Synthesis, Chemistry and Bioevaluation of Some Bioactive Heterocycles

Submitted in
Fulfillment of the requirements for the degree of
Doctor of Philosophy
by

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Dedicated to my Guruji
and Loving Parents

MALAVIYA NATIONAL INSTITUTE OF TECHNOLOGY, JAIPUR

DECLARATION

I, **Ritu Sharma**, declare that this thesis titled, "**Synthesis, Chemistry and Bioevaluation of Some Bioactive Heterocycles**" and the work presented in it, are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this university.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this university or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
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Date: 03-12-2019

Ms. Ritu Sharma
(2013RCY9527)

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis, Chemistry and Bioevaluation of Some Bioactive Heterocycles"** being submitted by Ms. **Ritu Sharma (2013RCY9527)** is a bonafide research work carried out under my supervision and guidance in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** in the Laboratory of Organic and Medicinal Chemistry, Department of Chemistry, Malaviya National Institute of Technology, Jaipur, India. The matter embodied in this thesis is original and has not been submitted to any other University or Institute for the award of any other degree.

Place: Jaipur
Date: 03-12-2019

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Ritu Sharma

Date: *Student ID: 2013RCY9527*

ABSTRACT

The thesis entitled "**Synthesis, Chemistry and Bioevaluation of Some Bioactive Heterocycles** study emphasis on the formation of bioactive heterocycles utilizing greener approach and carrying out their biological activities.

The present doctoral thesis has been framed into five chapters. The highlights and the results of all the five chapters are summarized below:

Chapter 1: This chapter focused on the concise studies towards the chemistry of benzo-fused nitrogen- and oxygen- containing heterocycles in which the methodology development of several moieties has been exploited efficiently vis metal as well as metal-free synthesis to construct bioactive heterocycles during the last five years (**2013-2018**). Hence, the detailed synthesis of selected biologically active heterocycles such as [1,4] benzoxazines and its related structures, Spirooxindoles, Coumarins, benzopyrans, (*2H*)-indazoles and their similar analogues have been discussed in detail in this chapter.

Chapter 2: This chapter describes the synthesis of a new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo [b] [1,4] oxazin-2-ones in excellent (upto 95%) yields. Several synthesized compounds were found to display greater *in vitro* antibacterial activity against gram positive and gram-negative bacteria i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli* as well as *in vitro* antifungal activity against fungal species i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei,* than standard drugs, ampicillin and chloramphenicol, as well as ketoconazole, respectively. *Cyclopropyl* and *cyclohexylphenyl* substructure moieties were identified to increase both antibacterial and antifungal activities. Electron-withdrawing group (i.e. NO_2 group) present at 2-oxo-benzo[1,4]oxazine nucleus increases antibacterial as well as antifungal activity. To the best of our knowledge, for the first time, pharmaceutically important substructures were incorporated into 2-oxo-benzo[1,4]oxazines, which displayed greater antimicrobial potency than standard drugs, ampicillin, chloramphenicol and ketoconazole.

Chapter 3: The first section of this chapter describes the ultrasonic-assisted synthesis of novel spirooxindolo-pyrrolidine as well as spirooxindolo-pyrrolizine derivatives in good yields by one-pot multi-component reaction of chalcones, isatin and substituted amino acids in methanol under ultrasonication in a sealed vial. The stereochemistry was confirmed by Single crystal X-ray diffraction. The compounds were also

evaluated for their *in vitro* antitubercular and antibacterial activity. The SAR studies were carried out which were validated out by carrying out *in silico* molecular docking studies.

The second section of this chapter focusses on the detailed investigation of the first clear experimental and theoretical evidence of the presence of new allotrope C9 and C12 appeared in nano-star fashion assisted by the organic functionality. In this, we found that the receptor is co-crystallized with the two new carbon forms C9 and C12, respectively.

Chapter 4: This chapter describes, for the first time, an intramolecular Palladium-catalyzed cross-dehydrogenative coupling reaction to synthesize the challenging heterocyclic scaffold i.e., coumarin-benzopyran fused heterocyclic skeleton. The details of the optimization studies are discussed in this chapter.

Chapter 5: This chapter describes the organocatalysed direct C-3 arylation of 2*H*-Indazole via using the stoichiometric amount of 2,3-dihydroxypyridine as an organocatalyst. Several differently substituted derivatives of C-3 arylated *2H*-indazoles were prepared in moderate yields with functional group tolerance.

Conclusion: The present thesis involves the synthesis of bioactive heterocycles via some novel methodologies that are simple and greener as well as the evaluation of the biological activities of the some of the synthesized compounds such as benzoxazine-2-ones, spirooxindole ring fused analogues etc.

AIMS AND OBJECTIVES

The origin of the present research plan has following specific objectives:

- Synthesis of novel analogues of bioactive heterocycles.
- Development of bioactive heterocycles of interest by adopting a greener and environmentally favourable approach.
- Characterization of structures of all novel compounds by their ESI-MS, ^{1}H NMR, ^{13}C NMR, IR, and HRMS spectral data analysis.
- Investigation of biological activity (antibacterial and antifungal, antitubercular activity) of thedifferently synthesized series of compounds.
- Validation of biological activity results *via in silico* molecular docking studies.

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LIST OF ABBREVIATIONS

aq.	Aqueous
a.u	atomic unit
$CDCl_3$	Deuterated Chloroform
DCE	1,2-Dichloroethane
dr	diastereomeric ratio
DMA	Dimethylacetamide
DMEDA	*N*,*N*′-Dimethylethylenediamine
DMF	Dimethylformamide
DMSO-d_6	Deuterated dimethyl sulphoxide
DTBP	Di-*tert*-butyl peroxide
EC_{50}	Half maximal effective concentration
ee	Enantiomeric excess
eq.	equivalents
GI_{50}	Growth inhibition of 50 %
HFIP	Hexafluoroisopropanol
HOMO	Highest Occupied Molecular Orbital
Hz	Hertz
IC_{50}	Inhibitory concentration
kJ	kilojoules
LUMO	Lowest Unoccupied Molecular Orbital
MS	Mass spectroscopy (in mass spectroscopy)
MHz	megahertz
m.p	Melting point
m	meta
MeOH	Methanol
mg	milligram
mL	millilitre
mM	millimoles
MIC	Minimum inhibitory concentration
min	Minutes
N	Normality

N_2	Nitrogen
nM	nanomolar
NMR	Nuclear Magnetic Resonance
o	ortho
p	para
ppm	parts per million
q	quartet
R_f	Retention factor
rt	Room temperature
s	singlet
t	triplet
TBHP	*tert*-Butyl hydroperoxide
TEAB	Tetraethylammonium bromide
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
THF	Tetrahydrofuran
TEA	Triethylamine
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography
TMS	Trimethylsilane
µg	microgram
µM	micromolar

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CHAPTER 1

Recent development in the chemistry of benzo-fused nitrogen- and oxygen-based bioactive heterocycles: An overview.

1.1 Introduction

Nitrogen and/or oxygen-based natural products (NPs), which are derived from higher plants and from terrestrial or marine organisms, have been identified as an ample source of therapeutic medicines, flavours, pesticides, dyes and fragrances for the survival of mankind.[1] Modern era of heterocyclic chemistry had revealed that, the pharmaceutical properties are present in the bioactive heterocyclic NPs and sometimes also serve as traditional medicines.[2] Now-a-days, the identification of lead molecules from bioactive heterocyclic NPs or their analogues, has been identified as a stimulating approach in the process of drug discovery.

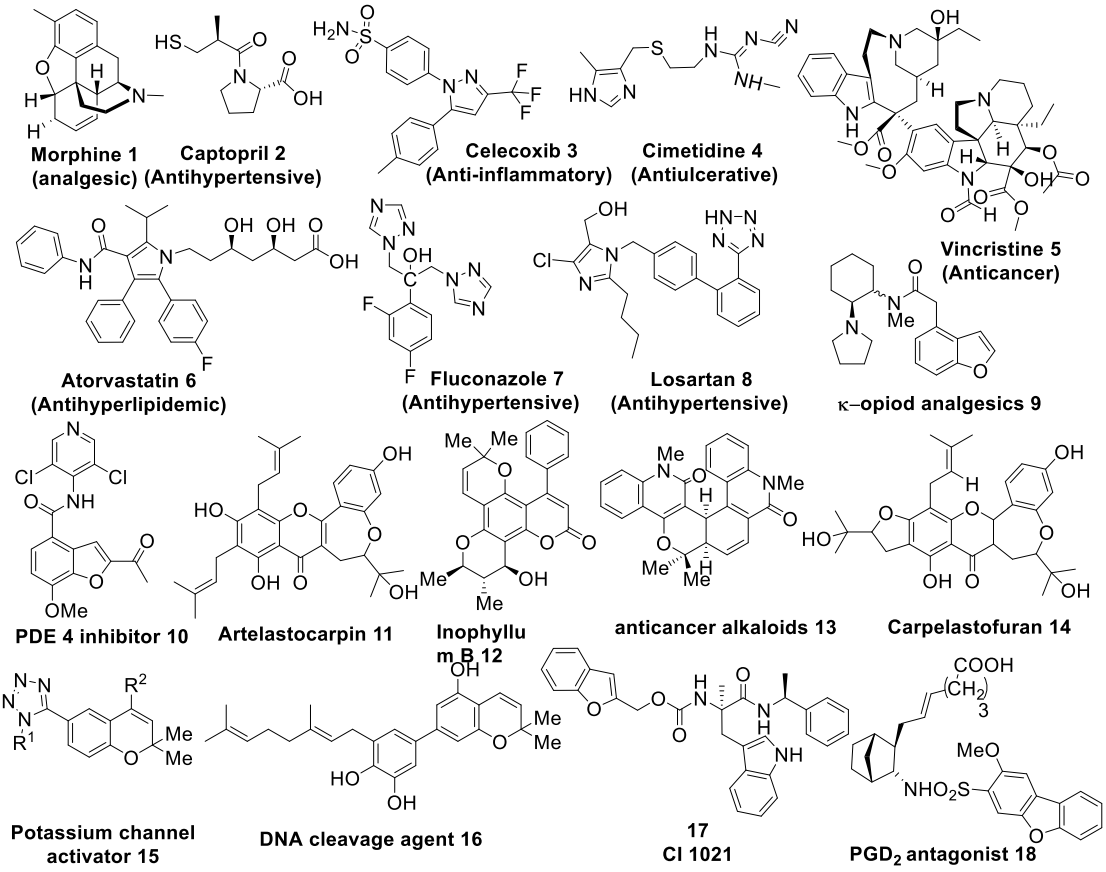


Figure 1. Structure of N-and O-containing commonly used drugs.

Nitrogen-and/or oxygen-based bioactive heterocycles are ubiquitous in both natural and synthetic compounds, most of them are fused polycyclic in nature. Among heterocycles, nitrogen- and/or oxygen-containing heterocyclic compounds have always been the point of attraction to synthetic chemist through decades of historical development of organic synthesis.[3] Nitrogen-and/or oxygen-containing heterocycles have been used as medicinal compounds for centuries, and form the basis for many common drugs such as Morphine **1**, Captopril **2**, anticancer Vincristine **3**, cholesterol reducing Atorvastatin **4**, anti-inflammatory Celecoxib **5**, antiulcerative Cimetidine **6**,

antifungal Fluconazole **7**, and antihypertensive Losartan **8** and several other biologically active synthetic and natural products **9-18**.[4] Thus, it can be revealed that these N- and O- containing heterocyclic moieties are interesting scaffolds in drug design and discovery (Figure 1).

Benzo-fused nitrogen-and/or oxygen-containing heterocycles occur in a diversity of natural products and drugs and are of great importance in a wide variety of applications. In particular, benzo-fused nitrogen- and/or oxygen-containing heterocycles such as **19** and **20** which are found in many natural products **21-30**. These display wide range of biological activities such as antibacterial,[5-6] antifungal,[7] anti-hypertensive,[8] anti-inflammatory,[9] antimalarial,[10] anti-cancer,[11] anti-relaxant,[12] anti-rheumatic,[13]potassium channel opener,[14] central nervous system activity,[15] potassium channel modulator,[16] vaso-dilating activity,[17] dopamine receptor[18] and neuroprotective[19] activities (Figure 2).

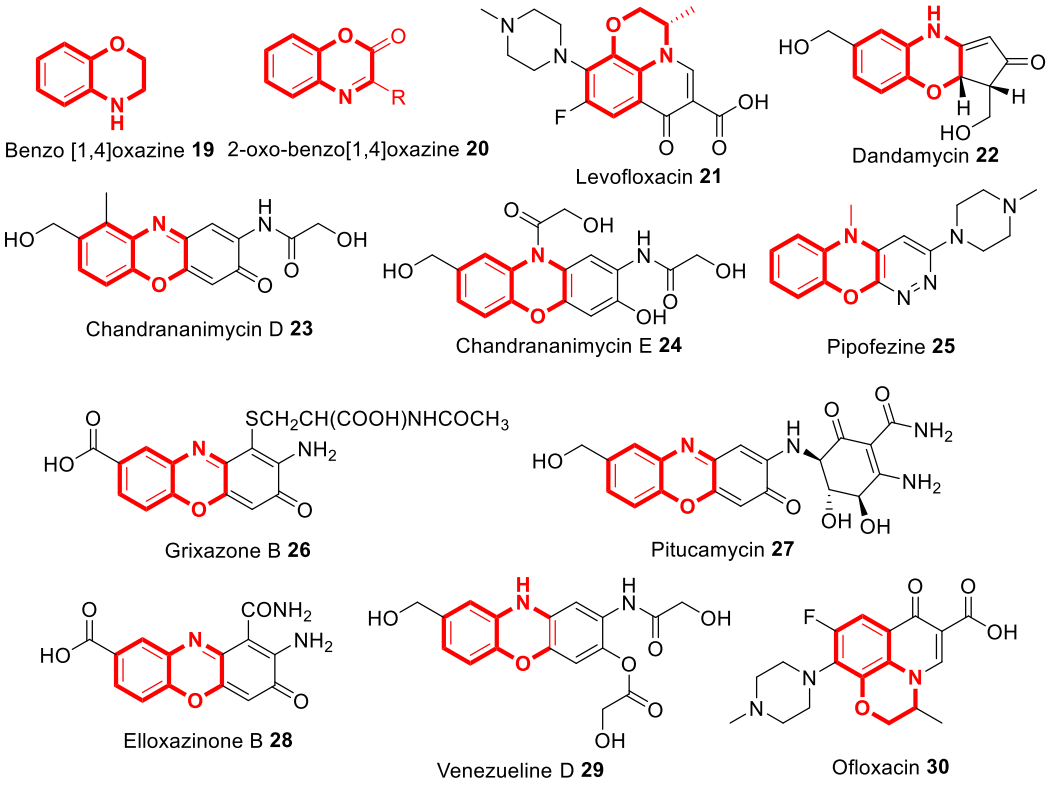


Figure 2. Structures of biologically active molecules **19-30** having benzo[1,4]oxazines moieties.

In this review, we have focused on the concise studies towards the chemistry of benzo-fused nitrogen- and oxygen- containing heterocycles in which the methodology development of several moieties has been exploited efficiently via metal as well as metal-free synthesis to construct bioactive heterocycles during the last five years

(**2013-2018**). Hence, the detailed synthesis of selected biologically active heterocycles such as [1,4] benzoxazines and its related structures, Spirooxindoles, Coumarins, benzopyrans, (*2H*)-indazoles and their similar analogues have been discussed in detail in this chapter.

1.2 Chemistry and Biology of selected biologically active heterocycles such as benzoxazinones, spirooxindoles, coumarins, benzopyrans, (*2H*)-indazoles and their similar analogues

1.2.1 [1,4]Benzoxazines and its related structures

1.2.1.1 Introduction- [1,4]Benzoxazines and its related structures

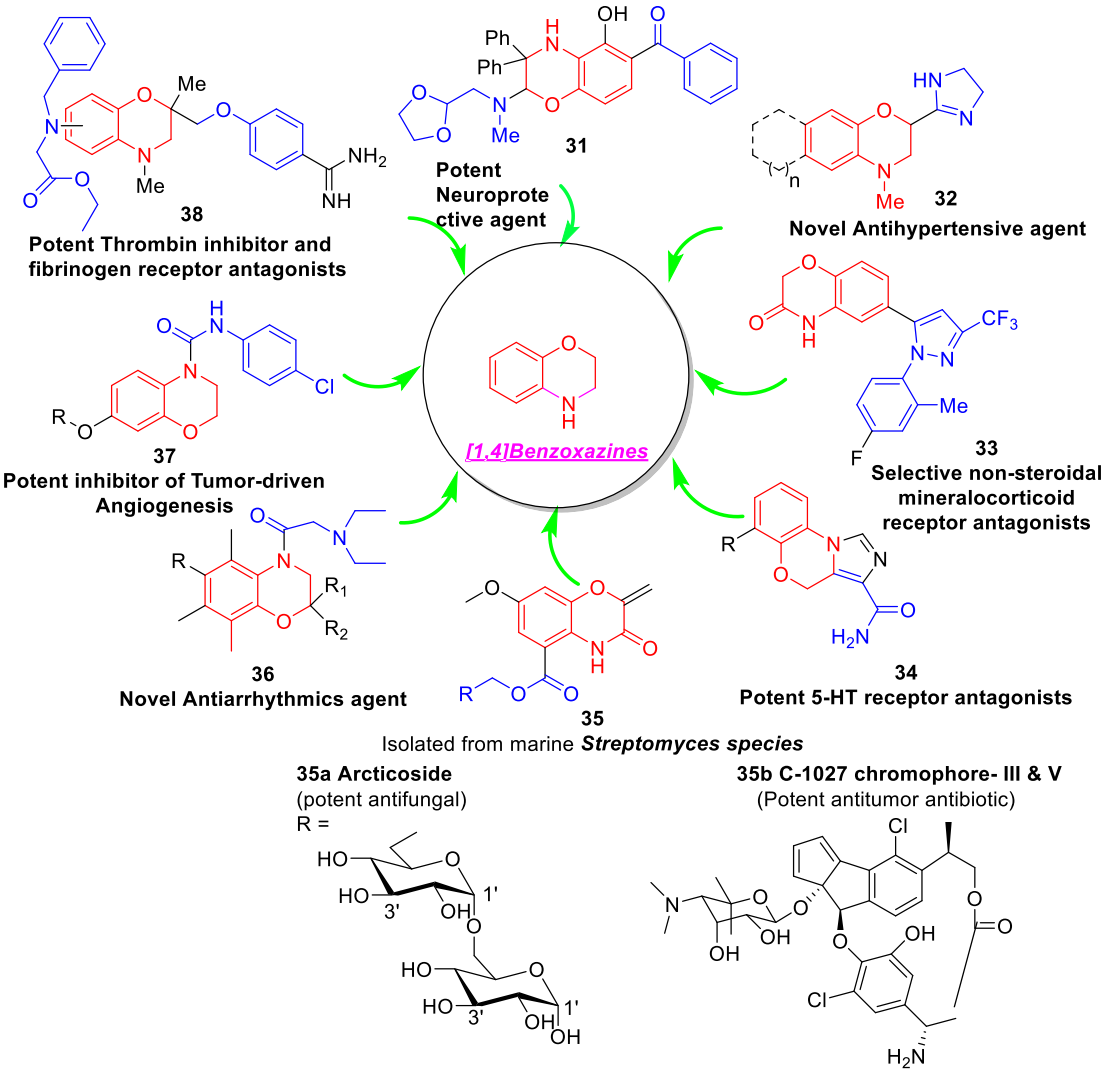


Figure 3. Structure of biologically active benzo [1, 4] oxazines **31-38.**

Benzo[1,4]oxazines **31-38**, a sub-class of benzo-fused heterocycles, had been recognised as biologically active moieties and are endowed with a wide range of activities such as anti-inflammatory,[20] analgesic,[21] antibacterial,[22] neuroprotective,[23]

D2 receptor antagonists,[24] antimycobacterial,[25] antihypertensive,[26] antifungal,[27] herbicidal,[28] antiarrhythmic,[29] thrombin inhibitor and fibrinogen receptor antagonists,[30] 5-HT receptor antagonists,[31] potent inhibitor of tumor-driven angiogenesis[32] and selective non-steroidal mineralocorticoid receptor antagonists[33] etc. Arcticoside 3**5a** (potent antifungal agent) and C-1027 chromophore- III & V **35b** (potent antitumor antibiotic),[34] are the marine secondary metabolites, which were isolated from a culture of an arctic marine actinomycete *Streptomyces strain*; and possesses benzo [1,4] oxazines substructures in their active scaffolds (figure 3).

Owing to the several biological activities having benzo [1,4] oxazines moieties in their scaffold or in whole molecule, several syntheses of benzo [1,4] oxazines and its related structural motifs have been reported in the literature using metal as well as metal-free catalyst.[35] Some of the selected synthesis are reported below:

1. Cu-catalyzed C-N coupling on **41**, which in turn can be prepared from base-catalyzed condensation of **39** with **40**, in the presence of DMEDA furnished benzo[1,4]oxazines **42** in excellent yields (figure 4).[35a]

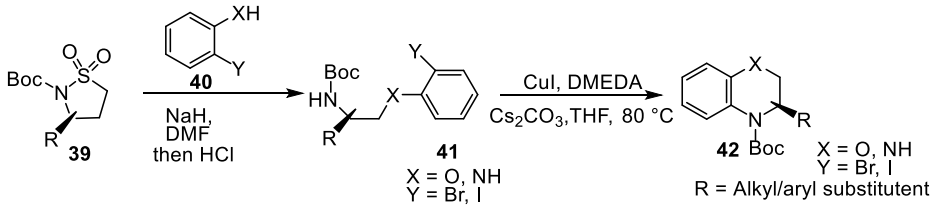


Figure 4. Synthesis of substituted benzo[1,4]oxazines derivatives.

2. The first synthesis of benzo[1,4]oxazines analogues i.e., 3,4-dihydro-1,4-benzoxazin-2-ones **46** have been reported by Kikelj et al. *via* catalytic hydrogenation of 4-benzyl-3,4-dihydro-1,4-benzoxazin-2-one **45**.[35b].[Figure 5]

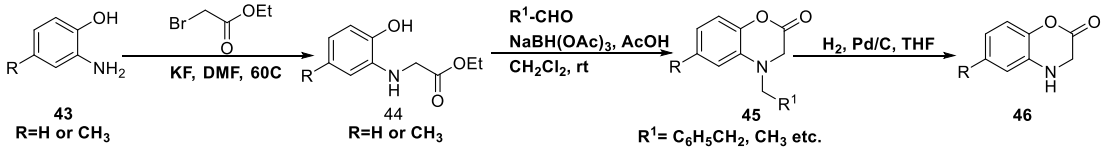
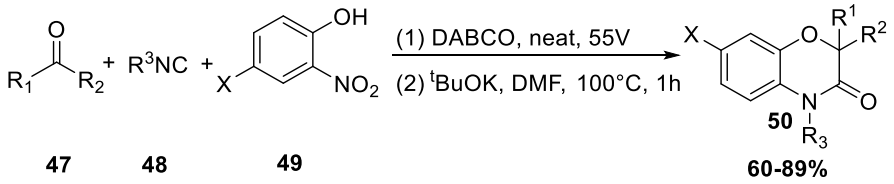


Figure 5. Synthesis of 3,4-dihydro- benzo[1,4]oxazinone derivative **46** by catalytic dehydrogenation of 4- benzyl 3,4-dihydro-1,4-benzoxazin-2-ones **45**.

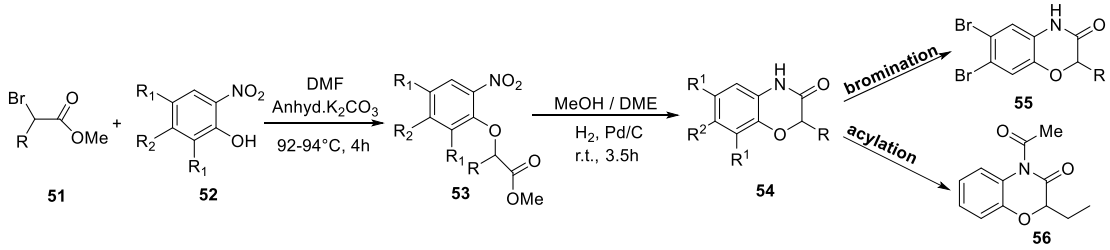
1.2.1.2 [1, 4] Benzoxazin-3-ones class of benzo-fused heterocycles.

Grimaud *et* al. reported a new isocyanide-based multicomponent one-pot access to benzoxazinones via the use of Passerini-Smiles rearrangement displaying a cascade of two Smiles rearrangements coupled with C–C bond formation. It has been proven that the nitro group plays an important role in triggering both Smiles rearrangement process before elimination at the end of the sequence (Scheme 1).[36]



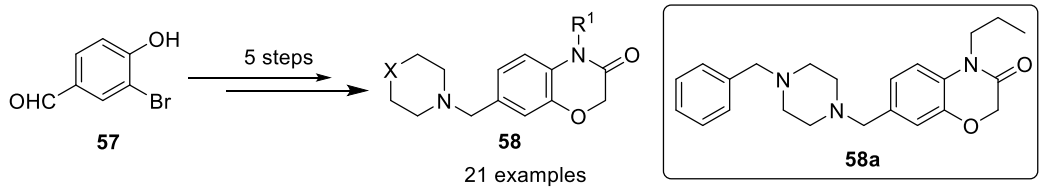
Scheme 1. Synthesis of 1.4-benzoxazin-3-ones by Passerini Smiles rearrangement.

Kwiecień *et.* al. reported a series of 2-alkyl-2H-1,4-benzoxazin-3(4H)-one via a two-step process. The initial step involves O-alkylation of 2-nitrophenols with methyl 2-bromoalkanoates to form 2-nitroester intermediates which on subjection to catalytic reductive cyclisation generates the 2-alkyl-2H-1,4-benzoxazin-3(4H)-one. These [1,4]-benzoxazin-3(4H)-one on further bromination and acylation generated the 2-alkyl-2H-benzo[b][1,4]oxazin-3(4H)-ones, **55** and **56**, respectively (Scheme2)[37]. **55** and **56** showed excellent antifungal activity against seven different strains of fungi.



Scheme 2. Synthesis of 2-alkyl-2H--2H–benzo[*b*][1,4]oxazin-3(4H)-ones.

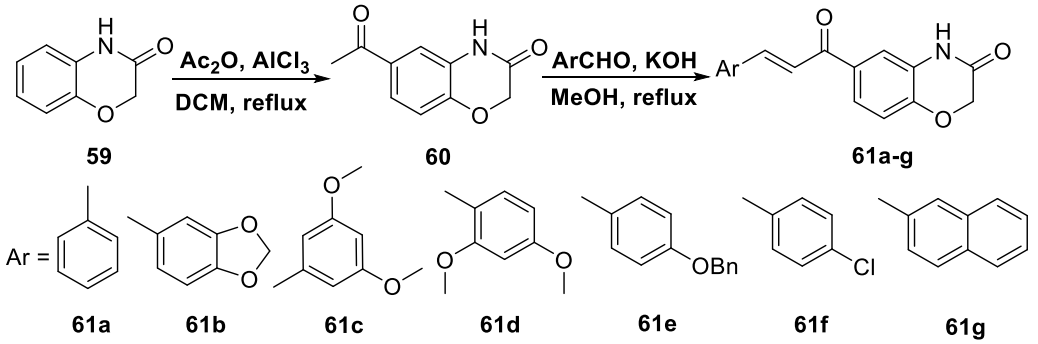
Liang-Feng *et* al. reported a novel series of benzo[*b*][1,4]oxazin-3(4H)-ones which were synthesized via Smiles rearrangement and were found to be potent platelet aggregation inhibitors (Scheme 3).[38] Compound **58a** (IC_{50}=9.20μM) was found to be the most active compound of the series compared with the standard drug aspirin (IC_{50} = 7.07μM). Molecular docking studies indicates that compound **58a** is an antagonist to GPIIb/IIIa receptor (PDB code: 2VDM) by binding to the amino acid residue Ser123 and carbonyl oxygen by H-bond.



Scheme 3. Synthesis of 4,7-disubstituted-2H-benzo[b][1,4]oxazin-3(4H)-ones.

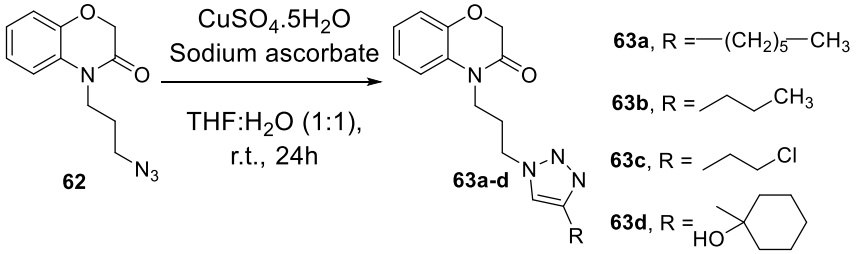
Jun-Ying-Mao and coworkers synthesized a series of compounds **61a-g** by the reaction of 2H-benzo[*b*][1,4]oxazin-3-(4H)-ones with the corresponding aromatic aldehydes(Scheme4)[39]. Compounds **61a-61d** were found to suppress the growth of A549 cancer cells by inducing autophagy and cell cycle arrest. Compound **61d** was

found to be most cytotoxic to the A549 cancer cell line at a dose of 10µM and caused a decrease in density to upto 24% compared to the control drug.



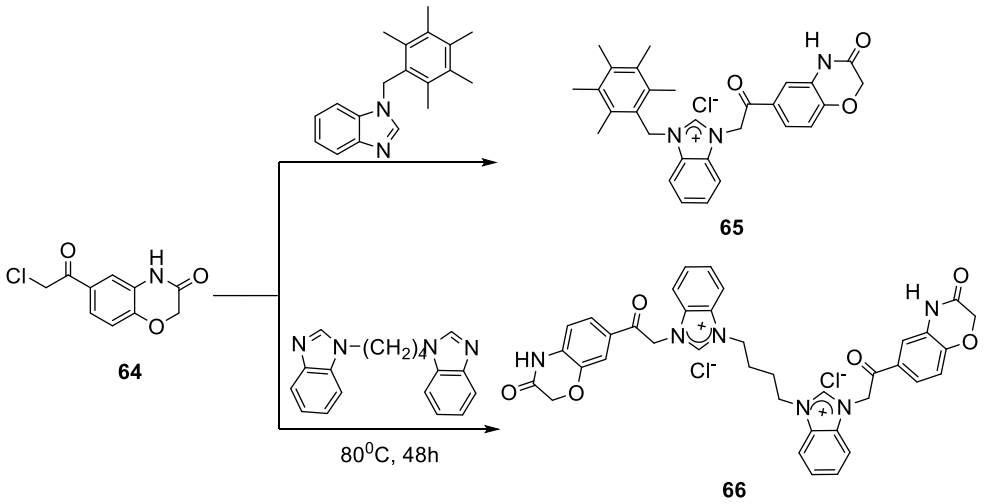
Scheme 4. Synthesis of 6-cinnamoyl-2H-benzo[b][1,4]oxazin-3(4H)-ones **61a-g**.

Nagarapu and co-workers synthesized a series of novel 1, 2, 3-triazole-benzoxazinone hybrids **63a-d** incorporating 1,2,3-triazole moiety with benzoxazinones moiety together (Scheme 5).[40] The hybrids were evaluated for their antiproliferative activity against various cancer cell lines like HeLa, MDA-MB-231, MIAPACA and IMR32 (cervical, breast, pancreatic and neuroblastoma, respectively) by SRB assay. Out of all, the compounds **63a-d** were found to be the most potent compounds of the series. Compound **63a** and **63d** were the most active with GI_{50} value ranging from 1.2-2.5µM and 0.1-1.1 µM, respectively against all the above cancer cell lines, while compound **63b** showed significant activity against MDA-MB-231 and IMR32 with GI_{50}values ranging from 1.1 and 1.4 µM.



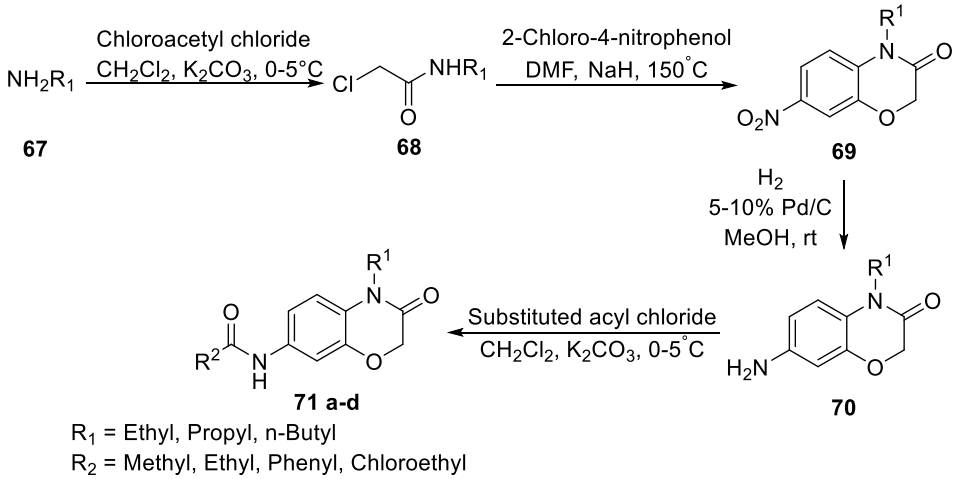
Scheme 5. Synthesis of a novel series of 1, 2, 3-triazole benzoxazinone hybrids **63a-d**.

Gencer and coworker's synthesized benzimidazolium and bisbenzimidazolium hybridized benzoxazinone compounds (**65** and **66**) and evaluated the compounds for the inhibition of human carbonic anhydrase (hCA I and hCA II). The compounds were found to exhibit good inhibitory activities as compared to their parent compound **64**, respectively. This could be a step to extend the medicinal importance of compound towards Glaucoma and epilepsy (Scheme 6).[41]



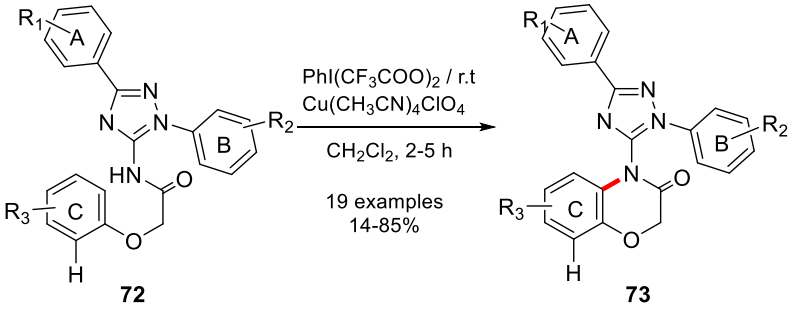
Scheme 6. Synthesis of benzimidazolium and bis (benzimidazolium) salts **65** and **66**.

Yang and Zhu *et* al. synthesised a series of novel platelet aggregation inhibiting 1,4-benzoxazin-3(4H)-one derivatives through Smiles rearrangement, reduction and acetylation reaction. The compound **71c** (IC_{50} = 8.99 μM) and **71d** (IC_{50} = 8.94 μM) proved to be the most potent compounds and it could be proved through molecular docking studies that these molecules were docked into the active site of GPIIb/IIIa receptor. Thus, these compounds could lead to the development of new platelet aggregation inhibitors (Scheme 7).[42]



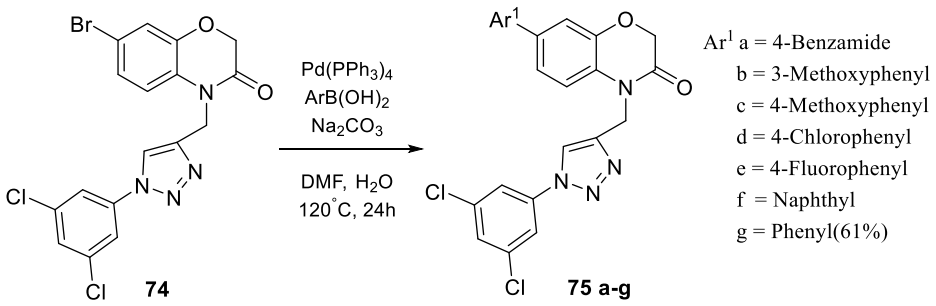
Scheme 7. Synthesis of 1,4-benzoxazin-3(4H)-one derivatives having antiplatelet activity.

Guang-Di Wang *et* al. developed a novel protocol for the synthesis of 2H-1, 4-benzoxazin-3-(4H)-one **73** from **72** by using inexpensive reagents and catalysts. The reaction performs intramolecular amidation of arenes in the presence of copper catalyst and forms the desired benzoxazinone compounds in excellent yields (Scheme 8).[43] The cytotoxicity of the compounds against two metastatic cancer cell lines confirms that the formed derivatives are important templates for the further construction of several small anticancer drug molecules.



Scheme 8. Copper-catalysed synthesis of 2H-1,4-benzoxazin-3-(4H)-one derivatives.

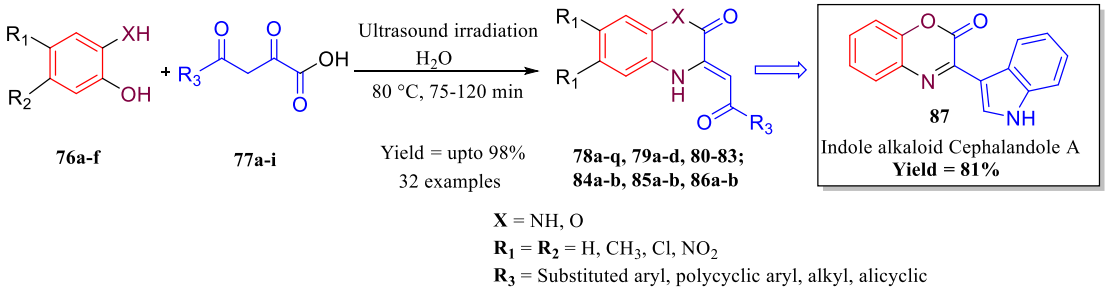
Nagavelli *et* al. synthesised a series of novel 1, 2, 3-triazolo 2H-benzo[*b*][1,4]oxazin-3(4H)-ones **75a-g** and screened for their anticancer activities against MCF-7 (breast) and HeLa (cervical) cell lines (Scheme9).[44] It was found that the compound **75b** and **75c** were found to be the most potent compounds of the series against the cancer cell lines. Compound **75c** was also tested *in vivo* in mice for its anticancer activity which was found comparable to the standard drug cisplatin.[44]



Scheme 9. Synthesis of series of 1, 2, 3-triazolo 2H-benzo[*b*][1,4]oxazin-3(4H)-ones **75a-g** as potent anticancer agents.

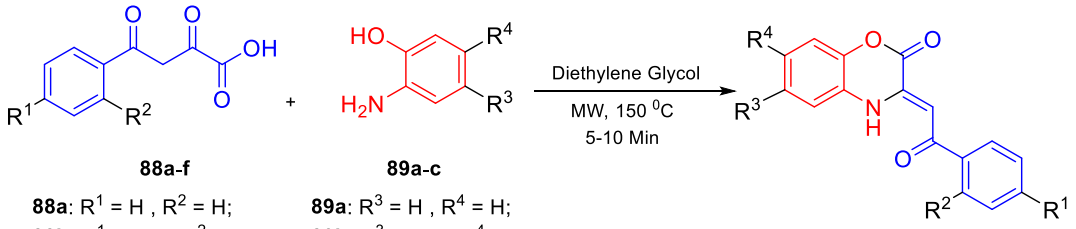
1.2.1.3 [1, 4] Benzoxazin-2-ones class of benzo-fused heterocycles.

Chaudhary *et* al. reported an efficient, simple, synthetic green protocol for the one-pot synthesis of functionalized 2-oxo-benzo [1, 4] oxazines **78-83** in water under ultrasound irradiation using substituted 2-aminophenol **76a-f** and substituted 2,4-dioxo-4-phenylbutanoic acid **77a-i**. As compared to conventional methods, the protocol avoids traditional chromatography and purification steps and furnished the target molecules in excellent yields (upto 98%) with no side products. The methodology was also demonstrated on gram scale synthesis. Moreover, functionalized 2-oxo-quinoxaline analogues **84-86**, another class of bioactive heterocyclic scaffolds, were also prepared using this method. For the first time, this protocol was successfully applied in the synthesis of the anticancer indole alkaloid, Cephalandole A **87** (Scheme 10).[45]



Scheme 10. "On water" ultrasonic-assisted synthesis of functionalized 2-oxo-benzo [1, 4] oxazines **78-86**.

Chaudhary *et* al. reported microwave-assisted, environmentally benign green protocol for the synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3, 4-dihydro-2H-benzo[*b*][1,4]oxazin-2-ones **90a-n** in excellent yields (upto 97%) and (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-ones **93a-h** (upto 96% yield) (Scheme11,12,13).[46]

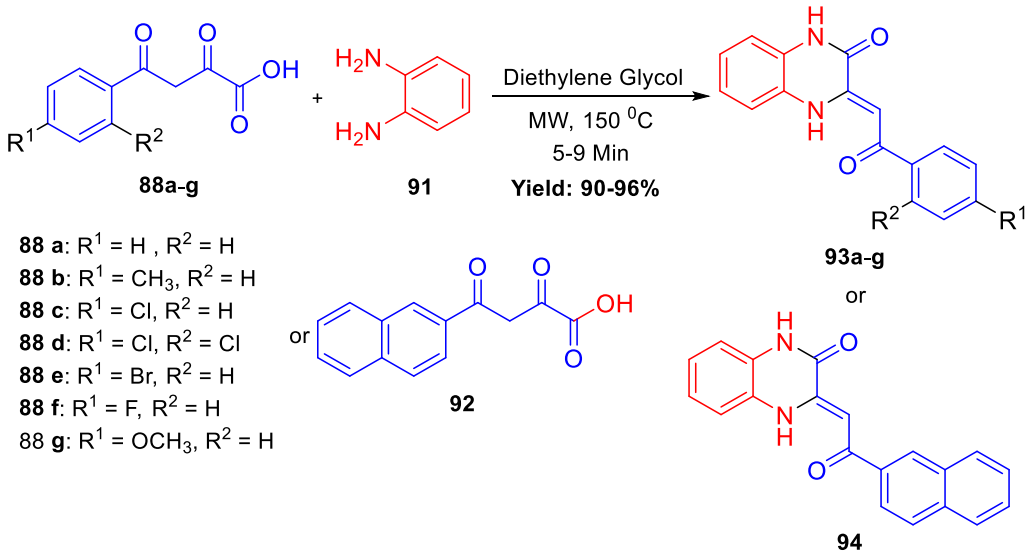


88a: R^1 = H , R^2 = H;
88b: R^1 = CH_3, R^2 = H;
88c: R^1 = Cl, R^2 = H;
88d: R^1 = Cl, R^2 = Cl;
88e: R^1 = F, R^2 = H;
88f: R^1 = Br, R^2 = H;

89a: R^3 = H , R^4 = H;
89b: R^3 = CH_3, R^4 = H;
89c: R^3 = NO_2, R^4 = H;

Scheme 11. Microwave-assisted synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3, 4-dihydro-2H-benzo[*b*][1,4]oxazin-2-ones **90a-n**.

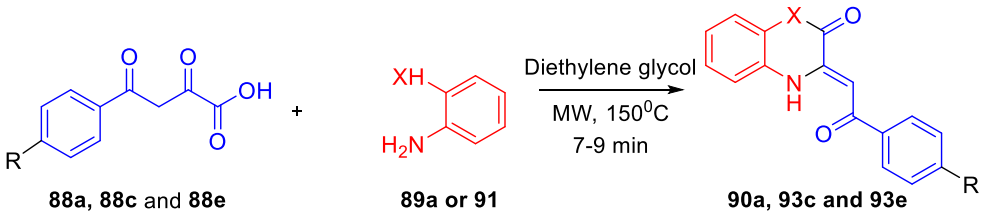
The synthesized compounds **90a-n** and **93a-h** were assessed for their *in vitro* antioxidant activities in DPPH radical scavenging and FRAP assay.



88 a: R^1 = H , R^2 = H
88 b: R^1 = CH_3, R^2 = H
88 c: R^1 = Cl, R^2 = H
88 d: R^1 = Cl, R^2 = Cl
88 e: R^1 = Br, R^2 = H
88 f: R^1 = F, R^2 = H
88 **g**: R^1 = OCH_3, R^2 = H

Scheme 12. Microwave-assisted synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1H)-ones **93a-g** and **94**.

In DPPH assay, compounds **90a**, **93c** and **93e**, the most active compounds of the series, were found to show IC_{50} value of 10.20 ± 0.08 µg/mL, 9.89 ± 0.15 µg/mL and 8.97 ± 0.13 µg/mL, respectively in comparison with standard reference (ascorbic acid, IC_{50} = 4.57 µg/mL). Whereas, in FRAP antioxidant assay seven compounds (**90c**, **90e**, **90i**, **90k**, **90l**, **93d** and **94**) displayed higher antioxidant activity in comparison to the reference standard BHT ($C_{0.5}$FRAP = 546.2 µM). Moreover, the cytotoxic studies of the compounds **90a**, **93c**, **93e** and **94** were found to be non-toxic in nature in $3T_3$ fibroblast cell lines using MTT assay.

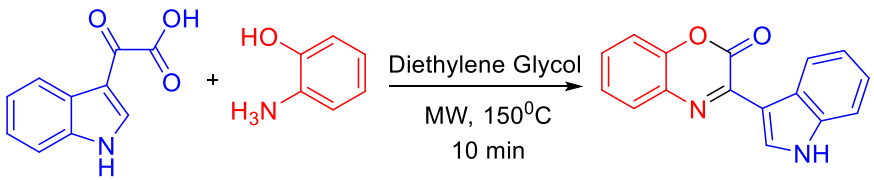


88a, 88c and **88e** | **89a or 91** | **90a, 93c and 93e**

88a: R = H; (2.00 g, 10.40 mmol) **89a**: X = O (1.13 g, 10.40 mmol) **90a**: R = H; X = O (2.49 g, 90.41%)
88c: R = Cl; (2.26 g, 10.00 mmol) **91**: X = NH (1.08 g, 10.00 mmol) **93c**: R = Cl: X = NH (2.60 g, 87.32%)
88e: R = Br; (2.71 g, 10.00 mmol) **93e**: R = Br: X = NH (3.09 g, 90.14%)

Scheme 13. Gram scale synthesis of **90a, 93c** and **93e.**

The practical applicability of the developed methodology were also confirmed by the gram scale synthesis of **90a**, **93c** and **93e**; synthesis of anticancer alkaloid Cephalandole A **96** (89% yield).

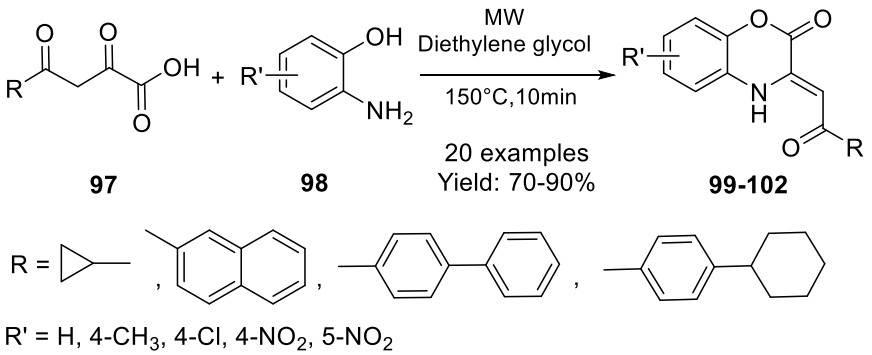


95 | **89a** | Cephalandole A **96** Yield = 89%

Scheme 14. Synthesis of Cephalandole A **96.**

Chaudhary *et* al. synthesized a new series of functionalized (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[b] [1,4]oxazin-2-ones **99-102**, incorporating pharmaceutically privileged substructures such as cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl in excellent yields under microwave irradiation conditions.[47] All the synthesized compounds were screened for their *in vitro* antibacterial activity against gram-(+)ve and gram-(-)ve bacterial species i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli* as well as *in vitro* antifungal activity against fungal species i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei,* respectively. In this study, compounds containing *cyclopropyl* and *cyclohexylphenyl* substructures were identified as promising antimicrobial agents than standard drug ampicillin and ketoconazole. SAR study illustrates that electron-withdrawing groups increases the antibacterial as well as

antifungal activity of 2-oxo-benzo[1,4]oxazines and vice versa. Compounds **99d-e** and **102d-e** are the most active compounds of the series which display promising antimicrobial activity than standard drug Ampicillin and Ketoconazole. The cytotoxic studies of the active compounds i.e. **99c-e**, **100e**, **101d** and **102d-e** were found to be non-toxic in nature in $3T_3$ fibroblast cell lines using MTT assay.



Scheme 15. Synthesis of (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-ones **99**-**102**.

1.2.2. Oxindole ring fused spirocyclic compounds

1.2.2.1. General introduction about spirooxindoles

Spirooxindoles class of heterocyclic frameworks are of great interest in the area of synthetic organic chemistry and medicinal chemistry like spirooxindole pyrrolidine, spirooxindole pyran, spirooxindole piperidine and spirooxindole pyrrolizidine derivatives and are endowed with a wide range of pharmacological activities such as aldose reductase inhibitors (ARIs), which help to treat and prevent diabetic complications arising from elevated levels of sorbitol,[48] act as poliovirus and rhinovirus3C proteinaseinhibitors,[49] inhibitors of Monoamine oxidase (MAO) in human urine and rat tissues,[50] inhibition of several enzymes such as acetylcholinesterase (AChE),[51] and atrial natriuretic peptides stimulated gyuanylase cyclase and potent antagonist of *in vitro* receptor binding by atrial natriuretic peptides,[52] Spirooxindole Schiff's bases and mannich base of isatin possess antibacterial,[53]antifungal,[54] antiviral,[55] anti-HIV,[56] antihelminthic,[57] and antiprotozoal activities.[58]

Most of the oxindoles are derived by the reaction at C-3 carbon of isatin, a highly reactive carbonyl group and is a prochiral centre as well. Various synthetic strategies have been reported in the literature for the synthesis of diverse spirooxindole heterocycles. The azomethine ylide represents one of the most reactive and versatile

classes of 1,3-dipoles and is readily trapped by a range of dipolarophiles forming substituted pyrrolidines.[59]

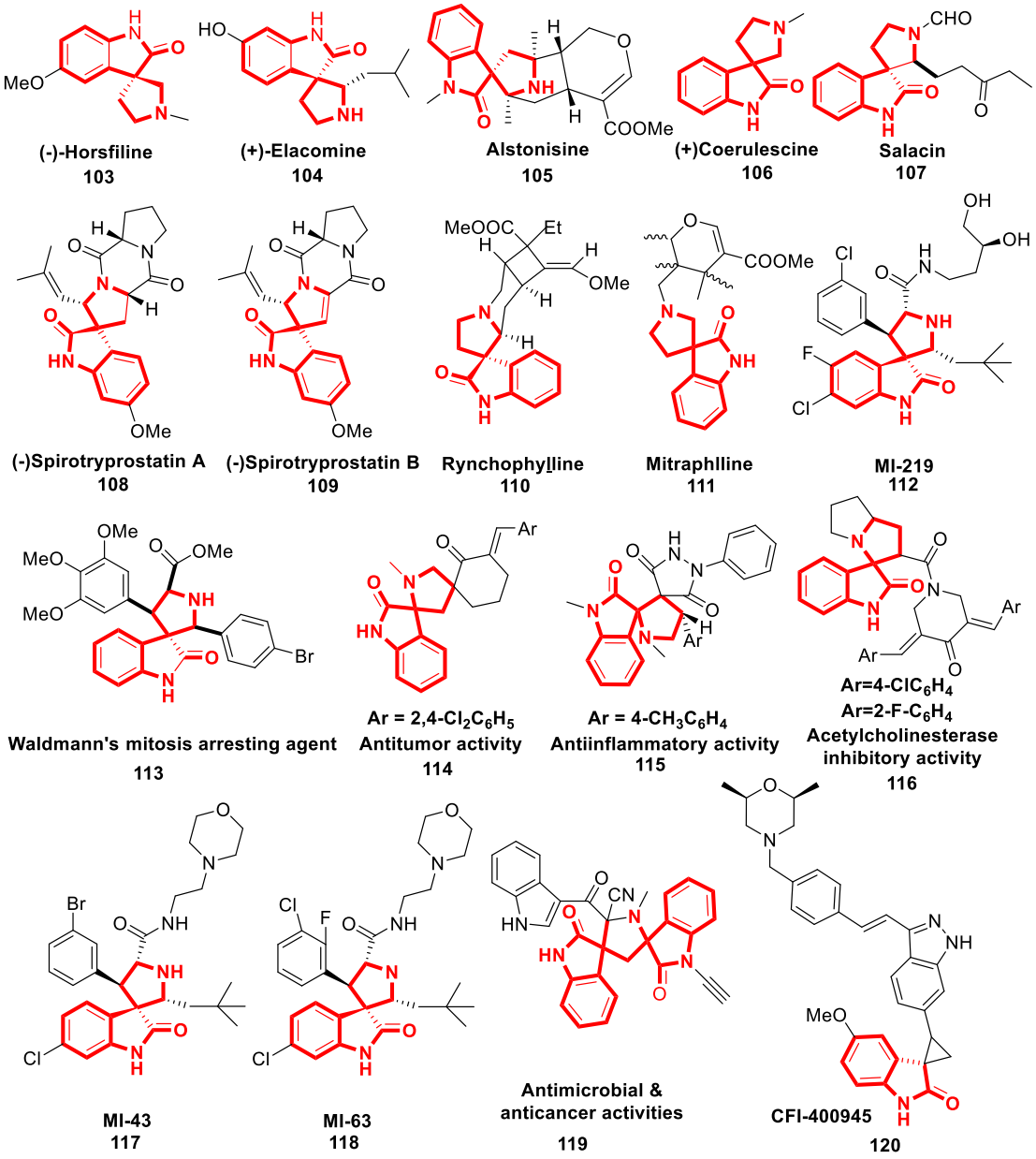


Figure 6. Structure of some biologically active spirooxindole class of heterocycles.

Wang *et* al. described MI-219 **112** as a potent MDM2 inhibitor.[60]Spirooxindole class of molecules are present in several natural products and form the backbones for numerous alkaloids like Horsfiline **103**, Elacomine **104**,[61] Alstonisine **105**, Spirotryptostatin A **108**, Belacomine etc and show wide range of biological activities such as antimicrobial,[62] anticancer,[63] antimycobacterial,[64] antimalarial,[65] anti-HIV,[66] AChE inhibitor,[67] β-secretase (BACE1) inhibitor,[68] p38alpha inhibitor,[69] NaV1.7 blocker,[70] p53 activity modulator,[71] and MDM2-p53 interaction inhibitor,[72] antitumoral, antibiotic agents and inhibitors of human NK-1receptor[73] (Figure 1).

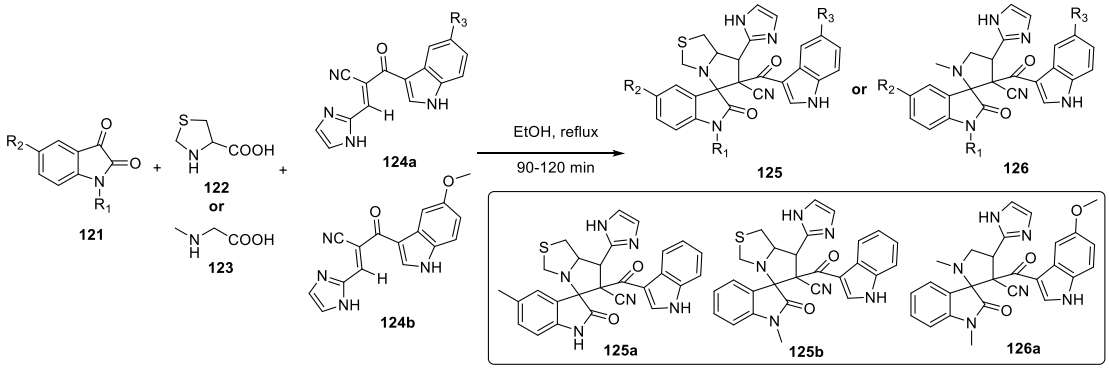
Mitraphylline **111** possess antitumor activity against human brain cancer cell lines neuroblastoma SKN-BE (2) and malignant glioma GAM.[74] Spirotryprostatins inhibit mammalian cell cycle at G2/M phase.[75] Rhyncophylline **110** is used as anti-hypertensive, antipyretic and as an anticonvulsant medications for the treatment of headache, vertigo and epilepsy,[76] use in traditional and modern medicine,[77] as well as also act as non-competitive NMDA receptor antagonist.[78] Fiduxosin is an adrenoreceptor antagonist and act as promising pharmaceutical agent for the treatment of benign prostatic hyperplasia (Figure1).

Multicomponent reactions– multicomponent reactions are the reactions in which three or more starting materials are brought together in a highly convergent approach to rapidly build up molecular structure and complexity and offers advantage over conventional.[79] Various multicomponent reactions by different mechanisms are employed for the synthesis spirooxindole compounds. [3+2] cycloaddition reaction are a kind of multi-component reaction used for the preparation of several spiro-N-heterocyclic compounds in a highly regio- and stereo-selective manner.[80] Various other strategies include the use of ultrasonic-assisted reactions,[81] microwave irradiations,[82] organocatalytic or metal catalysis, nanoparticle catalysis, ionic liquid mediated reactions, deep eutectic solvent mediated reaction etc.

In this section, we wish to focus on some recent studies carried out from **2013** onwards about the synthesis as well as biological activities of spiropyrrolidine and spiropyrrolizine ring-based spirocyclic compounds that were derived from isatin

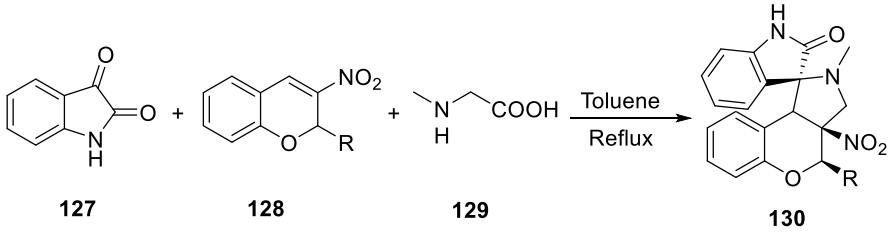
1.2.2.2 Synthesis and biological importance of spiropyrrolidines via 1, 3-dipolar cycloaddition reaction strategy.

Arun et al. reported the synthesis of novel spirooxindole pyrrolidine compounds via 1,3-dipolar cycloaddition of azomethine ylides generated by reaction of isatin **121** with sarcosine **123** or thioproline **122** with the dipolarophile 3-(1Himidazol-2-yl)-2-(1H-indole-3-carbonyl)acrylonitrile **124**. All the synthesized compounds were assessed for their *in vitro* anticancer activity against A549 human lung adenocarcinoma cancer cell line. Out of **29** tested compounds; **125a**, **125b** and **126a** were highly active with 66.3%, 64.8% and 66.3% at 25 mg/mL concentration against A549 lung adenocarcinoma cancer cell line (Scheme16).[83]



Scheme 16. Synthesis of spirooxindole pyrrolidines **125** and **126** from substituted isatin **121**, sarcosine **123** or thioproline **122** and dipolarophile **124a** or **124b**.

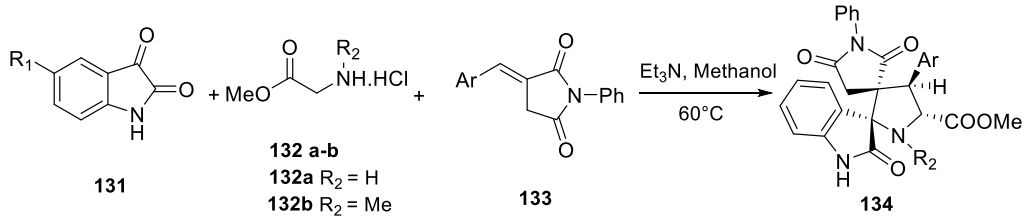
Raghunathan *et* al. synthesized 3-nitrochromane grafted novel pyrrolidinyl-spirooxindoles **130** by 1,3-dipolar cycloaddition reaction of azomethine ylides generated from isatin and secondary amino acids with 3-nitrochromenes (Scheme 17).[84] Through this reaction, the products were obtained regioselectively in high yields. The regioselectivity of the cycloaddition reaction was confirmed by X-ray diffraction.



Scheme 17. Synthesis of 3-nitrochromanes grafted pyrrolidinyl-spirooxindoles **130**.

Askri and coworker's synthesized a series of spiropyrrolidin-2, 3'-oxindoles **134a-r** via 1, 3-dipolar cycloaddition reaction via azomethine ylides generated *in situ* by exo attack of the dicarbonyl compounds on the azomethine ylide (Scheme 18).[85] Endo product is not formed in the reaction and a kinetically controlled product is formed as confirmed by X-ray diffraction and DFT calculations. The compounds were analyzed for their *in vitro* antibacterial, antifungal, antimalarial and antitubercular activities. The compound **134r** was found to be the most potent with a MIC of 62.5 µg/ml against MTCC 1688 in case of *Pseudomonas aeruginosa*. In case of *Staphylococcus aureus* and *Staphylococcus pyogens*, **134f** was found to be the most active compound with a MIC of 62.5µg/ml against MTCC 96. In addition, compounds **134n** and **134p** showed high order of antifungal activity as compared to antifungal drug Griseofulvin. Antimalarial activity of these compounds were determined against *P. falciparum* 3D7 Chloroquine sensitive strain taking Chloroquine and Quinine as reference drugs. Only **134q** was found to show much better IC_{50} value than quinine as a standard drug. It

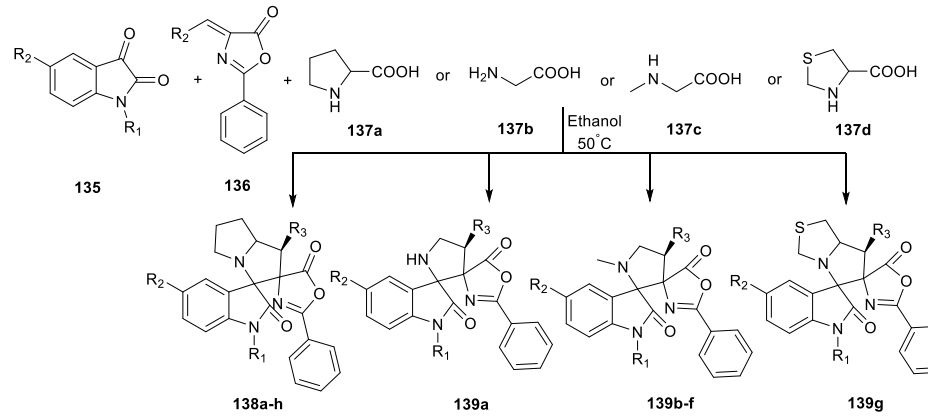
may be due to the fact that compound **134q** has sufficient H-bonding and desired lipophilicity or favourable steric hindrance. Compounds **134b**, **134g**, **134h** and **134m** showed best activity against *M. Tuberculosis* $H_{37}R_V$.



134a R_1 = H, R_2 = H, Ar = C_6H_5
134b R_1 = H, R_2 = H, Ar = *p*-MeC_6H_4
134c R_1 = H, R_2 = H, Ar = *p*-$OMeC_6H_4$
134d R_1 = H, R_2 = H, Ar = *p*-ClC_6H_4
134e R_1 = H, R_2 = H, Ar = *p*-BrC_6H_4
134f R_1 = Br, R_2 = H, Ar = C_6H_5
134g R_1 = Br, R_2 = H, Ar = *p*-MeC_6H_4
134h R_1 = Br, R_2 = H, Ar = *p*-$OMeC_6H_4$
134i R_1 = Br, R_2 = H, Ar = *p*-Cl-C_6H_4
134j R_1 = Br, R_2 = H, Ar = *p*-Br-C_6H_4
134k R_1 = NO_2, R_2 = H, Ar = C_6H_5
134l R_1 = NO_2, R_2 = H, Ar = *p*-MeC_6H_5
134m R_1 = NO_2, R_2 = H, Ar = *p*-$OMeC_6H_5$
134n R_1 = NO_2,R_2 = H, Ar = *p*-ClC_6H_5
134o R_1 = NO_2, R_2 = H, Ar = *p*-BrC_6H_5
134p R_1 = H, R_2 = Me, Ar = C_6H_5
134q R_1 = H, R_2 = Me, Ar = *p*-MeC_6H_4
134r R_1 = H, R_2 = Me, Ar = *p*-Cl-C_6H_4

Scheme 18. 1,3-Dipolar cycloaddition reactions for the synthesis of spiropyrrolidine oxindoles **134a-r**.

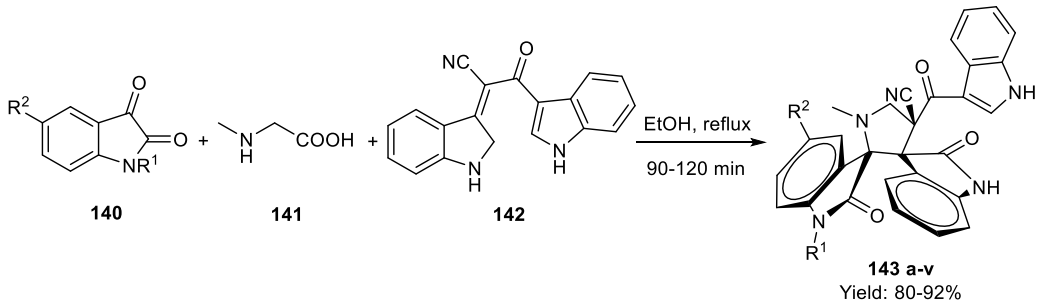
Ouyang and coworkers developed a facile method for the synthesis of oxazolones-grafted spirooxindole-pyrrolidine **138a-h**, pyrrolizidines **139a-f** and pyrrolothiazoles **139g** via 1,3-dipolar cycloaddition reaction of substituted benzylidene-2-phenyloxazolone **136** with azomethine ylides, which were generated *in situ* from diverse isatins **135** and amino acids **137a-d** under mild conditions(Scheme 19).[86] The generated 15-membered library was found to be regio- and stereoselective. After screening their anticancer activities against different cell-lines, compound **138h** was found to be the most potent apoptosis inducing antitumor agent. Hence, a rapidly constructed chemical library of oxazolones-grafted spirooxindoles could possibly be developed as a promising lead compound for further exploration.



138a R_1 = R_2 = H, R_3 = C_6H_5
138b R_1 = R_2 = H, R_3 = $3ClC_6H_4$
138c R_1 = R_2 = H, R_3 = 3,4-$Cl_2C_6H_3$
138d R_1 = $CH_2C_6H_5$, R_2 = H, R_3 = C_6H_5
138e R_1 = $CH_2C_6H_5$, R_2 = H, R_3 = 3,4-$Cl_2C_6H_5$
138f R_1 = H, R_2 = Cl, R_3 = 3-Cl-C_6H_4
138g R_1 = $CH_2C_6H_5$, R_2 = H, R_3 = 3,4,5-$(OCH_3)_3C_6H_2$
138h R_1 = R_2 = H, R_3 = 4-$CF_3C_6H_4$
139a R_1 = R_2 = H, R_3 = C_6H_5
139b R_1 = R_2 = H, R_3 = C_6H_5
139c R_1 = Methyl, R_2 = H R_3 = C_6H_5
139d R_1 = $CH_2C_6H_5$, R_2 = H R_3 = C_6H_5
139e R_1 = $CH_2C_6H_5$, R_2 = H, R_3 = 3,4-$Cl_2C_6H_5$
139f R_1 = $CH_2C_6H_5$ R_2 = H, R_3 = furan
139g R_1 = R_2 = H, R_3 = C_6H_5

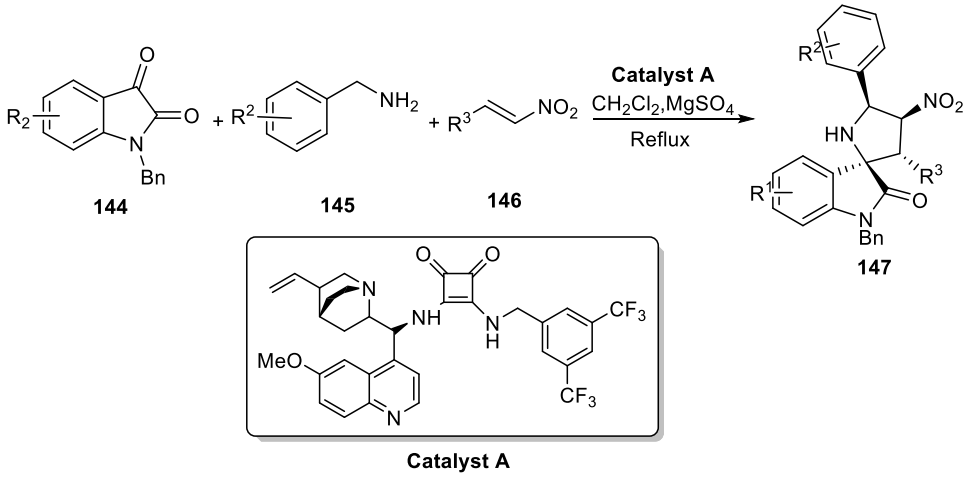
Scheme 19. Synthesis of oxazolones-grafted spirooxindole-pyrrolidine **138a-h**, pyrrolizidines **139a-f** and pyrrolothiazoles **139g** via 1,3-dipolar cycloaddition reaction strategy.

Perumal *et* al. synthesized a series of novel dispirooxindole pyrrolidines **143a-v** and assessed for their *in vitro* antifungal, antibacterial and anticancer activities. Compounds **143a-v** showed good anticancer activity against human lung adenocarcinoma cancer cell line (Scheme 20).[87]



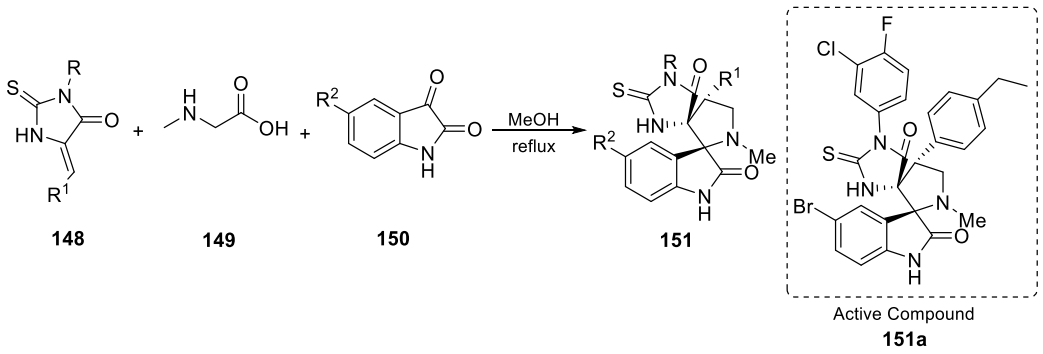
Scheme 20. Synthesis of spirooxindole derivatives **143a-v** from substituted isatin **140**, sarcosine **141** and 3-(1H-indol-3-yl)-3-oxo-2-(2-oxoindolin-ylidene) propanenitrile **142**.

Xu and co-worker's developed a new organocatalytic asymmetric multicomponent cascade reaction and synthesized spiro[pyrrolidin-3,2'-oxindoles] **147** using a bifunctional squaramide catalyst **A** in the presence of DCM and $MgSO_4$ via 1, 3-proton shift and [3+2] cycloaddition reaction in single step with high stereoselectivity (dr = upto 9:1 and 86% ee) (Scheme 21).[88]



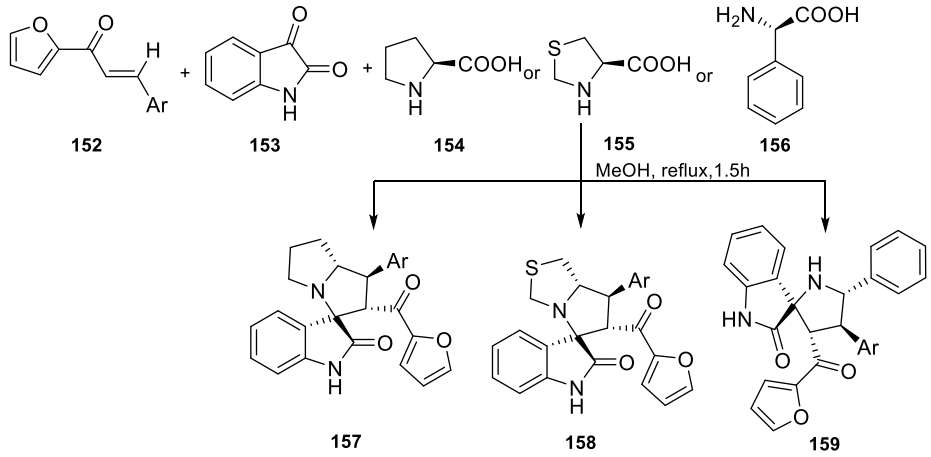
Scheme 21. Synthesis of spiro[pyrrolidin-3,2'-oxindoles] **147** via organocatalyzed asymmetric multicomponent reaction.

Ivanenkov *et* al. have carried out a regioselective synthesis of diastereomeric 2-thioxo-5H-dispiro [imidazolidine-4, 3-pyrrolidine-2, 3-indole]-2, 5(1H)-diones **151** (Scheme 22).[89] The compounds were tested against various cancer cell lines and their activity was studied against MDM2/p53 PPI. Only one compound **151a** showed significant activity against HCT116 ($p53^{+/+}$) and HCT116 ($P53^{-/-}$).



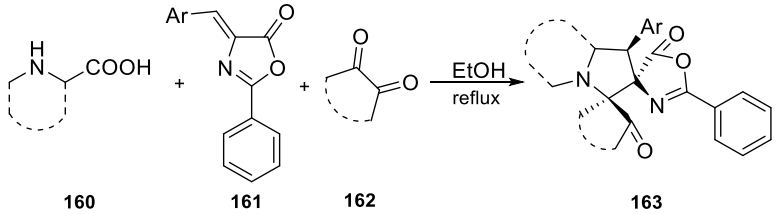
Scheme 22. Synthesis of 2-thioxo-5H-dispiro[imidazolidine-4,3-pyrrolidine-2,3-indole]-2,5[1H]-diones **151**.

Wu *et* al. synthesized spirooxindolo- pyrrolizines, pyrrolothiazoles, and pyrrolidines hybrid compounds **157**, **158** and **159**, respectively via regioselective, three-component, 1,3-dipolar cycloaddition reactions and assessed for their *in vitro* antibacterial activity (Scheme 23).[90] Compound with *p*-OCH_3 substituent was found to show the best activity particularly against *P. aeruginosa.* The molecular docking studies showed strong interactions with the active sites of lanosterol demethylase, dihydrofolate reductase, and topoisomerase II.



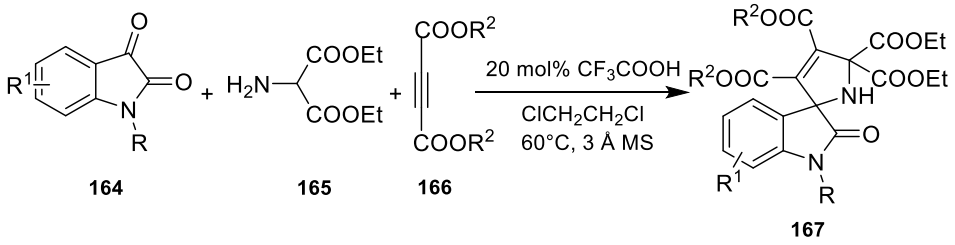
Scheme 23. 1,3-Dipolar cycloaddition reaction to synthesize pyrrolizines, pyrrolothiazoles and pyrrolidines **157**, **158** and **159** respectively.

Da-Qing Shi *et* al. synthesized some highly functionalized dispiropyrrolizidine derivatives as novel potential anticancer agents via one pot 1,3-dipolar cycloaddition reactions (Scheme 24).[81] Many compounds showed promising anticancer activities against various cancer cell lines.



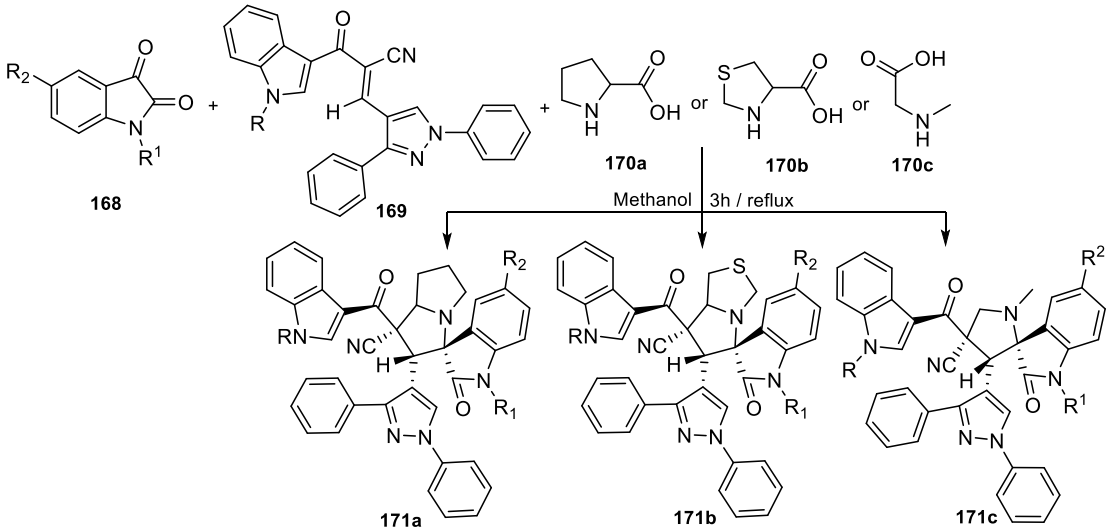
Scheme 24. Synthesis of functionalized dispiropyrrolizidines **163**.

Shi and Zhang and their coworker's synthesized a series of spirooxindole based 2,5-dihydropyrrole-based compounds **167** via three component 1, 3-dipolar cycloaddition reaction of isatin **164**, amino-ester **165** and alkynes **166** with structural diversity upto 99% yields (Scheme 25).[92] The compounds were assayed for their cytotoxicity studies against MCF-7 cancer cells. Out of all, sixteen compounds were found to show promising activity.



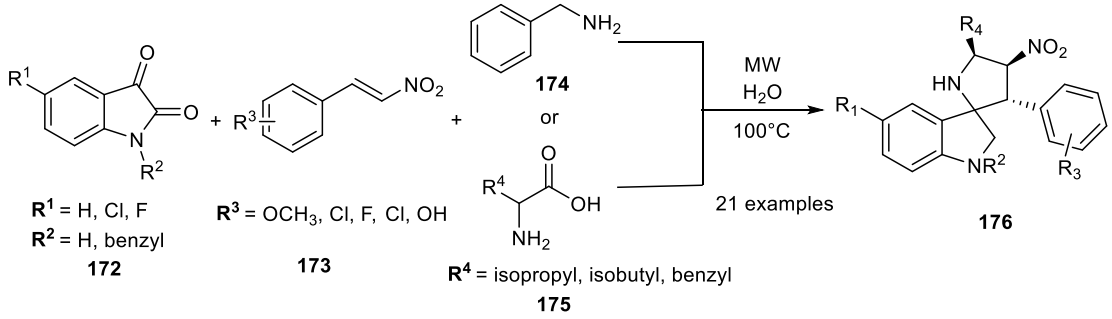
Scheme 25. Synthesis of Spirooxindole ring hybridized 2, 5-dihydropyrroles **167**.

Kathirvelan *et* al. reported a series of novel highly functionalized spiropyrrolidine oxindoles **171a-c** via 1,3-dipolar cycloaddition reaction employing substituted isatins **168**, various amino acids such as L-proline **170a**, thioproline **170b**, sarcosine **170c** etc. and the dipolarophile (E)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-(1H-indole-3-carbonyl) acrylonitrile **169** (Scheme 26).[93] The compounds were tested for their *in vitro* antimicrobial activity against nine bacteria, filamentous fungi and yeast by agar diffusion method. The compounds showed promising values against both bacteria and fungi.



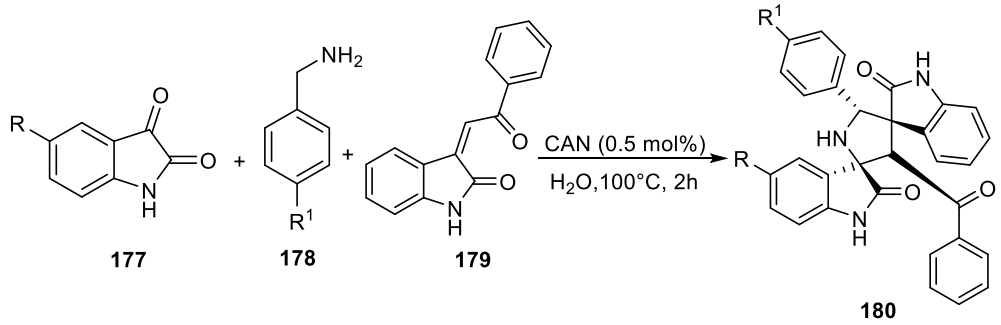
Scheme 26. Synthesis of highly functionalized spiropyrrolidine-oxindoles **171a-c**.

Meshram *et* al. reported the synthesis of spirooxindole derivatives **176** by a one-pot 3-component 1, 3-dipolar cycloaddition reaction involving substituted isatin **172**, substituted β-nitrostyrenes **173**, and benzyl amine **174** or α-amino acids **175** in water as a solvent under microwave irradiation with good diastereoselectivity (Scheme 27).[94] The synthesized compounds showed good antimicrobial activity against *Escherichia coli* ATCC 10536, *Candida tropicalis* ATCC 750, *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* ATCC 15442.



Scheme 27. Synthesis of spirooxindoles under MW irradiation in aqueous medium.

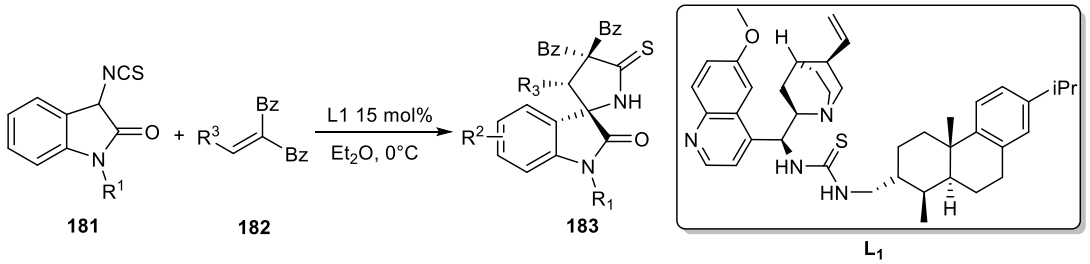
Ramesh *et* al. reported the synthesis of new spirooxindole-pyrrolidines **180** using 0.5 mol% CAN as catalyst in aqueous medium by reaction of substituted isatins **177**, substituted benzyl amines **178** and (Z)-3-(2-oxo-2-phenylethylidene)indolin-2-one **179** in a highly regioselective and diastereoselective manner.[95] All these compounds showed good *in vitro* antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus*, *Micrococcus luteus* (gram-positive), *Salmonella paratyphi, Pseudomonas aeruginosa and Salmonella typhi* (gram-negative) and *Candida Albicans* by Agar well diffusion method.



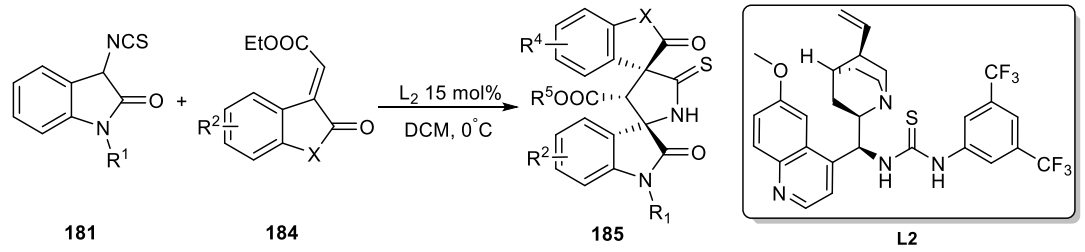
Scheme 28. Synthesis of novel spirooxindole-pyrrolidines in aqueous medium.

Rui-Wang *et* al. developed a highly efficient bifunctional thiourea-catalyzed (Catalyst L_1 and L_2) asymmetric Michael addition/cyclization reaction of isothiocyanato oxindoles which was utilized in the enantioselective synthesis of functionalized 3,2'-

pyrrolidinyl spirooxindole derivatives **185** and **186** in Diethyl ether as solvent at 0°C in upto 99% yield having diastereomeric ratio of >20:1 and with 96% ee which were having five contiguous stereocenters with two quaternary spirostereocenters (Scheme 29 and 30).[96] It was found that catalyst L_2 proved to be better because of low steric hindrance in the transition state formed between the catalyst and the substrate (because the bulkier methyleneindolinones derivative is used in place of olefin). This enantioselective structurally diverse synthesis offered an unprecedented platform for the studies of biologically important 3,2'-pyrrolidinyl spirooxindoles, as well as their bispirooxindole derivatives.

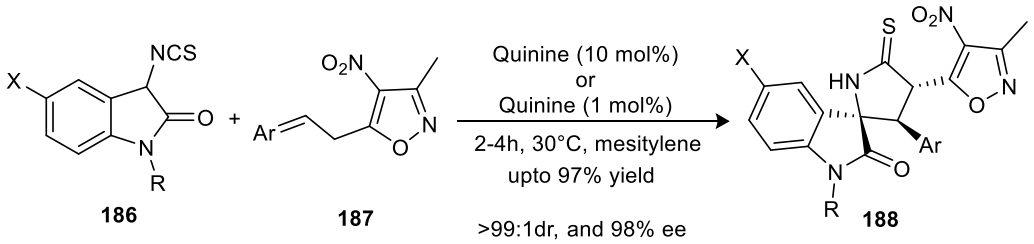


Scheme 29. Catalytic asymmetric synthesis of 3,2'-pyrrolidinyl spirooxindoles **183** using ligand L_1.



Scheme 30. Catalytic asymmetric synthesis of 3,2'-pyrrolidinyl spirooxindoles **185** using ligand L_2.

Wei-Cheng-Yuan *et* al. reported the synthesis of a wide array of diversified 3, 3'-thiopyrrolidinyl spirooxindoles **188** having three contiguous stereogenic centres via development of organocatalytic direct asymmetric aldol reaction of 3-isothiocyanato oxindoles **186** with isoxazoles **187** by using a quinine as catalyst.

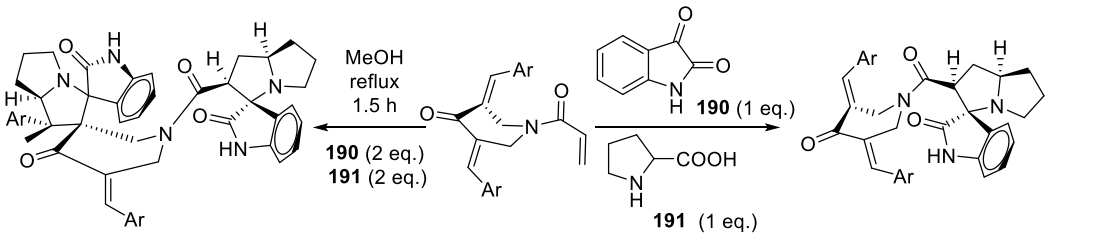


Scheme 31. Catalytic asymmetric Michael addition /Cyclization of isothiocyanato Oxindoles **188** to synthesize 3,2'-Pyrrolidinyl mono and bi-spirooxindole frameworks.

This reaction proceeded via domino Michael addition /cyclisation reaction between 3-isothiocyanato oxindoles and 3-methyl-4-nitro-5-alkenyl-isoxazoles taking quinine as the catalyst (Scheme 31).[97] The diversified 3, 3'-thiopyrrolidinyl spirooxindole

formed in this reaction in a highly enantio- and diastereo-selective manner (upto >99:1 dr and 98% ee) in high yields with high reactivity at lower catalyst loading.

Osman and coworker's reported the piperidone-grafted novel mono- and bis-spiro heterocyclic hybrids **192a-k** and **193a-k**, respectively, which were synthesized by 1, 3-dipolar cycloaddition reactions of a series of 1-acrylolyl-3,5-diarylidenepiperidin-4-ones **189**, isatin **190** and L-proline **191** in 1:1:1 and 1:2:2 ratios, respectively, in methanol (Scheme 32).[98]



193a C_6H_5 **193b** 2-$CH_3C_6H_4$ **193c** 2-$(OCH_3)C_6H_4$ **193d** 2ClC_6H_4 **193e** 2-FC_6H_4 **193f** 3-$(NO_2)C_6H_4$ **193g** 2,4-$Cl_2C_6H_3$ **193h** 4-$CH_3C_6H_4$ **193i** 4-ClC_6H_4 **193j** 4-F-C_6H_4 **193k** 1-Naphthyl

192a C_6H_5 **192b** 2-$CH_3C_6H_4$ **192c** 2-$(OCH_3)C_6H_4$ **192d** 2-ClC_6H_4 **192e** 2-FC_6H_4 **192f** 3$(NO_2)C_6H_4$ **192g** 2,4-$Cl_2C_6H_3$ **192h** 4-$CH_3C_6H_4$ **192i** 4-ClC_6H_4 **192j** 4-FC_6H_4 **192k** 1-Naphthyl

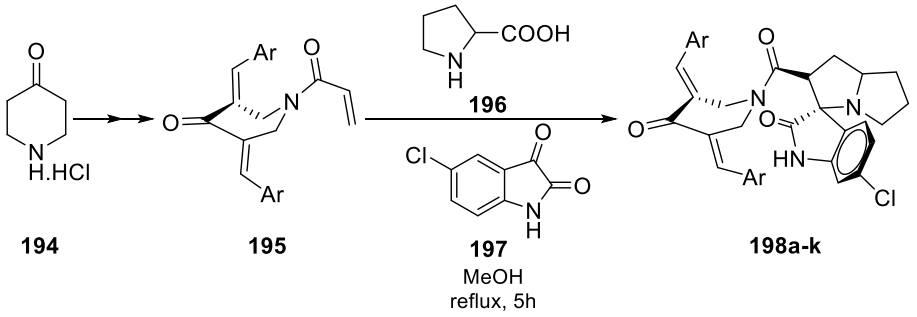
Scheme 32. Synthesis of mono and bisspiropyrrolizines **192a-k** and **193a-k**.

The monospiropyrrolizine derivatives were found to be more potent acetylcholinesterase inhibitors (AChE) as well as butylcholinesterase inhibitors (BuChE) than bisspiropyrrolizine derivatives. **192i** and **192k** were found to show the best cholinesterase inhibitory activity with the IC_{50} values of 3.36 μM and 3.50 μM, respectively, and showed comparable potency to AChE and were five times more potent than BuChE enzymes as compared to the standard drug Galanthamine.

Osman *et* al. synthesized a novel series of piperidine-grafted spiropyrrolizine derivatives **198a-k** by 3+2 cycloaddition reaction (Scheme 33).[99] Out of a series of compounds tested; the compounds **198e** and **198g** were found to be the most potent acetylcholinesterase inhibitors and butyl cholinesterase inhibitors with IC_{50} values of 3.33 and 3.13 μM, respectively.

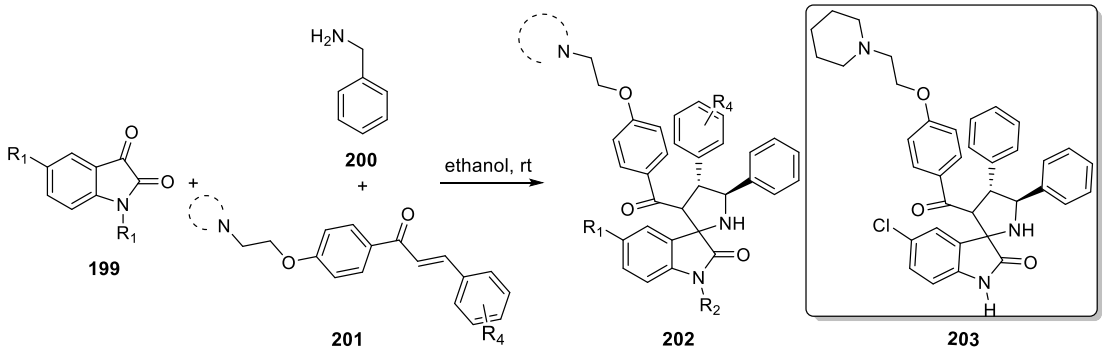
Kumar *et* al. reported the utility of 1,3-dipolar cycloaddition reaction on substituted isatins **199** with benzyl amine **200** and substituted chalcones incorporated with several alkylamino side chains **201**, to form a bioisosteres of spirooxindoles **202** (MI-63/219) as anti-breast cancer agents (Scheme 34).[100] All the synthesized compounds were

tested for cytotoxic activities in breast cancer cell lines MCF-7 and MDA-MB 231 cells by MTT assay.



Ar a = C_6H_5, b = 2-$CH_3C_6H_4$, c = 2-$(OCH_3)C_6H_4$, d = 2-ClC_6H_4, e = 2-FC_6H_4, f = 3-$(NO_2)C_6H_4$, g = 2,4-$Cl_2C_6H_3$, h = 4-$CH_3C_6H_4$, i = 4-ClC_6H_4, j = 4-FC_6H_4, k = 1-Naphthyl

Scheme 33. Synthesis of piperidine-grafted functionalized spiropyrrolizines **198a-k**.



Scheme 34. Synthesis of bioisosteres of spirooxindoles **202** (MI-63/219) as anti-breast cancer agents.

Compound **203** was found to be the most effective in causing loss of viability of breast cancer cells at concentrations that were comparable to Nutilin-3. The compound did not affect the growth of normal cells and were cytotoxic against cancer cells as tested in human kidney epithelial cells line (HEK-293) and monkey kidney epithelial cells (Vero). It proved the compound **203** to be more potent than Nutilin-3, OH-Tamoxifen in MDA-MB231, MCF-7 cell lines respectively. Since **203** was the best compound, it was selected for detailed studies on the mechanism in breast cancer cells and for *in vivo* efficacy in nude mice.

1.2.3. Coumarins-a general core of medicinally useful compounds.

1.2.3.1. General introduction about Coumarins

Coumarin, also known as benzopyran-2-one or 2-H-Chromen-2-one, constitutes the skeletal core of several naturally[101] occurring and synthetic pharmacologically active compounds and are endowed with a wide range of biological activities such as anti-inflammatory,[102] antiviral,[103] antioxidant,[104] antimicrobial,[105-107] antiHIV,[108] antidiabetic, anti-glycaemic,[109] anticancer etc.[110]

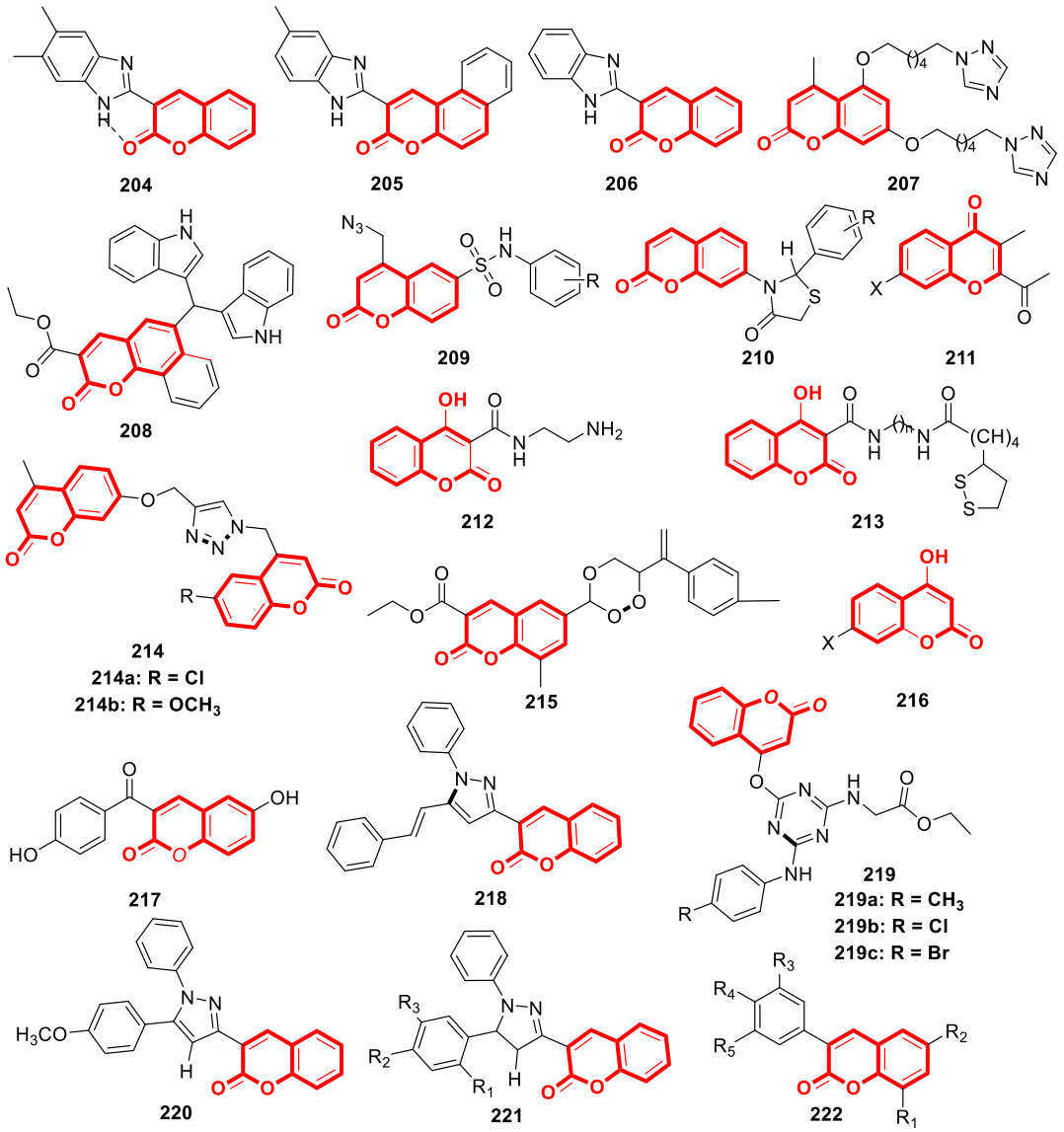


Figure 7. Structures of several biologically active Coumarins.

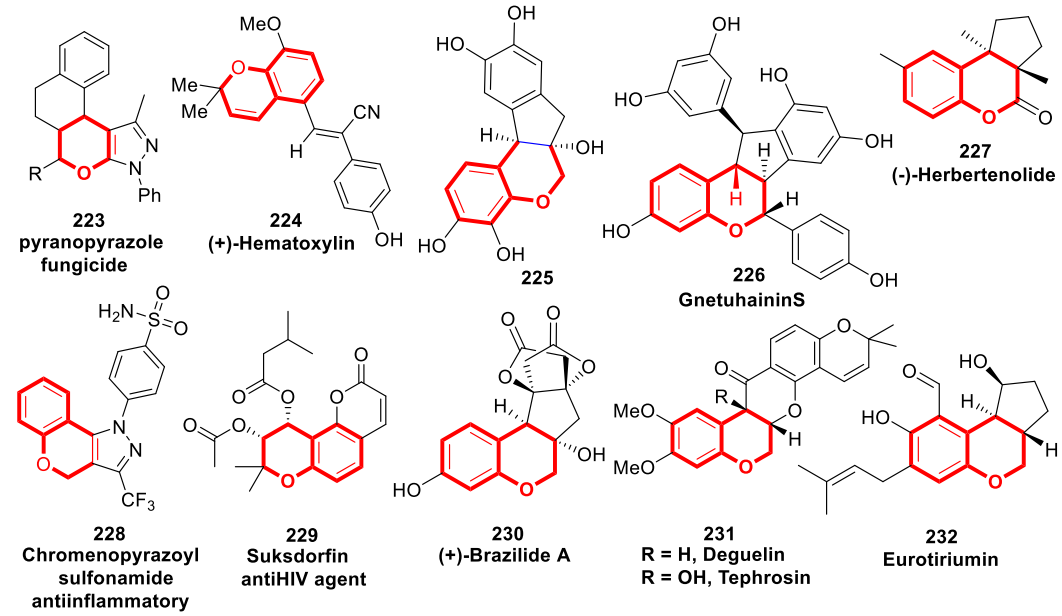
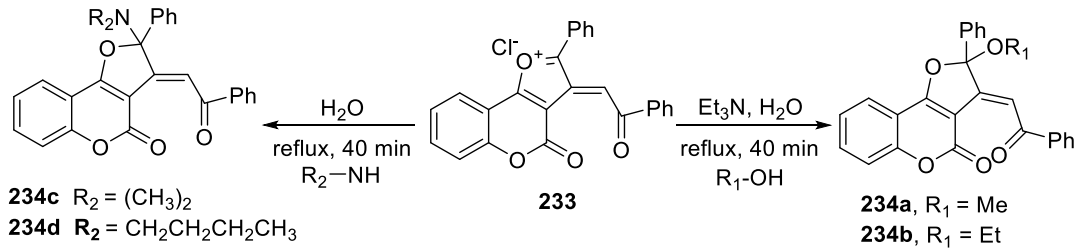


Figure 8. Structures of biologically active benzopyrans containing moieties.

Figure 7 illustrates some of the naturally occurring coumarins exhibiting a variety of pharmacological uses. It has also been found that coumarin skeleton is fused with other heterocyclic bioactive scaffolds to exhibit numerous pharmacological potencies to different extent. Among them, benzopyrans also constitute the family of heterocyclic scaffolds found in several pharmaceutically active compounds (Figure 8)**.**[111]

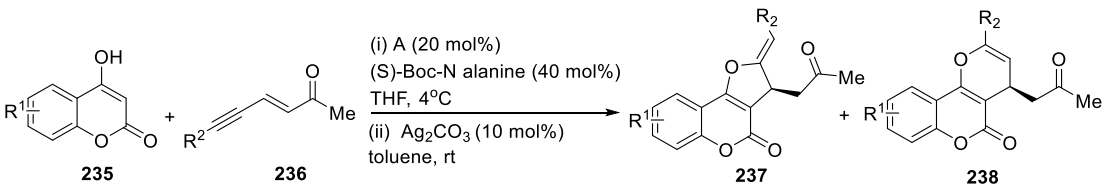
1.2.3.2 Synthesis and biological importance of Coumarin-fused heterocyclic compounds.

Mousa *et* al. reported the synthesis of a novel series of furo [3,2-c] coumarin derivatives **234a-d** and assessed for their *in vitro* antiproliferative activity in MCF-7 breast and HCT-15 colon cancer cell lines using Sulfo-rhodamine B (SRB) assay (Scheme 35).[112] Compounds **234b** and **234d** were found to better than compounds **234a** and **234c**, respectively.



Scheme 35. Synthesis of a new series of furo[3,2-c]coumarin analogues **234a-d** from the reaction of 4-oxo-3-[2-oxo-2-phenylethylidene]-2-phenyl-3H,4H-furo[3,2-c]chromene-1-ium chloride **233** with nucleophiles like alcohols and amines.

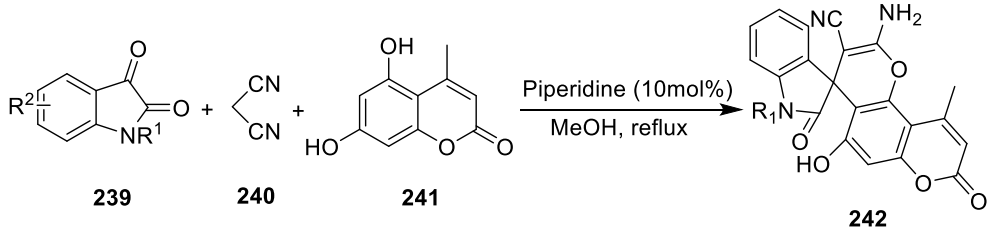
Dieter Enders and co-workers developed a highly stereoselective one-pot synthesis of five-membered annulated hydroxycoumarins **237** via Michael addition / hydroalkoxylation reaction catalyzed by organocatalysis and silver catalysis in excellent yields (upto 91%) with excellent enantioselectivities (upto 99% ee). This methodology was feasible also for the synthesis of annulated six-membered rings **238** depending on the substituents on the enones (Scheme 36).[113]



Scheme 36. One-pot synthesis of highly stereoselective five- and six-membered annulated hydroxycoumarins **237** and **238**.

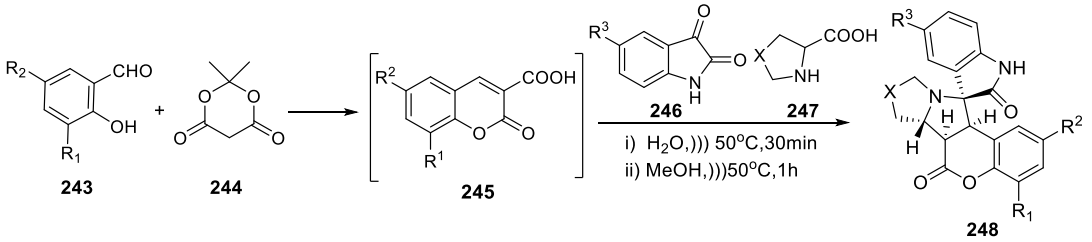
Choudhury and coworker's reported a three-component reaction of substituted isatin **239**, malononitrile **240** and 5,7-dihydroxy-4-methyl-2H-chromen-2-one **241** which

furnished pyranocoumarin fused spirooxindole derivatives **242** in a highly regiospecific manner (a single regioisomer is formed) using piperidine as organocatalyst (Scheme 37).[114] The noticeable features of this protocol are metal-free, regiospecific, easy purification, and its applicability to a wide range of isatins.



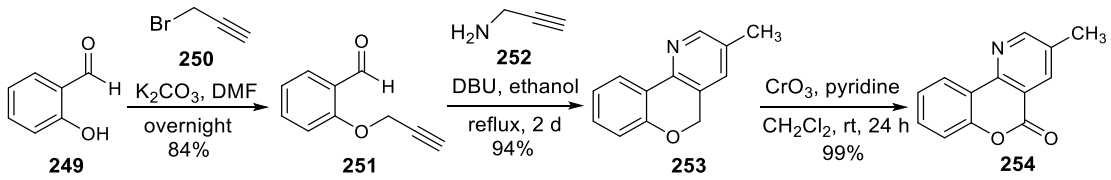
Scheme 37. Synthesis of pyranocoumarin-fused spirooxindoles **242**.

Ranjith Kumar *et* al. reported an ultrasonic-assisted one-pot sequential four component reactions of substituted salicylaldehydes **243**, 2,2-dimethyl-1,3-dioxane-4,6-dione **244**, substituted isatins **246**, and cyclic α-amino acids **247** to form a library of novel spirooxindole-pyrrolizine or pyrrolo[1,2-c]thiazole fused coumarin hybrids of prototype **248** (Scheme 38**)**.[115]



Scheme 38. Ultrasonic-assisted synthesis of spirooxindole-based coumarins of prototype **248**.

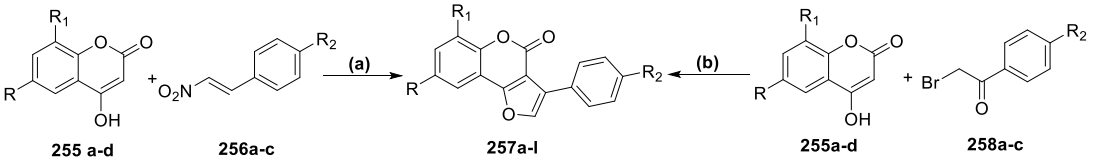
Balci and coworker's reported the synthesis of chromenopyridinone by the reaction of O-propargylated aromatic hydroxyaldehydes **251** with propargylamines **252**. The azadiene generated *in situ* undergoes intramolecular cycloaddition reaction with alkyne followed by subsequent 1,5-hydride shift generates chromenopyridine of prototype **253** which on oxidation with CrO_3 furnished chromenopyridinones **254** (Scheme 39).[116]



Scheme 39. Synthesis of Chromenopyridinones of prototype **254**.

Brahmbhatt *et* al. carried out a microwave-assisted synthesis of 3-aroyl-furo[3,2-c] coumarins **257a-l** by two different methods (Scheme 40).[117] In the first method, different 3-aryl-furo[3,2-c]coumarins **257a–l** have been synthesized by the Nef reaction of various 4-hydroxy coumarins **255a–d** with appropriate 2-aryl-1-nitro

ethenes **256a–c**; while in the second method, the same target compounds have been synthesized by the reaction of various 4-hydroxy coumarins **255a–d** with appropriate aroylmethyl bromides **258a–c** under Feist–Benary reaction conditions. It has been observed that the former method was found to be superior to the latter. All the compounds were screened for their *in vitro* antibacterial activity against two bacterial strains and the fungal strain *Candida albicans*. Compound **257h** showed the highest activity against *E.coli*. Compounds **257b**, **257c**, **257e-g**, **257i**, **257j** and **257l** showed activity better than streptomycin against *E.coli*. These compounds were found to be inactive against *Bacillus subtilis* and the fungal strain *Candida albicans*.

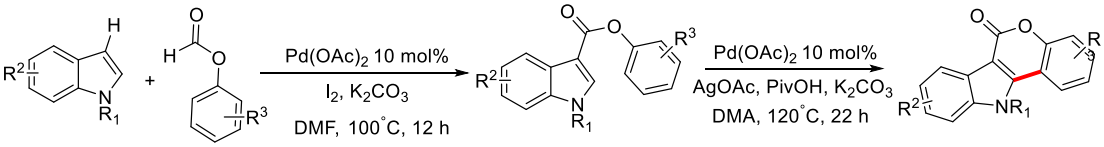


255 a-d 256a-c 257a-l 255a-d 258a-c

257a R = H, R_1 = H, R_2 = H
257b R = CH_3, R_1, = H, R_2 = H
257c R = H, R_1= CH_3, R_2 = H
257d R = Cl, R_1 = H, R_2 = H
257e R = H, R_1 = H, R_2 = CH_3
257f R = CH_3, R_1 = H, R_2 = CH_3
257g R = H, R_1 = CH_3, R_2 = CH_3
257h R = Cl, R_1= H, R_2 = CH_3
257i R = H, R_1 = H, R_2 = OCH_3
257j R = CH_3, R_1 = H, R_2 = OCH_3
257k R = H, R_1 = CH_3 R_2 = OCH_3
257l R = Cl, R_1 = H, R_2 = OCH_3

Scheme 40. Synthesis of 3-aroyl-furo[3,2-c]coumarins **257a-l**.

Lan *et* al. reported the synthesis of indolo[3,2-c] coumarins **261** via Pd-catalyzed C−H carbonylation of (hetero)arenes with aryl formates and intramolecular dehydrogenative oxidative C-H/C-H coupling in good yields (Scheme 41).[118]

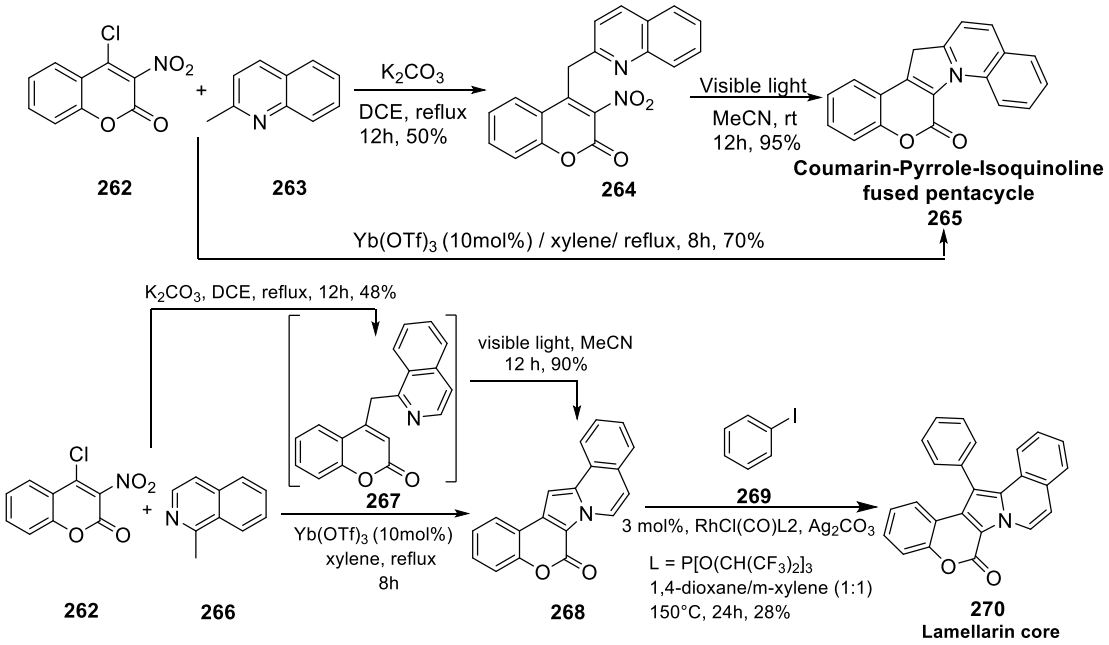


259a 259b 260 261

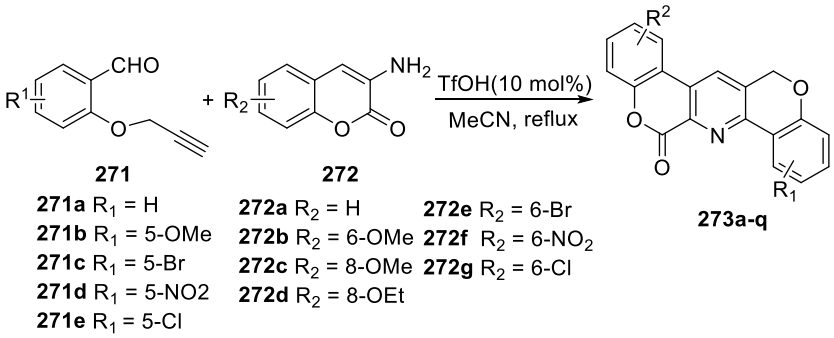
Scheme 41. Synthesis of indolo-[3,2-c]coumarins **261** by intramolecular oxidative coupling.

Yang *et* al. constructed coumarin-pyrrole-isoquinoline-fused pentacycle **265** via visible light-promoted cyclisation of 4-(isoquinolin-1-ylmethyl)-3-nitrocoumarin **262** with 2-methylisoquinoline **263** or $Yb(OTf)_3$-catalyzed coupling of 4-chloro-3-nitrocoumarin with 1-methylisoquinoline **266** followed by visible light treatment furnished pentacyclic core **268**. This Coumarin-Pyrrole-Isoquinoline-fused pentacyclic skeleton 268 on subject to Rhodium –catalysed C-C coupling with iodobenzene **269** furnished lamellarin core **270** in 28% yield. Hence, this strategy can be utilized for the synthesis of bioactive lamellarin core **270** in 3 steps via pentacyclic ring skeleton **265** or **268** (Scheme 42**)**.[119]

Khan *et* al. synthesized fused pyrido [2,3-c] coumarins **273a-q** by reacting 2-(propagyloxy) benzaldehydes **271a-e** with 3-aminocoumarins **272a-g** through an intramolecular Povarov reaction catalysed by 10 mol % triflic acid (Scheme 43).[120]



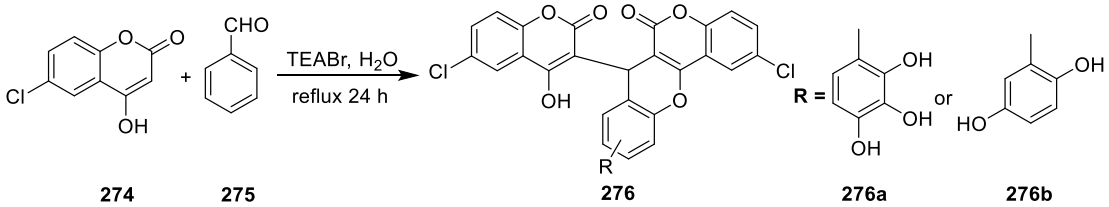
Scheme 42. Synthesis of coumarin-pyrrole-isoquinoline-fused pentacycle core **265** and Lamellerin core **270**.



Scheme 43. Synthesis of fused pyrido[3,2-c]coumarins **273a-q** by intramolecular Povarov reaction.

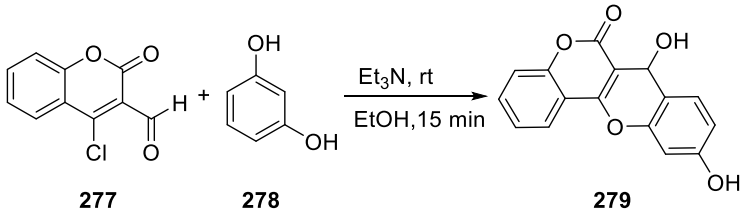
1.2.3.3 Synthesis of some benzopyranocoumarin heterocyclic compounds.

Ahmat *et* al. synthesized some benzopyranocoumarin derivatives **276** by the method shown in Scheme 44 .[121]



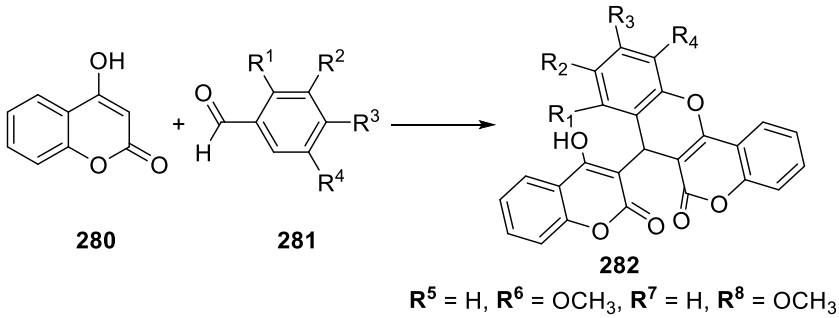
Scheme 44. Synthesis of benzopyrano coumarin derivatives of prototype **276**.

Gaddamanugu *et* al. reported a tandem base-promoted nucleophilic substitution on **277** by **278** which was then followed by intramolecular aromatic electrophilic cyclisation to synthesize novel benzopyrano [3, 2-c] coumarin **279** (Scheme 45).[122]



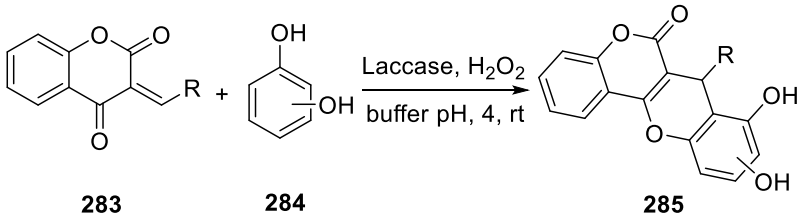
Scheme 45. Synthesis of benzopyrano[3,2-c]coumarin of prototype **279** by nucleophilic substitution on **277** by **278** and then followed by intramolecular electrophilic aromatic cyclisation.

Mintas *et* al. synthesized benzopyrano-fused coumarin derivatives of prototype **282** via reaction of 4-hydroxycoumarin **280** with substituted benzaldehyde **281** (Scheme46)[123] and carried out their *in vitro* antiviral studies. Compound **282** was active against feline herpes virus (50% effective concentration, EC_{50} = 5-8.1 μM), that is 4-7 times lower than the MCC (minimum cytotoxic concentration).



Scheme 46. Synthesis of benzopyranocoumarin derivatives of prototype **282**.

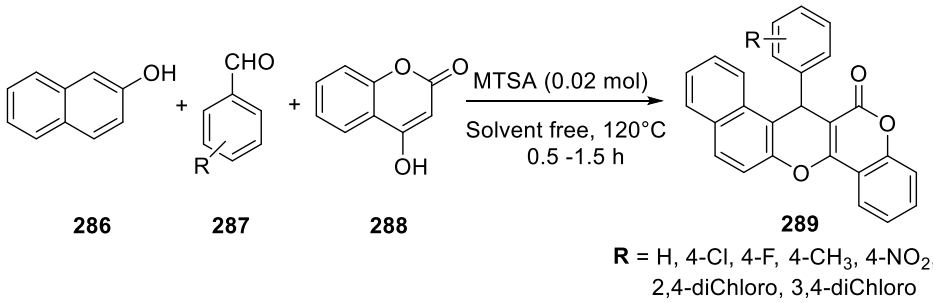
Kidwai *et* al. reported the synthesis of the substituted benzopyranocoumarins **285** via the domino reaction of α, β-unsaturated derivatives of coumarins **283** with catechol or 1, 4-hydroquinones of prototype **284** in aqueous medium using laccase from basidiomycetous fungus as a catalyst (Scheme 47).[124]



Scheme 47. Synthesis of benzopyranocoumarin derivatives of prototype **285** as HIV-protease inhibitors.

Wu *et* al. reported an efficient synthesis of 7-alkyl-6*H*,7H-naphtho-[1',2':5,6] pyrano [3,2-*c*] chromen-6-ones **289** via three-component reaction of β-naphthols **286**, 4-substituted aromatic aldehydes **287** and hydroxycoumarin **288** under solvent-free

conditions utilizing a reusable solid acidic catalyst, melamine trisulphonic acid (MTSA). The reaction underwent smoothly in good to excellent yields in shorter reaction time (Scheme 48).[125]



Scheme 48. Synthesis of 7-alkyl-6*H*, 7H-naphtho-[1',2':5,6] pyrano [3,2-*c*] chromen-6-ones (benzopyranocoumarin) **289** as HIV-protease inhibitors.

1.2.4. Indazoles-a general core of medicinally useful compounds.

1.2.4.1. General introduction about Indazoles.

Emil Fisher first studied indazoles as a pyrazole ring that is fused with a benzene ring. Indazoles are the aromatic system containing 10-π electron systems and contains two nitrogen atoms, also called as benzpyrazoles or azolones and resembles pyrroles and pyridines. Emil fisher and Kuzel attempted to obtain the anhydride of o-hydrazino cinnamic acid **293** applying the strategy that o-hydrazine benzoic acid **290** on dehydration gives indazolones **291** and isolated a product that did not contain any oxygen atom. It has been named as indazole in analogy with indole[126] (Figure 8). Indazole exist in form of the tautomer's and the phenomena is known as prototopic annular tautomerism (Figure 9). Naturally occurring indazole exist in the form of 1-H tautomer's mainly as described by the ^{1}H and ^{15}NNMR spectra of indazoles and its comparison with the ^{13}C and ^{1}H NMR spectra of 1-methyl and 2-methyl indazoles.[127] Nigellicine **298** is the first naturally occurring indazoles isolated from the plant *N. Sativa*.[128] Similarly, other oldest naturally occurring alkaloids having the same ring system such as Nigeglanine **296** and Nigellidine **297**, which exist in zwitter ionic forms, were isolated from the abstracts of *N. Glandulifera* and *N. Sativa*, respectively(Figure 10).

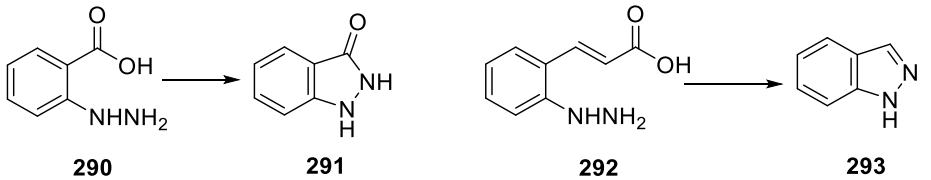
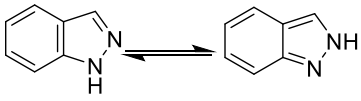
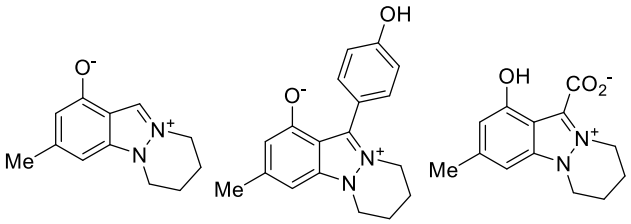


Figure 8. First reported synthesis of Indazoles **291** and **293**.



294 1*H*-Indazole **295** 2*H*-Indazole

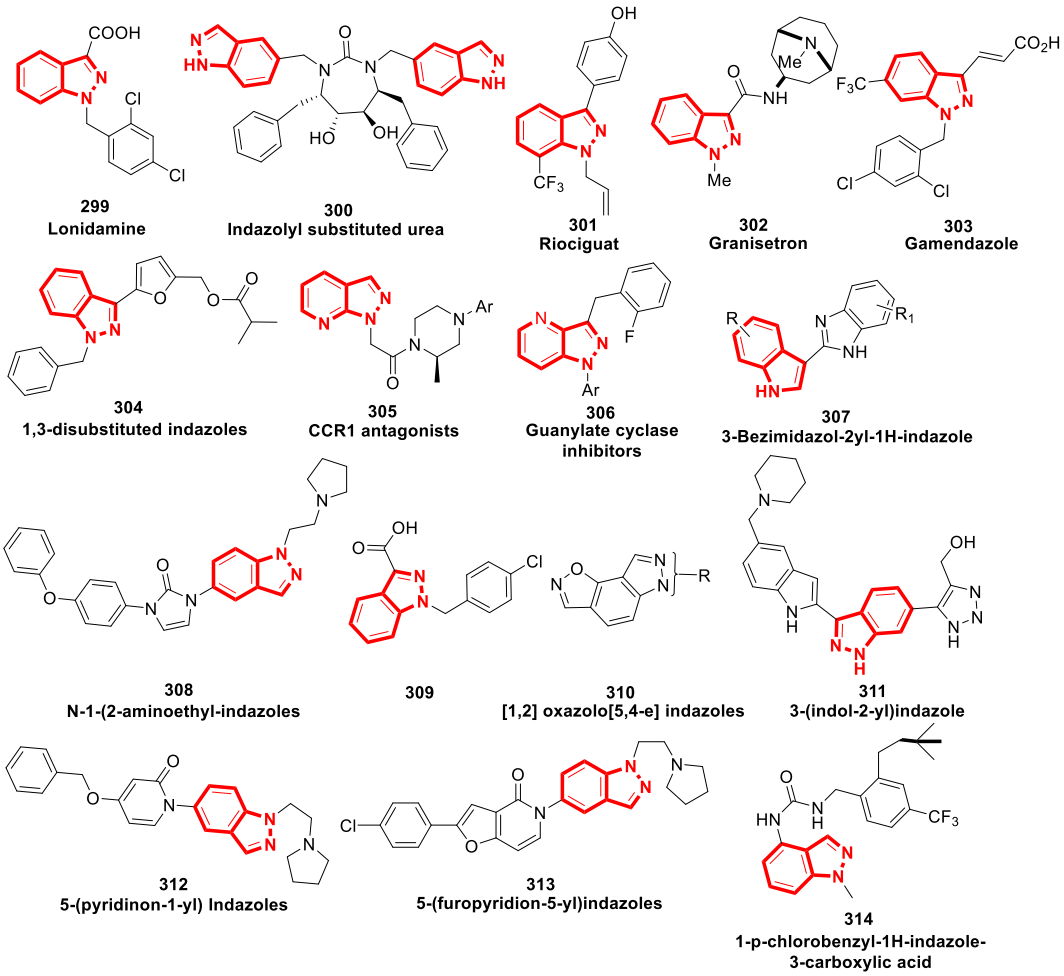
Figure 9. Annular prototopic tautomerism in Indazoles.



296 Nigeglanine **297** Nigellidine **298** Nigellicine

Figure 10. Structures of the oldest discovered naturally occurring indazole moieties of medicinal importance.

Azaindazoles and indazoles are biologically important heterocyclic molecules which are associated with a wide range of activities such as anti-tumour,[129] anti-HIV,[130] anti-microbial,[131] anti-depressant,[132] anti-platelet,[133] neuroprotective sodium channel modulator,[134] selective ligands for imidazoline I2,[135] estrogen[136] and 5-HT_{1A}[137] receptors (Figure11).



299 Lonidamine **300** Indazolyl substituted urea **301** Riociguat **302** Granisetron **303** Gamendazole

304 1,3-disubstituted indazoles **305** CCR1 antagonists **306** Guanylate cyclase inhibitors **307** 3-Bezimidazol-2yl-1H-indazole

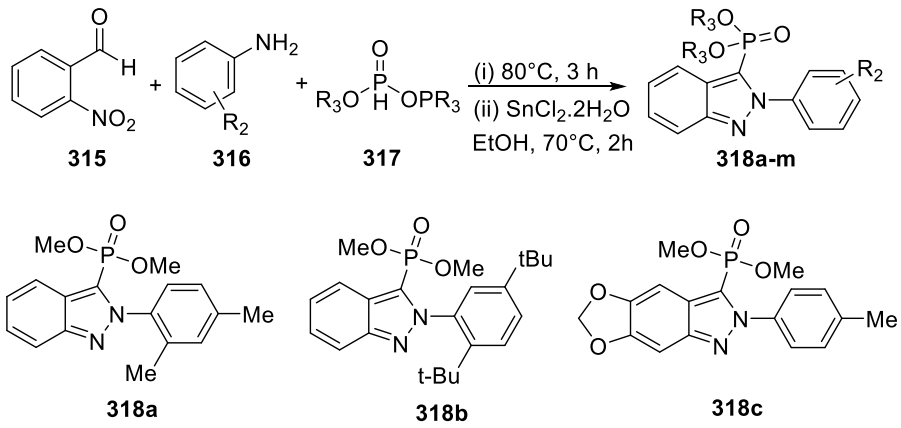
308 N-1-(2-aminoethyl-indazoles **309** **310** [1,2] oxazolo[5,4-e] indazoles **311** 3-(indol-2-yl)indazole

312 5-(pyridinon-1-yl) Indazoles **313** 5-(furopyridion-5-yl)indazoles **314** 1-p-chlorobenzyl-1H-indazole-3-carboxylic acid

Figure 11. Some biologically active indazole containing moieties

1.2.4.2. Synthesis of some biologically important Indazole skeletons.

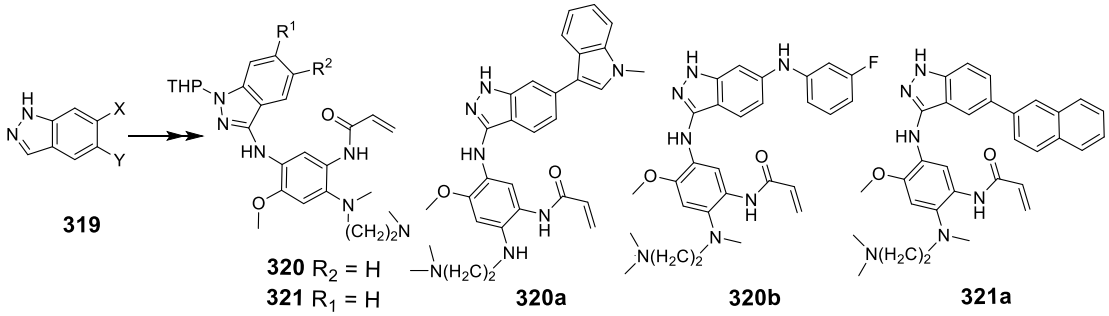
Perumal *et* al. reported $SnCl_2.H_2O$ catalysed one-pot, three-component reaction to synthesize several 2-aryl-2H-indazole-3-phosphonates **318a-m** via the reaction of o-nitrobenzaldehyde **315**, substituted anilines **316** and phosphoric acid **317** under transition metal-free and mild reaction conditions. This protocol display wide substrate scope in good yields and proceeds with high atom economy *via* the formation of *α*-aminophosphonates followed by the generation of indazole ring through N-N bond formation eliminating water as a by-product (Scheme 49).[138] All the compounds showed promising anticancer activity against A549 and HepG-2 cells. Compounds **318a-b** and **318c** showed cytotoxicity against HepG-2 cells and A549 cells, respectively. Intracellular visualization has also been carried out by using laser scanning confocal microscope. Apoptosis studies, western blot analysis, flow cytometry, colony formation and DNA fragmentation was also studied against A549 cell lines.



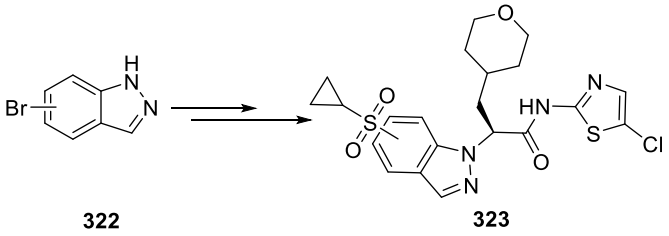
Scheme 49. Synthesis of 2-aryl-2H-indazole-3-phosphonates **318a-m**.

Rauh *et* al. reported novel indazole-based epidermal growth factor receptor (EFGR) inhibitors **320** and **321** which target the oncogenic mutant drug-resistant EGFR-L858R/T790M which alkylate Cys797 covalently to combat cancer (Scheme 50).[139] Compounds **320a** and **320b** affect the cellular viability of H1975 cells with effective concentrations of 417 and 191 nM respectively. These compounds moderately affect the cell viability of A431 wild type cells.

Cheruvallath *et* al. synthesized a new series of 1,4-disustituted indazole **323** from bromoindazole **322** compounds as a novel class of GKAs and activate Glucokinase in enzyme and cell assays (Scheme 51).[140] In rodent models with type 2 diabetes, OGTT efficacy was also demonstrated.

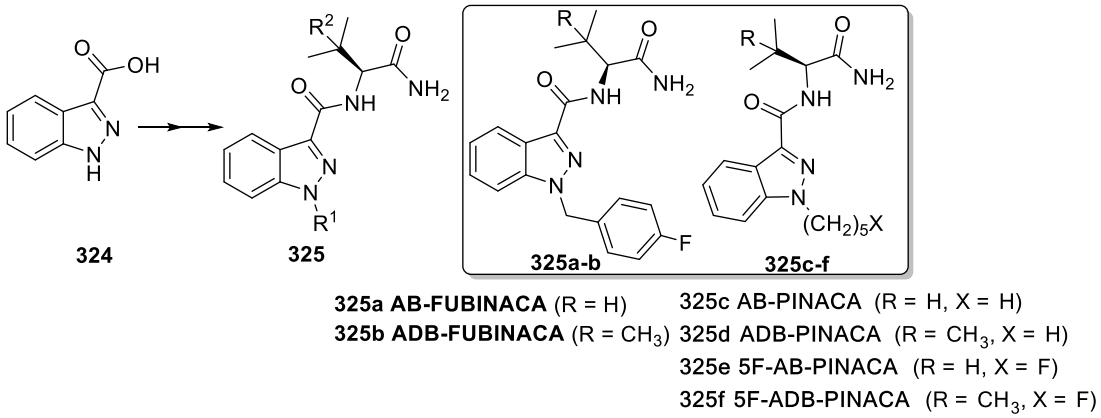


Scheme 50. Synthesis of Indazole-based EGFR inhibitors **320** and **321**.



Scheme 51. Synthesis of 1,4-disubstituted indazole derivatives.

Kassiou *et* al. reported the synthesis of indole-indazole scaffolds **325a-f** bearing novel synthetic cannabinoid (SC) drugs including L-valinamide or L-*tert*-leucine amide side chains Scheme 52.[141] The synthesized SCs were highly potent agonists of CB1 (EC_{50}-0.24-21nM) and CB2 (EC_{50}-0.24-21nM) receptors and 5F-ADB-PINACA **325f** acted was the most potent compound. AB-FUBINACA **325a** and AB-PINACA **325e** were evaluated *in vivo* in rats using biotelemetry.

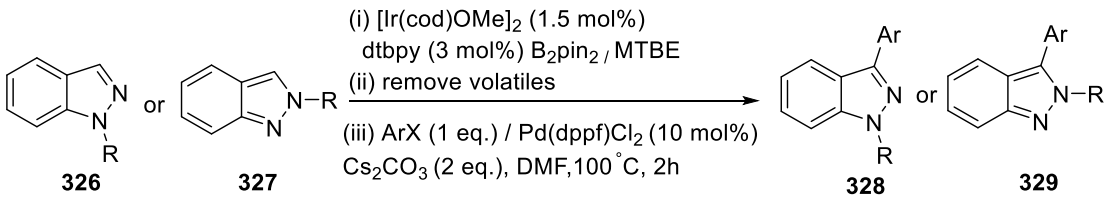


Scheme 52. Synthesis of indole-indazole based synthetic cannabinoids (SC) agents **325a-f**.

1.2.4.3. Representation of few examples depicting coupling reactions on some indazole derivatives.

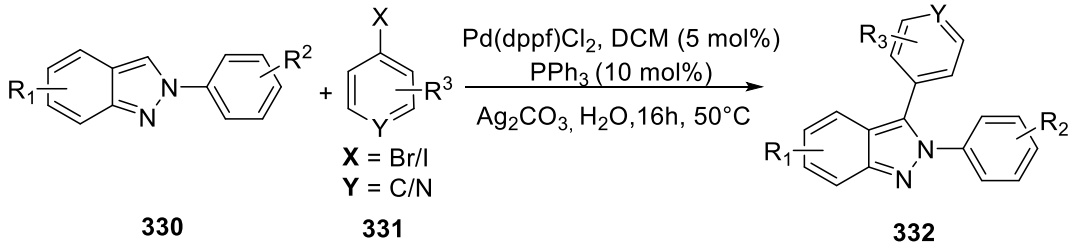
Steel *et* al. demonstrated and developed one-pot, iridium-catalysed C-H borylation of N-protected indazoles **326** or **327** selectively at C-3 position with diborance pinacolate in presence of MTBE. The borylated product was then subjected subsequently for palladium catalyzed C-H arylation of 1*H*- and 2*H*- indazoles with haloarenes in the presence of base such as Cs_2CO_3 in DMF at 100 °C which furnished

substituted indazoles **328** or **329** respectively, with multidirectional potential in good to moderate yields (Scheme 53).[142]



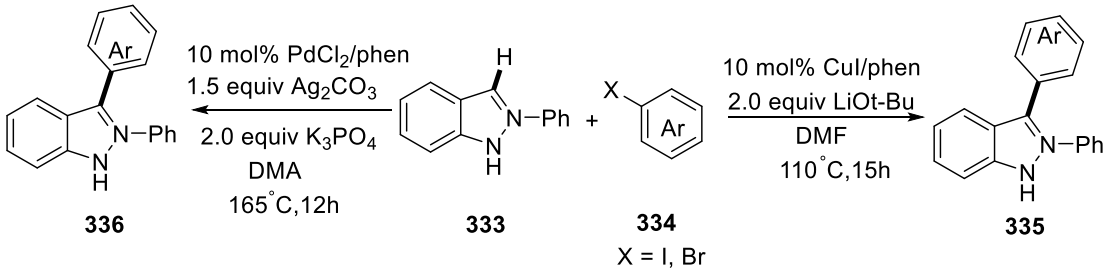
Scheme 53. Iridium-catalysed, one-pot, Suzuki Miyura cross-coupling of **1** and **2** substituted indazoles.

Greaney *et* al. reported the first Pd-catalysed direct C-3 arylation of 2*H*-indazoles under "on water" conditions. This reaction provides a direct access towards the synthesis of biologically important 2,3-diaryl indazoles of prototype **332** which were prepared via Palladium-catalyzed reaction of substituted N-arylated 2*H*-indazoles **330** with substituted haloarenes **331** (Scheme 54).[143]



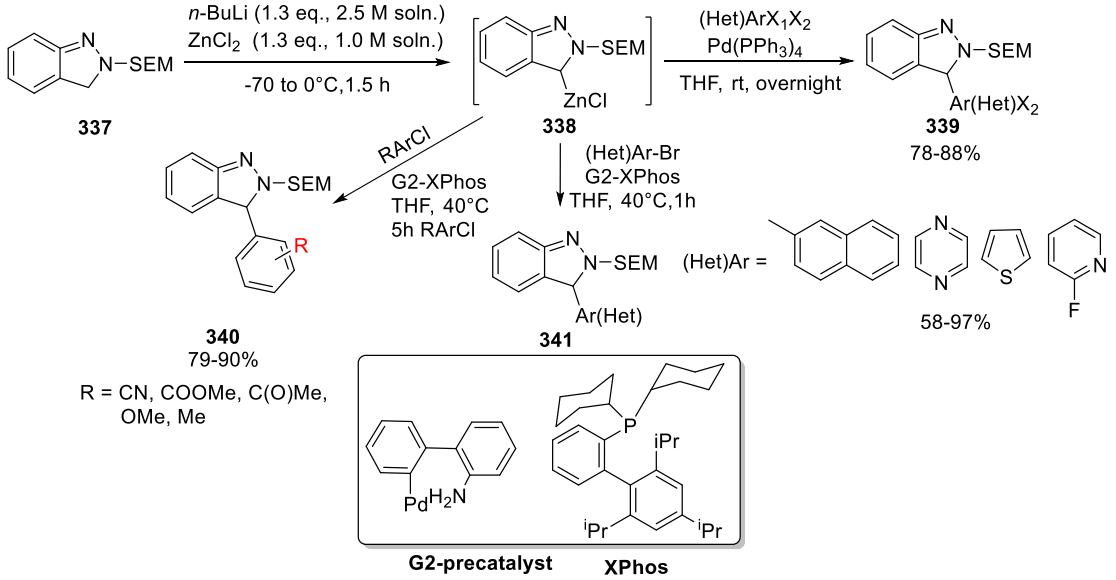
Scheme 54. Palladium-catalyzed direct arylation of 2-Phenylindazole on water.

Itami *et* al. reported the Cu- and Pd-catalyzed C-H arylation of 1*H*- and 2*H*-indazoles with haloarenes. The two catalytic systems, CuI/phen/LiO^{t}Bu and $PdCl_2$/phen/Ag_2CO_3/K_3PO_4, based on Cu and Pd, respectively were developed for the C-H arylation of 1*H*- and 2*H*-indazoles **333** with haloarenes **334** which furnished the 3-arylated 2H-indazoles **335** and **336**, respectively (Scheme 55).[144] The newly developed catalyst were utilized in the demonstration of rapid synthesis of an antitumor agent, **YC-1** and platelet anti-aggregating agent, **YD-3**. These new reactions represent important direct functionalization tools of indazoles, well-known bioisosteres of pharmaceutically important indole core.



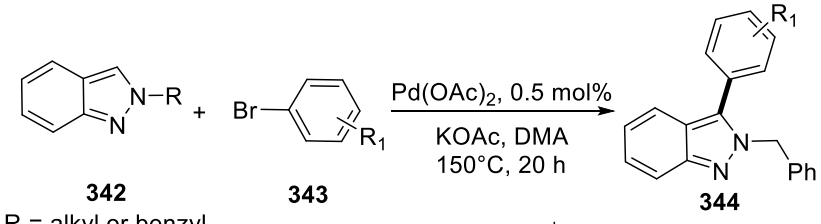
Scheme 55. Pd- and Cu-catalyzed C-H arylation of 1*H*- and 2*H*-indazoles with haloarenes.

Basu, Poirier and coworker's reported the Pd-mediated Negishi coupling to form biologically important 3-(Hetero)aryl substituted N(2)-SEM-protected indazoles **339**, **340** and **341** in high yields (Scheme 56).[145]



Scheme 56. Synthesis of 3-(hetero)aryl substituted N(2)-SEM-protected indazoles via Pd-mediated Negishi coupling strategy.

Soule and Doucet reported direct C-3 arylation of 2*H*-indazoles **342** with aryl/heteroaryl bromides **343** using only 0.5-0.1 mol% phosphine free $Pd(OAc)_2$ catalyst and KOAc as an inexpensive base which furnished C-3 arylated *2H*-indazoles **344** in good to excellent yields (Scheme 57).[146] The protocol proceeded well with several electron-deficient as well as electron-rich aryl/heteroaryl bromides. This reaction was also well demonstrated in cyclopentyl methyl ether as green solvent.



Scheme 57. Phosphine-free Pd-Catalyzed direct C3-arylation of *2H*-indazoles.

1.3 Summary

Since a variety of biologically active moieties are present in the form of benzoxazinones, spirooxindole-based heterocyclic compounds, coumarin-fused heterocyclic compounds and indazoles, this chapter include a brief review of some 1,4-benzoxazine-3-ones, 1,4-benzoxazin-2-ones, spiropyrrolidine and Spiro

pyrrolizine-based ring compounds, coumarin-fused compounds, benzopyran-fused Coumarins and *2H*-Indazoles. Many such kind of biologically active compounds have been represented in this chapter in the form of several figures. While reviewing these compounds, several reactions have been utilized such as smiles reaction, metal catalyzed coupling, and molecular hybridization with various other biologically active moieties such as 1,2,3-triazole, benzimidazole etc to form biologically active benzoxazinone derivatives. Microwave reactions and ultrasonic reactions which have advantages over conventional heating have also been reported to form benzoxazinone derivatives. Mainly multi-component reactions have been used to form spirooxindole derivatives and several organocatalysts have also been reported to form biologically active spirooxindole derivatives. Coumarins, benzopyrans and indazoles are also been reported by coupling in the presence or absence of metal and various cyclisation processes. Thus, keeping in view the medicinal importance of biologically active benzofused heterocycles and in concurrence with our work; their synthesis and medicinal activities have been reported in detail.

1.4 Conclusion

The heterocyclic compounds are endowed with a wide variety of biological activities which has been used in the treatment of infectious diseases, numerous methods have been developed over the decades to prepare numerous heterocyclic moieties and their medical investigation has also been done enormously. Various heterocyclic compounds are hybridised together to constitute two or more pharmacophores into one single structure that enhances their pharmaceutical value as done by their in vivo and in vitro analysis in labs. Several novel methodologies have been developed to synthesize or fuse the biologically active scaffolds into one molecular frame. For instance, oxindoles combined with pyrrolidine or pyrrolizine rings are endowed with increased biological functions. Coumarin rings are fused with several kinds of biologically active moieties to increase their biological importance. Several coupling reactions have been done on indazole moieties in presence of metals such as palladium, iridium or metal free conditions. Some reactions and biological values have been shown in this chapter that fulfils the aim of my thesis.

1.5 References:

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CHAPTER 2

Synthesis, antimicrobial activity, structure-activity relationship and cytotoxic studies of a new series of functionalized (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones

2.1 Introduction

Benzo[1,4]oxazines **1** and 2-oxo-benzo[1,4]oxazines **2**, an important bioactive heterocycle class, are found in many natural products and displays wide range of biological activities such as antibacterial,[1-2] antifungal,[3] anti-hypertensive,[4] anti-inflammatory,[5] antimalarial,[6] anti-cancer,[7] anti-relaxant,[8] anti-rheumatic,[9] potassium channel opener,[10] central nervous system activity,[11] potassium channel modulator,[12] vaso-dilating activity,[13] dopamine receptor[14] and neuroprotective[15] activities (figure 1). Several reports are available in the literature wherein compounds having benzo[1,4]oxazines as part of molecular architecture displayed promising antimicrobial activities.[1-3.]

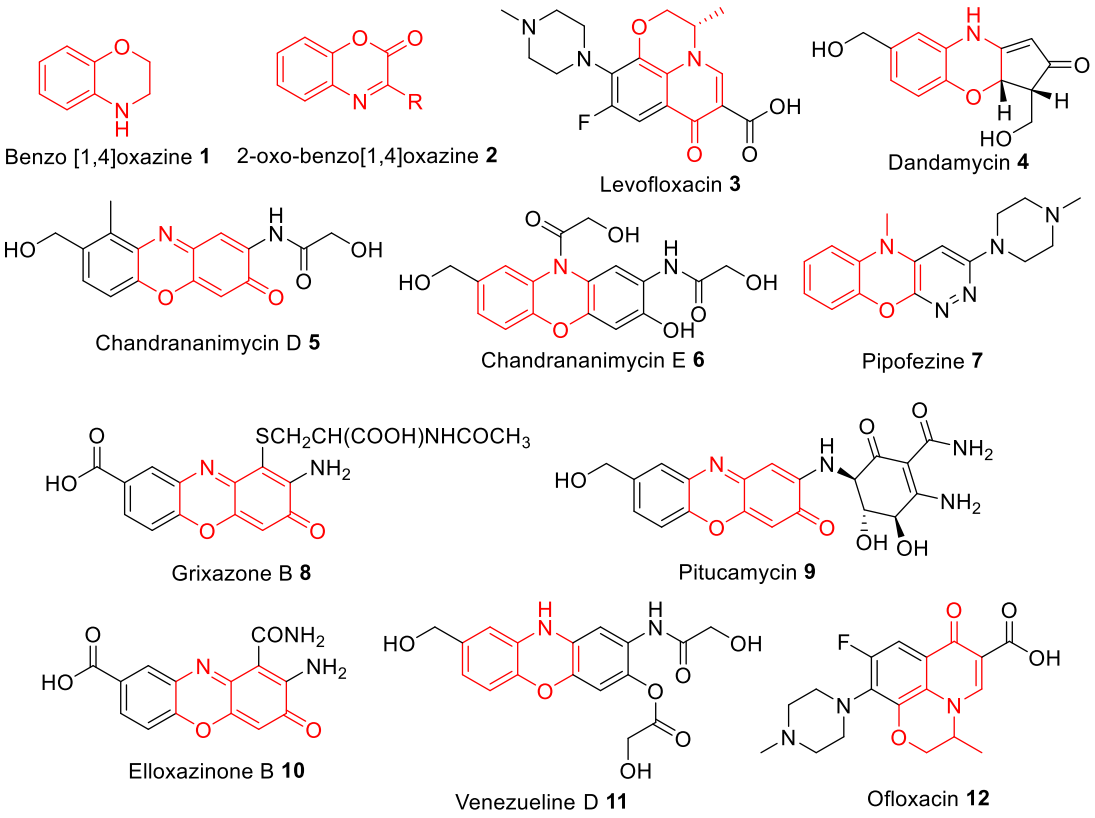


Figure 1. Structures of biologically active molecules having benzo[1,4]oxazine moieties.

Since it has also been observed that several pharmaceutically important substructure/scaffolds as well as some natural products containing pharmaceutically privileged substructures such as cyclopropyl,[16-18] naphthyl,[19] biphenyl[20] and cyclohexylphenyl[21] etc., as part of their molecular architecture, shows promising biological activities (figure 2).

In our research programme towards the search of new bio-active compounds and inspired by the promising role in improving biological activity of these substructures,

we prepared 2-oxo-benzo[1,4]oxazines **23-26** of *prototype I*, incorporating cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as pharmacologically privileged substructures and evaluated them for their *in vitro* antimicrobial activity in detail. (Figure 3)

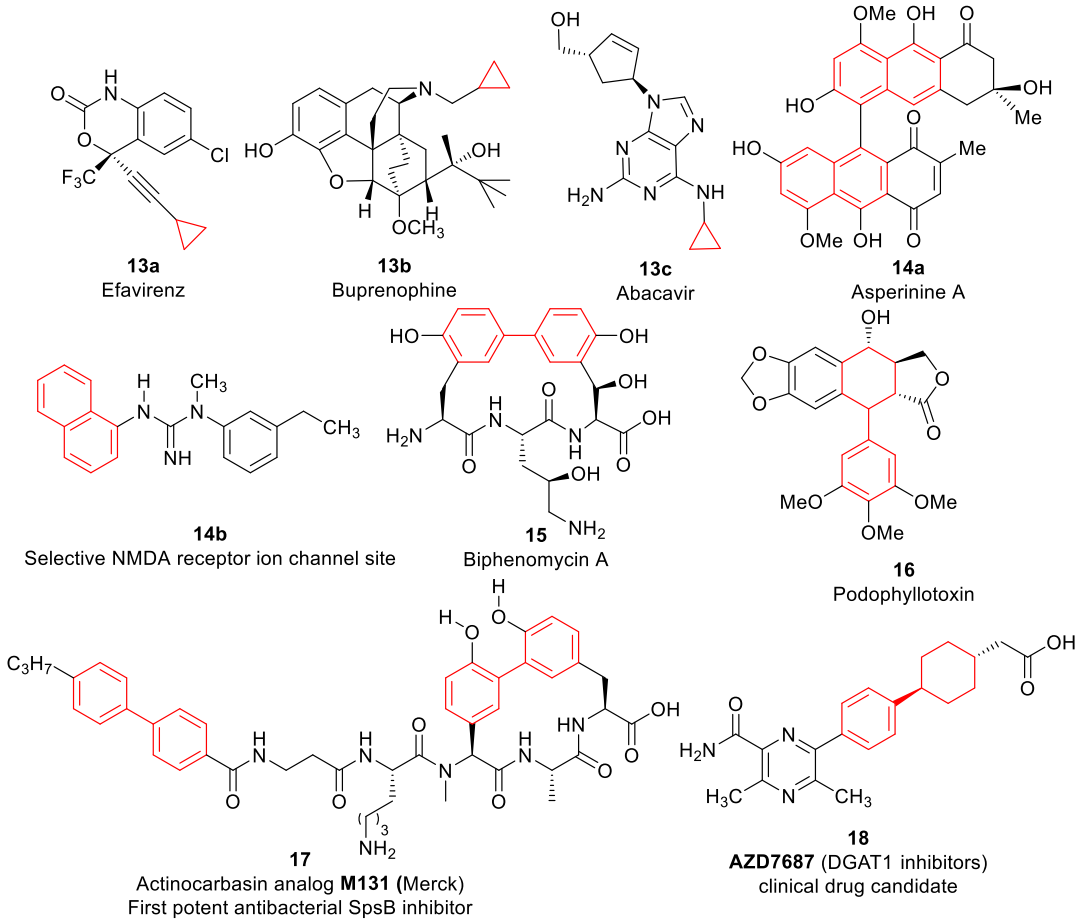


Figure 2. Structures of potent pharmacologically privileged molecules having cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl moieties.

Herein, we report the microwave-assisted synthesis and antimicrobial activity of a new series of functionalized (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo [b] [1,4] oxazin-2-ones **23-26** incorporating cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as pharmaceutically privileged substructures. The *in vitro* antibacterial activity against gram-positive and gram-negative bacteria (i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli*) as well as *in vitro* antifungal activity against fungal species (i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei*), respectively were performed in these antimicrobial studies. The standard drugs ampicillin and chloramphenicol as well as ketoconazole were used as standard references in antibacterial and antifungal assay, respectively. Out of all compounds, **23e** and **26e** have shown promising antimicrobial activity.

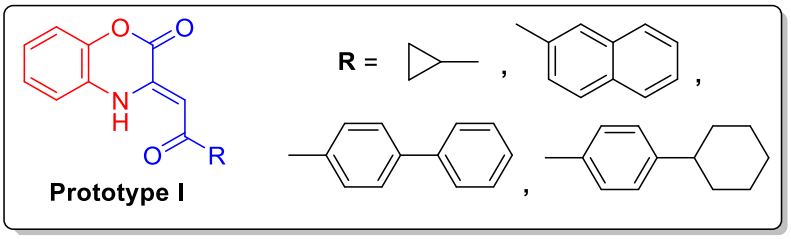


Figure 3. Structure of proposed prototype I.

To the best of our knowledge, for the first time, antimicrobial activity has been reported in 2-oxo-benzo[1,4]oxazines incorporating these pharmaceutically important substructures. We also report, for the first time, *in vitro* antimicrobial activity of some previously reported 2-oxo-benzo[1,4]oxazines **23a**,[22a] **25a**, and **26b-c**[22b]. Cytotoxic studies of active compounds, **23c-e**, **24e**, **25d** and **26d-e**, were also carried out using 3T3 fibroblast cell lines via MTT assay.

2.2 Results and Discussion

2.2.1 Chemistry

During the past few decades, Microwave-Assisted Organic Synthesis (MAOS) has been identified as an efficient green protocol for accelerating drug discovery process.[23a-d] Moreover, it is well documented that microwave irradiation (MW) is a form of electromagnetic energy having lower frequency (300 – 300000 MHz) and it has several advantages over conventional heating conditions such as: reduction of the reaction times as well as decreased side reactions, increased yields of desired products and improve reproducibility. Therefore, industrial as well as academic research groups are frequently using MAOS for rapid reaction optimization, accelerating the efficient synthesis of new chemical entities and also for the novel methodology development.[23e-g] Hence, utilizing this concept;[24a] herein, the analogues of ***prototype I*** were synthesized from the reaction of substituted diketoacid **21a-d** with substituted aminophenol **22a-e** under microwave irradiation condition**.** The required diketoesters **20a-d** incorporating pharmacologically privileged substructures such as cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl were prepared via base-catalyzed reaction of acetophenone **19a-d** with dimethyl oxalate at 0 ºC to 90 ºC in toluene for 4-5 h which furnished the desired diketoesters in 70-75% yields. Conversion of diketoesters **20a-d** to diketoacid **21a-d** with $LiOH.H_2O$ followed by coupling with substituted aminophenols **22a-e** furnished benzo[1,4]oxazines **23-26** incorporated cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as substructures. The desired 2-oxo-benzo

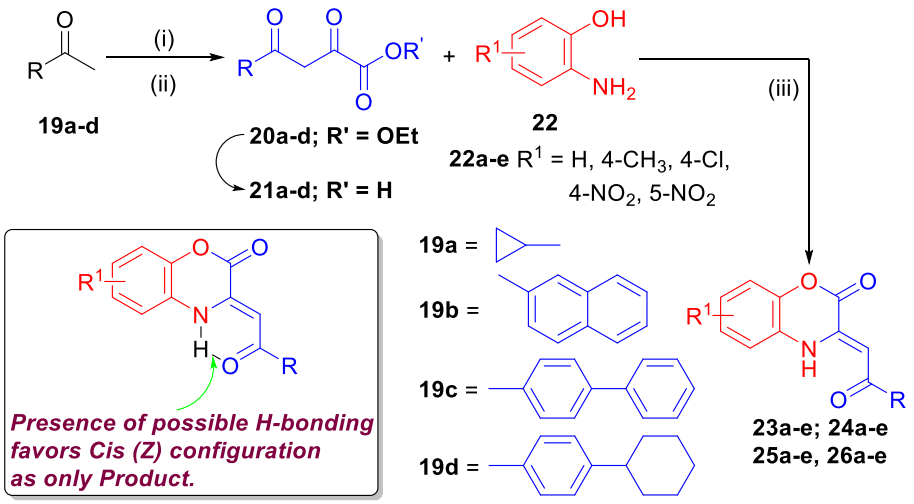
[1,4] oxazines **23-26** were purified either by flash column chromatography or by recrystalization. The geometry of exocyclic double bond was found to be *cis* due to H-bonding between hydrogen attached with benzoxazine nitrogen and oxygen of 2-oxo-2-phenylethylidenes substituent (Scheme 1).[24b-e] All the synthesized compounds were well characterized by ^{1}H NMR and ^{13}C NMR spectroscopy and HRMS analysis (Scheme 1).[25] The physicochemical data of all new functionalized 2-oxo-benzo[1,4]oxazine derivatives are given in Table 1. [25]

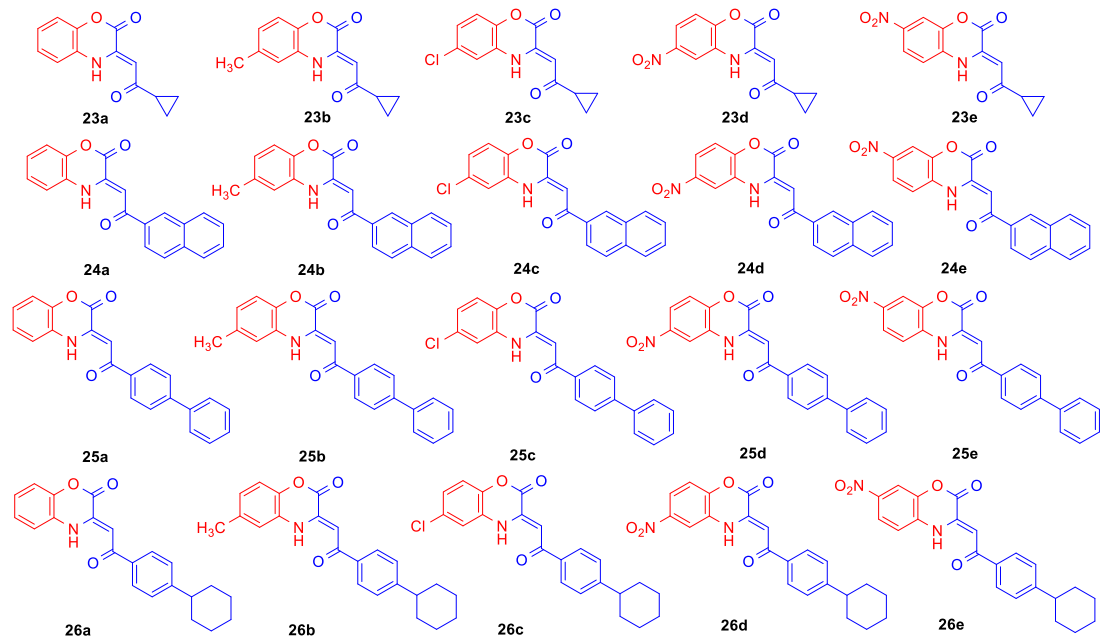
Table 1 Physicochemical data of new functionalized 2-oxo-benzo[1, 4]oxazine derivatives 23-26.

Compound	M.P. (^{o}C)	Method A[a]	
		Time (min.)	Yields[b] (%)
23a	154	10	90
23b	110	10	95
23c	108	10	89
23d	185	15	70
23e	218	15	74
24a	186	15	95
24b	218	15	93
24c	216	15	95
24d	288	15	75
24e	278	15	72
25a	212	20	80
25b	200	10	90
25c	212	10	85
25d	300	15	72
25e	264	15	72
26a	150	15	90
26b	140	15	95
26c	156	15	92
26d	240	15	74
26e	178	15	71

[a]Microwave heating. [b]Isolated yield after recrystalization/column chromatography.

It has been observed that nitro-based 2-oxobenzo[1,4]oxazines **23d-e**, **24d-e**, **25d-e** and **26d-e** were obtained in comparatively lesser yields (70-75%) in comparison with other substituted 2-oxo-benzo[1,4]oxazines (80-95%) **23a-c**, **24a-c**, **25a-c** and **26a-c**, respectively. This is due to poor solubility of nitro-based 2-oxobenzo[1,4]oxazines in ethylacetate which makes chromatographic purification of these compounds tedious and cumbersome. However, the most characteristic feature observed was that a broad range of functional groups, like Cl, Br, OMe and NO_2 are well tolerable under our optimized reaction conditions. Thus, these groups can further be functionalized to new therapeutic molecules.[24a,25]





Scheme 1. *Reagents and Conditions:* (i) NaH, $(COOCH_3)_2$, Toluene, 0-90 °C, (ii) $LiOH.H_2O$, MeOH:THF:H_2O = 7:2:1. rt, 4-5 h, (iii) Microwave 150 °C, 10-15 min.[25]

The plausible mechanism can be explained by taking representative example **23a** which is formed by reaction of **21a** with **22a** (Figure 4). The synthesis involves reaction under the optimized conditions via intermolecular condensation (**22aa** & **22ab**) followed by an intramolecular condensation which furnished (*Z*)-isomer (*cis*) as the only product. We speculate that the probable reason could be the existence of H-bonding between proton on ring B nitrogen atom and oxygen of α, β-unsaturated carbonyl group which stabilizes the Z-isomer as the only product **23a**.

2.2.2 Plausible mechanism for the formation of (Z)-3-(2-cyclopropyl-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (23a)

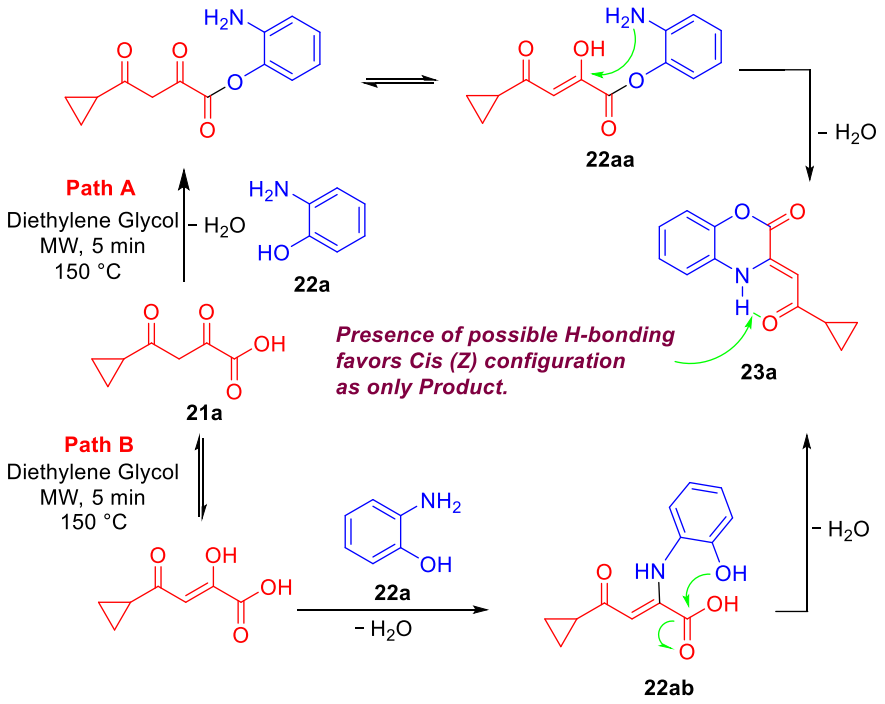


Figure 4. Proposed mechanism for the formation of 2-oxo-benzo[1, 4] oxazine **23a**.

2.2.3 Antimicrobial evaluation

Compounds (**23a-e**, **24a-e**, **25a-e** and **26a-e**) were initially screened for their *in vitro* antibacterial activity against Gram-positive bacterial strains, *Streptomyces griseus* [SG] (MTCC 4734), *Staphylococcus aureus* [SA] (MTCC 3381) and *Bacillus subtilis* [BS] (MTCC 10619), and Gram-negative bacterial strains *Escherichia coli* [EC] (MTCC 443), utilizing the agar diffusion assay.[26-28] The antibiotic ampicillin as well as chloramphenicol were taken as positive controls. Antibacterial screening of all the derivatives, **23-26** as well as positive controls were performed at a fixed concentration of 100 µg/mL. All twenty compounds exhibited antibacterial activity against both Gram-positive and Gram-negative bacterial strains with zones of inhibition (ZOI)

ranging from 4 mm to 24 mm; and also exhibited antifungal activities against four fungal strains. The minimum inhibitory concentration (MIC) values for all the 2-oxo-benzo[1,4]oxazines **23-26** and the positive control drugs, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[29,30]

2.2.3.1 Antibacterial Activity and Structure-Activity Relationship studies

2.2.3.1.1 Antibacterial Efficacy and Structure-Activity Relationship studies based on ZOI values.

Among all the screened gram-positive and gram-negative bacterial strains (Table 2); in the case of *Streptomyces griseus* [SG] strain; (Z)-3-(2-cyclopropyl-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one (**23e**) was found to be the most active compound showing $ZOI_{[SG]}$ = 28 mm as compared to standard drug Ampicillin ($ZOI_{[SG]}$ = 20 mm). However, it displayed equal potency showing $ZOI_{[SG]}$ = 28 mm when compared to Chloramphenicol ($ZOI_{[SG]}$ = 28 mm). Compounds, (Z)-3-(2-cyclopropyl-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazine-2-one (**23d**) and (Z)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one (**26d**), have shown $ZOI_{[SG]}$ values of 22 mm, respectively were found active to ampicillin but less active to Chloramphenicol. Compound (Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one (**25d**), was found to be equally active to ampicillin but displayed lesser zone of inhibition than Chloramphenicol. Compounds **23b**, **24d**, **25b**, **25e**, **26a** and **26e** have shown lesser ZOI than ampicillin as well as Chloramphenicol.

Similarly, in the case of *Staphylococcus aureus* [SA] strain; All the compounds displayed lesser zone of inhibition as compared to Chloramphenicol. While comparing with ampicillin, compounds, **25d** and **26d,** were found most active having ZOI of 20 mm, respectively as compared to Ampicillin ($ZOI_{[SA]}$ = 11 mm). (Z)-3-(2-cyclopropyl-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (**23b)** and (Z)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (**26e**), the next best compound against SA strain, show ZOI of 14 mm, respectively as compared to standard drug, (*Ampicillin*, $ZOI_{[SA]}$ = 11 mm). (Z)-6-chloro-3-(2-cyclopropyl-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4] oxazin-2-one (**23c**) and (Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-6-methyl-

3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (**25b**), were found to be slightly more or equal ZOI as compared to Ampicillin, respectively. Compounds **23a**, **23d**, and **24e** were found to show lesser ZOI as compared to standard drug.

Table 2. Zone of inhibition (ZOI) data for novel synthesized functionalized 2-oxo-benzo[1,4]oxazines **23-26** and positive control drugs against bacteria and fungi**.**

Analogs	**Zone of inhibition[a]**							
	Bacteria[b]			**Bacteria[c]**	**Fungi[d]**			
	SG	**SA**	**BS**	**EC**	**FO**	**AN**	**PF**	**TR**
23a	NA	4	NA	10	8	NA	NA	4
23b	6	14	NA	8	NA	NA	NA	NA
23c	NA	12	8	10	22	NA	NA	NA
23d	22	6	NA	14	22	6	NA	NA
23e	28	10	24	18	10	8	6	NA
24a	NA	NA	NA	NA	NA	4	NA	4
24b	NA	NA	NA	NA	NA	NA	NA	NA
24c	NA	NA	NA	NA	NA	NA	NA	NA
24d	4	NA	4	10	10	NA	NA	NA
24e	NA	8	NA	26	18	NA	20	18
25a	NA	NA	NA	14	NA	4	14	NA
25b	18	11	12	20	NA	NA	NA	NA
25c	NA	NA	NA	NA	NA	8	12	NA
25d	20	20	18	16	14	NA	16	10
25e	10	NA	8	12	18	NA	NA	NA
26a	10	NA	22	8	NA	6	6	NA
26b	NA	NA	NA	NA	12	NA	NA	NA
26c	6	NA	NA	NA	14	10	NA	16
26d	22	20	16	18	10	18	16	20
26e	10	14	24	18	20	8	14	12
AMP[e]	20	11	18	24	-	-	-	-
CAM[f]	28	22	26	32				
KET[g]	-	-	-	-	20	18	22	24

[a]Zone of inhibition was measured in mm at 100 µg/mL. [b]Gram-positive bacteria: SG, *Streptomyces griseus*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*. [c]Gram-negative bacteria: EC, *Escherichia coli*. [d]FO, *Fusarium oxysporium*; AN, *Aspergillus niger*; PF, *Penicillium funiculosum,* TR, *Trichoderma reesei.* [e]AMP: Ampicillin. [f]CAM: Chloramphenicol; [g]KET: Ketoconazole; NA = Compounds which were found Not Active.

Furthermore, in the case of another bacterial strain, *Bacillus subtilis* [BS]; All the compounds displayed lesser zone of inhibition as compared to Chloramphenicol. While comparing with ampicillin; two compounds, **23e** and **26e,** have shown greater ZOI as compared to Ampicillin ($ZOI_{[BS]}$ = 18 mm). Compound **26a**, (Z)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one, the second best compound against [BS], have shown $ZOI_{[BS]}$ = 22 mm as compared to Ampicillin. Compound **25d** was found equally effective to Ampicillin. 2-oxo-benzo

[1,4] oxazines **23c**, **24d**, **25b**, **25e**, and **26d** were found to display lesser $ZOI_{[BS]}$ as compared to Ampicillin.

In the case of gram-negative bacteria, All the compounds displayed lesser zone of inhibition as compared to Chloramphenicol. While comparing with ampicillin; only one compound, (Z)-3-(2-(naphthalen-2-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-one (**24e**), was found to show slightly more $ZOI_{[EC]}$ in comparison with ampicillin. While compounds **23a-e**, **25a-b**, **25d-e**, **26a**, **26d-e** show lesser $ZOI_{[EC]}$ than reference standard ampicillin; whereas remaining compounds i.e., **24a-d**, **25c**, **26b-c** were inactive.

Based on the data from the antibacterial studies against both gram-positive and gram-negative bacterial strains, SAR analysis shows that out of all the compounds, compound **23e**, **25b**, **25d**, **26d** and **26e** were found to exhibit ZOI against all the strains either more or slightly equal in comparison with the standard references. More importantly, compounds containing nitro group either at 4- or 5- position to the aminophenol moiety exhibits higher antibacterial activity with greater ZOI as compared to compounds containing 4-CH_3 or 4-Cl group. This infers that electron-withdrawing group at the aminophenol moiety increases antibacterial potency of the molecule, whereas electron-donating group decreases the antibacterial potency of the molecule. On the other side of the benzo[1,4]oxazine moiety; compounds having cyclohexylphenyl and cyclopropyl group exhibits greater antibacterial potency than compounds having naphthyl and biphenyl group.

2.2.3.1.2 Antibacterial Efficacy and Structure-Activity Relationship studies based on MIC values

The minimum inhibitory concentration (MIC) values for all the 2-oxo-benzo[1,4]oxazines **23-26** and the positive control drugs, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[29,30] As it can be seen from Table 3; **23e** was identified as the most potent antibacterial agents (MIC = 3.12 µg/mL) against [SG] and [BS] strains, respectively as it showed four times more activity than ampicillin against [SG] strain (MIC = 12.5 µg/mL) and eight times more potency than ampicillin against [BS] strains (MIC = 25 µg/mL). However, **23e** was found to be equally potent to chloramphenicol against [SG] strain (MIC = 3.12 µg/mL) whereas it displayed twice potency to chloramphenicol against [BS] strain (MIC = 6.25 µg/mL).

Table 3. Minimum inhibitory concentration values for novel functionalized 2-oxo-benzo [1,4]oxazines **23-26** and positive control drugs against bacteria and fungi.

Analogs	Minimum inhibitory concentration (MIC)[a]							
	Bacteria[b]			Bacteria[c]	Fungi[d]			
	SG	SA	BS	EC	FO	AN	PF	TR
23a	NA	100	NA	50	100	NA	NA	100
23b	100	12.5	NA	100	NA	NA	NA	NA
23c	NA	12.5	100	50	12.5	NA	NA	NA
23d	6.25	100	NA	50	12.5	100	NA	NA
23e	3.12	12.5	3.12	25	50	100	100	NA
24a	NA	NA	NA	NA	NA	100	NA	100
24b	NA	NA	NA	NA	NA	NA	NA	NA
24c	NA	NA	NA	NA	NA	NA	NA	NA
24d	100	NA	100	100	50	NA	NA	NA
24e	NA	100	NA	12.5	25	NA	25	25
25a	NA	NA	NA	25	NA	100	25	NA
25b	12.5	12.5	25	25	NA	NA	NA	NA
25c	NA	NA	NA	NA	NA	25	25	NA
25d	12.5	6.25	25	25	50	NA	25	100
25e	50	NA	100	25	25	NA	NA	NA
26a	50	NA	6.25	100	NA	50	50	NA
26b	NA	NA	NA	NA	50	100	NA	NA
26c	100	NA	NA	NA	25	25	NA	25
26d	12.5	6.25	12.5	25	50	25	25	25
26e	50	12.5	3.12	25	25	100	100	25
AMP[e]	12.5	12.5	25	12.5	-	-	-	-
CAM[f]	3.125	6.25	6.25	6.25				
KET[g]	-	-	-	-	12.5	12.5	25	25

[a]MIC of all compounds was measured at the range from 3.12-100 µg/mL. [b]Gram-positive bacteria: SG, *Streptomyces griseus*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*. [c]Gram-negative bacteria: EC, *Escherichia coli*. [d]Fungi: FO, *Fusarium oxysporium*; AN, *Aspergillus niger*; PF, *Penicillium funiculosum*; TR, *Trichoderma Reesei.* [e]AMP: Ampicillin. [f]CAM: Chloramphenicol. [g]KET: Ketoconazole; NA = Compounds which were found Not Active.

The next potent compound was **26e** which showed eight times greater potency than ampicillin (MIC = 25 µg/mL) and two times more activity than chloramphenicol

(MIC = 6.25 µg/mL) against [BS] strain, respectively. Furthermore, the next potent compounds found in this assay were **23d**, **25d**, **26a** and **26d** having MIC values of 6.25 µg/mL. **23d** showed greater potency than ampicillin but was found less active than chloramphenicol against [SG] strain. Compound **25d** showed two times greater potency than ampicillin and equal potency to chloramphenicol against [SA] strain. Similarly, **26a** exhibited four times greater potency than ampicillin and equal potency to chloramphenicol in [BS] strain. Likewise, **26d** was also found to show two times more activity than ampicillin but showed equal level of activity towards chloramphenicol against [SA] strain.

Compound **25b**, **25d** and **26d** against [SG] strains; **23b-c**, **23e**, **25b** and **26e** against [SA] strain; **26d** against [BS] strain and **24e** against [EC] strain showed equal potency to ampicillin but were less active than chloramphenicol. Similarly, **25b** and **25d** against [BS] strain; **23e**, **25a-b**, **25d-e**, **25d-e** against [EC] strain were found less active to both standard drugs, ampicillin and chloramphenicol. Finally, all those compounds which have shown (MIC = ≥25 µg/mL) in [SG], [SA] and [EC] strains were found to be either less active or no activity as compared to ampicillin and chloramphenicol. Overall, SAR study shows that nitro group either at 4-or 5- position and cyclopropyl and cyclohexylphenyl group have shown greater antibacterial activity (>MIC) than ampicillin and chloramphenicol except compound **23e** which showed equal potency to chloramphenicol against [SG] strain.

2.2.3.2 Antifungal Efficacy and Structure-Activity Relationship studies

2.2.3.2.1 Antifungal Efficacy and Structure-Activity Relationship studies based on ZOI values

All the derivatives (**23a-e**, **24a-e**, **25a-e** and **26a-e**) were also screened for their antifungal activity against four different fungal strains, i.e. *Fusarium oxysporium* [FO] (ATCC 62506), *Aspergillus niger* [AN] (ATCC 9029), *Penicillium funiculosum* [PF] (ATCC 11797) and *Trichoderma Reesei* [TR] (ATCC 13631) (Table 2). The antifungal drug, ketoconazole was used as a positive control.[26-28] The fungal strains were grown and maintained on Sabouraud glucose agar plates. The plates were incubated at 26 ºC for 72 h, and resulting ZOIs were measured. Antifungal screening of all the derivatives **23**-**26** has been performed at a fixed concentration of 100 µg/mL. Similarly, in case of *Fusarium oxysporium* [FO], Compound **23c** and **23d**, ($ZOI_{[FO]}$ = 22 mm each), were found to be the most active compound of the series against fungal

strains as compared to standard reference ketoconazole ($ZOI_{[FO]}$ = 20 mm). **26e** display equal potency to standard drug. Compounds (Z)-3-(2-(naphthalen-2-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one **24e** and (Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one **25e**; **25d** and (Z)-6-chloro-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one **26c**, were found to exhibit relatively lesser $ZOI_{[FO]}$ having values of 18 mm, and 14 mm, respectively. While compounds **23a**, **23e**, **24d**, **26b** and **26d** show lesser **$ZOI_{[FO]}$** than reference drug; whereas rest of the compounds i.e. **23b**, **24a-c**, **25a-c** and **26a** were found totally inactive.

Similarly, in case of *Aspergillus niger* [AN], compound **26d** was found equally active as compared to ampicillin ($ZOI_{[AN]}$ = 18 mm). Compounds **23d**, **23e**, **24a**, **25a**, **25c**, **26a** and **26e** show lesser $ZOI_{[AN]}$ than reference standard; whereas remaining compounds i.e., **23a-c**, **24b-e**, **25b**, **25d-e** and **26c** were found inactive.

Furthermore, in case of another fungal strain, *Penicillium funiculosum* [PF]; **24e**, **25a**, **25d**, and **26d-e** exhibited slightly lesser ZOI as compared to standard drug, whereas compounds **23a-d**, **24a-d**, **25b**, **25e**, **26b-c** were found inactive.

In the fourth fungal strain, *Trichoderma Reesei* [TR]; only one compound **26d,** shows $ZOI_{[TR]}$ = 20 mm, which is slightly lower than reference standard. Compound **23a**, **24a**, **24e**, **25d**, **26c** and **26e** were found to show lesser antifungal potency as compared to standard reference; while remaining compounds were found inactive in this strain.

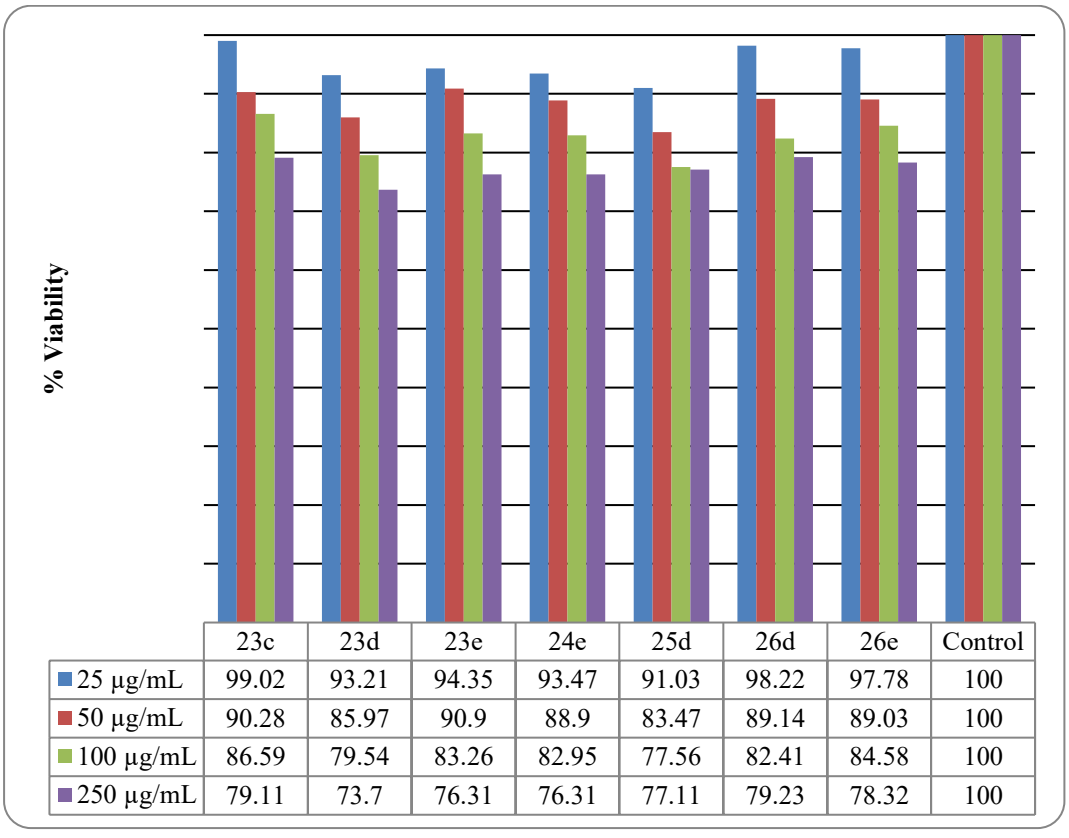
Based on the data from the antifungal studies against four fungal strains; Structure-activity relationship studies have been analysed and interpreted. *Cyclopropyl* group containing 2-oxo-benzo[1,4]oxazines (**23c** and **23d**) display greater potency in antifungal assay. Nitro group and *cyclohexylphenyl* substructure scaffold containing compound, **26d** and **26e,** exhibited antifungal potency against all the four antifungal strains as compared to ketoconazole ($ZOI_{[FO]}$ = 20 mm; $ZOI_{[AN]}$ = 18 mm; $ZOI_{[PF]}$ = 22 mm; $ZOI_{[TR]}$ = 24 mm). However, out of all, only compound **26d** was able to display equal level of antifungal potency ($ZOI_{[AN]}$ = 18 mm) in comparison to ketoconazole. Compound **24e**, containing nitro group and naphthyl substructure moiety, display relatively lower potency. Similarly, **25d** containing nitro group and biphenyl substructure scaffold, display lower potency than ketoconazole.

2.2.3.2.2 Antifungal Efficacy and Structure-Activity Relationship studies based on MIC values[29,30]

As can be seen from Table 3; compound **23c** and **23d**, have shown equal potency (MIC = 12.5 µg/mL) than ketoconazole (MIC = 12.5 µg/mL) in [FO] fungal strain. Moreover, compounds **24e**, **25e**, **26c** and **26e** in [FO] strain as well as compounds **25c**, **26c** and **26 d** in [AN] strain were found two times less active compared to ketoconazole. While compounds such as **24e**, **25a**, **25c**, **25d** and **26d**, were found equally active having MIC = 25 µg/mL in [PF] strains; whereas compounds **24e**, **26c**, **26d** and **26e** displayed equal potency in [TR] strains compared to ketoconazole. All those compounds which have shown (MIC = ≥50 µg/mL) in [FO], [AN], [PF] and [TR] strains were found to be either less active or no activity as compared to ketoconazole. Thus, SAR interpretation illustrates that nitro group containing cyclopropyl and cyclohexylphenyl substructure scaffolds in 2-oxo-benzo[1,4]oxazines increases antifungal activity.

2.2.4 Cytotoxicity studies

All the active compounds, **23c-e**, **24e**, **25d** and **26d-e**, were assessed for their cytotoxic studies in 3T3 fibroblast cell lines using MTT assay (Figure 5).[31] All the compounds were found non-toxic even at 250 µg/mL and shows acceptable values of cell viability.



	23c	23d	23e	24e	25d	26d	26e	Control
25 µg/mL	99.02	93.21	94.35	93.47	91.03	98.22	97.78	100
50 µg/mL	90.28	85.97	90.9	88.9	83.47	89.14	89.03	100
100 µg/mL	86.59	79.54	83.26	82.95	77.56	82.41	84.58	100
250 µg/mL	79.11	73.7	76.31	76.31	77.11	79.23	78.32	100

Figure 5. Percentage cell viability test of active compounds **23c-e**, **24e**, **25d** and **26d-e**.

2.3 Conclusions

In conclusion, a new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo [b] [1,4] oxazin-2-ones **23-26** were synthesized in excellent (upto 95%) yields. Several synthesized compounds were found to display greater *in vitro* antibacterial activity against gram positive and gram-negative bacteria i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli* as well as *in vitro* antifungal activity against fungal species i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei,* than standard drugs, ampicillin and chloramphenicol, as well as ketoconazole, respectively. *Cyclopropyl* and *cyclohexylphenyl* substructure moieties were identified to increase both antibacterial and antifungal activities. Electron-withdrawing group (i.e. NO_2 group) present at 2-oxo-benz[1,4]oxazine nucleus increases antibacterial as well as antifungal activity. To the best of our knowledge, for the first time, pharmaceutically important substructures were incorporated into 2-oxo-benzo[1,4]oxazines, which displayed greater antimicrobial potency than standard drugs, ampicillin, chloramphenicol and ketoconazole. Analogues of compounds **23e** and **26e** as well as compound **26d** were considered as lead molecules worthy of further structural optimization and development as potential antibacterial and antifungal agents, respectively, for the treatment of bacterial and fungal infections.

2.4 Experimental Section and Characterization data

2.4.1 General

All glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on complab melting point apparatus and are presented uncorrected. Microwave reactor (CEM Discover) was used for operation of reactions. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum 2 spectrophotometer ^{1}H NMR and ^{13}C NMR spectra were recorded on ECS 400 MHz (JEOL) NMR spectrometer using $CDCl_3$, CD_3OD and CD_3SOCD_3 as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S QTof (Waters, USA) Spectrometer. Column chromatography was performed over Merck silica gel (particle size: 60-120 Mesh) procured from Qualigens™ (India), flash silica gel (particle size: 230-400 Mesh). All chemicals and reagents were obtained from Sigma Aldrich (USA), Merck (India) or Spectrochem (India) and were used without further purification.

2.4.2 ***General procedure for the Synthesis of 2-oxo-benzo[1,4]oxazine derivatives*** **(23-26):** Diketoesters **20a-d**, which were obtained by base-mediated reaction of acetophenone **19a-d** with dimethyl oxalate, were dissolved in a mixture of MeOH:THF: H_2O (7:2:1) and LiOH.H_2O (1.2 eq.) was added to the reaction mixture after 10 min at room temperature. The reaction mixture was stirred for another 3 hours. The reaction mixture was quenched with 3N HCl and extracted with ethyl acetate (3 × 10 mL); washed with distilled water (3 × 20 mL); then with brine (3 × 10 mL). The combined organic layer was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The resultant crude product was further purified by recrystallization with EtOAc/Hexane that afforded pure diketo-acids **21a-d**. Then after, to a solution of the compound **21a-d** (substituted diketo-acid: 2.0 mmol) in diethylene glycol (2 mL) was added compound **22a-e** (substituted 2-aminophenol; 2.0 mmol). The reaction mixture was subjected to microwave irradiation at 150˚C temperature for about 10-20 min. The progress of the reaction was monitored by TLC (9:1 Hexane/ethyl acetate as an eluent). Then, the reaction mixture was extracted with ethyl acetate (3 × 50 mL); washed with distilled water (50 mL); then with brine (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant crude product were further purified by recrystallization by EtOAc/Hexane (v/v = 80:20) or by flash column chromatography technique over silica gel (using 9:1 Hexane/ethyl acetate as an eluent), which furnished the 2-oxo-benzo[1,4]oxazine derivatives (**23-26**) in 70-96 % yields range.

2.4.3 Characterization data of 2-oxo-benzo[1,4]oxazines (23a-e, 24a-e, 25a-e and 26a-e):

(Z)-3-(2-cyclopropyl-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (23a): Solid; yield: 90 %, m.p. 154 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 3093, 2929, 1758, 1628, 1571, 1263; ^{1}H NMR (400 MHz) δ 12.46 (s, 1H), 7.15 (t, J = 8.3 Hz, 2H), 7.05 (t, J = 7.7 Hz, 1H), 6.98 (d, J = 7.3 Hz, 1H), 6.45 (s, 1H), 2.04 – 1.97 (m, 1H), 1.15-1.11 (m, 2H), 1.01 – 0.96 (m, 2H); ^{13}C NMR (100 MHz) δ 202.3, 156.3, 141.0, 136.6, 125.9, 124.1, 123.5, 117.2, 115.6, 98.2, 22.1, 11.5; HRMS (ESI) calcd. for $C_{13}H_{11}NO_3$ $[M+H]^+$: 230.0739; found 230.0732.

(Z)-3-(2-cyclopropyl-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[b][1,4]

oxazin-2-one (23b): Solid; yield: 95 %, m.p. 110 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 2921, 1752, 1626, 1576, 1437, 1265; ^{1}H NMR (400 MHz) δ 12.39 (s, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.83 – 6.76 (m, 2H), 6.40 (s, 1H), 2.30 (s, 3H), 1.99 – 1.95 (m, 1H), 1.11 -1.09 (m, 2H), 0.97-0.94 (m, 2H).; ^{13}C NMR (100 MHz) δ 202.1, 155.6, 139.0, 136.7, 135.9, 124.3, 123.6, 116.8, 115.8, 97.9, 22.1, 21.1, 11.4; HRMS (ESI) calcd. for $C_{14}H_{13}NO_3$ $[M+H]^+$: 244.0895; found 244.0899.

(Z)-6-chloro-3-(2-cyclopropyl-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4] oxazin-2-one (23c): Solid; yield: 89 %, m.p. 108 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2932, 1757, 1629, 1491, 1376, 1258; ^{1}H NMR (400 MHz) δ 12.38 (s, 1H), 7.06 (d, J = 8.8 Hz, 1H), 6.99 – 6.97 (m, 2H), 6.44 (s, 1H), 2.03 – 1.96 (m, 1H), 1.13 -1.11 (m, 2H), 0.99-0.97 (m, 2H).; ^{13}C NMR (100 MHz) δ 202.5, 155.9, 139.5, 135.8, 131.1, 125.0, 123.3, 118.2, 115.3, 99.2, 22.3, 11.8; HRMS (ESI) calcd. for $C_{13}H_{10}ClNO_3$ $[M+H]^+$: 264.0349; found 264.0344.

(Z)-3-(2-cyclopropyl-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[*b*][1,4] oxazin-2-one (23d): Solid; yield: 70 %, m.p. 185 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 3026, 2934, 1766, 1619, 1456, 1270; ^{1}H NMR (400 MHz) δ 7.93 – 7.84 (m, 2H), 6.52 (s, 1H), 6.22 (s, 1H), 2.03 (s, 1H), 1.74 – 1.03 (m, 4H); ^{13}C NMR (100 MHz) δ 202.9, 155.2, 145.2, 144.7, 135.0, 124.9, 118.5, 117.9, 110.9, 100.5, 22.6, 12.3; HRMS (ESI) calcd. for $C_{13}H_{10}N_2O_5$ $[M+H]^+$: 275.0590; found 275.0595.

(Z)-3-(2-cyclopropyl-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[*b*][1,4] oxazin-2-one (23e): Solid; yield: 74 %, m.p. 218 °C; FT-IR (KBr, νmax/cm^{-1}) 3438, 3029, 2932, 1764, 1618, 1452, 1268; ^{1}H NMR (400 MHz) δ 8.08-8.04 (m, 2H), 7.05 (d, J = 8.4 Hz, 1H), 6.57 (s, 1H), 2.09-2.03 (s, 1H), 1.18-1.16 (m, 2H), 1.08-1.04 (m, 2H); ^{13}C NMR (100 MHz) δ 203.3, 155.1, 142.6, 140.1, 134.7, 129.9, 121.9, 115.2, 113.5, 101.6, 22.8, 12.5; HRMS (ESI) calcd. for $C_{13}H_{10}N_2O_5$ $[M+H]^+$: 275.0590; found 275.0596.

(Z)-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24a): Solid; yield: 95 %, m.p. 186 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2928, 1764, 1620, 1558, 1447, 1292; ^{1}H NMR (400 MHz) δ 8.55 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 8.00 -7.87 (m, 3H), 7.59- 6.55 (m, 2H), 7.23-7.19 (m, 3H), 7.15-7.12 (m, 2H); ^{13}C NMR (100 MHz) δ 191.5, 156.5, 141.4, 139.1, 135.7, 135.6, 132.8, 129.8, 129.3, 128.8, 128.5, 127.9, 126.9, 126.1, 124.1, 123.9, 123.7, 117.3, 116.1, 94.9; HRMS (ESI) calcd. for $C_{20}H_{13}NO_3$ $[M+H]^+$: 316.0895; found 316.0899.

(Z)-6-methyl-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24b): Solid; yield: 93 %, m.p. 218 °C; FT-IR (KBr, νmax/cm^{-1}) 3452, 3058, 1754, 1607, 1580, 1288, 1227; ^{1}H NMR (400 MHz) δ 8.54 (s, 1H), 8.09 – 7.87 (m, 4H), 7.59-7.55 (m, 2H), 7.21 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.94-6.89 (m, 2H); ^{13}C NMR (100 MHz) δ 191.4, 156.7, 139.4, 139.2, 136.2, 135.8, 135.5, 132.8, 129.7, 129.2, 128.7, 128.5, 127.9, 126.9, 124.9, 123.8, 123.5, 116.9, 116.3, 94.8; HRMS (ESI) calcd. for $C_{21}H_{15}NO_3$ $[M+H]^+$: 330.1052; found 330.1059.

(Z)-6-chloro-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24c): Solid; yield: 95 %, m.p. 216 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 3061, 1765, 1618, 1594, 1493, 1285; ^{1}H NMR (400 MHz) δ 8.54 (s, 1H), 8.08 – 7.87 (m, 4H), 7.62 – 7.55 (m, 3H), 7.15 – 7.13 (m, 2H), 7.07 – 7.05 (m, 1H); ^{13}C NMR (100 MHz) δ 190.2, 156.2, 140.6, 139.5, 136.0, 135.5, 133.0, 130.1, 129.7, 129.2, 129.1, 128.9, 128.1, 127.4, 126.1, 123.9, 123.5, 118.4, 116.9, 94.9; HRMS (ESI) calcd. for $C_{20}H_{12}ClNO_3$ $[M+H]^+$: 350.0506; found 350.0501.

(Z)-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24d): Solid; yield: 75 %, m.p. 288 °C; FT-IR (KBr, νmax/cm^{-1}) 3432, 2927, 1765, 1626, 1343, 1288; ^{1}H NMR (400 MHz) δ 8.71 – 8.70 (m, 2H), 8.17 (d, J = 7.6 Hz, 1H), 8.06 -7.88 (m, 4H), 7.65 – 7.56 (m, 2H), 7.41 (d, J = 8.8 Hz, 1H), 7.12 (s, 1H); ^{13}C NMR (100 MHz) δ 190.2, 155.9, 145.9, 145.0, 139.0, 135.9, 135.5, 133.0, 130.2, 129.3, 129.1, 128.9, 128.1, 127.4, 125.9, 123.9, 118.8, 117.8, 112.9, 95.7; HRMS (ESI) calcd. for $C_{20}H_{12}N_2O_5$ $[M+H]^+$: 361.0746; found 361.0743.

(Z)-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24e): Solid; yield: 72 %, m.p. 278 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 2929, 1763, 1629, 1344, 1290; ^{1}H NMR (400 MHz) δ 8.72 (s, 1H), 8.16 (d, J = 7.6 Hz, 1H), 8.04 -7.93 (m, 5H), 7.80 (d, J = 8.8 Hz, 1H), 7.64 – 7.56 (m, 2H), 7.18 (s, 1H); ^{13}C NMR (100 MHz) δ 190.8, 155.6, 142.5, 141.0, 138.6, 135.8, 135.6, 132.9, 131.2, 130.2, 129.6, 129.2, 129.1, 128.1, 127.4, 123.9, 121.4, 117.4, 112.6, 96.9; HRMS (ESI) calcd. for $C_{20}H_{12}N_2O_5$ $[M+H]^+$: 361.0746; found 361.0741.

(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (25a): Solid; yield: 80 %, m.p. 212 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 2922, 1761, 1602, 1623, 1572, 1283; ^{1}H NMR (400 MHz) δ 8.09 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.49 – 7.40 (m, 3H), 7.23 – 7.11 (m, 5H); ^{13}C NMR (100 MHz) δ 191.2, 156.5, 145.5, 141.4, 140.0, 139.2, 137.1,

129.1, 128.4, 128.3, 127.5, 127.4, 126.0, 124.1, 123.9, 117.3, 116.1, 94.8; HRMS (ESI) calcd. for $C_{22}H_{15}NO_3$ $[M+H]^+$: 342.1052; found 342.1059.

(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo [*b*][1,4] oxazin-2-one (25b): Solid; yield: 90 %, m.p. 200 °C; FT-IR (KBr, νmax/cm^{-1}) 3432, 3073, 2927, 1760, 1600, 1573, 1284; ^{1}H NMR (400 MHz) δ 8.10 – 8.08 (m, 2H), 7.25 – 7.64 (m, 4H), 7.49 – 7.39 (m, 3H), 7.10 – 7.08 (m, 2H), 6.93 – 6.89 (m, 2H), 2.36 (s, 3H); ^{13}C NMR (100 MHz) δ 191.0, 156.6, 145.4, 140.1, 139.4, 139.2, 137.2, 136.1, 129.1, 128.4, 128.3, 127.5, 127.4, 124.9, 123.5, 116.9, 116.3, 94.6, 21.2; HRMS (ESI) calcd. for $C_{23}H_{17}NO_3$ $[M+H]^+$: 356.1208; found 356.1203.

(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-6-chloro-3,4-dihydro-2H-benzo [*b*][1,4] oxazin-2-one (25c): Solid; yield: 85 %, m.p. 212 °C; FT-IR (KBr, νmax/cm^{-1}) 3438, 2926, 1765, 1619, 1595, 1494, 1274; ^{1}H NMR (400 MHz) δ 8.09 (d, J = 8.8 Hz, 2H), 7.74 – 7.64 (m, 4H), 7.48 – 7.41 (m, 3H), 7.14 – 7.08 (m, 4H); ^{13}C NMR (100 MHz) δ 189.8, 156.2, 144.8, 140.6, 139.6, 137.5, 129.6, 129.5, 129.3, 128.8, 128.6, 127.6, 127.4, 126.1, 123.5, 118.4, 117.0, 94.7; HRMS (ESI) calcd. for $C_{22}H_{14}ClNO_3$ $[M+H]^+$: 376.0662; found 376.0668.

(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo [*b*][1,4] oxazin-2-one (25d): Solid; yield: 72 %, m.p. 300 °C; FT-IR (KBr, νmax/cm^{-1}) 3431, 2923, 1767, 1636, 1598; 1574, 1276; ^{1}H NMR (400 MHz) δ 8.10 (d, J = 8.4 Hz, 2H), 8.03 – 7.97 (m, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.50 – 7.41 (m, 3H), 7.32 (d, J = 9.2 Hz, 1H), 7.19 (s, 1H); ^{13}C NMR (100 MHz) δ 189.8, 156.9, 145.9, 145.0, 144.9, 139.6, 139.1, 137.5, 129.6, 128.8, 128.7, 127.7, 127.5, 125.9, 118.8, 117.8, 112.9, 95.4; HRMS (ESI) calcd. for $C_{22}H_{14}N_2O_5$ $[M+H]^+$: 387.0903; found 387.0906.

(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo [*b*][1,4] oxazin-2-one (25e): Solid; yield: 72 %, m.p. 264 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2921, 1769, 1633, 1600, 1573, 1274; ^{1}H NMR (400 MHz) δ 8.09 (d, J = 8.8 Hz, 2H), 7.74 – 7.64 (m, 4H), 7.48 – 7.41 (m, 3H), 7.14 – 7.08 (m, 4H); ^{13}C NMR (100 MHz) δ 189.8, 156.2, 144.8, 140.6, 139.6, 137.6, 129.6, 129.3, 129.5, 128.8, 128.6, 127.6, 127.4, 126.1, 123.5, 118.4, 117.0, 94.7; HRMS (ESI) calcd. for $C_{22}H_{14}N_2O_5$ $[M+H]^+$: 387.0903; found 387.0909.

(Z)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[*b*][1,4] oxazin-2-one (26a): Solid; yield: 90 %, m.p. 150 °C; FT-IR (KBr, νmax/cm^{-1}) 3421,

2923, 2849, 1752, 1627, 1448, 1281; ^{1}H NMR (400 MHz) δ 7.94 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.18 (t, J =7.2 Hz, 2H), 7.10-7.05 (m, 3H), 2.57(t, J =6.8 Hz, 1H), 1.89 – 1.74 (m, 5H), 1.49 – 1.25 (m, 5H); ^{13}C NMR (100 MHz) δ 191.5, 156.5, 153.6, 141.3, 138.9, 136.1, 128.0, 127.4, 125.9, 124.0, 123.9, 117.2, 115.9, 94.9, 44.8, 34.3, 26.9, 26.2; HRMS (ESI) calcd. for $C_{22}H_{21}NO_3$ $[M+H]^+$: 348.1521; found 348.1527.

(*Z*)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo [*b*][1,4] oxazine-2-one (26b): Solid; yield: 95 %, m.p. 140 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2926, 2850, 1765, 1626, 1605, 1227; ^{1}H NMR (400 MHz) δ 7.94 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.08-7.04 (m, 2H), 6.90 – 6.86 (m, 2H), 2.57 – 2.56 (m, 1H), 2.35 (s, 3H), 1.89 -1.74(m, 5H), 1.52 – 1.24 (m, 5H); ^{13}C NMR (100 MHz) δ 191.4, 156.7, 153.5, 139.4, 139.0, 136.2, 136.1, 127.9, 127.4, 124.7, 123.6, 116.9, 116.2, 94.7, 44.8, 34.3, 26.9, 26.2, 21.1; HRMS (ESI) calcd. for $C_{23}H_{23}NO_3$ $[M+H]^+$: 362.1678; found 362.1673.

(*Z*)-6-chloro-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo [*b*][1,4] oxazin-2-one (26c): Solid; yield: 92 %, m.p. 156 °C; FT-IR (KBr, νmax/cm^{-1}) 3044, 2920, 2850, 1763, 1628, 1493, 1276; ^{1}H NMR (400 MHz) δ 7.95 - 7.92 (m, 2H), 7.33 – 7.31 (m, 2H), 7.13-7.01 (m, 4H), 2.57 – 2.56 (m, 1H), 1.87 -1.75(m, 5H), 1.46 – 1.24 (m, 5H); ^{13}C NMR (100 MHz) δ 191.6, 155.9, 153.9, 139.7, 138.1, 135.8, 131.1, 128.1, 127.4, 124.9, 123.6, 118.2, 115.7, 95.9, 44.8, 34.2, 26.8, 26.1; HRMS (ESI) calcd. for $C_{22}H_{20}ClNO_3$ $[M+H]^+$: 382.1132; found 382.1137.

(*Z*)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo [*b*][1,4] oxazin-2-one (26d): Solid; yield: 74 %, m.p. 240 °C; FT-IR (KBr, νmax/cm^{-1}) 3429, 2926, 1766, 1635, 1600, 1340, 1280; ^{1}H NMR (400 MHz) δ 8.00 - 7.94 (m, 4H), 7.35 – 7.29 (m, 3H), 7.13 (s, 1H), 2.59 – 2.57 (m, 1H), 1.87 -1.75(m, 5H), 1.46 – 1.24 (m, 5H); ^{13}C NMR (100 MHz) δ 191.6, 154.4, 144.9, 144.5, 137.4, 135.6, 128.3, 127.6, 124.9, 118.8, 117.9, 111.8, 111.4, 97.1, 44.9, 34.2, 26.8, 26.1; HRMS (ESI) calcd. for $C_{22}H_{20}N_2O_5$ $[M+H]^+$: 393.1372; found 393.1376.

(*Z*)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo [*b*][1,4] oxazin-2-one (26e): Solid; yield: 71 %, m.p. 178 °C; FT-IR (KBr, νmax/cm^{-1}) 3428, 2924, 1767, 1634, 1602, 1339, 1282; ^{1}H NMR (400 MHz) δ 8.12-8.07 (m, 2H), 7.95 (d, J = 8.0 Hz, 2H), 7.34 (d, J =8.4 Hz, 2H), 7.19-7.16 (m, 2H), 2.61-2.59(m, 1H), 1.87 – 1.75 (m, 5H), 1.49 – 1.25 (m, 5H); ^{13}C NMR (100 MHz) δ 192.2,

155.2, 154.7, 142.8, 140.3, 136.9, 135.5, 129.8, 128.4, 127.6, 121.9, 115.5, 113.6, 98.3, 44.9, 34.2, 26.8, 26.1; HRMS (ESI) calcd. for $C_{22}H_{20}N_2O_5$ $[M+H]^+$: 393.1372; found 393.1378.

2.5 Materials and Methods

2.5.1 Determination of antibacterial ZOI value using agar well diffusion method.[26-28]

In vitro antibacterial activity of samples was studied against gram positive and gram negative bacterial strains by the agar well diffusion method (Perez *et al,* 1990). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The compounds were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL. The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculums (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the compounds was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain controls were maintained where pure solvents were used instead of the compounds. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

2.5.2 Determination of antifungal ZOI value using agar well diffusion method.[27b]

Antifungal activity of the compounds was investigated by agar well diffusion method (Bonjar *et al*, 2005). The yeasts and saprophytic fungi were sub cultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°Cfor 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh compounds was administered to fullness for each well.

Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated.

2.5.3 Determination of antimicrobial MIC value using serial dilution method.[29,30]

Concentration of analogues and positive control drugs at 100 µg/mL were prepared in an appropriate solvent. Inoculums of the bacterial and fungal cultures were also prepared. Inoculum (0.2 mL) and sterile water (3.8 mL) were added to a series of tubes each containing 1 mL of test compound solution at the six different concentrations. The tubes were incubated for 24 h and carefully observed for the presence of turbidity. The minimum concentration at which no growth was observed was taken as the MIC. The MIC for all the analogues examined ranged from 3.12-100 µg/mL.

2.5.4 Determination of cytotoxic studies in 3T3 fibroblast cell lines using MTT assay

The cytotoxic effect of the active compounds prepared on cells was detected *in vitro* using the mitochondrial cytotoxic test according to Danihelová et al. (2013)[31] with modifications. Cell viability was evaluated using thiazolyl blue tetrazolium bromide (MTT), which indicates the metabolic activity of cells. The experiment was performed in 96-well microplates. The cells were seeded at a density of 3.5×10^3 3T3 fibroblast cells per well. Samples were dissolved in DMSO (stock solution 10 mM) and subsequently diluted in medium to the final concentration of 25 µM to 250 µM (concentration of DMSO 0.5%) and after 24 h they were added to the cells. Microplates were cultivated for 72 h in thermostat at 37 °C and 5% CO_2 atmosphere. After incubation thiazolyl blue tetrazolium bromide (3.33 mg/ml phosphate buffered saline, pH=7.4) was pipetted to each well and left to incubate for further two hours. Then the medium with MTT solution was removed. Formazan crystals in viable cells were dissolved in the lysis solution (4 mM HCl and 0.1% Nonidet P40 in ethanol). Microplates were shaken 15 min at 1500 rpm. Absorbance was measured at 540 nm and reference wavelength at 740 nm. Each value is the mean of 6 wells with standard deviation. Inhibition activity was expressed as percentages of control with DMSO.

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2.7 Characterization spectral data ([1]HNMR and [13]CNMR) of selected 2-oxo-benzo [1, 4] oxazines 23a, 23e, 24a, 24e, 25a, 25e, 26a, and 26e:

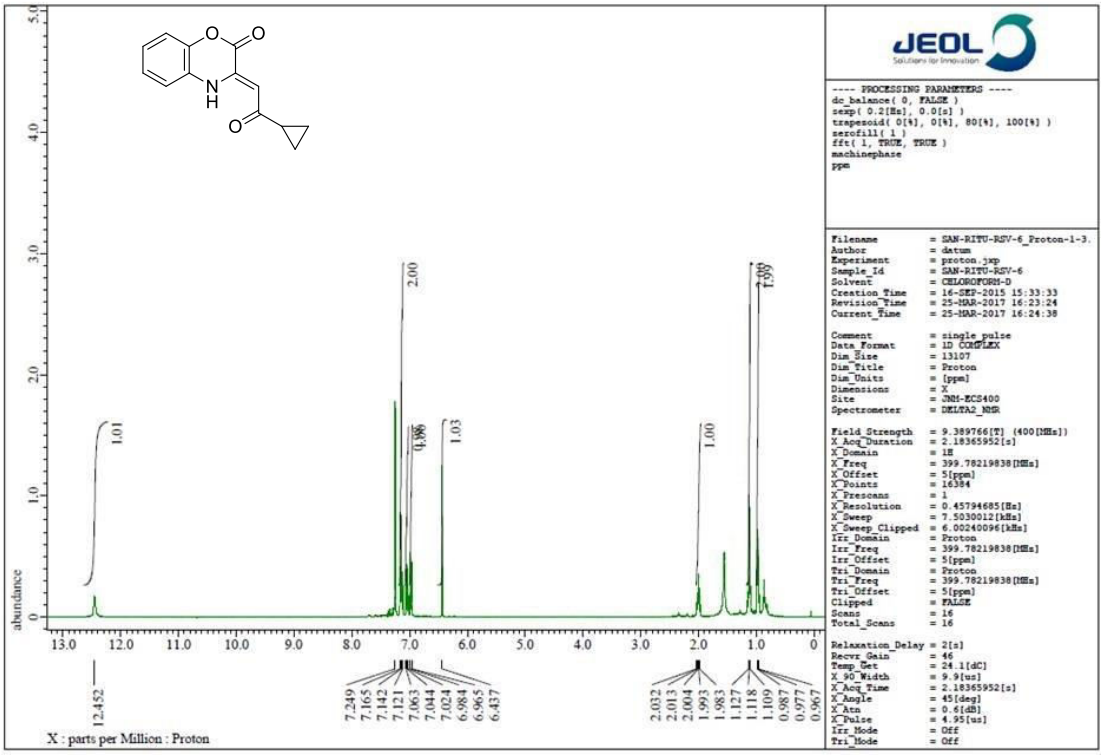


Figure 6. [1]H NMR Spectra of Compound **23a**.

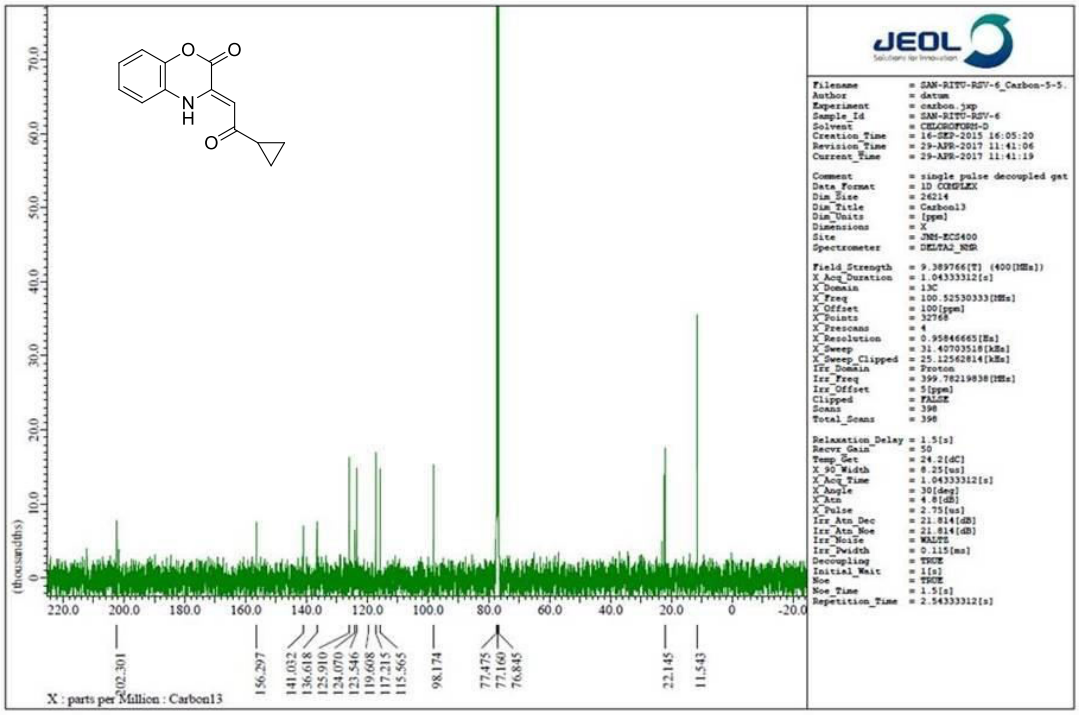


Figure 7. [13]C NMR Spectra of Compound **23a**.

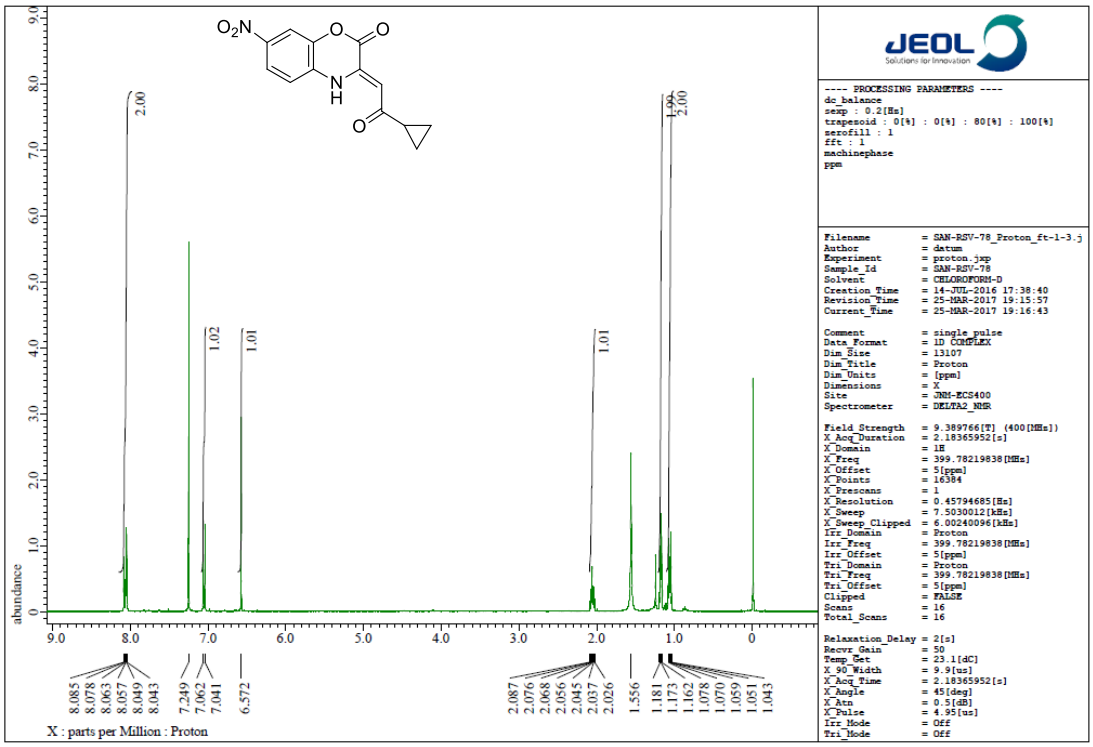


Figure 8. ^{1}H NMR Spectra of Compound **23e**.

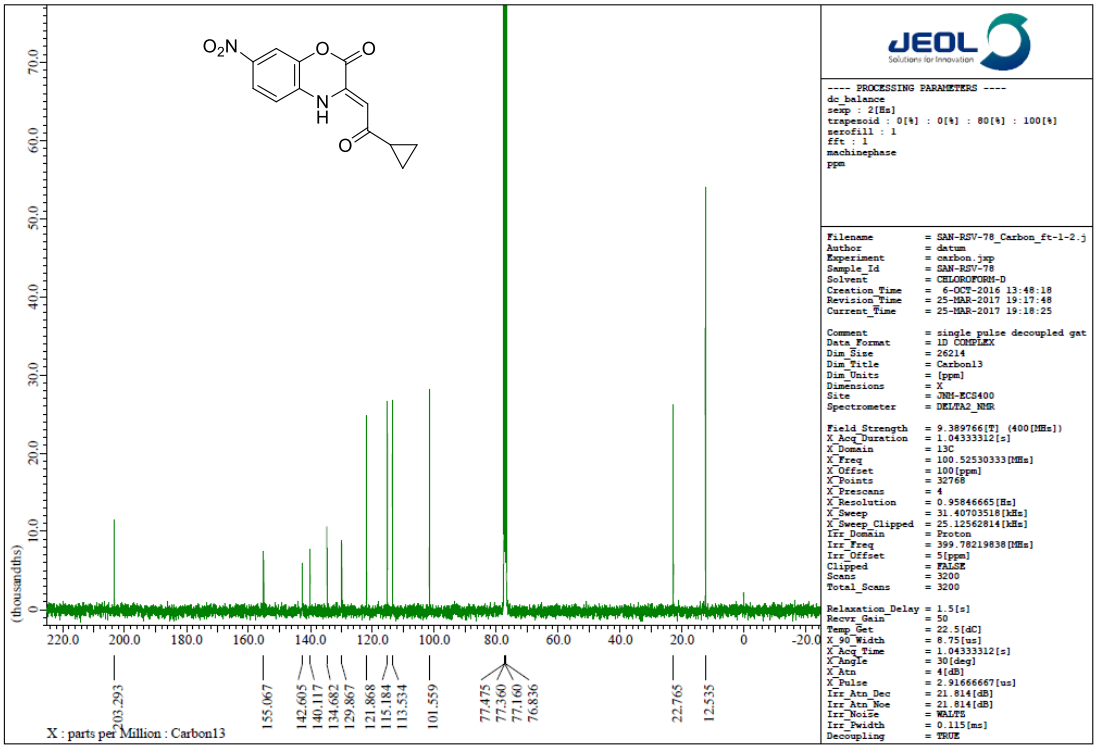


Figure 9. ^{13}C NMR Spectra of Compound **23e**.

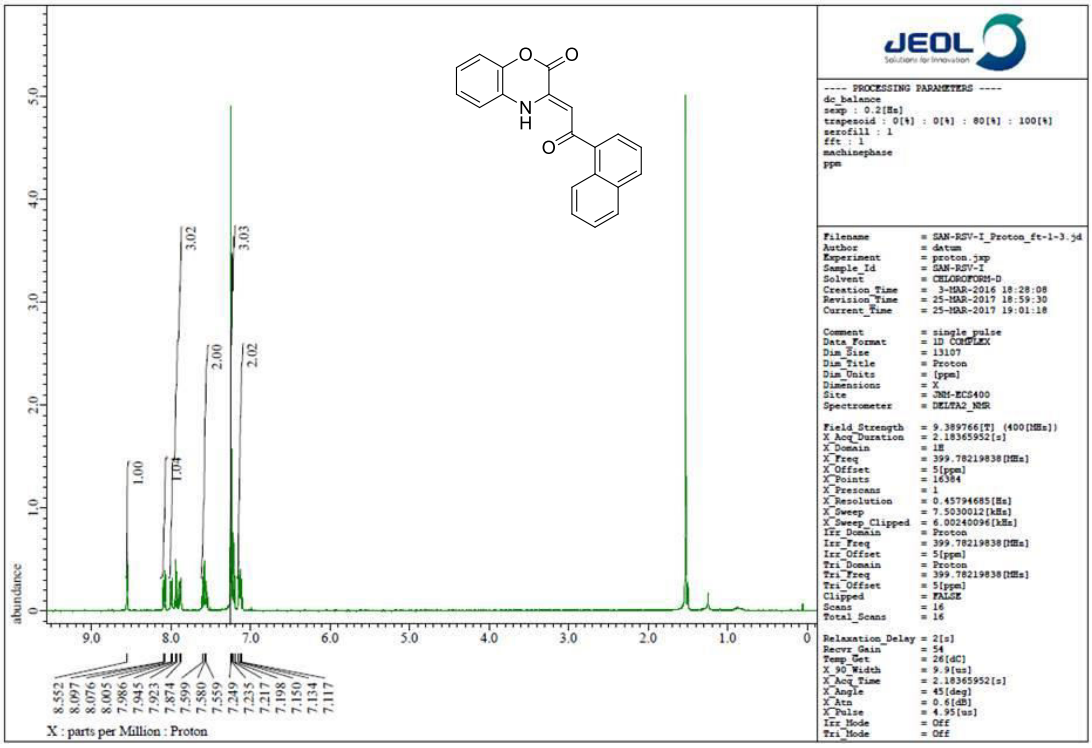


Figure 10. ^{1}H NMR Spectra of Compound **24a**.

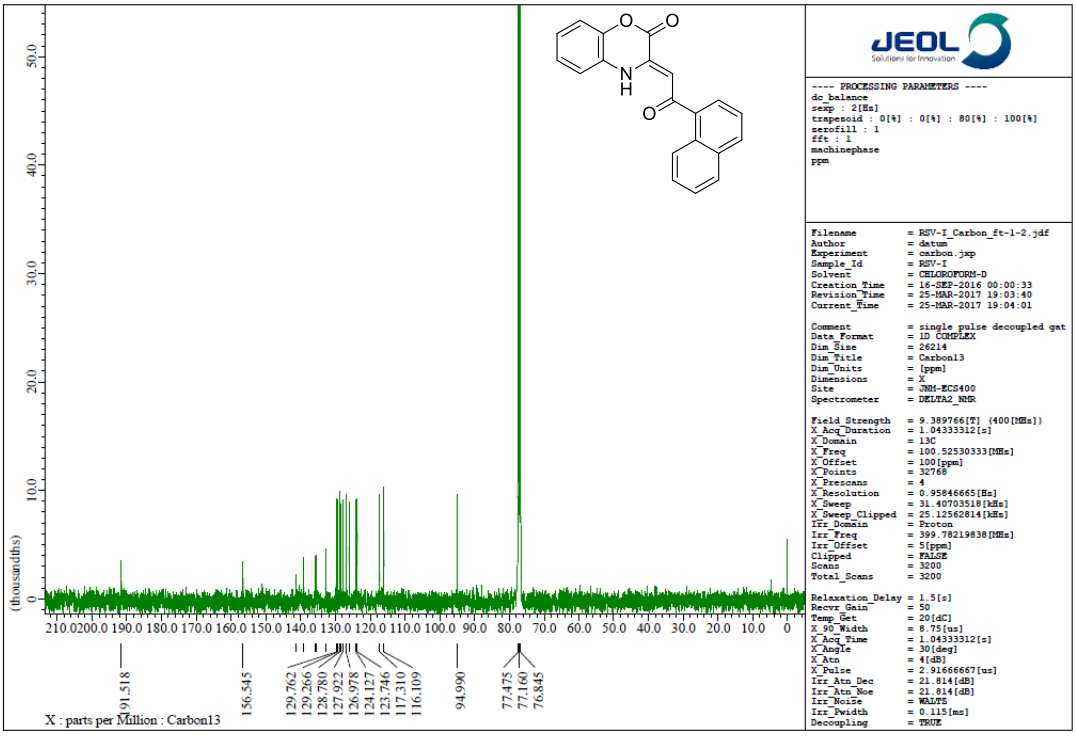


Figure 11. ^{13}C NMR Spectra of Compound **24a**.

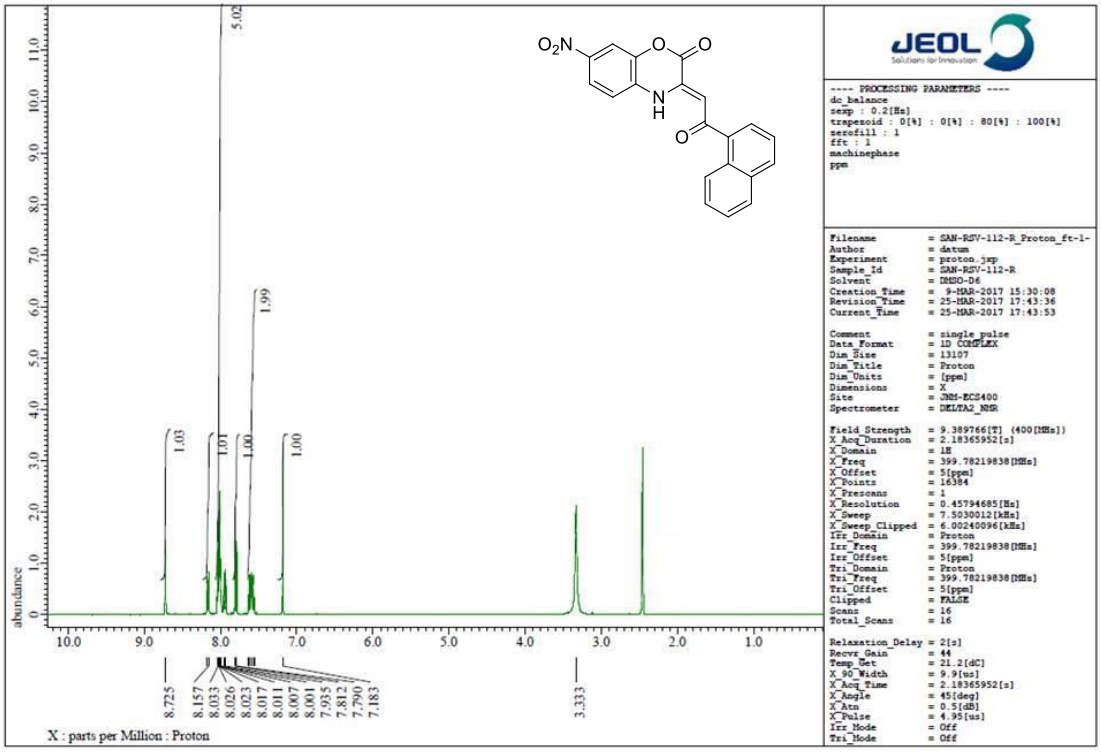


Figure 12. ^{1}H NMR Spectra of Compound **24e**.

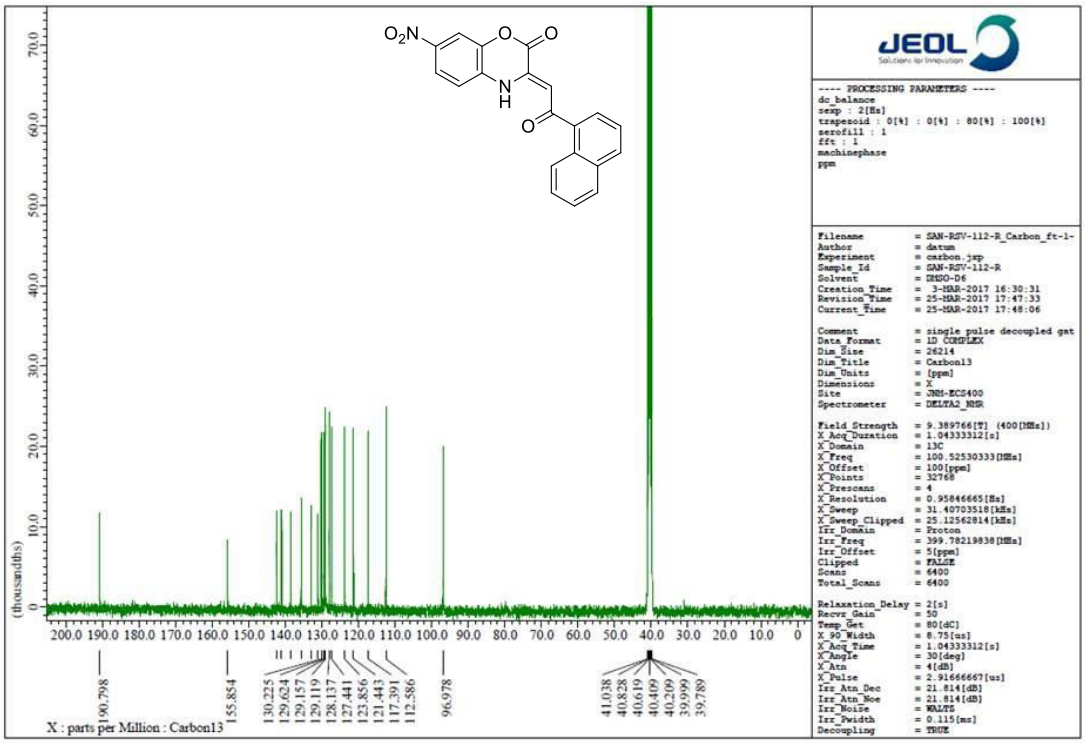


Figure 13. ^{13}C NMR Spectra of Compound **24e**.

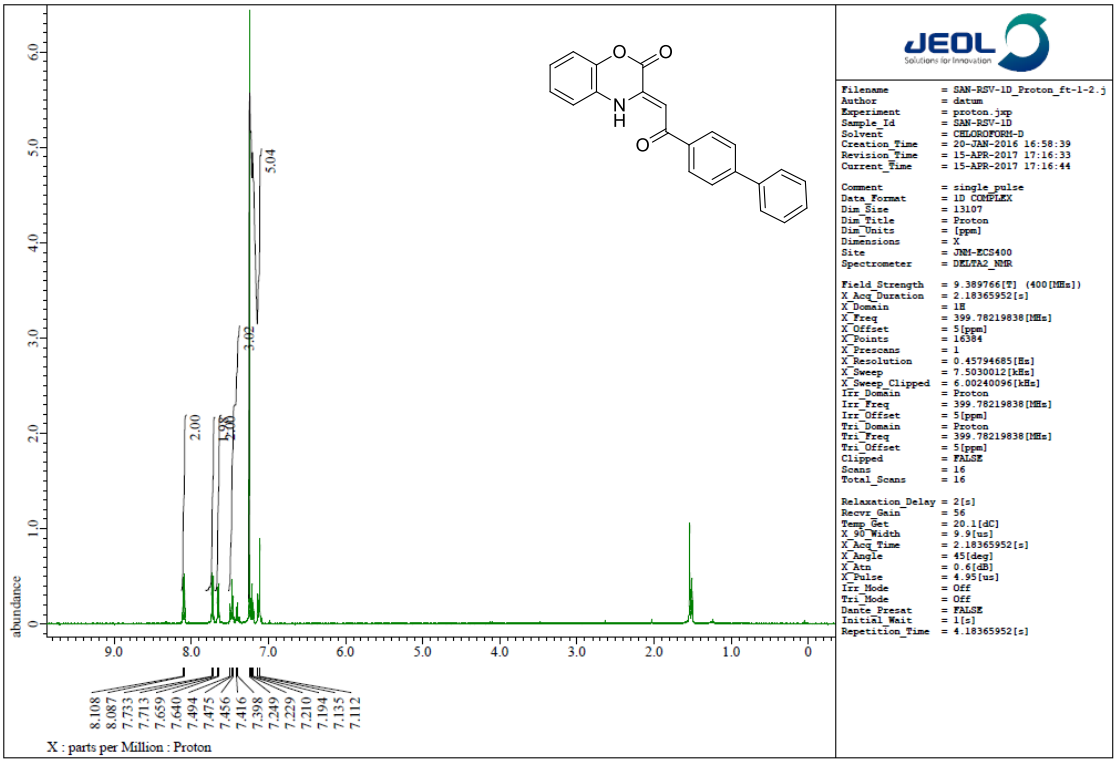


Figure 14. ^{1}H NMR Spectra of Compound **25a**.

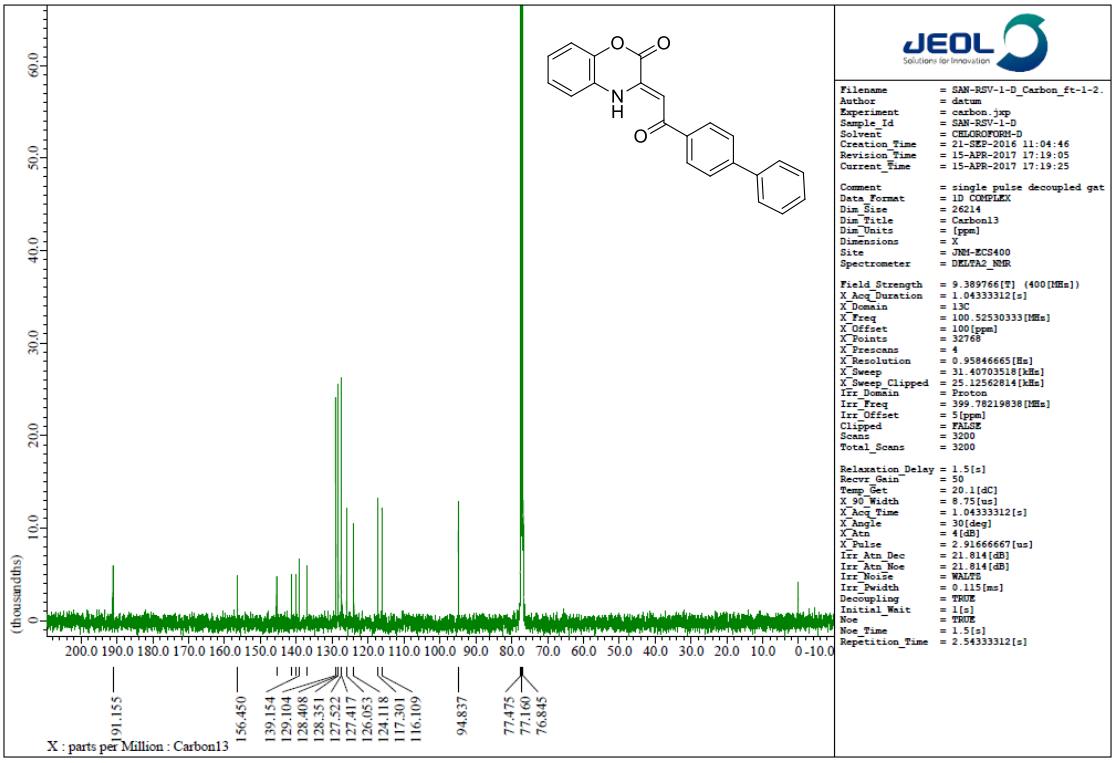


Figure 15. ^{13}C NMR Spectra of Compound **25a**.

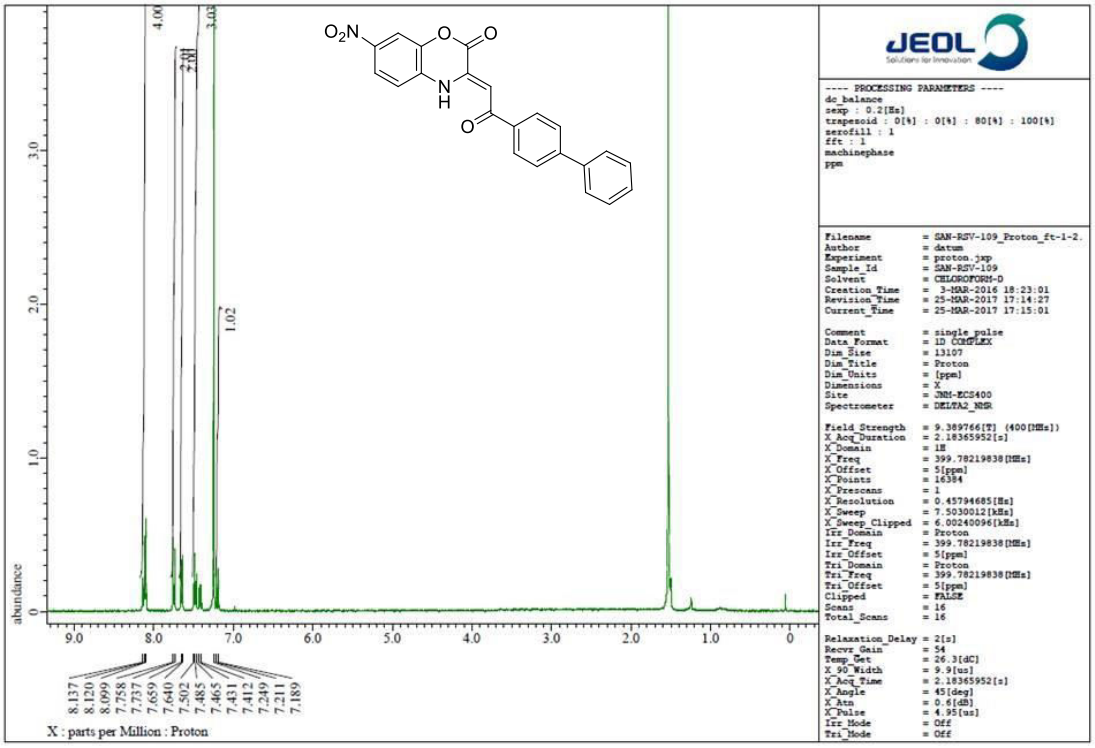


Figure 16. [1]H NMR Spectra of Compound **25e**.

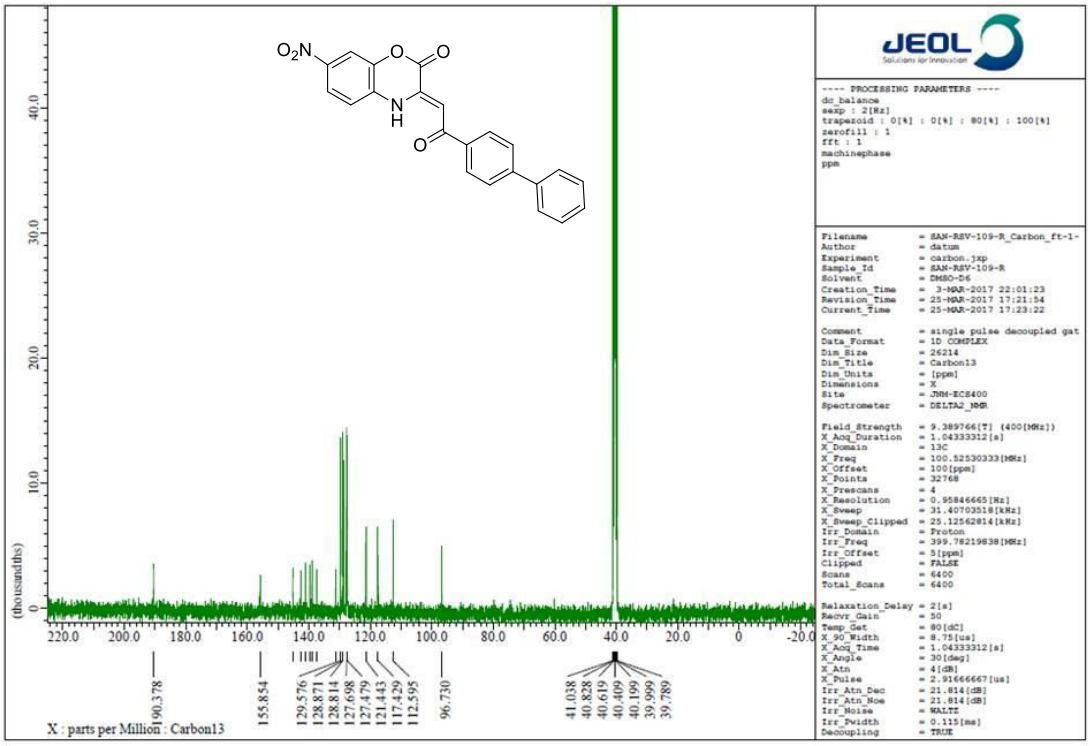


Figure 17. [13]C NMR Spectra of Compound **25e**.

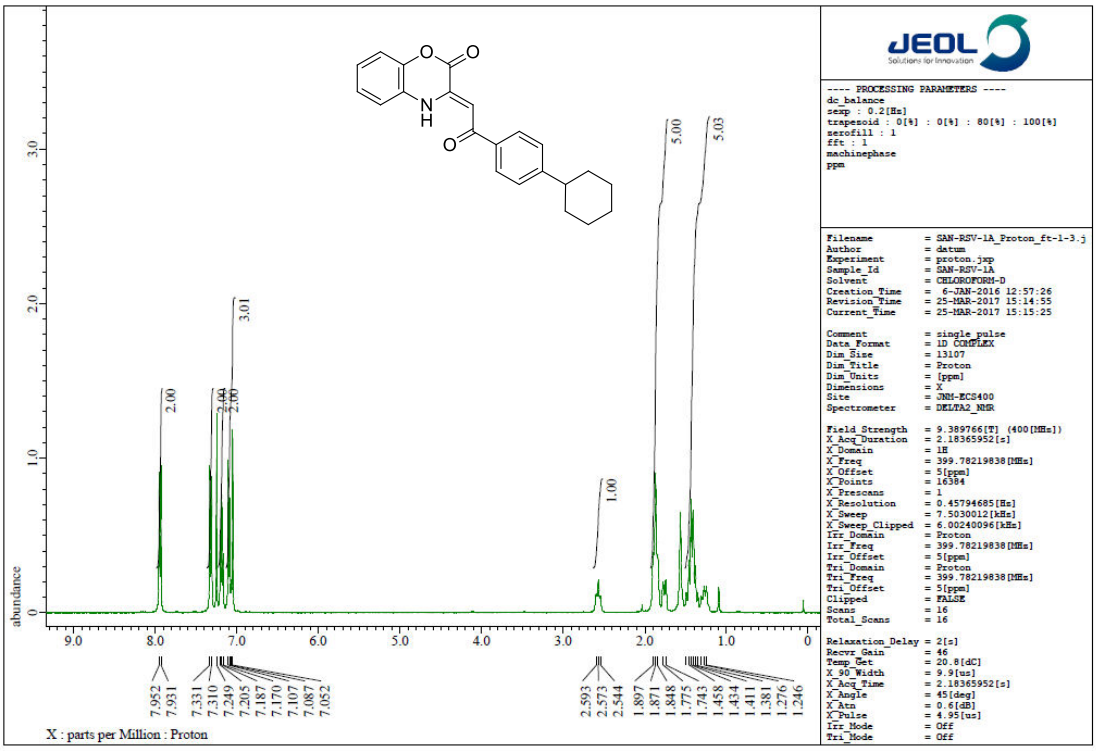


Figure 18. ^{1}H NMR Spectra of Compound **26a**.

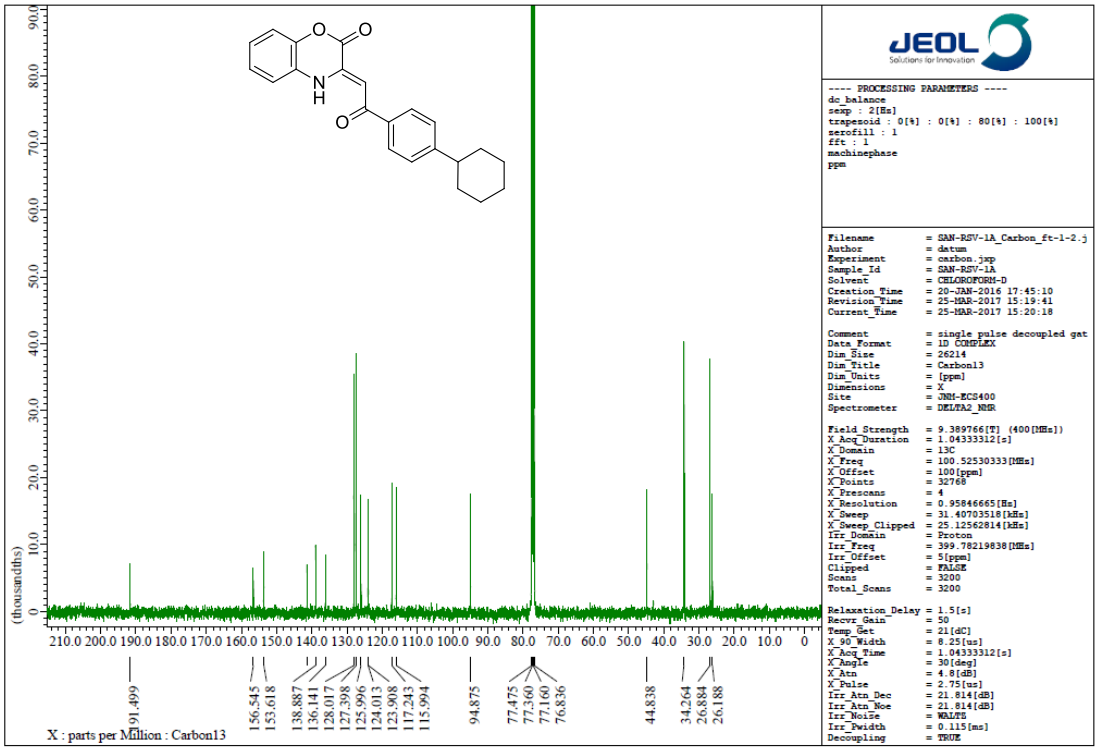


Figure 19. ^{13}C NMR Spectra of Compound **26a**.

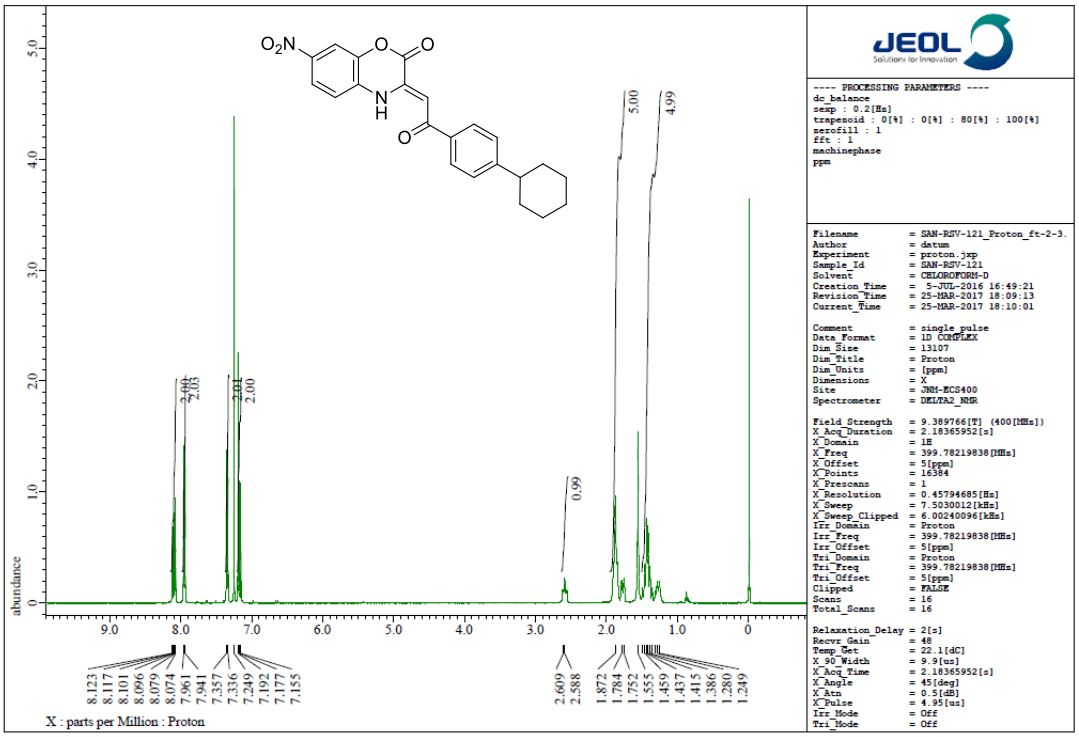


Figure 20. ^{1}H NMR Spectra of Compound **26e**.

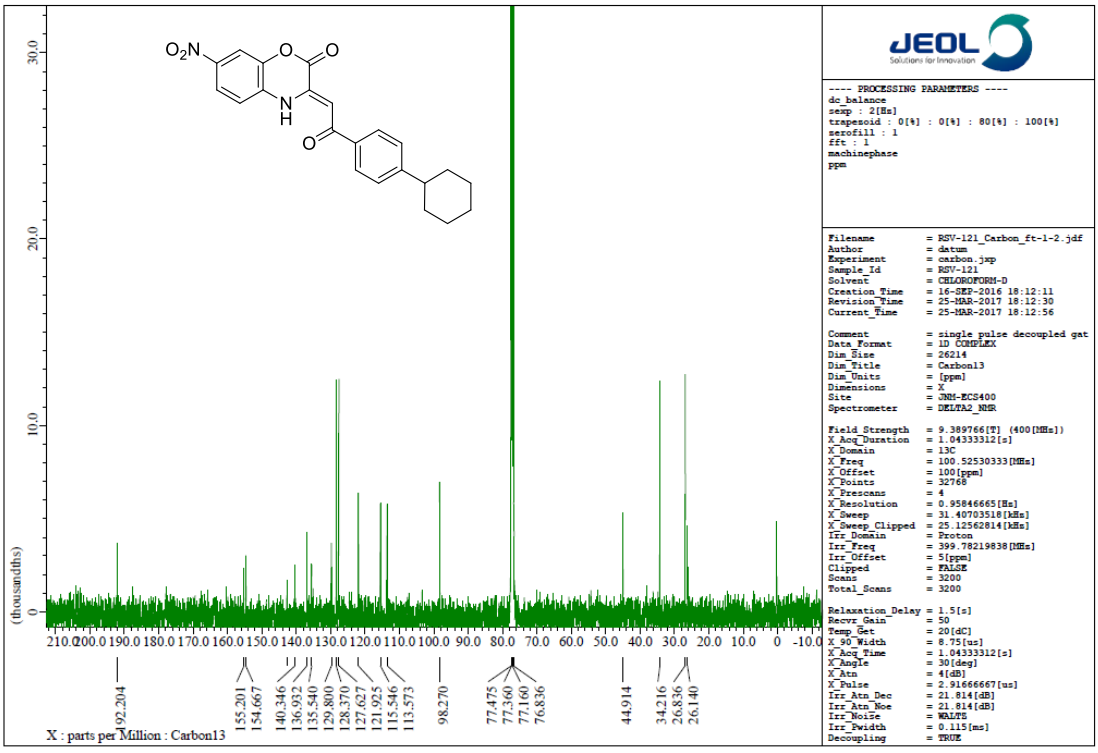


Figure 21. ^{13}C NMR Spectra of Compound **26e**.

CHAPTER 3

Ultrasound-assisted synthesis of novel spirooxindole derivatives via one pot three-component 1,3-dipolar cycloaddition reactions: Synthesis, stereochemical assignment, antimicrobial and antitubercular activities, and their SAR studies.

Section 3.1: ***Novel Spirooxindolo-pyrrolidines and Spirooxindolo-pyrrolizines as potent antimicrobial and antitubercular agents: Design, Ultrasonic-assisted Synthesis, X-ray determination, SAR and Molecular Docking Studies.***

3.1.1 Introduction

1,3-Dipolar cycloaddition reactions are a kind of multi-component reactions (MCRs) and have been recognized as the most powerful tool in organic, combinatorial and medicinal chemistry during the past decades.[1] From the medicinal/combinatorial chemistry point of view, several pharmacologically privileged molecules can be assembled into a single structurally complex molecule with more diversified and more enhanced biological activities that can target biological sites of interests in a specific manner to combat specific diseases. Through multi-component reactions, structurally complex molecules can be prepared in a cost-effective, atom economic and ecofriendly manner by the one-pot single step reaction of a library of structurally diverse small molecules in a regiospecific and sterospecific manner.

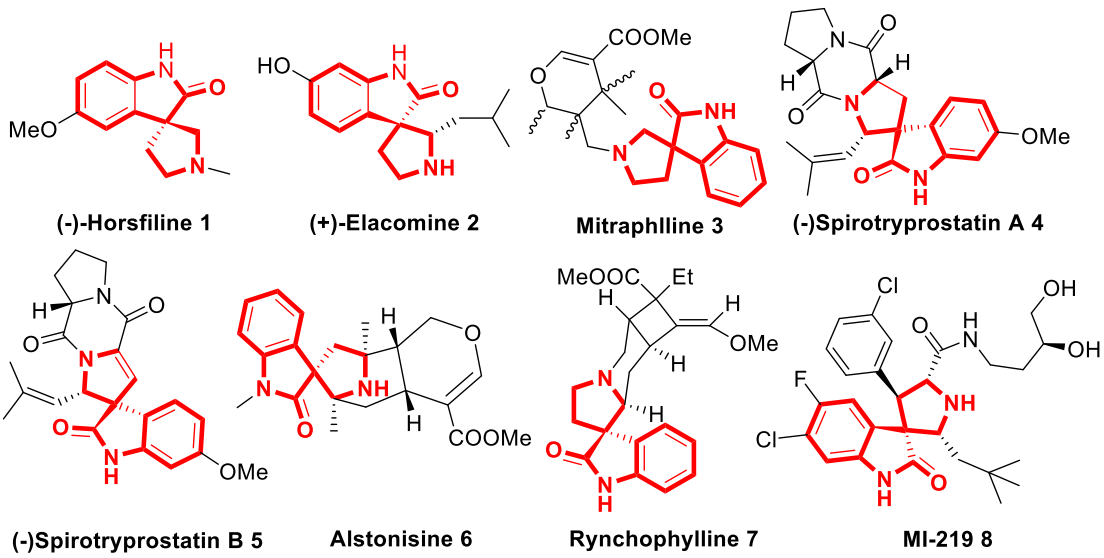


Figure 1. Structures of some pharmacologically active compounds with polycyclic spirooxindole skeleton.

Spirooxindole ring system, most commonly the spiro(pyrrolidin-2,3'-oxindole) core, is a privileged heterocyclic molecule which is highlighted in a large number of bioactive naturally occurring alkaloids and other natural products, drugs and therapeutics (Figure 1). These spiro-molecules are the central skeleton for numerous naturally occurring alkaloids and pharmacologically privileged compounds like horsfiline **1**,[2-7] elacomine **2**,[8] Mitraphylline **3**,[9] spiro-tryprostatine A **4** and B **5**,[10-11]

Alstonine **6**, rychnofilline **7**[12] and MI-219 **8**[13] etc. Spirooxindoles are well known for their wide biological applications such as antimicrobial[14], antitumoral,[15] anti-inflammatory,[16] antimycobacterial,[17] acetylcholinesterase-inhibitory activities,[18] anticancer activities[19] etc.

Hence, chemists have shown tremendous interest in the synthesis of novel compounds having spiro system with diverse functionalization. In our research programme towards the search of new bioactive compounds, we were interested to prepare a series of 3'-benzoyl-4'-phenylspiro [indoline-3, 2'-pyrrolidin]-2-one via 1, 3-dipolar cycloaddition of azomethine ylide (obtained by the reaction of isatin and amino acid) with various substituted chalcones.

Ultrasound irradiation has been recognized as a clean and advantageous greener approach to accelerate organic synthesis as the transmission and absorption of energy is entirely different as compared to the conventional thermal heating.[20] Moreover, this technique is capable to activate many organic synthetic transformations due to acoustic cavitational collapse. As conventional heating transfers thermal energy into the macro system, ultrasonic radiations lead to energy conservation by providing the activation energy to the microenvironment, which leads to an enhancement of its green significance. The noticeable features of the ultrasound-assisted reactions are higher reaction rates, enhanced formation of products in higher yield and selectivity, milder reaction conditions, reduced waste formation and shorter reaction time. Sometimes, it generally involves easy work-up procedures than the conventional methods.[20-21]

In an exploration towards novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds as antimicrobial and antitubercular agents, for the first time, we have prepared a new series of pharmacologically privileged substructures i.e., chalcone-isatin based spirooxindole compounds **12a-j**, **13a-e**, **14a-d** and **15-16** which were derived by the reaction of various substituted amino acids **11a-d**, respectively, substituted chalcone (Me, OMe, Cl) **10a-m** and isatins **9a-b** via 1,3-dipolar cycloaddition reaction. All the synthesized compounds were evaluated for their *in vitro* antibacterial and antitubercular activity. Herein, we report the ultrasonic-assisted synthesis of novel functionalized 3'-benzoyl-4'-phenylspiro [indoline-3,2'-pyrrolidin]-2-ones **12a-j**, **13a-e**, **14a-d** and **15** having pharmaceutically privileged groups. We also report the antibacterial and antitubercular activities, SAR and *in silico* molecular docking studies of **12a-j**, **13a-e**, **14a-d** and **15**. We also report, for the

first time, *in vitro* antimicrobial activity of some previously reported spiro compounds **12c**, **12f** and **12g**. The stereochemistry of the novel spirooxindole-pyrrolidine compounds were confirmed by carrying out the single crystal X-ray crystallography studies of bromo derivative i.e., **16** derived from 5-bromoisatin. All the synthesized cycloadducts **12a-j**, **13a-e**, **14a-d, 15** and **16** were confirmed by FT-IR, ^{1}H and ^{13}C NMR spectroscopic and HR-MS spectrometric studies.

3.1.2 Results and discussion

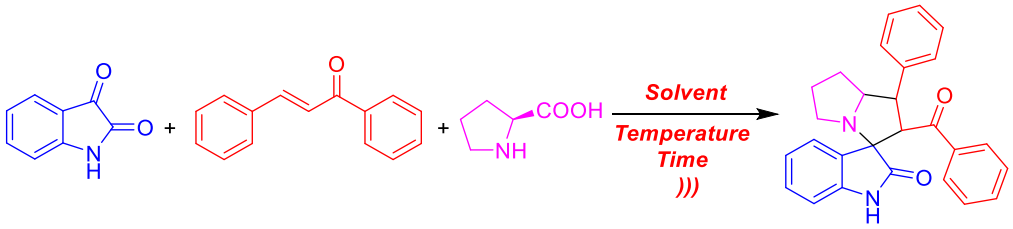
It has been well known that the 1,3-dipolar cycloaddition reaction of azomethine ylides has been utilized as a powerful tool for the construction of various types of complex polyheterocyclic frameworks.[22] Recently, the azomethine ylide has acquired a vibrant habitation in the synthesis of heterocyclic compounds as it serves as an important building block for the construction of N-containing heterocycles,[23] which are present, in part or as a whole, in many natural products and bioactive molecules.[24] It has also been well known that ultrasound irradiation has been used progressively as a fundamental tool for constructing heterocycles with interesting properties either in homogeneous or in heterogeneous liquid reaction systems.[25] In this chapter we have utilized the unseen potential of both ultrasonic irradiations as well as 1,3,-dipolar cycloaddition reaction strategy, for the first time, to construct diverse novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds **12a-j**, **13a-e**, **14a-d** and **15-16**.

3.1.2.1 Chemistry

We started our initial investigation taking isatin **9a**, chalcone **10a** and L-proline **11a** as starting materials for carrying out ultrasonic-assisted synthesis of spiroxindole-pyrrolidine **12a**. Initially, the reaction were attempted using reflux conditions. Therefore, the reaction was carried out taking **9a** (1 equiv.), **10a** (1.5 equiv.) and **11a** (1.5 equiv.) in methanol at 90 °C for 180 min which furnished the desired spiro compound **12a** in excellent (96%) yield (Table 1, entry 1). Since we get the promising yield; then, we analyzed the effect of number of equivalents of starting materials. Thus, the reaction was again carried out taking **9a** (1 equiv.), **10a** (1 equiv.) and **11a** (1 equiv.) in methanol at 90 °C for 120 min which furnished **12a** in 89% yield (Table 1, entry 2). It was then found that changing the number of equivalents do not further

improve the yield, instead there was no significant change in the yield of **12a** (Table 1, entry 3-4).

Table 1. ***Optimization study:*** Ultrasonic-assisted synthesis of spiroxindole-pyrrolidine **12a** from isatin **9a**, chalcone **10a** and L-proline **11a** as starting materials.



Entry	9a (equiv.)	10a (equiv.)	11a (equiv.)	Solvent	T (°C)	Time (min.)	[b]Yield (%)
[a]1	1	1.5	1.5	MeOH	90	180	96
[a]2	1	1	1	MeOH	90	120	89
[a]3	1	1.5	2	MeOH	90	180	96
[a]4	1	1.2	1.2	MeOH	90	120	91
5	1	1.5	2	MeOH	40	60	97
6	1	1.5	2	DMSO	40	60	95
7	1	1.5	2	Ethanol	50	60	95
8	1	1.5	2	Ethylene glycol	50	60	83
9	1	1.5	2	Diethylene glycol	40	60	95
10	1	1.5	2	AcCN	40	60	84
11	1	1.5	2	H_2O	40	60	12
12	1	1.2	1.2	H_2O/PEG (0.2 equiv.)	70	300	33
13	1	1.2	1.2	H_2O/SDS (1.7 equiv.), I_2 (0.2 equiv.)	70	300	30
14	1	1.2	1.2	H_2O/SDS (0.8 equiv.), I_2 (0.8 equiv.)	70	300	39
15	1	1.2	1.2	H_2O/SDS (4 equiv.), DBU (1 equiv.)	70	300	24
16	**1**	**1.5**	**2**	**MeOH**	**50**	**30**	**98**
17	1	1.5	2	MeOH	60	30	84

[a]Reflux condition, [b]Isolated yield after recrystalization/column chromatography

As mentioned in section 3.1.2; we were also interested to carry out our model reaction via ultrasonic-assisted 1,3-dipolar cycloaddition reaction. Along with using green technique, we were also interested to carry out the model reaction in some green solvent and that too in lower temperature as well as in less time. Thus, initially, the reaction was carried out taking **9a** (1 equiv.), **10a** (1.5 equiv.) and **11a** (2 equiv.) in methanol at 40 °C for 60 min under ultrasonic irradiations which furnished the desired spiro compound **12a** in 97% yield (Table 1, entry 5). Carrying out the same reaction

in DMSO and ethanol solvents furnished **12a** in 95% yields respectively (Table 1, entry 6-7). Furthermore, the reactions were also attempted in ethylene glycol, diethylene glycol and acetonitrile as solvents taking **9a** (1 equiv.), **10a** (1.5 equiv.) and **11a** (2 equiv.) at 40-50 °C for 60 min under ultrasonic irradiations which furnished the desired **12a** in 83%, 95% and 84% yields, respectively (Table 1, entry 8-10). We also attempted the reactions in water as it has been considered as a versatile green solvent in organic synthesis. Therefore, the reaction was carried out in water keeping all the conditions similar to that of entry 5; however, **12a** was obtained only in 12% yield (Table 1, entry 11). In order to increase the yield in water; several permutations and combinations were attempted with the use of other green solvents such as polyethylene glycol (Table 1, entry 12), and phase transfer catalysts[21] such as sodium dodecyl sulfate (SDS) in presence of I_2 or DBU (Table 1, entry 13-15) at 70 °C for 300 min under ultrasonic irradiations; the yield of **12a** was found to be reasonably better than entry 11 as **12a** was obtained in only 33%, 30%, 39% and 24% yields, respectively (Table 1, entry 12-15). Finally, the reaction was carried out taking **9a** (1 equiv.), **10a** (1.5 equiv.) and **11a** (2 equiv.) in methanol at 50 °C for 30 min under ultrasonic irradiations which furnished **12a** in 98% yield (Table 1, entry 16). Further increase in temperature do not have beneficial effect on the yield of the reaction (Table 1, entry 17).

*Overall, **9a** (1 equiv.), **10a** (1.5 equiv.) and **11a** (2 equiv.) in methanol at 50 °C for 30 min under ultrasonic irradiations were found to be the best optimized reaction conditions*.

3.1.2.2 Substrate scope and structure confirmation by single crystal X-ray diffraction studies.

Substituted isatins **9a-b**, substituted chalcones **10a-m** and various amino acids **11a-d** were subjected to ultrasonic-assisted 1,3-dipolar cycloaddition reactions in methanol at 50 °C for 30 minutes which furnished the desired chalcone-isatin-amino acid based spirooxindole compounds **12a-j**, **13a-e**, **14a-d** and **15-16** in excellent yields (upto 98%) in a highly diastereoselective manner (Scheme 1). In this reaction, [3+2] cycloaddition of substituted chalcones occurred with *in situ* generated azomethine ylides from thermal decarboxylative condensation of substituted isatins and various secondary amino acids. The structure of all the synthesized compounds were well characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and HRMS mass

spectrometric analysis. The physicochemical data of novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds **12a-j**, **13a-e**, **14a-d** and **15-16** are given in Table 2. Finally, the stereochemistry of the four chiral centres of the cycloaddition reaction was unequivocally determined by single crystal X-ray diffraction analysis of the cycloadduct **16** (Figure 2). The three-dimensional representation of the compound **16** shows that the compound has four chiral carbons with one carbon having R-configuration and the other three carbons having S-configurations. The crystal structure confirms that the *trans*-geometry of chalcone and the regioselectivity is also well established as a result of the concerted reaction of chalcones with the ylides.

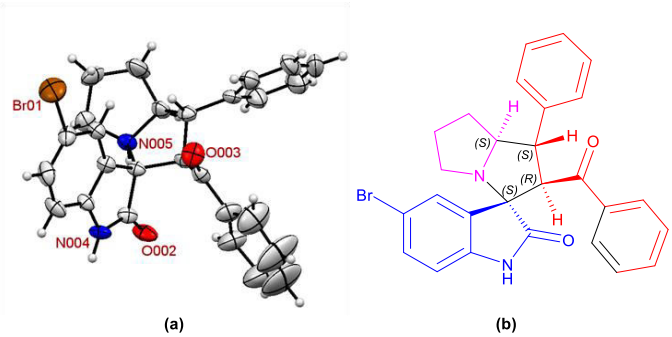


Figure 2. (a) ORTEP diagram of the cycloadduct **16**. (b) Structure of (1'S,2'R,3S,7a'S)-2'-benzoyl-5-bromo-1'-phenyl-1',2',5',6',7',7a'-hexahydrospiro[indolin-3,3'-pyrrolizin]-2-one **16**.

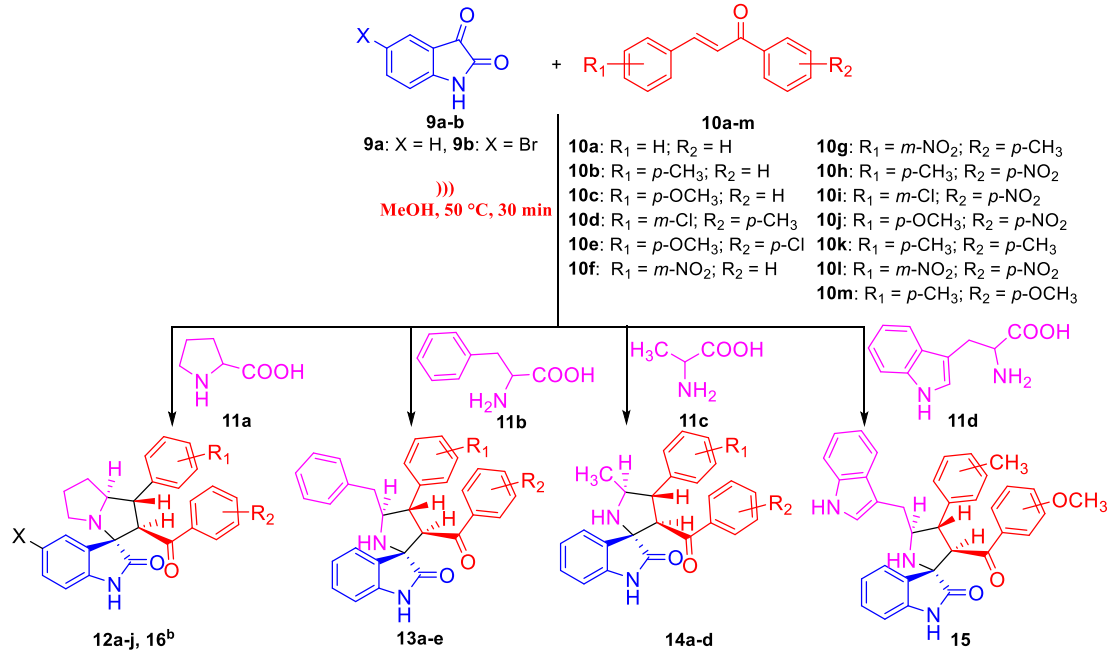
Table 2. Physicochemical data of novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds **12a-j**, **13a-e**, **14a-d** and **15-16**.

Compound	M.P. (°C)	Ultrasonic heating	
		Time (min.)	Yields[a] (%)
12a	165-167	30	98
12b	186-188	30	84
12c	164-166	30	84
12d	116-118	30	81
12e	150-152	30	94
12f	192-194	30	83
12g	162-164	30	58
12h	168-170	30	78
12i	140-142	30	74
12j	174-176	30	69
13a	210-212	30	55
13b	170-172	30	63
13c	161-163	30	48
13d	160-162	30	69
13e	192-194	30	78
14a	170-172	30	84
14b	138-140	30	73
14c	135-137	30	86
14d	165-167	30	96
15	130-132	30	74
16[b]	175-177	360	71

[a]Isolated yields by column chromatography, [b]Reflux.

As can be seen from Scheme 1 and Figure 3, all the synthesized novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds **12a-j**, **13a-e**, **14a-d** and **15-16** were obtained in 48-96% yield range. It was observed that electron-withdrawing or electron-donating group do not have much effect on the yield of the reaction. Among the spiro-pyrrolidine derivatives of L-proline i.e, spirooxindole-pyrrolizines **12a-j**, it was found that nitro analogues along with other substituents on the chalcone moiety i.e., disubstituted chalcones with nitro group led to a decreased formation of the product. The spiropyrrolidine derivatives of the amino acids L -phenylalanine i.e., **13a-e** were produced in lesser yield as compared to the L-proline derivatives **12a-j**. In addition, L-tryptophan derivative **15** was produced in lesser yields relative to L-proline derivatives (Figure 3). In case of tryptophan derivative, out of the two regioisomers, the lower diastereomer was found to be the major product. However, in the case of other members of the series, the upper diastereomer were found to be the major products.

Scheme 1. Ultrasonic-assisted synthesis of 3'-benzoyl-4'-phenylspiro[indoline-3,2'-pyrrolidin]-2-ones through 1,3-dipolar cycloaddition reaction.



12a: X = H, R_1 = H, R_2 = H
12b: X = H, R_1 = *p*-CH_3, R_2 = H
12c: X = H, R_1 = *p*-OCH_3; R_2 = H
12d: X = H, R_1 = *m*-Cl; R_2 = *p*-CH_3
12e: X = H, R_1 = *p*-OCH_3; R_2 = *p*-Cl
12f: X = H, R_1 = *m*-NO_2; R_2 = H
12g: X = H, R_1 = *m*-NO_2; R_2 = *p*-CH_3
12h: X = H, R_1 = *p*-CH_3; R_2 = *p*-NO_2
12i: X = H, R_1 = *m*-Cl; R_2 = *p*-NO_2
12j: X = H, R_1 = *p*-OCH_3; R_2 = *p*-NO_2
16: X = Br, R_1 = H, R_2 = H

13a: X = H, R_1 = *p*-CH_3; R_2 = *p*-CH_3
13b: X = H, R_1 = *p*-CH_3; R_2 = *p*-NO_2
13c: X = H, R_1 = *m*-NO_2; R_2 = *p*-CH_3
13d: X = H, R_1 = *p*-OCH_3; R_2 = *p*-NO_2
13e: X = H, R_1 = *m*-NO_2; R_2 = *p*-NO_2

14a: X = H, R_1 = H; R_2 = H
14b: X = H, R_1 = *p*-OCH_3; R_2 = H
14c: X = H, R_1 = *p*-CH_3; R_2 = *p*-OCH_3
14d: X = H, R_1 = *p*-CH_3; R_2 = *p*-NO_2

Reagents and conditions: a mixture of Chalcone (0.24 mmol, 1 equiv.), isatin (0.36 mmol, 1.5 equiv.), and amino acid (0.47 mmol, 2 equiv.) in dry methanol are heated in ultrasonicator for 30 min.

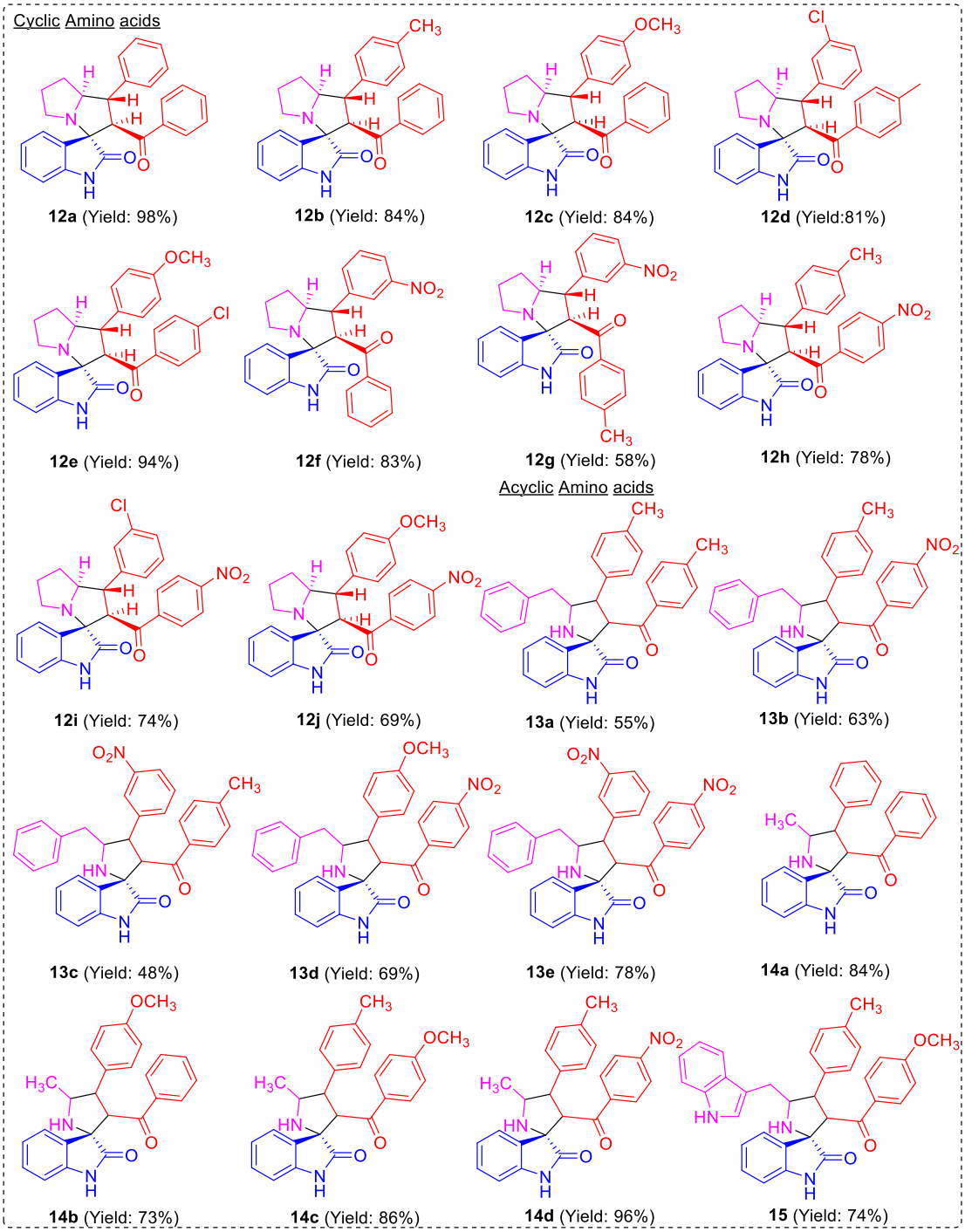


Figure 3. Structures of all synthesized novel spirooxindole-pyrrolidine/spirooxindole-pyrrolizine based compounds **12a-j**, **13a-e**, **14a-d** and **15**.

Regarding stereochemistry of all the synthesized compounds, the L-proline based compounds i.e., **12a-j** will exist in the same stereochemical environment as the compound **16** as it is also derived from L-proline. In all other acyclic amino acid containing spiro compounds i.e., **13a-e**, **14a-d** and **15**, the molecules may or may not have the same stereochemistry. Moreover, one can speculate that the stereochemistry

of isatin portion in **13a-e**, **14a-d** and **15** will have the same stereochemistry as in compound **16**.

3.1.2.3 Plausible mechanism for the formation of spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines.

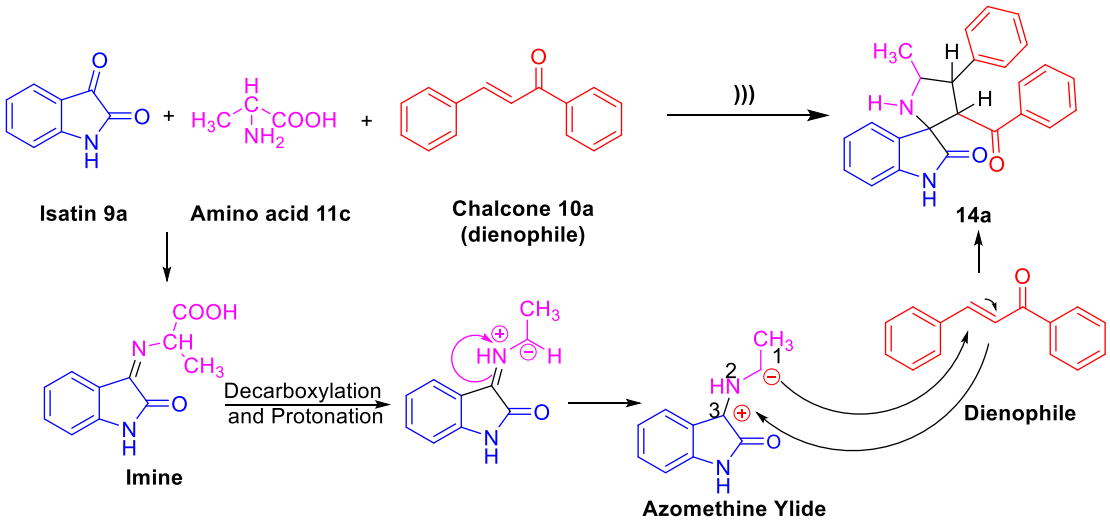


Figure 4. The proposed mechanism.

A plausible mechanism to rationalize the formation of spirooxindolo–pyrrolidines is depicted in figure 4 taking **14a** as representative example. Initially the reaction starts with the reaction of isatin **9a** with amino acid **11c** generates imine which undergoes in situ decarboxylation followed by protonation furnished the azomethine ylide which, then, undergoes chemoselective addition to the dienophile chalcone **10a** at the least hindered β-carbon of the enone moiety to form the desired spirooxindolo–pyrrolidine **14a**.

3.1.3 Antimicrobial and Antitubercular activity evaluation

3.1.3.1 Antimicrobial evaluation

Compounds (**12a-j**, **13a-e**, **14a-d** and **15**) were initially screened for their *in vitro* antibacterial activity against Gram-positive bacterial strains, *Streptomyces griseus* [SG] (MTCC 4734), *Staphylococcus aureus* [SA] (MTCC 3381) and *Bacillus subtilis* [BS] (MTCC 10619), and Gram-negative bacterial strains *Escherichia coli* [EC] (MTCC 443), utilizing the agar diffusion assay.[26] The antibiotic ampicillin was taken as positive control. Antibacterial screening of all the derivatives, **12a-j**, **13a-e**, **14a-d** and **15** as well as positive controls were performed at a fixed concentration of 100 µg/mL. All twenty compounds exhibited antibacterial activity against both gram-

positive and gram-negative bacterial strains; however, all compounds were found less or no activity against four fungal strains. The minimum inhibitory concentration (MIC) values for all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** and the positive control drugs, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[27]

All the derivatives (**12a-j**, **13a-e**, **14a-d** and **15**) were also screened for their antifungal activity against four different fungal strains, i.e. *Candida Albicans* [CA] (ATCC 10231), *Aspergillus niger* [AN] (ATCC 9029), *Penicillium funiculosum* [PF] (ATCC 11797) and *Trichoderma Reesei* [TR] (ATCC 13631) (Table 2). The antifungal drug, ketoconazole was used as a positive control.[26] The fungal strains were grown and maintained on Sabouraud glucose agar plates. The plates were incubated at 26 ºC for 72 h, and resulting ZOIs and MICs were measured. Antifungal screening of all the derivatives **12a-j**, **13a-e**, **14a-d** and **15** has been performed at a fixed concentration of 100 µg/mL.

3.1.3.2 Antibacterial and Antifungal Activity as well as Structure-Activity Relationship (SAR) studies

The minimum inhibitory concentration (MIC) values for all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** and the positive control drugs, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[27] As it can be seen from Table 3; all the compounds **12a-j**, **13a-e**, **14a-d** and **15** were found to be inactive against both [SG] and [SA] strains. However in the case of [BS] strains, as it can be seen from Table 3; **13c**, **13e**, **14a** and **15** were identified as the most potent antibacterial agents (MIC = 12.5 µg/mL) against [BS] strains, as it showed two times more potency than ampicillin against [BS] strains (MIC = 25 µg/mL). The next best compounds **12a**, **12e** and **14c** (MIC = 25 µg/mL) were found to be equally active in comparison to the standard drug ampicillin (MIC = 25 µg/mL) against [BS] strains. Further, the compounds **12b**, **12c** and **14b** (MIC = 50 µg/mL) as well as **13a** and **14d** (MIC = 100 µg/mL) were found to be less active than ampicillin (MIC = 25 µg/mL) against [BS] strains. The rest of the compounds did not show good activity against [BS] strains. In the case of gram-negative bacteria *E. coli* strain, compounds **12a-c**, **12e**, **12i**, **13c-d**, **14a-c** (MIC = 12.5 µg/mL) were found to be equally active as compared to ampicillin (MIC = 12.5 µg/mL) against [EC] strains. The compounds **12g-h**, **13b** and **15** (MIC =

25 µg/mL) as well as compounds **12d**, **12f, 12j**, **13a**, **13e** and **14d** (MIC = 50 µg/mL) were found to be less active than ampicillin (MIC = 25 µg/mL) against [EC] strains. The rest of the compounds were found to be inactive. Thus, among the bacterial strains, the compounds showed activity against only [BS] and [EC] strains.

Table 3. MIC values for novel functionalized all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** and positive control drugs against bacteria and fungi.

Analogs	Minimum inhibitory concentration (MIC)[a]							
	Bacteria[b]			Bacteria[c]	Fungi[d]			
	SG	SA	BS	EC	CA	AN	PF	TR
12a	NA	NA	25	12.5	NA	NA	NA	100
12b	NA	NA	50	12.5	NA	NA	100	50
12c	NA	NA	50	12.5	NA	NA	NA	NA
12d	NA	NA	NA	50	NA	NA	NA	NA
12e	NA	NA	25	12.5	NA	NA	NA	NA
12f	NA	NA	NA	50	NA	NA	NA	NA
12g	NA	NA	NA	25	NA	NA	NA	NA
12h	NA	NA	NA	25	NA	NA	NA	NA
12i	NA	NA	NA	12.5	NA	NA	NA	100
12j	NA	NA	NA	50	NA	NA	NA	NA
13a	NA	NA	100	50	NA	NA	NA	NA
13b	NA	NA	NA	25	NA	NA	NA	NA
13c	100	NA	12.5	12.5	NA	NA	NA	NA
13d	NA	NA	NA	12.5	NA	NA	NA	NA
13e	NA	NA	12.5	50	NA	NA	NA	NA
14a	100	NA	12.5	12.5	50	NA	50	25
14b	NA	NA	50	12.5	NA	NA	NA	100
14c	NA	NA	25	12.5	NA	NA	NA	NA
14d	NA	NA	100	50	NA	NA	NA	NA
15	NA	NA	12.5	25	NA	NA	NA	NA
AMP[e]	12.5	12.5	25	12.5	-	-	-	-
KET[f]	-	-	-	-	12.5	12.5	25	25

[a]MIC of all compounds was measured at 100 µg/mL. [b]Gram-positive bacteria: SG, *Streptomyces griseus*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*. [c]Gram-negative bacteria: EC, *Escherichia coli*. [d]FO, *Fusarium oxysporium*; AN, *Aspergillus niger*; PF, *Penicillium funiculosum,* TR, *Trichoderma Reesei.* [e]AMP: Ampicillin. [f]KET: Ketoconazole; NA = Compounds which were found Not Active.

As can be seen from Table 3; All the compounds were found to be inactive against all the four fungal strains except compound **14a** against [CA] strains, **12b** and **14a** against [PF] strains, and **12a-b**, **12i** and **14b** against [TR] strains in comparison to the standard drug ketoconazole (MIC = 12.5 µg/mL against [CA] and [AN] strains and 25

µg/mL against [PF] and [TR] strains). Compound **14a** (MIC = 12.5 µg/mL) have shown equal potency to ketoconazole in [TR] fungal strain. Overall, it can be interpreted that all the compounds have shown moderate antimicrobial activity. The compounds containing L-phenylalanine as well as L-tryptophan moieties were found to be most potent compounds of the series.

3.1.3.3 Antitubercular evaluation

Since it had been known that spirooxindole based compounds possesses antitubercular activity,[17] therefore, all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** were evaluated for their *in vitro* antitubercular activity against *M. tuberculosis* $H_{37}Ra$ using MABA method[28] at concentration ranging from 25 to 0.39 µg/mL.[29]

Table 4. *In vitro* antitubercular activity of functionalized spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** and positive control drugs.

Analogs	**MIC (**µg/mL)[a]
12a	25
12b	>25
12c	12.5
12d	>25
12e	6.25
12f	>25
12g	>25
12h	12.5
12i	6.25
12j	6.25
13a	>25
13b	**3.125**
13c	>25
13d	12.5
13e	25
14a	>25
14b	25
14c	25
14d	25
15	**1.56**
INH	0.1
Rifampicin	0.2

[a]MIC of all compounds was measured at concentration ranging from 25 to 0.39 µg/mL against the *Mycobacterium tuberculosis* bacterial strain.

The minimum concentrations of the compounds required for the complete inhibition of the bacterial growth per spot (minimum inhibitory concentrations, MIC) are

summarized in Table 4. The antibiotics isoniazid (INH) and Rifampicin were used as standard drugs.

3.1.3.4 Antitubercular activity as well as Structure-Activity Relationship (SAR) studies

Compound **15**, the most active compound of the series, have shown promising antitubercular activity with the MIC value of 1.56 µg/mL against avirulent strains (*M. Tuberculosis* $H_{37}Ra$). Compounds **13b**, the next most active compound of the series, (MIC = 3.125 µg/mL) exhibited antitubercular activity comparable to standard drugs INH (MIC = 0.1 µg/mL) and Rifampicin (MIC = 0.2 µg/mL). Compounds **12e** and **12i-j**, have also shown promising antitubercular activity with the MIC value of 6.25 µg/mL against avirulent strains (*M. Tuberculosis* $H_{37}Ra$). The rest of the compounds have shown less activity profile. Thus, it can be said that the compounds containing L-phenylalanine as well as L-tryptophan moiety were found to show better antitubercular activity as compared to the other compounds.

3.1.4 *In Silico* Molecular Docking Simulation Studies for antitubercular activity of 12a, 13b and 15 with standard drugs Isoniazid and Rifampicin.

3.1.4.1 Molecular modeling

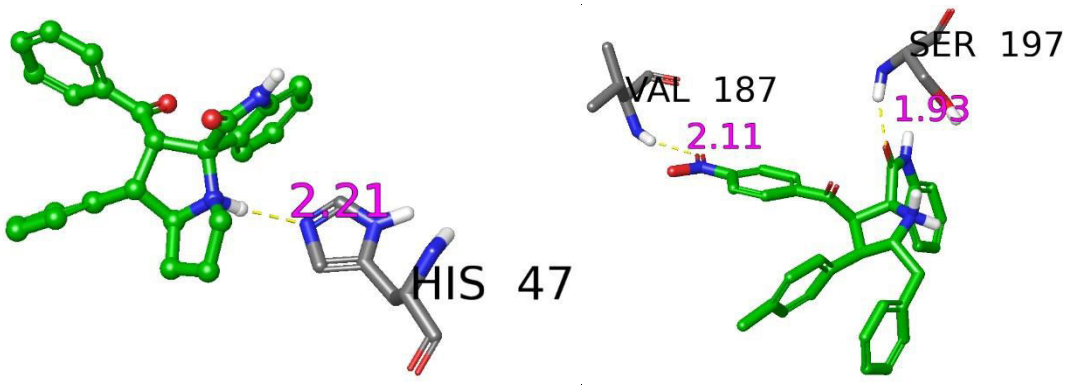
The activity in terms of MIC (MIC in microgram per milliliter) of the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a**, **13b** and **15** was converted to negative logarithmic concentration in moles. ChemBioDraw Ultra v12.0 (Cambridge Soft Corp., UK) software was used for sketching series of the studied compounds.[30] Molecules designing, geometry cleaning, and energy minimization was performed by (SYBYL-X 2.1, Tripos International, 1699 South Hanley Rd., St. Louis, MO, 63144, USA). Tripos force field and Gasteiger–Hückel charge were used for energy minimization. The 2D compound structures were converted to 3D by Concord 4.0 program. Geometry cleaning was carried out with MOPAC 6 program using the semi-empirical PM3 Hamiltonian method.[31]

3.1.4.2 *In Silico* Molecular Docking Studies

The docking study revealed the possible mechanisms of action of active all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a**, **13b** and **15** against antitubercular target InhA or ENR *of M. tuberculosis*. The docking results suggest that studied active inhibit the activity of ENR. We explored the binding orientation of

active functionalized spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines in terms of the docking score. Higher docking energy means higher binding affinity toward the target enzyme. The docking study of **12a**, **13b**, **15**, **isoniazid** and **rifampicin** with ENR revealed that standard ENR inhibitors triclosan similar binding active site and about 99% of binding pocket residues found similarity to that of ENR-triclosan complex.

The docking results for **12a** against *M. tuberculosis* (PDB ID: 1QSG) showed a high binding affinity docking score indicated by a total score of 4.6079 and form a H-bond of length 1.8Å to the hydrophobic aliphatic residue that is, side chain of Histidine-47. In the docking pose of the complex, the chemical nature of binding site residues within a radius of 3Å was nucleophilic (polar, hydrophobic), that is, Ser-196, Ser-197 (serine), Thr-39 Thr-291 (threonine); basic (polar, hydrophobic and positive charged), that is, His-47, His-44, His-135 (histidine), Val-143, Val-139 (valine), Leu-50 (leucine), Ile-168 (isoleucine), Lys-160 (lysine), Pro-38 (proline); polar amide, that is, Gln-164, Gln-72 (glutamine); Aromatic (hydrophobic), that is, Tyr-82 (tyrosine), Phe-157, Phe-136 (phenylalanine); acidic (polar, negative charged), for example, Asp-161 (Aspartic acid) and hydrophobic, for example, Gly-158 (Glycine), Met-40 (Methionine); as a result, the bound compound showed a strong hydrophobic interaction with *M. tuberculosis*, thus leading to more stability and activity in this compound (Figure 5).



(A) (B)

Figure 5. (A): Predicted interactions of **12a** with *M. tuberculosis* (PDB ID: 1QSG) with a docking total score of 4.6079, revealing a H-bonds of length 1.8Å, to the hydrophobic aliphatic residue i.e., side chain of Histidine-47. **(B)** Predicted interactions of **13b** with *M. tuberculosis* (PDB ID: 1QSG) with a docking total score of 8.1329, revealing a H-bonds of length 1.9 and 2.1Å to the hydrophobic nucleophilic residues that is, side chain of Serine-197 (backbone), and hydrophobic residue Val-187 (backbone).

Similarly, the docking results for **13b** against *M. tuberculosis* showed a high binding affinity docking score indicated by a total score of 8.1329 and form two H-bond of length 1.9 and 2.1Å to the hydrophobic nucleophilic residues that is, side chain of Serine-197 (backbone), and hydrophobic residue Val-187 (backbone). In the docking pose of the complex, the chemical nature of binding site residues within a radius of 3Å was nucleophilic (polar, hydrophobic), that is, Ser-196, Ser-197 (serine), Thr-39 Thr-186 (threonine); Aromatic (hydrophobic), that is, Tyr-82 (tyrosine), Phe-157, Phe-156 (phenylalanine); basic (polar, hydrophobic and positive charged), that is, Leu-50, Leu-280 (leucine), Val-139, Val-187(valine), Lys-160 (lysine), His-44, His-47 (histidine), Pro-38 (proline); Arg-198 (arginine); hydrophobic, for example, Gly-158, Gly-46 (Glycine), Met-195, Met-40 (Methionine); polar amide, that is, Gln-164(glutamine); and acidic (polar, negative charged), for example, Asp-161 (Aspartic acid) as a result, the bound compound showed a strong hydrophobic interaction with *M. tuberculosis*, thus leading to more stability and activity in this compound (Figure 5).

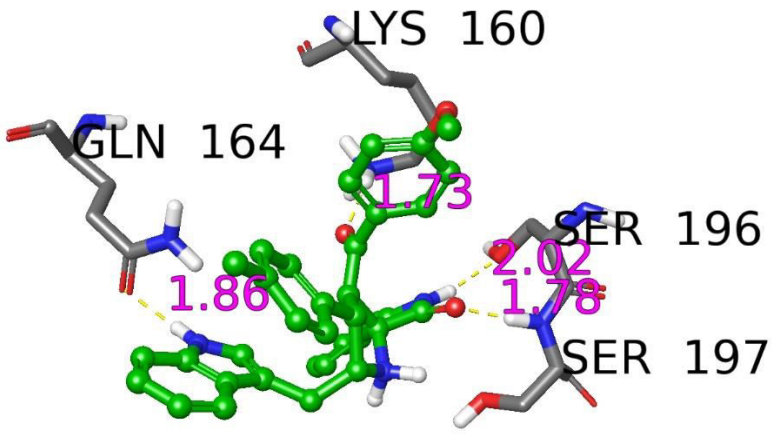
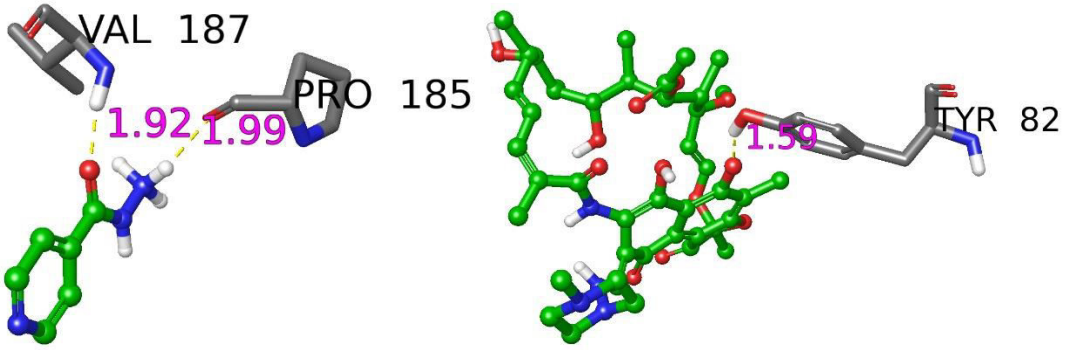


Figure 6.: Predicted interactions of **15** with *M. tuberculosis* (PDB ID: 1QSG) with a docking total score of 5.2059, revealing a four H-bond of length 1.9, 2.1, 2.3 and 1.7Å to the hydrophobic nucleophilic residues that is, side chain of Lysine-160 (backbone), Serine-197 (backbone), Serine-196 (side chain) and polar amide residue glutamine-164 (side chain).

Likewise, the docking results for the docking results for **15** against *M. tuberculosis* showed a high binding affinity docking score indicated by a total score of 5.2059 and form four H-bond of length 1.9, 2.1, 2.3 and 1.7Å to the hydrophobic nucleophilic residues that is, side chain of Lysine-160 (backbone), Serine-197 (backbone), Serine-196 (side chain) and polar amide residue glutamine-164 (side chain). In the docking pose of the complex, the chemical nature of binding site residues within a radius of 3Å was acidic (polar, negative charged), for example, Asp-161 (Aspartic acid);

hydrophobic, for example, Gly-158, Gly-46 (Glycine), Met-195, Met-40 (Methionine); Aromatic (hydrophobic), that is, Tyr-82 (tyrosine), Phe-157, Phe-156 (phenylalanine); basic (polar, hydrophobic and positive charged), that is, Leu-50 (leucine), Val-143 (valine), His-44, His-47 (histidine), Arg-198 (arginine), Pro-38 (proline); Arg-198 (arginine) and nucleophilic (polar, hydrophobic), that is, Thr-39 (threonine); as a result, the bound compound showed a strong hydrophobic interaction with *M. tuberculosis*, thus leading to more stability and activity in this compound (Figure 6).



(C) (D)

Figure 7.: Predicted interactions of control molecule isoniazid (C) and rifampicin (D) with *M. tuberculosis* (PDB ID: 1QSG) receptor protein with a docking total score of 4.0421 and 1.5110, respectively.

The docking results for the control molecule isoniazid (C) and rifampicin (D) with *M. tuberculosis* (PDB ID: 1QSG) receptor protein (Table 5) showed a low binding affinity docking score, indicated by a low total score of 4.0421 and 1.5110 respectively (Figure 7). The docking affinity of inactive compound **12a** was found to show docking total score of 4.6079 which is very close to the docking score of isoniazid (docking score = 4.0421). However, the docking score of active compounds **13b** and **15** were found to be 8.1329 and 5.2059, respectively which is much better than the standard drugs isoniazid (docking score = 4.0421) and Rifampicin (docking score = 1.5110). Thus, the docking procedure of Surflex-dock software (Sybyl-X 2.1) in reproducing the experimental binding affinity seems reliable, and therefore predicted as true positive.

3.1.5 Conclusion

In conclusion, we report, for the first time, the ultrasonic-assisted synthesis of novel functionalized the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** having pharmaceutically privileged groups. We also report, for

the first time, the antimicrobial and antitubercular activities, SAR studies of **12a-j**, **13a-e**, **14a-d** and **15**, which were validated by carrying out the *in silico* molecular docking studies of **12a**, **13b** and **15**.

Table 5. Comparison of binding affinity of the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a**, **13b**, **15**, **Isoniazid (INH)** and **Rifampicin** in terms of docking energy and binding site residues of antituberculosis target enoyl reductase (ENR; EnvM) (PDB: 1QSG)

Compound Name	Total Score	Amino acid involved in active pocket in 3Å	Involved group of Amino Acid	Length of H-bond Ă	No. of Hydrogen Bond
12a	4.6079	Leu-50, His-47, Ser-196, Ser-197, His-44, Tyr-82, His-135, Phe-136, Gln-72, Val-143, Val-139, Gln-164, Asp-161, Met-40, Ile-168, Thr-39, Lys-160, Gly-158, Phe-157, Pro-38	His-47 (side chain)	1.8	1
13b	8.1329	Arg-198, Gln-164, Ser-197, Ser-196, Met-195, Asp-161, Lys-160, Gly-158, Phe-157, Phe-156, Leu-280, His-44, Gly-46, His-47, Thr-186, Val-187, Leu-50, Pro-38, Thr-39, Met-40, Tyr-82, Val-139	Ser-197 (backbone) Val-187 (backbone)	1.9 2.1	2
15	5.2059	Arg-198, Ser-197, Ser-196, Met-195, Gln-164, Tyr-82, Asp-161, Lys-160, Gly-158, Phe-157, Phe-156, Leu-50, His-47, Gly-46, Pro-38, Thr-39, Met-40, His-44, Val-143	Lys-160 (backbone) Ser-197 (backbone) Ser-196 (side chain) Gln-164 (side chain)	1.9 2.1 2.3 1.7	4
INH	4.0421	Val-187, Pro-185, Thr-186, Val-184, His-44, Gly-46, His-47, Ala-49, Leu-50	Val-187 (backbone) Pro-185 (backbone)	1.9 1.9	2
Rifampicin	1.5110	Val-139, Val-142, Val-143, Leu-146, Ser-65, Phe-67, Asn-69, Gln-72, Pro-38, Thr-39, Met-40, Gly-41, His-44, Gly-46, His-47, Leu-50, Gln-164, Asp-161, Lys-160, Tyr-82, Gly-158, Phe-157, Leu-280, Arg-198, Ser-197, Ser-196	Tyr-82 (side chain)	1.5	1

We also report the *in vitro* antimicrobial and antitubercular activity of some previously reported spiro compounds **12c**, **12f** and **12g**. The stereochemistry of the novel spirooxindole-pyrrolidine compounds were confirmed by single crystal X-ray crystallography studies of **16**. All the synthesized spiro-pyrrolidines/pyrrolizines showed moderate antibacterial activity. The compounds **13c**, **13e**, **14a** and **15** (MIC = 12.5 µg/mL) were found two times more active as compared to standard drug ampicillin (MIC = 25 µg/mL) against the *Bacillus subtilis* [BS] bacterial strain. Compounds **12a** and **13c** (MIC = 25 µg/mL) were equipotent to ampicillin (MIC = 25µg/mL) the *Bacillus subtilis* [BS] bacterial strain. In antitubercular activity assay, the compounds **13b** (MIC = 3.125 µg/Ml) and **15** (MIC =1.56 µg/mL) exhibited promising antitubercular activity as compared to the standard drugs INH (MIC = 0.1 µg/mL) and Rifampicin (MIC = 0.2 µg/mL). Succinctly, it has also been justified that the spirooxindole-pyrrolidine derivatives of the amino acids L-phenylalanine as well as L-tryptophan were found to be more active as compared to other derivatives. The *in silico* molecular docking studies validates the biological activity as the docking score of the active compounds **13b** and **15** were found to be more than the standard drugs.

3.1.6 Experimental Details & Characterization Data

3.1.6.1 General experimental

All glass apparatus were used after oven drying. High quality reagents were purchased from commercial sources like Sigma Aldrich (USA), TCI Chemicals (Tokyo, Japan), Spectrochem (India) and were used without further purification. Laboratory grade commercial reagents and solvents were purified by standard procedures prior to use. Infrared spectra were recorded on a FT-IR Spectrum 2 (Perkin-Elmer) spectrophotometer and are quoted in cm^{-1}. NMR spectra were recorded on a Jeol resonance ECS 400 MHz spectrometer (operating at 400 MHz for ^{1}H; 100 MHz for ^{13}C). Chemical shifts (δ) are reported in parts per million from low to high field and referenced to residual solvent $CDCl_3$ as the internal reference ($CDCl_3$: δ = 7.249 ppm). The ^{13}C NMR (100 MHz) chemical shifts were given using $CDCl_3$ as the internal standard ($CDCl_3$: δ = 77.16 ppm). Tetramethylsilane (δ = 0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (δ 77.16 ppm) in ^{13}C NMR. Coupling constants (*J*) are reported in Hertz (Hz) with Standard abbreviations used as follows: m = multiplet, q = quartet, t = triplet, d = doublet, s = singlet, br =

broad. Electrospray emission mass spectrometry (ESI-MS) and high resolution mass spectra (HRMS) were obtained with a Gevo G-2 S Q-TOF (Waters). Melting points were recorded in open capillaries on Complab melting point apparatus and are presented uncorrected. Analytical thin layer chromatography was performed on Merck TLC Silica gel F_{254} plates with visualization under UV light (λ = 254 nm). Column chromatography was performed over Rankem silica gel (particle size: 100-200 Mesh) purchased from Rankem^{TM} (India).

3.1.6.2 General procedure (GP) for the synthesis of 3'-benzoyl-4'-phenylspiro[indoline-3,2'-pyrrolidin]-2-ones 12a-j, **13a-e**, **14a-d, 15** and **16**.

A mixture of Chalcone (0.24 mmol, 1 equiv.), isatin (0.36 mmol, 1.5 equiv.), and amino acid (0.47 mmol, 2 equiv.) in dry methanol or carbinol are heated in ultrasonicator for 30 min at a temperature of 50°C. The reaction was monitored by TLC. After completion of the reaction, the solvent (methanol) was evaporated under reduced pressure and the crude compound was purified through column chromatography using Ethyl acetate and hexane as an eluting solvent.

3.1.6.3 *In Vitro* Antitubercular MABA Activity assay against *M. tuberculosis* H_{37}Ra strain.[28, 29]

Briefly, the inoculum was prepared from fresh LJ medium re-suspended in 7H9-S medium (7H9 broth, 0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. 1, and diluted 1:20; 100 µl was used as inoculum. Each drug stock solution was thawed and diluted in 7H9-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well microliter plate using 100 µl 7H9-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at 37ºC in normal atmosphere. After 7 days incubation, 30 µl of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color.

3.1.6.4 Characterization data of all the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines 12a-j, 13a-e, 14a-d, 15 and 16.

2'-benzoyl-1'-phenyl-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**12a**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 70:30) White solid; Yield: 98%, R_f (EtOAc/hexane = 1:1) 0.48; m.p.: 165-167 ºC; FT-IR (KBr, ν max/cm^{-1}) 3427.99, 2923.05, 2859.3, 1706.41, 1606.02, 1091.24, 965.32, 759.37; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.65 (br, 1H), 7.50 (d, *J* = 7.3 Hz, 2H) 7.38 (d, *J* = 7.3Hz, 2H)**,** 7.34 – 7.28 (m, 3H), 7.24 – 7.08 (m, 5H), 7.02 – 6.98 (m, 1H), 6.53 (d, *J* = 7.7 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.26 – 4.21 (m, 1H), 3.92 – 3.87 (m, 1H), 2.71 – 2.57 (m, 2H), 2.06 – 1.96 (m, 1H), 1.94 – 1.81 (m, 2H), 1.77 – 1.69 (m, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 197.11 (C=O, ketone), 181.45 (C=O, amide), 140.85 (C), 139.89 (C), 137.10 (C), 133.00 (C), 129.52 (CH), 128.76 (2 × CH), 128.21 (2 × CH), 128.16 (2 × CH), 127.94 (2 × CH), 127.57 (CH), 127.05 (CH), 125.18 (CH), 122.36 (CH), 110.36 (CH), 73.95 (C), 72.23 (CH), 64.52 (CH), 53.02 (CH), 48.37 (CH_2), 30.90 (CH_2), 27.41 (CH_2). HRMS (ESI) calcd. for $C_{27}H_{25}N_2O_2$ $[M+H]^+$: 409.1911; found 409.1915.

2'-benzoyl-1'-(9p-tolyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**12b**): The product was obtained via the general procedure and isolated by column chromatography (DCM:EtOAc = 9:1) Pure white solid; Yield: 84%, R_f (EtOAc/hexane; 1:1) = 0.31; m.p.: 186-188 ºC; FT-IR (KBr, ν max/cm^{-1}) 3427.26, 2922.83, 2858.7, 1706.44, 1605.99, 1091.67, 964.98, 759.46; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.36 (m, 4H), 7.34-7.32 (m, 1H), 7.23 (s, 1H), 7.19-7.07 (m, 5H), 7.01-6.98 (m, 2H), 6.47 (d, *J* = 7.7 Hz, 1H), 4.91 (d, *J* = 11.5 Hz, 1H), 4.25 – 4.19 (m, 1H), 3.88 – 3.82 (m, 1H), 2.70 – 2.57 (m, 2H), 2.28 (s, 3H), 2.03 – 1.97 (m, 1H), 1.91-1.86 (m, 2H), 1.76 – 1.70 (m, 1H). ^{13}C NMR (100, MHz, $CDCl_3$) δ 197.18 (C=O, ketone), 180.56 (C=O, amide), 140.36 (C), 137.22 (C), 136.75(C) 136.62 (C), 132.85 (CH), 129.45 (2 × CH), 129.42 (CH), 128.16 (2 × CH), 128.05 (2 × CH), 127.97 (2 × CH),127.71 (CH) 125.22 (C),122.38 (CH), 109.82 (CH),73.59 (C),72.16 (CH), 64.66 (CH), 52.70 (CH), 48.37 (CH_2), 30.77 (CH_2), 27.34 (CH_2), 21.11 (CH_3). HRMS (ESI) calcd.for $C_{28}H_{27}N_2O_2$ $[M+H]^+$: 423.2067; found 423.2070.

2'-benzoyl-1'-(4-methoxyphenyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3, 3'-pyrrolizin]-2-one (**12c**): The product was obtained via the general procedure and isolated by column chromatography (hexane : EtOAc = 7:3) White solid; Yield: 84%, R_f(EtOAc/hexane; 1:1) = 0.33;m. p. 164-166 ºC; FT-IR (KBr, ν max/cm^{-1}) 3428.33, 2923.04, 2858.9, 1706.27, 1605.53, 1091.77, 965.05, 759.78; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.31 (m, 6H), 7.23 (s, 1H), 7.17 (t, *J* = 7.9 Hz, 2H), 7.12– 7.07 (m,

1H), 7.02 – 6.98 (m, 1H), 6.86 – 6.83 (m, 2H), 6.50 (d, *J* = 7.6 Hz, 2H), 4.87 (d, *J* = 11.5 Hz, 1H), 4.23 – 4.18 (m, 1H), 3.93 – 3.81 (m, 1H), 3.75 (s, 3H), 2.69 – 2.57 (m, 2H), 2.04 –1.98 (m, 1H), 1.93 – 1.82 (m, 2H), 1.76 – 1.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.19 (C=O, ketone), 181.24 (C=O, amide), 158.61 (C), 140.72 (C), 137.15 (C), 132.95 (CH), 131.81 (C), 129.46 (2 × CH), 129.13 (CH), 128.15 (2 × CH), 127.94 (2 × CH), 127.59 (CH), 125.21 (C), 122.33 (CH), 114.17 (2 × CH), 110.22 (CH), 73.84 (C), 72.09 (CH), 64.63 (CH), 55.30 (OCH_3), 52.30 (CH), 48.37 (CH_2), 30.84 (CH_2), 27.39 (CH_2). HRMS (ESI) calcd.for $C_{28}H_{26}N_2O_3$ $[M+H]^+$: 439.2016; found 439.2020.

1'-(3-chlorophenyl)-2'-(4-methylbenzoyl)-1',2',5',6',7',7a'-hexahydrospiro [indoline-3, 3'-pyrrolizin]-2-one (**12d**): The product was obtained via the general procedure and isolated by column chromatography (DCM:EtOAc = 93:7) White solid; Yield: 81%, R_f (EtOAc/hexane: 1:1) 0.56; m.p. 116-118 ºC; FT-IR (KBr, ν max/cm^{-1}) 3428.21, 2923.15, 2858.5, 1706.43, 1605.12, 1091.67, 965.15, 759.54; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 1H**)**, 7.37 (d, *J* = 7.5 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.24-7.20 (m, 2H), 7.17-7.15 (m, 1H), 7.11-7.08 (m, 1H), 7.00-6.96 (m, 3H), 6.52 (d, *J* = 7.7 Hz, 1H), 4.85 (d, *J* = 11.4 Hz, 1H), 4.23-4.17 (m, 1H), 3.87 (t, *J* = 10.6 Hz, 1H), 2.72– 2.65 (m, 1H), 2.62 – 2.57 (m, 1H), 2.25 (s, 3H), 2.06 – 1.98 (m, 1H), 1.93-1.84 (m, 2H), 1.75-1.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.36 (C=O, ketone), 180.59 (C=O, amide), 143.93 (C), 142.07 (C), 140.47 (C), 134.51 (C), 134.47 (C), 130.00 (CH), 129.51 (CH), 128.97 (2 × CH), 128.44 (CH), 128.12 (2 × CH), 127.67 (CH), 127.25, (CH), 126.38 (CH), 125.01 (C), 122.38 (CH), 110.03 (CH), 73.59 (C), 71.93 (CH), 64.33 (CH), 52.71 (CH), 48.34 (CH_2), 30.66 (CH_2), 27.26 (CH_2), 21.67 (CH_3). HRMS (ESI) calcd.for $C_{28}H_{26}ClN_2O_2$ $[M+H]^+$:457.1678; found 457.1675.

2'-(4-chlorobenzoyl)-1'-(4-methoxyphenyl)-1',2',5',6',7',7a'-hexahydrospiro [indoline-3,3'-pyrrolizin]-2-one (**12e**): The product was obtained via the general procedure and isolated by column chromatography (hexane: EtOAc = 75:25) Pale white solid; Yield: 94%; R_f (EtOAc/hexane: 1:1) = 0.31; m.p. 150-152 ºC; FT-IR (KBr, ν max/cm^{-1}) 3427.93, 2922.87, 2859.2, 1706.34, 1605.62, 1091.58, 965.26, 759.68; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.34 (d, *J* = 8.6, 2H), 7.25 – 7.22 (m, 1H), 7.14 – 7.10 (m, 3H), 7.03 – 6.99 (m, 1H), 6.84 (d, *J* = 8.7 Hz, 2H**),** 6.61 (d, *J* = 7.7 Hz, 1H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.22 – 4.16 (m, 1H), 3.86 – 3.81 (m, 1H), 3.74 (s, 3H), 2.69 – 2.58 (m, 2H), 2.05 – 1.95 (m, 1H), 1.94

– 1.81 (m, 2H), 1.75 – 1.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.98 (C=O, ketone), 180.97 (C=O, amide), 158.68 (C), 140.49 (C), 139.41(C), 135.47 (C), 131.56 (C), 129.59 (CH), 129.39 (2 × CH), 129.09 (2 × CH), 128.52 (2 × CH), 127.64 (CH) , 125.06 (C), 122.46 (CH), 114.21 (2 × CH), 110.25 (CH), 73.73 (C), 72.07 (CH), 64.64 (CH), 55.31 (OCH_3), 52.32 (CH), 48.35 (CH_2), 30.80 (CH_2), 27.39 (CH_2). HRMS (ESI) calcd.for $C_{28}H_{25}ClN_2O_3$ $[M+H]^+$: 473.1627; found 473.1629.

2'-benzoyl-1'-(3-nitrophenyl)-1',2', 5', 6', 7', 7a'-hexahydrospiro [indoline-3, 3'-pyrrolizin]-2-one (**12f**): The product was obtained via the general procedure and isolated by column chromatography (hexane: EtOAc = 70:30) White solid; Yield: 83%; R_f (EtOAc/hexane: 1:1) = 0.22; m.p. 192-194 ºC; FT-IR (KBr, ν max/cm^{-1}) 3428.09, 2922.91, 2857.6, 1705.78, 1604.69, 1092.03, 964.89, 759.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39– 8.38 (m, 1H), 8.06 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.99 (d, *J* = 5.5 Hz, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.36 –7.32 (m, 3H), 7.19 – 7.15 (m, 3H), 7.10 – 7.07 (m, 1H), 6.98 (t, *J* = 7.3 Hz, 1H), 6.51 (d, *J* = 7.7 Hz, 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.28 – 4.22 (m, 1H), 3.99 (t, *J* = 10.6 Hz, 1H), 3.72 – 3.70 (m, 1H), 2.85-2.83 (m, 1H), 2.04 – 1.98 (m, 1H), 1.95 – 1.85 (m, 2H), 1.76 – 1.68 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.92 (C=O, ketone), 180.83 (C=O, amide), 148.64 (C), 142.24 (C), 140.70 (C), 136.75 (C), 134.58 (CH), 133.24 (CH), 129.78 (CH), 129.74 (CH), 128.28 (2 × CH), 127.93 (2 × CH), 127.37 (CH), 124.75 (C), 123.32 (CH), 122.53 (CH), 122.26 (CH), 110.54 (CH), 73.59 (C), 71.89 (CH), 64.85 (CH), 52.63 (CH), 48.30 (CH_2), 30.69 (CH_2), 27.31 (CH_2). HRMS (ESI) calcd. for $C_{27}H_{24}N_3O_4$ $[M+H]^+$: 454.1762; found 454.1766.

2'-(4-methylbenzoyl)-1'-(3-nitrophenyl)-1', 2', 5', 6', 7', 7a'-hexahydrospiro [indoline-3, 3'-pyrrolizin]-2-one (**12g**): The product was obtained via the general procedure and isolated by column chromatography (hexane: EtOAc = 7:3) White solid; Yield: 58 %; R_f (EtOAc/hexane; 1:1) = 0.24; m.p.: 162-164 ºC; FT-IR (KBr, ν max/cm^{-1}) 3427.76, 2923.36, 2858.4, 1706.38, 1604.99, 1092.09, 965.16, 759.52; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (t, *J* = 1.7 Hz, 1H), 8.06 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.66 (s, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 7.4 Hz, 1H), 7.14 – 7.10 (m, 1H), 7.02 – 6.96 (m, 3H), 6.57 (d, *J* = 7.7 Hz, 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.28 – 4.22 (m, 1H), 4.04 – 3.98 (m, 1H), 2.73 – 2.67 (m, 1H), 2.64 - 2.59 (m, 1H), 2.24 (s, 3H), 2.07-1.98 (m, 1H), 1.96-1.85 (m, 2H), 1.77-1.69 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.26 (C=O, ketone), 180.52 (C=O, amide), 144.13 (C), 134.44 (C), 134.28 (C), 129.71 (C), 129.64 (C), 129.04 (2

× CH), 128.11 (2 × CH), 127.57 (CH), 126.57 (CH), 123.40 (CH), 122.49 (CH), 122.22 (CH), 109.98 (CH), 71.82 (C), 64.59 (CH), 52.68 (CH), 48.26 (CH), 30.66 (CH_2), 27.32 (CH_2), 21.69 (CH_3). HRMS (ESI) calcd.for $C_{28}H_{25}N_3O_4$ $[M+H]^+$: 468.1918; found 468.1921.

2'-(4-nitrobenzoyl)-1'-(p-tolyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**12h**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 78:22) Creamy white solid; Yield: 78%; R_f (EtOAc/hexane: 1:1) = 0.52; m.p.: 168-170 ºC; FT-IR (KBr, ν max/cm^{-1}) 3428.01, 2924.14, 2860.02, 1706.37, 1606.22, 1091.29, 965.47, 759.51; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.98 (m, 2H), 7.92 (br, 1H), 7.49 – 7.45 (m, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 7.4 Hz, 1H), 7.16 – 7.13 (m, 3H), 7.05-7.01 (m, 1H), 6.56 (d, *J* = 7.7 Hz, 1H), 4.88 (d, *J* = 11.4 Hz, 1H), 4.24-4.18 (m, 1H), 3.85 – 3.79 (m, 1H), 2.67 –2.56 (m, 2H), 2.28 (s, 3H), 2.05 – 1.97 (m, 1H), 1.92 – 1.83 (m, 2H), 1.75 – 1.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.15 (C=O, ketone), 180.89 (C=O, amide), 150.03 (C), 141.74 (C), 140.52 (C), 136.97 (C), 136.26 (C), 129.90 (CH), 129.60 (2 × CH), 128.91 (2 × CH), 127.99 (2 × CH), 127.57 (CH), 124.85 (C), 123.34 (2 × CH), 122.70 (CH), 110.42 (CH), 73.55 (C), 72.23 (CH), 65.37 (CH), 52.66 (CH), 48.30 (CH_2), 30.76 (CH_2), 27.34 (CH_2), 21.13 (CH_3); HRMS (ESI) calcd.for $C_{28}H_{26}N_3O_4$ $[M+H]^+$: 468.1918; found 468.1923.

1'-(3-chlorophenyl)-2'-(4-nitrobenzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (12i): The product was obtained via the general procedure and isolated by column chromatography (hexane: EtOAc = 4:1) Light brown solid; Yield: 74%; R_f (EtOAc/hexane: 1:1) = 0.67; m.p.: 140-142 ºC; FT-IR (KBr, ν max/cm^{-1}) 3428.31, 2924.59, 2857.92, 1705.93, 1604.84, 1092.13, 965.18, 759.23; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.99 (m, 2H), 7.74 (s, 1H), 7.51 – 7.45 (m, 3H), 7.39 (d, *J* = 7.6 Hz, 1H),7.28 – 7.24 (m, 1H), 7.21 – 7.12 (m, 3H), 7.04-7.00 (m, 1H), 6.54 (d, *J* = 7.7 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.23-4.18 (m, 1H), 3.85 – 3.80 (m, 1H), 2.68 – 2.57 (m, 2H), 2.06-1.98 (m, 1H), 1.93-1.84 (m, 2H), 1.74 – 1.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.98 (C=O, ketone), 180.46 (C=O, amide), 150.07 (C), 141.53 (C), 141.50 (C), 140.42 (C), 134.69 (C), 130.18 (CH), 130.05 (CH), 128.94 (2 × CH), 128.40 (CH), 127.56 (CH), 127.49 (CH), 126.35 (CH), 124.59 (C), 123.40 (2 × CH), 122.78 (CH), 110.38 (CH), 73.30 (C), 72.03 (CH), 65.44 (CH), 52.57 (CH), 48.26 (CH_2), 30.66 (CH_2), 27.27 (CH_2); HRMS (ESI) calcd. for $C_{27}H_{23}ClN_3O_4$ $[M+H]^+$: 488.1372; found 488.1371.

1'-(4-methoxyphenyl)-2'-(4-nitrobenzoyl)-1', 2', 5', 6', 7', 7a'-hexahydrospiro [indoline-3, 3'-pyrrolizin]-2-one (**12j**): The product was obtained via the general procedure and isolated by column chromatography (hexane: EtOAc = 7:3) White solid; Yield: 69%; R_f (EtOAc/hexane: 1:1) = 0.42; m.p. 174-176 ºC; FT-IR (KBr, ν max/cm^{-1}) 1246.97 (C-O stretch), 1349.49, 1523.96 (NO stretch), 1687.10 (Amide CO stretch), 1246.97 (C-O stretch), 1723.11 (Ketonic CO stretch), 1740.71, 1470.41, 752.40 (aromatic CH bend); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.8 Hz, 2H), 7.87 (s, 1H), 7.48 – 7.41 (m, 4H), 7.23 (d, *J* = 7.4 Hz, 1H), 7.17 – 7.10 (m, 1H), 7.05 – 7.01 (m, 1H), 6.86 (d, *J* = 8.68 Hz, 2H**)**, 6.55 (d, *J* = 7.7 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.22-4.17 (m, 1H), 3.85 – 3.78 (m, 1H), 3.75 (s, 3H), 2.67 – 2.56 (m, 2H), 2.05 – 1.97 (m, 1H), 1.91 – 1.83 (m, 2H), 1.74-1.66 (m, 1H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 196.23 (C=O, ketone), 181.02 (C=O, amide), 158.80 (C), 150.03 (C), 141.72 (C), 140.61 (C), 131.26 (C), 129.90 (CH), 129.09 (2 × CH), 128.91 (2 × CH), 127.54 (CH), 124.86 (C), 123.35 (2 × CH), 122.67 (CH), 114.30 (2 × CH), 110.49 (CH), 73.59 (C), 72.13 (CH), 65.47 (CH), 55.32 (OCH_3), 52.26 (CH), 48.30 (CH_2), 30.77 (CH_2), 27.34 (CH_2); HRMS (ESI) calcd.for $C_{28}H_{26}N_3O_5$ $[M+H]^+$:484.1867; found 484.1870.

5'-benzyl-3'-(4-methylbenzoyl)-4'-(p-tolyl)spiro[indoline-3,2'-pyrrolidin]-2-one (**13a**): The product was obtained via the general procedure A and isolated by column chromatography (hexane:EtOAc = 82:18) white solid; Yield: 55%; R_f (EtOAc/hexane: 1:1) = 0.69; m.p.: 210-212 ºC; FT-IR (KBr, ν max/cm^{-1}) 1524.14, 1348.23 (NO Stretch), 1620.42 (amide CO stretch), 1727.58 (ketone CO stretch), 3354.72 (amine NH stretch), 735.44, 700.23; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.7 Hz, 2H), 7.26 –7.24 (m, 4H), 7.20 – 7.14 (m, 5H), 7.04 (s, 1H), 6.96 – 6.90 (m, 3H), 6.86 – 6.78 (m, 2H), 6.37 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 10.9 Hz, 1H), 4.23 – 4.18 (m, 1H**)**, 3.83 (t, *J* = 10.8 Hz, 1H), 2.98 (dd, *J* = 13.8, 3.0 Hz, 1H), (2.67 (dd, *J* = 13.8, 7.5 Hz, 1H), 2.30 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.70 (C=O, ketone), 181.73 (C=O, amide), 143.57 (C), 139.65 (C), 138.11 (C), 136.68 (C), 136.42 (C), 134.79 (C), 129.81 (C), 129.65 (2 × CH), 129.55 (2 × CH), 129.02 (CH), 128.84 (2 × CH), 128.54 (2 × CH), 128.51 (2 × CH), 127.83 (2 × CH), 126.46 (CH), 125.87 (CH), 123.04 (CH), 109.13 (CH), 68.78 (C), 64.81 (CH), 63.20 (CH), 52.43 (CH), 38.63 (CH_2), 21.60 (CH_3), 21.15 (CH_3); HRMS (ESI) calcd.for $C_{33}H_{31}N_2O_2$ $[M+H]^+$: 487.2380; found 487.2384.

5'-benzyl-3'-(4-nitrobenzoyl)-4'-(p-tolyl)spiro[indoline-3, 2'-pyrrolidin]-2-one (13b): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 84:16) creamy white solid; Yield: 63%; R_f (EtOAc/hexane; 1:1) = 0.83; m.p. 170-172 ºC; FT-IR (KBr, ν max/cm^{-1}) 1524.59, 1348.35 (NO Stretch), 1620.21 (amide CO stretch), 1726.63 (ketone CO stretch), 3354.49 (amine NH stretch), 735.54, 699.69; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.8 Hz, 2H), 7.46 – 7.42 (m, 4H), 7.28 – 7.24 (m, 2H), 7.21 – 7.19 (m, 2H), 7.17 – 7.16 (m, 4H), 6.98 – 6.95 (m, 1H), 6.89 – 6.85 (m, 1H), 6.77 (d, *J* = 7.3 Hz, 1H), 6.35 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 10.7 Hz, 1H), 4.27 – 4.22 (m, 1H), 3.81 (t, *J* = 10.7 Hz, 1H), 3.00 – 2.96 (m, 1H), 2.69 – 2.64 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.25 (C=O, ketone), 181.81 (C=O, amide), 149.85 (C), 141.71 (C), 139.86 (C), 137.81 (C), 137.11 (C), 135.92 (C), 129.75 (2 × CH), 129.64 (2 × CH), 129.59 (CH), 129.20 (CH), 128.58 (4 × CH), 128.50 (2 × CH), 126.61 (CH), 125.68 (CH), 123.32 (2 × CH), 109.62 (CH), 68.59 (C), 64.89 (CH), 64.24 (CH), 52.23 (CH), 38.46 (CH_2), 21.19 (CH_3); HRMS (ESI) calcd.for $C_{32}H_{28}N_3O_4$ $[M+H]^+$: 518.2075 ; found 518.2079.

5'-benzyl-3'-(4-methylbenzoyl)-4'-(3-nitrophenyl)spiro [indoline-3,2'-pyrrolidin]-2-one (13c): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 81:19) brownish solid; Yield: 48%; (EtOAc/hexane; 1:1) = 0.55; m.p. 161-163 ºC; FT-IR (KBr, ν max/cm^{-1}) 1523.99, 1347.81 (NO Stretch), 1619.73 (amide CO stretch), 1727.54 (ketone CO stretch), 3354.02 (amine NH stretch), 735.34, 700.46; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (t, *J* = 1.8 Hz, 1H), 8.05-8.03 (m, 1H) 7.86 (d, *J* = 7.8 Hz, 1H), 7.47 (t, *J* = 7.9 Hz, 1H), 7.31 (brs, 1H), 7.27 (s, 1H), 7.23 – 7.11 (m, 6H), 7.00 – 6.96 (m, 1H), 6.93 (d, *J* = 8.1 Hz, 2H), 6.88-6.85 (m, 2H), 6.43 (d, *J* = 7.6 Hz, 1H), 4.52 (d, *J* = 10.7 Hz, 1H), 4.34-4.29 (m, 1H), 4.00 (t, *J* = 10.6 Hz, 1H), 2.92 (dd, *J* = 13.8, 4.5 Hz, 1H), 2.80 (dd, *J* = 13.8, 7.1 Hz, 1H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.30 (C=O, ketone), 182.13 (C=O, amide), 139.90 (C), 138.06 (C) , 137.19 (C), 136.77 (C), 136.37 (C), 132.80 (CH), 129.66 (2 × CH), 129.60 (2 × CH), 129.15 (CH), 128.54 (2 × CH), 128.14 (2 × CH), 127.65 (4 × CH), 126.51 (CH), 125.66 (C), 123.06 (CH), 109.41 (CH), 68.82 (C), 64.88 (CH), 63.41 (CH), 52.35 (CH), 38.58 (CH_2), 21.17 (CH_3); HRMS (ESI) calcd.for $C_{32}H_{28}N_3O_4[M+H]^+$: 518.2075; found 518.2079.

5'-benzyl-4'-(4-methoxyphenyl)-3'-(4-nitrobenzoyl)spiro[indoline-3,2'-pyrrolidin] -2-one (13d): The product was obtained via the general procedure and isolated by

column chromatography (hexane:EtOAc = 88:12) yellow solid; Yield: 69%; R_f (EtOAc/hexane; 1:1) = 0.85; m.p. 160-162 ºC; FT-IR (KBr, ν max/cm^{-1}) 1523.32, 1347.47 (NO Stretch), 1619.96 (amide CO stretch), 1726.78 (ketone CO stretch), 3353.84 (amine NH stretch), 734.52, 700.18; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.94 (m, 2H), 7.51 – 7.41 (m, 4H), 7.28 – 7.24 (m, 3H), 7.22 – 7.12 (m, 4H), 6.99 – 6.95 (m, 1H), 6.92 – 6.85 (m, 3H), 6.77 (d, *J* = 7.2 Hz, 1H), 6.35 (d, *J* = 7.7 Hz, 1H), 4.52 (d, *J* = 10.8 Hz, 1H), 4.24 – 4.19 (m, 1H), 3.82 – 3.76 (m, 4H), 2.98 (dd, *J* = 13.9, 3.4 Hz, 1H), 2.67 (dd, *J* = 13.9, 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.31 (C=O, ketone), 181.78 (C=O, amide), 158.90 (C), 149.85 (C), 141.69 (C), 139.80 (CH), 137.84 (CH), 130.90 (C), 129.61 (2 × CH), 129.59 (2 × CH), 129.22 (CH), 128.58 (2 × CH), 128.57 (2 × CH), 126.61 (CH), 125.70 (C), 123.32 (2 × CH), 114.45 (2 × CH), 109.59 (CH), 68.49 (CH), 64.82 (C), 64.25 (CH), 55.36 (OCH_3), 51.82 (CH), 38.52 (CH_2); HRMS (ESI) calcd.for $C_{32}H_{27}N_3O_5$ $[M+H]^+$: 534.2024; found 534.2028.

5'-benzyl-3'-(4-nitrobenzoyl)-4'-(3-nitrophenyl)spiro[indoline-3,2'-pyrrolidin]-2-one (13e): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 4:1) brownish solid; Yield: 78%; R_f (EtOAc/hexane; 1:1) = 0.70; m.p. 192-194 ºC; FT-IR (KBr, ν max/cm^{-1}) 1523.96, 1347.95 (NO Stretch), 1619.86 (amide CO stretch), 1726.14 (ketone CO stretch), 3353.53 (amine NH stretch), 734.14, 699.98; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36-8.35 (m, 1H), 8.08 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.98 (d, *J* = 8.9 Hz, 2H), 7.89 (d, *J* = 7.7 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.46 – 7.43 (m, 2H), 7.24 – 7.21 (m, 3H), 7.18 – 7.11 (m, 4H), 7.01– 6.98 (m, 1H), 6.92 – 6.88 (m, 1H**),** 6.84 (d, *J* = 7.0 Hz, 1H**)**, 6.39 (d, *J* = 7.7 Hz, 1H), 4.52 (d, *J* = 10.5 Hz, 1H), 4.38 – 4.33 (m, 1H), 3.97 (t, *J* = 10.5 Hz, 1H), 2.95 – 2.90 (m, 1H), 2.82 – 2.76 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.79 (CO, ketone), 181.51 (CO, amide), 149.99 (C), 148.67 (C), 141.60 (C), 141.22 (C), 139.93 (C), 137.13 (C), 134.96 (CH), 129.94 (2 × CH), 129.45 (2 × CH), 128.64 (2 × CH), 128.62 (2 × CH), 126.81 (CH), 125.57 (C), 123.67 (CH), 123.46 (CH), 123.41 (2 × CH), 122.50 (CH), 109.89 (CH), 68.53 (C), 64.79 (CH), 64.31 (CH), 52.28 (CH), 39.15 (CH_2); HRMS (ESI) calcd.for $C_{31}H_{25}N_4O_6$ $[M+H]^+$: 549.1769; found 549.1773.

3'-benzoyl-5'-methyl-4'-phenylspiro[indoline-3,2'-pyrrolidin]-2-one (**14a**): The product was obtained via the general procedure A and isolated by column chromatography (hexane:EtOAc = 70:30) white solid; Yield: 84%; R_f (EtOAc/hexane: 1:1) = 0.34; m.p. 170-172 ºC; FT-IR (KBr, ν max/cm^{-1}) 746.87

(aromatic CH bend), 1179.51, 1253.67 (C-O stretch), 1514.74, 1676.05 (Amide CO stretch), 1720.29 (Ketonic CO stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (brs, 1H), 7.50 (d, *J* = 7.3 Hz, 2H), 7.39 (d, *J* = 7.3 Hz, 2H), 7.33 – 7.28 (m, 3H), 7.20 (t, *J* = 7.4 Hz, 1H), 7.14 (t, *J* = 7.7 Hz, 3H), 7.04 – 6.91 (m, 2H), 6.47 (d, *J* = 7.5 Hz, 1H), 4.68 (d, *J* = 10.8 Hz, 1H), 4.01-3.94 (m, 1H), 3.73 – 3.68 (m, 1H), 2.12 (br, 1H), 1.22 (d, *J* = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.52 (C=O, Ketone), 181.81 (C=O, amide), 140.10 (C), 139.58 (C), 137.18 (C), 132.86 (CH), 129.37 (C), 129.27 (C), 128.79 (2 × CH), 128.32 (2 × CH), 128.22 (2 × CH), 127.68 (2 × CH), 127.11 (CH), 125.51 (CH), 123.13 (CH), 109.47 (CH), 69.29 (C), 63.63 (CH), 61.13 (CH), 56.83 (CH), 18.55 (CH_3); HRMS (ESI) calcd.for $C_{25}H_{22}N_2O_2$ $[M+H]^+$: 383.1754; found 383.1758

3'-benzoyl-4'-(4-methoxyphenyl)-5'-methylspiro[indoline-3,2'-pyrrolidin]-2-one (**14b**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 65:35) white solid; Yield: 73%; R_f (EtOAc/hexane: 1:1) = 0.5; m.p.: 138-140 ºC; FT-IR (KBr, ν max/cm^{-1}) 746.12 (aromatic CH bend), 1179.66, 1253.59 (C-O stretch), 1513.08, 1675.21 (Amide CO stretch), 1719.47 (Ketonic CO stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.43 – 7.37 (m, 4H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.16 – 7.12 (m, 3H), 7.01 – 6.91 (m, 2H), 6.87 – 6.84 (m, 2H), 6.44 (d, *J* = 7.4 Hz, 1H), 4.62 (d, *J* = 10.8 Hz, 1H), 3.96–3,90 (m, 1H), 3.75 (s, 3H), 3.68 – 3.62 (m, 1H), 1.20 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.67 (C=O, ketone), 181.96 (C=O, amide), 158.66 (C), 140.15 (C), 137.22 (C), 132.85 (CH), 131.52 (C), 129.34 (CH), 129.25 (2 × CH), 128.21 (2 × CH), 127.68 (2 × CH), 125.48 (CH), 123.10 (CH), 114.19 (2 × CH), 113.57 (CH), 109.51 (CH), 69.22 (C), 63.63 (CH), 60.89 (CH), 56.09 (CH), 55.31 (OCH_3), 18.55 (CH_3); HRMS (ESI) calcd. for $C_{26}H_{25}N_2O_3$ $[M+H]^+$: 413.1860; found 413.1863.

3'-(4-methoxybenzoyl)-5'-methyl-4'-(p-tolyl)spiro[indoline-3,2'-pyrrolidin]-2-one (**14c**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 65:35) white solid; Yield: 86%; R_f (EtOAc/hexane: 1:1) = 0.45; m.p.: 135-137 ºC; FT-IR (KBr, ν max/cm^{-1}) 746.68 (aromatic CH bend), 1178.27, 1254.19 (C-O stretch), 1514.32, 1676.75 (Amide CO stretch), 1721.71 (Ketonic CO stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.44 – 7.39 (m, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.03 – 6.99 (m, 1H), 6.95 – 6.91 (m, 1H), 6.64 – 6.60 (m, 2H), 6.50 (d, *J* = 7.6 Hz, 1H),

4.62 (d, *J* = 10.9 Hz, 1H), 3.95-3.90 (m, 1H), 3.71 (s, 3H), 3.70 – 3.64 (m, 1H), 2.28 (s, 3H), 2.03 (s, 1H), 1.20 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.66 (C=O, ketone), 181.76 (C=O, amide), 163.27 (C), 139.94 (C), 136.60 (C), 136.51 (C), 130.41 (C), 130.03 (2 × CH), 129.45 (2 × CH), 129.27 (CH), 128.16 (2 × CH), 125.63 (CH), 123.10 (CH), 113.39 (2 × CH), 109.41 (CH), 69.38 (C), 63.07 (CH), 60.95 (CH), 56.58 (CH), 55.40 (OCH_3), 21.12 (CH_3), 18.56 (CH_3); HRMS (ESI) calcd.for $C_{27}H_{27}N_2O_3$ $[M+H]^+$: 427.2016; found 427.2015.

5'-methyl-3'-(4-nitrobenzoyl)-4'-(p-tolyl)spiro[indoline-3, 2'-pyrrolidin]-2-one (**14d**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 65:35) white solid; Yield: 96%; R_f (EtOAc/hexane: 1:1) = 0.7; m.p. 165-167 ºC; FT-IR (KBr, ν max/cm^{-1}) 747.98 (aromatic CH bend), 1177.86, 1255.26 (C-O stretch), 1515.87, 1675.83 (Amide CO stretch), 1722.89 (Ketonic CO stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.96 (m, 2H), 7.73 – 7.67 (m, 1H), 7.49 – 7.46 (m, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.16– 7.11 (m, 3H), 7.05 (td, *J* = 7.6, 1.2 Hz, 1H), 6.99 – 6.93 (m, 1H), 6.46 (d, *J* = 7.6 Hz, 1H), 4.63 (d, *J* = 10.8 Hz, 1H), 4.00 – 3.93 (m, 1H), 3.64 (t, *J* = 10.7 Hz, 1H), 2.30 (s, 3H), 1.20 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.45 (C=O, ketone), 181.48 (C=O, amide), 149.88 (C), 141.72 (C), 139.95 (C), 137.02 (C), 135.98 (C), 129.79 (CH), 129.63 (2 × CH), 128.90 (C), 128.59 (2 × CH), 128.12 (2 × CH), 125.56 (CH), 123.39 (CH), 123.36 (2 × CH), 109.65 (CH), 68.98 (C), 64.45 (CH), 60.99 (CH), 56.30 (CH), 21.15 (CH_3), 18.49 (CH_3); HRMS (ESI) calcd. for $C_{26}H_{24}N_3O_4$ $[M+H]^+$: 442.1762; found 442.1765.

5'-(indolin-2-ylmethyl)-3'-(4-methoxybenzoyl)-4'-(p-tolyl) spiro[indoline-3, 2'-pyrrolidin]-2-one (**15**): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 62:38) light brown solid; Yield: 74%; R_f (EtOAc/hexane: 1:1) = 0.25; m.p.: 130-132º C; FT-IR (KBr, ν max/cm^{-1}) 745.43 (aromatic CH bend), 1172.34, 1259.82 (C-O stretch), 1471.58, 1599.17, 1716.18 (Ketonic CO stretch), 3329.83 (NH stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (br, 1H), 7.47 (t, *J* = 7.8 Hz, 3H), 7.36 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.21 (s, 1H), 7.16 – 7.11 (m, 3H), 7.06 (d, *J* = 1.8Hz, 1H), 7.04 – 7.00 (m, 1H), 6.94 – 6.89 (m, 1H), 6.78 (d, *J* = 4.32, 2H), 6.59 (d, *J* = 8.8 Hz, 2H), 6.37 (d, *J* = 7.7 Hz, 1H), 4.56 (d, *J* = 10.9 Hz, 1H), 4.31 – 4.27 (m, 1H), 3.94-3.88 (m, 1H), 3.70 (s, 3H), 3.15 (dd, *J* = 14.8, 3.0 Hz, 1H), 2.84 (dd, *J* = 14.9, 7.4 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.63 (C=O, Ketone), 182.04 (C=O, Amide),

163.21, 136.70, 136.62, 130.38, 130.03, 129.73, 129.52, 128.98, 128.57, 128.01, 125.61, 122.98, 122.93, 122.03, 119.44, 119.08, 113.33, 112.06, 111.11, 109.32, 69.20, 64.81, 63.25, 55.37, 52.92, 27.88, 21.18. HRMS (ESI) calcd.for $C_{35}H_{32}N_3O_3$ $[M+H]^+$: 542.2438; found 542.2440.

2'-benzoyl-5-bromo-1'-phenyl-1',2',5',6',7',7a'-hexahydrospiro[indoline-3, 3'-pyrrolizin]-2-one (16): The product was obtained via the general procedure and isolated by column chromatography (hexane:EtOAc = 70:30) creamy white solid; Yield: 71%; R_f (hexane:EtOAc = 3:2): 0.37; m.p.: 175-177 ºC; FT-IR (KBr, ν max/cm^{-1}) 3437.04 (NH stretch), 1740.57 (ketonic CO stretch), 1713.85, 1674.72 (amide CO stretch), 1469.14, 737.54 (aromatic CH bend), 692.44 (C-Br stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.1 (d, *J* = 7.6 Hz, 2H), 7.35 – 7.28 (m, 4H), 7.26 – 7.23 (m, 1H), 7.21-7.13 (m, 3H), 6.48 (d, *J* = 8.4 Hz, 1H), 4.92 (d, *J* = 11.6 Hz, 1H), 4.24-4.19 m, 1H), 3.90 – 3.83 (m, 1H), 2.69 – 2.58 (m, 2H), 2.04 – 1.73 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.72 (C=O, Ketone), 180.88 (C=O, Amide), 139.73 (C), 139.51 (C), 137.03 (C), 133.15(C), 132.46 (C), 130.49 (CH), 128.81 (2 × CH), 128.30 (2 × CH), 128.16 (2 × CH), 127.98 (2 × CH), 127.48 (CH), 127.16 (CH), 115.19 (CH), 111.63 (CH), 73.75 (C), 72.18 (CH), 64.59 (CH), 52.93 (CH), 48.28 (CH_2), 30.76 (CH_2), 27.52 (CH_2); HRMS (ESI) calcd for $C_{27}H_{24}BrN_2O_2^+$ ($M+H^+$): 487.1016; found, 487.1012.

3.1.6.5 Materials and methods for *In silico* molecular docking studies.[30-31]

Molecular modeling studies of functionalized the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines **12a-j**, **13a-e**, **14a-d** and **15** were carried out using molecular modeling software Sybyl-X 2.0, (Tripos International, St. Louis, Missouri, 63144, USA). Drawing of structures and simple geometry optimization were performed with Chem Bio-Office suite Ultra v12.0 (2012) (Cambridge Soft Corp., UK). Docking of all compounds was carried out on the *M. tuberculosis* (PDB ID: 1QSG). The Surflexdoc module in Sybyl was used to construct a 3D model of the structures. To find the possible bioactive conformations of functionalized the spirooxindolo–pyrrolidines and spirooxindole–pyrrolizines, molecular modeling studies were performed using the Sybyl X 2.0 interfaced for the synthesized compounds. Program automatically docks ligand into binding pocket of a target protein by using protomol-based algorithm and empirically produced scoring function. The protomol is very important and necessary factor for docking algorithm and works as a computational representation of proposed ligand that interacts into

binding site. Surflex-Dock's scoring function have several factors that play an important role in the ligand-receptor interaction, in terms of hydrophobic, polar, repulsive, entropic and solvation, and it is a worldwide well-established and recognized method. The most standard docking protocols have ligand flexibility into the docking process, while counts the protein as a rigid structure. Present molecular docking study involves the several steps *viz*., import of protein structure into Surflex and addition of hydrogen atoms; generation of protomol using a ligand-based strategy. During second step, two parameters first called *protomol–bloat*, which determines how far the site should extend from a potential ligand; and another called *protomol–threshold,* which determines deepness of the atomic probes, used to define the protomol penetration into the protein) were specified to form the appropriate binding pocket. Therefore, protomol–bloat and protomol–threshold was set to 0 and 0.50, respectively. In reasonable binding pocket, all the compounds were docked into the binding pocket and 20 possible active docking conformations with different scores were obtained for each compound. During the docking process, all of the other parameters were assigned their default values.

3.1.7 References

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3.1.6 Characterization spectral data (^{1}HNMR and ^{13}CNMR) of selected compounds spiro-pyrrolidine and spiro-pyrrolidines 12a, 13b, 13c, 13e, 14a and 15.

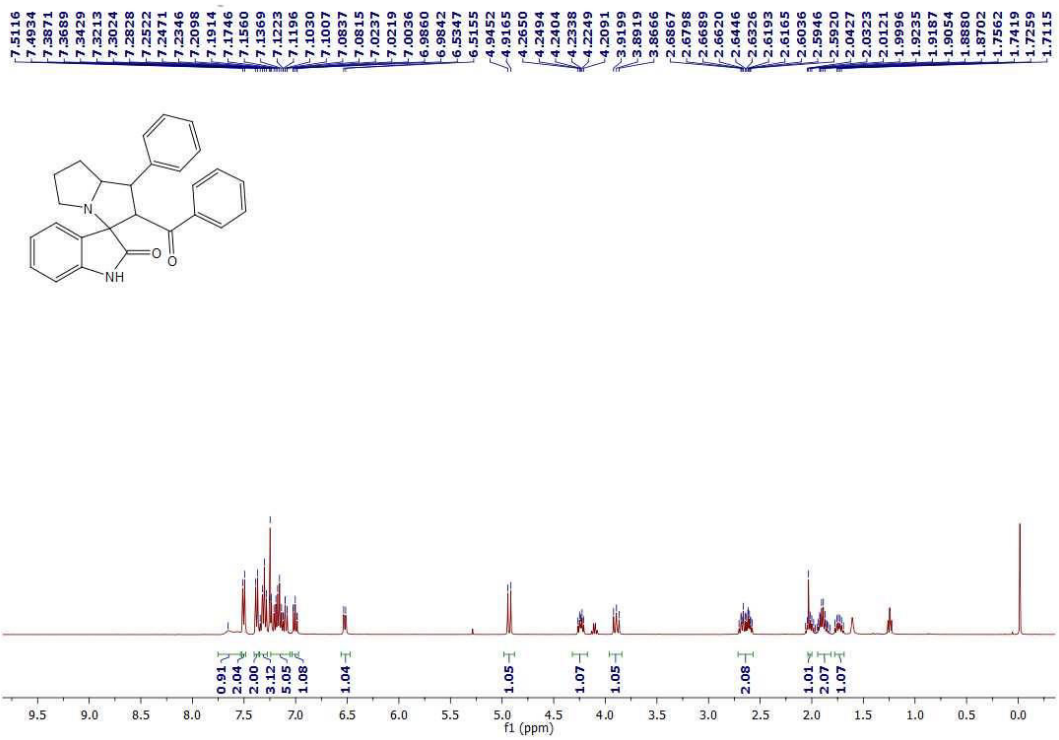


Figure 8: ^{1}H NMR spectra of compound **12a**.

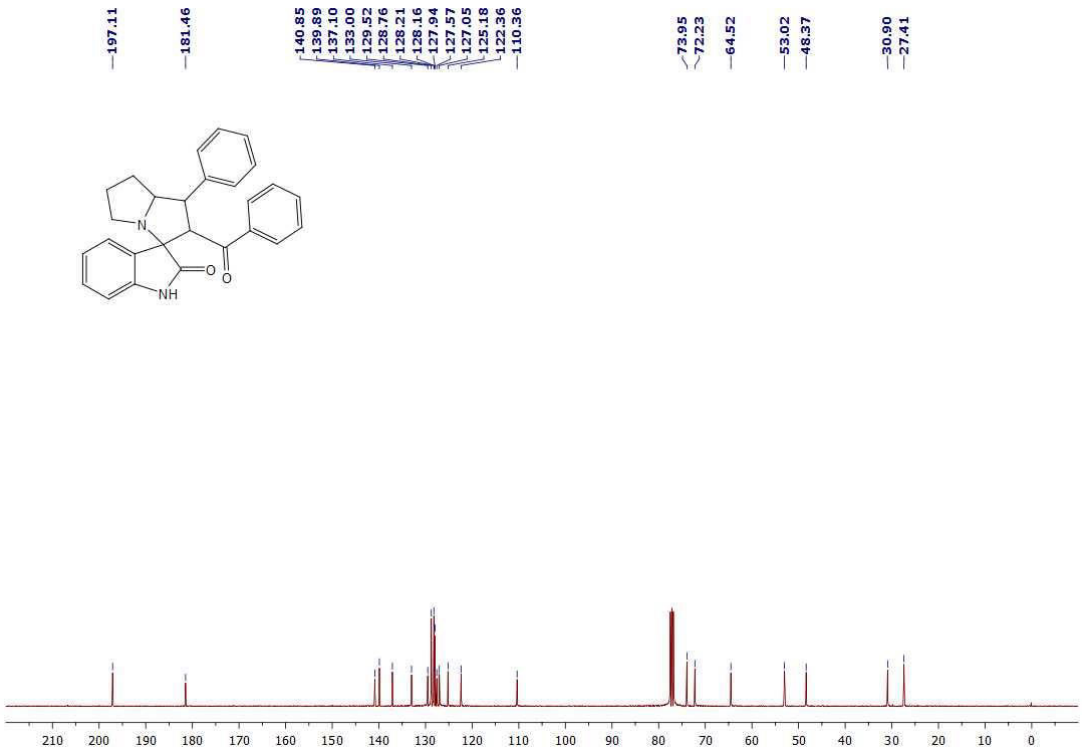


Figure 9: ^{13}C NMR spectra of compound **12a**.

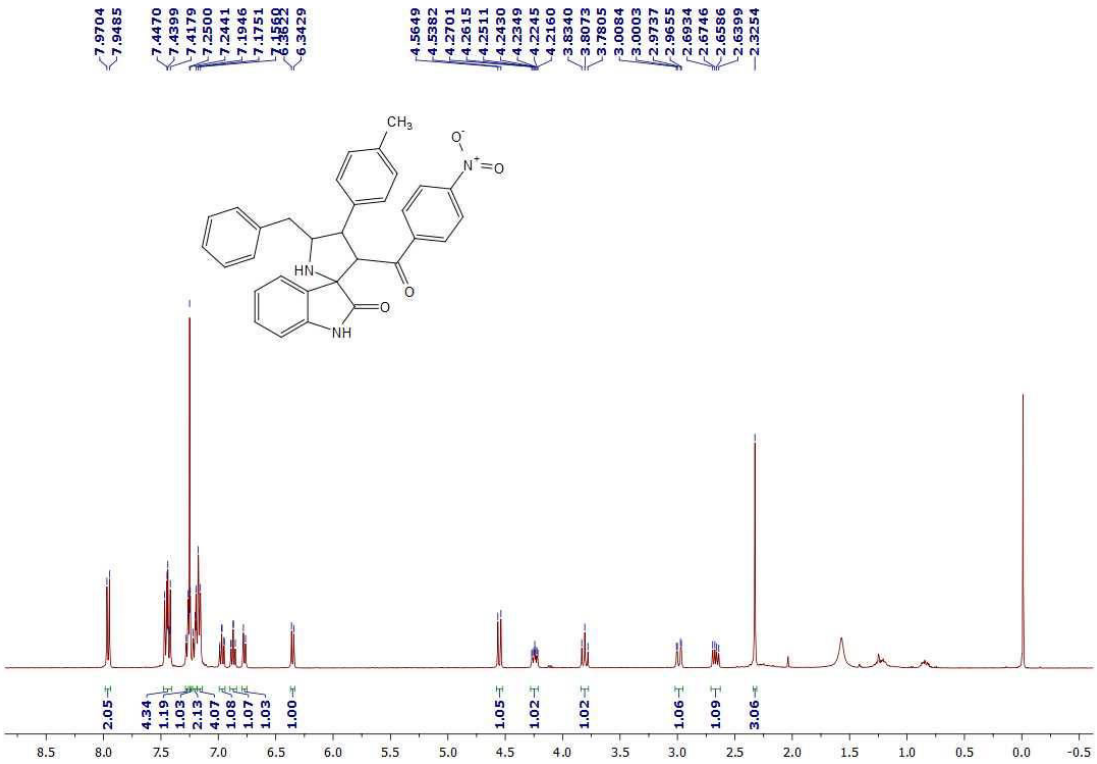


Figure 10: ^{1}H NMR spectra of compound **13b**.

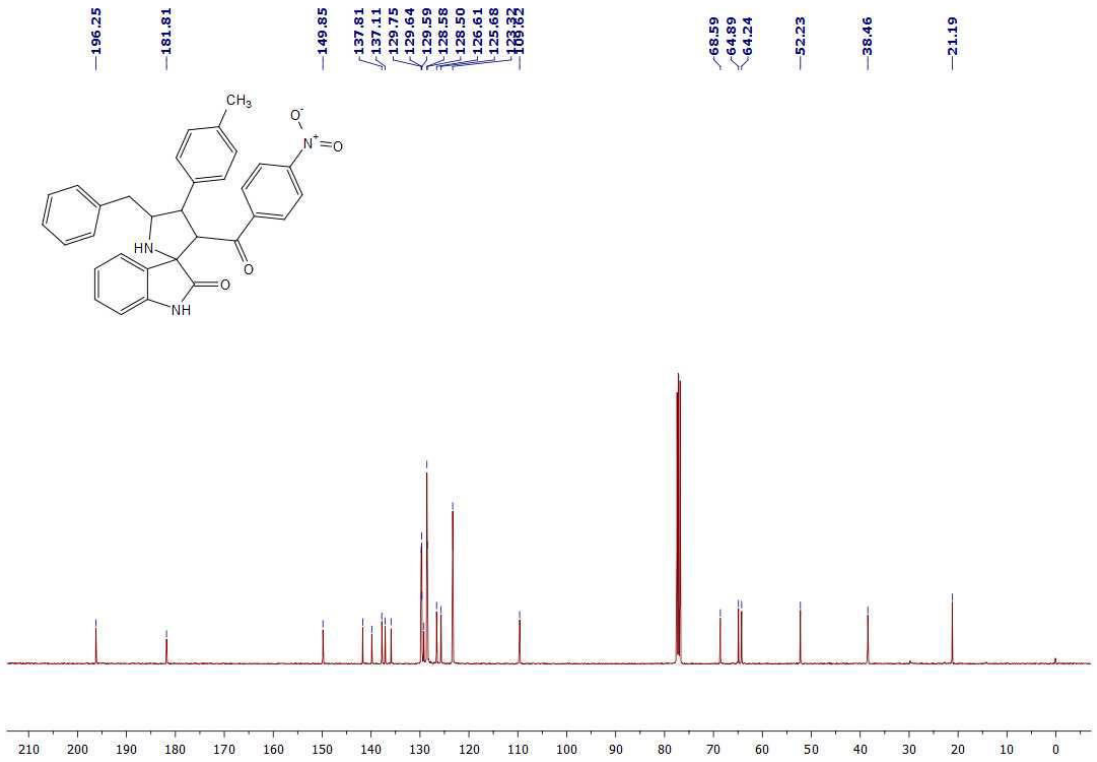


Figure 11: ^{13}C NMR spectra of compound **13b**.

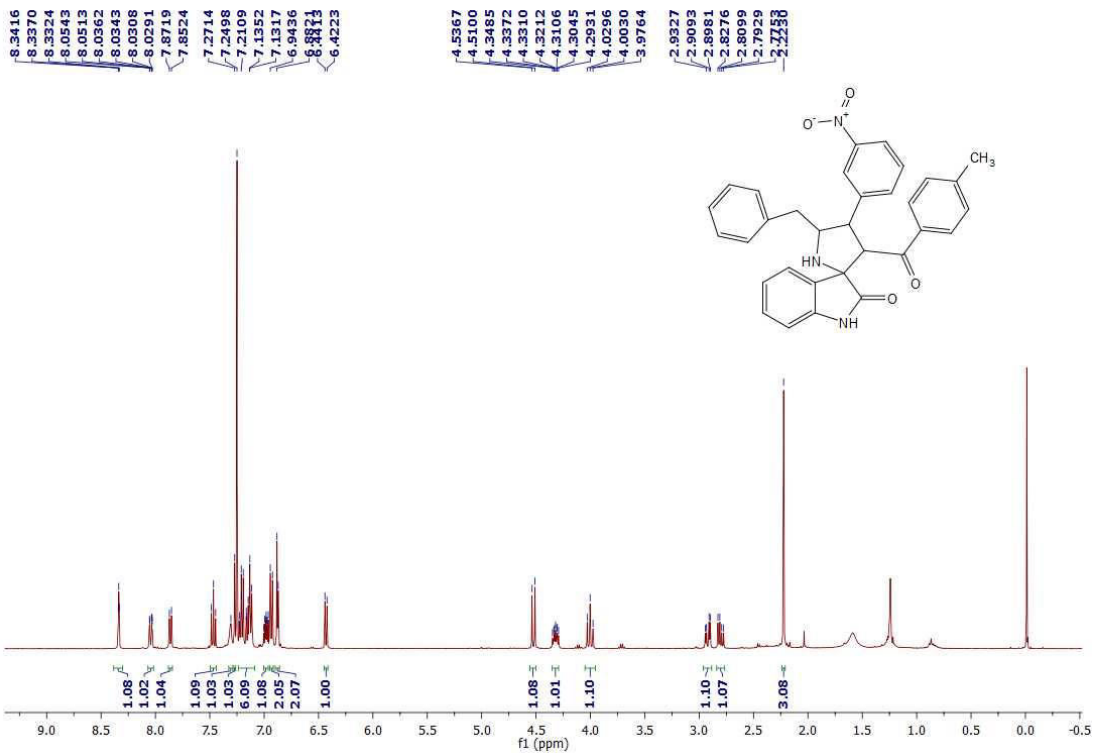


Figure 12: ^{1}H NMR spectra of compound **13c**.

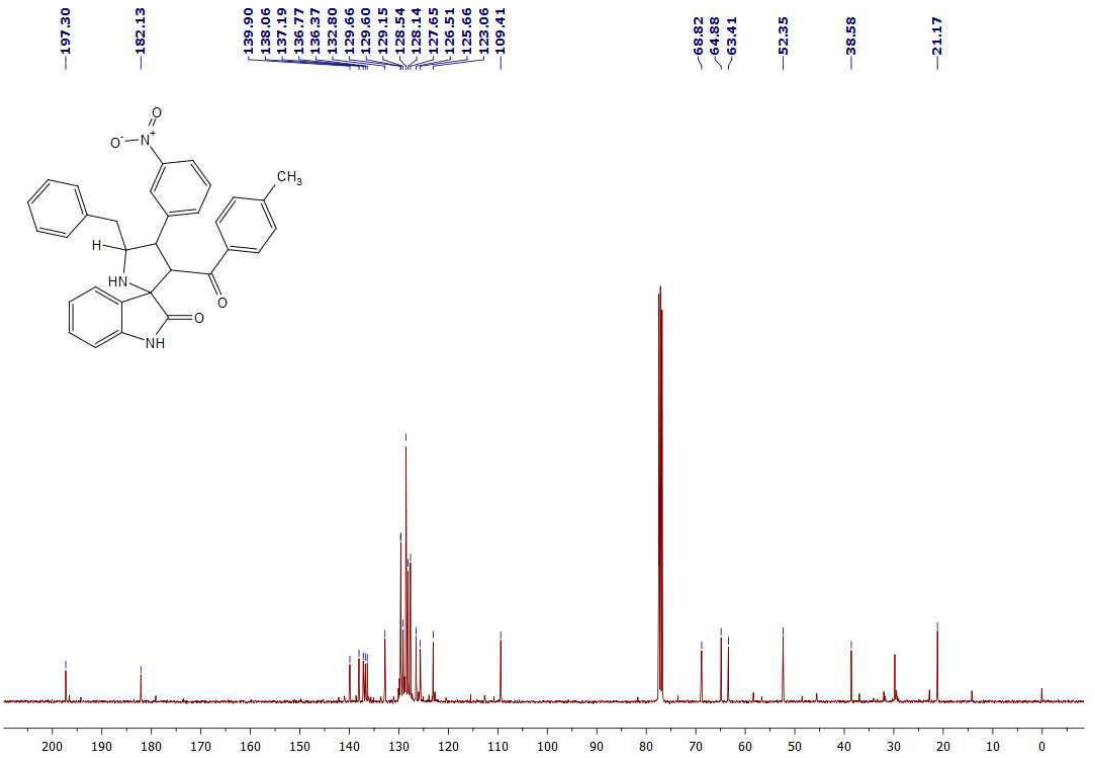


Figure 13: ^{13}C NMR spectra of compound **13c**.

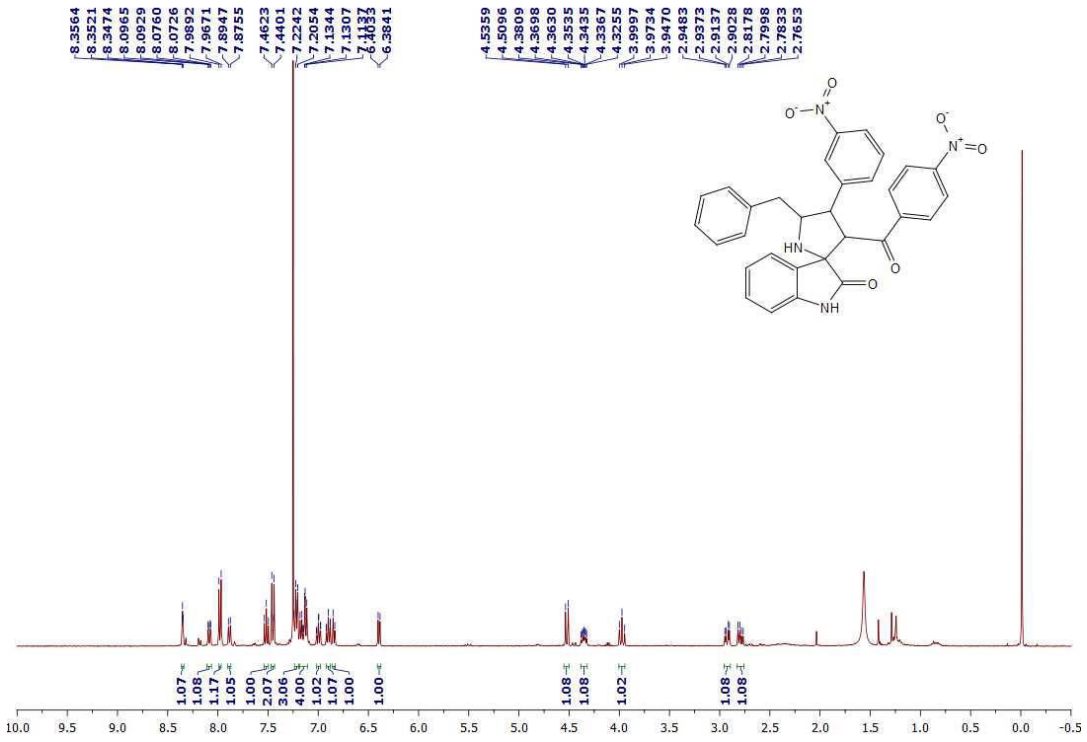


Figure 14: ^{1}H NMR spectra of compound **13e**.

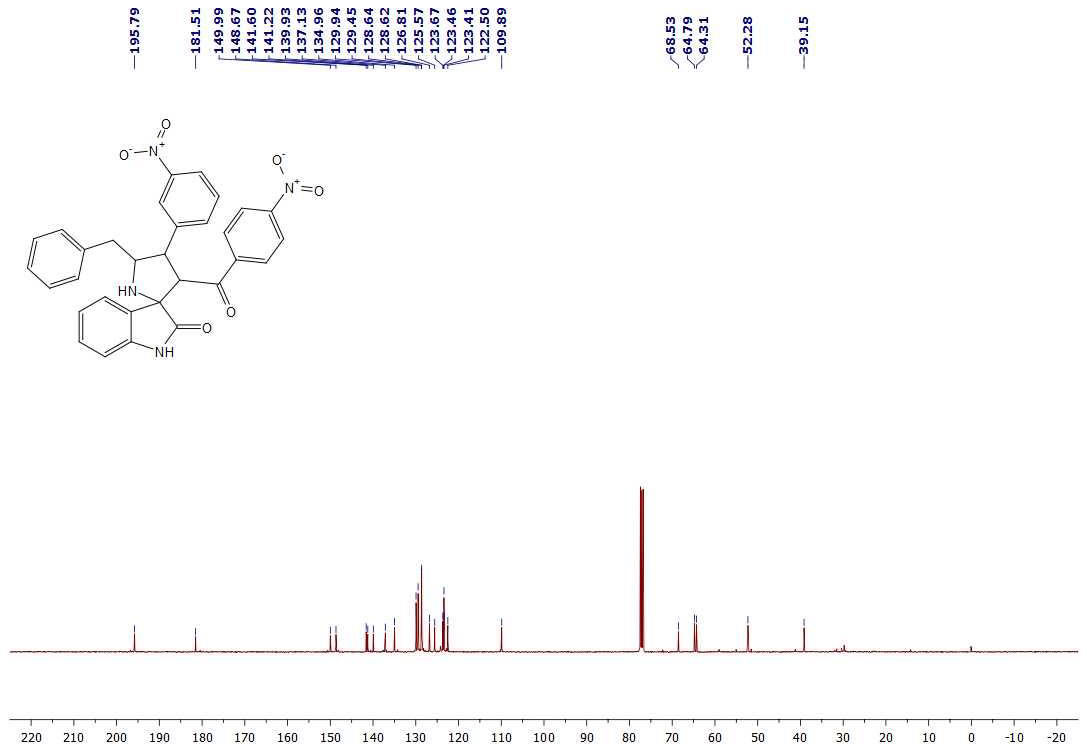


Figure 15: ^{13}C NMR spectra of compound **13e**.

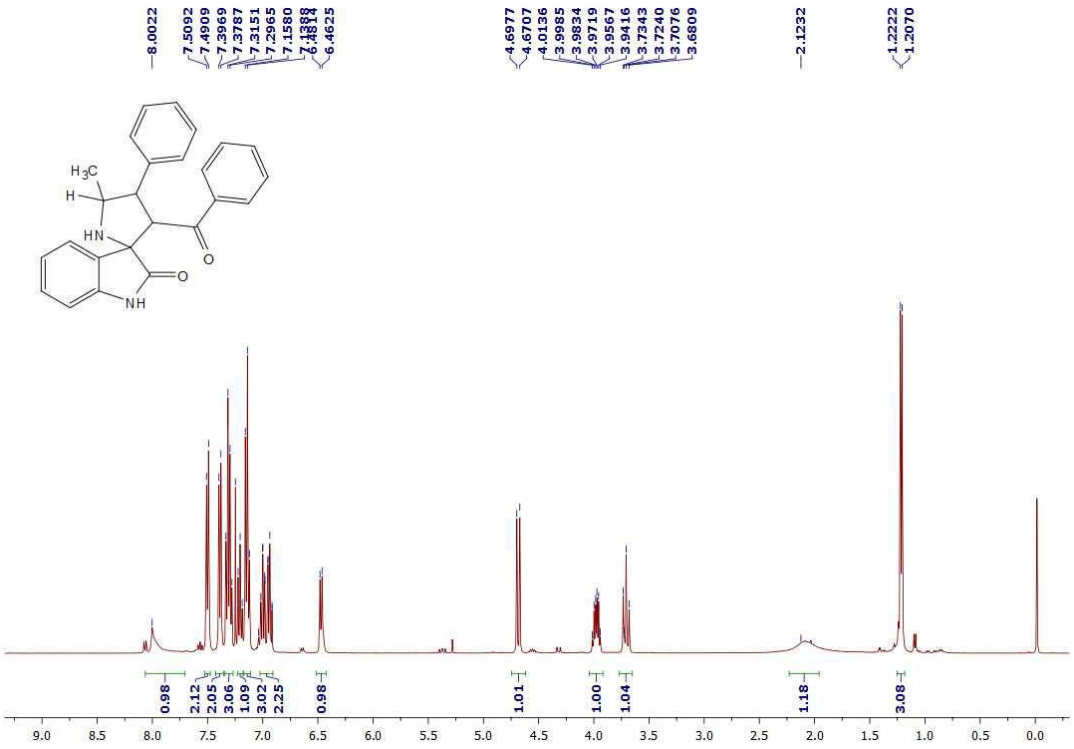


Figure 16: ^{1}H NMR spectra of compound **14a**.

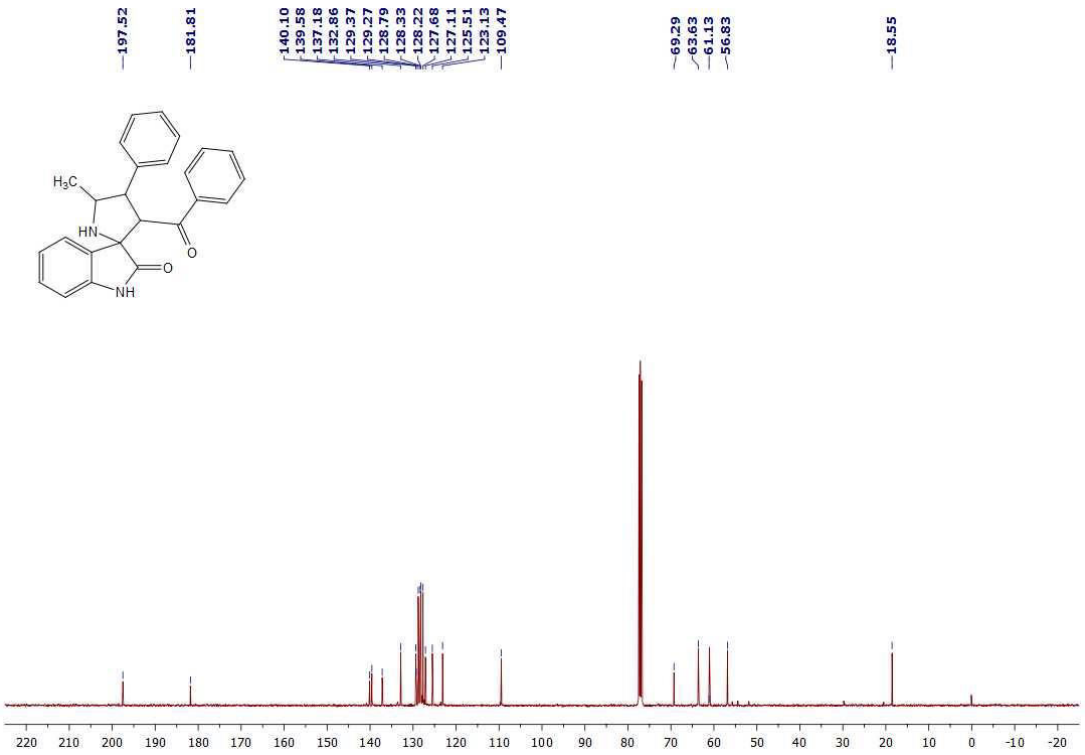


Figure 17: ^{13}C NMR spectra of compound **14a**.

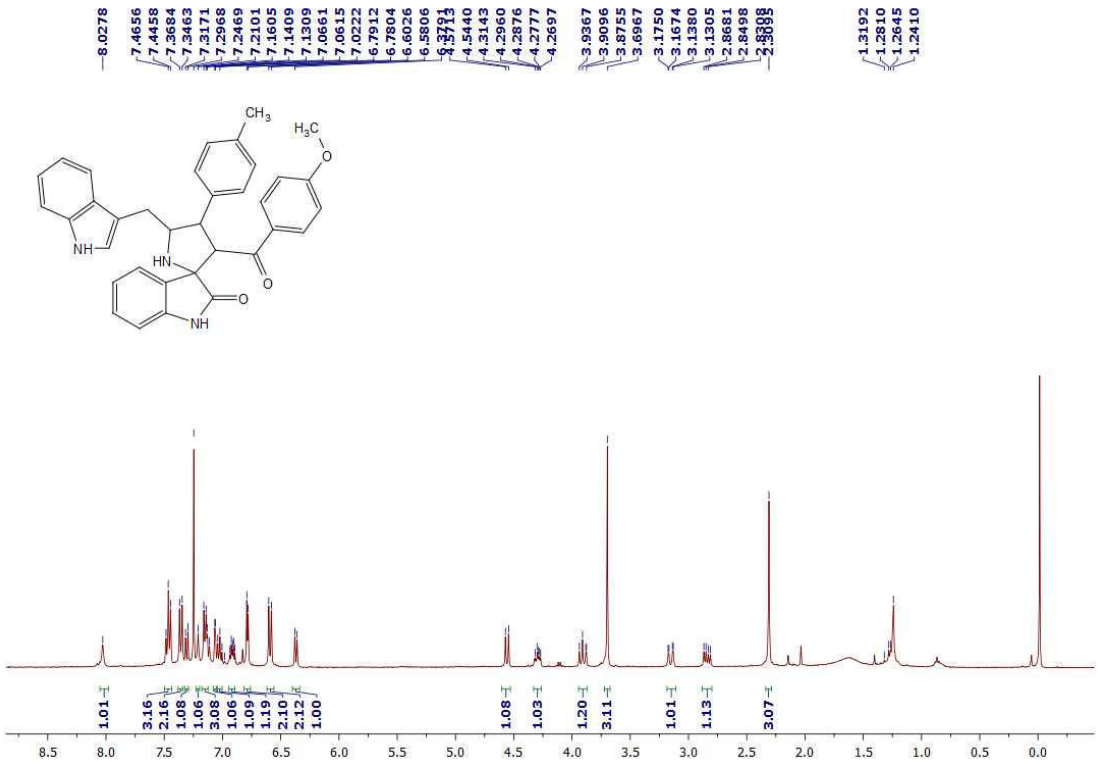


Figure 18: ^{1}H NMR spectra of compound **15**.

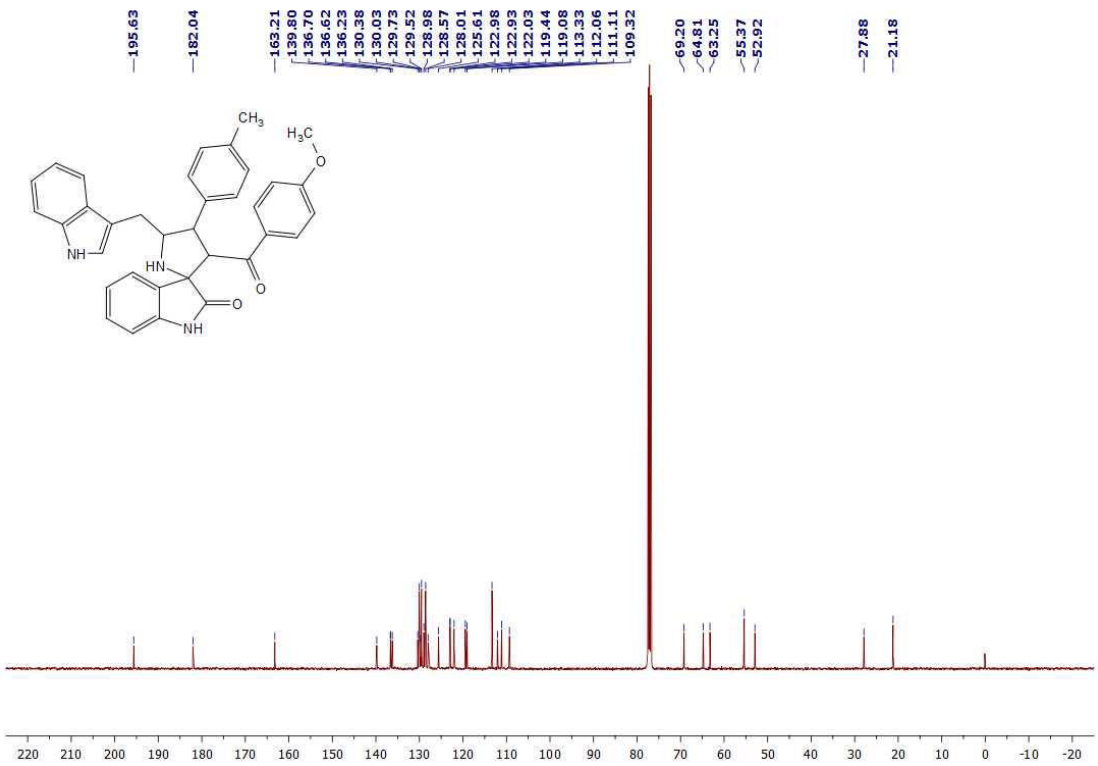


Figure 19: ^{13}C NMR spectra of compound **15**.

Section 3.2: Stereochemical Assignment and Confirmation of Novel Spirooxindoles via Formation of Nano-Star like Supramolecular Assembly: Discovery of C9 and C12 allotropes of Carbon by Single crystal X-ray crystallography studies.

3.2.1 Introduction

Advancement in the field of Supramolecular assembling at the molecular level always encourages the interest of material chemists around the world.[1] Nano-star supramolecular chemical architectures consisting of a spherical allotrope as a guest and large macrocyclic host assisted by the short contacts offers a marvelous functionality for the design and development of supramolecular assemblies.[2] The surrounding environment must have rigidity in a multiple triangular fashion and be able to allow the spherical allotrope inside the space through various non-covalent interactions.[3] Unlike the above fact, various substituted fullerenes, with their spherical shape and availability of π-electrons, push molecular crumbling.[4,5] As appearing like a molecular bowl for C9 and C12 core, short contact derived macrocyclic architecture have been used due to the presence of several interactions between the π-molecular systems.[6,7] Oda and Kawase reported the inclusion of C60 in nano-saturn by tuning the size of cycloparaphenylene-ethylene as a molecular ring.[2a, 4a-b, 5a] Recently, Toyota and co-workers reported the single crystal evidence for the inclusion of C60 as a guest in the cyclic anthracene oligomer (Figure 1).[3b] Earlier, it was noticed that π-π interaction plays a significant role in the stabilization of the allotropic guest with the organic host.[4c,8] On planet earth, the core radius is not considerable relative to the radius of the earth's crust (~6384 km or less).[9] In order to mimic this nature's feature precisely at the molecular level, we require curved molecules so as to pack in view of earth's crust. Giant supramolecular architecture for novel allotropic systems are remained covered till date.[10] Similarly, Kekulene designs of chemical structures are promising candidates for this use, because of the presence of C-H bonds are anisotropically targeted into the supramolecular host.[11] The contact area of the crust type surfaces in which *p*-orbitals are at right angle to the plane with the guest species, this might cause in weak supramolecular interactions.

Recently, the substituted allotropic carbon viz. cubane, Q-carbon and T-carbon were discovered.[12] Nonetheless, no report on the experimental-cum-theoretical calculations of C9 and C12 is available till date. However, to the best of our knowledge, single

crystal experimental evidence of C9 and C12 into such nano-star fashion remained uncovered.

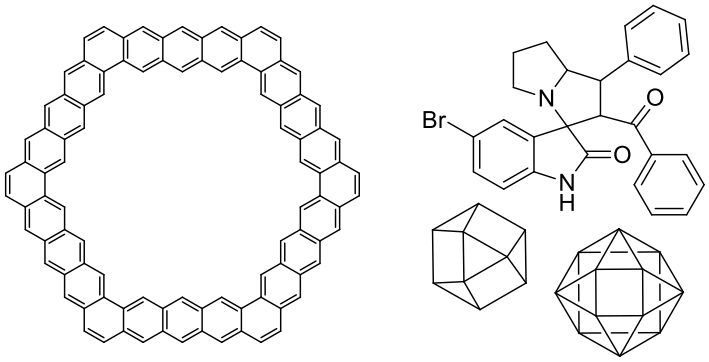


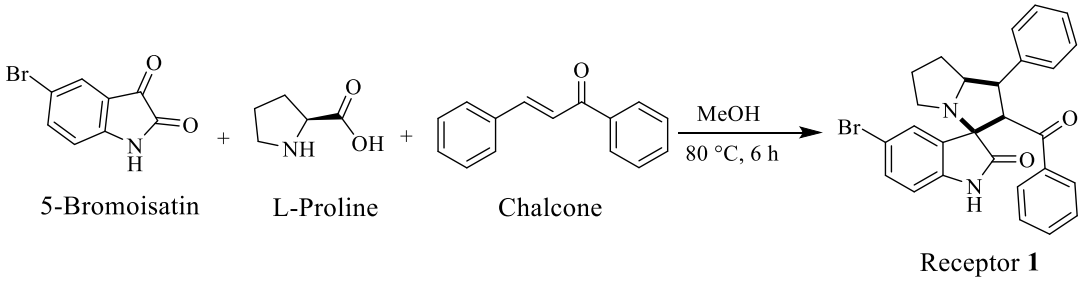
Figure 1. Schematic representation of the anthracyclic rings and receptor **1** withC9 and C12 guest.

Herein, we present the first clear experimental and theoretical evidence of the single crystal X-ray structure of receptor **1** along with the presence of new allotrope C9 and C12 appeared in nano-star fashion assisted by the organic functionality (Figure 1).

3.2.2 Results and discussion

3.2.2.1 Chemistry

Initially, we aimed to designed highly-functionalized indole derivatives using the multi-component reactions. During the synthesis of the receptor **1** with the available method (Scheme 1),[13] we found that the receptor **1** is co-crystallized with the two new carbon forms C9 and C12, respectively. Accidentally, we came up with the single crystal x-ray structure of the receptor **1** in which the C9 and C12 units are clearly shown following the structure and triggered the synthesis of spirooxindole–pyrrolizines in one step using the multi-component reaction (see experimental section). After screening over the series of other derivatives, we found that the specific receptor **1** prepared in one-step and obtained as off-white solid in 71% yield, which was further subjected for the crystallization; we were able to isolate the receptor **1** in ~10-11% yield using slow evaporation crystallization technique with EtOAc/hexane as a solvent system at low temperature.



Scheme 1. Synthesis of the spirooxindole–pyrrolizines receptor **1**.

3.2.2.2 Single Crystal X-ray crystallography studies of Receptor 1.

The receptor **1**@C9@C12 appeared as white block crystals that were soluble in a variety of organic solvents. After obtaining the single crystals of the receptor **1**, the raw data of receptor **1** was subjected for the solution using Olex2[14] and the crystal is crystallized in trigonal system in *R-3* space group (Table 1, 2, 3, 4, 5 and 6).

Table 1. Crystallographic parameters of the receptor **1**.

Identification code	SNDCH-1
CCDC No.	
Empirical formula	$C_{192}H_{138}Br_6N_{12}O_{12}$
Formula weight	*3284.54*
Temperature/K	*293(2)*
Crystal system	*trigonal*
Space group	*R-3*
a/Å	*22.6902(11)*
b/Å	*22.6902(11)*
c/Å	*25.8577(11)*
α/°	*90*
β/°	*90*
γ/°	*120*
Volume/Å^3	*11529.1(12)*
Z	*107*
ρ_{calc}g/cm^3	*1.419*
μ/mm^{-1}	*1.637*
F(000)	*5040.0*
Crystal size/mm^3	*0.5 × 0.5 × 0.5*
Radiation	*MoKα (λ = 0.71073)*
2Θ range for data collection/°	*7.814 to 49.99*
Index ranges	*$-26 \le h \le 26$, $-26 \le k \le 26$, $-30 \le l \le 30$*
Reflections collected	*46600*
Independent reflections	*4512 [R_{int} = 0.0710, R_{sigma} = 0.0424]*
Data/restraints/parameters	*4512/0/334*
Goodness-of-fit on F^2	*1.390*
Final R indexes [I>=2σ (I)]	*R_1 = 0.0927, wR_2 = 0.2075*
Final R indexes [all data]	*R_1 = 0.1210, wR_2 = 0.2201*
Largest diff. peak/hole / e Å^{-3}	*0.47/-0.34*

Table 2. Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for receptor **1**. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
Br01	4990.8(4)	4161.1(4)	6011.4(2)	74.7(4)
O002	4548.2(19)	3272.5(17)	3124.7(12)	46.8(9)

O003	3286.9(19)	3567.0(19)	4530.6(14)	48.5(9)
N004	5175(2)	4132.2(18)	3694.8(15)	32.7(9)
N005	4291.2(19)	2462.1(18)	4074.0(16)	32.9(9)
C006	5199(2)	4220(2)	4241.6(18)	28.7(10)
C007	3202(2)	2252(2)	4361.7(18)	32.5(11)
C008	4679(2)	3642(2)	4470.9(17)	29.3(10)
C009	3524(2)	2865(2)	3995.3(17)	30.4(11)
C00A	4284(2)	3110(2)	4052.6(16)	27.6(10)
C00B	4679(2)	3501(2)	3559.8(18)	31.6(11)
C00C	4603(3)	3621(3)	5000.2(19)	36.3(12)
C00D	3309(2)	3388(2)	4091(2)	33.3(11)
C00E	5584(3)	4753(3)	5048(2)	45.2(14)
C00F	5065(3)	4178(3)	5282(2)	40.9(13)
C00G	2437(2)	1829(2)	4339.7(19)	35.9(12)
C00H	5661(3)	4773(3)	4520(2)	39.9(12)
C00I	3604(2)	1899(2)	4225(2)	39.5(12)
C00J	4799(3)	2417(3)	4405(2)	43.6(13)
C00K	2046(3)	1962(3)	4680(2)	44.4(13)
C00L	2099(3)	1302(3)	3990(2)	55.2(16)
C00M	1351(3)	1599(3)	4669(3)	60.4(18)
C00N	3103(3)	3660(3)	3651(2)	51.7(15)
C00O	4424(3)	2000(3)	4868(2)	53.5(15)
C00P	3718(3)	1505(3)	4644(3)	64.9(18)
C00Q	1015(3)	1083(4)	4322(3)	66.3(19)
C00R	1395(4)	936(4)	3981(3)	74(2)
C00S	3234(4)	3579(4)	3136(3)	73(2)
C00T	2768(6)	4012(5)	3751(4)	109(3)
C00U	3015(6)	3833(5)	2735(3)	106(3)
C00V	2696(7)	4182(6)	2852(5)	135(4)
C00W	2541(9)	4250(7)	3342(6)	164(6)
C5	5986(19)	3220(20)	5720(20)	303(17)
C6	6086(16)	2608(12)	5705(16)	283(14)
C2	6401(8)	3479(10)	3800(7)	177(6)
C4	6041(10)	2690(13)	3284(11)	226(8)
C7	6219(15)	3100(20)	6132(13)	307(11)

Table 3. Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for receptor **1**. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	**U_{11}**	**U_{22}**	**U_{33}**	**U_{23}**	**U_{13}**	**U_{12}**
Br01	83.9(6)	98.6(6)	32.6(4)	-11.9(3)	-2.3(3)	38.7(4)
O002	56(2)	40(2)	26.2(19)	-2.5(16)	10.4(16)	10.7(18)

O003	57(2)	54(2)	44(2)	-15.9(18)	1.3(18)	34(2)
N004	36(2)	22(2)	30(2)	4.9(16)	10.0(17)	6.9(19)
N005	29(2)	23(2)	44(2)	3.1(17)	4.9(18)	11.0(17)
C006	27(2)	27(2)	32(2)	-3(2)	5(2)	13(2)
C007	33(3)	27(2)	29(2)	-1(2)	0(2)	9(2)
C008	33(3)	26(2)	29(2)	-2.0(19)	4(2)	15(2)
C009	34(3)	23(2)	27(2)	-0.1(19)	4(2)	9(2)
C00A	34(3)	21(2)	23(2)	-2.7(18)	4.3(19)	10(2)
C00B	34(3)	35(3)	28(3)	0(2)	5(2)	19(2)
C00C	35(3)	35(3)	34(3)	0(2)	1(2)	14(2)
C00D	32(3)	23(2)	42(3)	-1(2)	4(2)	11(2)
C00E	36(3)	47(3)	47(3)	-22(3)	-7(2)	16(3)
C00F	44(3)	52(3)	30(3)	-8(2)	1(2)	27(3)
C00G	35(3)	34(3)	33(3)	15(2)	5(2)	13(2)
C00H	34(3)	31(3)	46(3)	-5(2)	7(2)	10(2)
C00I	32(3)	28(3)	51(3)	4(2)	1(2)	10(2)
C00J	43(3)	36(3)	59(3)	-2(3)	-1(3)	25(3)
C00K	42(3)	42(3)	50(3)	5(3)	2(3)	22(3)
C00L	44(3)	57(4)	42(3)	-5(3)	1(3)	8(3)
C00M	41(4)	62(4)	86(5)	25(4)	18(3)	32(3)
C00N	69(4)	44(3)	52(4)	5(3)	-2(3)	36(3)
C00O	64(4)	60(4)	49(3)	19(3)	5(3)	40(3)
C00P	55(4)	49(4)	93(5)	38(3)	24(3)	27(3)
C00Q	34(3)	70(5)	89(5)	25(4)	-5(4)	21(3)
C00R	56(4)	64(4)	67(4)	-1(3)	-20(4)	3(4)

C00S	101(6)	80(5)	55(4)	17(4)	3(4)	58(5)
C00T	166(9)	138(8)	88(6)	-5(6)	-7(6)	125(8)
C00U	159(9)	117(7)	72(5)	26(5)	-8(5)	91(7)
C00V	201(12)	155(10)	116(9)	19(8)	-32(9)	138(10)
C00W	277(17)	224(14)	126(10)	-8(9)	-43(10)	227(14)
C5	230(30)	190(30)	500(50)	0(30)	-120(30)	110(30)
C6	200(30)	180(20)	480(40)	-90(30)	-30(20)	98(19)
C2	124(14)	148(15)	212(13)	6(13)	57(11)	33(8)
C4	127(15)	240(20)	295(19)	24(17)	-11(14)	82(10)
C7	280(20)	300(40)	360(30)	100(30)	105(19)	160(30)

Table 4. Bond Lengths for receptor **1.**

Atom	**Atom**	**Length/Å**	**Atom**	**Atom**	**Length/Å**
Br01	C00F	1.893(5)	C00L	C00R	1.384(9)
O002	C00B	1.212(5)	C00M	C00Q	1.366(10)
O003	C00D	1.216(6)	C00N	C00S	1.398(9)
N004	C006	1.425(6)	C00N	C00T	1.375(10)
N004	C00B	1.351(6)	C00O	C00P	1.537(10)
N005	C00A	1.480(6)	C00Q	C00R	1.384(10)
N005	C00I	1.492(6)	C00S	C00U	1.393(10)
N005	C00J	1.480(6)	C00T	C00W	1.397(15)
C006	C008	1.385(6)	C00U	C00V	1.347(14)
C006	C00H	1.369(7)	C00V	C00W	1.345(17)
C007	C009	1.532(6)	C5	$C6^{1}$	1.42(3)
C007	C00G	1.506(7)	C5	C6	1.53(3)
C007	C00I	1.528(7)	C5	C7	1.29(3)

C008	C00A	1.531(6)	C6	C5[2]	1.42(3)
C008	C00C	1.377(6)	C6	C7	1.48(4)
C009	C00A	1.532(7)	C6	C7[2]	1.96(5)
C009	C00D	1.511(7)	C2	C2[1]	1.42(3)
C00A	C00B	1.555(6)	C2	C2[2]	1.42(3)
C00C	C00F	1.379(7)	C2	C4[3]	1.31(3)
C00D	C00N	1.478(8)	C2	C4[1]	1.63(3)
C00E	C00F	1.385(8)	C4	C2[2]	1.63(3)
C00E	C00H	1.376(7)	C4	C2[4]	1.31(3)
C00G	C00K	1.385(7)	C4	C4[3]	1.462(18)
C00G	C00L	1.387(8)	C4	C4[4]	1.462(18)
C00I	C00P	1.507(8)	C7	C6[1]	1.96(5)
C00J	C00O	1.499(8)	C7	C7[2]	1.52(5)
C00K	C00M	1.367(8)	C7	C7[1]	1.52(5)

[1]1+Y-X, 1-X, +Z; [2]1-Y, +X-Y, +Z; [3]1/3+Y, 2/3-X+Y, 2/3-Z; [4]1/3-Y+X, -1/3+X, 2/3-Z

Table 5. Bond Angles for receptor**1.**

Atom	**Atom**	**Atom**	**Angle/°**	**Atom**	**Atom**	**Atom**	**Angle/°**
C00B	N004	C006	111.0(4)	C00S	C00N	C00D	123.1(5)
C00A	N005	C00I	109.6(3)	C00T	C00N	C00D	118.8(6)
C00J	N005	C00A	119.1(4)	C00T	C00N	C00S	118.1(6)
C00J	N005	C00I	107.8(4)	C00J	C00O	C00P	102.4(5)
C008	C006	N004	109.7(4)	C00I	C00P	C00O	105.2(4)
C00H	C006	N004	127.4(4)	C00M	C00Q	C00R	118.5(6)
C00H	C006	C008	122.9(4)	C00L	C00R	C00Q	121.1(6)
C00G	C007	C009	115.5(4)	C00U	C00S	C00N	120.7(7)

C00I	C007	C009	101.2(4)	C00N	C00T	C00W	120.0(9)
C00I	C007	C00G	117.2(4)	C00V	C00U	C00S	119.1(9)
C006	C008	C00A	109.2(4)	C00W	C00V	C00U	121.7(9)
C00C	C008	C006	119.4(4)	C00V	C00W	C00T	120.1(10)
C00C	C008	C00A	131.4(4)	C6	C5	$C6^{1}$	125(3)
C007	C009	C00A	102.2(4)	C7	C5	C6	63(2)
C00D	C009	C007	114.5(4)	C7	C5	$C6^{1}$	93(3)
C00D	C009	C00A	116.4(4)	C5	C6	$C5^{2}$	115(3)
N005	C00A	C008	117.6(4)	C5	C6	$C7^{2}$	88(2)
N005	C00A	C009	102.2(3)	$C5^{2}$	C6	$C7^{2}$	40.8(18)
N005	C00A	C00B	107.9(3)	C7	C6	C5	50.5(19)
C008	C00A	C009	117.6(4)	C7	C6	$C5^{2}$	90(3)
C008	C00A	C00B	100.7(3)	C7	C6	$C7^{2}$	50(3)
C009	C00A	C00B	110.6(4)	$C2^{2}$	C2	$C2^{1}$	59.997(6)
O002	C00B	N004	126.1(4)	$C4^{3}$	C2	$C2^{2}$	98(2)
O002	C00B	C00A	124.6(4)	$C4^{1}$	C2	$C2^{1}$	83.9(15)
N004	C00B	C00A	109.2(4)	$C4^{1}$	C2	$C2^{2}$	116.0(10)
C00F	C00C	C008	117.9(5)	$C4^{3}$	C2	$C2^{1}$	123.1(11)
O003	C00D	C009	120.0(4)	$C4^{3}$	C2	$C4^{1}$	58.2(10)
O003	C00D	C00N	120.2(4)	$C2^{4}$	C4	$C2^{2}$	119.5(19)
C00N	C00D	C009	119.7(4)	$C2^{4}$	C4	$C4^{3}$	72.0(19)
C00H	C00E	C00F	119.9(5)	$C2^{4}$	C4	$C4^{4}$	95(2)
C00C	C00F	Br01	119.2(4)	$C4^{4}$	C4	$C2^{2}$	49.8(15)
C00C	C00F	C00E	122.2(5)	$C4^{3}$	C4	$C2^{2}$	83(2)
C00E	C00F	Br01	118.6(4)	$C4^{3}$	C4	$C4^{4}$	117.0(13)

C00K	C00G	C007	119.7(5)	C5	C7	C6	66(2)
C00K	C00G	C00L	117.7(5)	C5	C7	C6[1]	46(2)
C00L	C00G	C007	122.6(5)	C5	C7	C7[1]	94(3)
C006	C00H	C00E	117.7(5)	C5	C7	C7[2]	121(3)
N005	C00I	C007	104.7(4)	C6	C7	C6[1]	98(3)
N005	C00I	C00P	106.3(4)	C6	C7	C7[2]	81(3)
C007	C00I	C00P	118.1(5)	C6	C7	C7[1]	119.4(17)
N005	C00J	C00O	106.8(4)	C7[1]	C7	C6[1]	48(2)
C00M	C00K	C00G	121.8(6)	C7[2]	C7	C6[1]	95.5(19)
C00R	C00L	C00G	120.2(6)	C7[2]	C7	C7[1]	60.002(14)
C00K	C00M	C00Q	120.7(6)				

[1]1+Y-X, 1-X, +Z; [2]1-Y, +X-Y, +Z; [3]1/3+Y, 2/3-X+Y, 2/3-Z; [4]1/3-Y+X, -1/3+X, 2/3-Z

Table 6. Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for receptor **1.**

Atom	***x***	***y***	***z***	**U(eq.)**
H004	5441	4439.61	3479.51	39
H007	3326.13	2426.7	4715.25	39
H009	3389.63	2693.76	3641.82	37
H00C	4252.31	3242.08	5162.88	44
H00E	5880.91	5124.64	5248.49	54
H00H	6015.06	5148.8	4356.76	48
H00I	3391.61	1600.36	3926.51	47
H00A	5149.97	2868.04	4511.19	52
H00B	5011.95	2201.89	4218.66	52
H00K	2262.72	2306.6	4923.52	53
H00L	2345.68	1193.89	3759.65	66
H00M	1103.36	1704.44	4900.32	73
H00D	4394.42	2281.45	5137.89	64

H00F	4640.68	1758.31	5006.45	64
H00G	3709.47	1104.91	4501.96	78
H00J	3370.7	1362.26	4908.96	78
H00Q	542.12	836.11	4313.71	80
H00R	1173.36	584.69	3743.43	89
H00S	3470.94	3353.01	3059.3	87
H00T	2693.07	4092.89	4090.74	131
H00U	3087.54	3762.82	2392.49	128
H00V	2580.17	4381.84	2586.07	162
H00W	2281.61	4454.38	3408.95	196

3.2.2.3 Mass spectral studies of Receptor 1.

The MS of **1** clearly revealed couple of molecular ion peaks at different *m/z* values are 113.9674, 144.9870, 489.0995 and 634.4462 which corresponds to C9, C12, Receptor **1** and Receptor **1**@C12, respectively (see experimental section, Figure 5-8).

The co-crystallized receptor **1**@C9@C12 gave additional information in ^{1}H and ^{13}C spectra, for example, the signal was observed as a singlet at δ~8.53 and the peak at δ~8.29 ppm may be assigned due to the supramolecular weak interactions.[3b] In ^{13}C spectra, two new peaks were observed at δ~110 ppm and δ~122 ppm which may corresponds to the allotropic carbon units, which further attributes to the presence of novel C9 and C12 functionality.[15] The receptor **1** and **1**@C9@C12 are totally different from each other in their chemical properties (see section 3.2.6, Figure 9-16).

3.2.2.4 Stereochemical assignment and structural confirmation of novel spirooxindoles: Formation of Nano-Star like Supramolecular Assembly

The single crystal x-ray structure of the receptor **1**@C9@C12 is shown in the figure 2a. The crystallized receptor **1**@C9@C12 has a hexagonal framework consisting six molecules in a circular fashion around the C9 and C12 functionality that takes non-planar circular conformations with the presence of short contacts in the alternate configuration. The outlying region is encircled by six molecules in approximately bisecting conformation due to the bending of the pyrrolizine units relative to the intermolecular short contacts. The molecular arrangement of the receptor **1** and its inside substituents results in a large cavity and its size is directly proportional from the distance between the carbon atoms at the opposite sides and the vanderwaals

radius of the carbon atom present in the ring. This size nearly equals to the vanderwaals radius of C9 and C12 functionality. It is clearly evident from the crystal structure that the stoichiometry of the receptor **1** with respect to C9 and C12 is found to be 1:1:1. This supramolecular macrocyclic host **1** with C9 and C12 was further confirmed by the NMR spectra (Figure 2b).

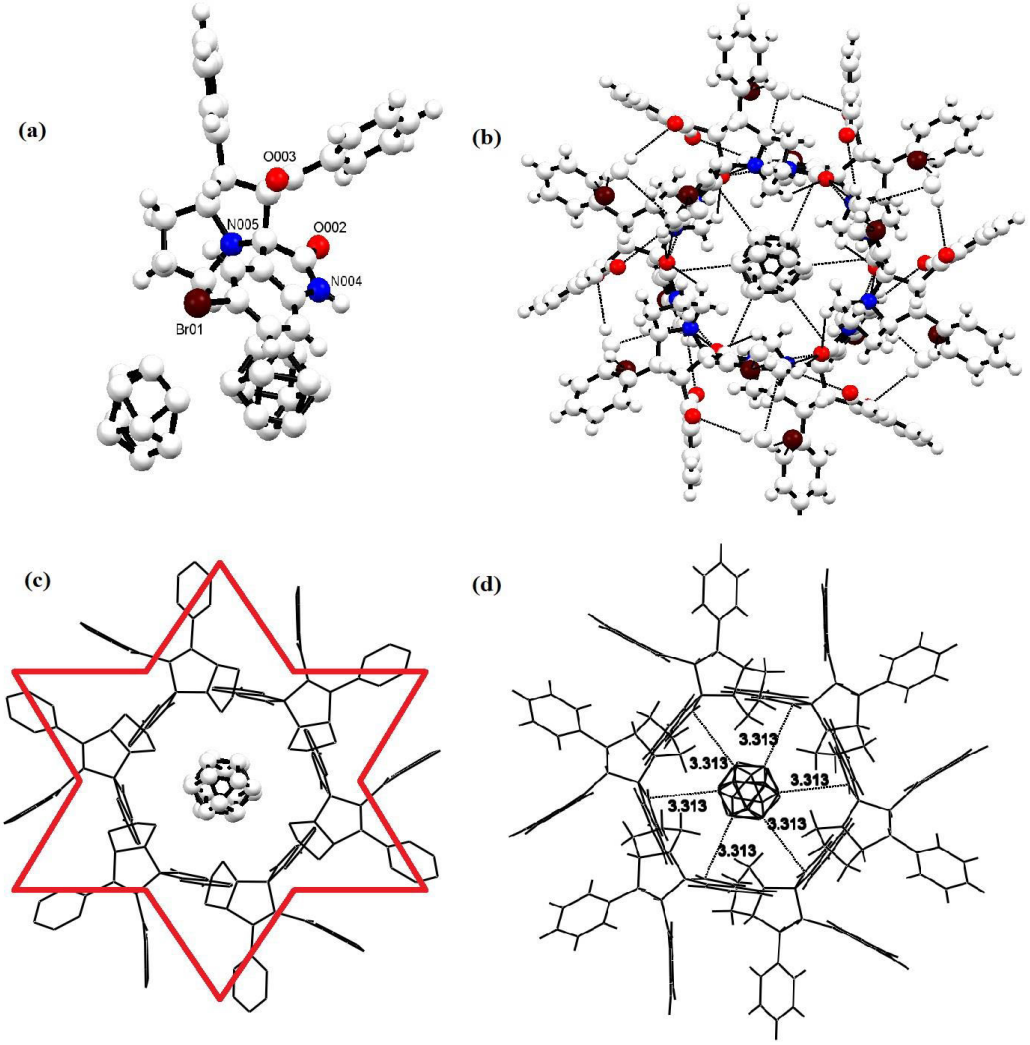


Figure 2. (a) ORTEP view of the receptor **1**. (b) Mesh structural representation of the receptor **1** assisted by C9 and C12 [Ball and Stick Model] (c) Star shaped crystal packing diagram of the receptor **1** with C9 and C12 obtained by Mercury 2.0 (d) Intramolecular short contacts present in the packing diagram of the receptor **1** [Wireframe Model].

From the mother liquor of the solution of receptor **1** in the EtOAc/hexane solvent by using the slow evaporation method, the single crystals of the receptor **1** was obtained, the structure of the receptor **1** was further confirmed by the single crystal x-ray analysis using the Oxford Diffaractometer (Figure 2). The single crystal raw data was subjected to the solution using Olex2[14] and ShelxT[16] with intrinsic phasing approach to finalize the structure of the receptor **1**. Initially, the solution revealed the presence

of C9 and C12 along the substituted indole moiety, in the crystal packing the molecule is shaped out to be as a nano-star and found that C9 and C12 is included at the center of the cavity of a macrocyclic host molecule forming a concerning nano-star like structure (Figure 2c). Although, the arrangement in the supramolecular assembly is like a ball structure, the cavity size of the ring is of the host molecule is not much affected by the inclusion. The distance between the intra-annular C atoms and the C9 and C12 atoms, are quiet comparable to the sum of the vanderwaals radius of C(sp^2) and C(sp^3), supporting the presence of short intermolecular contacts (Figure 2d).

3.2.2.5 Hirshfeld Surface Analysis and structural optimization of Receptor 1

In order to understand more about the intermolecular interactions of receptor **1**@C9@C12, Hirshfeld surface analysis using Crystal Explorer 3.1 software suite was used.[17] The 3D representation of short intermolecular contact can be provided by d_e and d_i mapped on Hirshfeld surface which corresponds to exterior and interior distances, respectively. The d_{norm}, shape index and curvedness of receptor **1** which roughly indicates the presence of strong intermolecular short contacts and stronger Vander Waals interactions (see experimental section). The 2D fingerplot of receptor **1** reveal significant interactions corresponding to C-C, H-C, H-H contributes 4.5%, 8.9%, 39.0% of the total hirshfeld surface, which again attributes to the presence of strong intermolecular interactions which is more prominent than that of π-π interactions (Figure 3).

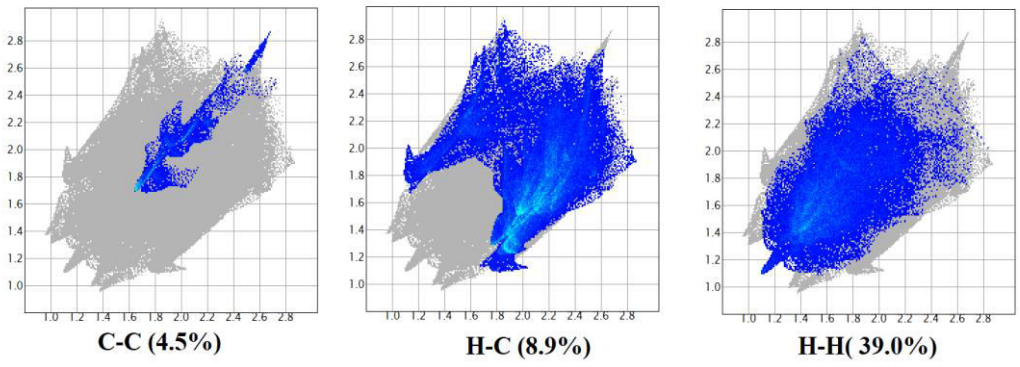


Figure 3. The 2D Finger plot of C-C, H-C, H-H of the receptor **1**.

In order to further understand the incorporation of C9 and C12 organic functionality and its complex formation, DFT calculations were performed by using R3BLYP methods at the 6-31G (d) level using Gaussian 09 Rev. software suite.[18] The optimization of the receptor **1** afforded the same geometry observed in the crystal

structure of approximately S6 symmetry at the global minimum value with an energy of -4670.518 a.u. and the dipole moment of 2.87 D. The **1**@C9@C12 complex is more stable than the independent guest and host molecules by the dissociation energy. Therefore, the presence of short contacts should be more significant in the attractive interactions. It is well established that intermolecular short contact having an energy of about 9-10 kJ/mol, for edge to face interaction in a T-shaped dimer of benzene molecule.[19] Further, these computational results suggested that the presence of multiple short contacts cooperatively encourage the formation and inclusion of C9 and C12 in the cavity of the receptor **1**, even though, it is always compromising the reproducibility of the computational[20] results of such supramolecular interactions by theoretical approaches due to several parameters such as basis set superposition error, solvation energy should be strictly approached.[21,22] In the Kohn-Sham orbital plots, the HOMO and LUMO are distribute over **1**@C9@C12, respectively in the figure 4.

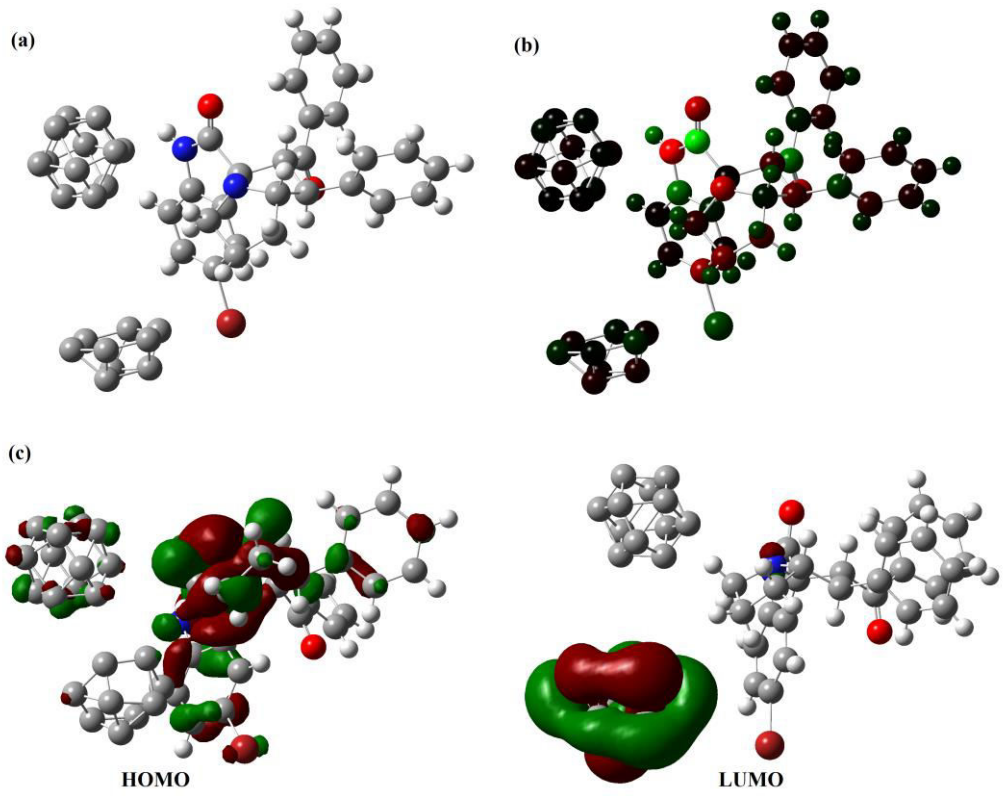


Figure 4. (a) Optimized structure of the receptor **1**@C9@C12 at RB3LYP/6-31(G) level. (b) Mulliken Charges representation separated by colors (c)Frontier HOMO and LUMO of the receptor 1@C9@C12 calculated by using R3BLY/6-31*G(d) level.

The mulliken charge on the C9, C12 and **1**@C9@C12 is quiet small at 0.66, 0.01-0.03 and 0.02-0.05, respectively. Further, this data revealed the presence of dispersion forces because of the intermolecular short contacts rather than induced and

electrostatic interactions should be more significant in the assembling of this supramolecular system.[23]

3.2.3 Conclusion

In summary, an indole functionalized substituted derivative was synthesized by the multi-component reaction which unambiguously came with the two different new allotropes of the carbon C9 or C12 from the mother liquor or in the solid state as determined by the single crystal X-ray crystallographic analysis and NMR, MS spectroscopy. Further, these results pave the way to open up the avenue for the theoretical prediction of the presence of two different allotrope C9 and C12 in nano-star fashion. The preferred complex formation is attributed to shape and size effect or to the presence of several intermolecular short contacts. The single crystal evidence of C9 and C12 allotrope again knocked the door of valence of carbon atom, the C9 allotrope has formed three and four bond among itself directly evidenced by X-ray methods. The present supramolecular system will act as a smart experimental model to elucidate the complex formation of allotrope with the different organic functionalities. With the bird's view on the development of functional materials, this supramolecular assembling with different guest allotrope will may leads to the creation of several nano-star in the galaxy of chemistry.[24]

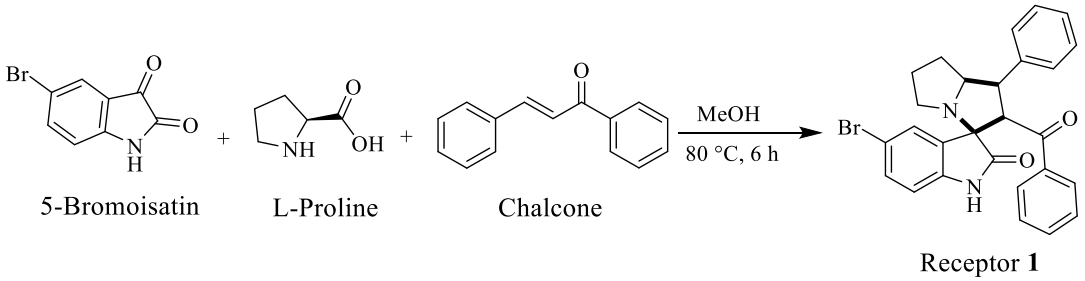
3.2.4 Experimental Section

3.2.4.1 General

All glass apparatus were used after oven drying. High quality reagents were purchased from commercial sources like Sigma Aldrich (USA), TCI Chemicals (Tokyo, Japan), Spectrochem (India) and were used without further purification. Laboratory grade commercial reagents and solvents were purified by standard procedures prior to use. Infrared spectra were recorded on a FT-IR Spectrum 2 (Perkin-Elmer) spectrophotometer and are quoted in cm^{-1}. NMR spectra were recorded on a Jeol *ECX* 400 MHz spectrometer (operating at 400 MHz respectively for ^{1}H; 100 MHz respectively for ^{13}C). Chemical shifts (δ) are reported in parts per million from low to high field and referenced to residual solvent $CDCl_3$ as the internal reference ($CDCl_3$: δ = 7.249 ppm). The ^{13}C-NMR (100 MHz) chemical shifts were given using $CDCl_3$ as the internal standard ($CDCl_3$: δ = 77.16 ppm). Tetramethylsilane (δ = 0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (δ 77.16 ppm) in ^{13}C NMR. Coupling constants (*J*) are reported in Hertz (Hz)

with Standard abbreviations used as follows: m = multiplet, q = quartet, t = triplet, d = doublet, s = singlet, br = broad. Electrospray Ionization mass spectrometry (ESI-MS) and high resolution mass spectra (HRMS) were obtained with a Gevo G-2 S Q-TOF (Waters). Melting points were recorded in open capillaries on Complab melting point apparatus and are presented uncorrected. Analytical thin layer chromatography was performed on Merck TLC Silica gel F_{254} plates with visualization under UV light (λ = 254 nm). Column chromatography was performed over Rankem silica gel (particle size: 100-200 Mesh) purchased from Rankem^{TM} (India).

3.2.4.2 General procedure (GP) for the synthesis of receptor 1



5-Bromoisatin L-Proline Chalcone

Receptor **1**

Scheme 1. Synthesis of the receptor **1**.

In an oven dried round bottomed flask, chalcone (0.2 g, 0.9603 mmol), 5-Bromoisatin (0.26 g, 1.1524 mmol) and L-proline (0.132 g, 1.1524 mmol) were dissolved in methanol and refluxed at 80 °C for 6 hours. The progress of the reaction was monitored by TLC. After completion of reaction, the solvent was evaporated under reduced pressure and purified through column chromatography using (hexane: EtOAc = 7:3 v/v) as an eluting solvent to get the desired product. R_f (hexane: EtOAc = 6:4): 0.30; Color: Off-white solid, Yield: 71% (329 mg, 0.68 mmol); m.p.: 175-177 °C; FT-IR (KBr, ν max/cm^{-1}) 3437.04 (NH stretch), 1740.57 (ketonic CO stretch), 1713.85, 1674.72 (amide CO stretch), 1469.14, 737.54 (aromatic CH bend), 692.44 (C-Br stretch); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.1 (d, *J* = 7.6 Hz, 2H), 7.35 – 7.28 (m, 4H), 7.26 – 7.23 (m, 1H), 7.21-7.13 (m, 3H), 6.48 (d, *J* = 8.4 Hz, 1H), 4.92 (d, *J* = 11.6 Hz, 1H), 4.24-4.19 m, 1H), 3.90 – 3.83 (m, 1H), 2.69 – 2.58 (m, 2H), 2.04 – 1.73 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.72 (C=O, ketone), 180.88 (C=O, amide), 139.73 (C), 139.51 (C), 137.03 (C), 133.15(C), 132.46 (C), 130.49 (CH), 128.81 (2 × CH), 128.30 (2 × CH), 128.16 (2 × CH), 127.98 (2 × CH), 127.48 (CH), 127.16 (CH), 115.19 (CH), 111.63 (CH), 73.75 (C), 72.18 (CH), 64.59 (CH), 52.93 (CH), 48.28 (CH_2), 30.76 (CH_2), 27.52 (CH_2); HRMS (ESI) calcd for $C_{27}H_{24}BrN_2O_2^+$ ($M+H^+$): 487.1016; found, 487.1012.

3.2.4.3 Single crystal X-ray structure of receptor 1: ORTEP view, crystal packing representation and Hirshfeld surfaces

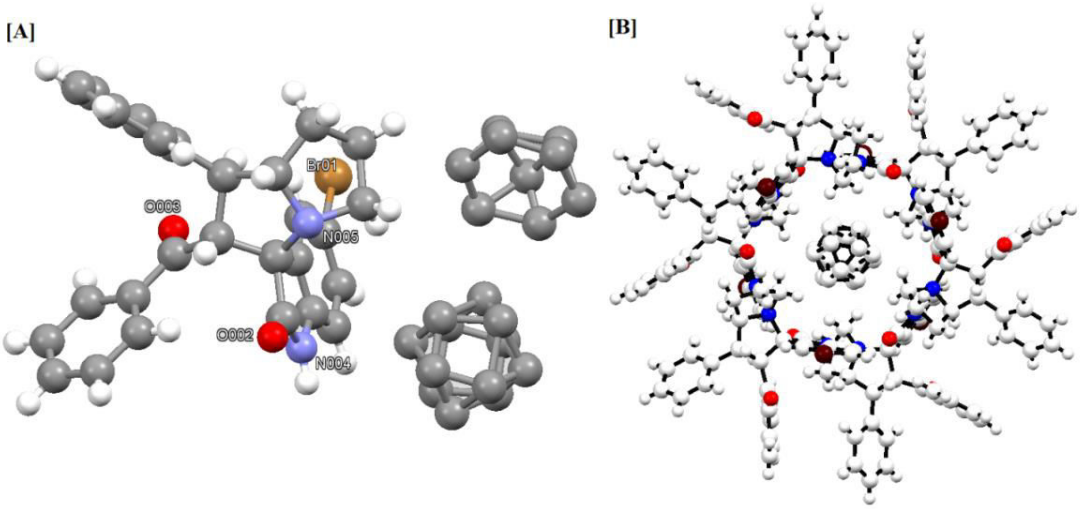


Figure 5. (a) ORTEP view of the receptor **1**.**(b)**Crystal packing representation of the receptor **1** with C9 and C12, respectively. Thermal Ellipsoids are at 50% probability level.

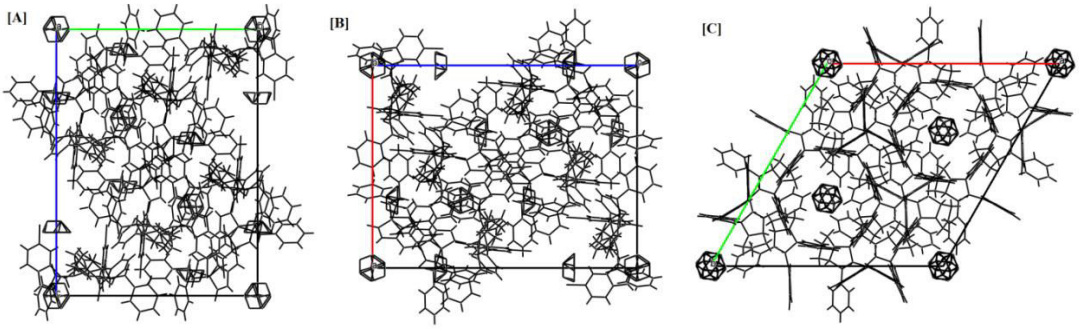


Figure 6. Crystal packing representation of receptor **1** along A-axis, B-axis, C-axis respectively. Thermal Ellipsoids are at 50% probability level.

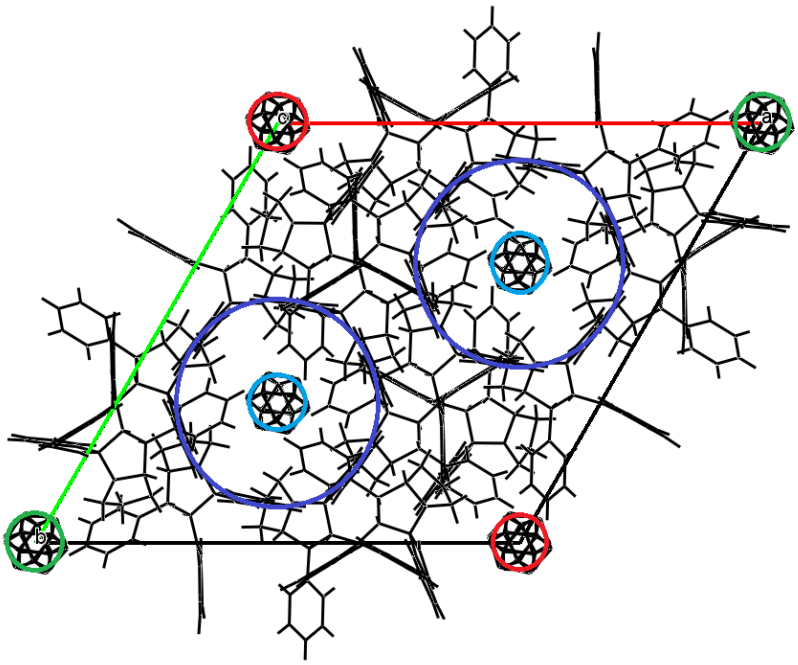


Figure 7. Crystal packing representation of receptor **1** along C-axis shadowed with unit cell. Thermal Ellipsoids are at 50% probability level.

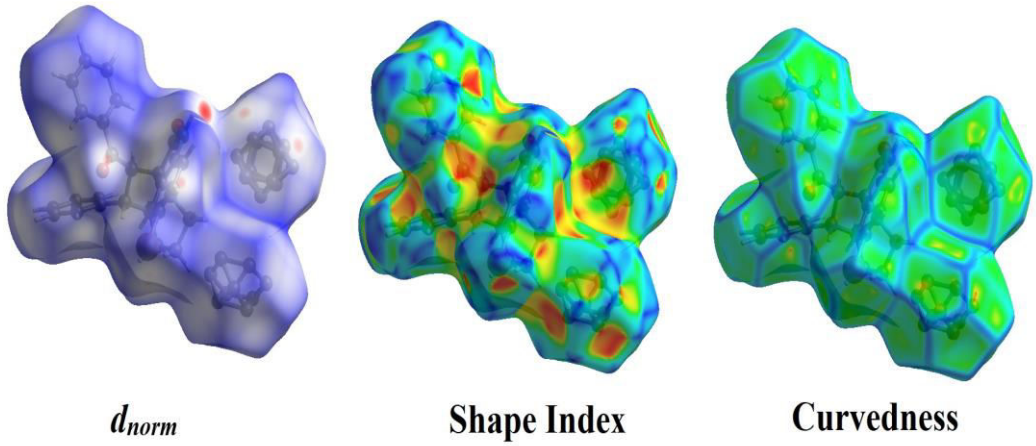


Figure 8. Hirshfeld surfaces mapped with (a) d_{norm} (b) shape index and (c) curvedness for the receptor **1**.

3.2.5 References

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24. CCDC No. 198676 contain the supplementary single crystal X-ray crystallographic data for the present manuscript. These data can be obtained free of cost by The Cambridge Crystallographic Data Centre.

3.2.6 *Characterization spectral data (^{1}H NMR and ^{13}C NMR and HRMS) of receptor 1, C9, C12 and receptor 1 + C12.*

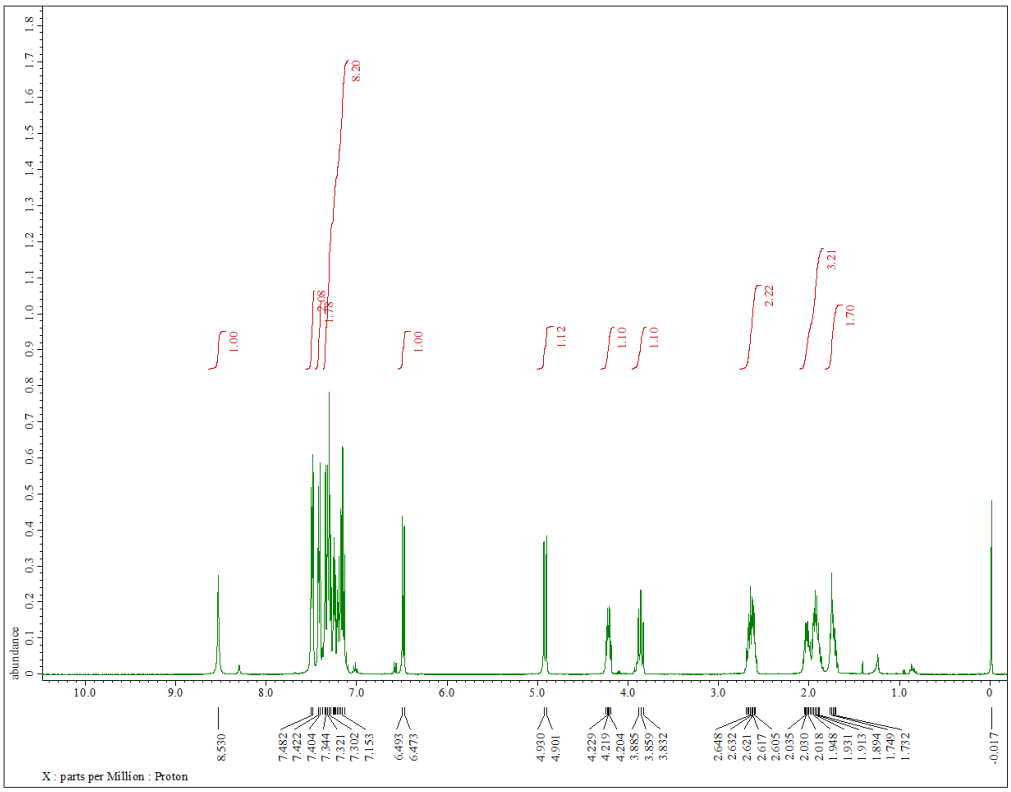


Figure 9. ^{1}H NMR spectrum of the receptor **1**.

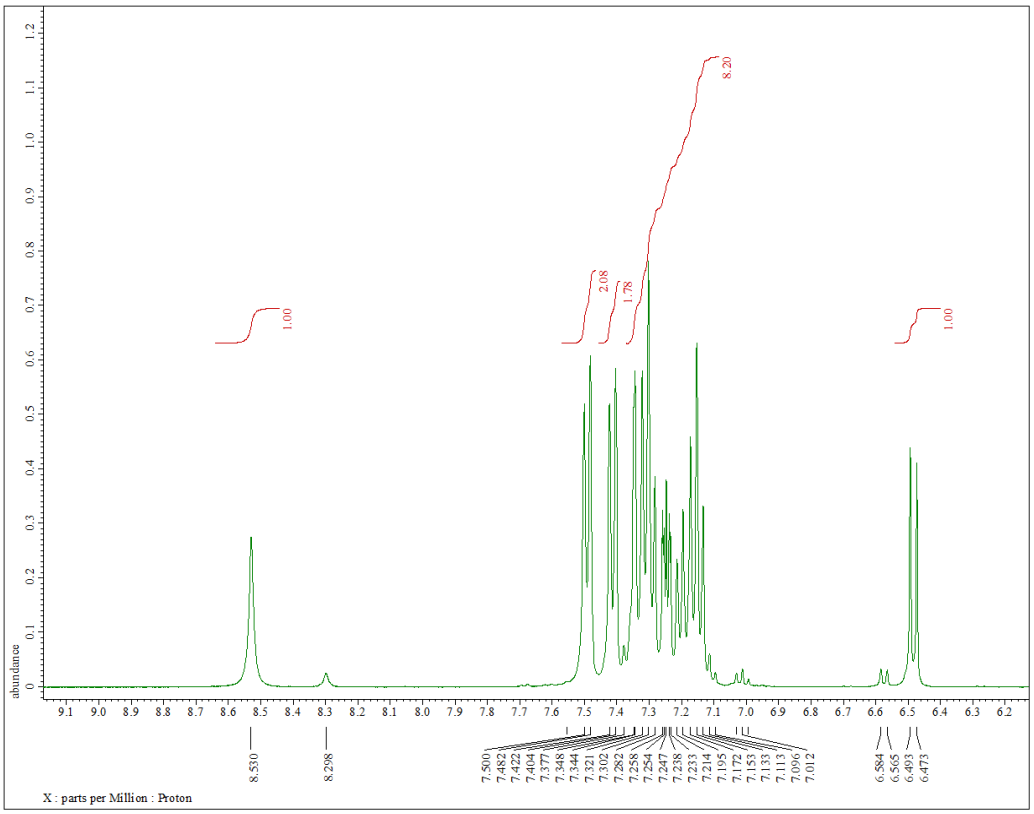


Figure 10. Partial ^{1}HNMR spectrum of the receptor **1**.

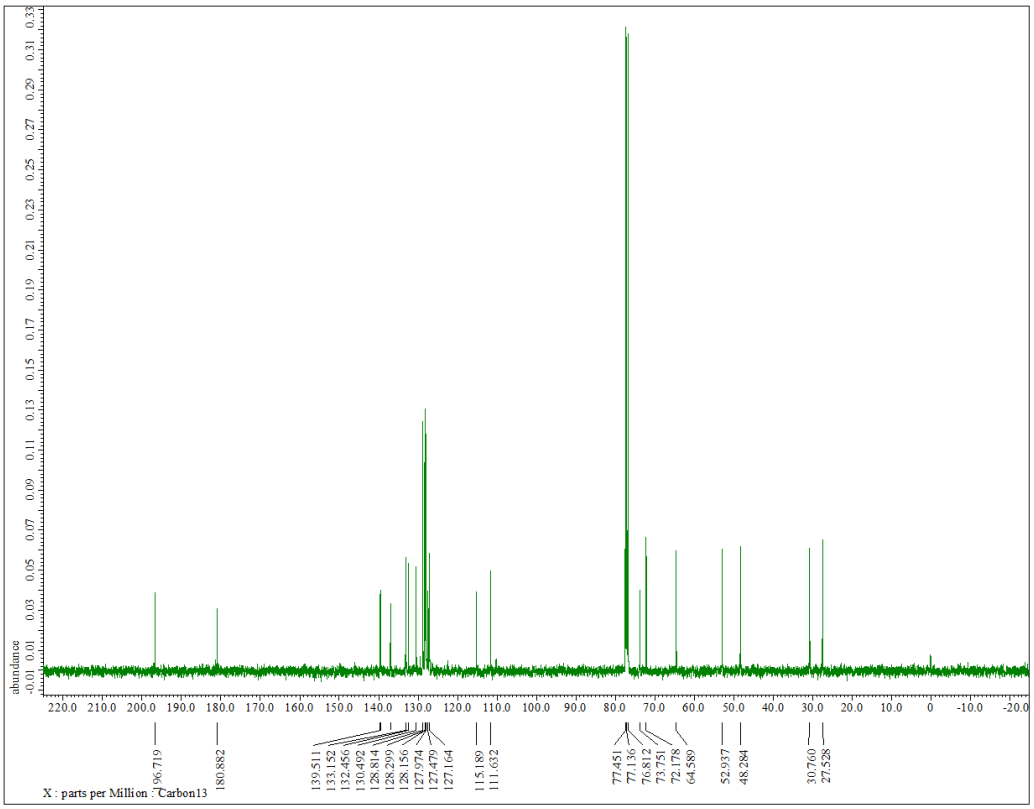


Figure 11. ^{13}C NMR spectrum of the receptor **1**.

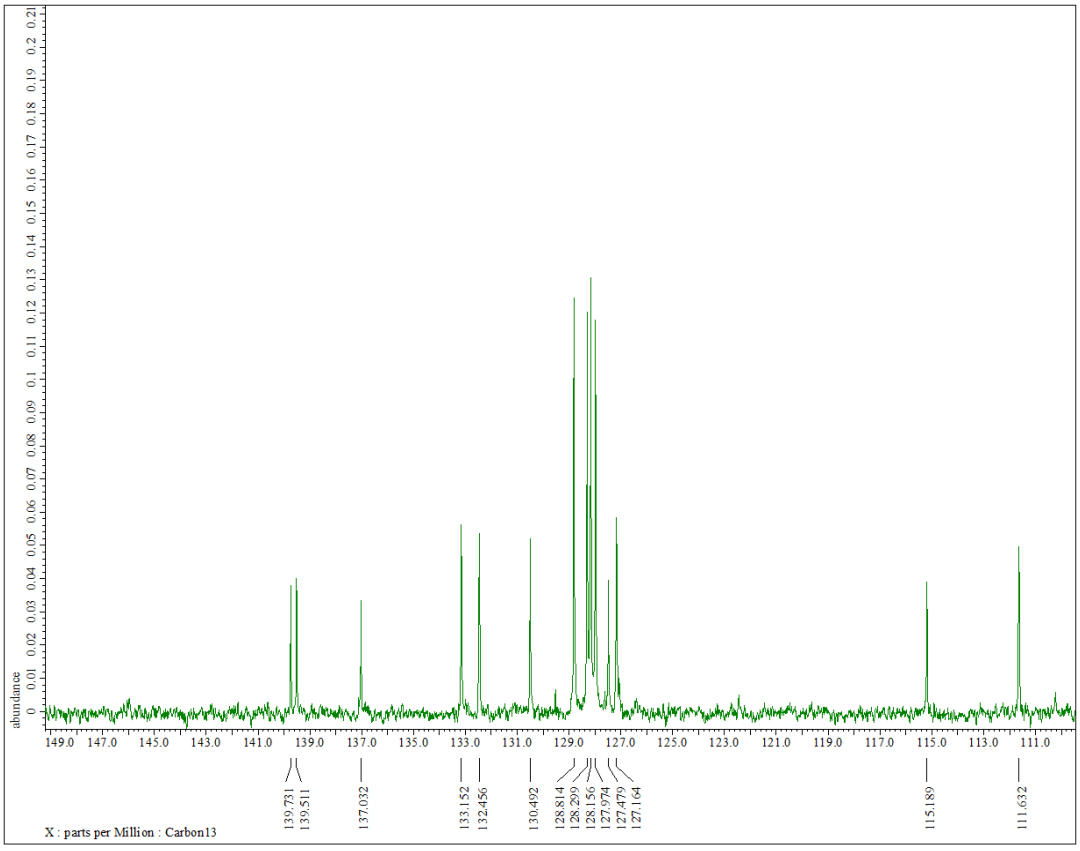


Figure 12. Partial ^{13}C NMR spectrum of the receptor **1**.

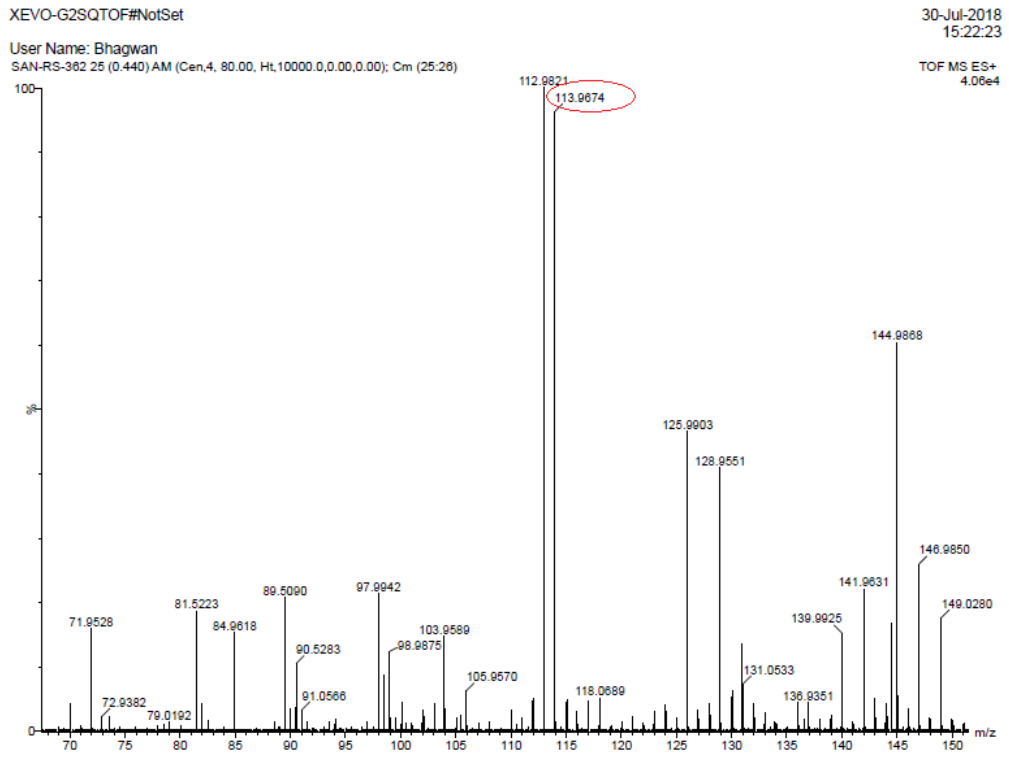


Figure 13. Mass Spectrum of the **C9** functionality.

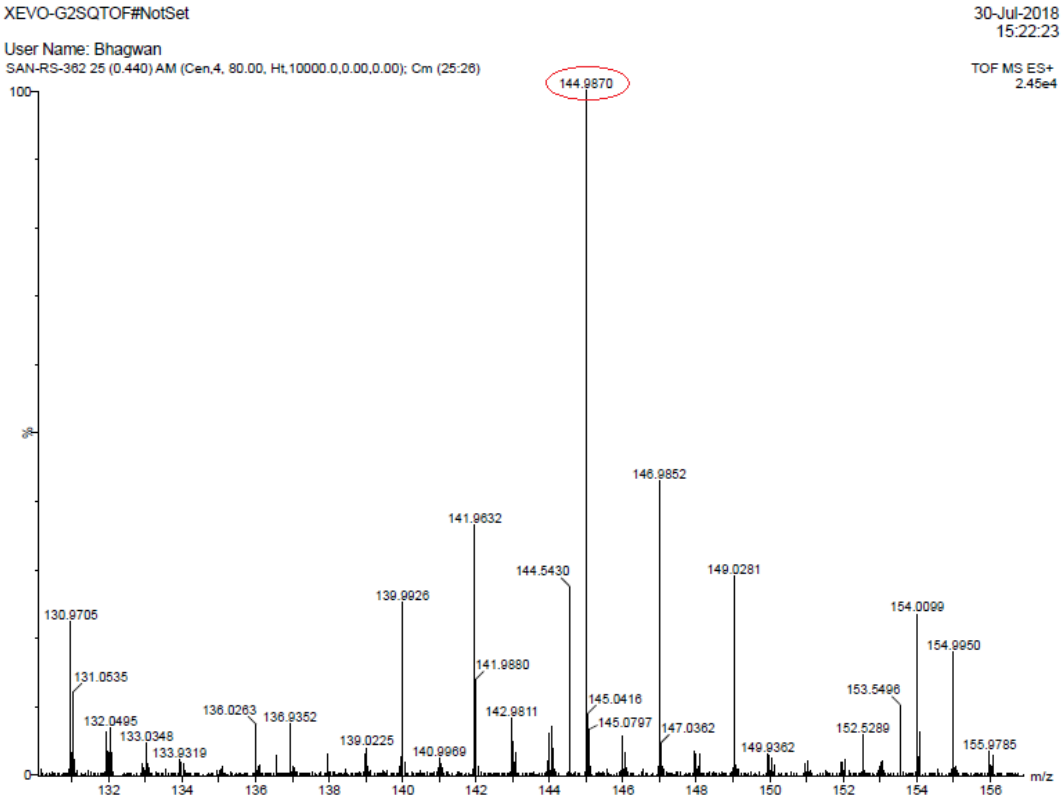


Figure 14. Mass Spectrum of the **C12** functionality.

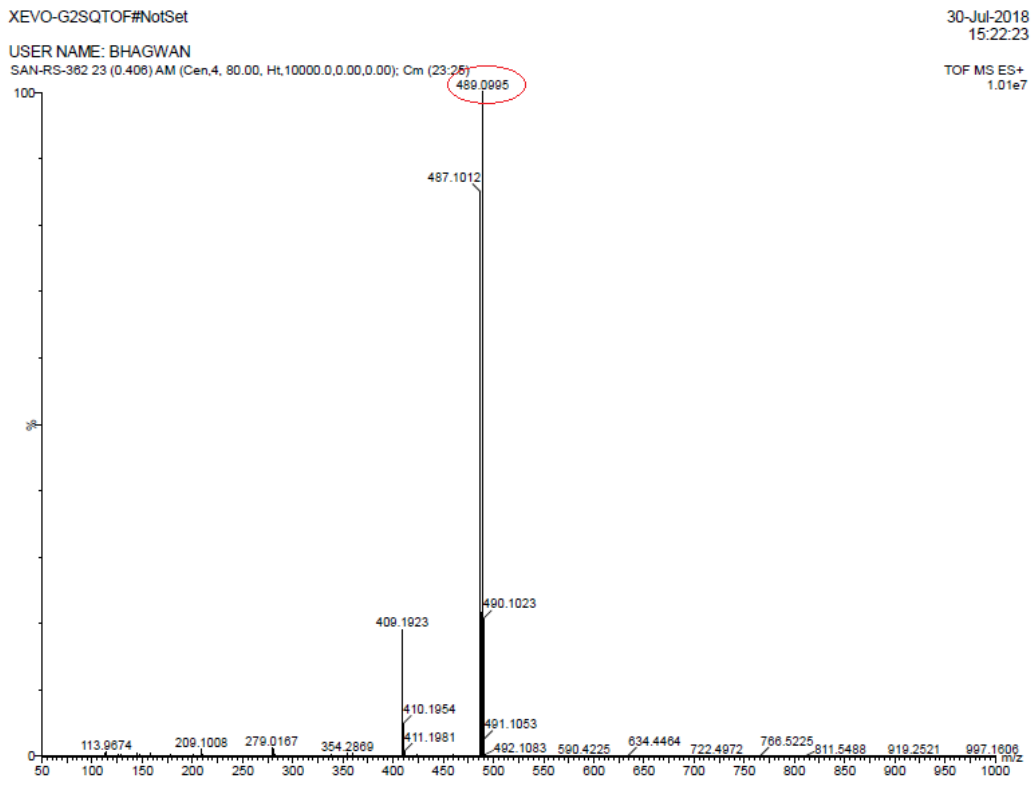


Figure 15. Mass Spectrum of the receptor **1**.

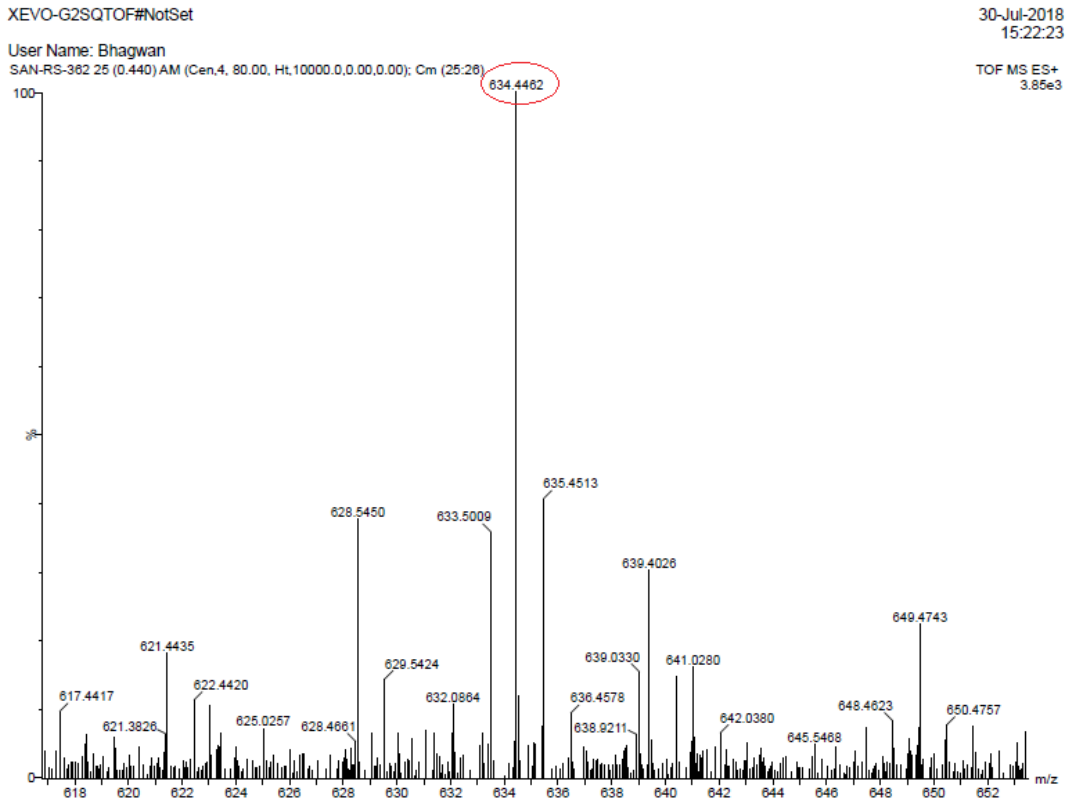


Figure 16. High Resolution Mass Spectrum of receptor **1+ C12**.

CHAPTER 4

Metal-catalyzed intramolecular cross-dehydrogenative coupling via C-H bond activation: Application to the synthesis of Coumarin-benzopyran fused heterocycles.

4.1 Introduction and objective of the work

Cross-dehydrogenative coupling, also known as "oxidative cross-coupling", is an important tool now-a-days to produce pharmaceutically important molecules. It's a coupling reaction in which a new bond is formed between two different molecules with the elimination of hydrogen atom from each of the molecules.[1] Hence, pre-functionalisation is not required in cross-dehydrogenative coupling reactions. Thus, the additional steps required to introduce functional groups into the molecules (such as -X, -OTf, -BR_2, -SnR_3,-ZnX, -MgX), is not required in this reaction as compared to other coupling reactions. Therefore, it leads to the minimization of the number of steps and by-products.[1]

1
2*H*-chromen-2-one
(**Coumarin**)

2
Isochromane
(**Benzopyran**)

Figure 1: General Structure of coumarin and benzopyran core.

Coumarin core belongs to the flavonoid class of plant secondary metabolites and are present in several natural products and biologically important molecules (Figure 1).[2] Various coumarin-based natural and synthetic derivatives are endowed with a wide spectrum of biological activities such as anticancer,[3] anti-HIV,[4] antimicrobial,[5] antioxidant and anti-inflammatory,[6a-c] antituberculosis,[7] anti-influenza,[8] anti-alzheimer,[9] antiviral,[10] and antihyperlipidemic[11a-b] etc. Literature survey revealed that the coumarin skeleton, either being hybridized or fused directly with various other heterocyclic rings, have tremendous biological significance and are important in terms of their medicinal and pharmaceutical interest (Figure 2). For example, the two naturally occurring coumarin analogues, scopoletin **3** and esculetin **4**, are associated with antiproliferative, antioxidant and anti-inflammatory activities. Cloricromene **5** (antiinflammatory), warfarin **6** (anticoagulant), acenocoumarol **7** (anticoagulant), armillarisin A **8** (antibiotic), hymecromone **9** (choleretic and antisplasmodic), carbochromen **10** (coronary disease), phenprocoumon **11** (anticoagulant), novobiocin **12** (antibiotic) and Geiparvin **13** (antiplatelet) are some of the most biologically active coumarin-containing compounds which are shown in Figure 1.

Benzopyrans (also known as "isochromane") constitute the core of several natural products and numerous drugs and pharmaceuticals (Figure 1). For example-

Compounds **14** and **15** (antibacterial),[12a] Fiduxosin **18** is an α_1 adrenoreceptor antagonist and shows an α_{1a}/α_{1b} selectivity for adrenoreceptors.[12b] Compound **24** is an anticoagulating agent (Figure 3).[12c] The rest of the compounds i.e., **16**, **17**, **19-23** shown in Figure 3, are some of the medicinally important compounds derived from natural products.[12d-e]

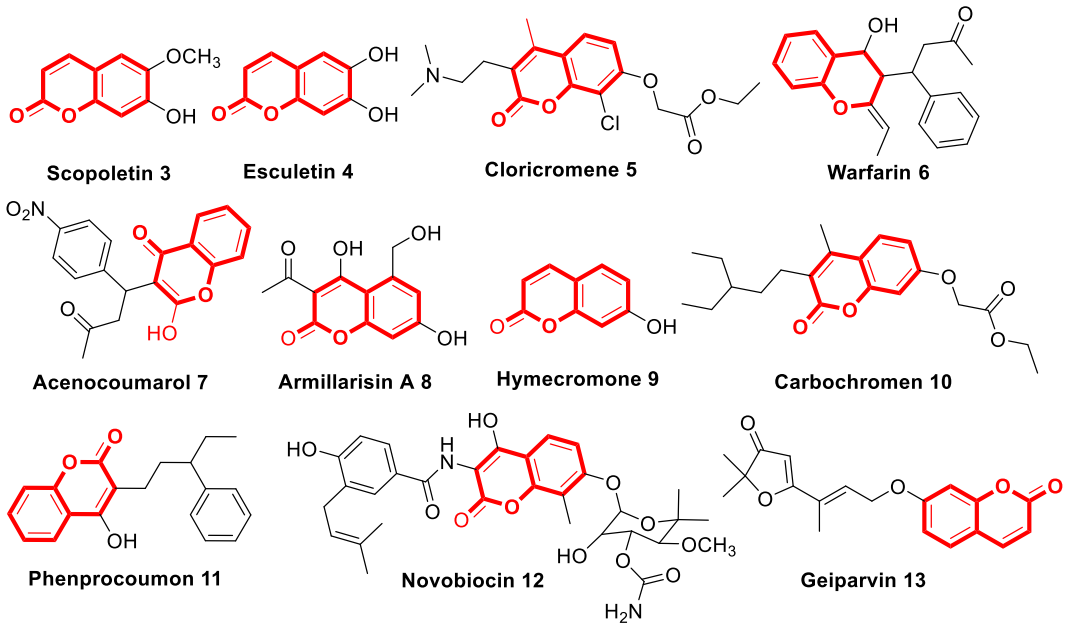


Figure 2. Some biologically active scaffolds containing coumarin moiety.

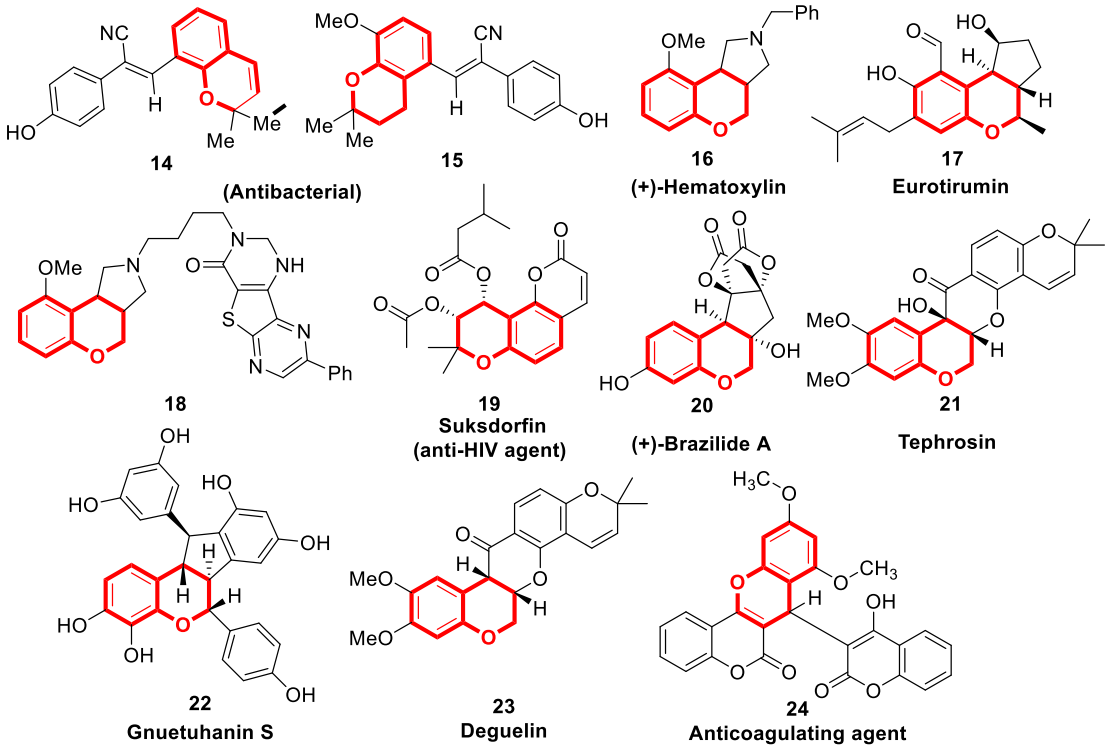
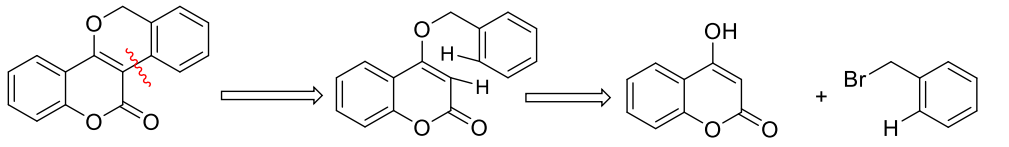


Figure 3. Some biologically active scaffolds containing benzopyran moiety.

Molecular hybridization is a novel approach that has emerged as an efficient technique to cumulate two pharmacophores into a single structure. The methodology

includes intramolecular metal-catalyzed cross-dehydrogenative coupling to form coumarin benzopyran scaffolds. Using this technique, several biologically active hybrid molecules such as indole-fused coumarins[13] and coumestans (benzofuran-fused coumarins)[14-22] have been reported in the literature via transition-metal catalysis and metal-free/oxidative coupling strategies including intramolecular CDC reaction strategy.

Since coumarins and benzopyrans have been identified as potent pharmaceutical agents and in our research programme towards the search of new bio-active compounds; it has been planned and decided to fuse these two bioactive moieties and prepare **prototype I** (6*H*,11*H*-isochromeno[4,3-c]chromen-11-one) via the development of an intramolecular CDC reaction and then study their biological activities such as antimicrobial, antimalarial etc. of all the synthesized coumarin-benzopyran fused tetracyclic frameworks. Therefore, its retro-analysis reveals that the tetracyclic prototype I **26** can be prepared by carrying out intramolecular cross-dehydrogenative coupling on 4-(benzyloxy)-2H-chromen-2-one **25** which, in turn, can be prepared from the reaction of 4-hydroxycoumarin with benzyl bromide. (Figure 4).



6*H*,11*H*-isochromeno[4,3-*c*]chromen-11-one

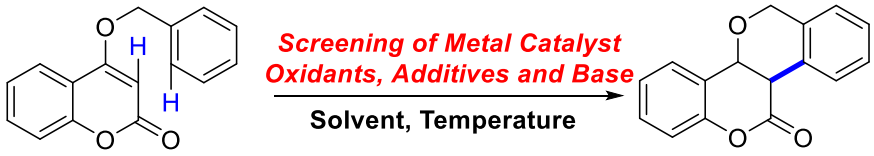
Figure 4. Retro-analysis of proposed prototype I.

So far, there has been no CDC reaction reported on coumarin derivatives to form coumarin-benzopyran fused scaffolds. Herein, in this chapter, we describe the development of a challenging intramolecular palladium-catalyzed cross-dehydrogenative coupling on 4-(benzyloxy)-2H-chromen-2-one *via* systematic screening of metal catalyst, solvents, ligands, oxidants and bases. After extensive investigation, 20 mol% $Pd(TFA)_2$, Ag_2CO_3 (2 equiv.), CH_3COONa (2 equiv.) in tert-amyl alcohol as solvent at 120 °C at 24 hrs was found to be the best optimized reaction condition for this challenging cyclization to form coumarin-benzopyran fused scaffolds from non-functionalized substrates. The reaction is simple, one pot requires simple substrates, has easy purification and is non-hazardous. To the best of our knowledge, for the first time, a new Pd-catalyzed intramolecular CDC reaction has

been developed for the synthesis of various coumarin-benzopyran fused tetracyclic frameworks.

4.2 Results and discussion

4.2.1 Initial investigation: Random identification of metal catalyst, oxidant and base.



25 **26**

We started our initial investigation on the model coupling precursor 4-(benzyloxy)-*2H*-chromen-2-one **25**. Since it has been well documented in the literature that, in general, cross-dehydrogenative coupling proceeds via C-H bond activation by the metal catalyst in the presence of oxidants and additives in some high boiling solvents above 100 ºC for 6-24 hours. Therefore, the initial attempts to optimize this challenging cyclization began with cheaper transition-metal catalysts such as Fe, Cu, Ni etc. The model substrate **25** was subjected to modified literature procedure[16] and was treated with $FeCl_3$ (0.2 equiv) in the presence of DTBP (2 equiv) in DMSO at 80 ºC for 24h did not furnish the desired cyclized product **26** (Table 1, entry 1). Further carrying out the reaction with increased amount of $FeCl_3$ as oxidant (8 equiv) at 110 ºC for 48h did not brought any improvement in the yield of the **26** (Table 1, entry 2). In continuation to this, the reaction was also carried out with $FeCl_3$ (0.1 equiv) in the presence of TBHP (3 equiv) and DABCO as base in benzene as solvent at 120 ºC for 21h; however, we did not obtained any product (Table 1, entry 3). We then switch over to mixed catalytic system and attempted the reaction with $Cu(OAC)_2$ (1.5 equiv) and $FeCl_3$ (0.1 equiv) in presence of K_2CO_3 (3 equiv) as base in DMF at 120 ºC for 24 hours; however, we did not obtained any product (Table 1, entry 4). Then we attempted the reaction with modified procedure[13a] with $Cu(OAC)_2$ (1.5 equiv) in the presence of oxidants (AgOAc, 3 equiv) and additives [CsOPiv, 3 equiv + PivOH, 40 mol%) in DMF at 130 ºC for 22h; **25** was not converted into **26** at all (Table 1, entry 5). Finally, we replaced the copper catalyst as used in entry no. 5 with nickel catalyst [$Ni(OAc)_2$, 20 mol%] and carried out the reaction in the presence of oxidants (AgOAc, 3 equiv) and additives [CsOPiv, 3 equiv + PivOH, 40 mol%) in DMF at 140 ºC for 17h; even though there is 0% formation of **26** (Table 1, entry 6). All these failure attempts inspire us to utilize traditional Palladium catalyst. Therefore, we

subjected the coupling precursor **25** with 20 mol% of $Pd(OAc)_2$ and TBHP (4 equiv) as oxidant) at 100 ºC for 24 hours; we were delighted to obtain the desired **26** in trace amount (Table 1, entry 7).

Table 1: Initial studies: Synthesis of 6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-ones **26** by intramolecular CDC of 4-(benzyloxy)-2-H-chromen-2-one **25.**

Entry	Catalyst (equiv.)	Oxidant (equiv.)	Additive (equiv.)	Base (equiv.)	Solvent (mL)	Temperature	Time (h)	Yield (%)
1.	$FeCl_3$ (0.2)	DTBP (2.0)	-	-	DMSO	80°C	24	0
2.	$FeCl_3$ (0.2)	DTBP (8.0)	-	-	DCE	110°C	48	0
3.	$FeCl_3$ (0.1)	TBHP (3.0)	-	DABCO	C_6H_6	120°C	21	0
4.	$Cu(OAc)_2$ (1.5) + $FeCl_3$ (0.1)	-	-	K_2CO_3 (3.0)	DMF	120°C	24	0
5.	$Cu(OAc)_2$ (1.5)	AgOAc (3.0)	Cs(OPiv) (3.0) + PivOH (0.4)	-	DMF	130°C	22	0
6.	$Ni(OAc)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0) + PivOH (0.4)	-	DMF	140°C	17	0
7.	$Pd(OAc)_2$ (0.2)	TBHP (4.0)	-	-	-	100°C	24	Traces
8.[a]	$Pd(OAc)_2$ (0.2)	-	-	K_2CO_3 (3.0)	PivOH	100°C	48	8
9.	$Pd(OAc)_2$ (0.2)	O_2 + TBHP	-	-	DMF	100°C	58	15
10.	$Pd(OAc)_2$ (0.2)	O_2	-	K_2CO_3 (2.0)	DMF	100°C	24	9
11.	$Pd(OAc)_2$	O_2			DMF	130°C	24	15
12.	$Pd(OAc)_2$ (0.2)	O_2		CH_3COONa (1.5)	DMF	130°C	24	8
13.	$Pd(TFA)_2$ (0.2)	AgOAc (2.0)			TFA	80°C	24	Traces
14.	$Pd(TFA)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0)		DCE	90-100°C	20	Traces
15.	$Pd(TFA)_2$ (0.2 eq)	AgOAc (3.0)	Cs(OPiv) (3.0)		DMF	90-100°C	22	8
16.	$Pd(TFA)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0)		PivOH	100°C	22	16
17.[b]	$Pd(TFA)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0)		DMF	130°C	17	16
18.[c]	$Pd(TFA)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0)		PivOH	130°C	42	15
19.	$Pd(TFA)_2$ (0.2)	AgOAc (3.0)	Cs(OPiv) (3.0)		DMF	140°C	24	4
20.	$PdCl_2$ (0.2)	Ag_2CO_3 (2.0)		CH_3COONa (2.0)	DMF	100°C	57	15
21.	$Pd(TFA)_2$ (0.2)	Ag_2CO_3 (2.0)		CH_3COONa (2.0)	DMF	120°C	24	18

[a]1 equiv. TEMPO was added. [b]0.4 equiv PivOH added. [c]Anhydrous DMF added to the reaction mixture after 18 hrs.

Adding K_2CO_3 (3 equiv) and PivOH as solvent to the reaction conditions as used in entry 7 provides the desired product **26** in 8% yield after 48h (Table 1, entry 8). Using modified literature procedure,[13b] Oxygen (O_2) along with 20 mol% of $Pd(OAc)_2$ and

TBHP (4 equiv) as oxidant in DMF at 100 ºC for 58h furnished **26** in only 15% yield (Table 1, entry 9). When the reaction has been carried out with O_2 and K_2CO_3 (2 equiv) as base at 100 ºC for 24h, the yield of **26** reduces to 9% (Table 1, entry 10). Furthermore, at 130ºC also, in the presence of oxygen, **26** was obtained in 15% yield (Table 1, entry 11). At 130ºC, in presence of O_2 and DMF solvent and CH_3COONa (1.5 equiv) along with 20 mol% of $Pd(OAc)_2$, 8% yield of the product was obtained (Table 1, entry 12). Thus, we failed in improving the yield of the final product beyond 15%. Hence, it was decided to change the metal catalyst and to use more activated Pd-catalyst i.e., $Pd(TFA)_2$. The reaction was carried out with 20 mol% of $Pd(TFA)_2$ in the presence of oxidant AgOAc (2 equiv) in TFA solvent at 80ºC for 24h, it leads to the formation of product in traces (Table 1, entry 13). Further, several reactions were attempted with Pd $(TFA)_2$ catalyst along with AgOAc (3 equiv) as oxidant and CsOPiv (3 equiv) as additive in different solvents such as DCE, DMF, PivOH and also at different temperature and time; there was no significant improvement in the yield (15-16%) of the desired coupled product (Table 1, entries 14-18). On increasing the temperature to 140ºC in the reaction conditions as used in entry 17, the yield dropped down dramatically to only 4% (Table 1, entry 19). Additionally, the reaction was also tried with $PdCl_2$ taking Ag_2CO_3 (2 equiv) and CH_3COONa (2 equiv) in DMF solvent at 100ºC for 57h; still there was no improvement in the yield and **26** was obtained in only 15% yield (Table 1, entry 20). Finally, the reaction was carried out with 20 mol% of Pd $(TFA)_2$ along with Ag_2CO_3 (2 equiv) and CH_3COONa (2 equiv) in DMF solvent at 120ºC for 24h; We were delighted by getting 18% of cyclized product **26**.

Therefore, after extensive screening of different metal catalysts, oxidants and bases, *20 mol% of $Pd(TFA)_2$, Ag_2CO_3 (2 equiv) and CH_3COONa (2 equiv)* was found to be the best metal catalyst, oxidant and base, respectively.

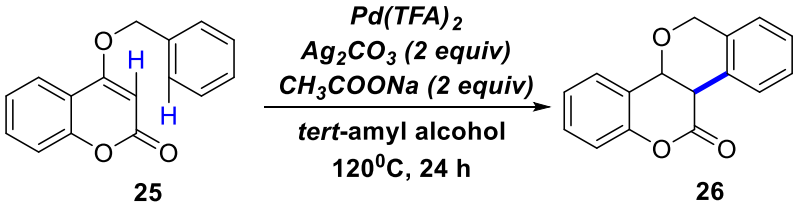
4.2.2 Further optimization studies towards different Pd-Catalyst screening and loading studies.

In order to ascertain the Pd-catalyst loading studies; the reaction was carried out with 10 mol% and 20 mol% of $Pd(TFA)_2$. The yield of the cyclized product **26** obtained in these two reactions were 23% and 45% respectively (Table 2, entry 1-2). Other Pd-catalyst such as $Pd(OAc)_2$**,** $PdCl_2$, Tetrakistriphenylphosphinepalladium (0) etc. were

also screening for their efficacy in this intramolecular CDC reaction (Table 2, entry 3-5). However, there is no significant change in % yields. Infact, yield drop down significantly when we use $PdCl_2$ (Table 2, entry 4).

Therefore, overall, after Pd-catalyst screening and loading, *20 mol% of $Pd(TFA)_2$* was found to be the best metal catalyst.

Table 2. Catalyst loading studies: Synthesis of 6*H*, 11*H*-isochromeno [4,3-c] chromen-11-ones **26** by CDC of 4-(benzyloxy)-2-H-chromen-2-one **25**.[a]



Entry	Catalyst Loading (equiv.)	Yield[b] (%)
1	$Pd(TFA)_2$ (0.1)	23
2	$Pd(TFA)_2$ (0.2)	45
3	$Pd(OAc)_2$ (0.2)	25
4	$PdCl_2$ (0.2)	Negligible
5	Tetrakistriphenylphosphinepalladium (0) (0.2)	23

[a]**Reaction conditions- 4-(benzyloxy)-2-H-chromen-2-one** (0.2 mmol, 0.05 g, 1 equiv) were added with Pd-catalyst, Ag_2CO_3 (0.3 mmol, 0.109 3g, 2 equiv), CH_3COONa (0.4 mmol, 0.0325 g, 2 equiv) and *tert*-amyl alcohol solvent (1 ml). The reaction mixture was purged with nitrogen, degasified and heated at 120ºC for 24 hrs.

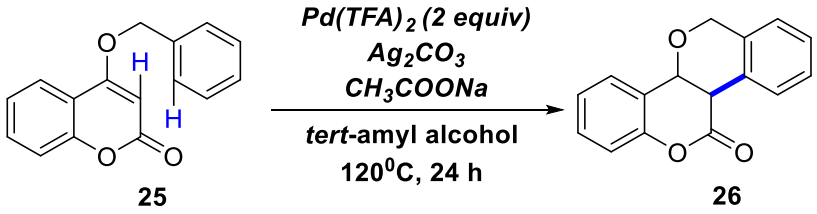
[b]Isolated yield after column chromatography.

4.2.3 Further optimization studies towards number of equivalents of different oxidants and screening of different bases.

In order to confirm the oxidants and bases; screening as well as loading studies were carried out (Table 3). Few silver-based oxidants such as Ag_2CO_3, $AgNO_3$, AgOAc were screened and their loading studies were also carried out. Among the oxidants tried, $AgNO_3$ gave negligible product, and AgOAc also gave lower yield as compared to Ag_2CO_3 which furnished the cyclized product in 45% yield (Table 3, entries 1-3). Few bases were also screened such as NaOPiv, Sodium benzoate, CH_3COONa in this reaction. Among the bases screened, trace formation of product formation was observed with NaOPiv and Sodium benzoate as compared to CH_3COONa (2 equiv.)/K_2CO_3 (0.5 equiv.) mixture as base where 26 was obtained in 16% yield. (Table 3, entries 4-6). Therefore, after extensive screening of different oxidants and

bases, *Ag_2CO_3 (2 equiv) and CH_3COONa (2 equiv)* was found to be the best oxidant and base, respectively.

Table 3. Screening of different oxidants and bases: Synthesis of 6*H*,11*H*-isochromeno [4,3-c] chromen-11-one **26** by intramolecular CDC of 4-(benzyloxy)-2-H-chromen-2-one **25**.[a]



25 **26**

Entry	Oxidant (equiv.)	Base (equiv.)	Yield[g] (%)
1	Ag_2CO_3 (2.0)	CH_3COONa (2.0)	45
2[b]	$AgNO_3$ (3.0)	CH_3COONa (2.0)	Traces
3[c]	AgOAc (3.0)	CH_3COONa (2.0)	16
4[d]	Ag_2CO_3 (2.0)	NaOPiv (2.0)	Traces
5[e]	Ag_2CO_3 (2.0)	Sodium benzoate (2.0)	Traces
6[f]	Ag_2CO_3 (2.0)	CH_3COONa (2.0) K_2CO_3 (0.5)	16

[a]**Reaction conditions: 4-(benzyloxy)-2-H-chromen-2-one** (0.2 mmol, 0.05 g, 1 equiv) were added with $Pd(TFA)_2$, (0.04 mmol, 0.0131 g, 0.2 equiv), oxidant (0.3 mmol, 2 equiv), base (0.4 mmol, 2 equiv) and tert-amyl alcohol solvent (1 ml), heated at 120ºC for 24 hrs, N_2 atmosphere

[b, c, d, e, f]0.1 equiv catalyst.

[g]Isolated yield.

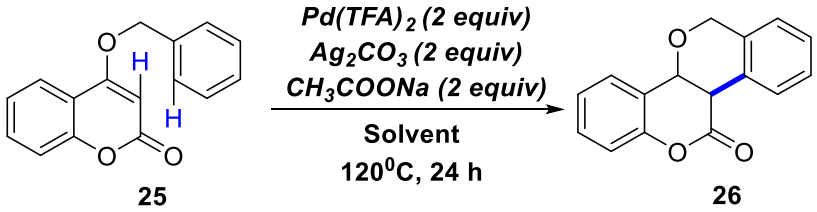
4.2.4 Optimization studies: Screening of different solvents and temperature.

After optimization of Pd-catalyst [$Pd(TFA)_2$], oxidant (Ag_2CO_3) and base (CH_3COONa), screening of different solvents such as DCE, DMSO, toluene, mesitylene, HFIP, 1,4-dioxane/DMF, chlorobenzene, DMF as well as temperature studies were carried out. The details of the studies are given in Table 4.

As can be seen from Table4; *tert*-amyl alcohol was found to be the best solvent for this reaction as the yield of the intramolecular CDC on **25** for the synthesis of **26** comes out to be 45% at 120ºC. While in mesitylene, HFIP and NMP solvents, the yield was dropped to 25% only; the yield of the reaction in DMF was observed only 18%. The reactions carried out in other solvents furnished the desired product **26** either in low yields or in traces.

Therefore, overall, after screening of different solvents and temperature, *tert-amyl alcohol and 120ºC* has been found to be the best solvent and temperature conditions, respectively.

Table 4: Optimization - Solvent screening: Synthesis of 6*H*,11*H*-isochromeno [4,3-c] chromen-11-ones **26** by intramolecular CDC of 4-(benzyloxy)-2-H-chromen-2-one **25**.[a]



25 **26**

Entry	Solvent	Temperature	Yield[d] (%)
1	DMSO	130°C	Trace
2	DMSO	130°C	Trace
3	mesitylene	120°C	25
4	HFIP	120°C	25
5	DCE	120°C	NR
6	*tert*-amyl alcohol	120°C	45
7	Chlorobenzene	120°C	NR
8	Toluene	130°C	Traces
9	1,4-dioxane/DMF	130°C	Traces
10[b]	*tert*-amyl alcohol	120°C	23
11	DMF	120°C	18
12[c]	NMP	120°C	25

[a]**Reaction conditions: 4-(benzyloxy)-2-H-chromen-2-one** (0.2 mmol, 0.05 g, 1 equiv) were added with $Pd(TFA)_2$, (0.04 mmol, 0.0131 g, 0.2 equiv), oxidant (0.3 mmol, 2 equiv), base (0.4 mmol, 2 equiv) and solvent (1 ml), heated at 120ºC for 24 hrs, N_2 atmosphere

[b, c] 0.1 equiv catalyst.

[d]Isolated yield.

4.2.5 Screening of phosphine and organic ligands:

After exhaustive optimization studies via screening of Pd-catalysts, oxidants, bases, solvents and temperature, we were successful in getting the yield of the desired **26** in 45% only. Thus, in order to further improve the yield of intramolecular CDC reaction, phosphine and organic ligands **I-XII** were also examined in this reaction (Figure 5). Hence, the intramolecular CDC reactions were carried out in the presence of organic ligands **I-XII**. The details of the studies are given in Table 5. Among the ligands screening, inorganic phosphine ligands I- IV furnished **26** upto 25% yield which was observed the same as that obtained without using the ligands (Table 2, entries 1-4). Among the organic ligands **V-XII**, only N-acetyl glycine **IX** and 2,6-lutidine **X** furnished **26** upto 25% yield (Table 2, entries 5, 7 and 8). No product formation was observed while using the remaining ligands. This study infers us that adding phosphine and organic ligands I-XII to the intramolecular CDC reaction do not have beneficial effect on the yield of the reaction. Infact, the use of these ligands have detrimental effects on this reaction.

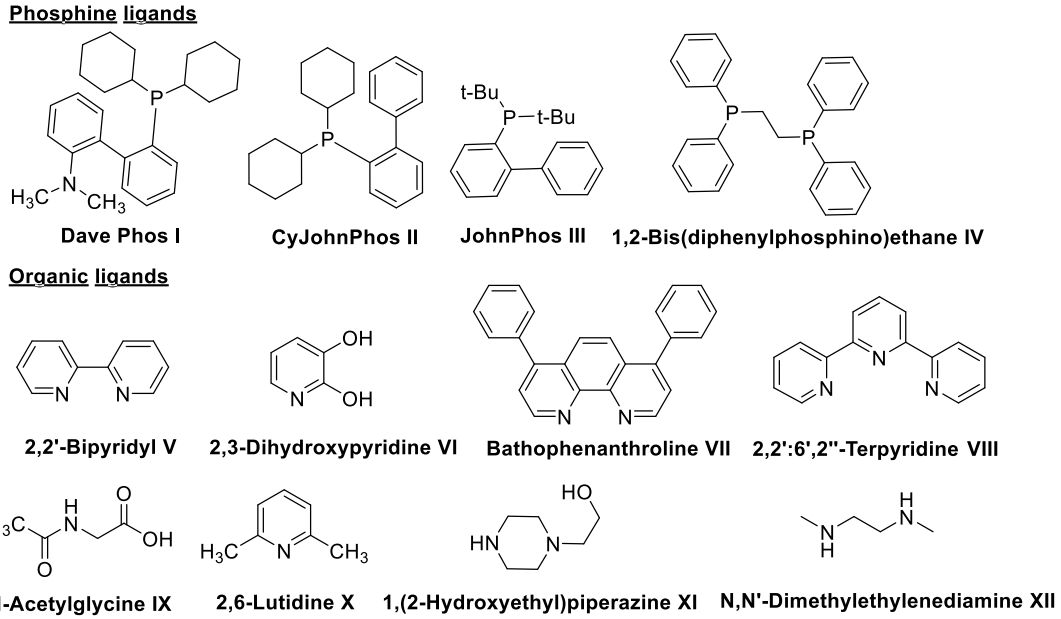
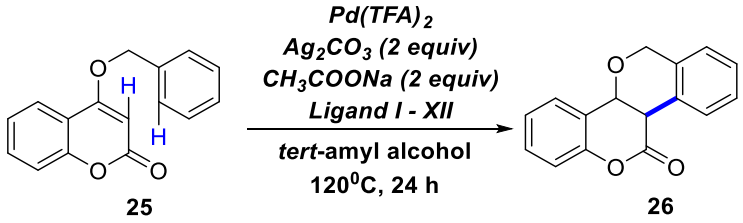


Figure 5. Structures of phosphine and organic Ligands I-XII used in the study.

Table 5- Screening of phosphine and organic ligands:: Synthesis of **26** by intramolecular CDC of **25**.[a]



Entry	Ligand (0.2 equiv.)	Yield[d]
1[b]	Jonphos	22
2	Davephos	18
3	CyJonphos	22
4	1,2-Bisbiphen.phosphinoethane	23
5[c]	N-acetyl glycine	22
6	2,2'-Bipyridyl	NR[e]
7	N-acetyl glycine	24
8	2,6-Lutidine	22
9	Bathophenanthroline	NR[e]
10	1,2-Hydroxyethylpiperazine	NR
11	2,3-Dihydroxypyridine	Traces
12	DMEDA	NR[e]
13	2,2': 6,2" Terpyridine	NR[e]

[a]**Reaction conditions- 4-(benzyloxy)-2-H-chromen-2-one** (0.2 mmol, 0.05 g, 1 equiv) were added with Pd-catalyst, Ag_2CO_3 (0.3 mmol, 0.109 3g, 2 equiv), CH_3COONa (0.4 mmol, 0.0325 g, 2 equiv) and *tert*-amyl alcohol solvent (1 ml). The reaction mixture was purged with nitrogen, degasified and heated at 120ºC for 24 hrs.

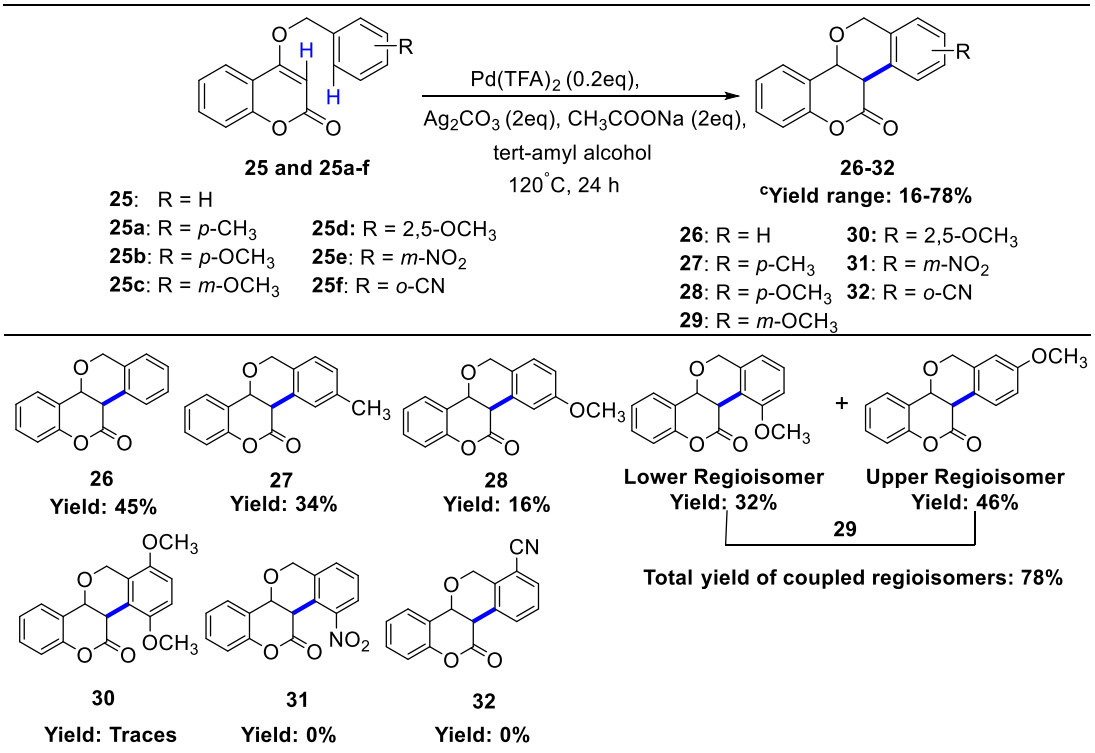
[b]K_2CO_3 added. [c]HFIP was used as a solvent. [d]Isolated yield after column chromatography.

Therefore, overall, *20 mol% of $Pd(TFA)_2$, Ag_2CO_3 (2 equiv), CH_3COONa (2 equiv) tert-amyl alcohol as solvent and 120ºC for 24h* has been found as the best optimized reaction conditions for the intramolecular cross-dehydrogenative coupling reaction.

4.3 Substrate scope

Several substituted 4-(benzyloxy)-2-H-chromen-2-ones **25** and **25a-f** having electron-withdrawing groups such as NO_2, CN etc. and electron-donating group such as CH_3, OCH_3 (synthesis of **25** and **25a-f** from substituted benzyl bromide is given in Scheme 2 of the experimental section) were subjected to intramolecular CDC reaction under optimized conditions which furnished the desired substituted 6*H*,11*H*-isochromeno [4,3-c] chromen-11-ones **26-32** in 16-78% yield range (Scheme 1). The starting precursor **25a** when subjected to intramolecular CDC under the optimized conditions furnished **27** in less (34%) yield. This infer us that electron-donating group have negative impact on the yield of the reaction.

Scheme 1: Substrate scope.[a]



[a]***Reaction conditions*: 4-(benzyloxy)-2-H-chromen-2-one derivatives (25 and 25a-f)** (0.2 mmol, 0.05 g, 1equiv.) were added with $Pd(TFA)_2$, (0.04 mmol, 0.0131 g, 0.2 equiv.), Ag_2CO_3 (0.3 mmol, 0.1093 g, 2 equiv.), CH_3COONa (0.4 mmol, 0.0325 g, 2 equiv.) and *tert*-amyl alcohol solvent (1 mL). The reaction mixture was purged with nitrogen, degasified and heated at 120ºC for 24 h. [b]reaction mixture heated for 48 h, [c]isolated yield after column chromatography.

Similarly, when **25b** having OCH_3 group at para position was subjected to optimized reaction conditions, further decrease in yield was observed as in compound **28**. Surprisingly, when the same OMe was introduced at *meta*-position (**25c**), drastic increase in the yield of **29** was obtained as a separable mixture of two regioisomers. In this case, due to the meta-OMe group, there is a formation of two regioisomers with a total yield of 78%. The formation of the two regioisomers was confirmed by ^{1}H and ^{13}C NMR spectral analysis. The upper regioisomer of **29** i.e., 8-methoxy-6*H*, 11*H*-isochromeno [4,3-c] chromen-11-one, was obtained in 46% yield (major) and the lower regioisomer of **29** (10-methoxy-6H, 11H-isochromeno [4,3-c] chromen-11-one) was obtained in 32% yield. Then we were interested to study the effect of introduction of two OMe groups. Therefore, we prepared **25d** and subjected it to intramolecular CDC under optimized conditions. Unexpectedly, trace amount of **30** was formed indicating that introduction of two OCH_3 groups have detrimental effect on the yield of the reaction. On the contrary, the starting precursors, **25e** and **25f**, having electron-withdrawing groups i.e., NO_2 and CN groups, respectively when subjected to the reaction did not furnish the desired compounds **31** and **32**, respectively. Overall, it can be inferred that the starting precursors having *m*-OMe group have beneficial effect. Electron-withdrawing groups have detrimental effect on the yield of the reaction. It also inferred that increasing the electron-donating group also do not have beneficial effect on the yield of the reaction.

4.4 Proposed Mechanism

To understand the activation sequence of two C-H bonds of the coumarin substrate in intramolecular cross-dehydrogenative coupling; Chen, Xu and co-workers carried out H/D exchange experimental study on the substrate analogous to our substrate and clearly explained that the initial palladation preferably occurs at C-3 position.[13a] A plausible mechanism for the intramolecular CDC has been proposed in Figure 6. Initial C-H activation takes place by deprotonation by the attack of $Pd(TFA)_2$ on the coumarin C-H bond of **25**. Then, the aryl palladium triflate complex A formed undergoes second ligand promoted C-H activation by CMD (cyclometallation and deprotonation step) to form a seven membered intermediate B. The intermediate B forms complex C by the release of triflic acid. Subsequently, the palladacycle undergoes reductive elimination by the release of Pd(0) and furnished the coupled

coumarin-benzopyran tetracyclic product **26**. The released Pd(0) is again oxidized by Ag(I) to regenerate Pd(II) and the catalytic cycle continues.

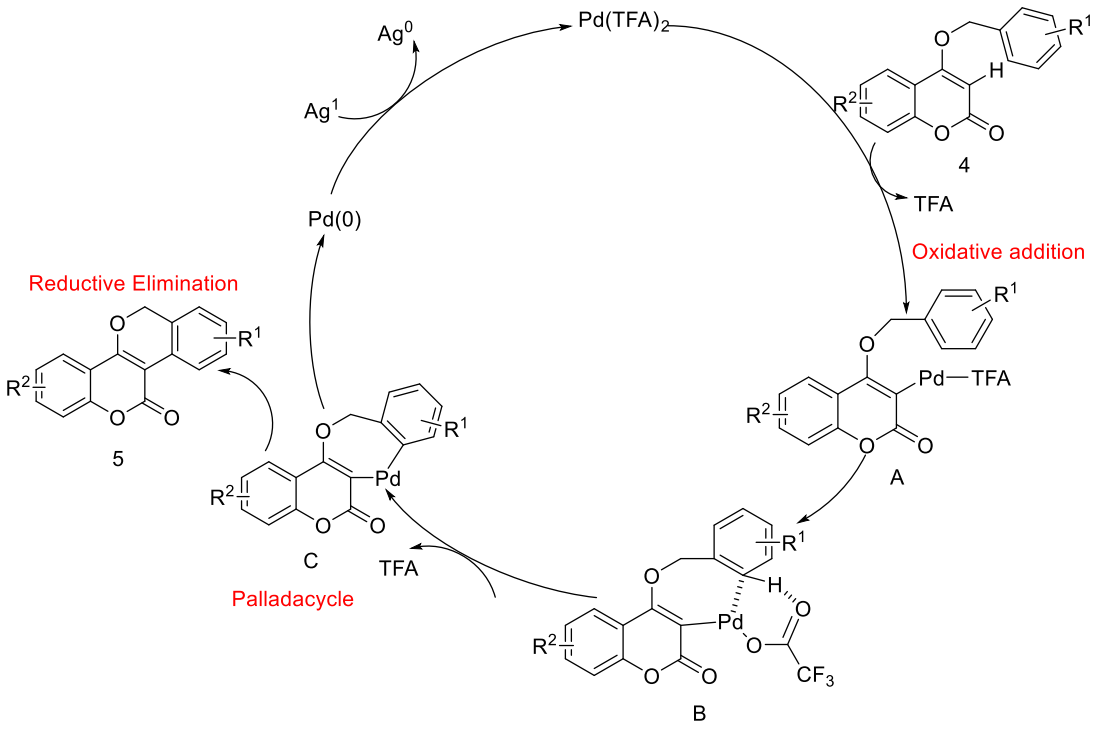


Figure 6. Proposed mechanism for the intramolecular cross-dehydrogenative coupling.

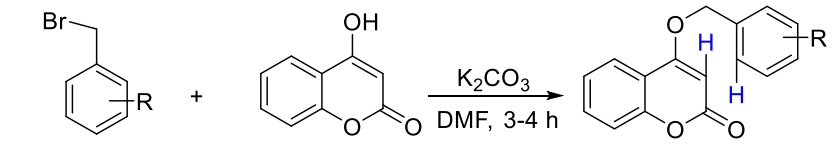
4.5 Conclusion

In conclusion, for the first time, Pd-catalyzed intramolecular cross-dehydrogenative coupling strategy has been developed to construct coumarin-benzopyran fused tetracyclic framework. Overall, the reaction is effectively better with single OMe group rather than two OMe group, but not at all with electron-withdrawing groups. The observation of increased yield in *m*-substituted OCH_3 and low yield in *p*-OCH_3 indicates that the electron-withdrawing inductive effect is favourable for the reaction. However, this possibility diminishes with the trace formation of cyclized product as in the case of nitro-substituted product. No further improvement in the yield has been observed in case of two OMe groups which infers that steric hindrance at ortho-position might be playing an important role in this CDC reaction. Though the reaction is simple, atom economic and no wasteful by-product is formed, this reaction can serve as a procedure in building a wide variety of coumarin-benzopyran fused tetracyclic framework for their further biological studies.

4.6 Experimental Section

4.6.1. General

All glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on complab melting point apparatus and are presented uncorrected. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum 2 spectrophotometer ^{1}H NMR and ^{13}C NMR spectra were recorded on ECS 400 MHz (JEOL) NMR spectrometer using $CDCl_3$, as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S QTof (Waters, USA) Spectrometer. Column chromatography was performed over Merck silica gel (particle size: 100-200 Mesh deactivated) procured from Qualigens (India), All chemicals and reagents were obtained from Sigma Aldrich (USA), Merck (India) or Spectrochem (India) and were used without further purification.



Substituted Benzylbromides | **4-Hydroxycoumarin** | **25 and 25a-f**

25: R = H
25a: R = *p*-CH_3 **25d:** R = 2,5-OCH_3
25b: R = *p*-OCH_3 **25e**: R = *m*-NO_2
25c: R = *m*-OCH_3 **25f**: R = *o*-CN

Scheme 2. Procedure for the synthesis of starting precursors **25** and **25a-f**.

4.6.2 General Procedure for the Synthesis of 4-(benzyloxy)-2-H-chromen-2-one derivatives (25 and **25a-f)[23]** 4-hydroxycoumarin (6 mmol, 1 equiv.) and K_2CO_3 (12.3 mmol, 2 equiv.) were stirred in DMF for 30 min and then substituted benzyl bromides (7 mmol, 1.2 equiv.) was added to the reaction mixture. The mixture was allowed to stir for 3-4 hours. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 20 mL); washed with distilled water (3 × 20 mL); then with brine (3 × 10 mL). The combined organic layer was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The resultant crude product was further purified by column chromatography using 9% ethylacetate/hexane which afforded pure **25** and **25a-f** in 60-70% yield range.

4.6.3 General Procedure for the Synthesis of 6*H*,11*H*-isochromeno [4,3-c] chromen-11-ones 26 in optimization study as given in table 1:

25 and **25a-f** (0.2 mmol, 0.05 g, 1 equiv.) were added with $Pd(TFA)_2$, (0.04 mmol, 0.0131 g, 0.2 equiv.), Ag_2CO_3 (0.3 mmol, 0.1093 g, 2 equiv.), CH_3COONa (0.4 mmol, 0.0325 g, 2 equiv.) and *tert*-amyl alcohol solvent (1ml). The reaction mixture was purged with nitrogen, degasified and heated at 120ºC for 24 hrs. The progress of

the reaction was monitored by TLC using 4:1 hexane/ethyl acetate as an eluent. After running the reaction for 24 hrs, the reaction mixture was filtered with ethyl acetate over celite. The organic layer was evaporated under reduced pressure to obtain the crude compound. The crude products were purified by column chromatography on 100-200 mesh deactivated silica either by chloroform or ethyl acetate/hexane mixture (upto 5:95) to obtain pure compounds **26-32** in 16-78% yield range.

4.6.4 Characterization data of all synthesized compounds 26-29.

Characterization data of 6*H*,11*H*-isochromeno [4, 3-c] chromen-11-ones (26-29)

6*H*,11*H*-isochromeno [4, 3-c] chromen-11-one (26)

Yellowish white solid; Yield: 45%; R_f (EtOAc/hexane: 20:80) = 0.69; m.p.: 162-170 ºC; FT-IR (KBr, νmax/cm^{-1}) 3427.99, 2923.05, 2859.3, 1706.41, 1606.02, 1091.24, 965.32, 759.37; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 7.88 Hz, 1H), 7.87 (d, *J* = 7.8, 1H), 7.56 (t, *J* = 7.28, 1H), 7.40 (t, *J* = 7.7, 1H), 7.35-7.28 (m, 3H), 7.13 (d, *J* = 7.28, 1H), 5.40 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.29, 160.24, 152.94, 132.60, 129.13, 128.32, 127.46, 126.74, 124.96, 124.15, 124.03, 123.20, 116.61, 115.29, 102.73, 69.83; HRMS (ESI) calcd. for $C_{16}H_{10}O_3$ $[M+H]^+$: 251.0703; found: 251.0705.

9-methyl-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one (27)

White solid; Yield: 34%; R_f (EtOAc/hexane:20:80) = 0.77; m.p.: 142-150 ºC; FT-IR (KBr, ν max/cm^{-1}) 3436.14, 2920.72, 2851.3, 1613.08, 1457.97, 1091.42, 758.85, 453.94. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.85 (dd, *J* = 7.9 Hz, 1.58 Hz, 1H), 7.57-7.53 (m, 1H), 7.34-7.26 (m, 2H), 7.12 (d, *J* = 6.92 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 5.36 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz,$CDCl_3$) 161.33, 160.31, 152.91, 138.99, 132.51, 128.95, 126.58, 125.5, 124.65, 124.12, 123.92, 123.20, 116.60, 115.39, 102.83, 69.81, 21.77; HRMS (ESI) calcd. for $C_{17}H_{12}O_3$ $[M+H]^+$: 265.0859; found: 250.0861.

9-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one (28)

White solid; Yield: 16%; R_f (EtOAc/hexane: 20:80) = 0.62; m.p.: 110-120 ºC; FT-IR (KBr, νmax/cm^{-1}) 3433.77, 2922.72, 2851.3, 1699.84, 1610.85, 1490.21, 1221.3, 1098.32, 1042.29, 986.39, 765.39; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 2.48 Hz, 1 H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 7.82 Hz, 1H), 7.35-7.29 (m, 2H), 7.04 (d, *J* = 8.24 Hz, 1H) 6.85 (dd, *J* = 8.28 Hz, 2.52 Hz, 1H), 5.35 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.30, 160.24, 152.98, 132.65, 128.05, 125.03,

124.15, 123.29, 119.60, 116.62, 114.50, 109.93, 69.68, 55.60; HRMS (ESI) calcd. for $C_{17}H_{12}O_4$ $[M+H]^+$: 281.0809; found: 281.0812.

8-methoxy-6*H*, 11*H*-isochromeno[4,3-c]chromen-11-one (29, upper regioisomer)

Yellowish white solid; Yield: 46%; R_f (EtOAc/hexane: 20:80) = 0.69; m.p.: 167-177 ºC; FT-IR (KBr, νmax/cm^{-1}) 3442.66, 2922.85, 2855.89, 1618.57, 1094.29, 1457.06; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, *J* = 8.76 Hz, 1H), 7.83 (dd, *J* = 7.92 Hz, 1.44 Hz, 1H), 7.54 - 7.50 (m, 1H), 7.33- 7.25 (m, 2H), 6.91 (dd, *J* = 8.78 Hz, 2.62 Hz, 1H) 6.65 (d, *J* = 2.52 Hz, 1H), 5.35 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$), 160.40, 159.74, 159.61, 152.57, 132.06, 129.34, 126.68, 124.092, 122.94.36, 119.24, 116.51, 115.43, 113.54, 110.28, 102.74, 69.66, 55.496; HRMS (ESI) calcd. for $C_{17}H_{12}O_4$ $[M+H]^+$: 281.0809; found: 281.0811.

10-methoxy-6*H*,11*H*-isochromeno[4,3-c]chromen-11-one (29, lower regioisomer)

Yellowish white solid; Yield: 30%; R_f (EtOAc/hexane: 20:80) = 0.38; m.p.: 158-168 ºC; FT-IR (KBr, ν max/cm^{-1}) 3453.94, 2922.11, 2855.89, 1727.73, 1602.22, 1468.46, 1272.09, 1079.89, 959.39, 751.17; ^{1}H NMR (400 MHz, $CDCl_3$) δ **7**.81 (dd, *J* = 7.92 Hz, 1.52 Hz, 1H), 7.54-7.50 (m, 1H), 7.34-7.24 (m, 3H), 7.00 (d, *J* = 8.36 Hz, 1H), 6.82 Hz (d, *J* = 7.4 Hz, 1H), 5.19 (s, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 162.182, 158.3, 155.47, 153.09, 132.52, 132.16, 129.36, 123.77, 122.67, 116.80, 116.58, 115.95, 115.23, 113.05, 104.09, 70.97, 56.15; HRMS (ESI) calcd. for $C_{17}H_{12}O_4$ $[M+H]^+$: 281.0809; found: 281.0892.

4.7 References

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4.8 Characterization spectral data (^{1}H NMR and ^{13}C NMR) of synthesized compounds 26-29.

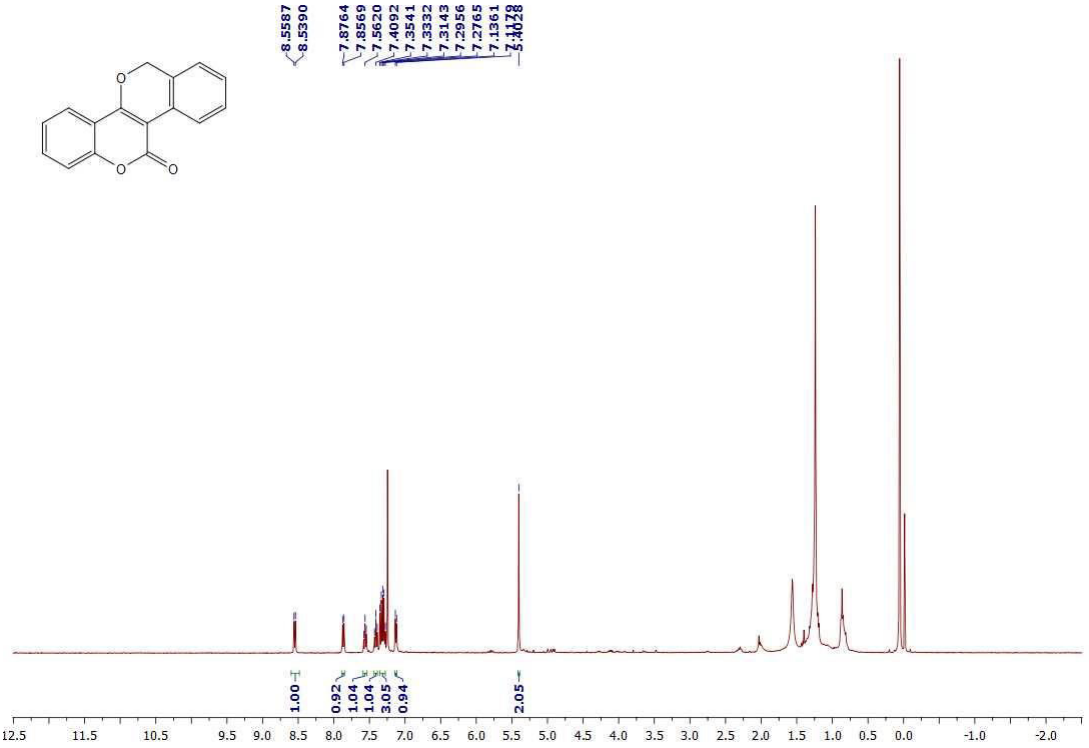


Figure 7: ^{1}H NMR spectra of 6H,11H-isochromeno [4, 3-c] chromen-11-one **26**.

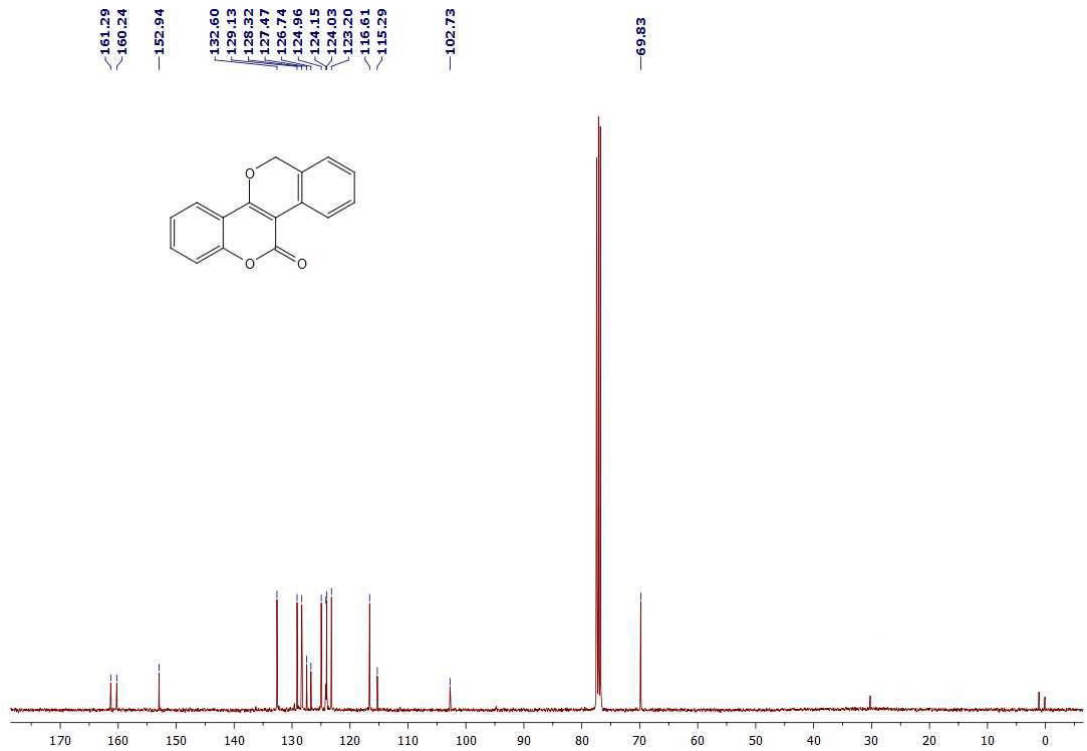


Figure 8: ^{13}C NMR spectra of 6H,11H-isochromeno [4, 3-c] chromen-11-one **26**.

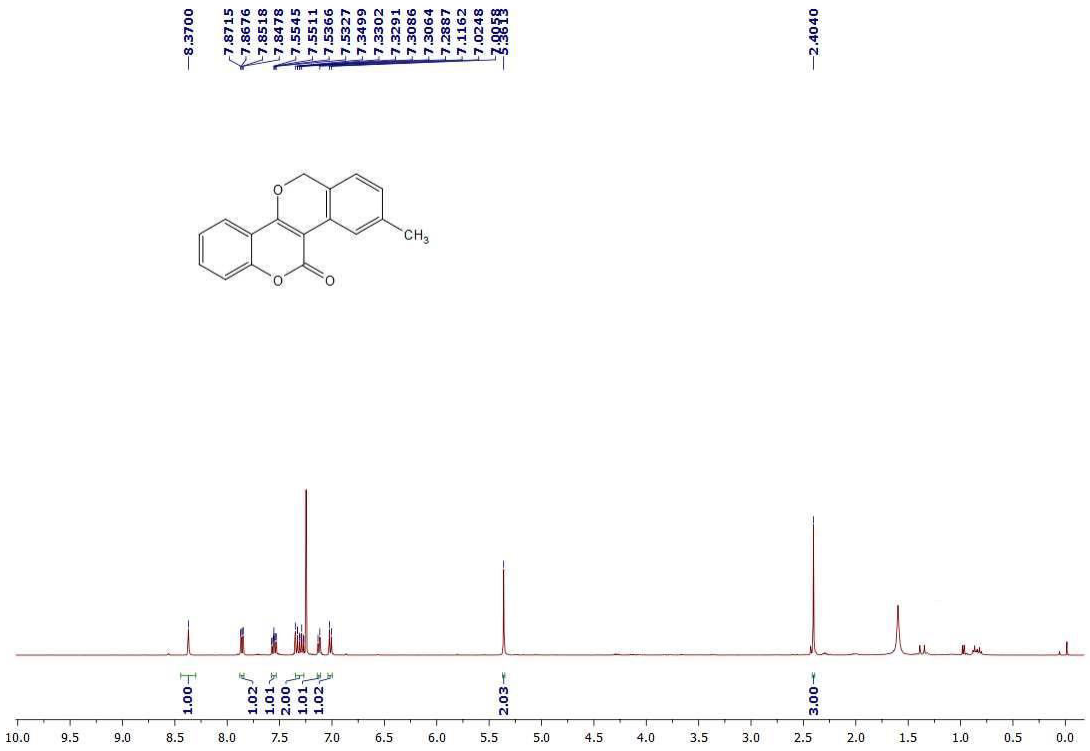


Figure 9: ^{1}H NMR spectra of 9-methyl-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **27**.

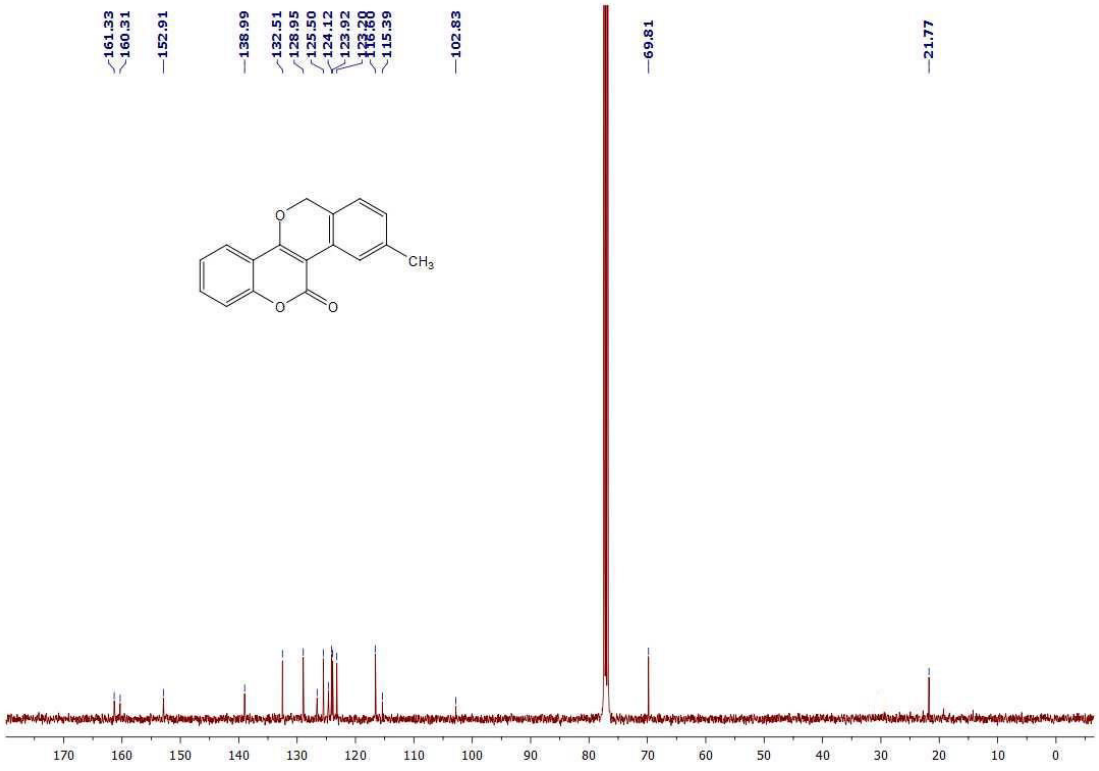


Figure 10: ^{13}C NMR spectra of 9-methyl-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **27**.

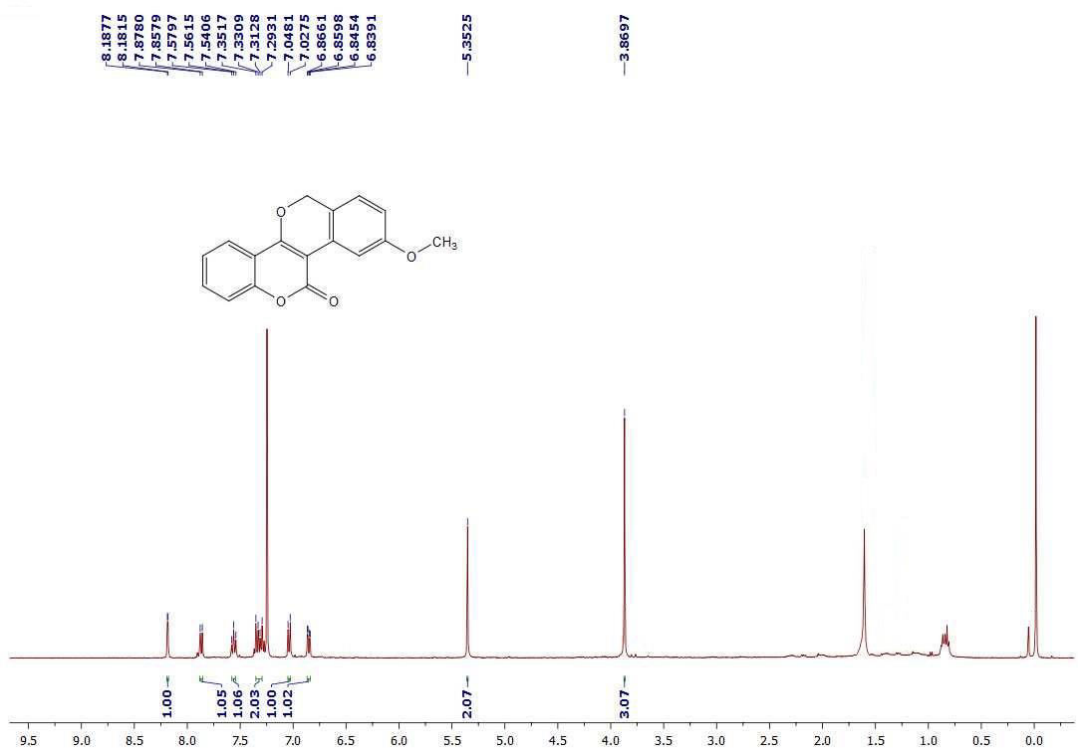


Figure 11: [1]H NMR spectra of 9-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **28**.

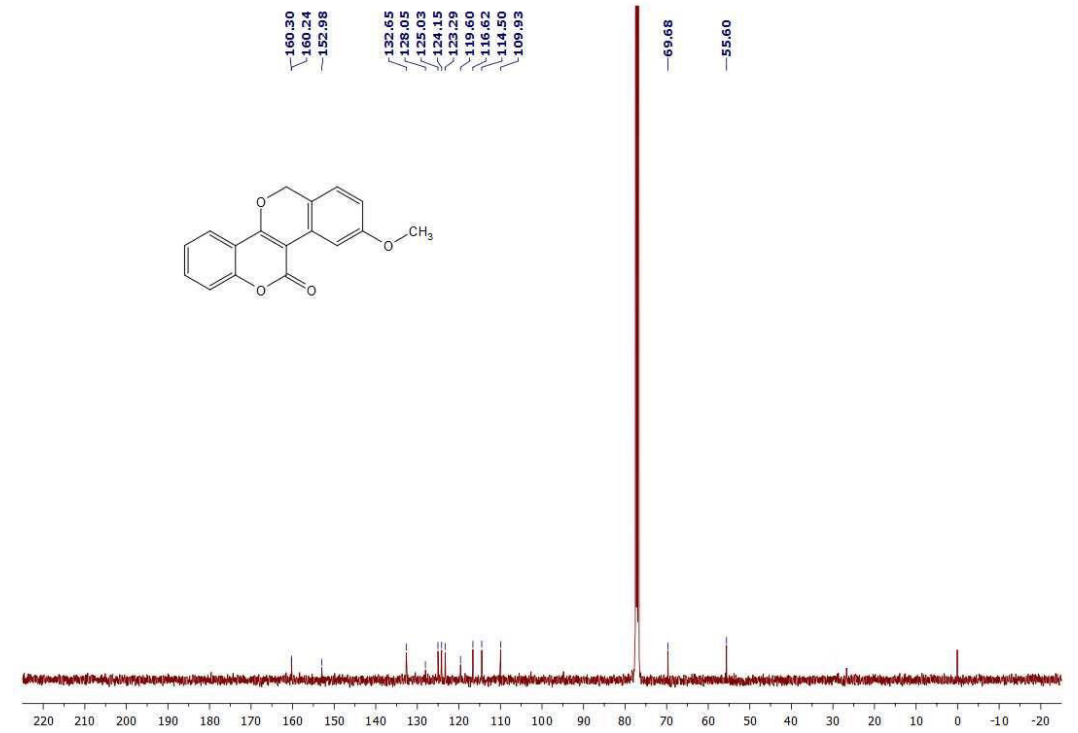


Figure 12: [13]C NMR spectra of 9-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **28**.

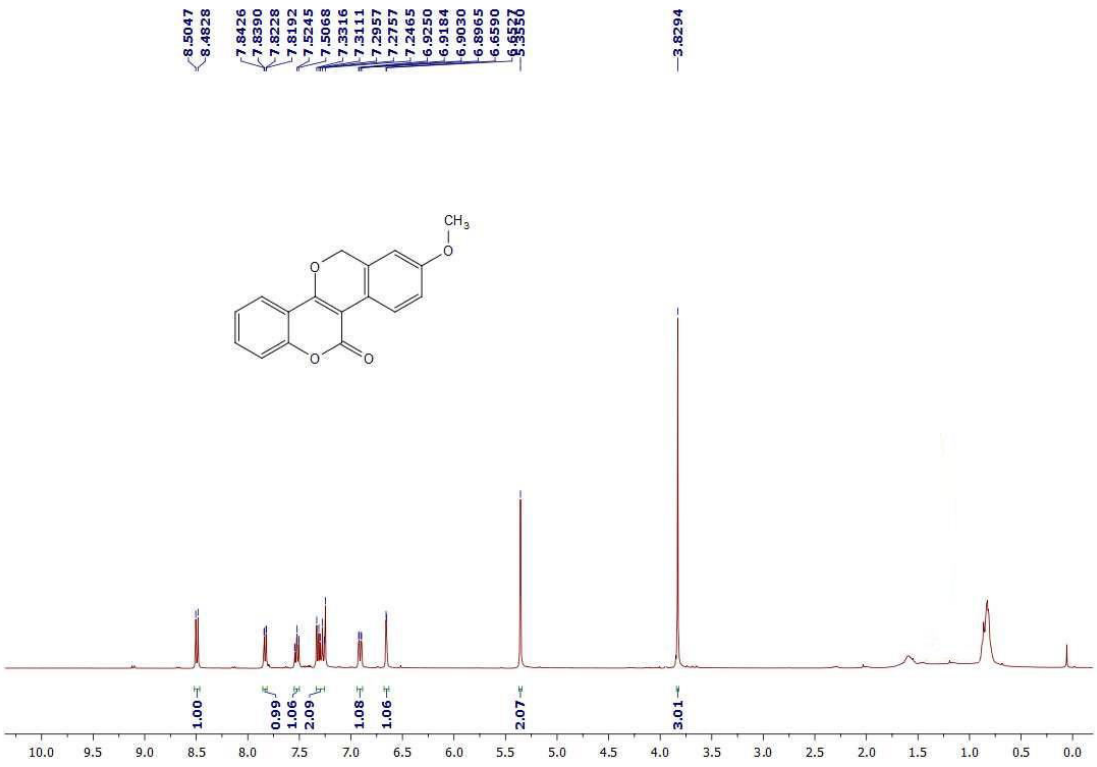


Figure 13: [1]H NMR spectra of 8-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **29 (Upper regioisomer)**.

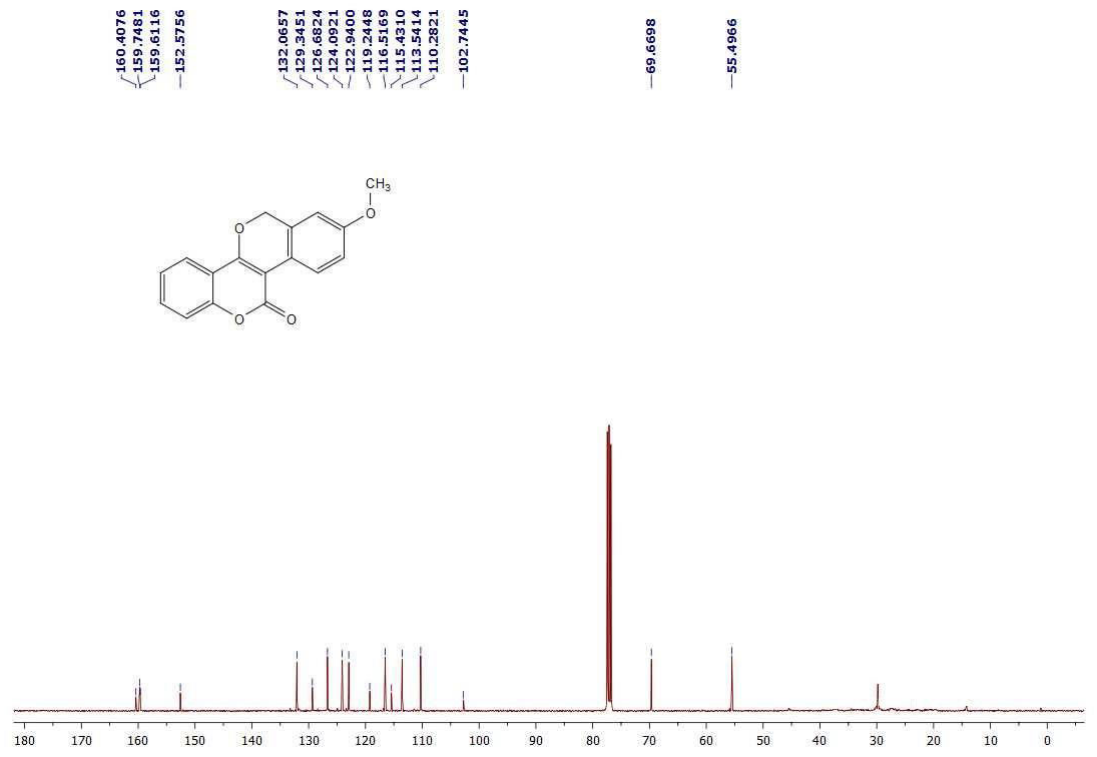


Figure 14: [13]C NMR spectra of 8-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **29 (Upper regioisomer)**.

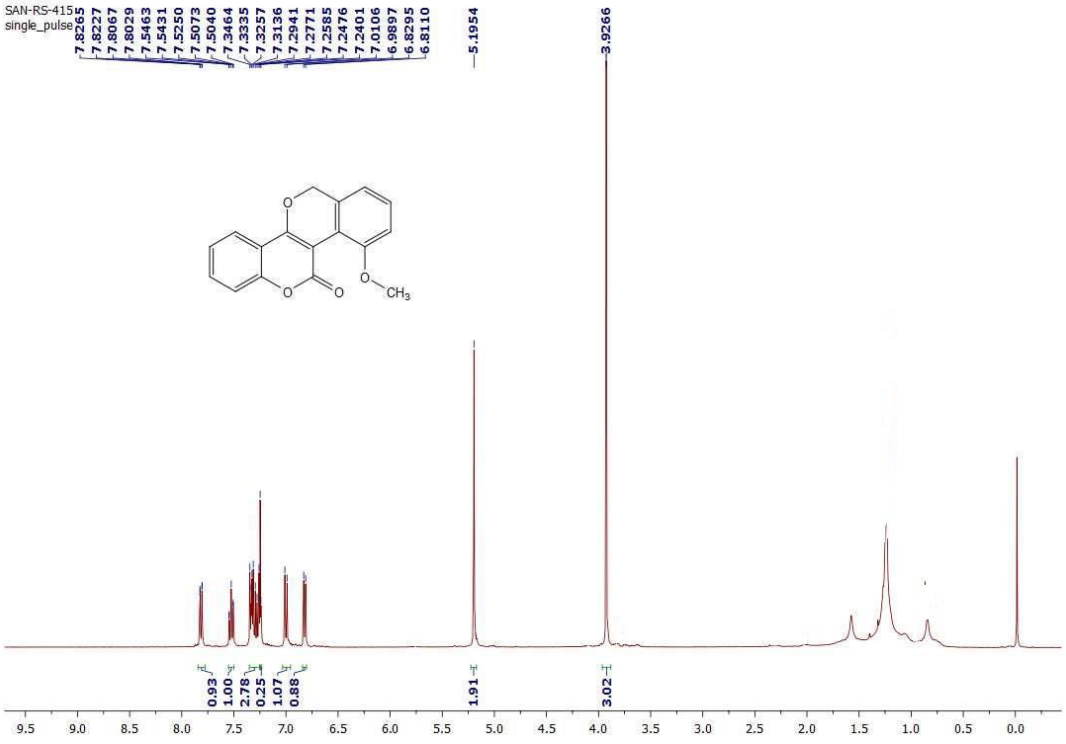


Figure 15: ^{1}H NMR spectra of 10-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **29 (Lower regioisomer)**.

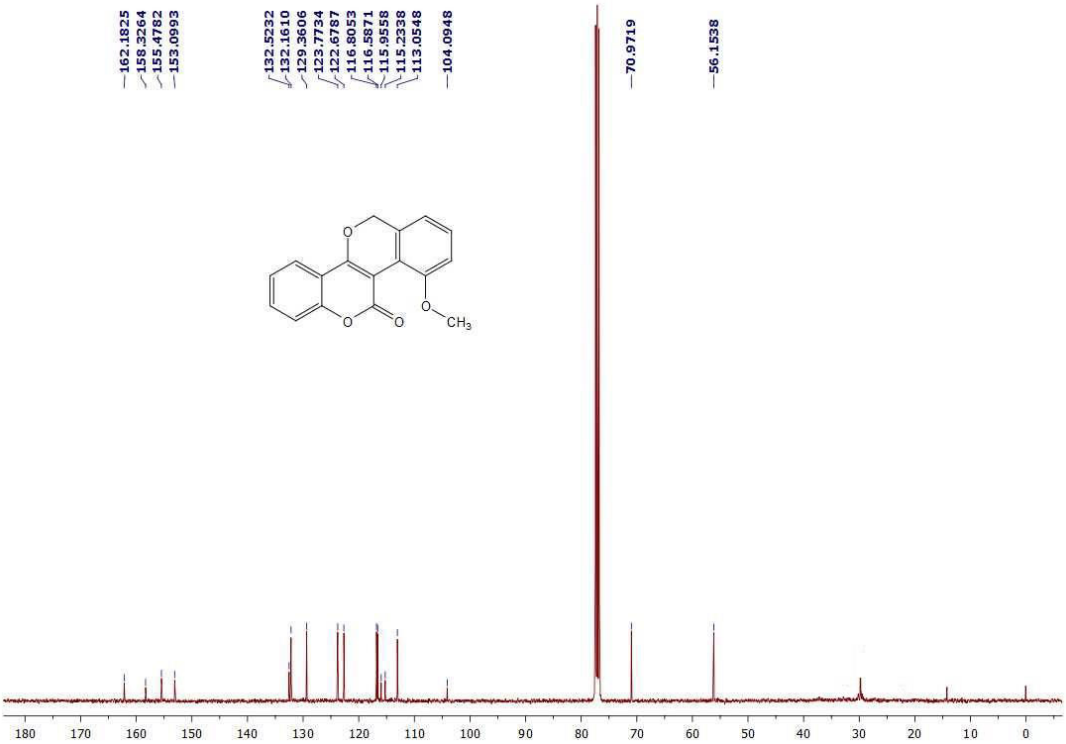


Figure 16: ^{13}C NMR spectra of 10-methoxy-6*H*, 11*H*-isochromeno [4, 3-c] chromen-11-one **29 (Lower regioisomer)**.

CHAPTER 5

Organo-catalyzed Direct C3-Arylation of 2H-Indazoles with haloarenes

5.1 Introduction

Transition metal-catalyzed direct arylation via C-H bond activation is a very effective and powerful tool for the construction of C-C bond formation in various pharmaceuticals, natural products, agrochemicals and bioactive molecules.[1] But now-a-days due to their toxicity and high cost complexes, synthetic community have been more attentive towards organocatalyzed direct C-H bond activation.[2] Using these greener catalysts, expensive transition metals and other organometallic reagents can be avoided while providing a strategic solution to construct pharmaceutically relevant molecular scaffolds that could serve as important moieties in the synthesis of pharmaceuticals, natural products, agrochemicals, and functional materials.[3]

Organocatalyzed direct C-H arylation has emerged as a rapidly growing area for chemical synthesis.[2] Few examples have been reported in the literature for direct C(sp^2)-H arylation via organocatalysis.[2] Hence, organocatalyzed C(sp^2)-H bond functionalization offers an alternative strategy to conventional cross-coupling methods.

Indazole is an important class of bioactive structural core and has been recognized as an effective pharmacophore present in various pharmaceuticals and natural products.[4] In particular, several 2*H*-indazole containing bioactive heterocycles **1**-**8** have attracted chemists tremendously due to their wide applications in drug discovery (Figure 1).[5]

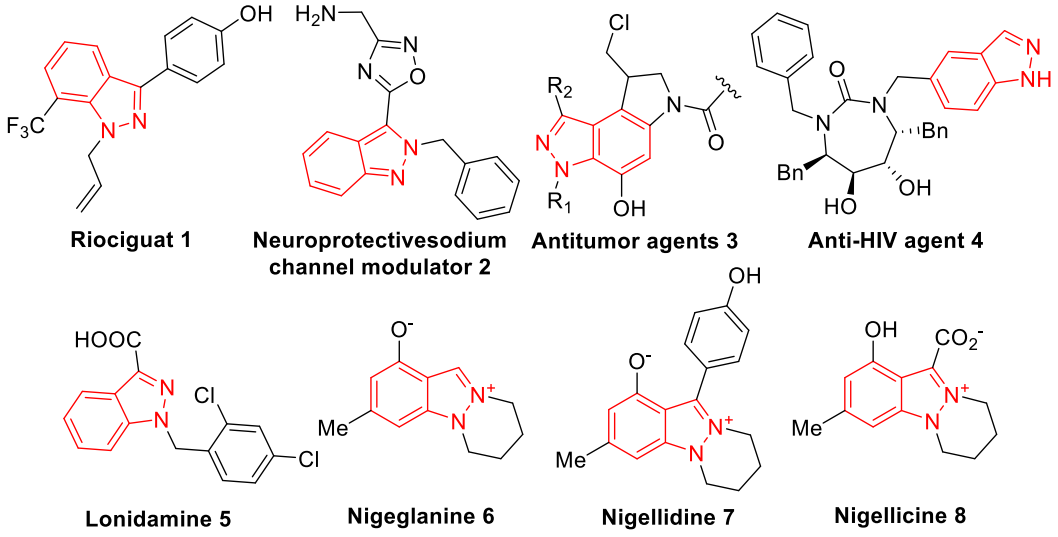
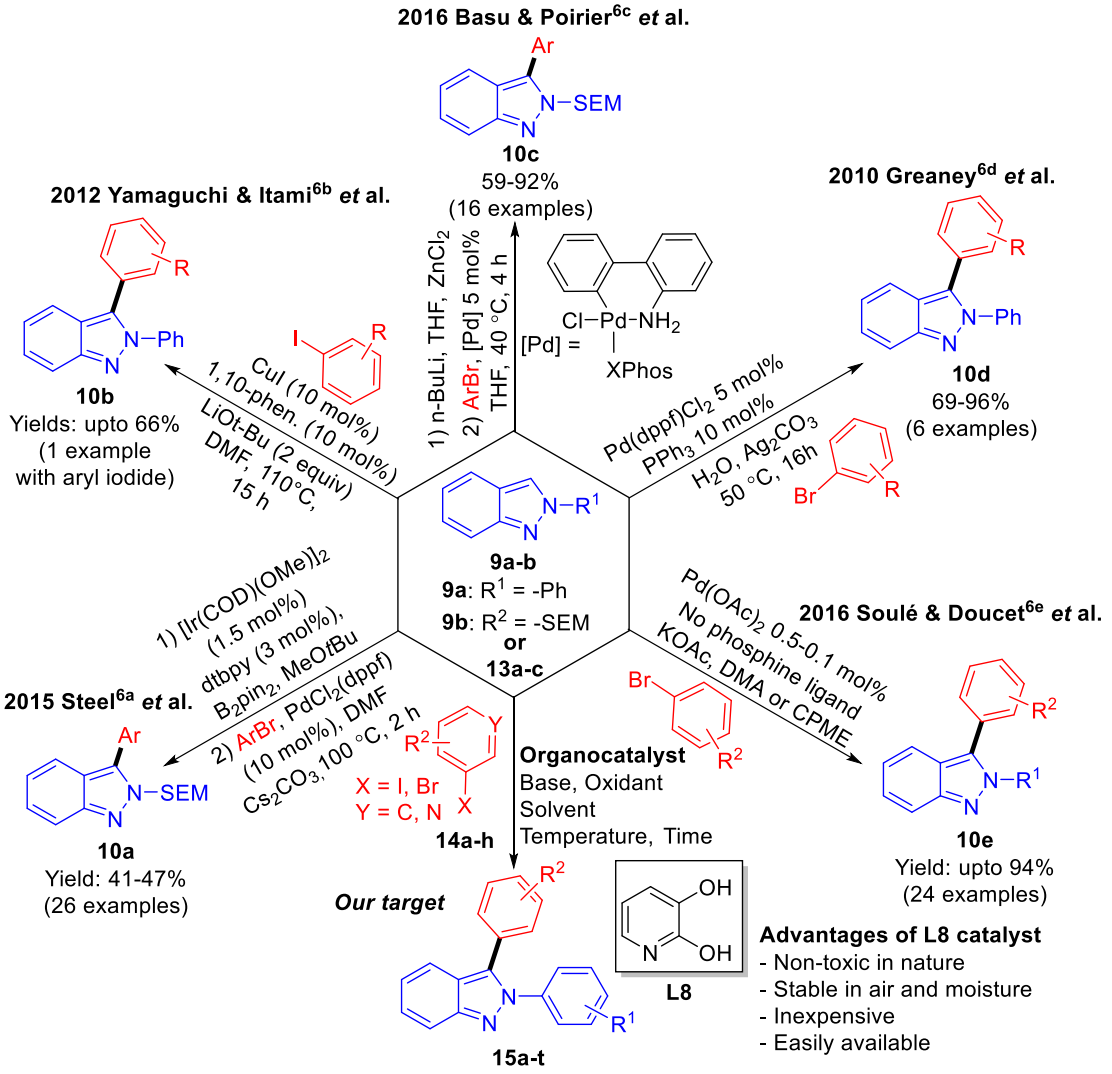


Figure 1. Representative examples of indazoles present in pharmaceuticals and natural products.

In our endeavor to prepare novel bioactive moieties for our structure-activity relationship studies as a part of drug discovery program, we were interested in an efficient preparation of a novel series of analogues of C-3 arylated 2*H*-indazoles for their anticancer properties.

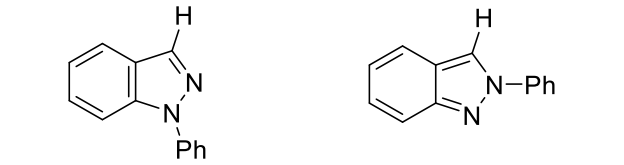
Consequently, a number of methods have been reported in the literature to construct arylated 2*H*-indazoles.[6a-e] Several metal-based catalytic systems such as Ir,[6a] Cu,[6b] and Pd[6c-e] have been reported for the synthesis of C-3 arylated *2H*-indazoles **10a-e** via direct C(sp^2)–H arylation at C-3 position of *2H*-indazoles **9a-b**. However, all these metal-based methodologies require transition metals, high temperatures (100-165 °C) and are incompatible with several sensitive functionalities. As a part of our drug discovery program, efficient access to 3-(hetero)aryl-*2H*-indazoles is highly needed to enable the preparation of a range of substituted indazoles.



Scheme 1. Schematic representation of existing strategies and our work.

Among the series of *1H*-indazoles **11** and *2H*-indazoles **12** (figure 2), C-3 position of 2*H*-indazoles is reported to be more active as compared to 1*H*-indazoles;[6a,6b,6d] various methods have been reported for C-H arylation[6a-e] using costly metal and metal complexes as catalyst along with costly ligands (Scheme 1). Moreover, metal salts

and complexes are toxic in nature and unstable to air and moisture, keeping this in mind, our study aimed at developing reliable organocatalyzed C-H arylation method for 2*H*-indazoles. Herein, we report an effective organocatalytic system based on 2,3-dihydroxypyridine as non-toxic, biodegradable, biologically active, easily available and inexpensive organocatalyst.



1-phenyl-*1H*-indazole **11** (1*H* series) 2-phenyl-*2H*-indazole **12** (2*H* series)

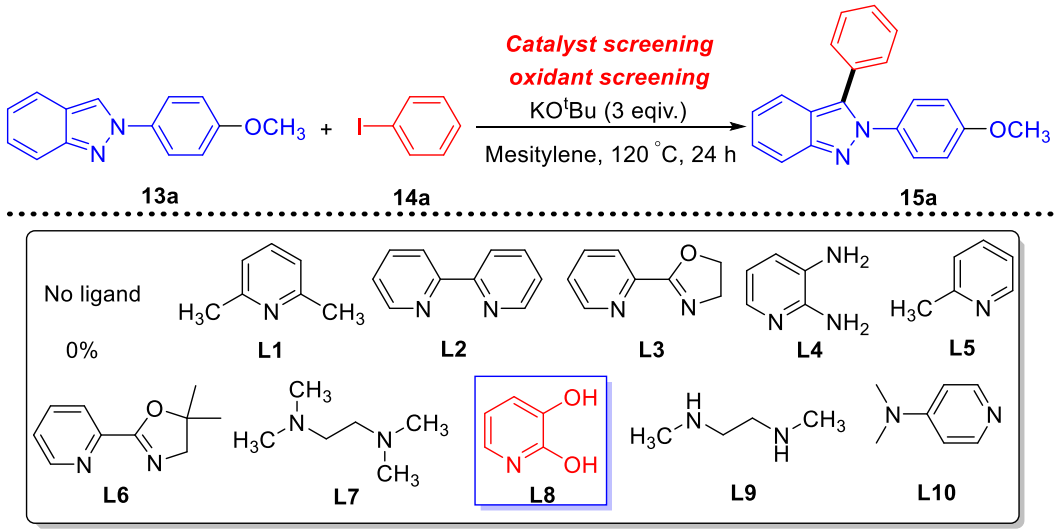
Figure 2. Structures of tautomeric 1-phenyl-*1H*-indazole and 2-phenyl-*2H*-indazole.

5.2 Results and Discussion

5.2.1 Initial investigation: Screening of organocatalysts L1-L10 and several oxidants taking KO^{t}Bu as strong base.

We started our initial investigation on the model substrate 2-(4-methoxyphenyl)-*2H*-indazole **13a** for the direct C-3 arylation with 4-methoxyiodoanisole **14a** taking KO^{t}Bu as strong base in DMSO solvent at 120 °C. However, the desired product **15a** could not be obtained (Table 1, entry 1). Further carrying out the same reaction in DMF and mesitylene solvents also did not furnish the desired product **15a**, respectively (Table 1, entry 2-3).

Table 1. Optimization study: Systematic Screening of organocatalysts **L1-L10** for the C-3 direct arylation reaction.[a, b]



Entry	Ligand/Oxidant	Solvent	Time (h)	Yield[c] (%)
1	-	DMSO	36	00
2	-	DMF	36	00
3	-	Mesitylene	36	00

4	L1	DMSO	36	21
5	L1	DMF	36	15
6	L2	DMSO	36	23
7	L3	DMSO	36	26
8	L4	DMSO	36	36
9	L5	DMSO	36	24
10	L6	DMSO	36	24
11	L7	DMSO	36	20
12	L8	DMSO	36	38
13	L9	DMSO	36	21
14	L10	DMSO	36	32
15	L8	DMF	36	30
16	L8	C_6H_6	36	30
17	L8	DMA	36	traces
18	L8	1,4-Dioxane	36	traces
19	L8	toluene	36	33
20	L8	benzene	36	30
21	L8	Acetonitrile	36	traces
22	L8	ethanol	36	traces
23	L8	mesitylene	36	45
24	L8	mesitylene	24	45
25	L8/DCP	mesitylene	36	traces
26	L8/TBHP	mesitylene	36	23
27	L8/DDQ	mesitylene	36	traces
28	L8/Oxone	mesitylene	36	traces
29	L8/$K_2S_2O_8$	mesitylene	36	37
30	L8/O_2 (balloon)	mesitylene	48	traces
31[d]	L8/$(NH_4)_2S_2O_8$	mesitylene	36	35
32	L8/DTBP	mesitylene	24	52
33	L8/DTBP	mesitylene	36	16

[a]Reaction conditions: 2-4-(methoxyphenyl)-2H-indazole **13a** (1equiv.), 4-methoxyiodoanisole **14a** (1.5 equiv.), Organic ligands **L1-L10** (20-100 mol%), KO^{t}Bu (3equiv.), oxidant in DMSO (0.5 mL), 120°C, 24 h. [b]Isolated yield after column chromatography. [c]Chromatographic yields. [d]LiOtBu (3.0 equiv.), 160 °C.

Once failed in getting the desired product; we started screening of the different organocatalysts **L1-L10**. Therefore, organic ligands **L1-L10** were screened one by one keeping the reaction conditions similar to entry no. 1 in DMSO/DMF solvents (Table 1 entries 4-14). However, **15a** was obtained in 38% yield with organocatalyst **L8** (Table 1, entry 12). Once we were successful in obtaining 15a in significant amount (entry 12); we then screened different solvents such as DMF, C_6H_6, DMA, 1,4-dioxane, toluene, acetonitrile, ethanol, mesitylene etc. taking **L8** as organocatalyst (Table 1, entries 15-24). It was noticed that in several solvents such as DMF, C_6H_6, DMA, 1,4-dioxane, toluene, acetonitrile and ethanol; **15a** was obtained either in traces or in lesser yields in comparison to the yield obtained in the reaction carried out in entry 12. However, we were delighted to obtain **15a** in 45% yield in mesitylene solvent when the reaction was allowed to stir for 36 hours. (Table 1, entry 23).

However, it was observed that 45% yield of **15a** was obtained when the reaction was stirred for only 24 hrs (Table 1, entry 24).

Having **L8** organocatalyst and mesitylene solvent in hand, we also screened different oxidants (Table 1, entries 25-33). While **15a** was obtained only in traces when the reactions were carried out using Dicumyl peroxide (DCP), DDQ, oxone or in oxygen balloon in the presence of L8 in mesitylene (Table 1, entries 25, 27, 28 and 30, respectively); 23%, 37%, 35% and 52% of **15a** was obtained when the reactions were carried out in the presence of TBHP, $K_2S_2O_8$, $(NH4)_2S_2O_8$ and DTBP, respectively (Table 1, entries 26, 29, 31 and 32). However, lower yield of the product was obtained with DTBP oxidant if the reaction was stirred for longer period of time (Table 1, entries 33). Therefore, several oxidants were screened while using **L8** in mesitylene out of which DTBP proved to be the best oxidant. After screening numerous variations of the reaction, the optimal selectivity was achieved at 120 °C using, 2,3-dihydroxy pyridine at a concentration of 100 mol% in 24 h. To assess the influence of the oxidant on the product yield, we have carried out the reaction in the absence of DTBP; the yield of **15a** has been decreased substantially. This indicates the need of DTBP upto 4 equivalents. It also infers that as the equivalent of DTBP increases, the yield of the desired product decreases. To our delight indazoles itself got dimerized when equivalents of DTBP reaches upto 8 equivalents.

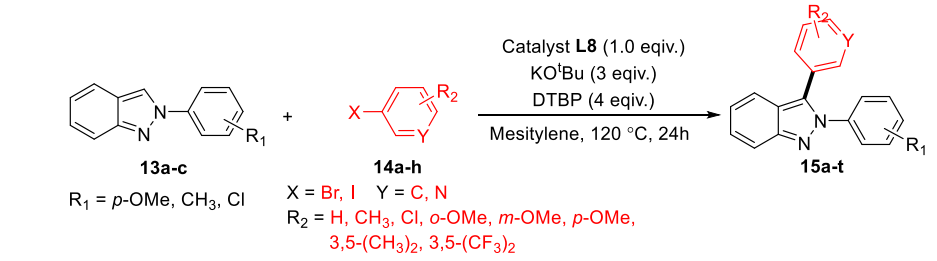
Finally, the optimized reaction condition is composed of *2,3-dihydroxypyridine (100 mol %), KO^{t}Bu (3 equiv) and DTBP (4.0 equiv) in mesitylene at 120 °C for 24h*.

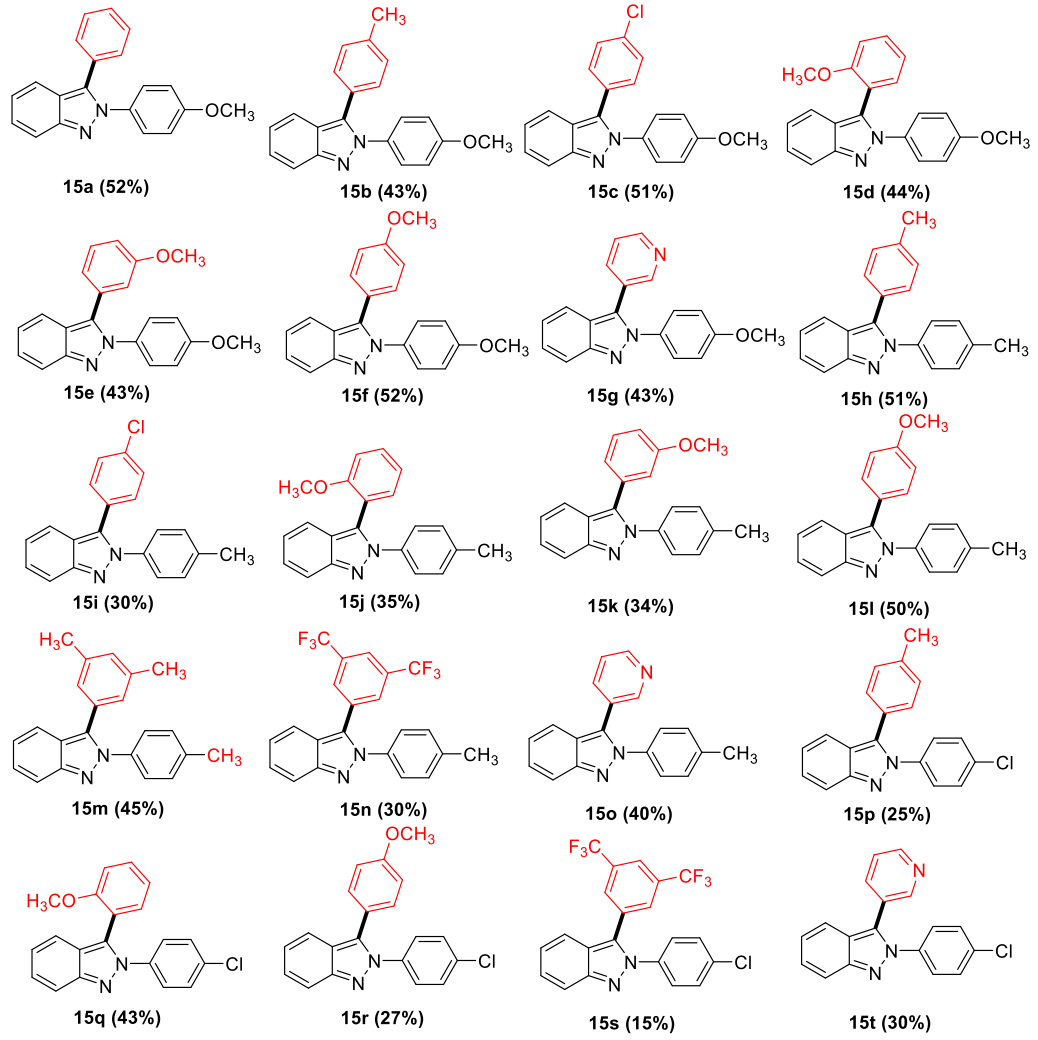
5.2.2 Substrate scope

Several substituted *2H*-indazoles **13a-c** (R_1 = *p*-OMe, CH_3, Cl) were reacted with substituted haloarenes **14a-h** [X = Br, I; Y = C, N; R_2 = H, CH_3, Cl, *o*-OMe, *m*-OMe, *p*-OMe, 3,5-$(CH_3)_2$, 3,5-$(CF_3)_2$] under the optimized reaction conditions which furnished the desired arylated 2H-indazoles **15a-t** in 15-52% yield range (Scheme 2). The starting precursor **13a** (R_1 = *p*-OMe) was further reacted with substituted halo arenes **14b-h** [X = Br, I; Y = C, N; R_2 = CH_3, Cl, *o*-OMe, *m*-OMe, *p*-OMe, 3,5-$(CH_3)_2$, 3,5-$(CF_3)_2$] under the optimized conditions furnished **15b-g** in 43%, 51%, 44%, 43%, 52%, and 43% yields, respectively. This infer us that C-3 arylation of substituted 2H-indazole with haloarenes having electron-donating (EDG) group or heteroaromatic haloarenes do not have much impact on the yield of the reaction. Similarly, The next starting precursor **13b** (R_1 = Me) was also reacted with substituted

halo arenes **14b-h** [X = Br, I; Y = C, N; R_2 = CH_3, Cl, *o*-OMe, *m*-OMe, *p*-OMe, 3,5-$(CH_3)_2$, 3,5-$(CF_3)_2$] under the optimized conditions furnished **15h-o** in 51%, 30%, 35%, 34%, 50%, 45%, 30% and 40% yields, respectively.

Scheme 2: Substrate scope.[a]



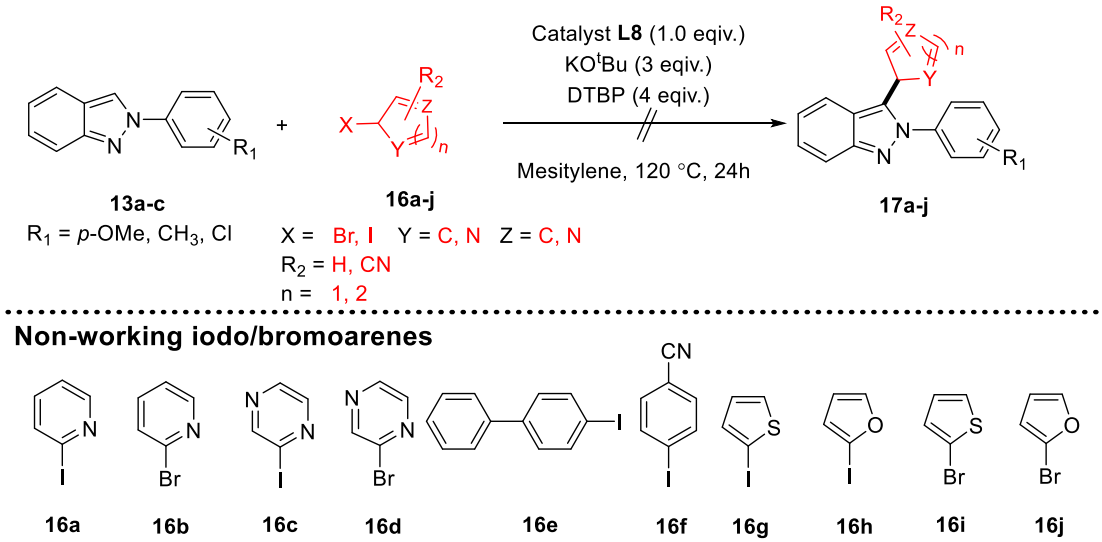


[a]*Reaction conditions*: Substituted *2H*-indazoles **13a-c** (0.2 mmol, 1 equiv.), substituted haloarenes **14a-h** (0.3 mmol, 1.5 equiv.), **L8** (100 mol %), KO^{t}Bu (0.6 mmol, 3 equiv.) and DTBP (0.8 mmol, 4 equiv.) in mesitylene (0.5 mL), heated at 120 °C for 24 h.

This also infers us that C-3 arylation of substituted 2H-indazole with haloarenes having electron-donating (EDG) group or heteroaromatic haloarenes do not show significant improvement on the yield of the reaction. Furthermore, the next starting

precursor **13c** (R_1 = Cl) was also reacted with substituted halo arenes **14b-h** [X = Br, I; Y = C, N; R_2 = CH_3, *o*-OMe, *p*-OMe, 3,5-$(CF_3)_2$] under the optimized conditions furnished **15p-t** in 25%, 43%, 27%, 15%, and 30% yields, respectively. This infers us that inductive effect of chlorine atom also do not have much impact on the yield of the reaction.

Furthermore, it has also been noted that this reaction does not work with substrates **16a-j** in which many of them belongs to the category of heterocyclic haloarenes which did not furnish the desired arylated *2H*-indazoles **17a-j** (Scheme 3).



Scheme 3. Non-working iodo/bromoarenes

5.3 Proposed reaction mechanism

The plausible reaction mechanism for the C-3 arylation reaction have been depicted in Figure 3. Initially, KO^{t}Bu coordinated to 2,3-dihydroxypyridine catalyst to form Complex which generates [Ar-I] radical anion via SET mechanism. [Ar-I] radical anion subsequently loses iodide anion to generate [Ar] free radical and [Complex] radical cation. The [complex] cation is regenerated with the loss of O^{t}Bu radical. ^{t}BuO radical formed, via thermal decomposition of DTBP, takes up C-3 hydrogen of 2*H*-indazole molecule to generate C-3 free radical of 2H-indazole. DTBP stabilizes the generated free radical. Both the free radicals ([Ar] free radical and the C-3 free radical of 2H-indazole) combines to form C-C bond at C-3 position of 2*H*-indazole.

5.4 Conclusion

In conclusion, we have described a concise and efficient method for the direct C-3 arylation of *2H*-indazoles using 2,3-dihydroxypyridine as an organocatalyst in the presence of KO^{t}Bu as an inexpensive base and DTBP as an oxidant in mesitylene at

120 °C for 24h. The methodology reported herein was found to show good functional group tolerance, and a wide range of differently substituted derivatives could be synthesized in good to excellent yields. This methodology could serve as an attractive and alternative pathway to the previously reported metal-catalyzed C-H arylation of 2*H*-indazoles. To the best of our knowledge, the present synthetic protocol has never been described so far.

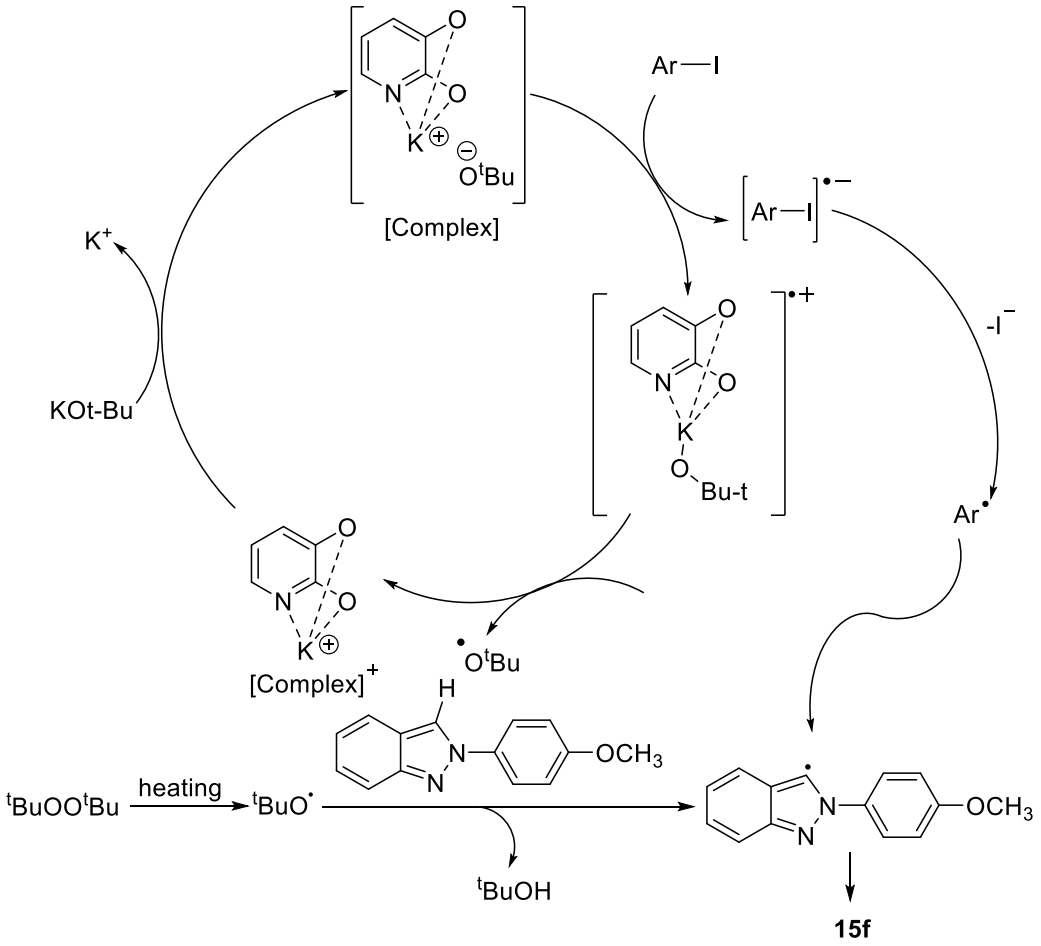


Figure 3. Plausible mechanism.

5.5 Experimental Section

5.5.1. General

All the reagents and catalysts used in the study were procured from Sigma-Aldrich, TCI and Spectrochem and used as received. IR spectra were recorded on a Perkin-Elmer FT IR spectrometer as thin films and indicated, with ν_{max} in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on ECS 400 MHz (JEOL) NMR spectrometer using $CDCl_3$ as solvent and NMR spectra were recorded in $CDCl_3$ on JEOL ECS 400 (400 MHz) instrument at a constant temperature of 300 K using tetramethylsilane (0.00 ppm) as an internal reference. Chemical shifts (δ) are reported in parts per million

(ppm) from lower to higher field and referenced to residual solvent ($CDCl_3$: δ 7.26/77.16, $^1H/^{13}C$ NMR) NMR shifts. Coupling constants (*J*) are reported in Hertz (Hz) and described as abbreviations: m = multiplet, q = quartet, t = triplet, d = doublet, s = singlet, br = broad. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S Q Tof (Waters, USA) Spectrometer. Melting points were taken in open capillaries on complab melting point apparatus and are presented uncorrected. For thin layer chromatography, TLC Silica gel 60 F_{254} (from Merck) used, compounds were visualized by irradiation with UV light. Column chromatography was performed using Rankem silica gel 100-200 mesh (approximately 15-20 g per 1 g of the crude product).

5.5.2 General procedure for the arylation of 2*H*-indazole (15a-t) taking 15e as representative example:

A 10 mL glass reaction vial was charged with substituted indazoles **13a-c** (1.0 equiv), substituted haloarenes **14a-h** (1.5 equiv), mesitylene (0.5 mL), organocatalyst **L8** (100 mol %), KO^{t}Bu (0.6 mmol, 3.0 equiv) and DTBP (0.8 mmol, 4 equiv). The reaction mixture was allowed to stir at 120 °C for 24 h. After completion of reaction, the reaction mixture was quenched with water (3 × 20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine (3 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (ethyl acetate /hexane) to afford the arylated 2H-indazole **15a-t** (Scheme 1).

5.5.3 Characterization data of organocatalyzed C-3 arylated 2*H*-indazoles (15a-15t):

2-(4-methoxyphenyl)-3-phenyl-2*H*-indazole (15a):

From 2-(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 1-iodobenzene (0.068 g, 0.3 mmol, 1.5 equiv), **15a** was obtained after purification by flash chromatography on silica gel (hexane: EtOAc = 95:5) in 41% (0.0275 g) yield as a muddy white solid (m.p.: 112-113 °C); FT-IR (ν max/cm^{-1}): 2939, 1604, 1511, 1246, 1025, 833, 754. ^1H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.41-7.33 (m, 8H), 7.14-7.11 (m, 1H), 6.89-6.87 (m, 2H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.41, 148.87, 135.36, 133.42, 130.07, 129.75, 128.84, 128.29, 127.26, 126.92, 122.44, 121.65, 120.56, 117.72, 114.22, 55.59.; HRMS calcd for $C_{20}H_{17}N_2O$ $[M+H]^+$: 301.1336, found: 301.1338.

2-(4-methoxyphenyl)-3-(*p*-tolyl)-2*H*-indazole (15b):

From 2,3-bis(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 1-iodo-4-methylbenzene (0.073 g, 0.3 mmol, 1.5 equiv), **15b** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 95:5) in 43% (0.030 g) yield as a white solid (m.p.: 140-141 °C); FT-IR (ν max/cm^{-1}): 2989, 1588, 1472, 1250, 1037, 825, 755. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.4 Hz, 1H) 7.69 (d, *J* = 8.4 Hz, 1H), 7.34-7.34 (m, 3H), 7.26-7.09 (m, 5H), 6.88 (d, *J* = 8.4 Hz, 2H) 3.82 (s, 3H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.39, 148.84, 138.30, 135.58, 133.48, 129.60, 127.28, 127.06, 126.94, 123.39, 122.27, 121.59, 120.69, 117.62, 114.21, 55.58, 21.45; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O$ $(M+H)^+$: 315.1492; Found: 315.1495.

3-(4-chlorophenyl)-2-(4-methoxyphenyl)-2*H*-indazole (15c):

From 2-(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 1-chloro-4-iodobenzene (0.079 g, 0.3 mmol, 1.5 equiv), **15c** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 96:4) in 51 % (0.038 g) yield as a white solid (m.p.: 149-150 °C); FT-IR (ν max/cm^{-1}): 2939, 1605, 1511, 1243, 1020, 829, 751. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.8 Hz, 1H) 7.66 (d, *J* = 8.4 Hz, 1H) 7.38-7.27 (m, 7H), 7.16-7.12 (m, 1H), 6.90 (d, *J* = 8.8Hz, 2H) 3.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.58, 148.88, 134.40, 134.05 133.12, 130.91, 129.19, 128.54, 127.27, 127.02, 122.79, 121.61, 120.16, 117.87, 114.36, 55.62. HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_2O$ $(M+H)^+$: 335.0946; Found: 335.0944.

3-(2-methoxyphenyl)-2-(4-methoxyphenyl)-2*H*-indazole (15d):

From 2-(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1.0 equiv) and 2-iodoanisole (0.078 g, 0.3 mmol, 1.5 equiv), **15d** was obtained after purification by flash chromatography on silica gel (hexane: EtOAc = 97:3) in 44% (0.0324 g) yield as a white solid (m.p.: 152-153 °C); FT-IR (ν max/cm^{-1}): 2936, 1602, 1512, 1249, 1022, 828, 751, 602. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.8 Hz, 1H), 7.54 (d, J = 8.4, 1H), 7.41-7.30 (m, 5H), 7.09-7.03 (m, 2H), 6.88-6.82 (m, 3H) 3.79 (s, 3H), 3.42 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.03, 156.88, 148.80, 134.63, 132.42, 131.84, 130.61, 126.63, 125.90, 121.52, 121.86, 120.88, 120.76, 119.22, 117.62, 113.80, 111.51, 55.56, 55.06; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O_2$ $(M+H)^+$: 331.1441; Found: 331.1441.

3-(3-methoxyphenyl)-2-(4-methoxyphenyl)-2*H*-indazole (15e):

From 2-(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 3-iodoanisole (0.078 g, 0.3 mmol, 1.5 equiv), **15e** was obtained after purification by

flash chromatography on silica gel (hexane: EtOAc = 97:3) in 43% (0.03164 g) yield as a white solid (m.p.: 127-128 °C); FT-IR (ν max/cm^{-1}): 2937, 1608, 1513, 1234, 1051, 830, 753. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.8 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.37-.7.27(m, 4H), 7.15-7.11 (m, 1H), 6.93-6.88 (m, 5H), 3.82 (s, 3H), 3.72 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.71, 159.43, 148.84, 135.19, 133.46, 131.25, 129.89, 127.23, 126.91, 122.46, 122.20, 121.63, 120.57, 117.73, 115.06, 114.21, 114.10, 55.62, 55.33; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O_2$ $(M+H)^+$: 331.1441; Found: 331.1445.

2,3-bis(4-methoxyphenyl)-2*H*-indazole (15f):

From 2,3-bis(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 4-iodoanisole (0.078 g, 0.3 mmol, 1.5 equiv), **15f** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 95:5) in 52 % (0.038 g) yield as a white solid (m.p.: 138-139 °C); FT-IR (ν max/cm^{-1}): 2933, 1607, 1513, 1232, 1050, 829, 752. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.35-7.32 (m, 3H), 7.28-7.24 (m, 2H), 7.12-7.08 (m, 1H), 6.93-6.87 (m, 4H), 3.83 (s, 3H), 3.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.58, 159.33, 148.81, 135.34, 133.51, 131.02, 127.25, 126.88, 122.34, 122.14, 121.50, 120.65, 117.63, 114.34, 114.20, 55.59, 55.38; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O_2$ $(M+H)^+$: 331.1441; Found: 331.1442.

2-(4-methoxyphenyl)-3-(pyridin-3-yl)-2*H*-indazole (15g):

From 2-(4-methoxyphenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 3-iodopyridine (0.069 g, 0.3 mmol, 1.5 equiv), **15g** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 94:6) in 43% (0.0288 g) yield as a white solid (m.p.: 138-139 °C); FT-IR (ν max/cm^{-1}): 2939, 1606, 1512, 1249, 1027, 831, 761. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (d, J = 2Hz, 1H), 8.59 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.62-7.59 (m, 1H), 7.39-7.30 (m, 4H), 7.20-7.16 (m, 1H) 6.91-6.89 (m, 2H) 3.82 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.78, 150.16, 149.21, 148.97, 136.74, 132.82, 131.86, 127.36, 127.14, 126.47, 125.41, 123.60, 123.26, 121.88, 119.80, 118.04, 114.53, 55.64.; HRMS (ESI): *m/z* calcd for $C_{19}H_{16}N_3O$ $(M+H)^+$: 302.1288; Found: 302.1290.

2, 3-di-p-tolyl-2*H*-indazole (15h):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 1-iodo-4-methylbenzene (0.0781 g, 0.36 mmol, 1.5 equiv), **15h** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 51 % (0.0365 g)

yield as a white solid (m.p.: 146-147 °C); FT-IR (ν max/cm^{-1}): 2987, 1587, 1471, 1250, 1033, 820, 751. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.36-7.30 (m, 3H), 7.25-7.23 (m, 2H), 7.20-7.09 (m, 5H) 2.38 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.94, 138.28, 138.23, 137.96, 135.51, 132.17, 129.62, 129.56, 127.14, 126.89, 125.84, 122.26, 121.71, 120.69, 117.73, 21.44, 21.26. HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2$ $(M+H)^+$: 299.1543; Found: 299.1547.

3-(4-chlorophenyl)-2-(*p*-tolyl)-2*H*-indazole (15i):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 1-chloro-4-iodobenzene (0.086 g, 0.36 mmol, 1.5 equiv), **15i** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 30% (0.023 g) yield as a white solid (m.p.: 168-169 °C); FT-IR (ν max/cm^{-1}): 2938, 1607 1513, 1245, 1172, 1027, 815, 748. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.37-7.33 (m, 3H), 7.29-7.27 (m, 4H) 7.19 (d, *J* = 8.0 Hz, 2H) 7.16-7.12 (m, 1H) 2.39 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.96, 138.63, 137.59, 134.42, 134.05, 130.93, 129.82, 129.18, 128.57, 127.05, 125.84, 122.82, 121.72, 120.18, 117.94, 21.27. HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_2$ $(M+H)^+$: 319.0997; Found: 319.1003.

3-(2-methoxyphenyl)-2-(*p*-tolyl)-2*H*-indazole (15j):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 2-iodoanisole (0.084 g, 0.36 mmol, 1.5 equiv), **15j** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 35% (0.0264 g) yield as a white solid (m.p.: 142-143 °C); FT-IR (νmax/cm^{-1}): 2938, 1606, 1512, 1244, 1170, 1027, 815, 749, 601. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.8Hz, 1H), 7.56(d, *J* = 8.4 Hz 1H), 7.41-7.29 (m, 5H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.08-7.03 (m, 2H), 6.86 (d, *J* = 8.4, 1H) 3.38 (s, 3H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.89, 148.85, 138.90, 137.68, 132.54, 131.81, 130.63, 129.25, 126.73, 124.49, 122.59, 121.93, 120.90, 120.81, 119.28, 117.67, 111.56, 54.99, 21.19; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O$ $(M+H)^+$: 315.1492; Found: 315.1493.

3-(3-methoxyphenyl)-2-(*p*-tolyl)-2*H*-indazole (15k):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1equiv) and 3-iodoanisole (0.084 g, 0.36 mmol, 1.5 equiv), **15k** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 34% (0.0256 g) yield as a white solid (m.p.: 118-119 °C); FT-IR (ν max/cm^{-1}): 2988, 1587, 1470, 1248, 1035, 822, 753, 693. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.8 Hz, 1H), 7.72 (d, *J* =

8.4 Hz, 1H), 7.37-7.27 (m, 4H), 7.18 (d, J = 8.4Hz, 2H), 7.15-7.13 (m, 1H), 6.93-6.89 (m, 3H), 3.71 (s, 3H), 2.37 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.69, 148.92, 138.36, 137.88, 135.21, 131.27, 129.88, 129.66, 126.96, 125.83, 122.50, 122.22, 121.73, 120.60, 117.80, 115.05, 114.16, 55.31, 21.27. HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2O$ $(M+H)^+$: 315.1492; Found: 315.1494.

3-(4-methoxyphenyl)-2-(*p*-tolyl)-2*H*-indazole (15l):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 4-iodoanisole (0.084 g, 0.36 mmol, 1.5 equiv), **15l** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 50 % (0.037 g) yield as a white solid (m.p.: 134-135 °C); FT-IR (ν max/cm^{-1}): 2940, 1607, 1513, 1247, 1172, 1028, 816, 751, 601. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87-7.85(m, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.36-7.26 (m, 5H), 7.17 (d, J = 8.0 Hz, 2H), 7.12-7.08 (m, 1H), 6.93-6.91 (m, 2H), 3.83 (s, 3H), 2.37 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.60, 148.91, 138.20, 137.94, 135.32, 131.03, 129.65, 126.89, 125.83, 122.39, 122.16, 121.63, 120.67, 117.72, 114.33, 55.38, 21.27. HRMS (ESI): *m/z* calcd for $C_{21}H_{18}N_2O$ $(M+H)^+$: 315.1492; Found: 315.1495.

3-(3,5-dimethylphenyl)-2-(*p*-tolyl)-2*H*-indazole (15m):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 1-iodo-3,5-dimethylbenzene (0.083 g, 0.36 mmol, 1.5 equiv), **15m** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 45% (0.00 g) yield as a white solid (m.p.: 122-123 °C); FT-IR (ν max/cm^{-1}): 2930, 1623, 1512, 1362, 1092, 970. 823, 755. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.48 Hz, 1H), 7.35-7.30 (m, 3H), 7.16 (d, *J* = 8.4Hz, 2H), 7.13-7.09 (m, 1H), 6.99 (s, 1H), 6.96 (s, 2H), 2.37 (s, 3H), 2.27(s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.90, 138.32, 138.13, 137.98, 135.72, 130.06, 129.91, 129.52, 127.52, 126.88, 125.75, 122.19, 121.79, 120.79, 117.69, 21.41, 21.25, HRMS (ESI): *m/z* calcd for $C_{22}H_{21}N_2$ $(M+H)^+$: 313.1699; Found: 313.1697.

3-(3,5-bis(trifluoromethyl)phenyl)-2-(*p*-tolyl)-2*H*-indazole (15n):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1equiv) and 1-iodo-3,5-bis(trifluoromethyl)benzene (0.122 g, 0.36 mmol, 1.5 equiv), **15n** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 30% (0.030 g) yield as a pale yellow solid (m.p.: 172-173 °C); FT-IR (ν max/cm^{-1}): 2961, 1668, 1550, 1441, 1272, 1114, 698. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H),

7.39-7.36 (m, 1H), 7.29 (d, J = 8.4 Hz, 2H), 7.21-7.15 (m, 2H), 2.39 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.02, H), 138.87, 137.47, 133.72 (d, J_{C-F} = 1.2 Hz), 133.59, 129.93, 129.90, 127.15, 125.87, 125.82 (d, J_{C-F} = 3.8 Hz), 125.76, 123.26, 121.93, 119.97, 118.09, 21.28; HRMS (ESI): *m/z* calcd for $C_{22}H_{15}F_6N_2$ $(M+H)^+$: 421.1134; Found: 421.1138.

3-(pyridin-3-yl)-2-(*p*-tolyl)-2*H*-indazole (15o):

From 2-(*p*-tolyl)-2*H*-indazole (0.050 g, 0.24 mmol, 1 equiv) and 3-iodopyridine (0.074 g, 0.36 mmol, 1.5 equiv), **15o** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 40% (0.0274 g) yield as a white solid (m.p.: 162-163 °C); FT-IR (ν max/cm^{-1}): 2920, 1624, 1512, 1361, 1092, 972, 823, 755. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 8.58 (d, *J* = 4.0 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.61 (dt, *J* = 1.6 Hz, 1H), 7.39-7.36 (m, 1H), 7.33-7.27 (m, 3H), 7.20-7.16 (m, 3H), 2.38 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.17, 149.21, 149.05, 138.96, 137.32, 136.77, 131.82, 129.82, 127.18, 126.50, 125.92, 123.60, 123.29, 121.99, 119.82, 118.10, 21.28; HRMS (ESI): *m/z* calcd for $C_{19}H_{16}N_3$ $(M+H)^+$: 286.1339; Found: 286.1343.

2-(4-chlorophenyl)-3-(*p*-tolyl)-2*H*-indazole (15p):

From 2-(4-chlorophenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 1-iodo-4-methylbenzene (0.073 g, 0.3 mmol, 1.5 equiv), **15p** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 25 % (0.0174 g) yield as a white solid (m.p.: 138-139 °C); FT-IR (ν max/cm^{-1}): 2939, 1605, 1511, 1247, 1170, 1025, 815, 747. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 1H), 7.40-7.34 (m, 5H), 7.25-7.18 (m, 4H), 7.14-7.10 (m, 1H), 2.40 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.18, 138.94, 138.92, 138.72, 135.74, 134.09, 129.78, 129.62, 129.27, 127.33, 127.20, 126.72, 122.62, 121.87, 120.71, 117.74, 21.46; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_2$ $(M+H)^+$: 319.0997; Found: 319.1002.

2-(4-chlorophenyl)-3-(2-methoxyphenyl)-2*H*-indazole (15q):

From 2-(4-chlorophenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 2-iodoanisole (0.077 g, 0.3 mmol, 1.5 equiv), **15q** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 95:5) in 43% (0.0315 g) yield as a pale yellow solid (m.p.: 130-131 °C); FT-IR (ν max/cm^{-1}): 2897, 1581, 1493, 1245, 1092, 836, 748. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.44-7.29 (m, 7H), 7.11-7.06 (m, 2H), 6.87 (d, J = 8.4 Hz, 1H), 3.39 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.62, 149.13, 140.02, 133.51, 132.66, 131.69,

130.96, 128.86, 127.10, 125.73, 122.70, 122.30, 121.09, 120.77, 118.80, 117.71, 116.57, 54.94; HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_2O$ $(M+H)^+$: 335.0946; Found: 335.0944.

2-(4-chlorophenyl)-3-(4-methoxyphenyl)-2*H*-indazole (15r):

From 2-(4-chlorophenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 4-iodoanisole (0.073 g, 0.3 mmol, 1.5 equiv), **15r** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 97:3) in 27 % (0.0197 g) yield as a white solid (m.p.: 145-146 °C); FT-IR (ν max/cm^{-1}): 2939, 1606, 1513, 1249, 1028, 831, 760.. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.40-7.33 (m, 5H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.86, 149.16, 138.92, 135.55, 134.04, 131.05, 129.27, 127.31, 127.18, 122.52, 121.91, 121.80, 120.69, 117.72, 114.55, 55.42. HRMS (ESI): *m/z* calcd for $C_{20}H_{16}ClN_2O$ $(M+H)^+$: 335.0946; Found: 335.0949.

3-(3,5-bis(trifluoromethyl)phenyl)-2-(4-chlorophenyl)-2*H*-indazole (15s):

From 2-(4-chlorophenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 1-iodo-3,5-bis(trifluoromethyl)-benzene (0.111 g, 0.3 mmol, 1.5 equiv), **15s** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 98:2) in 15% (0.0144 g) yield as a pale yellow solid (m.p.: 151-152 °C); FT-IR (ν max/cm^{-1}): 2960, 1670, 1552, 1440, 1273, 1115, 697; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.8 Hz, 1H), 7.69-7.65 (m, 3H), 7.46 (d, *J* = 8.0 Hz, 2H) 7.39-7.37 (m, 4H) 7.20-7.16 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.27, 138.44, 134.68, 133.74, 133.33, 129.93, 129.57, 127.55, 127.22, 126.05, 126.02, 123.61, 122.13, 119.97, 118.10, HRMS (ESI): *m/z* calcd for $C_{21}H_{12}ClF_6N_2$ $(M+H)^+$: 441.0588; Found: 441.0590.

2-(4-chlorophenyl)-3-(pyridin-3-yl)-2*H*-indazole (15t):

From 2-(4-chlorophenyl)-2*H*-indazole (0.050 g, 0.2 mmol, 1 equiv) and 3-iodopyridine (0.067 g, 0.3 mmol, 1.5 equiv), **15t** was obtained after purification by flash chromatography on silica gel (hexane:EtOAc = 96:4) in 30% (0.020 g) yield as a muddy white solid (m.p.: 157-158 °C); FT-IR (ν max/cm^{-1}): 2940, 1606, 1513, 1250, 1029, 833, 762. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.62 (d, *J* = 4.5 Hz, 1H) 7.80 (d, *J* = 8.8 Hz, 1H), 7.67(d, J = 8.4 Hz, 1H), 7.60(d, *J* = 7.6 Hz, 1H), 7.41-7.33 (m, 6H), 7.19 (t, *J* = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.13, 149.57, 149.30, 138.30, 136.79, 134.80, 129.64, 127.59, 127.27, 126.13, 123.75, 123.66,

122.21, 119.83, 118.11. HRMS (ESI): *m/z* calcd for $C_{18}H_{13}ClN_3$ $(M+H)^+$: 306.0793; Found: 306.0796.

5.6 References

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5.7 Characterization spectral data (^{1}H NMR and ^{13}C NMR) of arylated 2H-inazoles 15a, 15c, 15f, 15g, 15k, 15m, 15n, 15p,15q.

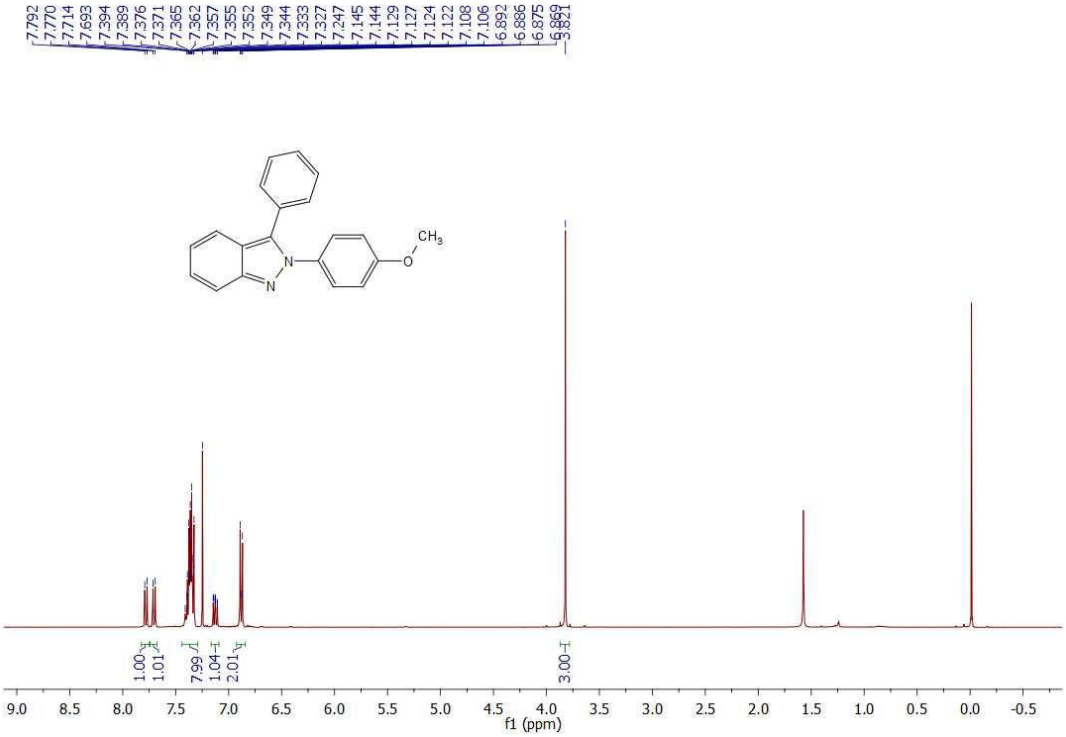

Figure 4. ^{1}H NMR Spectrum of 2-(4-methoxyphenyl)-3-phenyl-2*H*-indazole **15a**.

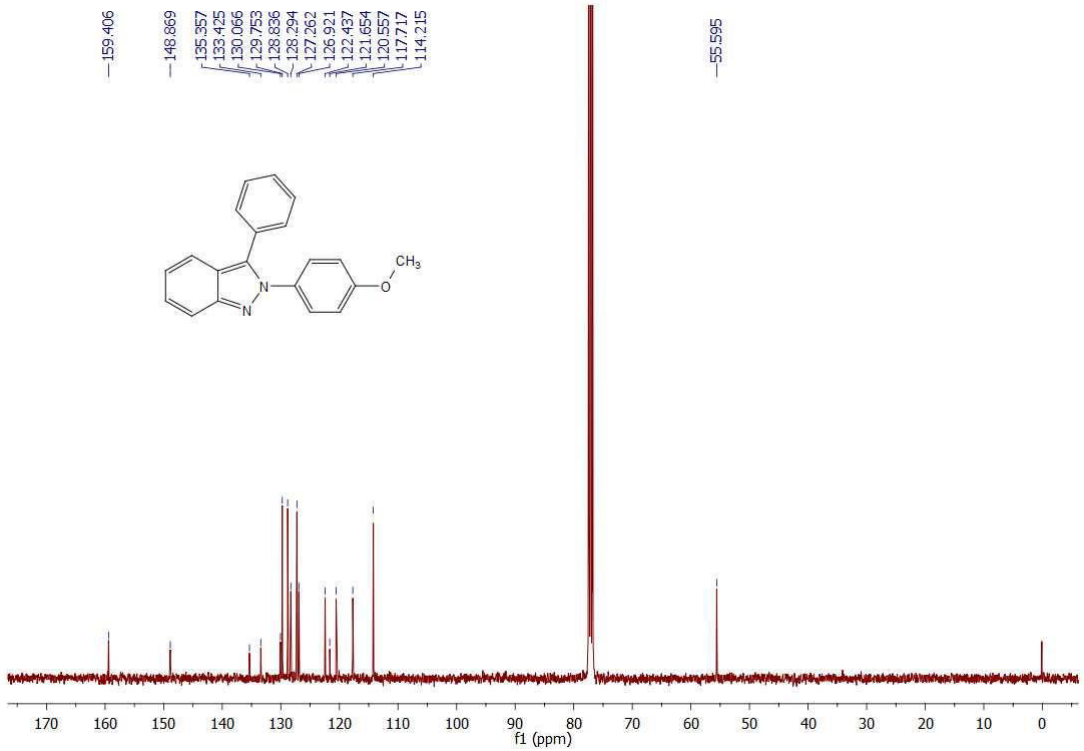

Figure 5. ^{13}C NMR Spectrum of 2-(4-methoxyphenyl)-3-phenyl-2*H*-indazole **15a**.

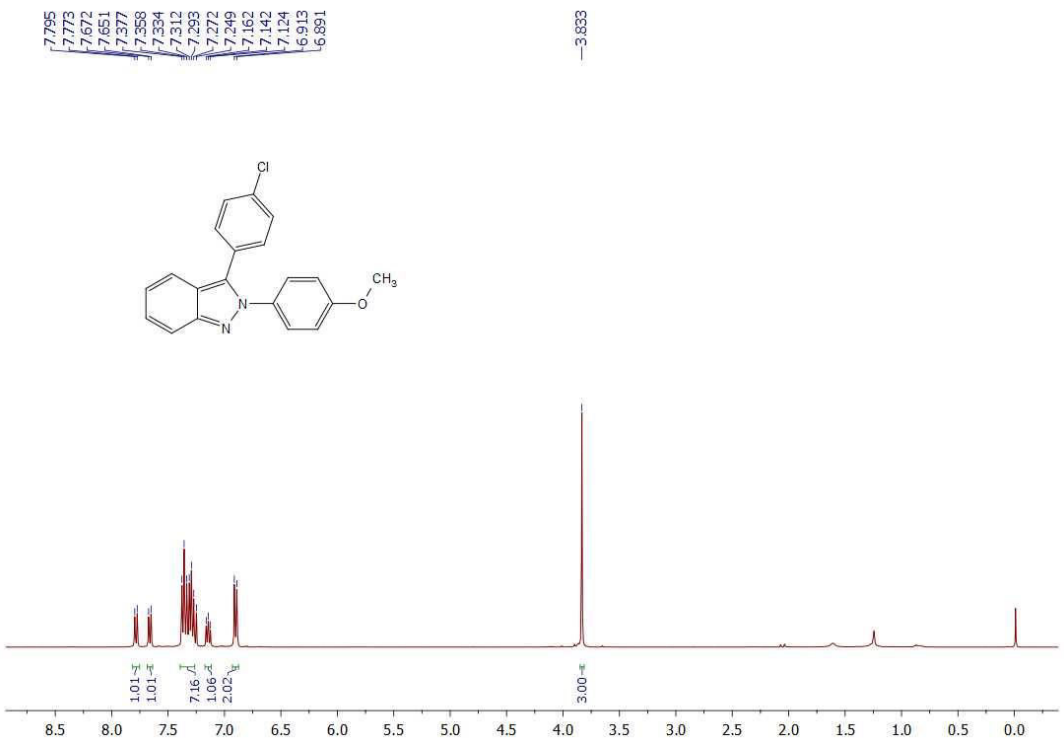

Figure 6. ^{1}H NMR Spectra of 3-(4-chlorophenyl)-2-(4-methoxyphenyl)-2*H*-indazole **15c**

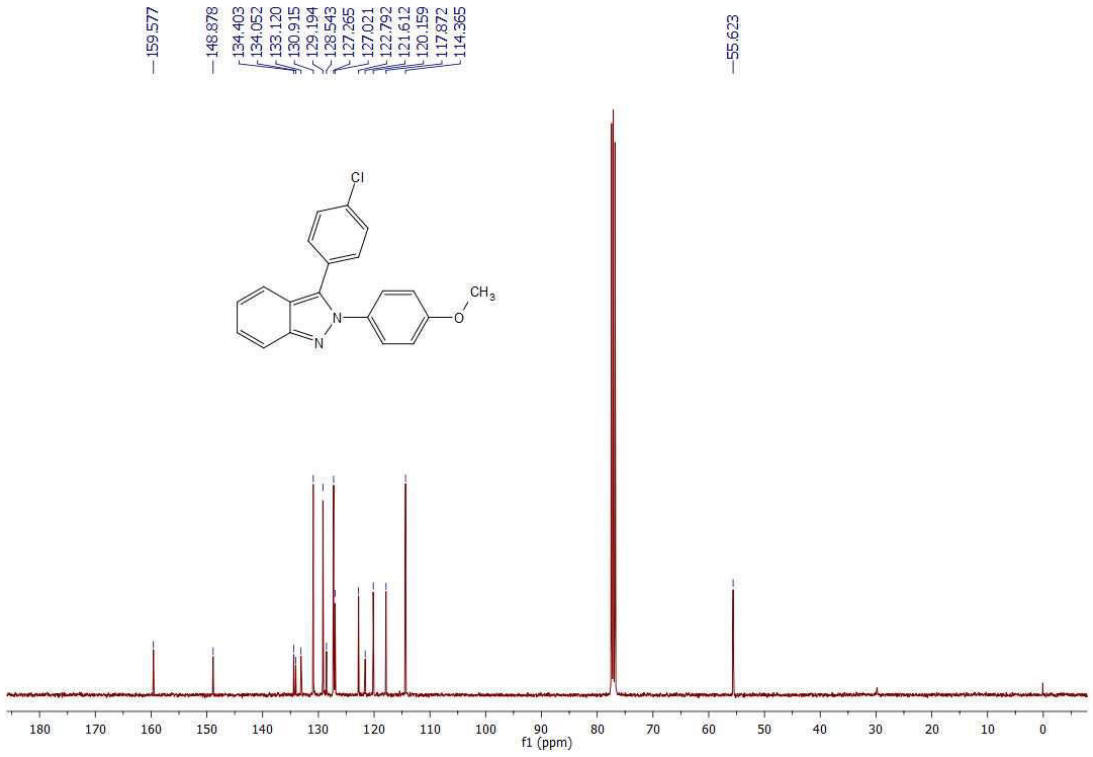

Figure 7. ^{13}C NMR Spectra of 3-(4-chlorophenyl)-2-(4-methoxyphenyl)-2*H*-indazole **(15 c)**

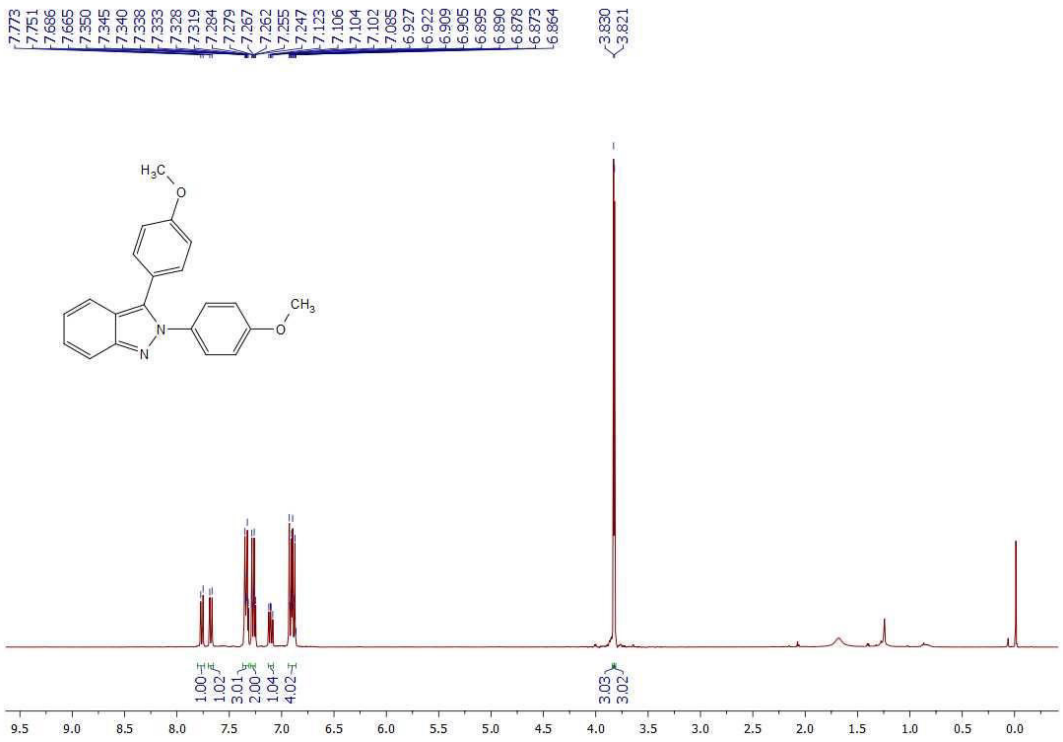

Figure 8. ^{1}H NMR Spectra of 2,3-bis(4-methoxyphenyl)-2*H*-indazole **15f**.

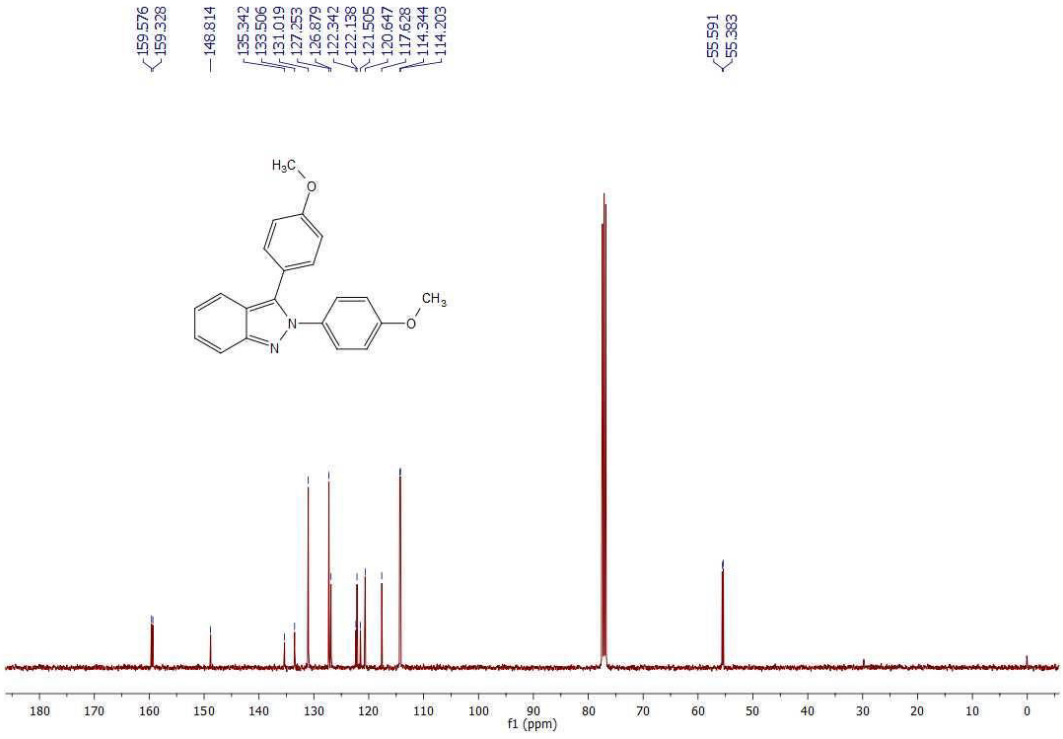

Figure 9. ^{13}C NMR Spectra of 2,3-bis(4-methoxyphenyl)-2*H*-indazole **15f**.

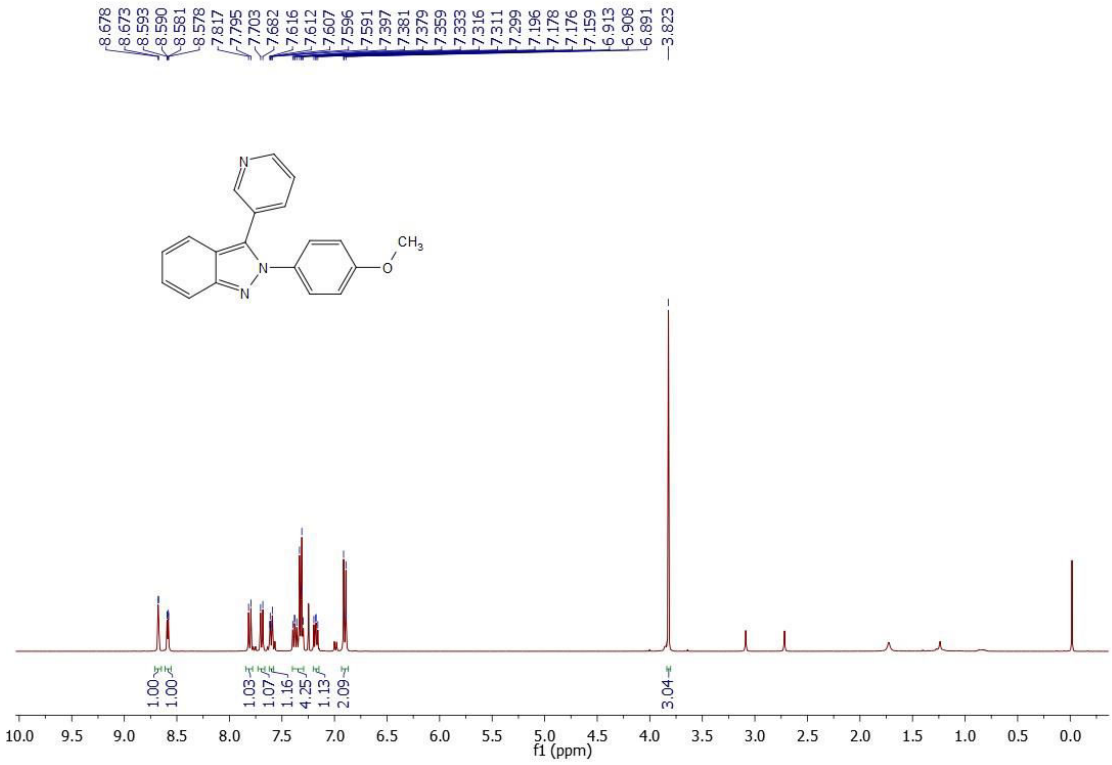

Figure 10. ^{1}H NMR Spectra of 2-(4-methoxyphenyl)-3-(pyridin-3-yl)-2*H*-indazole **15g**.

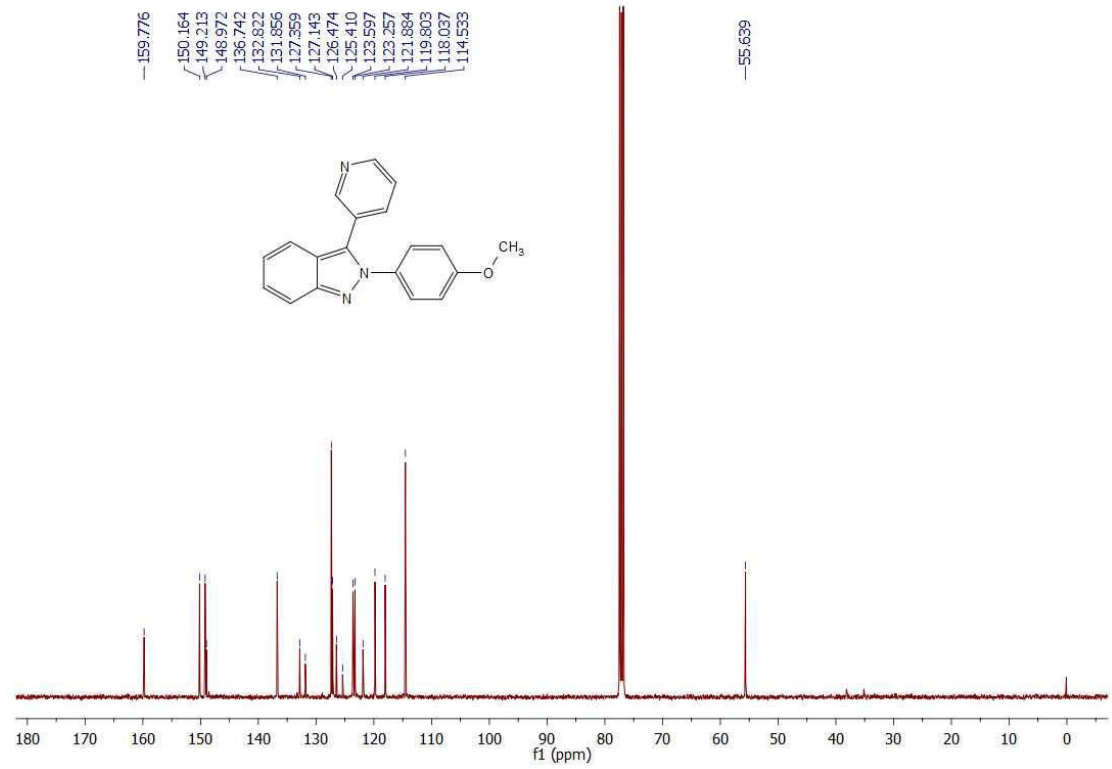

Figure 11. ^{13}C NMR Spectra of 2-(4-methoxyphenyl)-3-(pyridin-3-yl)-2*H*-indazole **15g**.

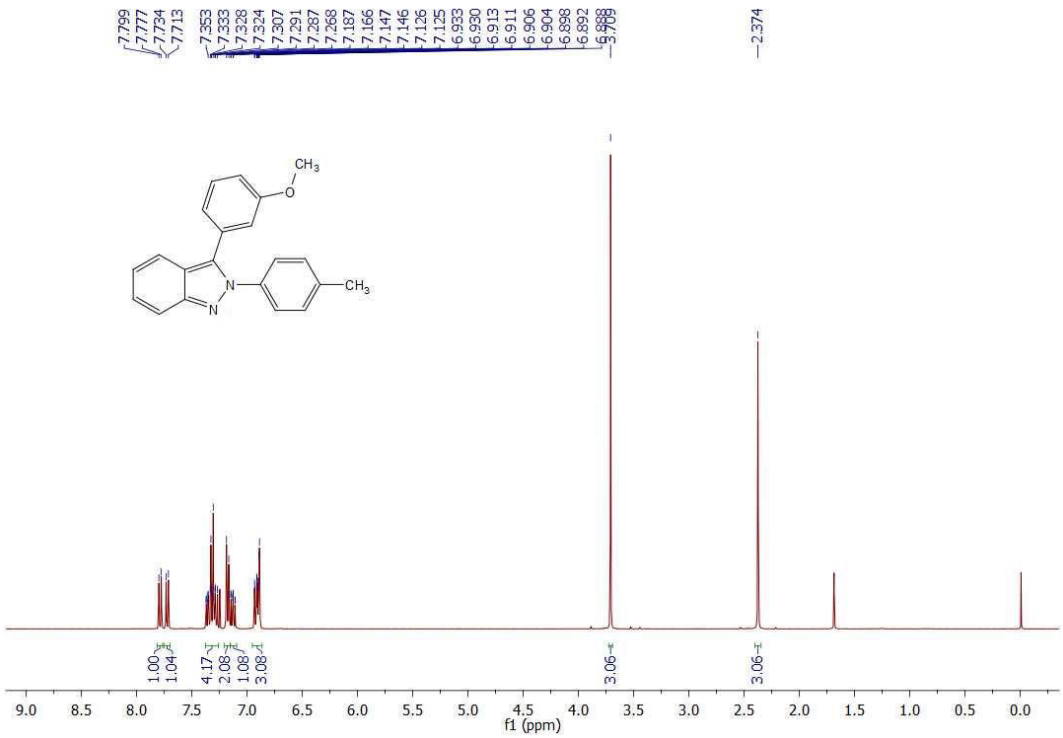

Figure 12. ^{1}H NMR Spectra of 3-(3-methoxyphenyl)-2-(*p*-tolyl)-2*H*-indazole **15k**.

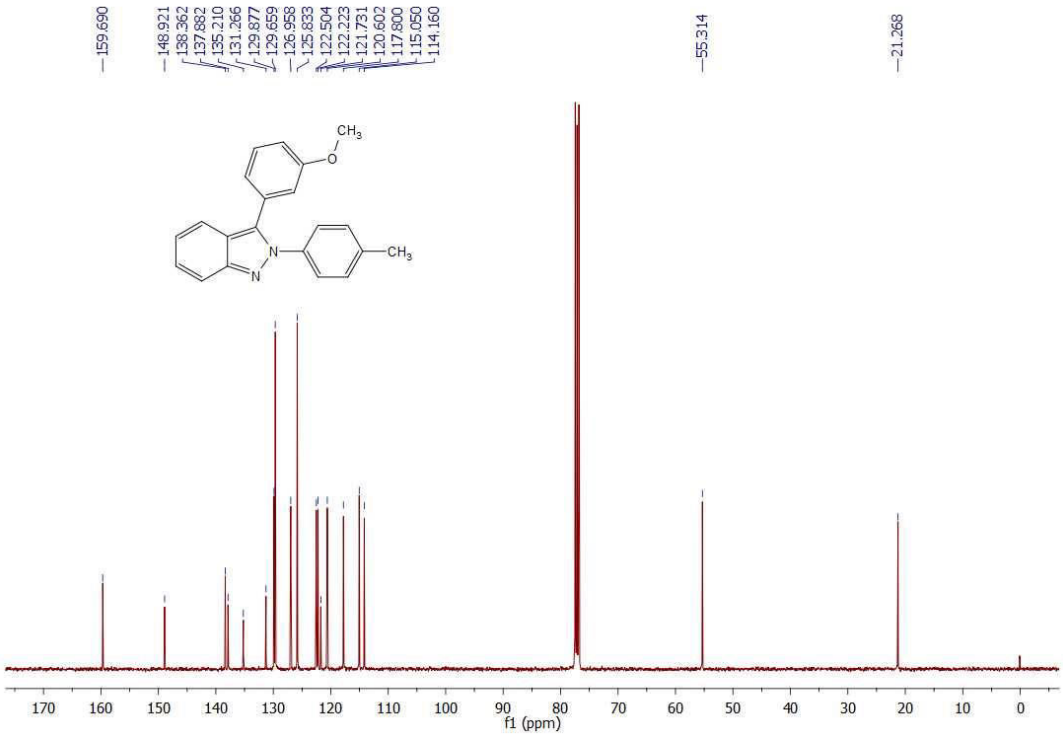

Figure 13. ^{13}C NMR Spectra of 3-(3-methoxyphenyl)-2-(*p*-tolyl)-2*H*-indazole **15k**.

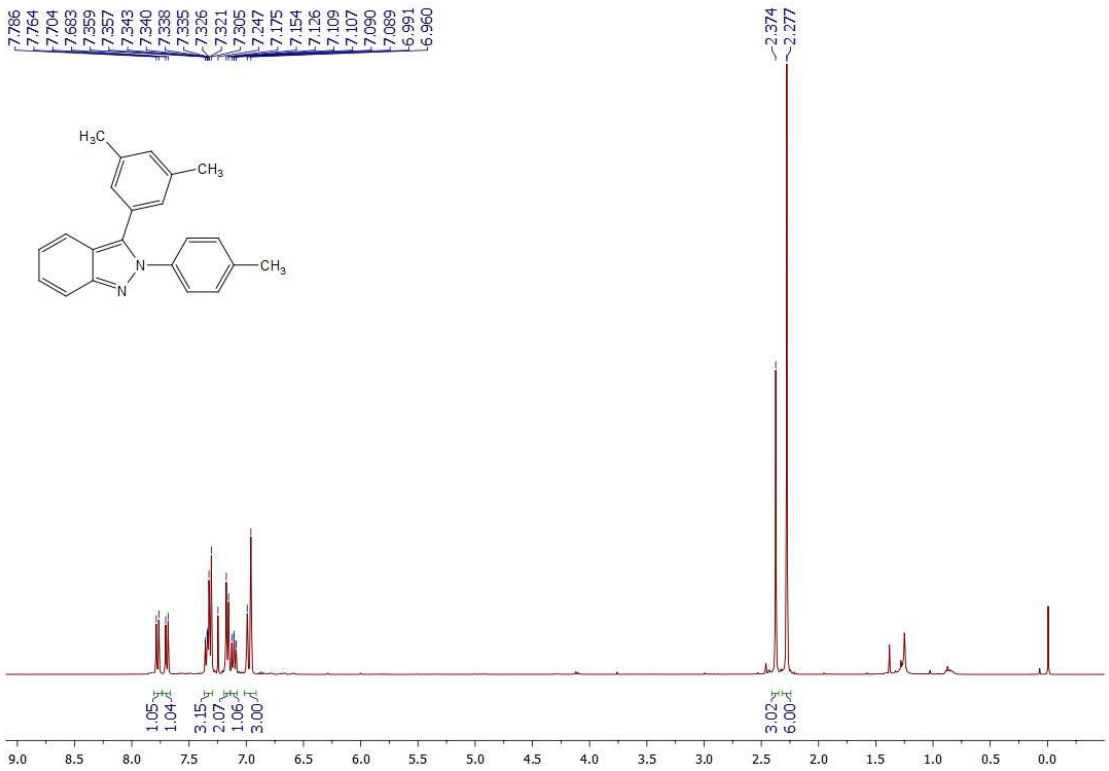

Figure 14. ^{1}H NMR Spectra of 3-(3,5-dimethylphenyl)-2-(*p*-tolyl)-2*H*-indazole **15m**.

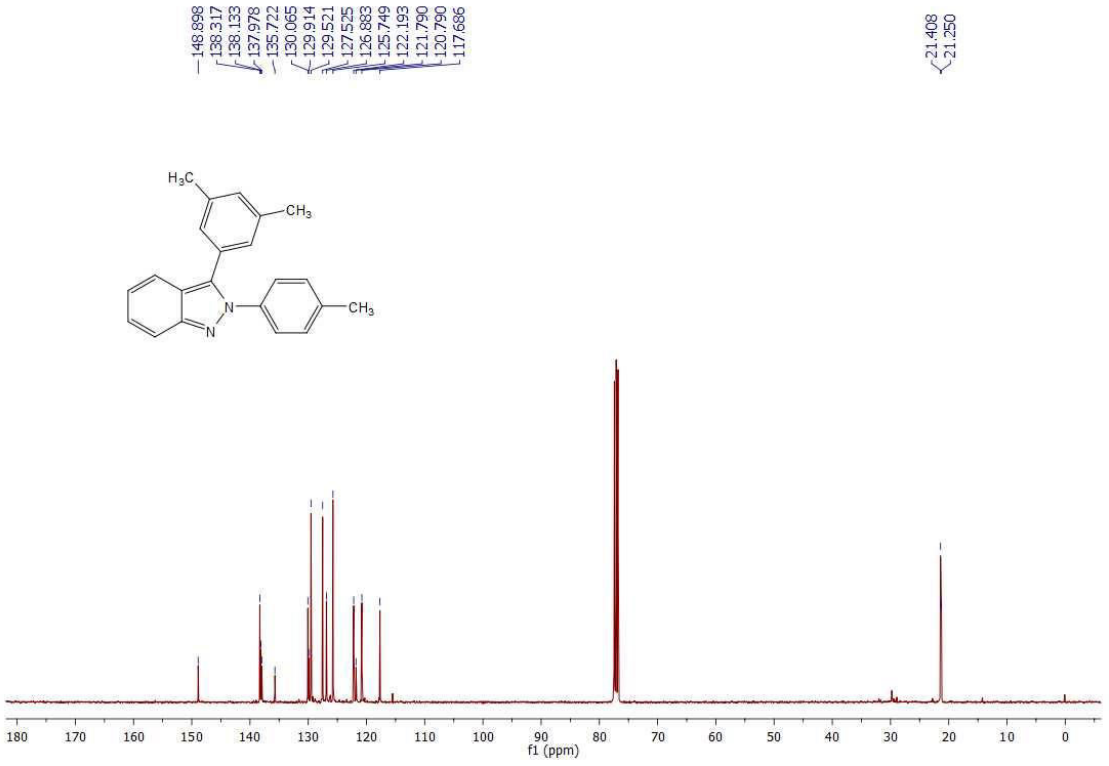

Figure 15. ^{13}C NMR Spectra of 3-(3,5-dimethylphenyl)-2-(*p*-tolyl)-2*H*-indazole **15m**.

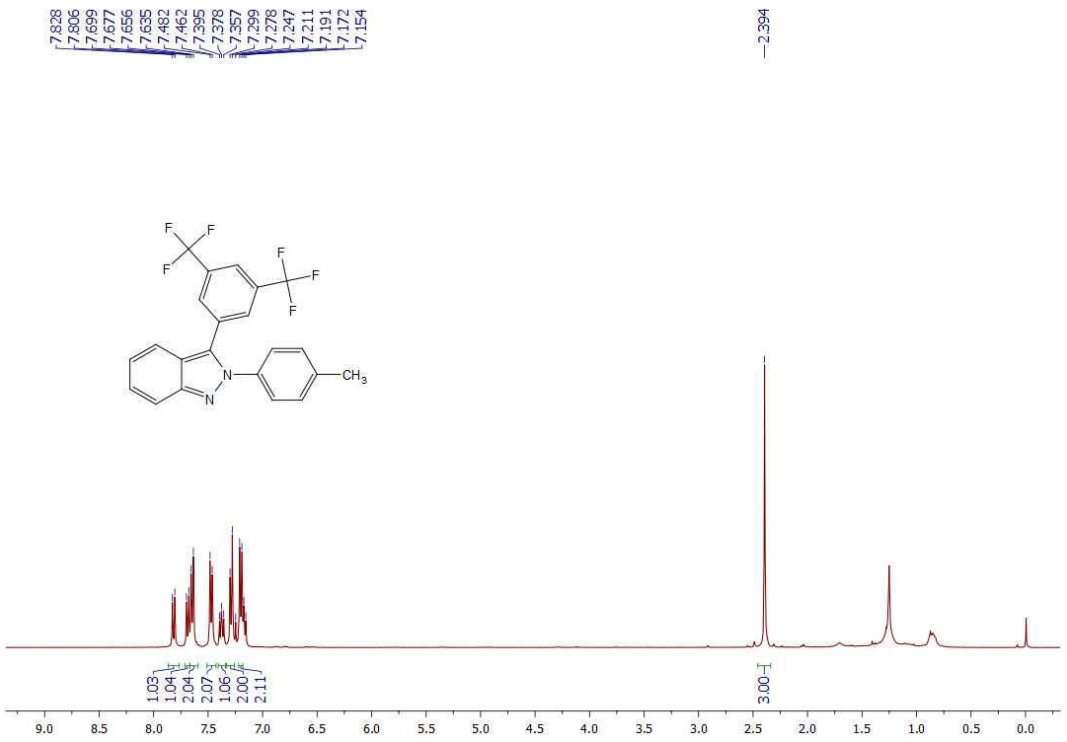

Figure 16. ^{1}H NMR Spectra of 3-(3,5-bis(trifluoromethyl)phenyl)-2-(*p*-tolyl)-2*H*-indazole **15n**.

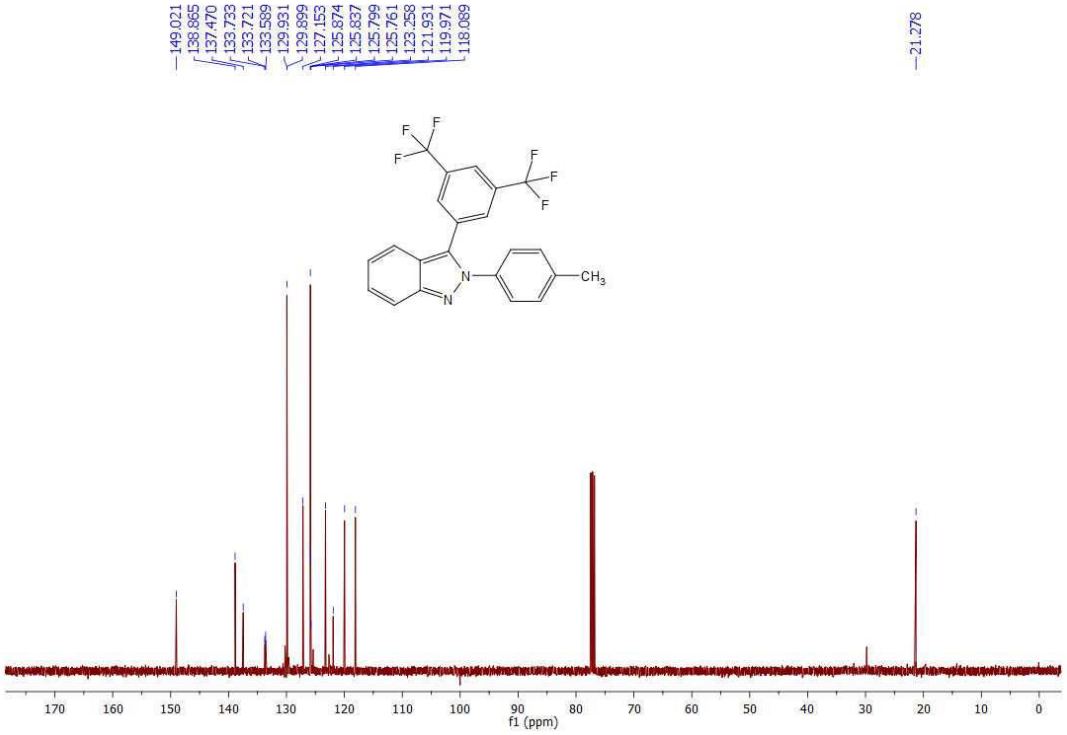

Figure 17. ^{13}C NMR Spectra of 3-(3,5-bis(trifluoromethyl)phenyl)-2-(*p*-tolyl)-2*H*-indazole **15n**.

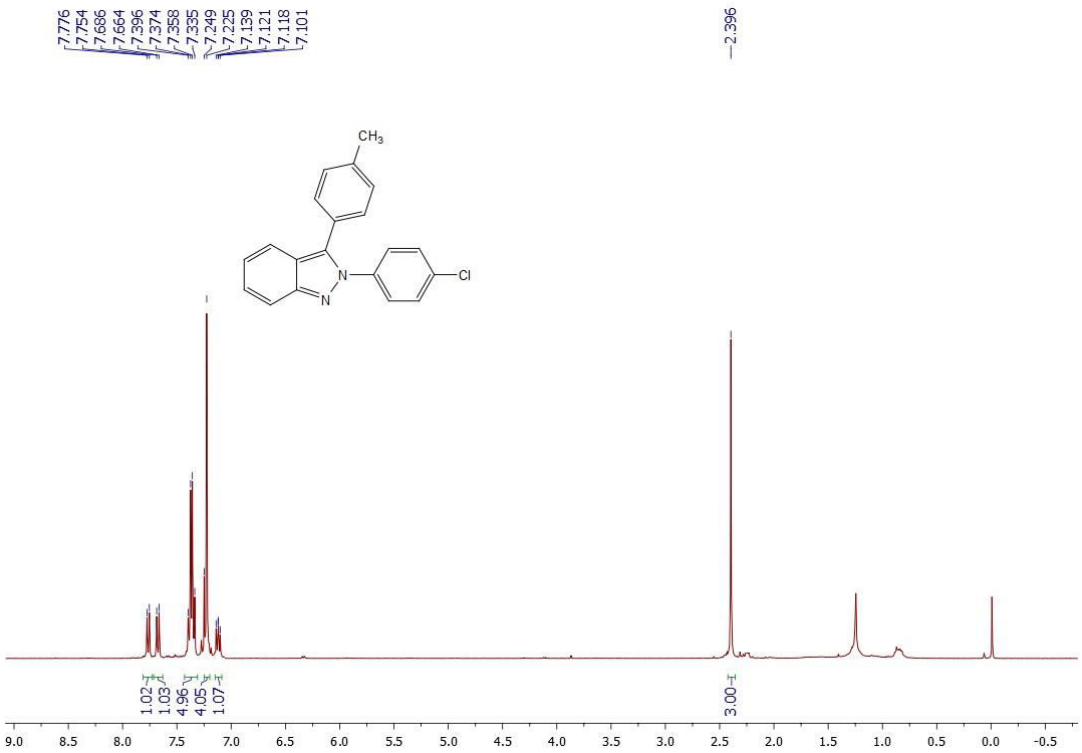

Figure 18. ^{1}H NMR Spectra of 2-(4-chlorophenyl)-3-(*p*-tolyl)-2*H*-indazole **15p**.

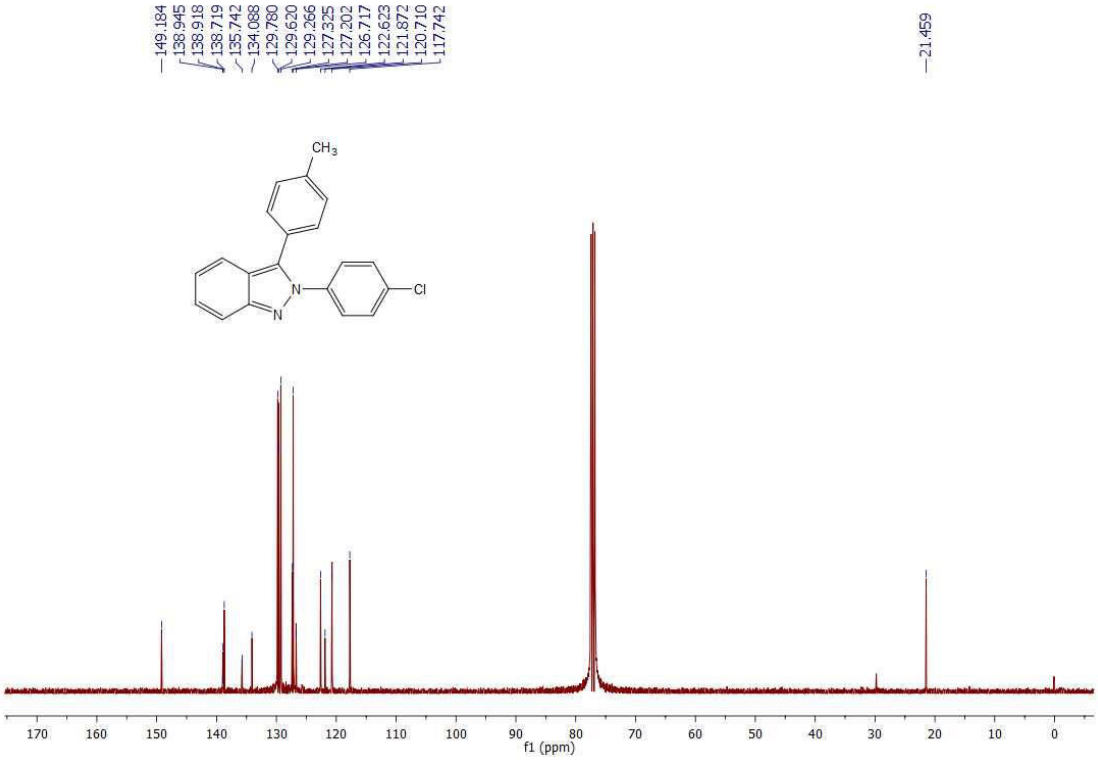

Figure 19. ^{13}C NMR Spectra of 2-(4-chlorophenyl)-3-(*p*-tolyl)-2*H*-indazole **15p**.

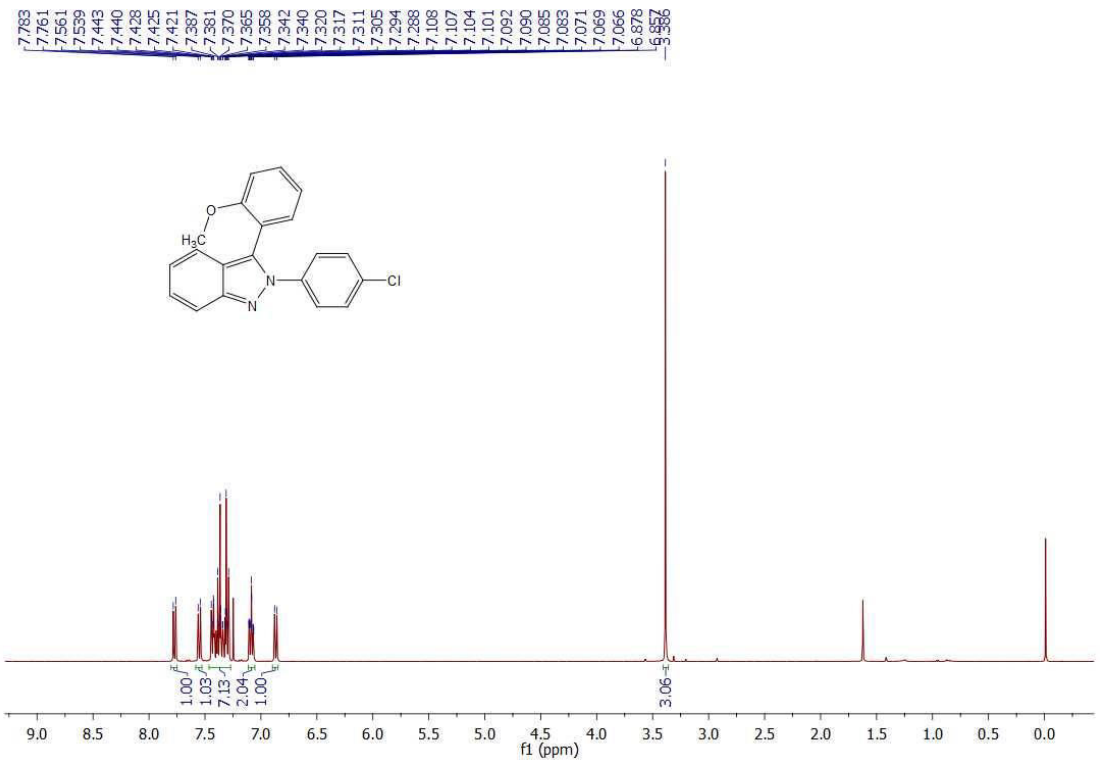

Figure 20. ^{1}H-NMR Spectra of 2-(4-chlorophenyl)-3-(2-methoxyphenyl)-2*H*-indazole **15q**.

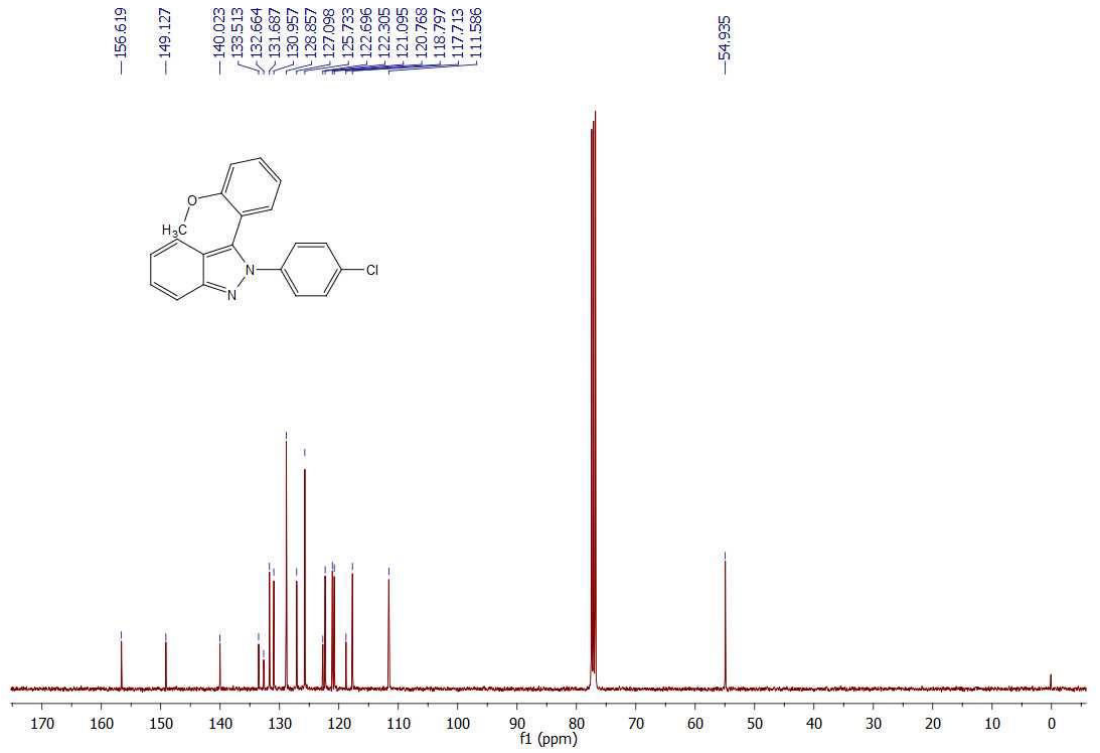

Figure 21. ^{13}C NMR Spectra of 2-(4-chlorophenyl)-3-(2-methoxyphenyl)-2*H*-indazole **15q**.

Publications, Conferences/Seminars/Workshops

&

Curriculum Vitae

(Appendix I & II)

LIST OF PUBLICATION

1. Sharma, R.; Yadav, L.; Lal, J.; Jaiswal,; P. K.; Mathur, M.; Swami, A. K.; Chaudhary, S.* *"Synthesis, antimicrobial activity, structure-activity relationship and cytotoxic studies of a new series of functionalized (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2H-benzo[b] [1,4]oxazin-2-one" Bioorg.Med. Chem. Lett.,* **2017**, *27(18)*, 4393-4398.
2. Sharma, R.; Prakash, K.; Yadav, L.; Pratap, R.;* Chaudhary, S.* "*Nano-Star: Single crystal X-ray evidence of an inclusion of C9 and C12 allotropes" Angew. Chem. Int. Ed.,* **2019**. (Manuscript under preparation)
3. Sharma, R.; Yadav, L.; Prakash, K.; Mathur, M.; Pratap, R.; Yadav, D. K.; Chaudhary, S.* "*Novel Spirooxindolo-pyrrolidines and Spirooxindolo-pyrrolizines as potent antibacterial and antitubercular agents: Design, Synthesis, Stereochemical Assignment, SAR and Molecular Docking Studies*" *ChemMedChem,* **2019**. (Manuscript under preparation)
4. Sharma, R.; Yadav, L.; Chaudhary, S.* "*Palladium-catalyzed intramolecular cross–dehydrogenative coupling (CDC) via C-H bond activation: First application to the synthesis of Coumarin-benzopyran fused heterocycles*" *Org. Lett.,* **2019**. (Manuscript under preparation)

Paper presented in Conferences with proceedings (Abstract published)

1. Sharma, R.; Yadav, L.; Lal, J.; Mathur, M.; Swami, A. K.; Chaudhary, S. *"Synthesis of a new series of 5'-(indoline-2-ylmethyl)-3'-(4-methylbenzoyl)-4'-(p tolyl)spiro[indoline-3,2'-pyrrolidin]-2-ones via 1,3-dipolar cycloaddition: x-ray crystal structure, SAR and biological evaluation"* 16th-18th May, **2018** Abstracts of papers ISBN 978-5-7944-3095-0 (Page-55) International Conference on "100 Years of Development of Chemistry: From Synthesis of Polyethylene to Stereodivergence", Department of Organic Chemistry, Perm State University, Perm, Russia Federation.
2. Sharma, R.; Yadav, L.; Lal, J.; Jaiswal, P. K.; Mathur, M.; Swami, A. K.; Chaudhary, S. "*Novel functionalized (Z)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ones: Synthesis, Antimicrobial Activity, Structure-activity Relationship and Cytotoxic Studies," International Conference on "Frontiers at the Chemistry-Allied Sciences Interface*" (FCASI-2017) 22-23

July **2017**. Abstract of paper at the Department of Chemistry, University of Rajasthan, Jaipur-302004, Rajasthan, India.

3. Sharma, R.; Lal, J.; Chaudhary, S. *"Microwave-assisted synthesis and antimicrobial activity of novel 2-oxo-benzo-[1,4]-oxazine analogues"* National Conference on "*Organic Chemistry in Sustainable Development: Recent Advances and Future Challenges*" (OCSD-2016) 30-31 Aug, **2016** Abstract of papers at the Department of Chemistry, Birla Institute of Science and Technology (BITS) Pilani, Pilani, India.
4. Sharma, R.; Jaiswal, P. K. and Chaudhary, S. (Nov, 2015), "*A metal free domino approach towards the microwave assisted direct synthesis of novel 2-oxo-benzo-(1, 4)-oxazine analogues as potential antimicrobial agents*" 23rd-25th Nov, **2015** Abstracts of papers ISBN No. 978-93-84869-93-9 (PP-151) International Conference on "*Current Challenges in Drug Discovery Research CCDDR-2015*", MNIT Jaipur-302017, Rajasthan, India.
5. Sharma, R.; Jaiswal, P. K. and Chaudhary, S. (Nov, 2015), "*A new insight towards the development of green synthetic protocol for Chalcone derived pharmaceutically demanding N-heterocycles and their biological activity evaluation*" 23rd-25th Nov, **2015** Abstracts of papers ISBN No. 978-93-84869-93-9 (PP-152) International Conference on "*Current Challenges in Drug Discovery Research CCDDR-2015*", MNIT Jaipur-302017, Rajasthan, India.

Curriculum Vitae

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Educational Qualifications

Ph.D Chemistry, (6th Jan, 2013-03 Dec, 2019): Malaviya National Institute of Technology (MNIT) Jaipur, India.

Ph.D. thesis title: "***Synthesis, Chemistry and Bioevaluation of Some Bioactive Heterocycles*" (**Supervisor: **Dr. Sandeep Chaudhary, MNIT, Jaipur, Rajasthan)**

M.Sc. Chemistry (2011): Department of Chemistry, Banaras Hindu University, Varanasi, India.

B.Sc (2009): Mahila Mahavidyalaya, Banaras Hindu University, Varanasi, India.

Scholastic Achievements

- Qualified **Ph.D Entrance Test** conducted by the department of Chemistry, Malaviya National Institute of Technology (MNIT) Jaipur, India for the award of Research Scholar in Dec, **2013**.
- Qualified Joint CSIR-UGC NET (Lecturer ship) examination in June, **2013** conducted by CSIR and UGC, New Delhi.
- Qualified GATE examination (GATE score = 483) in **2013** conducted by IIT Bombay on behalf of NCB-GATE for MHRD, Government of India.

Research experience

Pre-Ph.D Courses Work

- **Course 1**: *Heterocyclic Chemistry*
- **Course 2**: Stereochemistry, structure and reaction mechanism of Organic compounds
- **Course 3**: Research Methodology
- **Course 4:** *Chemical Dynamics and Surface Chemistry*
- **Course 5:** *Organic Synthesis*

Other research experiences

- Handling of Organic Peroxides such as H_2O_2 etc.
- Handling of Metal Hydrides such as $NaBH_4$ etc.
- Handling of Organometallics such as Grignard reagents, Reformatsky reagents, Wittig reagent.
- Analytical techniques, Column Chromatography including flash Chromatography, Chromatotron, Preparative Chromatography, TLC etc.
- Basic training for Chemical Laboratory Safety.
- Handling of Sophisticated Chromatographic Instruments such as HPLC etc.

List of Publications: **See appendix I**

Conferences/Seminars/Workshop: See appendix II

Computational Skill

- Working knowledge of *MS-Office*, *Chem Office Ultra (8.0)* including *Chem Draw Ultra 8.0*, *Chem 3D Ultra 8.0*, *Adobe Photoshop (7.0)*, *Internet Explorer*.
- Plotting of ^{1}H and ^{13}C NMR spectra using Jeol NMR and mestrenova software.
- Preparation of manuscripts for publication.

Research interests

- C—H bond activation
- Medicinal chemistry: Mechanism/Target based drug design and synthesis of new bioactive molecules.
- Drug Development-Lead generation and lead optimization, applying different approaches towards drug development.

Profile

- Strong background in basic organic chemistry including structural elucidation and spectroscopic interpretation of organic compounds.
- Conceptual experience in medicinal chemistry and drug discovery process viz. lead generation, lead optimization etc.
- Experience in synthesis and handling of organic molecules.
- Good written and oral communication skills, good computational skills.
- Gained experience in managing research projects from conception to completion.

References

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Synthesis, antimicrobial activity, structure-activity relationship and cytotoxic studies of a new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones

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ABSTRACT

A new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones **23**–**26**, incorporating pharmaceutically privileged substructures such as cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl were synthesized in excellent yields. All the synthesized compounds were screened for their *in vitro* antibacterial activity against gram-(+)ve and gram-(−)ve bacterial species i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli* as well as *in vitro* antifungal activity against fungal species i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei*, respectively. In this study, compounds containing *cyclopropyl* and *cyclohexylphenyl* substructures were identified as promising antimicrobial agents than standard drugs, ampicillin and chloramphenicol as well as ketoconazole. SAR study illustrates that electron-withdrawing groups increases the antibacterial as well as antifungal activity of 2-oxo-benzo[1,4]oxazines and vice versa. Compounds **23e** and **26e**, the most active compounds of the series, displayed promising antibacterial activity than Ampicillin and Chloramphenicol. Moreover, compound **26d** showed promising antifungal potency as compared to Ketoconazole. Cytotoxic studies of the active compounds i.e. **23c**–**e**, **24e**, **25d** and **26d**–**e** found to be non-toxic in nature in $3T_3$ fibroblast cell lines using MTT assay.

Benzo[1,4]oxazines **1** and 2-oxo-benzo[1,4]oxazines **2**, an important bioactive heterocycle class, are found in many natural products and displays wide range of biological activities such as antibacterial,[1,2] antifungal,[3] anti-hypertensive,[4] anti-inflammatory,[5] antimalarial,[6] anti-cancer,[7] anti-relaxant,[8] anti-rheumatic,[9] potassium channel opener,[10] central nervous system activity,[11] potassium channel modulator,[12] vaso-dilating activity,[13] dopamine receptor[14] and neuroprotective[15] activities (Fig. 1).

Several reports are available in the literature wherein compounds having benzo[1,4]oxazines as part of molecular architecture displayed promising antimicrobial activities.[1–3]

Since it has also been observed that several pharmaceutically important substructure/scaffolds as well as some natural products containing pharmaceutically privileged substructures such as cyclopropyl,[16–18] naphthyl,[19] biphenyl[20] and cyclohexylphenyl[21] etc., as part of their molecular architecture, shows promising biological activities (Fig. 2).

In our research programme towards the search of new bioactive compounds and inspired by the promising role in improving biological activity of these substructures, we prepared 2-oxo-benzo[1,4]oxazines **23**–**26** of *prototype I*, incorporating cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as pharmacologically privileged substructures and evaluated them for their *in vitro* antimicrobial activity in detail (Fig. 3).

Herein, we report the microwave-assisted synthesis and antimicrobial activity of a new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones **23**–**26** incorporating cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as pharmaceutically privileged substructures. The *in vitro* antibacterial activity against gram-positive and gram-negative bacteria (i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli*) as well as *in vitro* antifungal activity against fungal species

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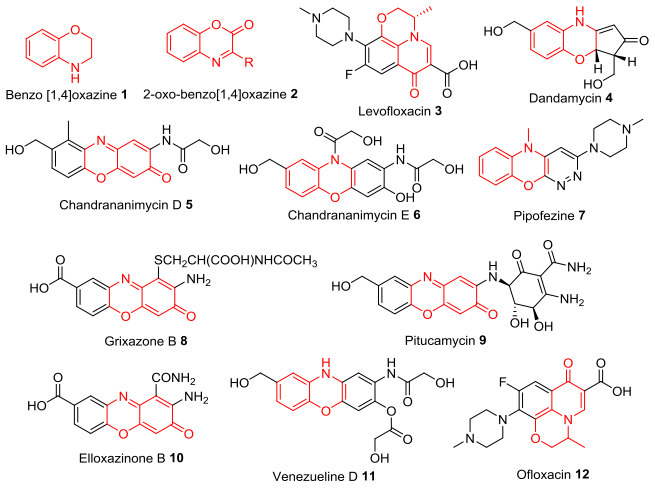


Fig. 1. Structures of biologically active molecules having benzo[1,4]oxazines moieties.

(i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei*), respectively were performed in these antimicrobial studies. The standard drugs ampicillin and chloramphenicol as well as ketoconazole were used as standard references in antibacterial and antifungal assay, respectively. Out of all compounds, **23e** and **26e** have shown promising antimicrobial activity. To the best of our knowledge, for the first time, antimicrobial activity has been reported in 2-oxo-benzo[1,4]oxazines incorporating these pharmaceutically important substructures. We also report, for the first time, *in vitro* antimicrobial activity of some previously reported 2-oxo-benzo[1,4]oxazines **23a**,[22a] **25a**, and **26b–c**.[22b] Cytotoxic studies of active compounds, **23c–e**, **24e**, **25d** and **26d–e**, were also carried out using 3T3 fibroblast cell lines via MTT assay.

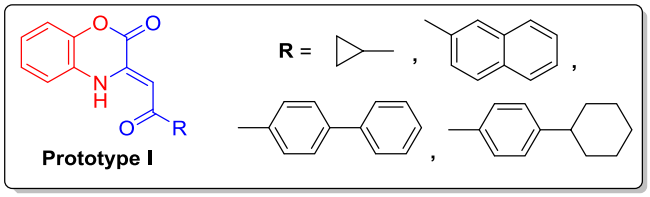


Fig. 3. Structure of proposed prototype I.

Using literature procedure,[23a] the analogues of ***prototype I*** were synthesized from the reaction of substituted diketoacid **21a–d** with substituted aminophenol **22a–e** under microwave irradiation condition. The required diketoesters **20a–d** incorporating pharmacologically privileged substructures such as cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl were prepared via base-catalyzed reaction of acetophenone **19a–d** with dimethyl oxalate at 0 °C to 90 ºC in toluene for 4–5 h which furnished the desired diketoesters in 70–75% yields. Conversion of the diketoesters **20a–d** to diketoacid **21a–d** with $LiOH{\cdot}H_2O$ followed by coupling with substituted aminophenols **22a–e** furnished benzo[1,4] oxazines **23–26** incorporated cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl as substructures. The desired 2-oxo-benzo[1,4] oxazines **23–26** were purified either by flash column chromatography or by recrystalization. The geometry of exocyclic double bond was found to be *cis* due to H-bonding between hydrogen attached with benzoxazin nitrogen and oxygen of 2-oxo-2-phenylethylidenes substituent (Scheme 1).[23b–d] All the synthesized compounds were well characterized by ^1H NMR and ^{13}C NMR spectroscopy and HRMS analysis (Scheme 1).[24]

The nitro compounds were obtained in slightly lesser yield (70–75%) in comparison with other substituted 2-oxo-benzo[1,4]oxazines (80–95%).

Compounds (**23a–e**, **24a–e**, **25a–e** and **26a–e**) were initially screened for their *in vitro* antibacterial activity against

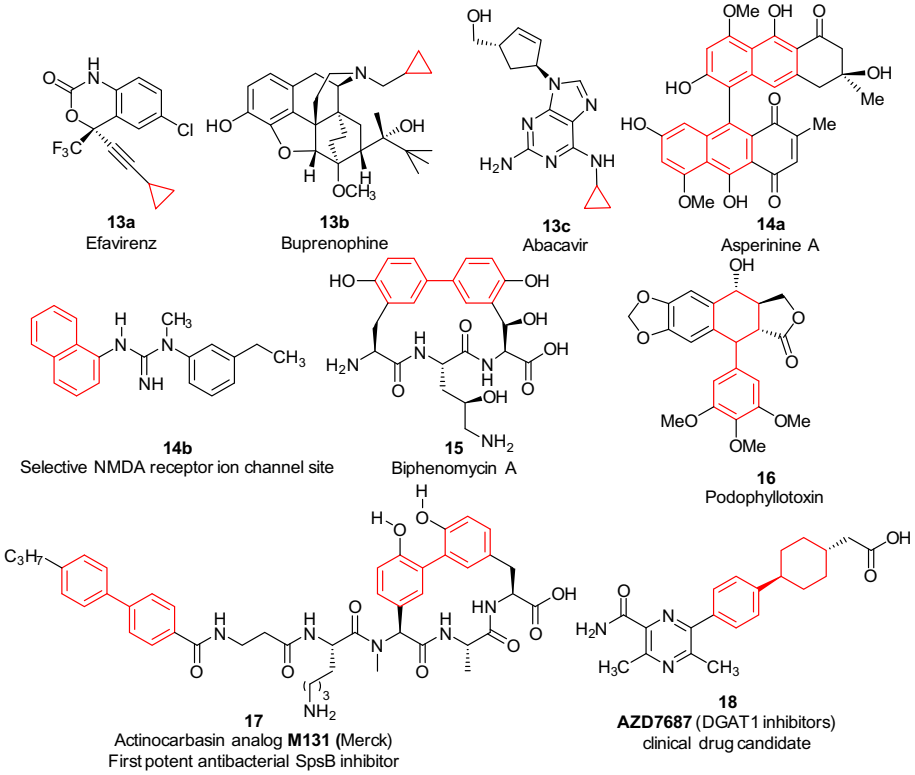
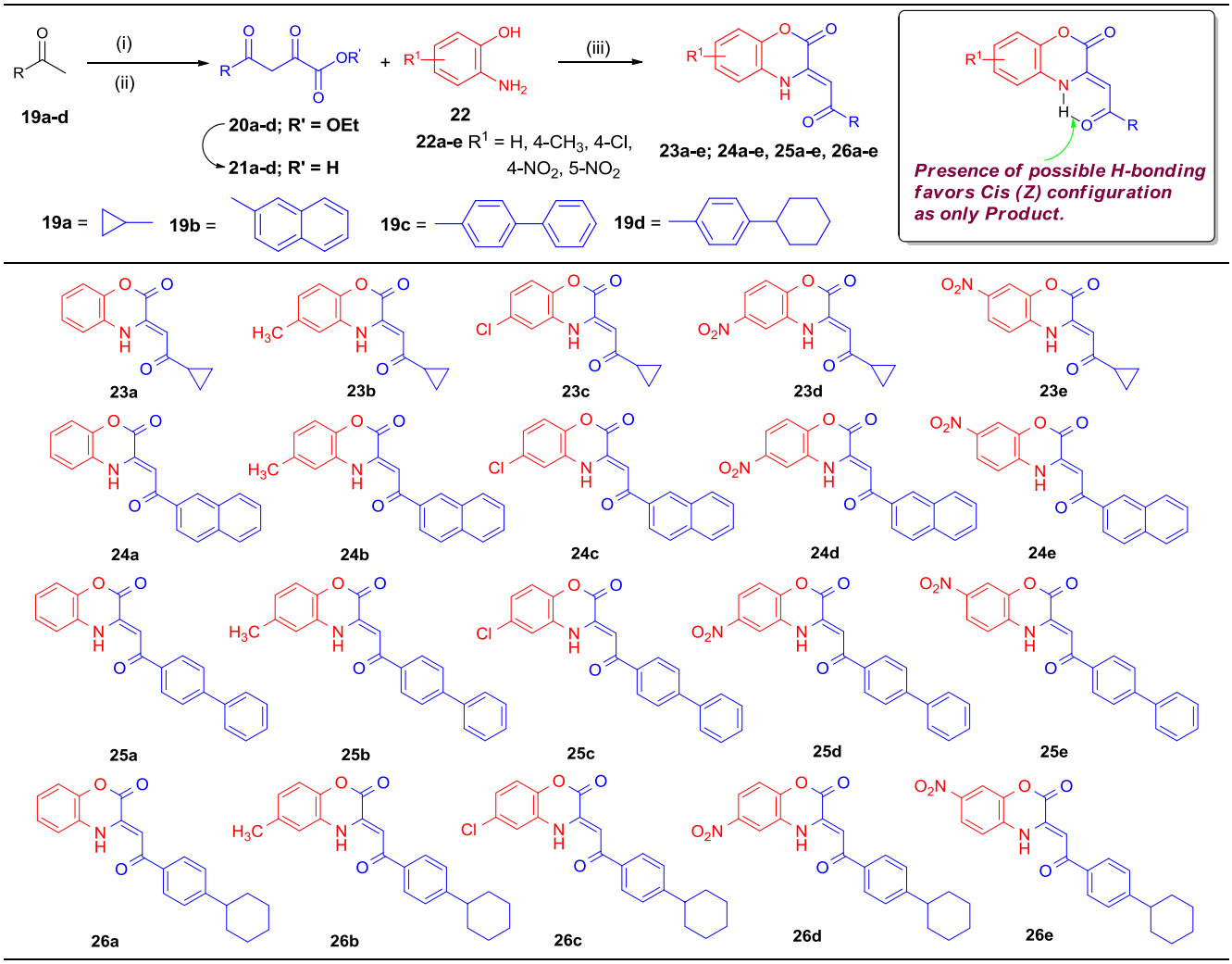


Fig. 2. Structures of potent pharmacologically privileged molecules having cyclopropyl, naphthyl, biphenyl and cyclohexylphenyl moieties.



Scheme 1. *Reagents and Conditions:* (i) NaH, $(COOCH_3)_2$, Toluene, 0–90 °C, (ii) $LiOH{\cdot}H_2O$, MeOH:THF:H_2O = 7:2:1. rt, 4–5 h, (iii) Microwave 150 °C, 10–15 min.[24]

Gram-positive bacterial strains, *Streptomyces griseus* [SG] (MTCC 4734), *Staphylococcus aureus* [SA] (MTCC 3381) and *Bacillus subtilis* [BS] (MTCC 10619), and Gram-negative bacterial strains *Escherichia coli* [EC] (MTCC 443), utilizing the agar diffusion assay.[25–27] The antibiotic ampicillin as well as chloramphenicol were taken as positive controls. Antibacterial screening of all the derivatives, **23**–**26** as well as positive controls were performed at a fixed concentration of 100 μg/mL. All twenty compounds exhibited antibacterial activity against both Gram-positive and Gram-negative bacterial strains with zones of inhibition (ZOI) ranging from 4 mm to 24 mm; and also exhibited antifungal activities against four fungal strains.[28]

The minimum inhibitory concentration (MIC) values for all the 2-oxo-benzo[1,4]oxazines **23**–**26** and the positive control drugs, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[29,30] As it can be seen from Table 1; **23e** was identified as the most potent antibacterial agents (MIC = 3.12 μg/mL) against [SG] and [BS] strains, respectively as it showed four times more activity than ampicillin against [SG] strain (MIC = 12.5 μg/mL) and eight times more potency than ampicillin against [BS] strains (MIC = 25 μg/mL). However, **23e** was found to be equally potent to chloramphenicol against [SG] strain (MIC = 3.12 μg/mL). whereas it displayed twice potency to chloramphenicol against [BS] strain (MIC = 6.25 μg/mL). The next potent compound was **26e** which showed eight times greater potency than ampicillin (MIC = 25 μg/mL) and two times more activity than chloramphenicol (MIC = 6.25 μg/mL) against [BS] strain, respectively. Furthermore, the next potent compounds found in this assay were **23d**, **25d**, **26a** and **26d** having MIC values of 6.25 μg/mL. **23d** showed greater potency than ampicillin but was found less active than chloramphenicol against [SG] strain. Compound **25d** showed two times greater potency than ampicillin and equal potency to chloramphenicol against [SA] strain. Similarly, **26a** exhibited four times greater potency than ampicillin and equal potency to chloramphenicol in [BS] strain. Likewise, **26d** was also found to show two times more activity than ampicillin but showed equal level of activity towards chloramphenicol against [SA] strain.

Compound **25b**, **25d** and **26d** against [SG] strains; **23b–c**, **23e**, **25b** and **26e** against [SA] strain; **26d** against [BS] strain and **24e** against [EC] strain showed equal potency to ampicillin but were less active than chloramphenicol. Similarly, **25b** and **25d** against [BS] strain; **23e**, **25a–b**, **25d–e**, **25d–e** against [EC] strain were found less active to both standard drugs, ampicillin and chloramphenicol. Finally, all those compounds which have shown (MIC = ≥25 μg/mL) in [SG], [SA] and [EC] strains were found to be either less active or no activity as compared to ampicillin and chloramphenicol. Overall, SAR study shows that nitro group either at 4- or 5-position and cyclopropyl and cyclohexylphenyl group have shown greater antibacterial activity (>MIC) than ampicillin and chloramphenicol except compound **23e** which showed equal potency to chloramphenicol against [SG] strain.

Table 1
Minimum inhibitory concentration values for novel functionalized 2-oxo-benzo [1,4]oxazines **23–26** and positive control drugs against bacteria and fungi.

Analogues	Minimum inhibitory concentration (MIC)[a]							
	Bacteria[b]			Bacteria[c]	Fungi[d]			
	SG	SA	BS	EC	FO	AN	PF	TR
23a	NA	100	NA	50	100	NA	NA	100
23b	100	12.5	NA	100	NA	NA	NA	NA
23c	NA	12.5	100	50	12.5	NA	NA	NA
23d	6.25	100	NA	50	12.5	100	NA	NA
23e	3.12	12.5	3.12	25	50	100	100	NA
24a	NA	NA	NA	NA	NA	100	NA	100
24b	NA	NA	NA	NA	NA	NA	NA	NA
24c	NA	NA	NA	NA	NA	NA	NA	NA
24d	100	NA	100	100	50	NA	NA	NA
24e	NA	100	NA	12.5	25	NA	25	25
25a	NA	NA	NA	25	NA	100	25	NA
25b	12.5	12.5	25	25	NA	NA	NA	NA
25c	NA	NA	NA	NA	NA	25	25	NA
25d	12.5	6.25	25	25	50	NA	25	100
25e	50	NA	100	25	25	NA	NA	NA
26a	50	NA	6.25	100	NA	50	50	NA
26b	NA	NA	NA	NA	50	100	NA	NA
26c	100	NA	NA	NA	25	25	NA	25
26d	12.5	6.25	12.5	25	50	25	25	25
26e	50	12.5	3.12	25	25	100	100	25
AMP[e]	12.5	12.5	25	12.5	–	–	–	–
CAM[f]	3.125	6.25	6.25	6.25				
KET[g]	–	–	–	–	12.5	12.5	25	25

[a] MIC of all compounds was measured at the range from 3.12 to 100 μg/mL.
[b] Gram-positive bacteria: SG, *Streptomyces griseus*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*.
[c] Gram-negative bacteria: EC, *Escherichia coli*.
[d] Fungi: FO, *Fusarium oxysporium*; AN, *Aspergillus niger*; PF, *Penicillium funiculosum*; TR, *Trichoderma Reesei*.
[e] AMP: Ampicillin.
[f] CAM: Chloramphenicol.
[g] KET: Ketoconazole; NA = Compounds which were found Not Active.

Compound **23c** and **23d**, have shown equal potency (MIC = 12.5 μg/mL) than ketoconazole (MIC = 12.5 μg/mL) in [FO] fungal strain. Moreover, compounds **24e**, **25e**, **26c** and **26e** in [FO] strain as well as compounds **25c**, **26c** and **26d** in [AN] strain were found two times less active compared to ketoconazole. While compounds such as **24e**, **25a**, **25c**, **25d** and **26d**, were found equally active having MIC = 25 μg/mL in [PF] strains; whereas compounds **24e**, **26c**, **26d** and **26e** displayed equal potency in [TR] strains; compared to ketoconazole. All those compounds which have shown (MIC = ≥50 μg/mL) in [FO], [AN], [PF] and [TR] strains were found to be either less active or no activity as compared to ketoconazole. Thus, SAR interpretation illustrates that nitro group containing cyclopropyl and cyclohexylphenyl substructure scaffolds in 2-oxo-benzo[1,4]oxazines increases antifungal activity.

All the active compounds, **23c–e**, **24e**, **25d** and **26d–e**, were assessed for their cytotoxic studies in 3T3 fibroblast cell lines using MTT assay.[31] The results are shown in Fig. 4. All the compounds

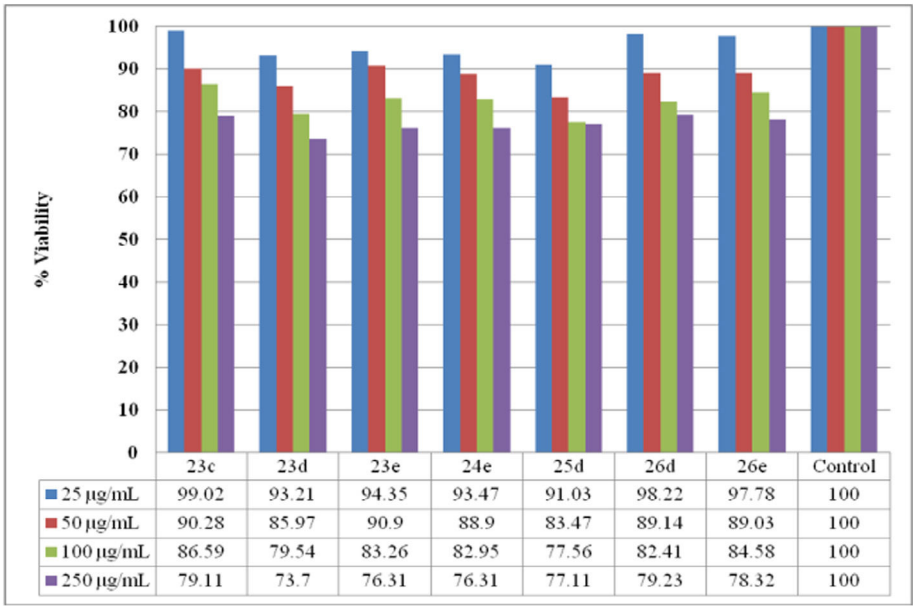


Fig. 4. Percentage cell viability test of active compounds **23c–e**, **24e**, **25d** and **26d–e**.

were found non-toxic even at 250 μg/mL and shows acceptable values of cell viability.

In conclusion, a new series of functionalized (*Z*)-3-(2-oxo-2-substituted ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-ones **23**–**26** were synthesized in excellent (upto 95%) yields. Several synthesized compounds were found to display greater *in vitro* antibacterial activity against gram positive and gram-negative bacteria i.e. *S. griseus*, *S. aureus*, *B. subtillis* and *E. coli* as well as *in vitro* antifungal activity against fungal species i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei*, than standard drugs, ampicillin and chloramphenicol, as well as ketoconazole, respectively. *Cyclopropyl* and *cyclohexylphenyl* substructure moieties were identified to increase both antibacterial and antifungal activities. Electron-withdrawing group (i.e. NO_2 group) present at 2-oxo-benz[1,4]oxazine nucleus increases antibacterial as well as antifungal activity. To the best of our knowledge, for the first time, pharmaceutically important substructures were incorporated into 2-oxo-benzo[1,4] oxazines, which displayed greater antimicrobial potency than standard drugs, ampicillin, chloramphenicol and ketoconazole. Analogues of compounds **23e** and **26e** as well as compound **26d** were considered as lead molecules worthy of further structural optimization and development as potential antibacterial and antifungal agents, respectively, for the treatment of bacterial and fungal infections.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.08.017.

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24. *General procedure for the Synthesis of 2-oxo-benzo[1,4]oxazine derivatives* (**23**–**26**): Diketoesters **20a**–**d**, which were obtained by base-mediated reaction of acetophenone **19a**–**d** with dimethyl oxalate, were dissolved in a mixture of MeOH:THF:H_2O (7:2:1) and LiOH.H_2O (1.2 eq.) was added to the reaction mixture after 10 min. The reaction mixture was stirred for another 3 h. The reaction mixture was quenched with 3 N HCl and extracted with ethyl acetate (3 × 10 mL); washed with distilled water (3 × 20 mL); then with brine (3 × 10 mL). The combined organic layer was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The resultant crude product was further purified by recrystallization with EtOAc/Hexane which afforded pure diketo-acids **21a**–**d**. Then after, to a solution of the compound **21a**–**d** (substituted diketo-acid: 2.0 mmol) in diethylene glycol (2 mL) was added compound **22a**–**e** (substituted 2-aminophenol; 2.0 mmol). The reaction mixture was subjected to microwave irradiated at 150 °C temperature for about 10–20 min. The progress of the reaction was monitored by TLC (9:1 Hexane/ethyl acetate as an eluent). Then, the reaction mixture was extracted with ethyl acetate (3 × 50 mL); washed with distilled water (50 mL); then with brine (3 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant crude product were further purified by recrystallization by EtOAc/Hexane (v/v = 80:20) or by flash column chromatography technique over silica gel (using 9:1 Hexane/ethyl acetate as an eluent), which furnished the 2-oxo-benzo[1,4]oxazine derivatives (**23**–**26**) in 70-96% yields range.(b) *Selected spectral data*: (i) *(Z)-3-(2-cyclopropyl-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one* (**23e**): Solid; yield: 74%, m.p. 218 °C; FT-IR (KBr, νmax/cm^{-1}) 3438, 3029, 2932, 1764, 1618, 1452, 1268; ^{1}H NMR (400 MHz) δ 8.08–8.04 (m, 2H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.57 (s, 1H), 2.09–2.03 (s, 1H), 1.18–1.16 (m, 2H), 1.08–1.04 (m, 2H); ^{13}C NMR (100 MHz) δ 203.3, 155.1, 142.6, 140.1, 134.7, 129.9, 121.9, 115.2, 113.5, 101.6, 22.8, 12.5; HRMS (ESI) calcd. for $C_{13}H_{10}N_2O_5$ [M+H]$^+$: 275.0590; found 275.0596. (ii) *(Z)-3-(2-(naphthalen-1-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one* (**24e**): Solid; yield: 72%, m.p. 278 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 2929, 1763, 1629, 1344, 1290; ^{1}H NMR (400 MHz) δ 8.72 (s, 1H), 8.16 (d, *J* = 7.6 Hz, 1H), 8.04–7.93 (m, 5H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.64–7.56 (m, 2H), 7.18 (s, 1H); ^{13}C NMR (100 MHz) δ 190.8, 155.6, 142.5, 141.0, 138.6, 135.8, 135.6, 132.9, 131.2, 130.2, 129.6, 129.2, 129.1, 128.1, 127.4, 123.9, 121.4, 117.4, 112.6, 96.9; HRMS (ESI) calcd. for $C_{20}H_{12}N_2O_5$ [M+H]$^+$: 361.0746; found 361.0741. (iii) *(Z)-3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one* (**25e**): Solid; yield: 72%, m.p. 264 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2921, 1769, 1633, 1600, 1573, 1274; ^{1}H NMR (400 MHz) δ 8.09 (d, *J* = 8.8 Hz, 2H), 7.74–7.64 (m, 4H), 7.48–7.41 (m, 3H), 7.14–7.08 (m, 4H); ^{13}C NMR (100 MHz) δ 189.8, 156.2, 144.8, 140.6, 139.6, 137.6, 129.6, 129.3, 129.5, 128.8, 128.6, 127.6, 127.4, 126.1, 123.5, 118.4, 117.0, 94.7; HRMS (ESI) calcd. for $C_{22}H_{14}N_2O_5$ [M+H]$^+$: 387.0903; found 387.0909. (iv) *(Z)-3-(2-(4-cyclohexylphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one* (**26e**): Solid; yield: 71%, m.p. 178 °C; FT-IR (KBr, νmax/cm^{-1}) 3428, 2924, 1767, 1634, 1602, 1339, 1282; ^{1}H NMR (400 MHz) δ 8.12-8.07 (m, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.19–7.16 (m, 2H), 2.61-2.59 (m, 1H), 1.87–1.75 (m, 5H), 1.49–1.25 (m, 5H); ^{13}C NMR (100 MHz) δ 192.2, 155.2, 154.7, 142.8, 140.3, 136.9, 135.5, 129.8, 128.4, 127.6, 121.9, 115.5, 113.6, 98.3, 44.9, 34.2, 26.8, 26.1; HRMS (ESI) calcd. for $C_{22}H_{20}N_2O_5$ [M+H]$^+$: 393.1372; found 393.1378.

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