.9%, 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): 0/70, 2/70, 9/98, 12/98,

15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 4.5 min; IR (KBr) ν_{max} (cm^{-1}): 3215, 2982, 2178, 1699, 957; MS (ESI) m/z: 379.1 [M+H].

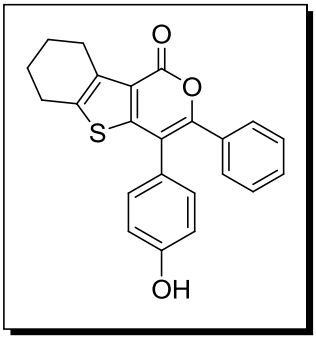
1-(Phenylethynyl)-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (**6f)**:



This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) and phenyl acetylene (0.07 mL, 0.70 mmol) according to a procedure similar to that of **6a**; Yield: 65% (0.138 g); light yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.66-1.69 (m, 4H), 1.81-1.90 (m, 2H), 2.80-2.88 (m, 2H), 3.21-3.29 (m, 2H), 7.27-7.34 (m, 3H), 7.37-7.50 (m, 5H), 8.16 (d, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.1, 27.6 (2C), 30.1, 32.5, 76.6, 77.0, 77.3, 82.4, 97.3, 97.6, 121.3, 122.4, 128.0, 128.2 (2C), 128.4, 130.2, 131.3 (2C), 131.8, 140.8, 142.0, 149.9, 155.7, 157.3; HPLC: 93.0%,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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.4 mL/min; UV 368 nm, retention time 5.2 min; IR (KBr) ν_{max} (cm^{-1}): 2938, 2214, 1723, 910; MS (ESI) m/z: 397.1[M +H].

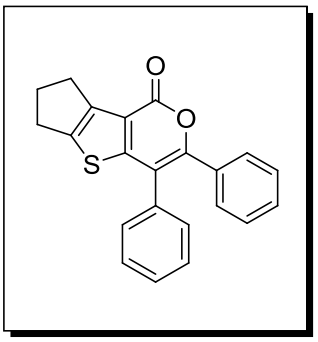
Synthesis of 7-aryl substituted-6-phenyl-4*H*-thieno[3,2-*c*]pyaran-4-one derivatives (7)

Typical procedure for the synthesis of 1-(4-hydroxyphenyl)-2-phenyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-one (7a):



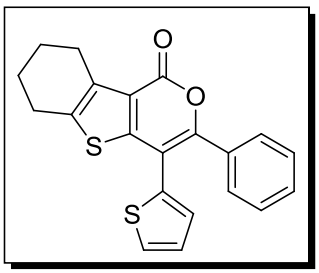
To a solution of compound **5a** (0.2 g, 0.49 mmol) in dry DMF (3 mL) was added 5 mol % $Pd(OAc)_2$ (0.001 g, 0.0049 mmol) and K_2CO_3 (0.13 g, 0.98 mmol) were added under a nitrogen atmosphere and the mixture was stirred for 10 min. To this was added, 4-hydroxy phenyl boronic acid (0.1 g, 0.73 mmol) and the mixture was allowed to stir at 80 °C for 3h. After completion of the reaction, the mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 7.5:2.5 hexane/ethyl acetate to afford the compound **7a**; yield: 52% (0.09 g); light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.89-1.91 (m, 4H), 2.07-2.09 (m, 4H), 5.97-5.99 (m, 2H), 6.29-6.31 (m, 2H), 6.48-6.49 (m, 5H), 8.91 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.9, 22.7, 25.1, 25.3, 115.9, 116.1 (2C), 121.4, 125.3, 127.8 (2C), 128.8 (2C), 130.7 (2C), 132.4, 134.9, 137.3, 149.8, 150.2, 153.4, 157.4, 158.2; HPLC: 96.8 %, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 6.9 min; IR (KBr) ν_{max} (cm^{-1}): 3403, 2924, 1732, 754, 688. MS (ESI) m/z: 375.4[M + H].

1,2-Diphenyl-4*H*-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[3,2-*c*]pyran-4-one (**7b)**:



This compound was prepared by using compound **5b** (0.2 g, 0.51 mmol) and phenyl boronic acid (0.09 g, 0.76 mmol) according to a procedure similar to that of **7a**; yield: 57% (0.14 g); light brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36-2.41 (m, 2H), 2.80 (t, *J* = 6.2 Hz, 2H), 2.92 (t, *J* = 7.0 Hz, 2H), 7.30-7.51 (m, 8H), 8.01-8.03 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.3, 29.0, 30.3, 127.9, 128.5 (3C), 128.8 (3C), 129.0 (2C), 129.2 (2C), 132.8, 136.6, 137.5, 140.8, 147.6, 157.5, 167.4, 197.1; HPLC: 93.1 %, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 5.2 min; IR (KBr) ν_{max} (cm^{-1}): 2854, 1615, 1501, 878; MS (ESI) m/z: 344.9 [M + H].

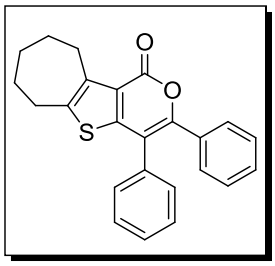
1-(Thiophen-2-yl)-2-phenyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-one (7c):



This compound was prepared by using compound **5a** (0.2 g, 0.49 mmol) and thiophen-2-ylboronic acid (0.09 g, 0.73 mmol) according to a procedure similar to that of **7a**; yield: 63% (0.11 g); light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.87-1.89 (m, 4H), 2.78-2.80 (m, 2H), 2.98-3.14 (m, 2H), 7.06 (dd, *J* = 5.4, 2.6 Hz, 1H), 7.27-7.33 (m, 2H), 7.39 (dd, *J* = 4.4, 1.9 Hz, 1H), 7.41- 7.49 (m, 2H), 7.84 (dd, *J* = 8.1, 1.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.0, 22.8, 25.2, 25.4, 108.5, 121.5, 125.1, 127.3, 127.5 (2C), 128.8 (2C), 128.9 (2C), 129.3, 132.1, 135.3, 135.4, 137.6, 152.8, 157.8 HPLC: 98.7%, 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): 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.1 mL/min; UV 368 nm, retention time 5.9 min; IR (KBr) ν_{max} (cm^{-1}): 2890, 1587, 1728, 854; MS (ESI) m/z: 365.3 [M + H].

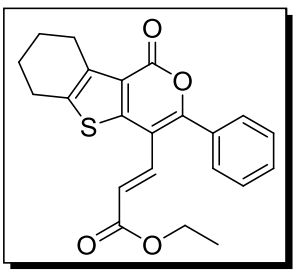
1,2-Diphenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (**7d**):



This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) and phenyl boronic acid (0.08 g, 0.70 mmol) according to a procedure similar to that of **7a**; yield: 53% (0.09 g); colorless semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.57-1.65 (m, 4H), 1.80-1.83 (m, 2H), 2.69-2.71 (m, 2H), 2.86-2.87 (m, 1H), 2.93-2.94 (m, 1H), 7.30-7.39 (m, 7H), 7.43-7.47 (m, 1H), 7.95-7.97(m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.1, 26.2, 28.4, 29.9, 114.5, 125.8 (2C), 126.1 (2C), 127.0 (2C), 128.1, 128.5, 129.1, 129.2 (2C), 129.7, 129.9, 130.6, 131.6, 135.0, 141.9, 143.8, 144.7; HPLC: 96.8%, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 7.2 min; IR (KBr) ν_{max} (cm^{-1}): 2894, 1643, 872; MS (ESI) m/z: 372.9 [M + H].

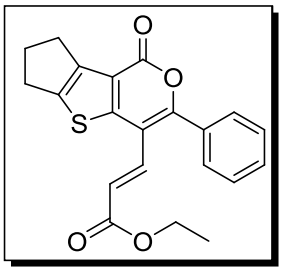
2A.7.7. Preparation of 7-alkenyl substituted-6-phenyl-4*H*-thieno[3,2-*c*]pyaran-4-one derivatives (8)

Typical procedure for the synthesis of (*E*)-ethyl-3-(4-oxo-2-phenyl-4*H*-5,6,7,8-tetrahydrobenzo[*b*]thieno[3,2-*c*]pyran-4-yl)acrylate (8a):



The reaction vessel was charged with compound **5a** (0.2 g, 0.49 mmol), ethyl acrylate (0.1 mL, 0.98 mmol), K_2CO_3 (0.13 g, 0.98 mmol), and the 5 mol % $Pd(OAc)_2$ (0.001 g, 0.0049 mmol) in *N*,*N*-dimethylformamide (2 mL). The reaction mixture was stirred at 110 °C for 4h. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc (20 mL), and washed with 1 N aq HCl and water. The organic phase was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using 8:2 hexane/ethyl acetate to afford the compound **8a**; yield: 63% (0.11 g); colorless semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (t, *J* = 6.3 Hz, 3H), 2.58-2.61 (m, 4H), 3.04-3.06 (m, 2H), 3.21-3.24 (m, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 6.65 (d, *J* = 16.5 Hz, 1H), 7.53-7.55 (m, 3H), 7.76-7.78 (m, 2H) 7.82 (d, *J* = 16.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 23.7, 27.3, 33.1, 38.7, 60.7, 121.0, 125.5, 128.4, 128.7, 129.9, 130.6, 130.1, 130.8, 131.4, 132.4, 133.2, 134.4, 138.5, 139.1, 166.6, 172.7; HPLC: 97.4%, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 8.2 min; IR (KBr) ν_{max} (cm^{-1}): 2971, 1738, 1536, 872; MS (ESI) m/z: 381.7 [M + H].

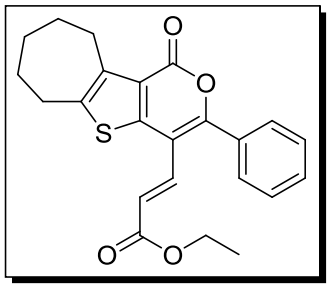
(*E*)-Ethyl-3-(4-oxo-2-phenyl-4*H*-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[3,2-*c*]pyran-4-yl)acrylate (8b):



This compound was prepared by using compound **5b** (0.2 g, 0.51 mmol) according to a procedure similar to that of **8a**; yield: 55% (0.10 g); light brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (t, *J* = 5.1 Hz, 3H), 1.86-1.92 (m, 2H), 2.88-2.81 (m, 2H), 3.09-3.02 (m, 2H), 4.29 (q, *J* = 5.3 Hz, 2H), 6.22 (d, *J* = 16.5 Hz, 1H), 7.50-7.51 (m, 3H), 7.58-7.60 (m, 2H), 7.64 (d, *J* = 16.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9, 27,3, 35.1, 68.1, 119.7, 121.0, 123.8, 125.1, 126.8, 129.9, 131.4, 133.2, 135.1, 137.4, 138.5, 140.0, 140.6, 142.5, 142.5, 143.2, 166.5; HPLC: 94.6%, 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):

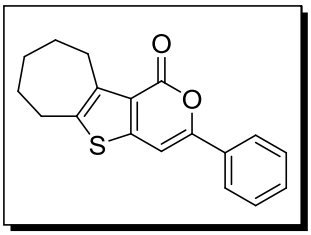
0/30, 2/30, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 6.6 min; IR (KBr) ν_{max} (cm^{-1}): 2983, 1742, 1522, 865; MS (ESI) m/z: 367.4 [M + H].

(*E*)-Ethyl-3-(4-oxo-2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-yl)acrylate (8c):



This compound was prepared by using compound **5c** (0.2 g, 0.47 mmol) according to a procedure similar to that of **8a**; yield: 60% (0.11 g); light brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (d, *J* = 5.2 Hz, 3H), 1.73-1.75 (m, 4H), 1.89-1.99 (m, 2H), 2.97-2.89 (m, 2H), 3.33-3.40 (m, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 6.64 (d, *J* = 16.5 Hz, 1H), 7.49-7.51 (m, 3H), 7.59-7.61 (m, 2H), 7.66 (d, *J* = 16.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 27.4, 27.6, 29.6, 29.8, 32.4, 60.7, 109.7, 121.1, 122.9, 128.4, 129.9 (2C), 130.5 (2C), 131.6, 138.5, 140.7, 141.7, 145.4, 156.7, 157.4, 166.5; HPLC 98.7%, 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, 9/98, 12/98, 15/30, 18/30; flow rate: 1.0 mL/min; UV 365 nm, retention time 7.3 min; IR (KBr) ν_{max} (cm^{-1}): 2983, 1701, 1554, 890; MS (ESI) m/z: 394.7 [M + H].

Synthesis of 2-phenyl-4*H*-6,7,8,9-tetrahydro-5*H*-cyclohepta[4,5]thieno[3,2-*c*]pyran-4-one (9):



To a solution of compound **5c** (0.2 g, 0.47 mmol) in 1,4-dioxane (2 mL) was added $Pd(OAc)_2$ (0.001 g, 0.0047 mmol), PPh_3 (0.006 g, 0.023 mmol), and MnO_2 (0.008 g, 0.094

mmol). The mixture was heated to reflux at 110 °C for 12 h. After completion of the reaction, the mixture was cooled to room temperature, and extracted with EtOAc (2 x 15 mL). The organic layers were collected, combined, washed with water (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue was then purified by column chromatography on silica gel using 8:2 hexane/ethyl acetate to afford the compound **9**; yield 58% (0.04 g); brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.65-1.76 (m, 4H), 1.87-1.96 (m, 2H), 2.82-2.91 (m, 2H), 3.33-3.35 (m, 2H), 7.04 (s, 1H), 7.35-7.49 (m, 3H), 7.80 -7.87 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.0, 27.6, 27.7, 29.9, 32.4, 98.2, 122.4, 125.0, 128.8 (2C), 129.7 (2C), 131.8, 140.4, 140.6, 147.1, 153.9, 158.6; IR (KBr) ν_{max} (cm^{-1}): 3016, 2931, 1680, 1743, 852; MS (ESI) m/z: 297.1[M + H].

Cell lines and culture conditions: Human metastatic breast cancer cells, MDA-MB 231, human chronic myeloid leukemia cells, K562, Hepatocellular carcinoma cells HEPG2 and human embryonic kidney cells, HEK293 (non cancerous cells), were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. All cell lines were maintained in humified condition at 5% CO_2, at 37 °C. Cells were subcultured twice a week.

MTT Assay: Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (5×10^3 cells/well) were seeded to 96-well culture plate and cultured with or without compounds at 10 µM concentration (five different concentrations i.e. 10, 5, 1, 0.5, 0.1 and 0.01 µM for dose response study) in duplicates for 24 h in a final volume of 200 µl. After treatment, the medium was removed and 20 µL of MTT (5 mg/mL in PBS) was added to the fresh medium. After 3h incubation at 37 °C, 100 µL of DMSO was added to each well and plates were agitated for 1 min. Absorbance was read at 570 nm on a multi-well plate reader (Victor3, Perkin Emler). Percent inhibition of proliferation was calculated as a fraction of control (without compound).

Reference:

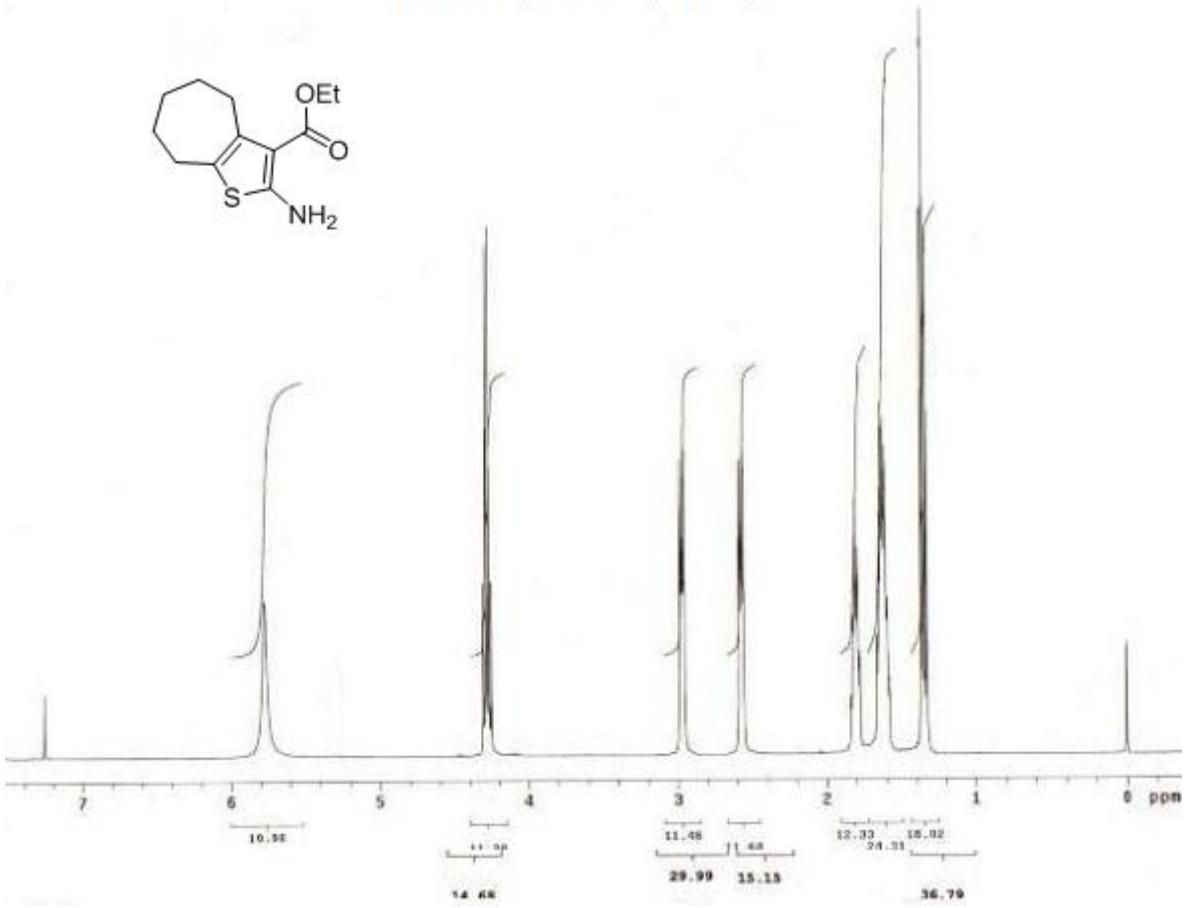
1. For leading reviews on 2-pyrone medicinal applications and natural products, see: (*a*) Dickinson, J.M. *Nat. Prod. Rep.* **1993**, *10*, 71. (*b*) McGlacken, G. P. and Fairlamb, I. J. S. *Nat. Prod. Rep*. **2005**, *22*, 369. for synthetic uses of 2-pyrones, see; (*c*) K. Afarinkia, K., Vinader, V., Nelson, T. D. and Posner, G. H. *Tetrahedron*, **1992**, *48*, 9111. (*d*) For recent synthetic applications see: Delaney, P. M., Browne, D. L., Adams, H., Plant, A., and Harrity, J. P. A. *Tetrahedron*, **2008**, *64*, 866.
2. Suzuki, K.; Kuwahara, A.; Yoshida, H.; Fujita, S.; Nishikiori, T.; Nishikiori, T. *J Antibiot*. **1997**, *50*, 314.
3. Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. *Cancer Lett*. **1998**, *126*, 29
4. Barrero, AF.; Oltra, JE.; Herrador, MM.; Cabrera, E.; Sanchez, JF.; Quílez, JF.; Rojas, F.; Reyes, JF. *Tetrahedron*, **1993**, *49*, 141.
5. Parker, SR.; Culter, HG.; Jacyno, JM.; Hillf, RA. *J. Agric. Food. Chem.* **1997**, *45*, 2774.
6. Altomare, C.; Perrone, G.; Zonno, M. C.; Evidente, A.; Pengue, R.; Fanti, F.; Polonelli, L. *J. Nat. Prod.* **2000**, *63*, 1131.
7. Larock, R.C.; Doty, M.J.; Xiaojun, H. *J. Org. Chem.* **1999**, *64*, 8770.
8. Yao, T.; Yue, D.; Larock, R.C. *J. Org. Chem*. **2005**, 70, 9985.
9. Raju, S.; Batchu, V.R.; Swamy, K. N.; Vasu Dev, R.; Sreekanth, B.R.; Moses Babu, J.; Vyas, K.; Rajender Kumar, P; Mukkanti, K.; Annamalaib, P.; Pal, M. *Tetrahedron*. **2006**, *62*, 9554.
10. Ngi, S.I.; Guilloteau, V.; Abarbri, M.; Thibonnet, J. *J. Org. Chem.* **2011**, *76*, 8347.
11. Estelle Marchal, E.; Uriac, P.; Legouin, B.; Toupet, L.; Weghe, P.V. *Tetrahedron.* **2007**, 63, 9979.
12. Chen, B.; Ma, S. *Org. Lett*. **2013**, *15* (15), 3884.
13. Li, Y.; Ding, Y.J.; Wang, J.Y.; Su, Y.M.; Wang, X.S. *Org. Lett.* **2013**, *15* (11), 2574.
14. Inamoto, K.; Kadokawa, J.; Kondo, Y. *Org. Lett.* **2013**, *15* (15), 3962.
15. Oikawa, T.; Sasaki, M.; Inose, M.; Shimamura, M.; Kuboki, H.; Hirano, S.-I.; Kumagai, H.; Ishizuka, M.; Takeuchi, T. *Anticancer Res.* **1997**, *17*, 1881.

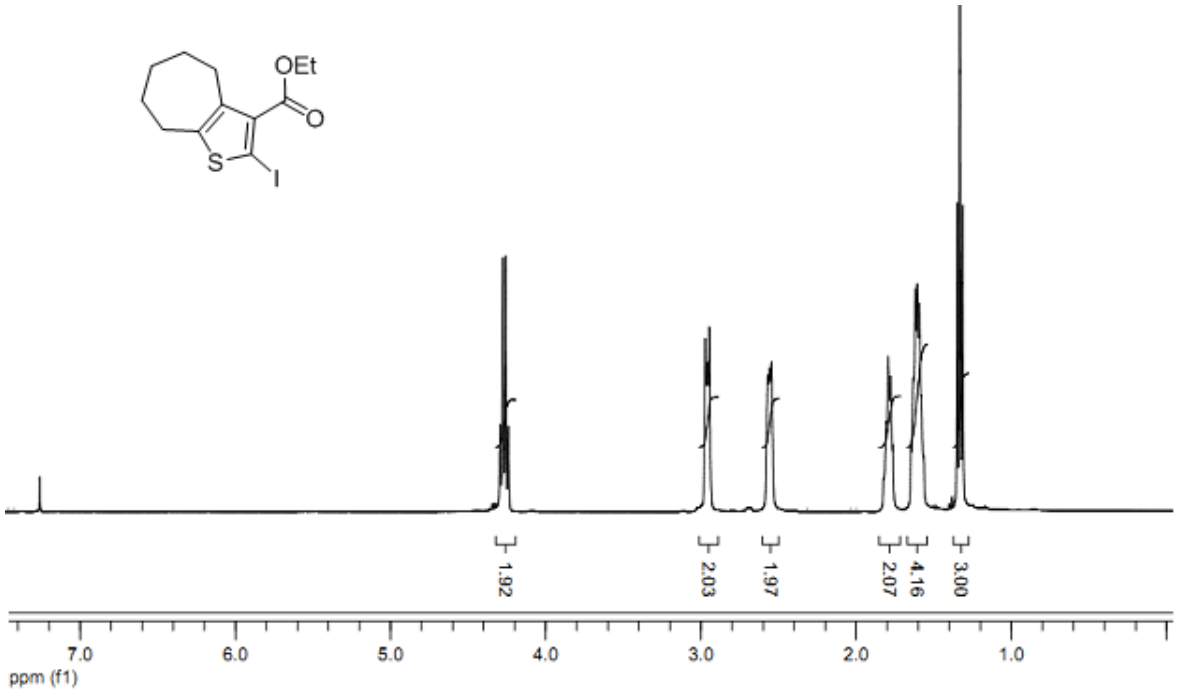
16. (a) Kalle, A. M.; Mallika, A.; Badiger, J.; Alinakhi.; Talukdar, P.; Sachchidanand *Biochem. Biophys. Res. Commun*. **2010**, *401*, 13. (b) Mulakayala, N.; Rambabu, D.; Raja, M. R.; Chaitanya, M.; Kumar, C. S.; Kalle, A. M.; Krishna, G. R.; Reddy, C. M.; Rao, M. V. B.; Pal, M. *Bioorg. Med. Chem.* **2012**, *20*, 759.
17. Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem.* **2001**, *276*, 38837.
18. Oikawa, T.; Sasaki, M.; Inose, M.; Shimamura, M.; Kuboki, H.; Hirano, S.-I.; Kumagai, H.; Ishizuka, M.; Takeuchi, T. *Anticancer Res.* **1997**, *17*, 1881.
19. Campaigne, E.; Katritzky, A. R., Ed.; Pergamon: New York, NY, *Comprehensive Heterocyclic Chemistry*, **1984**, *4*, 911.
20. Ram, V. J.; Goel, A.; Shukla, P. K.; Kapil, A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3101.
21. Raju, S.; Batchu, V. R.; Swamy, N. K.; Dev, R. V.; Sreekanth, B. R.; Babu, J. M.; Vyas, K.; Kumar, P. R.; Mukkanti, K.; Annamalai, P.; Pal, M. *Tetrahedron* **2006**, *62*, 955. (b) Raju, S.; Batchu, V. R.; Swamy, N. K.; Dev, R. V.; Babu, J. M.; Kumar, P. R.; Mukkanti, K.; Pal, M. *Tetrahedron Lett*. **2006**, *47*, 83.
22. (a) Gewald, K.; Schinke, E.; Böttcher, H. *Chem. Ber.* **1966**, *99*, 94. (b) Kumar, K. S.; Srinivas, C.; Vishweshwar, P.; Iqbal, J.; Pal, M. *Tetrahedron Lett*. **2010**, *51*, 3269. (c) Wang, T.; Huang, X.-G.; Liu, J.; Li, B.; Wu, J.-J.; Chen, K.-X.; Zhu, W.-L.; Xu, X.-Y.; Zeng, B.-B. *Synlett*, **2010**, 1351. (d) Sridhar, M.; Rao, R. M.; Baba, N. H. K.; Kumbhare, R. M. *Tetrahedron Lett* **2007**, *48*, 3171.
23. (a) Puterová, Z.; Andicsová, A.; Moncol, J.; Rabong, C.; Végh, D. *Tetrahedron Lett*, **2009**, *50*, 4670. (b) Perrior, T. R.; Tapolczay, D. J.; Whittle. A. J. US Patent No. 5077297, 31 Dec 1991.
24. Pal, M.; Synlett, **2009**, 2896.
25. (a) Yadav, J. S.; Reddy B. V. S.; Hashim, S. R. *J. Chem. Soc., Perkin Trans.* **2000**, *1*, 3025. (b) Yadav, J. S.; Reddy, B. V. S.; Premalatha, K.; Swamy, T. *Tetrahedron Lett.*, **2005**, *46*, 2687. (c) Yadav, J. S.; Reddy, B. V. S.; Rao, C. V.; Chand, P. K.; Prasad, A. R. *Synlett*, **2001**, 1638. (d) Bandyopadhyay, D.; Mukherjee, S.; Banik, B. K. *Molecules*, **2010**, *15*, 2520.
26. Barange, D. K.; Batchu, V. R.; Gorja, D.; Pattabiraman, V. R.; Tatini, L. K.; Babu, J. M.; Pal, M. *Tetrahedron* **2007**, *63*, 1775.

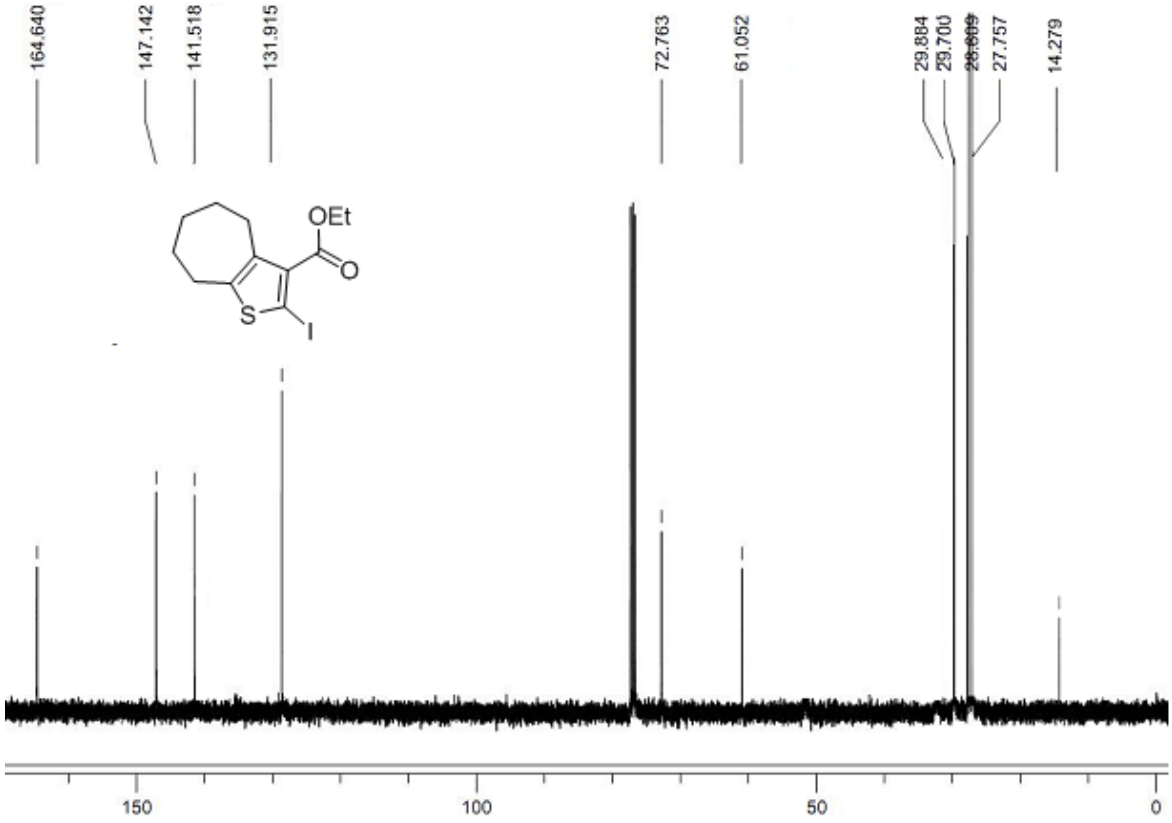
27. For recent and leading examples, see: (a) Mancuso, R.; Mehta, S.; Gabriele, B.; Salerno, G.; Jenks, W. S.; Larock, R. C. *J. Org. Chem.* **2010**, *75*, 897. (b) Verma, A. K.; Aggarwal, T.; Rustagi, V.; Larock, R. C. *Chem Comm.* **2010**, *46*, 4064. (c) Mehta, S.; Larock, R. C. *J. Org. Chem*. **2010**, *75*, 1652. (d) Cho, C.-H.; Neuenswander, B.; Larock, R. C. *J. Comb. Chem*. **2010**, *12*, 278.
28. Bondi, A. *J. Phys. Chem*. **1964**, *68*, 441.
29. Layek, M.; Kumar, Y. S.; Islam, A.; Karavarapu, R.; Sengupta, A.; Halder, D.; Mukkanti, K.; Pal, M. *Med. Chem. Commun*. **2011**, *2*, 478.
30. (a) Michan, S.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1; (b) Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D. *Annu. Rev. Biochem*. **2006**, *75*, 435; (c) Saunders, L. R.; Verdin, E. *Oncogene* **2007**, *26*, 5489; (d) Zhao, W.; Kruse, J. P.; Tang, Y.; Jung, S. Y.; Qin, J.; Gu, W. *Nature* **2008**, *451*, 587.

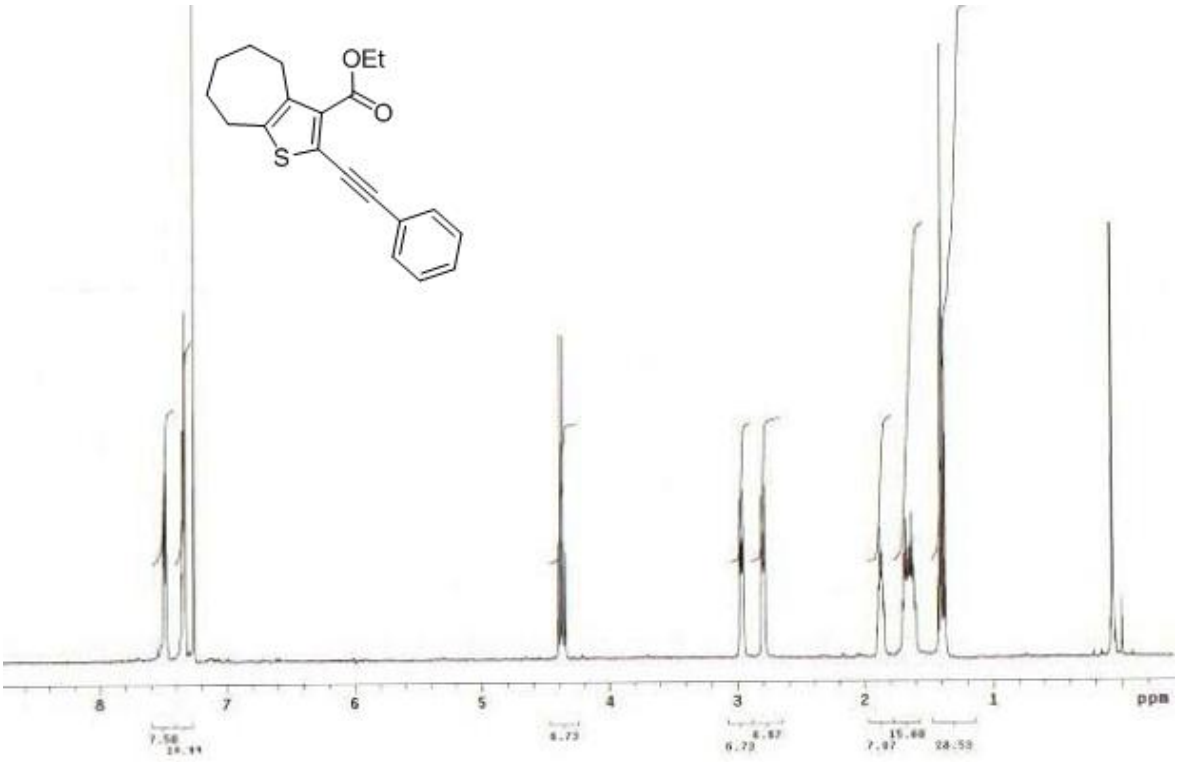
Appendix

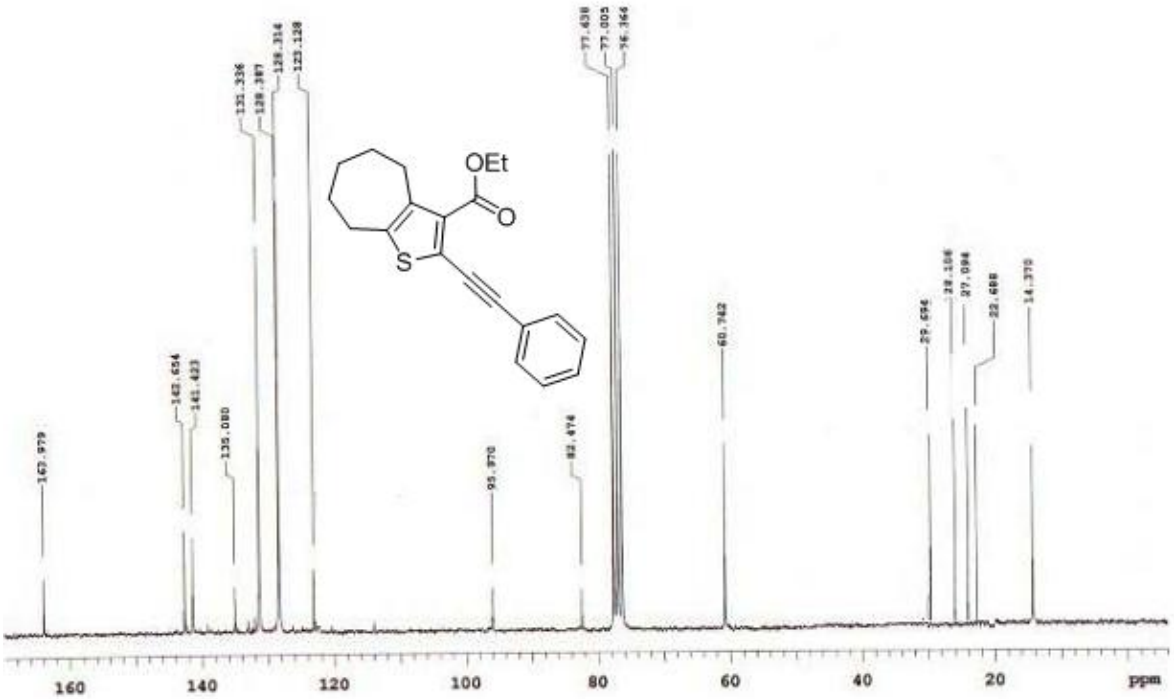
^{1}H NMR (400 MHz, $CDCl_3$)

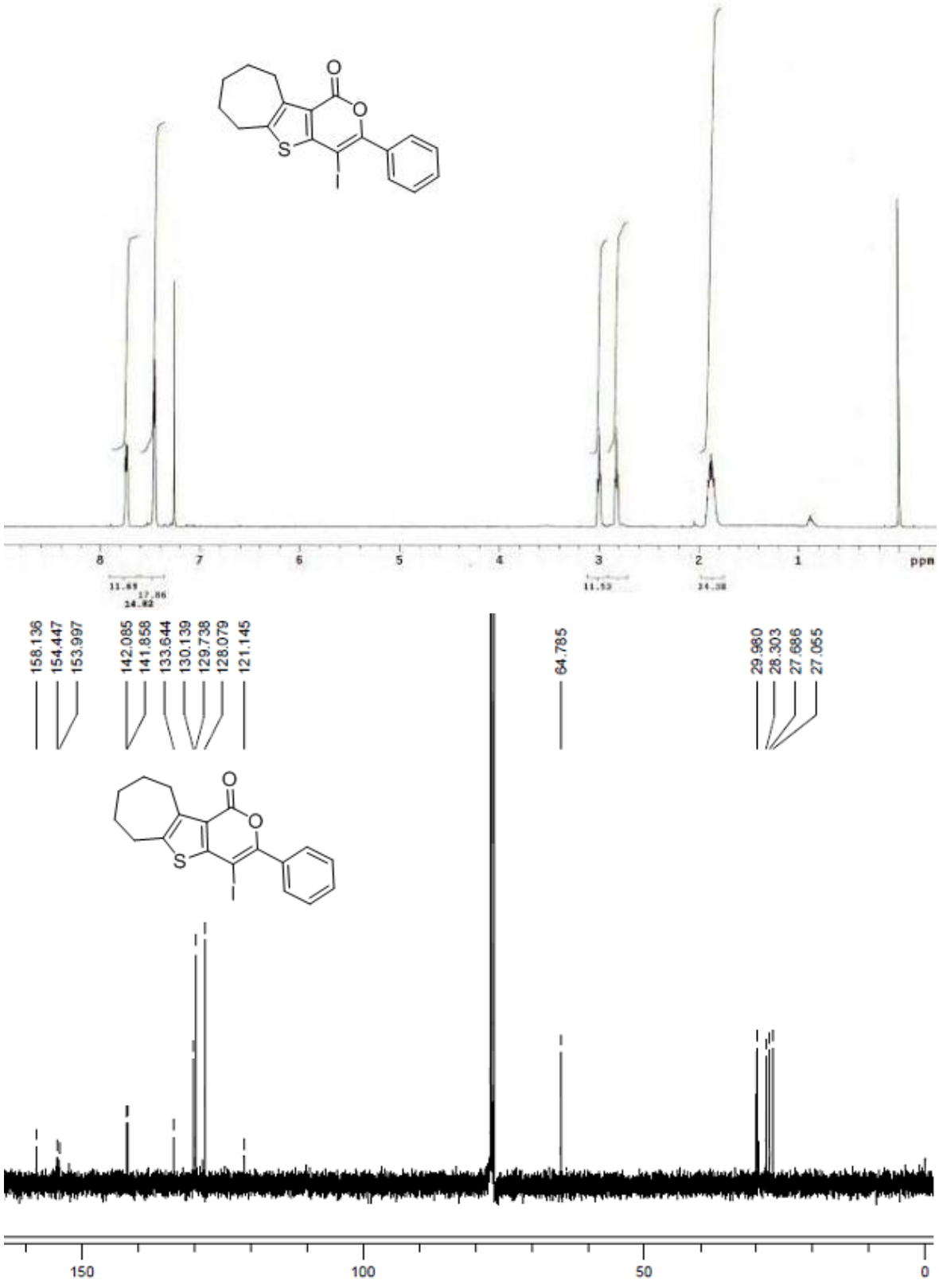


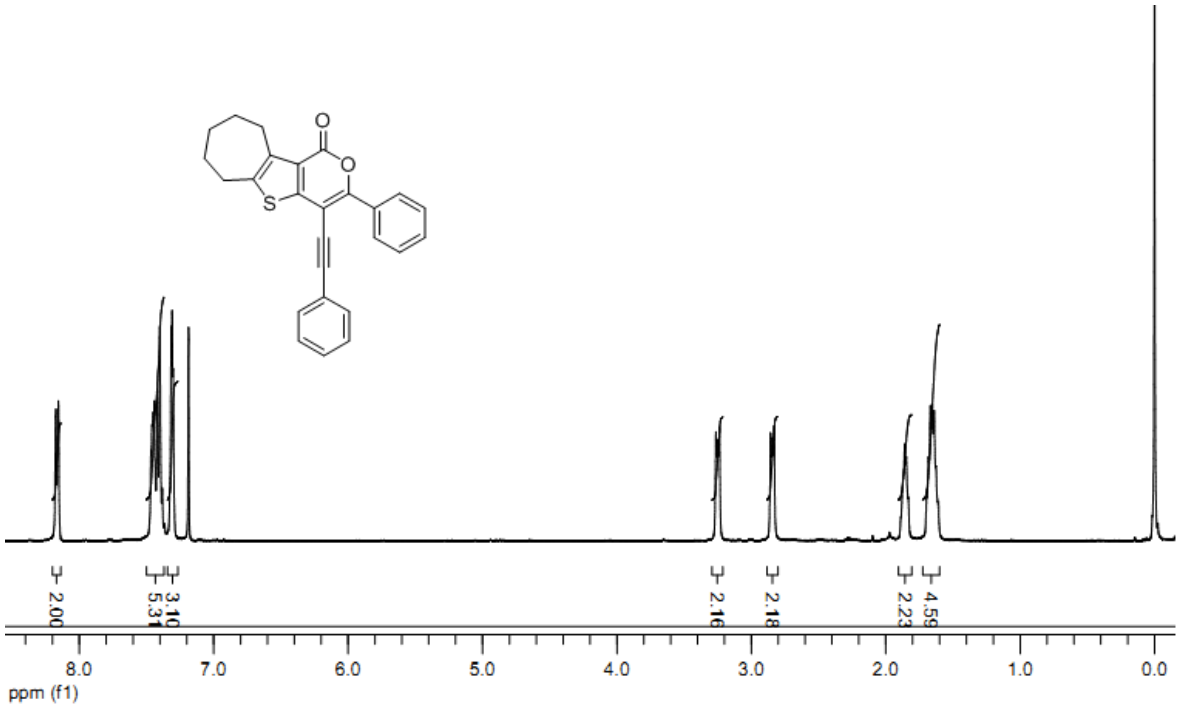

OEt
O
S
I
1.92
2.03
1.97
2.07
4.16
3.00
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

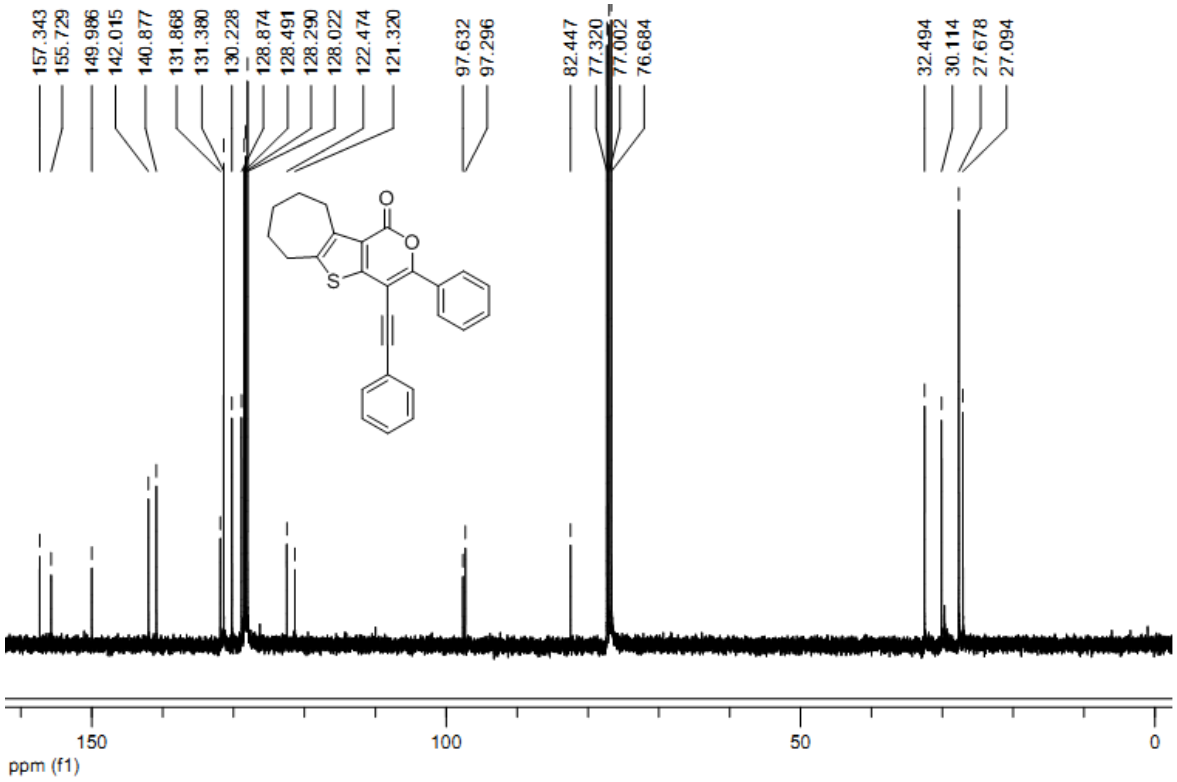

164.640
147.142
141.518
131.915
72.763
61.052
29.884
29.700
28.609
27.757
14.279
OEt
O
S
I
150
100
50
0

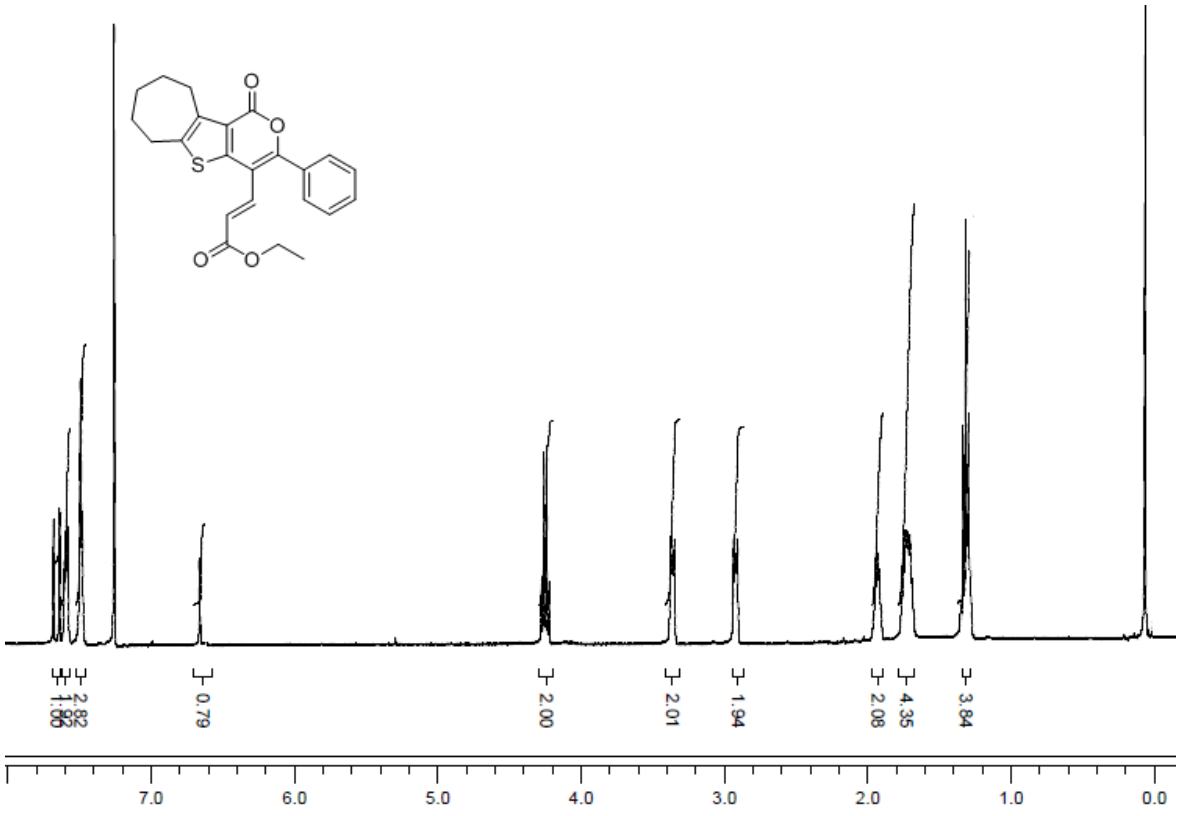

OEt
O
S
8
7
6
5
4
3
2
1
ppm

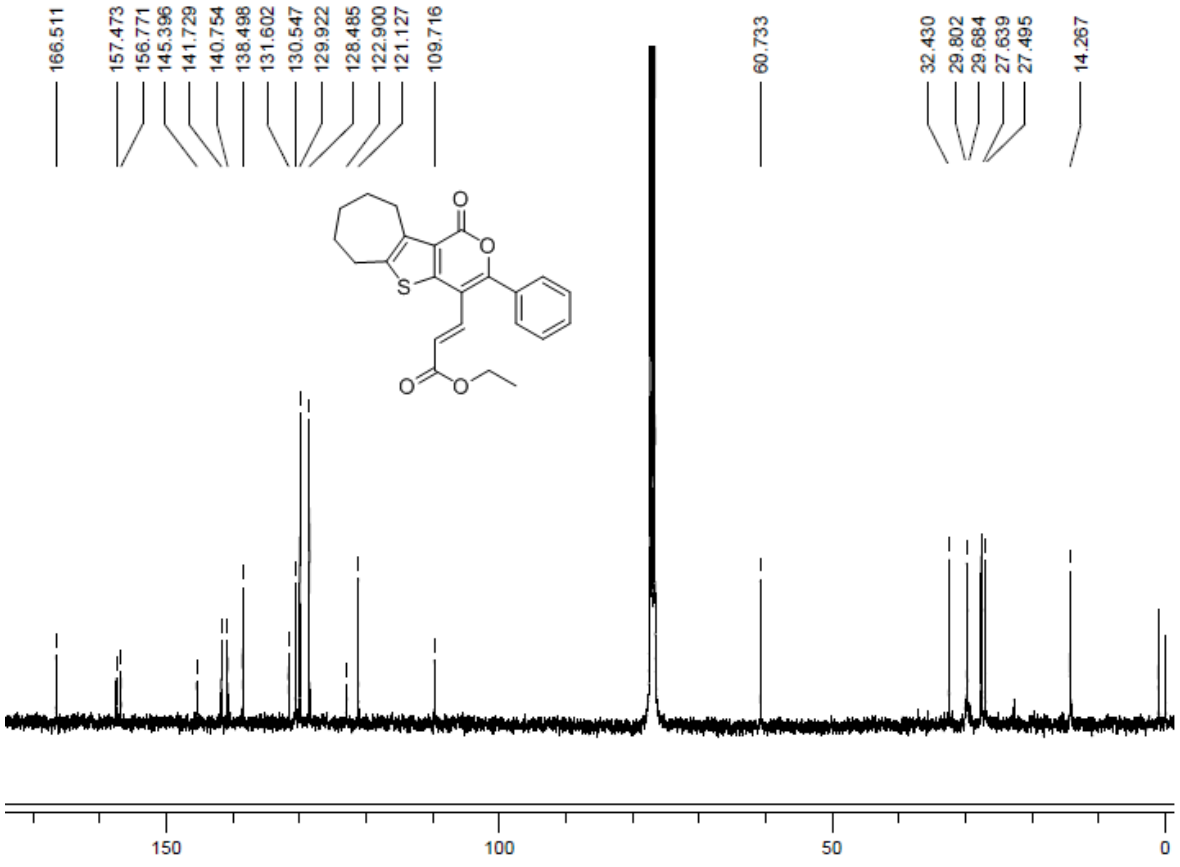

OEt
O
S
160
140
120
100
80
60
40
20
ppm

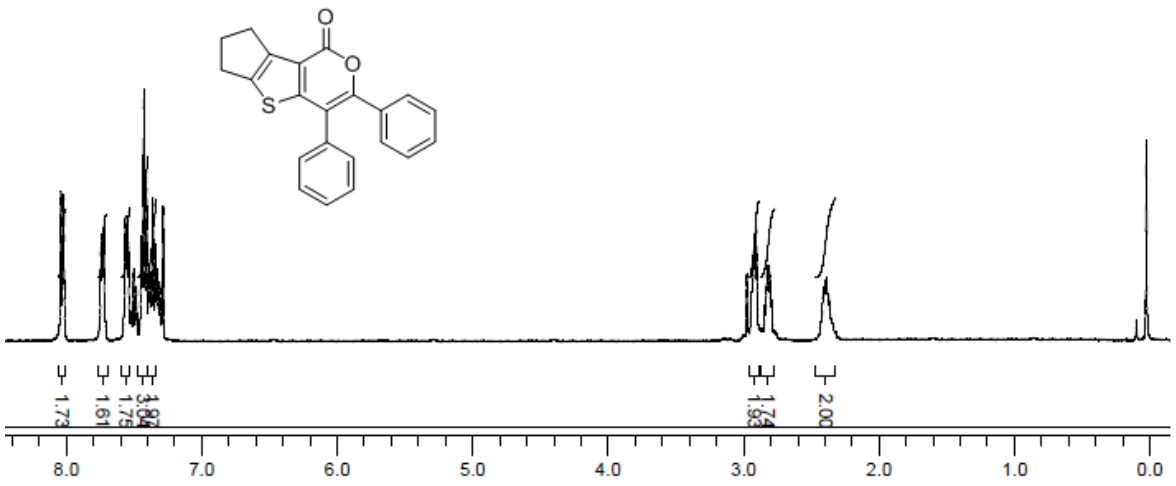

O
O
S
I
8
7
6
5
4
3
2
1
ppm
158.136
154.447
153.997
142.085
141.858
133.644
130.139
129.738
128.079
121.145
64.785
29.980
28.303
27.686
27.055
O
O
S
I
150
100
50
0

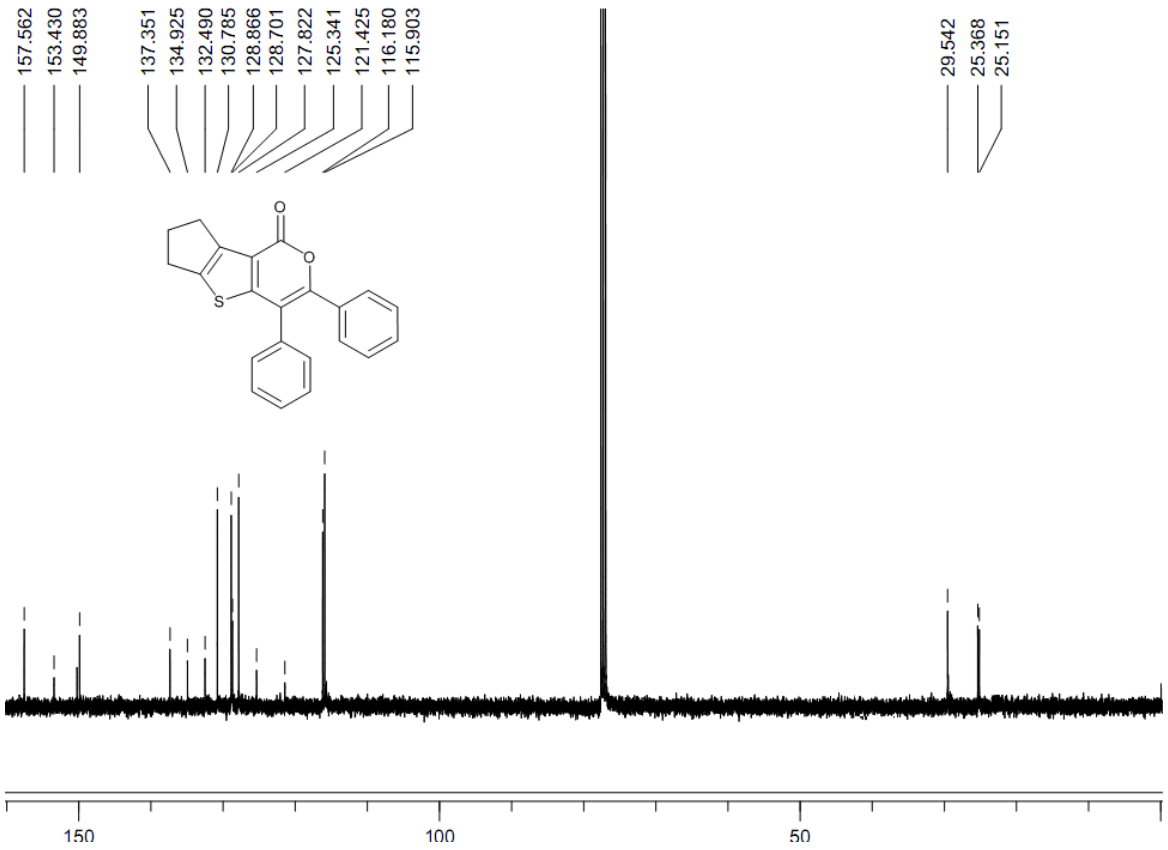

O
O
S
2.00
5.31
2.16
2.18
2.23
4.59
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

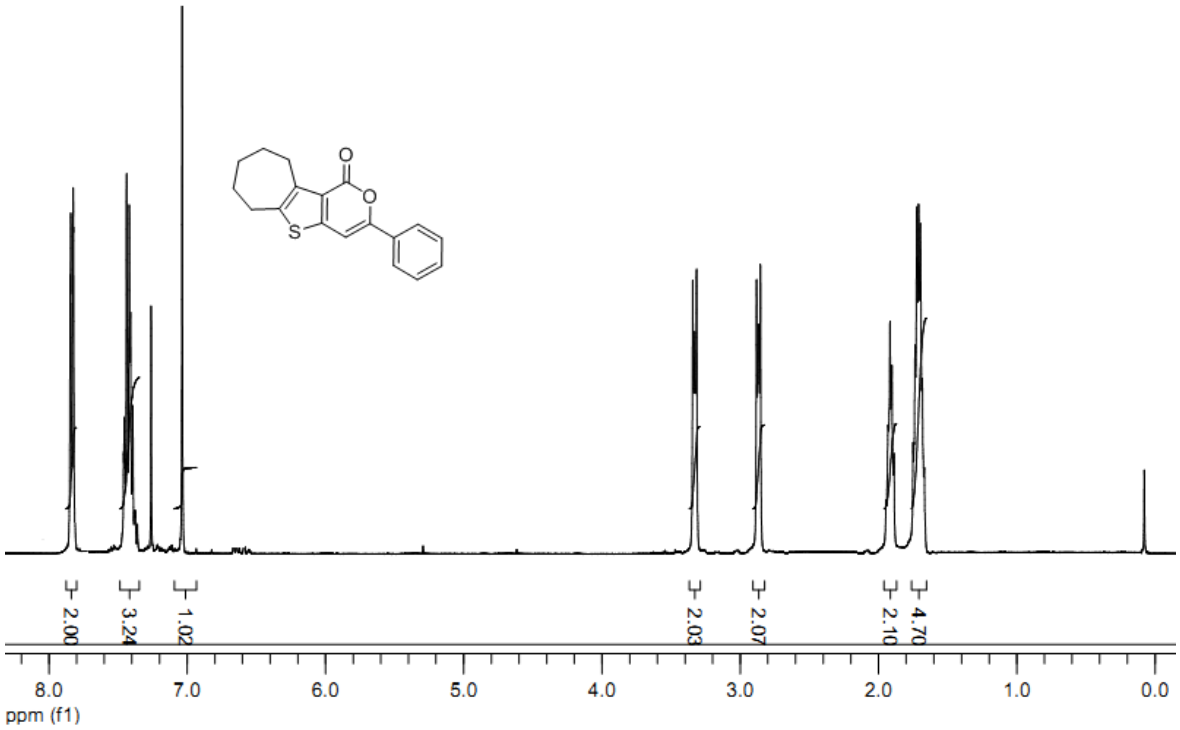

157.343
155.729
149.986
142.015
140.877
131.868
131.380
130.228
128.874
128.491
128.290
128.022
122.474
121.320
97.632
97.296
82.447
77.320
77.002
76.684
32.494
30.114
27.678
27.094
O
O
S
150
100
50
0
ppm (f1)

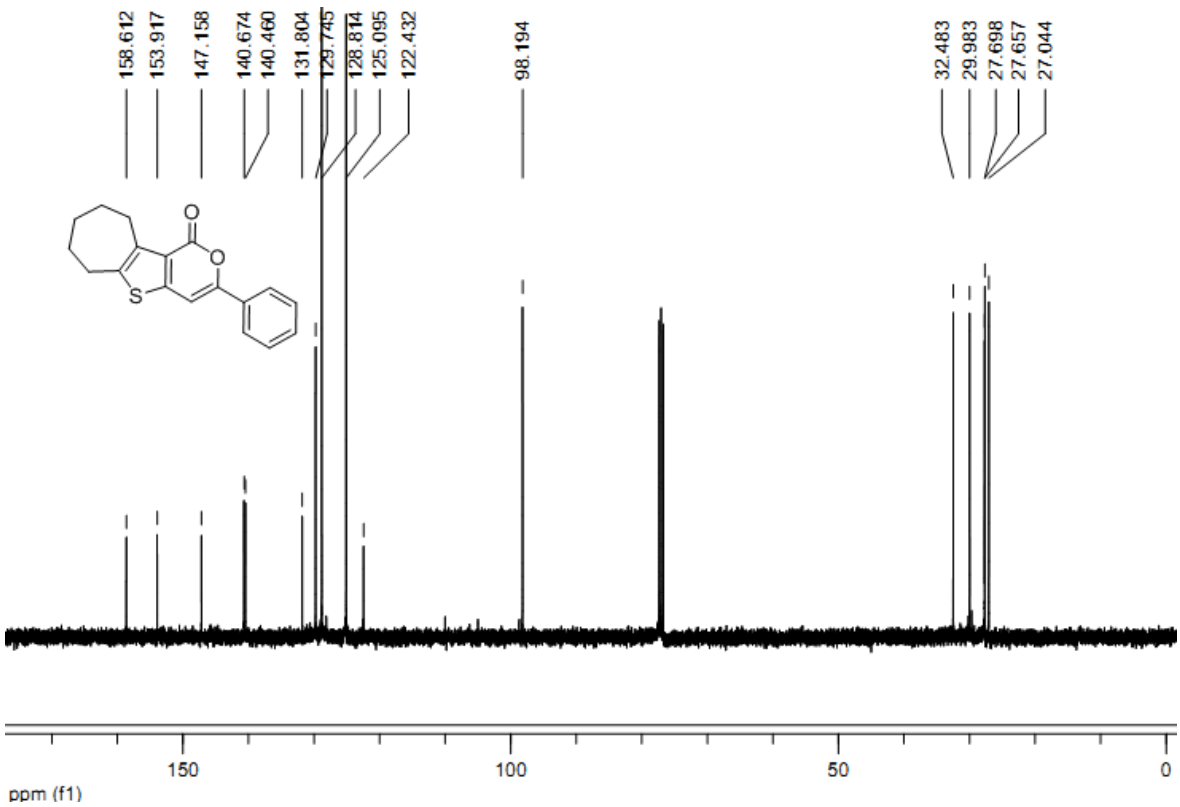

0.79
2.00
2.01
1.94
2.08
4.35
3.84
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


166.511
157.473
156.771
145.396
141.729
140.754
138.498
131.602
130.547
129.922
128.485
122.900
121.127
109.716
60.733
32.430
29.802
29.684
27.639
27.495
14.267
150
100
50
0


O
O
S
1.73
1.61
1.75
1.87
1.84
1.93
1.74
2.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


157.562
153.430
149.883
137.351
134.925
132.490
130.785
128.866
128.701
127.822
125.341
121.425
116.180
115.903
29.542
25.368
25.151
O
O
S
150
100
50

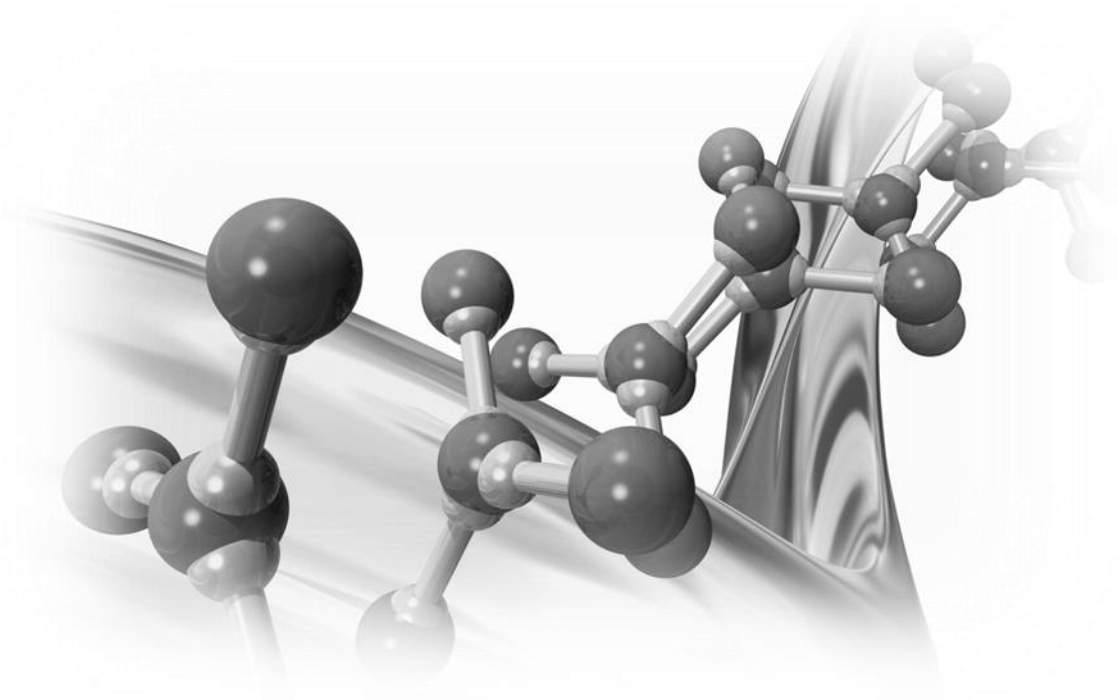

O
O
S
2.00
3.24
1.02
2.03
2.07
2.10
4.70
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


158.612
153.917
147.158
140.674
140.460
131.804
129.745
128.814
125.095
122.432
98.194
32.483
29.983
27.698
27.657
27.044
O
O
S
150
100
50
0
ppm (f1)

CHAPTER 2

CHAPTER 2B

Design and synthesis of novel pyrano [4,3-*b*]pyran-5(4*H*)-ones as potential inhibitors of sirtuins



2B.1. Introduction

Pyranocoumarins are an important class of heterocycles in which pyran is fused with coumarins are present in natural products as well as synthetic molecules, showing a broad spectrum of biological activities such as insecticidal, antifungal, anticancer, anti-HIV, antibacterial activities and anti-inflammatory.[1] In the same way pyranopyrones are another class of heterocyclic compounds where pyran fused with 2-pyrone ring a cyclic unsaturated six membered ester[2] **1** is found in microbial, bacterial, plant, insect and animal systems.[3] The 2-pyrones motifs can be found in simple to complex molecules such as Radicinin **2** which shows inhibitory activity towards the growth of some Gram positive bacteria, such as *Staphylococcus aureus* and *Clostridium* species. Camptothecin **3** is a pentacyclic alkaloid which is novel alkaloidal leukemia and tumor inhibitor [4] due to the inhibition of topoisomerase I, isolated from chinese tree *Camptotheca acuminate*. The natural product withaferin A **4** exhibits potent antitumor activity and other diverse pharmacological activities.[5] Another complex systems, such as bufadienolides **5** are some important natural products containing 2-pyrone sub unit.[6] The steroidal 2-pyrones poaefusarin **6** one of the toxins involved in alimentary toxic aleukia and sporofusarin **7** acute mammalian toxicity including skin inflammation. And also 2-Pyrones demonstrate a whole spectrum of bioactivity and have been shown to be neurotoxic, antifungal, antibiotic, cytotoxic and phytotoxic.[7]

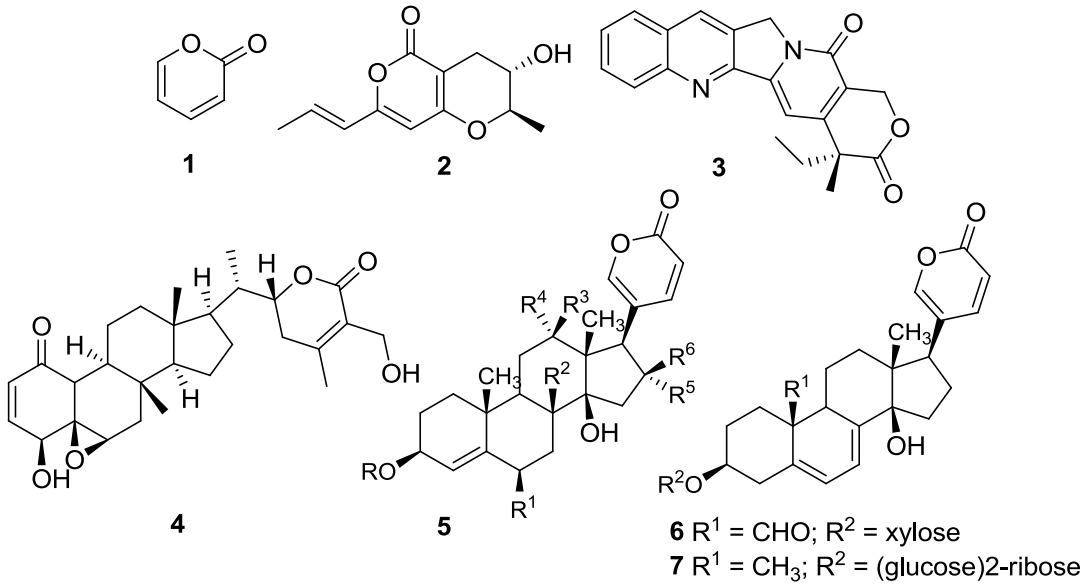
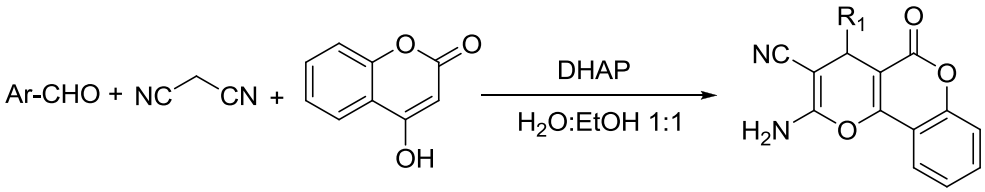


Figure 1. Natural products containing the 2-pyrone subunit.

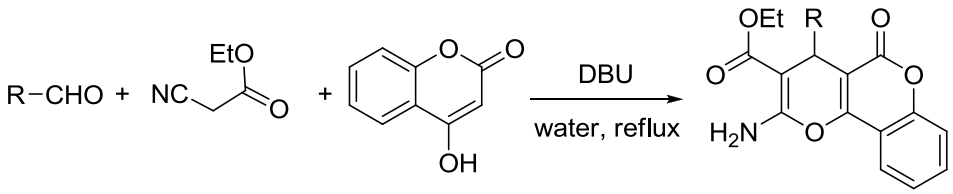
2B.2. Previous work

In 2007, Balalaie *et al.* showed that, diammonium hydrogen phosphate DAHP, $(NH_4)_2HPO_4$ efficiently catalyzes the three-component reaction of an aromatic aldehyde, malononitrile and 4-hydroxycoumarin in aqueous media under mild conditions at room temperature in one pot, to afford the corresponding dihydropyrano[*c*]chromenes.[8]



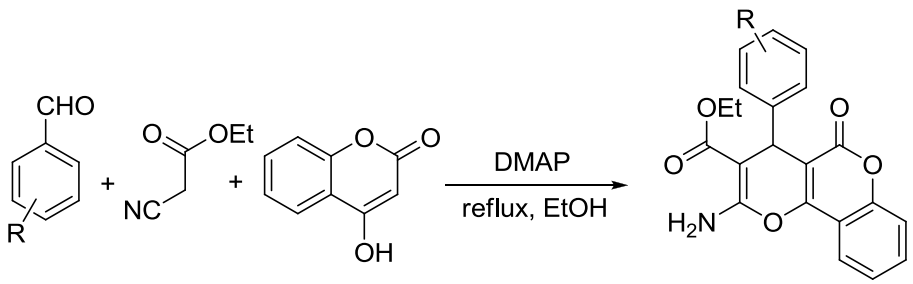
Scheme 1. Synthesis of 2-amino-4-aryl-3-cyano-5-oxo-4*H*, 5*H*-pyrano-[3,2-*c*]chromenes

Khurana and his group in 2010 reported DBU catalyzed one-pot synthesis of 3,4-dihydro pyrano[3,2-*c*]chromenes from aldehydes, ethyl cyanocacetate, and 4-hydroxycoumarin in water under reflux.[9]



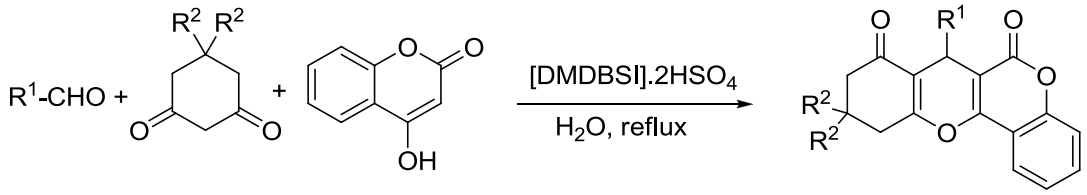
Scheme 2. DBU catalyzed synthesis of substituted dihydropyrano[3,2-*c*] chromene.

In 2011, Khan *et al.* devised the one-pot three-component reaction for the synthesis of pyran annulated heterocycles is reported by condensing aromatic aldehydes, ethyl cyanoacetate and C–H activated acidic compounds in the presence of catalytic amount of 4-(dimethylamino)pyridine (DMAP) in ethanol.[10]



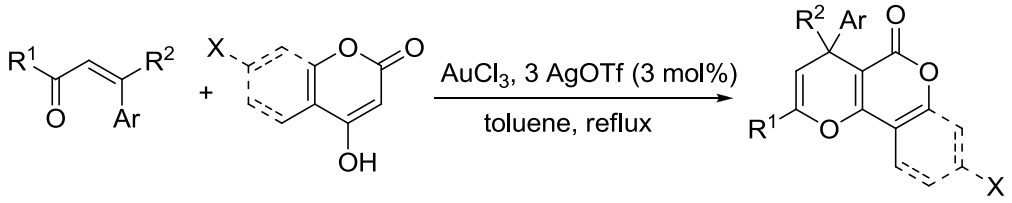
Scheme 3. Synthesis of dihydropyrano[3,2-*c*]chromenes

In the same year 2011, Su *et al.* has developed, three component reactions of 4-hydroxycoumarin, aldehydes, and cyclic 1,3-dicarbonyl compounds were prompted by novel sulfonic acid functionalized ionic liquids 1,3-dimethyl-2-oxo-1,3-b is(4-sulfobutyl)imidazolidine-1,3-diium hydrogen sulfate ([DMDBSI].2HSO_4) in water.[11]



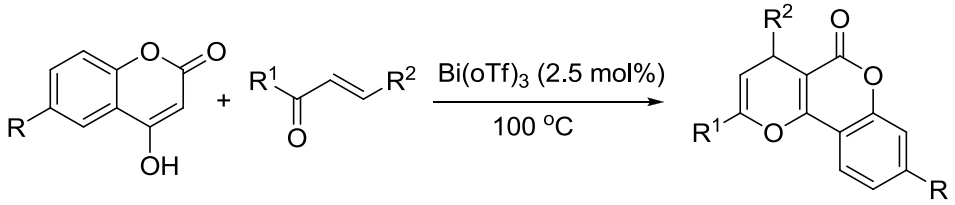
Scheme 4. Synthesis of 10,11-dihydrochromeno[4,3-*b*]chromene-6,8(7*H*,9*H*)-dione derivatives

In 2011 Liu *et al.* developed regioselective tandem conjugate addition /annulation of 4-hydroxy coumarins with α ,β -unsaturated ketones catalyzed by $AuCl_3$/3AgOTf, which furnished various functionalized pyrano-[3,2-*c*]coumarins.[12]



Scheme 5. Gold(III)-catalyzed synthesis of pyrano[3,2- *c*]coumarins

Bezuidenhoudt *et al.* in 2013 described $Bi(OTf)_3$ catalyzed tandem addition/annulation reaction is in order to synthesize pyrano[3,2-*c*]coumarins under solvent free conditions.[13]



Scheme 6. Synthesis of pyrano[3,2-*c*]coumarins by the reaction of chalcones and 4-hydroxycoumarins.

2B.3. Present work

α-pyrone and isocoumarin useful intermediates in the synthesis of a variety of important hetero and carbocyclic molecules and have high importance especially in drug discovery and pharmaceutical research because of their broad range of biological activities.[14] A good number of have been reported for the synthesis of isocoumarins in the literature that discloses a wide range of isocoumarin based small molecules of pharmacological importance including the clinical candidate NM-3,[15] (**A**, Fig. 2) analogue of natural product cytogenin inhibited the growth of human endothelial cells in culture and tumor angiogenesis in human tumor xenograft models. NM-3 was administered in combination with other chemotherapeutic agents and also after its discovery angiogenesis has become an important area of pharmaceutical research. Another important target for cancer research is Sirtuins (NAD^+ dependent class III histone deacetylases).[16] Splitomicin (**B**, Fig. 2) a pyrone containing molecule is micromolar inhibitor of yeast Sir2 (a homologue of Human SIRT1). In one of the study it was proved that hydrolytically unstable lactone ring of splitomicin is critically important for its activity.[17] Recently, we have reported 1,8-dioxo-octahydroxanthenes[18] (**C**, Fig. 2) and thieno[3,2-*c*]pyran-4-one based small molecules[19] (**D**, Fig. 2) as potential anticancer agents. While most of them showed promising growth inhibition when tested against a number of cancer cells. To understand the inhibition mechanism, one of the analogues of compound **D** tested against MDA-MB 231 (breast cancer cell lines) was tested for its inhibition property of sirtuin enzyme showed moderate inhibition. With this encouraging result, we intended to design a scaffold with all the structural features of previously reported molecules. As part of our ongoing efforts on the identification of novel anti cancer agents and their mode of action against sirtuins we became interested in evaluating the sirtuin inhibitory properties of small molecules based on pyrano[4,3-*b*]pyran-5(4*H*)-one (**E**, Fig. 2). The design of **E** was performed by incorporating some of the structural features of **A**, **B**, **C** and **D** i.e. substituted pyrone ring, insertion of oxygen atom in a single molecular entity. We envisioned that compounds based on **E** (pyrano[4,3-*b*]pyran-5(4*H*)-one) would show anticancer properties *via* inhibition of sirtuins.

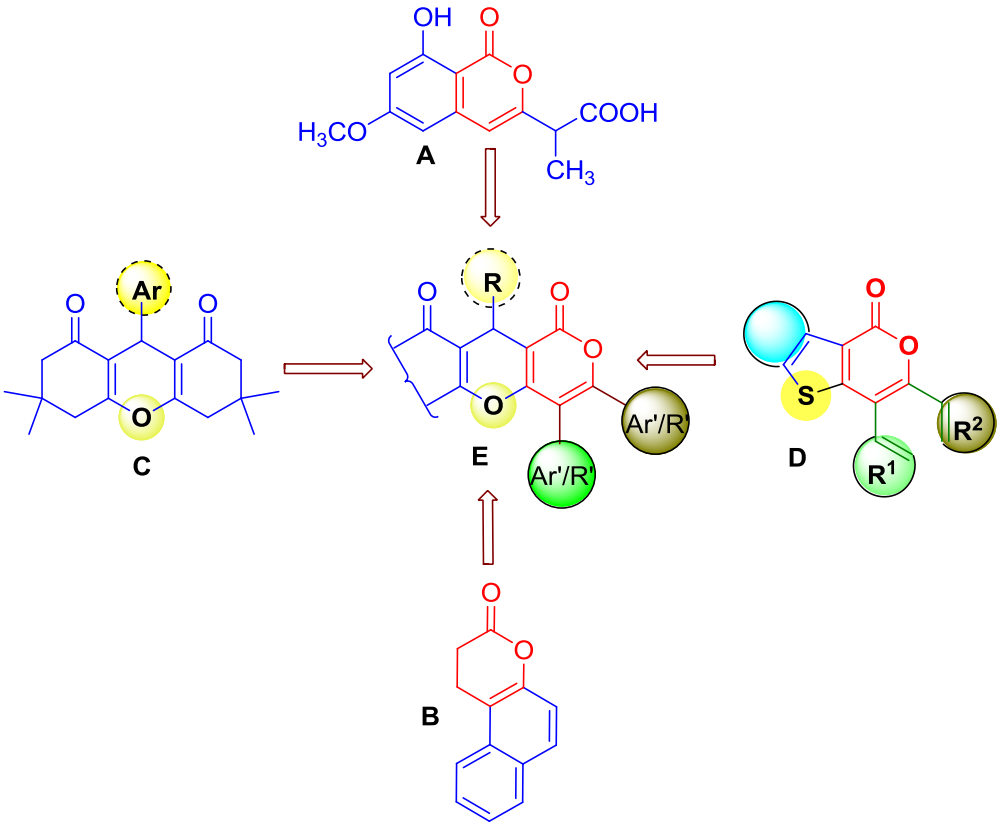
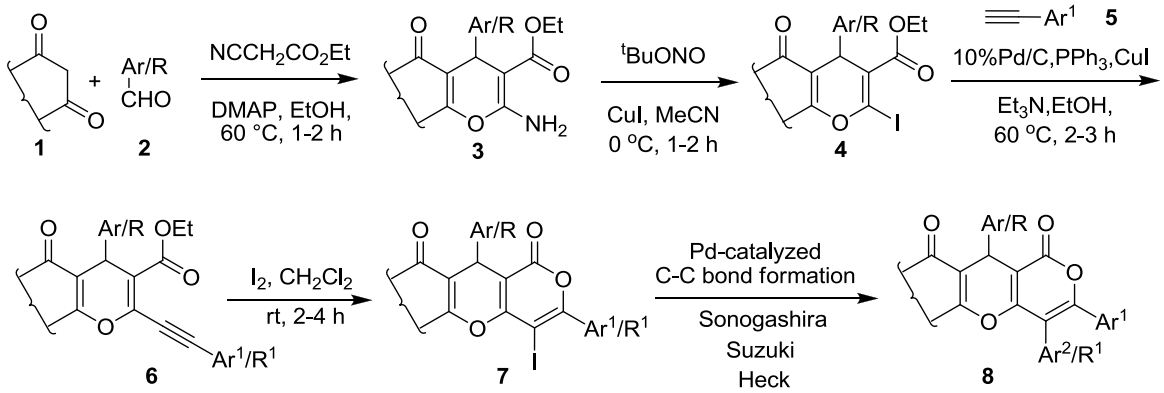


Figure 2. Design of novel pyrano[4,3-*b*]pyran-5(4*H*)-one based inhibitors (**E**) of sirtuins

The members of the Sir2 family, or sirtuins, have gained tremendous attention in biomedical research over the past decade. The first member of the family, yeast Sir2p, was originally identified in *Saccharomyces cerevisiae*. Sirtuins are class III NAD^+ dependent protein deacetylases[20] and mono-[ADP-ribosyl] transferases that catalyze the removal of acetyl group to generate deacetylated proteins, nicotinamide, and *o*-acetyl-ADP-ribose. They play important role in diverse biological processes such as transcriptional silencing, regulation of apoptosis by deacetylation of p53, fatty acid metabolism, cell cycle regulation, and aging.[21] Among the seven human sirtuins e.g. SIRT1–7, the SIRT1 has been studied well which has several substrates such as p53, Ku70, NF-κB, forkhead proteins etc.[22] Studies have shown that sirtuins are up-regulated in many cancers and inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. Thus, inhibition of sirtuins is being considered as a new approach for the discovery of novel anticancer drugs. While a number of inhibitors, e.g. nicotinamide, sirtinol, splitomicin, cambinol, tenovins, and EX527 have been reported none except EX527 (which is presently undergoing Phase 1a clinical trial for the treatment

of Huntington's disease) have progressed into clinical trials as anticancer agents.[23] This prompted us to explore pyrano[4,3-*b*]pyran-5(4*H*)-one (**C**) as potential and novel inhibitors of sirtuins.

The synthesis of our target compounds **7** and **8** (or **E**, Fig. 2) involved a multi-step sequence consisting of a multi-component reaction (MCR), Sandmeyer type iodination, Sonogashira type coupling followed by iodocyclization and then Pd-mediated various C-C bond forming reactions (Scheme 7).

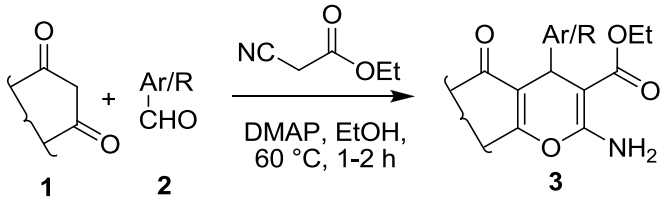


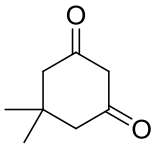
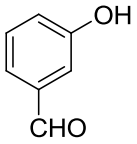
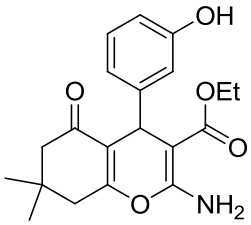
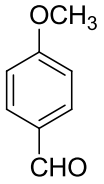
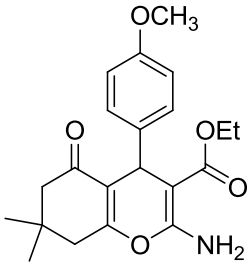
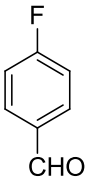
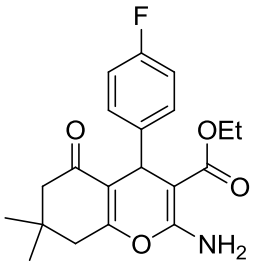
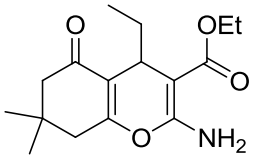
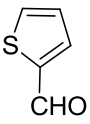
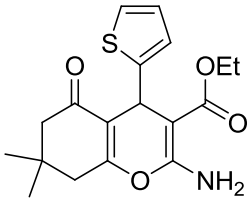
Scheme 7. Synthesis of novel pyrano[4,3-*b*]chromendione and pyrano[4,3-*b*]pyran based small molecules

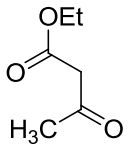
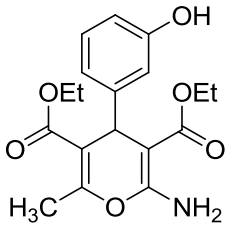
2B.4. Results and Discussion

The starting material **3** was obtained through the MCR of C-H activated compounds (**1**) 1,3-diketones or *β*-keto esters, aldehydes and ethyl cyanoacetate in the presence of catalytic amounts of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in ethanol as shown in Table 1.

Table 1. Synthesis of 2-aminochromene/pyran derivatives (**3**)[a].



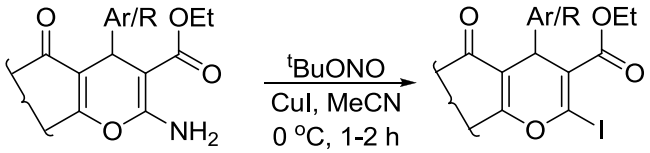
Entry	C-H activated compound (**1**)	Aldehyde (**2**)	Time (h)	2-aminochomene/ pyran derivative (**3**)	% of Yield [b]
1	 **1a**	 **2a**	1	 **3a**	78
2	**1a**	 **2b**	1	 **3b**	72
3	**1a**	 **2c**	2	 **3c**	66
4	**1a**	 **2d**	2	 **3d**	70
5	**1a**	 **2e**	1	 **3e**	80

6	 **1b**	**2a**	 **3f**	62

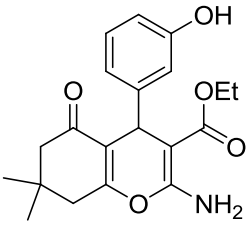
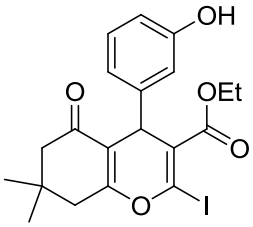
[a]All the reactions were carried out by taking aldehyde (**2**) (1 mmol), ethyl cyanoacetate or ethyl acetoacetate (**1**) (1 mmol) and C-H activated compound (1 mmol) in ethanol (3 mL) was added the DMAP (0.2 mmol) at 60°C. [b] Isolated yields.

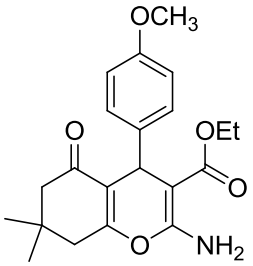
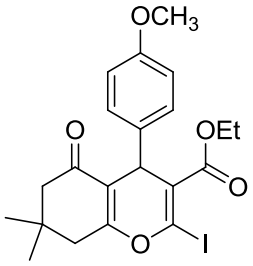
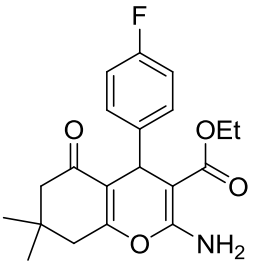
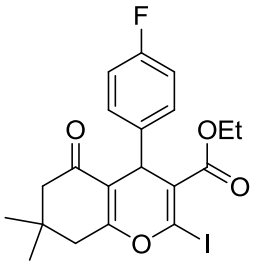
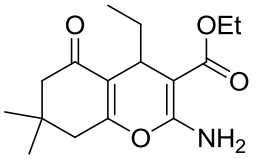
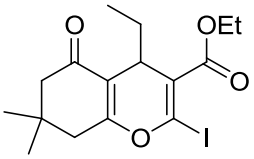
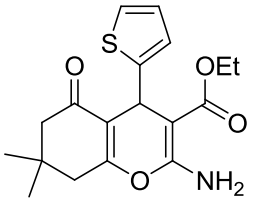
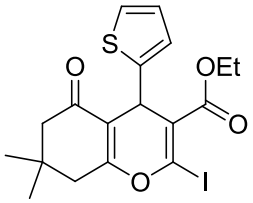
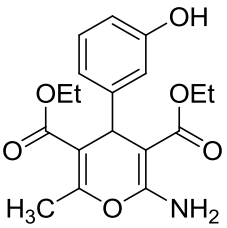
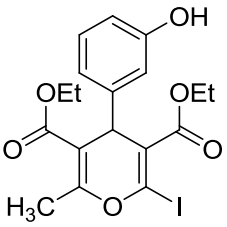
Compound **3** was then converted to the corresponding 2-iodochromene/pyran derivative **4** under a modified Sandmeyer conditions by convenient and rapid one-pot method was carried out which involves the sequential diazotization-iodination of 2-aminochromene/pyran with tertiary butyl nitrite, copper iodide, in acetonitrile the results of which are summarized in Table 2.

Table 2. Preparation of 2-iodo substituted chromeme/pyran-3-carboxylate derivatives (**4**).[a]



3 **4**

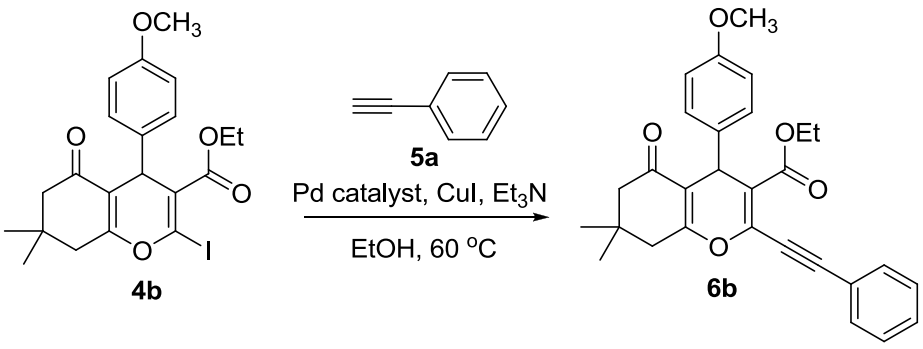
Entry	2-aminochromene/ pyran derivatives (**2**)	Time (h)	Product (**3**)	%Yield
1	 **3a**	1	 **4a**	82

2	 **3b**		1	 **4b**	88
3	 **3c**		2	 **4c**	85
4	 **3d**		1	 **4d**	82
5	 **3e**		1	 **4e**	75
6	 **3f**		2	 **4f**	70

[a]All the reactions were carried out using the amine **3**, [t]BuONO and CuI in MeCN at 0 ºC

The iodocompound **4** was then coupled with terminal alkynes **5** under Pd-Cu catalysis to give the desired internal alkyne **5**. Initially, we examined the coupling of **3b** with a terminal alkyne **4a** under two reaction conditions (Table 3). Since the use of Pd/C has been explored as an inexpensive, easily separable and recyclable catalyst for the alkynylation of aryl and heteroaryl halides[9] hence the initial coupling reaction of **3b** with **4a** was carried out in the presence of 10%Pd/C, PPh_3, CuI and Et_3N in ethanol. To our satisfaction, the desired product **5b** was isolated in 85% yield (entry 1, Table 3). Similarly, the use of $Pd(PPh_3)_2Cl_2$ in the presence of CuI was also found to be effective though the product yield was marginally low (entry 2, Table 3). Thus, a series of internal alkyne **5** were prepared in good to acceptable yields (Table 4) using either 10%Pd/C-PPh_3-CuI or $Pd(PPh_3)_2Cl_2$-CuI as catalyst system.

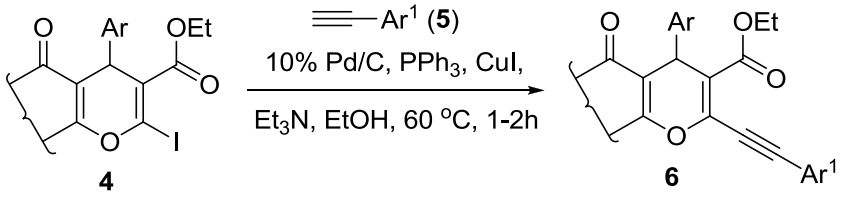
Table 3. Effect of reaction conditions on Sonogashira coupling of **4a** with phenylacetylene **5a**.[a]

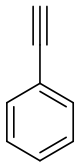
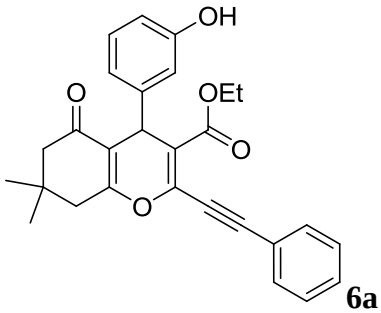
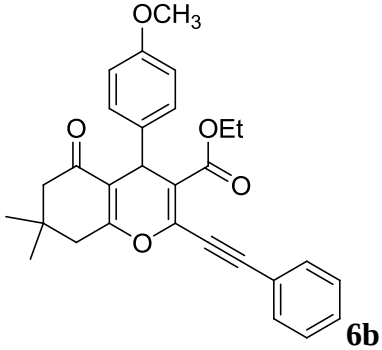
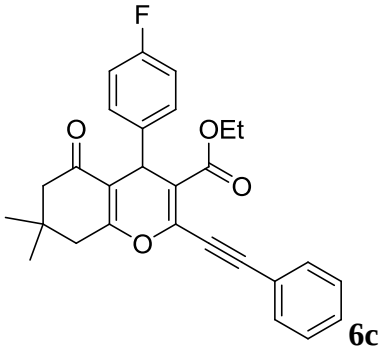


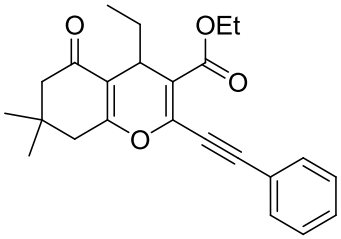
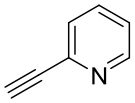
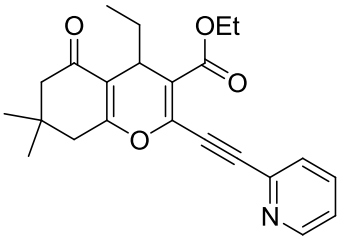
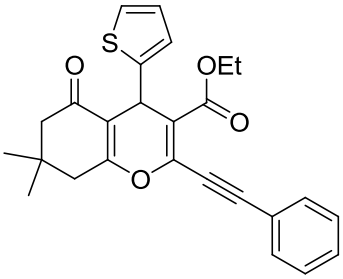
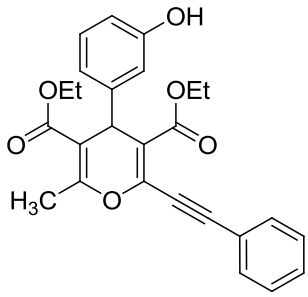
Entry	Pd-catalysts	Base	Time (h)	%Yield[b]
1.	10%Pd/C-PPh_3	Et_3N	2	85
2.	$Pd(PPh_3)_2Cl_2$	Et_3N	5	73

[a]All the reactions were carried out using compound **4b** (0.1 g, 0.2074 mmol), phenyl acetylene **5a** (0.02 mL, 0.2074 mmol), CuI (0.002 g, 0.0082 mmol), and Et_3N (0.85 mL, 0.6222 mmol), either 10% Pd/C (0.0020 mmol) & PPh_3 (0.001 g, 0.0040 mmol), or $Pd(PPh_3)_2Cl_2$, (0.0020 mmol) in ethanol (3mL) under nitrogen. [b]Isolated yield.

Table 4. Pd/C-mediated preparation of 2-alkynyl substituted 5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate ester derivatives (**5**).[a]



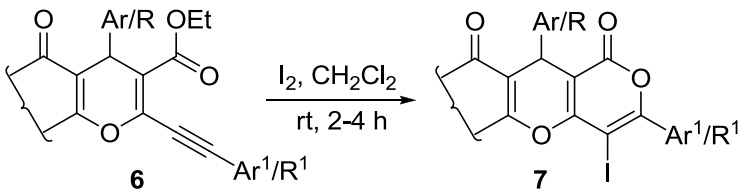
Entry	Compound (**3**)	Terminal alkyne (**4**)	Time (h)	Alkynyl ester (**6**)	Yield[c] (%)
1	**4a**	 **5a**	2	 **6a**	81
2	**4b**	**5a**	2	 **6b**	85
3	**4c**	**5a**	1	 **6c**	73

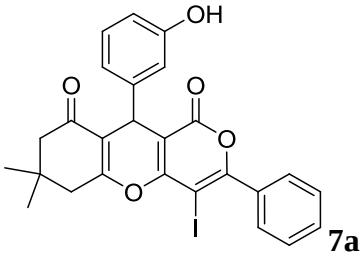
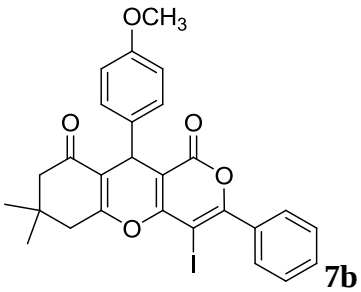
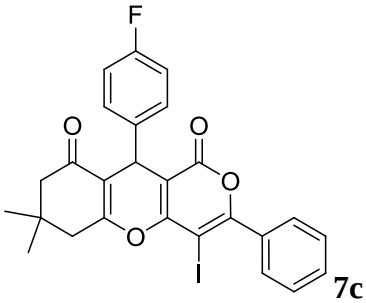
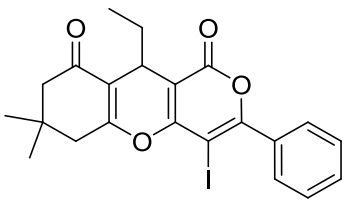
3	**4d**	**5a**	2	 **6d**	77
4	**4d**	 **5b**	2	 **6e**[b]	63[b]
5	**4e**	**5a**	1	 **6f**	82
6	**4f**	**5a**	1	 **6g**	85

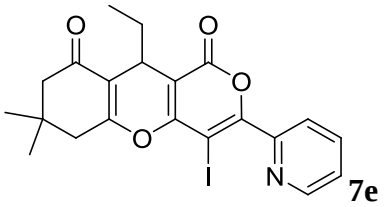
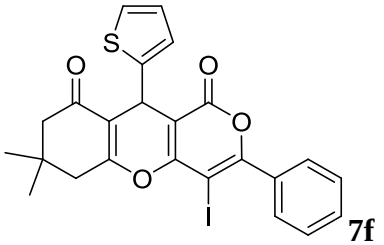
[a]Reactions were carried out compound **4** (0.2074 mmol), terminal alkyne (**5**) (0.2074 mmol), 10% Pd/C (0.0020 mmol), PPh_3 (0.0040 mmol), CuI (0.0082 mmol), and Et_3N (0.6222 mmol) in EtOH (5.0 mL) at 60 ºC. [b]$Pd(PPh_3)_2Cl_2$ was used instead of 10% Pd/C-PPh_3. [c] Isolated yield.

The use of iodine as an inexpensive, non-toxic and a readily available catalyst for various organic transformations has recently been well reviewed.[24] In recent years, I_2 or ICl-mediated intramolecular electrophilic cyclization of the alkynes in an *exo-dig* or *endo-dig* fashion has become convenient and economical method for the straightforward access of various iodo heterocycles.[25] The 2-alkynyl substituted chromene/pyran-3-carboxylate ester derivatives (**6**) thus synthesized were then subjected to I_2-mediated electrophilic cyclization which provided the pyrano[4,3-*b*]pyran-5(4*H*)-one core i.e. 4-iodo-7,8-dihydropyrano[4,3-*b*]chromenedione or 8-iodo-4,5-dihydropyrano[4,3-*b*]pyran (**7**) exclusively *via* a regioselective 6-*endo-dig* ring closure (Table 5).

Table 5. I_2 mediated synthesis of 4-iodo-7,8-dihydropyrano[4,3-*b*]chromenedione and 8-iodo-4,5-dihydropyrano[4,3-*b*]pyran (**7**).[a]



Entry	Alkynyl ester (**5**)	Time (h)	Product (**6**)	% of yield[b]
1	**6a**	2	 **7a**	93
2	**6b**	2	 **7b**	97
3	**6c**	2	 **7c**	88
4	**6d**	2	 **7d**	85

5	**6d**	4	 **7e**	53
6	**6e**	2	 **7f**	85

[a]All reactions were carried out by using alkynes **6** (0.9677 mmol) and I_2 (0.9677 mmol) in CH_2Cl_2 (3 mL). [b]Isolated yields.

All the iodo compounds (**7**) prepared were characterized by spectral data and the molecular structure compound **7c** i.e.10-(4-fluorophenyl)-4-iodo-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione was determined unambiguously by X-ray crystallographic analysis.[26]

Single crystals suitable for X-ray diffraction of compound **7c** were grown from EtOH/n-Hexane (3:7). Single crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Oxford XCalibur, Gemini diffractometer equipped with EOS CCD detector at 298 K. Monochromatic Mo Kα radiation (0.71073 Å) was used for the measurements. Absorption corrections using multi ψ-scans were applied. Structure was solved using SHELXS-97, and refined by full-matrix least squares against F^2 using SHELXL-97 software.[27] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms on the C atoms of compound **6d** were introduced on calculated positions and were included in the refinement riding on their respective parent atoms.

Crystal data of **7c:** Molecular formula = $C_{26}H_{20}FIO_4$, Formula weight = 542.32, Monoclinic, *C2/c*, a = 20.215 (8) Å, b = 12.168(2) Å, c = 20.673 (6) Å, V = 4500.00 (2) $Å^3$, T = 298 K, Z = 8, D_c = 1.601 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1}, 20983 reflections were measured with 3842 unique reflections (R_{int} = 0.0298), of which 3842 ($I > 2\sigma(I)$) were used for the structure solution. Final R_1 (w R_2) = 0.0229 (0.0567), 295 parameters.

The final Fourier difference synthesis showed minimum and maximum peaks of -0.434 and +0.301 e.Å^{-3} respectively. Goodness of fit is 1.034.

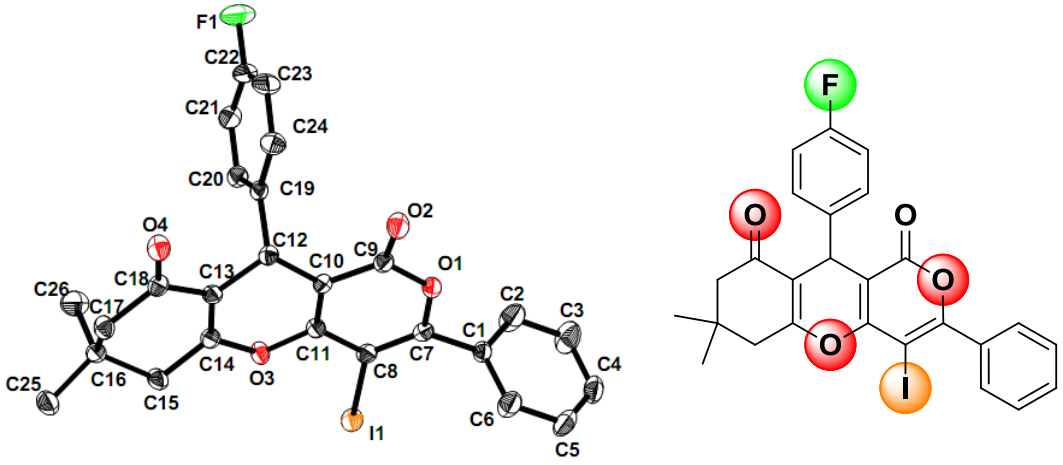
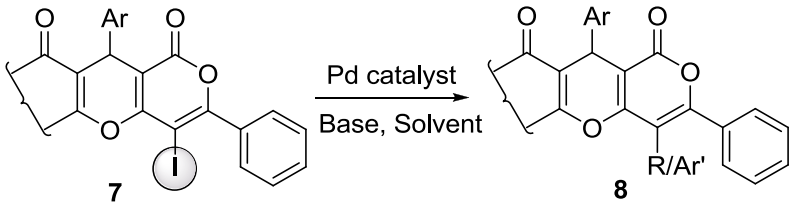
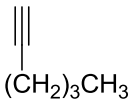
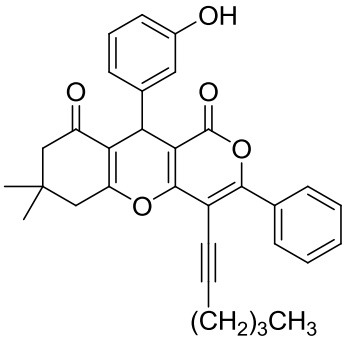
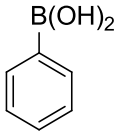
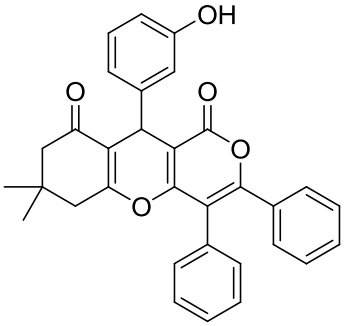
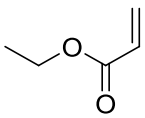
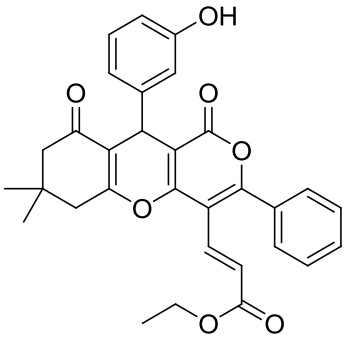


Figure 3. X-ray crystal structure of **7c** (ORTEP diagram). Thermal ellipsoidal diagram is drawn at 30% probability (hydrogen atoms are omitted for clarity).

After synthesizing the iodo derivatives **7** we then focused our attention on further structural elaboration of these compounds by using various Pd-catalyzed C-C bond forming reactions such as Sonogashira, Suzuki, and Heck coupling reactions. Thus, the compound **7a** was reacted with a terminal 1-hexyne in the presence of 10%Pd/C-PPh_3-CuI as catalysts and Et_3N as a base in EtOH to afford the corresponding 4-alkynyl substituted 7,8-dihydropyrano[4,3-*b*]chromene-1,9-(6*H*,10*H*)-dione derivative **8a** (Table 6). Similarly, Suzuki coupling of compound **7a** with pheylboronic acid provided **8b** in good yield (Table 6). The compound **7a** was subjected to Heck reaction condition with ethyl acrylate which afforded the corresponding alkene **8c** (Table 6).

Table 6. Functionalization of compounds (**7**) *via* Pd mediated C-C bond forming Sonogashira[a], Suzuki[b] and Heck[c] reactions.



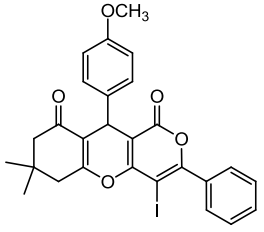
Entry	Iodo compound (**6**)	R = Alkyne/ Acrylate Ar^1 = Aryl boronic acid	Time (h)	Product (**7**)	yield[d] (%)
1	**7a**		2	 **8a**[a]	73
2	**7a**		1	 **8b**[b]	70
3	**7a**		1	 **8c**[c]	71

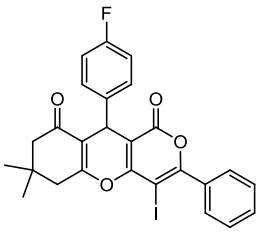
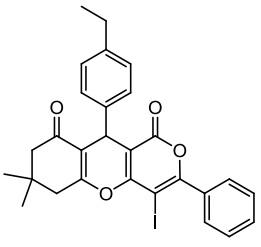
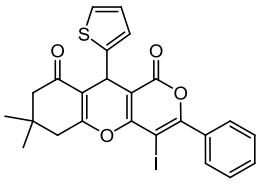
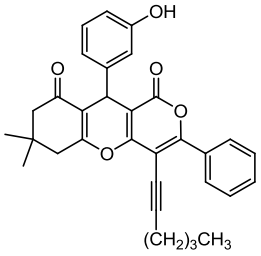
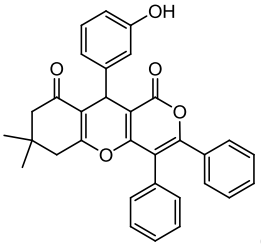
[a]Reaction were carried out using 10% Pd/C (0.0041 mmol), PPh_3 (0.0165 mmol), CuI (0.0041 mmol), Et_3N (0.82 mmol), and a terminal alkyne (0.7348 mmol) in EtOH (3 mL) at 60 °C. [b]Reactions were carried out by using $Pd(OAc)_2$ (0.0041 mmol, 5 mol %), K_2CO_3 (0.82 mmol) an appropriate boronic acid (0.61 mmol) in DMF (3 mL) at 80 °C. [c]Reaction was carried out by using $Pd(OAc)_2$ (0.0049 mmol, 5 mol %), K_2CO_3 (0.82 mmol), ethyl acrylate (0.82 mmol) in DMF (3 mL) at 80 °C. [d]Isolated yields.

2B.5. Pharmacology

After completing the synthesis of designed target molecules (**7** and **8**) we focused our interest towards sirtuin inhibition properties of some of the compounds. As he identification of novel inhibitors of sirtuin being the goal of the present work and due to our continuing interest in this area[28] we tested some of the synthesized compounds (**7** and **8**) *in vitro* by using yeast cell based reporter silencing assay. Compounds were tested without separating their individual steroisomers at the concentration of 50 µM for their ability to inhibit yeast sirtuin family NAD-dependent histone deacetylase (HDAC) sir 2 protein (a yeast homologue of mammalian SIRT1). Splitomicin,[29] a known inhibitor of sirtuin, was used as a reference compound. In this assay a yeast strain (TEL::URA3 strain (MATα ura3-52 lys2-801 ade2-101 trpΔ63 his3Δ200 leu3Δ200 leu2-Δ1 TEL adh4::URA) was used in which, a reporter gene URA3 was inserted in the silenced telomeric region where it is silenced by yeast Sir2 protein. Inhibition of sir2 protein by an inhibitor would allow the URA3 gene to be expressed thereby resulting in death of the yeast cell in presence of 5-FOA through the formation of toxic 5-fluorouracil. The results of our *in vitro* assay are summarized in Table 7. Among the compounds tested, **7b**, **7c**, **7d** and **7f** (entry 1, 2, 3, and 4, Table 7) showed inhibitory activities 32.3%, 49.5%, 43.9% and 43.2% against yeast sir2 at 50 µM respectively. In the case of compound **7c**, we observed moderate solubility. For this reason we have chosen compound **7d** for further studies. Whereas 8a and **8b** were inactive. In a dose response study the compound **7d** showed dose dependent inhibition of sir2 with an IC_{50} = 78.05 µM (Fig. 4).

Table 7. Yeast based *in vitro* assay for sir2 inhibition by compounds **7** and **8**.[a]

Entry	Compound	% of inhibition
1	 **7b**	32.38

2	 **7c**	49.53
3	 **7d**	43.90
4	 **7f**	43.20
5	 **8a**	1.01
6	 **8b**	3.30

[a]Data represent the mean values of three independent determinations. Splitomicin was used as a reference compound.

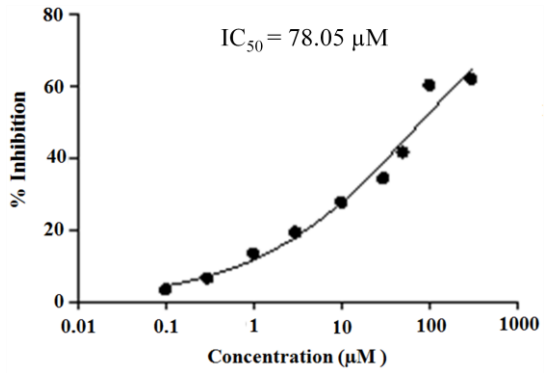


Figure 4. Dose dependent % inhibition of sir2 by the compound **7d**.

As compounds **7d** and **7f** have shown considerable inhibition of Sir2 we then turned our focus in understanding the binding mode of the compounds **7d** and **7f** with yeast Sir2 protein *in silico*. Docking was performed by using the crystal structure coordinating with NAD-dependent protein deacetylase (PDB ID: 2HJH).[30] Since two enantiomers are possible for both compounds **7d** and **7f**, docking studies was performed by using both *R* and *S* isomer individually.

In compound **7d** the phenyl group at C-3 position of *S* isomer was accommodated well into the hydrophobic pocket of the active site consisting of Arg 497, Gly 264, and Asp 273 residues and the dimethyl group at C-7 interacted with Lys 475 and Ser 473 (Fig. 5). While similar interactions were also observed in case of *R* isomer (Fig. 1.5) the *S* isomer however showed better score than the *R* antipode.

In compound **7f**, S isomer the carbonyl group of lactone ring makes hydrogen bonding with backbone amino group of Arg 275. And in the R isomer of **7f**, the same carbonyl binds in an opposite orientation to S isomer and makes a hydrogen bond with backbone amino group of Arg 497 (Fig. 6).

It is evident from the dock score that the (*S*)-isomer showed better interactions with sir2 protein than its (*R*)-antipode indicating that further SAR studies need to be performed around the (*S*)-isomer. Overall, the compound **7d** is of further interest as a novel small molecule based inhibitor of sirtuins. The dock score of individual isomer along with the contributing factors are listed in Table 8. Binding mode of **7d** docked into the catalytic site of Yeast Sir2 is shown in figure 1.7.

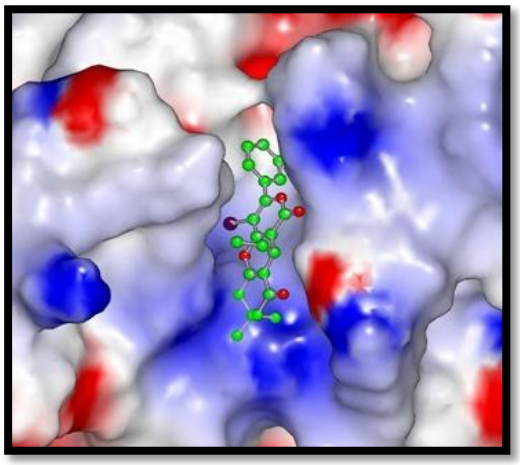


Figure 7. Binding mode of **7d** docked into the catalytic site of Yeast Sir2 (PDB ID: 2HJH).

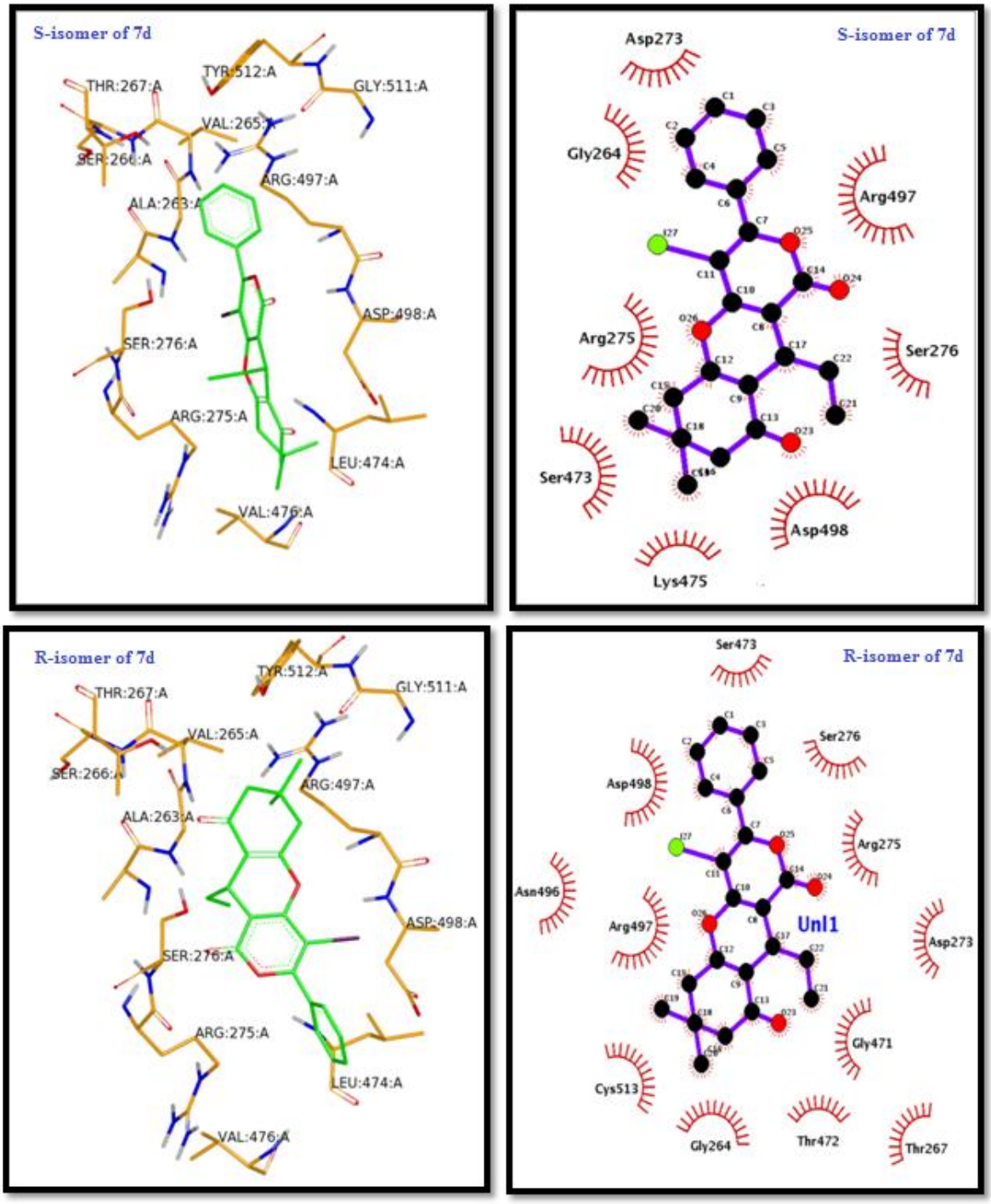


Figure 5. The binding mode of (*S*) and (*R*)-isomers of compound **7d** and their 2D interaction plot.

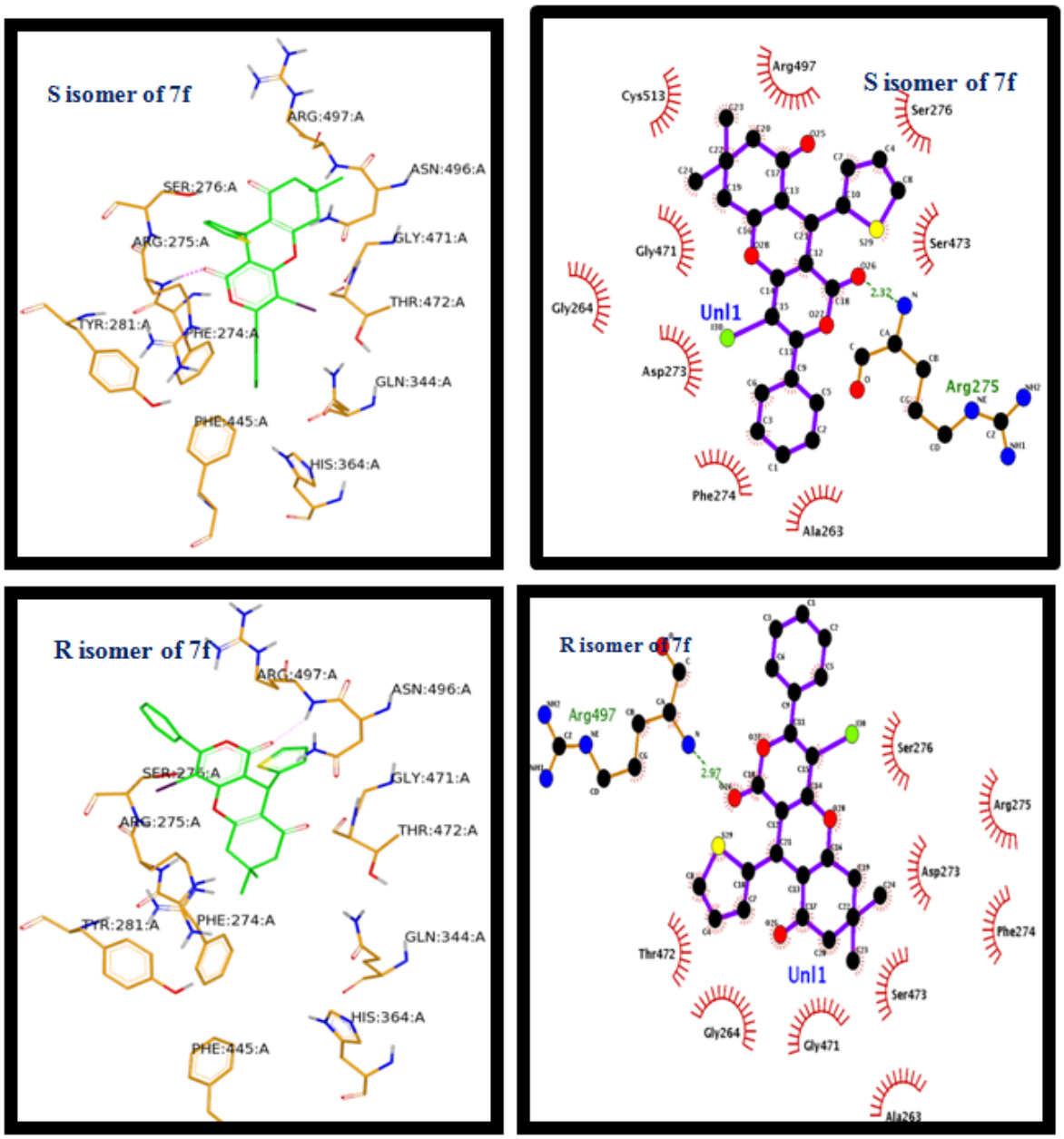


Figure 6. The binding mode of R and S isomers of compound **7d** and their 2d interaction

Table 8. Factors contributing docking score of (*S*) and (*R*)-isomers of compound **7d** and **7f** with sir2 protein.[a]

Molecules	Dock Score[a]	Steric	Protein Desolvation	Ligand Desolvation H-Bond	Ligand Desolvation	H-bond
Splitomicin	-8.5	-14.4	6.5	-0.5	0.5	-0.7
7d (S)	-6.7	-14.4	3.9	-0.01	1.3	0
7d (R)	-6.0	-17.9	4.8	-0.6	2.1	-0.6
7f (S)	-6.3	-12.3	10.3	-1.1	2.2	-0.6
7f (R)	-5.5	-12.4	7.9	-0.8	2.3	-0.5

[a] FRED Chemgauss4 score

2B.6. Conclusion

In conclusion, a series of novel pyrano[4,3-*b*]pyran-5(4*H*)-one based small molecules were designed as potential inhibitors of sir2. These compounds were obtained in good yields *via* an elegant multi-step method consisting of MCR (involving aldehydes, ethyl cyanoacetate and 1,3-diketone / β-keto ester), Sandmeyer type iodination, Sonogashira type coupling followed by iodocyclization and then Pd-mediated various C-C bond forming reactions. The crystal structure analysis of a representative iodolactonized product (**7c**) is presented. Some of the compounds synthesized showed encouraging inhibition of sir2 protein (a yeast homologue of mammalian SIRT1) when tested using yeast based assay. A representative compound **7d** showed dose dependent inhibition (IC_{50} = 78.05 μM) of yeast sir2 and good interactions *in silico* (dock score -6.7) when docked into this protein. Overall, the pyrano[4,3-*b*]pyran-5(4*H*)-one framework presented here could be an attractive template for the identification of novel sir2 inhibitors and the corresponding synthetic strategy described could be useful for generating diversity based library of small molecules related to this scaffold.

2B.7. Experimental Section

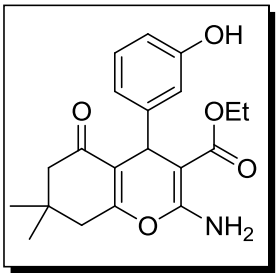
General methods

Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution by using 400 and 100 MHz spectrometers (VARIAN 400 MR), 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) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer (FT/IR-4200, JASCO). Melting points were determined by using melting point apparatus (Buchi melting point B-540) and are uncorrected. MS spectra were obtained on a mass spectrometer (AGILENT 6430 triple quardrupole LC-MS). 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.

General procedure for the synthesis of pyran annulated heterocyclic compounds (2a-2i).

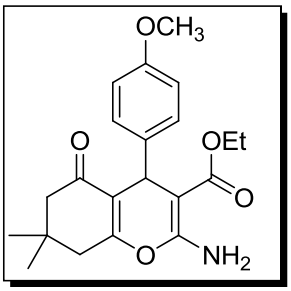
To a solution of aldehyde (1 mmol) and ethyl cyanoacetate or ethyl acetoacetate (1 mmol) in ethanol (3 mL) was added the DMAP (0.025 g, 0.2 mmol) and the mixture was stirred at room temperature. A solid was precipitated after 20–30 min. To this was added the dicarbonyl compound (1 mmol) and the mixture was stirred at 80 °C. Initially, the reaction mixture became a clear solution but after completion of the reaction, the solid was precipitated under hot conditions. The mixture was cooled to room temperature and the solid precipitate was filtered. The solid precipitate was then washed with methanol/n-hexane to obtain the desired product.

Ethyl-2-amino-4-(3-hydroxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (3a)



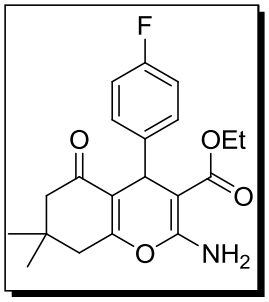
White solid; mp: 180-183 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.06 (t, *J* = 7.8 Hz, 1H), 6.84 (s, 1H), 6.80 (d, *J* = 7.6 Hz, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 6.18 (s, 2H), 5.79 (s, 1H), 4.68 (s, 1H), 4.04 (q, *J* = 7.0 Hz, 2H), 2.41 (s, 2H), 2.29-2.15 (m, 2H), 1.15 (t, *J* = 7.1 Hz, 3H), 1.09 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.0, 164.6, 162.4, 157.3, 156.1, 144.2, 134.2, 121.3 (2C), 116.1, 113.4, 111.8, 60.9, 50.7, 38.8, 38.1, 32.1, 27.1 (2C), 14.1; IR (KBr) ν_{max} (cm^{-1}): 3393, 3281, 2951, 1652, 1621, 1524; MS (ESI) m/z : 358.2 [M + H].

Ethyl-2-amino-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate[1] (3b)



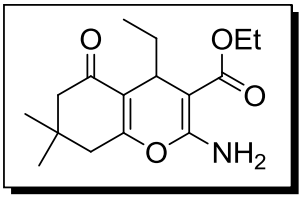
White solid; mp: 130-132°C (Lit[1] 131-134 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.17 (d, *J* = 8.6 Hz, 2H), 6.74 (d, *J* = 8.6 Hz, 2H), 6.22-6.00 (m, 2H), 4.65 (s, 1H), 4.12-3.96 (m, 2H), 3.74 (s, 3H), 2.41 (s, 2H), 2.27-2.07 (m, 2H), 0.97 (s, 3H), 1.17 (s, 3H).1.09 (s, 3H).

Ethyl-2-amino-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate[2] (3c)



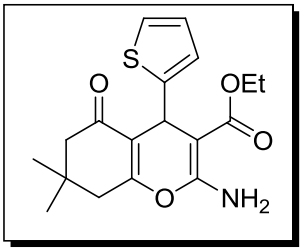
Light yellow; mp:157-159 °C (Lit[2] 150-155 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.22 (d, *J* = 8.3 Hz, 2H), 6.88 (t, *J* = 8.6 Hz, 2H), 6.17 (d, *J* = 1.1 Hz, 2H), 4.68 (s, 1H), 3.99-4.10 (m, 2H), 2.42 (s, 2H), 2.19 (q, *J* = 16.2 Hz, 2H), 1.15 (t, *J* = 7.1 Hz, 3H), 1.10 (s, 3H), 0.97 (s, 3H).

Ethyl-2-amino-4-ethyl-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (3d)



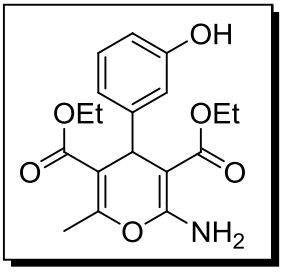
Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 2H), 4.28 (d, *J* = 7.1 Hz, 2H), 4.16 (m, 2H), 3.76 (t, *J* = 3.5 Hz, 1H), 2.37 (s, 2H), 2.28 (s, 2H), 1.54 (t, *J* = 10.1 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.09-1.07 (m, 6H), 0.68 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.2, 166.9, 163.9, 158.3, 156.8, 112.7, 105.2 (2C), 73.8, 63.0, 38.7, 32.0, 22.6 (2C), 14.3, 11.8; IR (KBr) ν_{max} (cm^{-1}): 2936, 1622, 1641, 1477; MS (ESI) m/z: 293.4 [M + H].

Ethyl-2-amino-7,7-dimethyl-5-oxo-4-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (3e)



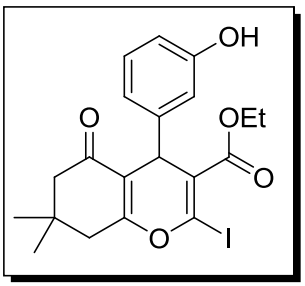
Light yellow solid; mp: 142-144 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.03 (d, *J* = 4.8 Hz, 1H), 6.93 (m, 2H), 6.77-6.38 (m, 2H), 5.09 (s, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.41 (s, 2H), 2.27 (s, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.11 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.9, 167.6, 163.9, 155.4, 141.1, 127.3 (2C), 125.0, 123.0, 113.3, 75.1, 61.1, 55.1, 38.4, 32.8, 27.8 (2C), 14.3; IR (KBr) ν_{max} (cm^{-1}): 2928, 2841, 1627, 1503; MS (ESI) m/z : 347.9 [M^+].

Diethyl-2-amino-4-(3-hydroxyphenyl)-6-methyl-4*H*-pyran-3,5-dicarboxylate(3f)



Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.06 (t, *J* = 7.8 Hz, 1H), 6.81 (t, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 2.1 Hz, 1H), 6.60 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.09 (d, *J* = 0.6 Hz, 2H), 5.37 (s, 1H), 4.65 (s, 1H), 4.13-4.03 (m, 4H), 2.33 (d, *J* = 4.8 Hz, 3H), 1.20-1.18 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.3, 165.2, 162.5, 157.3, 139.9 (2C), 120.3 (2C), 115.1, 112.3, 109.3, 73.7, 65.8(2C), 40.7, 16.9, 13.5 (2C); IR (KBr) ν_{max} (cm^{-1}): 2856, 1641, 1538, 1432; MS (ESI) m/z: 347.8 [M + H].

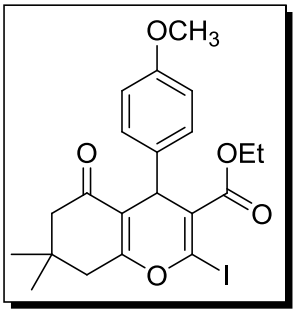
Ethyl-4-(3-hydroxyphenyl)-2-iodo-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4a)



To a solution of *tert*-butyl nitrite (0.13 mL, 0.84 mmol) and CuI (0.11 g, 1.53 mmol) in MeCN (4 mL) was added compound **2a** (0.2 g, 0.56 mmol). The reaction mixture was stirred for 10 min and then solvent was removed under low vacuum. The residue was treated with water (15 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and evaporated

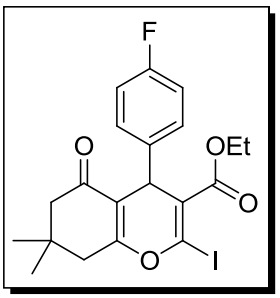
under low vacuum. The crude product was purified by column chromatography using 9:1 hexane/ethyl acetate to give light brown solid; mp: 143-145°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.11 (t, *J* = 7.9 Hz, 1H), 6.79 (d, *J* = 7.0 Hz, 2H), 6.64 (d, *J* = 7.2 Hz, 1H), 5.55 (d, *J* = 1.7 Hz, 1H), 4.88 (s, 1H), 4.15-4.10 (m, 3H), 2.47 (s, 2H), 2.23 (d, *J* = 7.3 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.12-1.09 (m, 3H), 0.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.0, 165.9, 156.3, 152.2, 130.2, 128.6 (2C), 128.1, 124.1(2C), 114.6, 114.1, 108.8, 60.6, 50.0, 41.3, 37.4, 31.7, 27.1 (2C), 13.7; IR (KBr) ν_{max} (cm^{-1}): 2831, 1647, 1540, 1422; MS (ESI) m/z : 468.9 [M + H].

Ethyl-2-iodo-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4b)



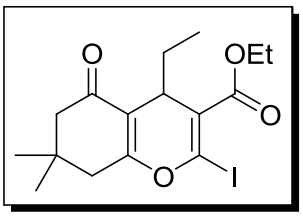
Brown solid; mp: 159-161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.78 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.5 Hz, 2H), 4.85 (s, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.75 (s, 3H), 2.46 (s, 2H), 2.20 (q, *J* = 6.4 Hz, 2H), 1.21 (t, *J* = 7.1 Hz, 3H), 1.10 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.5, 162.5, 154.4, 150.4, 142.5, 139.1, 137.1, 115.4, 114.9, 114.5, 114.3, 109.9, 62.7, 53.1, 51.4, 42.6, 36.7, 34.3, 31.3, 27.5 (2C), 14.5. IR (KBr) ν_{max} (cm^{-1}): 2928, 1613, 1521, 1501. MS (ESI) m/z: 482.8 [M + H].

Ethyl-4-(4-fluorophenyl)-2-iodo-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4c)



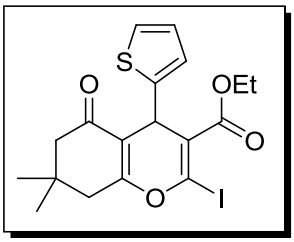
Off white solid; mp: 152-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26-7.17 (m, 2H), 6.93 (t, *J* = 8.6 Hz, 2H), 4.89 (s, 1H), 4.20-4.05 (m, 2H), 2.53-2.50 (m, 2H), 2.32-2.30 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.10 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.3, 164.3, 163.2, 138.7, 129.7, 129.6, 118.6, 115.0, 114.8, 114.1, 108.8, 50.5, 40.3, 36.2, 32.2, 26.9, 27.0 (2C), 13.8. IR (KBr) ν_{max} (cm^{-1}): 2929, 1623, 1548, 1463; MS (ESI) m/z: 471.1 [M + H].

Ethyl-4-ethyl-2-iodo-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4d)



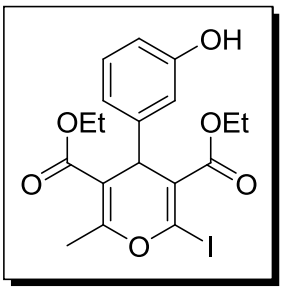
Light yellow solid; mp: 90-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.19-4.28 (m, 2H), 3.92 (t, *J* = 4.5 Hz, 1H), 2.42 (d, *J* = 8.8 Hz, 2H), 2.28 (s, 2H), 1.61-1.59 (m, 2H), 1.56-1.47 (m, 2H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.10 (s, 6H), 0.75 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.2, 165.3 (2C), 118.8 (2C), 113.1, 108.4, 61.1, 61.1, 50.9, 40.5, 32.2, 29.4, 27.2 (2C), 14.1. IR (KBr) ν_{max} (cm^{-1}): 2941, 1638, 1511, 1421. MS (ESI) m/z: 404.8 [M + H].

Ethyl-2-iodo-7,7-dimethyl-5-oxo-4-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4e)



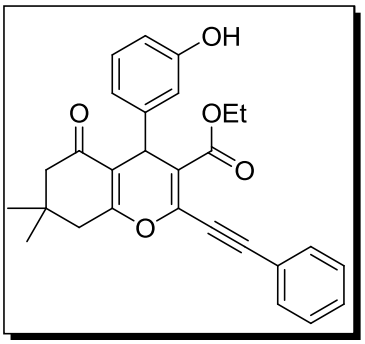
Offwhite solid; mp: 127-129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.11 (d, *J* = 4.7 Hz, 1H),6.89-6.83 (m, 2H), 5.27 (s, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 2.48 (s, 2H), 2.28 (s, 2H), 1.24 (t, *J* = 5.2 Hz, 3H), 1.10 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 194.1, 164.5, 156.8, 138.6, 128.1, 124.1, 123.2, 114.0, 113.8, 105.7, 60.5, 55.6, 34.6, 31.7, 27.7 (2C), 14.2; IR (KBr) ν_{max} (cm^{-1}): 2863, 1733, 1617, 1498; MS (ESI) m/z: 458.9 [M + H].

Diethyl-4-(3-hydroxyphenyl)-2-iodo-6-methyl-4*H*-pyran-3,5-dicarboxylate (4f)



Light yellow semi solid; ^{1}HNMR (400 MHz, $CDCl_3$) δ: 6.79 (d, *J* = 7.5 Hz, 1H), 6.72 (s, 2H), 6.68-6.63 (m, 1H), 5.59 (s, 1H), 4.86 (s, 1H), 4.17-4.09 (m, 4H), 2.37 (s, 3H), 1.25-1.20 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9 (2C), 163.9, 156.8, 147.8, 129.5, 121.8, 117.4, 109.9, 63.0, 42.1, 17.0, 13.4 (2C); IR (KBr) ν_{max} (cm^{-1}): 2817, 1633, 1618, 1472; MS (ESI) m/z: 458.9 [M + H].

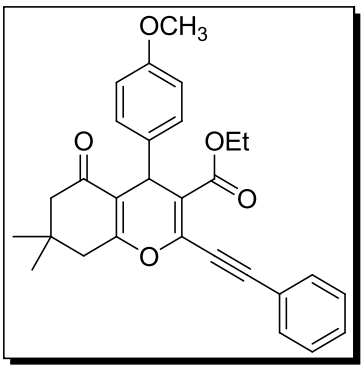
Ethyl-4-(3-hydroxyphenyl)-7,7-dimethyl-5-oxo-2-(phenylethynyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6a)



To a solution of compound **3a** (0.2 g, 0.44 mmol) in ethanol (3mL) was added 10% Pd/C (0.0004 g, 0.0044 mmol), PPh_3 (0.004 g, 0.02 mmol), CuI (0.008 g, 0.0044 mmol) and Et_3N (0.15 mL, 0.66 mmol) and the mixture was stirred for 15 min under nitrogen. Then, phenyl acetylene (**4a**) (0.05 mL, 0.44 mmol) was added and the mixture was stirred at 60 °C for 2h. After completion of the reaction, the mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford light yellow semi

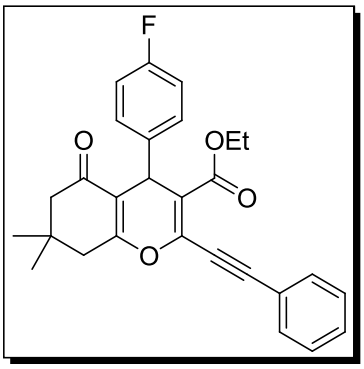
solid. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.63-7.54 (m, 2H), 7.39 (m, 3H), 7.20-7.06 (m, 1H), 6.90-6.76 (m, 2H), 6.68-6.59 (m, 1H), 5.42-5.30 (m, 1H), 4.87 (d, *J* = 5.1 Hz, 1H), 4.13 (d, *J* = 8.7 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.16 (s, 3H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.6, 165.2, 157.0, 153.7, 153.7, 149.6 (2C), 142.2, 132.5, 128.8 (2C), 120.7, 116.6, 112.6, 109.9, 93.5, 87.9, 62.3, 58.8, 41.1, 39.2, 33.2, 26.8 (2C), 13.6; IR (KBr) ν_{max} (cm^{-1}): 3345, 2835, 2227, 1641, 1578; MS (ESI) m/z: 443.2 [M + H].

Ethyl-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-2-(phenylethynyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6b)



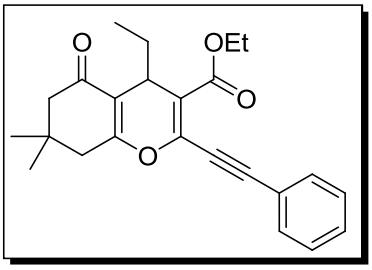
Yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 3.7 Hz, 2H), 7.37 (d, *J* = 6.9 Hz, 3H), 7.23 (d, *J* = 8.2 Hz, 2H), 6.78 (d, *J* = 7.8 Hz, 2H), 4.84 (s, 1H), 4.23-4.19 (q, *J* = 5.8 Hz, 2H 2H), 3.75 (s, 3H), 2.51 (s, 2H), 2.30-2.25 (m, 2H), 1.23 (t, *J* = 6.2 Hz, 3H)1.11 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 198.4, 152.2, 143.1, 141.7, 141.3, 139.5 (2C), 135.9, 134.8, 130.4, 130.2, 129.6 (2C), 128.1, 127.8, 124.1, 114.5, 112.9, 102.2, 94.7, 89.4, 60.6, 57.2, 51.7, 39.0, 31.6, 27.1 (2C), 13.7; IR (KBr) ν_{max} (cm^{-1}): 3352, 2931, 2235, 1614, 1578, 1456; MS (ESI) m/z: 457.2 [M + H].

Ethyl-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-2-(phenylethynyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6c)



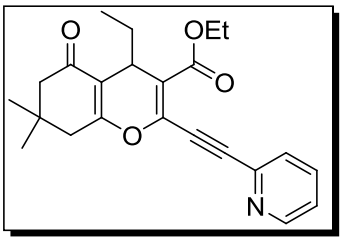
Light yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61-7.55 (m, 2H), 7.42-7.34 (m, 3H), 7.27 (d, *J* = 7.8 Hz, 3H), 6.93 (dt, *J* = 8.5, 4.4 Hz, 3H), 4.88 (s, 1H), 4.18-4.10 (m, 2H), 2.51 (s, 2H), 2.29-2.16 (m, 3H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.11 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.3, 164.6, 163.0, 160.4, 139.5, 132.0 (2C), 130.0, 129.9, 129.7 (2C), 128.4, 121.3, 116.9, 115.0, 114.8, 113.5, 97.7, 81.8, 60.9, 50.6, 40.7, 34.9, 32.2, 29.1, 27.2 (2C), 14.0; IR (KBr) ν_{max} (cm^{-1}): 2897, 2243, 1719, 1652, 1530; MS (ESI) m/z: 445.6 [M + H].

Ethyl-4-ethyl-7,7-dimethyl-5-oxo-2-(phenylethynyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6d)



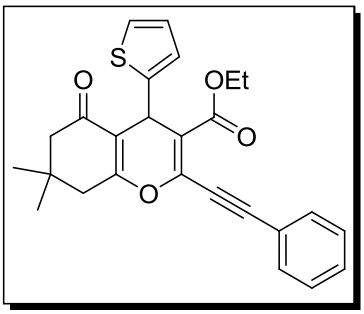
Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.52-7.62 (m, 2H), 7.29-7.45 (m, 3H), 4.34-4.21 (m, 2H), 3.91 (t, *J* = 4.6 Hz, 1H), 2.44 (s, 2H), 2.29 (d, *J* = 8.0 Hz, 2H), 1.61-1.65 (m, 2H), 1.29-1.36 (m, 3H), 1.11 (t, *J* = 3.1 Hz, 6H), 0.78 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.1, 165.4, 165.0, 140.5, 140.5, 132.0, 129.5, 128.4, 121.5, 116.9, 112.3, 96.6, 81.9, 60.9, 50.9, 40.8, 32.1, 30.3, 27.3 (2C), 14.3, 9.1; (KBr) ν_{max} (cm^{-1}): 2968, 2851, 2231, 1641, 1614, 1485. MS (ESI) m/z: 379.2 [M + H].

Ethyl-4-ethyl-7,7-dimethyl-5-oxo-2-(pyridin-2-ylethynyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6e)



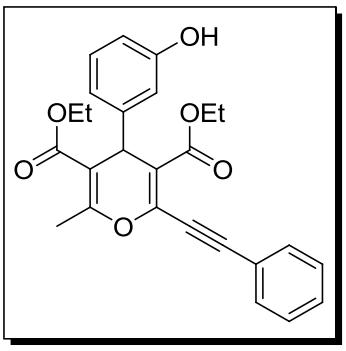
Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (d, *J* = 4.7 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.28-7.32 (m, 1H), 4.29 (2H), 3.92 (t, *J* = 4.5 Hz, 1H), 2.43 (s, 2H), 2.30 (s, 2H), 1.69-1.60 (m, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.12 (d, *J* = 2.1 Hz, 6H), 0.78 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.0, 165.0, 150.3, 142.0, 140.1, 136.2, 127.8, 123.8, 118.4, 112.2, 94.9, 81.0, 61.0, 50.9, 40.7, 32.1, 30.3, 29.3, 27.3 (2C), 14.2, 9.0; (KBr) ν_{max} (cm^{-1}): 2962, 2841, 1732, 1658, 1608, 1458; MS (ESI) m/z: 380.2 [M + H].

Ethyl-7,7-dimethyl-5-oxo-2-(phenylethynyl)-4-(thiophen-2-yl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (6f)



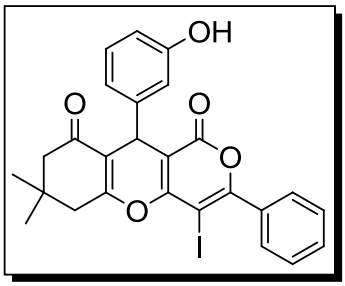
Off white semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60-7.55 (m, 2H), 7.43-7.34 (m, 3H), 7.10 (d, *J* = 5.0 Hz, 1H), 6.93 (d, *J* = 2.9 Hz, 1H), 6.86 (d, *J* = 4.9 Hz, 1H), 5.26 (s, 1H), 4.31-4.18 (m, 2H), 2.52 (s, 2H), 2.28 (d, *J* = 8.2 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.13 (s, 3H), 1.06 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.5, 163.5, 157.0, 146.7, 139.6, 138.6, 132.5 (2C), 130.5, 129.6, 128.7, 128.5, 127.0, 126.9 (2C), 113.5, 113.3, 93.1, 88.7, 63.1, 54.5, 38.1, 29.8, 27.8 (2C), 14.4; (KBr) ν_{max} (cm^{-1}): 2972, 2862, 1726, 1643, 1618, 1432; (ESI) m/z: 432.9 [M + H].

Diethyl-4-(3-hydroxyphenyl)-2-methyl-6-(phenylethynyl)-4H-pyran-3,5-dicarboxylate (6g)



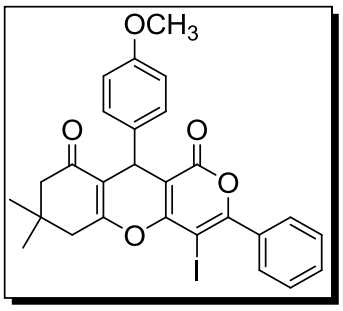
Yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, *J* = 6.4 Hz, 1H), 7.45-7.39 (m, 2H), 7.18-7.08 (m, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 6.83-6.75 (m, 2H), 6.71-6.63 (m, 1H), 4.86 (d, *J* = 6.5 Hz, 1H), 4.27- 4.20 (m, 4H), 2.48-2.40 (m, 3H), 1.31-1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.4, 166.5, 165.5, 162.6, 159.1, 155.6, 145.8, 143.6, 139.7, 132.0, 130.2, 129.3 (2C), 128.4, 122.9 (2C), 121.4, 114.0, 111.8, 96.8, 82.0, 61.1, 60.6, 38.4, 18.8, 14.1, 14.0; (KBr) ν_{max} (cm^{-1}): 2972, 2861, 1739, 1652, 1573, 1461; (ESI) m/z: 432.8 [M + H].

10-(3-Hydroxyphenyl)-4-iodo-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7a)



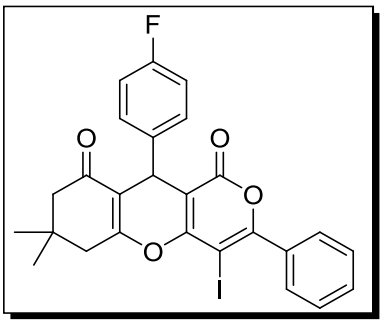
A solution of compound **5a** (0.2 g, 0.45 mmol) and I_2 (0.11 g, 0.45 mmol) in dichloromethane (3 mL) was placed in a round bottom flask and stirred for 2 h under a nitrogen atmosphere. The mixture was then diluted with ether (25 mL) and washed with aq $Na_2S_2O_3$ (20 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford the compound **7a** as a light brown solid; mp: 137-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 -7.64 (m, 2H), 7.49-7.41 (m, 3H), 7.13 (d, *J* = 7.8 Hz, 1H), 6.95 (s, 1H), 6.87 (d, *J* = 7.5 Hz, 1H), 6.68 (s, 1H), 5.61-5.59 (m, 1H), 4.90 (s, 1H), 2.65 (d, *J* = 7.8 Hz, 2H), 2.30 (d, *J* = 2.8 Hz, 2H), 1.16 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.3, 163.1, 142.2, 139.7, 132.0 (2C), 129.9 (2C), 128.4 (2C), 128.3, 121.2, 116.6 (2C), 113.3, 61.0, 50.6, 40.7, 35.2, 32.3, 29.1, 27.3 (2C); IR (KBr) ν_{max} (cm^{-1}): 3387, 2944, 2823, 1661, 1619, 1521; HPLC: 97.4%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.39 min. (ESI) m/z: 540.9 [M + H].

4-Iodo-10-(4-methoxyphenyl)-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7b)



Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71-7.62 (m, 2H), 7.44 (m, 3H), 7.29 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 4.87 (s, 1H), 3.75 (s, 3H), 2.65 (q, *J* = 3.4 Hz, 2H), 2.36-2.21 (m, 2H), 1.16 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 195.7, 162.7, 160.8, 156.6, 140.7, 133.3, 132.9 (2C), 130.9, 129.9, 129.5, 128.5, 128.2 (2C), 114.9, 104.7, 65.6, 63.2, 50.6, 40.6, 33.3, 32.3, 27.5 (2C); IR (KBr) ν_{max} (cm^{-1}): 2944, 1721, 1654, 1613, 1178; HPLC: 99.2%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.41 min. (ESI) m/z: 555.2 [M + H].

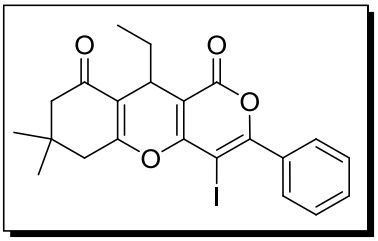
10-(4-Fluorophenyl)-4-iodo-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7c)



Off white solid; mp: 232-234 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 7.8 Hz, 2H), 7.47-7.52 (m, 3H), 7.35 (d, *J* = 8.5 Hz, 2H), 6.98 (t, *J* = 8.6 Hz, 2H), 4.91 (s, 1H), 2.29 (q, *J* = 5.4 Hz, 2H), 1.58 (s, 2H), 1.19 (s, 3H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 192.8, 162.5, 160.8 (2C), 156.5, 137.9, 133.3, 130.9, 130.1, 130.0, 129.4, 128.1, 115.3, 115.1, 115.0, 104.9, 6.6, 50.6, 40.6, 33.0, 32.3, 29.0, 27.4 (2C); IR (KBr) ν_{max} (cm^{-1}): 2949, 1718, 1643, 1501. HPLC: 97.5%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile

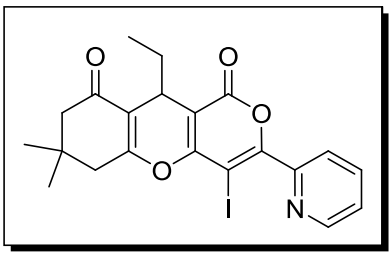
phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.72 min. (ESI) m/z: 542.9 [M + H].

10-Ethyl-4-iodo-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7d)



Off white solid; mp: 185-187 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 7.6 Hz, 3H), 7.54-7.41 (m, 2H), 3.99 (t, *J* = 3.9 Hz, 1H), 2.55 (q, *J* = 5.8 Hz, 2H), 2.42-2.24 (m, 2H), 1.79 (d, *J* = 4.4 Hz, 2H), 1.16 (d, *J* = 5.5 Hz, 6H), 0.76 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.5, 164.0, 161.4, 158.2, 133.5, 130.8 (2C), 129.5, 128.1(2C), 114.0, 104.2, 65.8, 50.9, 40.6, 32.1, 29.3, 28.5, 27.4 (2C), 25.0, 9.2; IR (KBr) ν_{max} (cm^{-1}): 2960, 1730, 1654, 1605, 1391; HPLC: 99.10%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.72 min; (ESI) m/z: 476.9.

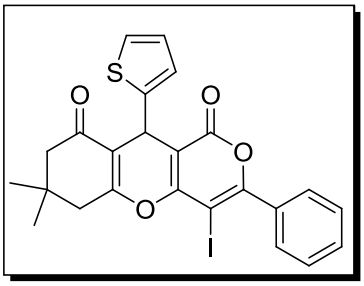
10-Ethyl-4-iodo-7,7-dimethyl-3-(pyridin-2-yl)-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7e)



Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.75-8.65 (m, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.85 (d, *J* = 1.6 Hz, 1H), 7.55-7.42 (m, 1H), 4.70 (q, *J* = 5.9 Hz, 1H), 4.11 (t, *J* = 5.2 Hz, 2H), 2.53-2.28 (m, 3H), 1.16-1.08 (m, 7H), 0.89-0.81 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.4, 166.5, 164.7, 156.9, 152.7, 148.9, 136.9, 127.3, 123.6, 121.9, 113.3, 110.8, 60.4, 50.9, 42.2, 40.6, 32.0, 29.4, 27.2, 14.0, 8.7; IR (KBr) ν_{max} (cm^{-1}): 2952, 2845, 1743, 1637, 1556, 1425; HPLC: 98.5%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile

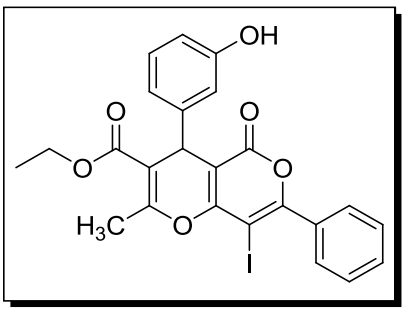
phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.63 min. (ESI) m/z: 477.9 [M + H].

4-Iodo-7,7-dimethyl-3-phenyl-10-(thiophen-2-yl)-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (7f)



Offwhite solid; mp: 212-214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (d, *J* = 6.1 Hz, 2H), 6.95-6.86 (m, 1H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.46 (q, *J* = 6.2 Hz, 3H), 5.30 (s, 1H), 2.66 (q, *J* = 3.4 Hz, 2H), 2.35 (s, 2H), 1.16 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 199.0, 163.7, 163.5, 157.0, 154.8, 139.6, 132.5 (2C), 130.5, 129.7, 128.5 (2C), 126.9, 122.0, 113.5, 100.0, 58.6, 54.5, 38.1, 32.2, 30.0, 27.8 (2C); IR (KBr) ν_{max} (cm^{-1}): 2916, 1723, 1673, 1541, 1338; HPLC: 95.2%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.49 min; (ESI) m/z: 430.9 [M + H].

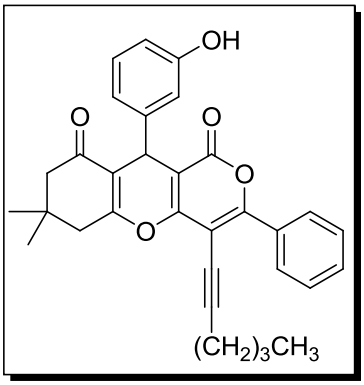
Ethyl-4-(3-hydroxyphenyl)-8-iodo-2-methyl-5-oxo-7-phenyl-4,5-dihydropyrano[4,3-*b*]pyran-3-carboxylate (7g)



Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 6.0 Hz, 2H), 7.45 (d, *J* = 7.2 Hz, 3H), 7.16 (d, *J* = 7.7 Hz, 1H), 6.94 (d, *J* = 7.3 Hz, 1H), 6.84 (s, 1H), 6.75-6.64 (m, 1H), 4.92 (s, 1H), 4.12 (d, *J* = 4.2 Hz, 2H), 2.43 (s, 1H), 2.58 (s, 3H), 1.24-1.17 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.7, 161.6, 160.6, 158.6, 156.9, 144.2, 133.3,

130.8, 129.5, 129.4, 128.1, 120.6, 114.4, 109.1, 103.9, 66.4, 60.9, 36.6, 18.5, 13.9; IR (KBr) ν_{max} (cm^{-1}): 3321, 2936, 1730, 1652, 1419; HPLC: 98.6%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.46 min; (ESI) m/z: 530.9 [M + H].

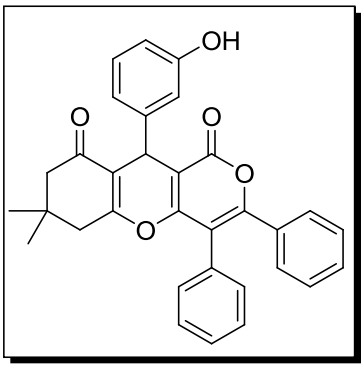
2B.5.1. 4-(Hex-1-ynyl)-10-(3-hydroxyphenyl)-7,7-dimethyl-3-phenyl-7,8-dihydropyrano[4,3-*b*]chromene-1,9(6*H*,10*H*)-dione (8a)



A mixture of compound **7a** (0.2 g, 0.41 mmol), 10% Pd/C (0.0004 g, 0.0041 mmol), PPh_3 (0.004 g, 0.0165 mmol), CuI (0.0009 g, 0.0041 mmol) and Et_3N (0.1 mL, 0.82 mmol) in ethanol (3.0 mL) was stirred for 15 min under nitrogen. Then, 1-hexyne (0.07 mL, 0.6150 mmol) was added and the mixture was stirred at 60 °C for 2 h. After completion of the reaction, the mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 9:1 hexane/ethyl acetate to afford the compound **8a** as a brown solid; mp: 99-101°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 8.0 Hz, 2H), 7.53-7.32 (m, 4H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.96 (s, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 4.87 (s, 1H), 2.68-2.55 (m, 2H), 2.49 (t, *J* = 6.8 Hz, 2H), 2.29 (d, *J* = 3.9 Hz, 2H), 1.66-1.58 (m, 3H), 1.56-1.46 (m, 2H), 1.15 (s, 3H), 1.08 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.2, 162.5, 161.3, 160.4, 157.8, 155.7, 144.0, 131.3 (2C), 130.9, 129.4, 128.3, 128.1 (2C), 120.5, 116.0, 114.8, 114.2, 104.6, 100.1, 96.2, 70.2, 50.6, 40.7, 32.9, 32.3, 30.2, 29.0, 27.6, 21.9, 19.5, 13.6; IR

(KBr) ν_{max} (cm^{-1}): 2953, 2871, 1738, 1633, 1610; HPLC: 93.7%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.47 min; (ESI) m/z: 494.9 [M + H].

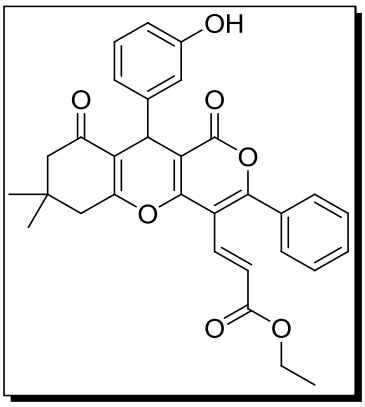
2B.5.2. 10-(3-Hydroxyphenyl)-7,7-dimethyl-3,4-diphenyl-7,8-dihydropyrano[4,3-b]chromene-1,9(6*H*,10*H*)-dione (8b)



To a solution of compound **7a** (0.2 g, 0.41 mmol) in dry DMF (3 mL) was added $Pd(OAc)_2$ (0.001 g, 0.0041 mmol, 5 mol %) and K_2CO_3 (0.1 g, 0.82 mmol) under a nitrogen atmosphere and the mixture was stirred for 10 min. To this 4-hydroxy phenyl boronic acid (0.1 g, 0.61 mmol) was added and the mixture was allowed to stir at 80 °C for 3h. After completion of the reaction, the mixture was cooled to room temperature, filtered through celite bed and the filtrate was concentrated under vacuum. The crude mass was diluted with dichloromethane (20 mL) and water (10 mL) and the mixture was extracted with dichloromethane (3 x 30 mL). The organic layers were collected, combined, washed with saturated aq NaCl (2 x 25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using 7.5:2.5 hexane/ethyl acetate to afford the compound **8b** as an off white solid; mp: 146-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40-7.36 (m, 3H), 7.26-7.20 (m, 4H), 7.16 (d, *J* = 7.6 Hz, 3H), 6.89 (d, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 1H), 5.61 (s, 1H), 4.96 (s, 1H), 7.03 (s, 1H), 2.36-2.30 (s, 2H), 2.26-2.24 (m, 2H), 1.09 (s, 3H), 1.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.3, 162.6, 161.6, 157.5, 155.8, 144.2, 131.6, 131.0, 130.9, 129.9, 129.4, 129.2, 129.1, 128.8, 128.5, 128.2, 127.9, 125.5, 120.3, 116.1, 114.7, 114.1, 112.3, 105.0, 50.6, 40.5, 33.0, 32.2, 29.6 (2C), 29.0, 27.4; IR (KBr) ν_{max} (cm^{-1}): 3354, 2962, 1731, 1647, 1451. HPLC: 89.9 %, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase

B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.23 min; (ESI) m/z: 490.8 [M + H].

2B.5.2. (*E*)-Ethyl-3-(10-(3-hydroxyphenyl)-7,7-dimethyl-1,9-dioxo-3-phenyl-1,6,7,8,9,10-hexahydropyrano[4,3-*b*]chromen-4-yl)acrylate (8c)



A mixture of compound **7a** (0.2 g, 0.41 mmol), ethyl acrylate (0.1 mL, 0.82 mmol), K_2CO_3 (0.11 g, 0.82 mmol), and $Pd(OAc)_2$ (0.001 g, 0.0049 mmol, 5 mol %) in *N*,*N*-dimethylformamide (2 mL) was stirred at 80 °C for 1h. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc (20 mL), and washed with 1 N aq HCl and water. The organic phase was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using 8:2 hexane/ethyl acetate to afford the compound **8c** as a off white solid; mp: 225-226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.53 (d, *J* = 7.5 Hz, 2H), 7.51-7.42 (m, 4H), 7.14 (t, *J* = 7.9 Hz, 1H), 6.95 (s, 1H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 6.2 Hz, 1H), 5.40 (s, 1H), 4.91 (s, 1H), 4.25 (q, *J* = 6.8 Hz, 2H), 2.63-2.60 (m, 3H), 1.32 (t, *J* = 7.12 Hz, 3H), 1.16 (s, 3H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 196.0, 166.6, 162.3, 162.0, 160.3, 155.7, 143.8, 134.6 (2C), 131.4, 130.9, 129.8 (2C), 123.1, 120.5, 115.9, 114.7, 114.3, 106.5, 105.5, 60.7, 50.7, 40.6, 32.8, 32.4, 29.0, 27.6 (2C), 14.2; (KBr) ν_{max} (cm^{-1}): 3351, 2948, 2863, 1733, 1709, 1642, 1451, 1322; HPLC: 93.3%, column: X-Bridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.22 min; (ESI) m/z: 514.8 [M + H].

Reference:

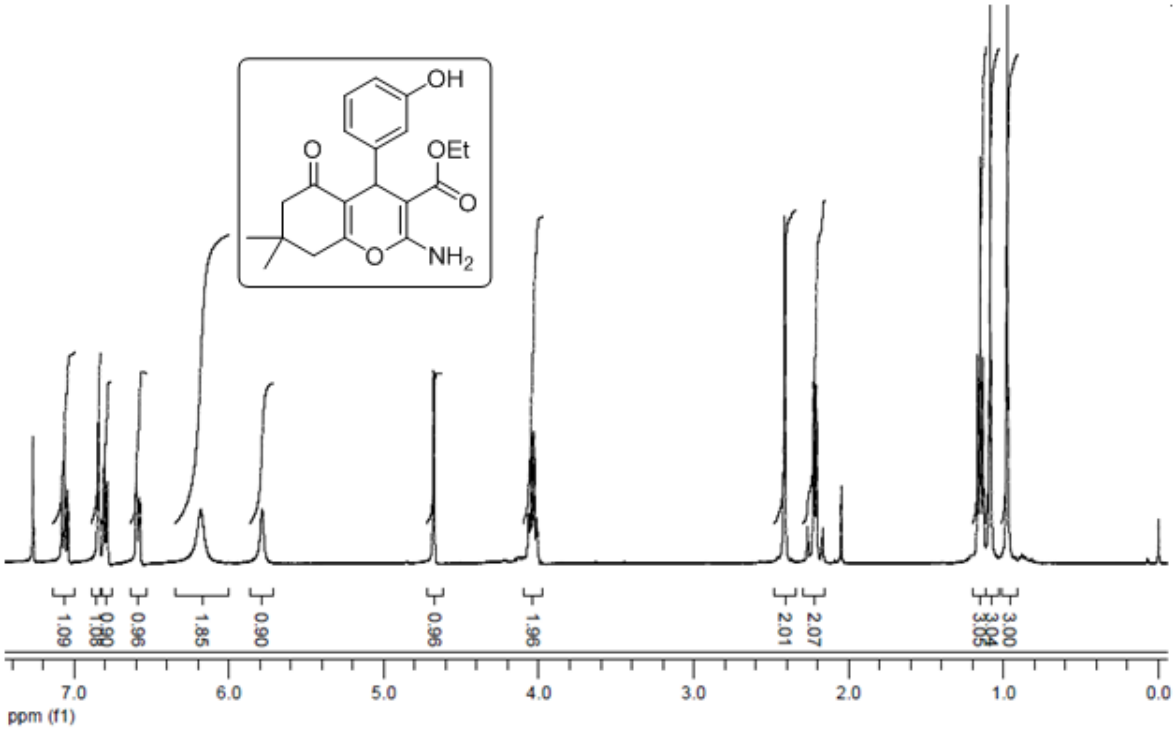
1. (a) Fong, W.F.; Shen, X.L.; Globisch, C.; Wiese, M.; Chen, G.Y.; Zhu, G.Y.; Yu, Z.L.; Tse, A. K.W.; Hu, Y.J. *Bioorg. Med. Chem.* **2008** , *16* , 3694. (b) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K.H. *J. Med. Chem.* **2001**, *44*, 664. (c) Nicolaides, D. N.; Gautam, D. R.; Litinas, K. E.; Hadjipavlou, L.D. J.; Fylaktakidou, K. C. *Eur. J. Med. Chem.* **2004**, 39, 323.
2. (a) Okamura, H.; Shimizu, H.; Iwagawa, T.; Nakatani, M. *Tetrahedron Lett.* **2000**, *41*, 4147. (b) Posner, G. H.; Lee, J. K.; White, M. W.; Hutchings, R. H.; Dai, H.; Kachinski, J.L.; Dolan, P.; Kensler, T. W. *J. Org. Chem.* **1997**, *62*, 3299.
3. Moreno-Manas, M.; Pleixats, R. *Naturally Occurring Oxygen Ring Systems*; Academic: New York, 1**992**; p 21.
4. (a) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888-3890. (b) Oberlies H. N.; Kroll, J.D. *J. Nat. Prod.* **2004**, *67*, 129.
5. Abbas K.S.; Tong, X.; Mukerji, R.; Zhang, H.; Barbara, N. T.; Cohen, M.S. *J. Nat. Prod.* **2010,** *73*, 1476.
6. Altomare, C.; Perrone, G.; Zonno, M. C.; Evidente, A.; Pengue, R.; Fanti, F.; Polonelli, L. *J. Nat. Prod.* **2000**, *63*, 1131.
7. Dickinson, J. M. *Nat. Prod. Rep*. **1993**, *10*, 7.
8. Abdolmohammadi, S.; Balalaie, S.*Tetrahedron Lett.* **2007,** *48*, 3299.
9. Khurana, J.M.; Nand, B.; Saluja, P. *Tetrahedron*, **2010**, *66*, 5637.
10. Khan, A.T.; Lal, M.; Ali, S.; Khan, M.M. *Tetrahedron Lett.* **2011**, 52, 5327.
11. Chen, Z.; Qiang Zhu, Q.; Weike Su, W. Tetrahedron Lett. **2011**, *52*, 2601.
12. Liu, Y.; Zhu, J.; Qian, J.; Jiang, B.; Xu, Z. *J. Org. Chem.* **2011**, *76*, 9096.
13. Gohain, M.; Tonder, J.H.; Bezuidenhoudt, B. C. B. *Tetrahedron Lett*. **2013**, *54*, 3773.
14. Pal, S.; Chatare, V.; Pal, M. *Curr. Org. Chem*. **2011**, *15*, 782.
15. Oikawa, T.; Sasaki, M.; Inose, M.; Shimamura, M.; Kuboki, H.; Hirano, S.; Kumagai, H.; Ishizuka, M.; Takeuchi, T. *Anticancer Res.* **1997**, *17*, 1881.
16. Bruzzone, S.; Parenti, M. D.; Grozio, A.; Ballestrero, A.; Bauer, I.; Rio, A.D.; Nencioni, A. *Current Pharmaceutical Design*, **2013**, *19*, 000

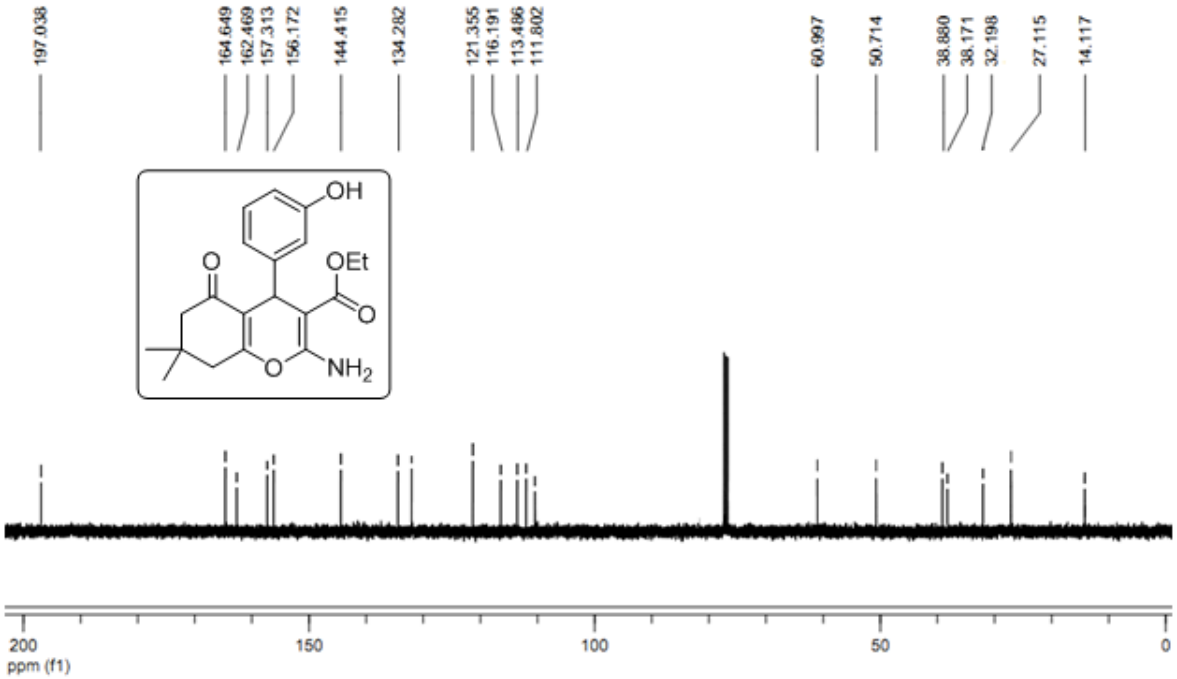
17. Posakony, J.; Hirao, M.; Stevens, S.; Simon, J.A.; Bedalov, A. *J. Med. Chem.* **2004,** *47*, 2635.
18. Mulakayala, N.; VNS Murthy, P.; Rambabu, D.; Aeluri, M.; Adepu, R.; Krishna, G. R.; Reddy, C. M.; Prasad, K. R. S.; Chaitanya, M.; Kumar, C. S.; Rao, M. V. B.; Pal, M. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 2186.
19. Nakhi, A.; Raju, A.; Rambabu, D.; Kishore, R.; Vanaja, G. R.; Arunasree M. K.; Pal, M. *Bioorg. Med. Chem. Lett.* **2012**, 22, 4418.
20. Imai, S.; Armstrong, C.M.; Kaeberlein, M.; Guarente, L. *Nature*. **2000**,*403*, 795.
21. Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D. *Annu. Rev. Biochem.* **2006**, *75*, 435.
22. Stunkel, W.; Campbell, R. M. *J. Biomol. Screen* **2011,** *16*, 1153.
23. (a) Milne, J. C.; Denu, J. M. *Curr. Opin. Chem. Biol.* **2008**, *12*, 11. (b) Heltweg, B.; Gatbonton, T.; Schuler, A. D.; Posakony, J.; Li, H.; Goehle, S.; Kollipara, R.; DePinho, R. A.; Gu, Y.; Simon, J. A.; Bedalov, A. *Cancer Res.* **2006**, *66*, 4368.
24. Parvatkar, P.T.; Parameswaran, P.S.; Tilve S.G. *Chem. Eur. J*. **2012**,*18*, 5460.
25. For a review on the use of iodine as a reagent, see: (a) Mphahlele, M. J. *Molecules*, **2009**, *14*, 5308. For leading references on iodocyclization, see: (b) Mancuso, R.; Mehta, S.; Gabriele, B.; Salerno, G.; Jenks, W. S.; Larock, R. C. *J. Org. Chem.* **2010**, *75*, 897. (c) Verma, A. K.; Aggarwal, T.; Rustagi, V.; Larock, R. C. *Chem Comm.* **2010**, *46*, 4064. (d) Mehta, S.; Larock, R. C. *J. Org. Chem*. **2010**, *75*, 1652. (e) Cho, C.-H.; Neuenswander, B.; Larock, R. C. *J. Comb. Chem*. **2010**, *12*, 278. (f) Barange, D. K.; Batchu, V. R.; Gorja, D.; Pattabiraman, V. R.; Tatini, L. K.; Babu, J. M.; Pal, M. *Tetrahedron* **2007**, *63*, 1775.
26. Crystallographic data (excluding structure factors) for **7c** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC No: 923597.
27. Sheldrick, G. M.; SHELX-97, Program for Crystal Structure Solution and Analysis, University of Gottingen, Gottingen, Germany, **1997**.
28. Layek, M.; Kumar, Y. S.; Islam, A.; Karavarapu, R.; Sengupta, A.; Halder, D.; Mukkanti, K.; Pal, M. *Med. Chem. Commun*. **2011**, 2, 478.
29. Splitomicin has been reported to inhibit yeast Sir2 with an IC_{50} value of 60 μM *in vitro*, see: (a) Bedalov, A.; Gatbonton, T.; Irvine, W. P.; Gottschling, D. E.; Simon,

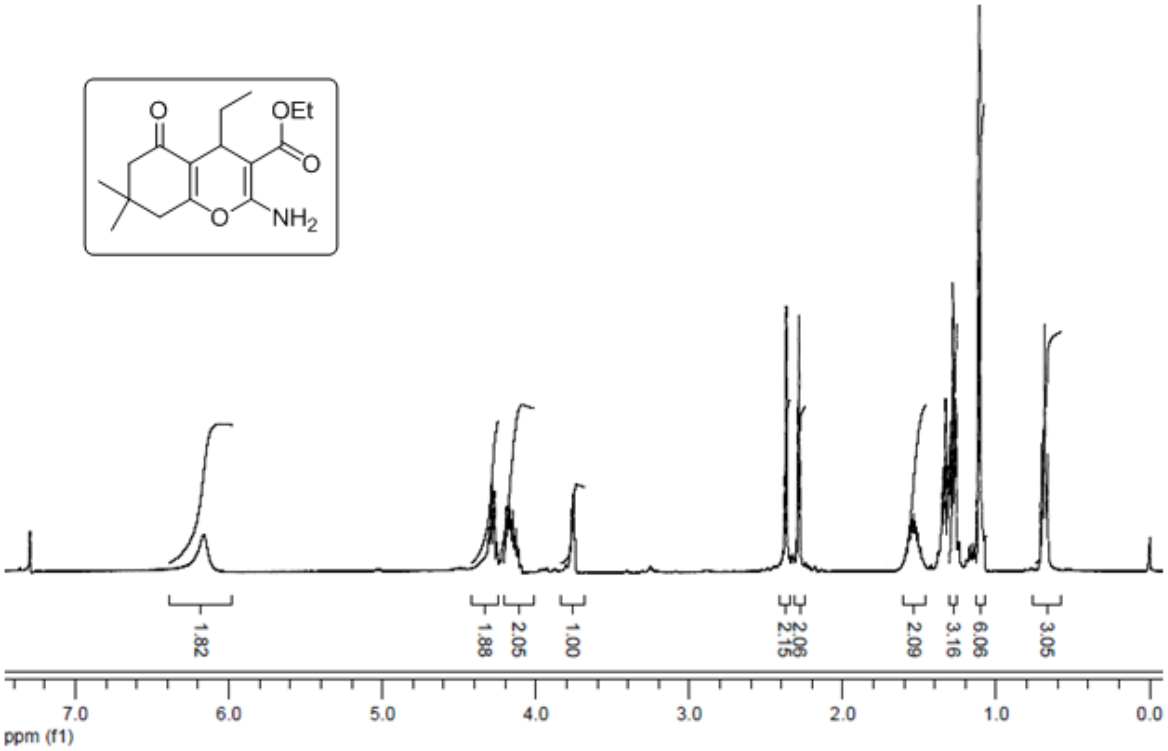
J. A. *Proc. Natl. Acad. Sci*. U.S.A. **2001**, 98, 15113; (b) Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem*. **2001**, *276*, 38837.

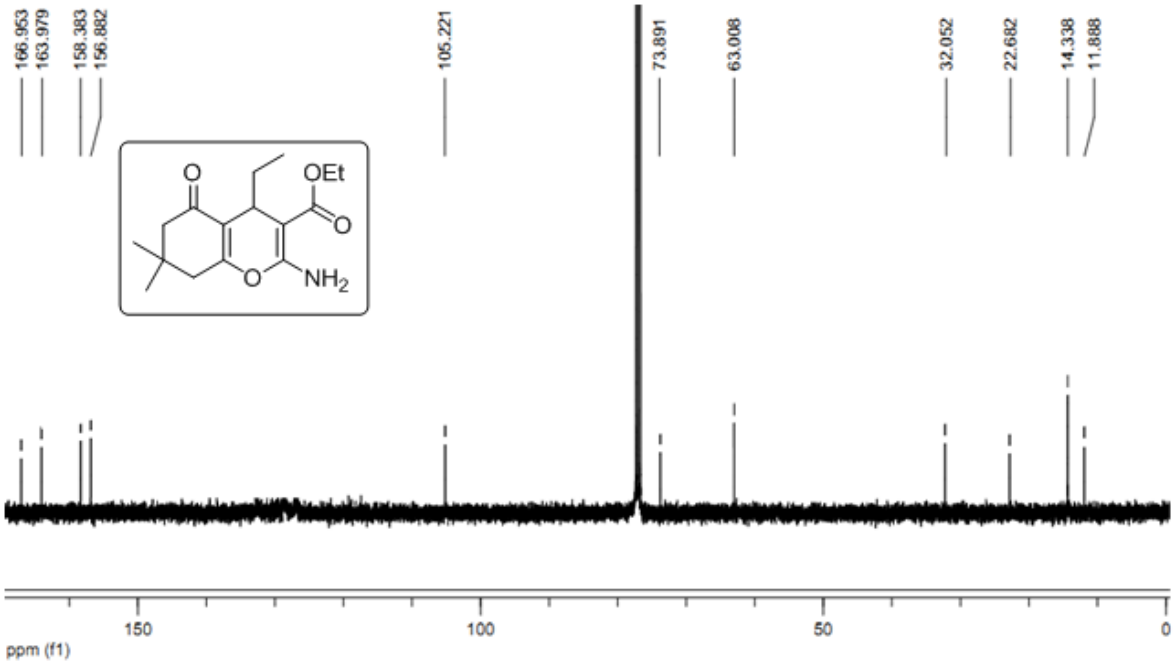
30. Hall, B. E.; Buchberger, J. R.; Gerber, S. A.; Ambrosio, A. L. B.; Gygi, S. P.; Filman, D.; Moazed, D.; Ellenberger, T. DOI: http://www.rcsb.org/pdb/explore/explore.do?structureId=2hjh

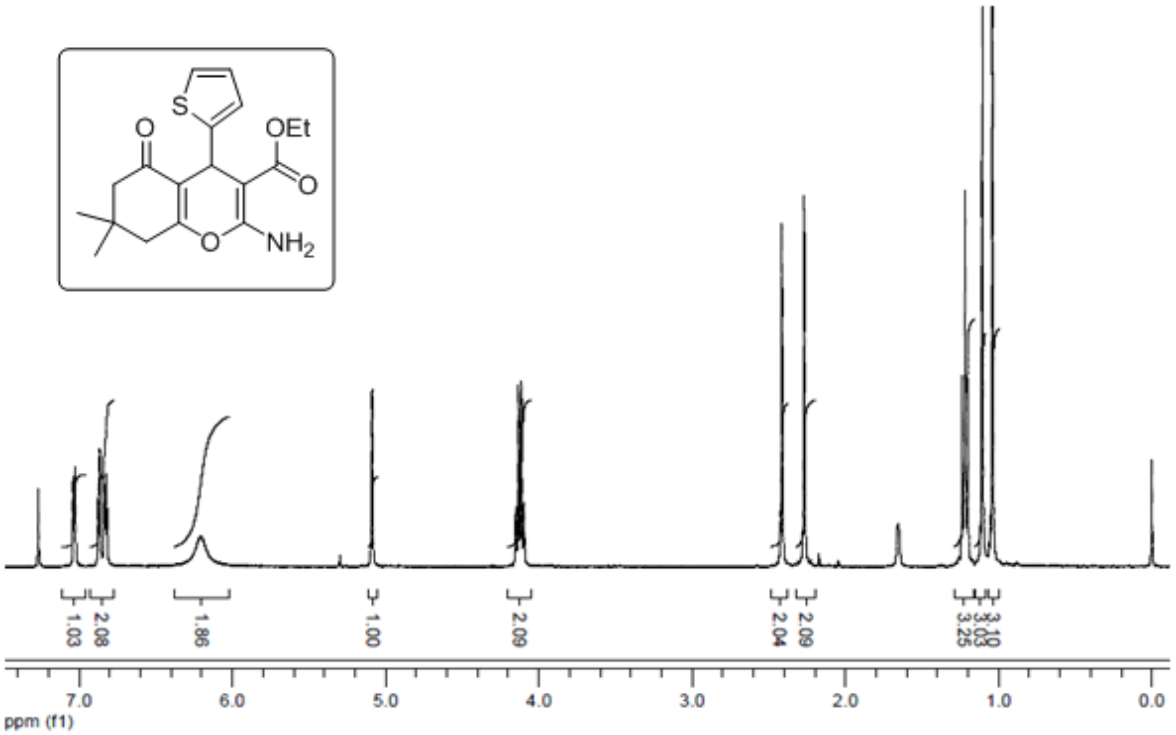
Appendix

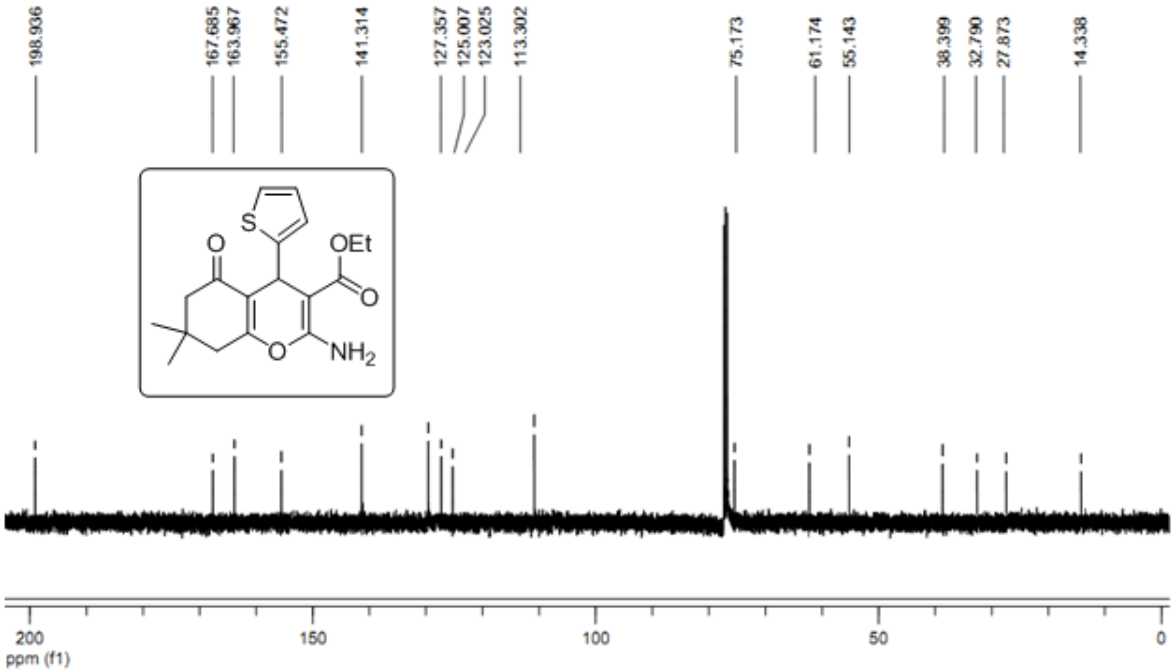


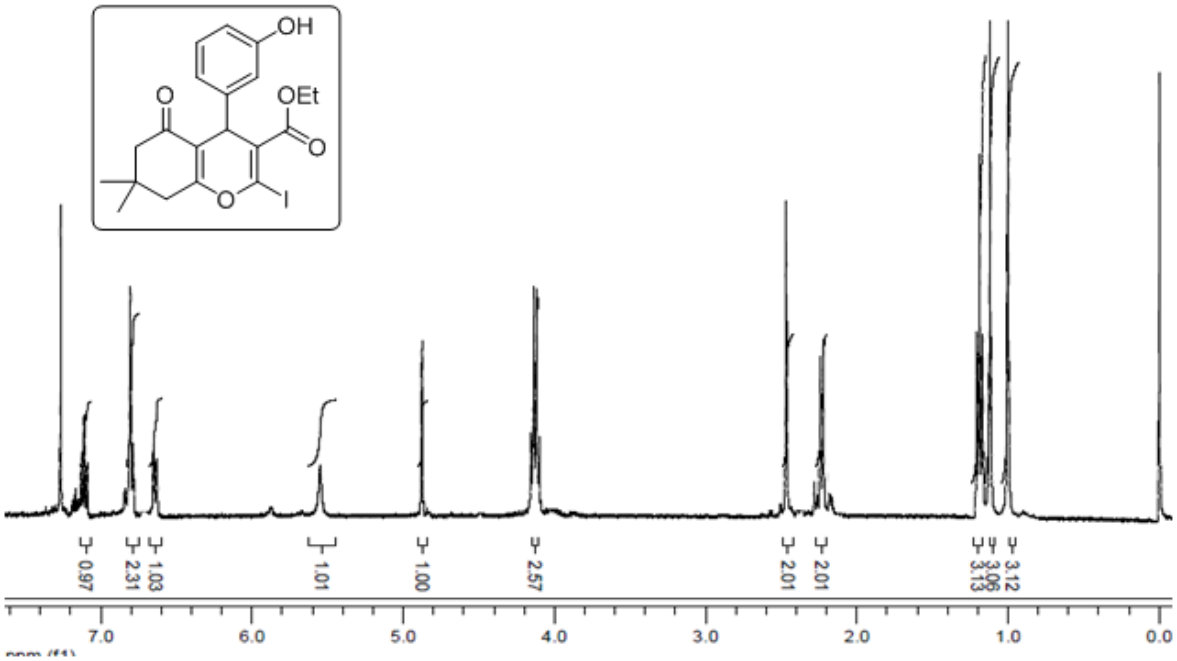

197.038
164.649
162.469
157.313
156.172
144.415
134.282
121.355
116.191
113.486
111.802
60.997
50.714
38.880
38.171
32.198
27.115
14.117
OH
O
OEt
O
O
NH2
200
150
100
50
0
ppm (f1)

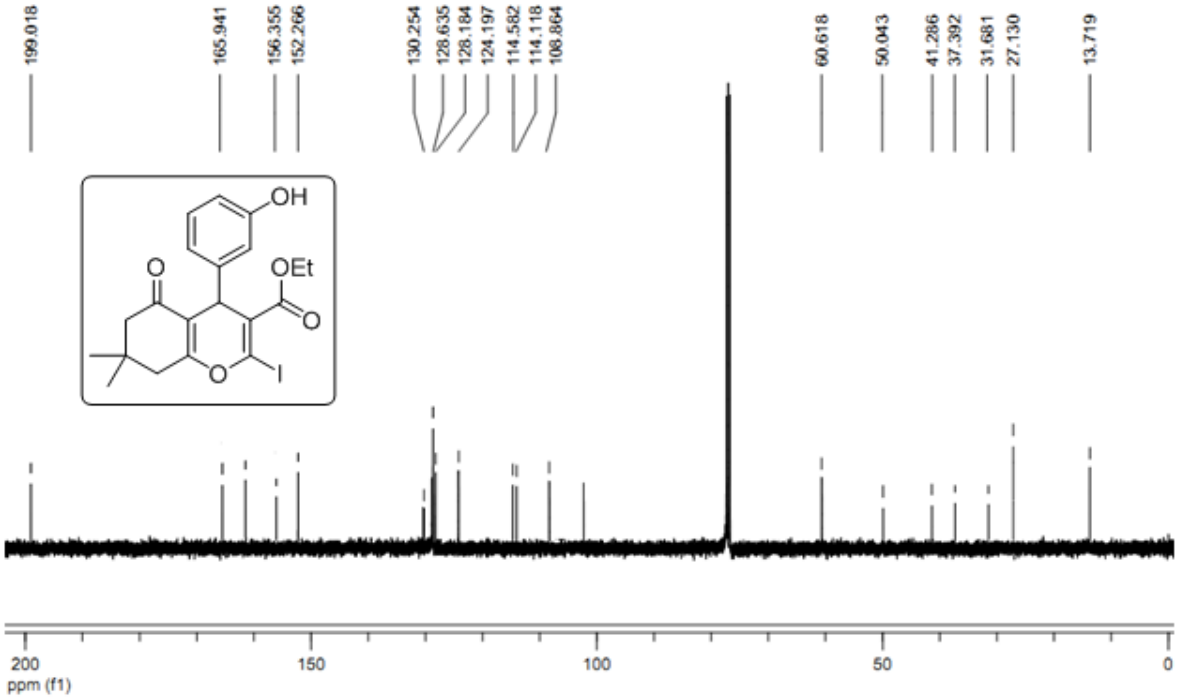

O
OEt
O
O
NH2
1.82
1.88
2.05
1.00
2.06
2.15
2.09
3.16
6.06
3.05
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

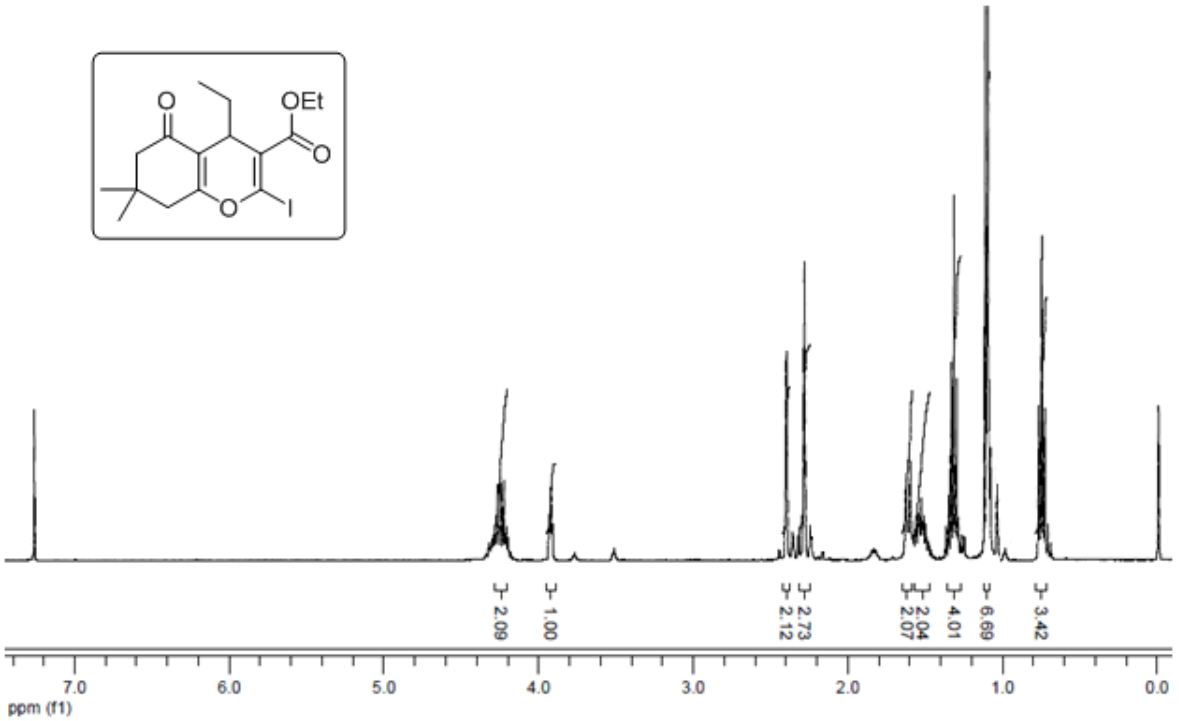

166.953
163.979
158.383
156.882
105.221
73.891
63.008
32.052
22.682
14.338
11.888
O
OEt
O
O
NH2
150
100
50
0
ppm (f1)

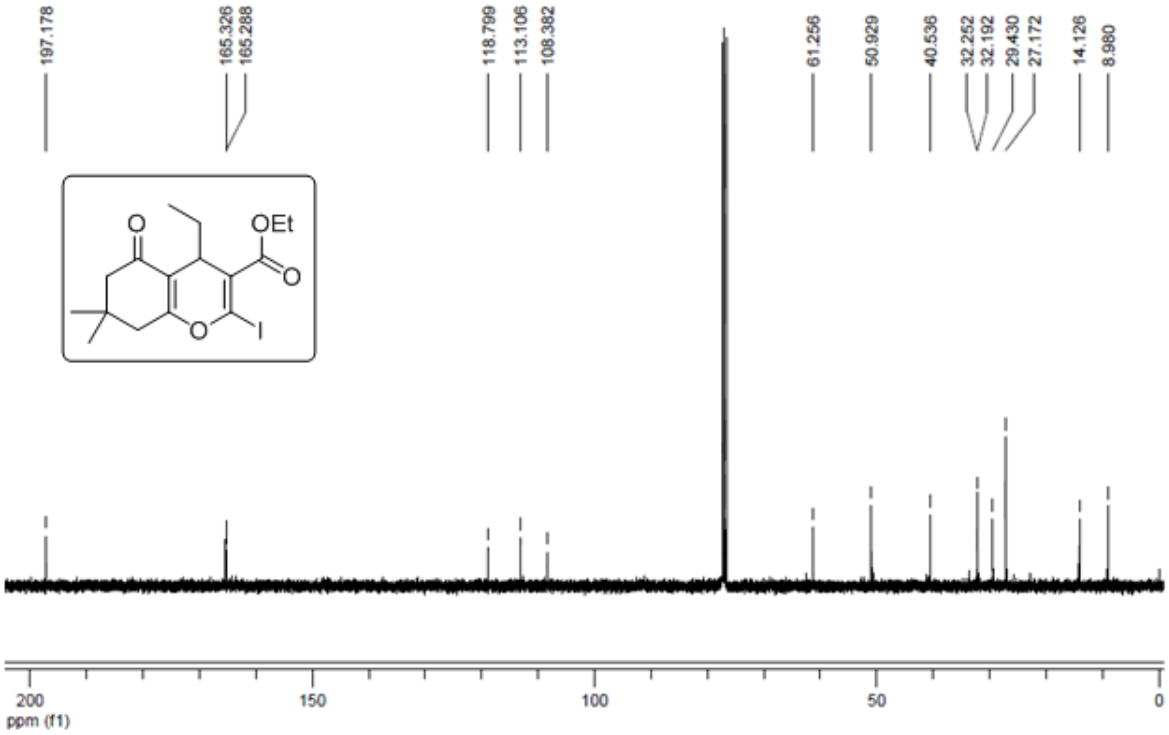

S
O
OEt
O
O
NH2
1.03
2.08
1.86
1.00
2.09
2.04
2.09
3.25
3.03
3.10
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

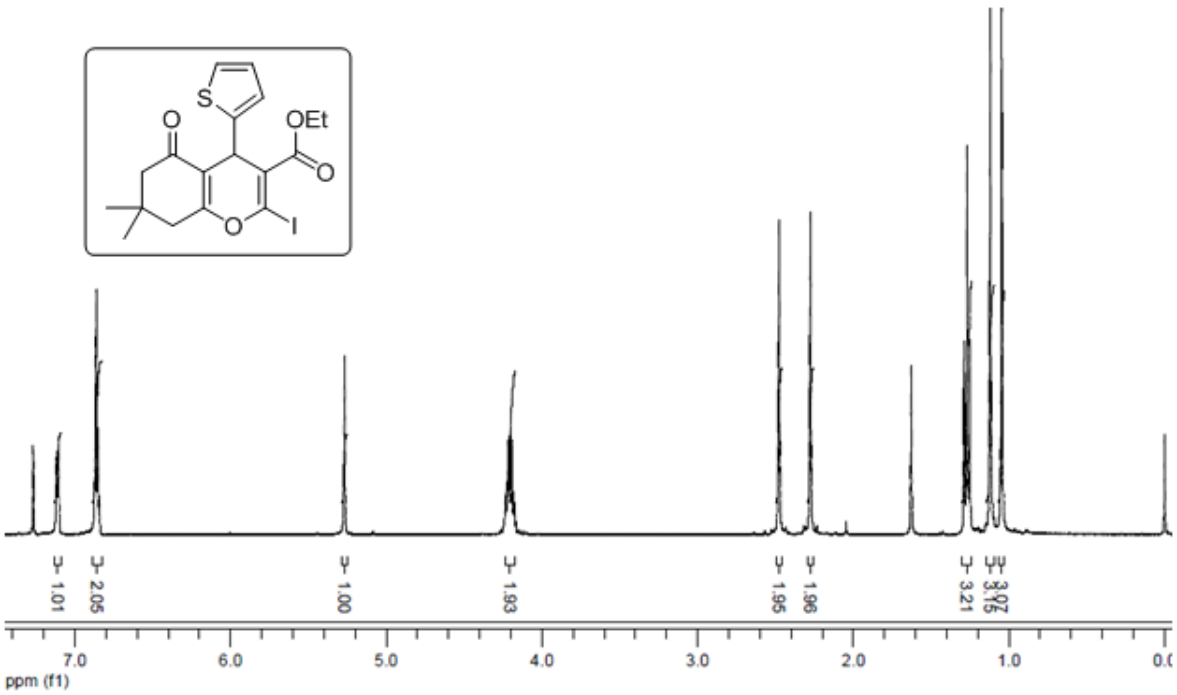

198.936
167.685
163.967
155.472
141.314
127.357
125.007
123.025
113.302
75.173
61.174
55.143
38.399
32.790
27.873
14.338
S
O
OEt
O
O
NH2
200
150
100
50
0
ppm (f1)

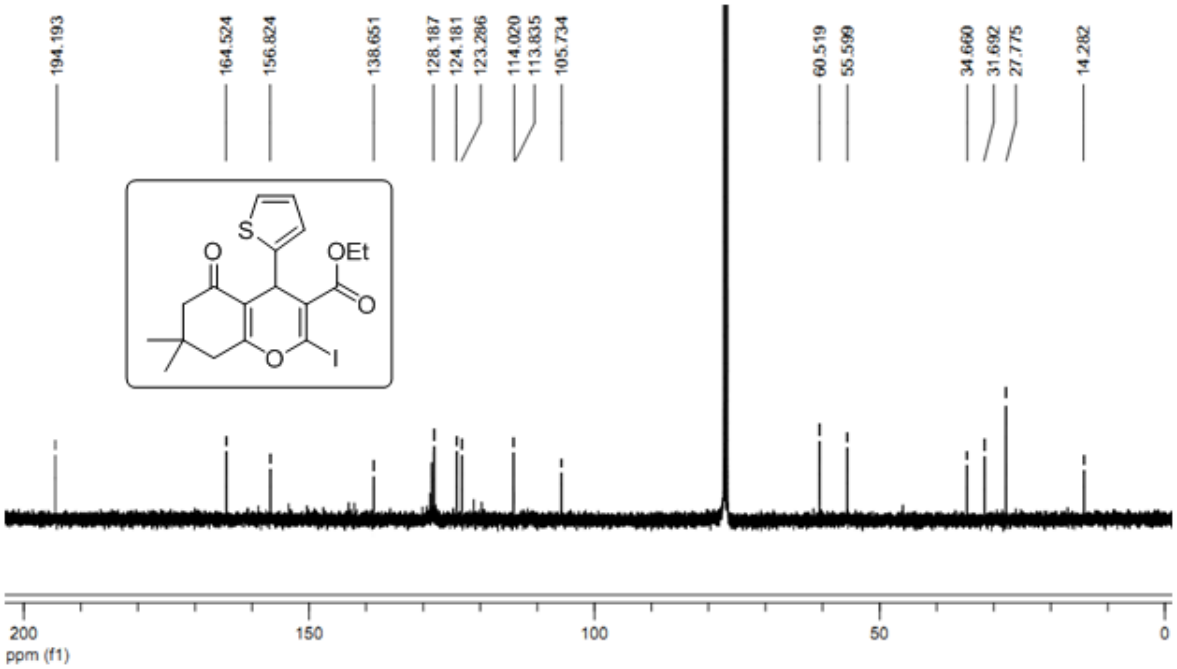

OH
O
OEt
O
O
I
0.97
2.31
1.03
1.01
1.00
2.57
2.01
2.01
3.13
3.06
3.12
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

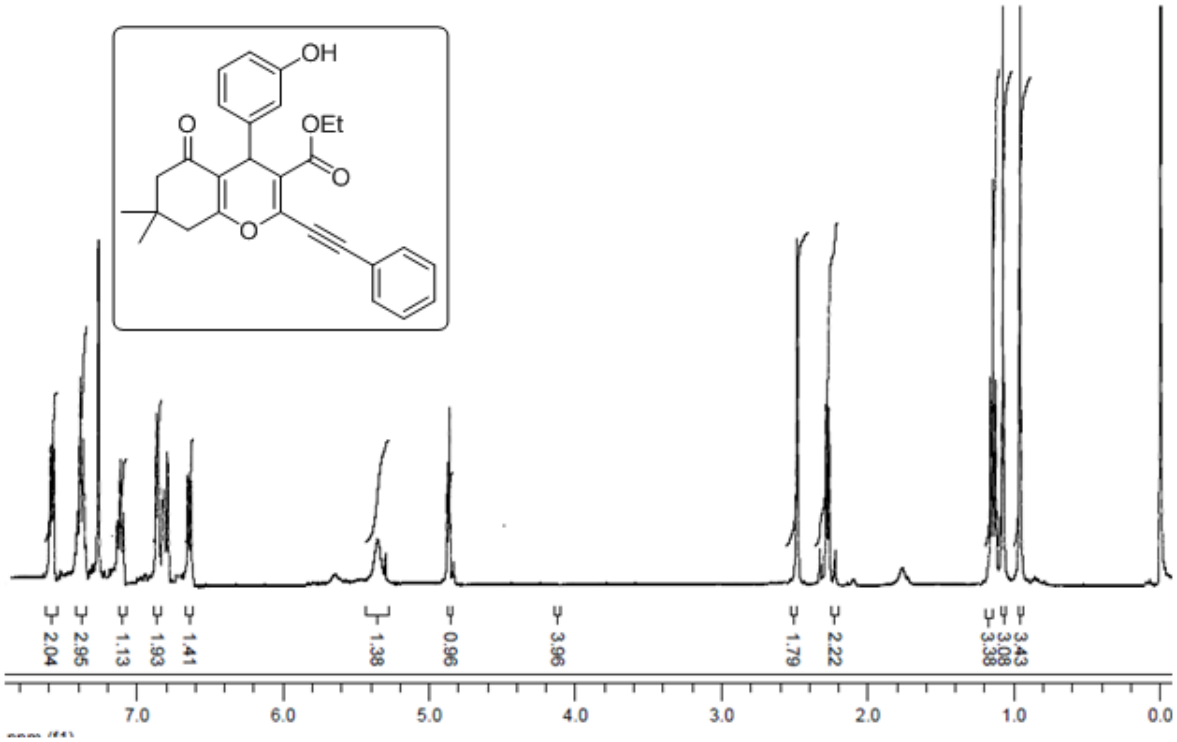

199.018
165.941
156.355
152.266
130.254
128.635
128.184
124.197
114.582
114.118
106.864
60.618
50.043
41.286
37.392
31.681
27.130
13.719
OH
O
OEt
O
O
I
200
150
100
50
0
ppm (f1)

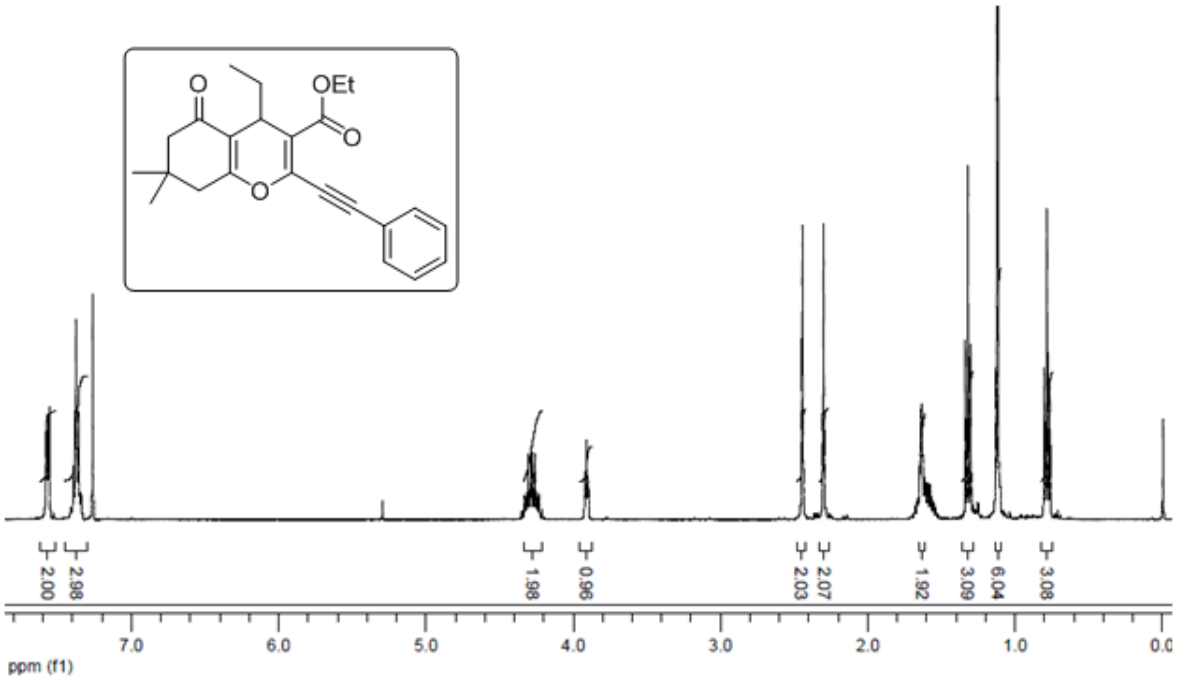

O
OEt
O
O
I
2.09
1.00
2.12
2.73
2.07
2.04
4.01
6.69
3.42
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

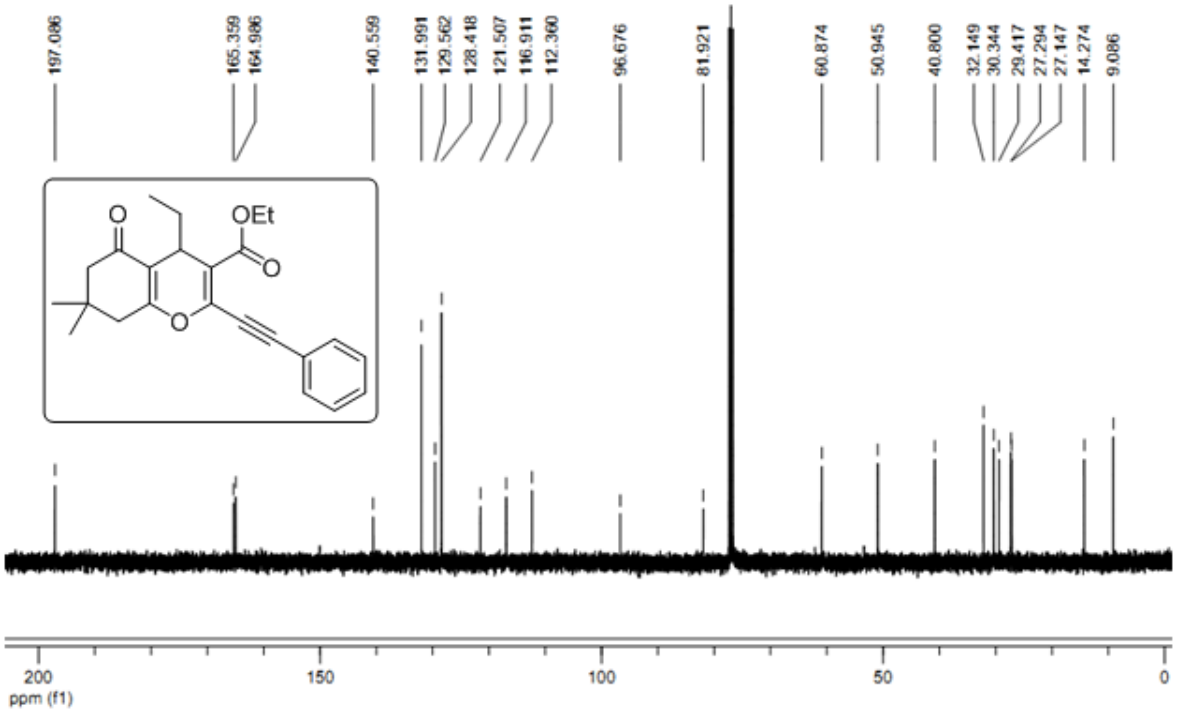

197.178
165.326
165.288
118.799
113.106
108.382
61.256
50.929
40.536
32.252
32.192
29.430
27.172
14.126
8.980
O
OEt
O
O
I
200
150
100
50
0
ppm (f1)

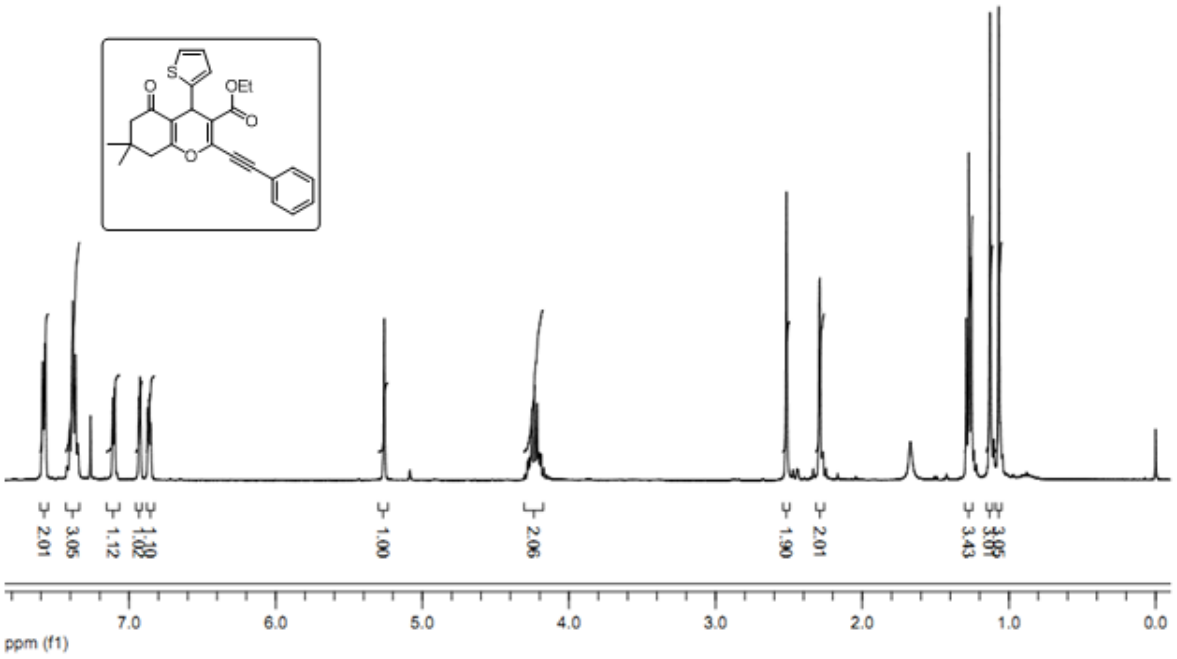

S
O
OEt
O
O
I
1.01
2.05
1.00
1.93
1.95
1.96
3.21
3.07
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

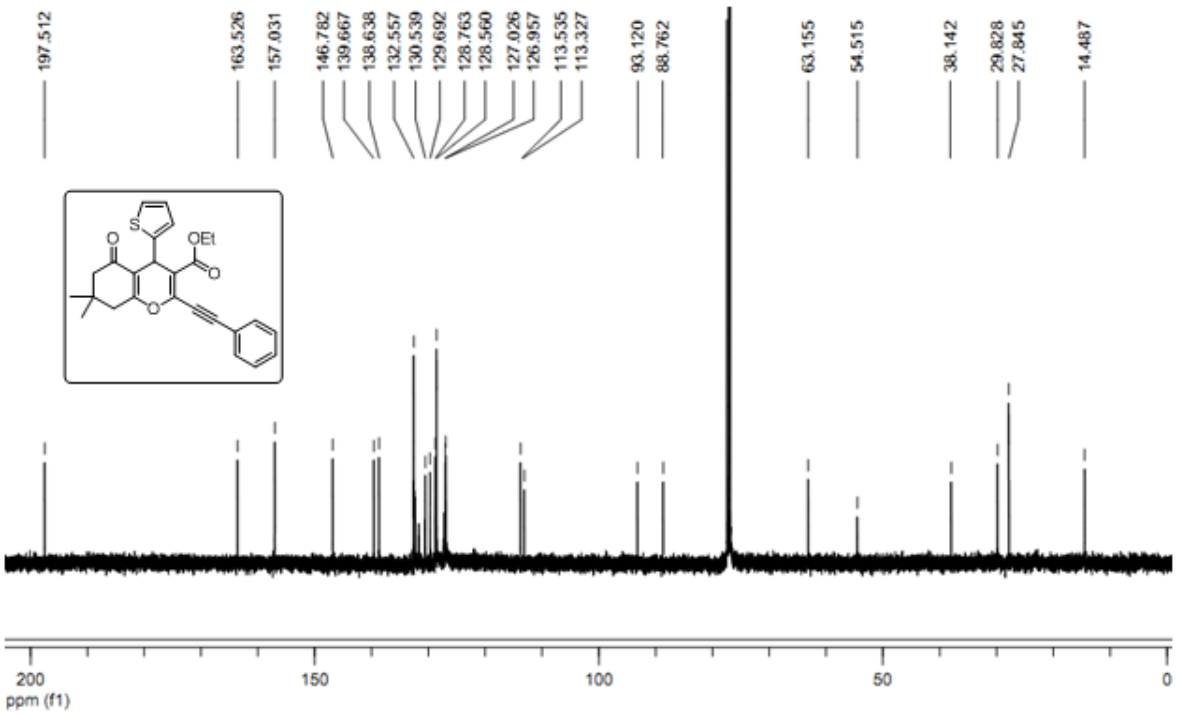

194.193
164.524
156.824
138.651
128.187
124.181
123.286
114.020
113.835
105.734
60.519
55.599
34.660
31.692
27.775
14.282
S
O
OEt
O
O
I
200
150
100
50
0
ppm (f1)

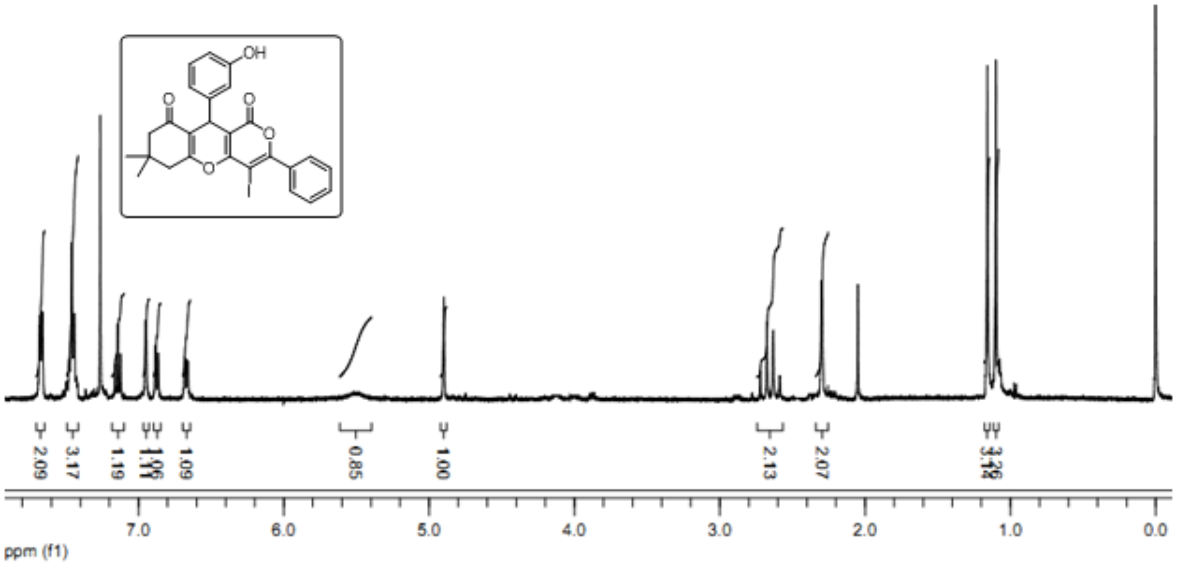

OH
O
OEt
O
O
2.04
2.95
1.13
1.93
1.41
1.38
0.96
3.96
1.79
2.22
3.38
3.08
3.43
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

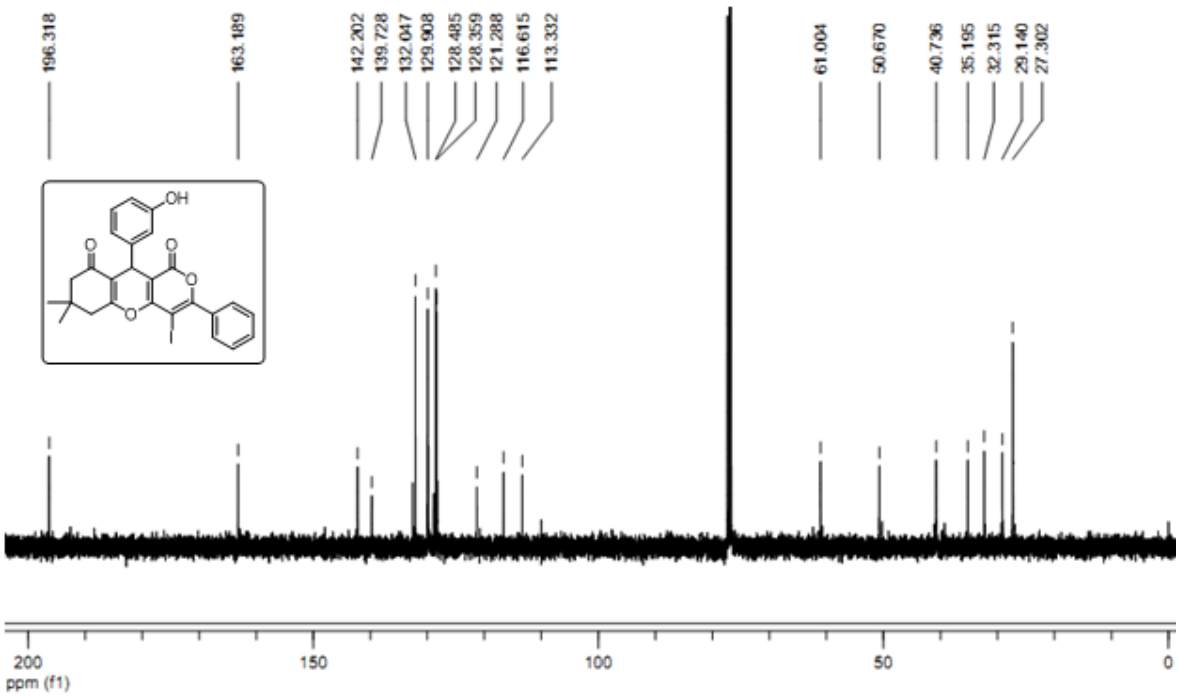

O
OEt
O
O
2.00
2.98
1.98
0.96
2.03
2.07
1.92
3.00
6.04
3.08
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

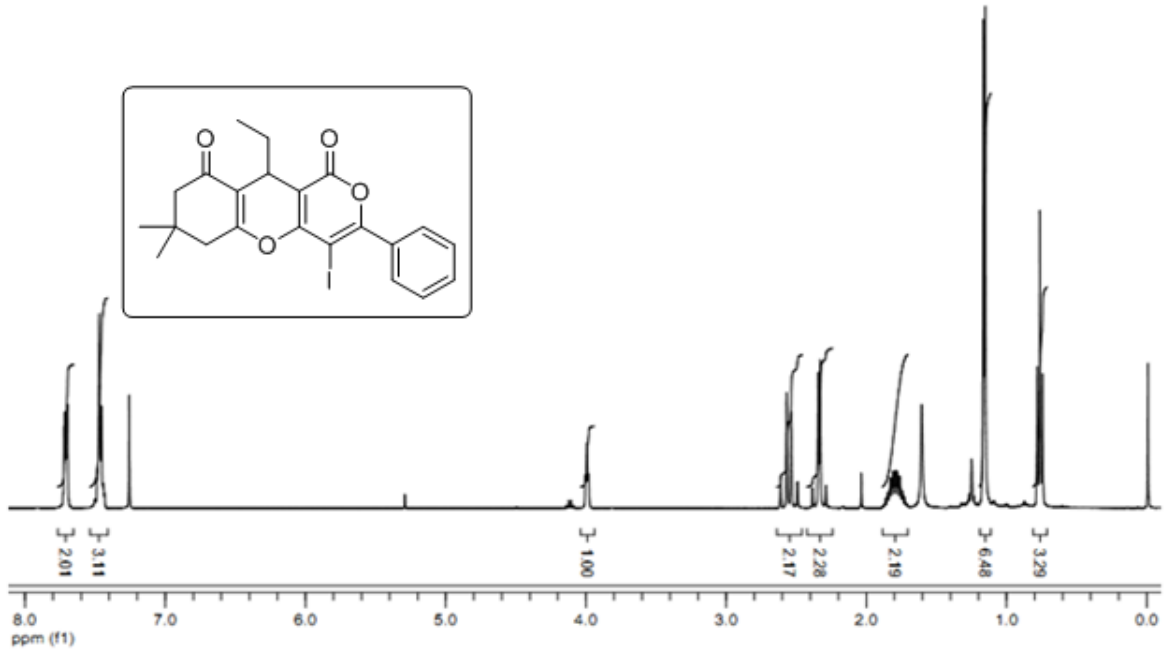

197.086
165.359
164.986
140.559
131.991
129.562
128.418
121.507
116.911
112.360
96.676
81.921
60.874
50.945
40.800
32.149
30.344
29.417
27.294
27.147
14.274
9.086
O
OEt
O
O
200
150
100
50
0
ppm (f1)

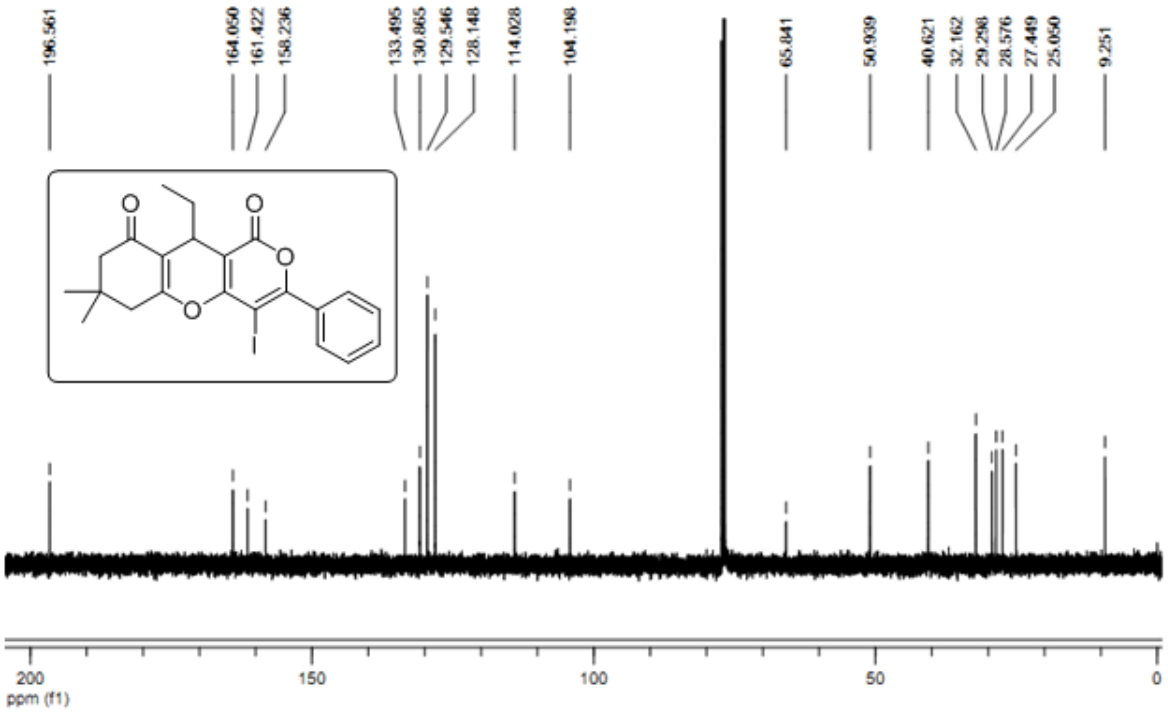

OEt
2.01
3.05
1.12
1.00
2.06
1.06
2.01
3.43
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

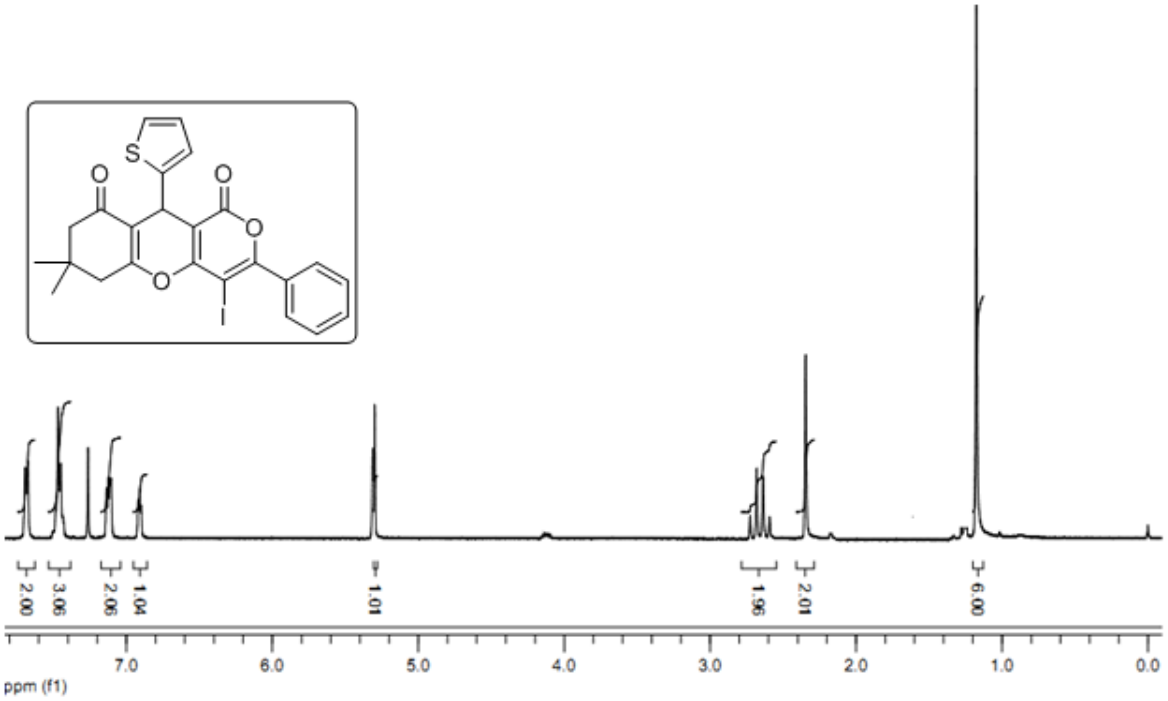

197.512
163.526
157.031
146.782
139.667
138.638
132.557
130.539
129.692
128.763
128.560
127.026
126.957
113.535
113.327
93.120
88.762
63.155
54.515
38.142
29.828
27.845
14.487
OEt
200
150
100
50
0
ppm (f1)

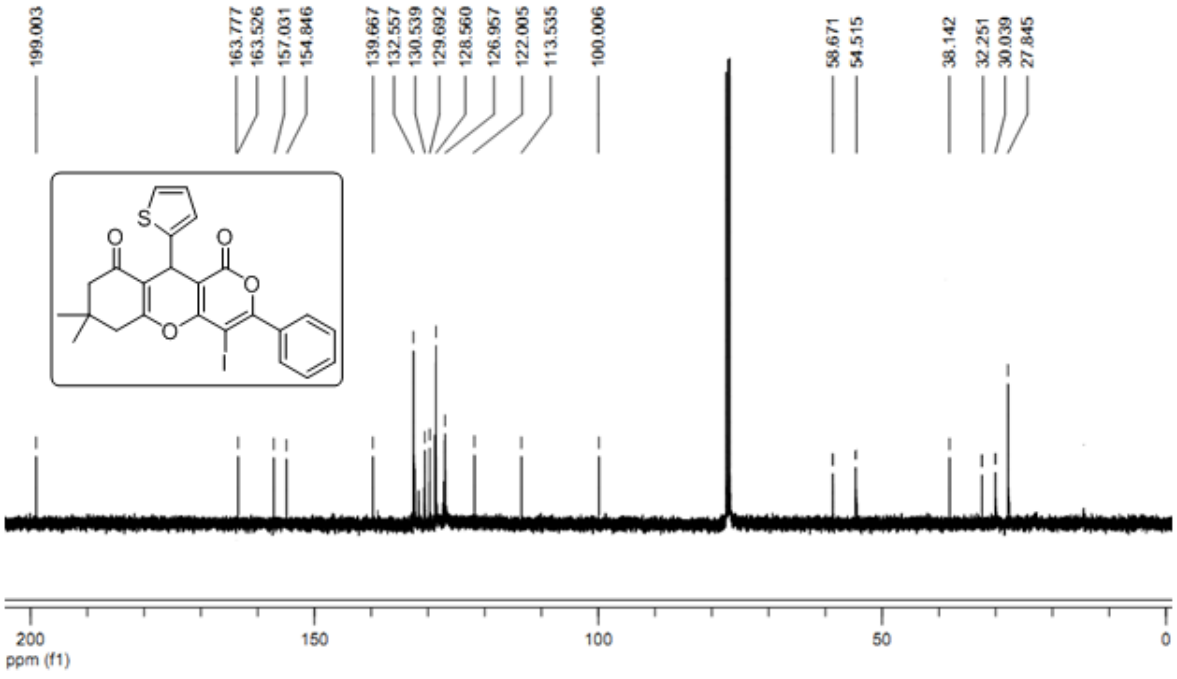

OH
O
O
O
O
2.09
3.17
1.19
1.06
1.11
1.09
0.85
1.00
2.13
2.07
3.49
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


196.318
163.189
142.202
139.728
132.047
129.908
128.485
128.359
121.288
116.615
113.332
61.004
50.670
40.736
35.195
32.315
29.140
27.302
OH
O
O
O
O
200
150
100
50
0
ppm (f1)


2.01
3.11
1.00
2.17
2.28
2.19
6.48
3.29
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

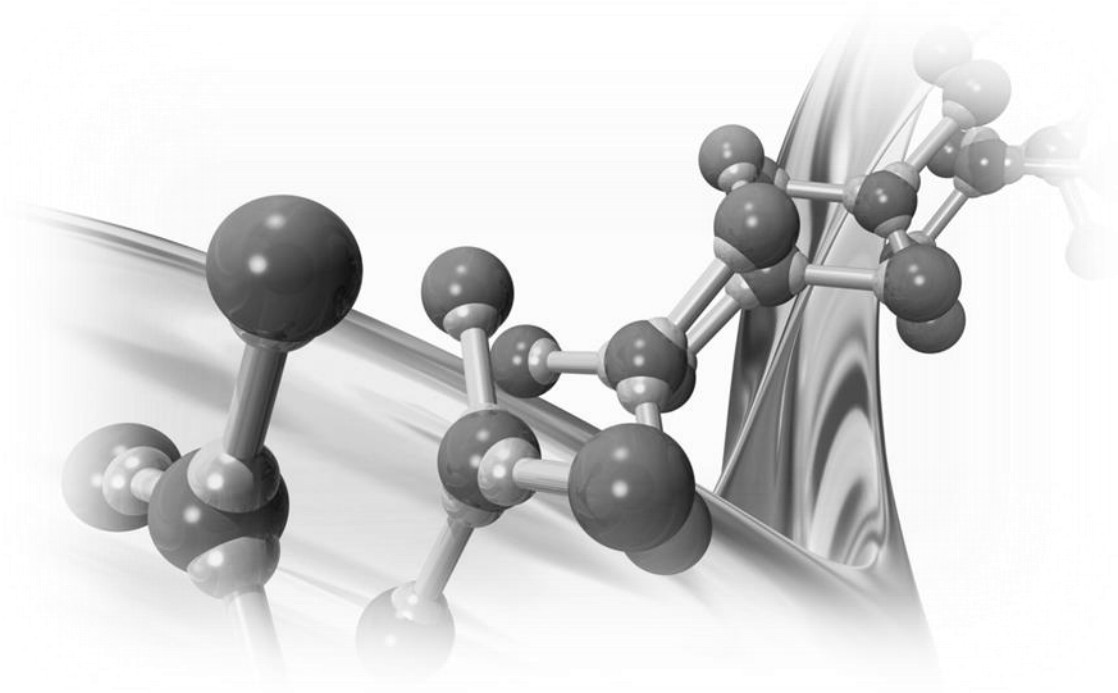

196.561
164.050
161.422
158.236
133.495
130.865
129.546
128.148
114.028
104.198
65.841
50.939
40.621
32.162
29.298
28.576
27.449
25.050
9.251
200
150
100
50
0
ppm (f1)


2.00
3.06
2.06
1.04
1.01
1.96
2.01
6.00
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)


199.003
163.777
163.526
157.031
154.846
139.667
132.557
130.539
129.692
128.560
126.957
122.005
113.535
100.006
58.671
54.515
38.142
32.251
30.039
27.845
200
150
100
50
0
ppm (f1)

CHAPTER 3

$AlCl_3$-mediated synthesis of functionalized olefins as potential inhibitors of sirtuins



3.1. Introduction

Functionalization of heteroarenes has drawn considerable attention in organic synthesis especially in the preparation of synthetic analogues of natural products and pharmaceuticals. Generally, Friedel–Crafts alkylations or acylations, nitrations, and halogenations are the classical methods that are used for the functionalization of arenes.[1] Friedel–Crafts alkylation or acylation of aromatic compounds is one of the fundamental methods for the introduction of C-C bond into the aromatic systems. The addition of an aromatic C–H bond to an unsaturated C–C bond that leads to the formation of multisubstituted olefins is an attractive strategy for the facile synthesis of aryl-substituted compounds from simple arenes without protection, deprotection steps[2] and prefunctionalization of arene such as halogenations.[3] In recent years, great attention has been paid to the metal-catalyzed hydroarylation of alkynes for the direct access of highly functionalized alkenes.[4a] Similarly, heteroarylation of aromatic and heteroaromatic compounds also attracted considerable interest.[4b]

The nitrogen heterocycles such as quinoxalines and its derivatives have a broad spectrum of biological activities.[5] For example, compound **A** is an inhibitor of p38 alpha MAP kinase[6] whereas compound **B** showed low nanomolar activity against JAK2 and suppressed proliferation of SET-2 cells *in vitro*.[7] **C** was identified as selective antagonist at human A(3) adenosine receptors.[8] Quinoxaline motif occurs in natural products like echinomycin & triostin-A. Some of the quinoxalines based bioactive molecules are shown in figure 1.

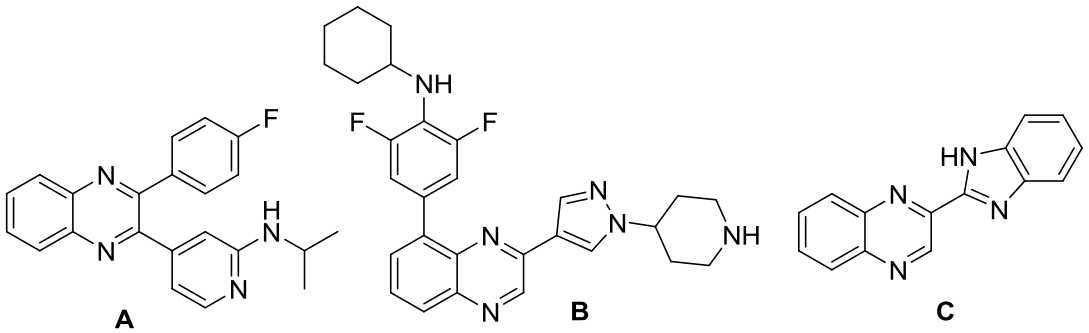
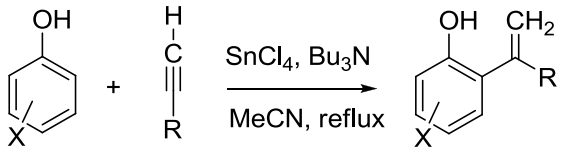


Figure 1. Quinoxaline-containing biologically active compounds.

3.2. Previous work

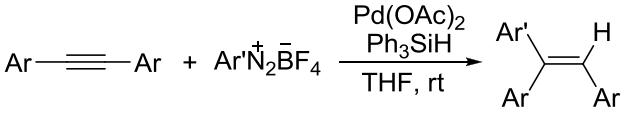
3.2.1. Hydroarylation reactions

Yamaguchi and co workers in 1995 described the direct *ortho*-vinylation and *ortho*-alkenylation of phenols by the reaction between 1-alkynes and phenols by using $SnCl_4$, and Bu_3N.[9]



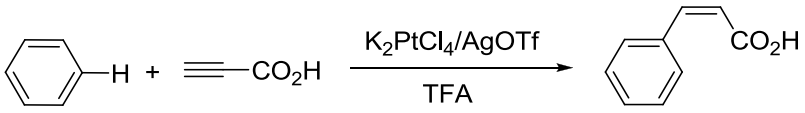
Scheme 1. *Ortho*-alkenylation of phenols with 1-alkynes.

In 2008, Cacchi and his co workers described the palladium-catalyzed hydroarylation of arenediazonium tetrafluoroborates with alkynes in the presence of triphenylsilane which afforded stereoselectively hydroarylation products in moderate to high yields.[10]



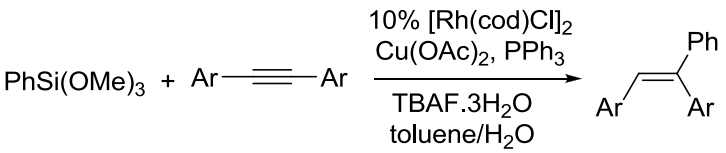
Scheme 2. Pd-catalyzed hydroarylation of diphenylacetylene with ArN_2BF_4.

In 2009, Kitamura *et al*. demonstrated that K_2PtCl_4/AgOTf catalyst has high hydroarylation activity toward less reactive benzene and toluene.[11]



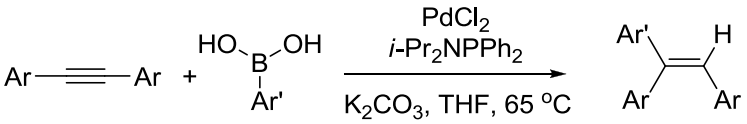
Scheme 3. Hydroarylation of propiolic acid with benzene K_2PtCl_4/AgOTf catalyst.

In the same year, Cheng *et al*. have developed an efficient and versatile rhodium catalyzed addition of $ArSi(OMe)_3$ to alkynes, providing the hydroarylation products.[12]



Scheme 4. Hydroarylation of alkynes with $PhSi(OMe)_3$

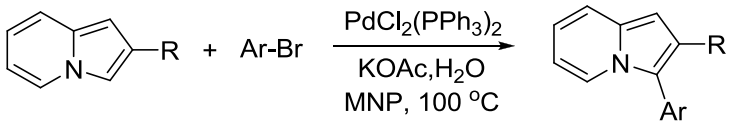
In 2010, Chen and co-workers have developed a palladium-catalyzed hydroarylation of symmetrical and unsymmetrical alkynes with aryl-boronic acids by using *i*-Pr_2NPPh_2 as the ligand and an inorganic base.[13]



Scheme 5. $PdCl_2$ -catalyzed hydroarylation of alkynes with arylboronic acids.

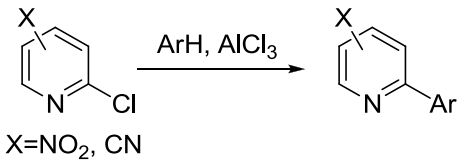
3.2.2. Heteroarylation reactions

Gevorgyan and co workers in 2004, developed Palladium-catalyzed regioselective C-3 aryl-and heteroarylation of indolizines.[14]



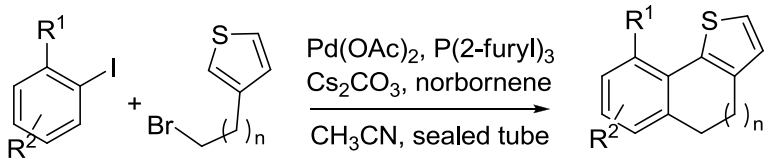
Scheme 6. Arylation and Heteroarylation of indolizines.

Pal *et. al.*, in 2005, showed the first $AlCl_3$-induced C-C bond-forming reaction between 2-halopyridines and arenes or heteroarenes, such reactions were only known by using transition metal catalysts.[15]



Scheme 7. $AlCl_3$-induced heteroarylation of arenes and heteroarenes.

In 2006, Lautens and his group developed a method for the synthesis of polycyclic sulfur containing heterocycles through a one-pot palladium-catalyzed ortho alkylation/direct heteroarylation reaction sequence.[16]



Scheme 8. Domino coupling of 3-(Bromoalkyl)thiophene.

3.3. Present work

The hydroarylation reactions of alkynes are valuable method for synthesis of aromatic alkenes. Transformations of aromatic C-H bonds are a burgeoning field in organic chemistry because it allows the efficient construction of organic building blocks.[17] Alkyne hydroarylation has received much attention in recent years because it allows an access for the synthesis of functionalized alkenes directly from simple arenes and alkynes.[18] The hydroarylation reactions of alkynes executed *via* aromatic C-H activation or through alkyne activation. On the other hand heteroarylation is also equally important process for the formation of C_{aryl}-C_{aryl} bonds. The traditional approach for this process is by employing Suzuki cross-coupling reaction which involves the use of expensive palladium catalyst and therefore may not be suitable for the large-scale preparation. The research on new and direct chemical methods which gives access to densely functionalized olefins is of fundamental interest for potential applications in chemical/pharmaceutical industries.[19] On the other hand highly substituted heteroaromatics has often played key roles in the early stage of drug discovery. Thus an appropriate assembly of alkene with a desired (hetero)arene in a single molecular motif is expected to show important pharmacological properties. This and our interest in novel heteroaromatics[20] prompted us to explore a new route to 2-(2,2-diarylvinyl)-3-arylquinoxaline (**E**, Fig. 2) as potential inhibitors of sirtuins. Sirtuins are NAD^+-dependent class III histone deacetylase proteins and are considered as promising targets for cancer therapeutics[21] as they are up regulated in various types of cancer. For example over expression of Sirtuin will suppress P^{53} a tumor suppressor gene. Inhibition of sirtuins leads to reduced growth of cancer cells due to the re-expression of silenced tumor suppressor genes. We designed compound **E** based on our earlier observation on activities of 3-enynyl flavones **D** which were tested against a panel of cancer cell lines. We envisioned that the designed[22] compound **D** could show anti cancer

property. Based on the above discussed concepts and our interest in $AlCl_3$ mediated reactions we carried out both hydro/heteroarylation reactions in step.

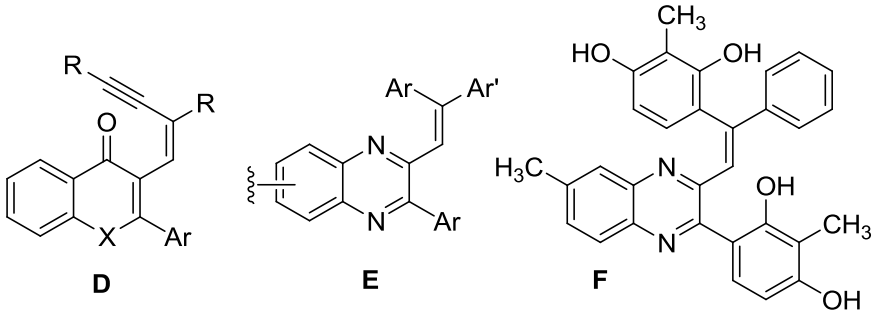
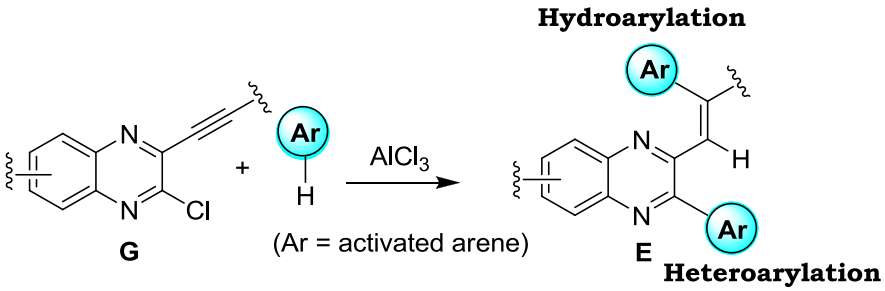


Figure 2. Design of **E**/**F** as novel inhibitors of sirtuins

Recently, in addition to olefin metathesis the transition metal mediated hydroarylation of alkynes have emerged as an effective tool for the quick access to substituted olefines.[23] And also different metal catalyzed reactions are reported in the literature.[24] These reports triggered us to envision the possibility of an $AlCl_3$-mediated hydroarylation of alkyne and heteroarylation of arene in a single pot leading to **E/F.** In this chapter we discuss the one pot hydroarylation / heteroarylation of 2-chloro-3-(arylethynyl)quinoxaline **G** leading to 2-(2,2-diarylvinyl)-3-arylquinoxaline **E** (Scheme 9).

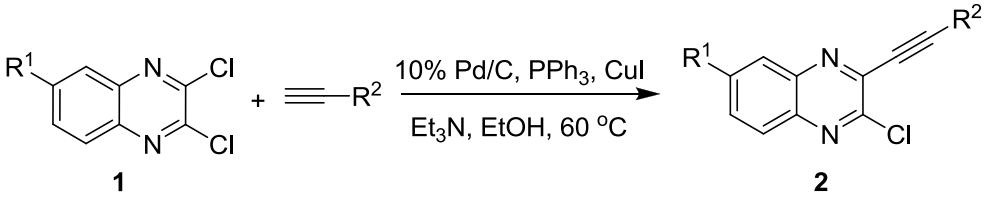


Scheme 9. $AlCl_3$-mediated hydroarylation / heteroarylation of **G**.

3.4. Results and Discussion

The key starting material **2/G** required was synthesized *via* a selective mono alkynylation of 2,3-dichloroquinoxaline (**1**) under Sonogashira coupling reaction conditions using Pd/C-Cu catalysis (Table 1).

Table 1. Synthesis of 3-alkynyl-2-chloroquinoxalines **2**.[a]

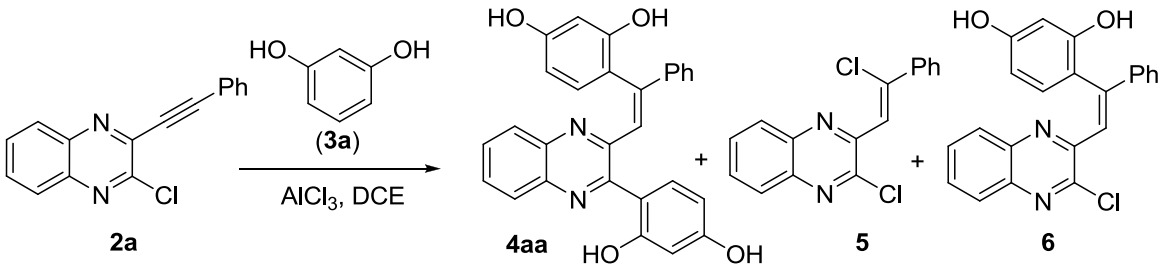


Entry	Halide (**1**); R^1 =	Alkyne; R =	Time (h)	Product (**2**)	Yield[b] (%)
1	**1a**; H	Ph	2	**2a**	76
2	**1a**	C_6H_4Me-*p*	3	**2b**	70
3	**1a**	CMe_3	3	**2c**	64
4	**1b**; Me	Ph	4	**2d**	66
5	**1b**	C_6H_4Me-*p*	4	**2e**	68
6	**1c**; NO_2	Ph	4	**2f**	63

[a]All reactions were performed by using **1** (1.256 mmol), terminal alkyne (1.256 mmol), 10% Pd/C (0.0125 mmol), PPh_3 (0.0502 mmol), CuI (0.0125 mmol), and Et_3N (1.8844 mmol) in EtOH (4mL). [c]Isolated yield.

While looking at the possibility of hydroarylation / heteroarylation reaction a mixture of **5** and **6** were obtained (entry 1 & 2, Table 2), when 2-chloro-3(phenylethynyl) quinoxaline **2a** was reacted with resorcinol **3a** in the presence of $AlCl_3$ (2 equiv) in 1,2-dichloroethane (DCE) at 50 °C for 15-60 min. The use of 3 equiv of $AlCl_3$ for 30-60 min did not change the outcome significantly (entry 3 & 4, Table 2). A further increase in $AlCl_3$ quantity to 4 equiv afforded the desired hydroarylation / heteroarylation product **4aa** in good yield (entry 5, Table 2). An attempt to perform the reaction at room temperature was not successful as the yield of **4aa** was decreased significantly (entry 6, Table 2). Thus the condition of entry 5 (Table 2) was found to be optimum and used for further studies.

Table 2. Effect of conditions on hydroarylation / heteroarylation of **2a.**[a]

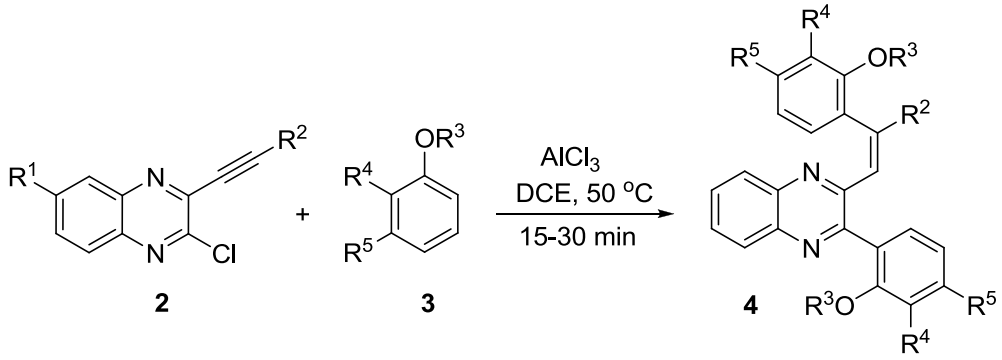


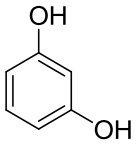
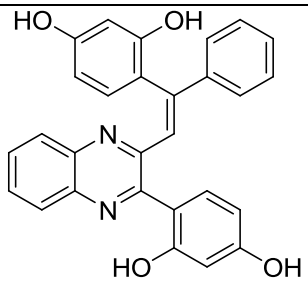
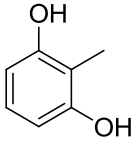
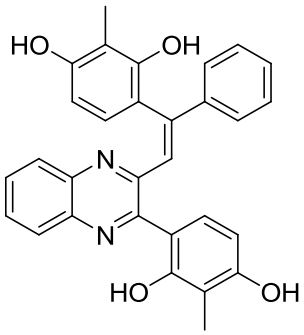
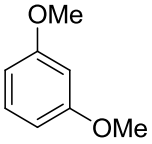
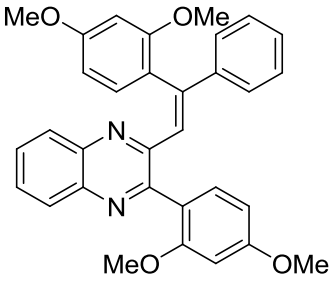
Entry	Arene **3a** (equiv)	$AlCl_3$ (equiv)	Time (min)	Yield[b] (%)		
				4aa	**5**	**6**
1	2	2	15	0	35	45
2	2	2	60	0	32	40
3	3	3	30	0	30	47
4	3	3	60	0	30	47
5	4	4	15	82	5	0
6	4	4	60[c]	48	10	0

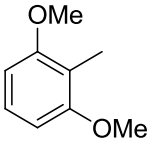
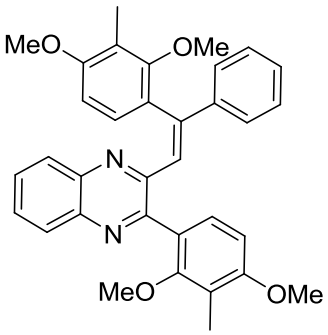
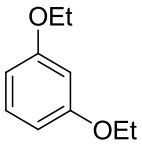
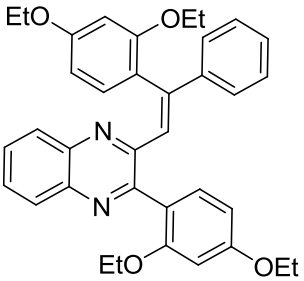
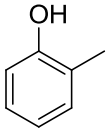
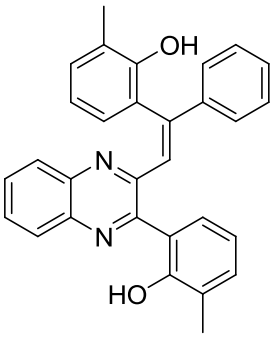
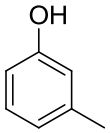
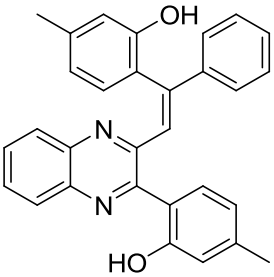
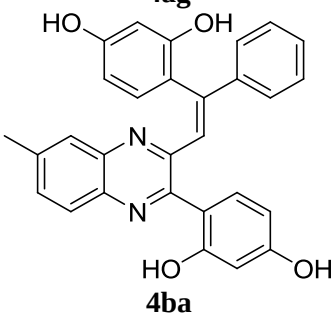
[a]Reaction conditions: **2a** (0.19 mmol), **3a** (mmol), $AlCl_3$ in DCE (2 mL). [b]Isolated yield. [c]Reaction was performed at room temperature.

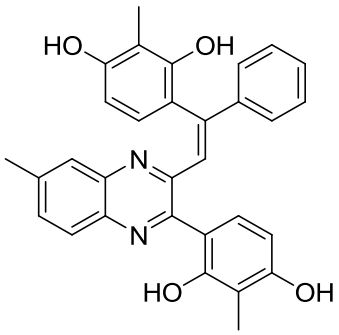
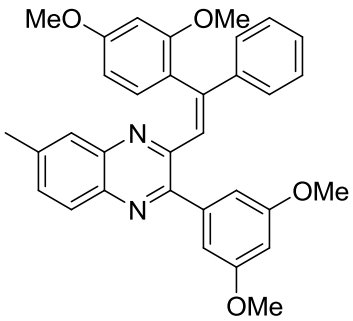
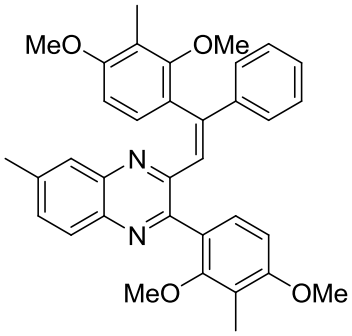
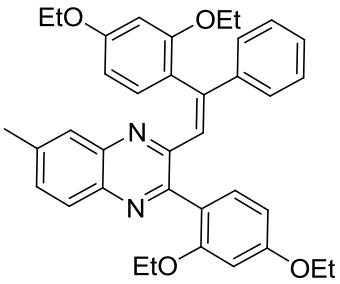
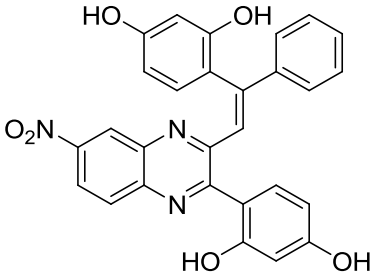
We then examined the substrate scope and generality of this methodolgy using 4 equiv of $AlCl_3$ and 4 equiv of activated arenes in presence of dichloroethane solvent at 50 °C (Table 3). The alkyne **2** containing contrasting groups e.g. CH_3 (**2b**) and NO_2 (**2c**) on the quinoxaline ring and aryl (**2a-e**) and alkyl (**2e**) substituents on the triple bond participated well in the reaction. However, the arene **3** containing electron donating groups only participated in the present reaction and the use of unactivated or deactivated arenes was not successful. Nevertheless, thirty new compounds were synthesized by this methodology.

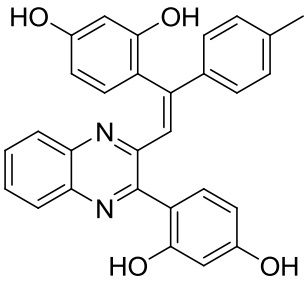
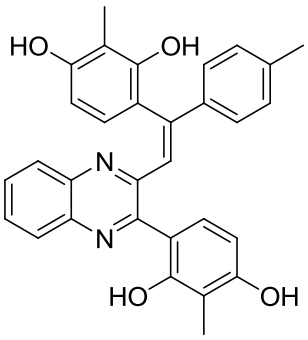
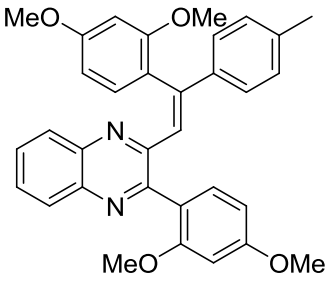
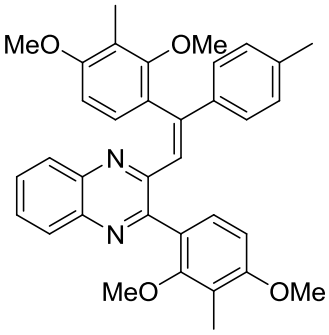
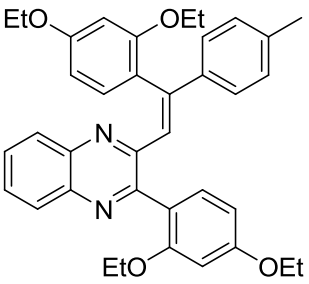
Table 3. Synthesis of 2-(2, 2-diarylvinyl)-3-arylquinoxaline **4**.[a]

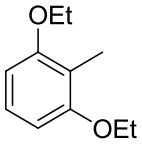
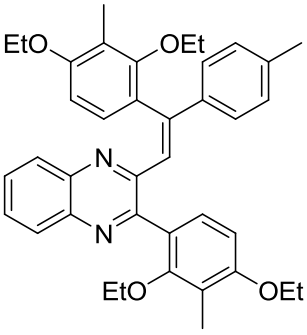
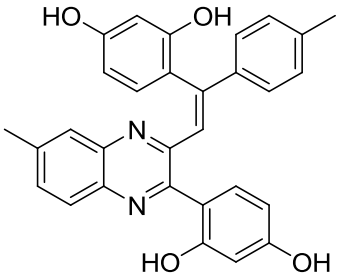
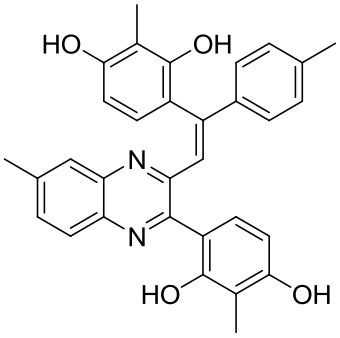
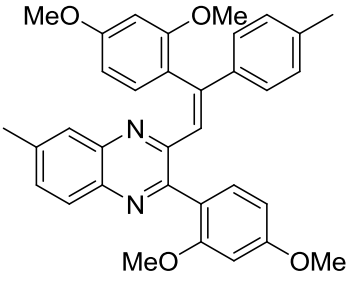
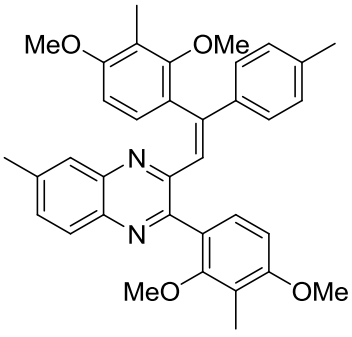


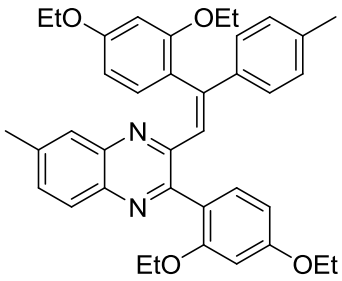
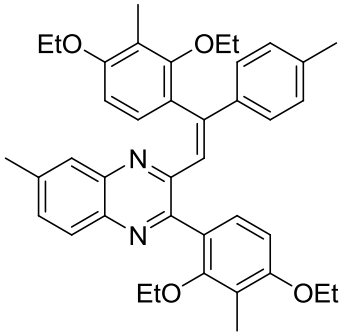
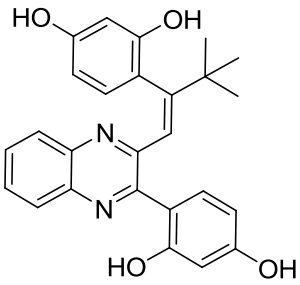
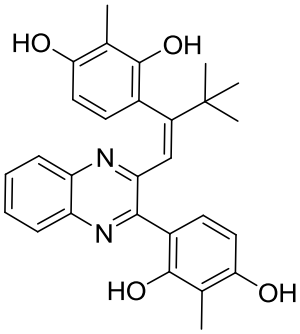
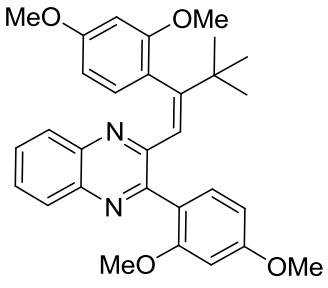
Entry	Alkyne (**2**) R^1, R^2	Arene (**3**)	Product (**4**)	Time (min)	Yield (%)[b]
1	H, Ph; **2a**	 **3a**	 **4aa**	15	82
2	**2a**	 **3b**	 **4ab**	15	79
3	**2a**	 **3c**	 **4ac**	15	64

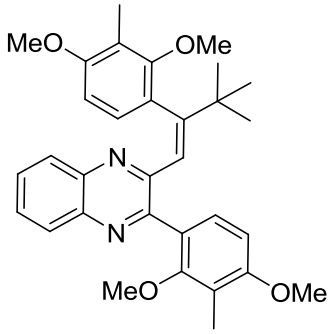
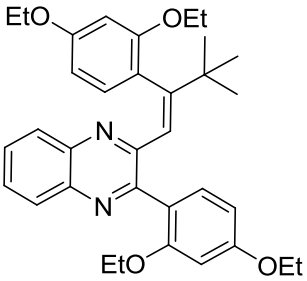
4	**2a**	 **3d**	 **4ad**	15	68
5	**2a**	 **3e**	 **4ae**	30	65
6	**2a**	 **3f**	 **4af**	30	62[c]
7	**2a**	 **3g**	 **4ag**	30	55[c]
8	CH_3, Ph; **2b**	**3a**	 **4ba**	15	80

9	**2b**	**3b**	 **4bb**	15	78
10	**2b**	**3c**	 **4bc**	15	73
11	**2b**	**3d**	 **4bd**	30	71
12	**2b**	**3e**	 **4be**	30	66
13	NO_2, Ph; **2c**	**3a**	 **4ca**	30	60

14	H,C_6H_4Me-*p*; **2d**	**3a**	 **4da**	15	71
15	**2d**	**3b**	 **4db**	15	69
16	**2d**	**3c**	 **4dc**	15	63
17	**2d**	**3d**	 **4dd**	30	67
18	**2d**	**3e**	 **4de**	30	58

19	**2d**	 **3h**	 **4dh**	30	55
20	CH_3, C_6H_4Me-*p*; **2e**	**3a**	 **4ea**	15	74
21	**2e**	**3b**	 **4eb**	15	71
22	**2e**	**3c**	 **4ec**	15	64
23	**2e**	**3d**	 **4ed**	15	60

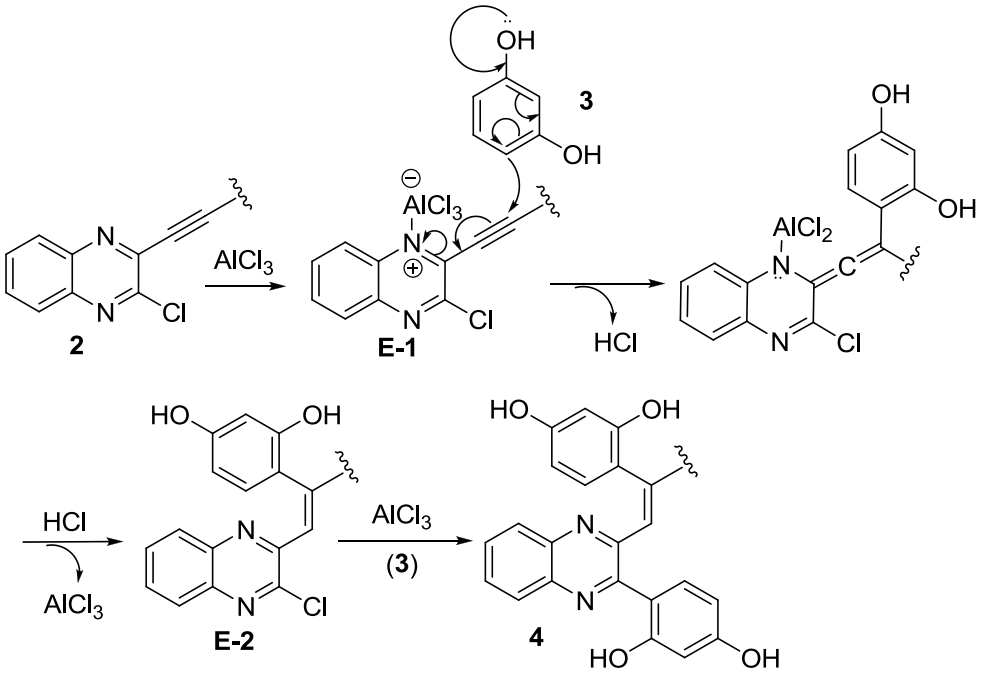
24	**2e**	**3e**	 **4ee**	30	56
25	**2e**	**3h**	 **4eh**	30	58
26	H, CMe_3; **2f**	**3a**	 **4fa**	30	55
27	**2f**	**3b**	 **4fb**	30	62
28	**2f**	**3c**	 **4fc**	30	60

29	**2f**	**3d**	 **4fd**	30	63
30	**2f**	**3e**	 **4fe**	30	61

[a]All reactions were carried out using **2** (0.3787 mmol), **3** (1.5151 mmol) $AlCl_3$ (1.5151 mmol) in DCE (4 mL) at 50 °C. [b]Isolated yield. [c]A 1:1 mixture of two regioisomers was isolated.

All compounds are characterized by spectral (NMR, IR and MS) data. The *Z*-geometry of vinylic sp^2 carbon was assigned by NOE experiment using **4aa** of which the vinylic H interacted with the Ph protons indicating they are on the same side of the double bond. (See experimental section).

Mechanism of this reaction (Scheme 10), proceeds through the complexation of $AlCl_3$ with *N*-4 of **2** that facilitates a nucleophilic attack by the arene **3** at the adjacent alkynyl moiety of **E-1** (the hydroarylation step). The release of $AlCl_3$ afforded **E-2** the second nitrogen of which on complexation with $AlCl_3$ followed by the attack of **3** at the adjacent carbon afforded the product **4.** Since nucleophilicity of the reacting arenes is crucial, the reaction therefore proceeded smoothly with electron rich arenes. While the alternative path i.e. heteroarylation followed by hydroarylation can not be ruled out completely. The isolation of **6** seems to support the former one. Moreover, the $AlCl_3$ mediated reaction of **5** with **3a** did not provide **4aa**.



Scheme 10. The proposed reaction mechanism.

3.5. Pharmacology

3.5.1. Sir2 reporter silencing assay in yeast cells (*in vitro*)

The compounds **4** and Splitomicin[25] (a known Sir2 inhibitor) were tested at 50 µM for their ability to inhibit Sir 2 protein [yeast sirtuin family NAD- dependent histone deacetylase (HDAC)] by estimating at telomeric locus, in the presence of 5-fluoroorotic acid (5-FOA).[26] As Sir2 protein is inhibited, the URA3 gene would be de-repressed resulting the death of yeast cell in the presence of 5-FOA. A parallel screen was performed in the absence of 5-FOA to check the cytotoxicity of compounds tested.

Among them compound **4bb** (**F**, Fig. 2) showed significant inhibition (~85%) in presence of 5-FOA and no significant toxic effect in the absence of 5-FOA (Fig.3). The dose dependent concentration of compound **4bb** showed IC_{50}~13.5 µM (Fig. 4) which is comparable to Splitomicin's IC_{50} 4.2 µM. The growth inhibition of yeast in presence of **4bb** which is due to inhibition of HDAC activity of Sir 2 protein. We then carried out dose response study of compound **4bb** on mammalian SIRT1 enzyme which showed the IC_{50}~32.9 µM, whereas the IC_{50} value of known inhibitor Splitomicin's 60 µM.[25]

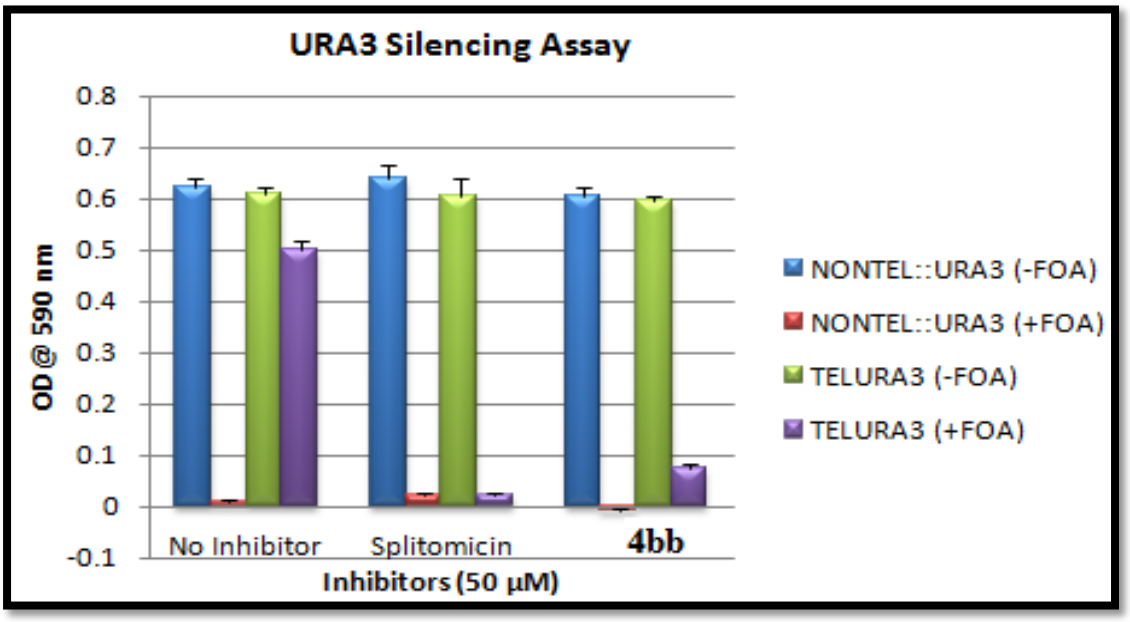


Figure 3. Inhibition of Sir 2 protein mediated transcriptional silencing at the telomeric locus in yeast by **4bb.** (NONTEL = non telomeric, TEL = telomeric, FOA = absence of FOA, +FOA = presence of FOA).

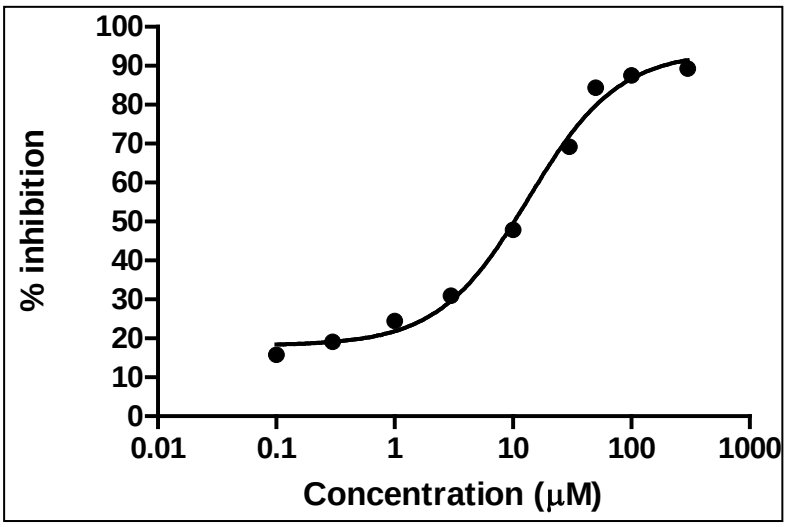


Figure 4. Dose dependent URA3 silencing assay of **4bb.**

3.5.2. Dose response study of compound 4bb on mammalian SIRT1

A dose response study was carried out with the compound, **4bb** using Invitro cyclex SIRT1/Sir2 Deacetylase Fluorometric Assay Kit to determine its IC_{50} using Graph pad software. IC_{50} for the compound **4bb** is found to be 32.92 µM (Fig.5).

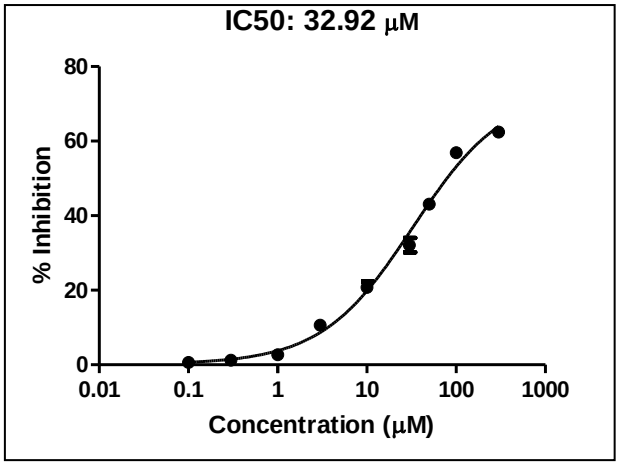


Figure 5. IC_{50} of compound **4bb** by *in vitro* assay on SIRT1 enzyme.

3.5.3. Treatment of HepG2 cancerous cells with inhibitor 4bb

The HepG2 Hepatic cells are seeded into three different 70 mm plates, one million cells in each. The cells are treated with the compound **4bb** at 50 µM and 100 µM concentrations for 48 h time point as shown in the figure 6. The untreated cells show a triangular morphology and are well adhered. In the treated cells, the growth of the cells is inhibited and rounded morphology was observed and the cells are released into media.

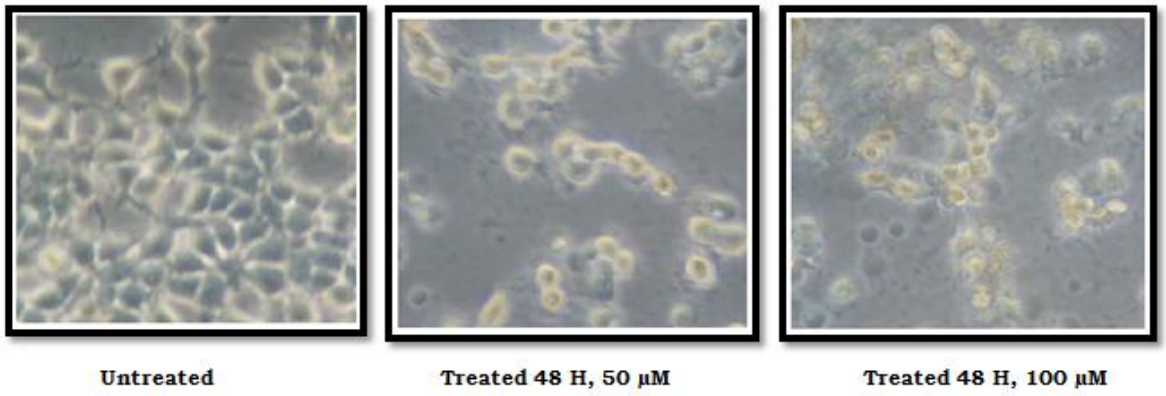


Figure 6. Treatment of HepG2 cancer cells with the compound **4bb**

3.5.4. Docking studies of 4bb with yeast Sir2 (*in silico*)

The docking analysis of molecules was performed using FRED, version 3.0.1 implemented from OpenEye Scientific Software.[27] The crystal structure coordinates of NAD-dependent protein deacetylase were obtained from the protein data bank (PDB ID: 2HJH).[28] The *in silico* binding studies of a representative compound **4bb** in the catalytic pocket of yeast Sir2 (Fig. 7) showed binding of **4bb** deep into the active site (docking score -8.0) along with two H-bond interactions of –OH group with the backbone –NH of Asp 498 and the side chain amino group of Asn 496. Contributing factors for docking score was shown in Table 4.

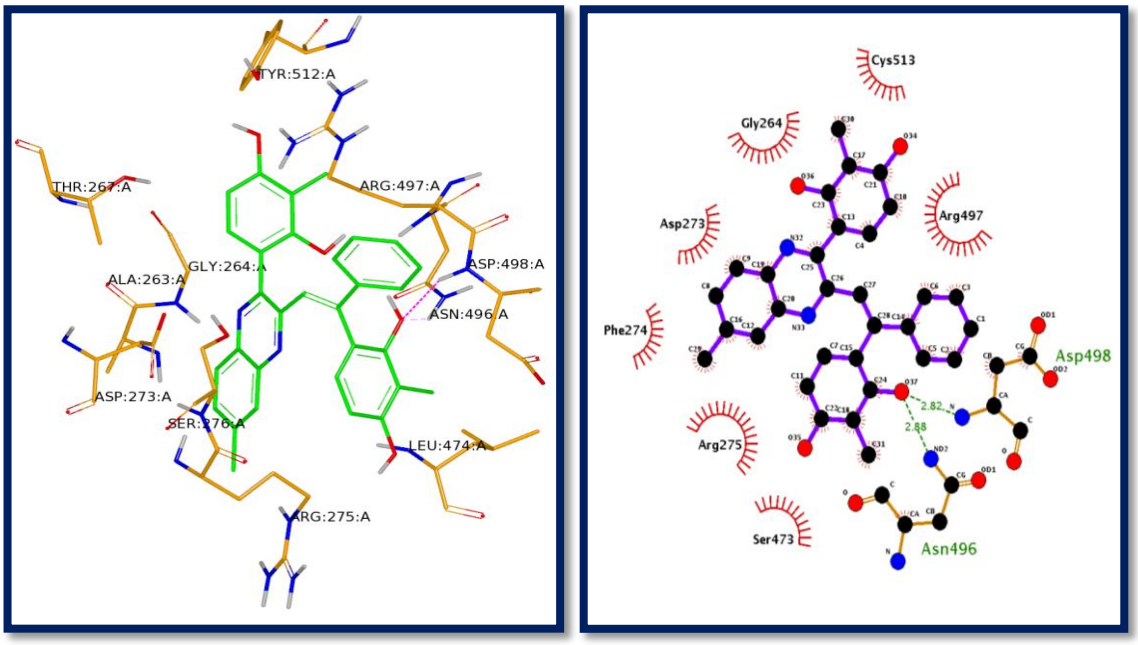


Figure 7. The binding mode of compound **4bb** and 2d interaction plot.

Table 4. Dock score and its contributing factors.

Molecule	Dock Score[a]	Shape	Protein Desolvation	Ligand Desolvation	Hydrogen bond
4bb	-8.0	-15.7	7.8	3.02	-3.09

[a]FRED Chemgauss4 score

3.5.5. *In vivo* Zebrafish embryo toxicity studies

Morphological evaluation was carried out by trained personnel in a blinded fashion.[29] All the embryos in control group were found normal. 3mM Phenobarbital was taken as positive control, where toxic effects were found. The compound was tested at concentrations starting from 10nM till 30µM (i.e. the highest soluble concentration). At all concentrations, the compound was found to be non toxic with no adverse effects. Mild liver, intestine and swim bladder toxicity were observed at high concentrations of 3µM and 10µM. No Observed Adverse Effect Level (NOAEL) was observed at 10 µM.

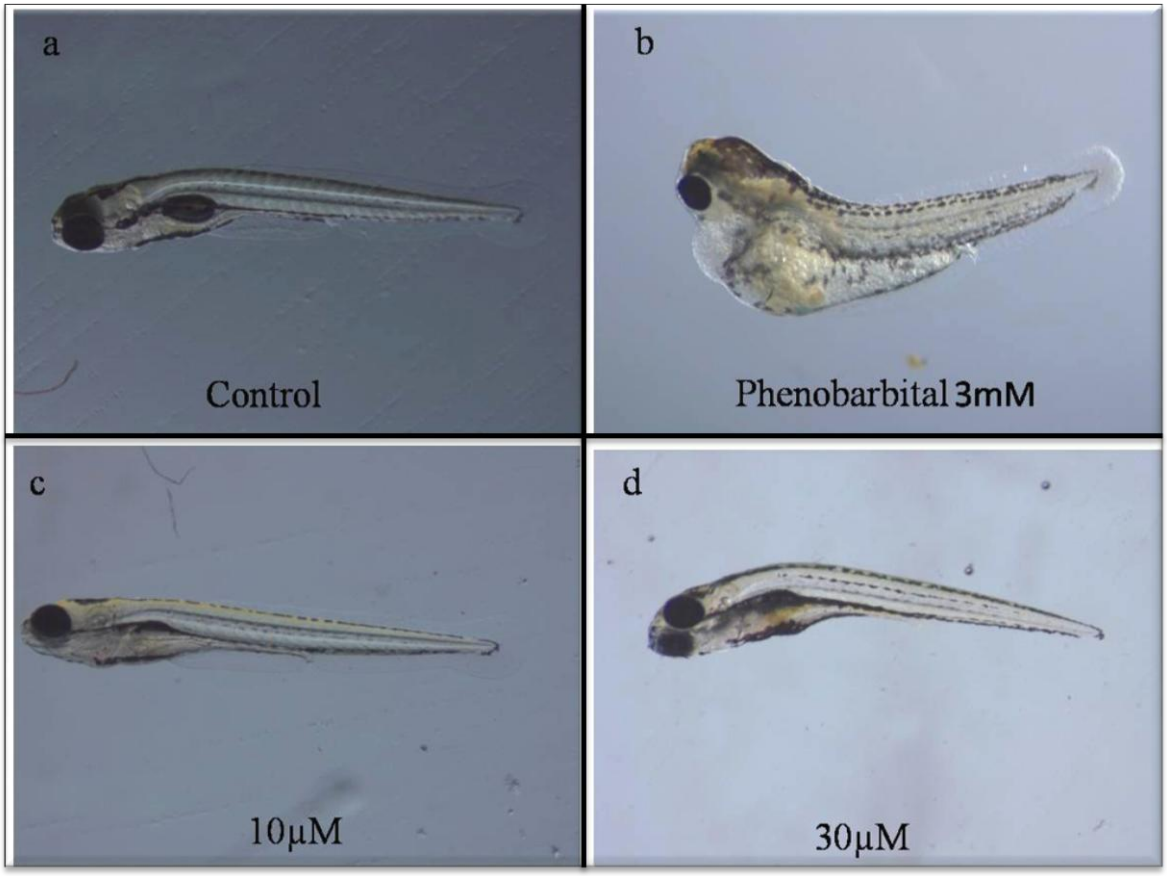


Figure 8. (a) Control zebrafish embryo showing normal body; Embryo treated with (b) Phenobarbital (positive control) showing severe abnormalities, body bent; (c) **4bb** (10 µM) showing slight abnormality at swim bladder; (d) **4bb** (30 µM) showing moderate abnormality at swim bladder and body shape.

No adverse effects observed when tested for toxicity in zebrafish embryo (Fig. 9) at a range 10 nM-30 µM [with No Observed Adverse Effect Level (NOAEL) ~ 10 µM].

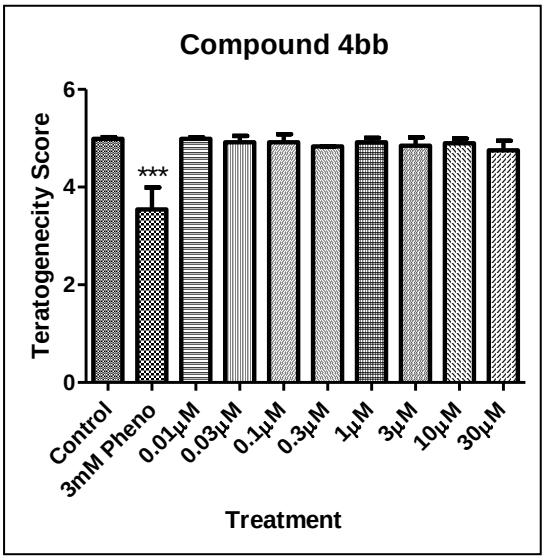


Figure 9. Toxicioty studies on zebrafish embryos with compound **4bb** at different concentrations.

3.6. Conclusion

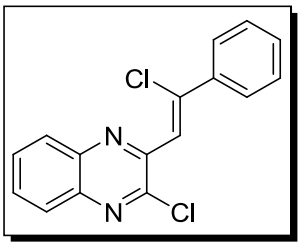
In conclusion, 2-(2,2-diarylvinyl)-3-arylquinoxalines are synthesized first time as novel and unique class of densely functionalized olefins *via* a $AlCl_3$-mediated hydroarylation / heteroarylation in a single pot. A representative compound showed promising sirtuin inhibiting property *in vitro* in a reporter gene assay. Compound **4bb** showed 84.91% inhibition of yeast Sir2. The dose responce study showed its IC_{50}~13.5 μM. It also showed IC_{50}~32.9 μM against mammalian SIRT1 protein. This was supported by the *in silico* binding studies. And also compound **4bb** was tested against human hepatocellular liver carcinoma (HepG2) cells for their anticancer property at 50 µM and 100 µM which showed the growth inhibition. Further *in vivo* toxicity studies has been carried out with compound **4bb** in Zebrafish embryo which showed no adverse effects. This compound can be a promising anticancer motiff for further studies.

3.7. Experimental Section

All starting materials **2a-2f** were prepared according to the procedure developed by our group earlier.[30]

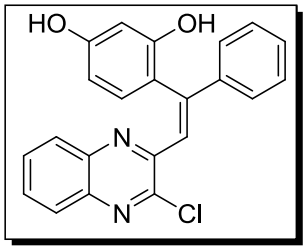
3.7.1. Analytical data of intermediate compounds 5 and 6

(*Z*)-2-Chloro-3-(2-chloro-2-phenylvinyl)quinoxalines (5)



Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.17 (dd, *J* = 6.2, 3.5 Hz, 1H), 8.03 (dd, *J* = 6.9, 2.7 Hz, 1H), 7.87-7.78 (m, 4H), 7.54 (s, 1H), 7.49-7.44 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 148.1, 147.0, 140.8, 140.7 (2C), 138.0, 131.0, 130.4, 130.0, 129.2, 128.6 (2C), 128.1, 127.2 (2C), 120.2; IR (KBr, cm^{-1}): 1604, 1563, 1451, 943; MS (ES mass): m/z 301.6 (M^+).

(*Z*)-4-(2-(3-Chloroquinoxalin-2-yl)-1-phenylvinyl)benzene-1,3-diol (6)

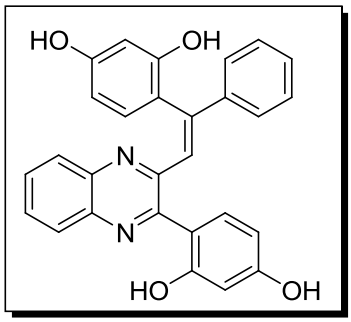


Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 9.14 (s, 1H), 8.92 (s, 1H), 7.87-7.86 (m, 2H), 7.62-7.58 (m, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.28 (m, 5H), 7.13 (s, 1H), 6.21 (s, 1H), 6.13 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 161.2 (2C), 161.0, 158.8, 149.8, 149.4, 140.3, 132.6, 132.5, 131.1, 129.9 (2C), 128.6, 127.3, 126.0 (2C), 125.9, 121.0, 114.1, 111.5, 108.1, 106.6; IR (KBr, cm^{-1}): 3341, 1610, 1533, 1427, 912; MS (ES mass): m/z 375.2 (M+1).

3.7.2. General procedure for the synthesis of 2-(2,2-diarylvinyl)-3-arylquinoxaline (4)

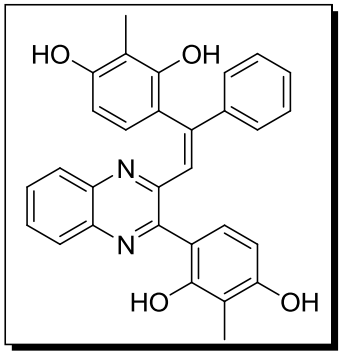
A mixture of alkyne (**2**) (0.37 mmol), and arene (**3**) (1.51 mmol) and anhydrous $AlCl_3$ (1.51mmol) in dichloroethane (4 mL) was stirred at 50 °C for 15-30 min under nitrogen atmosphere. The colour of the reaction gradually changed from yellow to dark yellow. After completion of the reaction as indicated by TLC, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel (Merck, 100–200 mesh) using *n*-hexane/ethyl acetate to afford the desired product.

(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-2-phenylvinyl)quinoxalin-2-yl)benzene-1,3-diol (4aa)



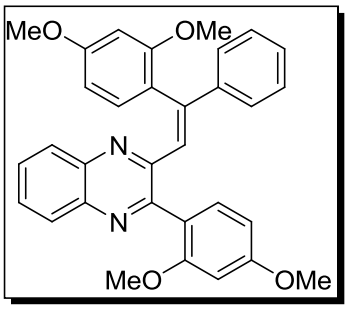
Yellow solid; mp: 206-208 °C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 10.31 (s, 1H), 9.61 (s, 1H), 9.13 (s, 1H), 8.78 (s, 1H), 7.97-7.85 (m, 1H), 7.65 (dd, *J* = 8.3, 3.5 Hz, 2H), 7.55-7.49 (m, 1H), 7.32-7.24 (m, 6H), 6.99 (s, 1H), 6.60 (d, *J* = 8.2 Hz, 1H), 6.38 (d, *J* = 2.1 Hz, 1H), 6.32 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.14 (d, *J* = 2.1 Hz, 1H), 6.08 (dd, *J* = 8.2, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 170.7, 159.9, 158.0, 157.2, 156.2, 154.0, 152.3, 144.7, 143.4, 140.2, 139.6, 132.3, 129.6, 129.5, 128.8, 128.6, 128.4, 128.2 (2C), 127.5 (2C), 125.5, 118.0, 116.6, 107.3, 106.5, 102.9, 102.8; HPLC: 99.6%; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.58 min; IR (KBr, cm^{-1}): 3357, 1613, 1508, 970; MS (ES mass): m/z 449.2 (M+1).

(*Z*)-4-(3-(2-(2,4-Dihydroxy-3-methylphenyl)-2-phenylvinyl)quinoxalin-2-yl)-2-methylbenzene-1,3-diol (4ab)



Brown semisolid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 11.15 (s, 1H), 9.78 (s, 1H), 9.12 (s, 1H), 8.25 (s, 1H), 8.00-7.93 (m, 1H), 7.70 (dd, *J* = 13.3, 5.8 Hz, 2H), 7.63-7.57 (m, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.36-7.22 (m, 5H), 7.13 (s, 1H), 6.24 (d, *J* = 8.4 Hz, 1H), 6.48 (d, *J* = 8.4 Hz, 2H), 2.08 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 156.8, 156.0 (2C), 154.4, 151.4, 147.0, 144.4, 143.2, 139.4, 138.2, 129.0 (2C), 128.6, 128.5 (2C), 128.4, 128.3, 128.0 (2C), 127.9 (2C), 127.3, 119.0, 113.8, 112.4, 111.7, 111.6, 106.7, 9.5, 9.1; HPLC: 98.1%; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.39 min; IR (KBr, cm^{-1}): 3373, 2853, 1605, 1489, 598; MS (ES mass): m/z 476.8 (M+1).

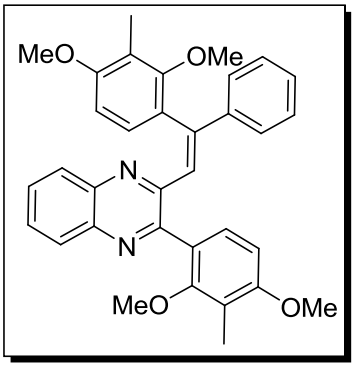
(*Z*)-2-(2,4-Dimethoxyphenyl)-3-(2-(2,4-dimethoxyphenyl)-2-phenylvinyl)quinoxalines (4ac)



Light brown semisolid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.04-7.94 (m, 1H), 7.73-7.68 (m, 1H), 7.65-7.53 (m, 3H), 7.06 (dd, *J* = 8.3, 9.0 Hz, 3H), 6.90-6.82 (m, 1H), 6.76 (d, *J* =

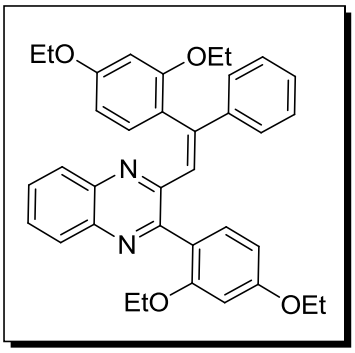
8.3 Hz, 1H), 6.31 (dd, *J* = 12.9, 4.9 Hz, 2H), 6.50 (d, *J* =13.3 Hz, 4H), 3.85-3.79 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 161.8, 160.5, 158.8, 158.0, 153.1, 152.3, 144.1, 142.7, 140.5, 140.3, 131.8, 131.6, 129.9, 129.7, 129.1, 128.8, 128.7, 128.2 (2C), 127.7, 126.9, 125.5, 121.1, 120.6, 105.9, 104.8, 98.8, 98.6, 56.0, 55.7, 55.6, 55.4; HPLC: 96.7%; column: Symmetry C-18 75*4.6 mm, 3.5µm, 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 220 nm, retention time 7.12 min; IR (KBr, cm^{-1}): 2926, 1616, 1398, 816; MS (ES mass): m/z 504.8 (M^+).

(*Z*)-2-(2,4-Dimethoxy-3-methylphenyl)-3-(2-(2,4-dimethoxy-3-methylphenyl)-2 phenylvinyl)quinoxalines (4ad)



Light yellow liquid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.98 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.75-7.68 (m, 2H), 7.51-7.45 (m, 1H), 7.32-7.26 (m, 4H), 6.95 (s, 1H), 7.22-7.17 (m, 2H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.73 (s, 1H), 6.63 (s, 1H), 3.85 (d, *J* = 7.9 Hz, 3H), 3.75 (s, 3H), 3.52 (s, 3H), 3.38 (s, 3H), 2.15 (d, *J* = 10.2 Hz, 3H), 1.99 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 182.9, 168.4, 158.1 (2C), 157.3, 156.9, 153.7, 151.5, 142.8, 140.6, 130.2, 130.0, 128.9, 128.8 (2C), 128.7(2C), 128.6 (2C), 128.5, 127.5, 127.2, 126.0, 125.1, 119.1, 118.6, 109.9, 106.9, 61.5, 60.0, 56.1, 55.9, 9.4 (2C); HPLC: 91.8 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.31 min; IR (KBr, cm^{-1}): 2913, 1604, 1381, 907; MS (ES mass): m/z 532.8 (M^+).

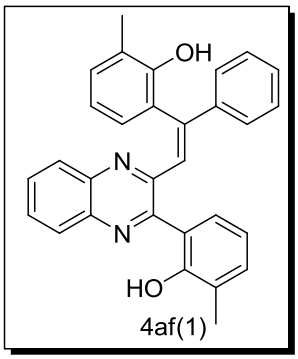
(*Z*)-2-(2,4-Diethoxyphenyl)-3-(2-(2,4-diethoxyphenyl)-2-phenylvinyl)quinoxalines (4ae)



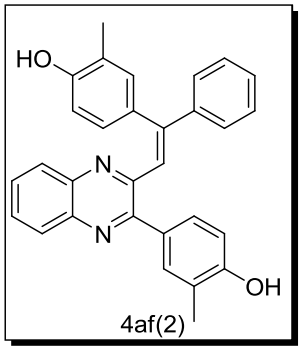
Light yellow liquid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.91 (t, J = 8.9 Hz, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.46-7.45 (m, 1H), 7.26 (s, 1H), 7.14 (dd, J = 8.3, 2.5 Hz, 1H), 7.06 (s, 1H), 6.98 (q, J = 7.2 Hz, 2H), 6.85 (s, 1H), 6.77 (dd, J = 7.7, 2.3 Hz, 1H), 6.49-6.41 (m, 3H), 6.39-6.29 (m, 1H), 6.25 (d, J = 1.8 Hz, 1H), 6.20 (dd, J = 8.3, 2.0 Hz, 1H), 4.03-4.01 (m, 8H), 1.46-1.31 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 160.7, 159.6, 158.0, 157.1, 152.2, 144.1, 143.8, 139.0, 132.2, 131.6, 131.5, 131.0, 129.0 (2C), 128.3, 127.7, 127.1 (2C), 127.0 (2C), 126.2 (2C), 126.1, 121.4, 105.4 (2C), 100.4, 99.5, 63.7, 63.5, 63.4, 63.3, 14.8 (2C), 14.7 (2C); HPLC: 92.6 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.28 min; IR (KBr, cm^{-1}): 2938, 1603, 1460, 823; MS (ES mass): m/z 561.2 (M+1).

(*Z*)-2-(3-(2-(2-Hydroxy-3-methylphenyl)-2-phenylvinyl)quinoxalin-2-yl)-6-methylphenol (4af (1)) and ***(Z)*-4-(3-(2-(4-hydroxy-3-methylphenyl)-2-phenylvinyl)quinoxalin-2-yl)-2-methylphenol (4af(2)):** A ~ 1:1 mixture of regioisomers i.e. **4af(1)** and **4af(2)** was isolated in this case which could not be separated by using standard column chromatography. The mixture was confirmed by HPLC analysis (see the data and the copy of the HPLC). While an attempt to separate the individual isomer by using preparative HPLC was failed, the LC-MS analysis indicated that both the component present in the mixture possess same mass.

All the spectral data presented here was recorded on 1:1 mixture of compounds.



4af(1)

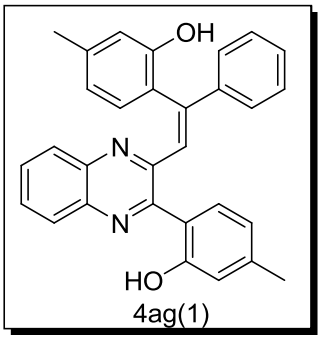


4af(2)

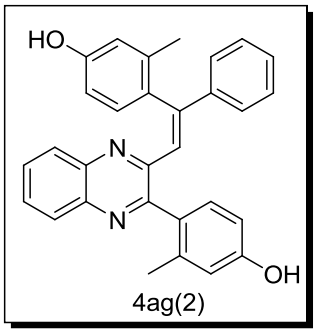
Yellow semi solid; 9.64 (s, 1H), 9.58 (s, 1H), 9.52 (s, 1H), 9.28 (s, 1H), 7.97-7.85 (m, 3H), 7.74-7.68 (m, 3H), 7.66 (s, 2H), 7.30 (dd, *J* = 13.2, 7.7 Hz, 7H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 6.5 Hz, 2H), 7.06 (dd, *J* = 12.8, 6.9 Hz, 5H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.76 (t, *J* = 7.5 Hz, 2H), 6.69 (t, *J* = 7.9 Hz, 3H), 6.36 (d, *J* = 8.1 Hz, 1H), 6.18 (d, *J* = 8.0 Hz, 1H), 6.04 (s, 1H), 2.12 (s, 3H), 2.08 (s, 6H), 1.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 156.8, 156.5, 156.4, 155.4, 154.1, 152.6, 151.7, 147.4, 147.1, 142.8, 140.3, 140.2, 140.1, 140.0, 139.9, 132.9, 132.3, 132.2, 132.0, 130.6, 130.1, 129.5, 129.1, 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.5, 128.4 (2C), 128.0, 127.9, 127.3, 127.0, 124.9, 124.2, 123.9, 123.7, 123.6, 114.8, 114.2, 113.8, 16.5, 16.4, 16.3, 16.2; HPLC (mixture): 48.1 and 45.9 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 13.5 and 13.9 min; IR (KBr, cm^{-1}): 3055, 2952, 1606, 1444, 820; MS (ES mass): m/z 444.5 (M^+).

(*Z*)-2-(3-(2-(4-Hydroxy-2-methylphenyl)-2-phenylvinyl)quinoxalin-2-yl)-5-methylphenol (4ag(1)) and ***(Z)*-4-(3-(2-(4-hydroxy-2-methylphenyl)-2-phenylvinyl)quinoxalin-2-yl)-3-methylphenol (4ag(2)):** A ~ 1:1 mixture of regioisomers i.e. **4ag(1)** and **4ag(2)** was isolated in this case which could not be separated by using standard column chromatography. The mixture was confirmed by HPLC analysis (see the data and the copy of the HPLC). While an attempt to separate the individual isomer by using preparative HPLC was failed, the LC-MS analysis indicated that both the component present in the mixture possess same mass.

All the spectral data presented here was recorded on 1:1 mixture of compounds.



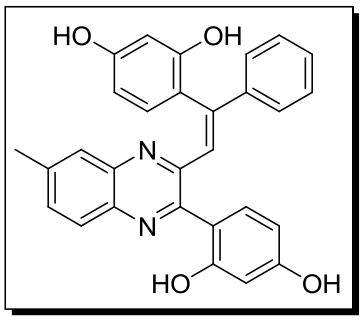
4ag(1)



4ag(2)

Yellow semi solid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 9.67 (s, 1H), 9.61 (s, 1H), 9.55 (s, 1H), 9.31 (s, 1H), 7.93 (s, 2H), 7.91-7.85 (m, 2H), 7.71 (d, *J* = 4.2 Hz, 1H), 7.65 (s, 2H), 7.33 (s, 3H), 7.25 (d, *J* = 12.6 Hz, 3H), 7.13 (d, *J* = 6.4 Hz, 2H), 7.05 (dd, *J* = 11.4, 6.9 Hz, 5H), 6.92 (d, *J* = 8.0 Hz, 1H), 6.78-6.73 (m, 2H), 6.69 (d, *J* = 6.6 Hz, 2H), 6.36 (d, *J* = 8.1 Hz, 1H), 6.17 (d, *J* = 8.0 Hz, 1H), 6.02 (s, 1H), 2.11 (s, 3H), 2.09 (s, 6H), 1.80 (s, 3H); HPLC (mixture): 46.7 and 43.1%; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/20, 3/20, 12/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 12.6 and 12.9 min; IR (KBr, cm^{-1}): 3362, 2941, 1608, 1421, 866; MS (ES mass): m/z 444.5 (M^+).

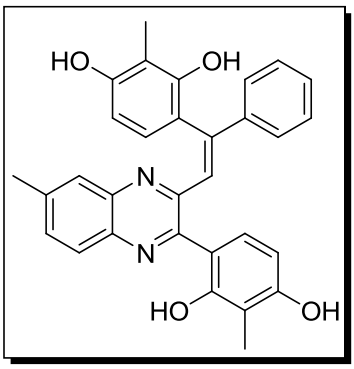
(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-2-phenylvinyl)-6-methylquinoxalin-2-yl)benzene-1,3-diol (4ba)



Light brown solid; mp: 158-160°C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 10.32 (s, 1H), 9.62 (s, 1H), 9.13 (s, 1H), 8.78 (s, 1H), 7.95-7.90 (m, 1H), 7.69-7.63 (m, 2H), 7.53 (d, *J* = 7.74 Hz, 2H), 7.28 (m, 5H), 7.00 (s, 1H), 6.61 (d, *J* = 8.2 Hz, 2H), 6.32 (d, *J* = 8.4 Hz,

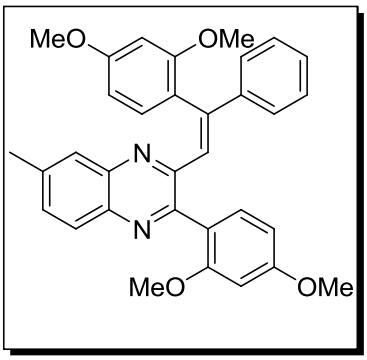
1H), 6.09 (d, *J* = 8.2 Hz, 1H) 2.69 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 159.6, 157.7, 156.8, 156.0, 153.9, 152.4, 144.7, 143.1, 140.1, 139.6, 132.3, 132.2, 129.8 (2C), 129.7, 128.7 (2C), 128.6, 128.4, 127.4 (2C), 125.5, 118.0, 116.7, 107.3, 106.4, 102.7, 102.6, 21.1; HPLC: 93.2 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.02 min; IR (KBr, cm^{-1}): 3410, 2929, 1611, 1416, 829; MS (ES mass): m/z 462.8 (M^+).

(*Z*)-4-(3-(2-(2,4-Dihydroxy-3-methylphenyl)-2-phenylvinyl)-6-methylquinoxalin-2-yl)-2-methylbenzene-1,3-diol (4bb)



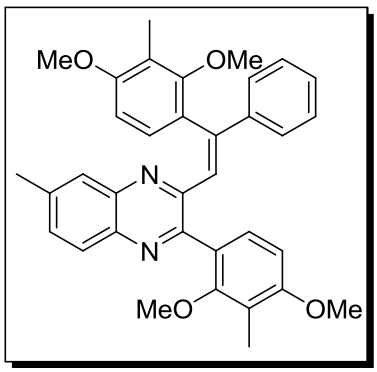
Yellow solid; mp: 168-170°C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 11.15 (s, 1H), 9.71 (s, 1H), 9.13 (s, 1H), 8.29 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.76 (s, 1H), 7.56-7.55 (m, 2H), 7.35-7.23 (m, 4H), 7.13 (s, 1H), 6.50 (d, *J* = 3.2 Hz, 1H), 6.46 (d, *J* = 8.4 Hz, 1H), 6.25 (dd, *J* = 8.2, 2.4 Hz, 1H), 2.50 (d, *J* = 5.1 Hz, 3H), 2.06 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 158.4, 156.9, 156.0, 151.1, 148.9, 145.2, 144.1 (2C), 140.4, 139.3, 137.4, 132.1, 129.0, 128.7 (2C), 128.6, 128.2 (2C), 127.6 (2C), 127.5, 126.5 (2C), 125.1, 118.4 (2C), 111.6, 106.8, 20.2, 11.6 (2C); HPLC: 99.8 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.32 min; IR (KBr, cm^{-1}): 3390, 2913, 1601, 1413, 832; MS (ES mass): m/z 490.8 (M^+).

(*Z*)-2-(3,5-Dimethoxyphenyl)-3-(2-(2,4-dimethoxyphenyl)-2-phenylvinyl)-6-methylquinoxaline (4bc)



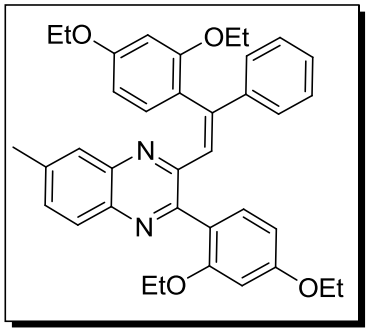
Yellow liquid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.83 (d, *J* = 8.5 Hz, 1H), 7.54 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.40 (s, 1H), 7.31-7.25 (m, 2H), 7.17 (d, *J* = 7.7 Hz, 2H), 7.11 (dd, *J* = 8.2, 4.8 Hz, 1H), 6.93 (d, *J* = 4.7 Hz, 1H), 6.74 (d, *J* = 7.0 Hz, 1H), 6.68-6.59 (m, 2H), 6.51 (s, 1H), 6.47-6.42 (m, 1H), 6.34 (dd, *J* = 8.3, 1.9 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.73 (s, 3H), 3.31 (s, 3H), 2.51 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 159.8, 159.7, 158.8, 157.7, 157.4, 156.7, 156.4, 155.9, 143.1 (2C), 142.9, 140.2, 137.7, 128.8 (2C), 128.7 (2C), 128.0, 127.5 (2C), 120.2, 119.8, 114.0, 107.2, 106.3, 104.5, 100.3, 99.4, 55.8 (4C), 21.2; HPLC: 92.7 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, 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 220 nm, retention time 6.22 min; IR (KBr, cm^{-1}): 2936, 1604, 1411, 795; MS (ES mass): m/z 518.9 (M^+).

(*Z*)-2-(2,4-Dimethoxy-3-methylphenyl)-3-(2-(2,4-dimethoxy-3-methylphenyl)-2-phenylvinyl)-6-methylquinoxaline (4bd)



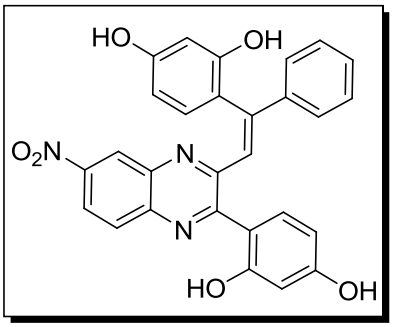
Yellow liquid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.98 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.76-7.66 (m, 2H), 7.49 (s, 1H), 7.34-7.26 (m, 5H), 7.22-7.16 (m, 2H), 6.95 (s, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.73 (s, 1H), 6.62 (d, *J* = 8.5 Hz, 2H), 3.85 (s, 3H), 3.75 (s, 3H), 3.52 (s, 3H), 3.38 (s, 3H), 2.15 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 168.4, 158.1, 157.3, 156.9, 153.7, 151.5, 142.7 (2C), 140.6 (2C), 130.2, 130.0, 129.3, 128.8 (2C), 128.7, 127.9, 127.5, 127.2 (2C), 126.0, 125.1(2C), 119.1, 118.6, 113.6, 109.9, 98.0, 61.5, 60.0, 56.1, 55.9, 21.4, 9.4 (2C); HPLC: 94.1 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.54 min; IR (KBr, cm^{-1}): 2941, 1599, 1411, 795; MS (ES mass): m/z 547.1 (M+1).

(*Z*)-2-(2,4-Diethoxyphenyl)-3-(2-(2,4-diethoxyphenyl)-2-phenylvinyl)-6-methylquinoxaline (4be)



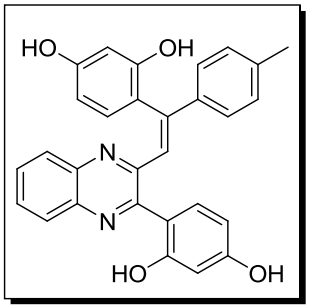
Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.08-7.98 (m, 1H), 7.80 (d, *J* = 9.7 Hz, 1H), 7.66-7.59 (m, 2H), 7.22-7.21 (m, 3H), 7.14 (d, *J* = 8.3 Hz, 1H), 7.07 (s, 1H), 6.98 (d, *J* = 5.4 Hz, 1H), 6.79 (d, *J* = 7.9 Hz, 2H), 6.69 (d, *J* = 8.3 Hz, 1H), 6.37 (d, *J* = 8.3 Hz, 1H), 6.26 (d, *J* = 6.2 Hz, 1H), 4.06-4.00 (m, 8H), 2.38 (s, 3H), 1.45-1.36 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 161.2, 157.9, 150.1, 149.7, 149.6, 144.7, 144.3, 140.5, 139.6, 138.9, 132.1(2C), 131.0, 130.7, 130.4 (2C), 130.3, 129.6, 129.4, 128.9 (2C), 128.5, 127.9, 127.1 (2C), 115.2, 111.0, 105.5, 63.8, 63.5 (2C), 63.3, 21.1, 14.8 (2C), 14.7 (2C); HPLC: 95.9 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.42 min; IR (KBr, cm^{-1}): 2937, 1589, 1424, 817; MS (ES mass): m/z 575.1 (M^+).

(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-2-phenylvinyl)-6-nitroquinoxalin-2-yl)benzene-1,3-diol (4ca)



Light yellow solid; mp: 234-236°C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 10.45 (s, 1H), 9.20 (s, 1H), 8.66 (s, 1H), 8.38 (s, 1H), 8.07 (s, 1H), 7.46-7.42 (m, 5H), 7.22-7.12 (m, 2H), 7.09-7.02 (m, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.87-6.80 (m, 1H), 6.79-6.69 (m, 1H), 6.55 (s, 1H), 6.48-6.29 (m, 1H), 6.23-6.04 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 160.3, 159.3, 158.9, 156.2, 147.5, 144.9, 144.3, 141.3, 139.9, 134.1, 129.7 (2C), 129.6, 129.0 (2C), 128.7, 128.6 (2C), 128.3, 127.9 (2C), 127.6 (2C), 112.3, 109.7, 107.6, 106.7, 104.7; HPLC: 80.3 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.42 min; IR (KBr, cm^{-1}): 3386, 2937, 1589, 1424, 817; MS (ES mass): m/z 493.8 (M^{+}).

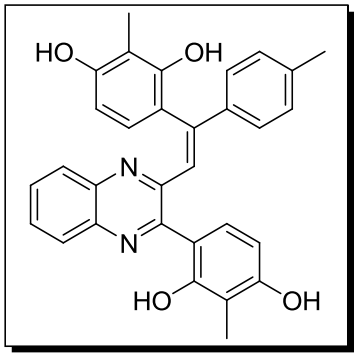
(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-2-*p*-tolylvinyl)quinoxalin-2-yl)benzene-1,3-diol (4da)



Dark brown solid; mp: 154-156°C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 10.36 (s, 1H), 9.64 (s, 1H), 9.14 (s, 1H), 8.77 (s, 1H), 7.94 (d, *J* = 6.2 Hz, 1H), 7.66 (d, *J* = 9.9 Hz, 2H),

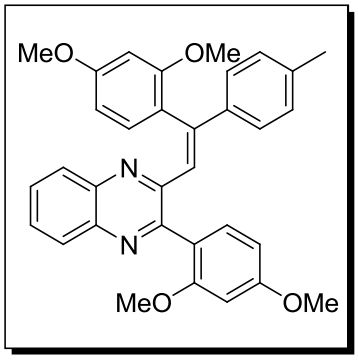
7.53 (d, *J* = 5.7 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 4H), 6.97 (s, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.41 (d, *J* = 2.1 Hz, 1H), 6.38-6.30 (m, 1H), 6.16 (d, *J* = 2.1 Hz, 1H), 6.11 (d, *J* = 8.2 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 159.9, 157.9, 157.2, 156.2, 154.0, 152.3, 144.6, 140.6, 140.2, 139.5, 137.6, 132.3 (2C), 129.5, 129.4, 129.3 (2C), 129.2, 128.7, 128.3, 127.5, 124.7, 118.1, 116.5, 107.2, 106.4, 102.9, 102.8, 21.1; HPLC: 94.0 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, 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 220 nm, retention time 6.1 min; IR (KBr, cm^{-1}): 3256, 2871, 1566, 1398, 802; MS (ES mass): m/z 462.9 (M^+).

(*Z*)-4-(3-(2-(2,4-Dihydroxy-3-methylphenyl)-2-*p*-tolylvinyl)quinoxalin-2-yl)-2-methylbenzene-1,3-diol (4db)



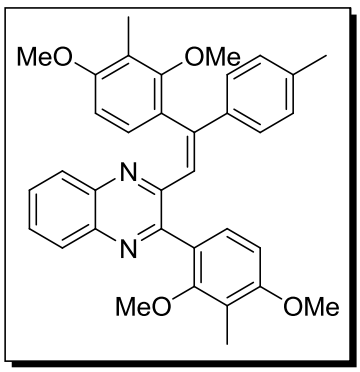
Yellow solid; mp: 160-162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 10.39 (s, 1H), 9.62 (s, 1H), 9.14 (s, 1H), 8.78 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.57-7.47 (m, 1H), 7.33 (d, *J* = 4.0 Hz, 1H), 7.18-7.08 (m, 4H), 6.95 (d, *J* = 4.1 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 6.32 (d, *J* = 8.3 Hz, 1H), 6.16 (d, *J* = 6.3 Hz, 1H), 6.12-6.07 (m, 1H), 2.50 (s, 6H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 159.9, 157.9, 157.2, 156.2, 154.0, 152.3, 144.6, 140.6, 140.2, 139.5, 137.6, 132.3, 129.5 (2C), 129.4, 129.3, 129.2 (2C), 128.7, 128.3, 127.5, 124.6, 118.1, 116.5, 107.2, 106.5, 102.9, 102.8, 21.2, 9.6 (2C); HPLC: 92.3 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.21 min; IR (KBr, cm^{-1}): 3345, 2931, 1610, 1421, 826; MS (ES mass): m/z 490.8 (M^+).

(*Z*)-2-(2,4-Dimethoxyphenyl)-3-(2-(2,4-dimethoxyphenyl)-2-*p*-tolylvinyl)quinoxalines (4dc)



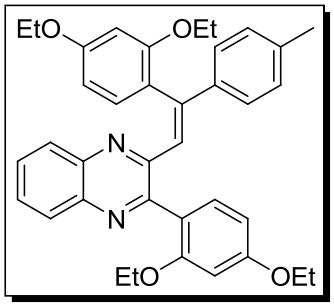
Brown solid; mp: 56-58 °C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 7.99 (d, *J* = 7.0 Hz, 1H), 7.69-7.65 (m, 1H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.18 (d, *J* = 5.5 Hz, 1H), 7.15 (s, 1H), 7.06 (d, *J* = 8.1 Hz, 2H), 7.03 (d, *J* = 7.8 Hz, 2H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.54 (d, *J* = 8.3 Hz, 2H), 6.51-6.48 (m, 1H), 6.34 (d, *J* = 2.4 Hz, 1H), 6.30 (d, *J* = 8.2 Hz, 1H), 3.85 (d, *J* = 6.8 Hz, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.34 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 160.0, 158.5 (2C), 158.0, 157.9, 144.1, 142.1, 141.0, 138.6 (2C), 137.5, 137.3, 129.1, 128.9 (2C), 128.7 (2C), 128.6, 128.3, 127.8, 127.5, 127.1, 126.2, 114.0, 105.2, 103.9, 99.2, 99.6, 66.4, 55.3, 55.2 (2C), 21.1; HPLC: 90.2 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.34 min; IR (KBr, cm^{-1}): 2953, 1604, 1433, 921; MS (ES mass): m/z 519.2 (M+1).

(*Z*)-2-(2,4-Dimethoxy-3-methylphenyl)-3-(2-(2,4-dimethoxy-3-methylphenyl)-2-*p*-tolylvinyl)quinoxalines (4dd)



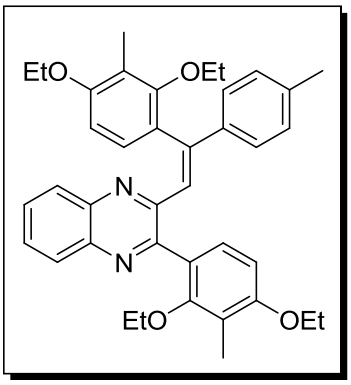
Brown solid; mp: 68-70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.00 (d, *J* = 7.7 Hz, 1H), 7.69-7.64 (m, 1H), 7.61-7.54 (m, 2H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 7.8 Hz, 2H), 7.06 (s, 1H), 7.04 (s, 2H), 6.77 (d, *J* = 8.3 Hz, 1H), 6.57-6.52 (m, 1H), 6.50 (s, 1H), 6.43 (d, *J* = 8.5 Hz, 1H), 6.34 (s, 1H), 6.31 (d, *J* = 8.3 Hz, 1H), 3.93-3.75 (m, 12H), 3.34 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 160.4, 158.8, 158.4, 140.9, 140.5, 140.3, 138.8, 132.6, 132.5, 131.1 (2C), 129.9 (2C), 128.6 (2C), 127.8, 127.3 (2C), 126.0, 125.9 (2C), 114.1, 111.5, 108.1, 106.6, 105.1, 102.8, 98.8, 56.2 (2C), 55.0 (2C), 21.1 (2C), 9.9; HPLC: 93.9 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.21 min; IR (KBr, cm^{-1}): 2964, 2857 1611, 1454, 870; MS (ES mass): m/z 547.1 (M+1).

(*Z*)-2-(2,4-Diethoxyphenyl)-3-(2-(2,4-diethoxyphenyl)-2-*p*-tolylvinyl)quinoxalines (4de)



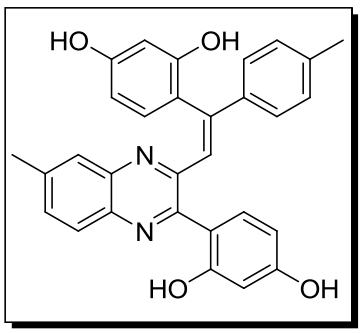
Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.63-7.58 (m, 2H), 7.12 (d, *J* = 7.9 Hz, 2H), 7.04 (t, *J* = 8.2 Hz, 4H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.73-6.67 (m, 1H), 6.64 (s, 1H), 6.50-6.39 (m, 2H), 6.26 (s, 1H), 6.24 (s, 1H), 4.08-3.98 (m, 8H), 2.32 (s, 3H), 1.46-1.37 (m, 9H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 160.1, 159.4, 158.9 (2C), 156.2, 153.8, 143.2, 142.3, 141.8, 138.4, 137.9, 131.2 (2C), 129.6 (2C), 128.0 (2C), 127.3 (2C), 125.8 (2C), 114.2, 109.9, 107.1, 105.5, 104.1, 100.9, 99.1, 63.3 (4C), 21.2, 14.1 (4C); HPLC: 93.6 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.38 min; IR (KBr, cm^{-1}): 2937, 2841, 1609, 1427, 843; MS (ES mass): m/z 575.3 (M+1).

(*Z*)-2-(2,4-Diethoxy-3-methylphenyl)-3-(2-(2,4-diethoxy-3-methylphenyl)-2-*p*-tolylvinyl)quinoxalines (4dh)



Light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.02 (d, *J* = 7.7 Hz, 2H), 7.59 (s, 1H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 10.8 Hz, 2H), 7.12 (s, 1H), 7.08 (d, *J* = 8.0 Hz, 3H), 6.69 (t, *J* = 9.6 Hz, 2H), 6.47 (d, *J* = 8.3 Hz, 2H), 4.01 (d, *J* = 6.8 Hz, 3H), 4.09-4.03 (m, 3H), 2.32 (s, 3H), 2.22 (s, 3H), 2.05 (s, 3H), 1.44-1.42 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.7, 158.9, 157.0, 155.1, 152.7, 143.9, 143.1, 140.4, 138.9 (2C), 136.8, 134.4, 129.4 (2C), 129.2, 125.9 (2C), 124.1, 123.5 (2C), 120.2, 113.2, 112.1, 110.7, 106.4 (2C), 105.4, 104.0, 65.5, 65.1, 64.8, 64.2, 21.4, 13.9 (4C), 9.7 (2C); HPLC: 94.8 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.58 min; IR (KBr, cm^{-1}): 2953, 2866, 1597, 1461, 912; MS (ES mass): m/z 603.1 (M+1).

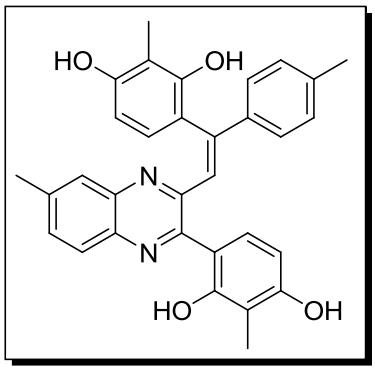
(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-2-p-tolylvinyl)-6-methylquinoxalin-2-yl)benzene-1,3-diol (4ea)



Yellow solid; mp: 230-232 °C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 11.19 (s, 1H), 9.79 (s, 1H), 9.13 (s, 1H), 8.24 (s, 1H), 7.98 (d, *J* = 7.56 Hz, 1H), 7.72 (d, *J* = 7.1 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 4H), 7.11 (s, 1H),

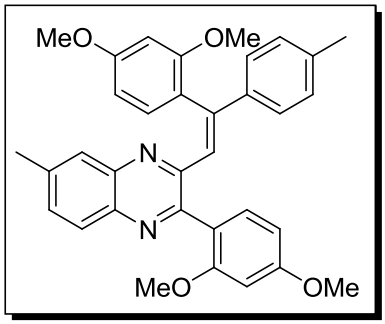
6.49 (d, *J* = 8.3 Hz, 2H), 6.26 (d, *J* = 8.2 Hz, 1H), 2.30 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 158.4, 156.9, 155.9, 154.4, 153.5, 151.4, 146.3, 144.3, 140.3, 139.3, 138.2, 137.8, 130.3, 129.9 (2C), 129.3, 128.8, 128.2, 128.1, 127.8, 127.6, 125.4, 119.2, 113.8, 111.6, 111.5, 109.9, 106.8, 21.1 (2C); HPLC: 94.2 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.21 min; IR (KBr, cm^{-1}): 3538, 2926, 2851, 1607, 1458, 845; MS (ES mass): m/z 476.9 (M+1).

(*Z*)-4-(3-(2-(2,4-Dihydroxy-3-methylphenyl)-2-p-tolylvinyl)-6-methylquinoxalin-2-yl)-2 methylbenzene-1,3-diol (4eb)



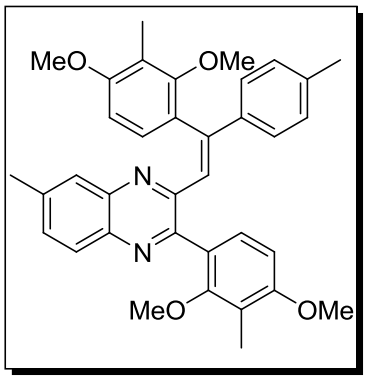
Brown solid; mp: 140-142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 11.22 (s, 1H), 9.75 (s, 1H), 9.11 (s, 1H), 8.29 (s, 1H), 7.90-7.84 (m, 2H), 7.55 (d, *J* = 8.2 Hz, 4H), 7.42-7.39 (m, 1H), 7.29 (d, *J* = 4.0 Hz, 1H), 7.12 (s, 1H), 6.52-6.47 (m, 1H), 6.46-6.41 (m, 1H), 6.27-6.21 (m, 1H), 2.48 (s, 6H), 2.28 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 157.1, 156.9, 156.2, 153.8, 142.3, 141.8, 140.4, 139.1, 138.4, 135.6, 134.3 (2C), 131.2, 128.0, 127.3 (2C), 125.8, 125.7, 125.0, 123.1, 121.7 (2C), 115.6, 114.2, 113.6, 112.7, 109.9, 106.3, 22.3 (2C), 9.2 (2C); HPLC: 93.7 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, 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 220 nm, retention time 8.10 min; IR (KBr, cm^{-1}): 3431, 2946, 2842, 1604, 1440, 871; MS (ES mass): m/z 504.8 (M^+).

(*Z*)-2-(2,4-Dimethoxyphenyl)-3-(2-(2,4-dimethoxyphenyl)-2-*p*-tolylvinyl)-6-methylquinoxaline (4ec)



Light brown solid; mp: 84-86 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.87 (d, *J* = 8.3 Hz, 1H), 7.48-7.37 (m, 2H), 7.20-7.12 (m, 3H), 7.08-6.99 (m, 3H), 6.77 (t, *J* = 10.1 Hz, 1H), 6.56-6.50 (m, 1H), 6.48 (t, *J* = 2.2 Hz, 1H), 6.33 (t, *J* = 2.7 Hz, 1H), 6.30 (d, *J* = 8.3 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 3.33 (s, 3H), 2.51 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 161.5, 160.2, 158.6, 158.0, 157.9, 152.5, 143.6, 140.9, 140.2, 139.2, 137.3, 131.9 (2C), 131.6, 129.1, 128.6, 128.4, 128.2, 128.0, 127.7, 127.6, 127.1, 125.1, 121.3, 105.1, 103.8, 98.5, 98.4, 55.4 (4C), 21.1 (2C); HPLC: 90.3 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.46 min; IR (KBr, cm^{-1}): 2937, 2830, 1615, 1416, 902; MS (ES mass): m/z 533.2 (M+1).

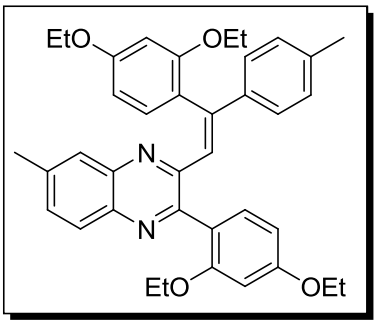
(*Z*)-2-(2,4-Dimethoxy-3-methylphenyl)-3-(2-(2,4-dimethoxy-3-methylphenyl)-2-*p*-tolylvinyl)-6-methylquinoxaline (4ed)



Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.98-7.87 (m, 2H), 7.47 (s, 2H), 7.16 (d, *J* = 5.7 Hz, 4H), 7.05 (s, 1H), 6.85-6.76 (m, 2H), 6.75-6.68 (m, 2H), 6.53 (s, 1H), 6.49-6.45 (m, 2H), 6.34 -6.32 (m, 2H), 3.80 (m, 12H), 2.32 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.2, 158.8, 157.7, 154.4, 144.1, 141.7, 139.4, 138.4, 134.3, 132.5, 129.9, 129.2, 128.6, 128.4 (2C), 127.3, 126.0, 125.7, 125.6 (2C), 123.0, 119.0, 117.7, 116.4, 110.8, 105.7, 104.5, 103.3, 68.7 (2C), 65.7 (2C), 21.2 (2C), 9.5 (2C); HPLC: 95.4 %;

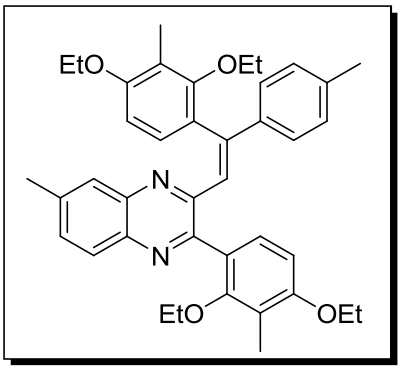
column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.31 min; IR (KBr, cm^{-1}): 2948, 2834, 1591, 1422, 907; MS (ES mass): m/z 561.2 (M+1).

(*Z*)-2-(2,4-Diethoxyphenyl)-3-(2-(2,4-diethoxyphenyl)-2-*p*-tolylvinyl)-6-methylquinoxaline (4ee)



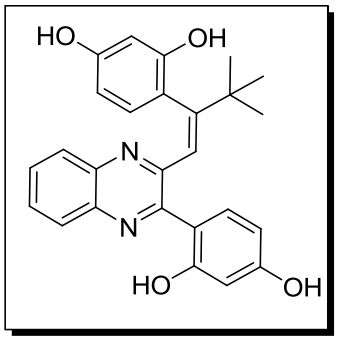
Light yellow; ^1H NMR (400 MHz, $CDCl_3$) ppm: 7.47-7.41 (m, 1H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.03-7.02 (m, 3H), 7.01 (s, 1H), 6.73-6.66 (m, 1H), 6.65-6.58 (m, 1H), 6.45 (s, 3H), 6.26 (s, 1H), 6.24-6.17 (m, 1H), 4.01 (m, 8H), 2.54 (s, 3H), 2.31 (s, 3H), 1.35-1.20 (m, 9H), 0.74 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.4, 156.2, 154.8, 151.9, 142.3, 140.4, 139.1, 137.9, 136.0, 135.9, 134.6, 134.3, 131.2, 128.0, 127.3, 125.8, 124.4, 123.5, 123.1, 121.7, 120.1, 114.2, 112.7, 111.8, 109.9, 106.4, 105.5, 104.1, 65.0 (2C), 64.5 (2C), 21.2 (2C), 14.3 (4C); HPLC: 92.7 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, 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 220 nm, retention time 6.28 min; IR (KBr, cm^{-1}): 2953, 2844, 1586, 1439, 802; MS (ES mass): m/z 589.1 (M+1).

(*Z*)-2-(2,4-Diethoxy-3-methylphenyl)-3-(2-(2,4-diethoxy-3-methylphenyl)-2-*p*-tolylvinyl)-6-methylquinoxaline (4eh)



Yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.90 (s, 1H), 7.80 (s, 1H), 7.48 (s, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 3H), 7.11 (s, 2H), 7.07 (d, *J* = 7.9 Hz, 2H), 6.73 (s, 1H), 6.67 (d, *J* = 8.5 Hz, 2H), 6.44 (d, *J* = 8.3 Hz, 2H), 4.03-4.02 (m, 8H), 2.32 (s, 3H), 2.23 (s, 3H), 1.02 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.4, 156.2, 154.8, 151.9, 142.3, 140.4, 139.1, 137.9, 136.0, 135.9, 134.6, 134.3, 131.2, 128.0, 127.3, 125.8, 124.4, 123.5, 123.1, 121.7, 120.1, 114.2, 112.7, 111.8, 109.9, 106.4, 105.5, 104.1, 65.0 (2C), 64.5 (2C), 21.2 (2C), 14.3 (4C), 9.7 (2C); HPLC: 94.8 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 7.12 min; IR (KBr, cm^{-1}): 2939, 2867, 1577, 1442, 817; MS (ES mass): m/z 617.2 (M+1).

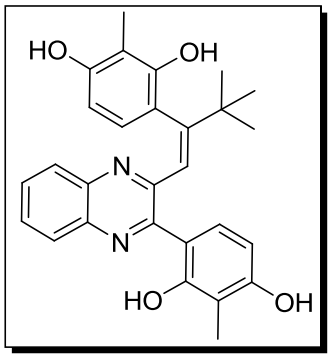
(*Z*)-4-(3-(2-(2,4-Dihydroxyphenyl)-3,3-dimethylbut-1-enyl)quinoxalin-2-yl)benzene-1,3-diol (4fa)



Yellow solid; mp: 208-210°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 10.56 (s, 1H), 9.72 (s, 1H), 8.91 (s, 1H), 8.46 (s, 1H), 7.94-7.80 (m, 1H), 7.63 (t, *J* = 7.0 Hz, 2H), 7.54-7.49 (m, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 6.72 (s, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 6.49-6.29 (m, 2H), 6.06 (d, *J* = 6.3 Hz, 2H), 1.09-1.01 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 160.1,

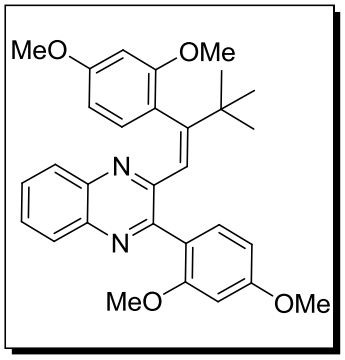
157.7, 156.9, 155.7, 153.6, 153.5, 152.4, 140.0, 139.0, 132.7, 131.4, 129.5, 129.3, 128.7 (2C), 123.9, 117.6, 115.8, 106.9, 105.4, 102.9, 102.4, 60.1, 29.7 (3C); HPLC: 95.1 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.31 min; IR (KBr, cm^{-1}): 3341, 2915, 2842, 1563, 1452, 826; MS (ES mass): m/z 428.9 (M^+).

(*Z*)-4-(3-(2-(2,4-Dihydroxy-3-methylphenyl)-3,3-dimethylbut-1-enyl)quinoxalin-2-yl)-2-methylbenzene-1,3-diol (4fb)



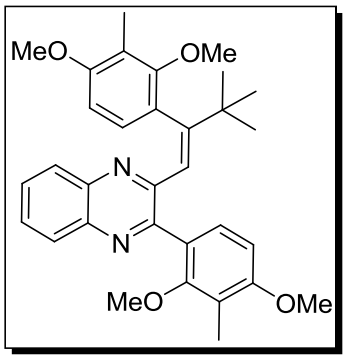
Light yellow solid; mp: 240-242 °C; ^1H NMR (400 MHz, $CDCl_3$) ppm: 11.73 (s, 1H), 11.05 (s, 1H), 9.93 (s, 1H), 8.97 (s, 1H), 7.95 (d, *J* = 6.1 Hz, 1H), 7.82 (s, 1H), 7.71 (d, *J* = 9.1 Hz, 1H), 7.64 (d, *J* = 9.1 Hz, 1H), 6.84 (s, 1H), 6.51 (d, *J* = 8.3 Hz, 2H), 6.21 (d, *J* = 8.4 Hz, 2H), 2.08 (s, 3H), 1.95 (s, 3H), 1.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.6, 156.9, 154.1, 145.1, 138.1, 137.7, 129.7, 129.4 (2C), 128.5, 128.2 (2C), 127.5, 127.4, 120.5, 120.4, 115.3, 112.2, 110.2, 108.1, 104.4, 101.1, 60.2, 29.6 (3C), 9.2 (2C); HPLC: 98.3 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.47 min; IR (KBr, cm^{-1}): 3347, 2927, 2834, 1542, 1419, 918; MS (ES mass): m/z 457.1 (M+1).

(*Z*)-2-(2,4-Dimethoxyphenyl)-3-(2-(2,4-dimethoxyphenyl)-3,3-dimethylbut-1-enyl)quinoxalines (4fc)



Yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.08-7.92 (m, 1H), 7.56 (d, *J* = 5.4 Hz, 3H), 7.28 (s, 1H), 6.72 (s, 1H), 6.65 (d, *J* = 8.2 Hz, 2H), 6.57 (d, *J* = 2.1 Hz, 1H), 6.39 (s, 1H), 6.34-6.23 (m, 1H), 3.91 (s, 3H), 3.80 (s, 3H), 3.75 (s, 3H), 3.72 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.7, 158.9, 158.2, 157.9, 156.5, 141.4, 140.5, 138.9, 132.1, 131.0, 130.7, 128.9, 128.5 (2C), 127.9, 127.1, 111.0, 108.7, 105.5, 105.1, 101.3, 99.5, 57.5, 56.1 (2C), 54.2 (2C), 29.6 (3C); HPLC: 91.4 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.46 min; IR (KBr, cm^{-1}): 2932, 2841, 1563, 1422, 913; MS (ES mass): m/z 484.8 (M^{+}).

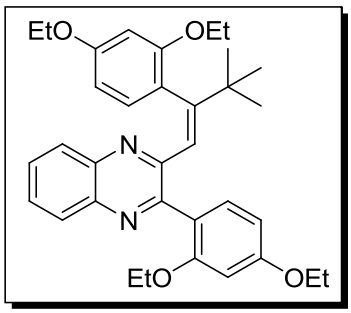
(*Z*)-2-(2,4-Dimethoxy-3-methylphenyl)-3-(2-(2,4-dimethoxy-3-methylphenyl)-3,3-dimethylbut-1-enyl)quinoxalines (4fd)



Yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.95 (d, *J* = 7.3 Hz, 1H), 7.58 (d, *J* = 4.2 Hz, 1H), 7.55-7.49 (m, 1H), 7.30-7.24 (m, 1H), 6.70 (s, 1H), 6.64 (d, *J* = 8.3 Hz, 1H), 6.56 (d, *J* = 2.1 Hz, 1H), 6.39 (s, 1H), 6.26 (d, *J* = 8.2 Hz, 1H), 3.90 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.72 (s, 3H), 1.25 (s, 6H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 161.7, 159.5, 158.1, 152.9, 140.9, 140.1, 131.8 (2C), 130.0, 128.9 (2C), 128.7 (2C),

128.5, 128.4, 128.0, 123.0 (2C), 121.1, 104.6, 102.7, 59.9, 55.4 (2C), 55.1 (2C), 37.4, 29.6 (3C), 9.6 (2C); HPLC: 90.6 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.37 min; IR (KBr, cm^{-1}): 2927, 2854, 1603, 1571, 1443, 804; MS (ES mass): m/z 512.9 (M^+).

(*Z*)-2-(2,4-Diethoxyphenyl)-3-(2-(2,4-diethoxyphenyl)-3,3-dimethylbut-1-enyl)quinoxalines (4fe)



Light yellow liquid; ^1H NMR (400 MHz, $CDCl_3$) ppm: 7.95 (d, *J* = 6.3 Hz, 1H), 7.68-7.59 (m, 1H), 7.58-7.48 (m, 2H), 7.27-7.19 (m, 1H), 6.78 (d, *J* = 6.2 Hz, 1H), 6.59 (d, *J* = 8.3 Hz, 1H), 6.55 (d, *J* = 4.3 Hz, 1H), 6.28 (s, 1H), 6.25 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 1H), 4.16-4.08 (m, 2H), 4.03 (q, *J* = 6.9 Hz, 2H), 3.96 (q, *J* = 6.9 Hz, 2H), 3.92 (q, *J* = 6.9 Hz, 2H), 1.50 (t, *J* = 6.8 Hz, 3H), 1.37 (t, *J* = 7.0 Hz, 3H), 1.28 (m, 6H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 160.9, 158.6 (2C), 157.7, 157.4, 153.1, 140.7, 132.2, 128.8 (2C), 128.5, 128.3(2C), 123.1 (2C), 121.2, 114.8, 112.8, 105.1 (2C), 103.3, 102.2, 63.6, 63.5, 63.2, 63.0, 37.4, 29.7 (3C), 14.8, 14.7 (2C), 14.6; HPLC: 96.4 %; column: Symmetry C-18 75*4.6 mm, 3.5µm, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B% : 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.53 min; IR (KBr, cm^{-1}): 2963, 2847, 1604, 1565, 1429, 811; MS (ES mass): m/z 540.9 (M^+).

Reference:

1) (a) Smits, R. A.; Lim, H. D.; Hanzer, A.; Zuiderveld, O. P.; Guaita, E.; Adami, M.; Coruzzi, G.; Leurs, R.; DeEsch, I. J. P. *J. Med. Chem*. **2008**, *51*, 2457; (b) Rong, F.;
Chow, S.; Yan, S.; Larson, G.; Hong, Z.; Wu, J. *Bioorg. Med. Chem. Lett*. **2007**, *17*,
1663; (c) Gao, H. L.; Yamasaki, E. F.; Chan, K. K.; Shen, L. L.; Snapka, R. M.*Mol.*
Pharmacol. **2003**, *63*, 1382; (d) Carta, A.; Loriga, M.; Paglietti, G.; Mattana, A.; Fiori, P. L.; Mollicotti, P.; Sechi, L.; Anetti, S. *Eur. J. Med. Chem*. **2004**, *39*, 195; (e)Hui, X.; Desrivot, J.; Bories, C.; Loiseau, P. M.; Franck, X.; Hocquemiller, R.; Figadere, *B. Bioorg. Med. Chem. Lett*. **2006**, *16*, 815;
2) Olah, G. A.; Krishnamurit, R.; Prakash, G. K. S. Friedel–Crafts Alkylations, In *Comprehensive Organic Synthesis*, Vol. 3; Trost, B. M.; Fleming, I., Eds.; Pergamon: Oxford, **1991**, 293–339.
3) (a) Jia, C. G.; Lu, W. T.; Oyamada, J.; Kitamura, T.; Matsuda, K.; Irie, M.; Fujiwara, Y. *J. Am. Chem. Soc*. **2000**, *122*, 7252. (b) Reetz, M. T.; Sommer, K. Eur. *J. Org. Chem*. **2003**, 3485.
4) (a) Ritleng, V.; Sirlin, C.; Pfeffer, M. *Chem. Rev*. **2002** , *102*, 1731; (b) Shilov; Shul.'pin, G. B. *Chem. Rev*. **1997** , *97*, 2879; (c) Kakiuchi, F.; Chatani, N. *Adv. Synth.Catal*. **2003**, *345*, 1077; (d) Kakiuchi, F.; Kochi, T. *Synthesis* **2008**, 301. (e) Kumar, K. S.; Rambabu, D.; Sandra, S.; Kapavarapu, R.; Krishna G. R.; Rao, M.V. B.; Kiranam Chatti, K.; Reddy, C. M.; Misra, P.; Pal, M. *Bioorg. Med. Chem.* **2012**, *20*, 1711.
5) (a) Hazeldine, S. T.; Polin, L.; Kushner, J.; Paluch, J.; White, K.; Edelstein, M.; Palomino, E.; Corbett, T. H.; Horwitz, J. P. *J. Med. Chem*. **2001**, *44*, 1758; (b) Rong, F.; Chow, S.; Yan, S.; Larson, G.; Hong, Z.; Wu, J. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 1663; (c) Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *J. Med. Chem*. **2005**, *48*, 2019; (d) Smits, R. A.; Lim, H. D.; Hanzer, A.; Zuiderveld, O. P.; Guaita, E.; Adami, M.; bCoruzzi, G.; Leurs, R.; de Esch, I. J. P. *J. Med. Chem*. **2008**, *51*, 2457; (e) Burguete, A.; Pontiki, E.; Hadjipavlou-Litina, D.; Villar, R.;

Vicente, E.; Solano, B.; Ancizu, S.; Perez-Silanes, S.; Aldana, I.; Monge, A. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 6439.

6) Koch, P.; Jahns, H.; Schattel, V.; Goettert, M.; Laufer, S. *J. Med. Chem*. **2010**, *53*, 1128.
7) Pissot-Soldermann, C.; Gerspacher, M.; Furet, P.; Gaul, C.; Holzer, P.; Mc Carthy, C.; Radimerski, T.; Regnier, C. H.; Baffert, F.; Drueckes, P.; Tavares, G. A.; Vangrevelinghe, E.; Blasco, F.; Ottaviani, G.; Ossola, F.; Scesa, J.; Reetz, *J. Bioorg.Med. Chem. Lett.* **2010**, *20*, 2609.
8) Novellino, E.; Cosimelli, B.; Ehlardo, M.; Greco, G.; Iadanza, M.; Lavecchia, A.; Rimoli, M. G.; Sala, A.; Da Settimo, A.; Primofiore, G.; Da Settimo, F.; Taliani, S.; La Motta, C.; Klotz, K.-N.; Tuscano, D.; Trincavelli, M. L.; Martini, C. *J. Med. Chem.***2005**, *48*, 8253.
9) Yamaguchi, M.; Hayashi, A.; Hirama, M. *J. Am. Chem. Soc*. **1995**,*117*, 1151.
10) Cacchi, S.; Fabrizi, G.; Goggiamani, A.; Persiani, D. *Org. Lett*, **2008**, *10*, 8,1597.
11) Oyamada, J.; Hashimoto, T.; Kitamura, T. *J. Organomet. Chem*. **2009**, *694*, 3626.
12) Lin, B.; Liu, M.; Ye, Z.; Zhang, Q.; Cheng, J. *Tetrahedron Lett.* **2009**, 50, 1714.
13) Xu, X.; Chen, J.; Gao, W.; Wu, H.; Ding, J.; Su, W. *Tetrahedron*. **2010**, *66*, 2433.
14) Park, C.-H.; Ryabova, V.; Seregin, I.V.; Sromek, A.W.; Gevorgyan, V. *Org. Lett*. **2004**, *6*, 7, 1159.
15) Pal, M.; Batchu, V.R.; Dager, I.; Kumara Swamy, N.; Padakanti, S. *J. Org. Chem*. **2005**, *70*, 2376.
16) Martins, A.; Alberico, D.; Lautens, M. *Org. Lett*. **2006**, *8*, 21, 4827.
17) For reviews, see: (a) Campeau, L.C.; Fagnou, K. *Chem. Commun*. **2006**, 1253. (b) Dick, A. R.; Sanford, M.S. *Tetrahedron.* **2006**, *62*, 2439. (c) Godula, K.; Sames, D. *Science*. **2006**, *312*, 67. (d) Alberico, D.; Scott, M. E.; Lautens, M. *Chem. Rev*. **2007**, *107*, 174 (e) Ackermann, L. Top. *Organomet. Chem*. **2007**, *24*, 35.
18) (a) Kakiuchi, F.; Murai, S. *Acc. Chem. Res*. **2002**, *35*, 826. (b) Nevado, C.; Echavarren, A. M. *Synthesis*. **2005**, 167. (c) Kakiuchi, F.; Kochi, T. *Synthesis* **2008**, 3013. (d) Kitamura, T. *Eur. J. Org. Chem*. **2009**, 1111.
19) For excellent reviews, see: (a) Schrock, R.R.; *Angew. Chem. Int. Ed. Engl*. **2006**, *45*, 3748. (b) Grubbs, R. H.; *Angew. Chem. Int. Ed. Engl*. **2006**, *45*, 3760. (c) For an anticancer drug, see: V. C. Jordan, *Nat. Rev. Drug Discov.* **2003**, *2*, 205.

20) (a) Kumar, K. S.; Kumar, P. M.; Kumar, K. A.; Sreenivasulu, M.; Jafar, A. A.; Rambabu, D.; Krishna, G. R.; Reddy, C. M.; Kapavarapu, R.; Kumar, K. S.; Priya, K. K.; Parsa, K. V. L.; Pal, M. *Chem. Commun*. **2011**, *47*, 5010 (b) 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. Commun*. **2013**, *49*, 190.

21) (a) Michan, S.; Sinclair, D. *Biochem. J*. **2007**, *404*, 1; (b) Bedalov, A.T.; Gatbonton, W. P.;. Gottschling Irvine, D. E.; Simon, J. A. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 15113.

22) Pal, M.; Dakarapu, R.; Parasuraman, K.; Subramanian, V. *J. Org. Chem.* **2005**, *70*, 7179

23) For in depth reviews, see: (a) Nevado, C.; Echavarren, A. M. *Synthesis.* **2005**, *167*; (b) Wang, X.; Zhou, L.; Lu, W. *Curr. Org. Chem.* **2010**, *14*, 289.

24) (a) Tsuchimoto, T.; Maeda, E.; Shirakawa.; Kawakami, Y.; *Chem. Commun*. **2000**, 1573. (b) Song, C. E.; Jung, D.-U.; Choung, S. Y.; Roh, E. J.; Lee, S.-G. *Angew. Chem. Int. Ed*. **2004**, *43*, 6183.

25) Bedalov, A.; Gatbonton, T.; Irvine, W. P.; Gottschling, D. E.; Simon, J. A. *Proc. Natl. Acad. Sci.* USA **2001**, *98*, 15113.

26) Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem.* **2001**, *276*, 38837.

27) (a) McGaughey, G.B.; Sheridan, R.P.; Bayly, C.I.; Culberson, J.C.; Kreatsolas, C.; Lindsley, S.; Maiorov, V.; Truchon, J.F.; Cornell, W.D. *J. Chem. Inf. Model.*, **2007**, *47*, 4, 1504. (b) McGann, M. *J. Chem. Inf. Model*., **2011**, *51*, 3, 578.

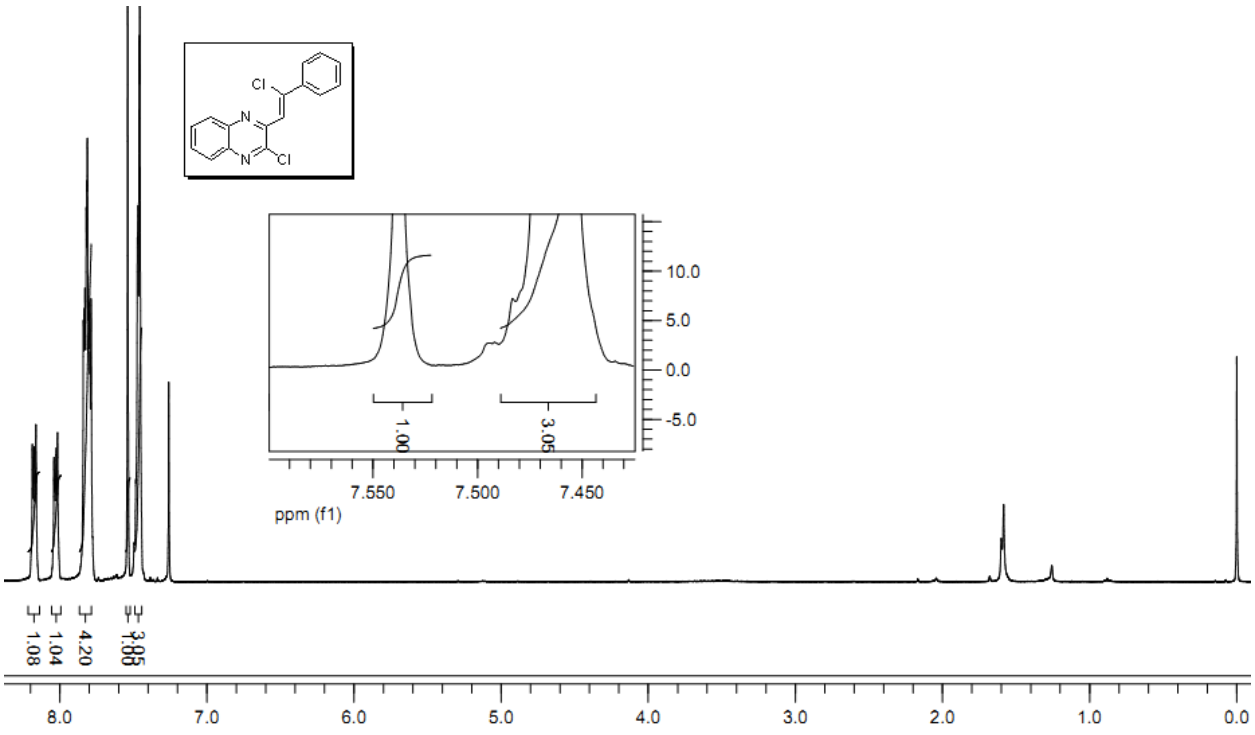
28) http://www.rcsb.org/pdb/explore/explore.do?structureId=2hjh

29) Westerfield, M. *A Guide for the Laboratory Use of Zebrafish* (Danio rerio). 4th edition. *Eugene, OR:* University of Oregon Press, **2000**.

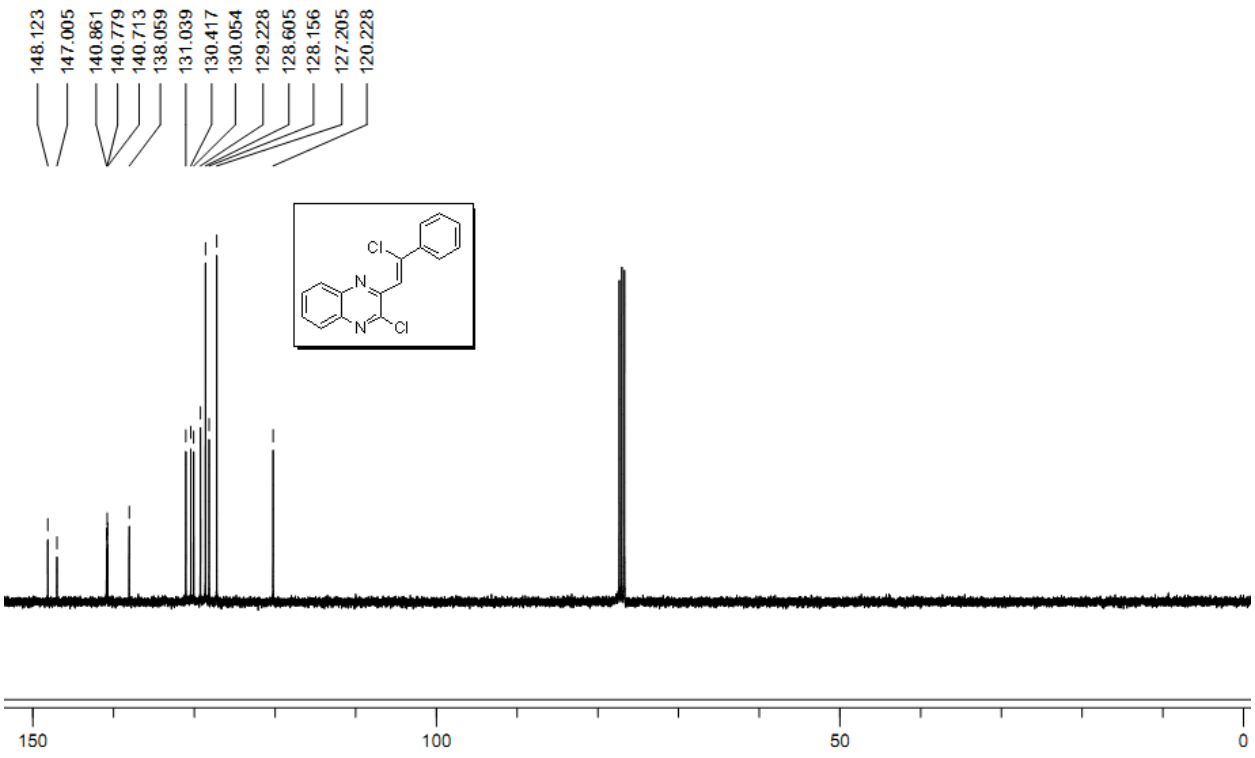
30) Nakhi, A.; Rahman, M. S.; Kishore, R.; Meda, C. L. T.; Deora, G. S.; Parsa, K. V. L.; Pal, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6433.

Appendix

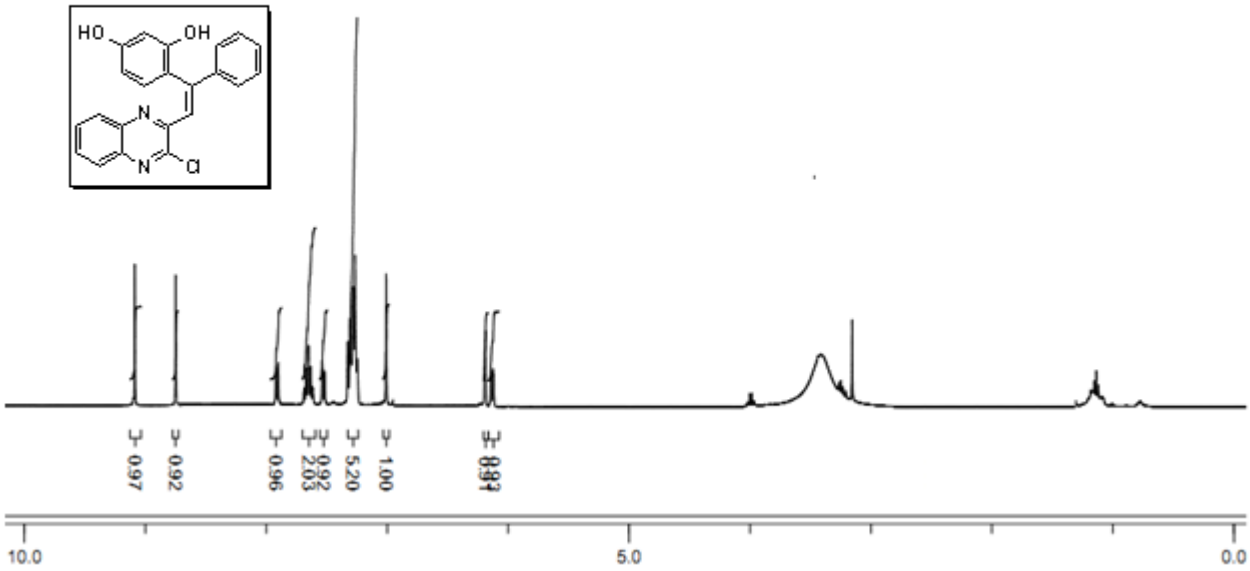
^{1}H NMR (400 MHz, $CDCl_3$)- **5**



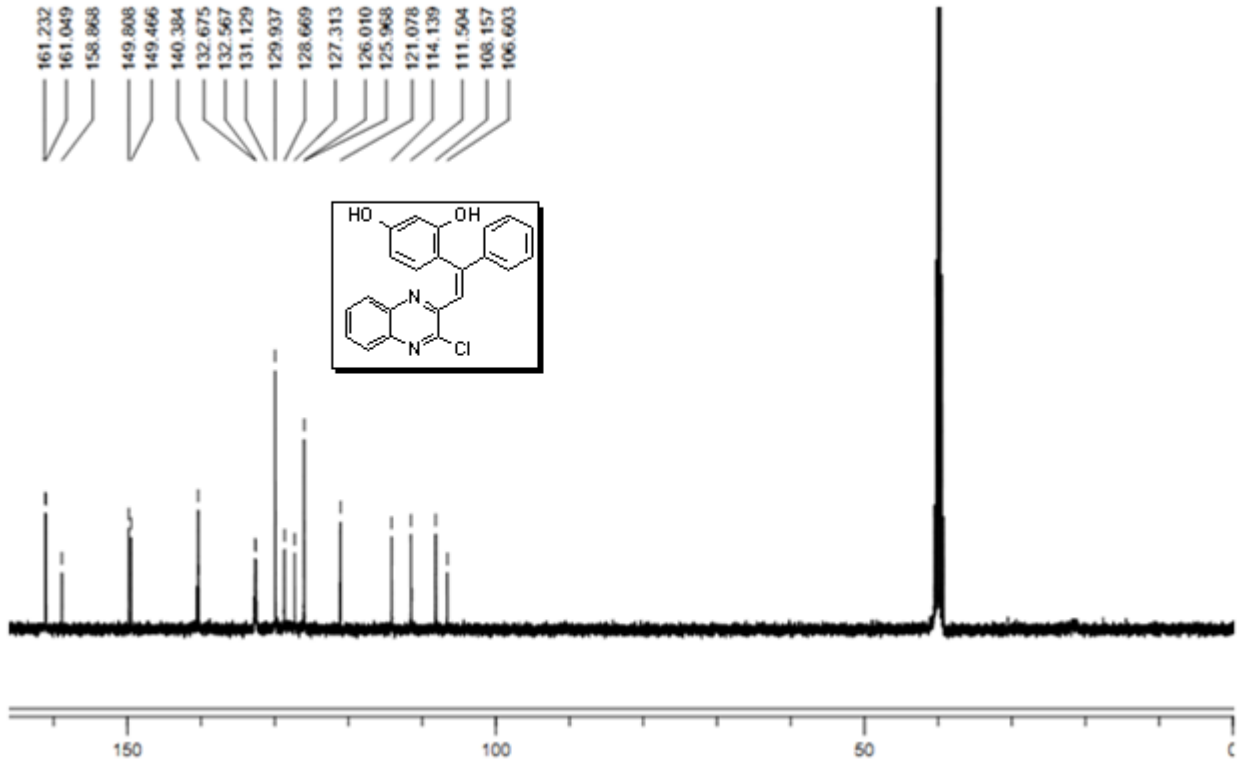
^{13}C NMR (100 MHz, $CDCl_3$)- **5**



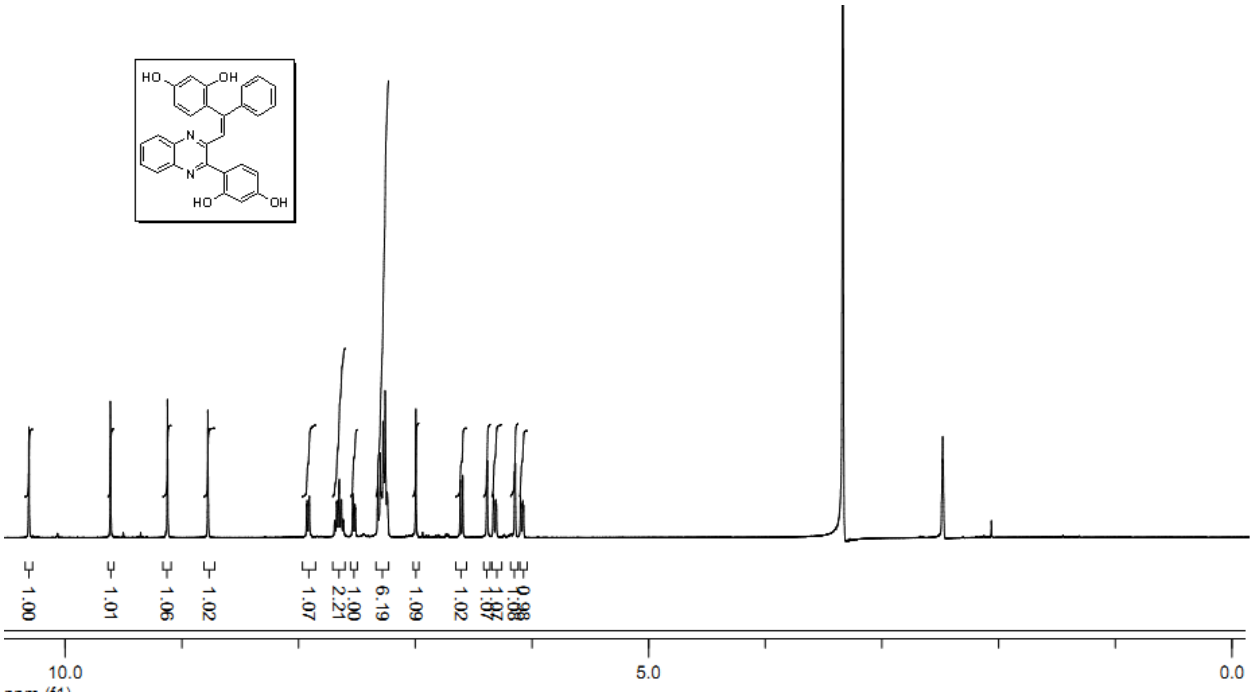
^{1}H NMR (400 MHz, DMSO-d_6) - **6**



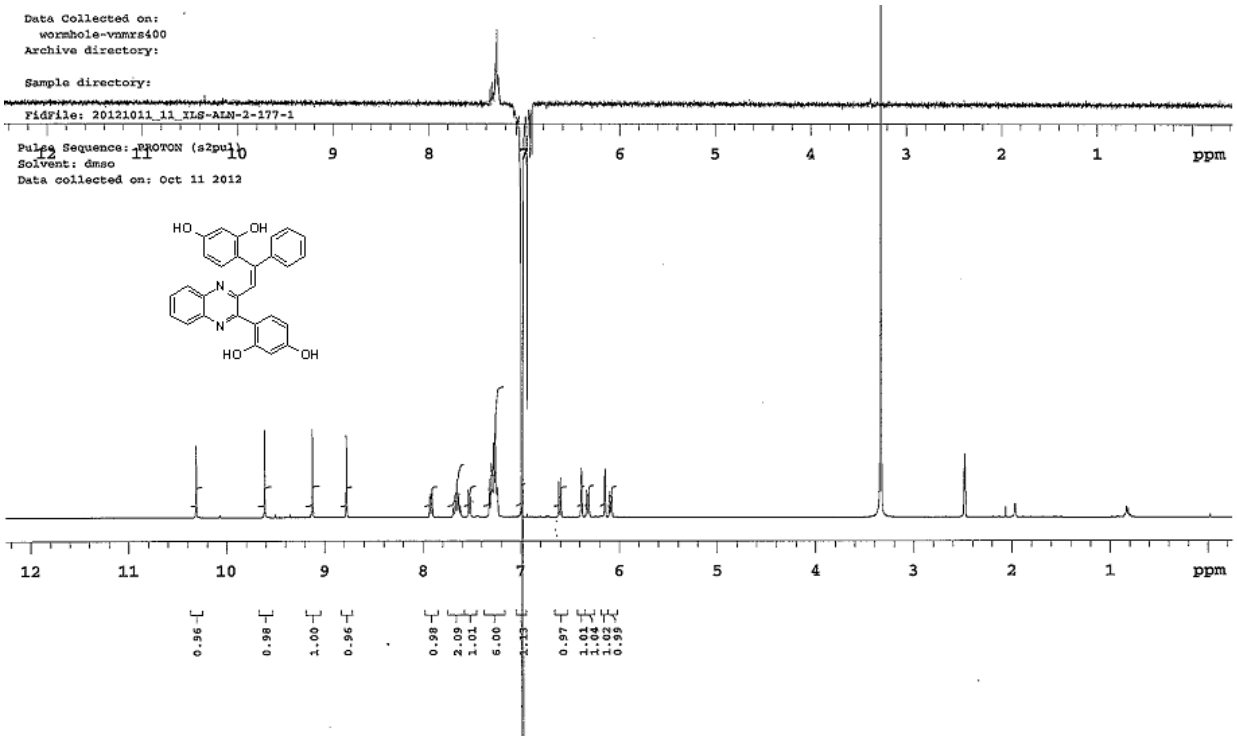
^{13}C NMR (100 MHz, DMSO-d_6)- **6**



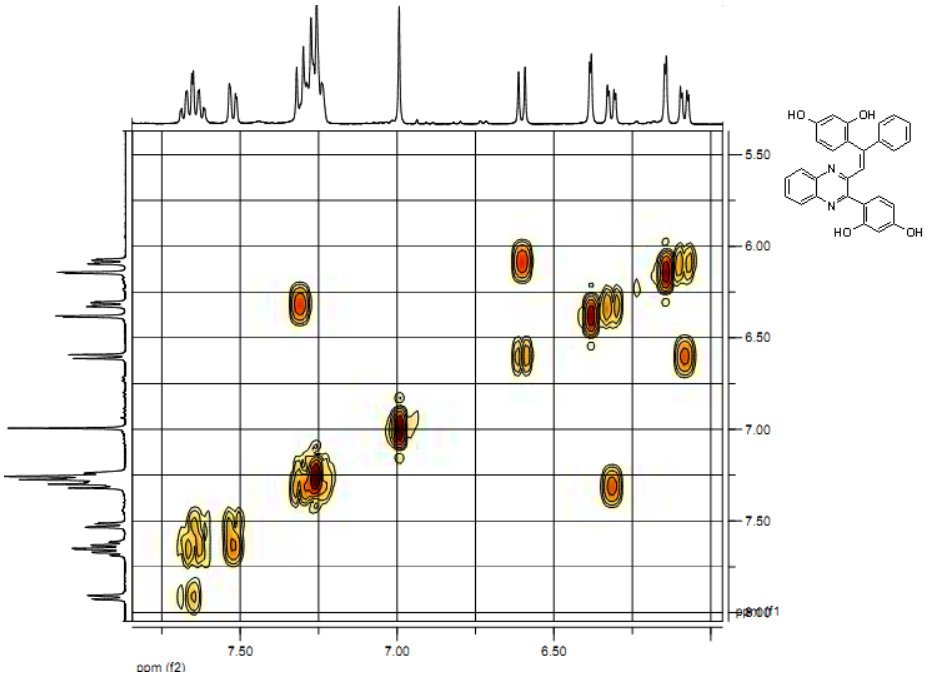
^{1}H NMR (400 MHz, DMSO-d_6) - **4aa**



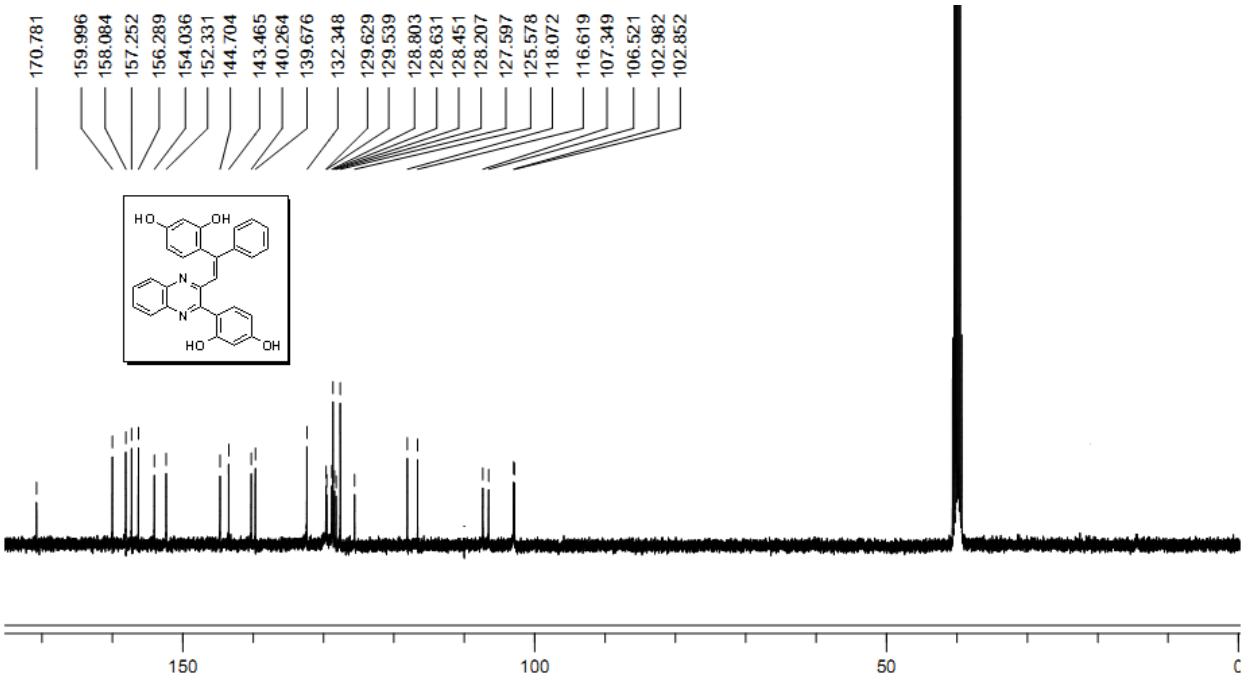
NOE- **4aa**



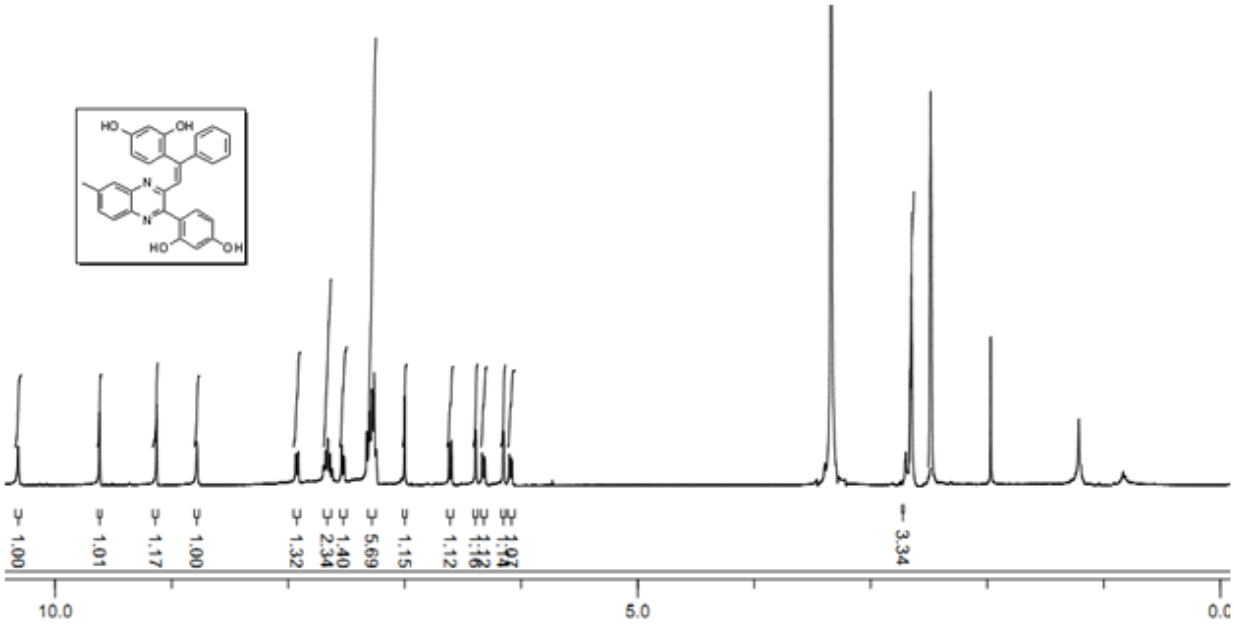
1D-COSY -**4aa**



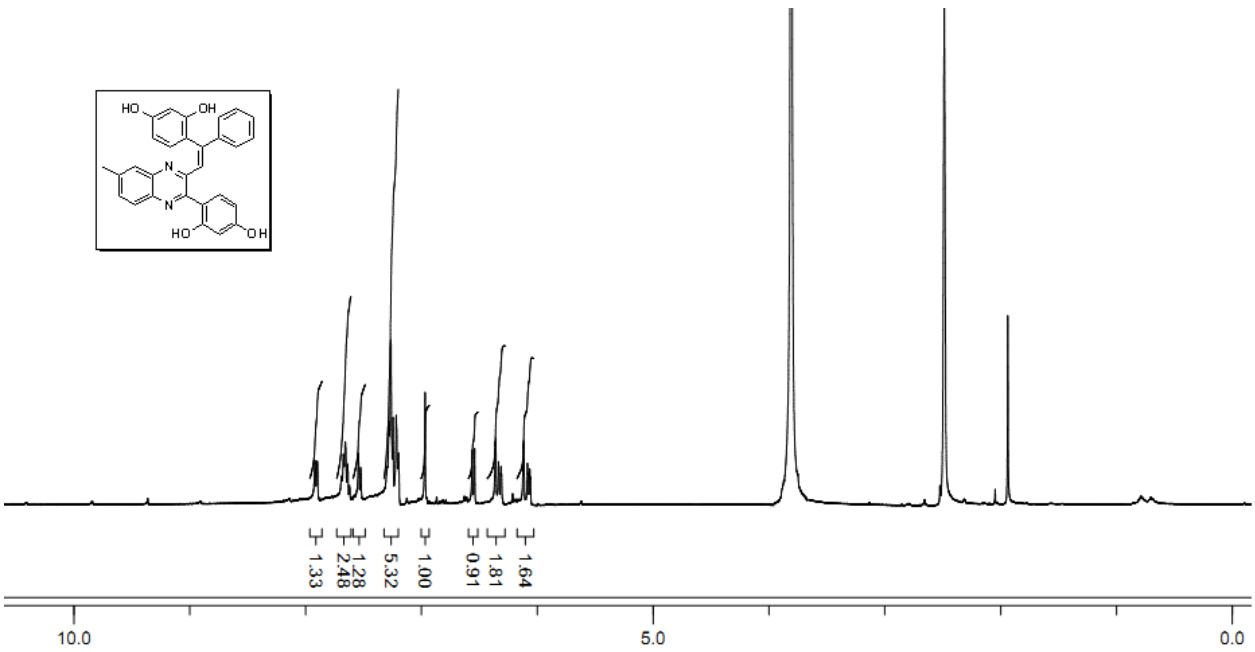
^{13}C NMR (100 MHz, DMSO-d_6) - **4aa**



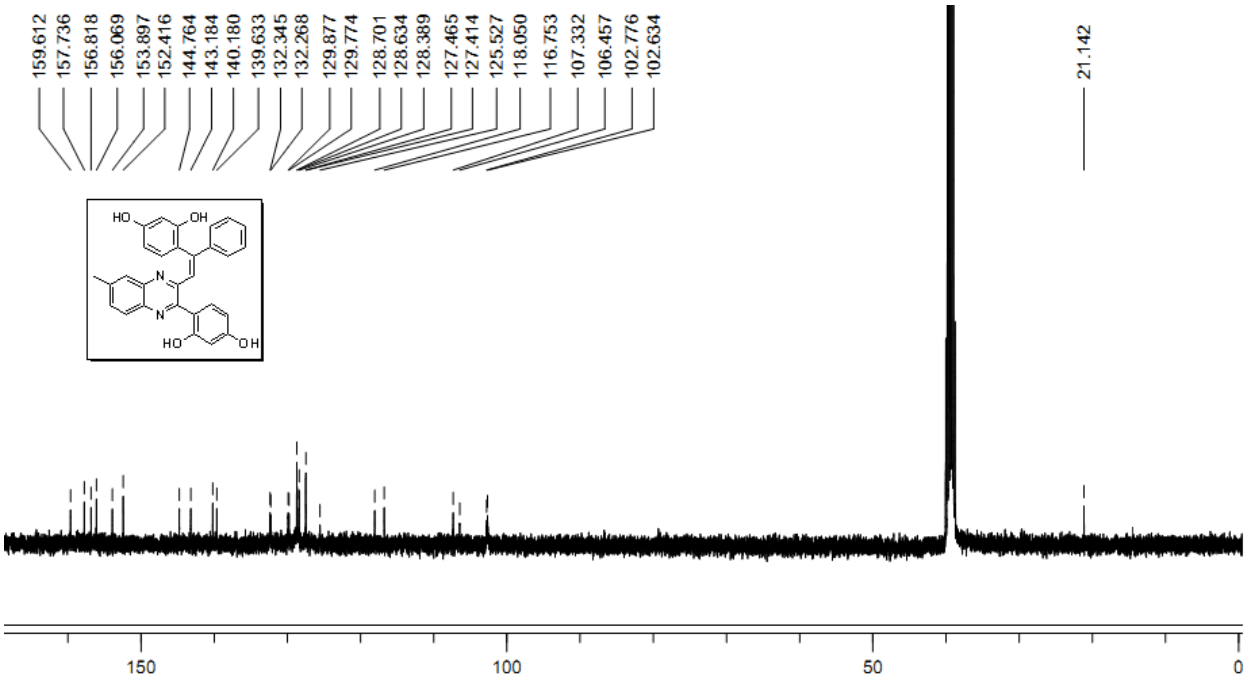
^{1}H NMR (400 MHz, DMSO-d_6) – **4ba**



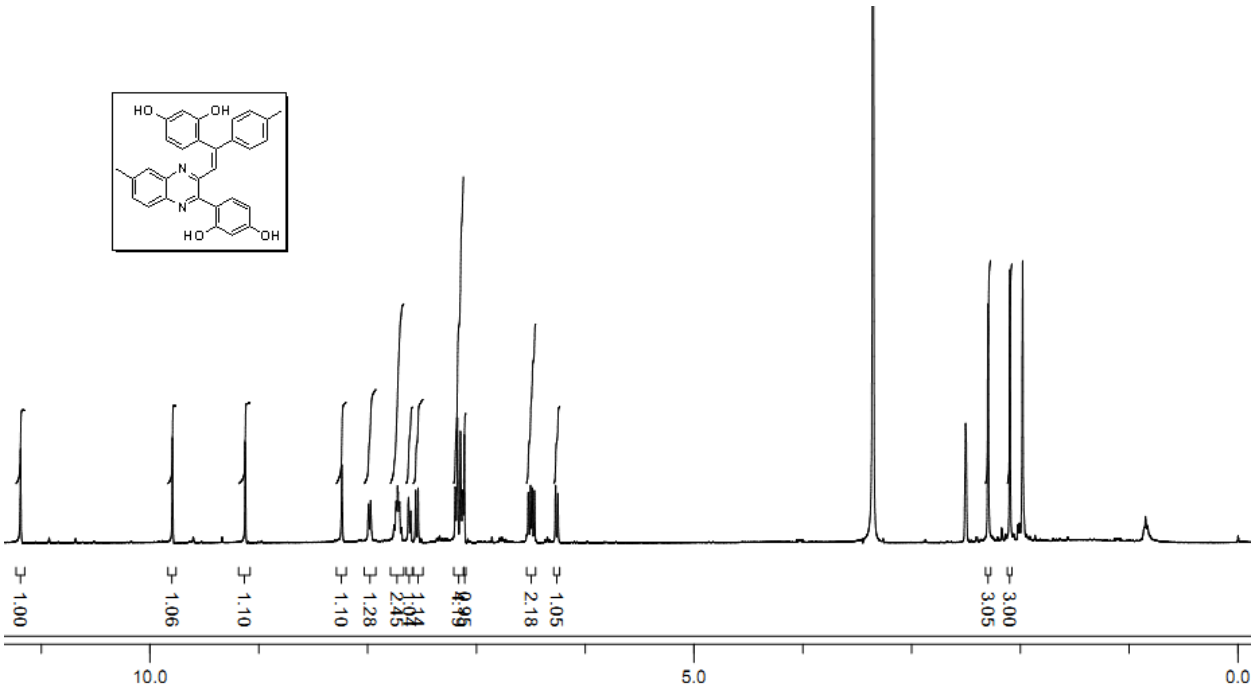
D_2O exchange - ^{1}H NMR (400 MHz, DMSO-d_6)– **4ba**



^{13}C NMR (100 MHz, DMSO-d_6)– **4ba**

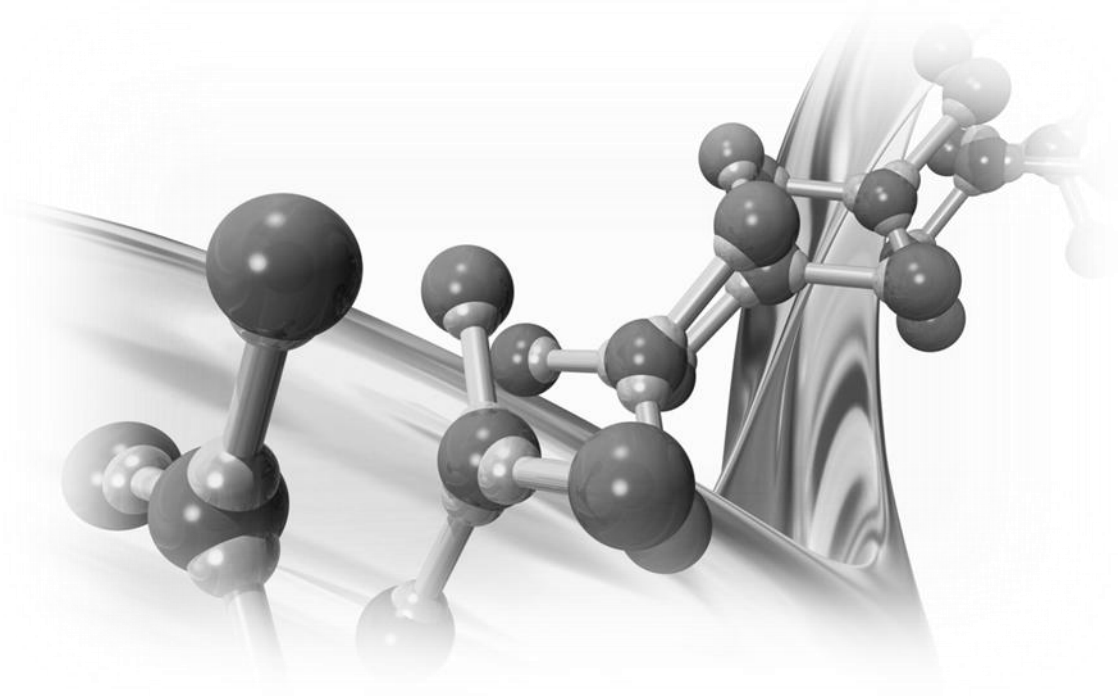


^{1}H NMR (400 MHz, DMSO-d_6) – **4ea**



CHAPTER 4

Synthesis of fused furan *N*-heterocycles as potential inhibitors of sirtuins



4.1. Introduction

Nitrogen containing quinoxalines and pyrazine motifs constitute an important class of heterocycles. Literature has revealed that quinoxalines possess numerous biological activities as in case of antibiotic echinomycin.[1] Quinoxaline hold up as a core unit in number of biologically active compounds including anticancer, antibacterial, antiviral, anti-inflammatory and anti HIV activity.[2] Naturally occurring quinoxalines are Quinomycins and Triostins.[3] These compounds show cytotoxic effects on the tumor cells. On the other hand, there is an increase in demand for synthetic methods of furan fused heterocycles. Furan fused nitrogen heterocycles are common structural motifs in biologically active compounds and drug candidates (Figure 1). For example, Elbfluorene (**A**) and its derivatives are interesting leads as cyclin-dependent kinase (CDK) inhibitors.[4] Benzofuropyrimidine (**B**) (MP-470)[5] is a novel multitarget tyrosine kinase inhibitor currently in Phase I clinical trials, while compound **C** and its analogues were found to be histamine H4 modulators.[6] Based on these facts and our continued interest in synthesizing heterocycle fused quinoxaline derivatives we planned to synthesize furan fused quinoxalines and pyrazines. Notably, benzo[*b*]furans have attracted considerable interest because of their presence in natural products,[7] biologically active compounds,[8] and other molecules of pharmaceutical interest.[9] These heterocycles also show wide spectrum of biological activities, which includes anticancer properties.[10]

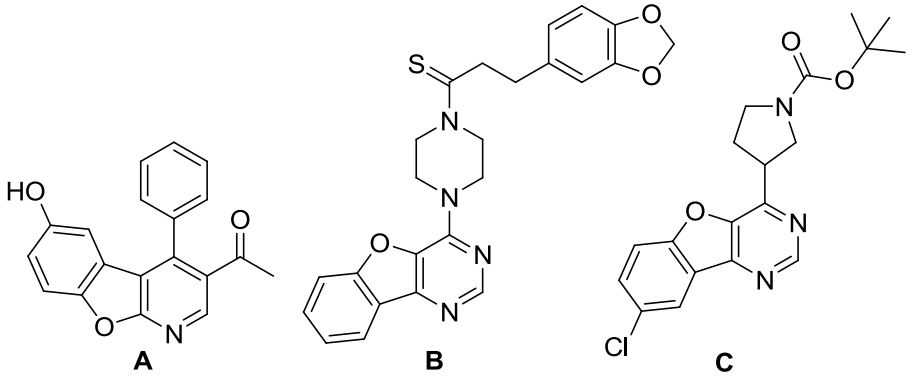
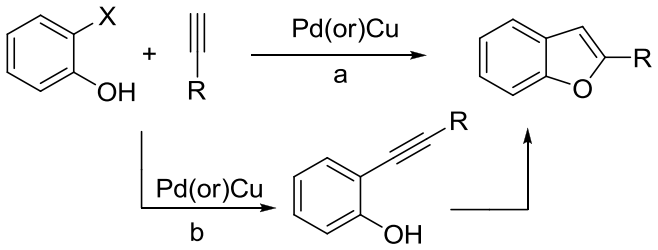


Figure 1. Biologically active furan fused nitrogen heterocycles.

These heterocycles is generally synthesized by metal-mediated heteroannulation of 2-halophenols with alkynes, as first demonstrated by Castro and co-workers in 1966. This method has been developed by the introduction of the Sonogashira procedure using 2-

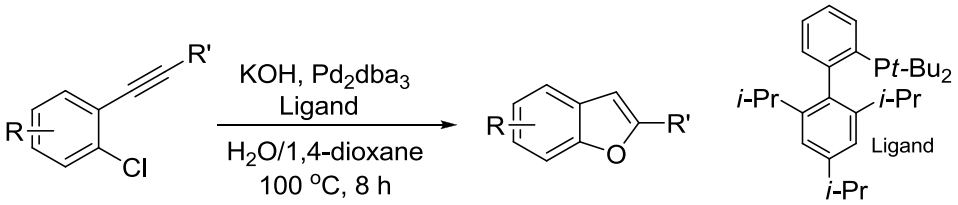
halophenols in a one-pot procedure (Scheme 1, path a) or *via* isolation of 2-alkynylphenol followed by subsequent cyclization (Scheme 1, path b).



Scheme 1. Metal-catalyzed Synthesis of 2-Substituted benzo[*b*]furans from 2-halophenols.

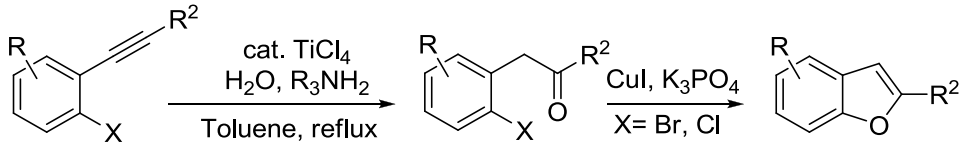
4.2. Previous work

In 2006, Buchwald and his group developed synthesis of substituted benzofurans starting from a 2-chloroaryl alkyne using Pd_2dba_3 catalyst system.[11]



Scheme 2. Synthesis of benzo[*b*]furans from 2-chloroaryl alkynes.

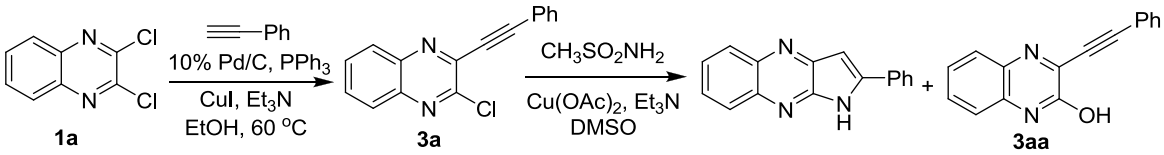
Ackermann and his group in 2007 reported $TiCl_4$-catalyzed indirect hydration reactions of unsymmetrically substituted internal and terminal alkynes, employing both aryl and alkyl amines.[12]



Scheme 3. Synthesis of benzo[*b*]furans *via* $TiCl_4$-catalyzed hydration of alkynes.

In 2012, we reported the $Cu(OAc)_2$ synthesis of pyrrolo[2,3-*b*]quinoxalines from 2-chloro 3-alkynylquinoxalines using methane sulfonamide ($CH_3SO_2NH_2$) as ammonia surrogate in DMF solvent. The formation of compound **3aa** 3-(phenylethynyl)quinoxalin-2-ol as a

major product while using DMSO solvent made us to explore the synthesis of furo[2,3-*b*]quinoxalines/pyrazines.[13]



Scheme 4. $Cu(OAc)_2$ mediated synthesis of 2-substituted-1*H*-pyrrolo[*2*,3-*b*]quinoxalines

The present work was mainly driven by our observation of formation of side product while performing the reaction for the synthesis of NH free pyrrolo[2,3-*b*]quinoxalines from 3-alkynyl-2-chloro quinoxalines (**3**) by using methane sulfonamide ($CH_3SO_2NH_2$) as ammonia surrogate in presence of $Cu(OAc)_2$ and Et_3N.[13] While selecting a suitable solvent for this reaction we made an attempt to use DMSO as solvent. Interestingly the use of DMSO provided 3-(phenylethynyl)quinoxalin-2-ol (**3aa**, Scheme 4) as a result of displacement of the chloro group of 3-alkynyl-2-chloro quinoxalines by a hydroxyl group the source of which seemed to be the traces of water present in DMSO used. We then repeated the same reaction without using any catalyst and we were able to isolate the product **3aa**. This gave us a clue that we can make use of this reaction condition for the synthesis of furo[2,3-*b*]quinoxalines/furo[2,3-*b*]pyrazine (**E**, Fig. 2).

4.3. Present work

Reaction media is of prime interest due to the worldwide concern of environmental safety and global warming as it plays a key role in deciding its environmental impact, cost, safety and health related issues. Use of 'green solvent' seems to open new vistas in synthetic organic chemistry. Thus the use of aqueous media in organic synthesis has gain high popularity as water is naturally abundant, safe and inexpensive which can be recycled.[14] Over the years, functionalized fused *N*-heteroaromatics have played key roles in the early stage of drug discovery and many of them have been marketed as successful drugs[15] e.g. blockbuster antibiotic levofloxacin. Furan fused *N*-heterocycles e.g. furoquinoxalines on the other hand have not been explored extensively as potential bioactive agents[16] perhaps due to their limited or complicated accessibility.

Our earlier observations on activities of pyrrolo[2,3-*b*]quinoxalines **D** against a panel of cancer cell lines[17] prompted us to design structurally similar scaffold **E** (Fig. 2). We then explored a greener route to furo[2,3-*b*]quinoxalines/furo[2,3-*b*]pyrazine (**E** Fig. 2). And these compounds were evaluated as potential inhibitors of sirtuins. Because of sirtuin up-regulation in various types of cancer, it is considered as promising target for cancer therapy.[18] Inhibition of sirtuins leads to reduced growth of cancer cells due to the re-expression of silenced tumor suppressor genes. Further, the inhibition property of sirtuin is supported by *in silico* binding studies in the catalytic pocket of yeast Sir2 followed by the toxicity studies in zebrafish embryo as *in vivo* model.

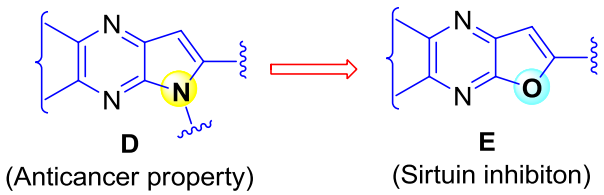


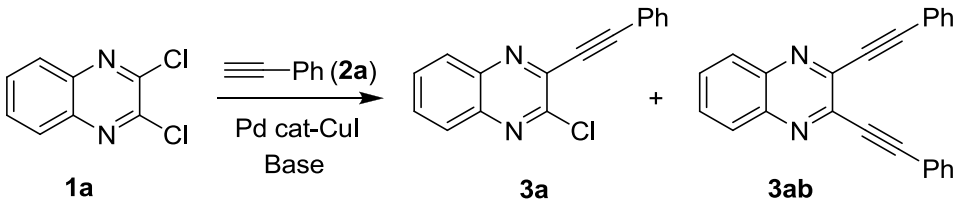
Figure 2. Design of **E** from **D** as novel inhibitors of sirtuins.

4.4. Results and Discussion

The synthesis of designed molecule **E** was started with the preparation of starting meterial **3**. The precursor of 3-alkynyl-2-chloro quinoxalines (**3**) i.e. 2,3-dichloro quinoxalines (**1**) were prepared by the condensation of phenylenediamines with diethyl oxalate to give the corresponding 1,4-dihydroquinoxaline-2,3-dione which on treatment with $POCl_3$ afforded the desired 2,3-dichloquinoxalines.[13] On coupling with various terminal alkynes (**2**) under a modified Sonogashira conditions the compound **1** afforded the required 3-alkynyl-2-chloro quinoxalines (**3**). To establish the optimized reaction conditions for selective mono alkynylation of **1** the dichloroquinoxaline (**1a**) was reacted with phenyl acetylene (**2a**) in the presence of various Pd catalysts and results are summarized in Table 1. The coupling reaction afforded the mono alkynylated product **3a** in 70% yield along with dialkynyl derivative **3aa** when 10% Pd/C-PPh_3-CuI was used as catalyst complex[20a] and Et_3N as a base (Entry 1, Table 1). The yield of **2a** was decreased when piperidine was used in place of Et_3N (Entry 2, Table 1). The use of other Pd catalysts e.g. $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$ resulted in a ~ 1:1 mixture of **3a** and **3ab** (Entries 3 and 4, Table 1). However, in

compared to $Pd(PPh_3)_2Cl_2$ or other Pd-catalysts the Pd/C has several advantages e.g. it is less expensive, stable, easily separable from the product by simple filtration and is recyclable. Thus, combination of 10% Pd/C-CuI-PPh_3 and Et_3N in EtOH was chosen as the preferred conditions for the present alkynylation reaction and was used to prepare compound **3** (Table 2, 3).

Table 1. Pd-mediated **c**oupling of **1a** with phenyl acetylene (**2a**) under various conditions.[a]



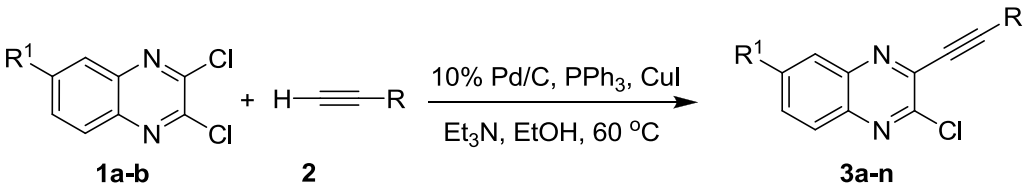
1a **3a** **3ab**

Entry	Palladium catalysts	Base	Time (h)	%Yield	
				3a	**3ab**
1	10%Pd/C[c]	Et_3N	2	70	18
2	10%Pd/C[c]	Piperidine	5	61	26
3	$Pd(PPh_3)_4$	Et_3N	4	45	47
4	$Pd(PPh_3)_2Cl_2$	Et_3N	5	48	42

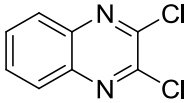
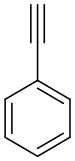
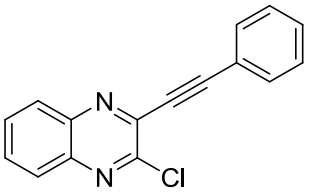
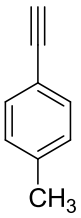
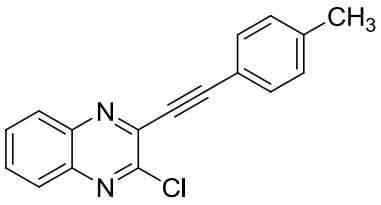
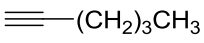
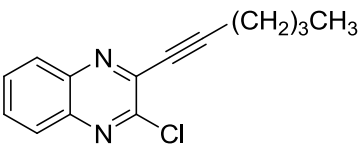
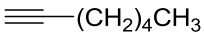
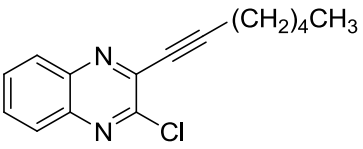
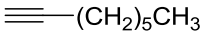
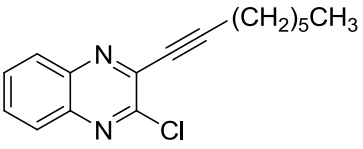
[a]All of the reactions were carried out using **1a** (1.256 mmol), phenyl acetylene **2a** (1.256 mmol), Pd catalyst (0.0125 mmol), CuI (0.0125 mmol) and base (1.8844 mmol) in EtOH (4 mL) at 60 ºC. [b]Isolated yield. [c]PPh_3 (0.0502 mmol) was used.

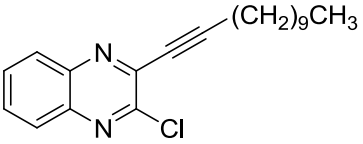
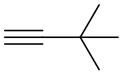
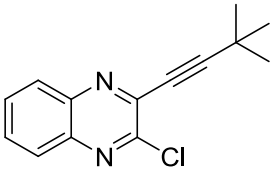
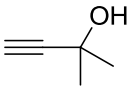
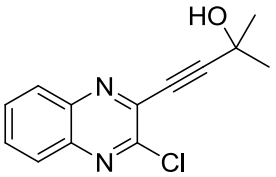
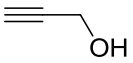
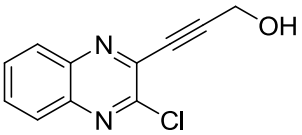
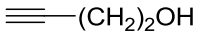
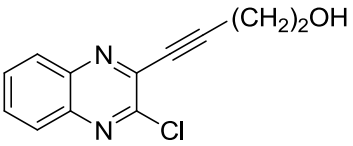
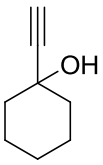
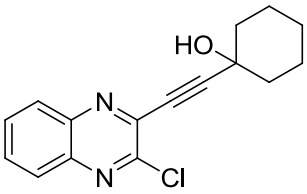
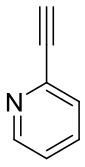
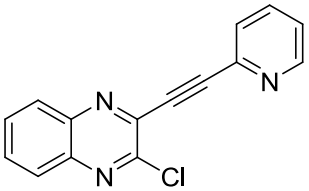
The key starting material **3** required was prepared *via* a selective mono alkynylation of 2,3-dichloroquinoxaline / pyrazine (**1**) under Pd-Cu catalysis (Table 2 and 3).

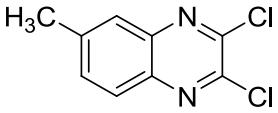
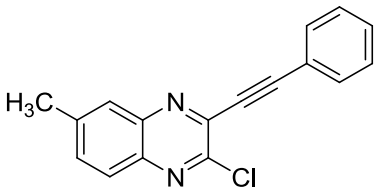
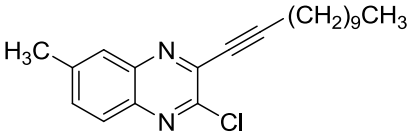
Table 2. Pd/C-CuI mediated synthesis of 3-alkynyl-2-chloroquinoxalines (**3a-n**)[a]



1a-b **2** **3a-n**

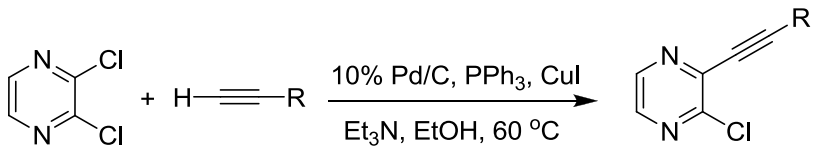
Entry	Halide (**1**)	Alkyne (**2**)	T (h)	Product (**3**)	Yield[b] (%)
1	 **1a**	 **2a**	2	 **3a**	70
2	**1a**	 **2b**	2	 **3b**	62
3	**1a**	 **2c**	2	 **3c**	65
4	**1a**	 **2d**	2	 **3d**	60
5	**1a**	 **2e**	3	 **3e**	66

6	**1a**	 **2f**	2	 **3f**	69
7	**1a**	 **2g**	3	 **3g**	65
8	**1a**	 **2h**	4	 **3h**	60
9	**1a**	 **2i**	4	 **3i**	68
10	**1a**	 **2j**	4	 **3j**	70
11	**1a**	 **2k**	4	 **3k**	68
12	**1a**	 **2l**	4	 **3l**	60

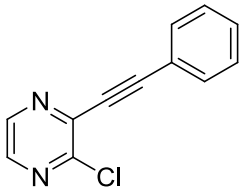
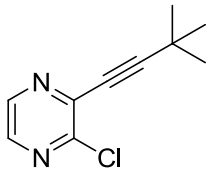
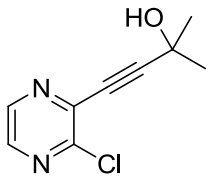
13	 **1b**	**2a**	3	 **3m**	63
14	**1b**	**2f**	2	 **3n**	67

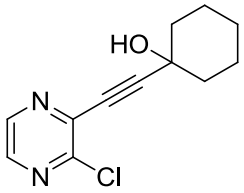
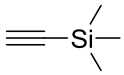
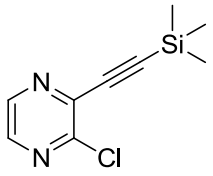
[a]All the reactions were carried out by using **1** (1.256 mmol), terminal alkyne **2** (1.256 mmol), 10% Pd/C (0.0125 mmol), PPh_3 (0.0502 mmol), CuI (0.0125 mmol), and Et_3N (1.8844 mmol) in EtOH (4 mL). [b]Isolated yield.

Table 3. Pd/C-CuI mediated synthesis of 3-alkynyl-2-chloropyrazines **3o-s**.[a]



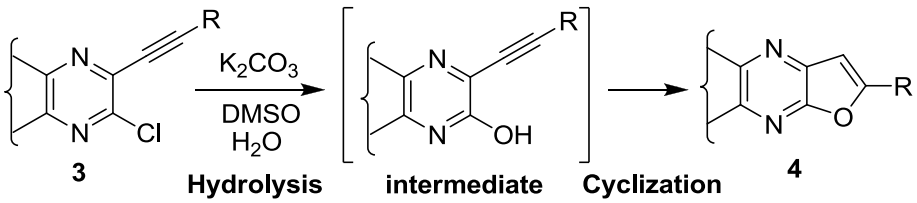
1c **2** **3o-s**

Entry	Alkyne (**2**)	T(h)	Product (**3**)	Yield[b] (%)
1	**2a**	2	 **3o**	64
2	**2g**	2	 **3p**	70
3	**2h**	4		70

4	**2k**	4	**3q** 	62
5	 **2m**	3	**3r**  **3s**	60[c]

[a]See footnote "a" of Table 1. [b]Isolated yield.[c]$Pd(PPh_3)_2Cl_2$ (0.0125 mmol) was used in place of Pd/C-CuI and PPh_3.

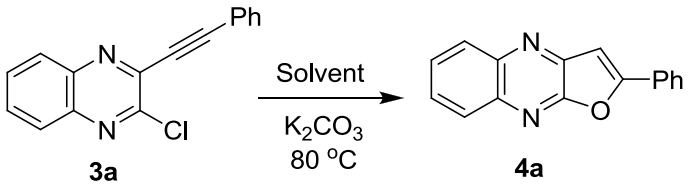
In this section our preliminary results on hydrolysis-cyclization strategy leading to desired furo derivatives **4** (or **E**, Scheme 5) is presented.



Scheme 5. Hydrolysis-cyclization strategy leading to furo derivatives **4**.

We then examined, the possibility of hydrolysis / cyclization of 2-chloro-3-(phenylethynyl) quinoxaline **3a** leading to **4a** in the presence of K_2CO_3. After assessing a range of solvents an appropriate combination of DMSO-H_2O was found to be effective perhaps due to the better solubility of **3a** (that was crucial for the success of the reaction) in this solvent system compared to other aqueous media including aqueous 1,4-dioxane.[7b] It was observed that only DMSO or water alone (entry 1 and 5, Table 4) was not effective whereas the best yield of **4a** was achieved when 2:8 DMSO-H_2O was used (entry 2, 3 and 4, Table 4). Moreover, we observed that the reaction was remarkably faster in aqueous DMSO rather than in aqueous 1,4-dioxane.

Table 4. Optimization of hydrolysis / cyclization of **3a**.[a]



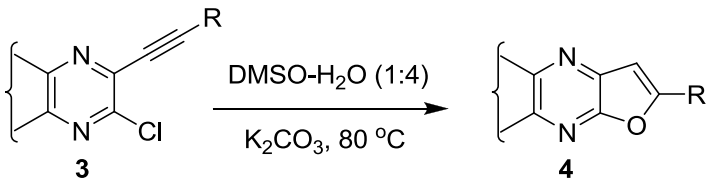
3a **4a**

Entry	Solvent	T/h	% Yield[b]
1	DMSO	4	65
2	DMSO/H_2O (1:1)	3	72
3	DMSO/H_2O (1:4)	1	82
4	DMSO/H_2O (1:8)	4	53
5	H_2O	8	28

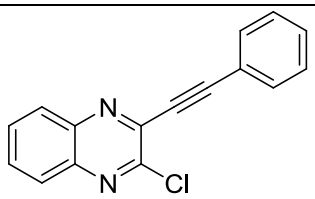
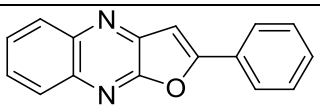
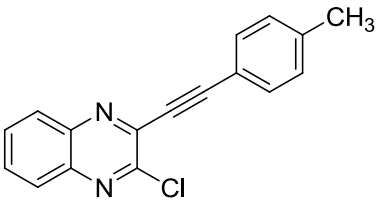
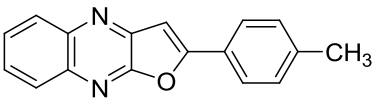
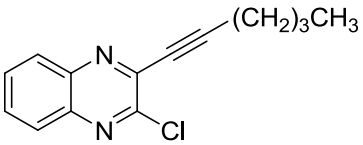
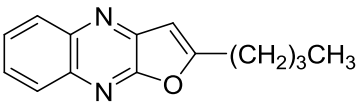
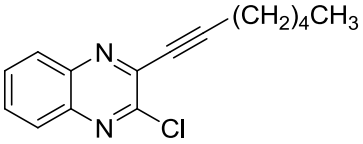
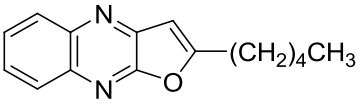
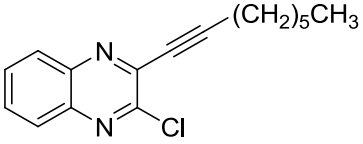
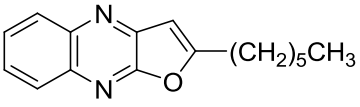
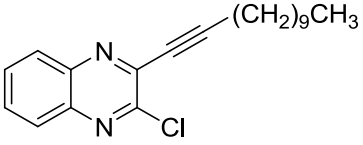
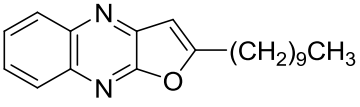
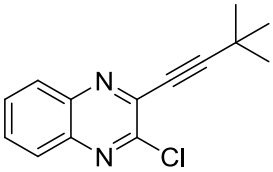
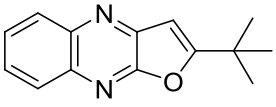
[a]Reactions were carried out using **3a** (0.7575 mmol), K_2CO_3 (0.3787 mmol) in solvent (3mL) at 80°C. [b]Isolated yield.

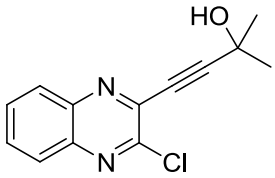
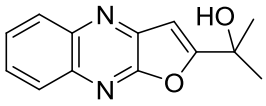
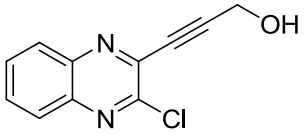
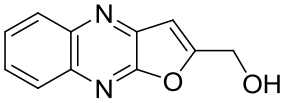
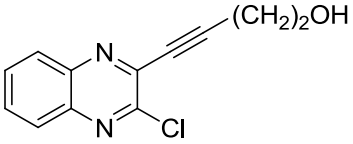
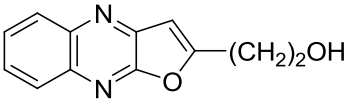
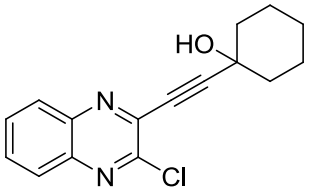
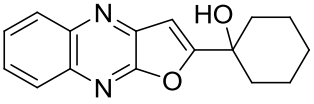
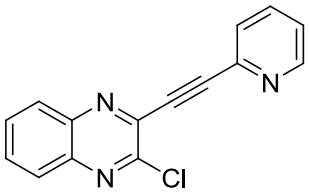
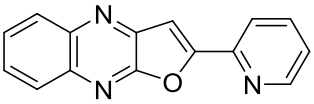
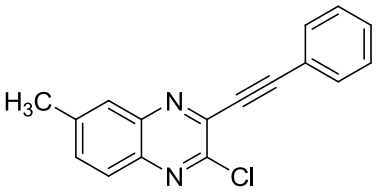
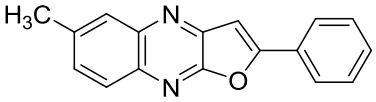
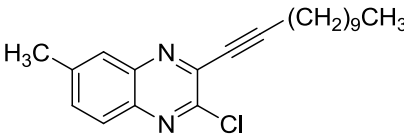
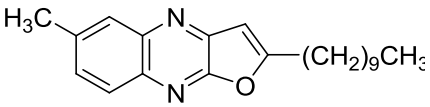
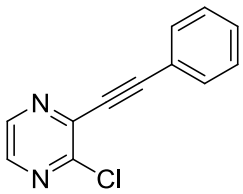
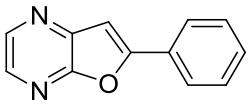
Thus the condition of entry 3 (Table 4) was found to be optimum and used for our further studies. We then examined the substrate scope and generality of this method (Table 5). The heteroaryl alkyne **3** containing contrasting groups e.g. aryl (**3a-b, 3m** and **3o**), heteroaryl (**3l**), alkyl (**3c-g**, **3p**, **3g** and **3n**), hydroxyalkyl (**3h-k**, **3q** and **3r**) and trimethylsilyl (**3s**) substituents on the triple bond participated well in the reaction. Both quinoxaline (**3a-n**) and pyrazine (**3o-s**) derivatives showed similar reactivities affording the desired products in acceptable yields.

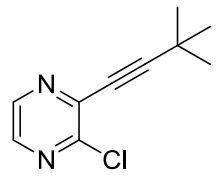
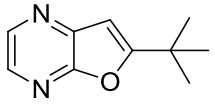
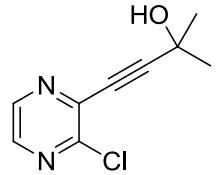
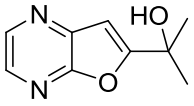
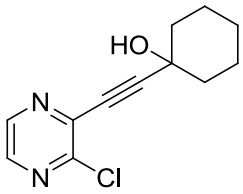
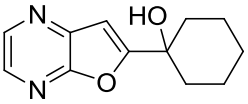
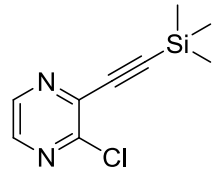
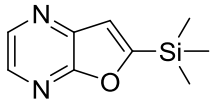
Table 5. Synthesis of furo[2,3-*b*]quinoxalines / pyrazines **4**[a].



3 **4**

Entry	Alkyne (**3**)	Time (h)	Product (**4**)	% Yield[b]
1	 **3a**	2	 **4a**	82
2	 **3b**	2	 **4b**	73
3	 **3c**	2	 **4c**	71
4	 **3d**	2	 **4d**	68
5	 **3e**	3	 **4e**	71
6	 **3f**	2	 **4f**	62
7	 **3g**	3	 **4g**	76

8	 **3h**	4	 **4h**	66
9	 **3i**	4	 **4i**	62
10	 **3j**	4	 **4j**	64
11	 **3k**	4	 **4k**	68
12	 **3l**	4	 **4l**	60
13	 **3m**	3	 **4m**	73
14	 **3n**	2	 **4n**	68
15		2	 **4o**	76

16	**3o**  **3p**	2	 **4p**	69[c]
17	 **3q**	4	 **4q**	66
18	 **3r**	4	 **4r**	62
19	 **3s**	3	 **4s**	58

[a]Reactions were carried out using **3** (0.7575 mmol), K_2CO_3 (0.3787 mmol) in DMSO–H_2O (1:4) (3 mL) at 80 °C. [b]Isolated yield. [c]KOH was used in place of K_2CO_3.

All the ninteen compounds synthesized by this method were well characterized by spectral (NMR, IR and MS) data. The molecular structure of a representative compound 2-phenylfuro[2,3-*b*]quinoxaline **4a** was further confirmed unambiguously by single crystal X-ray diffraction (Fig. 3).

Single crystal X-ray data for compound 4a

Crystals of compound **4a** (CCDC No. 935821) were grown from EtOAc. Single crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data obytained by using Bruker SMART APEX CCD single crystal diffractometer using graphite monochromated Mo-Kα radiation (0.71073 Å) at temperature 298 K. Structure was solved and refined by full-matrix least squares against

F^2 using SHELXL-97 software.[19] Crystal data of **4a:** Molecular formula = $C_{16}H_9N_2O$, Formula weight = 245.25, Monoclinic, C2/*c*, *a* = 25.558 (3) Å, *b* = 4.5239 (5) Å, *c* = 23.971 (3) Å, *V* = 2384.4 (5) $Å^3$, *T* = 298 K, *Z* = 8, D_c = 1.366 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1}, 9084 reflections were measured with 2122 unique reflections (R_{int} = 0.0316), of which 2122 ($I > 2\sigma(I)$) were used for the structure solution. Final R_1 (w R_2) = 0.0545 (0.1424), 172 parameters. The final Fourier difference synthesis showed minimum and maximum peaks of -0.239 and +0.557 e.$Å^{-3}$ respectively. Goodness of fit = 1.056

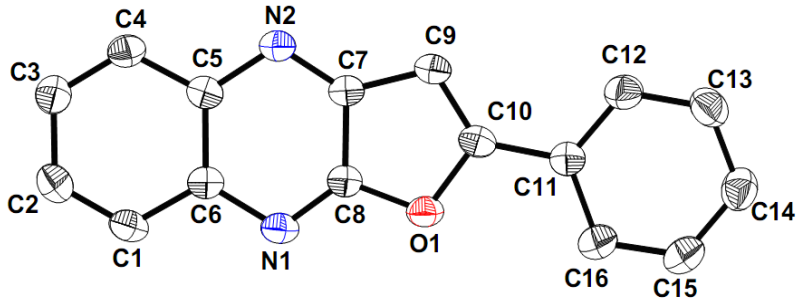


Figure 3. Ortep diagram of compound **4a** (30 % probability, Hydrogen atoms has been omitted for clarity).

The supramolecular interactions between nitrogen and hydrogen are within the range of 2.730 – 2.770 Å, it gives two types of interactions *i.e.* (N1···H15 = 2.734 Å, N2···H9 = 2.764 Å). Through these weak Vander-Waal interactions it gives a 1D network in its crystal packing, as shown in Figure 4.

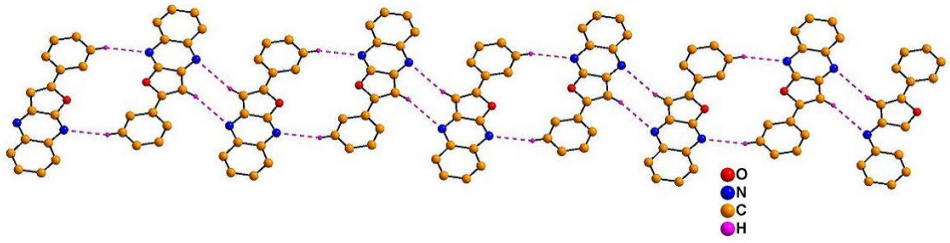
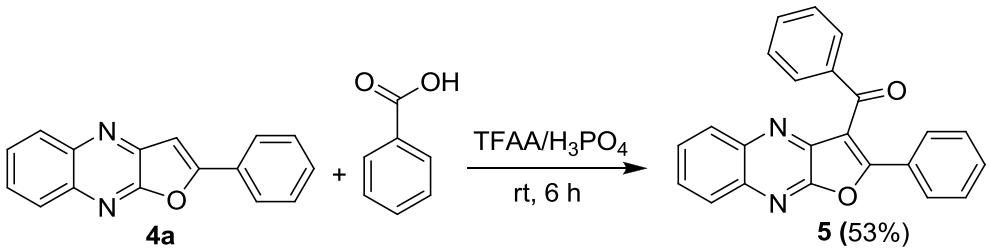


Figure 4. Supramolecular interactions and crystal packing of compound **4a.**

It is evident that the present transition metal[11] free hydrolysis-cyclization of alkyne **3** proceeds *via* a 2-hydroxy-3-alkynyl quinoxaline / pyrazine intermediate (Scheme 4).[20] To gain further evidence 3-(phenylethynyl)quinoxalin-2-ol (**3aa**) prepared from **3a** was iosalted and treated under the condition of entry 3 of Table 4 when **4a** was isolated in good yield. The scope of the present strategy was expanded further by using TFAA/H_3PO_4 mediated C-3 benzoylation of **4a** (Scheme 6).



Scheme 6. TFAA/H_3PO_4 mediated C-3 acylation of **4a**.

Finally, after synthesis and charecterization of all compounds, we focused our interest to pharmacological properties againest sirtuin protien which is discussed in this section.

4.5. Pharmacology

4.5.1. Yeast cell based *in vitro* URA3 reporter silencing assay

Sirtuins are NAD^+-dependent class III histone deacetylase (HDAC). Sirtuins are over expressed in some types of cancer cell, it can be a good target to treat cancer. With this reason we carried out screening of most of the compounds **4** along with Splitomicin[21] (known inhibitor of yeast Sir2) at 50 μM for their ability to inhibit Sir2 protein by estimating inhibition of growth of yeast strain containing URA3 gene at telomeric locus, in the presence of 5-fluoroorotic acid (5-FOA). As Sir2 protein is inhibited, the URA3 gene would be expressed resulting the death of yeast cell in the presence of 5-FOA. A parallel screen was performed in the absence of 5-FOA to check the cytotoxicity of compounds tested. Among them **4c**, **4d** and **4e** (entry 3,4 and 5, Table 5) showed significant inhibition (55%, 56% and 65%, respectively) in the presence of 5-FOA (Fig 5) and no significant toxic effect in the absence of 5-FOA. Compound **4e** showed IC_{50}~15.02 µM (Fig. 6) (comparable to Splitomicin's 4.2 μM) and **4d** showed IC_{50} 39.59 µM (see experimental section) in a dose respone study. The compound **4e** showed IC_{50}~23.5 µM against mammalian SIRT1 protien (Fig. 7). MTT assay **4e** strongly inhibited the cell growth when tested against human hepatocellular liver carcinoma (HepG2) and Cervical Cancer (Hela) cells at 50 µM concentration.

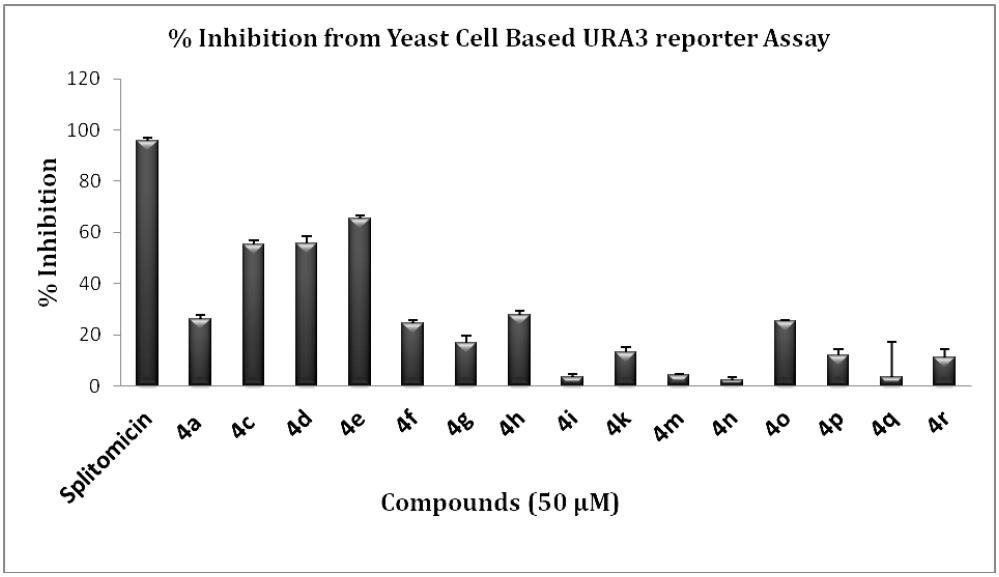


Figure 5. URA3 reporter assay of known inhibitor Splitomicin and **4**.

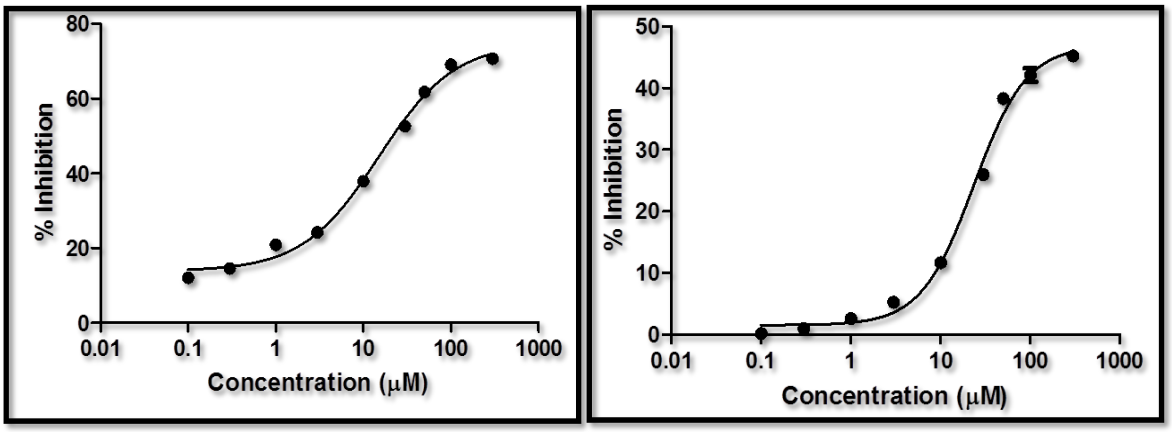


Figure 6. Dose dependent URA3 reporter assay of **4e** (IC_{50} 15.02 µM).
Figure 7. Dose dependent mammalian SIRT1 assay of **4e** (IC_{50} 23.5 µM).

4.5.2. Docking studies of compounds 4c, 4d and 4e (*in silico*)

Docking was performed by using the crystal structure coordinating with NAD-dependent protein deacetylase (PDB ID: 1Q1A).[22] The docking analysis of molecules was performed using FRED,[23a] version 3.0 implemented from OpenEye Scientific Software. All molecules were sketched in 3D format with VIDA program of OpenEye. Omega module[23b] was used to produce maximum conformers of the molecules and charges were added from mmff94s force field. The protein is preprocessed by removal of water molecules and assigning bond orders. Hydrogens were added to the protein from the program Reduce version 3.1. The final protein was obtained by optimizing the added hydrogens using conjugate gradient algorithm from SZYBKI version 1.7. The grid for molecular docking was generated with bound co-crystallized ligand. Interpretation of the docking results of the molecules **4c**, **4d** and **4e** reveal that these molecules binds in an orientation by positioning their furo[2,3-*b*]quinoxaline moiety near adeninine binding site and thereby passing aliphatic chain into nicotinamide binding site. All three compounds made conserved hydrogen bonding with two nitrogen of quinoxalines moiety and the backbone amino groups of SER12 and GLY 30 respectively (Fig. 8). There is a significant change in dock scores with increase in the length of the hydrophobic tail (Table 6). This could be due to the van der waals interactions made by this hydrophobic tail with TYR 48, ILE 118 and PHE 63. The hydrophobic surface view of the protein receptor based on Eisenberg potential shows the hydrophobic tail of **4c**, **4d** and **4e** aligning well with the hydrophobic surface area of the receptor (Fig. 8).

Table 6. Factors contributing docking scores compound **4c**, **4d** and **4e**

Molecules	Dock Score[a]	Steric	Protein Desolvation	Ligand Desolvation H-Bond	Ligand Desolvation	Hydrogen bond
4e	-10.2	-15.7	9.0	-4.4	2.9	-2.6
4d	-8.7	-14.5	8.6	-4.1	2.9	-2.1
4c	-7.8	-13.4	8.0	-4.0	2.9	-1.8

[a] FRED Chemgauss4 score.

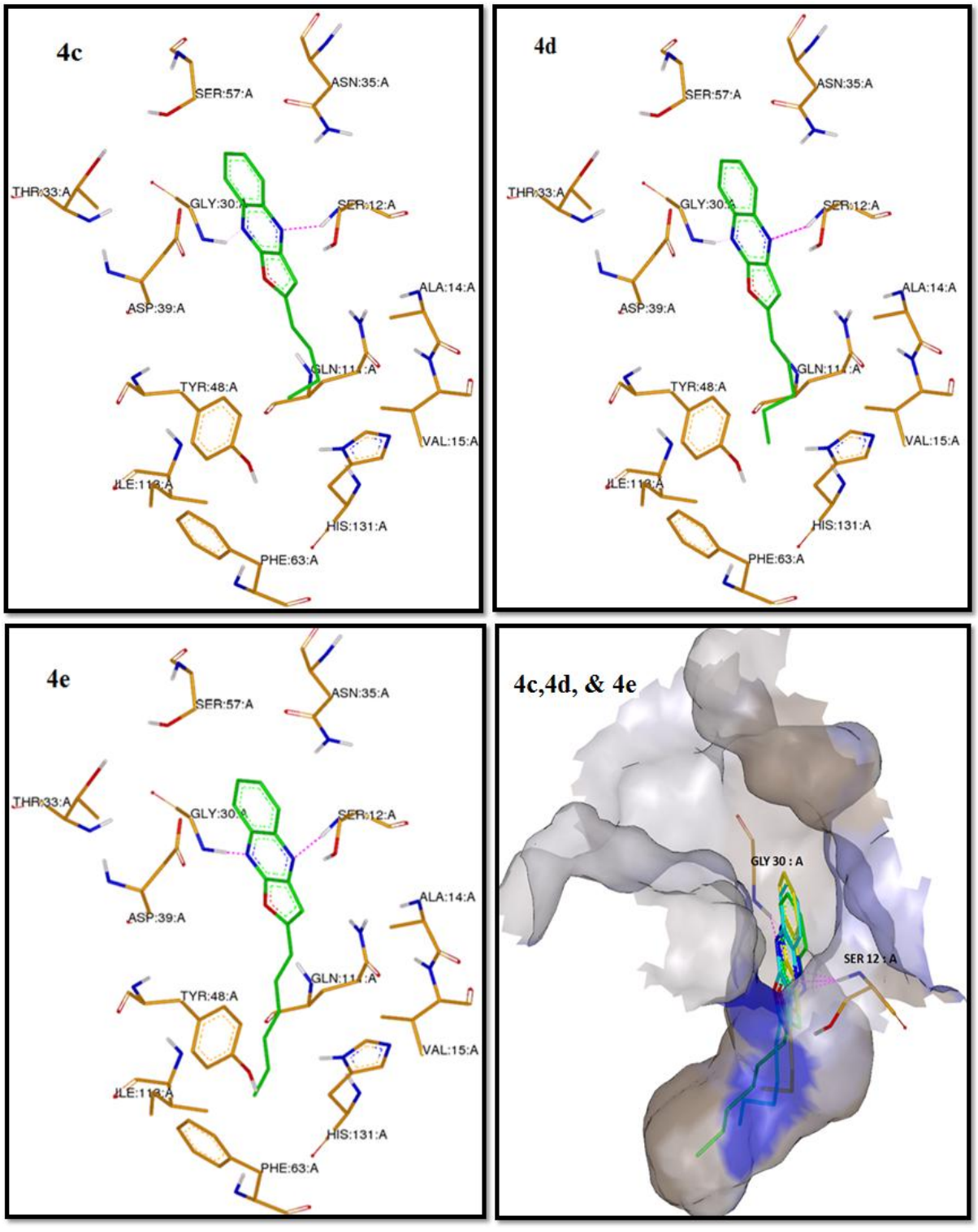


Figure 8. The binding mode of **4c**, **4d** and **4e** in the catalytic site. **4c**, **4d** and **4e** along binding mode of **4c** (yellow), **4d** (cyan) and **4e** (green) at the catalytic site represented as surface and mapped on Eisenberg potential hydrophobicity.

4.5.3. Toxicity studies in Zebrafish embryo (*in vivo*)

Having the preliminary *in vitro* data we then focused on testing these compounds (**4c**, **4d** and **4e**) for their toxicity evaluation using zebrafish embryos as an *in vivo* model.[24] The experiment was carried out at lowest statistically significant toxic concentrations 30µM and 50µM for the compounds **4d** and **4c** respectively. But the lowest statistically toxic concentration for test compound **4e** could not be established in the present study and is hence reported as > 50µM. All embryos survived the entire duration of the study and the toxic effects observed in the positive control group i.e. Phenobarbital (3mM) were consistent with our in-house control data. The results of the statistical analysis of mean morphological scores are depicted in Figure 8. Considering both statistical and biological relevance the NOAEL (No Observed Adverse Effect Level) for **4d** and **4e** were 1µM and 30µM respectively. Once again the NOAEL for test compound **4c** could not be established in the present study and is hence reported as < 1 µM. The MTC for the three compounds **4c**, **4d** and **4e** were 1µM, 10µM and 50µM respectively.

Compound **4e** has showed no adverse effects till 30 µM when tested for toxicity in zebrafish embryo (Fig. 7). Notably, **4c** and **4d** showed low to severe abnormalities in zebrafish embryo (Fig. 7). From this study it can be concluded that compound **4e** is the safest compound.

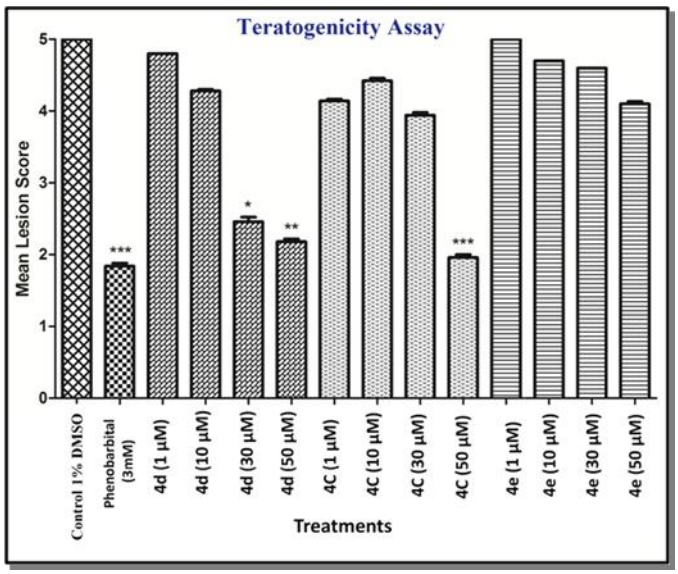


Figure 8. Evaluation of teratogenic effects of the compounds in Zebrafish embryos. (*p<0.05, **p<0.01 and ***p<0.001

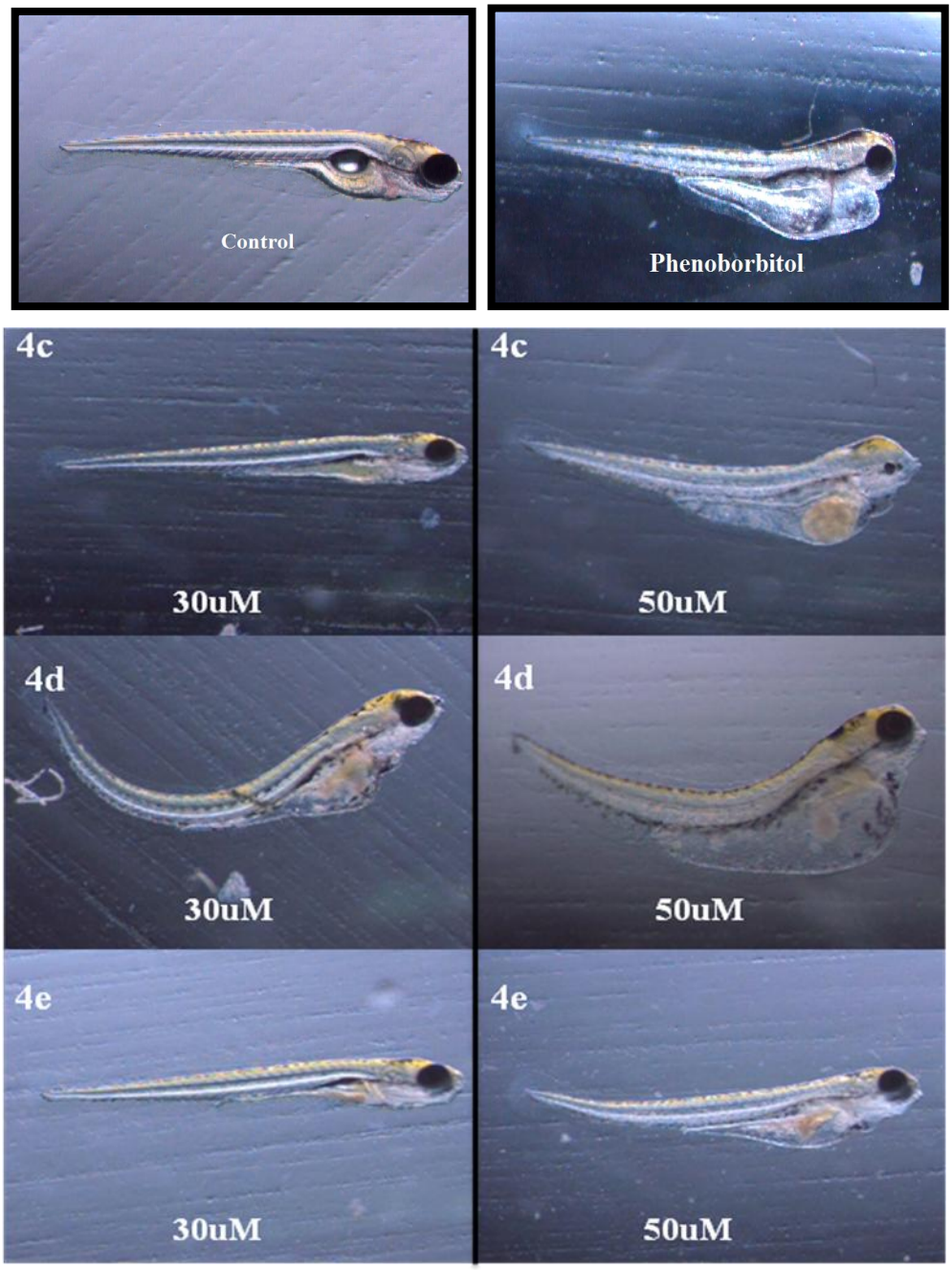


Figure 9. (a) Control embryo showing normal body; Embryo treated with (b) Phenobarbital (positive control) showing severe abnormalities, body bent; Compound **4c** and **4e** showing slight /no abnormalities at 30 μM and moderate abnormalities at 50 μM whereas **4d** showing moderate and severe abnormalities at 30 and 50 μM respectively.

4.6. Conclusion

In conclusion, furo[2,3-*b*]quinoxalines / pyrazines are synthesized as novel and unique class of fused furo *N*-heterocycles *via* a tandem hydrolysis-cyclization strategy in a single pot. Out of three hit compounds **4c**, **4d** and **4e** (55%, 56% and 65% Sir2 inhibition respectively) tested *in vitro* the compound **4e** i.e., 2-hexylfuro[2,3-*b*]quinoxaline showed promising yeast Sir2 inhibition and also mammalian SIRT1 inhibition at 50 µM. The dose response concentrations in yeast Sir2 and mammalian SIRT1 are IC_{50}~15.02 µM and IC_{50}~23.5 µM repctively. In MTT assay **4e** strongly inhibited the cell growth when tested against human hepatocellular liver carcinoma (HepG2) and Cervical Cancer (Hela) cells at 50 μM. Further this *in vitro* data is supported by *in silico* docking studies of these three compounds with Sir2 crystal structure (PDB ID: 1Q1A) which is corelating with enzyme inhibition data. Finally these compounds are tested for their toxicity properties using zebrafish embryos as *in vivo* model. These studies reveals that compound **4e** is the safest compound with no adverse effect levels. With this priliminary data we can conclude that compound **4e** can be a potentialanticancer agent with sirtuin inhibiting propperty which can taken forward for further studies.

4.7. Experimental Section

Chemistry

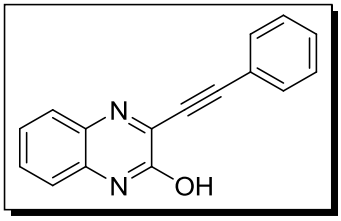
General methods: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solution by using a 400 MHz spectrometers (VARIAN 400 MR). 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), dd (doublet of doublet), td (triplet of doublet) and m (multiplet) as well as bs (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer (FT/IR-4200, JASCO). Melting points were determined by using melting point apparatus (Buchi melting point B-540) and are uncorrected. MS spectra were obtained on a mass spectrometer

(AGILENT 6430 triple quardrupole LC-MS). 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.

General procedure for the preparation of 3:

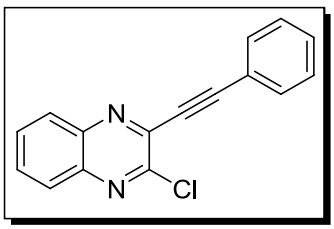
In a round bottom flask 2,3-dichloro quinoxalines/pyrazine (**1**) compound (1.256 mmol), 10% Pd/C (0.0125 mmol), CuI (0.0125 mmol), PPh_3 (0.05 mmol) and Et_3N (1.8844 mmol) in EtOH (4 mL) was added and stirred for 15 min. Then terminal alkyne (**2**) (1.256 mmol), was added in N_2 atmosphere and stirred at 60 ºC for 2-4 h. After the reaction over, the reaction mixture was extracted with EtOAc and H_2O. The organic layer was then dried with Na_2SO_4 and concentrated in vacuum. The reaction mixture was then purified with column chromatography using EtOAc and *n*-Hexane solvent system.

3-(Phenylethynyl)quinoxalin-2-ol (3aa)



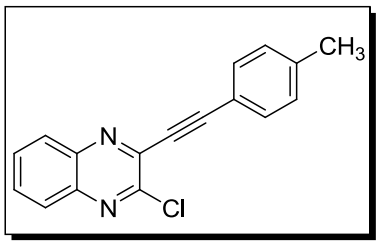
Off white semi solid; ^{1}H NMR (400 MHz, DMSO-d_6) ppm: 12.86-12.85 (m, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.68-7.61 (m, 2H), 7.56 (dd, *J* = 9.2, 3.9 Hz, 1H), 7.52-7.43 (m, 3H), 7.35-7.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 154.4, 143.5, 132.7, 132.5 (2C), 132.2 (2C), 131.7, 130.6, 129.4 (2C), 124.1, 121.2, 115.9, 95.3, 86.8; IR (KBr) ν_{max} (cm^{-1}): 3226, 2138, 1461, 673. MS (ESI) m/z: 246.9 [M + H].

2-Chloro-3-(phenylethynyl)quinoxaline (3a)



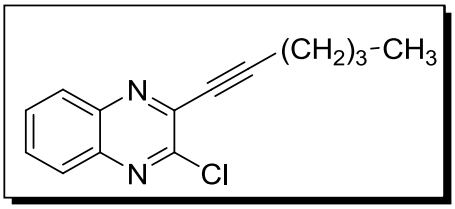
Light yellow solid; mp: 103-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.19-7.87 (m, 2H), 7.87-7.57 (m, 4H), 7.57-7.31 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 147.9, 140.7, 140.2, 138.5 (2C), 132.9, 131.9, 130.2 (2C), 129.4, 129.4, 128.9, 128.6, 121.1, 97.1, 85.4; IR (KBr) ν_{max} (cm^{-1}): 2213, 1497, 1193, 721; MS (ESI) m/z: 265.5 [M + H].

2-Chloro-3-(p-tolylethynyl)quinoxaline (3b)



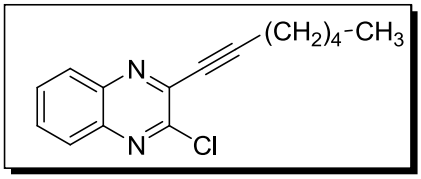
Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.14-8.08 (m, 1H), 8.05-8.00 (m, 1H), 7.83-7.75 (m, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.27-7.21 (m, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 144.3, 140.2, 140.0 (2C), 139.9 (2C), 137.7, 133.5, 129.9 (2C), 128.8, 128.7 (2C), 128.0, 90.3, 85.2, 21.2; IR (KBr) ν_{max} (cm^{-1}): 2891, 2218, 1522, 677; MS (ESI) m/z: 278.9 [M + H].

2-Chloro-3-(hex-1-ynyl)quinoxaline (3c)



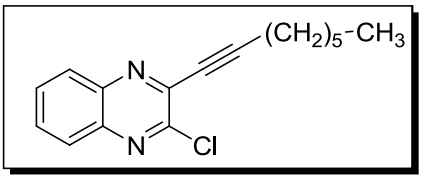
Brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.51 (d, *J* = 6.2 Hz, 1H), 8.24 (d, *J* = 7.2 Hz, 1H), 7.77-7.72 (m, 2H), 2.04-2.02 (m, 2H), 1.54-1.50 (m, 2H), 1.42-1.38 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 151.9, 148.7, 144.8, 143.9, 126.8, 126.5, 119.7, 117.3, 81.5, 79.7, 34.3, 23.5, 18.9, 13.3; IR (KBr) ν_{max} (cm^{-1}): 2938, 2853, 2198, 1546, 701; MS (ESI) m/z: 245.7 [M + H].

2-Chloro-3-(hept-1-ynyl)quinoxaline (3d)



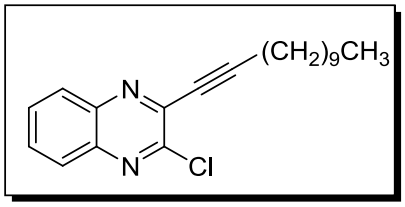
Light yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.11 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.44-7.41(m, 2H), 2.20-2.18 (m, 2H), 2.16-2.15 (m, 2H), 1.88-1.78 (m, 2H), 1.53-1.51 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 156.9, 149.0, 148.9, 129.0, 128.9 (2C), 123.8, 115.3, 112.8, 91.6, 79.3, 37.3, 30.0, 28.1, 14.2; IR (KBr) ν_{max} (cm^{-1}): 2957, 2830, 2213, 1504, 677; MS (ESI) m/z: 259.1 [M + H].

2-Chloro-3-(oct-1-ynyl)quinoxaline (3e)



Light yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.96 (d, *J* = 6.4 Hz, 1H), 7.89 (d, *J* = 6.5 Hz, 1H), 7.67-7.66 (m, 2H), 2.51 (t, *J* = 7.0 Hz, 2H), 1.75-1.74 (m, 2H), 1.44-1.42 (m, 2H), 1.32-1.31(m, 4H), 0.89 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 156.0, 144.4, 139.5, 129.4, 128.3 (2C), 126.9, 125.7, 106.6, 79.2, 72.0, 30.8, 29.5, 28.2, 27.3, 14.6; IR (KBr) ν_{max} (cm^{-1}): 2973, 2851, 2218, 1521, 768; MS (ESI) m/z: 273.2 [M + H].

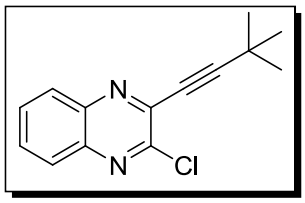
2-Chloro-3-(dodec-1-ynyl)quinoxaline (3f)



Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.07 (d, *J* = 7.4 Hz, 1H), 8.00 (d, *J* = 7.4 Hz, 1H), 7.80-7.70 (m, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 1.76-1.67 (m, 2H), 1.58-1.46 (m, 2H), 1.30-1.24 (m, 12H), 0.87 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 148.0, 140.5, 140.1, 138.8, 131.0, 130.4, 128.6, 128.1, 100.2, 78.9, 31.8, 29.5, 29.4,

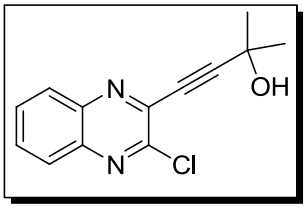
29.3, 29.0, 28.9, 27.9, 22.6, 19.7, 14.1; IR (KBr) ν_{max} (cm^{-1}): 2961, 2844, 2235, 1468, 647; MS (ESI) m/z : 329.1 [M + H].

2-Chloro-3-(3, 3-dimethylbut-1-ynyl) quinoxaline (3g)



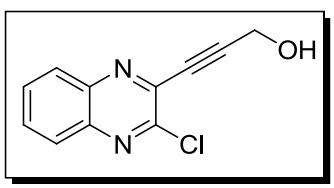
Light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.14-7.89 (m, 2H), 7.81-7.56 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) ppm: 151.9, 144.8, 140.9, 140.3, 129.5(2C), 128.1, 91.5, 79.7, 30.2, 29.7 (3C); IR (KBr) ν_{max} (cm^{-1}) : 2893, 2264, 1531, 784; MS (ESI) m/z: 245.6 [M + H].

4-(3-Chloroquinoxalin-2-yl)-2-methylbut-3-yn-2-ol (3h)



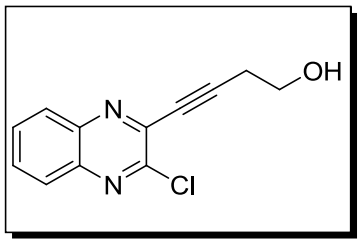
Light green solid; mp: 77-79°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.07 (d, J = 6.2 Hz, 1H), 8.03 (d, J = 6.8 Hz, 1H), 7.82-7.74 (m, 2H), 2.61 (s, 1H), 1.73 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 150.9, 147.1, 144.0, 141.7, 141.3, 129.3 (2C), 127.8 (2C), 96.2, 73.5, 50.2, 31.4; IR (KBr) ν_{max} (cm^{-1}): 3358, 2858, 2217, 1527, 1102, 677; MS (ESI) m/z: 247.4 [M + H].

3-(3-Chloroquinoxalin-2-yl)prop-2-yn-1-ol (3i)



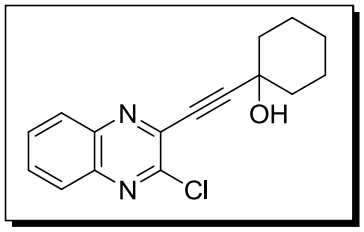
Brown semi solid; mp: 153-155°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.08-7.96 (m, 2H), 7.81-7.74 (m, 2H), 2.17 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 151.1, 140.5, 140.2, 140.1, 130.6, 129.8 (2C), 129.5, 85.7, 84.9, 51.0; IR (KBr) ν_{max} (cm^{-1}): 3443, 2231, 1529, 697; MS (ESI) m/z : 219.3 [M + H].

4-(3-chloroquinoxalin-2-yl)but-3-yn-1-ol (3j)



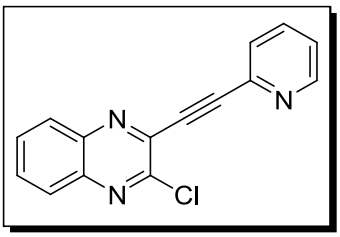
Light green semi solid; ^{1} H NMR (400 MHz, $CDCl_3$) ppm: 8.13 (d, J = 6.4 Hz, 1H), 8.19 (d, J = 6.3 Hz, 1H), 7.73-7.71 (m, 2H), 4.15-4.13 (m, 2H), 3.20-3.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 153.1, 152.9, 145.5, 144.4, 143.7, 132.1, 131.3, 127.7, 99.4, 78.9, 63.0, 23.0; IR (KBr) ν_{max} (cm^{-1}): 3301, 2938, 2217, 1478, 718; MS (ESI) m/z : 232.9 [M + H].

1-((3-Chloroquinoxalin-2-yl)ethynyl)cyclohexanol (3k)



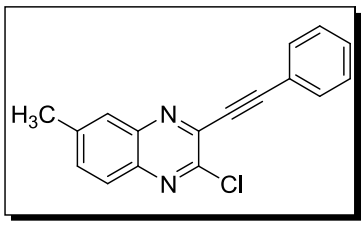
Off white solid; mp: 134-136°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.07 (d, J = 6.4 Hz, 1H), 7.99 (d, J = 6.4 Hz, 1H), 7.82-7.80 (m, 2H), 2.42 (s, 1H), 2.13 (t, J = 9.4 Hz, 2H), 1.83-1.66 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 142.0 (2C), 141.8 (2C), 139 (2C), 135.0 (2C), 101.6, 69.1, 39.6 (2C), 39.6, 39.4, 25.0, 23.1; IR (KBr) ν_{max} (cm^{-1}): 3342, 2217, 1478, 718; MS (ESI) m/z: 287.1 [M + H].

2-Chloro-3-(pyridin-2-ylethynyl)quinoxaline (3l)



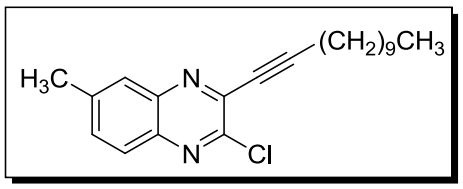
Light gray solid; mp: 134-136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.72 (d, *J* = 4.7 Hz, 1H), 8.17 (d, *J* = 4.4 Hz, 1H), 8.07-7.99 (m, 1H), 7.85-7.80 (m, 2H), 7.75-7.73 (m, 2H), 7.39-7.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 151.6, 150.5, 146.6, 143.4, 141.1, 139.7, 132.8, 130.1, 129.9, 128.7, 128.2, 127.1, 109.9, 92.6, 84.2; IR (KBr) ν_{max} (cm^{-1}): 2203, 1518, 677; MS (ESI) m/z: 266.2 [M + H].

2-Chloro-6-methyl-3-(phenylethynyl)quinoxaline (3m)



Light yellow semi solid; ^{1}HNMR (400 MHz, $CDCl_3$) ppm: 8.15-8.08 (m, 1H), 8.06-7.98 (m, 1H), 7.84-7.76 (m, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.27-7.20 (m, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 148.0, 140.7, 140.6, 140.2, 138.7, 132.4, 131.2, 130.6, 129.3, 128.8, 128.2, 118.1, 97.7, 85.1, 21.7; IR (KBr) ν_{max} (cm^{-1}): 2934, 2221, 1450, 791; MS (ESI) m/z : 278.9 [M + H].

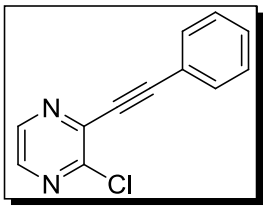
2-Chloro-3-(dodec-1-ynyl)-6-methylquinoxaline (3n)



Light brown liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.91 (d, *J* = 8.5 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.79 (s, 1H), 7.73 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 2.56-2.55 (m, 4H), 1.75-1.66 (m, 2H), 1.56-1.46 (m, 4H), 1.32-1.30 (m, 8H), 0.87 (t, *J* = 6.7 Hz, 3H); ^{13}C

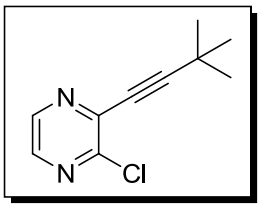
NMR (100 MHz, $CDCl_3$) ppm: 141.9, 141.1, 133.2, 132.7, 128.1, 127.6, 127.5, 127.0, 99.8, 87.7, 31.8, 29.5, 29.4, 29.2, 29.0, 28.1, 28.0, 22.6, 21.8, 19.7, 14.0; IR (KBr) ν_{max} (cm^{-1}): 2934, 2852, 2213, 1530, 761; MS (ESI) m/z : 342.9 [M + H].

2-Chloro-3-(phenylethynyl)pyrazine (3o)



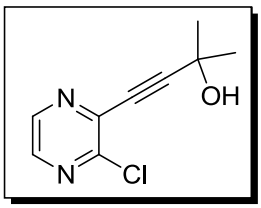
Light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.50 (d, *J* = 3.1 Hz, 1H), 8.29 (d, *J* = 2.8 Hz, 1H), 7.65 (d, *J* = 7.3 Hz, 2H), 7.47-7.36 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 159.3, 146.8, 146.7, 139.9, 132.0, 128.4 (2C), 115.4, 113.9, 106.9, 97.0, 81.9; IR (KBr) ν_{max} (cm^{-1}): 2221, 1508, 674; MS (ESI) m/z: 214.9 [M + H].

2-Chloro-3-(3, 3-dimethylbut-1-ynyl)pyrazine (3p)



Light green liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.42 (d, *J* = 2.9 Hz, 1H), 8.23 (t, *J* = 3.6 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 150.7, 141.9, 141.1, 139.9, 108.0, 75.5, 30.3 (3C), 28.4; IR(KBr) ν_{max} (cm^{-1}): 2949, 2237, 1494, 701; MS (ESI) m/z: 195.1 [M + H].

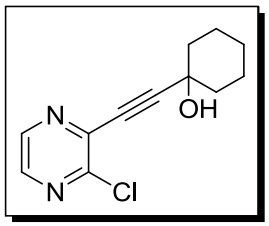
4-(3-Chloropyrazin-2-yl)-2-methylbut-3-yn-2-ol (3q)



Light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.47 (d, *J* = 3.1 Hz, 1H), 8.32 (d, *J* = 3.4 Hz, 1H), 2.46 (s, 1H), 1.68 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 154.1, 144.8,

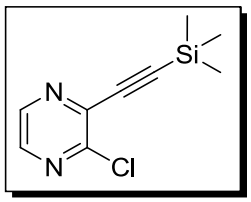
141.7, 138.3, 99.7, 78.8, 34.0, 28.0 (2C); IR (KBr) ν_{max} (cm^{-1}): 3392, 2853, 2211, 1460, 655; MS (ESI) m/z: 196.1 [M + H].

1-((3-Chloropyrazin-2-yl)ethynyl)cyclohexanol (3r)



Brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.46 (d, *J* = 2.4 Hz, 1H), 8.29 (d, *J* = 2.4 Hz, 1H), 2.44 (s, 1H), 2.09-2.07 (m, 2H), 1.77-1.74 (m, 4H), 1.61 (d, *J* = 9.1 Hz, 2H), 1.33-1.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 150.7, 142.0, 141.8, 139.0, 80.2, 79.6, 39.4 (3C), 25.0 (2C), 23.1; IR (KBr) ν_{max} (cm^{-1}): 3432, 2853, 2243, 1529, 701; MS (ESI) m/z : 236.9 [M + H].

2-Chloro-3-((trimethylsilyl)ethynyl)pyrazine (3s)



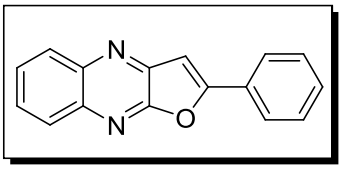
Light yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.87 (d, *J* = 4.6 Hz, 1H), 8.06-7.95 (d, *J* = 4.4 Hz, 1H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 147.8, 140.3, 140.2, 137.8, 104.5, 99.4, -0.71 (3C); IR (KBr) ν_{max} (cm^{-1}): 2938, 2210, 1546, 632; MS (ESI) m/z: 211.2 [M + H].

General procedure for the preparation of 4

In round bottom flask compound **3** (0.75 mmol) in 3 mL DMSO-H_2O (1:4), and K_2CO_3 (0.38 mmol) and stirred at 80°C for 1-6 h. After the reaction over, the crude mass was diluted with ethyl acetate (20 mL) and water (10 mL) and the mixture was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered

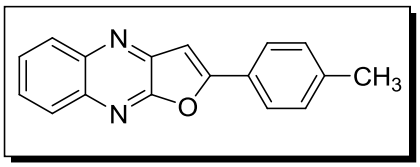
and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using EtOAc and *n*-Hexane solvent system.

2-Phenylfuro[2,3-*b*]quinoxaline (4a) [25]



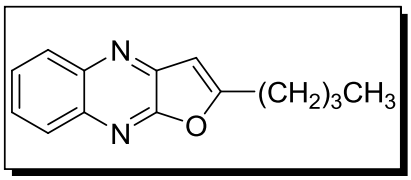
White solid; mp: 258–260 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.21–8.20 (m, 2H), 8.12–8.10 (m, 2H), 7.85–7.83 (m, 2H), 7.66–7.61 (m, 3H), 7.23 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 161.1, 147.9, 140.7, 140.1, 138.8, 134.2 (2C), 131.1 (2C), 130.6, 128.7 (2C), 128.2 (2C), 114.3 (2C); HPLC: 97.1 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.2 min; IR (KBr) ν_{max} (cm^{-1}): 1563, 1498, 771; MS (ESI) m/z: 246.9 [M + H].

2-*p*-Tolylfuro[2,3-*b*]quinoxaline (4b)



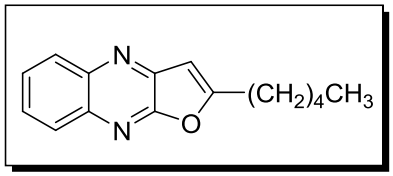
Light Yellow solid; mp: 205-207 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.18 (d, *J* = 5.6, 1H), 8.12 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.80-7.70 (m, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 2.47 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 158.9, 155.9, 145.6, 143.0 (2C), 141.9, 130.2 (2C), 129.1, 128.8 (2C), 126.8, 124.3, 121.1, 120.6, 115.6, 20.9; HPLC: 98.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.43 min; IR (KBr) ν_{max} (cm^{-1}): 2837, 1481, 792; MS (ESI) m/z: 261.1 [M + H].

2-Butylfuro[2,3-*b*]quinoxaline (4c)



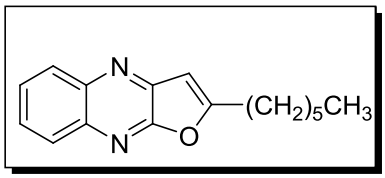
Brown solid; mp: 103-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.16 (d, *J* = 6.7 Hz, 1H), 8.10 (d, *J* = 5.6 Hz, 1H) 7.78-7.77 (m, 2H), 6.02 (s, 1H), 2.88-2.67 (m, 2H), 2.55-2.40 (m, 2H), 2.16-1.89 (m, 2H), 0.88 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 153.7, 152.5, 145.4, 142.0 (2C), 136.1, 129.9, 129.6, 127.0, 124.5, 96.3, 31.6, 22.5, 19.6, 13.9; HPLC: 96.9%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.61 min; IR (KBr) ν_{max} (cm^{-1}): 2941, 2868, 1477, 735; MS (ESI) m/z : 226.8 [M + H].

2-Pentylfuro[2,3-*b*]quinoxaline (4d)



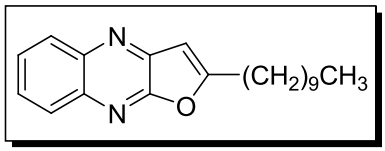
Light yellow semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.09 (d, *J* = 5.7 Hz, 1H), 8.06 (d, *J* = 5.6 Hz, 1H), 7.65 (d, *J* = 6.4 Hz, 2H), 6.63 (s, 1H), 2.86 (t, *J* = 7.5 Hz, 2H), 1.78–1.77 (m, 2H), 1.43-1.25 (m, 4H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 150.2, 145.4 (2C), 136.2, 136.1, 129.4 (2C), 127.1, 125.0, 108.4, 30.5, 30.3, 29.7, 22.5, 13.9; HPLC: 99.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, 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 229 nm, retention time 4.44 min; IR (KBr) ν_{max} (cm^{-1}): 2936, 2871, 1463, 784; MS (ESI) m/z: 240.8 [M + H].

2-hexylfuro[2,3-*b*]quinoxaline (4e)



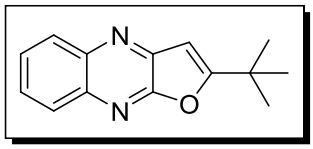
Dark brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.24 (d, *J* = 6.8 Hz, 1H), 8.14 (d, *J* = 6.7 Hz, 1H), 7.80-7.78 (m, 3H), 2.95 (t, *J* = 7.5 Hz, 2H), 1.93-1.91 (m, 2H), 1.47-1.46 (m, 2H), 1.40-1.37 (m, 4H), 0.93 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 156.0, 155.6, 141.7, 138.3, 128.6 (2C), 128.4 (2C), 128.0, 102.5, 31.4, 29.6, 28.8, 28.7, 22.4, 14.0. HPLC: 99.1%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.78 min; IR (KBr) ν_{max} (cm^{-1}): 2943, 2856, 1494, 692; MS (ESI) m/z: 254.9 [M + H].

2-Decylfuro[2,3-b]quinoxaline (4f)



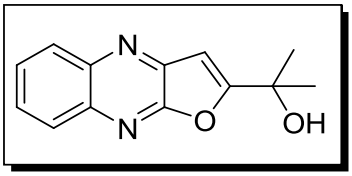
Brown solid; mp: 130-132°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.20 (d, *J* = 7.2 Hz, 1H), 8.12 (d, *J* = 7.2 Hz, 1H), 7.73-7.72 (m, 2H), 6.71 (s, 1H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.90-1.81 (m, 2H), 1.50-1.42 (m, 2H), 1.41-1.35 (m, 2H), 1.29-126 (m, 10H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 170.0, 154.1, 144.8, 141.7, 138.3, 128.6 (2C), 128.4, 128.0, 102.6 (2C), 31.8, 29.6, 29.5, 29.4, 29.(2C), 26.7, 22.6, 14.1; HPLC: 99.2%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.18 min; IR (KBr) ν_{max} (cm^{-1}): 2921, 2835, 1439, 721; MS (ESI) m/z : 310.9 [M + H].

2-*tert*-Butylfuro[2,3-*b*]quinoxaline (4g)



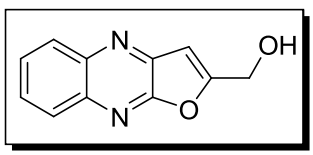
Light yellow solid; mp: 98-100 °C (found); 98°C (lit 26); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.17-8.12 (m, 1H), 8.10-8.05 (m, 1H), 7.73-7.67 (m, 2H), 6.65 (s, 1H), 1.46 (s, 9H); HPLC: 98.9%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.15 min; IR (KBr) ν_{max} (cm^{-1}): 2945, 2866, 1563, 1498, 771; MS (ESI) m/z: 227.1 [M + H].

2-(Furo[2,3-*b*]quinoxalin-2-yl)propan-2-ol (4h)



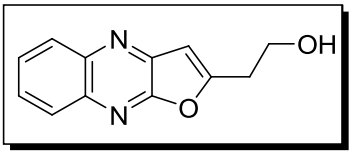
Off white semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.14 (d, J = 6.2 Hz, 1H), 8.01 (d, J = 6.1 Hz, 1H), 7.78-7.77 (m, 2H), 7.43 (s, 1H), 2.04 (s, 1H), 1.66 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 163.3, 155.6, 141.6, 139.7, 129.7 (2C), 128.1 (2C), 123.7, 98.5, 60.7, 30.9 (2C). HPLC: 97.5%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.0 min; IR (KBr) ν_{max} (cm^{-1}): 3328, 2837, 1566, 692; MS (ESI) m/z: 229.1 [M + H].

Furo[2,3-*b*]quinoxalin-2-ylmethanol (4i)



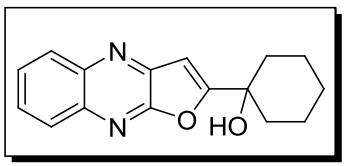
Light brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.16-7.91 (m, 1H), 7.78-7.77 (m, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.18 (s, 1H), 6.95 (s, 1H), 2.99 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 145.4, 136.2, 136.1, 129.4, 127.0, 125.0, 124.5, 115.7, 114.1, 108.4, 63.5; HPLC: 98.8%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 4.9 min; IR (KBr) ν_{max} (cm^{-1}): 3353, 2857, 1491, 724; MS (ESI) m/z: 200.9 [M + H].

2-(furo[2,3-*b*]quinoxalin-2-yl)ethanol (4j).



Light green semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.19-8.18 (m, 1H), 8.10-8.09 (m, 1H), 7.74-7.72 (m, 2H), 6.83 (s, 1H), 4.17 (t, *J* = 6.0 Hz, 2H), 3.21 (t, *J* = 6.0 Hz, 2H), 2.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 166.4, 153.9, 144.1, 141.5, 138.2, 128.7, 128.6, 128.1, 104.3, 59.5, 33.1, 29.6; HPLC: 95.7%, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.22 min; IR (KBr) ν_{max} (cm^{-1}): 3355, 2951, 2869, 1537, 1441, 818; MS (ESI) m/z: 214.9 [M + H].

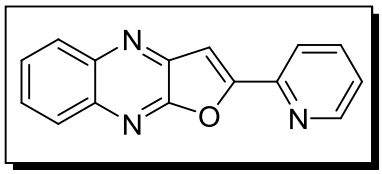
1-(Furo[2,3-*b*]quinoxalin-2-yl)cyclohexanol (4k).



Light yellow solid; mp: 178-180°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.24-8.17 (m, 1H), 8.15-8.07 (m, 1H), 7.75-7.73 (m, 2H), 6.94 (s, 1H), 2.27 (s, 1H), 2.18 (m, 2H), 1.97 (d, *J* = 9.3 Hz, 2H), 1.88-1.87 (m, 2H), 1.70 (t, *J* = 9.5 Hz, 2H), 1.55-1.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_{3)}$ ppm: 154.0, 144.2, 141.9, 138.5, 128.8 (2C), 128.7, 128.6, 128.2, 101.2, 71.2, 35.7 (2C), 25.1, 21.4 (2C); HPLC: 96.9%, column: Zorbax XDB C-18 150 x

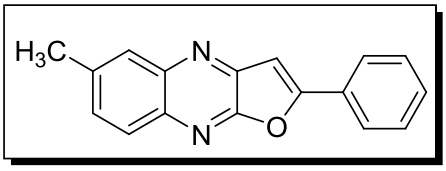
4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.54 min; IR (KBr) ν_{max} (cm^{-1}): 3367, 2923, 2852, 1546, 1432, 751; MS (ESI) m/z: 268.8 [M + H].

2-(Pyridin-2-yl)furo[2,3-*b*]quinoxaline (4l)



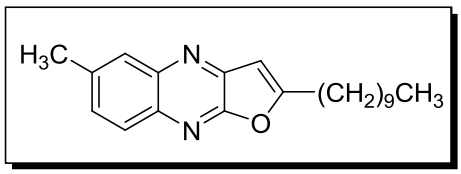
Light green solid; mp: 220-222°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.79 (d, J = 7.8 Hz, 1H), 8.23 (s, 1H), 8.13 (d, J = 7.0 Hz, 2H), 7.91 (d, J = 7.1 Hz, 1H), 7.77-7.75 (m, 3H), 7.41 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_{3)}$ ppm: 162.5, 154.4, 150.4, 147.4, 144.1, 142.5, 139.1, 137.1, 129.2 (2C), 129.0, 128.7, 128.5, 121.1, 104.1; HPLC: 97.18 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.05 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.41 min; IR (KBr) ν_{max} (cm^{-1}): 3355, 2951, 2869, 1537, 1441, 818; MS (ESI) m/z : 247.9 [M + H].

6-Methyl-2-phenylfuro[2,3-*b*]quinoxaline (4m)



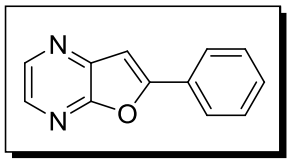
Light yellow; mp: 182-184°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm**:** 8.04-8.07 (m, 2H), 7.94 (d, J = 7.2 Hz, 1H), 7.46-7.58 (m, 5H), 7.27 (s, 1H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 140.7, 138.6, 131.1, 131.0, 130.6, 129.1(2C), 128.2, 128.1(2C), 127.5 (2C), 127.6, 126.0 (2C), 100.8, 21.8; HPLC: 98.9 %, column: Zorbax XDB C-18 150 x 4.6 mm 5, mobile phase A: 0.1 % formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.42 min; IR (KBr) ν_{max} (cm^{-1}): 2843, 1533, 1428, 718; MS (ESI) m/z : 261.1 [M + H].

2-Decyl-6-methylfuro[2,3-*b*]quinoxaline (4n)



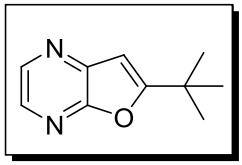
Dark brown solid; mp: 74-76°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.01 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 6.68 (s, 1H), 2.91 (t, *J* = 7.5 Hz, 2H), 2.59 (s, 3H), 1.84 (d, *J* = 7.5 Hz, 2H), 1.45 (d, *J* = 5.3 Hz, 2H), 1.35-1.26 (m, 12H), 0.88 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 158.9, 155.9, 145.6 (2C), 139.4, 135.8, 129.1 (2C), 126.8, 120.6, 115.4, 94.6, 39.2, 33.5, 28.5, 28.4, 27.5, 24.3, 22.9, 18.7, 14.0; HPLC: 99.0 %; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.42 min; IR (KBr) ν_{max} (cm^{-1}): 2918, 2857, 1471, 677; MS (ESI) m/z: 325.1 [M + H].

6-Phenylfuro[2,3-*b*]pyrazine (4o)



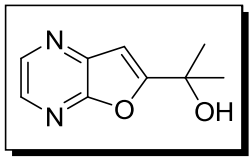
Off white solid; mp: 107-109°C (found); 112–113°C (lit 27); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.54 (d, *J* = 1.9 Hz, 1H), 8.25 (d, *J* = 2.4 Hz, 1H), 7.99 (d, *J* = 7.1 Hz, 2H), 7.65-7.64 (m, 3H), 7.28 (s, 1H); HPLC: 99.1 %; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.34 min; IR (KBr) ν_{max} (cm^{-1}): 2941, 1563, 1452, 788; MS (ESI) m/z: 196.8 [M + H].

6-*tert*-Butylfuro[2,3-*b*]pyrazine (4p)



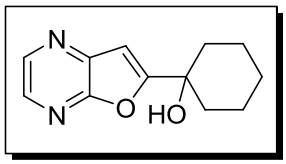
Off white solid; mp: 75-77° (found); 73-75 (lit 28); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 7.96 (d, *J* = 2.1 Hz, 1H), 7.85 (d, *J* = 2.0 Hz, 1H), 7.62 (s, 1H), 1.35 (s, 9H); HPLC: 96.7 %; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 8.34 min; IR (KBr) ν_{max} (cm^{-1}): 2963, 1572, 1461, 659; MS (ESI) m/z: 177.1 [M + H].

2-(Furo[2,3-*b*]pyrazin-6-yl)propan-2-ol (4q)



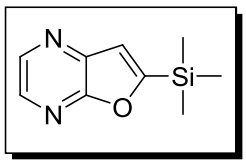
Light brown semi solid; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.48 (d, *J* = 2.7 Hz, 1H), 8.19 (d, *J* = 2.7 Hz, 1H), 6.93 (s, 1H), 3.08 (s, 1H), 1.72-1.67 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 162.3, 145.0 (2C), 144.0, 141.4, 96.5, 62.3, 33.8, 29.6; HPLC: 88.1 %; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.23 min; IR (KBr) ν_{max} (cm^{-1}): 3136, 2940, 1501, 1411, 723; MS (ESI) m/z: 179.1 [M + H].

1-(Furo[2,3-*b*]pyrazin-6-yl)cyclohexanol (4r)



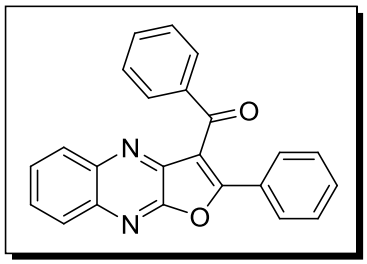
Off white solid; mp: 105-107°C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 8.50 (d, *J* = 2.6 Hz, 1H), 8.21 (d, *J* = 2.6 Hz, 1H), 6.87 (s, 1H), 2.24-2.13 (m, 2H), 2.13-2.01 (m, 2H), 1.99-1.90 (m, 2H), 1.86-1.74 (m, 2H), 1.70-1.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 155.1, 141.8, 141.2, 137.2, 101.6, 84.0, 70.9, 36.0 (2C), 25.1, 21.5 (2C); HPLC: 96.8%; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 6.55 min; IR (KBr) ν_{max} (cm^{-1}): 3039, 2963, 2952, 2880, 1530, 1454; MS (ESI) m/z : 218.9 [M + H].

6-(Trimethylsilyl)furo[2,3-*b*]pyrazine (4s)



Yellow semi solid.[1] H NMR (400 MHz, $CDCl_3$) ppm: 8.74 (d, *J* = 3.1 Hz, 1H), 8.58 (d, *J* = 2.9 Hz, 1H), 7.61 (s, 1 H), -0.62 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 162.0, 144.8, 141.4, 140.4, 110.7, 108.9, -0.24 (3C); HPLC: 92.8%; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 5.11 min; IR (KBr) ν_{max} (cm^{-1}): 2941, 1530, 1252; MS (ESI) m/z : 192.9 [M + H].

Phenyl(2-phenylfuro[2,3-*b*]quinoxalin-3-yl)methanone (6)



Brown solid; mp: 152-154°C (found); 149-151°C (lit 29); ^1H NMR (400 MHz, $CDCl_3$) ppm: 7.96 (d, *J* = 7.2 Hz, 1H), 7.78-7.76 (m, 2H), 7.54 (d, *J* = 7.1 Hz, 1H), 7.29-7.21 (m, 5H), 7.19 (d, *J* = 6.4 Hz, 2H), 6.92-6.89 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 185.9, 160.7, 159.9, 156.2, 152.3, 144.7, 143.4, 140.2, 132.3 (2C), 131.7, 129.9, 129.6 (2C), 129.5, 128.8, 128.6 (2C), 128.4, 128.2, 127.5, 125.5, 125.2; HPLC: 91.6 %; column: XBridge C-18 150*4.6 mm 5µ, mobile phase A: 0.1 % Formic Acid in water mobile phase B: CH_3CN (gradient) T/B%: 0/50, 0.5/50, 4/98, 10/98, 10.5/50, 12/50; flow rate:1.0 mL/min; UV 229 nm, retention time 7.59 min; IR (KBr) ν_{max} (cm^{-1}): 1621, 1522, 1431, 764; MS (ESI) m/z : 351.1 [M + H].

Dose dependent URA3 assay of compound 4d

The synthesized compounds (**4**) were tested *in vitro* by using yeast cell based reporter silencing assay. Compounds were tested at the concentration of 50 µM for their ability to inhibit yeast sirtuin family NAD-dependent histone deacetylase (HDAC) sir 2 protein (a yeast homologue of mammalian SIRT1). Splitomicin, a known inhibitor of sirtuin, was used as a reference compound. In this assay a yeast strain (TEL::URA3 strain (MATα ura3-52 lys2-801 ade2-101 trpΔ63 his3Δ200 leu3Δ200 leu2-Δ1 TEL adh4::URA) was used in which, a reporter gene URA3 was inserted in the silenced telomeric region where it is silenced by yeast Sir2 protein. Inhibition of sir2 protein by an inhibitor would allow the URA3 gene to be expressed thereby resulting in death of the yeast cell in presence of 5-FOA through the formation of toxic 5-fluorouracil.

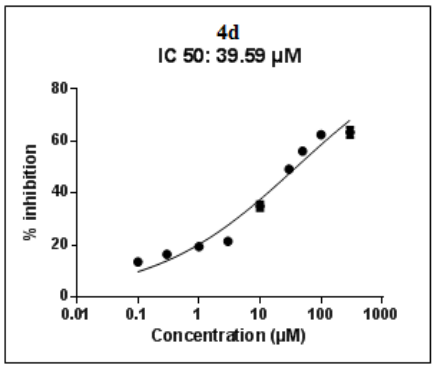


Figure 8. Dose dependent URA3 reporter assay of **4d**.

Zebrafish embryo toxicity evaluation

Aquaculture

All procedures for experimentation were as per Guidelines for Use of Zebrafish in the NIH Intramural Research Program (http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf) and the Zebrafish Book (Westerfield, 2000).[12] Indigenous wild type zebrafish strains were obtained from Vikrant Aquaculture, Mumbai, India. Fish were maintained in a recirculation system with polysulphone housing tanks containing purified water (Millipore ELIX system grade) with 0.2% sea salt at 28°C under a 14:10 h light and dark cycle (Westerfield M, 2000). Fish were fed three times daily with live hatched brine shrimp and

dry food. Males and females were separated for four days before they were allowed to spawn. On day five they were allowed to span at optimal conditions at the ratio of two males to one female. Collected embryos were grown in E3 medium at 28.5°C.

Toxicity Evaluation

Preliminary safety evaluation of test compounds **4c**, **4d** and **4e** was carried out in a zebrafish embryo model based on the protocol and morphological score assessment by Panzica-Kelly et al, 2010.[13] Five embryos/treatment/concentration were exposed in a twenty-four well plate to the vehicle, positive control (3mM Phenobarbital) and test compounds (each test compound at four concentrations viz. 1, 10, 30 and 50 µM) from day 1 post fertilization to day 5 post fertilization and observed on day 5. Morphological evaluation was carried out by trained personnel in a blinded fashion. Each embryo was assigned a lesion score (5-0, 5 being non-toxic) for various parameters and mean morphological assessment scores were used to conduct the statistical evaluation using the Kruskal–Wallis one-way analysis of variance by ranks followed by Dunn's post hoc test. However, the determination of No Observed Adverse Effect Level (NOAEL) and Minimum Toxic Concentration (MTC) were based on both statistical and biological significance.

Reference:

1) Dell, A.; William, D.H.; Morris, H.R.; Smith, G.A.; Feeney, J.; Roberts, G.C.K. *J. Am. Chem. Soc.***1975**, *97*, 2497.
2) Suresh, M.; Lavanya, P.; Sudhakar, D.; Vasu, K.; Venkata, C. *J.Chem.Pharm.Res*. **2010**, *2* (1), 497.
3) Gerchakov, S.; Pershing, H. S. *J. Med. Chem.*, **1969**, *12* (1), 141.
4) Voigt, B.; Meijer, L.; Lozach, O.; Schachtele, C.; Totzke, F.; Hilgeroth, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 823.
5) Hurley, L. H.; Mahadevan, D.; Han, H.; Bearss, D. J.; Vankayalapati, H.; Bashyam, S.; Munoz, R. M.; Warner, S. L.; Della, C. K.; Von Hoff, D. D.; Grand, C. L. *PCT Int. Appl.* WO 2005/037825, **2005**.

6) Harris, N.; Higgs, C.; Wren, S.; Dyke, H. J.; Price, S.; Cramp, S. *PCT Int. Appl.* WO 2006/050965, **2006**.

7) (a) McCallion, G. D. Curr. Org. Chem. **1999**, *3*, 67. (b) Sheen, W.S.; Tsai, I.L.; Teng, C.M.; Chen, I.S. Phytochemistry **1994**, *36*, 213.

8) (a) Moss, G. P. Pure Appl. Chem. **2000**, *72*, 1493. (b) Zareba, K. M. Drugs Today **2006**, *42*, 75.

9) Gordaliza, M.; Castro, M.; Miguel del Corral, J. M.; Lopez-Vazquez, M.; San Feliciano, A.; Faircloth, G. T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2781.

10) Lambert, J. D.; Meyers, R. O.; Timmermann, B. N.; Dorr, R. T. *Cancer Lett.* **2001**, *171*, 47.

11) Anderson, K.W.; Ikawa, T.; Tundel, R. E.; Buchwald, S.L. *J. Am. Chem. Soc*. **2006**, *128*, 10694

12) Ackermann, L.; Kaspar, L.T. *J. Org. Chem*. **2007**, *72*, 6149.

13) Nakhi, A.; Rahman, M.S.; Kishore, R.; Meda, C.T.L.; Deora, G.S.; Parsa, K.V. L; Pal, M. *Bioorg. Med. Chem. Lett.* **2012,** *22*, 6433.

14) (a) Li, C.J.; Chen, L. *Chem. Soc. Rev*. **2006**, *5*, 68; (b) Li, C.J.; *Chem. Rev*. **2005**, *105*, 3095.

15) Lednicer, D. **2009** Strategies for Organic Drug Synthesis and Design,2nd Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA.

16) Ismail, M. M.; Ammar, Y. A.; Ibrahim, M. K.; ElZahaby, H. S.; Mahmoud, S. S. *Arzneimittel forschung* **2005**, *55*, 738.

17) Prasad, B.; Kumar, K. S.; Babu, P. V.; Anusha, K.; Rambabu, D.; Kandale, A.; Vanaja, G.R.; Kalle, A. M.; Pal, M. *Tetrahedron Lett*. **2012**, *53*, 6059.

18) (a) Michan, S.; Sinclair, D.; *Biochem. J. 2007, 404*, 1; (b) Bedalov, A.; Gatbonton, T.; Irvine, W.P.; Gottschling, D. E.; Simon, J. A. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 15113.

19) G. M. Sheldrick, SHELX-97, *Program for Crystal Structure Solution and Analysis*, University of Gottingen, Gottingen, Germany, **1997**.

20) (a) For a Pd-nanoparticle mediated cyclization of 2-hydroxyaryl alkynes leading to benzofurans, see: Witham, C. A.; Huang, W.; Tsung, C.K.; Kuhn, J. N.; Somorjai, G. A.; Toste, F. D. *Nature Chem*. **2010**, *2*, 36. (b) For the cleavage of O-Si bond of *o*-triisopropylsiloxyphenylacetylenes leading to benzofurans, see: Ito, Y.; Aoyama, T.; Shioiri, T. *Synlett*, **1997**, 1163.

21) Bedalov, A.; Gatbonton, T.; Irvine, W. P.; Gottschling, D. E.; Simon, J. A. *Proc. Natl. Acad. Sci. USA* **2001**, **98**, 15113.

22) Zhao, K.; Harshaw, R.; Chai, X.; Marmorstein, R. *Proc Natl Acad Sci USA* **2004**, *101*, 8563.

23) McGann, M.; *J. Chem. Inf. Model.*, **2011**, *51* (3), 578. (b) Paul, C.D.; Hawkins, A.; Skillman, G.; Warren, G.L.; Benjamin, A; Matthew, T. S**.** *J. Chem. Inf. Model*., **2010**, *50*, 572.

24) Basu, S.; Sachidanandan, C. *Chem. Rev.*, **2013**, *113* (10), 7952.

25) Ames, Donald E.; Mitchell, John C.; Takundwa, Chisango C. *J. Chem. Res, Miniprint,* **1985** , *5*, 1683.

26) Westerfield M*: The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio). 4th edition. Eugene.*

27) Panzica, K.J.M.; Zhang, C. X.; Danberry, T. L.; Flood, A.; DeLan, J. W.; Brannen, K. C.; Augustine-Rauch, K. A. *Birth Defects Research* (Part B) **2010,** *89*, 382.

28) Iijima, H.; Zasshi, Y. *Chem.Abstr*. **1972**, *77*, 88434.

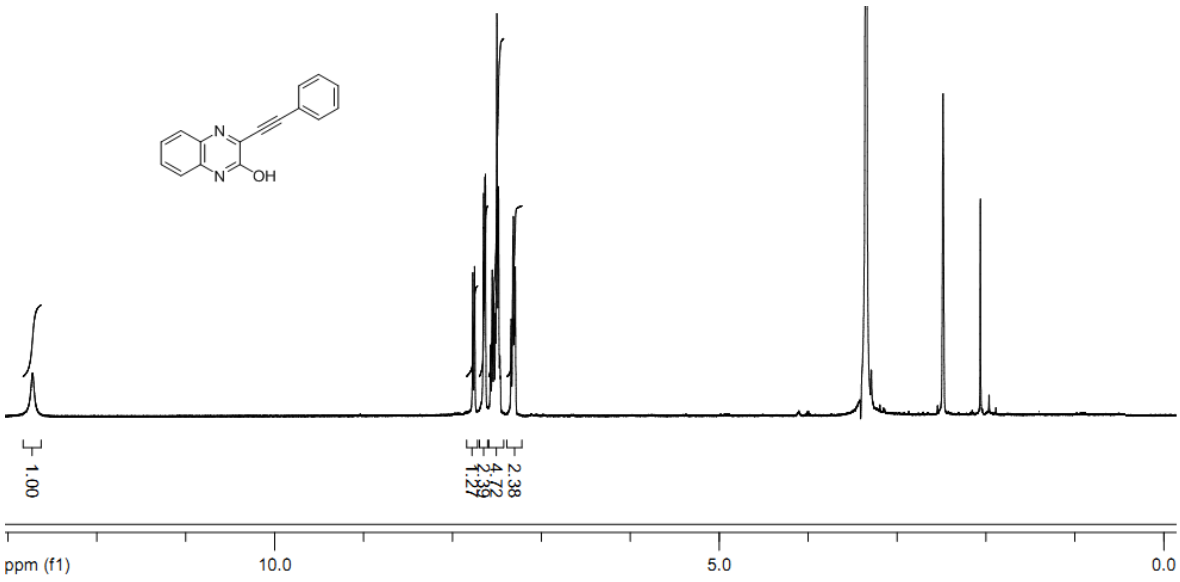
29) Chekmarev, D. S.; Shorshnev, S. V.; Stepanov, A. E.; Kasatkin, A. N. *Tetrahedron*, **2006**, 62, *42*, 9919.

30) Timmermans, P.B.M.W.M.; Recueil des, T. C. **1978**, 97, *3*, 81.

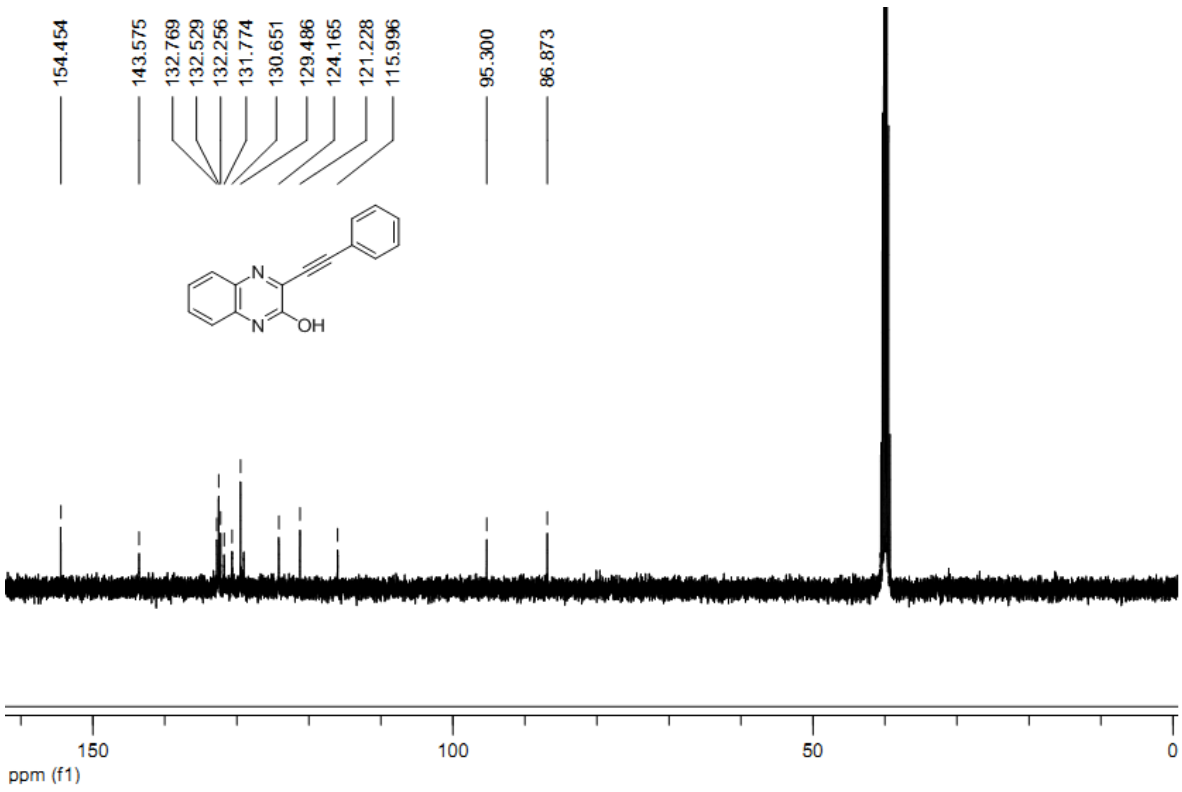
31) Terpetschnig, E.; Ott, W.; Kollenz, G.; Peters, K.; Peters, E. M.; Schnering, H.; Von, G. *Monatshefte fuer Chemie*, **1988** , *119*, 367.

Appendix

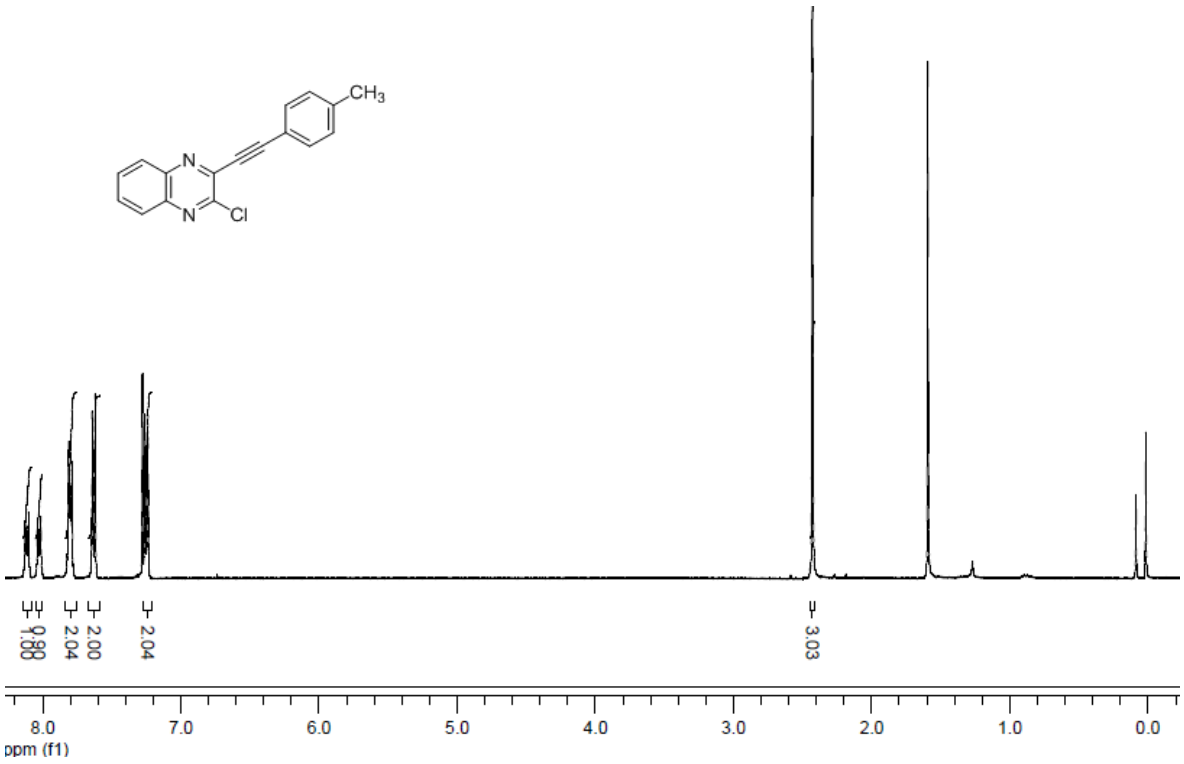
^{1}H NMR (400 MHz, DMSO-d_6) - **3aa**



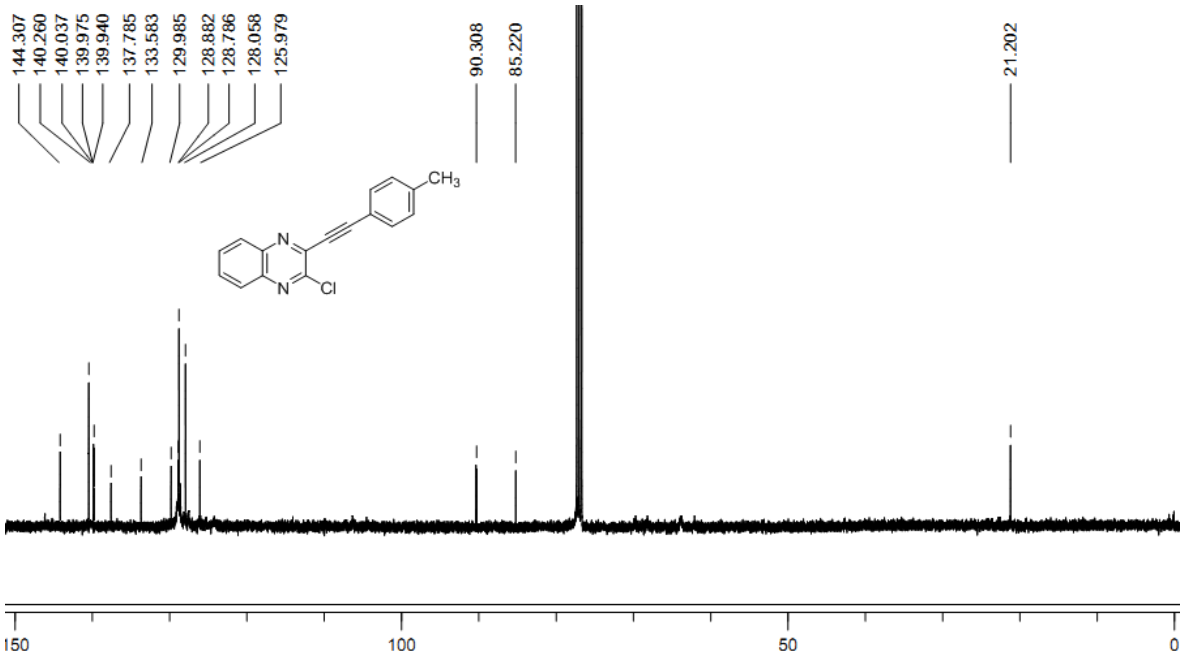
^{13}C NMR (100 MHz, DMSO-d_6) – **3aa**



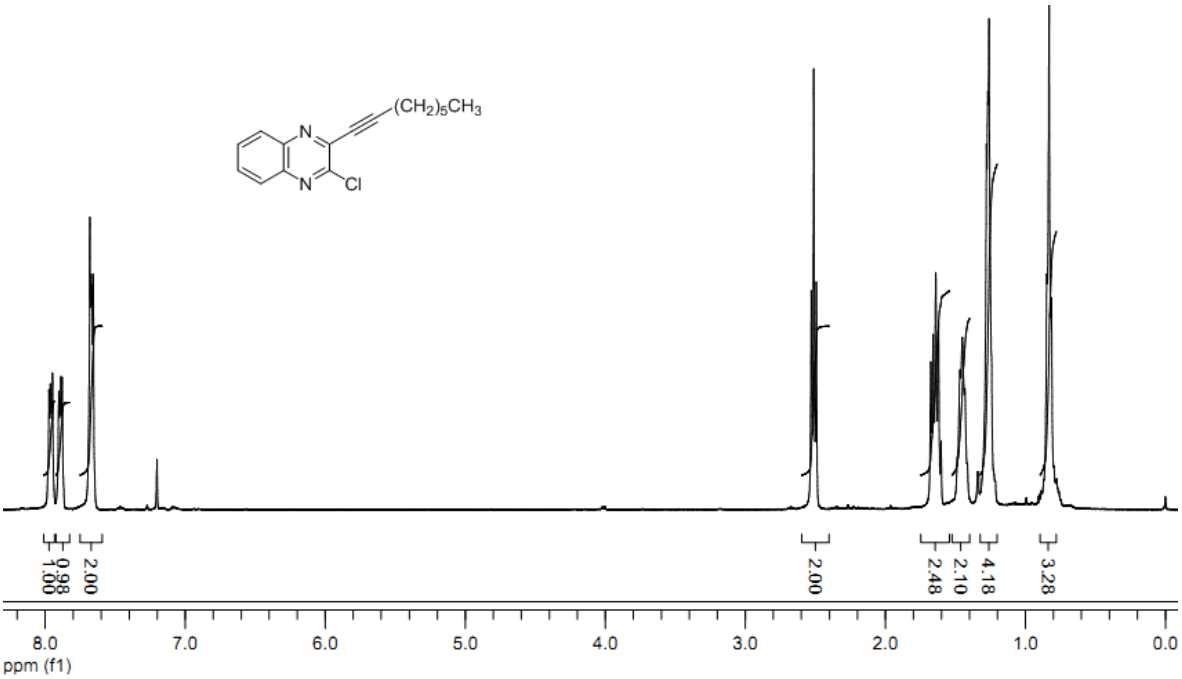
^{1}H NMR (400 MHz, $CDCl_3$) – **3b**



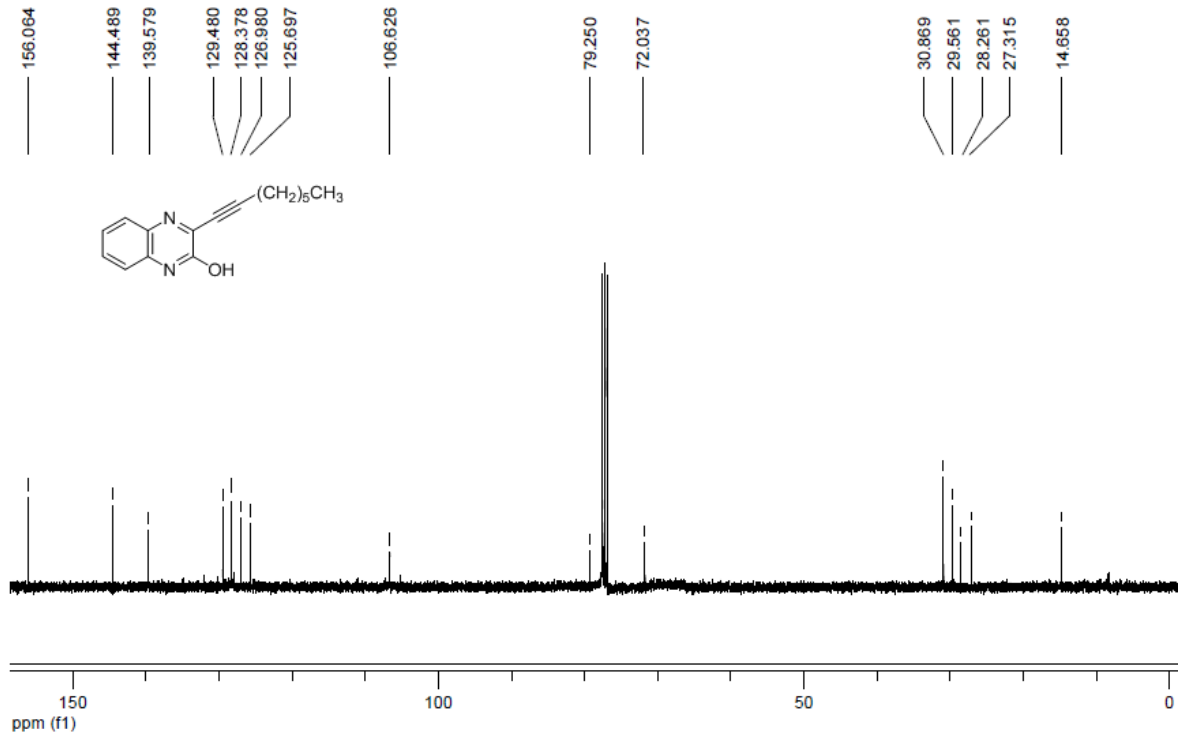
^{13}C NMR (100 MHz, $CDCl_3$)-**3b**



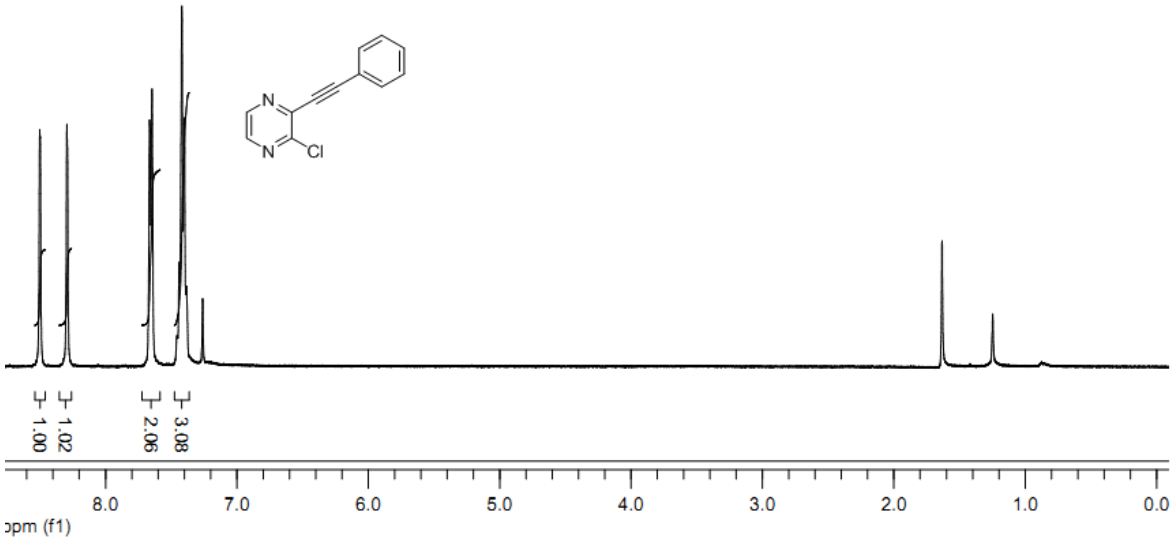
^{1}H NMR (400 MHz, $CDCl_3$) – **3e**



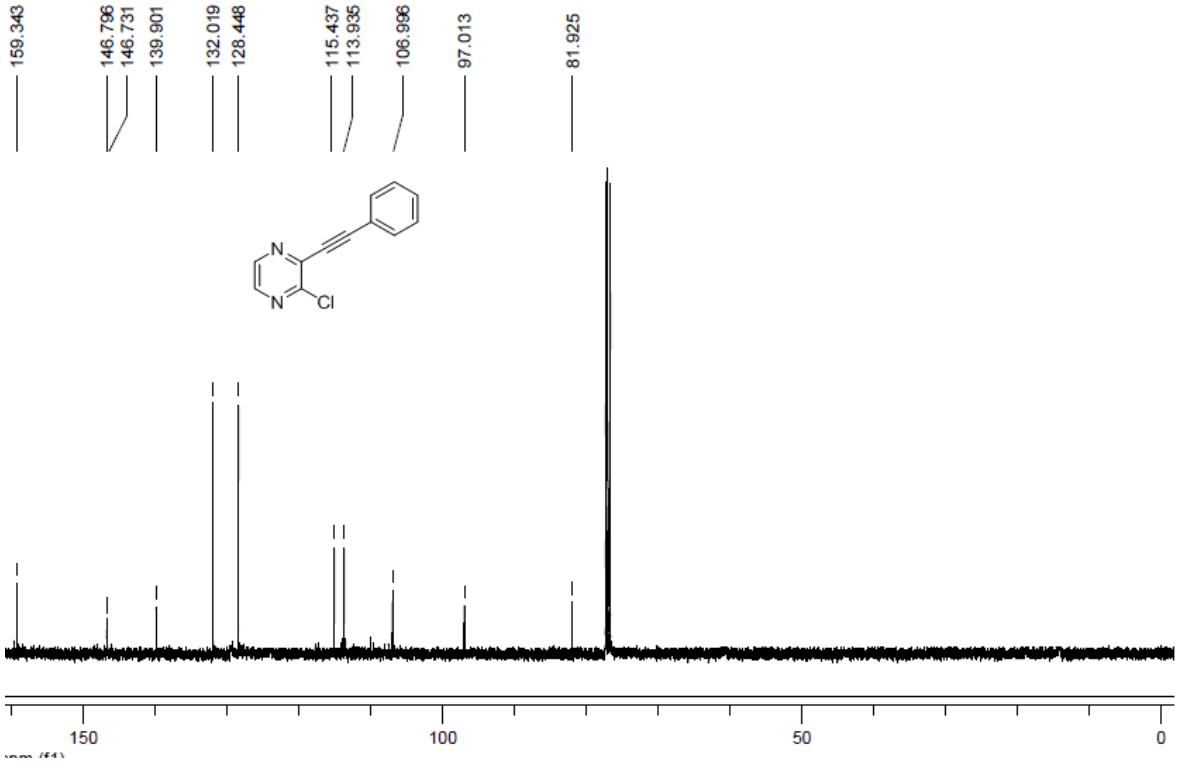
^{13}C NMR (100 MHz, $CDCl_3$)-**3e**



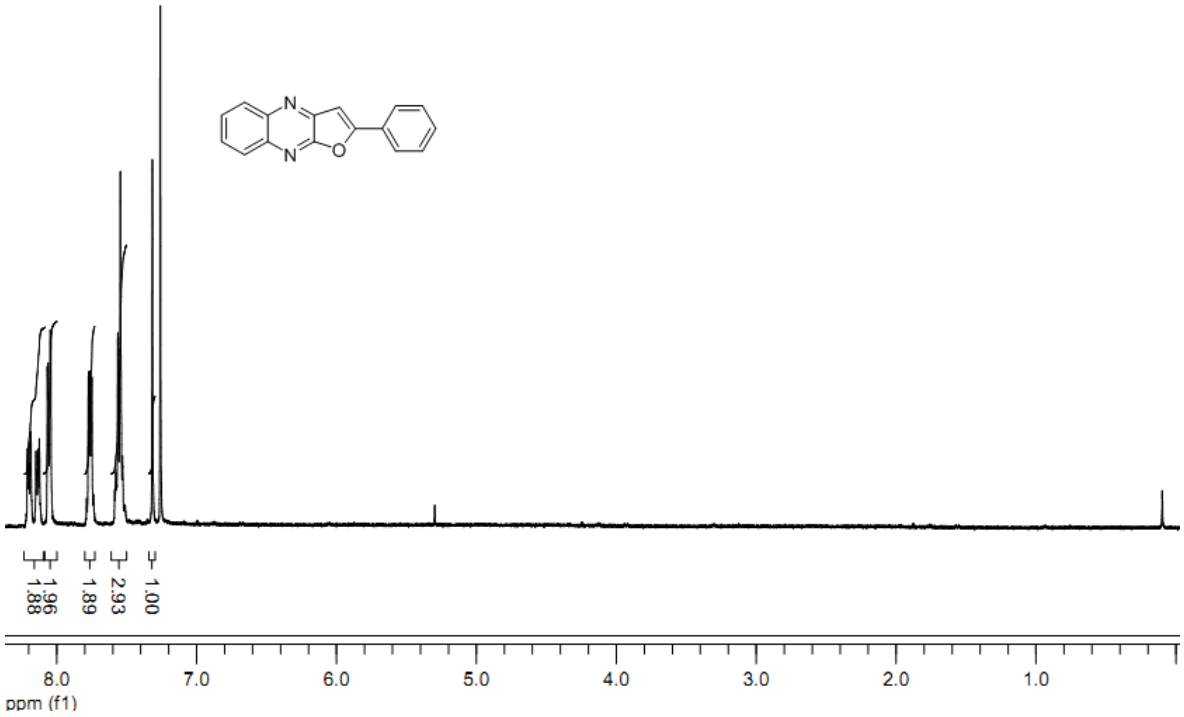
^{1}H NMR (400 MHz, $CDCl_3$) – **3o**



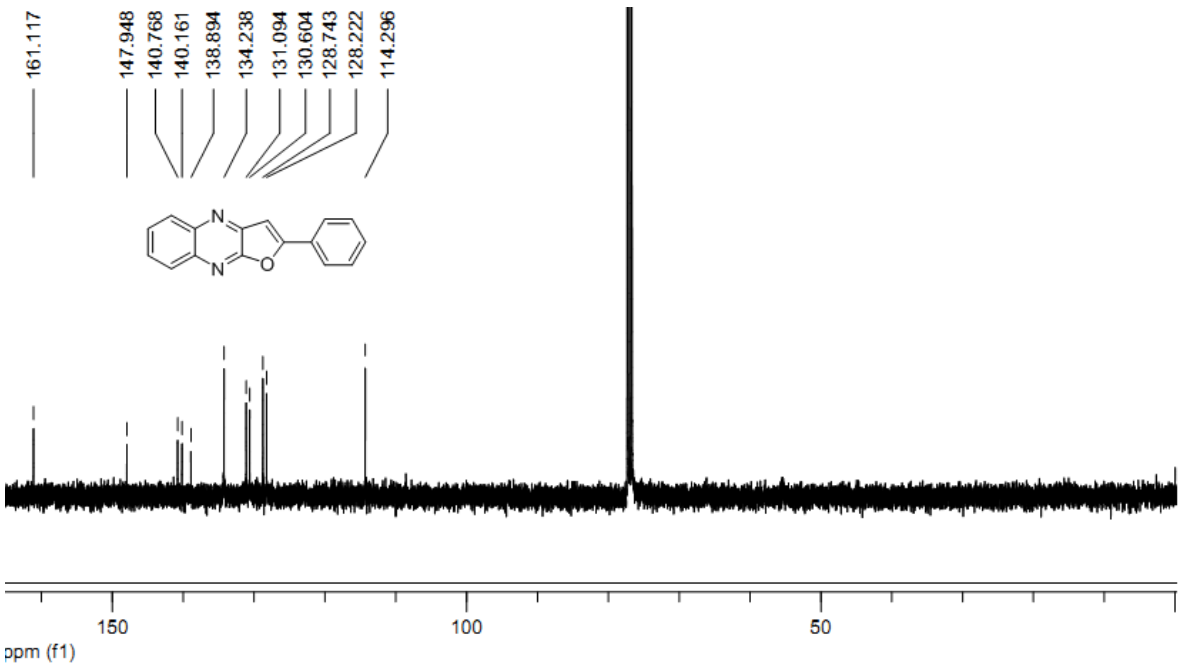
^{13}C NMR (100 MHz, $CDCl_3$) – **3o**



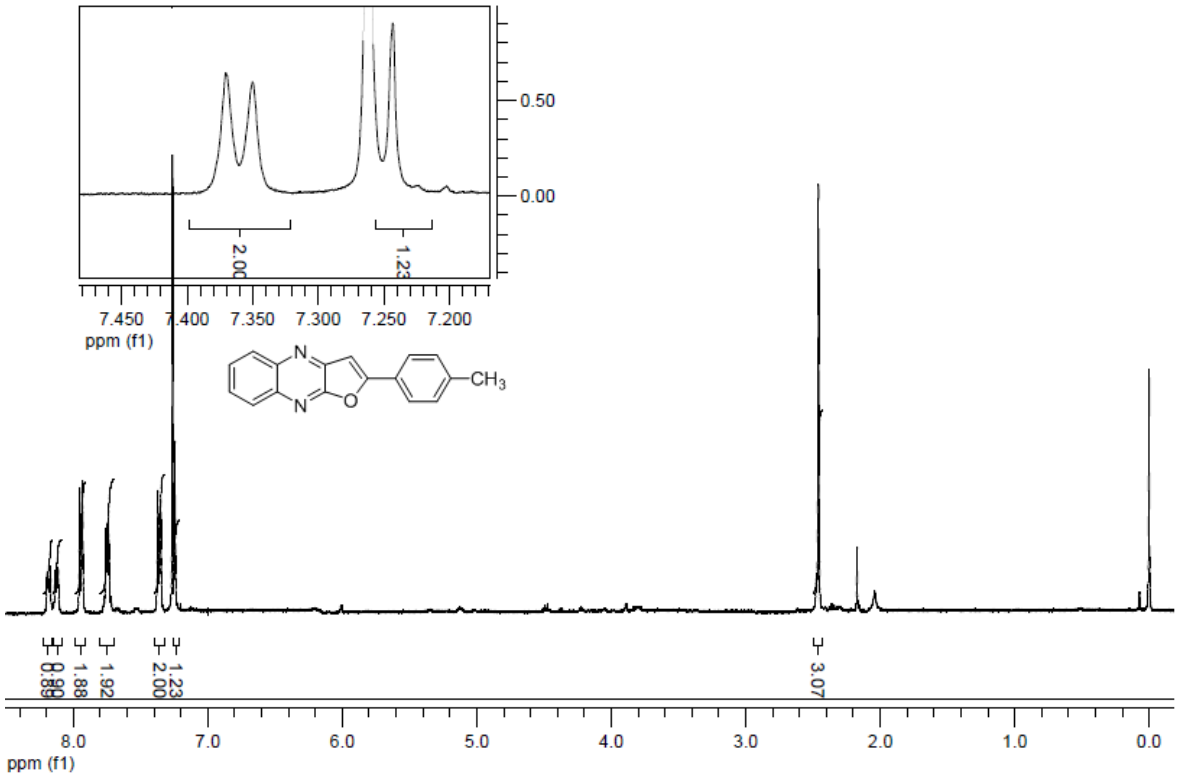
^{1}H NMR (400 MHz, $CDCl_3$) - **4a**



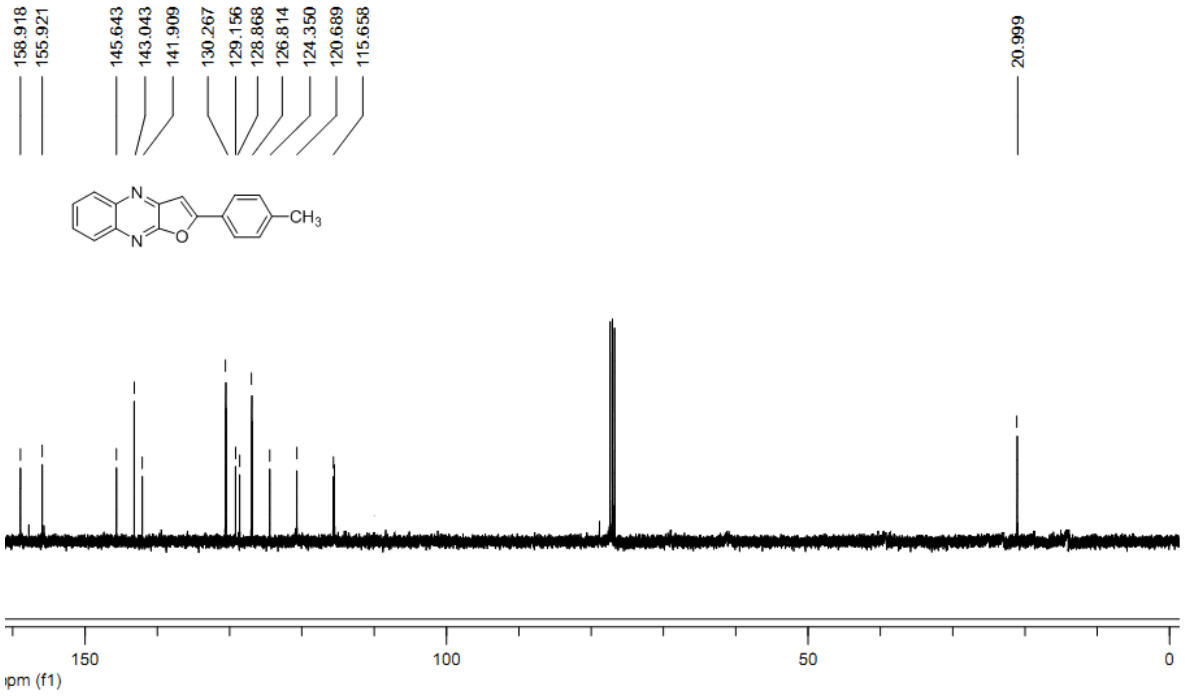
^{13}C NMR (100 MHz, $CDCl_3$) – **4a**



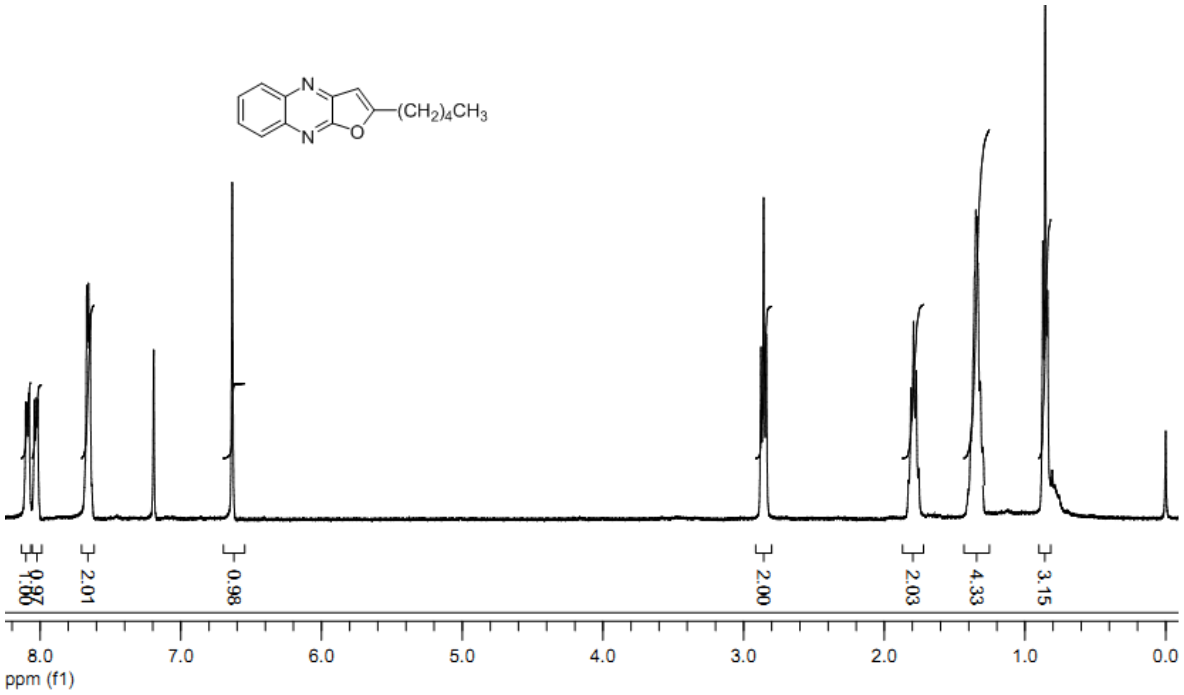
^{1}H NMR (400 MHz, $CDCl_3$) – **4b**



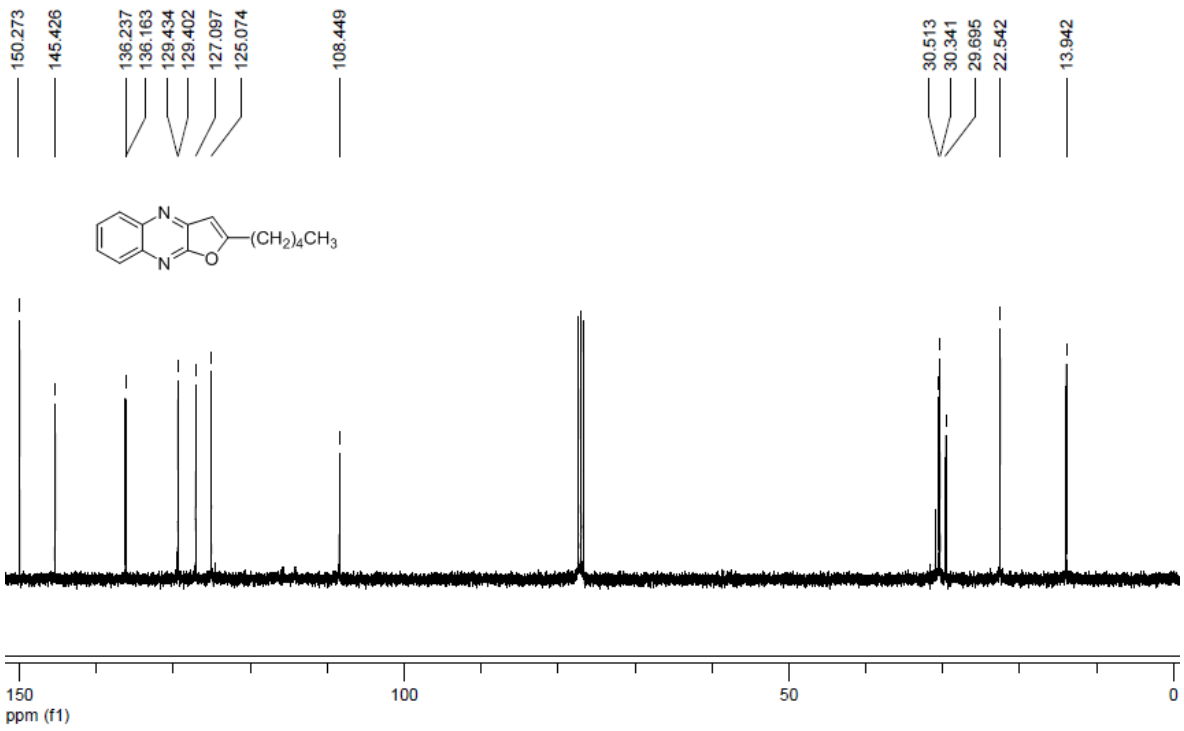
^{13}C NMR (100 MHz, $CDCl_3$) – **4b**



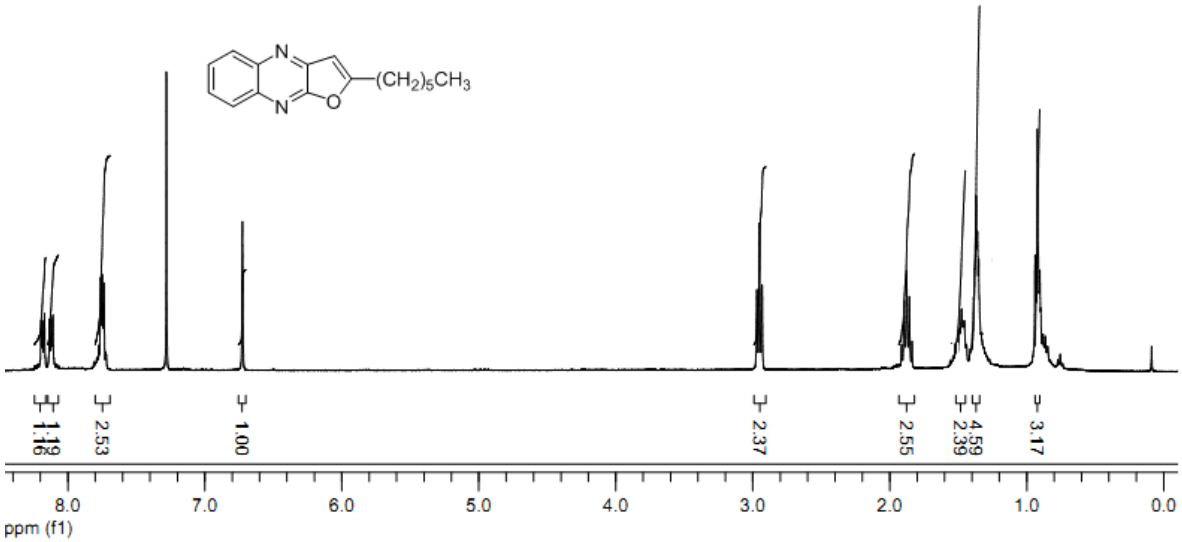
^{1}H NMR (400 MHz, $CDCl_3$) – **4d**



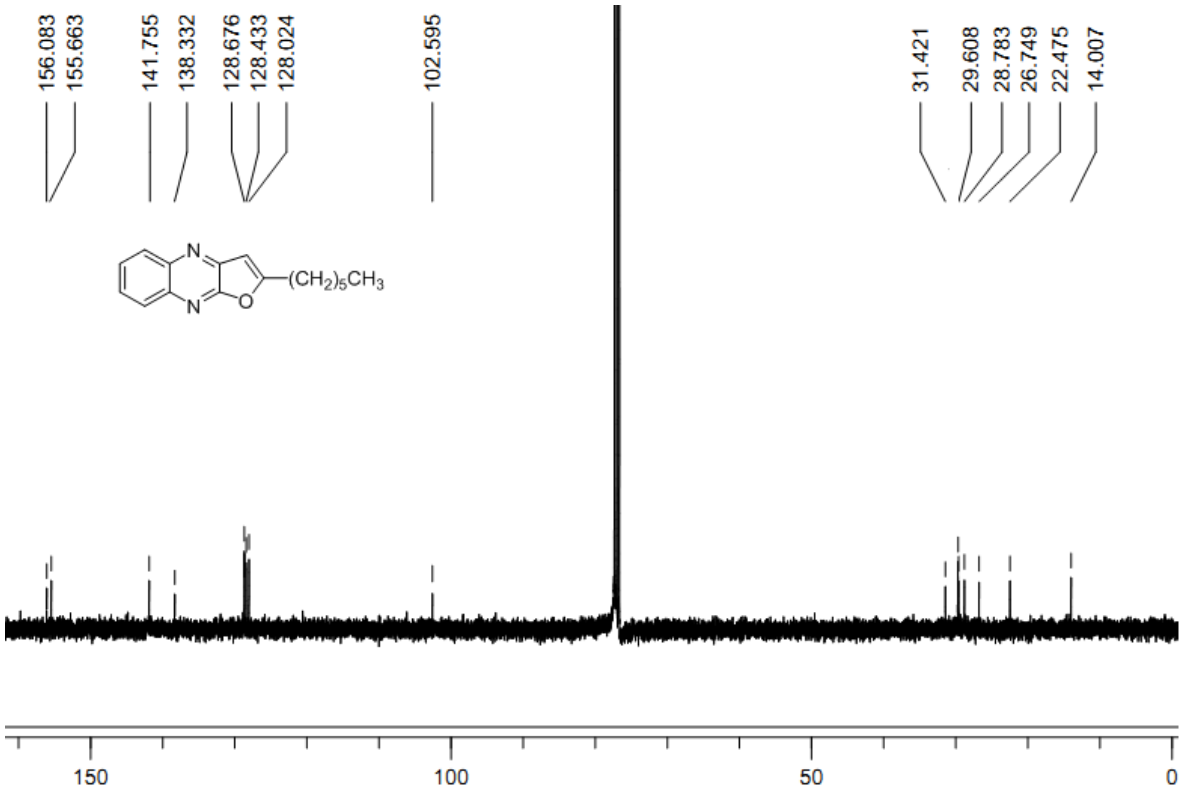
^{13}C NMR (100 MHz, $CDCl_3$) – **4d**



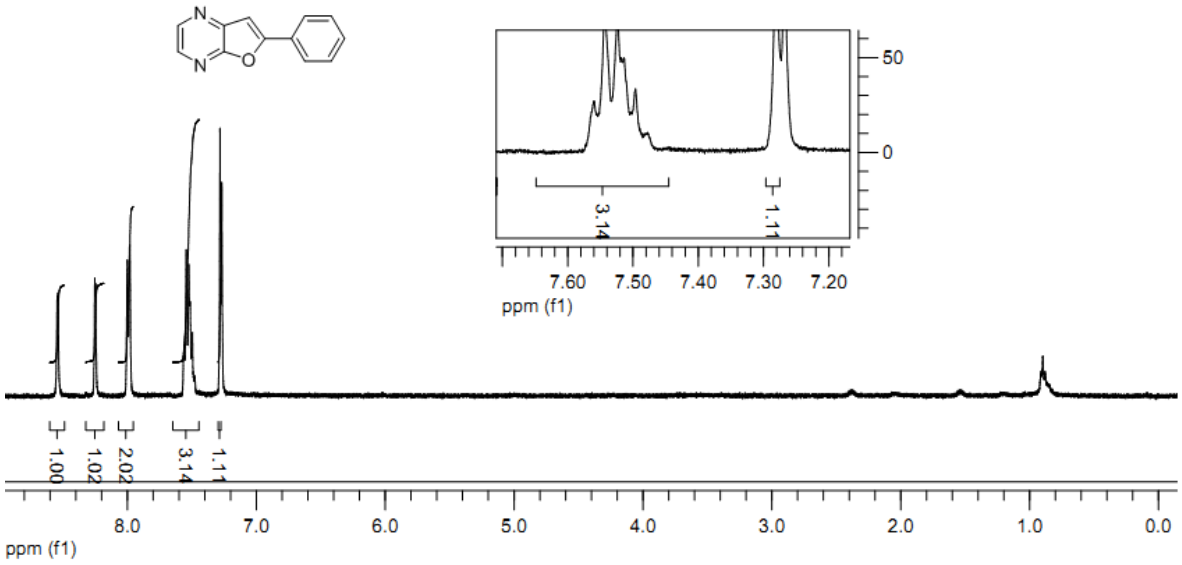
^{1}H NMR (400 MHz, $CDCl_3$) – **4e**



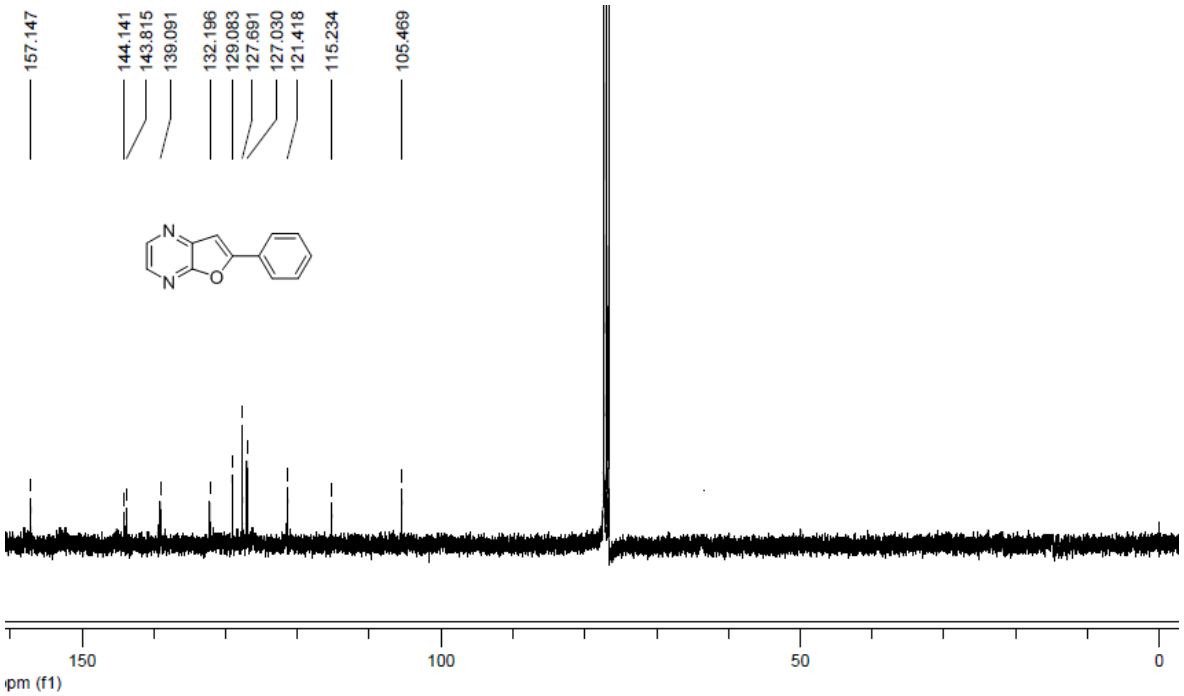
^{13}C NMR (100 MHz, $CDCl_3$) – **4e**



^{1}H NMR (400 MHz, $CDCl_3$) – **4o**



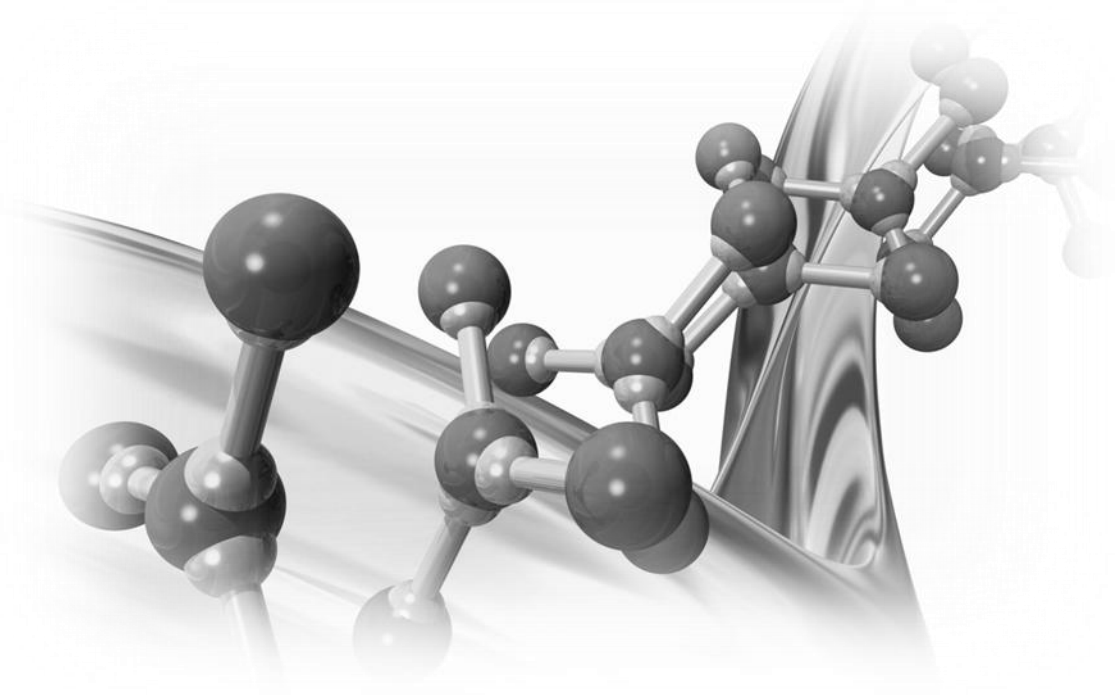
^{13}C NMR (100 MHz, $CDCl_3$) – **4o**



CHAPTER 5

CHAPTER 5A

Synthesis of 2-(hetero)aryl indoles via coupling/desilylation–coupling / cyclization under Pd/C-Cu catalysis



5A.1. Introduction

Heterocycles are ubiquitous building blocks in natural products, bioactive compounds and materials. Amongst the variety of heterocycles indole motif occur widely in nature as partial structures of alkaloids and have unique feature of alkaloid natural products and represents a privileged structural element for pharmaceutically active compounds.[1] Various 2-substituted indoles exhibit interesting pharmacological properties such as antithrombatic,[2] anticancer,[3] histamine H3 receptor antagonism.[4] The indole moiety is present in a number of drugs currently on the market. Most of these belong to triptans[5] which are used mainly in the treatment of migraine headaches for example, Sumatriptan (Fig.1, **1**) for the treatment of migraines, Zolmitriptan (Fig.1, **2**) to treat acute migraine attacks and cluster headaches. All members of this group are agonists of migraine associated 5HT1B and 5HT1D serotonin receptors (Serotonin is one of the neurotransmitters present in the central and peripheral nervous system, which plays an important role in normal brain function and regulates sleep, mood, appetite, sexual function, memory, anxiety, and many others). Indoles are also present in the complex natural products Vinblastine (Fig.1, **3**) and Vincristine (Fig.1, **4**) and are widely recognized members of the Vinca alkaloids as antitumor drugs. On the other hand Lecanindoles acts as Progesterone receptor (PR) agonists. Monaspiloindole (Fig.1, **5**) and Monaspyroindole (Fig.1, **6**) are indole containing fungal (Monascus) metabolites which are used in production of red yeast rice by the fermentation of rice (Oryza sativa). These metabolites are also used for medicinal purposes like improved food digestion and blood circulation.[6] In this view, interest in the synthesis of these compounds has sparked the development of numerous methods for the construction of indole moieties and their derivatives.[7] Classical methods include (to name a few) the Fischer indole synthesis, the Batcho-Limgruber synthesis from *o*-nitrotoluenes and dimethylformamide acetals, the Gassman synthesis from *N*-haloanilines, the Gassman synthesis from *N*-haloanilines, the reductive cyclization of *o*-nitrobenzyl ketones, and the Madelung cyclization of *N*-acylotoluidines.

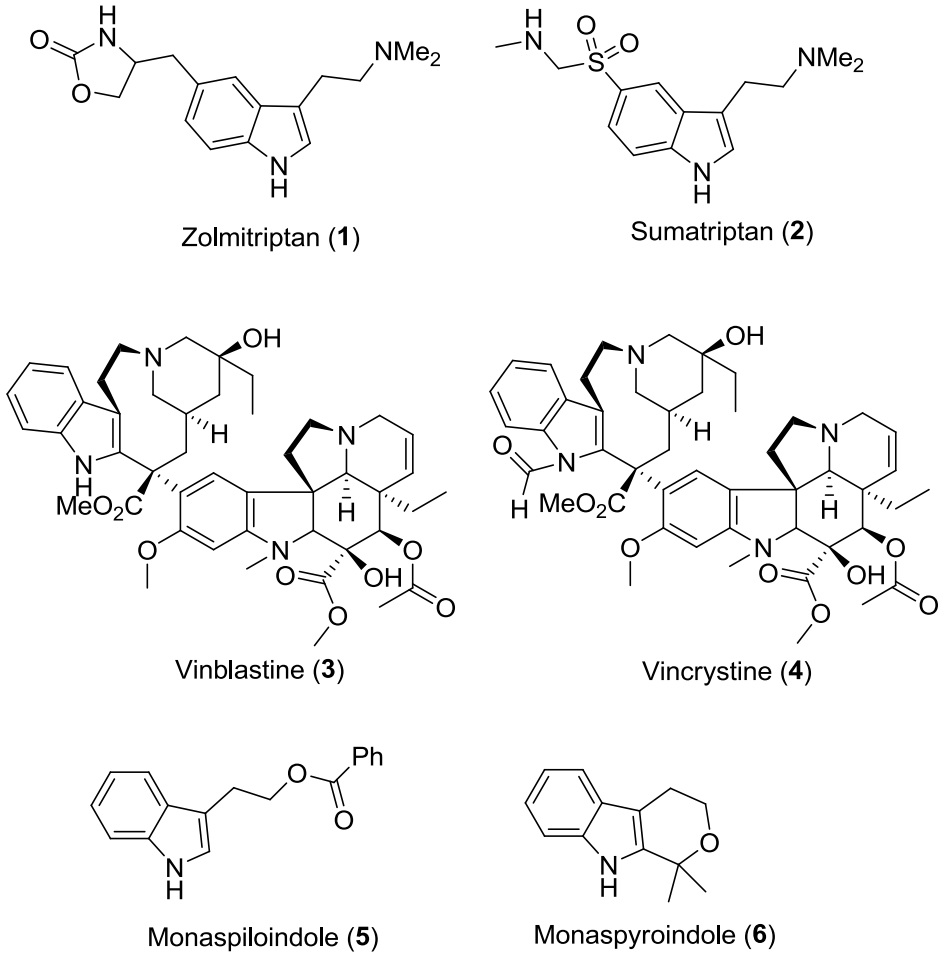
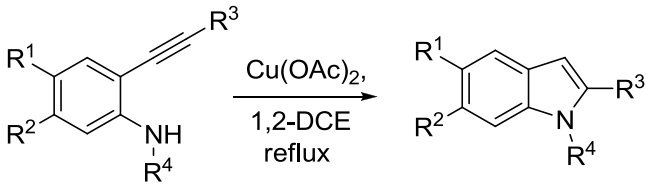


Figure 1. Natural products containing Indole motif.

5A.2. Previous Work

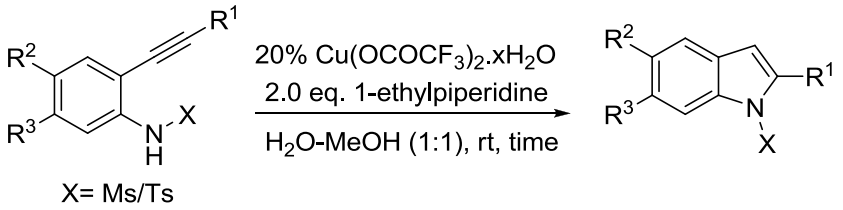
Until now, many kinds of catalysts have been reported for the synthesis of indole derivatives from 2-ethynyl-aniline derivatives.[8] Despite the plethora of methodologies developed for synthesis of this heterocyclic system,[9] efficient general methods for regioselective preparation of highly substituted indoles are limited.[10] Some of them are discussed below.

Hiroya in 2004, developed a relatively general and efficient method for indole via cyclization reactions of 2-ethynyl aniline derivatives.[11]



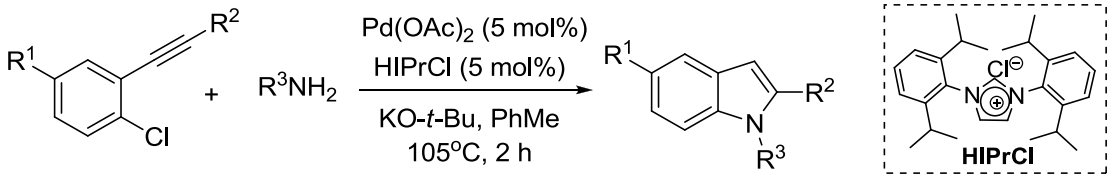
Scheme 1. Cu catalyzed synthesis of indoles.

In the next year same group reported the cyclization reaction of 2-ethynylaniline derivatives to indoles catalyzed by copper (II) salts.[12]



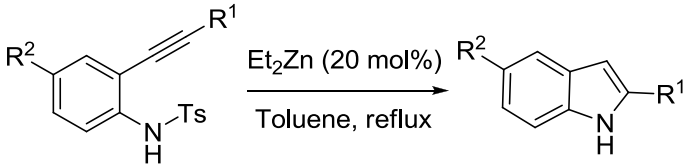
Scheme 2. Reaction of 2-ethynylaniline derivatives to indoles catalyzed by copper (II) salt.

In 2005, Ackermann and his group developed a general and efficient approach to the indole framework starting from o-alkynylhaloarenes is presented.[13]



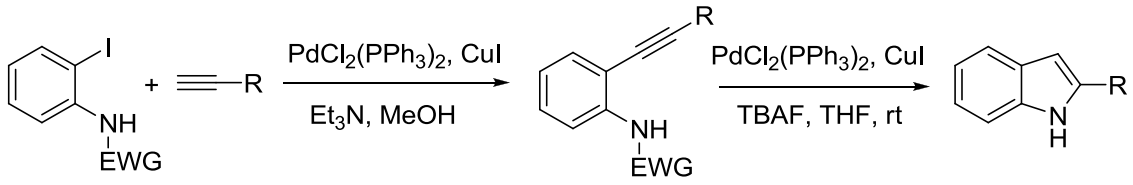
Scheme 3. Palladium catalyzed synthesis of indoles

In the year 2007, Zhao *et al*. developed a new and efficient method for the cyclization of alkynyl amides to form the corresponding nitrogen containing heterocycles mediated by Et_2Zn.[14]



Scheme 4. Zinc mediated synthesis of indoles from alkynyl amides

Yasuhara and his group developed cyclization reaction of various 2-ethynylanilines, which were synthesized from 2-haloanilines by the palladium-catalyzed reaction with terminal alkynes, with tetrabutylammonium fluoride (TBAF) to yield 2-substituted indoles.[15]



Scheme 5. Palladium mediated synthesis of 2-substituted indoles in presence of TBAF

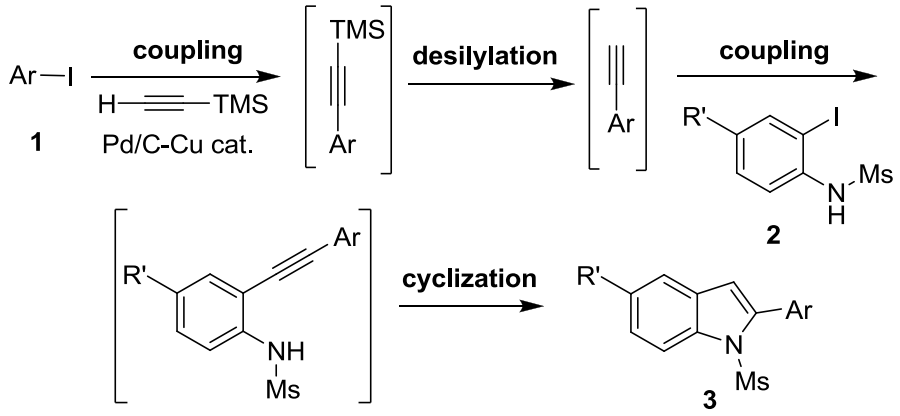
5A.3. Present Work

Among the plethora of heterocycles, Indole has gained prior importance because of its abundance in bioactive compounds and emerged as privileged structure, especially in medicinal chemistry.[16] On the other hand 2-substituted indole is one of the key structural frameworks found in many naturally occurring alkaloids, bioactive compounds and drugs.[17-19] Medicinal chemistry approach for different therapeutic areas like metabolic disorders and cancer focus on modulation of certain enzymes/proteins. One of such protein is Sirt1 (Silent information regulation type1),[20] an NAD^+ dependent class III histone deacetylase enzyme. SIRT1 is a principal modulator of pathways downstream of calorie restriction that produces beneficial effects on glucose homeostasis and insulin sensitivity. SIRT1 regulates glucose and lipid homeostasis in the liver, modulates insulin secretion in pancreatic islets, controls insulin sensitivity and glucose uptake in skeletal muscle.[21]Recent studies have suggested that reduced sirtuin action is correlated with type 2 diabetes. Both over nutrition and aging, which are two major risk factors for diabetes, lead to decreased sirtuin function and result in abnormal glucose and lipid metabolism. Therefore, restoring normal levels of sirtuin action in type 2 diabetes may be a promising method of treating diabetes. Recently carbazole based inhibitor of Sirtuin e.g. EX527 (6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide) is undergoing Phase1 clinical trial.[22] On the other hand Sirtuin activators like Resveratrol a major component in the red wine had proven its positive effect in increasing life span and produces a metabolic profile desirable for treating

diseases of ageing such as type 2 diabetes in mammals.[23] Milne and his co workers reported small molecule activator of SIRT1 which is 1000 folds more potent than resveratrol that is SIRT1720 an imidazo[2,1-*b*]thiazole based structure as therapeutics for the treatment of type 2 diabetes.[24] Due to our continuous interest in synthesis of modulators of Sirtuin protein we focused on the synthesis of activators which can be promising anti diabetic drugs. We envisaged that indole can be a good motif towards SIRT1 activity. Many synthetic methods including transition metal catalyzed reactions have been reported for the construction of indole ring.[25] One of the commonly used methods for the synthesis of 2-substituted indoles involves two steps e.g. Sonogashira coupling of 2-aminoaryl halide with a terminal alkyne followed by cyclization of the resulting 2-alkynylanilines. The cyclization step can be carried out in the presence of a range of catalysts or promoters such as Cu(I)salt,[26] metal alkoxide,[27] fluorides,[28] Lewis acids,[29] gold(III),[30] and iodine.[31] Alternatively, 2-substituted indoles can be prepared directly *via* a single step method using Pd/C-Cu mediated coupling-cyclization of *o*-iodoanilides with terminal alkynes.[32] However, to obtain an indole derivative possessing a specific aryl group at C-2 position by the use of appropriate terminal alkyne containing the corresponding aryl moiety was necessary in all these cases. Moreover, many aryl alkynes are either expensive or commercially not available or their preparation requires cumbersome methods.[33-34] Their isolation in the pure form is also sometimes problematic as the terminal alkynes are prone to undergo rapid dimerization like in Glaser coupling reaction. Thus a more effective method was necessary to prepare 2-aryl substituted indoles of our interest.

Herein we developed Pd/C-Cu mediated sequential synthesis of 2-aryl substituted indoles (Scheme 1) *via* four steps in a single pot.

(i) C-C coupling reaction (Sonogashira coupling reaction)
(ii) C-Si bond cleavage reaction (Desilylation reaction)
(iii) C-C coupling reaction (Sonogashira coupling reaction)
(iv) C-N bond forming reaction.

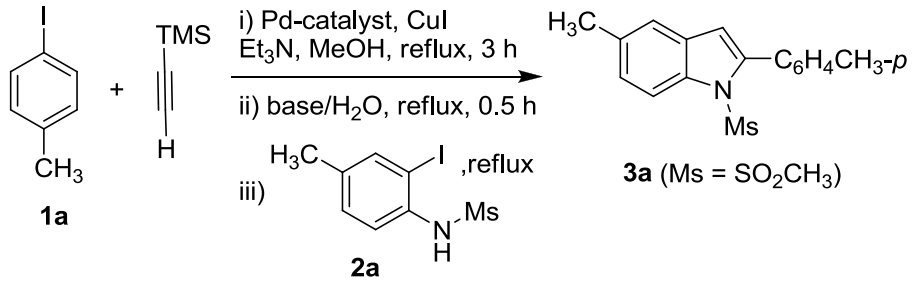


Scheme 7. Strategy to synthesize 2-aryl indoles in a single pot.

5A.4. Results and discussion

We anticipated that various terminal alkynes can be synthesized *in situ* from alkynes endcapped with trimethylsilyl (TMS) and removal of trimethylsilyl (TMS) group in subsequent iterations would be more facile. Based on the idea that desilylation of 2-aryl substituted trimethylsilyl alkynes could be achieved easily by using K_2CO_3 in MeOH-H_2O we planned to generate a range of terminal alkynes of our choice in situ. We anticipated that once generated these alkynes could undergo further Pd-mediated coupling reaction with an appropriate *o*-substituted arylhalide in the same pot. To this end, we assessed the reaction sequence involving the use of 1-iodo-4-methyl benzene **1a**, (trimethylsilyl) acetylene (TMSA) and *o*-iodoanilide **2a** under various reaction conditions (Table 1).

Table 1. The reaction of **1a**, TMSA and **2a** under various reaction conditions[a]



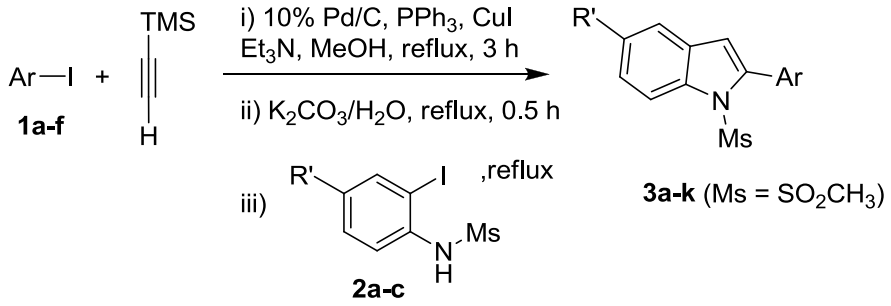
Entry	Catalysts	Base	Time[b] (h)	% Yield[c]
1.	$Pd(PPh_3)_2Cl_2$	K_2CO_3	4	78
2.	10%Pd/C-PPh_3	K_2CO_3	6	80
3.	10%Pd/C-PPh_3	KOH	6	75
4.	10%Pd/C	K_2CO_3	6	12
5.	PPh_3	K_2CO_3	8	55
6.	10%Pd/C-PPh_3[d]	K_2CO_3	8	43

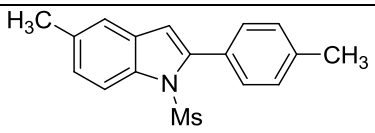
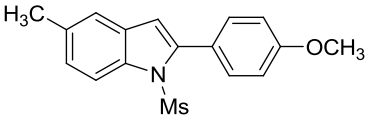
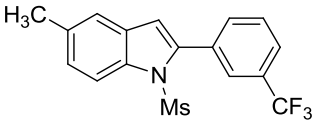
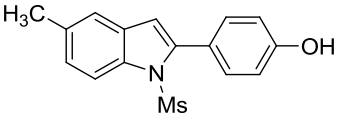
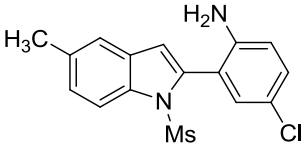
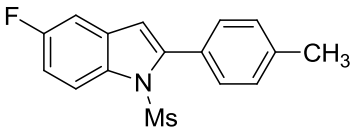
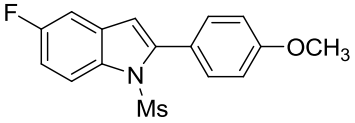
[a]All the reactions were carried out using iodide **1a** (2.137 mmol), TMSA (4.274 mmol), Pd catalyst (0.02137 mmol), CuI (0.02137 mmol), [PPh_3 (0.04274 mmol)] and Et_3N (0.5 mL) in MeOH (5.0 mL), then a base (4.274 mmol) in water (0.5 mL) and MeOH (1.5 mL) and finally o-iodoanilides (**2a**). [b]After adding **2a**. [c]Isolated yield. [d]The reaction was without CuI.

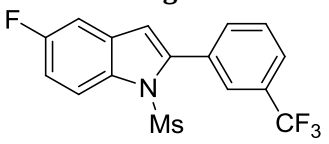
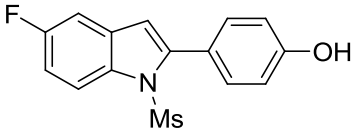
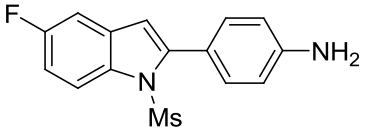
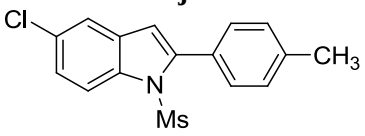
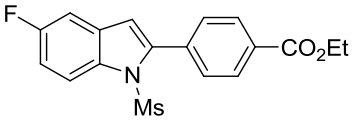
To optimize the reaction we performed Sonogashira coupling[35] of **1a** with TMSA using a Pd catalyst, CuI and Et_3N in methanol at refluxing temperature. The resulting crude reaction mixture was then directly treated with a solution of an inorganic base in MeOH/H_2O (3:1). After stirring this mixture at refluxing temperature for 0.5 h, *o*-iodoanilides **2a** were added and the mixture was stirred at refluxing temperature. Initially, we used $(PPh_3)_2PdCl_2$ as a catalyst and K_2CO_3 as a base (for desilylation step) when the desired product **3a** was isolated in good yield (entry 1, Table 1). However, due to our long term interest in the Pd/C-mediated alkynylation reaction[36] we conducted the present reaction using 10%Pd/C-PPh_3 as a catalyst system. Additionally, Pd/C is cheaper, stable, easy to handle and separable from the product and has potential to be recycled. The reaction proceeded well affording **3a** in good yield (entry 2, Table 1). Though the use of KOH was found to be equally effective for desilylation step (entry 3, Table 1) we however preferred milder base K_2CO_3. Omission of any component of the catalyst 10%Pd/C-PPh_3-CuI decreased the product yield (entries 4, 5 and 6, Table 1).

With this optimized reaction condition in hand we then decided to expand the generality and scope of this methodology i.e., sequential (i) C-C coupling followed by (ii) C-Si bond cleavage and subsequent (iii) C-C and (iv) C-N bond forming reactions *via* employing other iodoarenes and the results of this study are summarized in Table 2.

Table 2. One pot synthesis of 2-aryl indoles[a]



Entry	ArI (**1**; Ar =)	**2**; R' =	Product (**3**)	Time[b] (h)	%Yield[c]
1	**1a**; $C_6H_4CH_3$-*p*	**2a**; CH_3	 **3a**	6	80
2	**1b**; $C_6H_4OCH_3$-*p*	**2a**	 **3b**	5	85
3	**1c**; $C_6H_4CF_3$-*m*	**2a**	 **3c**	5	78
4	**1d**; C_6H_4OH-*p*	**2a**	 **3d**	8	65
5	**1e**; $C_6H_3NH_2$(*o*)Cl (*m*)	**2a**	 **3e**	4	69
6	**1a**	**2b**; F	 **3f**	4	60[d]
7	**1c**	**2b**		5	83

8	**1c**	**2b**	**3g** 	8	68
9	**1d**	**2b**	**3h** 	8	68
10	**1f**	**2b**	**3i** 	11	63
11	**1a**	**2c**; Cl	**3j** 	6	84[d]
12	**1g**; $C_6H_4CO_2Et$	**2b**	**3k**  **3l**	5	70

[a]All the reactions were carried out using iodide **1** (2.137 mmol), TMSA (4.274 mmol), 10%Pd/C (0.02137 mmol), CuI (0.02137 mmol), PPh_3 (0.04274 mmol) and Et_3N (0.5 mL) in MeOH (5.0 mL), then K_2CO_3 (4.274 mmol) in water (0.5 mL) and MeOH (1.5 mL) and finally *o*-iodoanilides (**2**). [b]After adding **2**. [c]Isolated yield. [d]Known compound.

The indole **3a** was characterized by spectral data and this was supported by the molecular structure being confirmed by X-ray analysis (Fig. 2).[37]

Single crystal X-Ray Diffraction (SXRD): X-ray data for the single crystal of **3a** has been collected at room temperature on Rigaku AFC-7S diffractometer equipped with mercury CCD detector using graphite monochromated Mo-K_α (λ = 0.7107 Å) radiation. Data collection, indexing, initial cell refinements, frame integration and final cell refinements were carried out using CrystalClear SM 1.3.6 software. The crystal structure was solved with direct methods (SIR2004)[38] and refined using least squares procedure (CRYSTALS)[39] using the CrystalStructure 3.8.1 software. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms bonded to carbons were positioned geometrically and refined in the riding model approximation with C–H =

0.95 Å, and with U(H) set to 1.2U_{eq}I.

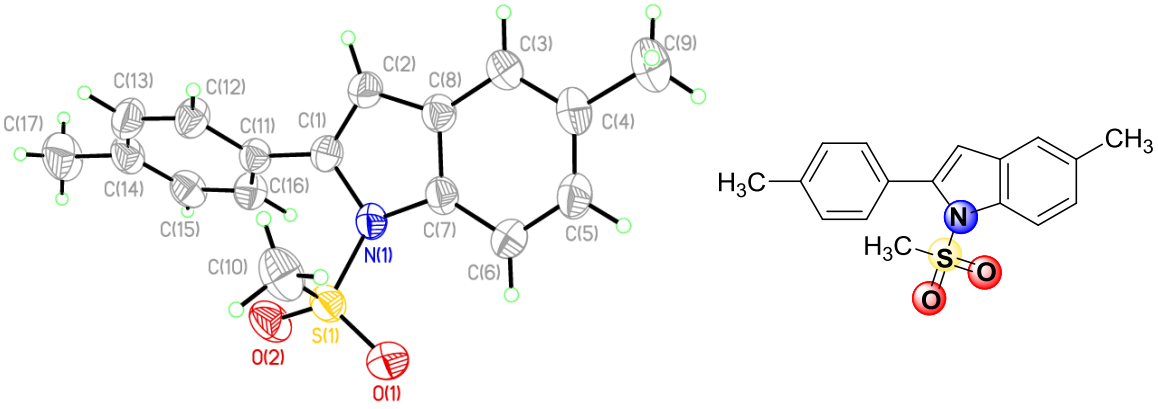
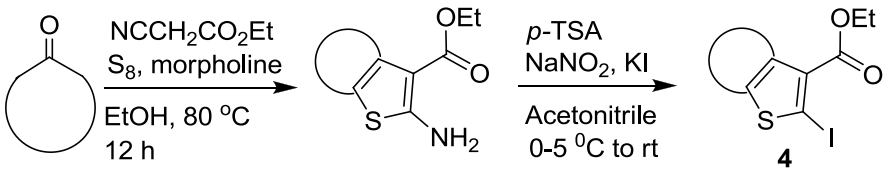


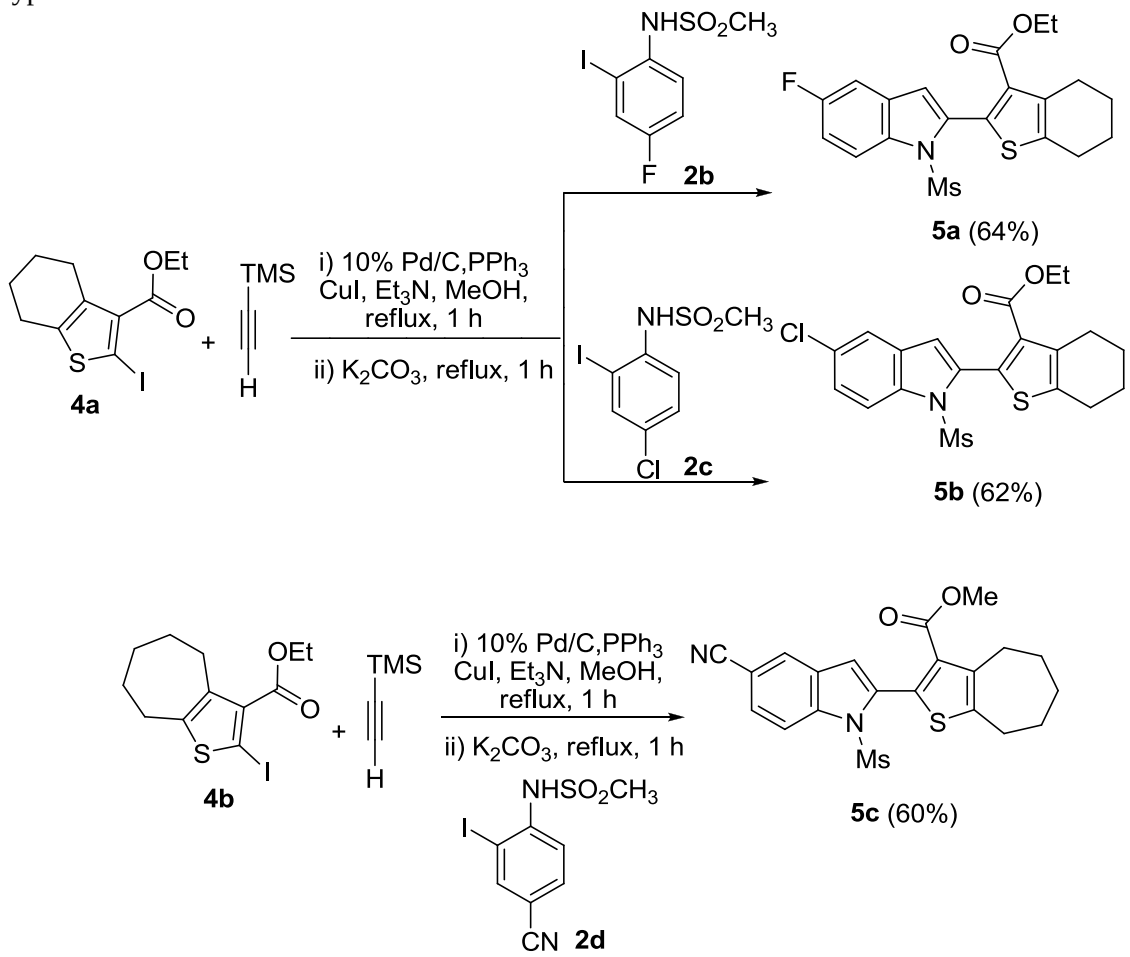
Figure 2. X-ray crystal structure of **3a** (ORTEP diagram). Displacement ellipsoids are drawn at 50% probability level for non-hydrogen atoms.

Crystal data of 3a: Molecular formula = $C_{17}H_{17}NO_2S$, Formula weight = 299.39, Triclinic, *P*ī, a = 5.805(4)Å, b = 10.234(6)Å, c = 13.495(8)Å, V = 750.8(8)Å^3, T = 298 K, Z = 2, D_c = 1.324 Mg m^{-3}, μ(Mo-Kα) = 0.219 mm^{-1}, 8318 reflections measured, 2988 unique reflections, 2515 observed reflections [I>2.0σ(I)], R_1_obs = 0.057, Goodness of fit = 1.089. Crystallographic data (excluding structure factors) for **3a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 784742.

The present four-step reaction in a single pot proceeded well with a number of iodoarenes affording a variety of indoles in good yields. Both electron donating groups such as Me (**1a**), OMe (**1b**), OH (**1d**) and NH_2 (**1e** and **1f**) or electron withdrawing group CF_3 (**1c**) present on the iodoarene ring were well tolerated. A further application of this methodology was demonstrated in the preparation of 2-heteroaryl indoles **5a-c** from 2-iodothiophene derivative **4** (Scheme 8). The iodo compound **4** was prepared from an appropriate cyclic ketone according to the Scheme 7.[40] Thus condensation of the cyclic ketone with a α-cyanoester in the presence of elemental sulfur under Gewald reaction conditions provided an 2-aminothiophene derivative which was converted to the 2-iodo derivative **4** under Sandmeyer conditions.



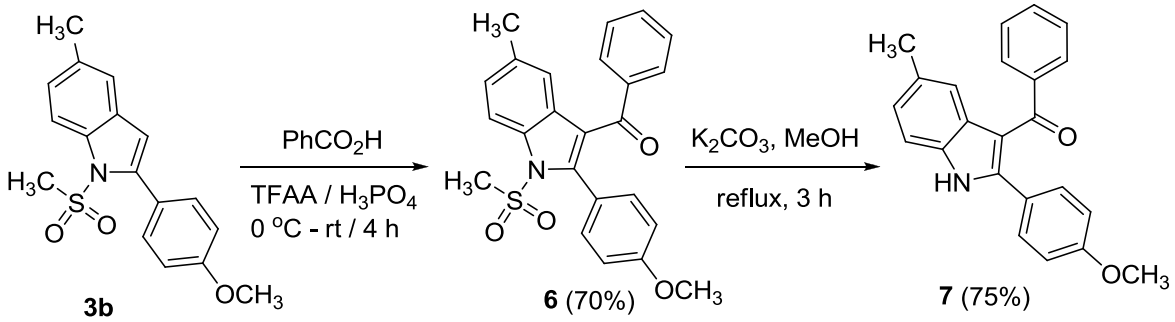
Scheme 7. Synthesis of 2-iodo thiophene ester *via* Gewald reaction followed by Sandmeyer type reaction.



Scheme 8. One pot synthesis of 2-heteroaryl indoles

The reaction of **4** with TMSA and subsequently with *o*-iodoanilide (**2b** or **2c** or **2d**) under the optimized contions (entry 2, Table 1) provided the desired 2-heteroaryl indoles (**5a-c**) in 60-64% yield. Notably, *N*-demesylation as well as trans-esterification was observed during the preparation of indole **5c** in the same pot. Overall, the present four-step method does not require the addition of any additional amounts of the Pd/Cu-catalyst to facilitate the final coupling cyclization step.

Due to our continued interest in bioactive molecules[41] particularly in modulators of Sirtuin protien, we then synthesized compound **6** *via* TFAA-H_3PO_4 mediated[42] benzoylation. This process does not require the use of moisture sensitive acid chloride and the corresponding acid therefore can be used directly. Notably, TFAA-H_3PO_4 mediated acylation of indole is not common in the literature. Finally we carried out deprotection of the resulting *N*-mesyl product (**7**) in presence of K_2CO_3 in MeOH (Scheme 9).



Scheme 9. Synthesis of 2-aryl-3-aroylindole

And further we focused on synthesizing compound (**8**) structurally similar to SIRT 1720 (fig. 3), a quinaxoline derivative that has been reported as a potent activator of human SIRT1.[24] Based on this, compound **3e** was converted to an indole based quinaxoline derivative (**8**) (Scheme 10) via the reaction with quinoxaline-2-carboxylic acid in the presence of coupling reagents e.g. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI.HCl) and 1-hydroxy-benzotriazole (HOBt) under mild conditions.

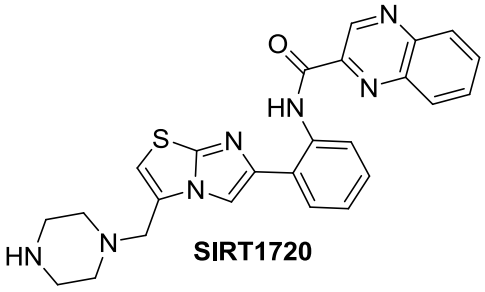
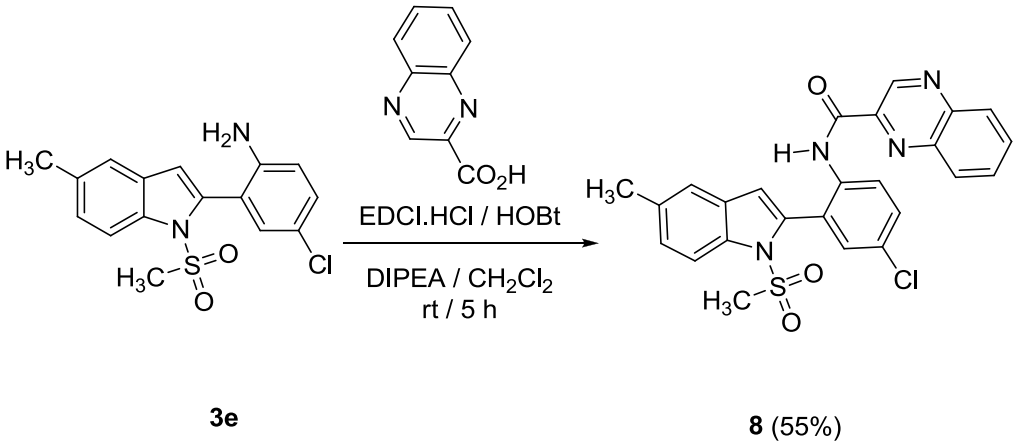
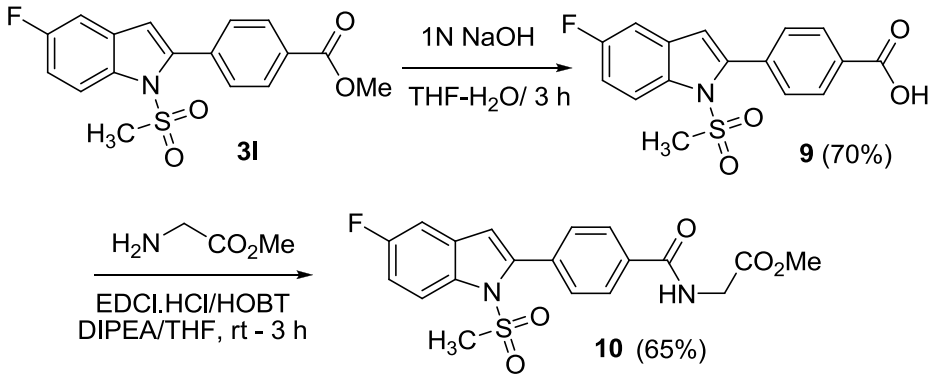


Figure 3. Structure of SIRT1720



Scheme 10. Synthesis of quinoxaline derivative.

Indole **3l** was converted to α-amino acid derivative **10**, *via* the corresponding acid **9** (Scheme 11). Thus, hydrolysis of **3l**, followed by the reaction of the resultant acid **9** with the methyl ester of glycine in the presence of coupling reagents, afforded the desired compound, **10**.



Scheme 11 Synthesis of α-amino acid analogue of indole **3l**.

5A.5.Pharmacology

Because of our interest in the identification of activators of SIRT1 (silent information regulator) we screened some of the compounds synthesized for their SIRT1 activating properties *in vitro*.[43] SIRT1 activators have been reported to be beneficial for the potential treatment of type 2 diabetes via anti-aging effect.[24] The *in vitro* activity was determined by using SIRT1 fluoresence activity assay kit from Cyclex Inc according to a known method.[44] Thus bacterially purified human SIRT1 enzyme was incubated with the fluorophore labeled substrate peptide (25 µM) and cofactor, NAD^+ (25 µM) in the presence or absence of compounds. Suramin, a known inhibitor of SIRT1 was also used in this assay. Among all the compounds tested 2-aryl-3-benzoylindole derivative **7** was found to be an activator of human Sirt 1 when tested at 10 μM in vitro whereas suramin showed significant inhibition. No significant activation of Sirt 1 was observed when 2-arylindole derivatives (**3**) were used at the same concentration.

***In vitro* assay for measuring SIRT1 activation:** The activity of the small molecules on SIRT1 was determined by using SIRT1 fluorescence activity assay kit from Cyclex Inc. according to manufacturer's protocol. Briefly, bacterially purified hSIRT1 enzyme was incubated with the fluorophore labeled substrate peptide (25 uM) and cofactor, NAD^+ (25 uM) in presence or absence of 10 µM compounds (suramin, an inhibitor of Sirt 1 along with compound **7**) for 15 min at 37 °C. Then 50 µL of stock solution was added and incubated for 45 min at room temperature. Fluorescence was read at Ex: 360 nm and Em: 450 nm. Blank consists of all components of the reaction mixture except enzyme. The difference between the blank and control reading gives the enzyme activity. Blank value is subtracted from all the sample readings. The compound control contains all the components of reaction mixture including the compound but no enzyme. So the reading obtained in the compound control indicates the autofluorescence of the compound and this is also substracted from the reading. Finally a graph is plotted against the samples on X-axis and absorbance value after substracting blank and autofluorescence values from the sample. Absorbance/Fluorescence is directly proportional to the enzyme activity (Fig.4).

Docking studies

In order to understand its interaction with Sirt 1 the indole **7** was docked into the active site of Sirt 1 (Fig. 3). Indeed, the binding energy (i.e. -5.81 Kcal/mol) of interaction of compound **7** with the activator domain of Human Sirt 1 indicates that it binds well with this NAD^+-dependent protein deacytylase. The key interacting amino acids were found to be Pro2, Leu3, Glu20, Asp5 and Ile27.

Homology Model of Human SIRT1 (144-217): The three dimensional model of hSIRT1 (uniprot code: Q96EB6, 144-217 amino acid residues) was developed by threading method using PRIME homology modeling program (Schrödinger L.L.C., USA). The multi step Schrödinger's Protein preparation tool (PPrep) has been used for final preparation of receptor model. Hydrogen's were added to the model automatically via the Maestro interface.[45] PPrep neutralizes side chains and residues which are not involving in salt bridges.[46] This step is then followed by restrained minimization using docking procedure. The compound **7** was sketched by using chemdraw and converted them to their 3D representation. The compound **7** and protein (homology model of hSIRT1) were prepared for docking (i.e. adding hydrogen's, gasteiger charge addition, and energy minimization) by using Chimera program. Autodock 4.0 program was used for docking. The best model of activator domain of hSIRT1 was developed and validated. The receptor grid was generated with co ordinates X: 43.804; Y: 47.333; Z: 29.948. The best 5 poses and corresponding scores have been evaluated by autodock 4.0 program.

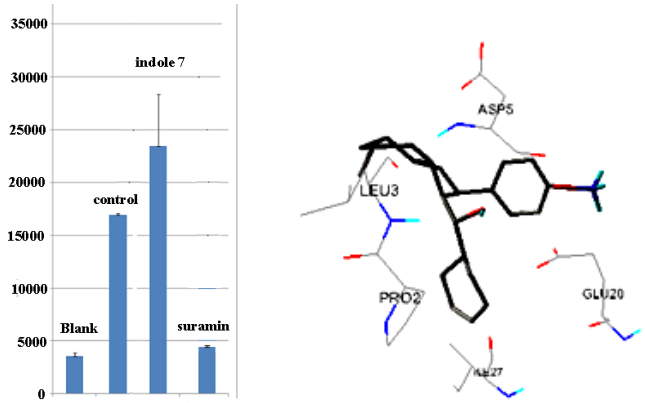


Figure 4. Sirt 1 activation and docking study of indole **7.**

5A.6. Conclusions

In conclusion, we have demonstrated a Pd/C-mediated new one-pot synthesis of 2-(hetero)aryl indoles *via* sequential C-C coupling followed by C-Si bond cleavage and subsequent tandem C-C / C-N bond forming reaction in the same pot. A variety of indole derivatives were prepared by using this methodology. Its application has been demonstrated in preparing compound with promising Sirt1 (Silent information regulator tupe1) activating property. The methodology does not involve the use of any expensive reagents, Palladium catalysts or solvents. In spite of a number of reports on efficient palladium-mediated synthesis of 2-(hetero)aryl indoles, no successful examples of indole synthesis conducting four steps in a single pot under Pd/C-Cu catalysis have been reported. Due to the operational simplicity and potential for introducing complexicity into an indole framwork the present methodology could become a useful alternative to the previously reported methods. The methodology therefore would find wide application in generating diversity based library of indoles of potential medicinal value.

5A.7. Experimental section

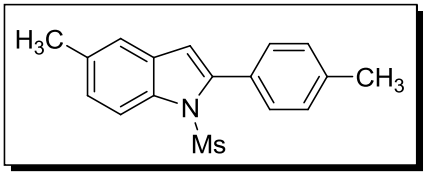
General methods: Unless stated otherwise, reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F_{254}), visualizing with ultraviolet light or iodine spray. Column chromatography was performed on silica gel (60-120 mesh) using distilled petroleum ether and ethyl acetate. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ solution using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.0) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FTIR spectrometer. Melting points were determined by using a Buchi melting point B-540 apparatus and are uncorected. MS spectra were obtained on a mass spectrometer. HRMS was determined using waters LCT premier XETOF ARE-047 apparatus. Elemental analyses were performed using C,H,N elemental analyzer.

General procedure for the preparation of 2-aryl indoles (3a-l):

To a solution of aryl iodide (**1a,** 0.46 g, 2.137 mmol), 10%Pd/C (0.003 g, 0.0214 mmol), CuI (0.004 g, mmol), and PPh_3 (0.012 g, 0.04274 mmol) in methanol (5.0 mL) was added triethylamine (0.5 mL, 1.26 mmol,) and (trimethylsilyl)acetylene (0.42 g, 4.274 mmol) at room temperature with stirring. The mixture was stirred at refluxing temperature for 3h (the reaction was monitored by TLC) and a solution of K_2CO_3 (0.59 g, 4.274 mmol) dissolved in water (0.5 mL) and methanol (1.5 mL) was added at the same temperature. The mixture was stirred at refluxing temperature for additional 0.5 h and *o*-iodoanilide (**2a,** 0.65 g, 2.137 mmol) was added. The stirring was allowed for additional 4-11 h (the reaction was monitored by TLC) at the same temperature. After completion the reaction mixture was quenched with saturated NH_4Cl solution (60 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were collected, combined, washed with 2.0 N HCl (50 mL) followed by water (50 mL), and saturated NaCl solution (50 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (EtOAc/*n*-Hexane) compound **3.**

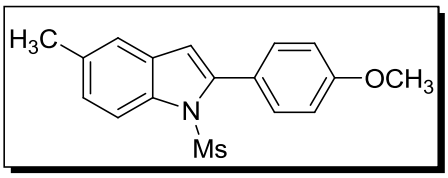
Spectral data of 2-aryl indoles (3a-l)

5-Methyl-1-(methylsulfonyl)-2-*p*-tolyl-1*H*-indole(3a):



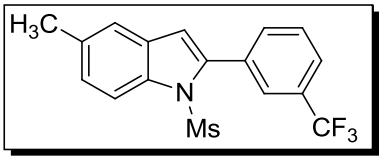
Yield: 0.51 g (80%), off white solid; mp: 178-180 °C; R_f = 0.71 (20% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.40 (s, 3H), 2.45 (s, 3H), 2.66 (s, 3H), 6.6 (s, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 7.6 Hz, 2H), 7.36 (s, 1H), 7.44 (d, *J* = 8 Hz, 2H), 7.97 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.2, 21.4, 38.9, 112.6 (2C), 115.6, 120.8 (2C), 126.2, 128.4, 129.1, 129.91, 130.6, 134.2, 136.2, 138.7, 142.2; MS (ES mass): *m*/*z* 300.3 (M+1, 40%); HRMS: calcd for $C_{17}H_{18}NO_2S$ (M+H): 300.1058, found 300.1056; Elemental Analysis found C, 68.43; H, 5.70; N, 4.44; $C_{17}H_{17}NO_2S$ requires C, 68.20; H, 5.72; N, 4.68.

2-(4-Methoxyphenyl)-5-methyl-1-(methylsulfonyl)-1*H*-indole (3b):



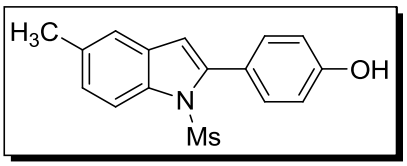
Yield: 0.57 g, (85%), pale yellow solid; mp: 184-186 °C; R_f = 0.57 (20% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.45 (s, 3H), 2.67 (s, 3H), 3.85 (s, 3H), 6.58 (s, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.8 Hz, 1H), 7.35 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.4, 39.1, 55.4, 112.4 (2C), 113.3, 115.8, 120.8, 124.4, 126.3, 130.8, 131.5, 134.3, 136.3 (2C), 142.2, 160.2; MS (ES mass): *m*/*z* 316.4 (M+1, 70%); HRMS: calcd, for $C_{17}H_{18}NO_3S$ (M+H): 316.1007, found: 316.0995; Elemental Analysis found: C, 64.97; H, 5.42; N, 4.32; $C_{17}H_{17}NO_3S$ requires C, 64.74; H, 5.43; N, 4.44.

5-Methyl-1-(methylsulfonyl)-2-(3-(trifluoromethyl)phenyl) -1*H*-indole (3c):



Yield: 0.59 g (78%), white solid; mp: 158-165 °C; R_f = 0.37 (10% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.47 (s, 3H), 2.68 (s, 3H), 6.72 (s, 1H), 7.22 (dd, J_1 = 1.2 Hz, J_2 = 1.2 Hz, 1H), 7.40 (s, 1H), 7.51-7.81 (m, 4H), 7.98 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.2, 39.8, 114.2, 115.5, 121.2, 125.3, 125.3, 126.1, 126.6, 128.0, 129.9, 130.4, 132.8, 133.9, 134.6, 136.4, 140.4; MS (ES mass): *m*/*z* 354.1 (M+1, 50%); HRMS: calcd for $C_{17}H_{15}F_3NO_2S$ (M+H): 354.0776, found 354.0746; Elemental Analysis found C, 57.54; H, 3.97; N, 4.19; $C_{17}H_{14}F_3NO_2S$ requires C, 57.78; H, 3.99; N, 3.96.

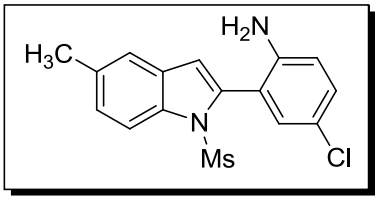
4-(5-Methyl-1-(methylsulfonyl)-1*H*-indol-2-yl)phenol(3d):



Yield: 0.42 g (65%), white solid; mp 190-192 °C; R_f = 0.4 (10% EtOAc-*n*-Hexane); ^{1}H

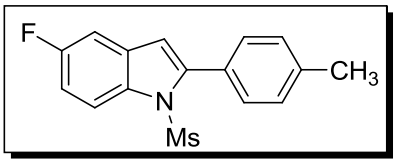
NMR (400 MHz, $CDCl_3$) ppm: 2.30 (s, 3H), 2.56 (s, 3H), 6.8 (s, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 7.12-7.20 (m, 2H), 7.5 (d, *J* = 8.0 Hz, 1H) 7.90 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.2, 39.8, 114.1, 115.5, 121.9, 125.8 (2C), 125.3, 126.6, 130.4, 132.8, 133.5, 134.6, 136.4 (2C), 162.5; MS (ES mass): *m*/*z* 302.3 (M+1, 50%). HRMS: calcd for $C_{16}H_{16}NO_3S$ (M+H): 302.0851, found: 302.0875; Elemental Analysis found: C, 63.56; H, 5.05; N, 4.79; $C_{16}H_{15}NO_3S$ requires C, 63.77; H, 5.02; N, 4.65.

4-Chloro-2-(5-methyl-1-(methylsulfonyl)-1*H*-indol-2-yl)aniline (3e):



Yield: 0.49 g (69%), light brown solid; mp 171-173 °C; R_f = 0.4 (20% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.46 (s, 3H), 2.88 (s, 3H), 3.90 (bs, 2H), 6.63 (s, 1H), 6.68 (d, *J* = 8.0, 1H), 7.16-7.25 (m, 3H), 7.4 (s, 1H), 7.96 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.2, 39.8, 113.1, 114.9, 116.6, 119.5, 121.1, 122.4, 126.7, 130.2, 130.3, 133, 134.1, 135.6, 136.9, 144.9; MS (ES mass): *m*/*z* 335.3 (M+1, 100%); HRMS: calcd for $C_{16}H_{16}N_2O_2SCl$ (M+H): 335.0621, found 335.0639; Elemental Analysis found: C, 57.57; H, 4.50; N, 8.29; $C_{16}H_{15}ClN_2O_2S$ requires C, 57.40; H, 4.52; N, 8.37.

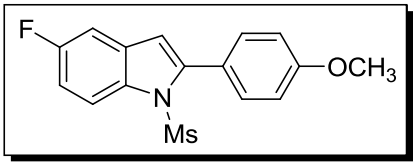
5-Fluoro-1-(methylsulfonyl)-2-*p*-tolyl-1*H*-indole [31a] (3f):



Yield: 0.39 g (60%), white solid; mp 203-205 °C (lit[31a] 209-210 °C). R_f = 0.47 (10% EtOAc-*n*-Hexane); White solid, mp 190 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.41 (s, 3H), 2.74 (s, 3H), 6.6 (s, 1H), 7.16 (s, 1H), 7.30 (d, *J* = 2 Hz, 2H), 7.32 (d, *J* = 1.6 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.55 (d, *J* = 2 Hz, 1H), 8.07-8.09 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.4, 39.9, 111.7, 116.9 (2C), 120.4, 125.0, 128.5, 129.1 (2C), 130.1, 131.5,

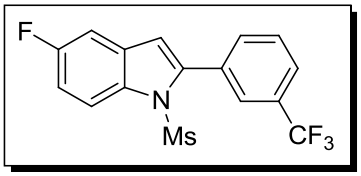
134.2, 136.2, 139.3, 143.5; IR (cm^{-1}): 3139,1272, 1360; MS (ES mass): *m*/*z* 304.6 (M+1, 100%); HRMS: calcd for $C_{16}H_{15}FNO_2S$: 304.2546, found 304.2462.

5-Fluoro-2-(4-methoxyphenyl)-1-(methylsulfonyl)-1*H*-indole (3g):



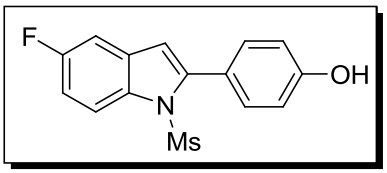
Yield: 0.56 g (83%), off white solid; mp 198-200 °C R_f = 0.55 (10% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.72 (s, 3H), 3.86 (s, 3H), 6.59 (s, 1H), 6.95 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 2 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 1.6 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 39.8, 55.3, 111.5, 113.3, 116.9 (2C), 120.3, 123.5, 124.9, 130.2 (2C), 131.5, 131.6, 136.2, 143.3, 160.4; MS (ES mass): *m*/*z* 320.3 (M+1, 10%); HRMS: calcd for $C_{16}H_{15}FNO_3S$: 320.0757, found 320.0746; Elemental Analysis found C, 60.04; H, 4.40; N, 4.47; $C_{16}H_{14}FNO_3S$ requires C, 60.18; H, 4.42; N, 4.39.

5-Fluoro-1-(methylsulfonyl)-2-(3-(trifluoromethyl)phenyl)-1*H*-indole (3h):



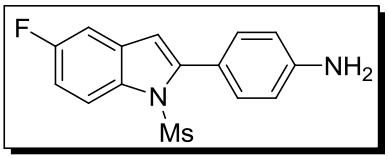
Yield: 0.52 g (68%), off white solid; mp 121-123 °C; R_f = 0.35 (10% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.72 (s, 3H), 6.83 (s, 1H), 7.17 (dd, J_1 = 1.6 Hz, J_2 = 2.0 Hz, 2H), 7.51-7.87 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.1, 112.1, 121.8, 122.6, 123.3, 124.5, 125.3, 126.2, 128.3, 129.6, 130.1, 131.4, 132.7, 135.4, 137.6, 142.2; MS (ES mass): *m*/*z* 358.9 (M+1, 10%); HRMS: calcd for $C_{16}H_{12}F_4NO_2S$: 358.0525, found 358.0536; Elemental Analysis found C, 53.55; H, 3.08; N, 4.05; $C_{16}H_{11}F_4NO_2S$ requires C, 53.78; H, 3.10; N, 3.92.

4-(5-Fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)phenol (3i):



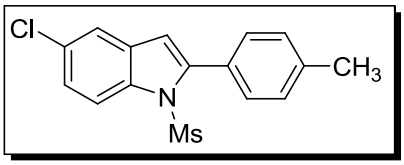
Yield: 0.44 g (68%), white solid; mp 152-155 °C; R_f = 0.42 (20% EtOAc-*n*-Hexane) ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.57 (s, 3H), 6.79 (s, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 7.20-7.79 (m, 3H), 7.88 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 40.3, 112.4, 113.3, 114.9 (2C), 120.8, 123.5, 128.7, 129.8 (2C), 131.9, 132.6, 136.3, 138.5, 161.8; MS (ES mass): *m*/*z* 306.3 (M+1, 10%); HRMS: calcd for $C_{15}H_{13}FNO_3S$ (M+H): 306.0600, found 306.0623; Elemental Analysis found: C, 59.19; H, 3.95; N, 4.45; $C_{15}H_{12}FNO_3S$ requires C, 59.01; H, 3.96; N, 4.59.

4-(5-Fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)aniline (3j):



Yield: 0.41 g (63%), light brown solid; mp 133-135 °C; R_f = 0.45 (20% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.72 (s, 3H), 4.10 (bs, 2H), 6.55 (s, 1H), 6.71 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 1.6 Hz, 1H), 8.03 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.3, 112.4, 113.3, 115.7 (2C), 120.8, 123.5, 128.7, 129.8 (2C), 131.9, 132.6, 136.3, 138.5, 146.8; MS (ES mass): *m*/*z* 305.6 (M+1, 10%); HRMS: calcd for $C_{15}H_{14}FN_2O_2S$ (M+H): 305.0760, found 305.0778; Elemental Analysis found: C, 59.43; H, 4.30; N, 9.02; $C_{15}H_{13}FN_2O_2S$ requires C, 59.20; H, 4.31; N, 9.20.

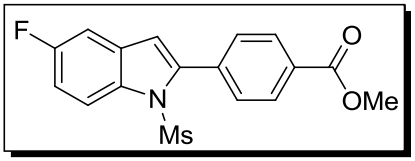
5-Chloro-1-(methylsulfonyl)-2-*p*-tolyl-1*H*-indole [31a] (3k):



Yield: 0.57 g (84%), white solid, mp 206-208 °C. R_f = 0.65 (20% EtOAc-*n*-Hexane); Brown solid, mp 145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.41 (s, 3H), 2.73 (s, 3H), 6.61

(s, 1H), 7.24 (d, *J* = 8.8 Hz, 2H), 7.30 (d, *J* = 1.6 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.55 (d, *J* = 1.6 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.4, 39.8, 111.7, 115.7 (2C), 120.4, 125.0, 128.5, 128.9 (2C), 130.1, 131.5, 134.2, 136.2, 139.3, 143.5; IR (cm^{-1}): 3258, 1434, 1634 ; MS (ES mass): *m*/*z* 320.1 (M+1, 10%); HRMS: calcd for $C_{16}H_{15}ClNO_2S$: 320.0210, found 320.0064.

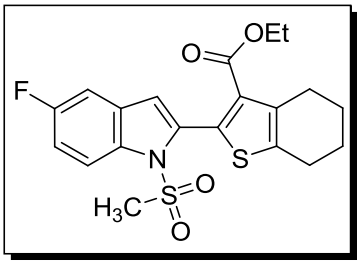
Ethyl 4-(5-fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)benzoate(3l).



Yield 0.54 g (70%); white solid, mp 155℃; *R*f = 0.65 (20% EtOAc/*n*-Hexane); 1H NMR(400 MHz, $CDCl_3$) ppm: 1.37–1.49 (t, *J* = 6.9 Hz, 3H), 2.72 (s, 3H), 4.41–4.44 (q, *J* = 6.9 Hz, 2H), 6.76 (s, 1H), 7.13 (t, *J* = 9.7 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.63–7.64 (d, *J* = 7.9 Hz, 2H), 8.16–8.04 (m, 3H); 13C NMR (100 MHz, $CDCl_3$) ppm: 14.3, 39.3, 61.1, 107.0, 113.2 (2C), 113.7, 117.2, 129.4 (2C), 130.9, 131.2, 134.5, 135.8, 142.7, 159.2, 161.6, 166.1; IR (cm-1): 3016, 1719, 1609, 1481; MS (ES mass): *m*/*z* 362.1 (M + 1)+, (100%); HR-MS: calcd for C18H17FNO4S: 362.1019, found 362.1012.

Preparation of 2-heteroaryl indoles (5)

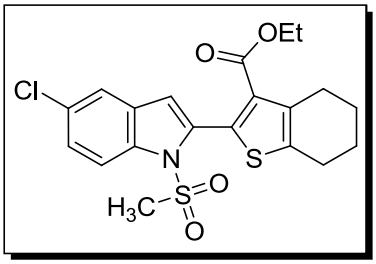
Ethyl-2-(5-fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (5a).



A mixture of ethyl 2-iodo-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate (**4a**, 0.2g, 0.593 mmol), 10%Pd/C (0.003g, 0.023 mmol), PPh_3 (0.01 g, 0.041 mmol), CuI (0.008 g, 0.041 mmol) and triethylamine (0.09g, 0.13 mL, 0.890 mmol) was stirred in MeOH (3 mL) at room temperature for 15 min. Then trimethylsilylacetylene (0.058 g, 0.084 mL, 0.593 mmol) was added and refluxed for 1h. After starting material was consumed

K_2CO_3 (0.082 g, 0.593 mmol) dissolved in 2:1 MeOH-H_2O (3 mL) was added and the mixture was refluxed for another 1h. Then *N*-(4-fluoro-2-iodophenyl)methane sulfonamide (**2b**, 0.187 g, 0.593 mmol), was added and the mixture was allowed to reflux for 2 h. Upon completion of the reaction, the mixture was diluted with saturated aqueous NH_4Cl and extracted with ethyl acetate (3 x 25 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography using *n*-Hexane/EtOAc (7:3) to give the desired product as white solid; Yield: 0.16 g (64%), R_f = 0.4 (20% EtOAc-*n*-Hexane); mp: 220-228 °C; IR (KBr, cm^{-1}) 2930, 1718, 1274; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 1.09 (t, *J* = 7.3 Hz, 3H), 1.87-1.89 (m, 4H), 2.78-2.79 (m, 2H), 2.88-2.89 (m, 2H), 3.09 (s, 3H), 4.12-4.17 (m, 2H), 6.63 (s, 1H), 7.36 (d, *J* = 2.4 Hz, 1H), 7.61 (s, 1H), 8.02 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 14.1, 22.4, 22.8, 25.2, 26.3, 29.6, 40.9, 60.5, 113.3, 116.5, 126.7, 129.7, 133.2, 134.6, 135.7, 136.5, 137.0, 138.2, 148.7, 168.5; MS (ES mass): *m*/*z* 420.8 (M-1, 100%); HRMS: calcd for $C_{20}H_{20}NO_4S_2F$: 421.0818, found 421.0829.

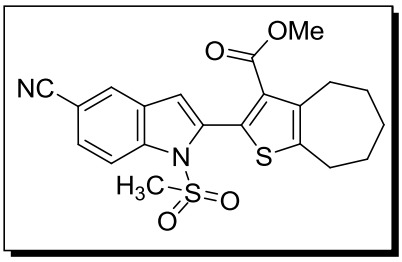
Ethyl-2-(5-chloro-1-(methylsulfonyl)-1*H*-indol-2-yl)-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate (5b).



A mixture of ethyl 2-iodo-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate (**4a**, 0.2 g, 0.593 mmol), 10%Pd/C (0.003g, 0.023 mmol), PPh_3, (0.01 g, 0.041 mmol), CuI (0.008g, 0.041 mmol) and triethylamine (0.09g, 0.13 mL, 0.890 mmol) was stirred in MeOH (3 mL) at room temperature for 15 min. Then (trimethylsilyl)acetylene (0.058 g, 0.084 mL, 0.593 mmol) was added and the mixture was refluxed for 1h. After starting material was consumed K_2CO_3 (0.082 g, 0.593 mmol) dissolved in 2:1 MeOH-H_2O (3 mL) was added and the mixture was refluxed for another 1h. Then *N*-(4-chloro-2-iodophenyl)methanesulfonamide (**2c**, 0.203 g, 0.593 mmol), was added and the mixture

was heated to reflux for 2 h. Upon completion of the reaction, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with ethyl acetate (3 x 25 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography using *n*-Hexane/EtOAc (7:3) to give the desired product as off white solid; Yield: 0.16 g (62%), mp > 200 °C; R_f = 0.3 (20% EtOAc-*n*-Hexane); IR (KBr, cm^{-1}): 2926, 1714, 1333; ^1H NMR (400 MHz, $CDCl_3$) ppm: 1.07 (t, *J* = 7.3 Hz, 3H), 1.84-1.85 (m, 4H), 2.77-2.79 (m, 2H), 2.86-2.87 (m, 2H), 3.07 (s, 3H), 4.10-4.16 (m, 2H), 6.60 (s, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 7.56 (s, 1H), 7.97 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 13.9, 22.4, 22.8, 25.2, 26.2, 29.7, 40.6, 60.4, 112.2, 115.5, 120.6, 125.3, 129.6, 130.3, 132.4, 133.9, 135.1, 136.1, 138.7, 163.4; MS (ES mass): *m*/*z* 438.8 (M+1, 100%); HRMS: calcd for $C_{20}H_{21}NO_4S_2Cl$ (M+H): 438.0601, found 438.0596.

Methyl 2-(5-cyano-1*H*-indol-2-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (5c).

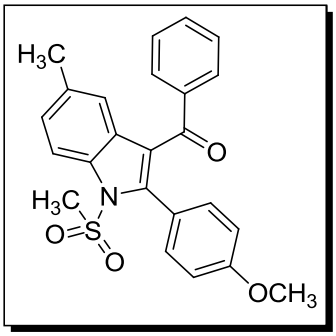


A mixture of ethyl 2-iodo-5,6,7,8-tetrahydr-4*H*-cyclohepta[*b*] thiophene-3-carboxylate (**4b**, 0.1 g, 0.2857 mmol), 10%Pd/C (0.002g, 0.011 mmol), PPh_3 (0.005 g, 0.019 mmol), CuI (0.004 g, 0.019 mmol), triethylamine (0.044 g, 0.07 mL, 0.428 mmol), were stirred in MeOH (3 mL) at room temperature for 15 min. Then trimethylsilylacetylene (0.027 g, 0.04 mL, 0.2857 mmol) was added and the mixture was heated to reflux for 1.0 h. After starting material was consumed K_2CO_3 (0.02 g, 0.2857 mmol) dissolved in 2:1 methanol-water (3 mL) was added and the mixture was stirred at refluxing temperature for another 1.0 h. Then *N*-(4-cyano-2-iodophenyl) methanesulfonamide (**2d,** 0.092 g, 0.2857 mmol), was added and stirring continued for 2 h at refluxing temperature. Upon completion of the reaction, the mixture was diluted with saturated aqueous NH_4Cl 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. The residue was purified by silica gel column chromatography using 7:3 *n*-hexane: EtOAc to give the desired product as off white solid; Yield: 0.06 g (60%), mp 150-152 °C; R_f = 0.3 (20% EtOAc-*n*-Hexane); IR (KBr, cm^{-1}): 3316, 2921, 2847, 1694, 1439; ^1H NMR (400 MHz, $CDCl_3$) ppm: 1.68-1.66 (m, 4H), 1.89-1.88 (m, 2H), 2.82-2.80 (m, 4H), 3.9 (s, 3H), 6.78 (s, 1H), 7.44 (d, *J* =3.5 Hz, 2H), 7.91 (d, *J* =3.5 Hz, 1H), 10.6 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 27.1, 27.6, 29.2, 29.5, 32.2, 52.6, 102.9, 103.1, 112.2, 120.8, 125.2, 125.8, 127.8, 129.1, 133.4, 133.6, 137.7, 141.3, 141.6, 167.7; MS (ES mass): *m*/*z* 348.9 (M-1, 100%); HRMS: calcd for $C_{20}H_{18}N_2O_2S$: 350.1089, found 350.1054.

Synthesis of 2-aryl-3-aroylindole

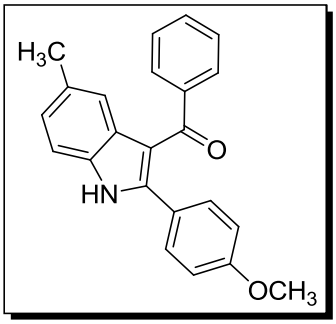
Preparation of (2-(4-methoxyphenyl)-5-methyl-1-(methylsulfonyl)-1*H*-indol-3-yl) (phenyl) methanone (6):



A mixture of TFAA (270 mg, 12.83 mmol) and benzoic acid (360 mg, 3.2 mmol) was stirred for 20 min until all the solids were dissolved. After stirred for additional 20 min, the indole **3b** (1.10 g, 3.52 mmol) was added in one portion. To this mixture was added 85% H_3PO_4 (58 mg, 0.59 mmol,) drop wise for a duration of 20 min. The mixture was then stirred for 4 h (monitored by TLC) and excess of TFA/TFAA was distilled out at atmospheric pressure. The remaining liquid was partitioned between $CHCl_3$ (40 mL) and H_2O (20 mL). The organic layer was separated and washed with 5% NaOH (10 mL) and then brine (10 mL). The mixture was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography using EtOAc-hexane to give the desired product as pale yellow solid; Yield: 1.03 g (70%), mp 90-92 °C; R_f = 0.69 (20% EtOAc-*n*-Hexane); IR (KBr, cm^{-1})**:** 2923, 1727, 1377,

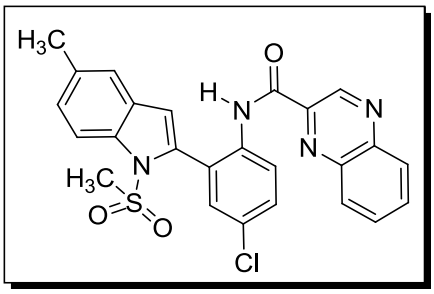
1253, 1177; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.44 (s, 3H), 2.78 (s, 3H), 3.71 (s, 3H), 6.68 (d, *J* = 8.8 Hz, 2H), 7.19-7.37 (m, 6H), 7.52 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.3, 40.4, 55.2, 112.9, 115.3 (2C), 120.8, 121.8, 122.6, 127.3 (2C), 128.0 (2C), 129.4 (2C), 130.5, 132.6, 133.1, 134.9, 135.1, 137.5, 142.4, 160.5, 193.4; MS (ES mass): *m*/*z* 420.2 (M+1, 100%); HRMS: calcd for $C_{24}H_{22}NO_4S$ (M+H): 420.1270, found 420.1291.

Preparation of (2-(4-methoxyphenyl)-5-methyl-1*H*-indol-3-yl) (phenyl) methanone (7):



A mixture of compound **6** (0.993 g, 2.37 mmol) and K_2CO_3 (166 mg, 1.2 eq) in MeOH (5 mL) was refluxed for 3 h. After completion, the reaction mixture was filtered and the residue was washed with MeOH (5 mL). The methanol filtrates were collected, combined and concentrated under reduced pressure. The residue was purified by column chromatography using EtOAc/*n*-Hexane to give the desired product as off white solid; Yield: 0.61 g (75%), mp 160-162 °C; R_f = 0.5 (20% EtOAc-*n*-Hexane); IR (KBr, cm^{-1}) 2917, 1729, 1248, 1174; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.44 (s, 3H), 3.74 (s, 3H), 6.71 (d, *J* = 8.4 Hz, 2H), 7.04-7.20 (m, 3H), 7.22-7.27 (m, 4H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.77 (s, 1H), 8.39 (bs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 21.3, 55.2, 112.9, 114.2 (2C), 120.8, 121.8, 122.6, 127.1 (2C), 127.4 (2C), 128.9 (2C), 130.5, 132.6, 133.1, 134.9, 135.1, 137.5, 142.4, 160.5, 193.4; MS (ES mass): *m*/*z* 342.1 (M+1, 100%); HRMS: calcd for $C_{23}H_{20}NO_2$ (M+H) 342.1494 found 342.1479.

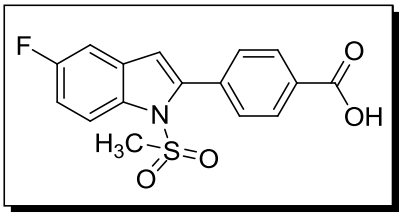
Preparation of *N*-(4-chloro-2-(5-methyl-1-(methylsulfonyl)-1*H*-indol-2-yl)phenyl) quinoxaline-2-carboxamide (8):



To a mixture of 2-quinoxaline carboxylic acid (11 mg, 0.06 mmol) and compound **3e** (20 mg, 0.06 mmol) in CH_2Cl_2 (5 mL) was added EDCI.HCl (13.5 mg, 0.071 mmol), HOBT (10 mg, 0.071 mmol) and DIPEA (16 mg, 0.119 mmol) under a nitrogen atmosphere. The mixture was stirred at room temperature for 5 h. After completion of reaction (monitored by TLC), the mixture was poured in cold water (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography using EtOAc/*n*-Hexane to give the desired product as a gummy mass; Yield: 0.016 g (55%), R_f = 0.22 (20% EtOAc/*n*-Hexane); IR (KBr, cm^{-1}): 3327, 2924, 1690, 1677, 1367, 1173; ^{1}H NMR (400 MHz, $CDCl_3$) ppm: 2.48 (s, 3H), 2.83 (s, 3H), 6.69 (s, 1H), 7.25 (s, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.31 (bs,1H), 7.38-7.41 (m, 2H), 7.43-7.45 (m, 2H), 7.98 (d, *J* = 8.8 Hz, 2H), 8.11 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm: 22.6, 40.3, 113.9, 114.4, 114.4, 115.4, 117.4, 118.2, 121.7, 122.1, 122.7, 123.4, 125.4, 128.5, 129.0, 131.9, 132.0, 132.1, 132.4, 134.1, 137.8, 138.3, 138.5, 140.9, 161.6; MS (ES mass): *m*/*z* 491.8 (M+1, 100%); HRMS: calcd for $C_{25}H_{20}N_4ClO_3S$ (M+1): 491.0945 found 491.0952.

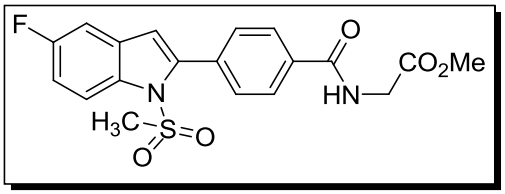
Synthesis of α-amino acid analogue

4-(5-fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)benzoic acid (9):



To the solution of **3l** 0.16 g (0.44 mmol) in THF and water 5 ml (3:1), NaOH 0.04 g (0.968 mmol) was added and refluxed for 3h. The progress of the reaction was checked by TLC. After completion of the reaction the reaction mixture was neutralized by 1N HCl, extracted with ethyl acetate, washed with water and brine solution, dried over anhydrous sodium sulfate. The reaction mixture was concentrated under reduced pressure. Crude was purified by washing with hexane to get the product white solid. mp: 266°C. ^{1}H NMR (400 MHz, *acetone*D_6) ppm: 2.91 (s, 3H), 6.98-6.85 (m, 1H), 7.25-7.16 (m, 1H), 7.49-7.36 (m, 1H), 7.77-7.62 (m, 2H), 8.20-8.01 (m, 3H), 10.95 (s, 1H). ^{13}C NMR (100 MHz, *acetone*D_6) ppm: ppm 40.9, 107.0, 107.3, 113.1, 113.3, 117.1(2C), 125.2, 129.0, 130.2, 131.2(2C), 134.2, 142.8, 158.6, 161.0. IR (cm^{-1}): 3430, 3014, 1691, 1608. MS (ES mass): *m*/*z* 331.9 $(M-1)^+$, 100%); HRMS: calcd for $C_{16}H_{13}FNO_4S$ is 334.1100 found 334.1102.

Methyl 2-(4-(5-fluoro-1-(methylsulfonyl)-1*H*-indol-2-yl)benzamido)acetate(10):



To a mixture of **9** (0.1 g, 0.3 mmol) and compound methyl glycine ester (0.038 g, 0.3 mmol) in THF (5 mL) was added EDCI.HCl (0.07 g, 0.36 mmol), HOBT (0.05 g, 0.36 mmol) and DIPEA (0.78 g, 0.6 mmol) under a nitrogen atmosphere. The mixture was stirred at room temperature for 3 h. The progress of the reaction was checked by TLC. After completion of reaction the mixture was poured in cold water (10 ml) and extracted with EtOAc (3 x 20 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography using EtOAc-*n*-Hexane (6:4) to give the desired product as white solid. mp: 230-232 °C 1H NMR (400 MHz, *acetone*D_6) ppm: 3.02 (s, 3H), 3.71 (s, 3H), 4.17 (t, *J* = 5.6 Hz, 2H), 6.92 (s, 1H), 7.23 (dd, *J* = 13.0, 5.3 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 2H), 7.92 -8.03 (m, 3H), 8.07 (dd, *J* = 9.0, 4.3 Hz, 1H), 8.25 (s, 1H). ^{13}C NMR (100 MHz, *acetone*D_6) ppm: 39.5, 41.1, 51.3, 106.5, 106.7, 112.9, 113.0, 116.8, 116.9, 126.5 (2C), 130.2 (2C), 134.3, 135.1, 142.8, 161.2, 166.5, 170.2. . IR (cm^{-1}): 3072, 2951, 1739, 1341. MS (ES mass): *m*/*z* 404.7 $(M+1)^+$,(100%); HRMS: calcd for $C_{19}H_{18}FN_2O_5S$ is 405.1221 found 405.1209.

Reference:

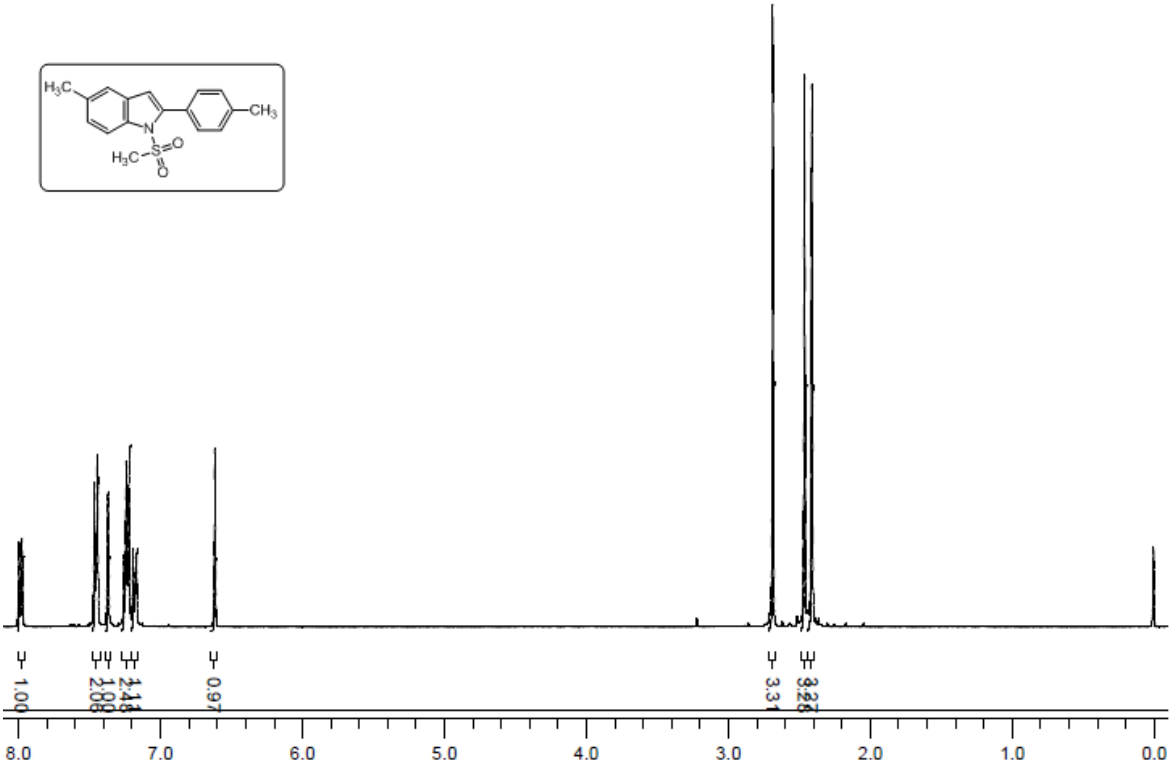
1. (a) Somei, M.; Yamada, F. *Nat. Prod. Rep.* **2005**, *22*, 73–103 and reference cited for the previous review. (b) Gribble, G. W. J. *Chem. Soc., Perkin Trans. 1* **2000**, 1045.
2. Young, W. B.; Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spencer, J.; Elrod, K.; Cregar, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2253.
3. Mackman, R. L.; Hui, H. C.; Breitenbucher, J. G.; Katz, B. A.; Luong, C.; Martelli, A.; McGee, D.; Radika, K.; Sendzik, M.; Spencer, J. R.; Sprengeler, P. A.; Tario, J.; Verner, E.; Wang, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2019.
4. Chai, W.; Breitenbucher, J. G.; Kwok, A.; Li, X.; Wong, V.; Carruthers, N. I.; Lovenberg, T. W.; Mazur, C.; Wilson, S. J.; Axe, F. U.; Jones, T. K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1767.
5. Karamyan, A. J.; Hamann, M. T. *Chem. Rev.* **2010**, *110*, 4489.
6. Ma, J., Li, Y., Ye, Q., Li, J. *J. Agric. Food Chem.* **2000**, *48*, 5220.
7. (a) Islam, M. S.; Brennan, C.; Wang, Q. W.; Hossain, M. M. *J. Org. Chem.* **2006**, *71*, 4675. (b) Taber, D. F.; Tian, W. W. *J. Am. Chem. Soc.* **2006**, *128*, 1058.
8. (a) Hiroya, K.; Matsumoto, S.; Sakamoto, T. *Org. Lett.* **2004**, *6*, 2953. (b) Kamijo, S.; Sasaki, Y.; Yamamoto, Y. *Tetrahedron Lett.* **2004**, *45*, 35. (c) Koradin, C.; Dhohle, W.; Rodriguez, A. L.; Schmid, B.; Knochel, P. *Tetrahedron*. **2003**, *59*, 1571.
9. (a) Joucla, L.; Djakovitch, L. *Adv. Synth. Catal.* **2009**, *351*, 673. (b) Humphrey, G. R.; Kuethe, J. T. *Chem. Rev.* **2006**, *106*, 2875.
10. For palladium-catalyzed regioselective indolization methods, see: (a) Larock, R. C.; Yum, E. K. *J. Am. Chem. Soc.* **1991**, *113*, 6689. (b) Larock, R. C.; Yum, E. K.; Refvik, M. D. *J. Org. Chem.* **1998**, *63*, 7652.
11. Hiroya, K.; Itoh, S.; Sakamoto, T. *J. Org. Chem.* **2004**, *69*, 1126.
12. Hiroya, K.; Itoh, S.; Sakamoto, T. *Tetrahedron.*, **2005,** *61*, 10958.
13. Ackermann, L. *Org. Lett.*, **2005**, *7*, 3, 439.
14. Yin, Y.; Ma, W.; Chai, Z.; Zhao, G. *J. Org. Chem.* **2007**, *72*, 5731.

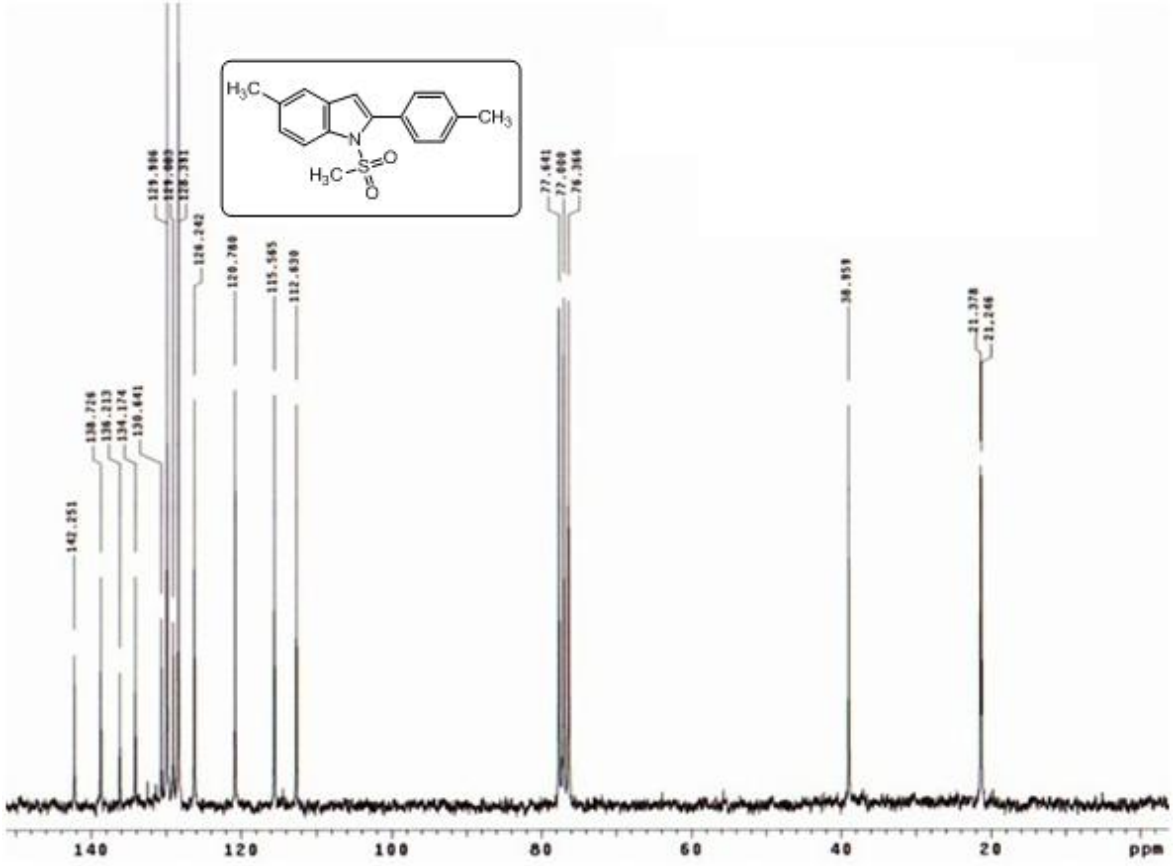
15. Yasuhara, A.; Kanamori, Y.; Kaneko, M.; Numata, A.; Kondo, A.; Sakamoto, T. *J. Chem. Soc., Perkin Trans. 1*, **1999**, 529.
16. Lounasmaa, M.; Tolvanen, A. *Nat. Prod. Rep.* **2000**, *17*, 175.
17. Young, W. B.; Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spencer, J.; Elrod, K.; Cregar, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2253.
18. Mackman, R. L.; Hui, H. C.; Breitenbucher, J. G.; Katz, B. A.; Luong, C.; Martelli, A.; McGee, D.; Radika, K.; Sendzik, M.; Spencer, J. R.; Sprengeler, P. A.; Tario, J.; Verner, E.; Wang, J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2019.
19. Chai, W.; Breitenbucher, J. G.; Kwok, A.; Li, X.; Wong, V.; Carruthers, N. I.; Lovenberg, T. W.; Mazur, C.; Wilson, S. J.; Axe, F. U.; Jones, T. K.; *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1767.
20. Carles Canto, C.; Auwerx, J. *Pharmacol. Rev.* **2012**, *64*, 166 –187.
21. (a) Bordone, L.; Guarente, L. *Nature Rev. Mol. Cell Biol.* **2005**, *6*, 298. (b) Cohen, H. Y. *Science.* **2004**, *305*, 390. (c) Imai, S.; Armstrong, C. M.; Kaeberlein, M.; Guarente, L.; *Nature.* **2000**, *403*, 795.
22. Napper, A.D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J.F.; Barker, J.; Yau, W.T.; Amouzegh, P.; Flegg, A.; Estelle Hamelin, E.; Thomas, R. J.; Kates, M.; Jones, S.; Navia, M. A.; Saunders, J.O.; DiStefano, P.S.; Curtis, R. *J. Med. Chem.*, **2005**, *48* (25), 8045.
23. Blum, C. A.; Ellis, J. L.; Loh, C.; Yee, P.; Perni, R. B.; Stein, R. L. *J. Med. Chem.* **2011**, *54*, 417.
24. Milne, J.C.; Lambert, P.D.; Schenk, S.; Carney, D.P.; Smith J.J.; Gagne, D.J.; Jin, L.; Boss, O.; Perni, R. B.; Vu, C. B.; Bemis, J. E.; Xie, R.; Disch, J. S.; Ng, P.Y.; Nunes, J. J.; Lynch, A.V.; Yang, H.; Galonek, H.; Israelian, K.; Choy, W.; Iffland, A.; Lavu, S.; Medvedik, O.; Sinclair, D. A.; Olefsky, J. M.; Jirousek, M. R.; Elliott, P. J.; Westphal1, C. H. *Nature*. **2007**, *450*, 712.
25. (a) S. Cacchi, S.; Fabrizi, G. *Chem. Rev.* **2005,** *105*, 2873. (b) Chen, Y.; Markina N. A.; Larock, R. C. *Tetrahedron* 2009, **65**, 8908.
26. Castro, C. E.; Gaughn, E. J.; Owsley, D. C. *J. Org. Chem.* **1966**, *31*, 4071.

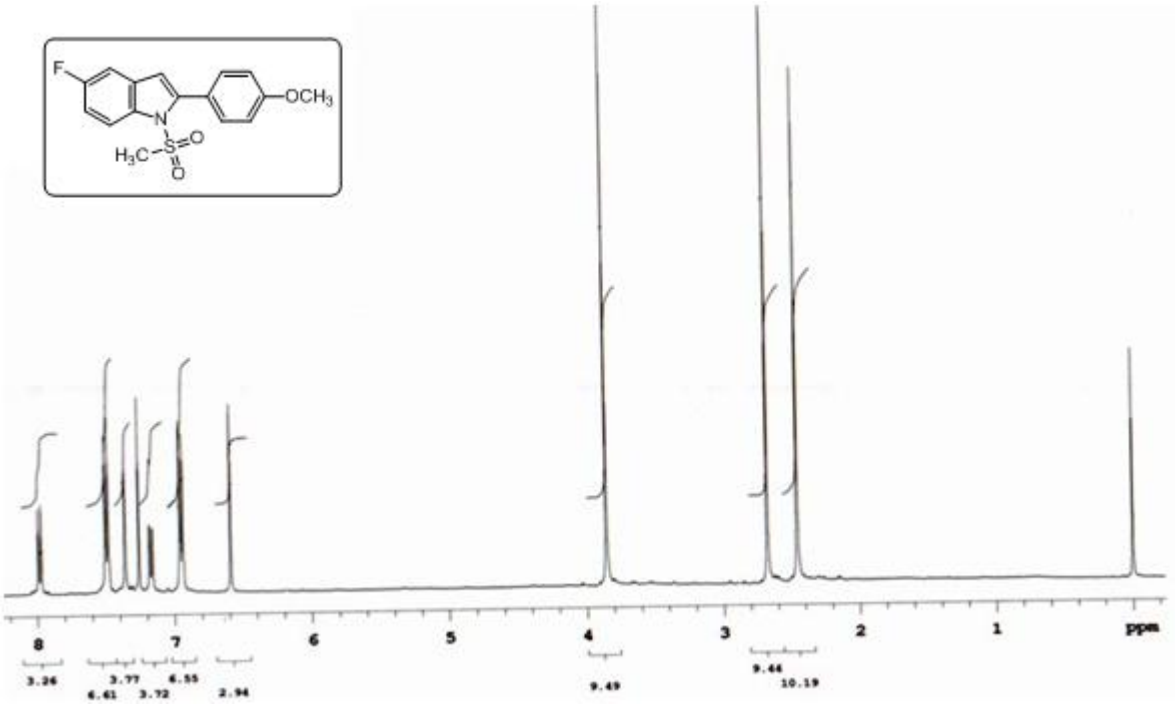
27. (a) Dai, W.M.; Sun, L.P.; Guo, D.S. *Tetrahedron Lett.* **2002**, *43*, 7699; (b) Dai, W.M.; Guo, D.S.; Sun, L.P. *Tetrahedron Lett.* **2001**, *42*, 5275; (c) Rodriguez, A.L.; Koradin, C.; Dohle, W.; Konchel, P. *Angew. Chem., Int. Ed.* **2000**, *39*, 2488.
28. Yasuhara, M. C.; Kanamori, Y.; Kaeko, M.; Numata, A.; Kondo, Y.; Sakamoto, T. *J. Chem. Soc., Perkin Trans. 1* **1999**, 529.
29. Hiroya, K.; Itoh, S.; Ozawa, M.; Kanamori, Y.; Sakamoto, T. *Tetrahedron Lett.* **2002**, *43*, 1277.
30. Arcadi, A.; Bianchi, Marinelli, G. F. *Synthesis* **2004**, *4*, 610.
31. (a) Amjad, M.; Knight, D. W. *Tetrahedron Lett.* **2004**, *45*, 539; (b) Yue, D.; Larock, R.C. *Org. Lett.* 2004, **6**, 1037.
32. (a) Layek, M.; Lakshmi, U.; Kalita, D.; Barange, D. K.; Islam, A.; Mukkanti, K.; Pal, M. *Beilstein J. Org. Chem.* **2009**, *5*, 46. (b) Pal, M.; Subramanian, V.; Batchu, V. R.; Dager, I. *Synlett* **2004,** 1965.
33. (a) Auria, M. D.; *Synth. Commun.* **1992**, *22*, 2393. (b) Mio, M. J.; Kopel, L. C.; Braun, J. B.; Gadzikwa, T. L.; Hull, K. L.; Brisbois, R. G.; Markworth, C. J.; Grieco, P. A. *Org. Lett.* **2002**, *4*, 3199.
34. (a) Nagy, A.; Novak, Z.; Kotschy, A. *J. Organomet. Chem.* **2005**, *690*, 4453. (b) Yi, C.; Hua, R.; Zeng, H.; Huang, Q. *Adv. Synth. Catal.* **2007**, *349*, 1738.
35. (a) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett*. **1975**, *16*, 4467. (b) Batchu, V. R.; Subramanian, V.; Parasuraman, K.; Swamy, N. K.; Kumar, S.; Pal, M. *Tetrahedron* **2005**, *61*, 9869. (c) Bhattacharya, S.; Sengupta, S. *Tetrahedron Lett.* **2004**, *45*, 8733.
36. Pal, M. *Synlett* 2009, 2896.
37. Crystallographic data (excluding structure factors) for **3a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 784742.
38. Burla, M. C.; Caliandro, R.; Camalli, M.; Carrozzini, B.; Cascarano, G. L. L.; Caro De, C.; Polidori, G. G.; Spagna, R. *J. Appl. Cryst*. **2005**, *38*, 381.
39. Betteridge, P. W.; Carruthers, J. R.; Copper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Cryst*. **2003**, *36*, 1487.

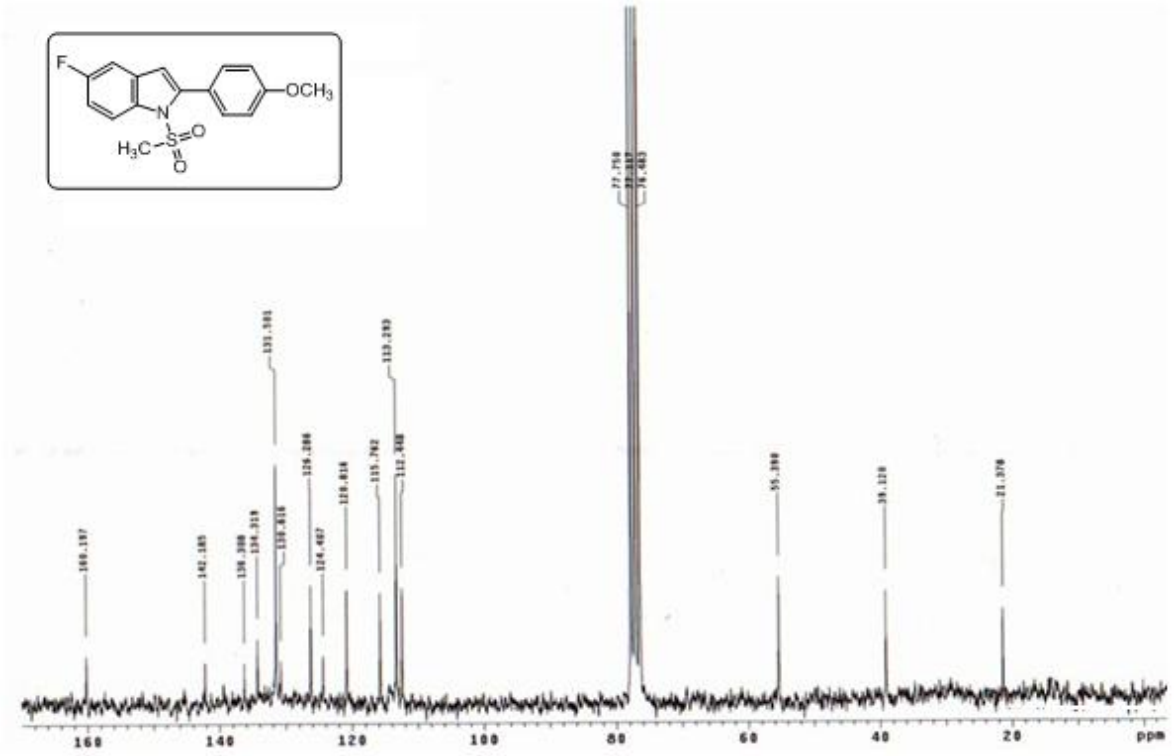
40. (a) Kumar, K. S.; Chamakuri, S.; Vishweshwar, P.; Iqbal, J.; Pal, M. *Tetrahedron Lett.* **2010**, *51*, 3269. (b) Puterová, Z.; Andicsová, A.; Moncol, J.; Rabong, C.; Végh, D. *Tetrahedron Lett.* **2009**, *50*, 4670.
41. (a) Pal, M. *Drug Disc. Today*. **2009**, *14*, 784 (b) Pal, M. *Curr. Med. Chem.* **2009**, *16*, 3858. (c) Havale, S. H.; Pal, M. *Bioorg. Med. Chem.* **2009**, *17*, 1783. (d) Kodimuthali, A.; Jabaris, S. S. L.; Pal, M. *J. Med. Chem.* **2008**, *18*, 5471; (e) Pal, M.; Angaru, S.; Kodimuthali, A.; Dhingra, N. *Curr. Pharm. Des.* **2009**, *15*, 1008.
42. Veeramaneni, V. R.; Pal, M.; Yeleswarapu, K. R. *Tetrahedron.* **2003**, *59*, 3283.
43. Layek, M.; Reddy M. A.; Rao, A. V. D.; Alvala, M.; Arunasree, M. K.; Islam, A.; Mukkanti, K.; Iqbal, J.; Pal, M. *Org. Biomol. Chem.* **2011**, *9*, 1004.
44. Shaday, M.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1.
45. L. L. C. Schrodinger, http://www.Schr¨odinger.com, (accessed: 24. 04. 2007)
46. Michel, F. Sanner. Python: A programming language for software integration and development. *J. Mol,. Graphics Mod.*, **1999**, *17*, 57.

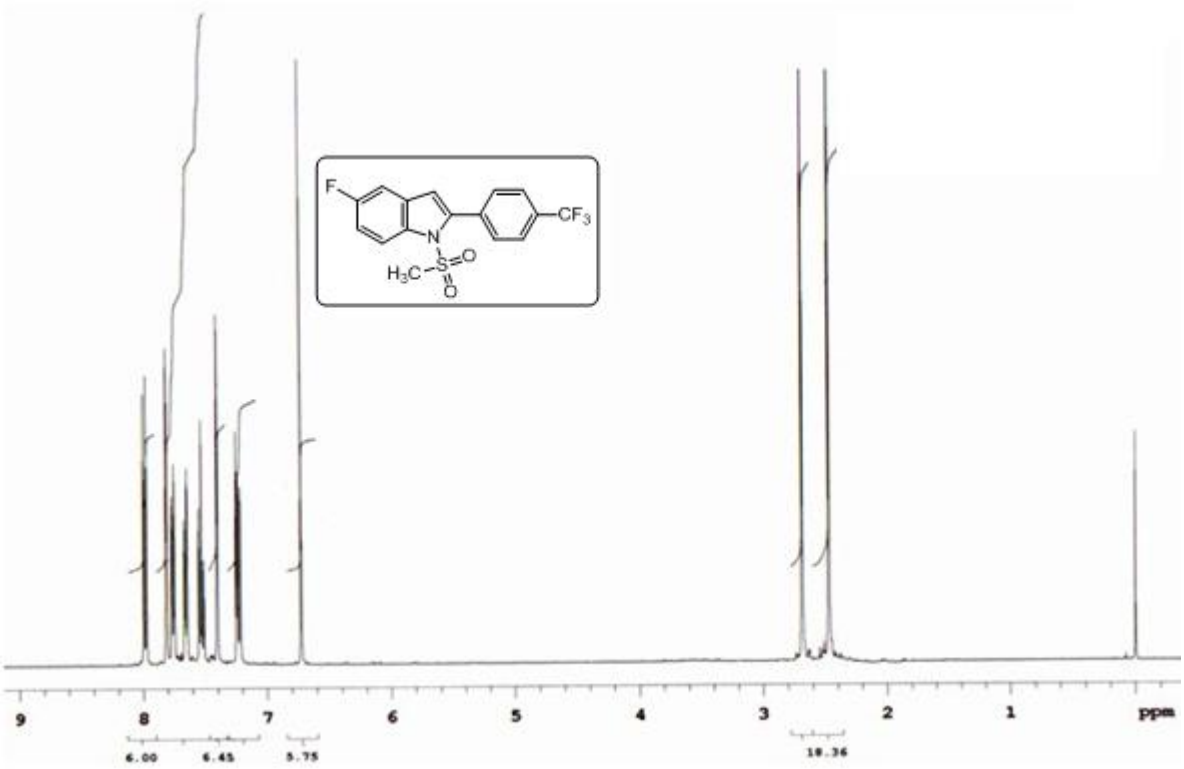
Appendix

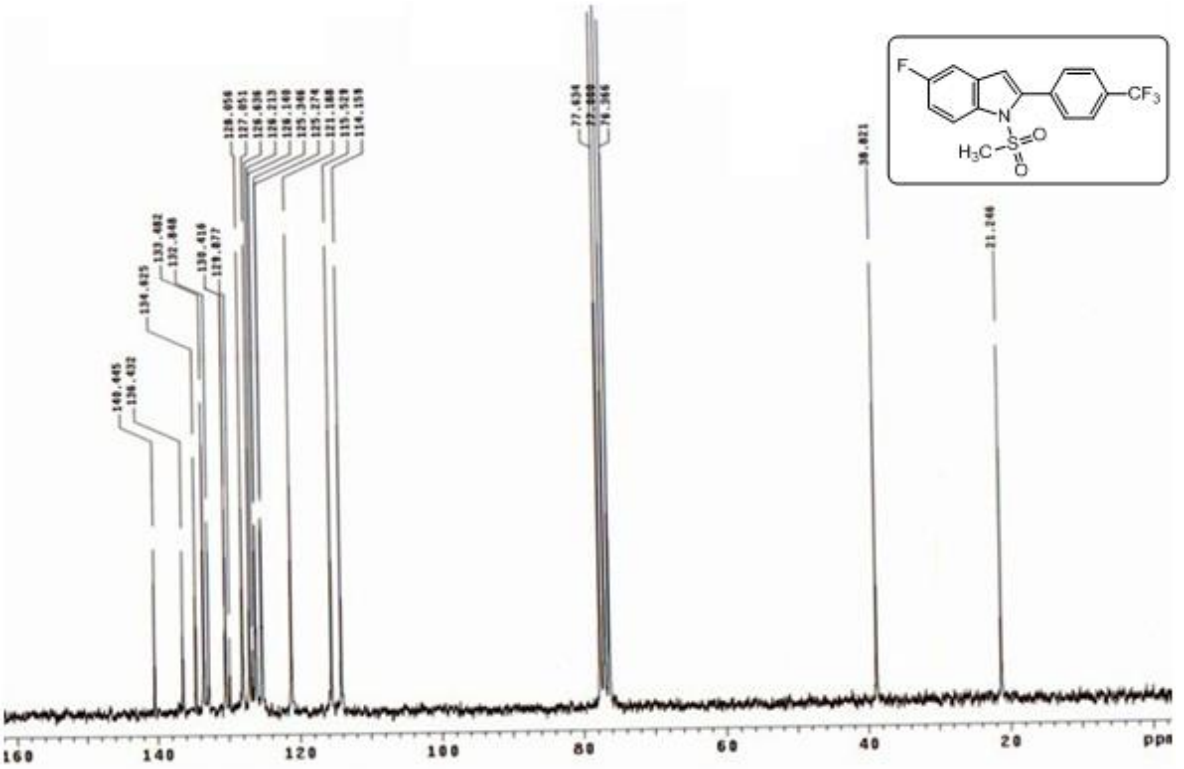

1.00
2.08
1.00
1.11
1.18
0.97
3.31
3.26
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

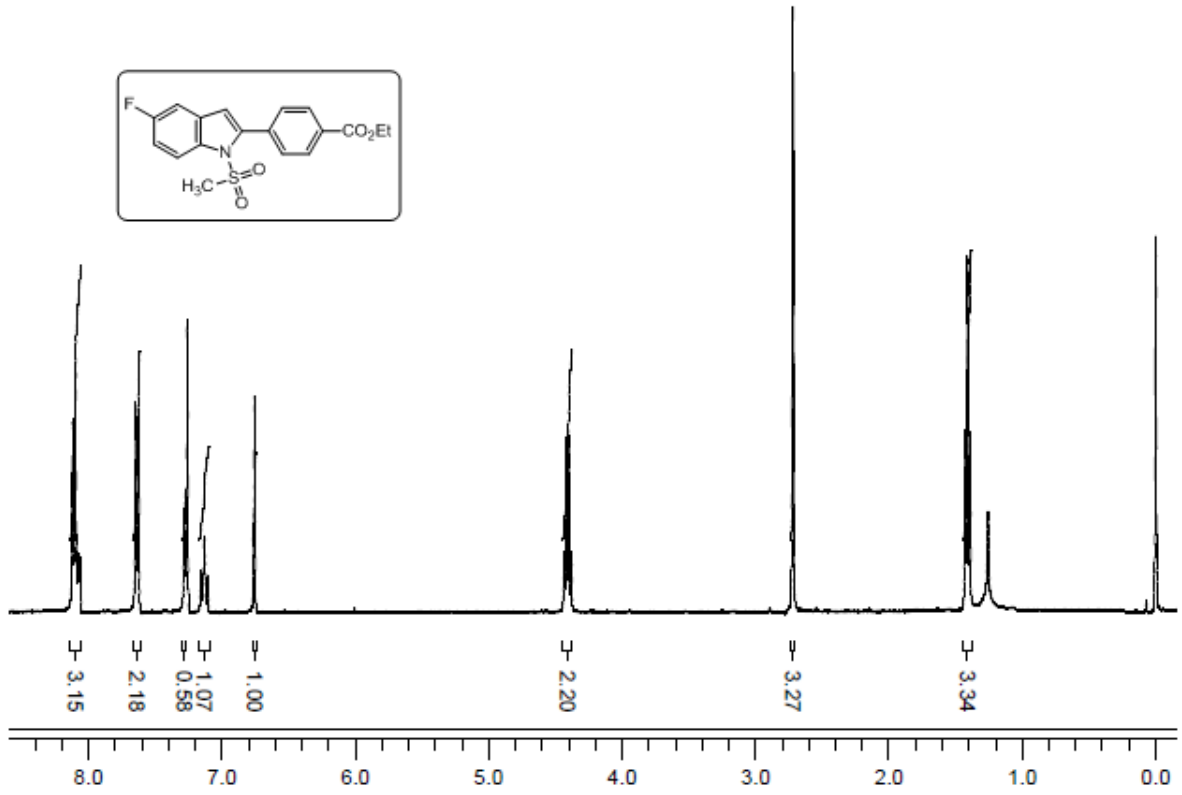

142.251
138.716
136.213
134.174
130.441
129.986
129.903
128.331
126.242
120.780
115.565
112.630
77.441
77.000
76.366
38.959
21.378
21.246
140
120
100
80
60
40
20
ppm

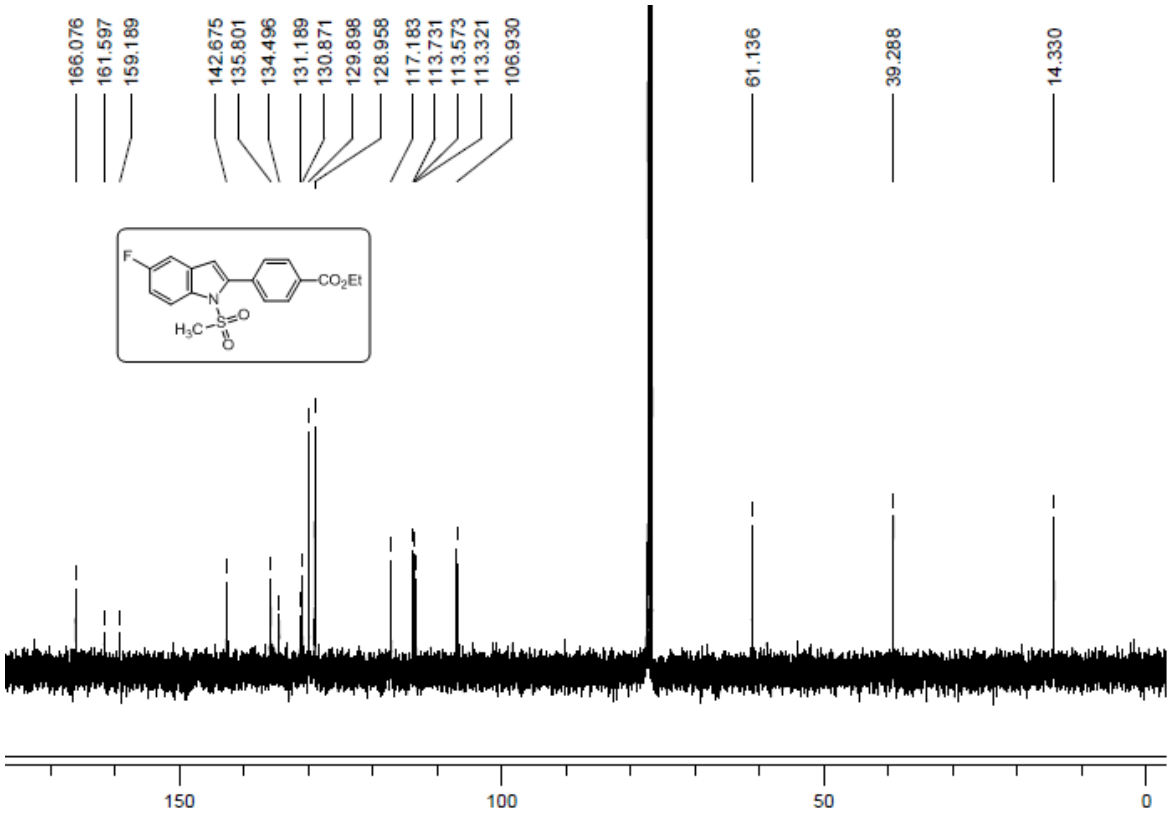

F
OCH3
N
S
O
H3C
O
8
7
6
5
4
3
2
1
ppm
3.26
3.77
6.61
3.72
6.55
2.94
9.49
9.44
10.19

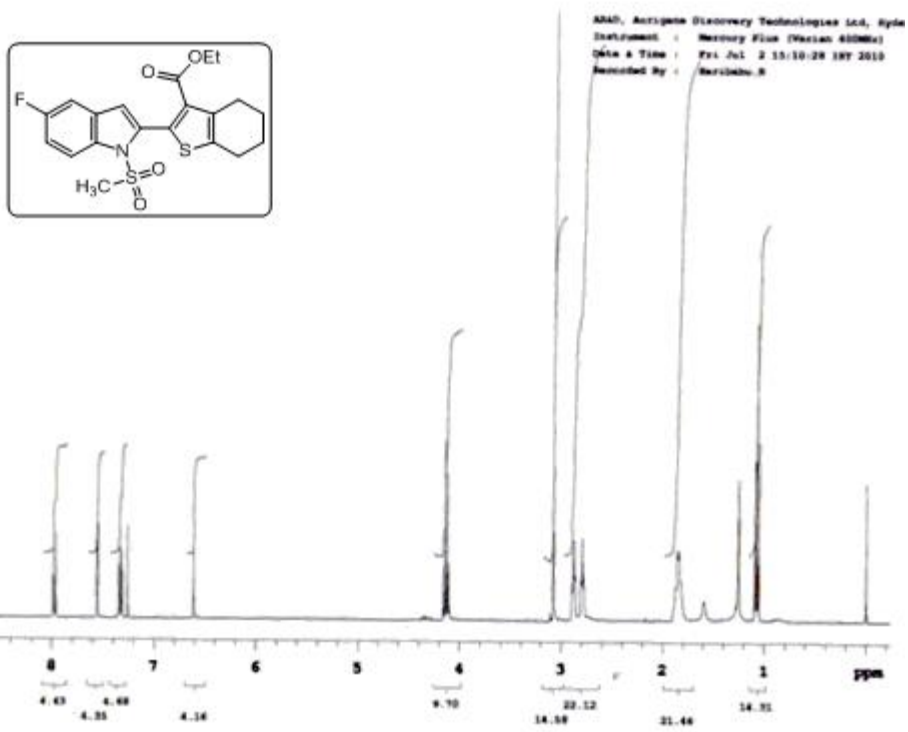

F
OCH3
N
S
O
H3C
O
160
140
120
100
80
60
40
20
ppm

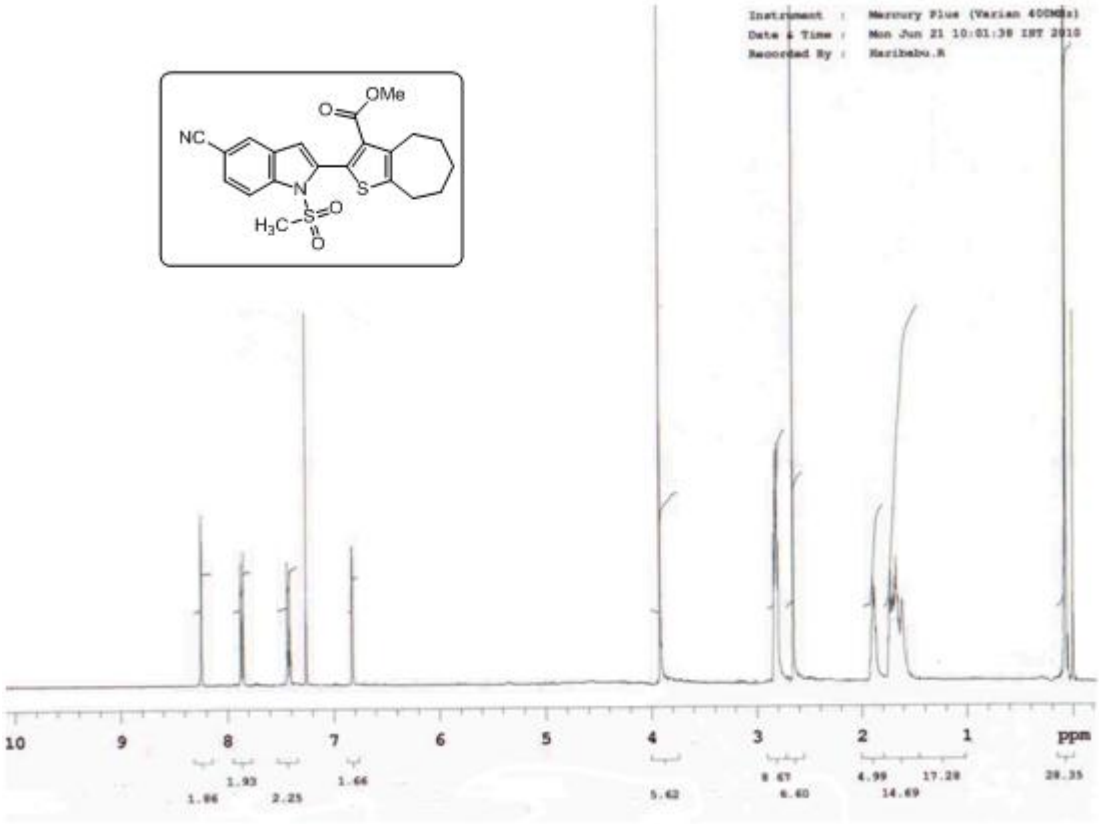

F
CF3
N
H3C
S
O
O
9
8
7
6
5
4
3
2
1
ppm
6.00
6.45
5.75
10.36

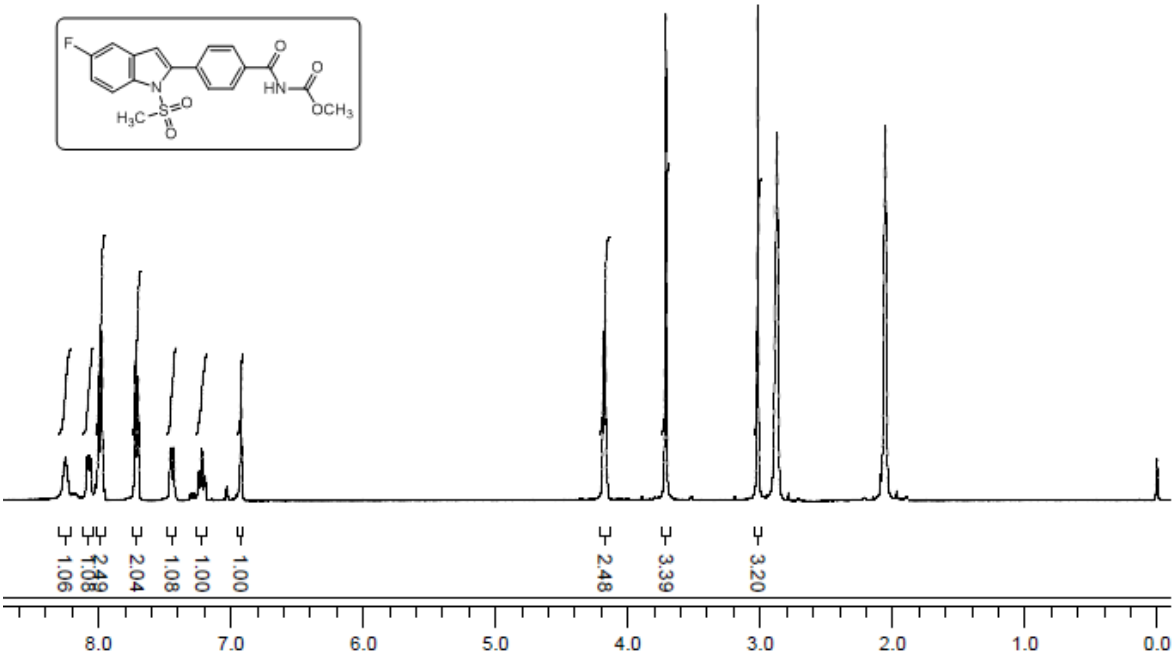

F
CF3
N
H3C
S
O
O
160
140
120
100
80
60
40
20
ppm

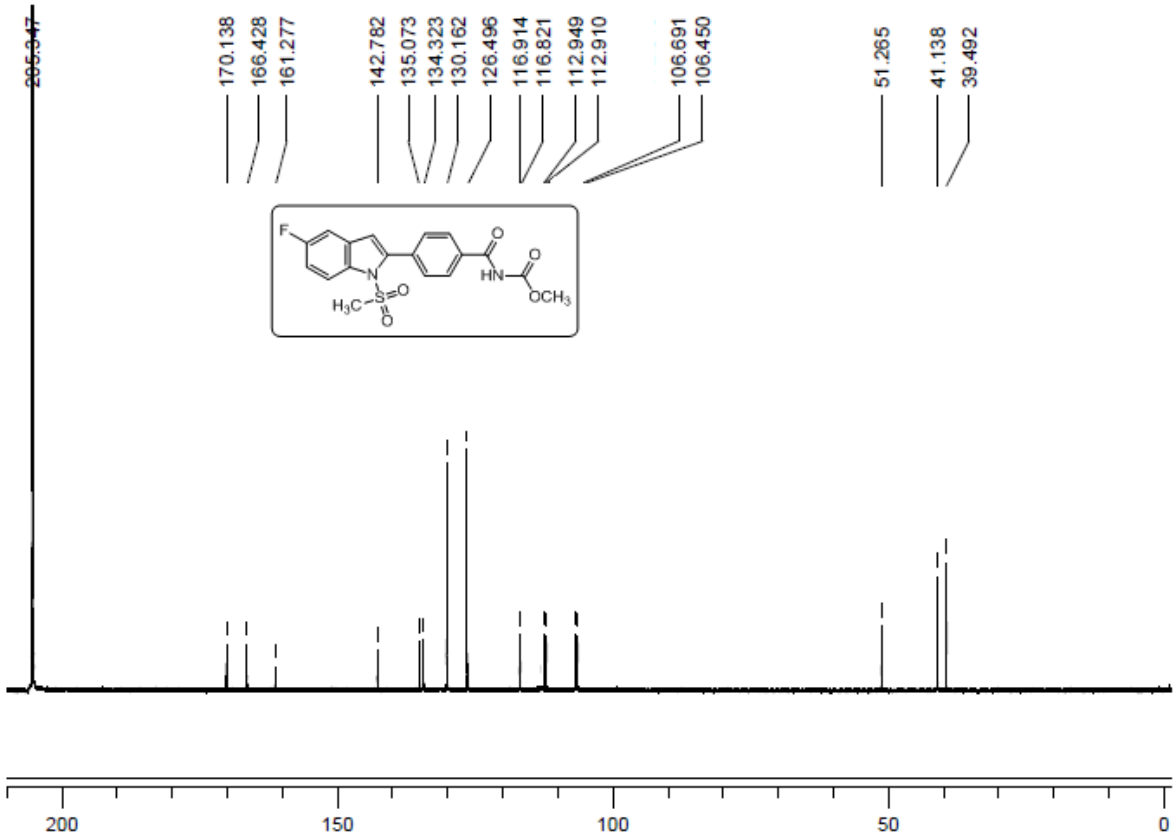

F
CO2Et
N
S
O
O
H3C
3.15
2.18
0.58
1.07
1.00
2.20
3.27
3.34
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


166.076
161.597
159.189
142.675
135.801
134.496
131.189
130.871
129.898
128.958
117.183
113.731
113.573
113.321
106.930
61.136
39.288
14.330
F
CO2Et
N
S
O
O
H3C
150
100
50
0


OEt
O
F
N
S
O
H_3C
S
O
O
ppm


OMe
O
NC
N
S
O
H_3C
S
O
O
ppm

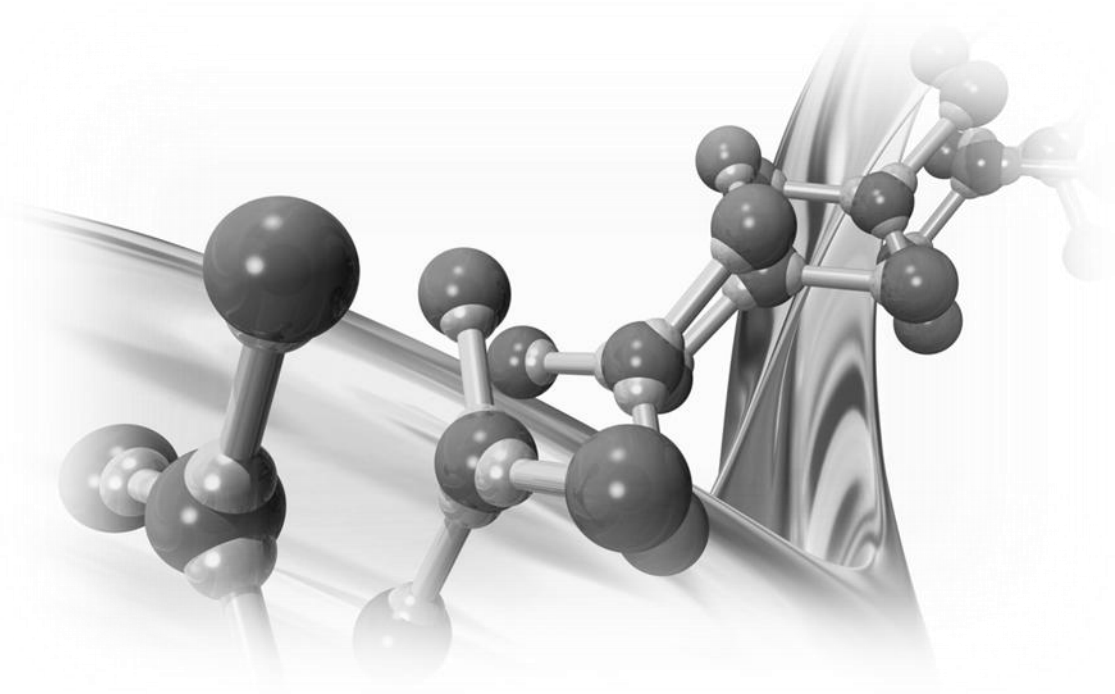

F
N
S
O
O
H3C
HN
O
O
OCH3
1.90
1.88
2.40
2.04
1.08
1.00
1.00
2.48
3.39
3.20
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


295.347
170.138
166.428
161.277
142.782
135.073
134.323
130.162
126.496
116.914
116.821
112.949
112.910
106.691
106.450
51.265
41.138
39.492
F
N
S
O
O
H3C
HN
O
O
OCH3
200
150
100
50
0

CHAPTER 5

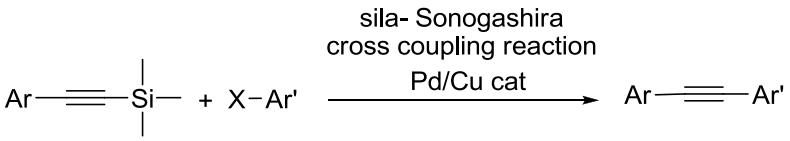
CHAPTER 5B

Pd/C-Cu catalyzed one pot synthesis of 2-substituted indoles



5B.1. Introduction

An ample number of transition-metal-catalyzed cross-coupling reactions from the literature has become cornerstones in the field of organic synthesis.[1] Out of all transition metal, Palladium catalyzed cross coupling reaction i.e., Csp^2-Csp bond formation reaction has attained most importance. In this method, aryl alkynes and conjugated enynes[2] are prepared by the reaction between aryl/alkenyl halides/triflates and terminal alkynes, in the presence or absence of a copper(I) as co-catalyst. In 1975, Heck[3] and Cassar[4] reported palladium catalyzed cross coupling reactions independently. Heck reaction is a palladium-catalyzed C-C coupling between aryl halides or vinyl halides and activated alkenes in the presence of phosphane-palladium complex as a catalyst and triethylamine or piperidine as a base. Cassar's procedure involved the use of a phosphane-palladium catalyst in combination with sodium methoxide as a base and DMF as a solvent. Both methods generally required high temperature (up to 100 °C). In 1975, Heck–Cassar independently reported the palladium catalyzed coupling of aryl halides with terminal alkynes in the presence of a stoichiometric amount of base.[5] In the same year, Sonogashira and Hagihara reported that addition of a catalytic amount of copper(I) iodide greatly accelerated the reaction, thus enabling alkynylation of aryl/heteroaryl moieties at room temperature.[6] Undoubtedly, Sonogashira reaction is one of the most important synthetic strategies for the preparation of symmetrical and unsymmetrical internal alkynes based on the palladium/copper catalyzed cross-coupling reactions of (hetero)aryl halides with terminal alkynes in the presence of a base. However, despite these improvements the use of Sonogashira reactions to prepare di(hetero)aryl alkynes often requires systematic silane protection and deprotection to afford the required terminal alkyne coupling partner. In order to overcome the necessity of protecting group removal and to avoid the use of acetylene, trimethylsilylacetylene (TMSA) and silylated acetylene equivalents such as ethynyltrimethylsilane (ETS) or 1,2-bis(trimethylsilyl)ethyne (BTSE) have been directly used as nucleophilic partners *via* sila-Sonogashira reaction (Scheme 1).[7,8]



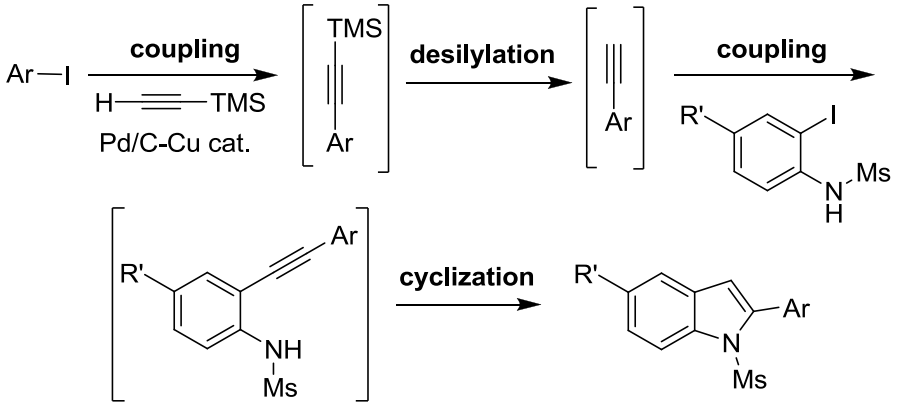
Scheme 1. Synthesis of internal alkynes *via* sila Sonogashira reaction.

A further distinct advantage of the use of TMS-protected alkynes instead of unprotected terminal alkynes resides in the suppression of the formation of diynes and enynes, which, as stated are typical byproducts of classical Sonogashira protocols. Based on these reports, we envisaged that synthesis of indoles can be achieved *via* formation of terminal alkyne by using a particular iodoarene *in situ* in a single pot. The details of this work are presented below.

5B.2. Present work

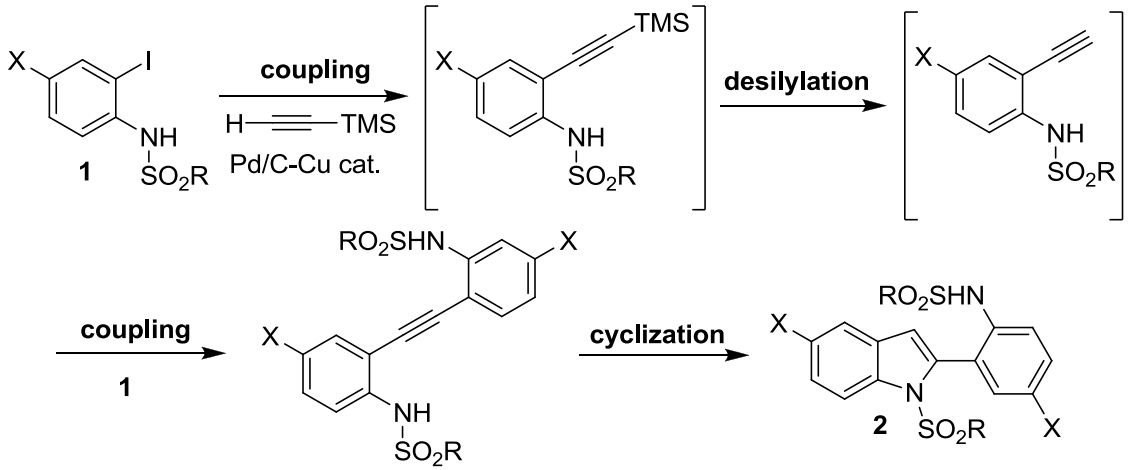
The indole ring is considered as one of the privileged structures in the area of drug discovery. Indole derivatives display a range of valuable pharmacological properties.[9] For example, indole (Scheme 1) containing an *o*-($MeSO_2NH$)C_6H_4 group at C-2 has been reported to be useful for the potential treatment of multiple disorders.[10] While a number of methods are available for the preparation of 2-aryl indoles,[11] a direct and general method for the construction of the key indole moiety of **2** has not been reported yet. The existing method for **2** requires a multi-step process.[10] Recently, because of their environmental and economic advantages one-pot multi component reactions have gained considerable interest which do not require the typical purification and isolation of product in each step. While investigation of our work (Scheme 2, chapter 3A) i.e. a one-pot, four-step synthesis of 2-substituted indoles[12] we have observed that coupling of *o*-iodoanilide with trimethylsilylacetylene (TMSA) directly provides the self coupled indole derivatives unexpectedly without the requirement of any additional catalyst or reagent for the removal of Me_3Si- group or cyclization step, respectively. This resulted in the development of first Pd/C-mediated one-pot synthesis of indoles possessing a *o*-(RSO_2NH)C_6H_4 group at C-2 (**2**, Scheme 3) *via* a Sila-Sonogashira based strategy.[13] The Sila-Sonogashira reaction though found applications in the preparation of internal alkynes its use in heterocyclic synthesis remained unexplored.

Previous work: (Chapter 5A)



Scheme 2. Strategy to synthesize 2-aryl indoles in a single pot

Present work:

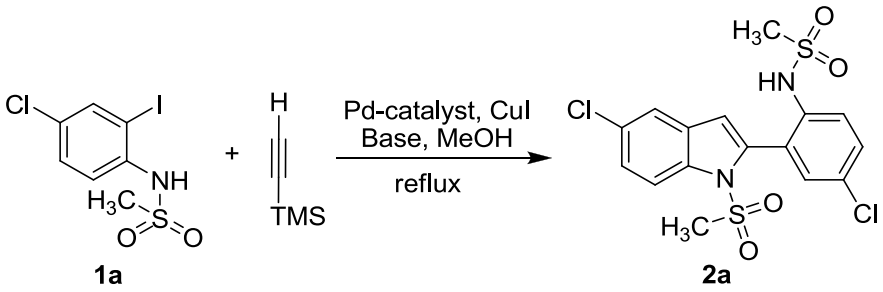


Scheme 3. Strategy to Pd/C catalyzed one-pot synthesis of indole containing a *o*-$(RSO_2)C_6H_4$ group at C-2.

5B.3. Results and Discussion

Based on the earlier report that trimethylsilyl group participates in Pd-mediated C-C bond forming reaction we examined the reaction of TMSA (trimethylsilylacetylene) with *N*-(4-chloro-2-iodophenyl)methanesulfonamide (**1a**) under Pd/C-Cu catalysis. While the formation of a 1,2-arylethyne *via* a Sila-Sonogashira type reaction was expected. However the isolated product was identified as a indole derivative **2a** (Table 1). To this end, we conducted the reaction of **1a** with TMSA under various conditions to establish the optimized condition (Table 1).

Table 1. Effect of reaction conditions on coupling of **1a** with TMSA[a]

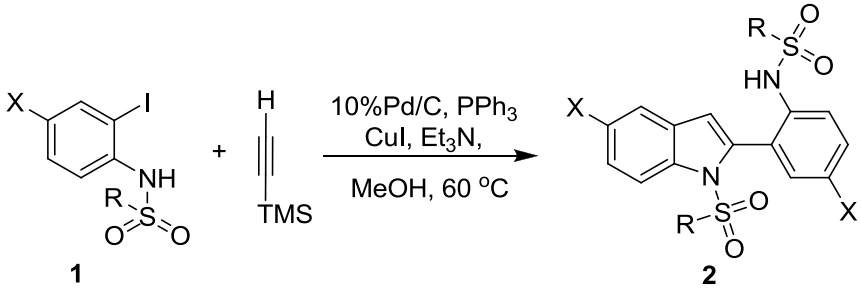


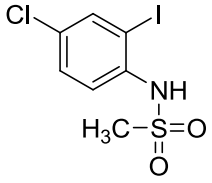
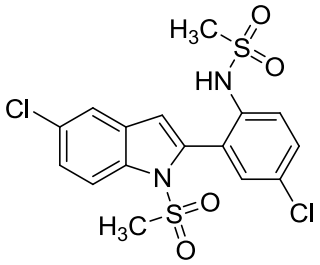
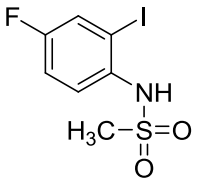
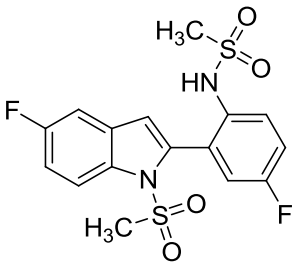
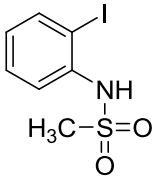
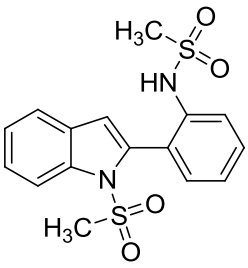
Entry	Pd-catalysts	Base	Time[b] (h)	%Yield[c]
1	10%Pd/C-PPh_3	Et_3N	4	39
2	10%Pd/C-PPh_3	Et_3N	6	62
3	10%Pd/C	Et_3N	6	15
4	PPh_3	Et_3N	6	33
5	10%Pd/C-PPh_3[d]	Et_3N	8	10
6	$Pd(PPh_3)_2Cl_2$[d]	Et_3N	6	18
7	$Pd(PPh_3)_2Cl_2$[d]	Piperidine	6	55

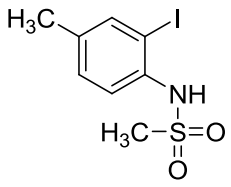
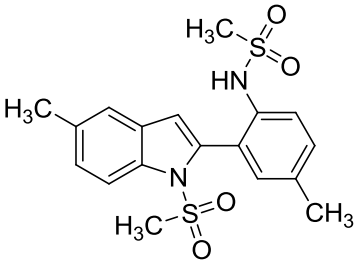
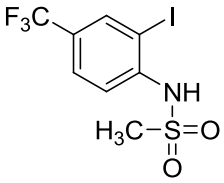
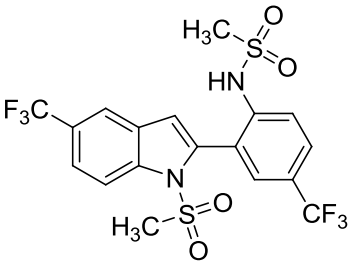
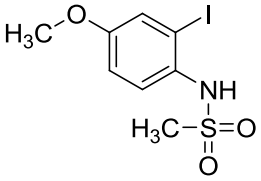
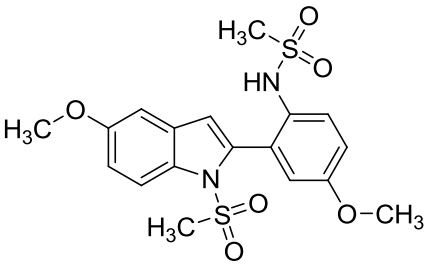
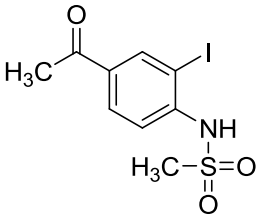
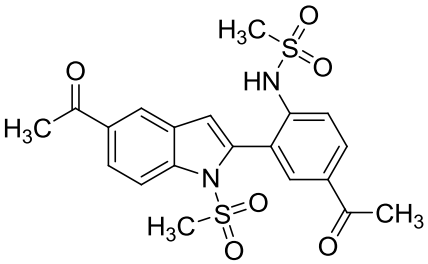
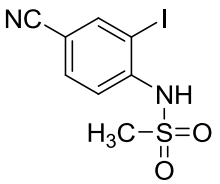
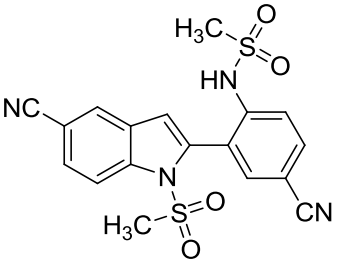
[a] All the reactions were carried out using iodide **1a** (1.6835 mmol), TMSA (6.734 mmol), Pd-catalyst (0.0168 mmol) and base (4.0287 mmol) in MeOH (5.0 mL), at 60 ºC; [b]After adding **2a**. [c]Isolated yield. [d]The reaction was carried out without CuI.

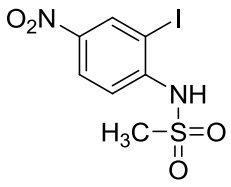
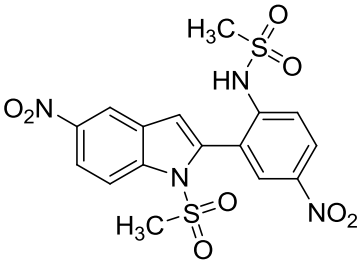
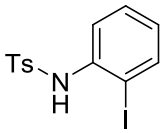
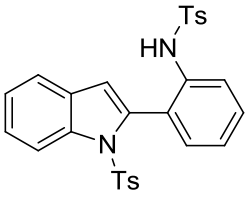
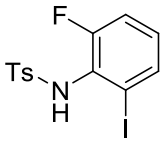
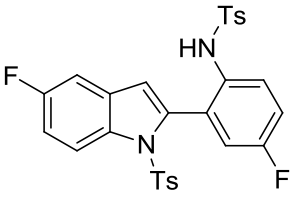
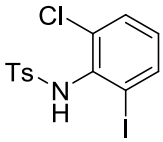
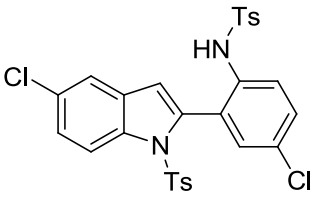
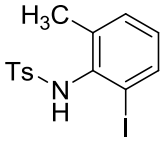
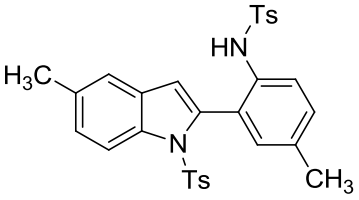
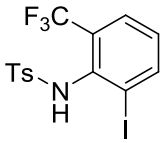
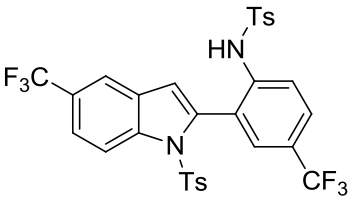
A series of experiments were performed with different Pd catalysts. We carried out the coupling of **1a** with TMSA using a Pd catalyst, CuI and a base in methanol at 60 °C. Due to our interest in the Pd/C-mediated alkynylation reaction[14] we conducted the reaction using 10%Pd/C-PPh_3 as a catalyst system. The reaction afforded **2a** in low yield after 4h (entry 1, Table 1). However, a longer reaction time i.e. 6h improved the product yield significantly (entry 2, Table 1). The omission of any component of the catalyst 10%Pd/C-PPh_3-CuI decreased the product yield significantly (entries 3, 4 and 5, Table 1). Notably, the use of $Pd(PPh_3)_2Cl_2$ was found to be effective even in the absence of CuI when piperidine was used in place of Et_3N (entry 6 *vs* 7, Table 1). To assess the generality of this reaction we treated TMSA with other iodoarenes (Scheme 1, Table 2).

Table 2. Synthesis of indole containing a *o*-($RSO_2NH)C_6H_4$ group at C-2 (Scheme 1).[a]



Entry	*o*-Iodoanilides (**1**) X; R =	Indoles (**2**)	Time (h)	Yield[b] (%)
1	 **1a**	 **2a**	6	62
2	 **1b**	 **2b**	5	65
3	 **1c**	 **2c**	8	60

4	 **1d**	 **2d**	6	63
5	 **1e**	 **2e**	7	60
6	 **1f**	 **2f**	6	58
7	 **1g**	 **2g**	9	56
8	 **1h**	 **2h**	9	64

9	 **1i**	 **2i**	8	55
10	 **1j**	 **2j**	4	64
11	 **1k**	 **2k**	6	58
12	 **1l**	 **2l**	4	60
13	 **1m**	 **2m**	7	63
14	 **1n**	 **2n**	5	62

[a]All the reactions were carried out using *o*-iodoanilide **1** (1.6835 mmol), TMSA (6.734 mmol), 10% Pd/C (0.0168 mmol), PPh_3 (0.0673 mmol), CuI (0.1685 mmol), and Et_3N (4.0287 mmol) in MeOH (5.0 mL), at 60 °C; [b]Isolated yield.

The present single pot tandem reaction proceeded well affording a variety of indoles. Both electron donating groups such as Cl (**1a**, **1l**), F (**1b, 1k**), Me (**1d, 1m**) and OMe (**1f**) or electron withdrawing groups e.g. CF_3 (**1e, 1n**), $COCH_3$ (**1g**), CN

(**1h**), NO_2 (**1i**) were tolerated. The structure of the representative compound **2e** was confirmed by X-ray analysis (Fig. 2).

Crystal structure of 2e :

Single crystals suitable for X-ray diffraction of **2e** were grown from dichloromethane. Single 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 $Mo_{K\alpha}$ 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 Broker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[15] The structure was solved by direct methods and all the non-hydrogen atoms were refined anisotropically while the hydrogen atoms, except hydrogens on N which were refined by picking electron density peaks, fixed in the predetermined positions by Shelxs-97[16] and Shelxl-97 packages respectively.

Single crystal X-ray data for compound 2e: Molecular formula = $C_{18}H_{14}F_6N_2O_4S_2$, Formula weight = 500.45,Crystal system = Monoclinic, Space group = Pn, a = 9.922 (5) Å, b = 14.243 (7) Å, c = 14.847 (7) Å, V = 2092.7 (18) Å3, T = 296 K, Z = 4, Dc = 1.550 Mg m–3, μ(Mo-Kα) = 0.71073 mm–1, 10680 reflections measured, 5285 independent reflections, 3766 observed reflections [I > 2.0 σ (I)], R1_obs = 0.072, Goodness of fit =1.357. Crystallographic data (excluding structure factors) for **2e** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 818602.

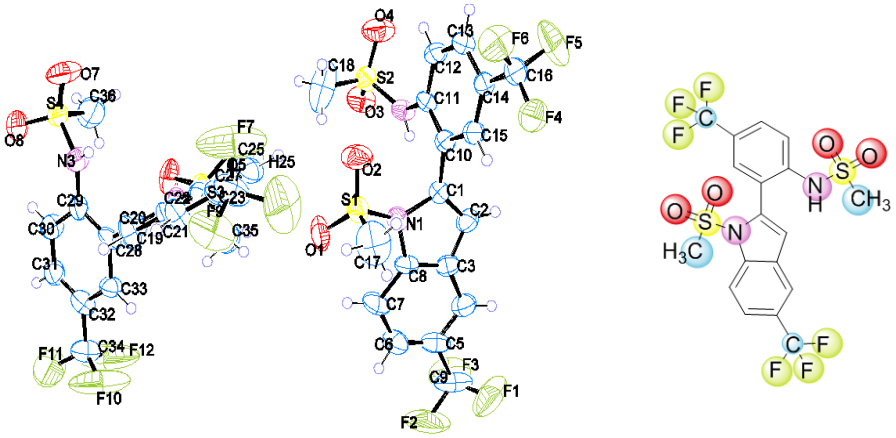
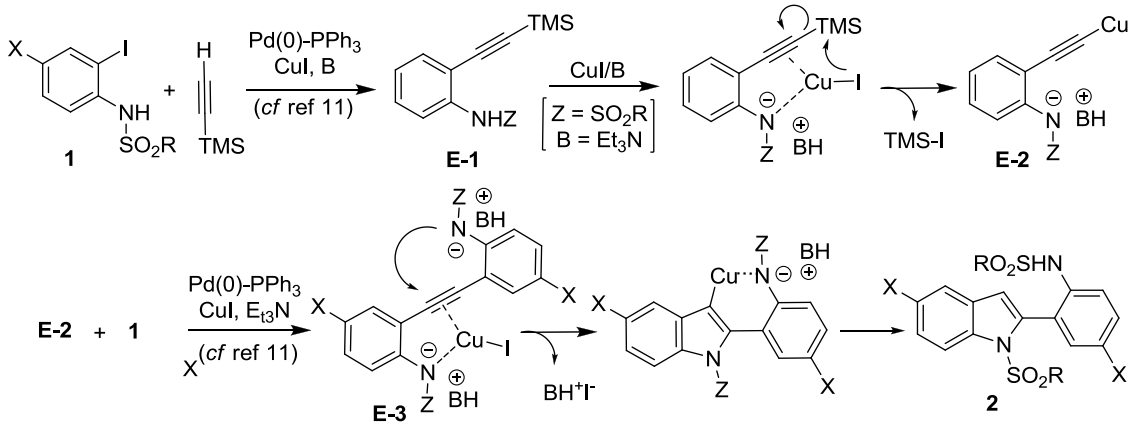


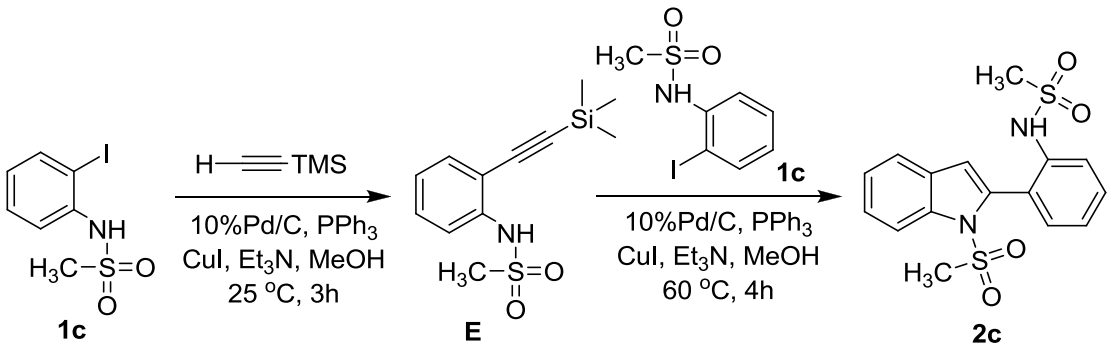
Figure 2. X-ray crystal structure of **2e** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Mechanistically, the present reaction seems to proceed via Pd/C-Cu mediated[11] Sonogashira followed by subsequent cyclization step in a single pot (Scheme 4). Thus, the alkyne **E-1** generated *in situ via* Pd(0) mediated stepwise formation of C–C bond between **1** and TMS-acetylene undergoes the cleavage of C-Si bond facilitated by CuI to generate the corresponding Cu-acetylide **E-2** which on subsequent coupling with **1** provides the internal alkyne **E-3**. The Cu-mediated ring closure of **E-3** in an intramolecular fashion provides the desired indole (**2**). Notably, the *o*-sulfonamide moiety facilitated CuI-mediated desilylation of **E-1** leading to the geneation of Cu-acetylide (**E-2**) in the present case, whereas a seperate desilylation step in the presence of a suitable reagent was required when this substituent was absent at *o*-position in aryl iodides employed.[12] In other words 1,2-diphenylethyne was not isolated when iodobenzene was reacted with TMSA under Pd/C–Cu catalysis in the presence of Et_3N in MeOH at refluxing temperature.



Scheme 4. Proposed mechanism for the synthesis of indoles (**2**) *via in situ* desilylation–Sonogashira strategy.

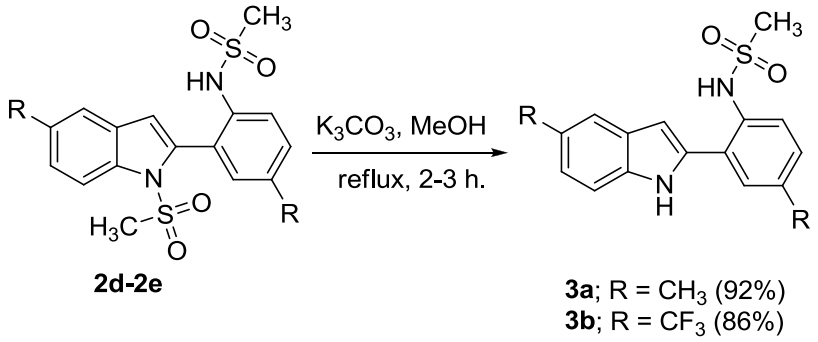
Nonetheless, to gain further evidence on the intermediacy of **E-1** the reaction of **1c** with TMSA was carried out at 25 °C for 3h under the condition of Entry 2 of Table 1 when the uncyclized product i.e. *N*-[2-{(trimethylsilyl)ethynyl} phenyl]methanesulfonamide (**E**) was isolated (Scheme 5). The alkyne **E** when reacted with **1c** in the presence of 10% Pd/C, PPh_3, CuI, and Et_3N in MeOH at 60 °C for 4h provided **2c**.



Scheme 5. Synthesis of indole **2c** *via* isolating the intermediate **E**.

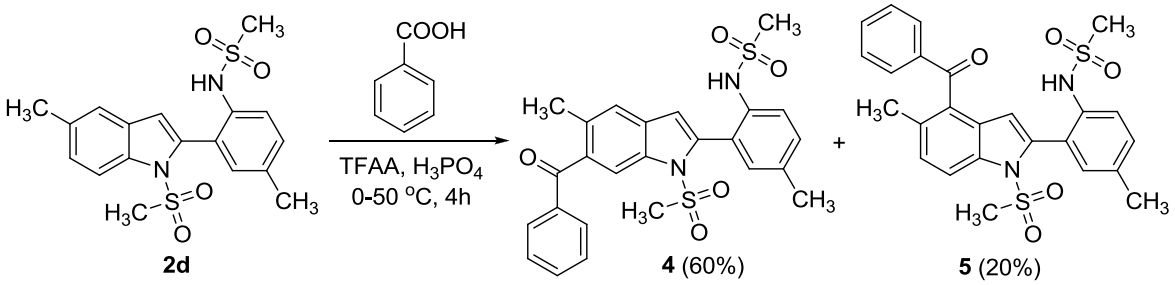
5B.4. Scope of the reaction

In order to demonstrate the further scope of this methodology *N*-mesyl indoles **2d** and **2e** were converted to indole **3a** and **3b** (Scheme 6).



Scheme 6. *N*-demesylation of indole ring.

Compound **2d** was subjected to TFAA-H_3PO_4 mediated [17] benzoylation to give **4 as** a major product and **5** as a minor product which is a regioisomer of **4** (*N* -(2-(4-benzoyl-5-methylsulfonyl)-1*H*-indol-2-yl)-4-methylphenyl)methanesulfonamide (Scheme 7).



Scheme 7. Synthesis of **4** *via* TFAA/H_3PO_4 mediated benzoylation.

The molecular structure of **4** was confirmed by ^{1}H and ^{13}C NMR, mass spectrometry and nfrared spectral data. Further molecular structure was unambiguoulsly determind by X-ray analysis (Fig. 3).

Single crystal X-ray data for compound 4:

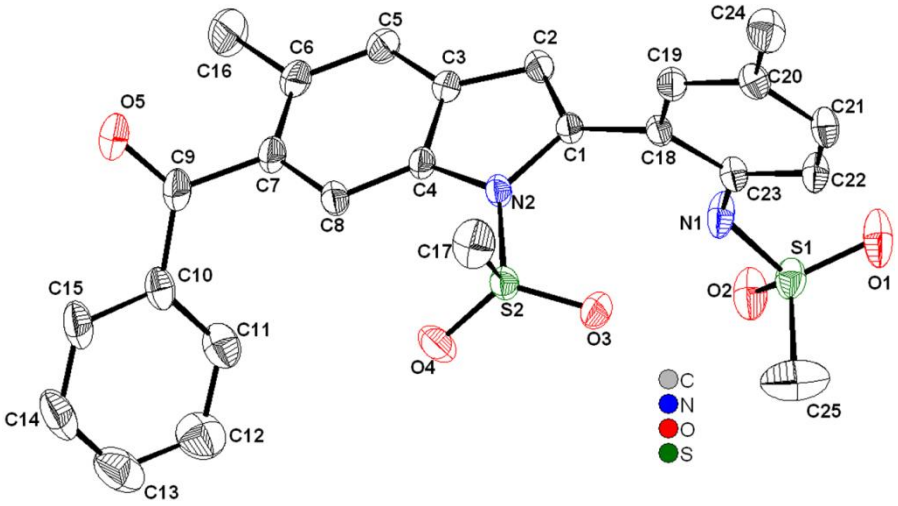
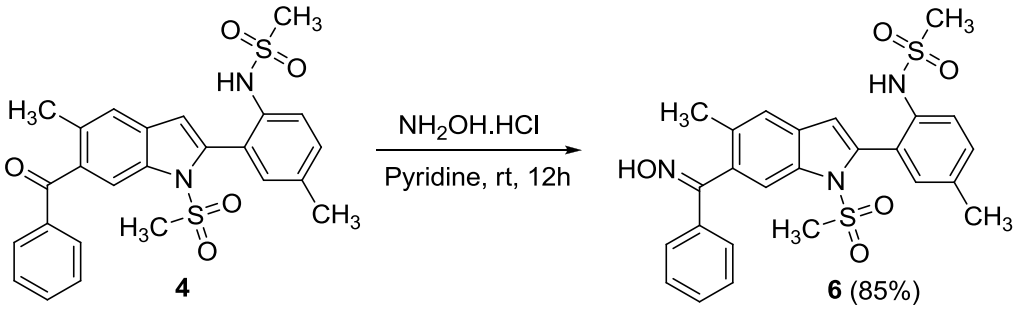


Figure 3. X-ray crystal structure of **4** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Crystal data of 4: Molecular formula = $C_{25}H_{24}N_2O_5S_2$, Formula weight = 496.58, Triclinic, Space group = *P-1*, a = 8.271 (4) Å, b = 12.010 (6) Å, c = 13.310 (6) Å, V = 1237.00 (10) Å^3, T = 298 K, Z = 2, D_c = 1.333 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1}, 8168 reflections were measured with 4219 unique reflections (R_{int} = 0.0199), of which 4219 ($I > 2\sigma(I)$) were used for the structure solution. Final R_1 (w R_2) = 0.0429 (0.1053), 311 parameters. The final Fourier difference synthesis showed minimum and maximum peaks of -0.273 and +0.246 e.Å^{-3} respectively. Goodness of fit = 1.061.

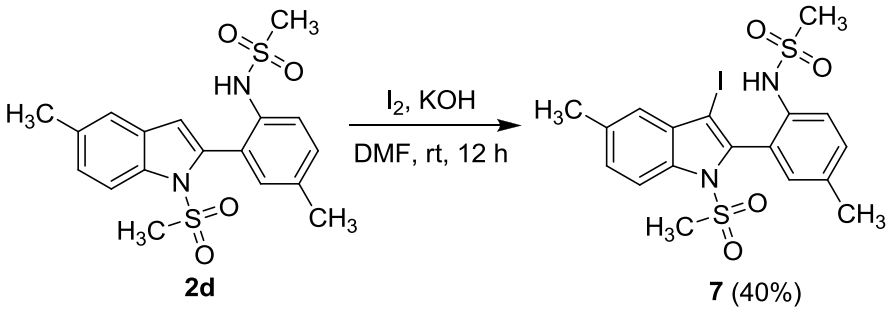
Crystallographic data (excluding structure factors) for **4** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 818615.

Further compound **4** was reacted with hydroxyl amine to give an oxime derivative **6** (Scheme 8).



Scheme 8. Synthesis of oxime derivative **6** from compound **4.**

Iodination of **2d** provided 3-iodoindole derivative **7** for further functionalization *via* transition metal catalyzed reactions (Scheme 9).[18]



Scheme 9. Synthesis of 3-iodo substituted indole derivative **7**

5B.5. Conclusion

In conclusion, we have developed a new facile and general one-pot synthesis of novel indoles containing an *o*-$(RSO_2NH)C_6H_4$ group at the C-2 position *via* Pd/C-mediated tandem coupling–cyclization strategy. This reaction proceeds well without the requirement of any additional catalyst for alkyne coupling or reagent for the removal of Me_3Si- group or cyclization step, respectively. Due to the operational simplicity this

methodology could become an application in generating a diversity based library of compounds.

5B.6. Experimental

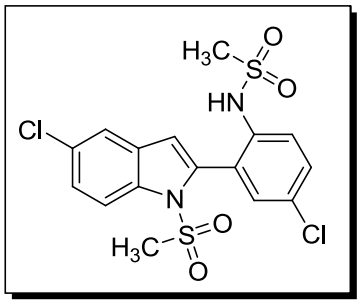
General methods: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were recodred in $CDCl_3$ or DMSO-d_6 solution by using 400 and 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) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in Hz. 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 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.

General method for the preparation of indole 2

To a stirred solution of *o*-iodoanilide **1** (1.6835 mmol) in methanol (5 mL), 10% Pd/C (0.002 g, 0.0168 mmol), PPh_3 (0.018 g, 0.0673 mmol), CuI (0.032 g, 0.1685 mmol), and Et_3N (0.406 g, 4.0287 mmol) were added under a nitrogen atmosphere. The reaction mixture was allowed to stir at room temperature for 15 min, and then the reaction temperature was increased slowly to 40 °C. To this was added trimethylsilyl acetylene (0.662 g, 6.734 mmol) slowly and portion wise maintaining the reaction mixture at 40 ºC. Then the reaction mixture was stirred at 60 ºC. The Progress of the reaction was monitored by TLC. 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

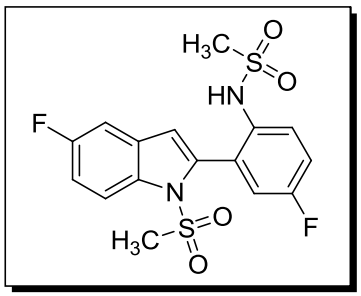
Na_2SO_4, filtered and concentrated under a reduced pressure. The residue was purified by column chromatography over silica gel using ethylacetate - hexane.

_N_-(4-chloro-2-(5-chloro-1(methylsulfonyl)-1_H_-indol-2-yl)phenyl)methanesulfonamide (2a):



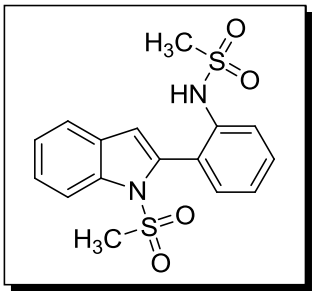
Yellow solid; mp: 142-146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05-7.96 (d, *J* = 8.5 Hz, 1H), 7.69-7.59 (m, 2H), 7.49-7.36 (m, 2H), 7.28 (s, 1H), 6.75 (s, 1H), 6.66 (s, 1H), 2.98 (s, 3H), 2.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.6, 131.0, 130.8, 130.7 (2C), 130.5, 129.7, 126.4, 124.9, 121.5 (2C), 121.1, 116.3, 113.9, 40.8, 40.2; IR (KBr, cm^{-1}): 3252, 2298, 1582, 1333; HPLC purity 97.82%; MS (ES mass): *m*/*z* 432.8 (M+1); HRMS: calcd for $C_{16}H_{14}Cl_2N_2O_4S_2$ M^+: 432.9650, found 432.9649.

_N_-(4-fluoro-2-(5-fluoro-1(methylsulfonyl)-1_H_-indol-2-yl)phenyl)methanesulfonamide (2b):



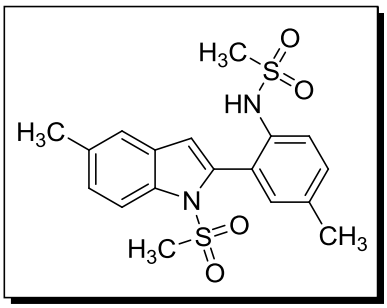
White solid; mp: 220-222 °C; ^{1}H-NMR (400MHz, $CDCl_3$) δ/ppm: 8.05 (dd, *J* = 9.07, 4.27 Hz, 1H), 7.67 (dd, *J* = 8.92, 4.99 Hz, 1H), 7.30 (dd, *J* = 8.13, 1.97 Hz, 1H), 7.24-7.15 (m, 2H), 7.08 (dd, *J* = 8.10, 2.64 Hz, 1H), 6.74 (s, 1H), 6.53 (s, 1H), 2.89 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ/ppm: 159.1, 158.1, 136.4, 132.7, 130.5, 123.9, 123.8, 117.8, 117.6, 116.6, 116.5, 114.4, 114.3, 107.2, 40.5, 40.2; IR (KBr, cm^{-1}): 3250, 2929, 1370, 1159;HPLC purity 99.7%; MS (ES mass): *m*/*z* 398.8 (M-1); HRMS: calcd for $C_{16}H_{14}F_2N_2O_4S_2$: 399.1021, found: 399.1043.

***N*-(2-(1-(methylsulfonyl)-1*H*-indol-2-yl)phenyl)methanesulfonamide (2c):**



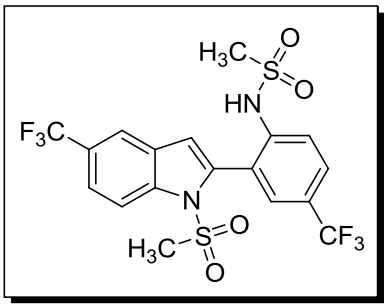
White solid; mp: 180-181 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 8.3 Hz, 1H), 7.73 (d, *J* = 8.3 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.52 – 7.31 (m, 4H), 7.23 (dd, *J* =13.3, 5.60 Hz, 1H), 6.75 (s, 1H), 6.58 (s, 1H), 2.99 (s, 3H), 2.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ/ppm: 137.3, 137.0, 135.5, 131.1, 131.0, 129.7, 125.9, 124.7, 124.2, 123.4, 121.4, 129.6, 115.3, 114.4, 40.6, 40.0; IR (KBr, cm^{-1}): 3374, 2983, 1576, 1084; HPLC purity 99.4%; MS (ES mass): *m*/*z* 362.8 (M-1); HRMS: calcd, for $C_{16}H_{17}N_2O_4S_2$ (M+H): 365.0630, found: 365.0638.

***N*-(4-methyl-2-(5-methyl-1(methylsulfonyl)-1*H*-indol-2-yl)phenyl)methane sulfonamide (2d):**



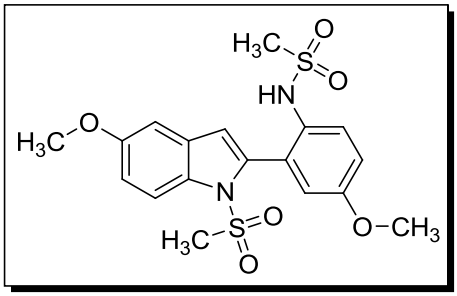
White solid; mp: 134-136 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.96 (d, *J* = 8.5 Hz,1H), 7.58 (d, *J* = 8.5 Hz,1H), 7.40 (s, 1H), 7.27 -7.23 (m, 2H), 7.15 (s, 1H), 6.66 (s, 1H), 6.56 (s, 1H), 2.89 (s, 3H), 2.82 (s, 3H), 2.48 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.3, 135.6, 134.5, 134.0 (2C), 131.4, 131.3, 130.0, 127.2, 124.5, 121.3, 121.1, 115.1, 114.4, 40.0, 39.9, 21.2, 20.7; IR (cm^{-1}): 3280, 2930, 1365, 1169; HPLC purity 83.3%; MS (ES mass): *m*/*z* 393.1 (M+1) ; HRMS: calcd, for $C_{18}H_{19}N_2O_4S_2$ (M-H): 391.0786, found: 391.0796.

_N_-(2-(1-(methylsulfomyl)-5-(trifluoromethyl)-1_H_-indol-2-yl)-4-(trifluromethyl) phenyl) methane sulfonamide (2e):



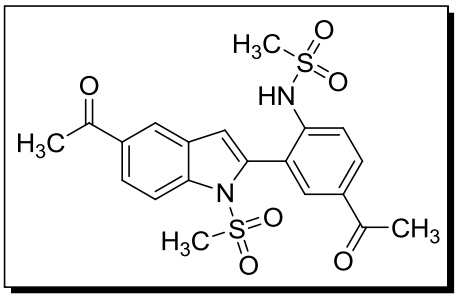
Brown solid; mp: 158-160 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, *J* = 8.8 Hz, 1H), 7.96 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 8.83 Hz, 1H), 7.71 (d, *J* = 8.84 Hz, 1H), 7.56 (s, 1H), 6.89 (s,1H), 6.75 (s, 1H), 3.07 (s, 3H), 2.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 140.5, 138.7, 135.2, 129.1, 128.5, 128.4, 127.9, 127.5, 123.0, 122.9, 121.9, 119.2, 119.1, 117.8, 115.5, 114.5, 41.6, 40.2; IR (KBr, cm^{-1}): 3260, 2934, 1332, 1158; HPLC purity 98.5%; MS (ES mass): *m*/*z* 498.8 (M-1); HRMS: calcd, for $C_{18}H_{13}N_2O_4F_6S_2$ (M-H): 499.0221, found: 499.0256.

_N_-(4-methoxy-2-(5-methoxy-1(methylsulfonyl)-1_H_-indol-2-yl)phenyl)methane sulfonamide (2f):



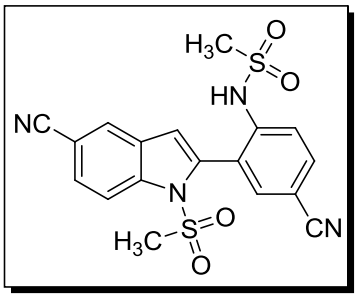
Brown solid; mp 171-173 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64 (s, 1H), 7.49 – 7.46 (m, 2H), 7.31 (s, 1H), 7. 22 (d, *J* = 8.4 Hz, 1H), 7.0 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.58 (s, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.02 (s, 3H), 2.85 (s, 3H); ^{13}C NMR (100MHz, $CDCl_3$) δ: 158.7, 140.8, 137.8, 129.0, 128.4, 123.4, 121.7, 117.4, 115.4, 114.2, 113.9, 113.2, 109.7, 99.6, 55.7, 55.7, 40.3, 39.8; IR (KBr, cm^{-1}): 3312, 2932, 1365, 1159; HPLC purity 86.3%; MS (ES mass): *m*/*z* 425.1 (M+1) ; HRMS : Calcd for $C_{18}H_{19}N_2O_6S_2$ (M-H):423.0685, found:423.0692.

***N*-(4-acetyl-2-(5-acetyl-1(methylsulfonyl)-1*H*-indol-2-yl)phenyl)methanesulfonamide (2g):**



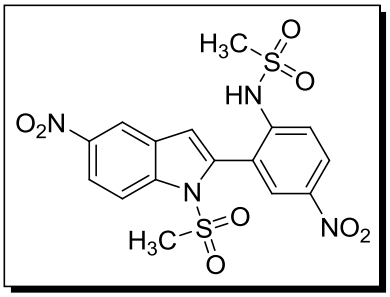
White solid; mp: 234- 236 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.40 (s, 1H), 8.26 (s, 1H), 8.04-7.90 (m, 3H), 7.69 (d, *J* = 8.53 Hz, 1H), 7.52 (d, *J* = 3.61 Hz, 1H), 6.81 (s, 1H), 3.09 (s, 3H), 2.65 (s, 3H), 2.55 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 197.9, 197.0, 142.3, 139.5, 136.9, 132.9, 132.8, 131.8, 130.6, 129.8, 124.7, 123.7, 122.9, 119.5, 114.5, 113.5, 42.4, 40.9, 27.2 27.0; IR (KBr, cm^{-1}): 3156, 2930, 1669, 1338; HPLC purity 90.9%; MS (ES mass): *m*/*z* 448.9 (M+1); HRMS (EI): Calcd for M^+ $C_{20}H_{20}N_2O_6S_2$ 447.2016, found 447.2019.

***N*-(4-cyano-2-(5-cyano-1(methylsulfonyl)-1*H*-indol-2-yl)phenyl)methanesulfonamide (2h):**



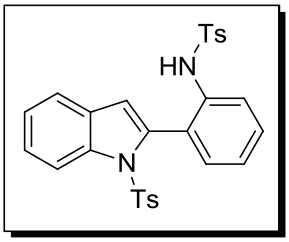
White solid; mp: 175-177 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.79 (s, 1H), 8.24 (s, 1H), 8.07 (d, *J* = 8.60 Hz, 1H), 7.93 (s, 1H), 7.90-7.77 (m, 2H), 7.69 (d, *J* = 8.55 Hz, 1H), 6.95 (s, 1H), 3.37 (s, 3H), 3.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 138.5, 137.7, 136.0, 134.4, 131.0, 129.7, 128.6, 127.7, 127.0, 126.5, 123.6, 119.6, 119.5, 119.3, 115.4, 105.8, 42.2, 40.6. IR (KBr, cm^{-1}): 3267, 3127, 2923, 2232, 1341; HPLC purity 97.6 %; MS (ES mass): *m*/*z* 412.8 (M-1); HRMS (EI): calcd for $C_{18}H_{14}N_4O_4S_2$ 413.0050, found: 413.0066.

***N*-(2-(1-(methylsulfonyl)-5-nitro-1*H*-indol-2yl)-4-nitrophenyl)methanesulfonamide (2i):**



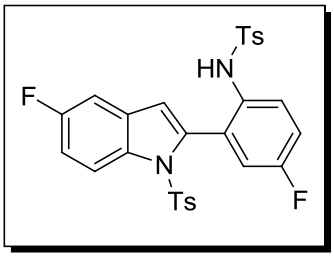
Yellow solid; mp: 230-232 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.99 (s, 1H), 8.68 (s, 1H), 8.32-8.28 (m, 2H), 7.81 (s, 1H), 7.14 (s, 1H), 6.78-6.82 (m, 2H), 3.37 (s, 3H), 3.14 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ/ppm: 143.4, 141.6, 139.6, 136.3, 129.3, 127.4, 125.6, 123.2, 122.2, 119.4, 118.2, 117.3, 114.5, 113.3, 42.3, 40.4; IR (KBr, cm^{-1}): 3362, 2926, 1513, 1343; HPLC purity 97.24%; MS (ES mass): *m*/*z* 452.7 (M-1) ; HRMS Calcd, for $C_{16}H_{13}N_4O_8S_2$ (M-H): 453.0175, found 453.0194.

4-methyl-*N*-(2-(1-tosyl-1*H*-indol-2-yl)phenyl)benzenesulfonamide (2j):



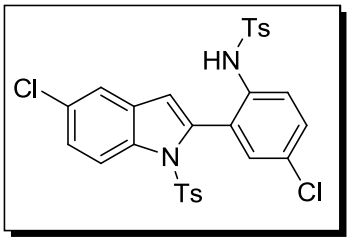
White solid; mp: 210-212 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.46-7.40 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 4 Hz, 2H), 7.20-7.13 (m, 3H), 7.04 (d, *J* = 8.4 Hz, 2H) 6.94-6.86 (m, 4H), 5.71 (s, 1H), 2.30 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 145.2, 143.1, 137.7, 136.8, 136.2, 135.7, 134.2, 131.2, 130.1 (2C), 129.4 (2C), 129.2 (2C), 126.7 (2C), 126.7 (2C), 126.4, 125.4, 125.1, 124.7, 124.3, 120.6, 116.2, 114.7, 21.5, 21.4; IR (KBr,cm^{-1}): 3381, 3067, 1596,1090; HPLC purity 98.0%; MS (ES mass): *m*/*z* 515 (M-1,100%); HRMS: calcd for: $C_{28}H_{25}N_2O_4S_2$ (M+H) 517.1256, found: 517.1249.

***N*-(4-fluoro-2-(5-fluoro-1-tosyl-1*H*-indol-2-yl)phenyl)-4methylbenzenesulfonamide (2k):**



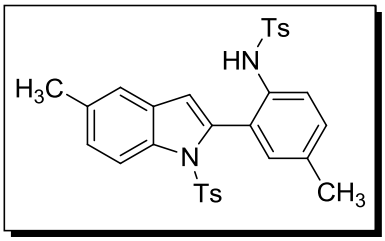
Brown solid; mp: 207-209 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38 (d, *J* = 9.2 Hz, 1H), 7.80 (d, *J* = 8.8 Hz 1H), 7.39-7.30 (m, 5H), 7.21 (d, *J* =8.0 Hz, 2H), 7.08 (m, 2H), 7.03 (d, *J* = 9.6 Hz, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.66 (d, *J* = 10.8 Hz, 1H), 5.74 (s, 1H) 2.44 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.2, 158.8, 145.7, 143.1, 137.0, 136.7, 133.6, 129.6 (2C), 129.1 (2C), 128.9 (2C), 126.6 (2C), 117.7, 117.5, 117.4, 117.2, 117.0, 114.9, 114.8, 113.7, 113.4, 106.3, 106.1, 21.5, 21.3; IR (KBr, cm^{-1}): 3389, 3076, 1463,1089; HPLC purity 98.36%; R_f =0.30 (20 % EtOAc-*n*-Hexane); MS (ES mass): *m*/*z* 551.1 (M-1, 100%) HRMS: calcd, for $C_{28}H_{23}N_2O_4S_2F_2$ (M+H): 553.1067 found: 553.1076.

_N_-(4-chloro-2-(5-chloro-1-tosyl-1_H_-indol-2-yl)phenyl)-4-methylbenzenesulfonamide (2l):



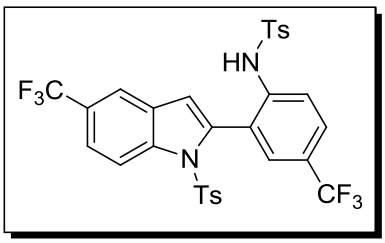
White solid; R_f =0.25 (20 % EtOAc-*n*-Hexane); mp: 212 -214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.26 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.45-7.39 (m, 2H), 7.34 (d, *J* = 7.74 Hz, 2H), 7.14 (m, 5H), 6.95 (d, *J* = 7.4 Hz, 2H), 6.86 (s, 1H), 6.71 (s, 1H), 5.68 (s, 1H), 2.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 145.9, 143.5, 136.7, 136.1, 136.0, 134.4, 133.9, 131.0, 130.9, 130.8, 130.4, 130.2 (2C), 129.7 (2C), 129.3 (2C), 127.1, 126.8 (2C), 126.7 (2C), 125.9, 120.3, 117.2, 113.9, 21.6, 21.4; HPLC purity 80.8%; MS (ES mass): *m*/*z* 582.8 (M-1): IR (KBr, cm^{-1}): 3262, 3070, 1482, 1074; HRMS: calcd, for $C_{28}H_{22}Cl_2N_2O_4S_2$: 584.1257 found: 584.1267.

4-methyl-*N*-(4-methyl-2-(5-methyl-1-tosyl-1*H*-indol2yl)phenyl)benzenesulfonamide (2m):



White solid; mp: 204-206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.32 (s, 1H), 7.29 (d, *J* = 8.8 Hz, 2H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.15 (d, *J* = 8.4, 2H) Hz, 7.05-7.03 (m, 3H), 6.86 (d, *J* = 8.0, 2H), 6.57 (s, 1H), 2.46 (s, 3H), 2.30 (s, 3H), 2.29 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 145.0, 142.8, 137.0, 136.6, 135.9, 135.1, 134.3, 133.9, 133.0, 131.8, 130.8, 130.5, 129.2 (2C), 129.1 (2C), 126.9, 126.8 (2C), 126.7 (2C), 126.6, 125.7, 120.4, 115.9, 114.4, 21.5, 21.4, 21.2, 20.7; IR (KBr, cm^{-1}): 3300, 2921, 1456, 1039; HPLC purity 95.87%; MS (ES mass): *m*/*z* 542.9 (M-1, 100%): HRMS: calcd, for $C_{30}H_{29}N_2O_4S_2$(M+H): 545.1569 found: 545.1567.

4-methyl-*N*-(2-(1-tosyl-5-(trifluoromethyl)-1*H*-indol-2-yl)-4-(trifluoromethyl)phenyl) benzenesulfonamide (2n):

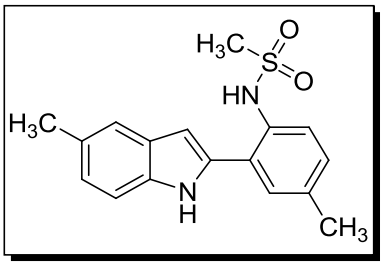


Yellow solid; mp: 224-228 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.47 (d, *J* = 8.8 Hz, 1H), 7.75-7.68 (m, 4H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 4H), 6.95 (s, 1H), 6.92 (s, 1H), 6.05 (s, 1H), 2.37 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 146.3, 144.1, 139.6, 139.1, 136.4, 135.6, 134,2, 129.9, 129.8 (2C), 129.7 (2C), 129.6, 129.2, 128.6, 127.0 (2C), 126.6 (2C), 124.4, 122.6, 122.5, 122.4, 118.5, 118.4, 118.3, 116.2, 114.2, 21.5, 21.4; IR (KBr, cm^{-1}): 3256, 2925, 1596,1079; HPLC purity: 92.4%; MS (ES mass): *m*/*z* 650.9 (M-1, 100%) HRMS: calcd, for $C_{30}H_{22}F_6N_2O_4S_2$: 652.0927, found: 652.0918.

Preparation of indole 3a and 3b

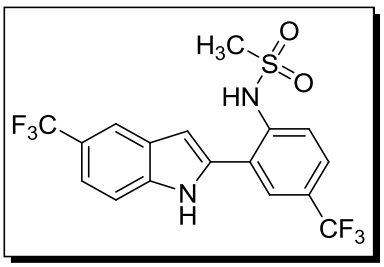
A mixture of compound **2d** or **2e** (0.4008 mmol) and K_2CO_3 (0.083 g, 0.6012 mmol) in MeOH (5 mL) was refluxed for 3 h. After completion, the reaction mixture was filtered and the residue was washed with MeOH (5 mL). The filtrates were collected, combined and concentrated under reduced pressure. The residue was purified by column chromatography using 0-20% EtOAc - hexane to give the desired product.

***N*-(4-methyl-2-(5-methyl-1*H*-indol-2-yl)phenyl)methanesulfonamide (3a) :**



Brown solid; mp: 212-213 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.43 (s, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.19 (d, 8.4 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.96 (s, 1H), 6.56 (s, 1H), 2.94 (s, 3H), 2.46 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.7, 135.1, 133.7, 131.4, 130.6, 130.1, 130.1, 129.0, 125.8, 124.6, 122.1, 120.4, 110.9, 102.4, 39.8, 21.4, 20.8; MS (ES mass): *m*/*z* 312.9 $(M-1)^+$; IR (KBr, cm^{-1}): 3246, 2956, 1660, 1157; HRMS: calcd for $C_{17}H_{18}N_2O_2S$ is 314.1086 found 314.1074.

***N*-(4-(trifluoromethyl)-2-(5-(trifluoromethyl)-1*H*-indol-2-yl)phenyl)methane sulfonamide (3b):**



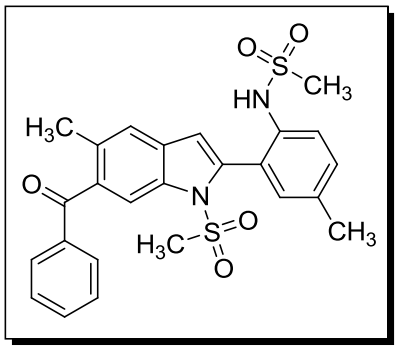
White solid; mp: 204-206 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm: 8.78 (s, 1H), 7.97 (s, 1H), 7.84 (s, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.51 (s, 1H), 6.82 (s, 1H), 3.11 (s, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ/ppm: 139.5, 139.6, 135.2, 128.1,

128.5, 128.4, 126.8, 127.5, 122.9, 122.9, 121.9, 119.2, 119.1, 117.8, 115.5, 114.5, 40.2; IR (KBr, cm^{-1}): 2934, 1332, 1158; MS (ES mass): *m/z* 420.8 (M-1); HRMS: calcd for $C_{17}H_{13}N_2F_6O_2S$ (M+H): 423.0602, found: 423.0600.

Benzoylation of indole

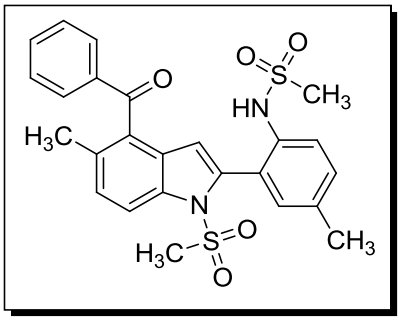
_N_-(2-(6-benzoyl-5-methyl-1-(methylsulfonyl)-1_H_-indol-2-yl)-4methylphenyl) methane sulfonamide (4):

A mixture of TFAA (0.85 g, 4.085 mmol) and benzoic acid (0.052 g, 0.46 mmol) was stirred at 0 ºC for 20 min till all the solids are dissolved. To this was added indole **2d** (0.2 g, 0.51 mmol) with stirring followed by 85 % H_3PO_4 (0.008 g, 0.08 mmol). Then the reaction was allowed to stir at 0 ºC for 20 min and at 50 ºC for 4 h. The progress of the reaction was monitored by TLC. After completion of the reaction the TFA/TFAA mixture was distilled off at atmospheric pressure. The remaining liquid was partitioned between $CHCl_3$ and water. The organic layer was separated and washed with 5% NaOH and then brine. The mixture was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography on silica gel using EtOAc-hexane to give the compound **4**.



Yellow solid; mp: 198-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 7.88 (d, *J* = 1.4 Hz, 2H), 7.60 (d, *J* = 6.3 Hz, 2H), 7.52-7.49 (m, 3H), 7.32 (d, *J* = 6.8 Hz,1H), 7.15 (s,1H), 6.71 (S, 1H), 6.53 (s, 1H, D_2O Exchange), 2.96 (s, 3H), 2.86 (s, 3H), 2.43 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz,$CDCl_3$) δ: 19.4, 20.6, 39.8, 40.6, 112.9, 116.4, 121.2, 123.9, 128.1, 128.4, 128.8, 129.7 (2C), 131.3 (2C), 131.6, 132.0, 133.9, 134.0, 134.1, 134.5, 135.6, 137.0, 137.1, 197.4; MS (ES mass): *m/z* 497.1 (M+1); HRMS: calcd for $C_{25}H_{24}N_2O_5S_2$ is 497.1210 found 497.1205.

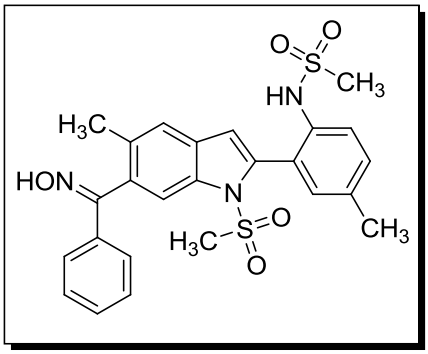
_N_-(2-(4-benzoyl-5-methyl-1-(methylsulfonyl)-1_H_-indol-2-yl)-4-methylphenyl)methane sulfonamide (5) :



White solid; mp: 190-192 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 7.2 Hz, 2H), 7.53 -7.51 (m, 2H), 7.45 (t, *J* = 7.9 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.24 (s, 1H), 7.07 (s, 1H), 6.5 (s,1H) , 6.37 (s, 1H), 2.86 (s, 3H), 2.84 (s, 3H), 2.33 (s, 3H), 2.32 (s,3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 197.5, 137.2, 137.1, 135.6, 134.6, 134.1, 134.0, 113.1, 132.1, 131.7, 131.4, 129.8 (2C), 128.9 (2C), 128.5, 128.2, 124.0, 121.2, 116.5, 113.1, 40.6, 39.9, 20.6, 19.4; IR (KBr,cm^{-1}): 3281, 2928, 1662, 1367, 1163; MS (ES mass): *m*/*z* 496.9 $(M+1)^+$: HRMS: calcd for $C_{25}H_{24}N_2O_5S_2$ is 497.1208 found 497.1213.

Preparation of (_E_)-_N_-(2-(4-((hydroxyimino)(phenyl)methyl)-5-methyl-1 (methylsulfonyl) -1_H_-indol-2-yl)-4-methylphenyl)methanesulfonamide (6) :

To the stirred solution of pyridine (0.35 g, 4.4 mmol) and hydroxylamine hydrochloride (0.05 g, 0.88 mmol), was added the ketone **4** (0.2 g, 0.44 mmol) and the mixture was stirred at room temperature for 12 h. Upon completion of the reaction, the mixture was diluted with cold water (15 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography over alumina using 2: 8 ethylacetate hexane to give the desired product (**5**).

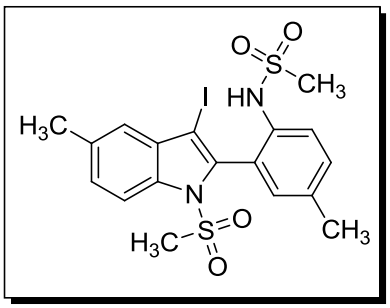


White solid; mp: 122-124 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (bs, 1H D_2O Eexchangable), 7.87 (s, 1H), 7.61 (d, *J* = 8.4 Hz,1H), 7.52-7.57 (m, 3H), 7.35 -7.38 (m, 3H) 7.29 (d, *J* = 8.4Hz, 1H), 7.1 (s, 1H), 6.7 (s, 1H), 6.5 (s, 1H, D_2O Exchangeable), 3.00 (s, 3H), 2.96 (s, 3H), 2.38 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 136.7, 135.5, 134.4, 134.2, 132.7, 131.7, 131.5, 131.3, 130.2, 130.0, 129.7, 128.6 (2C), 127.0(2C), 123.7, 122.3, 120.5, 120.4, 114.5, 113.7, 40.8, 39.9, 20.7, 19.5; IR (KBr, cm^{-1}): 3425, 3275, 2943, 1710, 1152; MS (ES mass):*m*/*z* 511.9 $(M+1)^+$; HRMS: calcd for $C_{25}H_{26}N_3O_5S_2$ (M+H): 512.1314, found:512.1314.

Iodination of indole at C3 position

Preparation of *N*-(2-(3-iodo-5-methyl-1-(methylsulfonyl)-1*H*-indol-2-yl)-4-methylphenyl) methanesulfonamide (7):

To the solution of indole **2d** (0.2 g, 0.51 mmol) in DMF (5 mL) was added, KOH (0.12 g, 2.04 mmol) and iodine (0.26 g, 1.01 mmol) at room temperature. The mixture was allowed stir for 12 h, at room temperature. After completion of the reaction solvent was removed under vacuum. The residue was treated with ethylacetate (15 mL), washed with 0.1% sodium bisulfate solution (10 mL), water (10 mL) and brine (10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography over alumina using 1: 9 ethylacetate-hexane to give the desired product (**7**).



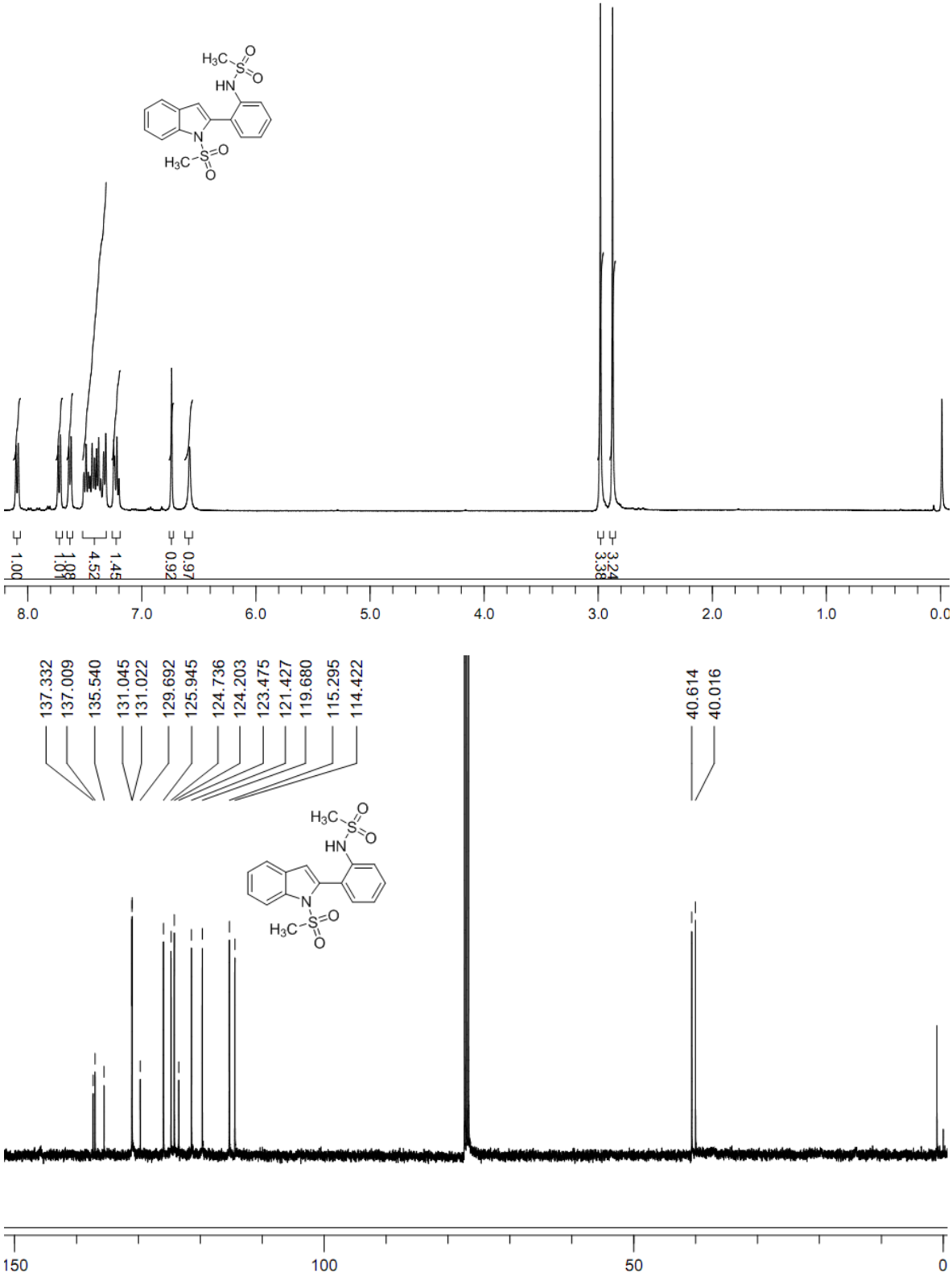
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8 Hz, 1H), 7.65 (d, *J* = 8 Hz, 1H),7.30 -7.35 (m, 3H), 7.1 (s, 1H), 6.32 (s, 1H), 2.94 (s, 3H), 2.93 (s, 3H), 2.53 (s, 3H), 2.4 (s, 3H); ^{13}C NMR (100 M Hz, $CDCl_3$) δ: 134.7, 134.5, 132.6, 130.0, 129.8, 129.5, 128.1, 127.8, 124.7, 122.8, 121.5, 119.5, 108.3, 101.9, 39.5, 39.2, 20.9, 20.4; IR (KBr, cm^1) : 3269, 1665, 1225, 1150; MS (ES mass):*m*/*z* 518.5 $(M+1)^+$; HRMS: calcd for $C_{18}H_{19}IN_2O_4S_2$ is 517.9827 found 517.9835.

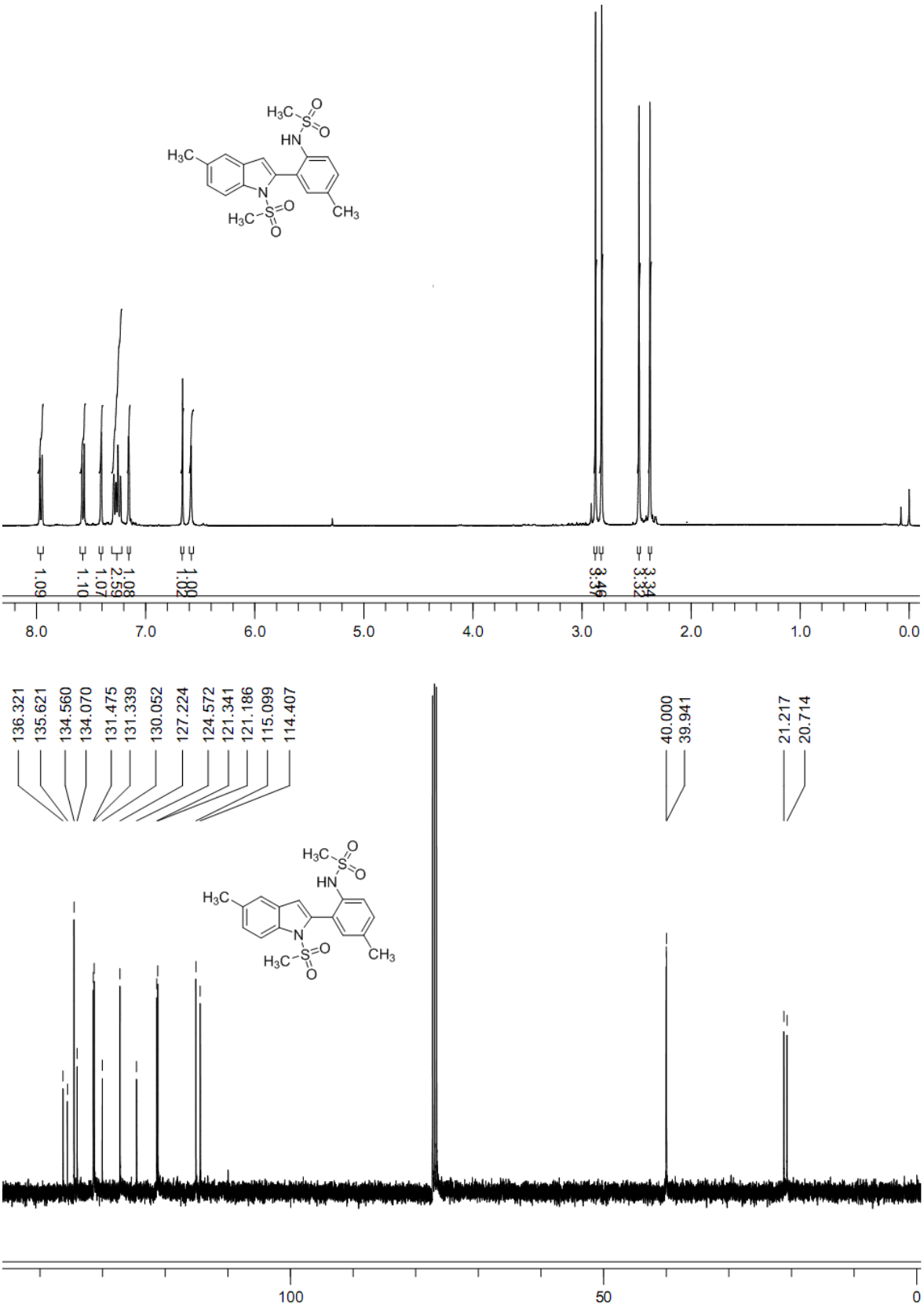
Reference:

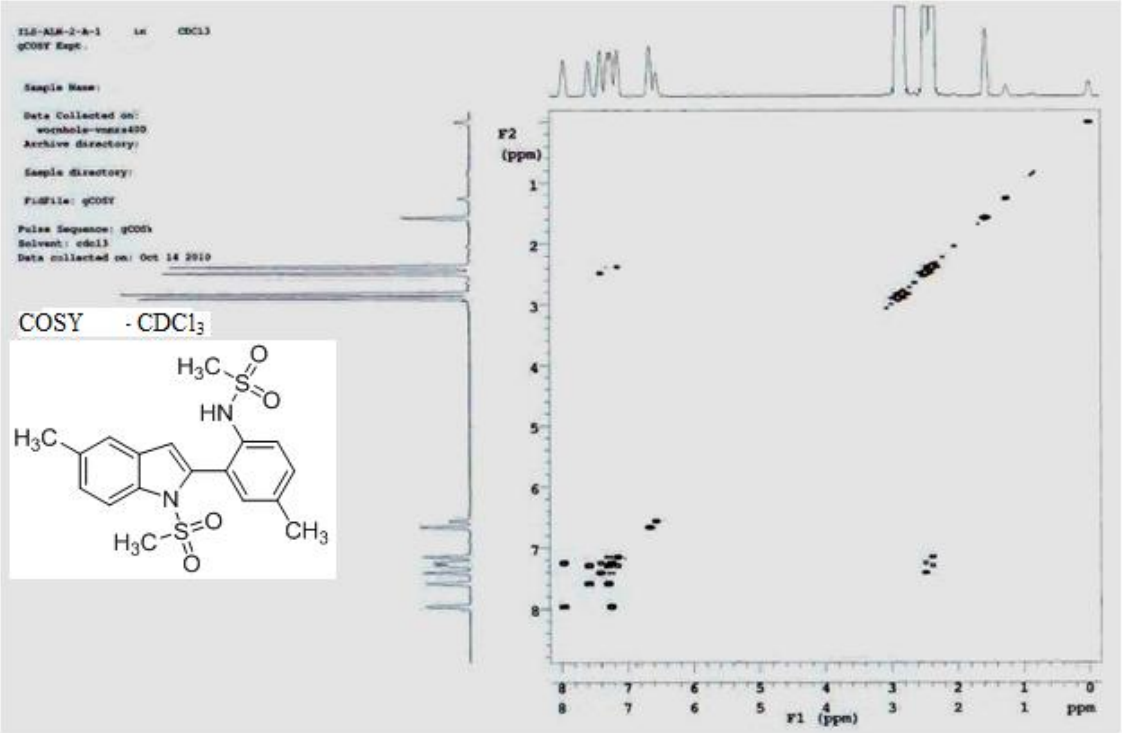
1. (a) Zapf., A.; Beller, M. *Top. Catal.* **2002**, *19*, 101. (b) Tucker, C. E.; de Vries, J. G. *Top. Catal.* **2002**, *19*, 111. (c) Bra¨se, S.; Kirchhoff, J. H.; Ko¨bberling, J. *Tetrahedron* **2003**, *59*, 885. (d) van de Weghe, P. *Lett. Org. Chem.* **2005**, *2*, 113.
2. (3) (a) Brandsma, L. *Synthesis of Acetylenes, Allenes and Cumulenes:Methods and Techniques*; Elsevier: Oxford, 2004; p 293. (b) Sonogashira, K. In *Metal-Catalyzed Cross-Coupling Reactions*;Diederich, F., de Meijera, A., Eds.; Wiley-VCH: Weinheim, 2004;Vol. 1, p 319. (c) Tykwinski, R. R. *Angew. Chem., Int. Ed.* **2003**,*42*, 1566. (d) Negishi, E.; Anastasia, L. *Chem. Re*V. **2003**, *103*, 1979.(e) Sonogashira, K. In *Handbook of Organopalladium Chemistry for Organic Synthesis*; Negishi, E., de Meijere, A., Eds.; Wiley-Interscience: New York, 2002; p 493. (f) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46. (g) Rossi, R.; Carpita, A.; Bellina,F. *Org. Prep. Proced. Int.* **1995**, *27*, 127. (h) Sonogashira, K. In *Comprehensi*V*e Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 3, p 521.
3. Diek, H. A.; Heck, F. R. *J. Organomet. Chem.* **1975**, *93*, 259.
4. Cassar, L. *J. Organomet. Chem.* **1975**, *93*, 253.
5. (a) Cassar, L. *J. Organomet. Chem.* **1975**, *93*, 253. (b) Dieck, H. A.; Heck, F. R. *J. Organomet. Chem.* **1975**, *93*, 259.
6. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *16*, 4467.
7. (a) Mio, M.J.; Kopel, L. C.; Braun, J. B.; Gadzikwa, T. L.; Hull, K. L.; Brisbois, R. G.; Markworth, C. J.; Grieco, P. A. *Org. Lett.* **2002**, *4*, 3199. (b) Halbes, U.; Pale, P. *Tetrahedron Lett.* **2002**, *43*, 2039. (c) Li, G.; Wang, X.; Wang, F. *Tetrahedron Lett.* **2005**, *46*, 8971. (d) Sommer, W. J.; Weck, M. *Adv. Synth. Catal.* **2006**, *348*, 2101.

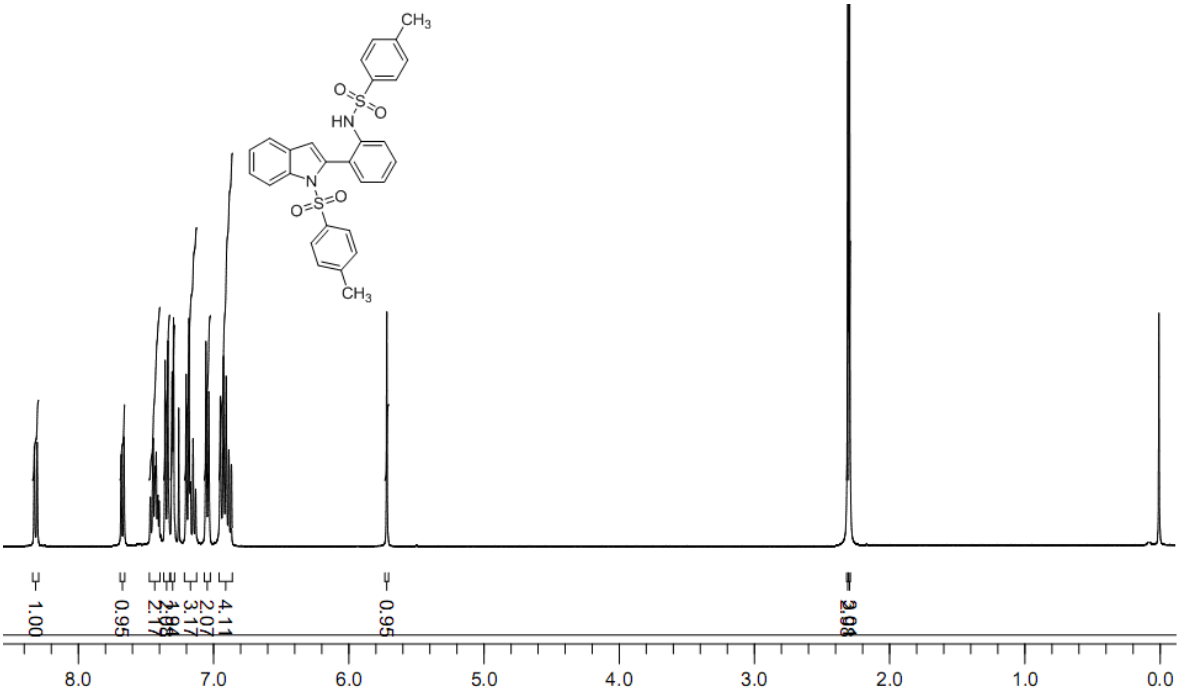
8. Nishihara, Y.; Noyori, S.; Okamoto, T.; Suetsugu, M.; Iwasaki, M. *Chem. Lett.* **2011**, *40*, 972.
9. For recent reviews, see: (a) Humphrey, G. R. and Kuethe, J. T. *Chem. Rev.*, **2006**, *106*, 2875; (b) Cacchi, S.; Fabrizi, G. *Chem. Rev.*, **2005**, *105*, 2873; (c) Gribble, G. W. in *Comprehensive Heterocyclic Chemistry II, ed*. Katrizky, A. R.; Rees, C. W. Scriven, E. F. V. *Pergamon*, *Oxford*, **1996**, *vol. 2*, p. 20.
10. Purandare, A. V.; Wan, H.; Huynh, T. N.; US2006/235037, 2006, A1.
11. For selected references, see: (a) Fukuoka, S.; Naito, T.; Sekiguchi, H.; Somete, T.; Mori, A. *Heterocycles*, **2008**, *78*, 819; (b) Leogane, O. Lebel, H. *Angew. Chem.*, *Int. Ed*., **2008**, *47*, 350; (c) McLaughlin, M.; Palucki, M. Davies, I. W. *Org. Lett.*, **2006**, *8*, 3307; (d) Layek, M.; Reddy, M. A.; Rao, A. V. D.; Alvala, M.; Arunasree, M. K.; Islam, A.; Mukkanti, K.; Iqbal, J.; Pal, M. *Org. Biomol. Chem.*, **2011**, *9*, 1004.
12. Rao, R. M.; Reddy U.CH.; Nakhi, A.; Mulakayala, N.; Alvala, M.; Arunasree, M. K.; Poondra, R. R.; Iqbal, J.; Pal, M. *Org.Biomol.Chem.*, **2011**, *9*, 3808.
13. For selected references, see: (a) Hatanaka, Y.; Matsui, K.; Hiyama, T. *Tetrahedron Lett.*, **1989**, *30*, 2403; (b) Chang, S.; Yang, S. H.; Lee, P. H.; *Tetrahedron Lett.*, **2001**, *42*, 4833; (c) Li, G.; Wang, X.; Wang, F. *Tetrahedron Lett*., **2005**, *46*, 8971; (d) Nishihara, Y.; Inoue, E.; Ogawa, D.; Okada, D.; Noyori, S.; Takagi, K. *Tetrahedron Lett.*, **2009**, *50*, 4643.
14. Pal, M.; *Synlett*, 2009, 2896.
15. Bruker SADABS V2008-1, Bruker AXS.: Madison, WI, USA (2008).
16. G. M. Sheldrick, SHELX93, Program for Crystal Structure Determination, University of Göttingen (1997).
17. (a) Veeramaneni, V. R.; Pal, M.; Yeleswarapu, K. R. *Tetrahedron*, 2003, 5(9), 3283; (b) Pal, S.; Bindu, P.; Vinna, P. R.; Dubey, P. K. *Lett. Org. Chem.*, 2007, 4, 292;
18. For Suzuki and Sonogashira coupling of 2-aryl-3-iodoindoles, see: Worlikar, S. A.; Neuenswander, B.; Lushington, G. H.; Larock, R. C. *J. Comb. Chem.*, **2009**, *11*, 875.

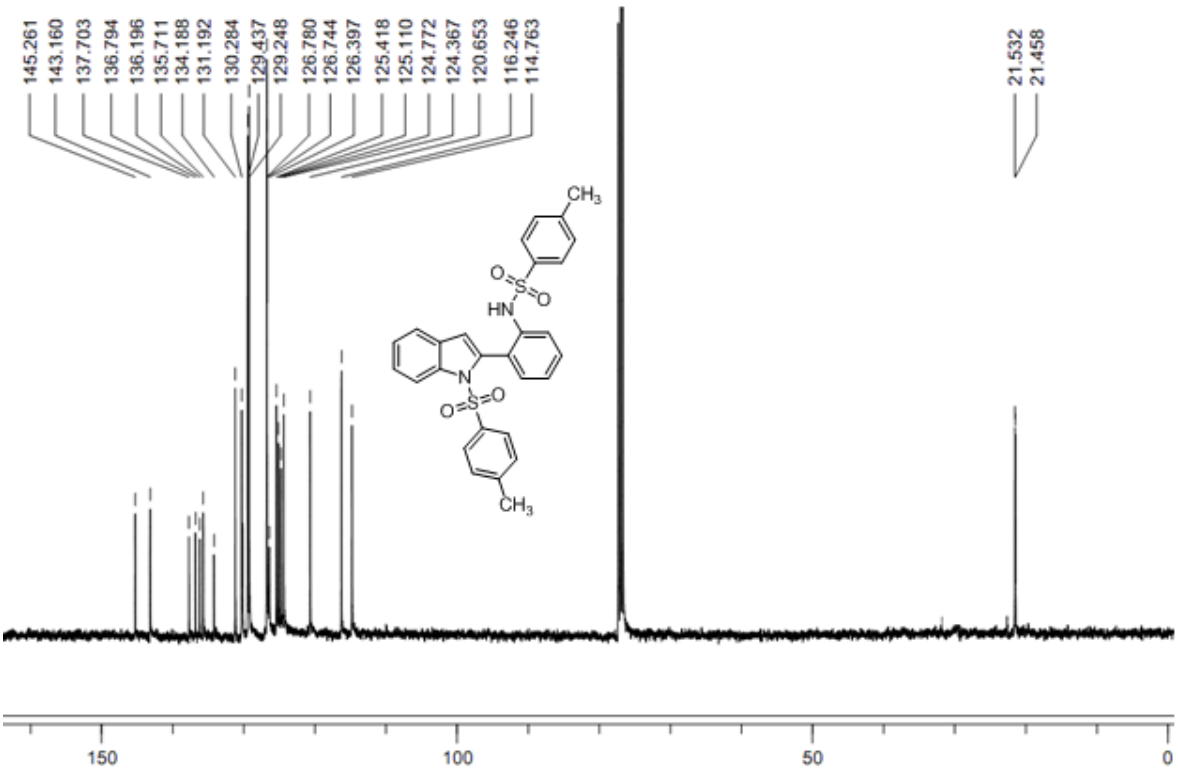
Appendix

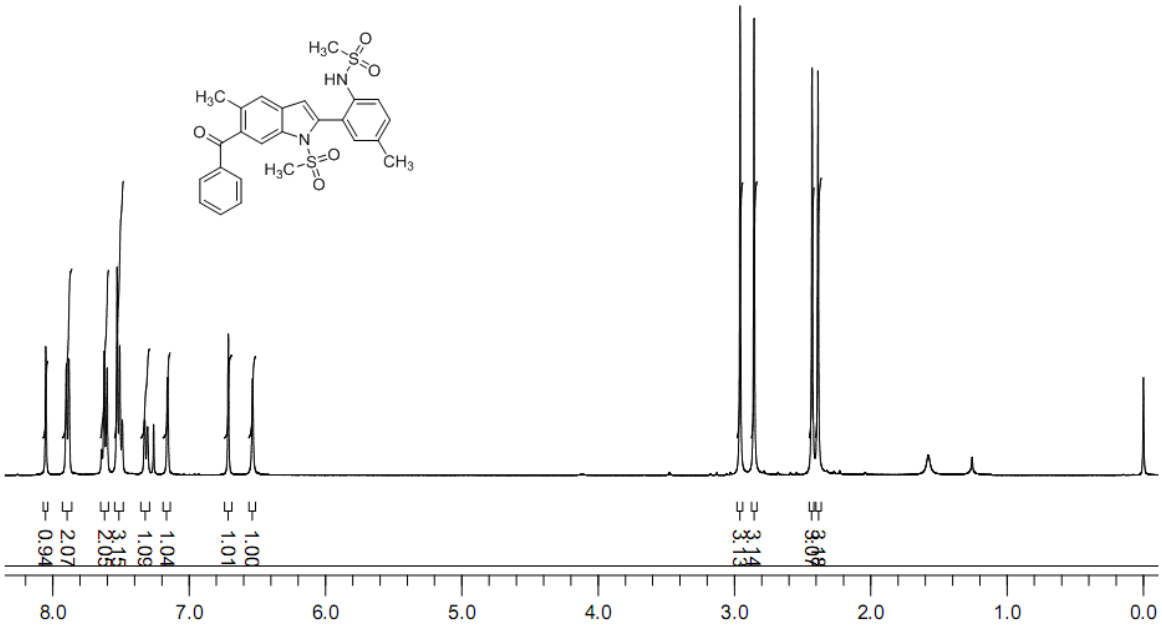

1.00
1.01
1.08
4.52
1.45
0.92
0.97
3.38
3.24
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
137.332
137.009
135.540
131.045
131.022
129.692
125.945
124.736
124.203
123.475
121.427
119.680
115.295
114.422
40.614
40.016
150
100
50
0

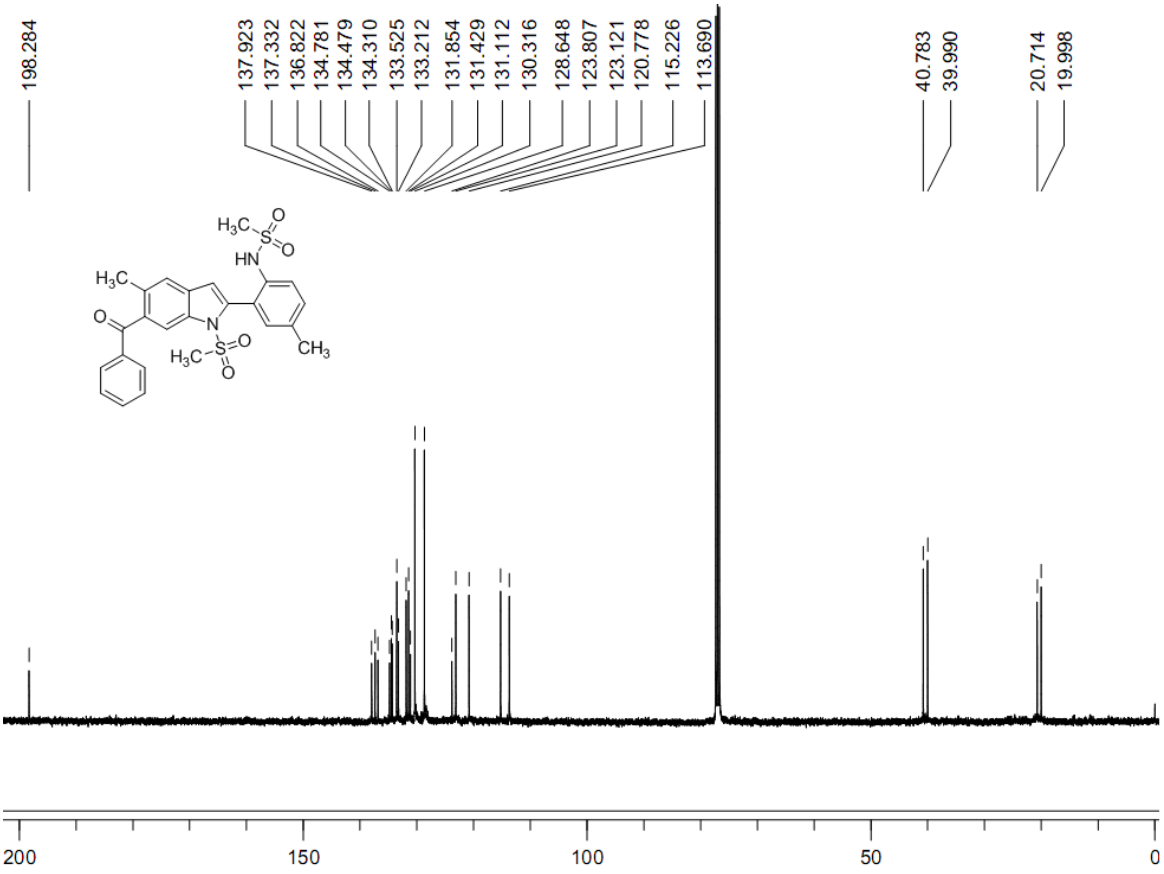

1.09
1.10
1.07
2.99
1.08
1.02
3.37
3.32
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
136.321
135.621
134.560
134.070
131.475
131.339
130.052
127.224
124.572
121.341
121.186
115.099
114.407
40.000
39.941
21.217
20.714
100
50
0

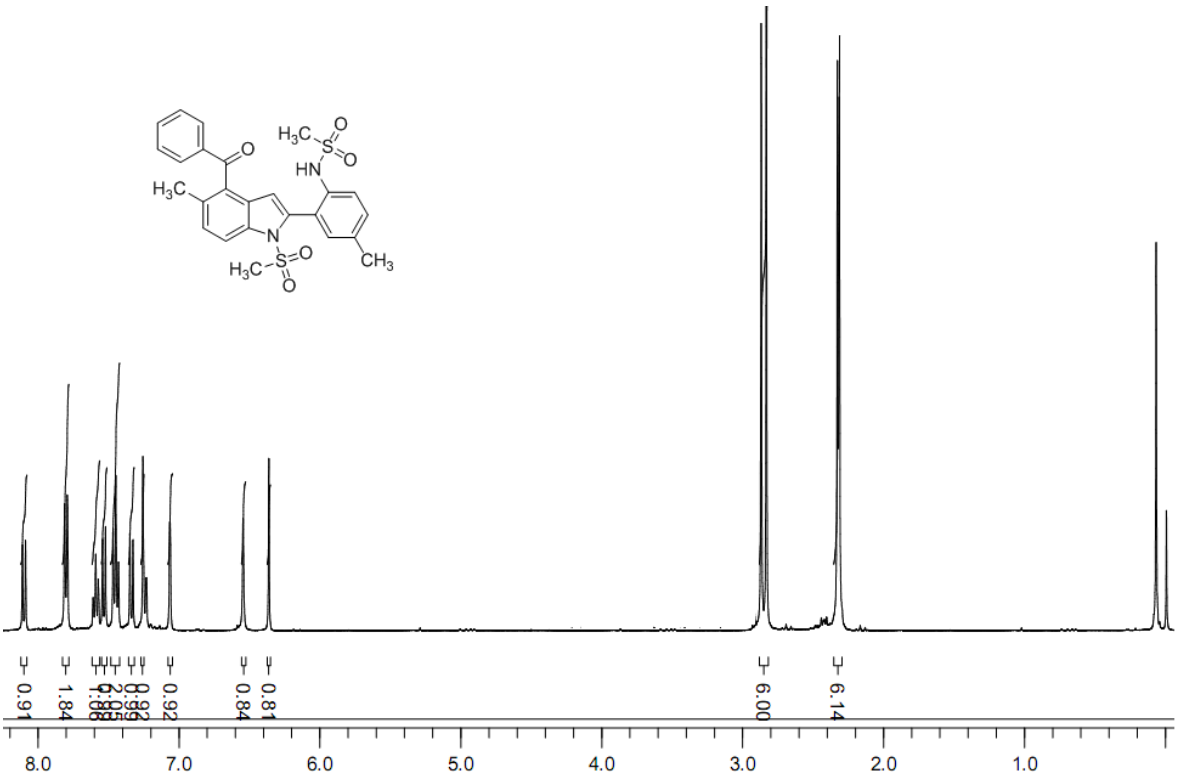

COSY - $CDCl_3$
F2 (ppm)
F1 (ppm)
ppm

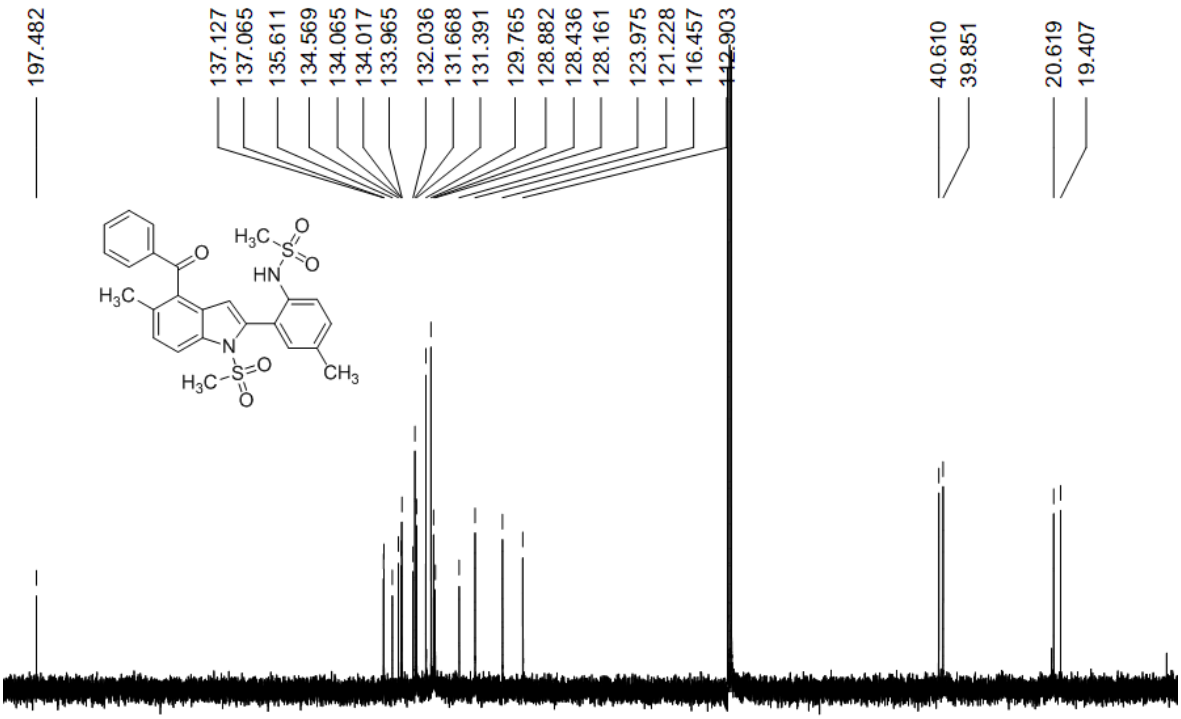

CH3
O
S
O
HN
N
O
S
O
CH3
1.00
0.95
2.17
1.84
3.17
2.07
4.11
0.95
2.08
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

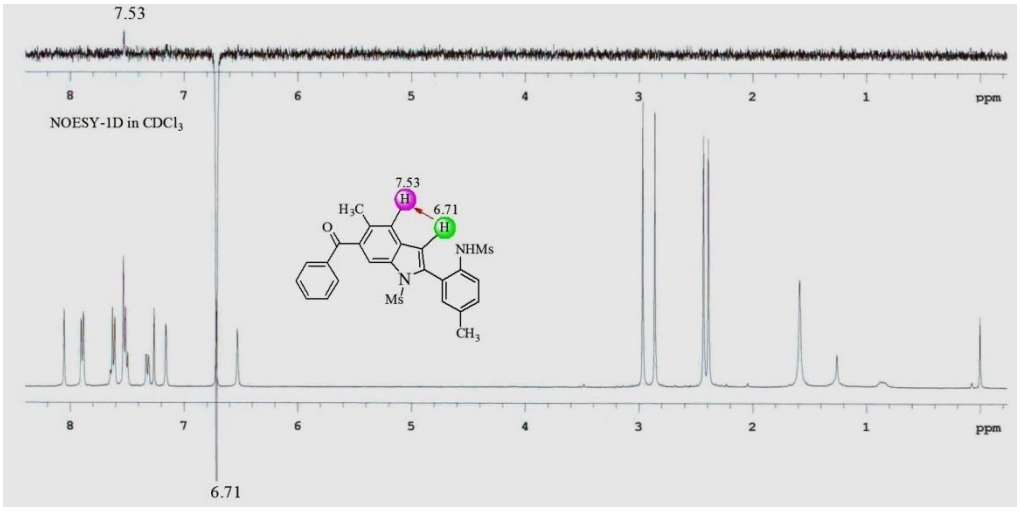

145.261
143.160
137.703
136.794
136.196
135.711
134.188
131.192
130.284
129.437
129.248
126.780
126.744
126.397
125.418
125.110
124.772
124.367
120.653
116.246
114.763
21.532
21.458
CH3
O
S
O
HN
N
O
S
O
CH3
150
100
50
0

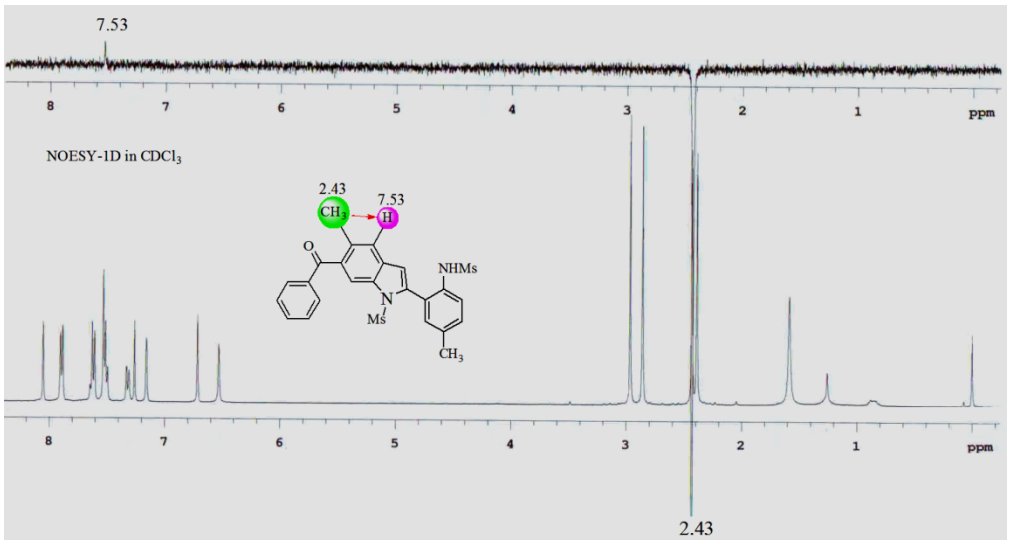

0.94
2.07
3.15
2.05
1.09
1.04
1.01
1.00
3.13
3.18
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

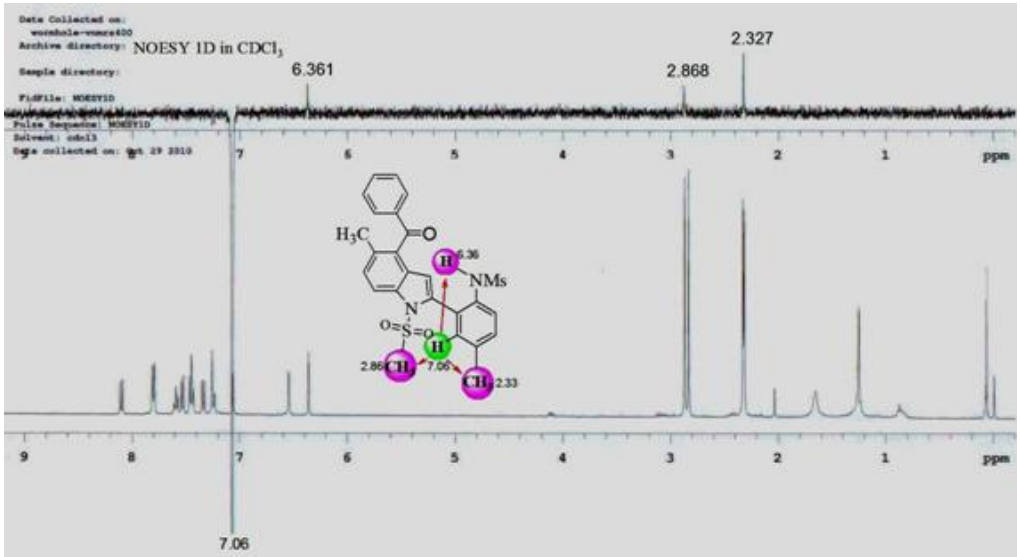

198.284
137.923
137.332
136.822
134.781
134.479
134.310
133.525
133.212
131.854
131.429
131.112
130.316
128.648
123.807
123.121
120.778
115.226
113.690
40.783
39.990
20.714
19.998
200
150
100
50
0


0.91
1.84
0.92
0.84
1.81
6.00
9.14
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0


197.482
137.127
137.065
135.611
134.569
134.065
134.017
133.965
132.036
131.668
131.391
129.765
128.882
128.436
128.161
123.975
121.228
116.457
112.903
40.610
39.851
20.619
19.407


7.53
NOESY-1D in CDCl3
7.53
6.71
NHMs
Ms
CH3
6.71
ppm


7.53
NOESY-1D in CDCl3
2.43
7.53
NHMs
Ms
CH3
2.43
ppm


NOESY 1D in CDCl3
6.361
2.868
2.327
NMs
7.06
ppm