Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bio-Active Alkaloids

Ph.D. Thesis

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Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bio-Active Alkaloids

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by

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DECLARATION

I, **Vashundhra Sharma**, declare that this thesis titled, "**Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bio-Active Alkaloids**" 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.
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(2012RCY9526)

CERTIFICATE

This is to certify that the thesis entitled **"Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bio-Active Alkaloids"** being submitted by Ms. **Vashundhra Sharma (2012RCY9526)** 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: 22-12-2018

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ABSTRACT

The thesis entitled "***Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bioactive Alkaloids***'' study emphasize on the formation of bioactive alkaloid from the cross coupling reactions utilizing greener approach.

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

Chapter 1: This chapter presents a concise overview of the development of several coupling (C-C/C-N/C-O) reactions such as Kharasch, Tsuji, Trost, Kumada, Tamao, Sonogashira, Stille, Heck, Negishi, Hiyama and Suzuki etc. using numerous transition metals such as Pd, Ru, Rh, Cu, Fe, Co and Ni to synthesize bioactive alkaloids. It also highlights the concise overview towards the applications of coupling methodologies in which the C–H bond has been exploited efficiently to construct C-C, C–O and C–N bonds in bioactive natural alkaloids syntheses.

Chapter 2: This chapter represents the synthetic and biological utility of the benzo[1,4]oxazine class of compounds. In benzo[1,4]oxazines, a sub-class of benzo fused heterocycles, are endowed with a wide range of biological activities such as anti-inflammatory, analgesic, antibacterial, neuroprotective, D2 receptor antagonists, antimycobacterial, antifungal, herbicidal, antiarrhythmic, and selective non-steroidal mineralo-corticoid receptor antagonists etc. Some marine secondary metabolites such as, Arcticoside (potent antifungal agent) and C-1027 chromophore- III & V (potent antitumor antibiotic), which were isolated from a culture of an arctic marine actinomycete *Streptomyces strain*; possess as benzo [1,4] oxazines substructures in their active scaffolds.

Chapter 3: This chapter describes the discovery of an unprecedented organocatalyst for the synthesis of pyridine-fused analogues. Pyridine nucleus constitutes the core of a large family of natural products, bioactive pharmaceuticals, vitamins, alkaloids, chiral ligands and functional materials. Substituted pyridine-alicyclic fused bioactive heterocycles, as a whole or as a subunit, are present in several natural products, and are endowed with a wide range of biological activities such as antitubercular, anti-fungal, D2 receptor antagonists, anticancer, anti-microbial, anti-HIV, Na+/H+ exchange inhibitors, atypical antipsychotic, H1-antihistamine, cytotoxic and antidepressant etc.

Chapter 4: This chapter describe the design, synthesis, antioxidant and antiplatelet activities, and their SAR studies of novel aporphine analogues and their SAR studies. These alkaloids are isolated from several natural sources (i.e. *Hernandiaceae*, *Lauraceae*, *Annonaceae, Menispermaceae*, *Monimiaceae*, and other plant families) and displayed a wide range of pharmacological activities. Therefore the design of a retro-synthetic route for the synthesis of novel analogues of aporphine class of alkaloids in a highly efficient method leading to excellent yield (*ca.* 84%). *via* metal catalyzed as well as organocatalytic approach is reported.

Chapter 5: In this chapter, we have carried out The synthesis, antimicrobial and antiplatelet activities of novel *Cephalandole A* analogues are reported. Cephalandole A was isolated from the Taiwanese orchid *Cephalanceropsis gracilis* (Orchidaceae) in 2006. The crude extract of this plant exhibited excellent activity against lung (NCI-H460; IC_{50} = 7.8 μM), breast (MCF-7; IC_{50} = 7.57 μM) and CNS (SF-268; IC_{50} = 12.2 μM) carcinoma cell lines.

Conclusion: The present thesis involves focused on the development of novel green synthetic methodologies via C-C/C-O/C-N coupling reactions. The developed methodologies are were also utilized directly in the synthesis of bioactive alkaloids; such as 2-oxo-benzo[1,4]oxazines, alicyclic[*b*]-fused pyridine molecular frameworks, aporphines and Cephalandole A analogues.

AIMS AND OBJECTIVES

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

- To design and synthesize novel analogues of bioactive alkaloids.
- Development of operationally simple and environmentally benign approach for the synthesis of targeted bioactive alkaloids.
- Characterization of structures of all novel compounds by their ESI-MS, ^{1}H NMR, ^{13}C NMR, IR, and HRMS spectral data analysis.
- Bio-evaluation (antioxidant, antiplatelet, antibacterial and antifungal activity) of synthesized libraries of compounds.
- Validation of biological activity results *via insilico* molecular docking studies.

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LIST OF ABBREVIATIONS

MIC	Minimum inhibitory concentration
min	Minutes
ml	Millilitre
mmol	Millimole
mp	Melting point
MS	Mass spectroscopy (in Mass Spectrometry)
MeOH	Methanol
N_2	Nitrogen
N	Normality
NMR	Nuclear Magnetic Resonance
o	Ortho
p	Para
m	meta
ppm	Parts per million
q	Quartet
R_f	Retardation factor
rt	Room temperature
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TEA	Triethylamine
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography
TMS	Ttrimethylsilane
µg	Microgram
µM	Micromolar
aq.	Aqueous
$CDCl_3$	Deuterated Chloroform
DMSO-d_6	Deuterated dimethylsulphoxide
IC_{50}	Inhibitory concentration

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Chapter 1

Recent advances in bioactive alkaloid synthesis via coupling reactions - Current and its future perspective

1.1 Introduction

Natural products (NPs), derived from terrestrial as well as from marine organism and higher plants, have been recognized as an abundant sources of therapeutic medicines, dyes, pesticides, flavours, and fragrances by human being.[1] Modern era of natural products chemistry had revealed that, the bioactive NPs are substantially responsible for the pharmaceutical properties of botanical pesticides and traditional herbal medicines.[2] Nowadays, the identification of lead motifs from bioactive natural products or their analogues, has been found to be a robust and challenging approach in new drug discovery. Moreover, the chemistry on natural products has been accelerated by the development of modern separation and purification techniques, improvements in spectroscopic analysis, and by the use of newly-introduced modern sophisticated equipment/instruments and infrastructure in synthetic organic chemistry.[3]

The pharmaceutically potent alkaloids are an important class of bioactive natural products and are widely isolated from various plants and marine organisms.[4] The term "alkaloid" had been coined by Meissner in 1819, and it had been frequently used since 1882, after the report by Jacobson in a review.[5] In the same year i.e. 1882, the structure of the first alkaloid "xanthine" was established, and in 1886 Ladenburg reported the first synthesis of an alkaloid "(+)-coniine".[6] The determination of absolute structure of alkaloids always remains a tedious task for chemists due to their complex architecture. Therefore, although the "strychnine (a indole alkaloid) was first isolated in 1818, its structure was fully characterized in 1947, and its first synthesis was developed by Woodward in 1954.[6,7]

The alkaloids, isolated from marine invertebrates and microorganisms, are rich sources of secondary metabolites of several drugs or lead drug candidates and are being used in the treatment of several diseases.[1,4] They are known to exhibit wide range of structural diversity and complexity which are being generally introduced by the incorporation of several bioactive scaffolds such as terpenes,[8] steroids,[9] mono and poly-saccharides,[10] amino acids[11] and other naturally chiral molecules.[12] It has been portrayed that alkaloids do large interaction with several biological targets and thus, they exhibited various potent activities. In addition, they have also provided inspiration for the synthesis of drugs or drugs like candidates. Among these natural products, quinoline,[13] isoquinoline,[14] coumarine,[15] naphthols,[16] imidazole,[17] oxazole[18] as well as thiazole[19] substructure-containing alkaloids are the diverse class of bioactive alkaloids; which exhibited several significant biological activities, including

antitumor, anti-bacterial, anti-viral, anti-malarial, immunosuppressive activities, etc. (Figure 1[20] and 2[21])

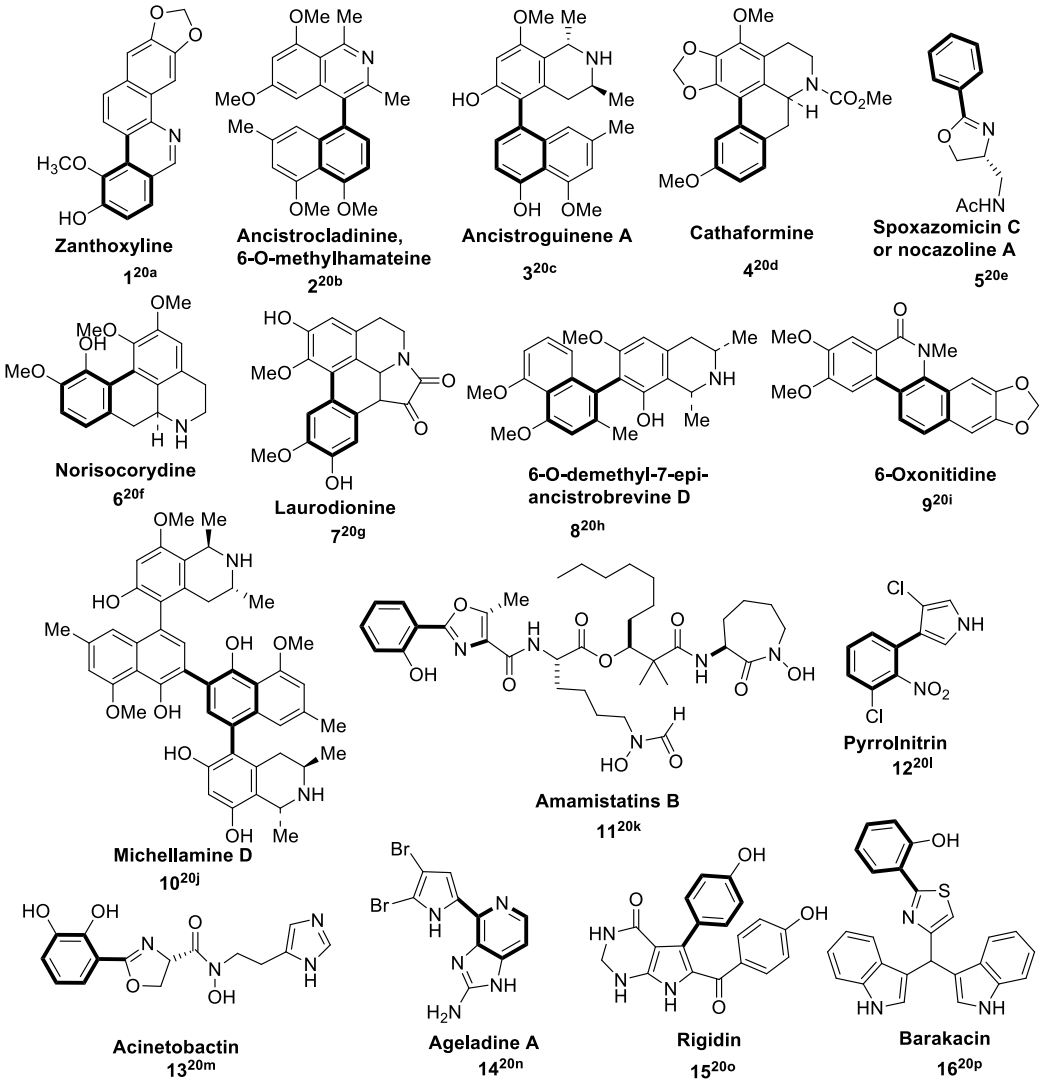


Figure 1. Structure of some biaryl subunits present in biologically active alkaloids.[20]

As shown in Figures 1 and 2; it is clearly observed that these bioactive molecules can be easily synthesized via carbon-carbon (C-C) coupling reactions, perhaps applying on suitable substrates and it is important in pharmaceutical industries.[20-21] Therefore, there is a challenge to develop a mild and simple; a highly efficient and synthetically useful methods to synthesize these biologically active alkaloids via coupling reactions. In this context, numerous transition metals such as Pd, Ru, Rh, Cu, Fe, Co and Ni were used for the synthesis of several biologically privileged scaffolds including biaryl and its related analogues.

In this review, we have focused on the concise studies towards the applications of coupling methodologies in which the C–H bond has been exploited efficiently to construct C-C, C–O and C–N bonds in bioactive natural alkaloids syntheses. Selected examples were taken to illustrate the potential of the coupling (C-C, C–O and C–N)

reactions and its synthetic application towards the synthesis of biologically privileged natural product and its analogues.

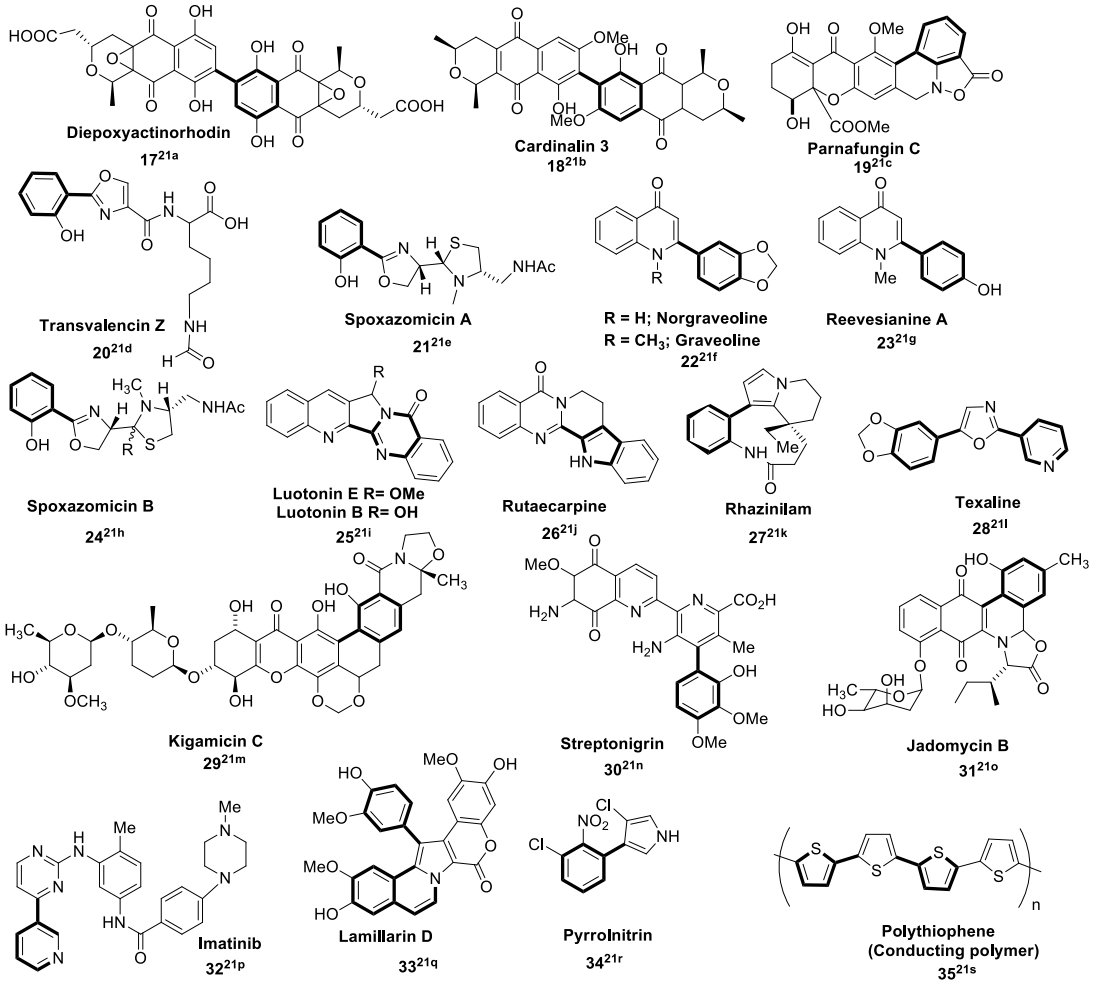


Figure 2. Some biaryl subunits having biologically privileged scaffolds.[21]

1.2 Development of coupling (C-C/C-N/C-O bond) approach: A concise overview

Total synthesis of bioactive natural products is necessary and demanding for synthetic chemist to synthesize highly constrained molecular architecture in the laboratory due to its high efficacy, occurrence in small amount from natural sources, multi-step synthesis. In recent years, C-C/C-N/C-O coupling reactions via C–H bond functionalization have been used extensively to prepare these NPs efficiently in a single high-yielding step. Moreover, it has been well documented that the coupling reaction is a powerful tool to synthesize the target compound involving a variety of reactions where two hydrocarbon fragments are coupled by the use of suitable catalyst and reaction conditions.

The basic coupling reaction involves the reaction between a organometallic compound i.e. RM (R = organic fragment, M = main group centre) with an organic halide i.e. R'X and forms a new C - C bond in the product i.e. R-R', and thus in this way the transition metal catalysed biaryl cross-coupling reactions occurs.[22,23] The

transition-metal (TM) catalysed carbon-hetero (i.e. C – X) bond forming reactions have also received huge attention to synthetic chemists towards coupling reactions, due to their extensive medicinal as well as industrial applications.

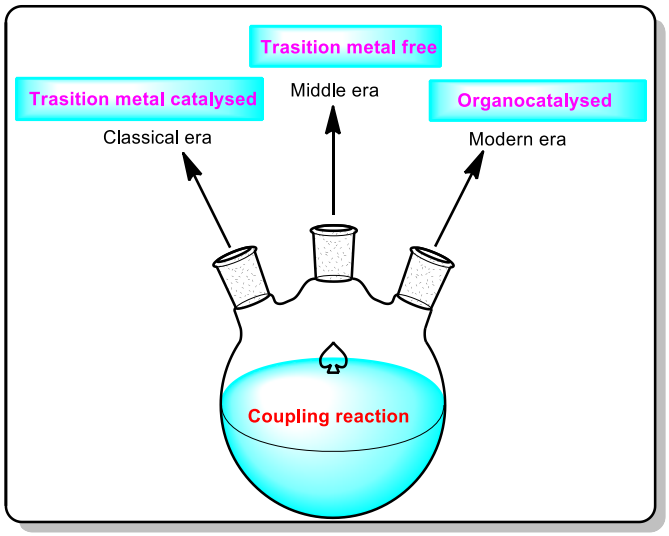


Figure 3. Schematic representation of types of coupling reactions.

Based on whether the reaction is catalyzed via transition metal or not; the coupling reactions has been classified in three major categories (Figure 3):

1. Transition metal catalyzed Coupling reactions.
2. Transition metal free Coupling reactions.
3. Organo-catalyzed Coupling reactions

1.3 Different Era's of coupling reactions

The d-block transition metals have been utilised in several areas of organic synthesis and medicinal chemistry including biological processes, signalling pathways, sensing as well as in catalysis. In the area of synthetic chemistry, it has been found that transition metals have excellent properties with respect to interaction with substrates through their vacant d-orbitals, activating functional group transformations, as well as polarization and rearrangements of bonds. The main differences between the several methodologies, which had been developed by various research groups working in the area of the coupling reactions, lies in the type of transition metals used because of its major effects on the reactivity of the substrates as well as the functional groups. Therefore, on the basis of the nature and reactivity of transition metals used as catalyst, various types of coupling reactions had been developed (Figure 4).

The development of transition metal-catalysed cross-coupling reactions had been delineated by pioneer work done by Kharasch [24] and, then later by, Tsuji,[25] Trost,[26] Kumada,[27] Tamao,[27] and Sonogashira[28] etc. However, the significance of these palladium-catalysed processes became fully delighted after contributions made by the

late Stille,[29] Heck,[30] Negishi,[31] Hiyama[32] and Suzuki[33] in 1970 to late 1980. Kumada et. al.[27] reported that the coupling reaction between an aryl halide i.e. R-X with Grignard reagent i.e. RMgX (moisture sensitive) to form cross-coupled biaryl products. Negishi et al. [31] developed a carbon-carbon coupling reaction between an organo-zinc chloride with an organo halide (X = Br, I) in the presence of a catalytic amounts of a Pd-phosphine complex. Stille and his co-workers[29] reported the formation of C-C coupling products via the reaction of organotin compounds with organohalides using Pd catalyst and phosphine ligands. This developed reaction has the limited scope in the synthesis of bioactive natural products due the highly toxic nature of tin metal.

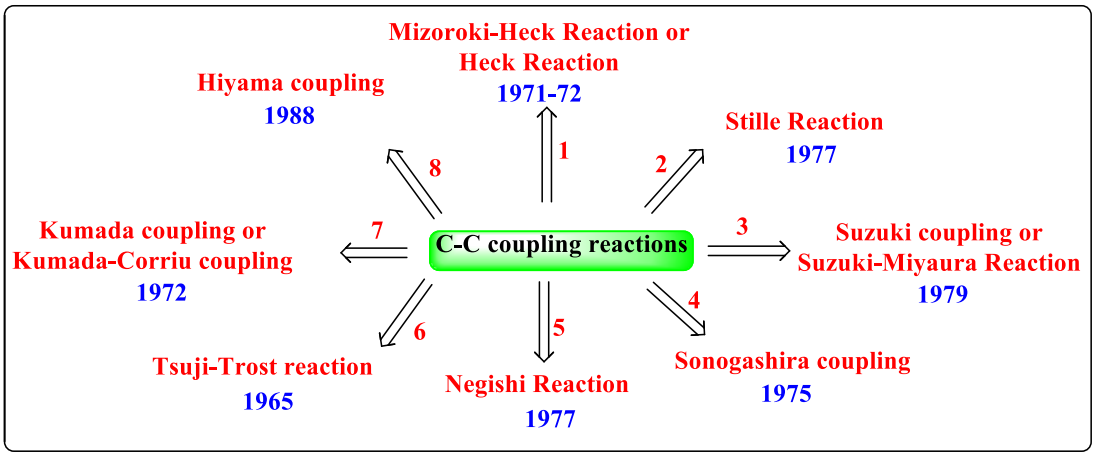


Figure 4. Era of coupling reactions.

Furthermore, the coupling reaction of organosilicon compounds with organic halides using Pd catalyst and a fluorine source (such as KF, CsF etc.) was reported by Hiyama and his co-worker`s.[32] The organoboronic acids/esters were utilised by Suzuki et al.[33] for the selective formation of C-C bonds in the synthesis of biaryl moiety.[34] This methodology has a wide applicability due to the excellent thermal stability and inertness, with respect to water and oxygen, of organoboronic compounds. Thus, Suzuki reaction is frequently utilised without taking any critical precautions in the synthesis of numerous bioactive scaffolds and its analogues.[35,36]

Finally, due to the excellent and most significant contribution in development of C – C coupling reaction methodology, Richard Heck, Ei-ichi Negishi, and Akira Suzuki have been awarded the Nobel Prize in Chemistry in 2010.[37,3]

1.3.1 Cross-coupling reactions *via* transition metal catalytic approach

The most important and challenge task in coupling reactions is selectivity of the transition metal towards inert C–H bond with respect to generate a reactive C–M bond. This selective activation is achieved by a directing group (DG), which

coordinates the catalyst and delivers very selectively to a proximal C–H bond. Therefore, specific transition metal has specific interaction with a selective directing group (DG). On the basis of the transition metals selectivity, various research groups have developed different type of coupling methodologies, which is highlighted in figure 5.[39]

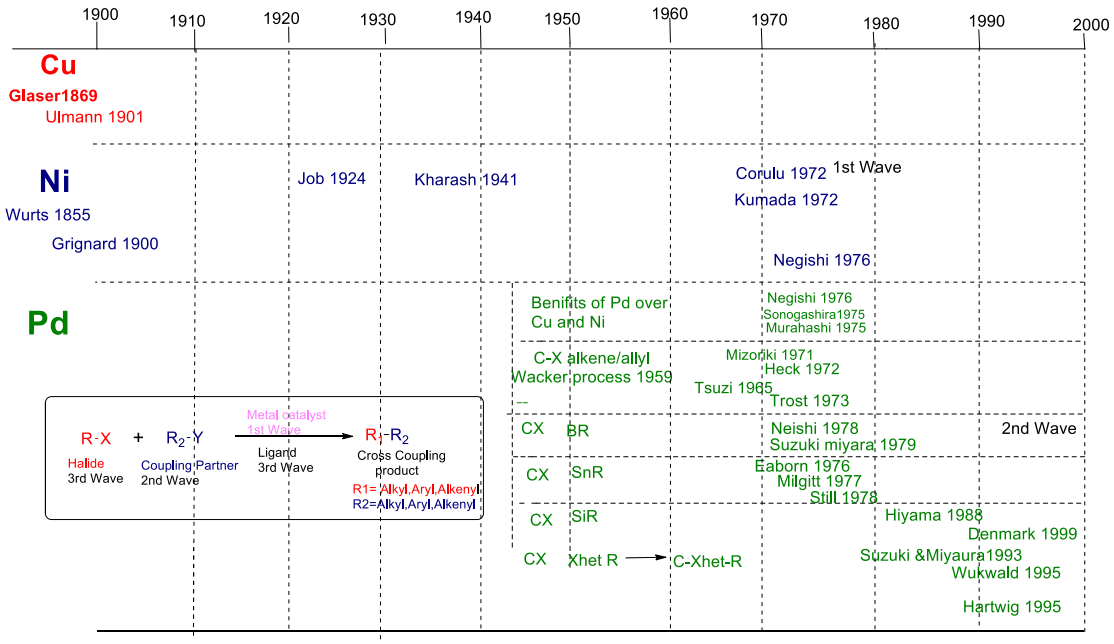


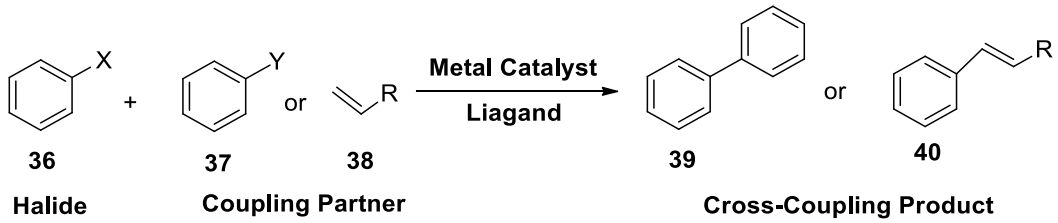
Figure 5. Timeline of the discovery and development of metal-catalysed cross-coupling reactions.

As depicted in figure 5; the transition metal catalyzed coupling reactions have been categories in three major categories:

(1) The investigation of the suitable metal catalysts (which is capable of promoting the coupling reactions in a selective manner).

(2) The selection of suitable coupling partners, and

(3) The improvement and selection of more effective ligand with a suitable catalytic system. The schematic representation of general C-C coupling reaction is shown in scheme-1:[39]



Scheme 1: General representation of C-C coupling reaction

To enhance the rate of coupling reactions, various directing groups have been developed for C–H functionalization; mostly they are O- or N-centered heterocycles or some functional groups (FG).[40] The synthetic utility and efficacy of C-H bond

functionalization were found to be excellent, when the directing group present within an existing FG on the substrates. In other words, the directing groups were firstly installed in the substrate and then removed after coupling reaction. Some directing groups may be utilised in the transformation into other functionalities to get the bioactive scaffolds. In the general mechanistic pathway of metal-catalyzed coupling reactions, it involves a sequence of coordinations, followed by C–M bond formation via cyclo-metalation and further C–M bond functionalization via other coupling partner, which affords the coupled product.[39] This common strategy has been used extensively in the metal-catalyzed coupling reactions via the formation of C–O, C–N, C–X (halogen), C–S, and C–C bonds. This strategy has also been modified by different research groups to get the good results in coupling reaction approach towards the design and efficient synthesis of bioactive natural alkaloids and its analogues.

1.3.1.1 Pd-catalysed C-C/C-N coupling reaction

Prior to the discovery of Pd and other transition metals catalysts, the coupling reactions have limited applications. For example, they mostly involves Grignard reagents and organo-alkali species (M = Li, Na, K); which reacts with unhindered alkyl (sp^3) substrates in a general fashion, but their use in coupling reactions with the substrates having unsaturated carbon atoms (sp^2 - sp^2 or sp^2 - sp bonds) were limited and least explored. These problems were overcome by the development of TM-catalyzed coupling reactions over the three decades (1965-1988), and it has been growing at an extremely higher rate. The coupling reaction methodologies were primarily centered on Pd-catalyzed reactions between aryl or alkenyl halides with organo-metallic substrate containing Al, Zn, Zr (Negishi reaction)[31] (Suzuki reaction),[33] Sn (Stille reaction),[29] Si (Hiyama reaction) [32] and terminal alkenes (Heck reaction)[30] or alkynes (Sonogashira reactions).[28]

The widely accepted mechanism for Pd-catalyzed coupling reactions of organometals R^1M with electrophiles R^2X is depicted in Figure 6.[39] It involves three steps: (i) oxidative addition (OA) of R^2X to $Pd(0)L_n$ species, where L_n represents coordinating ligands, (ii) trans-metallation between $R_2Pd(II)L_nX$ and R^1M, and (iii) reductive elimination (RE) of $R^1R^2Pd(II)L_n$ to give R^1 - R^2 *via* C–C bond formation, along with the regeneration of Pd(0) species for continuation of the catalytic cycle. The catalytic cycle is applicable for a wide range of Pd-catalyzed coupling reactions between R_1M and various organic halides R_2X (where, R = allyl, propargyl, benzyl, acyl, alkenyl,

alkynyl, aryl). The catalytic cycle in figure **6** serves as a representative schematic presentation for others coupling reactions catalysed by other Transition Metals.

Furthermore, the reaction models have also been proposed using concepts in frontier molecular orbital (FMO) theory developed by Fukui,[41] the conservation of orbital symmetry demonstrated by Woodward and Hoffman[42] and synergistic bonding as explained by Dewar.[43] These models explains that at least two critical factors are responsible in C–C couplings catalysed by d-block transition metals:

(i) The presence of (filled) non-bonding orbitals on the organometallic species along with one or more (unfilled) valence orbitals on the acceptor species, which serves as highest occupied (HOMO) and lowest unoccupied molecular orbitals (LUMO), respectively, and (ii) Available redox states to support oxidative and reductive routes in the same reaction conditions.

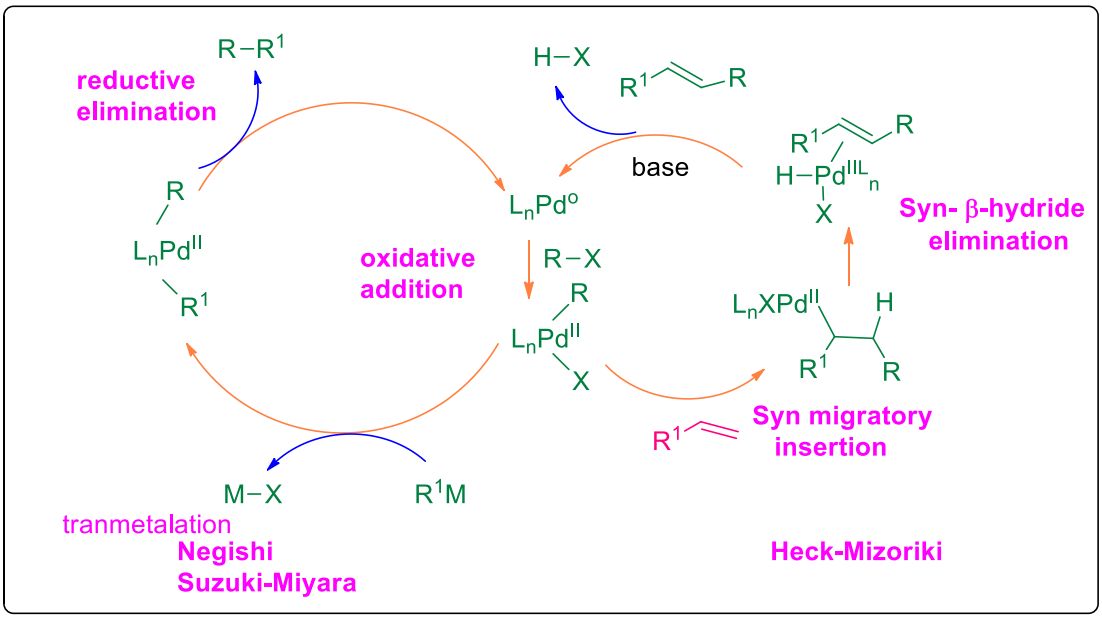
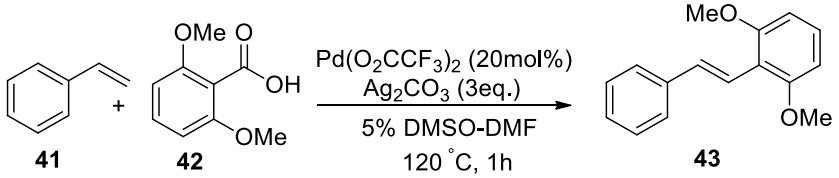


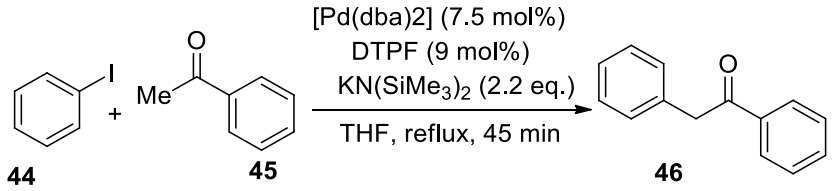
Figure 6. General mechanism of Pd-catalysed cross coupling reaction

The direct arylation *via* direct C–H bond functionalization has emerged as an efficient methodology for cross-coupling reaction, and is being increasingly utilised in the synthesis of bioactive alkaloids.[44] Myers et al.[45] reported decarboxylative C-C cross-coupling reaction employing palladium (II) salt as catalyst for the synthesis of functionalized stilbene using phenyl styrene and substituted carboxylic acid.



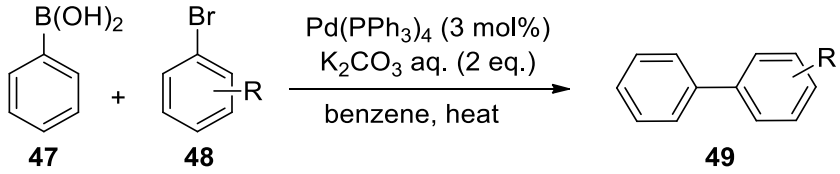
Scheme 2: Direct arylation *via* direct C–H bond functionalization.

Hartwig and co-worker's reported an efficient synthesis [46] of 1,2-diphenylethanone *via* the reaction of acetophenone and iodobenzene using Pd-catalyst in THF solvent.



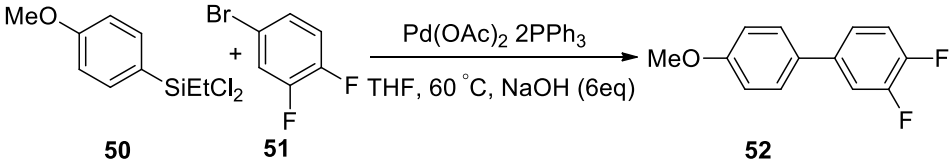
Scheme 3: Synthesis of 1, 2-diphenylethanone.

Singh et al. reported[47, 48] the synthesis of functionalized biaryls via Suzuki-Miyaura cross coupling reaction conditions utilizing Pd (0) as catalyst.



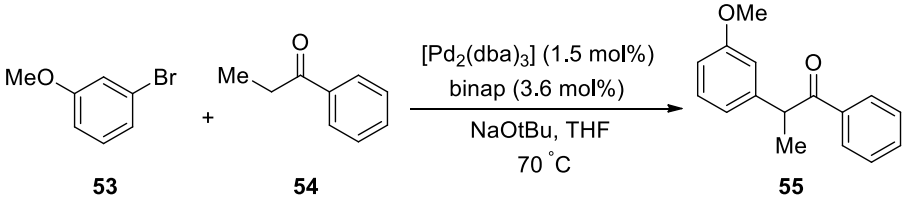
Scheme 4: Synthesis of functionalized 1, 1'-biphenyl.

Hiyama and co-worker's utilized organo-chlorosilanes & NaOH as the base (avoiding fluoride activator base i.e.TBAF) for the synthesis of fluorinated biaryl. [49, 50, 51]



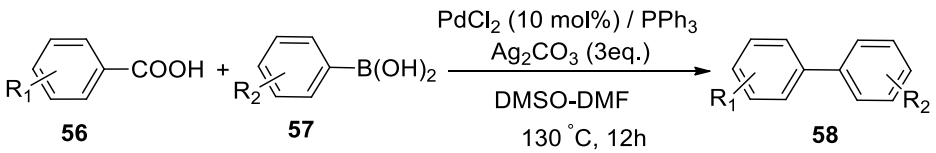
Scheme 5: Synthesis of fluorinated biaryl.

Buchwald et al.[52] developed an efficient methodology for the arylation *via* sp^3 C-H bond functionalization alkyl aryl ketone and haloarenes.



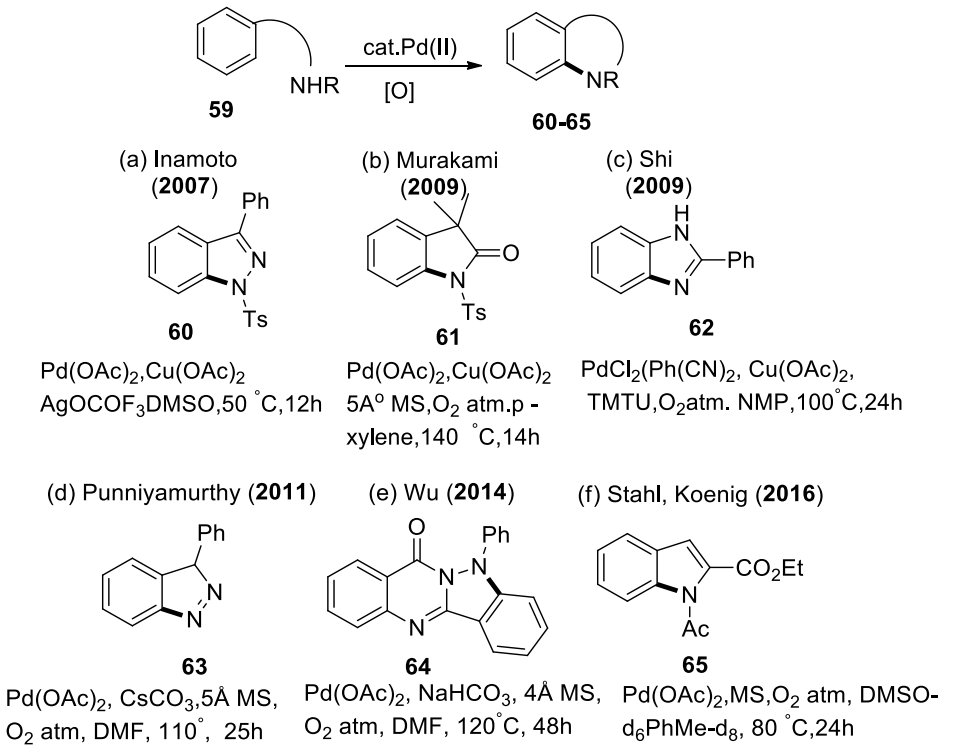
Scheme 6: Synthesis of 2-(3-methoxyphenyl)-1-phenylpropan-1-one.

Moreover, the synthesis of unsymmetrical biaryls *via* Pd-catalyzed decarboxylative arylation of functionalized benzoic acids with phenylboronic acid have been reported [53] by Ze Tan and his co-worker`s.



Scheme 7: Synthesis of unsymmetrical biaryls.

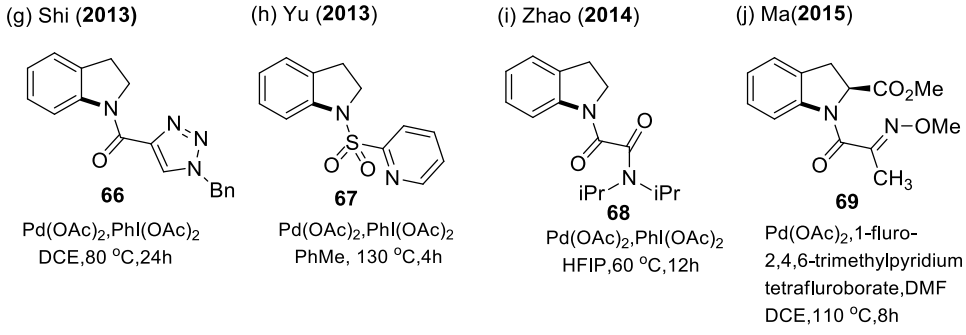
In addition to C-C coupling reactions, the inter- as well as intra-molecular C-N coupling reactions have been also developed using Pd-catalyst for the synthesis of N-heterocyclic bioactive scaffolds.



Scheme 8: Synthesis of N-heterocyclic bioactive scaffolds.

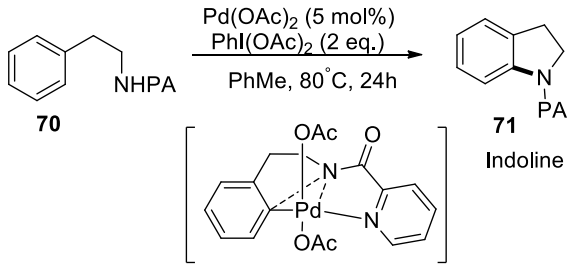
Thus, an extensive study had been carried out by numerous research groups to improve the reaction conditions with respect to higher yields and good selectivity. Earlier, Buchwald et al. reported the synthesis of azaheterocycles *via* the formation of C-N bond using oxidative cross dehydrogenative coupling (CDC) methodology.[54] After that, various heterocyclic bioactive scaffold had been synthesized such as indoles,[55,56] oxindoles,[57] indazoles,[58] imidazoles,[59] benzotriazoles,[60] as well as quinazolinones based analogues (scheme 8).[61]

Similarly, indoline synthesis under various conditions had also been reported by several research groups, which is shown in scheme 9.[62]



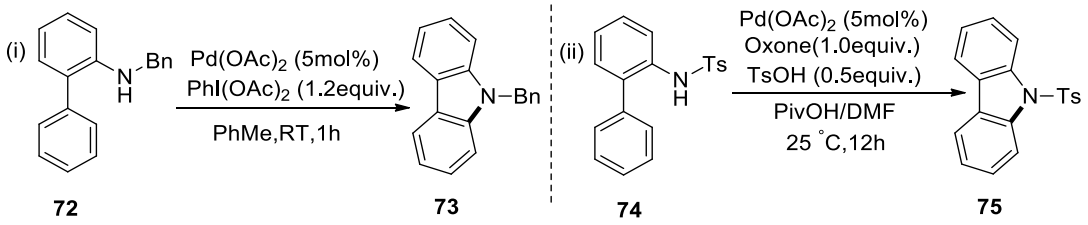
Scheme 9: Synthesis of indoline.

Daugulis et al. also developed an efficient protocol for the synthesis of protected indolines using $PhI(OAc)_2$ as oxidant in the following way: [63,64]



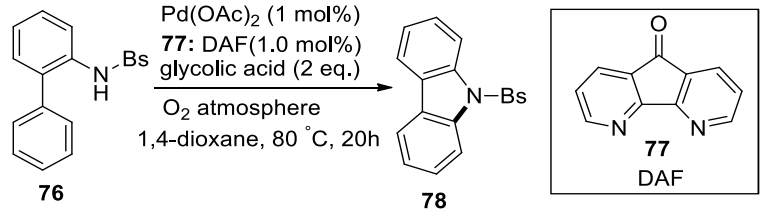
Scheme 10: Daugulis et al. approach of indolines Synthesis.

Gaunt et al. in 2008, [Scheme 11 (i)] reported a mild synthetic approach to synthesize carbazoles using $PhI(OAc)_2$ as the oxidant.[65] Whereas, Youn and co-workers modified this protocol by the use of oxone as a oxidant [scheme- 11 (ii)], in the place of $PhI(OAc)_2$.[66]



Scheme 11: Synthesis of carbazoles.

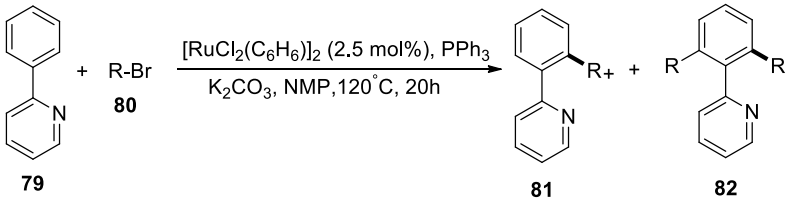
Furthermore, the synthesis of carbazoles from N-protected [1,1'-biphenyl]-2-amine was reported by Stahl and his co-workers using glycolic acid and O_2 as an oxidant.[67]



Scheme 12: Synthesis of carbazoles using glycolic acid and O_2 as an oxidant.

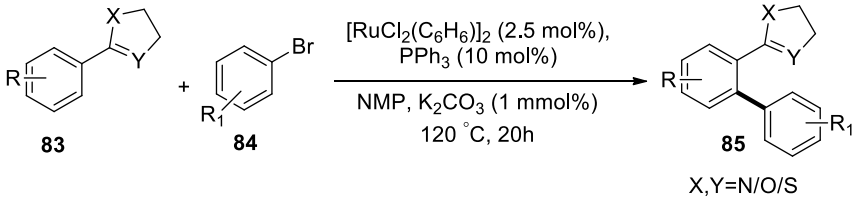
1.3.1.2 Ru-catalysed C-C coupling reaction

The mono-arylation of substituted 2-pyridylarenes were selectively obtained in good yields. The arylation and alkenylation reactions showed that the 2-pyridyl group was an efficient directing group for functionalization of sp^2 C−H bonds at the neighbouring ortho-position.[68]



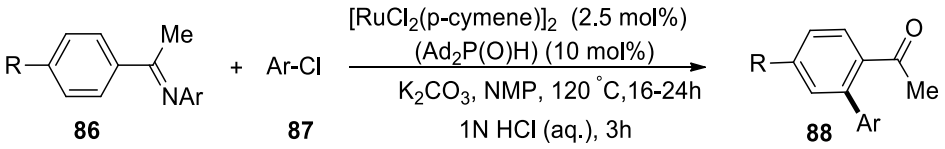
Scheme 13: Mono-arylation of substituted 2-pyridylarenes.

Oi and co-workers reported the coupling of 2-aryloxazolines and 2-arylimidazoles with halo-benzene using Ru-catalyst, at 120 °C temperature.[68]



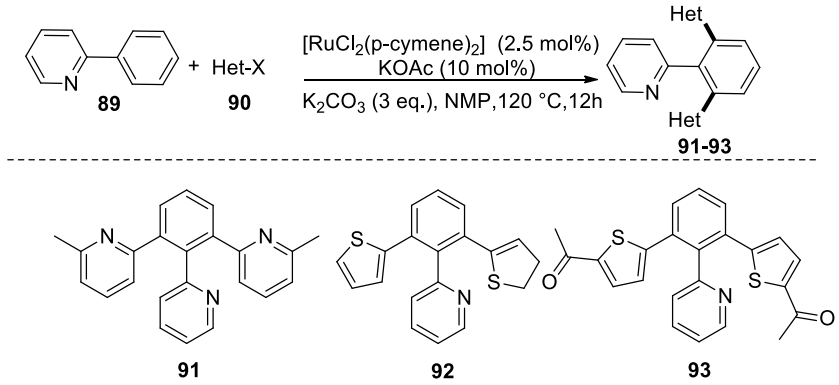
Scheme 14: Coupling of 2-aryloxazolines & 2-arylimidazoles.

This Ru-catalysed mono-arylation of ketimines with arylchlorides was further developed and the ketimines was hydrolyzed after coupling reaction into the corresponding ketones. [68]



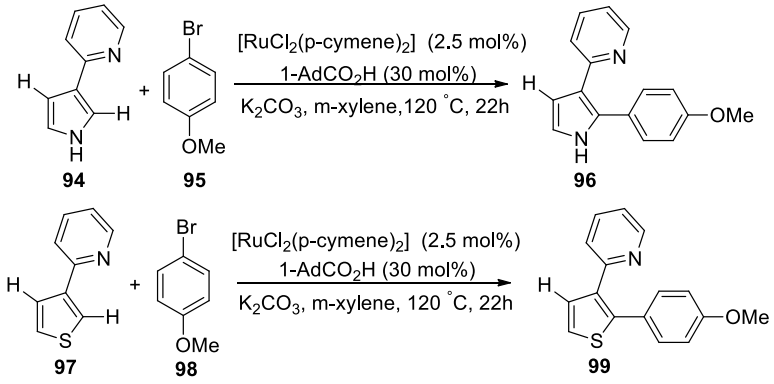
Scheme 15: Formation of ketones *via* Ru-catalysed mono-arylation.

The synthesis of poly-heterocyclic compounds *via* the diarylation of 2-aryl pyridine was also reported by the use of [$RuCl_2$(p-cymene)$_2$] catalyst. [69]



Scheme 16: Synthesis of poly-heterocyclic compounds.

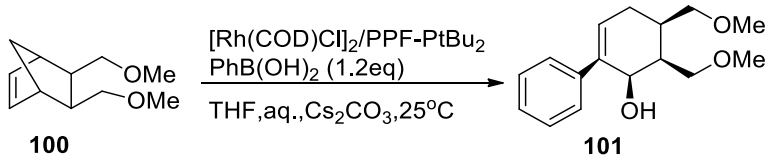
The selective arylation of 2-pyridyl pyrrole/thiophene was reported by Ackermann et al. using [$RuCl_2$(p-cymene)$_2$] catalyst under basic condition at 120 °C temperature.[70]



Scheme 17: Selective arylation of 2-pyridyl pyrrole/thiophene.

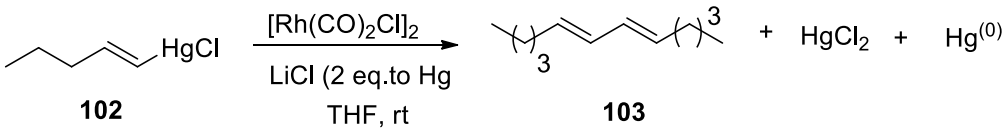
1.3.1.3 Rh-catalysed C-C coupling reaction

The stereo-selective arylation of 1,4-dihydro-1,4-epoxynaphthalene with phenylboronic acid under basic condition using Rh-catalyst had been reported by Mark Lautens et al. [71]



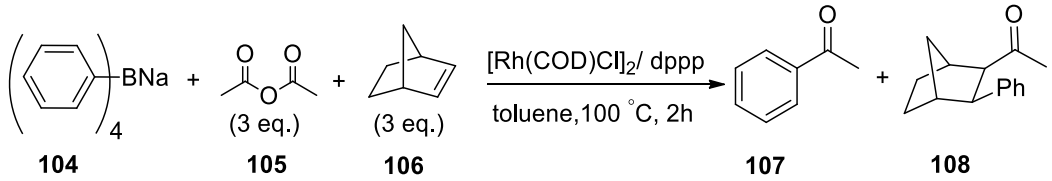
Scheme 18: Stereo-selective arylation of 1,4-dihydro-1,4-epoxynaphthalene.

Larock et al.[72] developed an efficient methodology for the synthesis of biaryl compounds from arylmercurials using $[Rh(CO)_2Cl]_2$ as catalyst and lithium chloride (excess) at room temperature.



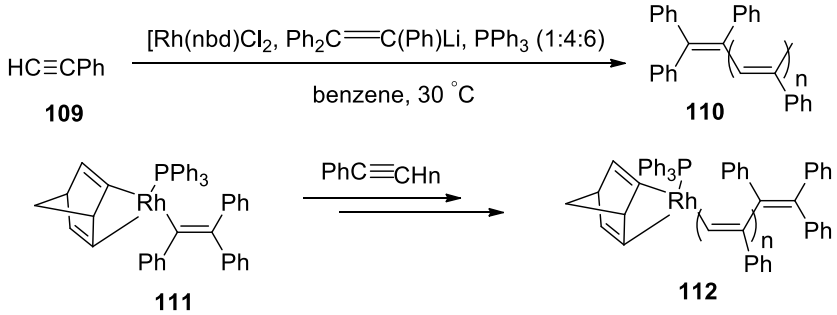
Scheme 19: Synthesis of biaryl compounds from arylmercurials.

Miura et al. had developed a three-component coupling reaction[73] for the synthesis of arylated bicyclic analogues using borylated sodium salt of benzene, acetic anhydride and norbornene along with Rh-catalyst at 100 °C temperature.



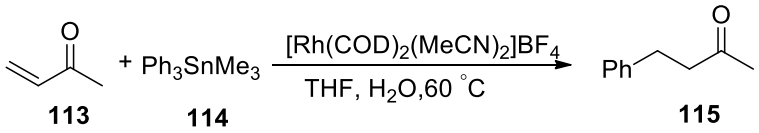
Scheme 20: Synthesis of arylated bicyclic analogues.

Masuda et al. [74] reported the polymerisation of phenylacetylene catalysed *via* Rh-(I) complex gives functionalised polymerisation.



Scheme 21: Polymerisation of phenylacetylene.

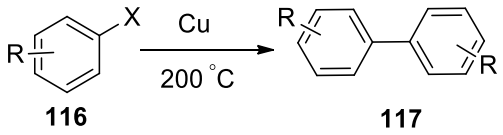
Oi et al. reported an efficient synthesis of 4-phenylbutanone by the reaction of tributylstannane and 2-butenone using tetrafluoroborate Rh-catalyst under aqueous conditions.[75]



Scheme 22: Synthesis of 4-phenylbutanone tetrafluoroborate Rh-catalyst.

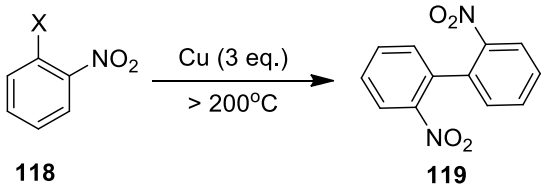
1.3.1.4 Cu-catalysed C-C coupling reaction

In 1901, Fritz Ullmann[76] reported, the first example of biaryl synthesis via the homo-coupling of two aryl halides. In this developed methodology, two equivalents of an aryl halide and one equivalent of copper were utilised at 200ºC temperature to construct biaryl compounds.



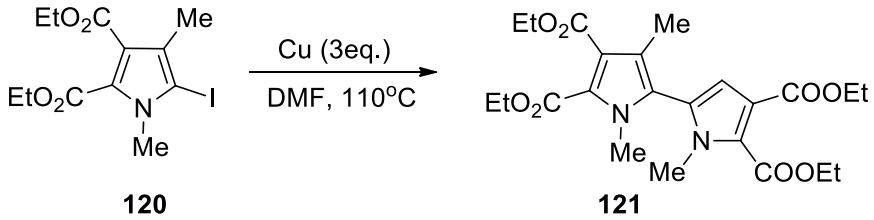
Scheme 23: Fritz Ullmann approach.

Later on, a number of methods have been developed in the field of coupling reactions using Cu metal or Cu-based metal catalysts in different reaction conditions. Based on Ullmann reaction conditions, Glaser developed similar type of coupling reaction for the synthesis of substituted biphenyl between two equiv. of o-halo nitrobenzene.[77]



Scheme 24: Synthesis of substituted biphenyl.

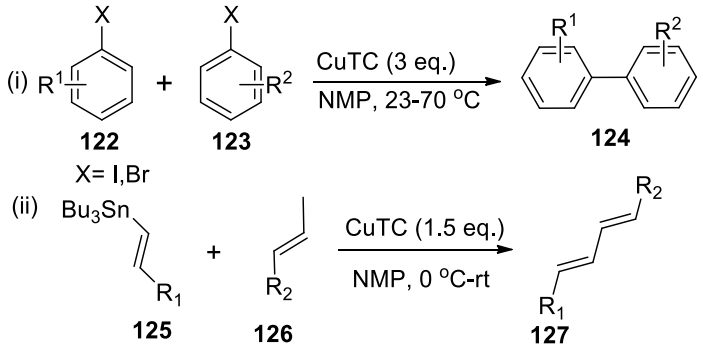
Sessler et al.[78] developed an efficient protocol for the intermolecular coupling between two moles of functionalized iodo-pyrrole using copper metal as catalyst at lower temperature comparison than Ullmann reaction.[76]



Scheme 25: Intermolecular coupling using copper metal.

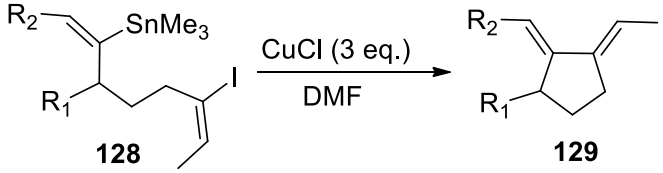
Liebeskind and his co-worker's[79] utilised activated Cu (0) as catalyst (which was synthesize from the reduction of CuI with potassium), for the synthesis of substituted 2,2'-bipyrrole [Scheme 26 (i)]. The same research group also reported the intermolecular coupling of the vinyl iodides, aryl and heteroaryl with organo-

stannanes for the synthesis of conjugated 1,3-dienes in the presence of copper(I)-thiophene carboxylate complex in stoichiometric amount. [Scheme 26 (ii)] [80]



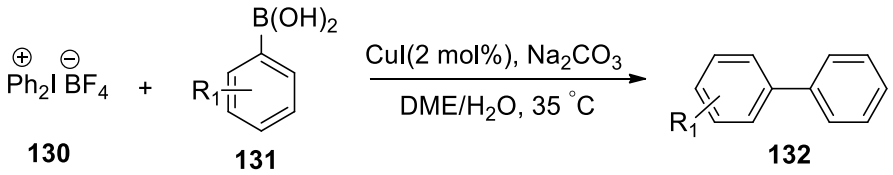
Scheme 26: Synthesis of substituted 2,2'-bipyrrole & conjugated 1,3-dienes.

Cu-mediated, an efficient intramolecular coupling of vinyl iodide with vinyl tin derivatives was reported by Piers and his co-workers, which furnished conjugated cyclic dienes via C (sp^2)-C(sp^2) coupling reaction.[81]



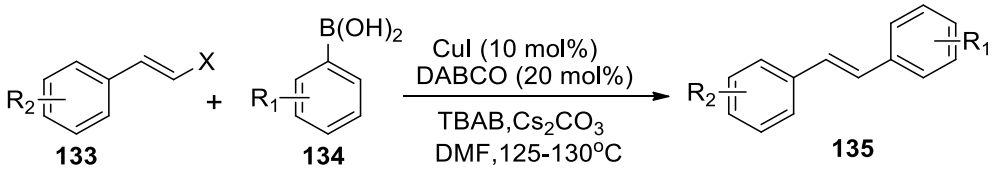
Scheme 27: Cu-mediated intramolecular coupling of vinyl iodide.

In 1996, Kang et al. reported the synthesis of biaryls at room temperature *via* the coupling reaction between boronic acid derivatives and iodonium salts along with CuI in DME/H_2O solvent.[82]



Scheme 28: Synthesis of biaryls with CuI

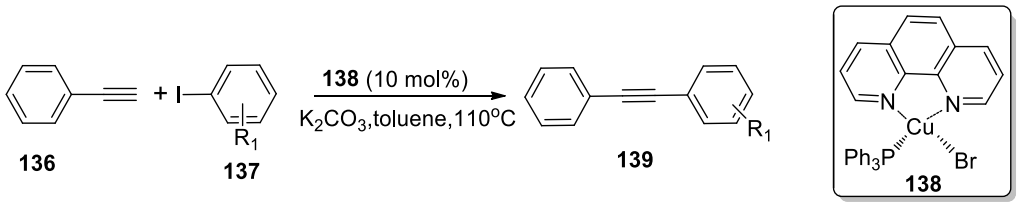
Li et al.[83] modified the reaction using vinyl halides or aryl halides in the place of iodonium salts with boronic acid derivatives and TBAB (tetrabutyl ammonium bromide), which furnished the phenyl styrene analogues in moderate to good yield.



Scheme 29: Synthesis of phenyl styrene analogues.

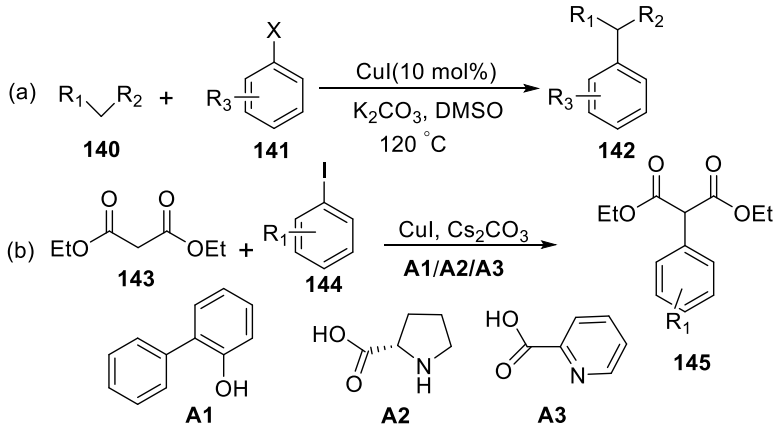
Venkataraman and his co-worker's [84] reported that the Sonogashira type reaction for the synthesis of 1,2-diphenylethyne *via* the reaction between phenyl acetylene and

aryl halides in the presence of 1,10-phenanthroline and copper bromide in toluene at 110 °C temperature.



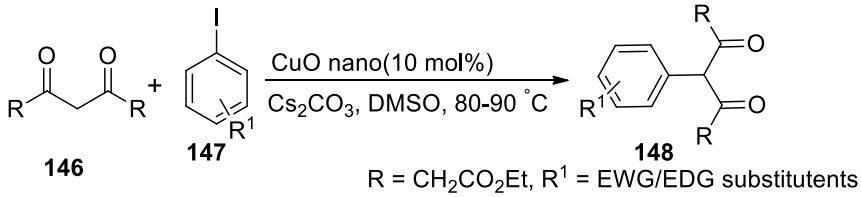
Scheme 30: Synthesis of 1, 2-diphenylethyne

In 1993, Miura et al. developed a ligand-mediated copper-catalyzed synthesis, via the coupling reaction between alkynes with vinyl or aryl halides, which furnished the bond of aryl-alkynes and vinyl-alkynes respectively.[85]



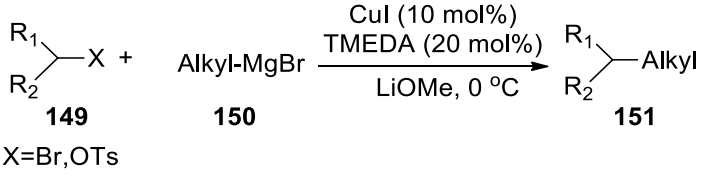
Scheme 31: ligand-mediated copper-catalyzed synthesis.

The recyclable CuO nanoparticle catalysed coupling between 1, 3-diketones with aryl halides for the synthesis of 2-aryl substituted 1,3-diketone was reported by Kidwai et al. reported.[86]



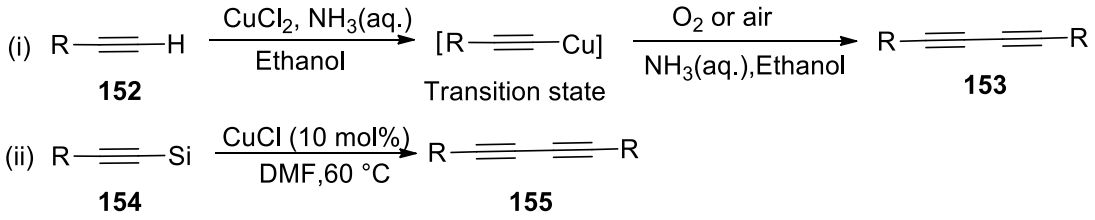
Scheme 32: CuO nanoparticle catalysed coupling.

Liu et al.[87] reported the C-C bond formation by using CuI, as catalyst, and TMEDA, as an additive, for the cross-coupling reaction between Grignard reagents and non-activated secondary alkyl halides or pseudo halides.



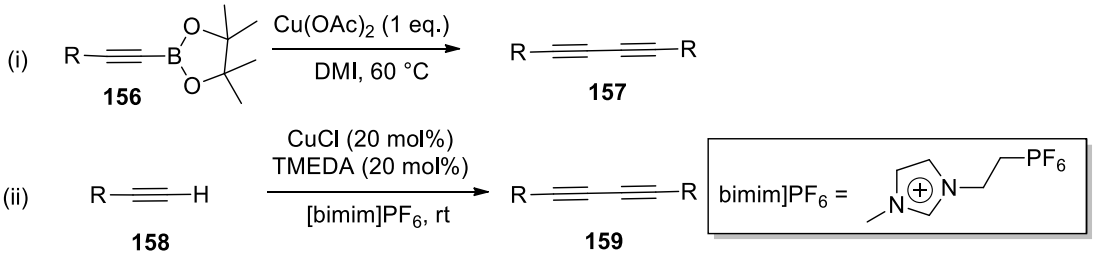
Scheme 33: Cross-coupling reaction between Grignard reagents & pseudo halides.

Glaser et al.[88] reported that the synthesis of 1, 4-disubstituted conjugated alkynes *via* the copper mediated synthesis from phenyl acetylene. [Scheme 34 (i)] Moreover, Mori et al.[89] modified this approach using silylated phenyl acetylene in the place of phenyl acetylene for the synthesis of 1, 4-disubstituted conjugated alkynes. [Scheme 34 (ii)]



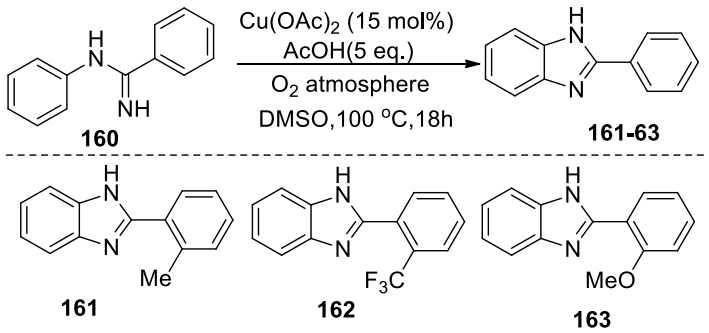
Scheme 34: Glaser et al. and Mori et al. synthesis of conjugated alkynes.

Nishihara et al.[90] utilized the borylated phenyl acetylene for the synthesis of 1, 4-disubstituted conjugated alkynes in the presence of $Cu(OAc)_2$ catalyst [Scheme 35 (i)] whereas, Yadav et. al. [91] utilized unactivated phenyl acetylene in ionic liquid and CuCl catalyst for the synthesis of 1,4-disubstituted conjugated alkynes. [Scheme 35 (ii)]



Scheme 35: Nishihara et al. and Yadav et. al synthesis of conjugated alkynes.

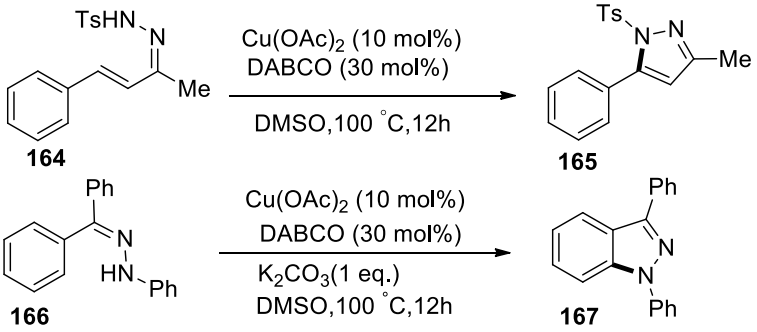
Buchwald et al.[92] reported the synthesis of bio-active benzimidazoles using $Cu(OAc)_2$, as catalyst, in DMSO and AcOH.



Scheme 36: Synthesis of bio-active benzimidazoles.

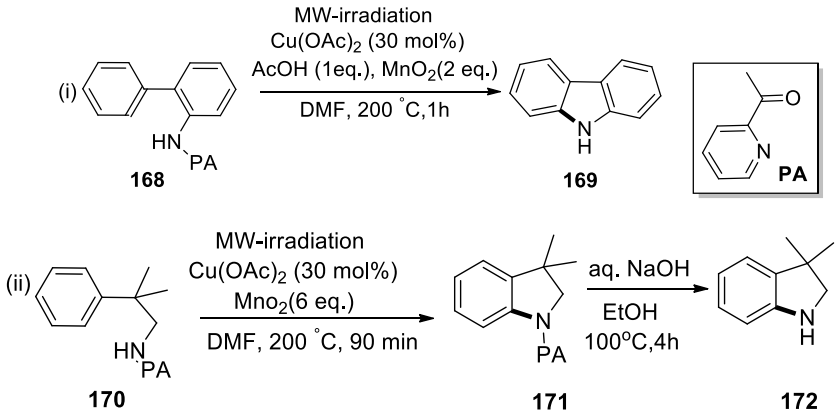
Jiang et al.[93] reported a direct access to indazoles and pyrazoles synthesis *via* Cu-catalysed oxidative C−H amidation reaction under O_2 atmoshphere. In this developed methodology, phenyl or vinyl-substituted arylhydrazones were cyclized to the corresponding aza heterocyclic products at 100 °C temperature. Notably, they have found

that there is no any effect on the reaction rate in the presence of any radical scavengers, which proves that it is not a free radical mediated reaction methodology.



Scheme 37: Indazoles and pyrazoles synthesis.

Miura et al. reported the synthesis of carbazoles [94] via the intramolecular C−H amination of picolinamide (PA)-protected 1, 1'-biphenyl-2-amine by using a copper catalyst. The reaction conditions required a stoichiometric amount of manganese oxide under microwave irradiation condition at high temperature. [Scheme 38 (i)]

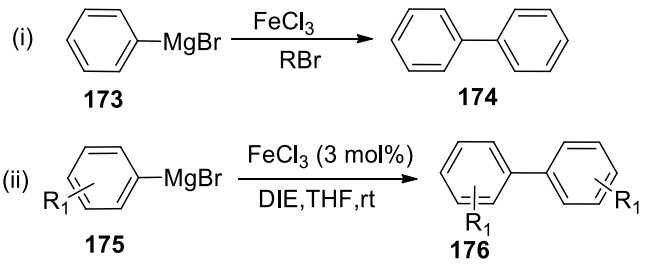


Scheme 38: Synthesis of carbazoles *via* the intramolecular C−H amination.

Further, Miura et al. utilized the same reaction conditions for the synthesis of indolines [95] using picolinamide (PA)-protected 2-methyl-2-phenylpropan-1-amine in the place of 1,1'-biphenyl-2-amine. [Scheme 38 (ii)]

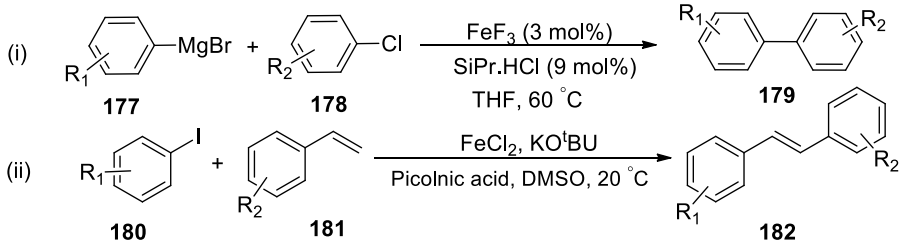
1.3.1.5 Fe-Catalysed C-C coupling reaction

Fe-catalysed homocoupling of aryl Grignard reagent, to the synthesis of symmetrical biaryls, was the first report given by Kharasch et al. in 1941 [Scheme 39 (i)].[24]



Scheme 39: Fe-catalysed homocoupling of aryl Grignard reagent.

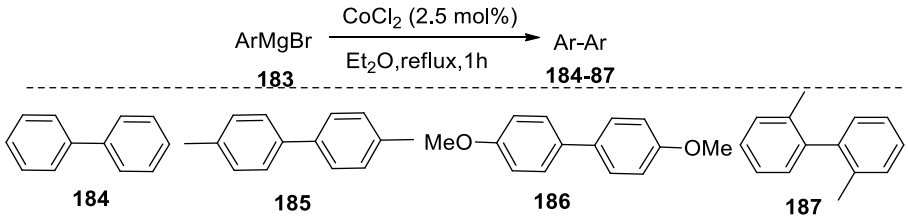
Subsequently, inspired by this methodology, Cahiez et al.[96] reported the Fe-catalysed synthesis of substituted biaryl compounds using same catalyst at room temperature. [Scheme 39 (ii)]. Nakamura et al.[97] also discovered the catalytic ability of Fe-catalyst in the synthesis of unsymmetrical biaryl moiety via cross-coupling reaction of aryl Grignard reagent. [Scheme 40 (i)] Moreover, Vogel et al.[98] explored the iron-catalyzed coupling reaction towards the synthesis of functionalized styrenes via the reaction of aryl/heteroaryl halide with substituted aryl styrenes. [Scheme 40 (ii)]



Scheme 40: Fe-catalyst in the synthesis of unsymmetrical biaryl moiety.

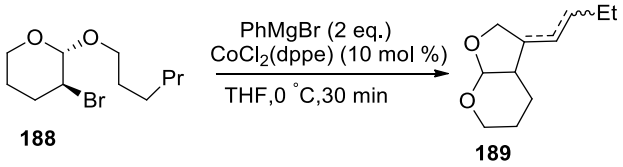
1.3.1.6 Co-catalysed C-C coupling reaction

Kharasch et al. [24] reported an efficient synthetic protocol for the synthesis of biaryl moiety using $CoCl_2$ under refluxing condition in ether

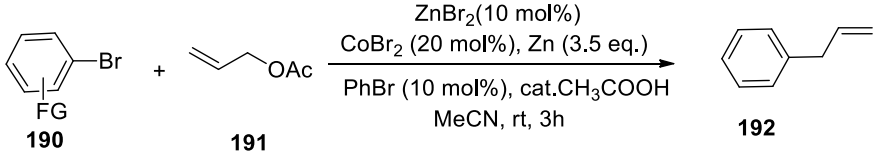


Scheme 41: $CoCl_2$ catalysed synthesis of biaryl moiety.

Oshima et al.[99] has been reported the synthesis of fused tetrahydrofuran by the cobalt-catalyzed C-C bond forming approach.



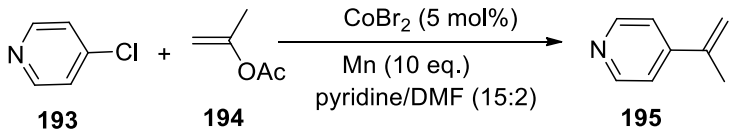
Scheme 42: Synthesis of fused tetrahydrofuran.



Scheme 43: Cobalt-catalyzed C-C cross-coupling.

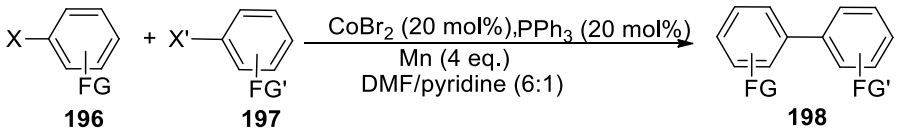
Pe´richon et al.[100] developed the cobalt-catalyzed C-C cross-coupling of allyl acetates with arylhalides in the presence of a catalytic amount of $CoBr_2$ and $ZnBr_2$; whereas zinc metal was used as reductant.

In **2005**, Pe´richon et al. reported the cross-coupling reaction of isopropenyl acetate with chloropyridine in the presence of a catalytic amount of cobalt bromide (5%) and stoichiometric amount of manganese.[101]



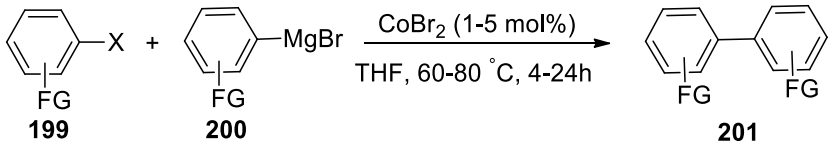
Scheme 44: Synthesis of 4-(prop-1-en-2-yl)pyridine.

Gosmini et al.[102, 103] developed a methodology for the synthesis of unsymmetrical biaryl moiety *via* the coupling of two aromatic halides ArX and Ar′X.



Scheme 45: Synthesis of unsymmetrical biaryl moiety.

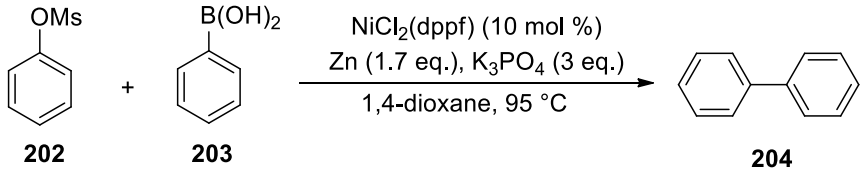
Nakamura et al. reported the synthesis[104] of functionalized biaryl analogues *via* the Cross-coupling reaction between non-activated aryl halides or heteroaryl bromides with aromatic Grignard reagent.



Scheme: 46 Synthesis of functionalized biaryl analogues.

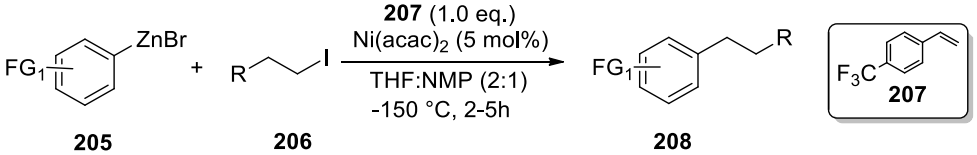
1.3.1.7 Ni-catalysed C-C coupling reaction

Percec et al. reported[105] Ni-catalysed synthesis of biaryls using the reaction of aryl mesylates with aryl boronic acid via Suzuki-Miyaura cross coupling reaction.



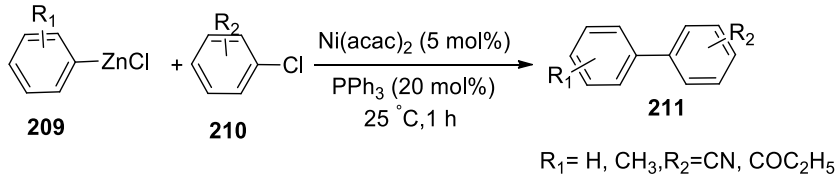
Scheme 47: Ni-catalysed synthesis of biaryls.

Giovannini et al. reported an efficient synthesis of alkyl substituted benzene *via* the Ni-catalysed C–C cross-coupling reaction of phenyl zinc bromide with iodoalkanes.[106]



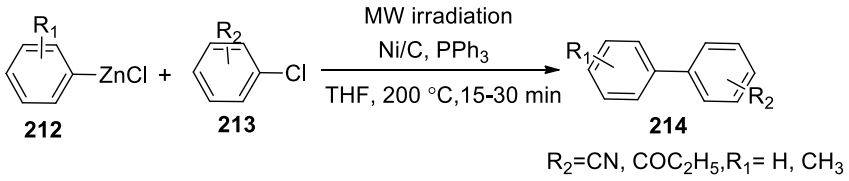
Scheme 48: Synthesis of alkyl substituted benzene *via* the Ni-catalysed.

Tucker and his co-worker's [107] reported the Ni-catalysed C–C cross-coupling reaction for the synthesis of biaryl moiety using PPh_3 ligand at room temperature (Scheme 49).



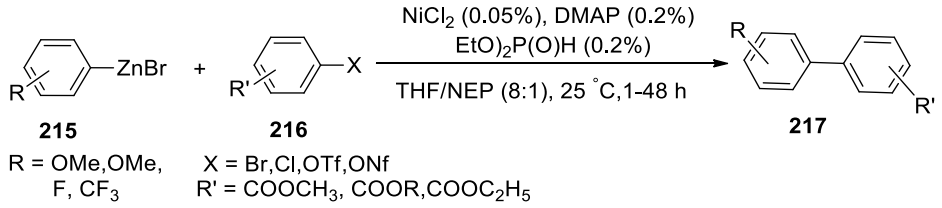
Scheme 49: Ni-catalysed synthesis of biaryl moiety.

Lipshutz et al. explored the coupling reaction for the synthesis of biaryl using Ni-on-charcoal (Ni/C) as a heterogeneous catalyst.[108]



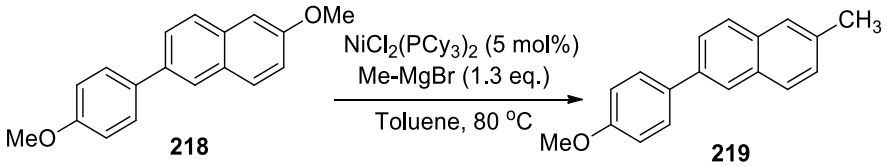
Scheme 50: Synthesis of biaryl using Ni-on-charcoal (Ni/C).

In **2006**, Knochel et al.[109] had been reported the Ni-catalysed C – C cross-coupling reaction for biaryl synthesis via the reaction of the aryl or alkenyl triflates with nonaflates.



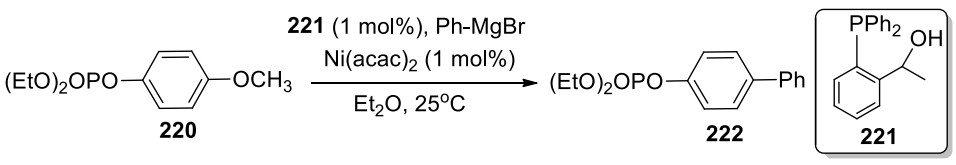
Scheme 51: Knochel et al. for biaryl synthesis.

Moreover, Shi et al.[110] had been reported the formation of C - C bond (Sp^3-Sp^2) by the use of methyl magnesium bromide with aryl ether *via* demethoxylation reaction.



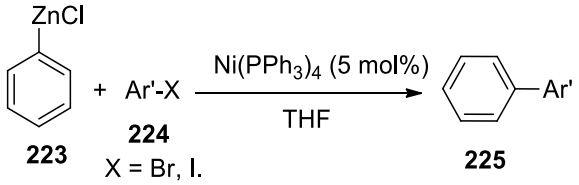
Scheme 52: Shi et al. approach for the formation of C-C bond.

Nakamura et al.[111] reported the Ni-catalysed Kumada coupling for the synthesis of biaryl phosphonate ester *via* the reaction of aryl phosphate with hydroxy phosphine ligand.



Scheme 53: Nakamura et al. approach for synthesis of biaryl phosphonate ester

Ni-catalysed regio- and chemo-selective biaryl synthesis, [112] by the use of only 5 mol % of Ni (0) catalyst with tetrakis-triphenyl phosphine ligand, had been reported by Negishi et al.



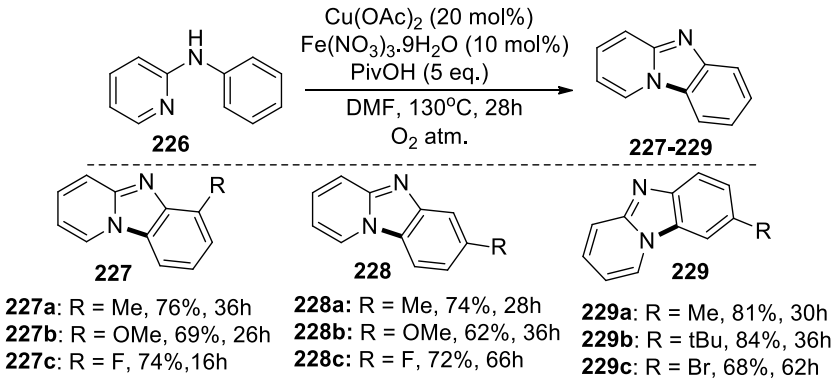
Scheme 54: Negishi et al. approach for the synthesis of biaryls

1.3.1.8 Synergistic as well as mixed catalytic approach in coupling reaction

Synergistic catalysis is a specific approach where at least two different catalysts act on two dissimilar substrates simultaneously to permit the reaction forward between the two activated substrates. While a catalyst works at lower energy of reaction than the other, and overall, a reaction having synergistic catalytic system work together to enhance the energy level of HOMO of one of the substrate and lowers the LUMO of other substrate.[113] This concept has been extensively utilised to enhance the rate of reaction as well as to develop the newer synthetic pathways.

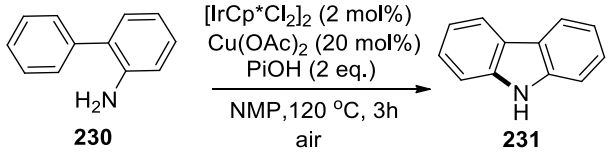
Synergistic catalytic system has been utilized in several reactions, where, both substrates need some kind of significant activation either with stoichiometric amount or in a catalytic amount of an activator or additive. Synergistic catalysis differs from other multi-catalytic systems by the nature that one catalyst somehow activates one substrate, while the other catalyst activates a different substrate. Some other types of multi-catalytic systems are also being utilised; where two catalysts are necessary to activate one substrate or cascade catalytic system: where one catalyst first transforms a substrate into other conjugated form and this conjugated form is activated by a second catalyst to react.[114-116] Synergistic catalytic systems are very common in biological systems[117] such as: the synthesis of tetrahydrofolate by the use of enzyme dihydrofolate reductase. Firstly, the dihydrofolate reductase enzyme catalytically activates dihydrofolate by protonation of the imine, while NADPH (essentially a hydride source activated by the cofactor $NADP^+$) then come and add a hydride to the imine to afford the product.[118] Thus, several research groups have also utilized the same concept to develop the efficient coupling methodology.

Zhu and his co-workers developed a synthetic route for the synthesis of pyrido [1, 2-]benzimidazoles using synergistic catalytic system of copper/iron along with pivalic acid as an additive under O_2 atmosphere.[119]



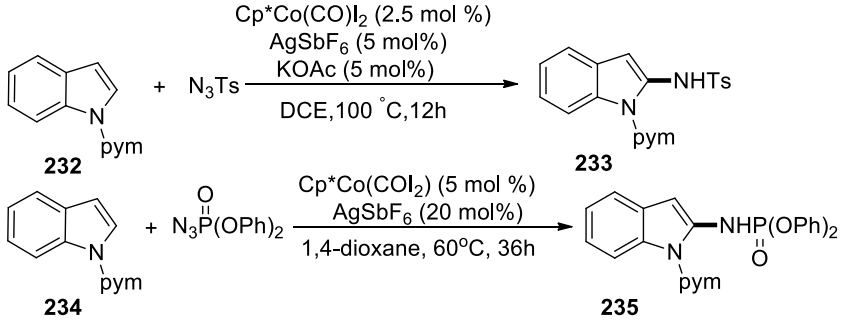
Scheme 55: Zhu et al. approach for the synthesis of pyrido [1, 2-]benzimidazoles.

Miura et al. utilized the Ir/Cu synergistic catalytic system for the synthesis of carbazoles in one pot reaction conditions.[120]



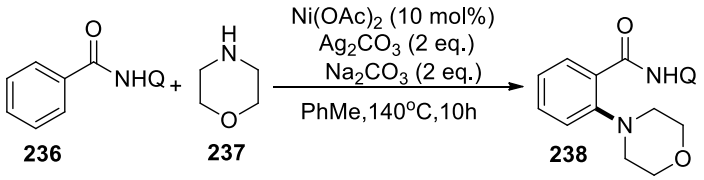
Scheme 56: Miura et al. approach for the synthesis of carbazoles.

Kanai et. al. reported the synthesis of N-protected 2-amino indole using Co/Ag synergistic catalytic system along with tosyl/phosphoryl azides.[121]



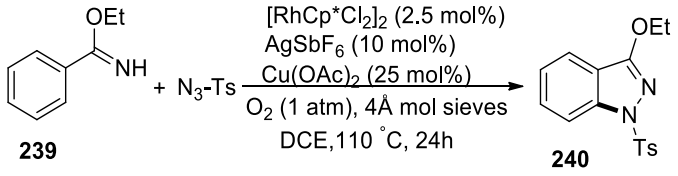
Scheme 57: Kanai et al. report for synthesis of N-protected 2-amino indole.

Zhang and his co-workers[122] used Ni/Ag synergistic catalytic system for the synthesis of N-arylated morpholine *via* C-N bond forming approach in toluene at 140 °C.



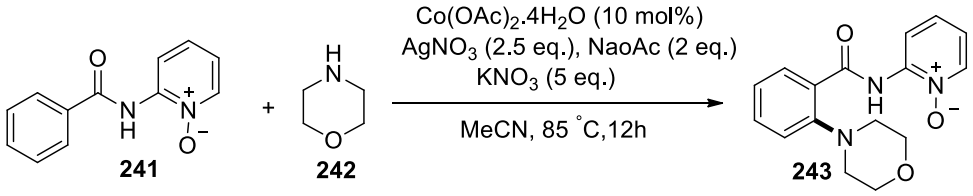
Scheme 58: Zhang et al. approach for the synthesis of morpholine.

Glorius et al. developed a one-pot synthetic protocol for the synthesis of functionalized *1H*-indazoles by tandem Rh-catalysed C−H amidation of arylimidates followed by a Cu-mediated coupling reaction.[123]



Scheme 59: Glorius et al. approach for synthesis of *1H*-indazoles.

In 2016 Niu and Song et. al. developed a novel protocol for the synthesis of N-arylated morpholine using Co/Ag as a mixed catalytic approach.[124]

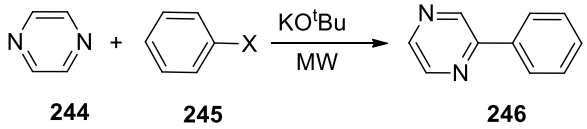


Scheme 60: Niu and Song et al. approach for synthesis of arylated morpholine.

1.3.2 Transition metal free coupling reactions

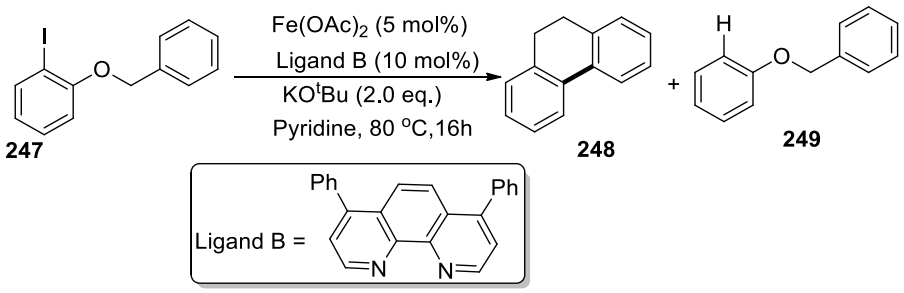
The transition metal catalysed coupling reactions have received tremendous attention by organic chemist to catalyse several C-C, C-N coupling reactions over the past three decades. However, use of these transition metals as catalyst causes severe hazards to environments as well as toxic to the living organisms. Therefore, transition metal free coupling approaches are an alternate way to carry out coupling reactions in an eco-friendly manner. In the recent years, several research groups have developed the metal free coupling approach *via* the direct functionalization of inert C-H bonds.

Itami and his co-workers (**2008**), [125] developed a metal free C–C coupling methodology for the synthesis of 2-aryl bioactive pyrazine scaffold under microwave irradiation conditions.



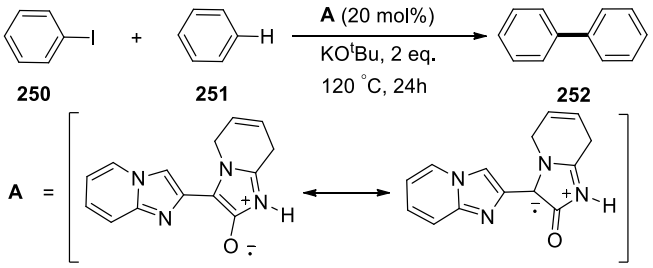
Scheme 61: Itami et al. approach for synthesis of bioactive pyrazine scaffold.

Charette et. al. reported a similar transition metal free KO^{t}Bu-mediated intramolecular radical arylation reaction.[126]



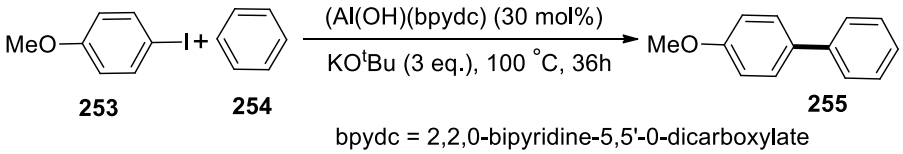
Scheme 62: Charette et al. approach of arylation reaction.

In **2011**, Yong et al.[127] reported, direct arylation *via* unactivated aromatic C−H bond functionalization in the presence of KO^{t}Bu using stable zwitter ionic radical catalyst at 120 °C temperature.



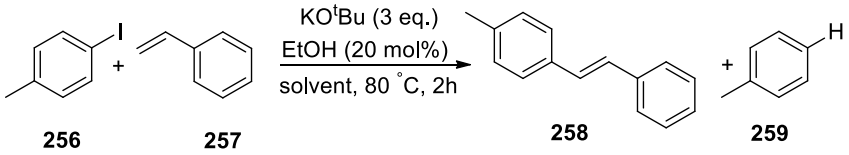
Scheme 63: Direct arylation *via* unactivated aromatic C−H bond functionalization.

In **2012**, Li et al. reported the transition metals free cross-coupling reaction of un-activated arenes with aryl halides using MOFs (metal−organic frameworks) as heterogeneous catalytic system.[128]



Scheme 64: Li et al. approach of unactivated arenes.

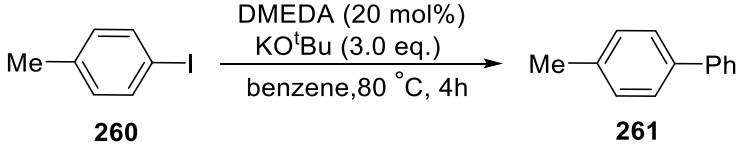
Moreover, Hayashi et. al. presented a transition-metal-free Mizoroki−Heck type reaction *via* KO^{t}Bu-promoted C–C cross coupling reaction for the synthesis of styrenes.[129]



Scheme 65: Hayashi et al. approach for synthesis of styrene analogue.

1.3.3 Organocatalytic coupling reaction

Organocatalyzed coupling methods have received tremendous attention now-a-days due to its advantage (such as less toxicity, environmentally benign nature, easy to handle etc.) over transition metal catalytic system.

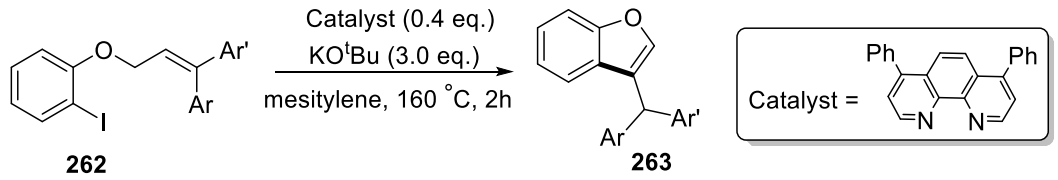


Scheme 66: Kwong et al. approach for biaryl synthesis.

Several research groups have utilised various natural as well as synthetic organocatalyst to investigate the efficacy of these organocatalyst in coupling

reactions. Kwong et al. discovered that DMEDA had been identified as an efficient organocatalyst for C – C coupling reaction to the synthesis of biaryl moieties.[130]

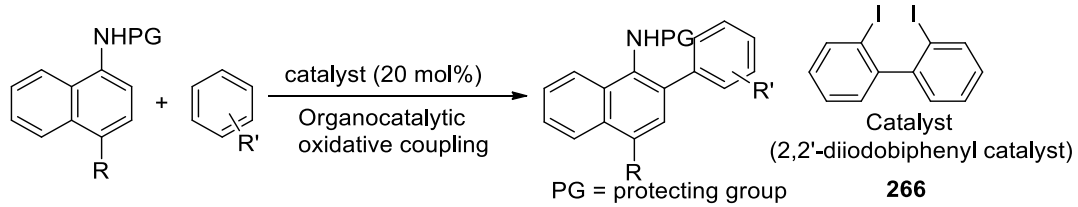
Rueping and his co-worker's.[131] reported the organocatalytic intramolecular C – C bond formation using 1, 10-phenanthroline to form the biologically privileged heterocycles.



262 **263**

Scheme 67: Rueping et.al. approach for biaryl synthesis.

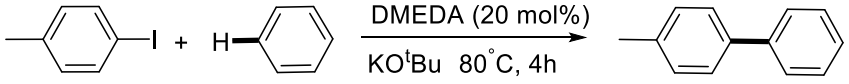
Ito et al. reported a novel C-C cross-coupling of aniline analogues with substituted benzene by the use of 2,2`-diiodobiphenyl organocatalyst.[132]



264 **265** **266**

Scheme 68: Ito et al. approach for biaryl synthesis.

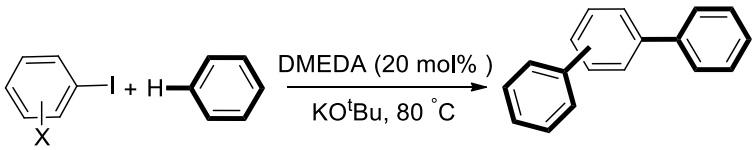
Liu et al. had been developed a very efficient organocatalytic methodology [130] for the direct arylation of halobenzene with substituted benzene using DMEDA as an organocatalyst.



267 **268** **269**

Scheme 69: Liu et al. approach for synthesis of biaryl.

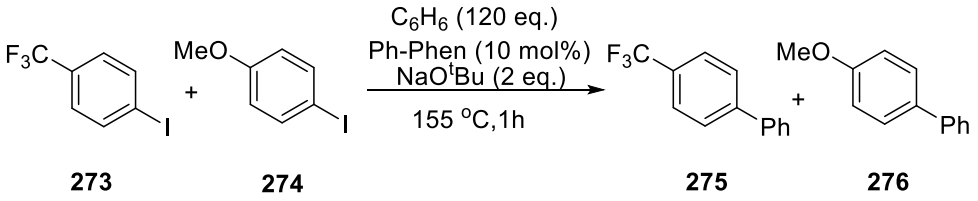
Similarly, Wei Liu et al. also utilised the same organocatalytic condition[130] for the diarylation of dihalobenzene [Scheme 70].



270 X = I, Br, Cl **271** **272**

Scheme: 70 Wei et al. organocatalytic approach for biaryl synthesis.

Eiji Shirakawa et al. demonstrated that the more electron-deficient aryl iodide as well as electron-withdrawing substituents having aryl bromides/chlorides selectively reacts with benzene in the presence of bathophenanthroline as an organocatalyst.[133]

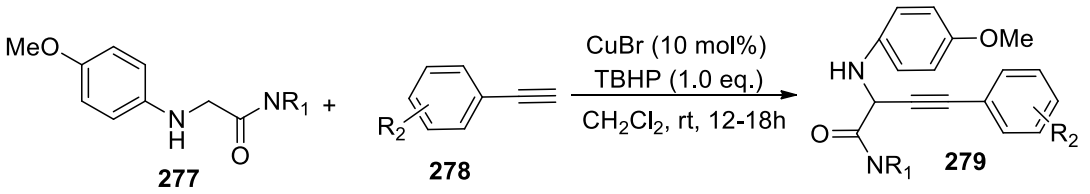


Scheme 71: Organocatalytic approach of *Eiji Shirakawa* for biaryl synthesis.

1.3.4 CDC cross coupling reaction

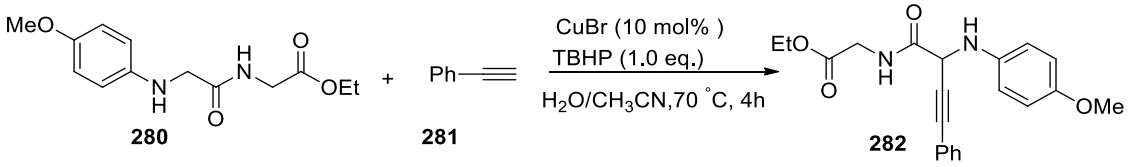
In recent years, CDC reaction gained attention to construct C-C bond *via* C-H bond functionalization under oxidative conditions. CDC reactions are advantageous due to non- requirement of pre-functionalization of substrates and higher atom economy of the substrates. In CDC reactions, transition metal is usually required in combination with an oxidant. Some selected examples of CDC couplings are given below:

Zhao et al. utilized CDC approach to the synthesis of unnatural amino acid analogues via the reaction of p-methoxyphenyl (PMP)–protected glycine amides with substituted phenylacetylenes at room temperature.[134]



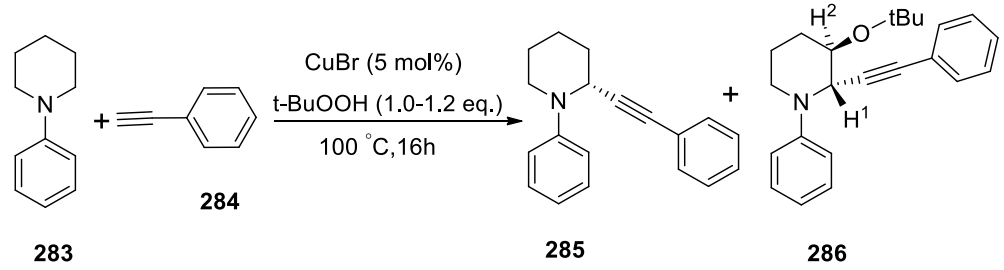
Scheme: **72** Synthesis of unnatural amino acid.

This method was further modified for direct and site-selective peptide functionalization, at lower temperature (70 °C).[134]



Scheme73: Site-selective peptide functionalization.

Moreover, similar type of reaction methodology was also utilised for the synthesis of functionalized piperidine analogues *via* CDC reaction approach.[135]



Scheme 74: Synthesis of functionalized piperidine analogue.

1.4 Application of coupling reactions in the synthesis of bioactive alkaloid

Coupling reactions are being used to synthesize diverse class of pharmaceutically privileged scaffolds and other N-containing alkaloids[136] as well as in paints, dyes, agrochemicals, biosensors, and industrial fields.

1.4.1 Different class of bioactive alkaloids

The structures of some representative class of alkaloids are given below-

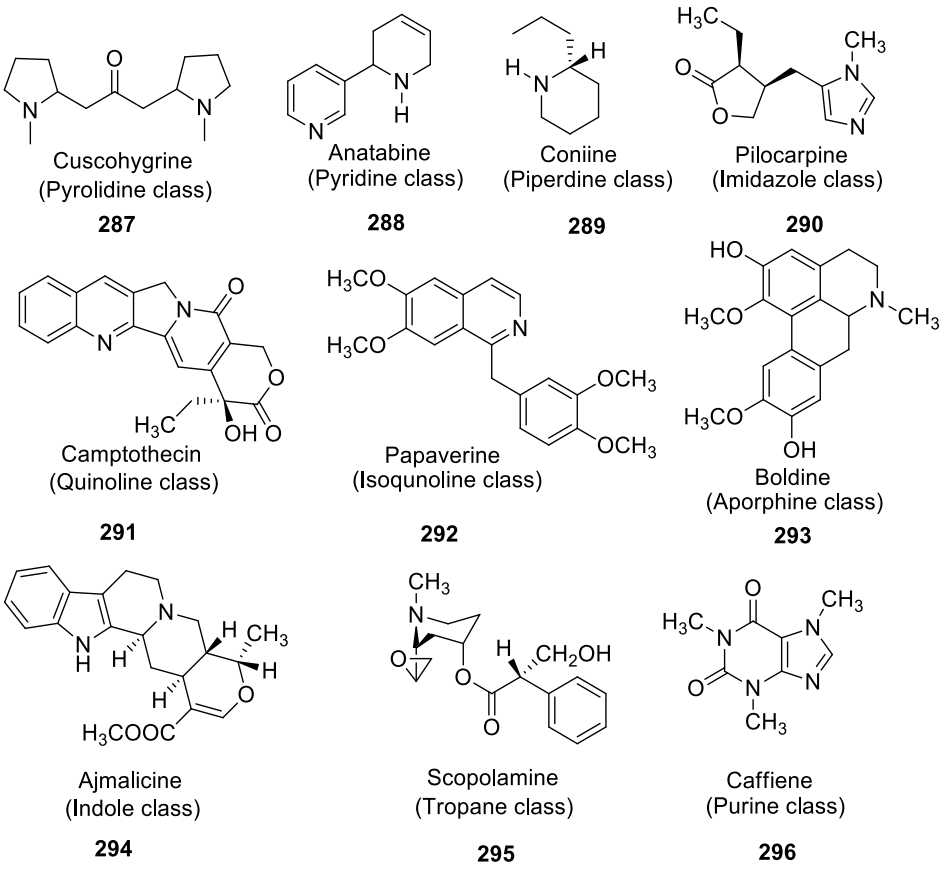
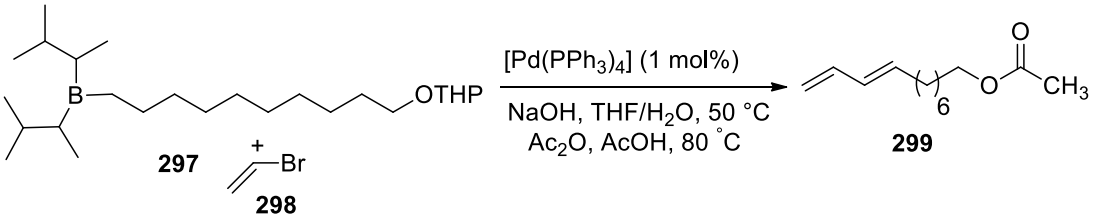


Figure 7. Class of alkaloids along with selected examples

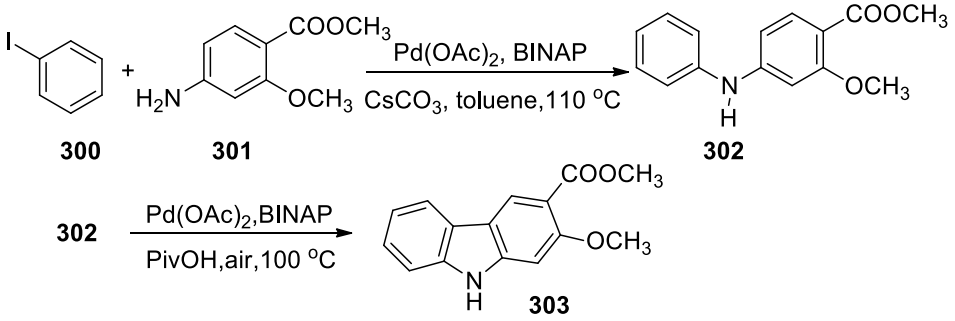
1.4.2 Synthesis of bioactive alkaloid utilising coupling reactions

Indole and indoline class of alkaloids are present widely in nature and display several biological activities.[136] Due to their biological importance, several research groups are involved to develop novel transition metal catalysed as well as transition metal-free methodologies. Rossi and his co-workers reported [137] the first application of Suzuki cross-coupling reaction methodology in the synthesis of an insect pheromone **299**, which was isolated from *Diparopsis castanea*.



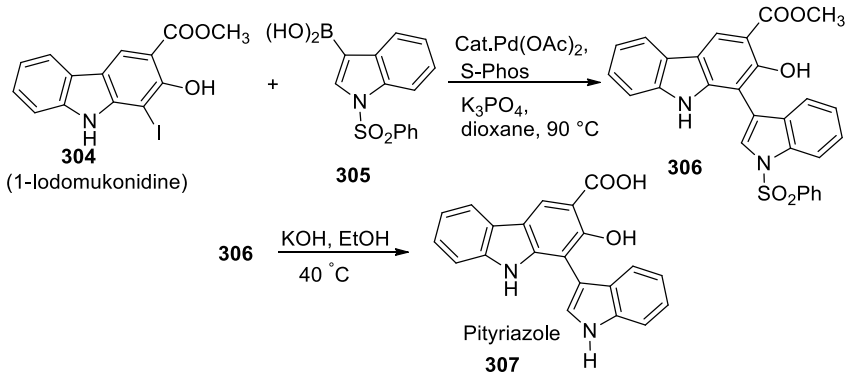
Scheme 75: Synthesis of an insect pheromone.

Forke et al. reported the synthesis of Clausine-L by the Pd-catalyzed amination of iodobenzene with substituted anilines followed by oxidative cyclization of the resulting N, N-diarylamine afforded clausine-L.[138]



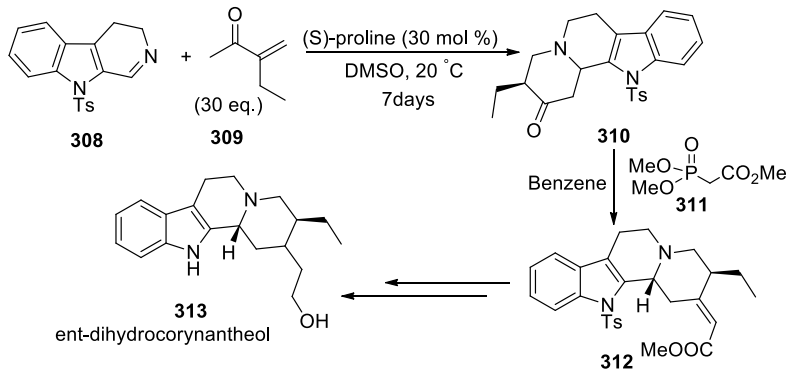
Scheme 76: Synthesis of Clausine-L.

Forke et al. reported the synthesis of **Pityriazole** *via* the Suzuki-Miyaura coupling of 1-iodomukonidine with N-(phenylsulfonyl)indol-3-ylboronic acid followed by hydrolysis under basic conditions.[138]



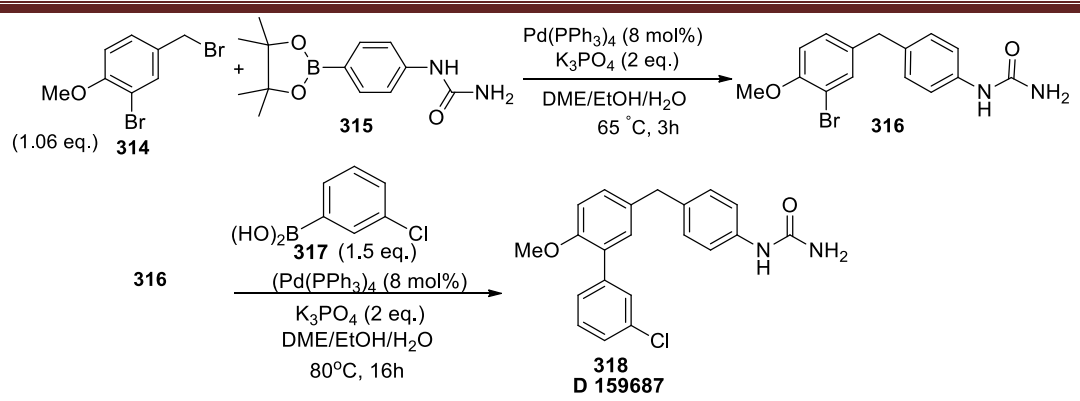
Scheme 77: Synthesis of Pityriazole.

Itoh et al. **(2006)** reported stereoselective total synthesis of indole alkaloid **ent-dihydrocorynantheol** starting from N-tosyl protected carbazole.[139]



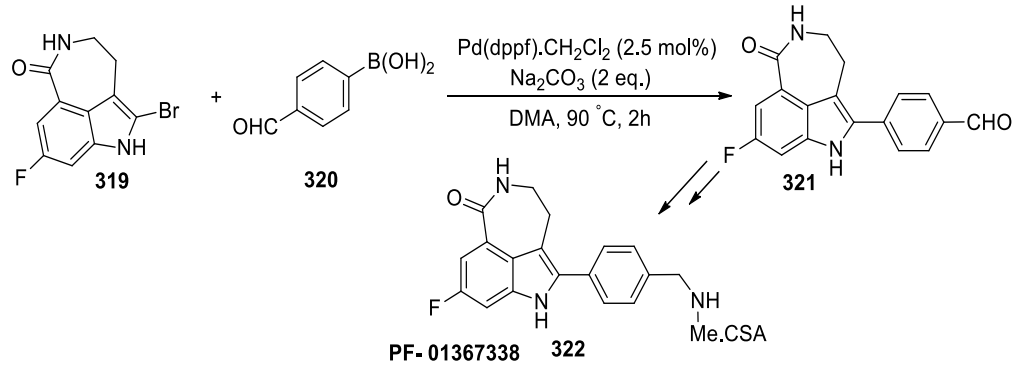
Scheme 78: Total synthesis of indole alkaloid ent-dihydrocorynantheol.

Dalby et al.[140] reported a concise synthesis of selective negative allosteric modulator **D159687** *via* Pd-catalysed Suzuki-Miyaura cross coupling reaction between Organoboron compound and a halide substrate.



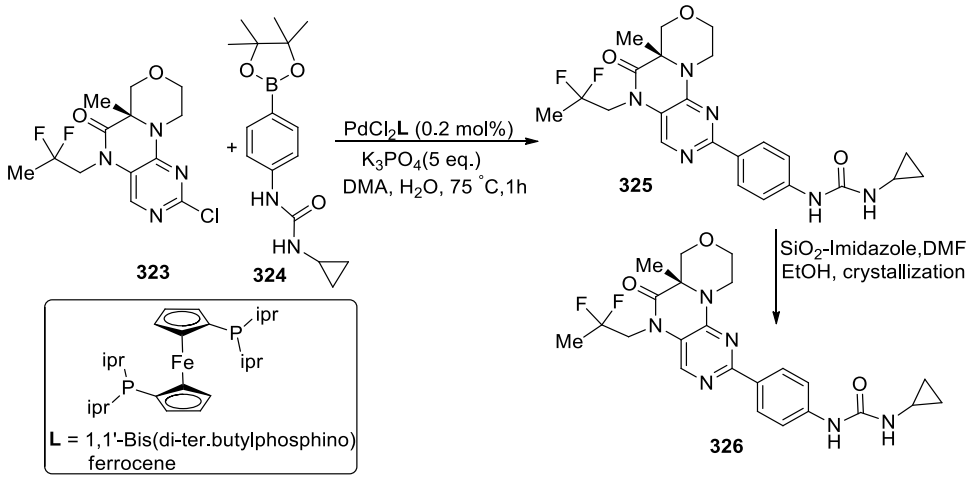
Scheme 79: Synthesis of selective negative allosteric modulator D159687**.**

Gillmore et al. developed a synthetic protocol for the synthesis of PF-01367338 (a drug candidate for breast and ovarian cancers) in Kilogram-scale.[141]



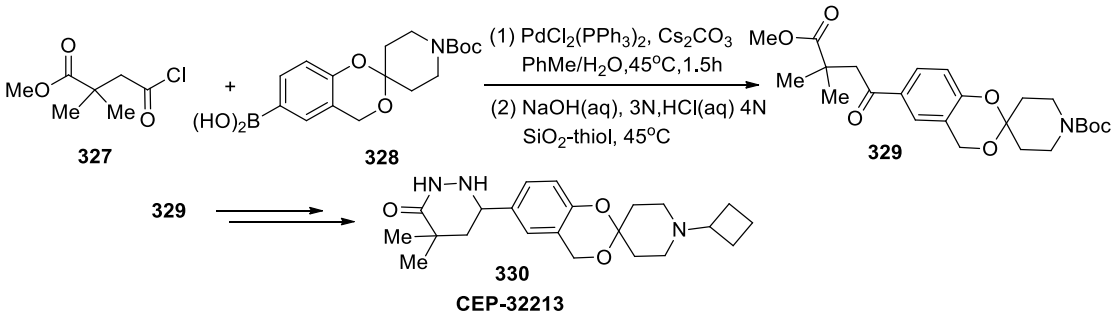
Scheme 80: Synthesis of PF-01367338.

Hicks et al. reported the synthesis of compound **326**; which is related to PI3k and AKT/PKB signalling pathway that inhibits cell proliferation and tumour growth.[142]



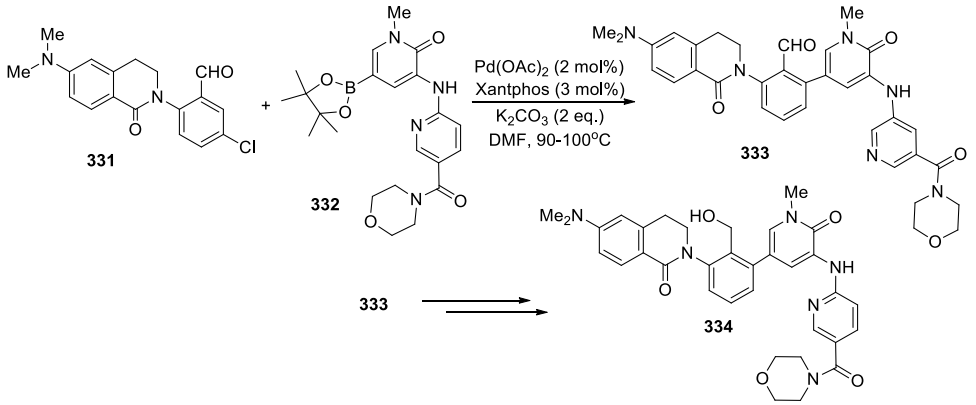
Scheme 81: Synthesis of PI3k & AKT/PKB.

A process chemistry development, utilising Suzuki-type coupling of acid chloride and aryl boronic acid, was performed for the synthesis of **CEP-32215** (an antagonist of histamine H-3 receptor).[143]



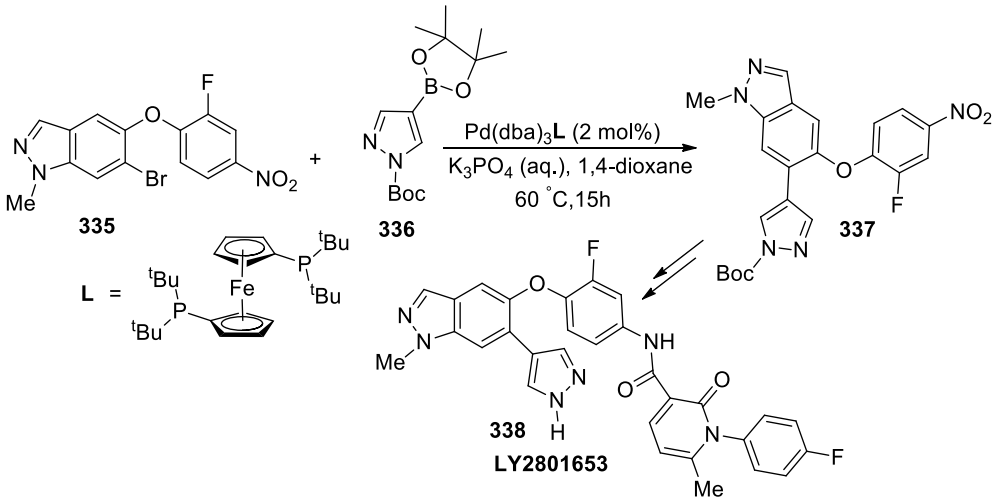
Scheme 82: Synthesis of CEP-32215.

Hong et al. developed the synthesis [144] of Bruton's Tyrosine Kinase (BTK) inhibitor, which is a cell signalling molecule related in early β-lymphocytes growth.



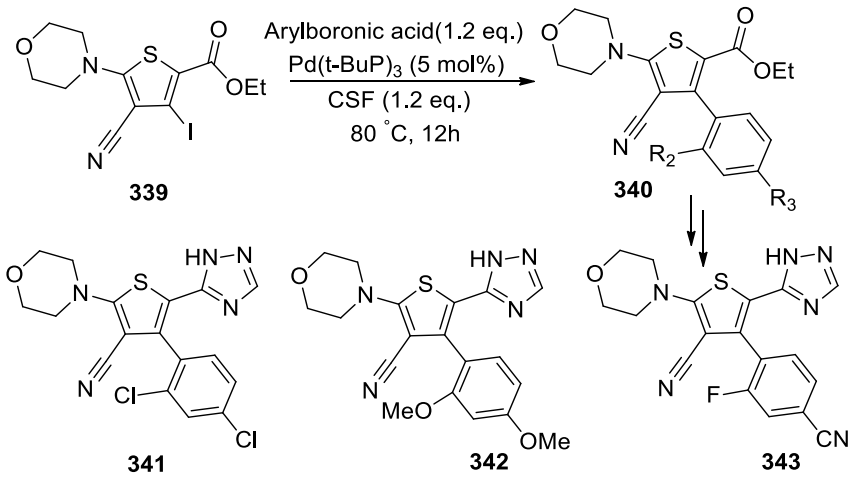
Scheme 83: Synthesis of Bruton's Tyrosine Kinase (BTK) inhibitor.

Kallman et al. developed a Pd- catalysed methodology for the synthesis of **LY-2801653**, which is a receptor of tyrosine kinase family.[145]



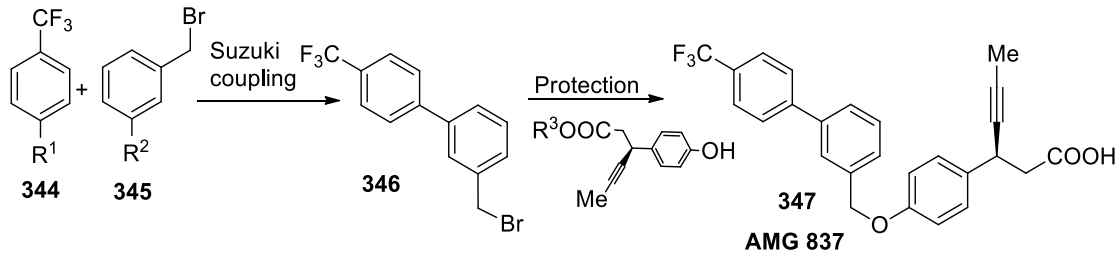
Scheme 84: Synthesis of LY-2801653.

Huang et al. developed the synthesis of tetra-substituted thiophene analogues, which are very prominent candidates for inhibition of PI3K receptors.[146]



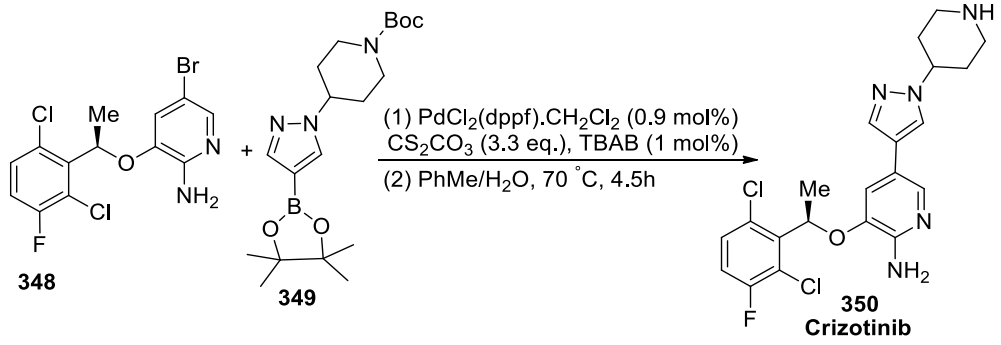
Scheme 85: Synthesis of tetra-substituted thiophene analogue.

Walker et al. reported the synthesis of **AMG 837**[147] (which is GPR40 receptor antagonist and associated with type 2-diabetes) via from Suzuki cross coupling reaction.



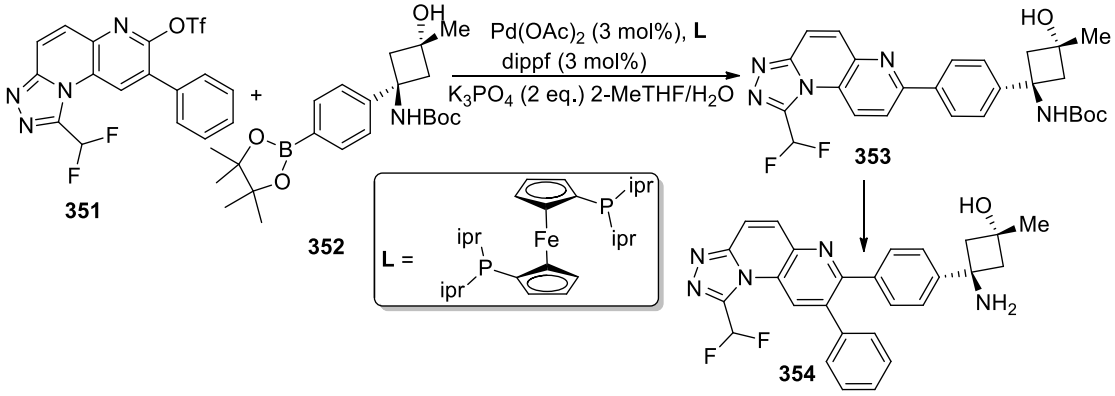
Scheme 86: Synthesis of AMG 837.

De Koning et al. reported the large scale synthesis of an anti-cancer drug **Crizotinib** *via* Suzuki coupling, which acts as an ALK and ROS1 inhibitor.[148]



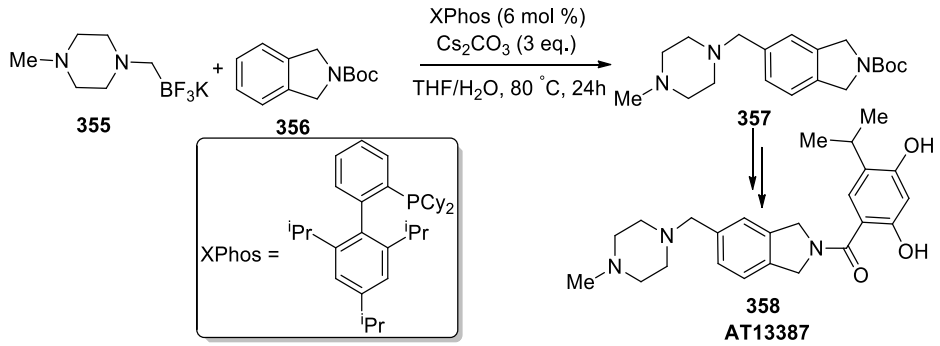
Scheme 87: Synthesis of an anti-cancer drug Crizotinib.

Grongsaard et al. developed a scalable synthetic route of polycyclic AKT kinase inhibitor **354** (AKT kinase is an enzyme that is responsible for several types of cancer, such as: colon, breast, brain, lung and prostate) via Suzuki reaction between a pyridyl chloride and an arylboronate.[149]



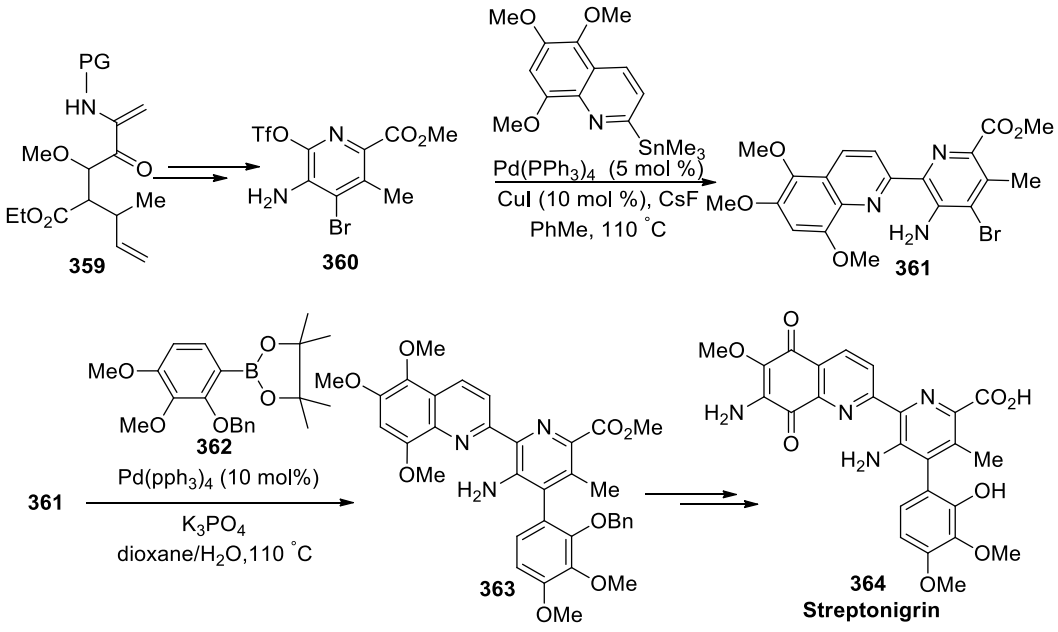
Scheme 88: Synthesis of AKT kinase inhibitor.

Patel et al. reported the total synthesis of **AT-13387** (a molecule that is capable of interacting with chaperones and essential for the survival of cancer cells) via sp^3-sp^2 C – H arylation via Suzuki cross-coupling reaction under Molander approach. [150,151]



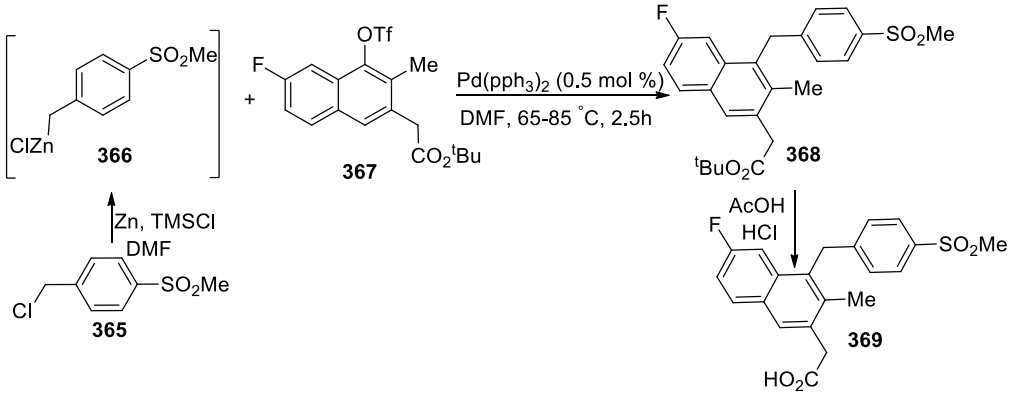
Scheme 89: Total synthesis of AT-13387.

Donohoe et al. reported the a total synthesis of the antitumor antibiotic **(±)-streptonigrin** via step-wise cascade approach using transition metal-catalysed reactions.[152]



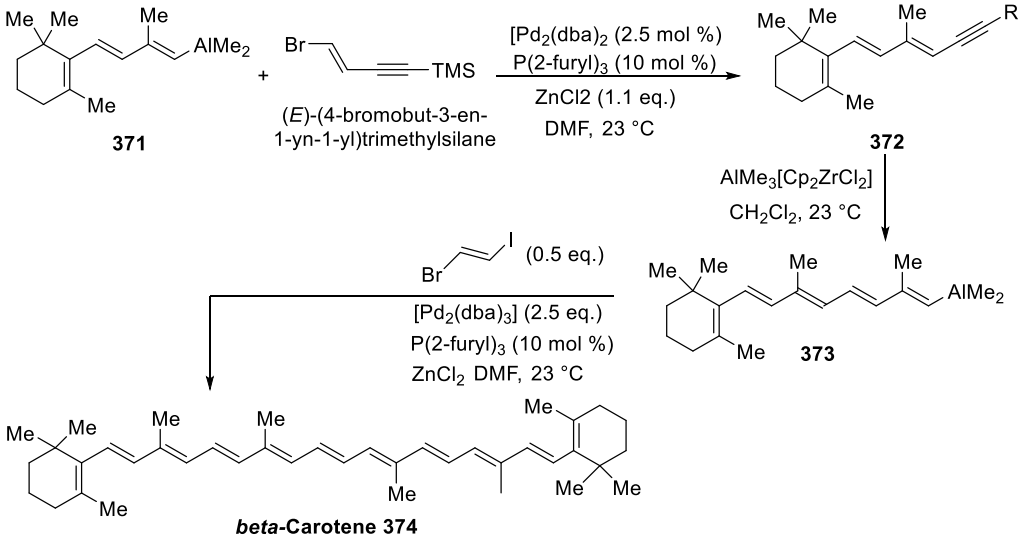
Scheme 90: Total synthesis of the antitumor antibiotic (±)-streptonigrin**.**

Shu and Co-workers [153] utilised Negishi coupling reaction for the synthesis of compound **369**, which is CHRT2 receptor antagonist.



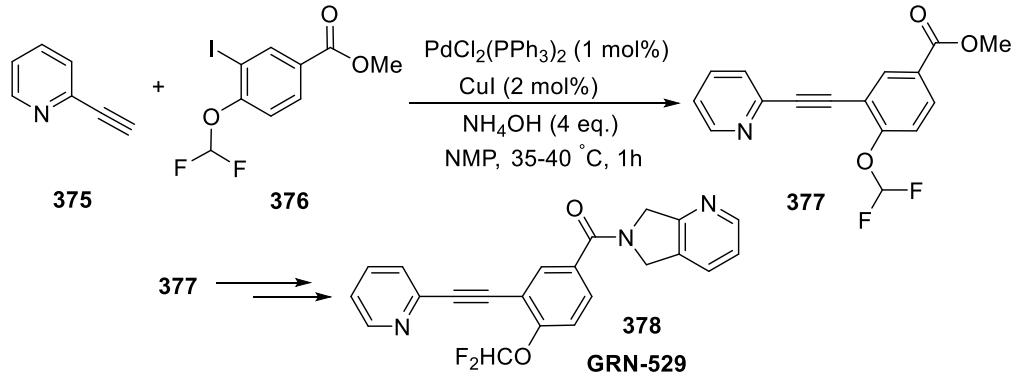
Scheme 91: Synthesis of CHRT2 receptor antagonist.

Negishi et al. developed an efficient stereoselective synthesis of **carotenoids** (β-carotene) *via* transition metal catalysed step-wise synthetic approach.[154]



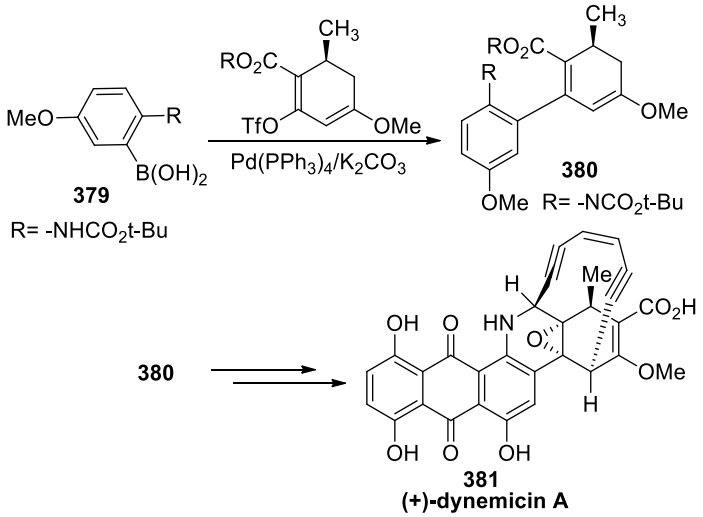
Scheme 92: Synthesis of β-Carotene.

Wyeth Research lab **(2012)** reported the synthesis of **GRN-529** (a highly selective mGluR5 negative allosteric modulator) utilising Sonogashira reaction between the 2-pyridyl acetylene and substituted iodobenzene as a key step.[155]



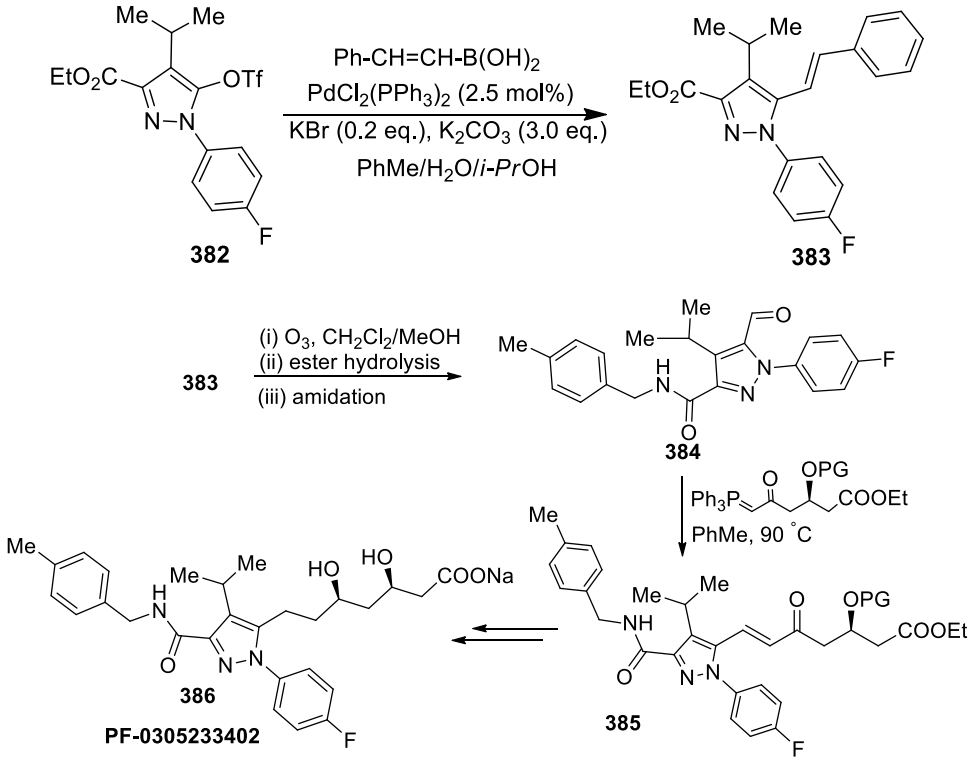
Scheme 93: Synthesis of GRN-529.

Myers et al. had utilized Suzuki coupling reaction, for the synthesis of the antitumor agent **(+)-dynemicin A 381** as an important step.[156]



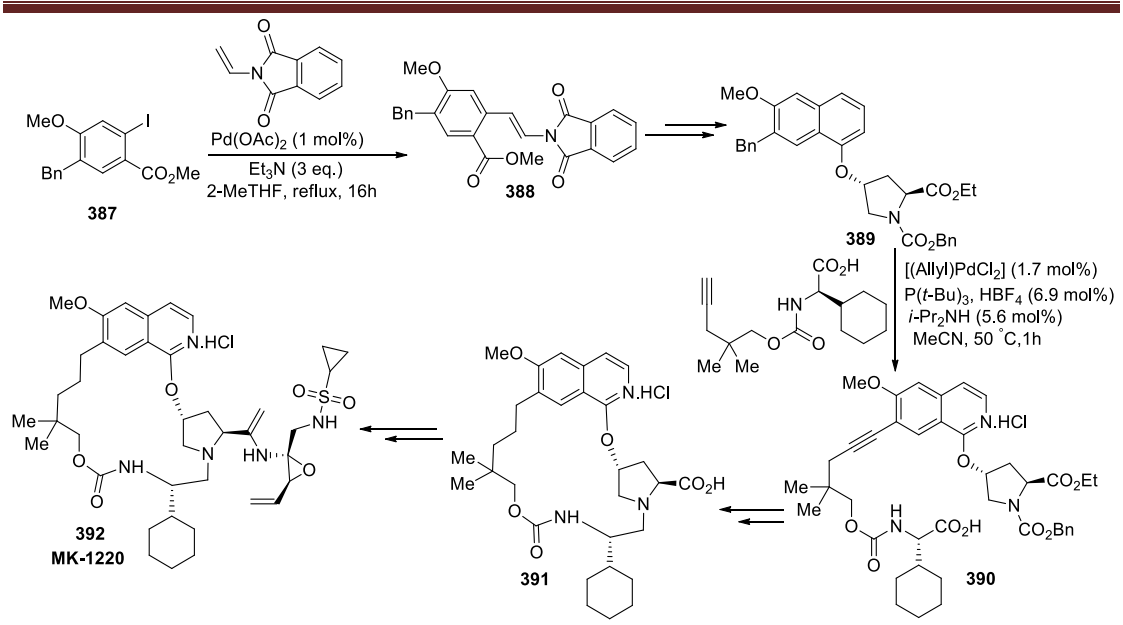
Scheme 94: Synthesis of the antitumor agent (+)-dynemicin.

Bowles et al. developed a synthetic protocol for the synthesis of PF-0305233402 (which display reduced risk of musculo-skelton side effects in the treatment of coronary diseases) via the suzuki cross coupling between substituted pyrazole triflates with aryl vinyl boronic acid.[157]



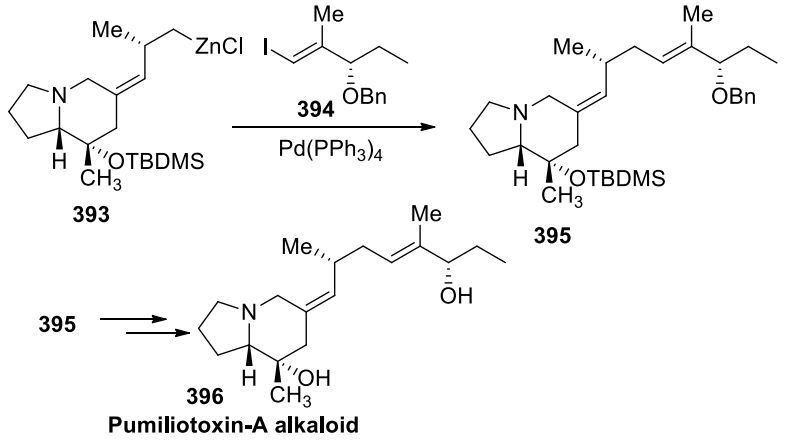
Scheme 95: Synthesis of PF-0305233402.

Merck recently reported the total synthesis of a macro-cyclic compound **MK-1220** (a potent new drug candidate against hepatitis C virus) employing Heck and Sonogashira reaction as the key step.[158]



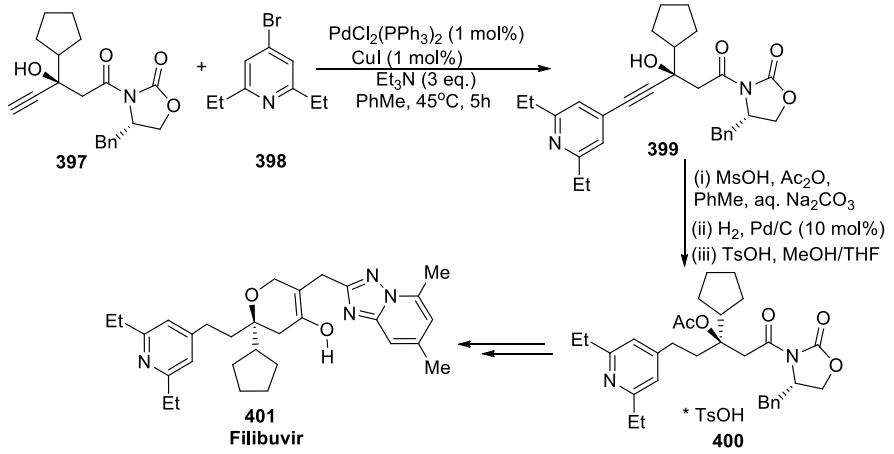
Scheme 96: Synthesis of MK-1220.

Negishi coupling had been utilised by Kibayashi et. al. in the synthesis of **Pumiliotoxin A 396**,[159] which is found in the skin of frogs (Dendrobatidae family).



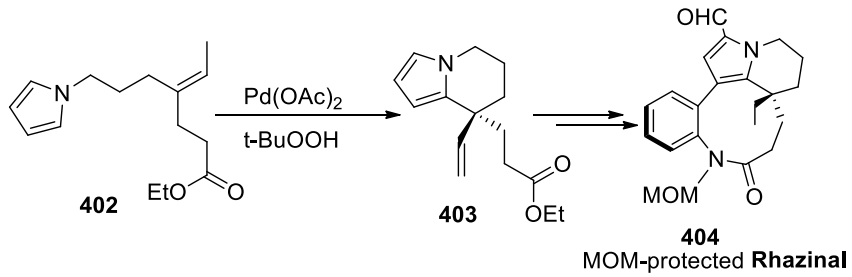
Scheme 97: Synthesis of Pumiliotoxin A.

The Sonogashira coupling reaction has also been utilized in the synthesis of **Filibuvir** (a potential drug for hepatitis C, and also acts as an inhibitor of RNA-polymerase).[160]



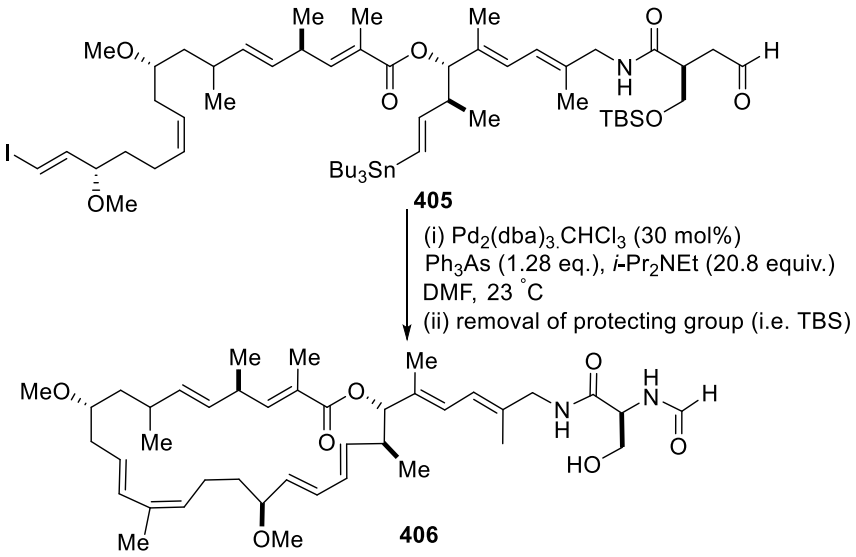
Scheme 98: Synthesis of Filibuvir.

The synthesis of **Rhazinal** alkaloid (obtained from the stem extract of Kopsia teoi), an antimitotic agent, had been accomplished using C-C coupling approach as a key step by the use of Pd-catalyst.[161, 162]



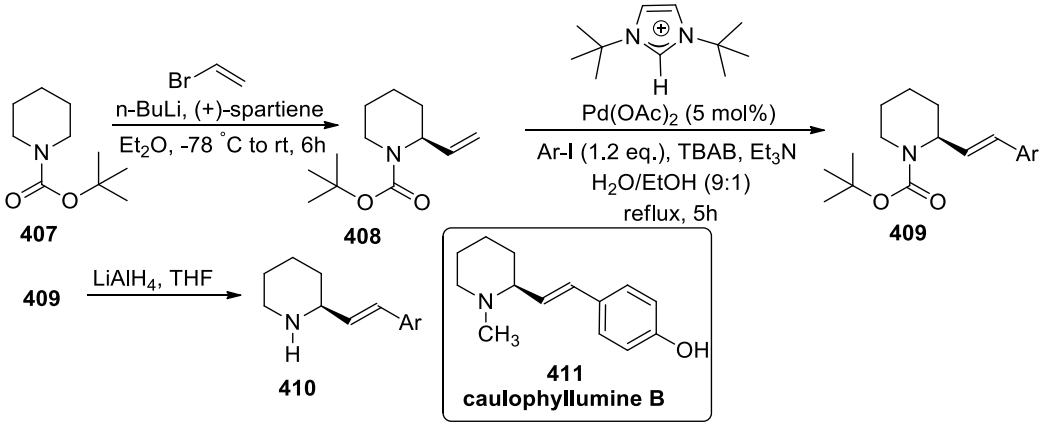
Scheme 99: Total synthesis of Rhazinal alkaloid.

A Pd-catalyzed intramolecular Stille cross-coupling was utilized as a key step in the total synthesis of an anticancer compound **Jimalide B**.[163]



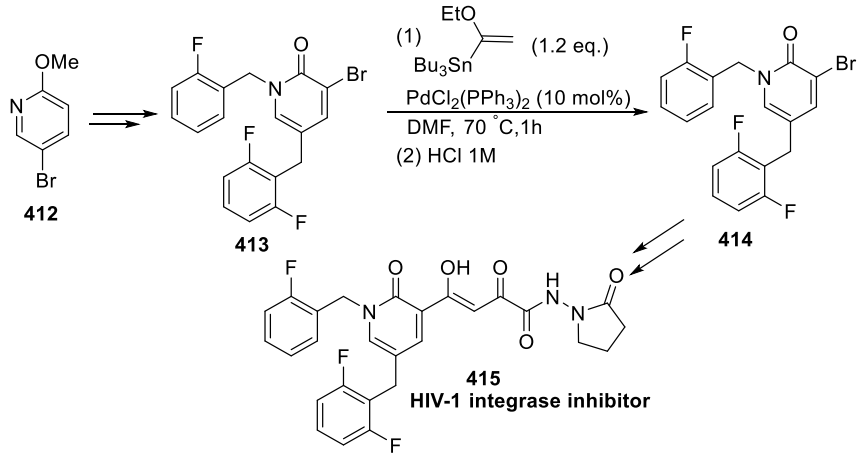
Scheme 100: Total synthesis of an anticancer compound Jimalide B.

A facile Pd-catalysed coupling approach was developed in the synthesis of anticancer piperidine alkaloid **(+)-caulophyllumine** using N-Boc-piperidine as starting material.[164]



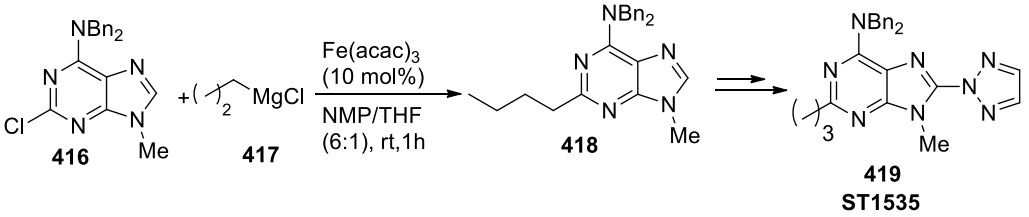
Scheme 101: Synthesis of anticancer piperidine alkaloid (+)-caulophyllumine.

In **2013**, the total synthesis of the HIV-1 integrase inhibitor **415** was reported *via* Stille coupling using 3-bromopyridine as a starting material in overall 18% yield after nine steps.[165]



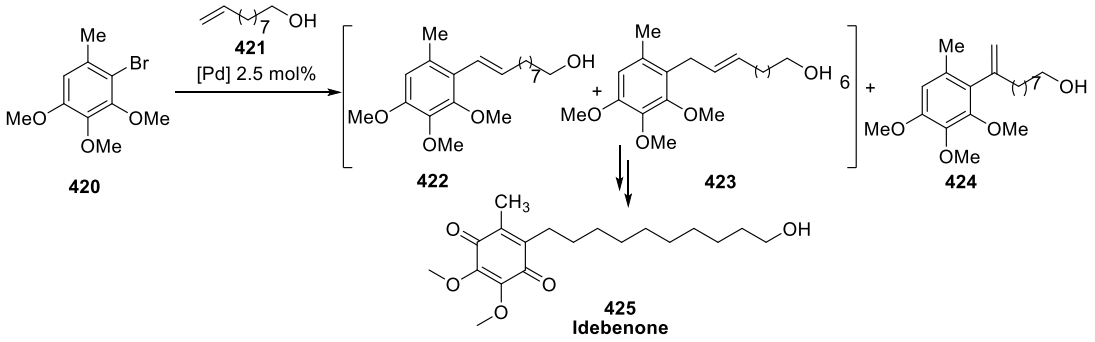
Scheme 102: Total synthesis of the HIV-1 integrase inhibitor.

In **2014**, the synthesis of a highly selective adenocine A2A receptor antagonist **ST1535** (a potential new drug candidate for Parkinson's disease) was reported *via* Kumada coupling reaction using Grignard reagent and functionalized purine base under Fe-catalysed reaction conditions.[166]



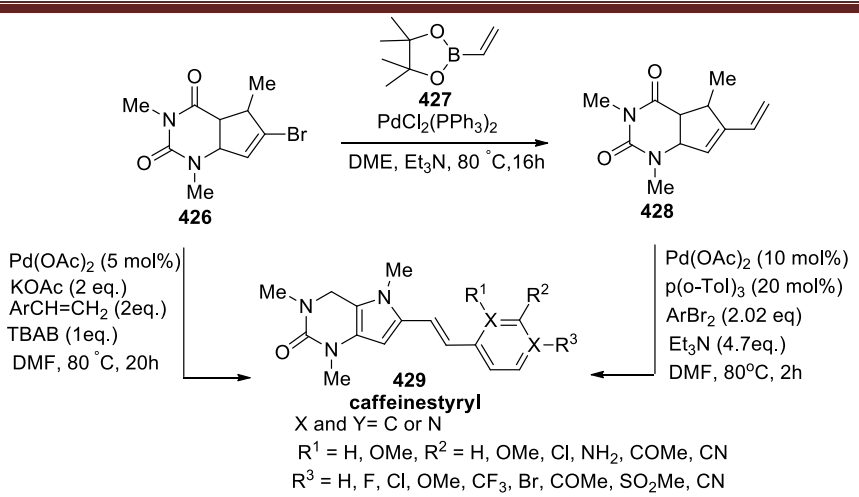
Scheme 103: Synthesis of receptor antagonist **ST1535.**

The total synthesis of **Idebenone**, a drug candidate for Alzheimer's and Parkinson's diseases, was reported using 2-bromo-3,4,5-trimethoxy-1-methylbenzene as starting substrate under Heck coupling reaction approach.[167]



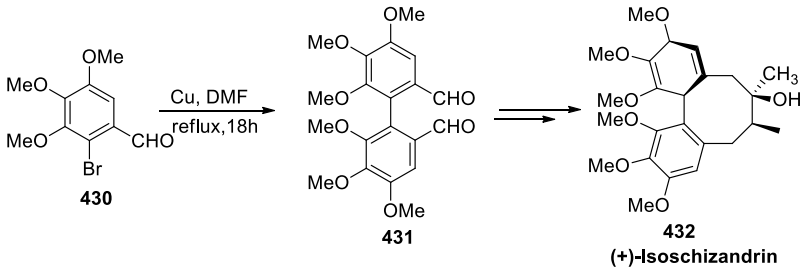
Scheme 104: Total synthesis of Idebenone**.**

Two Heck-coupling strategies were applied in the synthesis of caffeine-styryl hybrid compound **429**, which have dual A2A antagonist/MAO-B inhibitiory activity as well as potential activity against Parkinson's disease.[168]



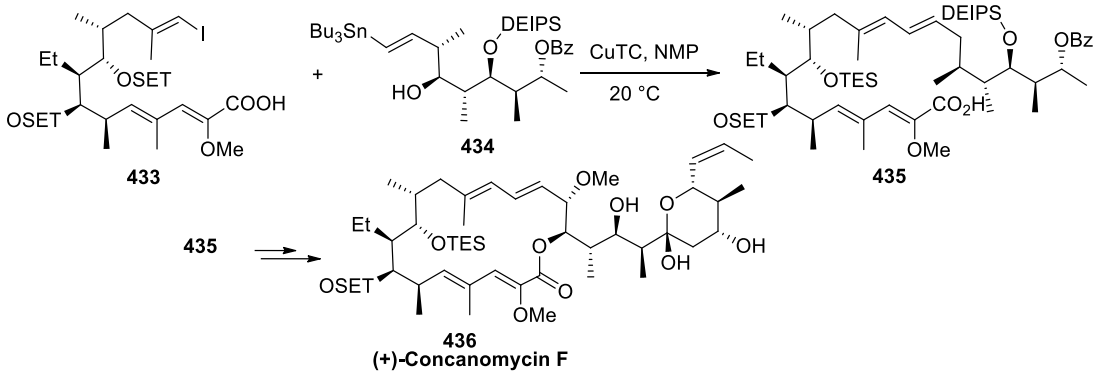
Scheme 105: Synthesis of caffeine-styryl using heck-coupling reaction.

The total synthesis of **(+)-isoschizandrin**, was reported by the use of copper-mediated Ullmann coupling of the corresponding haloaldehyde as the key step.[169]



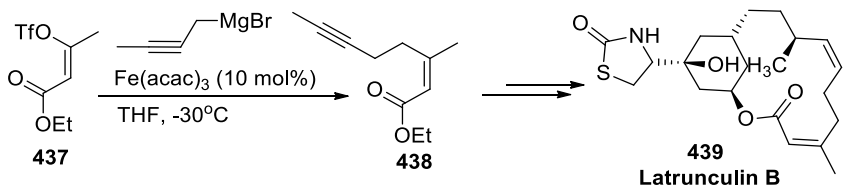
Scheme 106: Total synthesis of (+)-isoschizandrin.

Peterson et al.[170] reported the synthesis of **Concanamycin F** using copper-catalyzed cross-coupling as the key intermediate step, as shown in below scheme.



Scheme 107: Total synthesis synthesis of Concanamycin F.

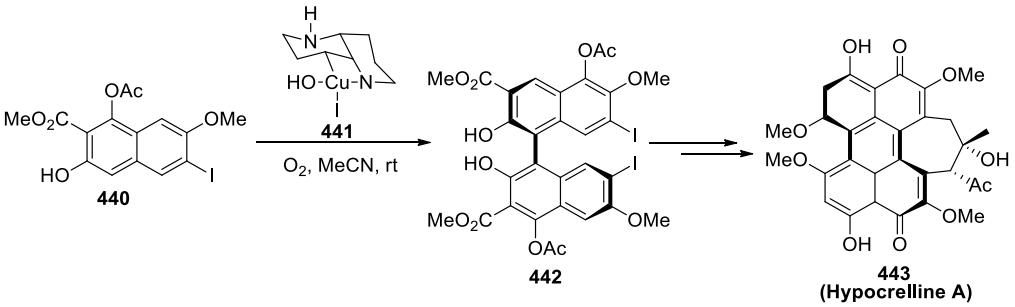
Fürstner et al.[171] reported the Fe-catalysed coupling of alkenyl electrophiles with organomagnesium reagents, as a key step, in the total synthesis of **latrunculin B**.



Scheme 108: Total synthesis of latrunculin B.

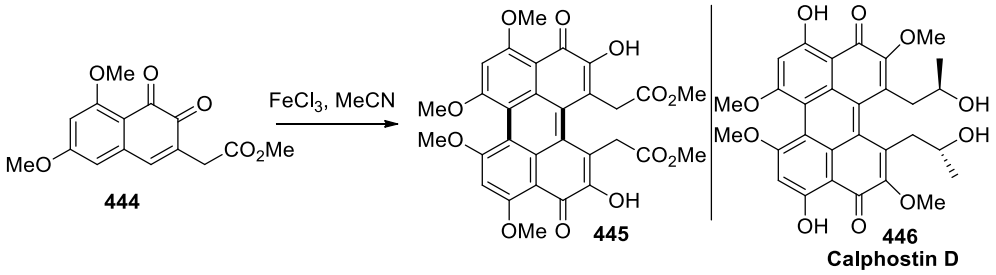
1.4.3 Oxidative coupling and visible light-mediated photocatalytic addition reactions in the synthesis of bioactive alkaloids

Kozlowski et al. reported the synthesis of **hypocrellin A** under oxidative coupling via dimerization of functionalized naphthol analogues.[172]



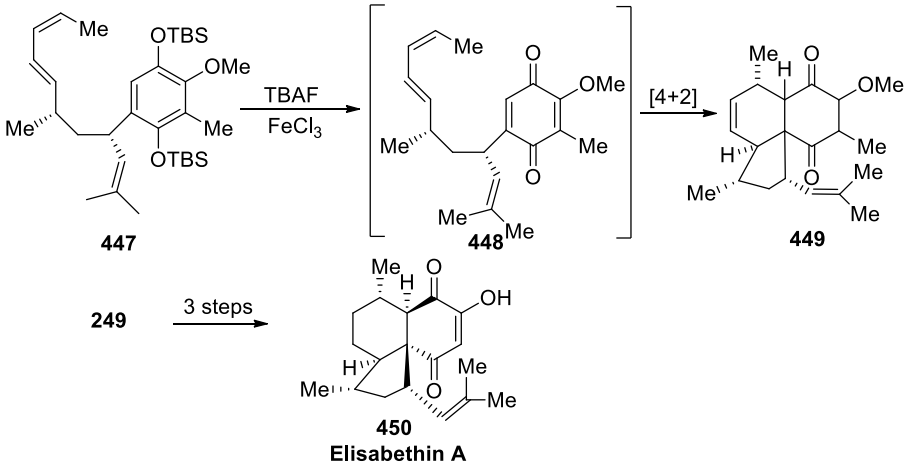
Scheme 109: Synthesis of hypocrellin A.

Merlic et al.[173] had been reported an efficient binaphthyl couplings involving Fe-catalytic system for the synthesis of **Calphostin D**, an inhibitors of protein kinase C.



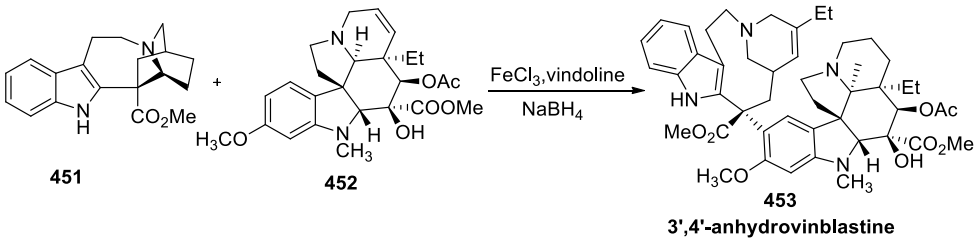
Scheme 110: Synthesis of Calphostin D.

Mulzer and his co-workers utilized the $FeCl_3$-oxidation followed by intramolecular Diels–Alder reaction of functionalized dihydroquinone to the tricyclic alkaloid **Elisabethin A**.[174]



Scheme 111: Total synthesis of tricyclic alkaloid Elisabethin A.

In 1988, Kutney et al. reported the oxidative coupling of catharanthine with vindoline in the presence of $FeCl_3$ catalyst, which furnished the vinblastine precursor anhydrovinblastine.[175]



Scheme 112: Synthesis of vinblastine precursor Anhydrovinblastine.

Visible light mediated photoredox catalysis has also been utilised to synthesis of wide range of bioactive alkaloids via utilising single electron transfer (SET) mechanism under mild reaction conditions.

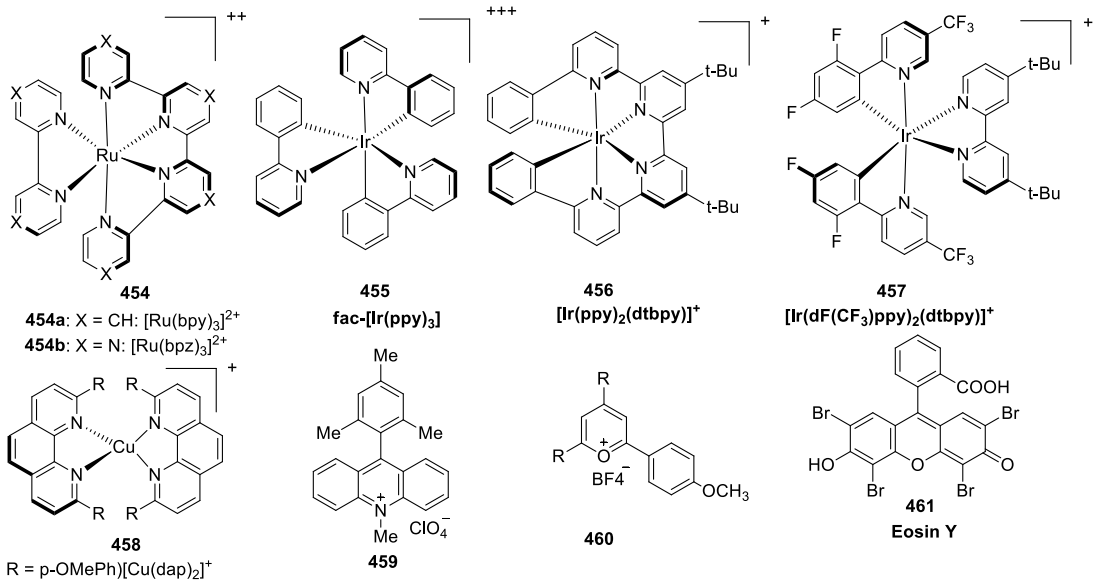
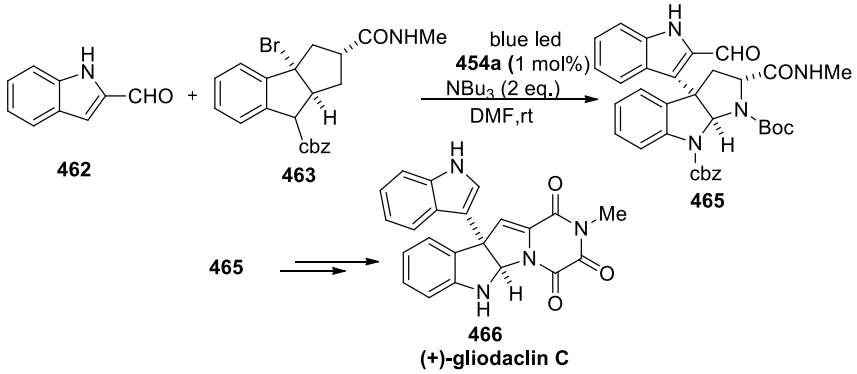


Figure 8. Structures of transition metal as well as organic dyes based photo-catalysts.

In photoredox catalysis, the organic molecules do not normally absorb the light in the visible range (390–700 nm). The energy of visible light is absorbed by organic or transition metal-based photocatalysts [as shown in Figure. 8] [176] that, activated to their excited states, and mediates SET oxidation and/or reduction routes.

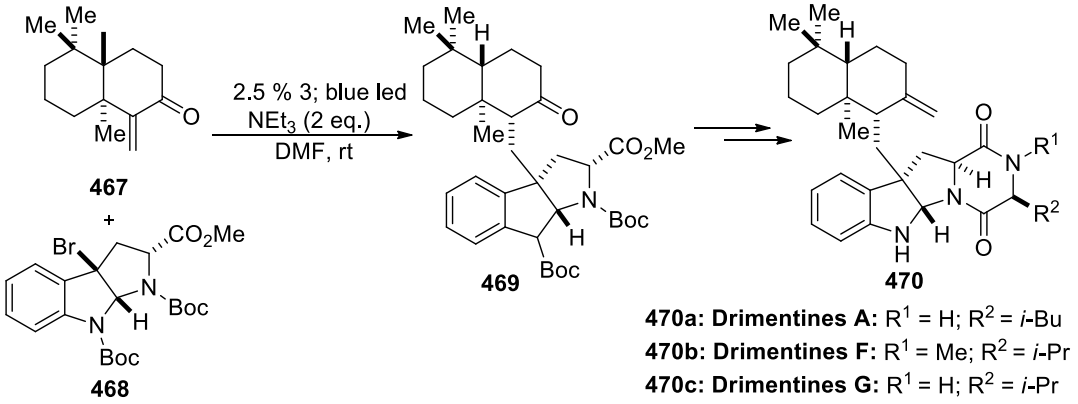


Scheme 113: Asymmetric synthesis of **(+)-Gliocladin C.**

In **2011**, Stephenson et al. reported a Sn-free debromination of C3-bromopyrroloindolines by reductive photoredox catalysis for the asymmetric

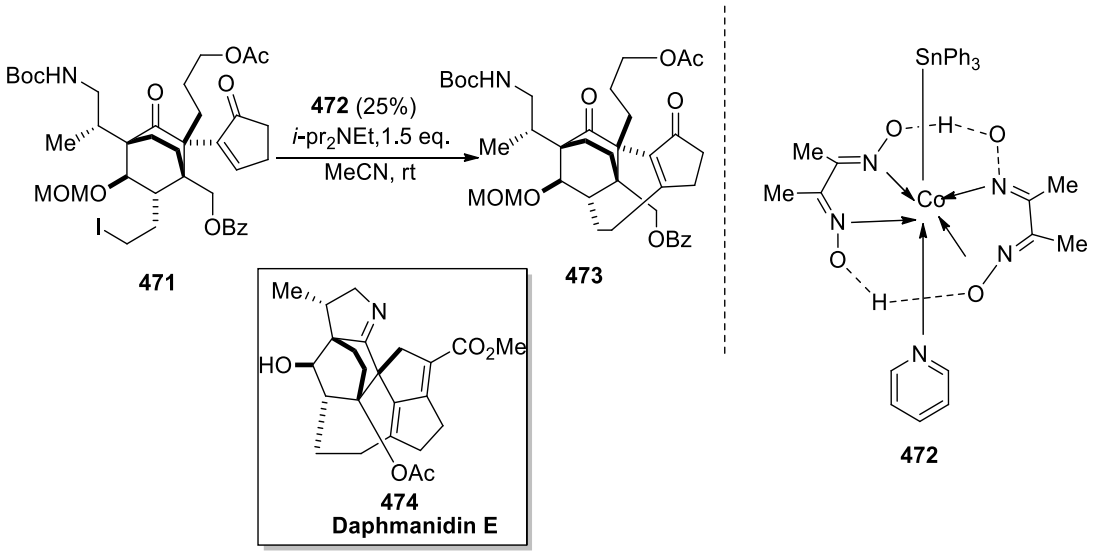
synthesis of **(+)-Gliocladin C** (a complex natural product with interesting cytotoxic activity) via the use of blue light source.[177].

Recently, Li et al. utilised a similar methodology i.e. de-bromination of C3-bromopyrroloindolines for the total syntheses of the bio-active alkaloids **Drimentines A**, **F** and **G**. via a reductive photoredox methodology.[178]



Scheme 114: Total syntheses of the bio-active alkaloids Drimentines.

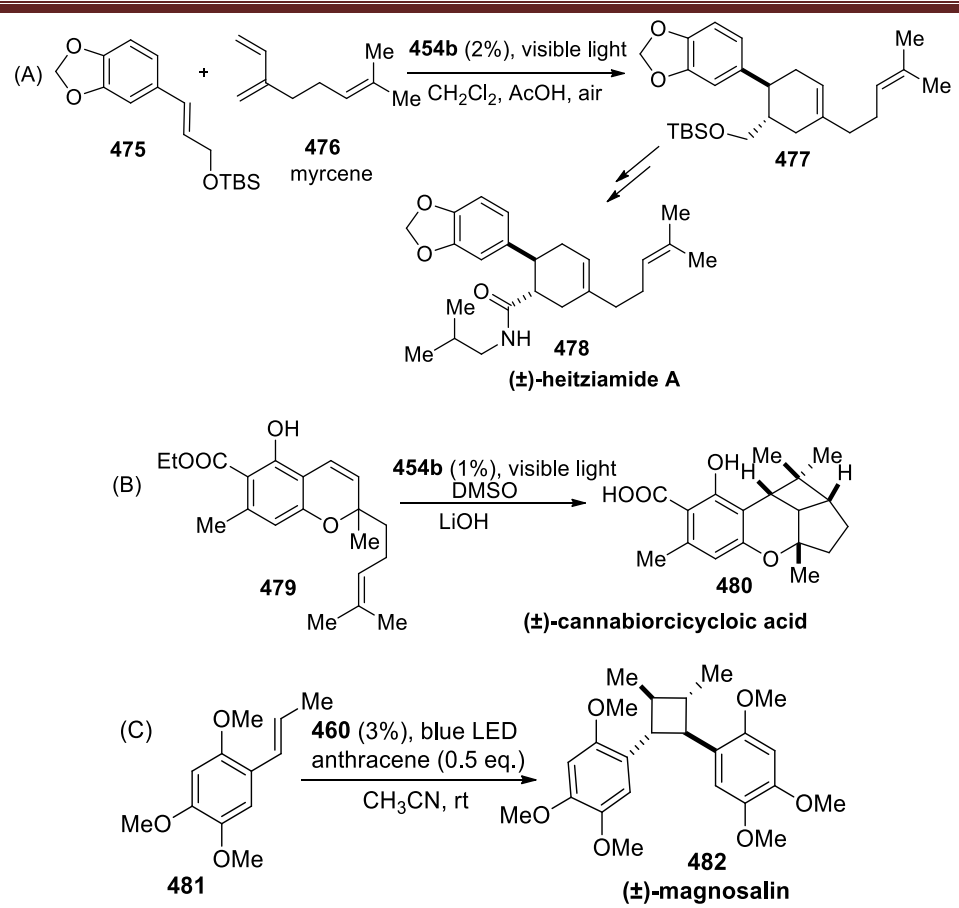
Carreira et al. reported a Heck-type reaction *via* cobalt-mediated intramolecular cyclisation of alkyl iodides with alkenes employing visible light photocatalytic approach to the total synthesis of **(+)-Daphmanidin E**.[179]



Scheme 115: Total synthesis of **(+)-Daphmanidin E.**

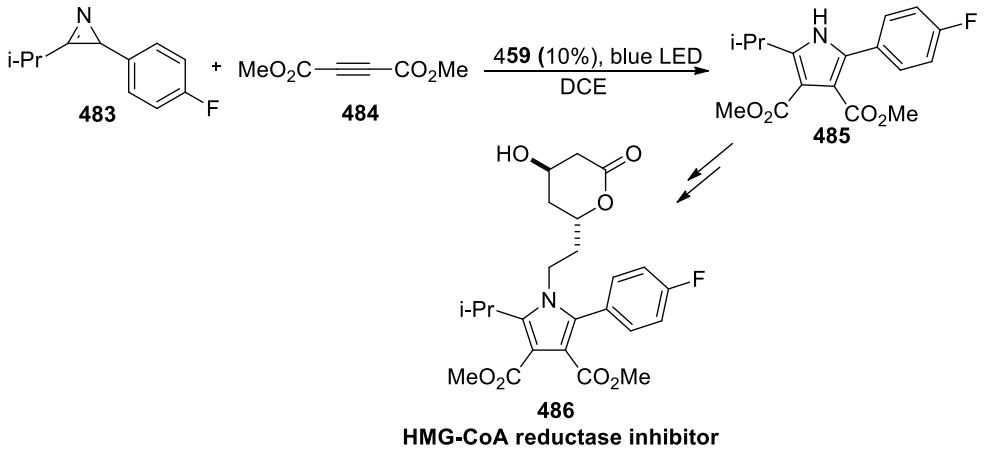
In **2011**, Yoon et. al. reported a photoredox-promoted SET oxidation via [4 + 2]-cycloaddition of functionalized styrene with myrcene to the concise synthesis of (–)-**heitziamide A** and cannabinoid natural product **cannabiorcicycloic acid**.

Notably, in the same year, Nicewicz et al. also developed an intermolecular photoredox promoted [2 + 2] cycloaddition approach for the total synthesis of the lignin and neolignan cyclobutane natural products **magnosalin**.[180, 182]



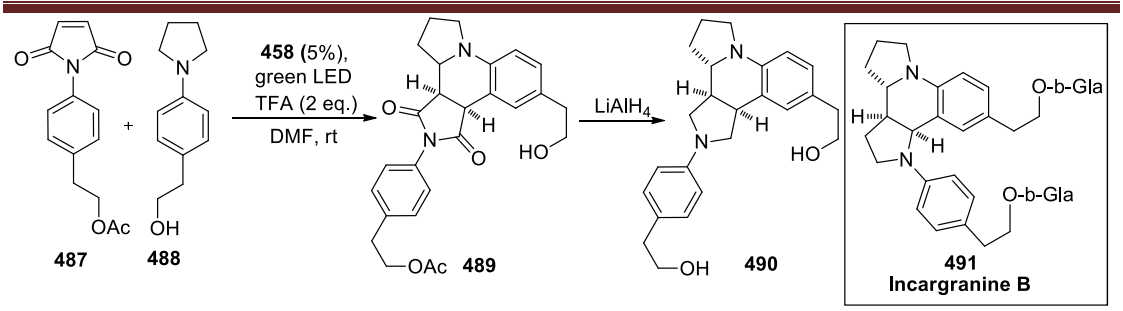
Scheme 116: Total synthesis of natural products Magnosalin.

Recently, Xiao et. al. reported formal [3 + 2]-cycloaddition of 2*H*-azirines with Michael acceptors, using photoredox-catalysed SET oxidation approach under blue led light, for the synthesis of the HMG-CoA reductase inhibitor.[183]



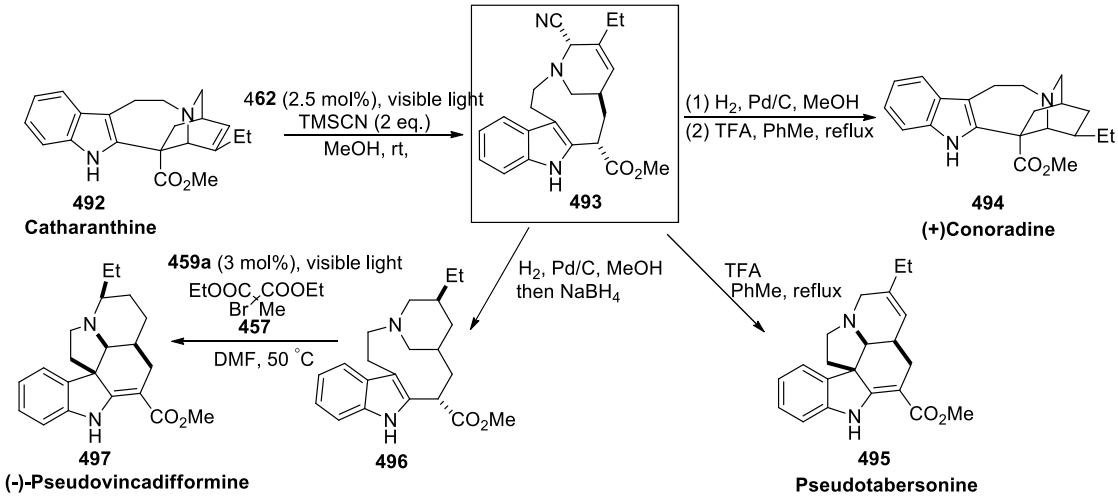
Scheme 117: Synthesis of the HMG-CoA reductase inhibitor.

Bissember et al. developed a tandem (TFA)/Cu(dap)$_2$-photocatalytic system via the direct annulation of N-arylmaleimide and N-arylpyrrolidine followed by reduction with $LiAlH_4$, for the synthesis novel aglycone analogue of the natural product **Incargranine B**.[184]



Scheme 118: Synthesis of natural product Incargranine B.

Stephenson et. al. used photoredox catalytic approach as a key step for the synthesis of complex natural products (+)-coronaridine, (–)-pseudotabersonine and (–)-pseudovincadifformine.[185]



Scheme 119: Formation of indole alkaloids via photoredox catalytic approach.

In this approach, alkaloid (+)-catharanthine was converted into a common intermediate **493** *via* iridium-based photoredox catalytic approach, which was further diversified other indole alkaloids **494**, **495**, **496, 497**.

1.5 Summary

In this review, the different era's of cross coupling reactions (C-C/C-N/C-O) were highlighted and a shift from transition-metal catalyzed reactions to metal-free reactions and then, ultimately, to organo-catalyzed/Cross dehydrogenative coupling reactions (CDC) were discussed. The applications towards the synthesis of natural alkaloids and drug leads by using coupling reactions have been summarized. The conventional metal catalysed cross coupling reactions (C-C/C-N/C-O) such as: Suzuki, Stille, Miyura, Kumada, Heck and Negishi etc. are the most powerful approaches for the synthesis of natural alkaloids as well as biologically privileged motifs. Moreover, the use of organocatalysts as well as metal-free approaches also drove the interest of synthetic/medicinal chemist due to their economic and environmental benign nature of reaction approach. Moreover, these methodologies

were successfully applied in the synthesis of several natural products such as Clausine-L **303**, Pityriazole **307**, ent-dihydrocorynantheol **313**, carotenoids (β-carotene) **374**, (+)-isoschizandrin **432**, Concanamycin F **436**, latrunculin B **439**, hypocrellin A **443**, Calphostin D **446**, tricyclic alkaloid elisabethin A **450**, vinblastine precursor anhydrovinblastine **453**, (+)-gliocladin C **466**, Drimentines A, F and G **470a-c**, heitziamide A **478**, Incargranine B **491**, and indole alkaloids **494, 495, 497**. Interestingly, some potent drug components/candidates were also synthesized utilizing the cross coupling approach such as: selective negative allosteric modulator D159687 **318**, PF01367338 (a drug candidate for breast and ovarian cancers) **322**, PI3k and AKT/PKB signalling pathway that inhibitor **326**, CEP-32215 (an antagonist of histamine H-3 receptor) **330**, Bruton's Tyrosine Kinase (BTK) inhibitor **334**, LY-2801653, a receptor of tyrosine kinase family **338**, inhibitor of PI3K receptors **341-343**, AMG 837: a GPR40 receptor antagonist associated with type 2-diabetes **347**, anti-cancer drug Crizotinib **350**, AKT kinase inhibitor **354**, AT-13387 (a molecule that is capable of interacting with chaperones and essential for the survival of cancer cells) **358**, antitumor antibiotic (±)streptonigrin **364**, CHRT2 receptor antagonist **369**, GRN-529 (a highly selective mGluR5 negative allosteric modulator) **378**, antitumor agent (+)-dynemicin A **381**, PF-0305233402 (which display reduced risk of musculo-skelton side effects in the treatment of coronary diseases) **386**, HIV1 integrase inhibitor **415** and ST1535 (a potential new drug candidate for Parkinson's disease) **419** etc.

1.6 Conclusion

The formation of new bonds (C-C/C-N/C-O) is of utmost importance in the synthesis of organic compounds. Cross-coupling reactions merge two fragments and lead to the formation of new bonds, often catalysed by metal as well as metal free conditions facilitates the reaction. In recent years, a lot of work has been done to improve the reaction conditions necessary for cross-coupling such as better functional group tolerance, expansion of the substrate scope, lower catalyst loadings etc via metal as well as metal-free/organocatalytic environmentally benign approaches. The current coupling era shifted to metal-free, organocatalytic or cross-dehydrogenative coupling strategies in order to accelerate the reactions and minimizing the typical purification processes. Therefore, in this chapter, we highlighted the various developments in coupling via C-H bond activation which will encourage further advancement in the development of green coupling reactions (C-C/C-N/C-O) and these will be applied towards the synthesis of biologically active molecules and drug candidates.

Succinctly, the outcomes of new green methods had been, so far, multi-directional with an unlimited potential impact on synthetic developments of bioactive alkaloids/therapeutics which ultimately benefits society and mankind.

1.7 References

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Chapter 2

Novel Functionalized 2-oxo-benzo[1,4]oxazines and its congeners: MW- and Ultrasonic-assisted Synthesis, Chemistry, Biological Evaluation, SAR and Molecular Docking Studies."

Section 2.1

MW-assisted Synthesis, Chemistry, Biological Evaluation, and SAR studies.

2.1.1 Introduction

Benzo[1,4]oxazines **1-8**, a sub-class of benzo fused heterocycles, are endowed with a wide range of biological activities such as anti-inflammatory,[1] analgesic,[2] antibacterial,[3] neuroprotective,[4] D2 receptor antagonists,[5] antimycobacterial,[6,7] antihypertensive,[8] antifungal,[9] herbicidal,[10] antiarrhythmic,[11] thrombin inhibitor and fibrinogen receptor antagonists,[12] 5-HT receptor antagonists,[13] potent inhibitor of tumor-driven angiogenesis[14a] and selective non-steroidal mineralocorticoid receptor antagonists[14b] etc. Some marine secondary metabolites such as, Arcticoside **5a** (potent antifungal agent) and C-1027 chromophore- III & V **5b** (potent antitumor antibiotic),[15] which were isolated from a culture of an arctic marine actinomycete *Streptomyces strain*; possess as benzo [1,4] oxazines substructures in their active scaffolds (figure 1).

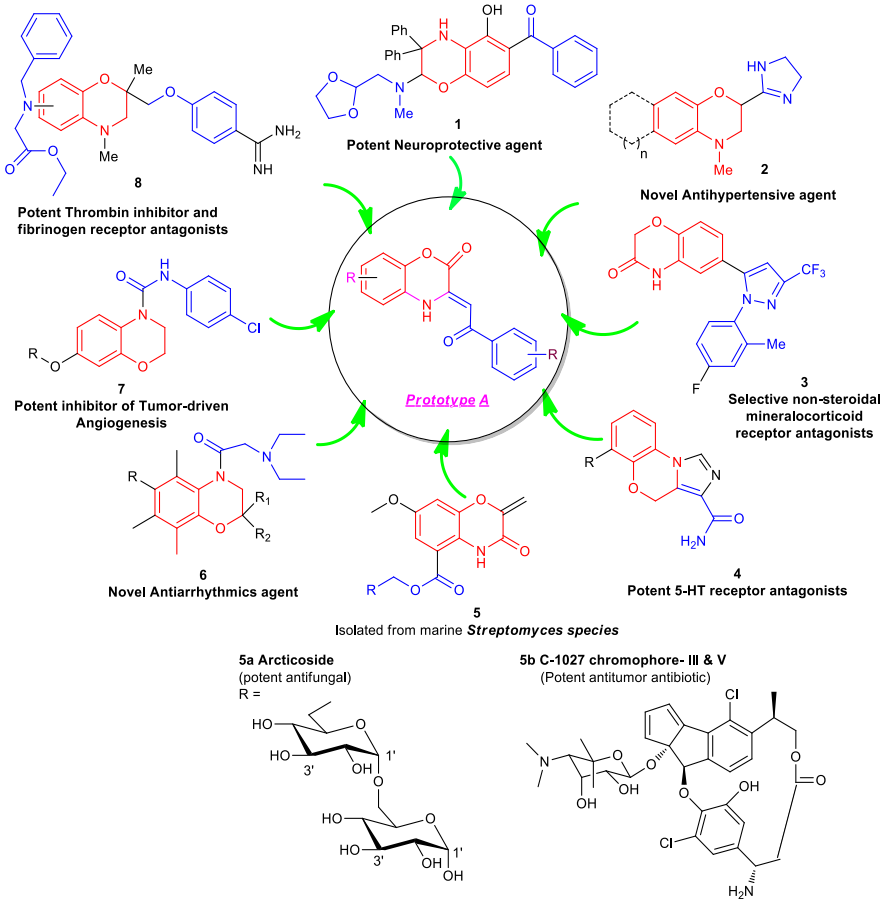


Figure 1. Structure of biologically active benzo [1, 4] oxazines **1-8.**

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, 2-oxobenzo [1,4] oxazines and its related structural motifs have been reported in the literature using metal as well as metal-free catalyst. [16-18] However, none of the reported methodologies were environmentally benign and economic, because these were associated with several drawbacks such as the use of toxic catalysts, toxic starting materials, hazardous organic solvents, multistep and complicated reaction assembly, limited number of appropriate substrates for diverse synthesis, tedious workup and low yields etc. Therefore, an efficient, environmentally benign and more greener approach for the synthesis of benzo[1,4]oxazines is still a challenging area of research.

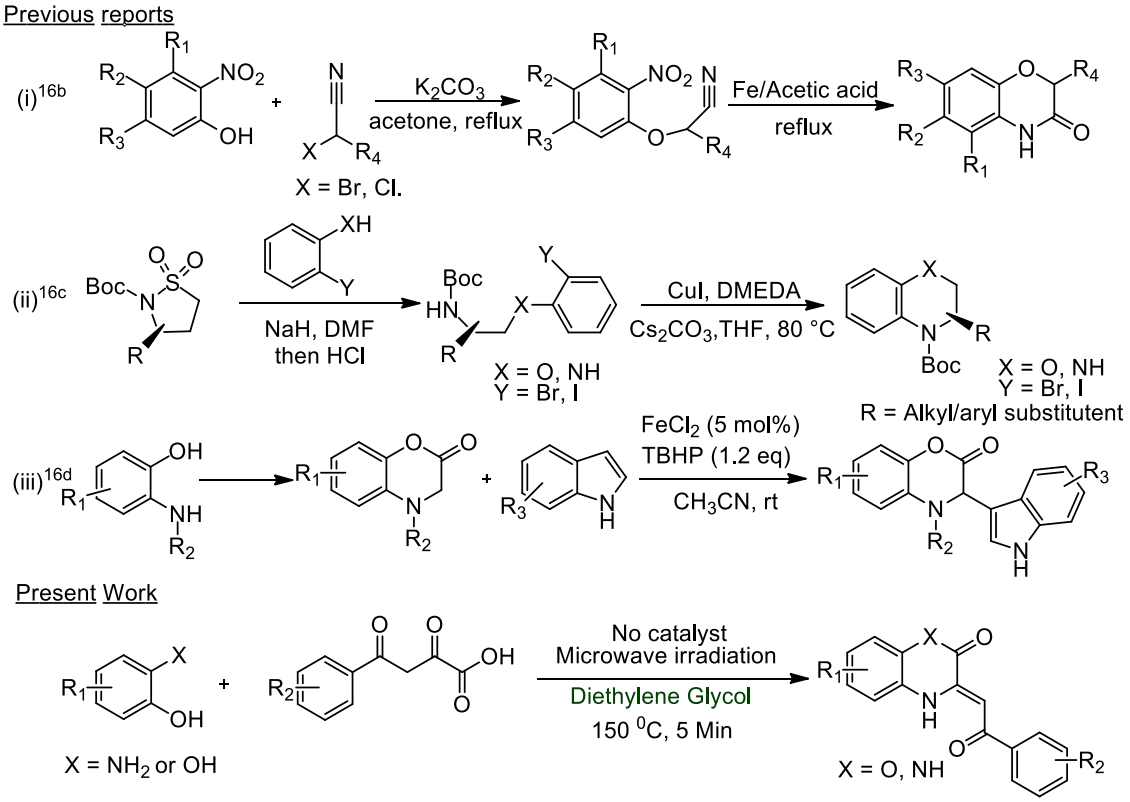


Figure 2. Previous and present reports for the synthesis of substituted benzo[1,4]oxazines derivatives.

Earlier, Kikelj et al. reported the first synthesis of 3-unsubstituted 3,4-dihydro-1,4-benzoxazin-2-ones *via* catalytic hydrogenation of 4-benzyl-3,4-dihydro-1,4-benzoxazin-2-one.[16a] Since then, several metal-catalysed synthesis of substituted benzo[1,4]oxazines have been reported in the literature[16b-d] [figure 2; entry 1-3].

2-aminophenols or substituted 2-nitrophenols[16f] or 2-halophenols,[16g-j] were most commonly used as starting materials towards the synthesis of benzo[1,4]oxazine derivatives. With 2-aminophenols as the starting substrate, various protection and deprotection steps are required.[16k-l] Xia et al. (2008) reported sulphamic acid as an efficient catalyst for the synthesis of benzo[1,4]oxazines derivatives in one pot reaction condition providing good yield.[16e] In spite of this efficient methodology, sulphamic acid is associated with several drawbacks with respect to its hazardous nature towards animals as well as environment, such as the high toxicity of sulphamic acid in animals (LD_{50} = 1312 mg/kg in mouse via oral route; LD_{50} = 3160 mg/kg in rat via oral route; toxicity value of LC_{50} = 70.3 mg/l in fish *Pimephales promelas* species); acute oral and inhalation toxicity to human etc. Its disposal also induces toxicity to the environment.[16f] Thus, none of the reported methodologies were environmentally benign as these were associated with several drawbacks such as the use of toxic catalysts, toxic starting materials, hazardous organic solvents, multistep and complicated reaction assembly, limited number of appropriate substrates for diverse synthesis, tedious workup and low yields etc.[19-21] Therefore, an efficient, environmentally benign and more green approach for the synthesis of benzo[1,4]oxazines is still a challenging area of research.

Moreover, it has also been observed that several chalcones and its analogues,[22a-c] quinolines,[22d-f] and coumarin-derived scaffolds,[22g,h] which have 2-oxobenzo[1,4]oxazines like substructure in their scaffold, were found to be potent antioxidants under several *in vitro* antioxidant assays. So, in our endeavour to search for new class of potent antioxidants; we developed inclinations towards 2-oxo-benzo[1,4]oxazine class of molecules as these class have never been studied as potential antioxidants (prototype A: figure 1), because it has almost similar substructure as present in Coumarine, chalcones and quinolone or its analogues. In this context, we were interested to explore the green synthesis and antioxidant activity of non-naturally occurring 2-oxobenzo[1,4]oxazines derived analogues.

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

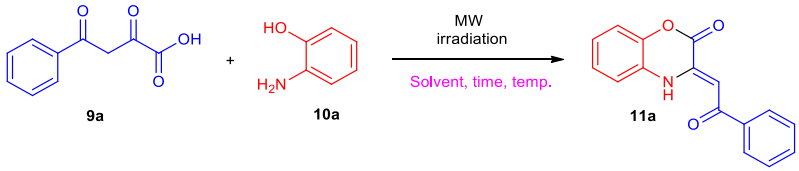
advantages over conventional heating conditions such as: reduction of the reaction times as well as decrease side reactions, increase yields of desired products and improve reproducibility. Therefore, industrial as well as academic research groups are frequently using MAOS for rapid reaction optimization, accelerating the efficient synthesis of new chemical entities and also for the novel methodology development.[23e-g] Hence, utilizing this concept; herein, we report a very simple, mild and highly efficient green protocol for the synthesis of highly functionalized 2-oxobenzo[1,4]oxazines **11a-n** (upto 97% yield) and 2-oxoquino[1,4]oxalines **14a-h** (upto 96% yield) under microwave irradiations using readily available starting materials. The main advantage of this protocol is the avoidance of any toxic reagent, solvent or catalyst. Although, compounds **11a-i** and **14a-h** are already reported in the literature, but they were prepared by other routes,[17] and their antioxidant activities have never been evaluated till now. Therefore, for the first time, we have evaluated the *in vitro* antioxidant activities of all the synthesized compounds **11a-n, 14a-h** and **16** in DPPH radical scavenging assay using ascorbic acid (IC_{50} = 4.57 μg/mL) as standard reference and ferric reducing antioxidant power (FRAP) assay taking BHT (546.0 ± 13.6 μM) as standard reference.

To the best of our knowledge, this is the first report of microwave-assisted synthesis and *in vitro* antioxidant activities of functionalized 2-oxobenzo[1,4]oxazines **11a-n,** 2-oxoquino[1,4]oxalines **14a-h** and **16** in excellent yields having high level of functional group compatibility.

2.1.2 Results and Discussion

We started our initial investigation towards the development of an environmentally benign, sustainable protocol for the synthesis of 2-oxobenzo[1,4]oxazine with a typical model reaction between 2,4-dioxo-4-phenylbutanoic acid **9a** and 2-aminophenol **10a** in isopropanol (1.0 mL) under nitrogen atmosphere at room temperature for 3 h which furnished the condensation product **11a** in only 18% yield (entry 1, Table 1). Carrying out the above reaction at 90 °C for 3 h afforded **11a** in better yield [(45%), entry 2, Table 1]. The product obtained was fully characterized by its spectroscopic data (^{1}H and ^{13}C NMR, HRMS and IR).

Table 1 Optimization study[a]: Synthesis of 2-oxobenzo[1,4]oxazines **11a** by the reaction of 2,4-dioxo-4-phenylbutanoic acid **9a** and 2-aminophenol **10a**.



Entry	Solvent	Temp(°C)	Method A[b]		Method B[c]	
			Time (min)	Yield[d] (%)	Time (min)	Yield[d] (%)
1	Isopropanol	rt	180	18	--	---
2	Isopropanol	90	180	45	10	52
3	Isopropanol	90	300	55	30	58
4	DMF	90	180	51	30	64
5	DMF	120	180	58	20	62
6	DMF	150	120	50	15	69
7	DMSO	150	180	59	10	77
8	DMSO	150	240	61	5	51
9	DMSO	150	300	67	15	72
10	DMSO	180	120	65	2	56
11	**Diethylene glycol**	**150**	180	61	**5**	**94**
12	Diethylene glycol	150	120	54	3	80
13	Diethylene glycol	150	300	67	7	93
14	Diethylene glycol	170	180	64	2	85
15	Diethylene glycol	160	180	65	2	82
16	Diethylene glycol	170	300	63	5	81

[a]Reaction conditions: **9a** (0.1 mmol), **10a** (0.1 mmol) in solvent (1.0 mL), 5-300 min, N_2 atmosphere. [b]Method A: Conventional heating; [c]Method B: Microwave Irradiation; [d]Isolated yield after recrystalization/column chromatography.

Since we observed an increase in the yield of **11a** as we change solvent from isopropanol to DMF; we switched over to more polar DMSO solvent. Thus, the above reaction was carried out in DMSO solvent, at 150 °C for 10 min under microwave irradiation, which furnished **11a** in 77% yield (entry 7, Table 1). Decreasing or increasing the time for the above reaction in DMSO solvent was not found to be fruitful. (Entries 8-10, Table 1). Then, we planned to perform our model reaction in more polar diethylene glycol as solvent. To our surprise, after 5 min at 150 °C, we obtained **11a** in 94% yield (entry 11, Table 1). Furthermore, in spite of increasing the reaction temperature from 150 °C to 170 °C or increasing/ decreasing the reaction time; we were successful in obtaining **11a** in the yield range of 81-93% (entries 12-16,

Table 1). Finally, based on above screening studies, diethylene glycol as solvent, 150 °C temperature for 5 min was found to be the best optimized reaction condition under microwave irradiations (entry 11, Table 1).

Plausible mechanism for the formation of (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11a)

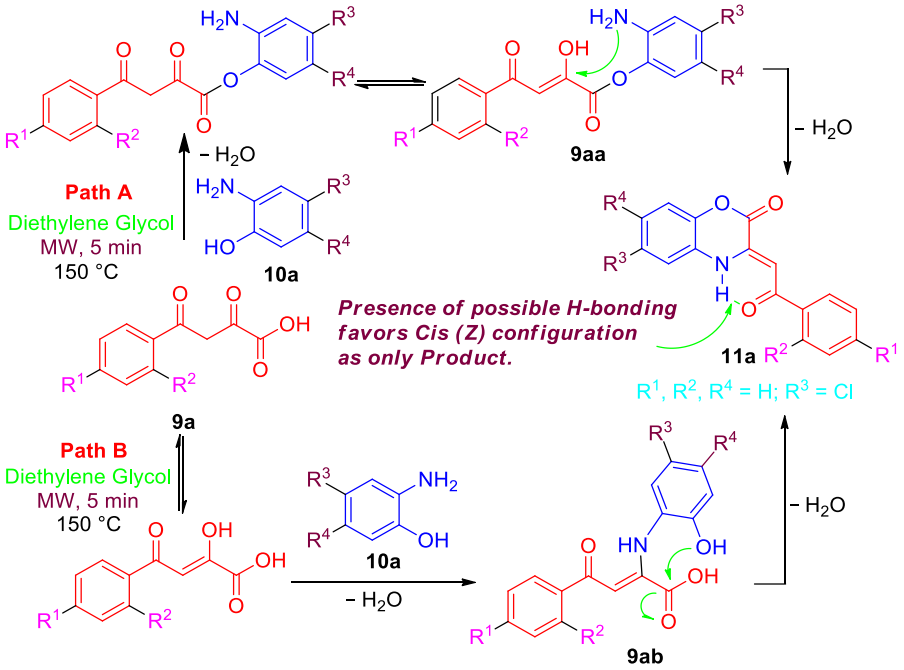


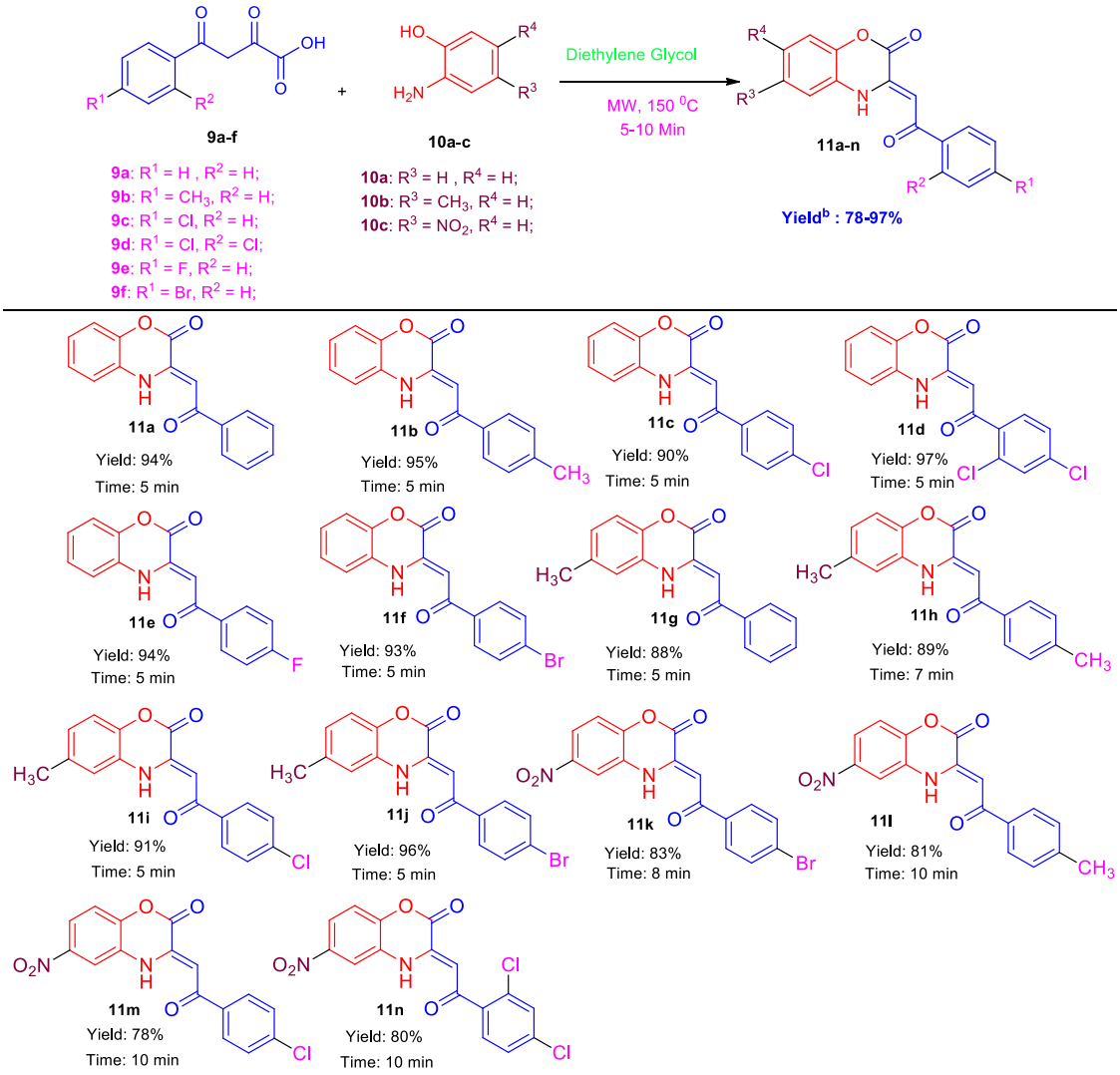
Figure 3. Possible pathway for the formation of 2-oxo-benzo[1, 4] oxazine **11a**.

A plausible mechanism for the formation of the desired 2-oxo-benzo[1,4]oxazine **11a**, which was formed by the reaction between **9a** and **10a** under the optimized conditions involves intermolecular condensation followed by an intramolecular condensation and we obtained (*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 **11a**.After optimization study, we further investigated the scope and generality of this reaction. Several alkyl/ alkoxy/ halide/ nitro-substituted 2,4-dioxo-4-phenylbutanoic acids **9a-f** were reacted with alkyl/halide/nitro-substituted 2-aminophenol **10a-c** in diethylene glycol under our optimized conditions (Scheme 1). The desired 2-oxobenzo[1,4]oxazines **11a-n** were purified either by flash column chromatography method or by recrystallization (see experimental section).

As evident from scheme 1; substituted 2, 4-dioxo-4-phenylbutanoic acid **9a-f** reacted smoothly with substituted 2-aminophenol **10a-c**, and furnished substituted 2-oxobenzo[1,4]oxazines **11a-n** in 78-97% yield range. It has been observed that nitro-

based 2-oxobenzo[1,4]oxazines **11k-n** were obtained in comparatively lesser yields (78-83%) with rest of the compounds **11a-j**. This is due to poor solubility of nitro-based 2-oxobenzo[1,4]oxazines **11k-n** in ethyl acetate which makes the purification of these compounds via column chromatography very tedious and cumbersome. In this study, the most characteristic feature observed was that a broad range of functional groups, like Cl, Br, OMe and NO_2 are well compatible under our optimized reaction conditions. Thus, these groups can further be manipulated to obtain new therapeutic molecules.

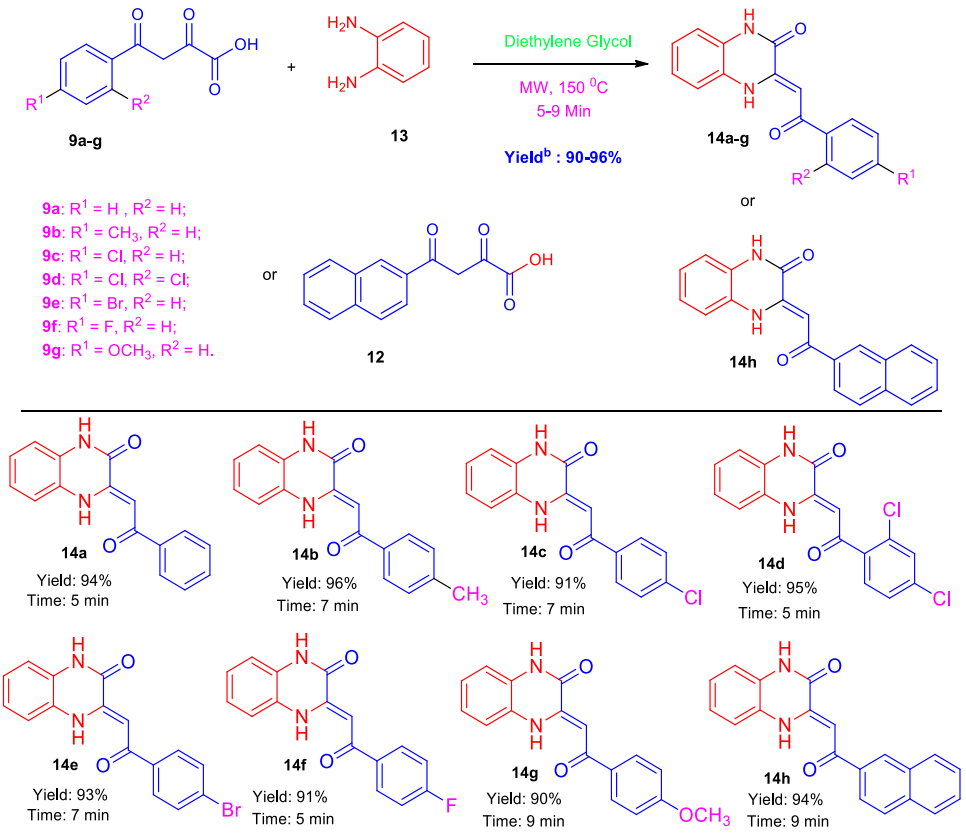
Scheme 1. Microwave-assisted one-pot green synthesis of 2-oxobenzo[1,4]oxazines analogues (**11a-n**).[a & b]



[a]unless otherwise mentioned, all the reactions were carried out with substrates **9a-f** (0.2 mmol), substituted 2-aminophenols **10a-c** (0.2 mmol) in diethylene glycol (2.0 mL) at 150 °C temperature under microwave irradiation. [b]Isolated yield.

After successful implementation of our methodology on **11a-n** series; we extended its synthetic application towards the synthesis of its congener class of bioactive heterocycles i.e. 2-oxoquino[1,4]oxalines **14a-h;** which were synthesized from **9a-g** with phenyl-1,2-diamine **13** using our optimised methodology in the excellent yield (90-96%), as depicted in Scheme 2.

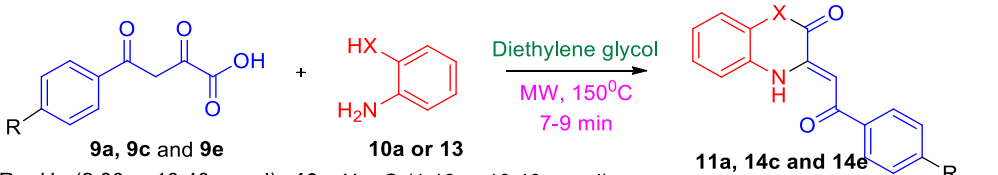
Scheme 2. Microwave-assisted one-pot synthesis of functionalized 2-oxoquino[1,4]oxalines **14a-h**.[a]



[a]unless otherwise mentioned, all the reactions were carried out with substrates **9a-g** or **12** (0.2 mmol) and 1,2-diamino benzene **13** (0.2 mmol) in diethylene glycol (2.0 mL) at 150 °C under microwave irradiation. [b]Isolated yield.

Furthermore, the practicality of this methodology was demonstrated via gram scale synthesis of compounds **11a, 14c** and **14e**.

Scheme 3. Gram scale synthesis of **11a, 14c** and **14h.**



9a: R = H; (2.00 g, 10.40 mmol)
9c: R = Cl; (2.26 g, 10.00 mmol)
9e: R = Br; (2.71 g, 10.00 mmol)

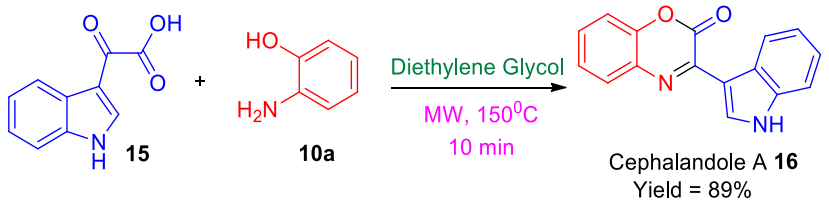
10a: X = O (1.13 g, 10.40 mmol)
13: X = NH (1.08 g, 10.00 mmol)

11a: R = H; X = O (2.49 g, 90.41%)
14c: R = Cl: X = NH (2.60 g, 87.32%)
14e: R = Br: X = NH (3.09 g, 90.14%)

Thus, the reaction of **9a** (2.00 g, 10.40 mmol), **9c** (2.26 g, 10.00 mmol) or **9e** (2.71 g, 10.00 mmol) with either **10a** (1.13 g, 10.40 mmol) or **13** (1.08 g, 10.00 mmol) in diethylene glycol under MW irradiation at 150 °C for 7-9 min furnished the target compounds, **11a** (2.49 g, 90.41%); **14c** (2.60 g, 87.32%) and **14e** (3.09 g, 90.14%), respectively (Scheme 3).

We have further demonstrated practicality of our developed methodology for the synthesis of anticancer indole alkaloid, Cephalandole A, which was isolated from Taiwanese orchid *Cephalanceropsis gracilis* (Orchidaceae). It showed good activity against CNS (SF-268; IC_{50} = 12.2 µM), breast (MCF-7; IC_{50} = 7.57 µM) and lung (NCI-H460; IC_{50} = 7.8 µM) carcinoma cell lines.[24] 3-indoleglyoxylic acid **15** on reaction with aminophenol **10a** in diethylene glycol under MW at 150 °C for 10 min furnished indole alkaloid Cephalandole A **16** in 89% yield (Scheme-4). The spectral data was found to be the same as the literature data.

Scheme 4. Synthesis of Cephalandole A **16.**



2.1.3 Material and methods

2.1.3.1 *In vitro* antioxidant DPPH radical scavenging activity[24a-e]

In DPPH radical scavenging method the synthesized compound at different concentrations ranging from 10 to 100 µg mL^{-1} was mixed with 1.5 mL of a DPPH methanolic solution (20 mg L^{-1}). Pure methanol was taken as control and ascorbic acid (vitamin C), was used as a reference compound. The percent of DPPH decoloration of the sample was calculated according to the formula.

Decoloration % = [1 − (Abs sample / Abs control)] × 100

The decoloration was plotted against the sample concentration and a logarithmic regression curve was established in order to calculate the IC_{50}. The results are expressed as antiradical efficiency (AE), which is 1000-fold inverse of the IC_{50} value AE=1000/ IC_{50}.

2.1.3.2 *In vitro* Ferric Reducing Antioxidant Power (FRAP) Assay[24f]

The FRAP reagent was prepared by mixing freshly prepared 10.0 mM of ferric-tripyridyltriazine (TPTZ) solution, 20.0 mM $FeCl_3.6H_2O$ solution and 300 mM

sodium acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). Sample was added to 3 mL of FRAP reagent and this reaction mixture was incubated for 30 min at 37 °C temperature. The absorbance of prepared reaction mixture was measured at 593 nm. A freshly prepared solution of $FeSO_4$ was used for calibration of standard curve. The FRAP antioxidant capacities were expressed in terms of $C_{0.5}$FRAP (the concentration of samples with respect to the antioxidant ability equivalent to that of $FeSO_4$ at 0.5 mmol/L).

2.1.4 Antioxidant Activity: *In vitro* antioxidant DPPH radical scavenging assay, FRAP assay and structure-activity relationship[25]

The DPPH radical scavenging assay is generally utilised for the screening of antioxidant activity of diverse heterocycles.[25a] DPPH is a stable free radical, which can easily accept an electron or a hydrogen radical to become a stable molecule. Literature reports illustrate that DPPH assay works in two ways; a single electron transfer (SET) or a hydrogen atom transfer (HAT) mechanism.[25b] DPPH in the methanolic medium has odd electron configuration which shows a strong absorption band at 515 nm and the absorbance decreases in the presence of free radical scavengers which results in the colour change from deep purple to yellow.[25c-d] The radical quenching ability strongly depends on the structural accessibility of the radical trapping site. The electron density as well as steric hindrance plays a vital role in the antioxidant activity because they may prevent the test molecule from reaching to the DPPH radical site and thus results in lower activity.[25e]

The FRAP assay was measured using the method as described by Benzie and Strain.[25f] This methodology demonstrates that the antioxidant molecule reacts to a complex of ferric tripyridyltriazine [Fe^{3+}-TPTZ] and produces a colored ferrous tripyridyltriazine [Fe^{2+}-TPTZ] complex. Generally, the reducing nature of antioxidant molecule is associated with their action by breaking the free radical chain via donating a hydrogen atom. All the synthesized 2-oxobenzo[1,4]oxazines **11a-n,** 2-oxoquino[1,4]oxalines **14a-h** and Cephalandole A **16** were screened for their *in vitro* antioxidant activities using DPPH radical scavenging activity assay using ascorbic acid as standard reference as well as in FRAP assay (Table 2).[25,26]

The *in vitro* antioxidant screening of compounds **11a-f,** having no substitution at benzoxazine aromatic ring; it had been observed that the compound **11a** was found to be the most active compound having IC_{50} value of 10.20 ± 0.08 µg/mL (entry 1) in

comparison with standard reference ascorbic acid (IC_{50} = 4.57 µg/mL). When mono-halo substituents were present at side chain of aromatic ring as in case of **11c**, **11e** and **11f** having −Cl,−F and −Br substituent respectively; these molecules exhibited slightly lesser antioxidant activity in comparison with **11a** (Table 2; entry 3, 5 and 6). Further, 2-oxobenzo[1,4]oxazine having di-halo substituents on side chain of aromatic ring (**11d**) exhibited decrease of antioxidant activity drastically (Table 2; entry 4) in comparison to mono-halo substituted analogues **11c**, **11e** and **11f**. In addition, electron-donating substituents at side chain of aromatic ring in compound **11b** showed better antioxidant activity in comparison to halo-substituted 2-oxobenzo[1,4]oxazine analogues **11c-11f**.

Table 2. Antioxidant activity of synthesized compounds **11a-n, 14a-h** and **16** by DPPH radical scavenging and FRAP assay.[25]

S. No.	Compound No.	Antioxidant activity[a]	
		DPPH assay (IC_{50})(µg/mL)	FRAP assay ($C_{0.5FRAP}$ µM)
1	**11a**	10.20 ± 0.08	611.5 ± 23.2
2	**11b**	19.70 ± 0.31	763.2 ± 38.1
3	**11c**	29.80 ± 0.17	306.8 ± 25.8
4	**11d**	65.32 ± 0.97	>1000
5	**11e**	25.02 ± 0.21	421.7 ± 37.9
6	**11f**	23.45 ± 0.14	845.9 ± 35.1
7	**11g**	67.40 ± 0.28	>1000
8	**11h**	78.50 ± 1.41	921.6 ± 29.6
9	**11i**	34.42 ± 0.62	291.7 ± 23.1
10	**11j**	42.98 ± 0.76	>1000
11	**11k**	21.27 ± 0.11	489.2 ± 18.5
12	**11l**	56.12 ± 1.03	348.8 ± 31.4
13	**11m**	15.70± 0.14	598.5 ± 23.4
14	**11n**	78.76 ± 1.43	>1000
15	**14a**	27.36 ± 0.44	638.4 ± 37.6
16	**14b**	91.36 ± 2.04	>1000
17	**14c**	9.89 ± 0.15	612.8 ± 17.8
18	**14d**	28.24 ± 0.46	498.4 ± 22.4
19	**14e**	8.97 ± 0.13	689.3 ± 30.0
20	**14f**	43.54 ± 0.88	592.7 ± 41.6
21	**14g**	38.97 ± 0.97	>1000
22	**14h**	14.27 ± 0.23	358.3 ± 17.7
23	**16**	11.87± 0.14	ND[b]
24	**Ascorbic acid**	4.57	---
25	**BHT**	---	546.0 ± 13.6

[a]Results are expressed as a mean ± standard deviation (n = 3). DPPH radical scavenging activities are expressed as IC_{50} concentrations of the compounds (µg/mL) required to inhibit 50 % of the radicals and the maximum inhibition values; [b]ND means not done.

Moreover, in 2-oxobenzo[1,4]oxazines having $-CH_3$ or $-NO_2$ substituent at para-position of benzoxazine aromatic nucleus (**11g-11n**); the antioxidant activity was found to be lesser (Table 2; entry 7-14) in comparison with unsubstituted analogues

11a-11f, except compound **11k** and **11m**; which exhibited better activity profile (Table 2; entry 11 and 13). Whereas, 2-oxobenzo[1,4]oxazine having di-chloro substituents at side chain of aromatic ring (Table 2; entry 14); **11n** was found to be the least active compound among 2-oxobenzo[1,4]oxazine series. Furthermore, the $-CH_3$ substituent at benzoxazine nucleus along with electron-donating methyl substituent or halo-substituent at side chain of aromatic ring as in compounds **11g-j**; these were found to show moderate to poor antioxidant activities (Table 2; entry 7-10) in comparison to other analogues of the series.

In the case of 2-oxoquino[1,4]oxalines **14a-h** derivatives, compounds **14c** and **14e** were found to be the best compounds of this series and have shown the antioxidant activities having the IC_{50} value of 9.89 ± 0.15 μg/mL and 8.97 ± 0.13 μg/mL, respectively, in comparison to ascorbic acid (Table 2; entry 17 and 19). The 2-oxoquino[1,4]oxalines **14f** having fluoro substituent showed lesser antioxidant activity profile (IC_{50} value of 43.54 ± 0.88 μg/mL). It can be speculated that due to larger electronegativity of fluorine atom, which accumulates the electron density, restricts the delocalization of bonds due to which, the free electrons of **14f** are not easily available for quenching of DPPH radical. Furthermore, extending the side chain of phenyl ring to more electron rich naphthyl ring in **14h (**IC_{50} = 14.27 ± 0.23 μg/mL) showed promising activity. In addition, the un-substituted 2-oxoquino[1,4]oxaline **14a** and dichloro-substituted side chain of aromatic ring having 2-oxo-quino[1,4]oxaline **14d** showed lesser activity (Table 2; entry 15 and 18) in comparison with **14c** and **14e**. The electron-donating substituents at side chain of aromatic ring (compound **14b** and **14g**) showed poor activity profile (Table 2: entry 16 and 21) in comparison with their corresponding halo-substituted analogues **14c** and **14e**.

All the synthesized compounds were also assessed in the ferric to ferrous reduction assay (FRAP assay) taking BHT as standard reference. (Table 2) In the present study, the trend for ferric ion reducing activities of all the compounds **11a-n** and **14a-h**, with respect to standard reference BHT indicates that the seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**) were found more potent antioxidant than BHT. The compound which have the mono-halo (such as: F, Cl, Br) substituent at the side chain of aromatic ring in 2-oxobenzo[1,4]oxazin (compound **11c**, **11e**, **11i**, **11k** and **11l**) exhibited higher antioxidants activity than BHT, whereas 2-oxoquino[1,4]oxaline **14d** and **14h**, which have 2,4-dichloro substituent at side chain of aromatic ring or

naphthyl substituent displayed better antioxidant activity than standard reference BHT. Compounds **11a**, **11m**, **14c** and **14f** showed comparable FRAP antioxidant activity than standard reference BHT. Rest of the compounds showed moderate to low FRAP antioxidant activity. Moreover, for the first time the antioxidant activity of Cephalandole A **16** was also evaluated and found to possess moderate antioxidant activity having IC_{50} value of 11.87± 0.14 µg/mL in comparison to ascorbic acid (Table 2; entry 23) in DPPH radical scavenging assay.

These results showed that the mono-halo substitution at side chain of aromatic ring in nitrogen congener of 2-oxobenzo[1,4]oxazines i.e. 2-oxoquino[1,4]oxalines **14c** and **14e** along with un-substituted 2-oxobenzo[1,4]oxazine **11a** were found to be the most active compounds of the series showing promising antioxidant activities in DPPH radical scavenging. Furthermore, in the FRAP antioxidant assay, seven compounds (**11c**, **11e**, **11i**, **11k**, **11l, 14d** and **14h**), which have mono-halo substitution at side chain of aromatic ring in 2-oxobenzo[1,4]oxazine (**11c**, **11e**, **11i** and **11k**, **11l)** and dihalo substituent as well as naphthyl substituent at 2-oxoquino[1,4]oxalines (**14d** and **14h**), showed higher antioxidant activity in comparison with BHT, respectively.

2.1.5 Cell toxicity study

Compounds **11a**, **14c**, **14e** and **14h** (which displayed good antioxidant activity in DPPH radical scavenging assay) were also assessed for their cytotoxic studies using MTT assay taking 25-250 µg/mL concentration in $3T_3$ fibroblast cell lines.[27] The screening results showed that these compounds were found non-toxic even at 250 µg/mL and displays allowable values of cell viability. (Figure 3)

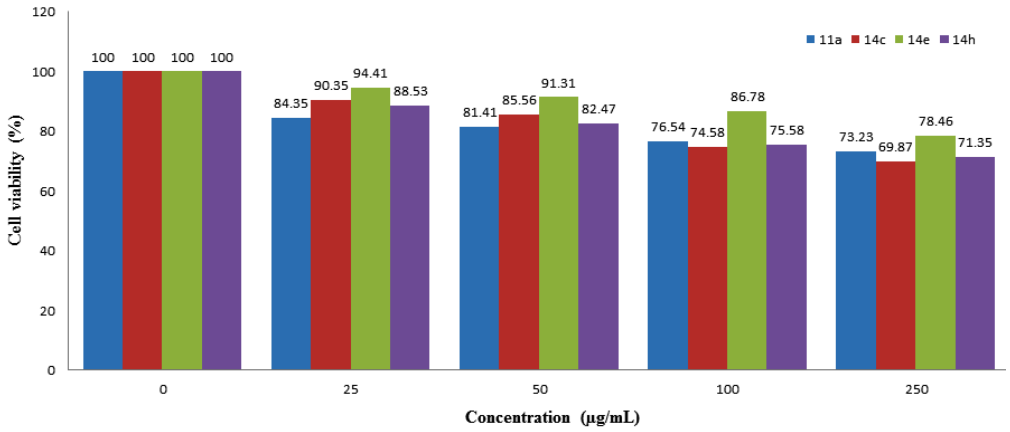


Figure 4. Percentage cell viability test.

2.1.6 Conclusions

In summary, we have developed a simple and highly efficient MW-assisted protocol for the synthesis of functionalized 2-oxobenzo[1,4]oxazines **11a-n** and 2-oxoquino[1,4]oxalines **14a-h** in excellent yields. This reaction tolerates a broad range of substrates, and provides a straightforward access to functionalized 2-oxobenzo[1,4]oxazines and 2-oxoquino[1,4]oxalines. The practical applicability of developed methodology was confirmed by the gram scale synthesis of **11a**, **14c** and **14e,** along with the synthesis of Cephalandole A **16** (89% yield). All the synthesized compounds were screened for their *in vitro* antioxidant activities using DPPH radical scavenging and FRAP assays. Compounds **11a, 14c** and **14e**, 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 as compared to standard reference ascorbic acid (IC_{50} = 4.57 μg/mL) in DPPH assay, whereas in FRAP assay, seven compounds (**11c**, **11e**, **11i**, **11k**, **11l**, **14d** and **14h**) exhibited higher antioxidant activity in comparison with BHT. Cytotoxic studies revealed that the non-toxic nature of compounds **11a**, **14c**, **14e** and **14h** even at 250 μg/mL concentration. To the best of our knowledge, this is the first report of microwave-assisted synthesis and *in vitro* antioxidant activities of functionalized 2-oxobenzo[1,4]oxazines **11a-n**; and 2-oxoquino[1,4]oxalines **14a-h** and Cephalandole A **16** in excellent yields. The potential *in vitro* antioxidant activity combined with ease of preparation qualifies these compounds as candidates for further lead optimization studies.

2.1.7 Experimental Details & Characterization Data

2.1.7.1 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.

2.1.7.2 General Procedure for the Synthesis of (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11a) in optimization study as given in table 1:

(1) Method A (conventional heating condition)

A solution of **9a** (19.2 mg, 0.10 mmol) and **10a** (10.9 mg, 0.10 mmol) in given solvent (1.0 mL) was heated at given time and temperature (as shown in Table 1). The progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired 2-oxobenzo[1,4]oxazine **11a** having good yields (18-67%).

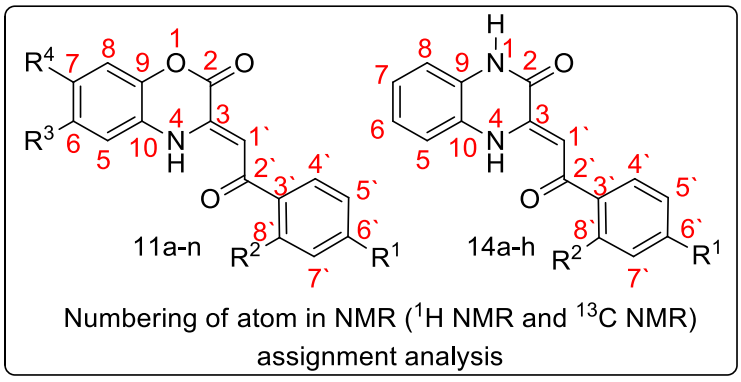
(2) Method B (microwave irradiation condition)

To a solution of **9a** (19.2 mg, 0.10 mmol) in given solvent (1.0 mL) was added **10a** (10.9 mg, 0.10 mmol), and the reaction mixture was irradiated under microwave at given temperature and time (as shown in Table 1). The progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired 2-oxobenzo[1,4]oxazine **11a** product having good yields (51-94%).

2.1.7.3 General Procedure for the synthesis of functionalized (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones (11a-n) and (*Z*)-3-(2-oxophenylethylidene)-3,4-dihydroquinoxalin-2(1*H*)-ones (14a-h) as given in scheme 1 and scheme 2:

To a solution of compound **9a-f** (0.20 mmol; 1 eq., as given in Scheme 1) or **9a-g** (0.20 mmol; 1 eq. as given in Scheme 2) in diethylene glycol (2.0 mL) was added compound **10a-c (**0.20 mmol; 1 eq., as given in Scheme 1**)** or **12** and **13 (**0.20 mmol; 1 eq., as given in Scheme 2**)** and the reaction mixture was irradiated under microwave at 150˚C temperature for about 5-10 min depending upon the substrate utilized. The progress of the reaction was monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography method over silica gel using 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones **11a-n** and (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1*H*)-ones **14a-h** having good yields (78-97%).

2.1.7.4 Characterization data of (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-ones, (*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro quinoxalin-2(1*H*)-ones (11a-n and 14 a-h) and Cephalandole A



Numbering of atom in NMR (^{1}H NMR and ^{13}C NMR) assignment analysis

(*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11a)[17a-c]

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 185-186 °C; FT-IR (KBr, vmax/cm^{-1}) 3434, 1754, 1614, 1594, 1270; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 7.4 Hz, 2H, C4`H, C8`H), 7.55 – 7.46 (m, 3H, C1`H, C5`H, C7`H), 7.21 – 7.05 (m, 5H, C5H, C6H, C7H, C8H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 191.6 (>C=O), 156.3 (O=C-O-), 141.3 (C9), 139.1 (C3`), 138.3 (C6`), 132.8 (C3), 128.8 (C10), 127.7 (C4`,

C8`), 126.0 (C5`, C7`), 124.0 (C6), 123.8 (C7), 117.2 (C5), 116.0 (C8), 94.7 (-C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}NO_3$ $[M+H]^+$: 266.0739; found 266.0734.

(*Z*)-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11b)[17b,d]

Yellow solid; yield: 95%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 160-162 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 2925, 1759, 1622, 1110; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 7.6 Hz, 2H, C4`H, C8`H), 7.27 (m, 2H, C5`H, C7`H), 7.20-7.16 (m, 2H, C5H, C8H), 7.10-7.06 (m, 2H, C6H, C7H), 7.03 (d, J = 1.2 Hz, 1H, C1`H), 2.41 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 191.4 (>C=O), 156.5 (O=C-O-), 143.6 (C6`), 141.2 (C9), 138.8 (C3`), 135.7 (C3), 129.5 (C10), 127.9 (C4`, 8`), 125.9 (C5`, C7`), 123.9 (C6), 123.8 (C7), 117.2 (C5), 115.9 (C8), 94.8 (-C=C-), 21.8 (-CH_3); HRMS (ESI) calcd. for $C_{17}H_{13}NO_3$ $[M+H]^+$: 280.0895; found 280.0899.

(*Z*)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11c)[17a,d]

Yellow solid; yield: 90%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 155-157 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 1759, 1633, 1585, 752; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 8.4 Hz, 2H, C4`H, C8`H), 7.44 (d, J = 8.4 Hz, 2H, C5`H, C7`H), 7.22 – 7.09 (m, 4H, C5H, C6H, C7H, C8H), 6.98 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.1 (>C=O), 156.1 (O=C-O-), 141.4 (C6`), 139.4 (C9), 139.1 (C3`), 136.6 (C3), 129.1 (C10), 129.0 (C4`, C8`), 126.0 (C5`, C7`), 124.3 (C6), 123.6 (C7), 117.3 (C5), 116.1 (C8), 94.2 (-C=C-); HRMS (ESI) calcd. for $C_{16}H_{10}ClNO_3$ $[M+2H]^+$: 301.0349; found 301.0345.

(*Z*)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11d)

Yellow solid; yield: 97%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 160-162 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 1758, 1620, 1577, 1101; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.85 (s, 1H, -NH-), 7.53 (d, J = 7.6 Hz, 1H, C4`H), 7.46 (s, 1H, C8`H), 7.32 (d, J = 8.0 Hz, 1H, C5`H), 7.20 – 7.12 (m, 5H, C6H, C7H, C8H, C7`H), 6.76 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, $CDCl_3$) 191.1 (>C=O), 155.1 (O=C-O-), 140.8 (C9), 138.4 (C6`), 136.8 (C3`), 136.6 (C4`), 131.8 (C10), 130.0 (C3), 129.9 (C8`), 126.7 (C5`), 125.4 (C7`), 123.9 (C6), 122.7 (C7), 116.6 (C5), 115.6 (C8), 97.5 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_9Cl_2NO_3$ $[M+2H]^+$: 334.9959; found 334.9953.

(*Z*)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11e)[17e,i]

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 152-154 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 2925, 1757, 1622, 1596, 1156; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J = 5.6, 8.8 Hz, 2H, C4`H, C8`H), 7.22 – 7.11 (m, 6H, C5H, C6H, C7H, C8H, C5`H, C7`H), 7.00 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.1(>C=O), 166.9 (C6`), 156.2 (O=C-O-), 141.3 (C9), 139.2 (C3`), 134.6 (C3), 130.3 (C10), 125.9 (C4`, C8`), 124.6 (C6), 123.7 (C7), 117.3 (C5), 116.0 (C5`, C7`), 115.8 (C8), 94.3 (C1`:-C=C-) ; HRMS (ESI) calcd. for $C_{16}H_{10}FNO_3$ $[M+H]^+$: 284.0645; found 284.0649.

(*Z*)-3-(2-(4-bromophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11f)[17i]

Yellow solid; yield: 93%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 200-202 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 1754, 1624, 1585, 1277, 1111; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.0 Hz, 2H, C4`H, C8`H), 7.60 (d, J = 8.0 Hz, 2H, C5`H, C7`H), 7.24 – 7.09 (m, 4H, C5H, C6H, C7H, C8H), 6.97 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.3 (>C=O), 156.1 (O=C-O-), 141.4 (C9), 139.4 (C3`), 137.1 (C3), 132.1 (C5`, C7`), 129.2 (C10), 127.8 (C4`, C8`), 126.0 (C6`), 124.3 (C6), 123.6 (C7), 117.3 (C5), 116.1 (C8), 94.2 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{10}BrNO_3$ $[M+2H]^+$: 344.9844; found 344.9849.

(*Z*)-6-methyl-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11g)[17c]

Yellow solid; yield: 88%, R_f (EtOAc/hexane; 20:80) = 0.90; m.p. 157-158 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 1750, 1618, 1572, 1123, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00-7.98 (m, 2H, C4`H, C8`H), 7.56-7.45 (m, 3H, C1`H, C5`H, C7`H), 7.07 – 7.02 (m, 2H, C8H, C6`H), 6.88 (d, J = 8.5 Hz, 2H, C5H, C7H), 2.34 (s, 3H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5 (>C=O), 156.5 (O=C-O-), 139.4 (C9), 139.2 (C3`), 138.4 (C6), 136.1 (C3), 132.7 (C6`), 128.8 (C4`, C8`), 127.7 (C5`, C7`), 124.8 (C7), 123.4 (C10), 116.8 (C5), 116.2 (C8), 94.5 (C1`: -C=C-), 21.08 (-CH_3); HRMS (ESI) calcd. for $C_{17}H_{13}NO_3$ $[M+H]^+$: 280.0895; found 280.0899.

(*Z*)-6-methyl-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11h)[17e]

Yellow solid; yield: 89%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 162-164 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 1762, 1602, 1313, 1047; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.1 Hz, 2H, C4`H, C8`H), 7.27 (d, J = 8.0 Hz, 2H, C5`H, C7`H), 7.06 (d, J = 8.1 Hz, 1H, C8H), 7.02 (s, 1H, C1`H), 6.88 (d, J = 9.4 Hz, 2H, C5H, C7H), 2.42 (s, 3H, C6`: -CH_3), 2.35 (s, 3H, C6: -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3 (>C=O), 156.6 (O=C-O-), 143.5 (C6`), 139.3 (C9), 139.0 (C3`), 136.0 (C6), 135.8 (C3), 129.5 (C5`, C7`), 127.8 (C4`, C8`), 124.6 (C7), 123.5 (C10), 116.8 (C5), 116.1 (C8), 94.6 (C1`: -C=C-), 21.7 (C6: -$CH_3$), 21.0 (C6`: -CH_3); HRMS (ESI) calcd. for $C_{18}H_{15}NO_3$ $[M+H]^+$: 294.1052; found 294.1055.

(*Z*)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11i)[17h]

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 145-147 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 1767, 1629, 1582; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94-7.92 (m, 2H, C4`H, C8`H), 7.46-7.43 (m, 2H, C5`H, C7`H), 7.08 (d, J = 9.2 Hz, 1H, C8H), 6.97 (s, 1H, C1`H), 6.92-6.90 (m, 2H, C5H, C7H), 2.35 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.1 (>C=O), 156.3 (O=C-O-), 139.5 (C9), 139.4 (C6`), 139.0 (C3`), 136.7 (C6), 136.1 (C3), 129.1 (C4`, C8`), 129.0 (C5`, C7`), 125.0 (C7), 123.2 (C10), 116.9 (C5), 116.3 (C8), 94.1 (C1`: -C=C-), 21.1 (-CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}ClNO_3$ $[M+2H]^+$: 315.0506; found 315.0509.

(*Z*)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11j)

Yellow solid; yield: 96%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 179-181 °C; FT-IR (KBr, νmax/cm-1) 3435, 2923, 1763, 1624, 1543, 1052; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.84 (m, 2H, C4`H, C8`H), 7.63 – 7.59 (m, 2H, C5`H, C7`H), 7.08 (d, J = 9.2 Hz, 1H, C8H), 6.96 (s, 1H, C1`H), 6.92 – 6.90 (m, 2H, C5H, C7H), 2.35 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.2 (>C=O), 156.3 (O=C-O-), 139.5 (C9), 139.4 (C6`), 137.1 (C3`), 136.2 (C6), 132.0 (C3), 129.2 (C5`, C7`), 127.7 (C4`, C8`), 125.1 (C7), 123.2 (C10), 117.0 (C5), 116.3 (C8), 94.0 (C1`: -C=C-), 21.1 (-CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}BrNO_3$ $[M+H]^+$: 358.0001; found 358.0007.

(*Z*)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11k)

Yellow solid; yield: 83%, R_f (EtOAc/hexane; 20:80) = 0.70; m.p. 195-197 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2925, 1759, 1525, 1023; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03

– 7.97 (m, 2H, C4`H, C8`H), 7.89 – 7.86 (m, 2H, C5`H, C7`H), 7.66 – 7.63 (m, 2H, C5H, C7H), 7.32 (d, J = 9.2 Hz, 1H, C8H), 7.07 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.8 (>C=O), 154.9 (O=C-O-), 145.3 (C6), 145.0 (C9), 138.0 (C3`), 136.5 (C3), 132.3 (C5`, C7`), 129.4 (C4`, C8`), 128.6 (C6`), 124.5 (C10), 119.2 (C7), 118.0 (C5), 111.6 (C8), 96.4 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_9BrN_2O_5$ $[M+2H]^+$: 389.9695; found 389.9699.

(*Z*)-6-nitro-3-(2-oxo-2-(p-tolyl) ethylidene)-3, 4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (11l)

Yellow solid; yield: 81%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 220-223 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 1760, 1624, 1524, 1109; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, *J* = 2.4 Hz, 1H, C4`H), 7.96-7.92 (m, 3H, C5`H, C7`H, C8`H), 7.46- 7.37 (m, 3H, C5H, C7H, C8H), 6.95 (s, 1H, C1`H), 2.40 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) 189.9 (>C=O), 155.7 (O=C-O-), 145.9 (C6), 143.8 (C9), 139.2 (C6`), 138.5 (C3`), 135.2 (C3), 130.1 (C4`, C8`), 128.1 (C5`, C7`), 119.5 (C10), 118.9 (C7), 117.7 (C5), 112.6 (C8), 94.8 (C1`: -C=C-), 21.7 (-CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_5$ $[M+H]^+$: 325.0746; found 325.0741.

(*Z*)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (11m)

Yellow solid; yield: 78%, R_f (EtOAc/hexane; 20:80) = 0.70; m.p. 239-240 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2924,1622, 1525, 1272; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (s, 1H, C5H), 8.03 (d, *J* = 7.2 Hz, 2H, C4`H, C8`H), 7.91 (d, *J* = 9.1 Hz, 1H, C7H), 7.59 (d, *J* = 7.2 Hz, 2H, C5`H, C7`H), 7.42 (d, *J* = 9.0 Hz, 1H, C8H), 6.90 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 188.9 (>C=O), 156.0 (O=C-O-), 146.0 (C6), 144.7 (C9), 139.8 (C6`), 138.2 (C3`), 137.1 (C3), 129.9 (C4`, C8`), 129.6 (C5`, C7`), 125.8 (C10), 119.0 (C7), 117.8 (C5), 113.1 (C8), 94.4 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_9ClN_2O_5$ $[M+2H]^+$: 346.0200; found 346.0204.

(*Z*)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (11n)

Yellow solid; yield: 80%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 185-187 °C; FT-IR (KBr, νmax/cm^{-1}) 3588, 2930, 1769, 1585, 1685, 1108; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.99 (m, 2H, C7H, C8`H), 7.55(d, J = 8.4 Hz, 1H, C5`H, C7`H), 7.48 (d, J = 2.0 Hz, 1H, C7`H), 7.37 – 7.33 (m, 2H, C5H, C8H), 6.89 (s, 1H, C1`H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4 (>C=O), 154.5 (O=C-O-), 145.2 (C6), 145.1 (C9), 138.0

(C6`), 137.6 (C3`), 136.9 (C4`), 132.7 (C3), 131.0 (C8`), 130.8 (C5`), 127.6 (C7`), 124.2 (C10), 119.4 (C7), 118.1 (C5), 111.7 (C8), 100.4 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_8Cl_2N_2O_5$ $[M+2H]^+$: 379.9810; found 379.9815.

(*Z*)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14a)[17a,b]

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 268-269 °C; FT-IR (KBr, νmax/cm^{-1}) 3060, 1688, 1619; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.0 (s, 1H, -NH-), 8.07-8.05 (m, 2H, C4`H, C8`H), 7.55 – 7.48 (m, 3H, C5`H, C6`H, C7`H), 7.21 – 7.12 (m, 4H, C5H, C6H, C7H, C8H); 7.03 (s, 1H, C1`H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.9 (>C=O), 155.2 (-NH-C=O), 145.4 (C3), 138.4 (C3`), 131.2 (C6`), 128.2 (C10), 126.5 (C5`, C7`), 123.9 (C4`, C8`), 123.6 (C9), 123.1 (C6), 116.1 (C7), 115.1 (C5), 114.9 (C8), 89.0 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{12}N_2O_2$ $[M+H]^+$: 265.0899; found 265.0893.

(*Z*)-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14b)[17k]

Yellow solid; yield: 96%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 221-222 °C, FT-IR (KBr, νmax/cm^{-1}) 3045, 1677, 1615; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (s, 1H, -NH-), 7.96 (d, J = 8.0 Hz, 2H, C4`H, C8`H), 7.31 – 7.29 (m, 2H, C5`H, C7`H), 7.21 – 7.12 (m, 4H, C5H, C6H, C7H. C8H); 7.01 (s, 1H, C1`H), 2.43 (s, 3H, -$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 190.5 (>C=O), 158.0 (-NH-C=O), 144.6 (C6`), 142.8 (C3), 136.3 (C3`), 130.3 (C10), 129.4 (C5`,C7`), 127.7 (C4`, C8`), 125.6 (C9), 124.9 (C6), 123.9 (C7), 116.2 (C5), 115.9 (C8), 90.9 (C1`: -C=C-), 21.7 (C6`: -CH_3); HRMS (ESI) calcd. for $C_{17}H_{14}N_2O_2$ $[M+H]^+$: 279.1055; found 279.1059.

(*Z*)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-quinoxalin-2(1*H*)-one (14c)[17a,k]

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 267-268 °C, FT-IR (KBr, νmax/cm^{-1}) 3052, 1686, 1614; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.8 (s, 1H, -NH-), 7.98 (d, J = 8.8 Hz, 2H, C4`, C8`), 7.57 – 7.44 (m, 3H, C5`H, C7`H, C8H), 7.19 – 7.14 (m, 3H, C5H, C6H, C7H); 6.80 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 187.5 (>C=O), 156.1 (-NH-C=O), 146.7 (C3), 138.1 (C6`), 137.3 (C3`), 129.3 (C10), 127.5 (C4`, C8`), 124.8 (C5`,C7`), 124.2 (C9), 124.1 (C6), 117.3 (C7), 117.2 (C5), 115.9 (C8), 89.9 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}ClN_2O_2$ $[M+2H]^+$: 300.0509; found 300.0503.

(*Z*)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14d)[17l]

Yellow solid; yield: 95%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 260-262 °C, FT-IR (KBr, νmax/cm^{-1}) 3054, 1682, 1618; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, 1H, -NH-), 7.63-7.49 (m, 4H, C4`H, C5`H, C7`H, C8H), 7.19 – 7.15 (m, 3H, C5H, C6H, C7H), 6.44 (s, 1H, C1`H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.8 (>C=O), 154.8 (-NH-C=O), 145.2 (C6`), 138.2 (C3), 134.7 (C3`), 130.7 (C8`), 130.3 (C4`), 129.2 (C10), 127.1 (C5`), 126.6 (C9), 123.8 (C7`), 123.5 (C6), 123.1 (C7), 116.3 (C5), 114.9 (C8), 92.8 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{10}Cl_2N_2O_2$ $[M+2H]^+$: 334.0119; found 334.0113.

(*Z*)-3-(2-(4-bromophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14e)[17g]

Yellow solid; yield: 93%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 281-282 °C, FT-IR (KBr, νmax/cm^{-1}) 3044, 1678, 1606; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H, -NH-), 7.92-7.89 (m, 2H, C4`H, C8`H), 7.71 – 7.69 (m, 2H, C5`H, C7`H), 7.44 (s, 1H, C8H), 7.18 – 7.14 (m, 3H, C5H, C6H. C7H); 6.79 (s, 1H, C1`H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 186.5 (>C=O), 155.1 (-NH-C=O), 145.7 (C3), 137.5 (C3`), 131.2 (C5`, C7`), 128.5 (C10), 126.5 (C4`, C8`), 125.1 (C6`), 123.8 (C9), 123.1 (C6), 116.2 (C7), 115.1 (C5), 114.9 (C8), 88.8 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}BrN_2O_2$ $[M+2H]^+$: 344.0004; found 344.0009.

(*Z*)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14f)[17g]

Yellow solid; yield: 91%, R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 252-253 °C, FT-IR (KBr, νmax/cm^{-1}) 3053, 1680, 1614; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.0 (s, 1H, -NH-), 8.02-8.00 (m, 2H, C4`H, C8`H), 7.48 (s, 1H, C8H), 7.33 – 7.28 (m, 2H, C5`H. C7`H), 7.12 – 7.11 (m, 3H, C5H, C6H, C7H); 6.76 (s, 1H, C1`H);$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 187.1 (>C=O), 164.4 (C6`), 155.7 (-NH-C=O), 145.7 (C3), 135.3 (C3`), 129.9 (C4`, 8`), 129.8 (C10), 126.7 (C9), 124.1 (C6), 123.7 (C7), 116.6 (C5), 113.8 (C8), 115.4 (C5`,C7`), 88.9 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{16}H_{11}FN_2O_2$ $[M+H]^+$: 283.0805; found 283.0809.

(*Z*)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14g)[16e]

Yellow solid; yield: 90%, R_f (EtOAc/hexane; 20:80) = 0.75; m.p. 241-242 °C, FT-IR (KBr, νmax/cm^{-1}) 3058, 1689, 1618; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.95 (s, 1H, -NH-), 7.95 (d, J = 9.2 Hz, 2H, C4`H, C8`H), 7.45 – 7.44 (m, 1H, C8H), 7.14 – 7.03

(m, 5H, C5H, C6H, C7H, C5`H, C7`H); 6.77 (s, 1H, C1`H), 3.83 (s, 3H, -$OCH_3$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 187.7 (>C=O), 162.3 (C6`), 155.9 (-NH-C=O), 144.9 (C3), 131.3 (C4`,C8`, C10), 129.2 (C3`), 126.5 (C9), 124.3 (C6), 123.7 (C7), 116.3 (C5), 115.3 (C8), 113.9 (C5`, C7`), 88.9 (C1`: -C=C-), 55.4 (-OCH_3); HRMS (ESI) calcd. for $C_{17}H_{14}N_2O_3$ $[M+H]^+$: 295.1004; found 295.1009.

(*Z*)-3-(2-(naphthalen-2-yl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (14h)[17m]

Yellow solid; yield: 94%, R_f (EtOAc/hexane; 20:80) = 0.85; m.p. 263-264 °C, FT-IR (KBr, νmax/cm^{-1}) 3093, 1694, 1614; ^1H NMR (400 MHz, DMSO-d_6) δ 11.67 (s, 1H, -NH-), 8.17-8.05 (m, 2H, C4`H, C9`H), 7.81 – 7.72 (m, 3H, C5`H, C6`H, C8`H), 7.52 – 7.39 (m, 4H, C6H, C8H, C7`H, C10`H); 7.25 – 7.14 (m, 2H, C5H, C7H), 6.89 (s, 1H, C1`H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.3 (>C=O), 156.3 (-NH-C=O), 146.5 (C3), 144.1 (C3`), 139.9 (C12`), 138.3 (C11`), 129.6 (C9`), 129.4 (C10), 128.5 (C4`), 128.4 (C5`), 127.5 (C9), 127.3 (C8`), 125.3 (C7`), 125.1 (C6`), 124.7 (C6), 124.4 (C7), 117.1 (C10`), 116.3 (C5), 115.9 (C8), 90.1 (C1`: -C=C-); HRMS (ESI) calcd. for $C_{20}H_{14}N_2O_2$ $[M+H]^+$: 315.1055; found 315.1059.

Synthesis of Cephalandole A 16: To a solution of 3-Indoleglyoxylic acid **15** (226.9 mg, 1.20 mmol) in diethylene glycol was added **10a** (130.8 mg, 1.20 mmol) and the reaction mixture was irradiated under MW at 150 ˚C temperature for 10 min and the progress of reaction was monitored by TLC. After that, the reaction mixture was extracted with ethyl acetate (3 × 50 mL) and distilled water. The organic layer was combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude product was further purified by flash column chromatography method over silica gel using hexane/ethyl acetate (8:2; v/v) as an eluent which afforded the pure desired Cephalandole A **16** having good yield (280.5 mg, 89%). Yellowish solid; m.p. 232-233 °C; ^1H NMR[24c] (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.76 – 8.74 (m, 1H), 8.69 (s, 1H), 7.85 (d, J = 6.4 Hz, 1H), 7.54 – 7.39 (m, 4H), 7.27 – 7.25 (m, 2H); HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_2$ $[M+H]^+$: 263.0742; ; found 263.0749.

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2.1.9 Characterization spectral data (^{1}HNMR and ^{13}CNMR) of selected compounds:

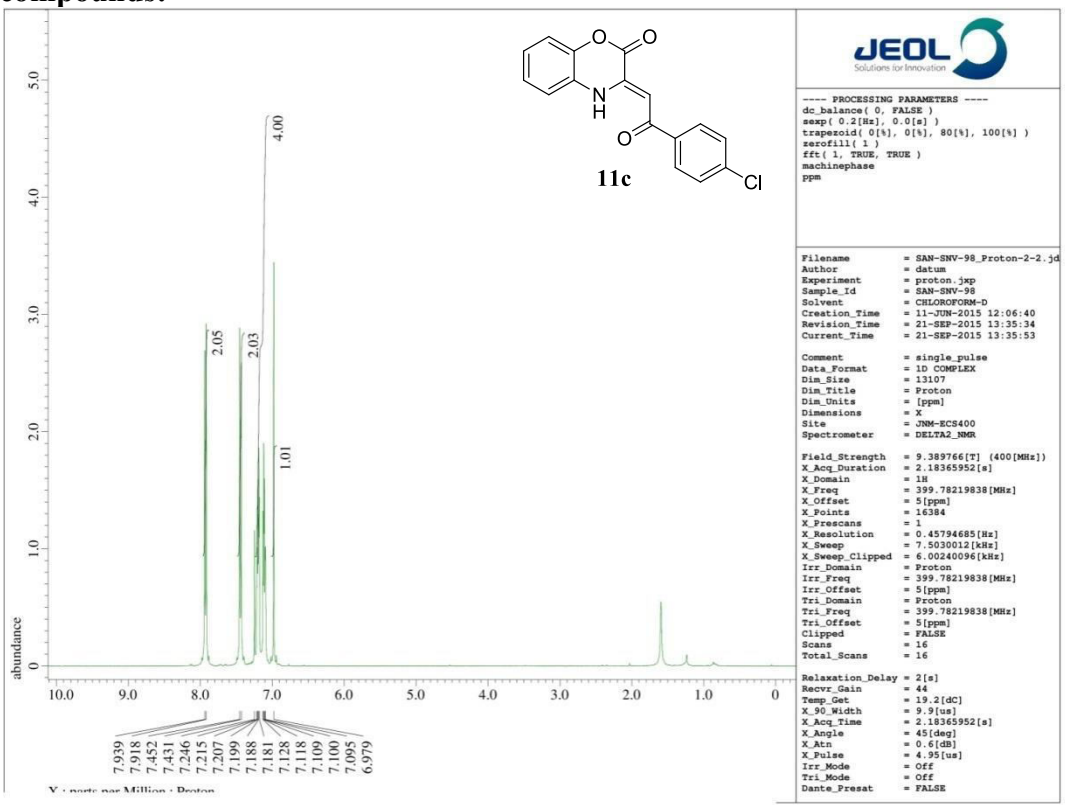


Figure 5. ^{1}H NMR Spectra of Compound **11c**.

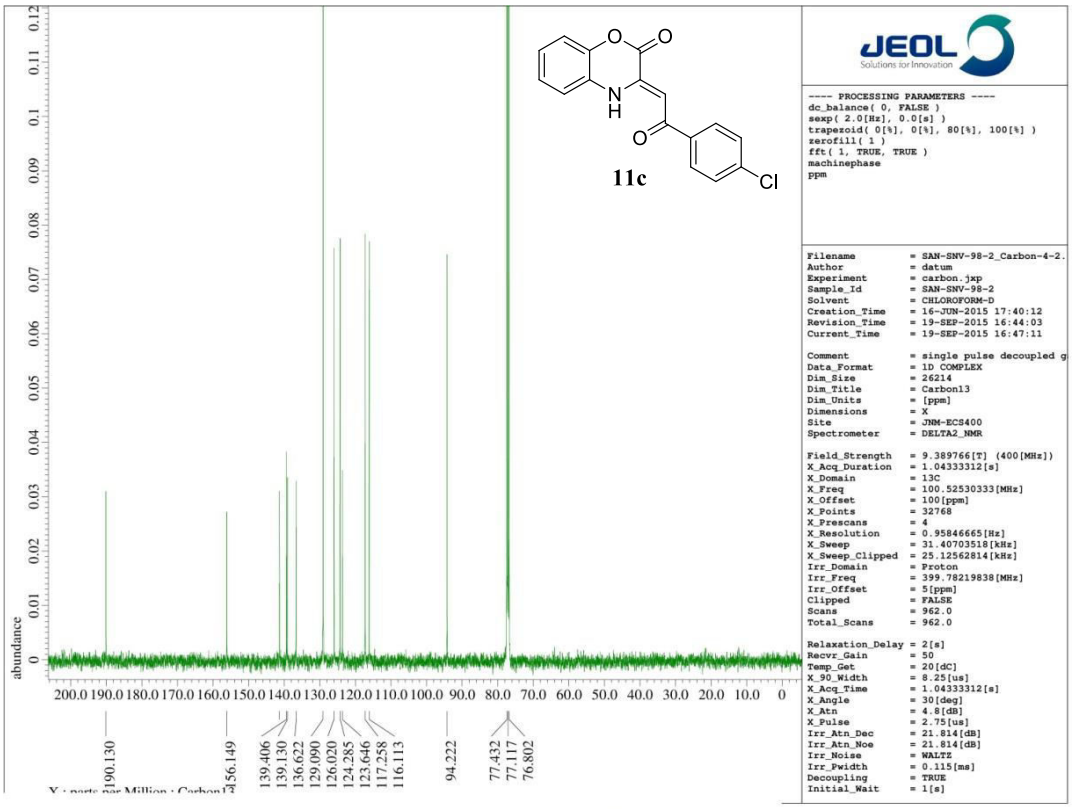


Figure 6. ^{13}C NMR Spectra of Compound **11c**.

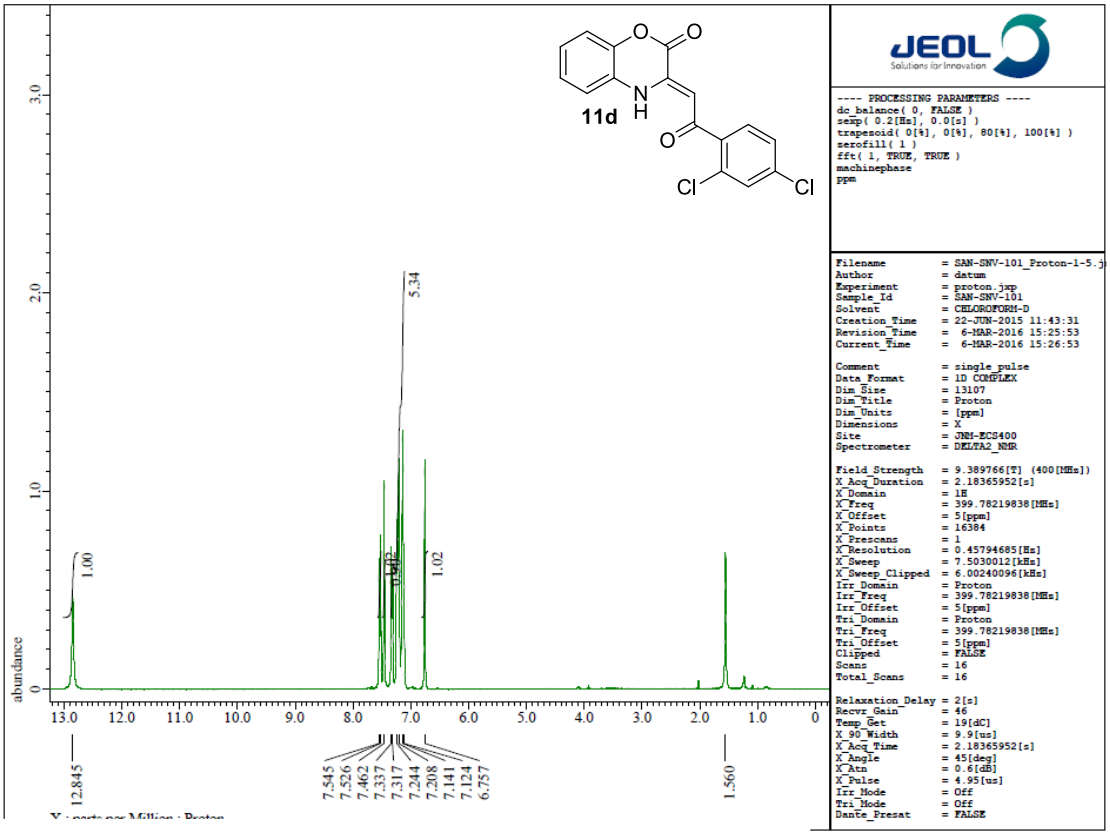


Figure 7. ^{1}H NMR Spectra of Compound **11d**.

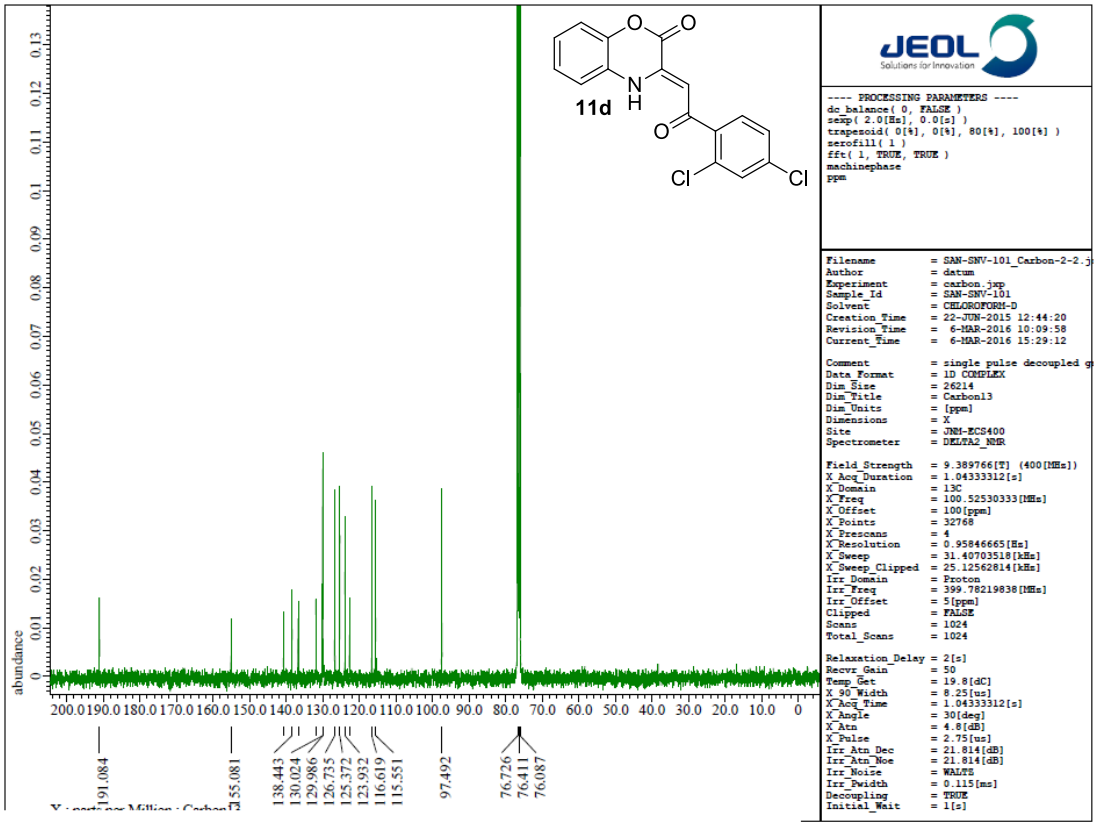


Figure 8. ^{13}C NMR Spectra of Compound **11d**.

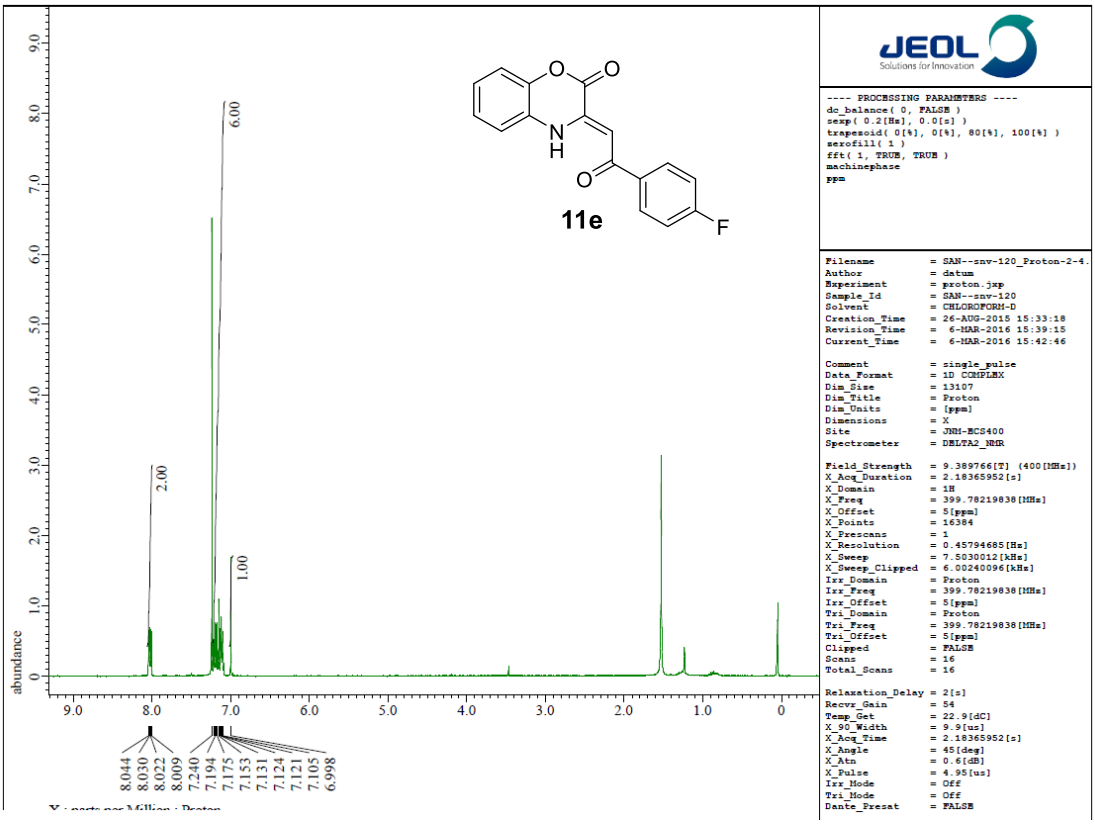


Figure 9. ^{1}H NMR Spectra of Compound **11e**.

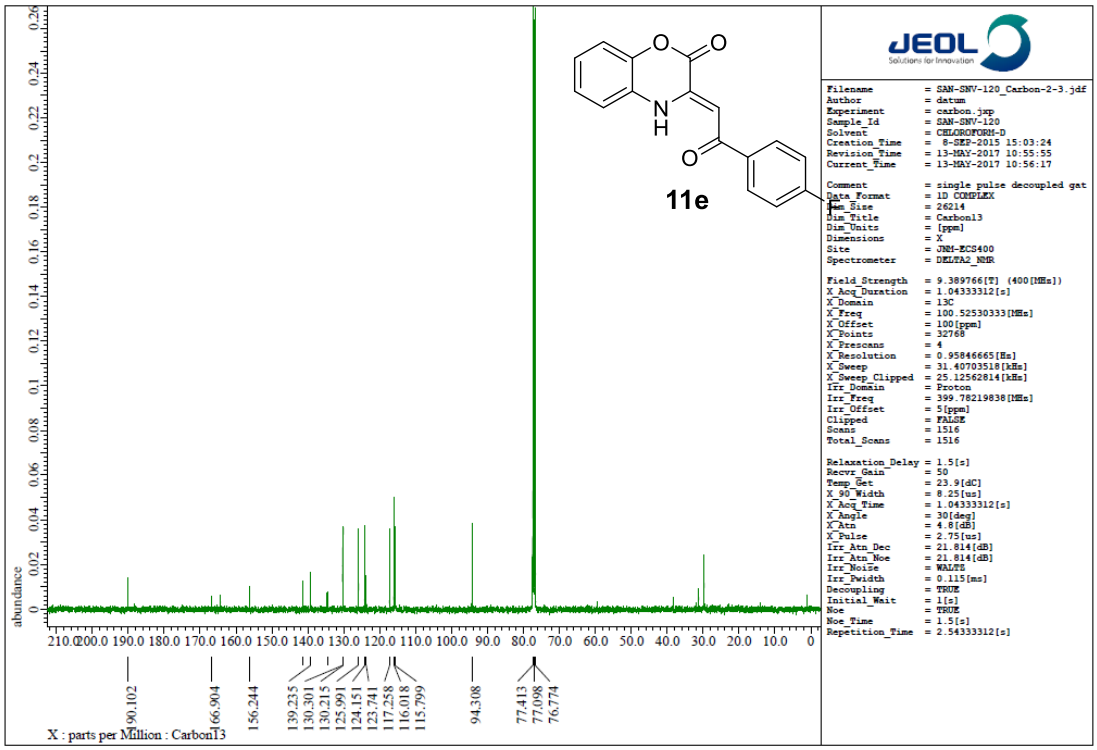


Figure 10. ^{13}C NMR Spectra of Compound **11e**.

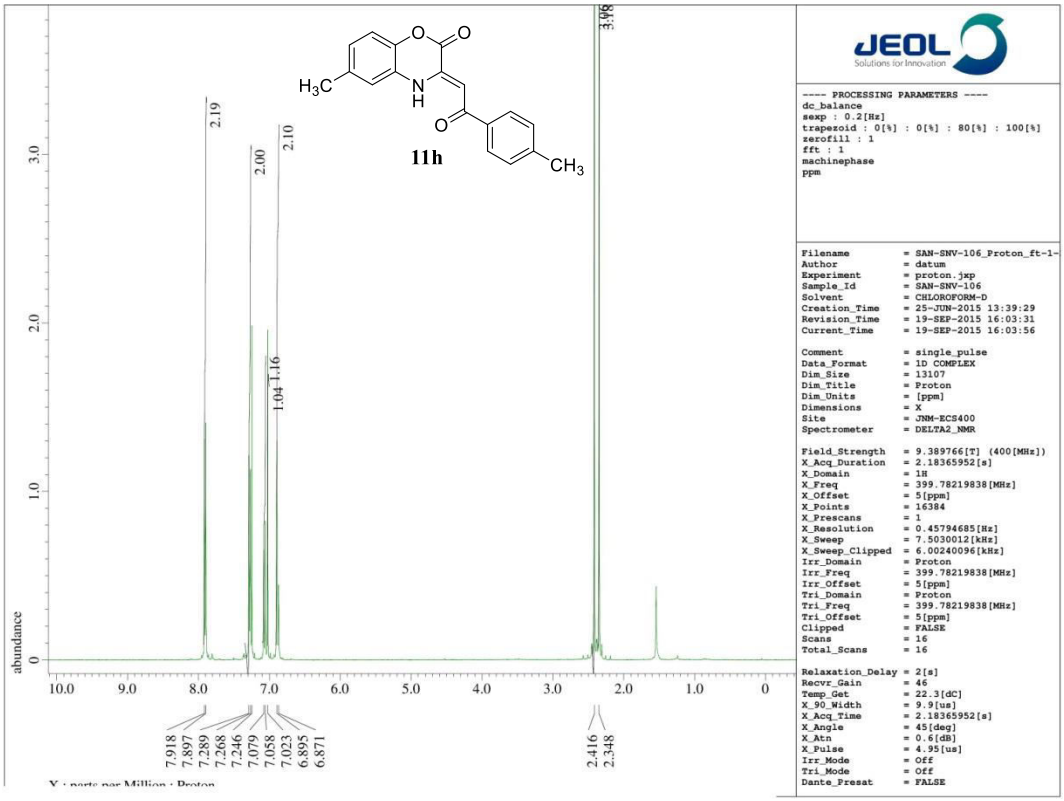


Figure 11. ^{1}H NMR Spectra of Compound **11h**.

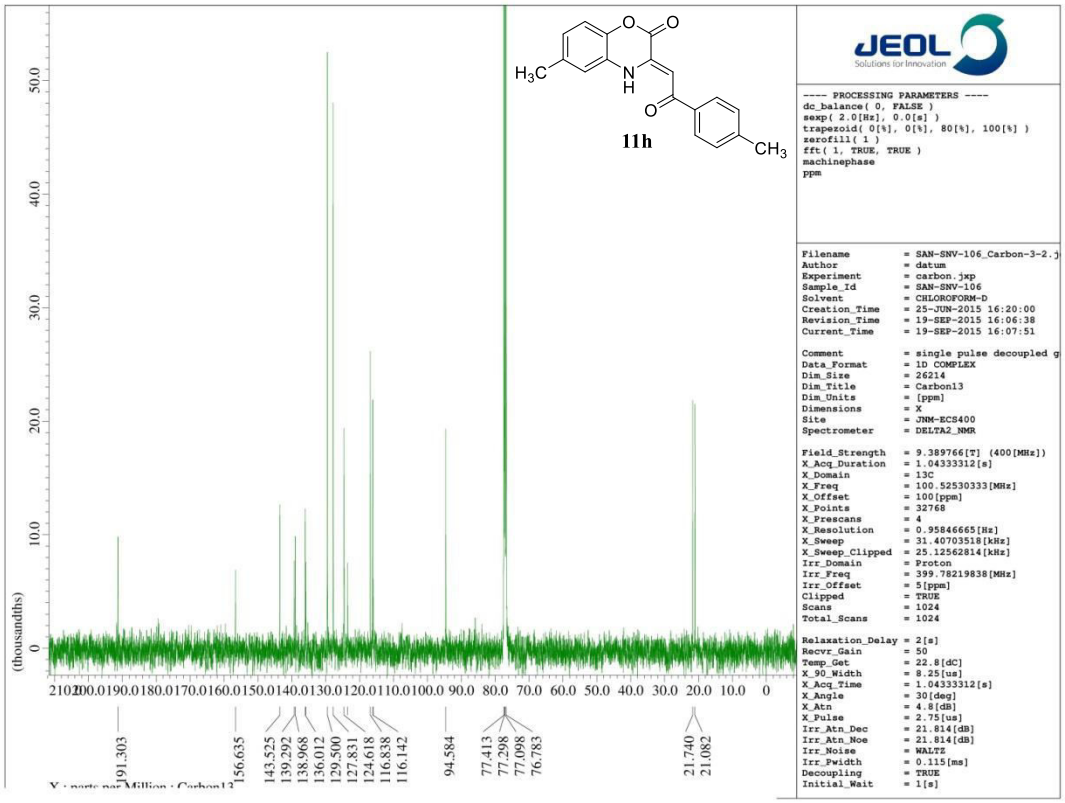


Figure 12. ^{13}C NMR Spectra of Compound **11h**.

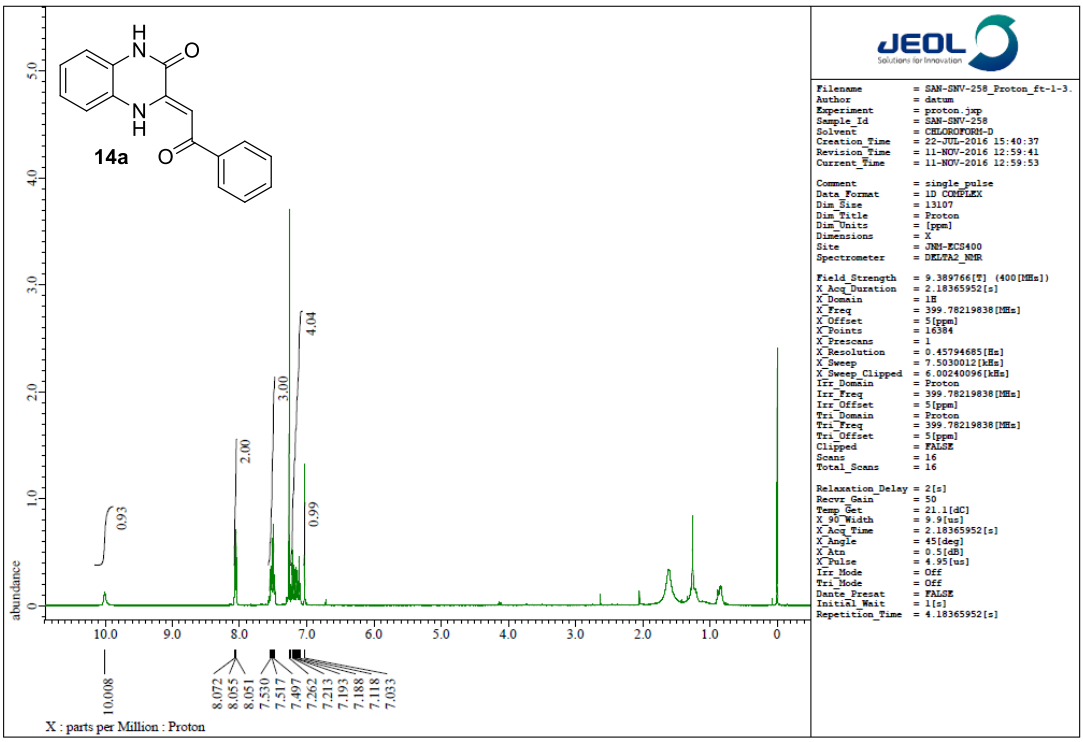


Figure 13. ^{1}H NMR Spectra of Compound **14a**.

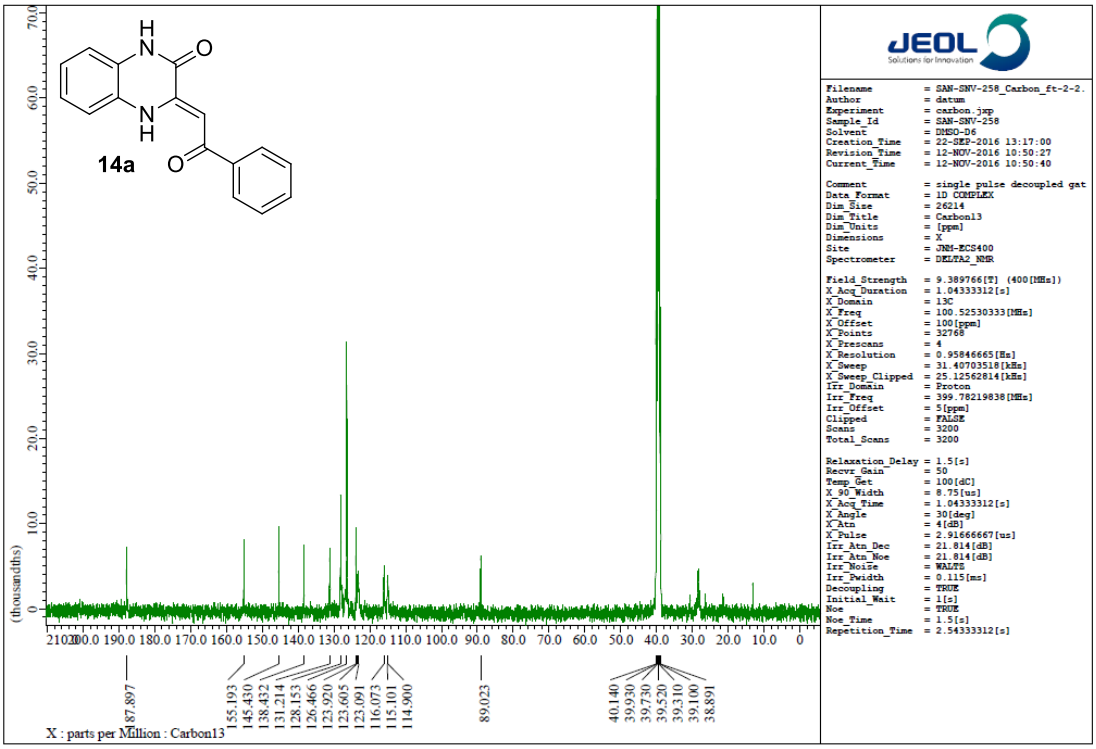


Figure 14. ^{13}C NMR Spectra of Compound **14a**.

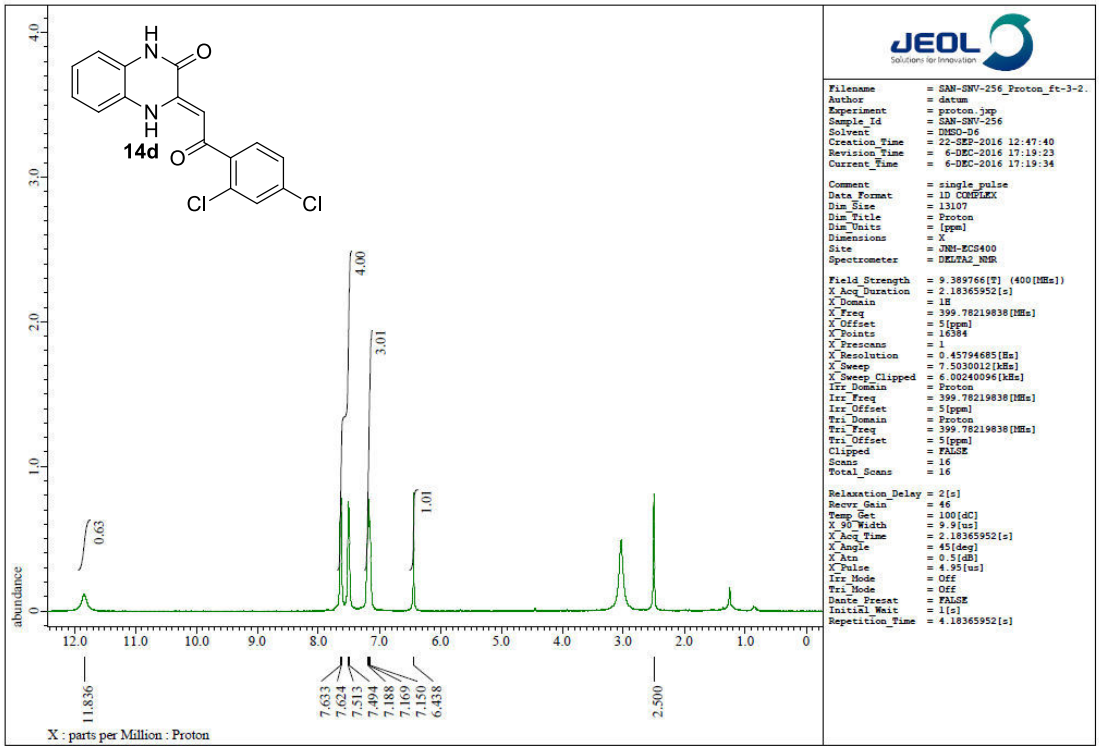


Figure 15. ^{1}H NMR Spectra of Compound **14d**.

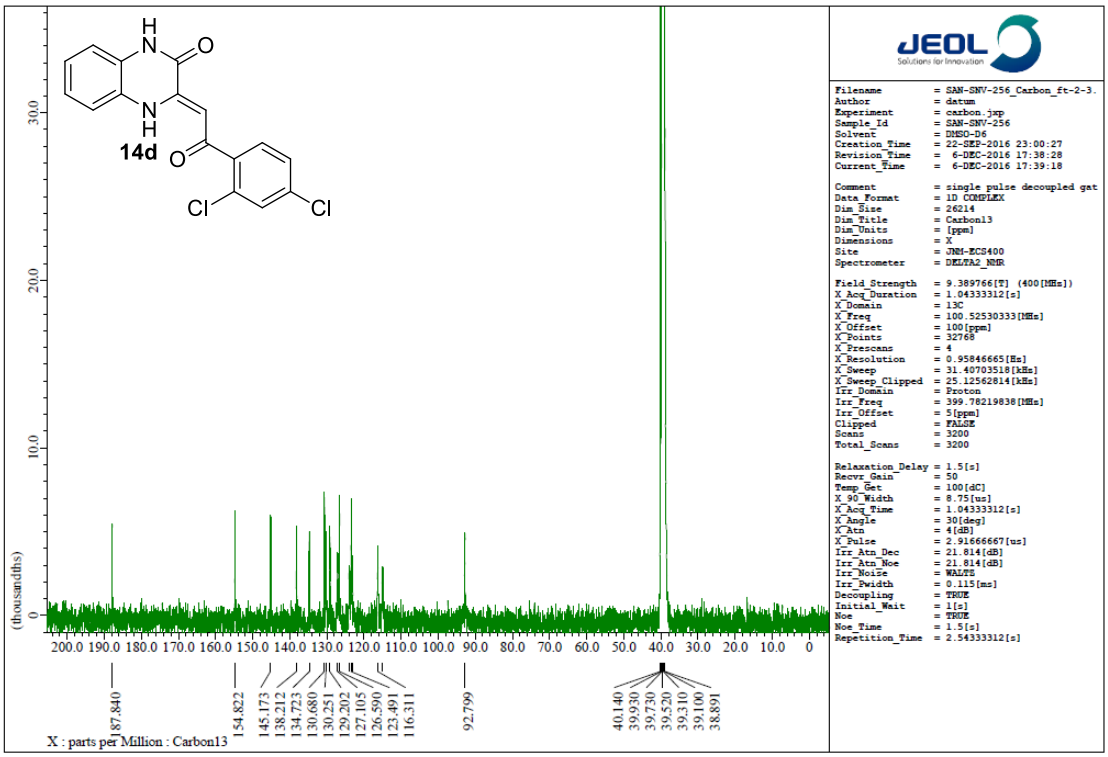


Figure 16. ^{13}C NMR Spectra of Compound **14d**.

Section 2.2
Development of Ultrasound-assisted Green Synthesis of 2-oxo-benz(1,4)oxazines under aqueous media.

2.2.1 Introduction

Water is a versatile solvent for many chemical reactions and becomes very valuable for organic chemists, because it is safe, cheap, environmentally benign, readily available, non-flammable, non-toxic and also in respect of isolation procedure of desired products (because most of the organic compounds are insoluble in water and they may be isolated in pure form only by filtration and/or recrystalization for avoiding column chromatography with hazardous solvents).[1] "On water" synthesis, firstly given by Barry K. Sharpless (2005),[2] is a unique concept of showing higher reactivity by the organic compounds in aqueous medium. Generally, reactions in water require vigorous stirring of water-insoluble reagents as they are not miscible in water due to the absence of organic co-solvents. This concept introduces the fact that reaction those occur in high yields in water, is due to the dual role played by water as a solvent and as a catalyst, in catalyzing the reaction.[1b-c, 3]

Ultrasound irradiation has been recognized as a clean and advantageous greener approach to accelerate organic reactions.[4] The salient features of the ultrasound-assisted reactions are higher reaction rates, formation of products in higher yield and selectivity, milder reaction conditions and shorter reaction time. Moreover, this technique is capable to activate many organic synthetic transformations due to acoustic cavitational collapse. In comparison to conventional heating, which provides thermal energy into the macro system; ultrasound irradiation reduces reaction time, increases yield, minimize waste and involve energy conservation by providing the activation energy to micro environment which emphasizes its greener significance.[5]

Benzo[1, 4]oxazines **1** and 2-oxo-benzo[1, 4]oxazines **2** are an important class of heterocycles which are found in many natural products as well as in several biologically active and medicinally important molecules.[6] Natural products such as blepharin **3**,[7] Cephalandole A **4**,[8a-d] C-1027-chr **5**,[9] and other pharmaceuticals incorporating 2H-1,4-benzoxazine-3(4H)one key scaffolds **6-9**, exhibit a wide range of biological activities such as potential activity against several diseases including heart disease,[10] an inhibitor of bacterial histidine protein kinase,[11] serotonin-3(5-HT_3) receptor antagonists,[12] neurodegenerative agents,[13] antihypertensive agents,[14]

inflammatory agents,[15] analgesic,[16] D2 receptor antagonists,[17] antimycobacterial,[18] and antifungal agents.[19] (Figure 1)

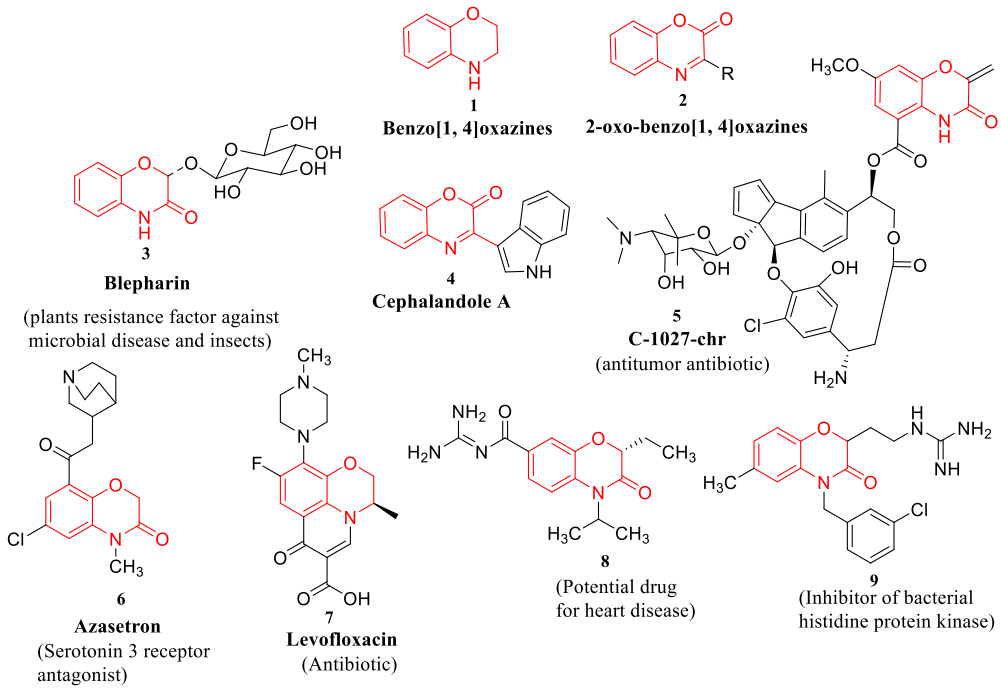


Figure 1. Structures of some natural products **3-5** and pharmaceutically active compounds **6-9** possessing 1, 4-benzoxazine scaffolds.

A number of synthetic approaches for the synthesis of benzo[1,4]oxazines, 2-oxo-benzo[1,4]oxazines and similar moieties have been reported over the past few decades.[20-24] In general, 2-oxo-benzo[1,4]oxazines and similar derivatives are synthesized by the treatment of *o*-aminophenols with either *α*-ketoesters,[24a-c] or with domino reaction of substituted β-nitroacrylates[24h] or with alkyl propiolates.[24f] Another method involves synthesis of the 2-oxo-3-aryl-benzo[1,4]oxazines by condensation of aryl acetates with methyl-o-quinonemonoximes.[24d-e] Furthermore, 4-alkyl- and 4-benzyl-3,4-dihydro-1,4-benzoxazin-2-one derivatives were also synthesized from reaction between an aldehydes and ethyl 2-(2-hydroxyphenylamino)acetate.[24g] All these methodologies were associated with several drawbacks such as the use of toxic catalysts[23e] and starting materials, hazardous organic solvents, somewhat multistep and requirement of complicated reaction assembly, limited number of appropriate substrates for diverse synthesis,[25a-b] tedious workup and low yields etc. Hence, green protocols having wide substrate scope and versatile nature for the synthesis of such bioactive moieties are in great demand. So far, there is no green protocol available in the literature using ultrasonic-assisted "On water" synthesis of these benzo[1,4]oxazines and related molecules.

A sequential two-step reaction involving $R_4N^+F^-$ directed ring opening of epoxides **11** with aryl sulfonamides **10** followed by *in situ* cyclization of **12** have also been reported to synthesize 2-substituted 3,4-dihydro-2H-1,4-benzoxazines **13**.[24i&j] (Scheme 1, reaction–i)

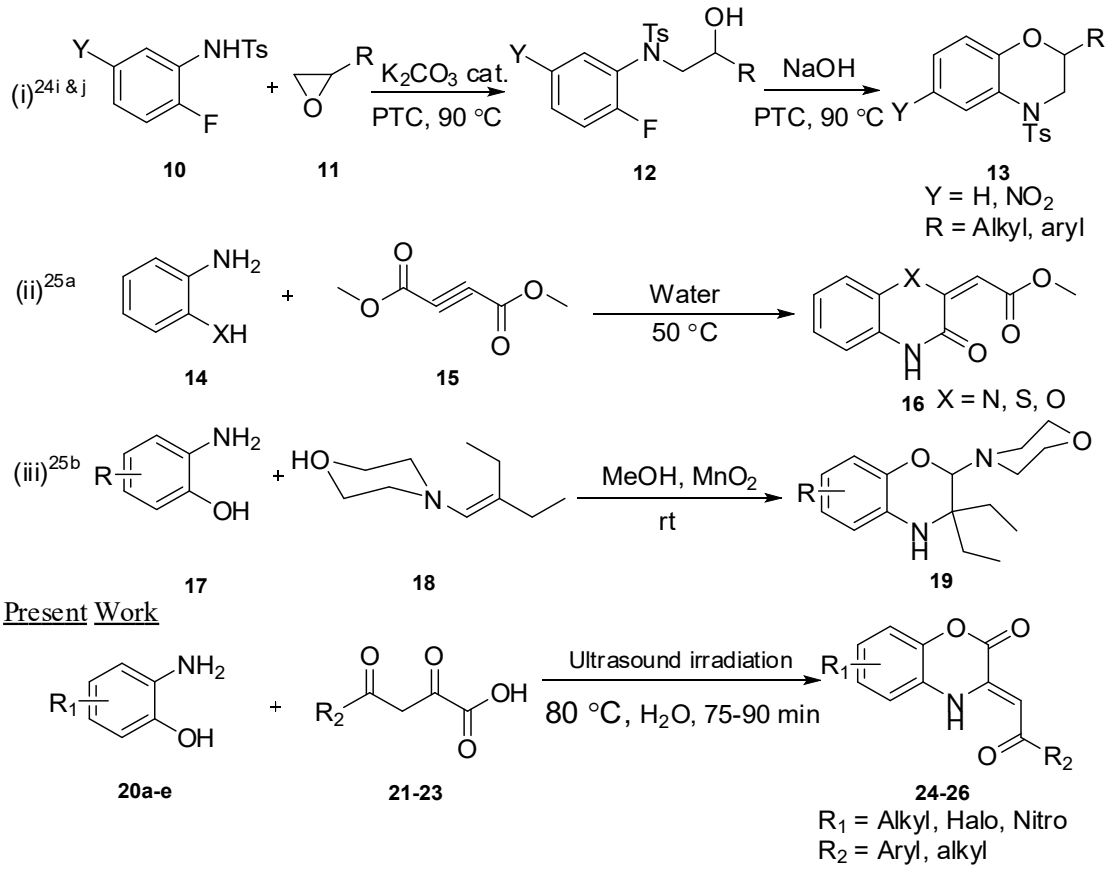


Figure 2. Previous and present report for the synthesis of 1, 4-benzoxazine scaffold under greener approach.

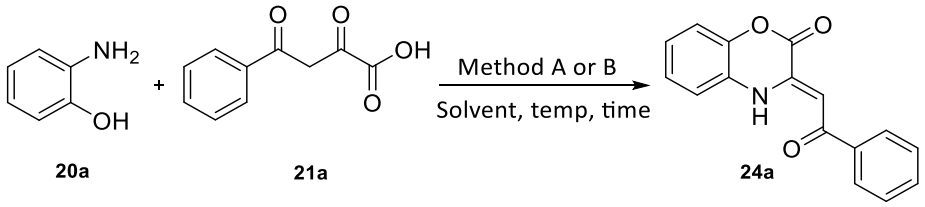
Recently, a green protocol for the synthesis of 3-oxo-1,4-benzoxazine derivatives **16** have also developed via the reaction of o-substituted anilines **14** with dimethyl but-2-ynedioate **15**.[25a] However, this methodology is associated with limited diversification (Scheme-1, reaction–ii). MnO_2 catalyzed tandem oxidation- inverse electron demand Diels Alder (IEDDA) reaction of o-aminophenol derivatives **17** with enamine **18** had also been reported to synthesize substituted 1,4-benzoxazine cycloadduct **19** with complete regio-chemical control.[25b] (Scheme 1, reaction–iii). Herein, we report a simple, efficient, "*on water*'' ultrasound-assisted, catalyst free, diverse one-pot synthesis of highly functionalized 2-oxo-benzo[1,4]oxazines **24-29** (Scheme 1, reaction–iv). This protocol involve one-pot reaction of substituted 2-aminophenol

20a-f and substituted 2, 4-dioxo-4-phenylbutanoic acid **21-23** *on water* under ultrasound irradiation at 80 °C for 75-120 min, which furnished substituted 2-oxo-benzo[1,4]oxazines **24-29**, respectively upto 98% yields. Compounds **24a-g**, **24i-j**, **31a-b** had been prepared earlier by known procedures.[21] To the best of our knowledge, this is the first report of "*on water*" ultrasonic-assisted green synthesis of functionalized 2-oxo-benzo[1, 4]oxazines and its derivatives in excellent yields.

2.2.2 Results and discussion

Initially, we performed the model reactions between 2-aminophenol **20a** (0.1 mmol) and 2,4-dioxo-4-phenylbutanoic acid **21a** (0.1 mmol) in several polar solvents (2.0 mL) at room temperature for 120 min. The condensation product **24a** in isopropanol was isolated in only 16% yield; but when the same reaction was performed under ultrasound irradiation for 45 min at room temperature then, to our surprise, we obtained **24a** in 46 % yield (entry 1, Table 1).

Table 1. Optimization study[a]: Synthesis of 2-oxo-benzo[1,4]oxazines **24a** by the reaction of 2-aminophenol **20a** and 2, 4-dioxo-4-phenylbutanoic acid **21a.**



Entry	Solvent	Temp (°C)	Method A[b]		Method B[c]	
			Time (min)	Yield[d] (%)	Time (min)	Yield[d] (%)
1	Isopropanol	rt	120	16	45	46
2	Isopropanol	90	120	39	45	62
3	EtOH	rt	120	22	45	44
4	EtOH	80	120	52	45	70
5	DMF	80	120	61	45	78
6	DMSO	80	120	64	45	73
7	Diethylene glycol	80	120	60	45	81
8	H_2O	rt	120	12	45	62
9	H_2O	80	120	69	45	86
10	H_2O	100	120	73	45	89
11	H_2O	80	240	78	60	94
12	H_2O	80	300	82	**75**	**98**
13	H_2O	80	360	80	90	97

[a] *Reaction condition*: **20a** (0.1 mmol), **21a** (0.1 mmol) in solvent (2.0 mL), at given time and temp under method A or B; [b] **Method A**: Conventional heating; [c] **Method B**: Ultrasound Irradiation; [d]Isolated yield after recrystallization/column chromatography.

Further for getting the better yield, we subjected the same reaction at 90°C under conventional as well as in ultrasound irradiation for 120 min and 45 min respectively, which afforded **24a** in 39 % and 62 % yield, respectively (entry 1, Table 1). After obtaining the improved yield under ultrasound irradiation, we anticipated that ultrasound irradiation could potentially accelerate the reaction and increase yields. Thus, these results prompted us to investigate in detail the effect of sonication, temp, time and solvent on the rate and yield of the reaction. The obtained condensed product **24a** was fully characterized by their spectroscopic data (^{1}H and ^{13}C NMR, HRMS and IR).

In order to increase the yield of the desired 2-oxo-benzo[1,4]oxazine **24a**, we did this reaction in EtOH, DMF, DMSO, Diethylene glycol, water etc. at different temperature using method A as well as method B. As can be seen from the table 1; yield was significantly improved under ultrasonic conditions (method B) as compared to conventional method (method A) conditions (entries 3-8; Table 1). So, we performed our model reaction in water as solvent only (entries 8-13). When we performed our model reaction on water at room temp for 120 min and under ultrasound irradiation for 45 min, we obtained **24a** in 12 % and 62 % yield, respectively (entry 8, Table 1). Then, we carried out the model reaction under heating at 80 °C under conventional as well as under ultrasound irradiation conditions; **24a** was obtained in 69 % and 86 % yield respectively (entry 9, Table 1). Either on further increasing the reaction temperature from 80°C to 100 °C or increasing /decreasing the reaction time, under conventional heating, there was slight improvement in yields ranging from 73-80 % (entries 10-13, Table 1). But, to our delight, the yield of desired product **24a** was increased up to 98 % when time was extended from 45 to 75 min. under ultrasonic irradiation conditions (entries 10-12, Table 1). On further extending the time from 75 min to 90 min at the same reaction conditions, i.e. at 80 °C temp under ultrasound irradiation condition, yield of **24a** dropdown slightly to 97 % (entries 13, Table 1). Thus, based on above screening studies, water as solvent, 80°C temperature for 75 min was found to be the best optimized reaction condition under ultrasound irradiation for the synthesis of desired 2-oxo-benzo[1,4]oxazines **24a** (entry 12, Table 1).

The proposed mechanism of this optimized reaction might involve intermolecular condensation followed by an intramolecular condensation *via* either **path A** or **path**

B. This can be explained by taking **24a** as reference example, which is formed by the reaction between **20a** and **21a,** (Figure 3).

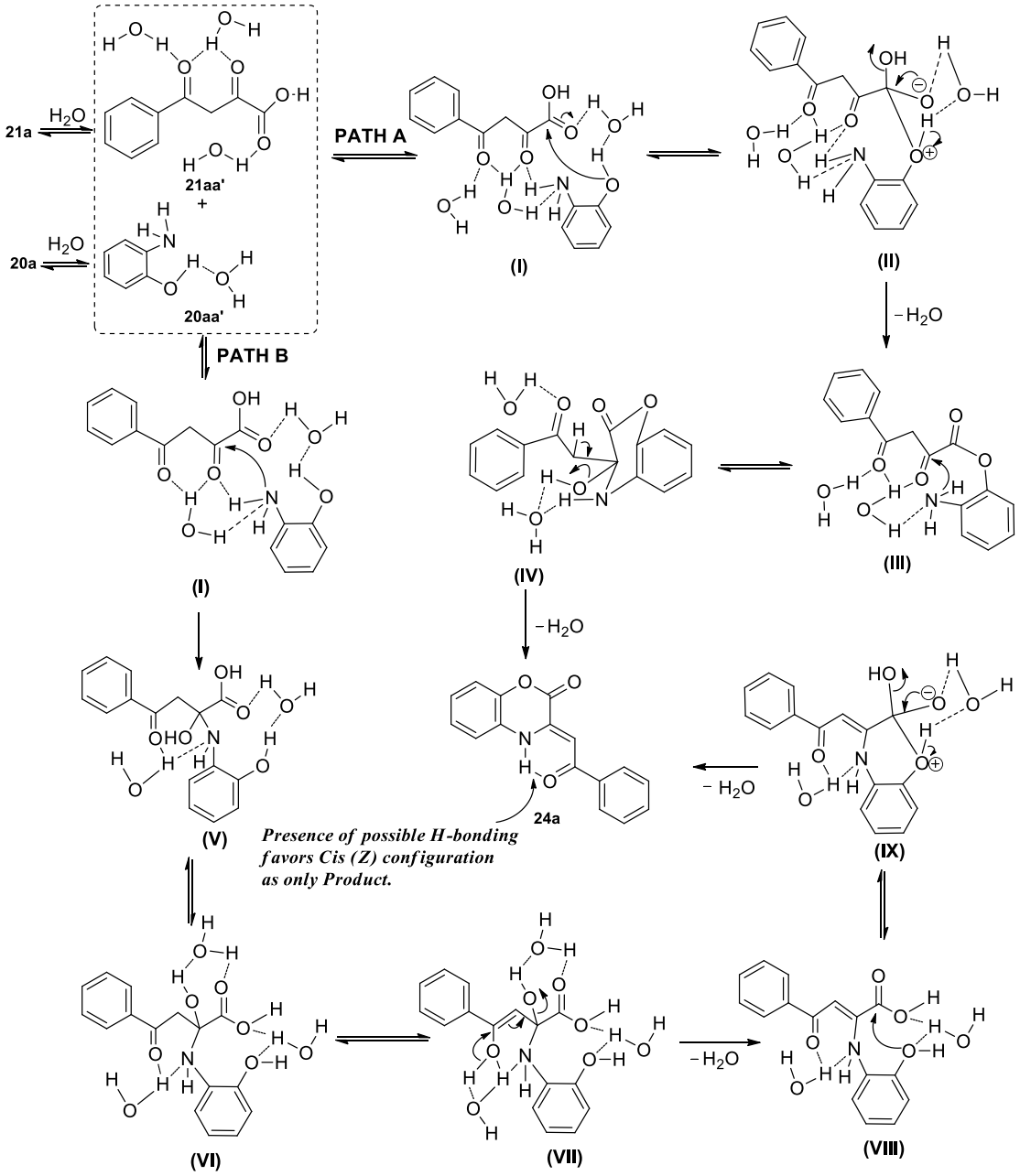


Figure 3. Probable mechanism for the synthesis of 2-oxo-benzo[1,4]oxazine **24a**.

The H-bonding between the oxygen atom of water and the phenolic hydrogen of 2-aminophenol increased the nucleophilicity of the oxygen atom of the 2-aminophenol (**20aa'**). On the other hand, the hydrogen bond between hydrogen atom of water and carboxylic oxygen increased the electrophilic character of carbon adjacent to the carbonyl group (**21aa'**).Therefore, according to **path A**; in the probable intermediate (**I)**, the nucleophilic attack at the carboxylic carbon atom by the phenolic oxygen (**II**)

followed by removal of water led to conjugated acyclic adduct (**III**). Then, the second nucleophilic attack of nitrogen atom to the carbonyl carbon adjacent to newly formed ester linkage of this acyclic adduct took place (**IV**) and the corresponding 2-oxo-benzo[1,4]oxazine **24a** was obtained followed by the removal of second water molecule. Whereas, contrary to this, as depicted in **path B**; firstly the nucleophilic attack by nitrogen of aminophenol **20aa'** at the carbonyl carbon (adjacent to the carboxylic group) of **21aa'** led to the intermediate **(V)**, which on removal of the water molecule gets converted to the conjugated acyclic adduct (**VIII**). Finally, the desired product 2-oxo-benzo[1,4]oxazine **24a** was obtained after the second nucleophilic attack by phenolic oxygen at carboxylic carbon followed by removal of second water molecule from intermediate **(IX).**

2.2.3 Substrate scope and versatility

The generality and versatile nature of our optimized reaction conditions was then investigated. Several alkyl/halide/nitro-substituted 2-aminophenol **20a-f** was reacted with alkoxy/ alkyl/halide/nitro-substituted 2, 4-dioxo-4-phenylbutanoic acid **21a-i** and **22-23** in water in excellent yields (upto 98%) under our optimized conditions furnished the desired 2-oxo-benzo[1,4]oxazines **24a-q, 25a-d** and **26-29**, respectively (Scheme 1, figure 4).[26] All the compounds were purified either by flash column chromatography method or by recrystalization method (see experimental section).

The electron donating substituents present (**20b** or **20e**) on 2-aminophenol increases the yields, while the electron withdrawing substituents (as in **20c, 20d** and **20f**) slightly decreases the yields of products. The same trend of isolated yield was also obtained with electron donating (**21a-h**) and electron withdrawing substituents (**21i**), which was present on 2,4 -dioxo-4-phenylbutanoic acid **21a-i**. In the case of polycyclic (aromatic as well as aliphatic) diketo acids i.e., **22** and **23a** and aliphatic diketo acid **23b**; **25a-d, 26** and **27** were obtained in excellent yields (86-93%) whereas **28** and **29** was obtained in 79% and 76% yields, respectively. Thus, these result shows that the aromatic diketo acids, **21a-i** and **22**, afforded the target 2-oxo-benzo[1,4]oxazines in high yields (upto 98%) as compared with that obtained from alicyclic diketo acids **23a** and alkyl diketo acids **23b**.It has been also observed that the several functional groups, like F, Cl, Br, OMe and NO_2 are well tolerable under our

reaction conditions as the desired products were obtained in high isolated yields indicating the versatility of the methodology.

Scheme 1. Ultrasound-assisted one-pot green synthesis of 2-oxo-benzo[1,4]oxazines **24a-q**, **25a-d** and **26-29**.[a]

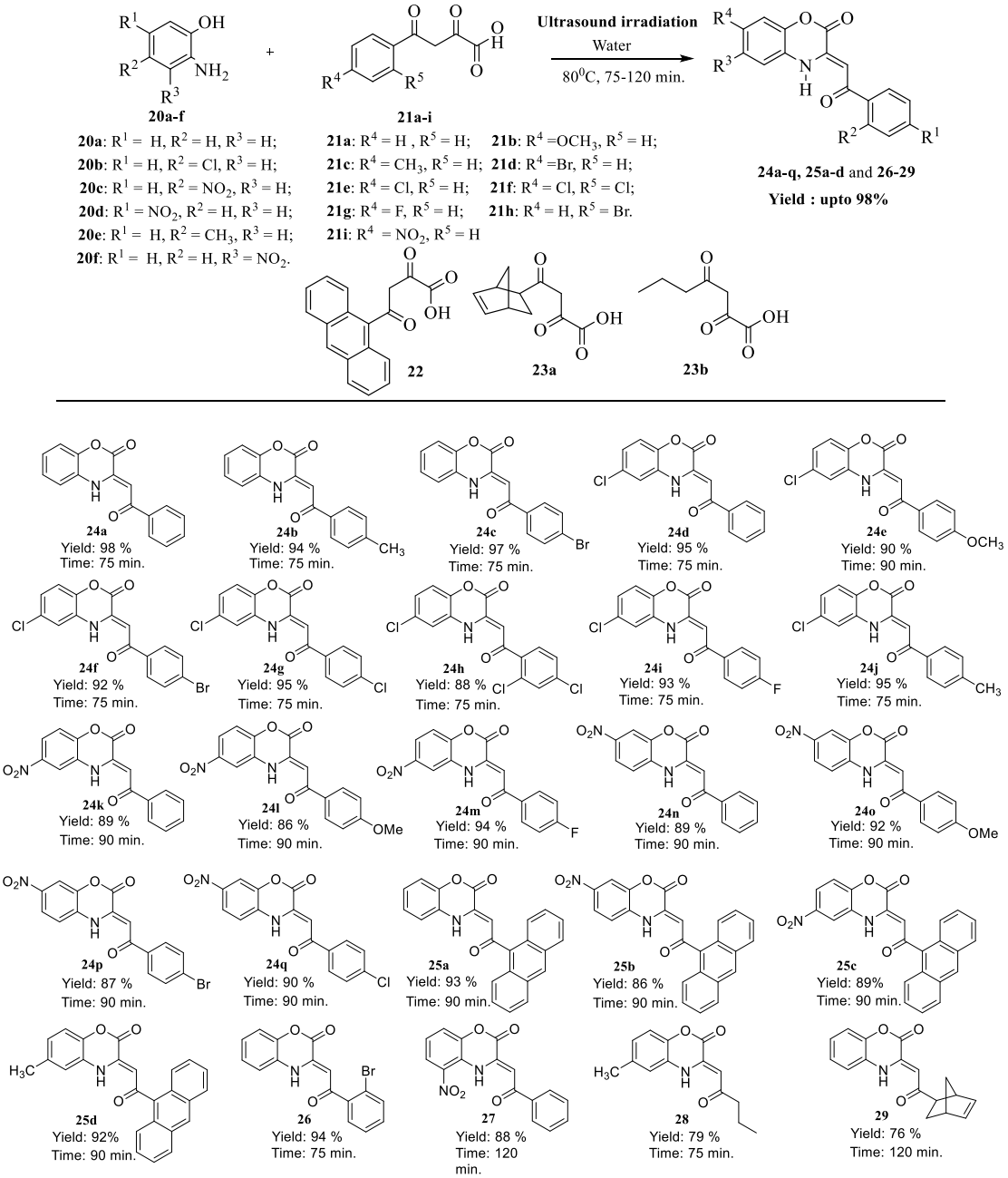
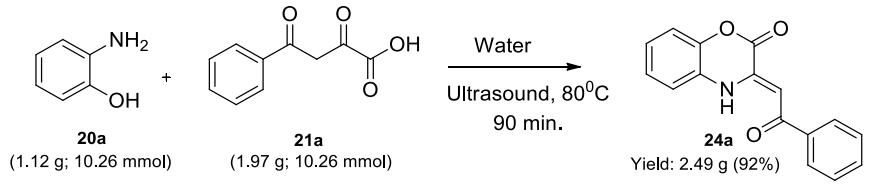


Figure 4. Structures of all synthesized 2-oxo-benzo[1,4]oxazines. [a]Unless otherwise mentioned, all the reactions were carried out with substrates aminophenols **20a-f** (0.2 mmol) and **21- 23** (0.2 mmol) in water (2.0 mL) at 80 °C temperature at given time under ultrasound irradiation. [b]Isolated yield.

Furthermore, we demonstrated its practicality by performing the model reaction in gram scale. The gram scale synthesis was performed taking **24a** as representative example. The heterogeneous reaction mixture of 2-amino phenol (**20a**, 1.12 g, 10.26

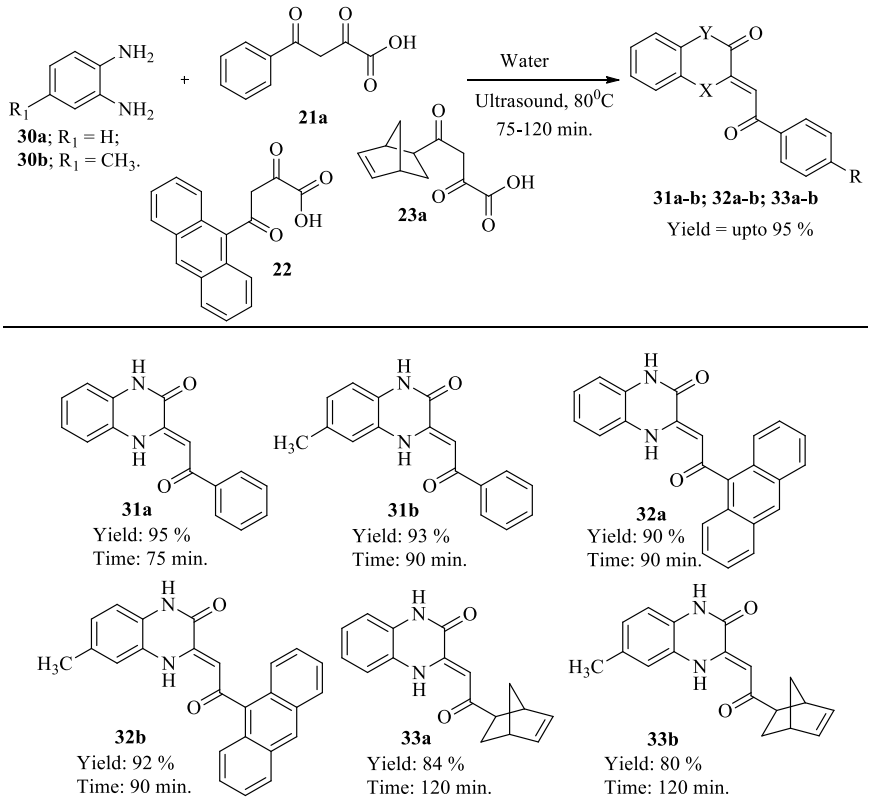
mmol) and 2,4-dioxo-4-phenylbutanoic acid (**21a**, 1.97 g, 10.26 mmol) in water was stirred and heated at 80 °C for 90 min under ultrasonic irradiations (monitored by TLC). The solid 2-oxo-benzo[1,4]oxazines **24a** was precipitated out, which was easily isolated in 92 % yield by simple filtration followed by washing and recrystalization with EtOH (Scheme 2).

Scheme 2. Ultrasound-assisted gram scale synthesis of 2-oxo-benzo[1,4]oxazines **24a.**



After successful application of developed methodology for 2-oxo-benzo[1,4]oxazines class of molecules, the study was further extended to 2-oxo-quinoxaline class of molecules, an important class of heterocyclic bioactive compound present in various natural products.

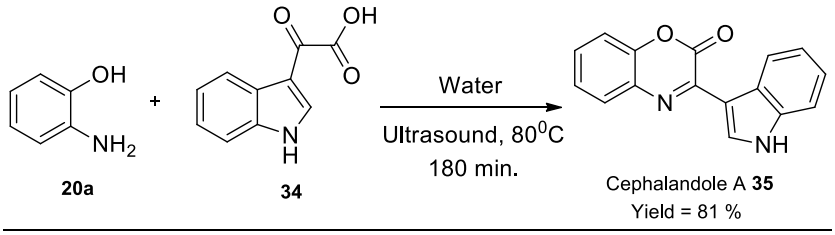
Scheme 3. Ultrasound-assisted one-pot green synthesis of 2-oxo- to 2-oxo-quinoxaline **31**-**33**.[a]



[a]Unless otherwise mentioned, all the reactions were carried out with substrates **30a-b** (0.2 mmol) and **21a, 22** and **23a** (0.2 mmol) in water (2.0 mL) at 80 °C temperature at given time under ultrasound irradiation. [b]Isolated yield.

Functionalized 1,2-Diphenyl amine **30a-b** on reaction with several 2,4-dioxo-4-aryl/alicyclic acid **21a, 22** and **23a** furnished the corresponding substituted 2-oxo-quinoxaline analogues (**31a-b, 32a-b & 33a-b**) in excellent 80-95% yields under our optimized reaction conditions (Scheme 3).[27]

Scheme 4. Synthesis of Cephalandole A **35.**



Finally, we extended the application of our developed methodology in the synthesis of 2H-benzo[b][1,4]oxazin-2-one-based anticancer indole alkaloid, Cephalandole A. It was isolated from the Taiwanese orchid *Cephalanceropsis gracilis* (Orchidaceae) in 2006.[8a-d] The crude extract of this plant exhibited activity against lung (NCI-H460; IC_{50} = 7.8 μM), breast (MCF-7; IC_{50} = 7.57 μM) and CNS (SF-268; IC_{50} = 12.2 μM) carcinoma cell lines. Aminophenol **20a** on reaction with 3-indoleglyoxylic acid **34** in water under our optimized conditions furnished Cephalandole A **35** in 81 % yield.[28] (Scheme-4). The spectral data of **35** was found identical with the literature data.

2.2.4 Conclusions

In summary, we have developed a simple, highly efficient green protocol for the synthesis of functionalized 2-oxo-benzo[1,4]oxazines **24a-q, 25a-d,** and **26-29** in excellent yields upto 98% under ultrasound irradiation conditions at 80 °C in 75-120 min. Moreover, this methodology tolerates a broad range of functional groups and their positions under simple reaction conditions, and provides a straightforward access to a library of functionalized 2-oxo-benzo[1, 4]oxazines analogues from readily available starting substrates. To the best of our knowledge, this is the first report of ultrasound assisted synthesis of functionalized 2-oxo-benzo[1,4]oxazines in water. In addition, functionalized 2-oxo-quinoxaline analogues **31a-b**, **32a-b** and **33a-b** were also synthesized utilizing this methodology in excellent yields (upto 95%). Gram scale synthesis and synthesis of Cephalandole A **35**, highlights the practicality of this methodology.

2.2.5 Experimental Details & Characterization Data

2.2.5.1 General experimental

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. Ultrasonic irradiation was performed in a Elmasonic S 30 (H) ultrasonic water bath cleaner and the reaction vessel was positioned in the maximum energy area in the cleaner and the removal or addition of water was used to control the temperature of the water bath. 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 $CDCl_3$, $CD_3ODandCD_3SOCD_3$ as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S Q Tof (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.

2.2.5.2 General procedure for the Synthesis of 2-oxo-benzo[1,4]oxazines (24a-q, 25a-d and 26-29): To a solution of the compound 0.2 mmol of **20a-f** in water was added 0.2 mmol of compound **21a-i**, **22** or **23**; and the reaction mixture was irradiated under ultrasonic Sonicator at 80 ˚C temperature for about 75-120 min. (depending upon the substrate employed). The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was filtered off, washed with distilled water (3 × 50 ml) and dried it under vacuum, which furnished the crude product. The crude product were purified either by recrystallization using EtOAc/hexane; or by flash column chromatography method over silica gel using hexane/ethyl acetate as an eluent which afforded the pure desired 2-oxo-benzo[1,4]oxazines **24a-q**, **25a-d** and **26-29**, respectively in excellent yield (76-98 %).

2.2.5.3 General procedure for the Synthesis of 2-oxo-quinoxaline analogues (31a-b, 32a-b and 33a-b): To a solution of benzene-1,2-diamine (**30a-b**, 0.2 mmol) in water was added substituted diketo-acid (**21a**, **22** or **23a;** 0.2 mmol each); and the reaction mixture was irradiated under ultrasonic Sonicator at 80 ˚C for about 75-120 min. (depending upon the substrate employed). The progresses of the reaction were

monitored by TLC. After completion of the reaction, the reaction mixture was filtered off, washed with distilled water (3 × 50 ml) and dried it under vacuum, which furnished the crude product. The crude product were then purified either by recrystallization method or by flash column chromatography method over silica gel using 9:1 EtOAc/hexane as an eluent which afforded the pure desired 2-oxo-quinoxaline analogues (**31a-b**, **32a-b** and **33a-b**) having excellent yield (80-95 %).

2.2.5.4 Characterization data of 2-oxo-benzo[1,4]oxazines (24a-q, 25a-d and 26-29) and 2-oxo-quinoxaline analogues (31a-b, 32a-b and 33a-b):

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[*b*][1,4]oxazin-2-one (24a)

Yellowish solid; yield: 53.2 mg (98 %), R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 185-186 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 1754, 1614, 1594, 1270; ^{1}H NMR (400 MHz) δ 8.00 (d, *J* = 7.4 Hz, 2H), 7.56 – 7.47 (m, 3H), 7.21 – 7.06 (m, 5H); ^{13}C NMR (100 MHz) δ 191.6, 156.3, 141.3, 139.1, 138.3, 132.8, 128.8, 127.7, 126.0, 124.0, 123.8, 117.2, 116.0, 94.7; HRMS (ESI) calcd. for $C_{16}H_{11}NO_3$ $[M+H]^+$: 266.0739; found 266.0744.

(Z)-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24b)Yellowish solid; yield: 54.1 mg (94 %), R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 160-162 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 2925, 1759, 1622, 1110; ^{1}H NMR (400 MHz) δ 7.91 (d, *J* = 7.6 Hz, 2H), 7.27 (m, 2H), 7.20-7.16 (m, 2H), 7.10-7.06 (m, 2H), 7.03 (d, *J* = 1.2 Hz, 1H), 2.41 (s, 3H); ^{13}C NMR (100 MHz) δ 191.4, 156.5, 143.6, 141.2, 138.8, 135.7, 129.5, 127.9, 125.9, 123.9, 123.8, 117.2, 115.9, 94.8, 21.8; HRMS (ESI) calcd. for $C_{17}H_{13}NO_3$ $[M+H]^+$: 280.0895; found 280.0896.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24c)

Yellowish solid; yield: 67.7 mg (97 %), R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 200-202 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 1754, 1624, 1585, 1277, 1111; ^{1}H NMR (400 MHz) δ 7.85 (d, *J* = 8.0 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.09 (m, 4H), 6.97 (s, 1H); ^{13}C NMR

(100 MHz) δ 190.3, 156.1, 141.4, 139.4, 137.1, 132.1, 129.2, 127.8, 126.0, 124.3, 123.6, 117.3, 116.1, 94.2; HRMS (ESI) calcd. for $C_{16}H_{10}BrNO_3$ $[M+2]^+$: 344.9844; found 344.9847.

(Z)-6-chloro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24d)

Yellowish solid; yield: 57.2 mg (95 %), R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 185-187 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 1761, 1555, 1622, 1174; ^{1}H NMR (400 MHz) δ 8.00 – 7.98 (m, 2H), 7.58 – 7.55 (m, 1H), 7.50-7.47 (m, 2H), 7.13 – 7.03 (m, 4H); ^{13}C NMR (100 MHz) δ 191.8, 155.8, 139.8, 138.4, 138.1, 133.0, 131.1, 128.9, 127.8, 124.8, 123.8, 118.3, 115.8, 95.7; HRMS (ESI) calcd. for $C_{16}H_{10}ClNO_3$ $[M+2]^+$: 301.7085; found 301.7089.

(Z)-6-chloro-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24e)

Yellowish solid; yield: 62.6 mg (90 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 178-180°C; FT-IR (KBr, νmax/cm^{-1}) 3434, 1764, 1628, 1594, 1018; ^{1}H NMR (400 MHz) δ 7.99-7.97 (m, 2H), 7.10-7.06 (m, 2H), 7.02 – 6.99 (m, 2H), 6.97-6.94 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz) δ 190.5, 163.7, 156.1, 139.6, 137.8, 131.1, 130.9, 130.1, 125.0, 123.4, 118.2, 115.6, 114.1, 95.7, 55.6; HRMS (ESI) calcd. for $C_{17}H_{12}ClNO_4$ $[M+2]^+$: 331.7345; found 331.7349.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-chloro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24f)

Yellowish solid; yield: 71.5 mg (92 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 175-177 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 2924, 1755, 1632, 1583, 1007; ^{1}H NMR (400 MHz) δ 7.85 (d, *J* = 7.9 Hz, 2H), 7.62 (d, *J* = 7.9 Hz, 2H), 7.13 – 7.00 (m, 4H); ^{13}C NMR (100 MHz) δ 190.5, 155.6, 139.8, 138.8, 136.8, 132.1, 131.2, 129.3, 128.1, 124.6, 124.0, 118.3, 115.9, 95.2; HRMS (ESI) calcd. for $C_{16}H_9BrClNO_3$ $[M+2]^+$: 377.9454; found 377.9458.

(Z)-6-chloro-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24g)

Yellowish solid; yield: 65.2 mg (95 %); R_f (EtOAc/hexane; 80:20) = 0.90; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 182-185 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 1761, 1631, 1586, 1088; ^{1}H NMR (400 MHz) δ 7.93 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.13 – 7.00 (m, 4H); ^{13}C NMR (100 MHz) δ 190.4, 155.7, 139.8, 139.4, 138.7, 136.3, 131.2, 129.2, 129.1, 124.6, 124.0, 118.3, 115.9, 95.3; HRMS (ESI) calcd. for $C_{16}H_9Cl_2NO_3$ $[M+2]^+$: 334.9959; found 334.9956.

(Z)-6-chloro-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one(24h)

Yellowish solid; yield: 65.8 mg (88 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 135-137 °C; FT-IR (KBr, νmax/cm-1) 3432, 3075, 2923, 1626, 1583, 1105; ^{1}H NMR (400 MHz) δ 7.54 (d, J = 8.4 Hz, 1H), 7.47 (d, J = 1.6 Hz, 1H), 7.34 (dd, J = 1.6 Hz, 8.4 Hz, 1H), 7.17-7.09 (m, 3H), 6.82 (s, 1H); ^{13}C NMR (100 MHz) δ 192.1, 155.4, 139.9, 138.4, 137.7, 137.2, 132.7, 131.3, 130.8, 130.7, 127.6, 124.4, 118.4, 116.1, 99.3; HRMS (ESI) calcd. for $C_{16}H_8Cl_3NO_3$ $[M+2]^+$: 368.9570; found 368.9577.

(Z)-6-chloro-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one(24i)

Yellowish solid; yield: 57.8 mg (93 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 155-157 °C; FT-IR (KBr, νmax/cm^{-1}) 3434,1754,1634,1601, 1495, 1226,1160; ^{1}H NMR (400 MHz) δ 8.06-8.02 (m, 2H), 7.20-7.13 (m, 4H), 7.08-7.04 (m, 2H);^{13}C NMR (100 MHz) δ 190.4, 155.8, 139.8, 138.6, 131.3, 130.5, 130.4, 124.8, 123.9, 118.4, 116.2, 115.9, 115.8, 95.4; HRMS (ESI) calcd. for $C_{16}H_9ClFNO_3$ $[M+H]^+$: 318.0255; found 318.0259.

(Z)-6-chloro-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one(24j)

Yellowish solid; yield: 60.7 mg (95 %); R_f (EtOAc/hexane; 80:20) = 0.90; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 160-162 °C; FT-IR

(KBr, νmax/cm-1) 3434, 2925, 1624, 1766, 1494, 1178; ^{1}H NMR (400 MHz) δ 7.94-7.92 (m, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.14-7.04 (m, 4H), 2.44 (s, 3H);^{13}C NMR (100 MHz) δ 191.6, 156.0, 144.0, 139.8, 138.2, 135.6, 131.2, 129.7, 128.0, 125.0, 123.7, 118.3, 115.8, 95.9, 21.8; HRMS (ESI) calcd. for $C_{17}H_{12}ClNO_3$ $[M+H]^+$: 314.0506; found 314.0509.

(Z)-6-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24k)

Yellowish solid; yield: 57.2 mg (89 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by recrystallization using EtOAc/hexane; m.p. 198-200 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 2928, 1762, 1625, 1581, 1142; ^{1}H NMR (400 MHz) δ 8.03-7.96 (m, 4H), 7.61-7.49 (m, 3H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.15 (s, 1H); ^{13}C NMR (100 MHz) δ 192.1, 155.1, 145.2, 144.9, 137.7, 137.6, 133.4, 128.9, 127.9, 124.7, 118.9, 118.0, 111.5, 96.9; HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_5$ $[M+H]^+$: 311.0590; found 311.0593.

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (24l)

Yellowish solid; yield: 60.4 mg (86 %); R_f (EtOAc/hexane; 80:20) = 0.75; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 195-197 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2926, 1599, 1758, 1633, 1594; ^{1}H NMR (400 MHz) δ 8.02 – 7.93 (m, 4H), 7.29 (d, *J* = 8.9 Hz, 1H), 7.10 (s, 1H), 6.98 (d, *J* = 8.7 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR (100 MHz) δ 190.7, 164.0, 155.3, 145.2, 144.9, 137.0, 130.7, 130.3, 124.9, 118.6, 117.8, 114.2, 111.2, 97.0, 55.7; HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_6$ $[M+H]^+$: 341.0695; found 341.0692.

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (24m)

Yellowish solid; yield: 62.2 mg (94 %); R_f (EtOAc/hexane; 80:20) = 0.75; Purification of crude product was done by recrystallization using EtOAc/hexane; m.p. > 250 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 3107, 1759,1622, 1594, 1156; ^{1}H NMR (400 MHz) δ 8.73 (s, 1H), 8.11 (d, *J* = 5.2 Hz, 2H), 7.92 (d, *J* = 6.4 Hz, 1H), 7.44 – 7.36 (m, 3H), 6.92 (s, 1H); ^{13}C NMR (100 MHz) δ 188.7, 156.0, 145.9, 144.6, 139.5, 135.1, 130.9, 125.8, 118.9, 117.7, 116.6, 116.4, 113.0, 94.5; HRMS (ESI) calcd. for $C_{16}H_9FN_2O_5$ $[M+H]^+$: 329.0495; found 329.0490.

(Z)-7-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24n)

Yellowish solid; yield: 57.5 mg (89 %); R_f (EtOAc/hexane; 80:20) = 0.70; Purification of crude product was done by recrystallization using EtOAc/hexane; m.p. 240-242 °C; FT-IR (KBr, νmax/cm-1) 3436, 1763, 1622, 1596, 1268; 1H NMR (400 MHz) δ 8.06 - 8.00 (m, 4H), 7.83 – 7.81 (m, 1H), 7.62 (t, J = 7.3 Hz, 1H), 7.54 (t, J = 7.5 Hz, 2H), 6.99 (s, 1H); 13C NMR (100 MHz) 190.7, 156.1, 142.2, 141.1, 139.2, 138.3, 133.6, 131.3, 129.6, 128.0, 121.4, 117.4, 112.6, 96.0; HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_5$ $[M+H]^+$: 311.0590; found 311.0595.

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one(24o)

Yellowish solid; yield: 60.8 mg (92 %); R_f (EtOAc/hexane; 80:20) = 0.70; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (8:2) as an eluent; m.p. 218-220 °C; FT-IR (KBr, νmax/cm-1) 3437, 2927, 2854, 1632, 1517; ^{1}H NMR (400 MHz) δ 8.07-8.02 (m, 4H), 7.73 (d, J = 10.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 2H), 7.02 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz) δ 189.1, 163.4, 155.8, 141.7, 140.6, 138.1, 131.1, 130.7, 130.1, 121.2, 116.8, 114.5, 112.2, 95.9, 55.8; HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_6$ $[M+H]^+$: 341.0695; found 341.0699.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24p)

Yellowish solid; yield: 69.6 mg (87 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by recrystallization using EtOAc/hexane; m.p. 230-232 °C; FT-IR (KBr, νmax/cm-1) 3435, 3093, 1769, 1619, 1521; ^{1}H NMR (400 MHz) δ 8.09-7.96 (m, 4H), 7.83-7.44 (m, 3H), 7.01(d, J = 4.8 Hz, 1H); ^{13}C NMR (100 MHz) δ 188.9, 154.7, 141.8, 140.1, 137.9, 136.6, 131.5, 130.0, 129.1, 126.6, 120.7, 116.5, 111.4, 95.7; HRMS (ESI) calcd. for $C_{16}H_9BrN_2O_5$ $[M+2]^+$: 389.9695; found 389.9691.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (24q)

Yellowish solid; yield: 63.2 mg (90 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 205-207 °C; FT-IR (KBr,

νmax/cm-1) 3432, 2925, 2860, 1633, 1525, 1776, 1075; ^{1}H NMR (400 MHz) δ 8.08 (d, J = 8.4 Hz, 4H), 7.89-7.87 (m, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.01 (s, 1H); ^{13}C NMR (100 MHz) δ 188.9, 155.4, 141.9, 140.7, 138.9, 136.5, 130.7, 129.8, 129.5, 129.2, 120.9, 117.0, 112.1, 95.2; HRMS (ESI) calcd. for $C_{16}H_9ClN_2O_5$ $[M+H]^+$: 345.0200; found 345.0207.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (25a)

Yellowish solid; yield: 68.8 mg (93 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. > 250 °C; FT-IR (Neat, νmax/cm^{-1}) 2969,1756,1601,1549,1170; ^{1}H NMR (400 MHz) δ 8.52 (s, 1H), 8.07 – 8.02 (m, 4H), 7.51 – 7.47 (m, 4H), 7.23 – 7.14 (m, 4H), 6.75 (s, 1H); ^{13}C NMR (100 MHz) δ 198.8, 155.9, 141.6,138.4, 135.7, 131.3, 129.0, 128.8, 127.9, 126.8, 126.1, 125.6, 125.2, 124.5, 123.7, 117.3, 116.3, 101.7; HRMS (ESI) calcd. for$C_{24}H_{15}NO_3$ $[M+H]^+$: 366.1052; found 366.1059.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (25b)

Reddish solid; yield: 69.2 mg (86 %); R_f (EtOAc/hexane; 80:20) = 0.70; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (8:2) as an eluent; m.p. > 250 °C; FT-IR (Neat, νmax/cm^{-1}) 2924, 2852,1766,1624,1437,1129; ^{1}H NMR (400 MHz) δ 8.72 (s, 1H), 8.17 – 8.04 (m, 6H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.58 – 7.55 (m, 4H), 6.56 (s, 1H); ^{13}C NMR (100 MHz) δ 196.6, 154.8, 141.9, 140.3, 136.8, 135.2, 130.4, 130.1, 128.4, 128.2, 126.8, 126.5, 125.3, 124.1, 116.8, 111.8, 111.6, 102.2; HRMS (ESI) calcd. for$C_{24}H_{14}N_2O_5$ $[M+H]^+$: 411.0903; found 411.0909.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (25c)

Reddish solid; yield: 74.3 mg (89 %); R_f (EtOAc/hexane; 80:20) = 0.70; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (8:2) as an eluent; m.p. > 250 °C; FT-IR (Neat, νmax/cm^{-1}) 2919, 2853,1728,1605,1461, 1193; ^{1}H NMR (400 MHz) δ 8.52 (s, 1H), 8.07 – 7.96 (m, 6H), 7.51 – 7.25 (m, 5H), 6.82 (s, 1H); ^{13}C NMR (100 MHz) δ 195.6, 154.6, 150.0, 144.9, 144.2, 136.9, 135.2, 130.4, 128.2, 128.0, 127.8, 126.8, 126.2, 124.9,

124.4, 117.9, 116.8, 112.3; HRMS (ESI) calcd. for $C_{24}H_{14}N_2O_5$ $[M+H]^+$: 411.0903; found 411.0907.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (25d)

Reddish solid; yield: 71.1 mg (92 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 180-182 °C; FT-IR (Neat, νmax/cm^{-1}) 2925, 1745,1611,1570,1448,1148; ^{1}H NMR (400 MHz) δ 8.49 (s, 1H), 8.07 – 8.00 (m, 4H), 7.49 – 7.46 (m, 4H), 7.08 – 6.89 (m, 3H), 6.72 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz) δ 198.5, 156.1, 139.6, 138.5, 136.1, 135.8, 131.3, 128.9, 128.7, 127.9, 126.7, 125.6, 125.3, 125.2, 123.2, 116.9, 116.5, 101.4, 21.0; HRMS (ESI) calcd. for $C_{25}H_{17}NO_3$ $[M+H]^+$: 380.1208; found 380.1203.

(Z)-3-(2-(2-bromophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (26)

Yellowish solid; yield: 65.8 mg (94 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p.= 140-142 °C; FT-IR (Neat) 3041, 1747, 1620, 1571, 1497, 1131; 1H NMR (400 MHz) δ 7.61 (d, J = 7.2 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.37 (t, J = 6.4 Hz, 1H), 7.28 (t, J = 6.4 Hz, 1H), 7.19-7.08 (m, 4H), 6.71 (s, 1H); 13C NMR (100 MHz) δ 194.0, 155.9, 141.4, 141.1, 138.8, 134.0, 131.8, 129.6, 127.6, 126.0, 124.4, 123.5, 119.7, 117.2, 116.2, 98.3; HRMS (ESI) calcd. for C16H10BrNO3 [M+2]+: 344.9844; found 344.9849.

(Z)-5-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (27)

Yellowish solid; yield: 55.7 mg (88 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p.= 210-212 °C; FT-IR (Neat) 3054, 1766, 1622, 1584, 1461, 1041; 1H NMR (400 MHz) δ 8.16 (d, J = 8.8 Hz, 1H), 8.08 (d, J = 7.2 Hz, 2H), 7.59 (t, J = 7.6 Hz, 1H), 7.52-7.47 (m, 3H), 7.29 (s, 1H), 7.15 (t, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz) δ 191.5, 155.1, 142.6, 137.9, 135.5, 134.0, 133.5, 128.9, 128.3, 123.0, 122.8, 122.6, 121.6, 99.7; HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_5$ $[M+H]^+$: 311.0590; found 311.0596.

(Z)-6-methyl-3-(2-oxopentylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (28)

Sticky yellowish solid; yield: 39.5 mg (79 %); R_f (EtOAc/hexane; 90:10) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; FT-IR (Neat) 3026, 2923, 1731, 1571, 1491, 1100; 1H NMR (400 MHz) δ 7.02 (d, J = 8.0 Hz, 1H), 6.85-6.81 (m, 2H), 6.28 (s, 1H), 2.49 (t, J = 7.2 Hz, 2H), 2.32 (s, 3H), 1.71-1.65 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); 13C NMR (100 MHz) δ 203.2, 156.6, 139.1, 137.5, 135.9, 124.4, 123.6, 116.8, 115.9, 97.7, 45.4, 21.1, 18.8, 13.9; HRMS (ESI) calcd. for C14H15NO3 [M+H]+: 246.1052; found 246.1059.

(Z)-3-(2-bicyclo[2.2.1]hept-5-en-2-yl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (29)

Yellowish solid; yield: 43.4 mg (76 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 75-77 °C; FT-IR (Neat, νmax/cm^{-1}) 2965,2870,1753,1637,1501,1447,1104; ^{1}H NMR (400 MHz) δ 12.40 (s, 1H), 7.17 – 6.99 (m, 2H), 6.39 (s, 1H), 6.19 – 6.16 (m, 2H), 3.03 – 2.95 (m, 2H), 2.53 – 2.49 (m, 1H), 2.00 – 1.95 (m, 1H), 1.57 – 1.45 (m, 2H), 1.40 – 1.24 (m, 3H); ^{13}C NMR (100 MHz) δ 205.1, 156.5, 140.9, 138.5, 137.2, 136.1, 125.9, 124.0, 123.6, 117.2, 115.6, 95.6, 51.6, 47.0, 46.0, 42.0, 29.7; HRMS (ESI) calcd. for :$C_{17}H_{15}NO_3$ $[M+H]^+$: 282.1052; found 282.1058.

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (31a)

Yellowish solid; yield: 51.4 mg (95 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by Recrystallization using EtOAc/hexane; m.p. 240-242°C;^{1}H NMR (400 MHz) δ 10.0 (s, 1H), 8.07-8.05 (m, 2H), 7.55 – 7.48 (m, 3H), 7.21 – 7.12 (m, 4H), 7.03 (s, 1H); ^{13}C NMR (100 MHz) δ 187.9, 155.2, 145.4, 138.4, 131.2, 128.2, 126.5, 123.9, 123.6, 123.1, 116.1, 115.1, 114.9, 89.0; HRMS (ESI) calcd. for $C_{16}H_{12}N_2O_2$ $[M+H]^+$: 265.0899; found 265.0893.

(Z)-6-methyl-3-(2-oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (31b)

Yellowish solid; yield: 52.7 mg (93 %); R_f (EtOAc/hexane; 80:20) = 0.85; Purification of crude product was done by recrystallization using EtOAc/hexane; m.p. 225-227 °C; FT-IR (Neat, νmax/cm^{-1}) 2915, 2861, 1735, 1685,1490,1138;^{1}H NMR (400 MHz) δ 11.56 (s, 1H), 7.99-7.94 (m, 2H), 7.52 – 7.50 (m, 3H), 7.29 – 6.95 (m, 3H), 6.79 (t, J = 8.4 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz) δ 186.8, 155.2,

154.9, 145.7, 138.4, 133.4, 132.7, 130.9, 127.7, 126.5, 126.2, 124.2, 121.9, 88.5, 19.9; HRMS (ESI) calcd. for$C_{17}H_{14}N_2O_2$ $[M+H]^+$: 279.1055; found 279.1059.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (32a)

Yellowish solid; yield: 64.6 mg (90 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. > 250 °C; FT-IR (Neat, νmax/cm^{-1}) 2920,1671,1596,1455,1131; ^{1}H NMR (400 MHz) δ 11.86 (s, 1H), 8.66 (d, *J* = 6.0 Hz, 1H), 8.13 – 8.09 (m, 4H), 7.59 – 7.47 (m, 5H), 7.20 – 7.19 (m, 3H), 6.37 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz) δ 193.7, 155.1, 144.5, 136.2, 130.5, 128.2, 127.9, 127.3, 126.8, 126.7, 125.9, 125.8, 125.0, 124.9, 124.7, 123.8, 116.3, 96.2; HRMS (ESI) calcd. for $C_{24}H_{16}N_2O_2$ $[M+H]^+$: 365.1212; found 365.1218.

(Z)-3-(2-(anthracen-9-yl)-2-oxoethylidene)-6-methyl-3,4-dihydroquinoxalin-2(1*H*)-one (32b)

Yellowish solid; yield: 70.3 mg (92 %); R_f (EtOAc/hexane; 80:20) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 220-222°C; FT-IR (Neat, νmax/cm^{-1}) 2920,2852,1602,1536,1486,1135; ^{1}H NMR (400 MHz) δ 11.81 (s, 1H), 8.65 (s, 1H), 8.21 – 8.11 (m, 4H), 7.53 – 7.39 (m, 5H), 7.12 – 7.04 (m, 2H), 6.35 (d, *J* = 3.6 Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz) δ 192.8, 155.1, 144.6. 136.2, 133.7, 132.9, 130.5, 128.1, 127.9, 127.2, 127.1, 126.9, 126.7, 125.8, 125.7, 124.9, 124.5, 124.3, 123.6, 121.7, 116.4, 115.3, 95.9, 20.2; HRMS (ESI) calcd.for$C_{25}H_{18}N_2O_2$ $[M+H]^+$: 379.1368; found 379.1361.

(Z)-3-(2-bicyclo[2.2.1]hept-5-en-2-yl)-2-oxoethylidene)-3,4-dihydroquinoxalin-2(1*H*)-one (33a)

Yellowish solid; yield: 48.2 mg (84 %); R_f (EtOAc/hexane; 90:10) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 190-192 °C; FT-IR (Neat, νmax/cm^{-1}) 2932,2866,1739,1678,1121,1027; ^{1}H NMR (400 MHz) δ 11.52 (s, 1H), 7.28 – 7.22 (m, 1H), 7.09 – 7.00 (m, 2H), 6.15 – 6.07 (m, 2H), 2.99 – 2.86 (m, 4H), 2.46 – 2.39 (m, 1H),1.91 – 1.82 (m, 1H), 1.48 – 1.22 (m, 3H); ^{13}C NMR (100 MHz) δ 201.3, 155.3, 142.9, 137.3, 135.8, 125.9, 124.1, 122.9, 115.2, 92.7, 49.6, 46.2,

46.0, 45.2, 40.9, 40.8, 29.1; HRMS (ESI) calcd. for : $C_{17}H_{16}N_2O_2$ $[M+H]^+$: 281.1212; found 281.1218.

(Z)-3-(2-bicyclo[2.2.1]hept-5-en-2-yl)-2-oxoethylidene)-6-methyl-3,4-dihydro-quinoxalin-2(1*H*)-one (33b)

Yellowish solid; yield: 46.2 mg (80 %); R_f (EtOAc/hexane; 90:10) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9.8:0.2) as an eluent; m.p. 165-167°C; FT-IR (Neat, νmax/cm^{-1}) 2968,2866,1679,1574,1190; ^{1}H NMR (400 MHz) δ 11.27-11.12 (m, 1H), 7.03 – 6.81 (m, 2H), 6.32 – 6.30 (m, 1H), 6.17 (s, 1H), 3.32 – 2.94 (m, 2H), 2.53 – 2.31 (m, 4H), 2.02 – 1.24 (m, 6H); ^{13}C NMR (100 MHz) δ 203.6, 158.4, 142.9, 138.3, 136.3, 132.2, 125.7, 122.9, 115.8, 93.9, 50.9, 50.2, 47.3, 46.1, 42.0, 30.0, 28.5, 21.1; HRMS (ESI) calcd. for$C_{18}H_{18}N_2O_2$ $[M+H]^+$: 295.1368; found 295.1362.

2.2.5.5 Synthesis of 3-(1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (35)

To a solution of the compound **20a** (130.8 mg, 1.20 mmol) in water was added 3-Indoleglyoxylic acid **34** (226.9 mg, 1.20 mmol); and the reaction mixture was irradiated under ultrasonic sonicator at 80 ˚C temperature for about 180 min. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was filtered off, washed with distilled water (3 × 50 ml) and dried it, which furnished the crude product. The crude product was further purified by recrystalization using EtOAc/hexane and EtOH; which afforded the pure desired Cephalandole A **35** having good yield (255.4 mg, 81 %). Yellowish solid; yield: 255 mg (81) %, m.p. 230-232 °C; FT-IR (KBr, νmax/cm-1) 3292, 3053, 2921, 2852, 1713, 1604, 1428, 1151; ^{1}H NMR (400 MHz) δ 11.99 (s, 1H), 8.77 – 8.75 (m, 1H), 8.70 (s, 1H), 7.84 (d, J = 6.4 Hz, 1H), 7.55 – 7.53 (m, 1H), 7.48 – 7.38 (m, 3H), 7.29 – 7.24 (m, 2H); ^{13}C NMR (100 MHz) δ 152.1, 147.9, 144.9, 136.6, 133.8, 131.9, 128.7, 127.7, 126.0, 125.3, 123.1, 122.9, 121.6, 115.9, 112.2, 110.6; HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_2$ $[M+H]^+$: 263.0742; found 263.0749.

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2.2.7 Characterization spectra (^{1}H and ^{13}C NMR) of selected compounds:

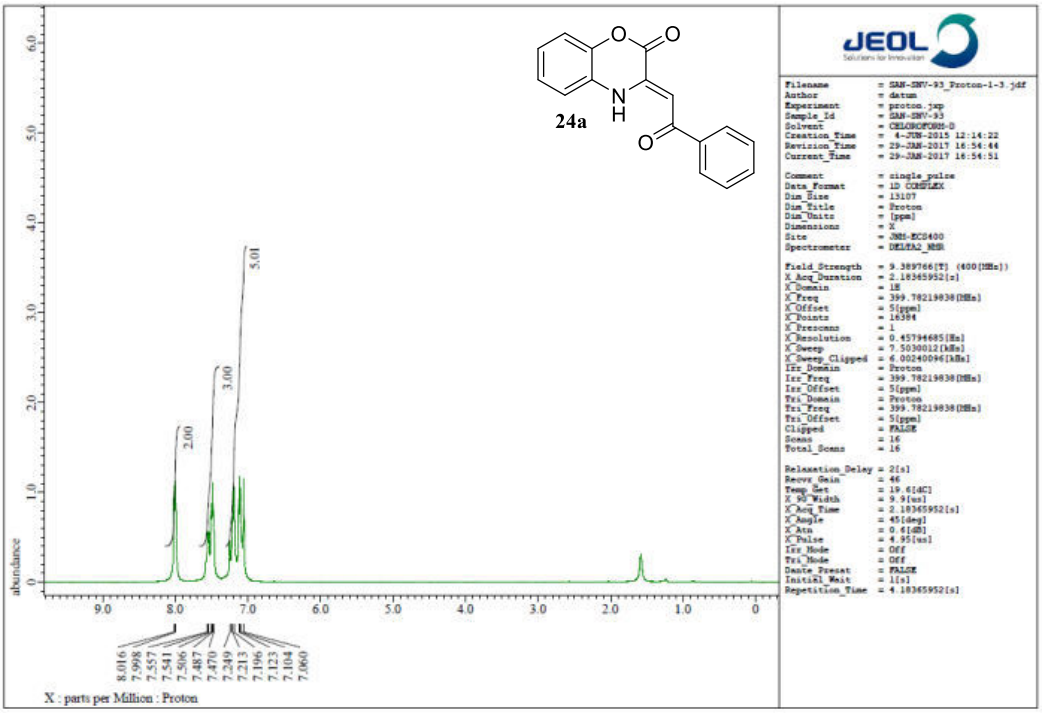


Figure 5.^{1}H NMR Spectra of Compound **24a**.

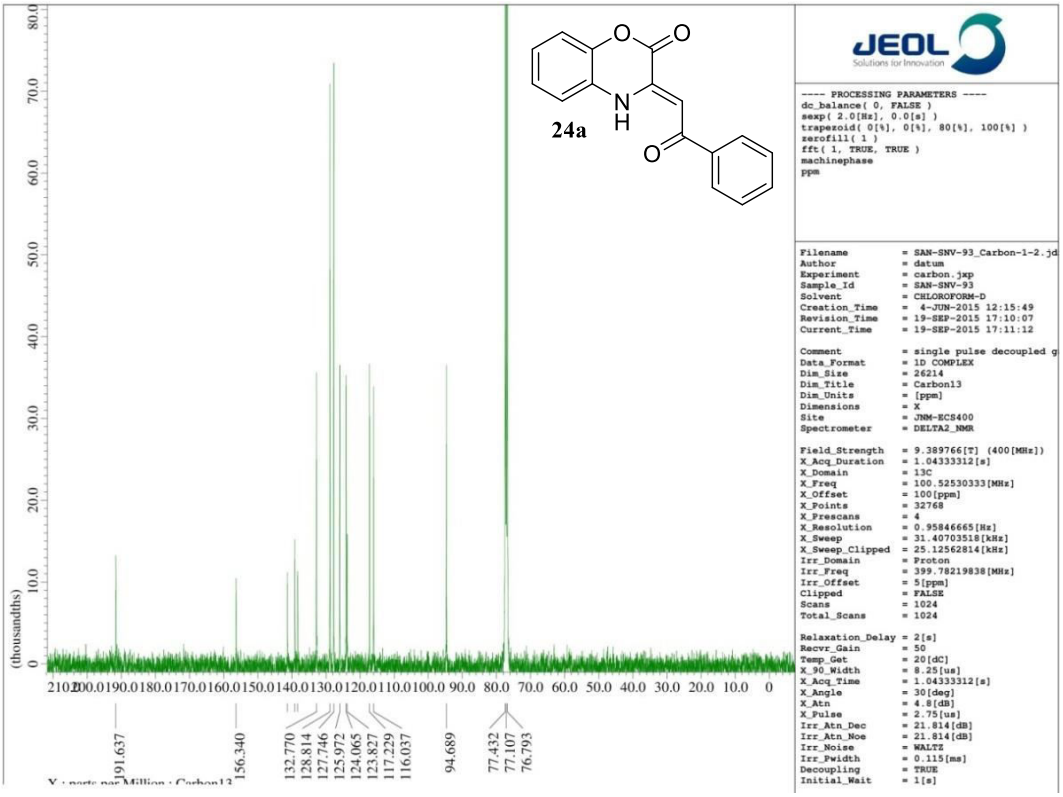


Figure 6. ^{13}C NMR Spectra of Compound **24a**.

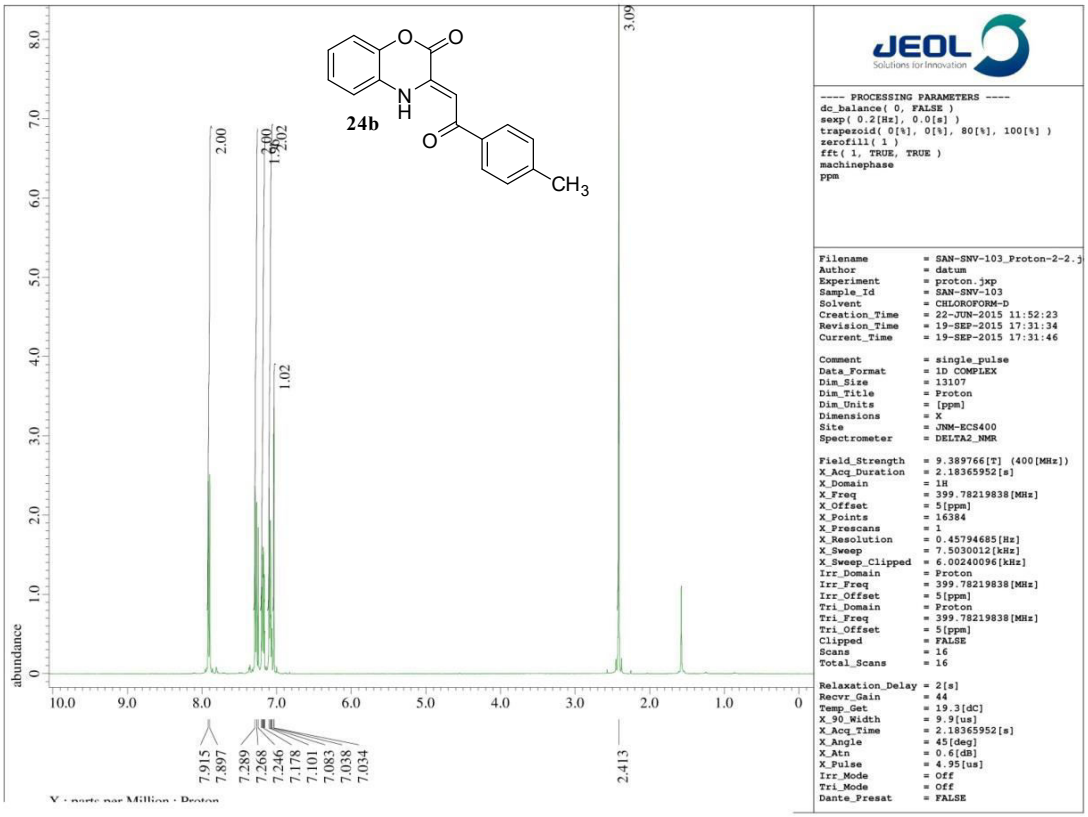


Figure 7.[1]H NMR Spectra of Compound **24b**.

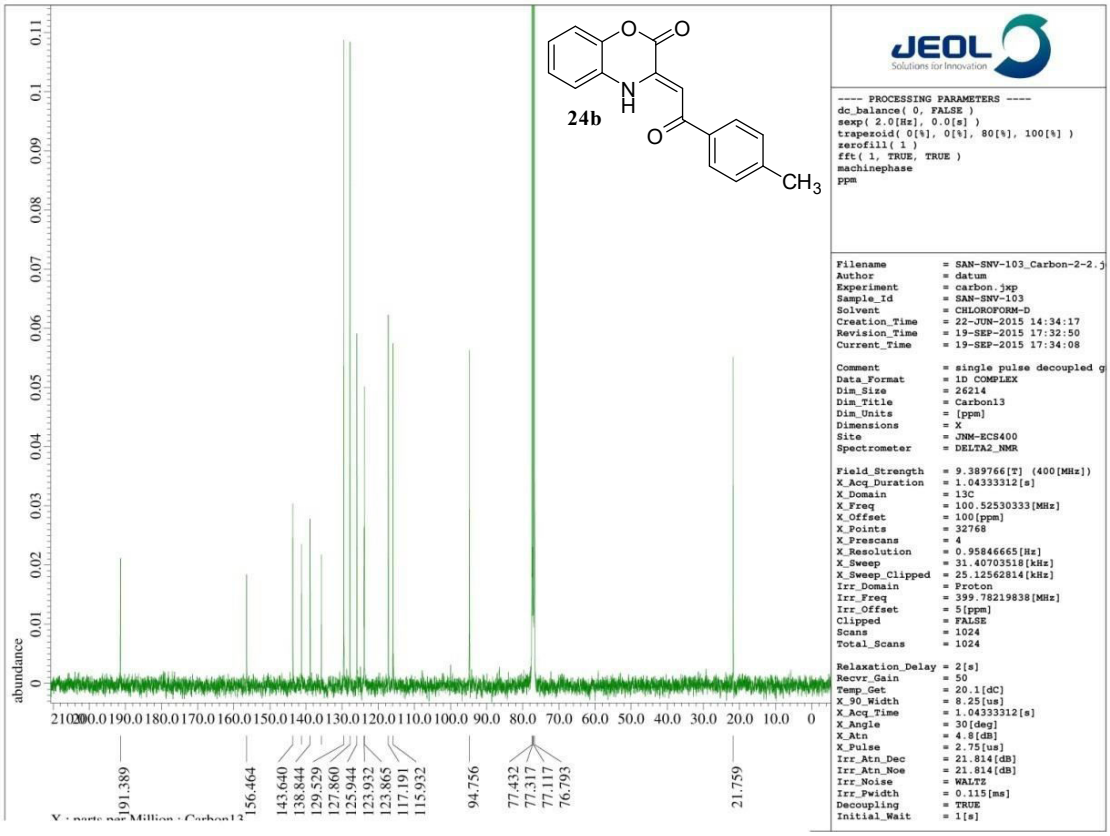


Figure 8.[13]C NMR Spectra of Compound **24b**.

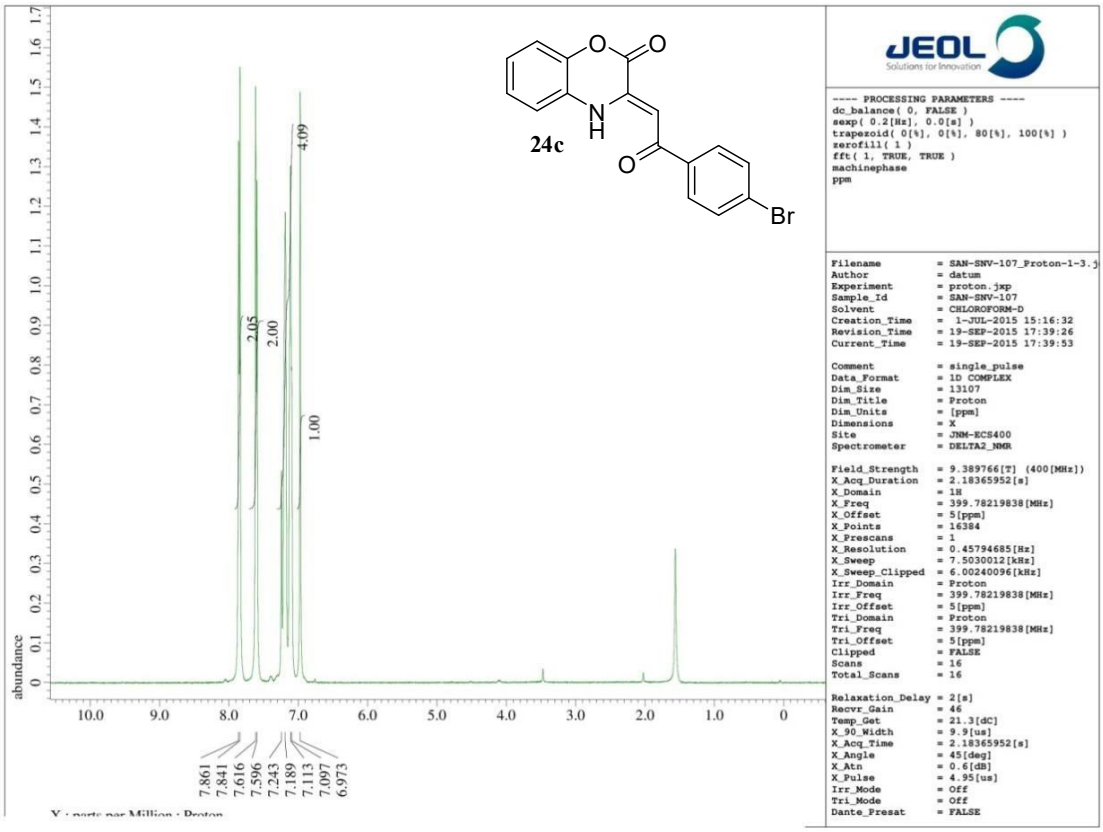


Figure 9.[1]H NMR Spectra of Compound **24c**.

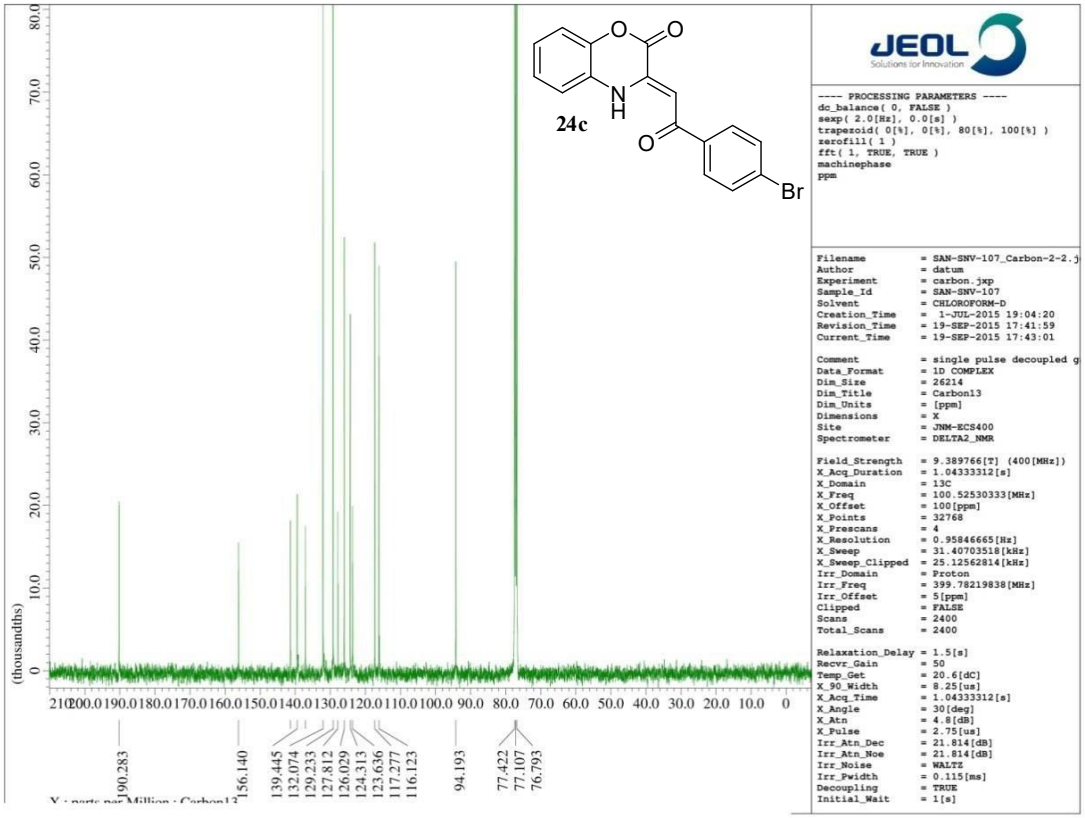


Figure 10.[13]C NMR Spectra of Compound **24c**.

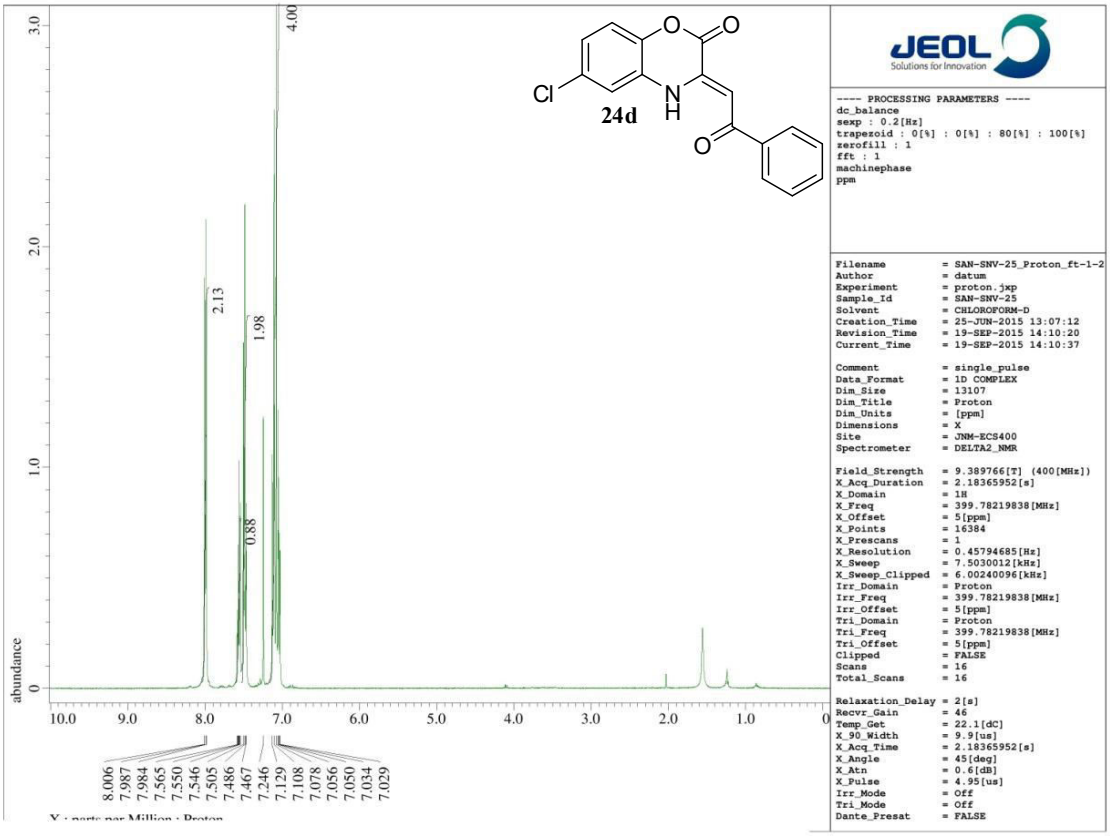


Figure 11.[1]H NMR Spectra of Compound **24d**.

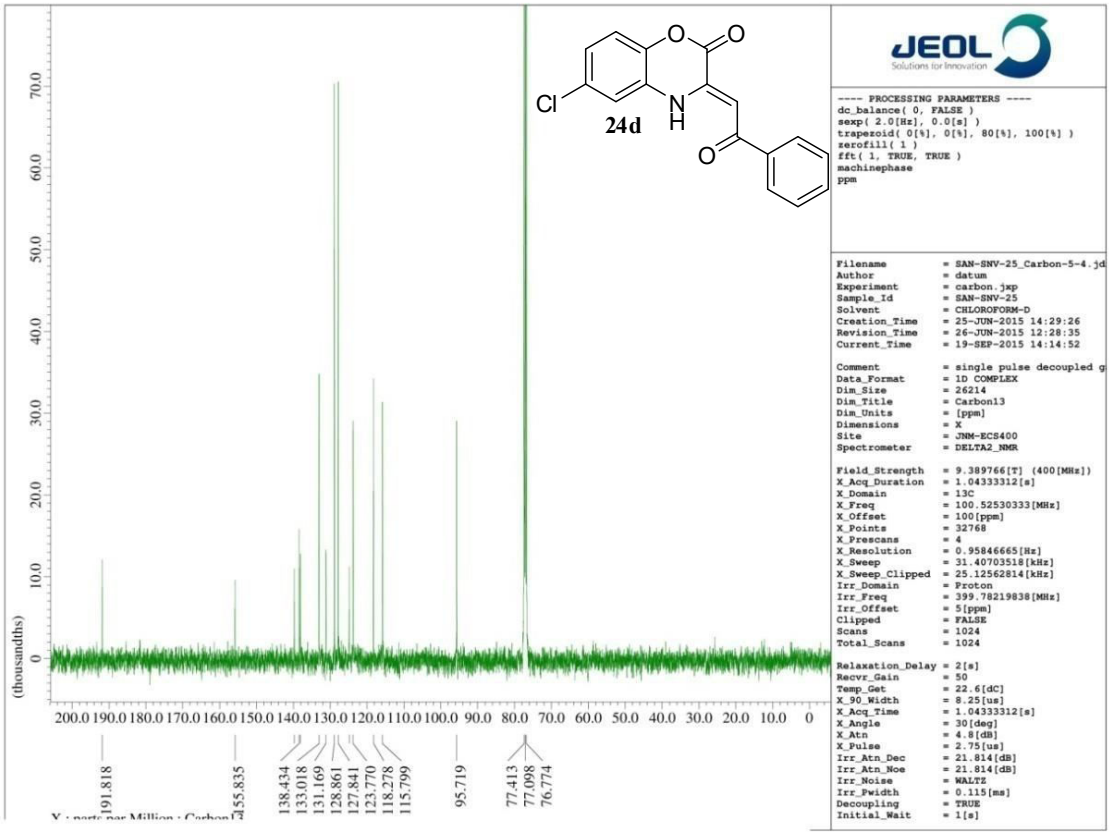


Figure 12.[13]C NMR Spectra of Compound **24d**.

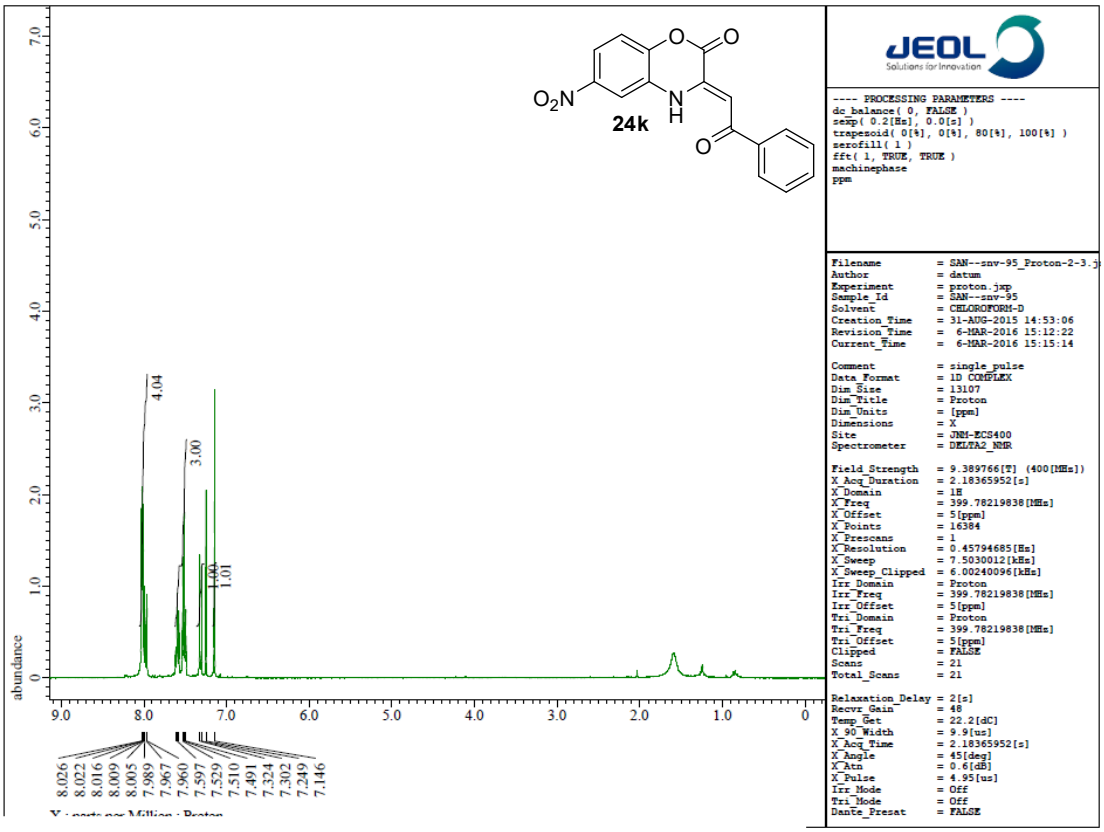


Figure 13. ^{1}H NMR Spectra of Compound **24k**.

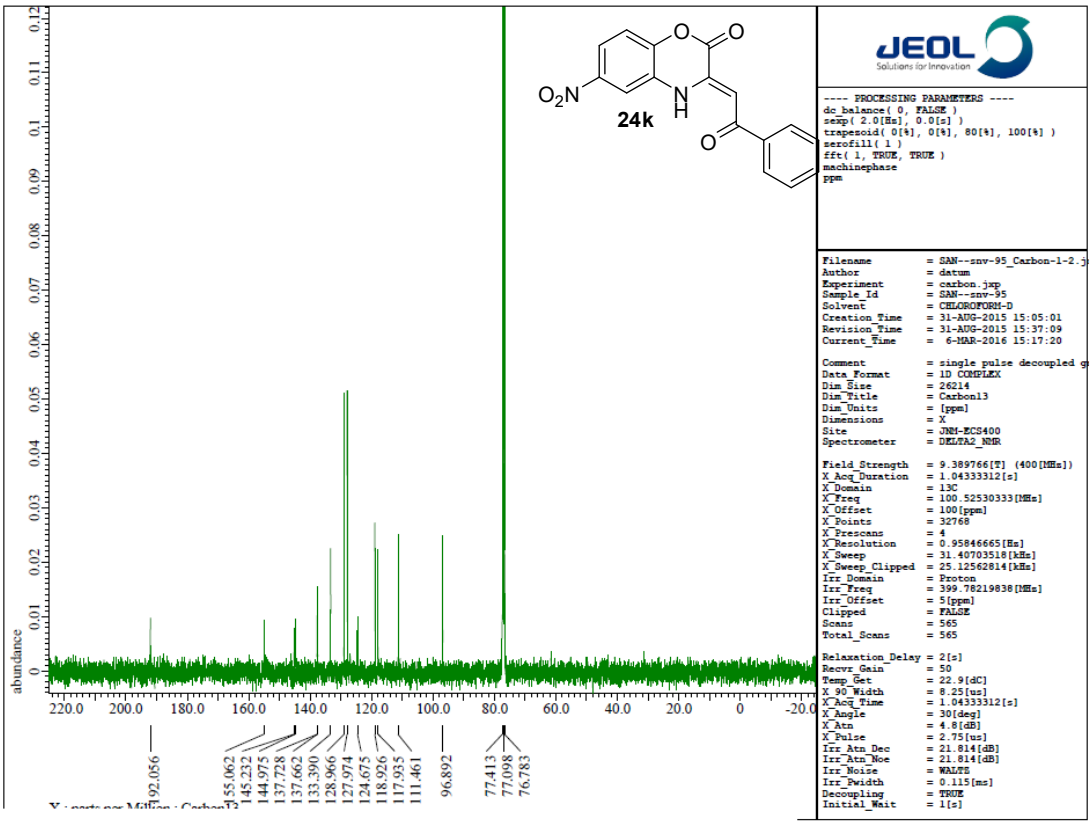


Figure 14. ^{13}C NMR Spectra of Compound **24k**.

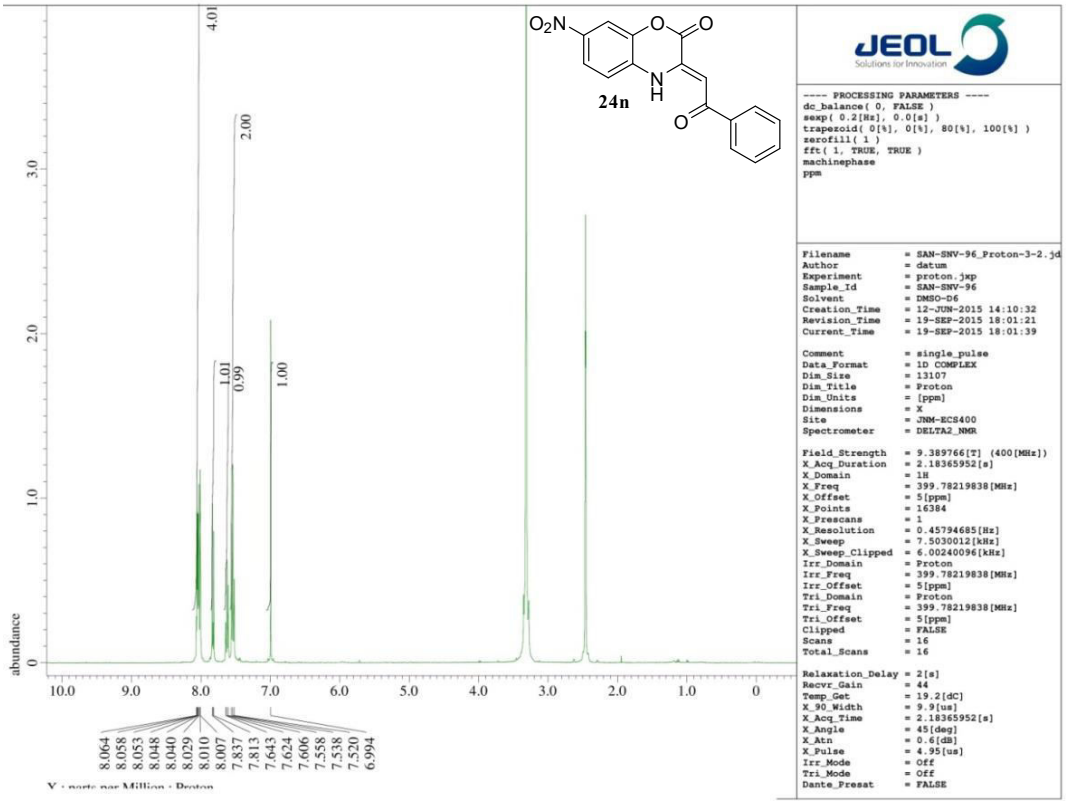


Figure 15. ^{1}H NMR Spectra of Compound **24n**.

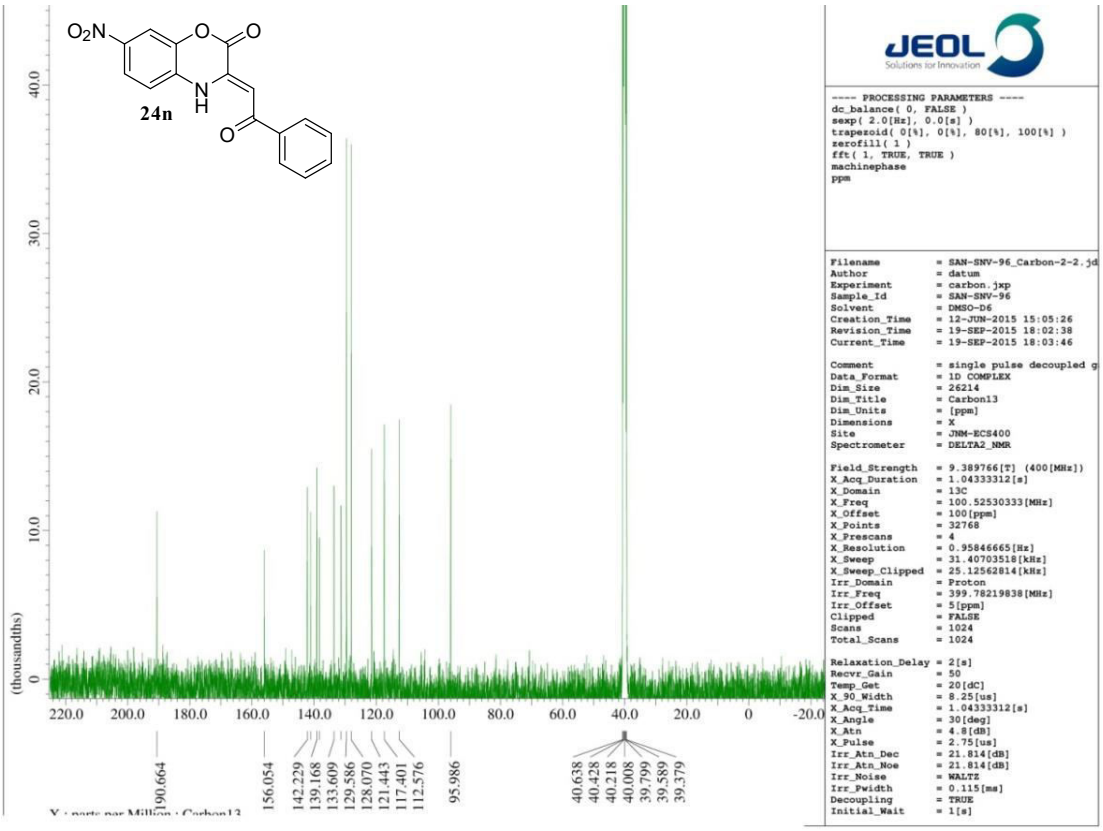


Figure 16. ^{13}C NMR Spectra of Compound **24n**.

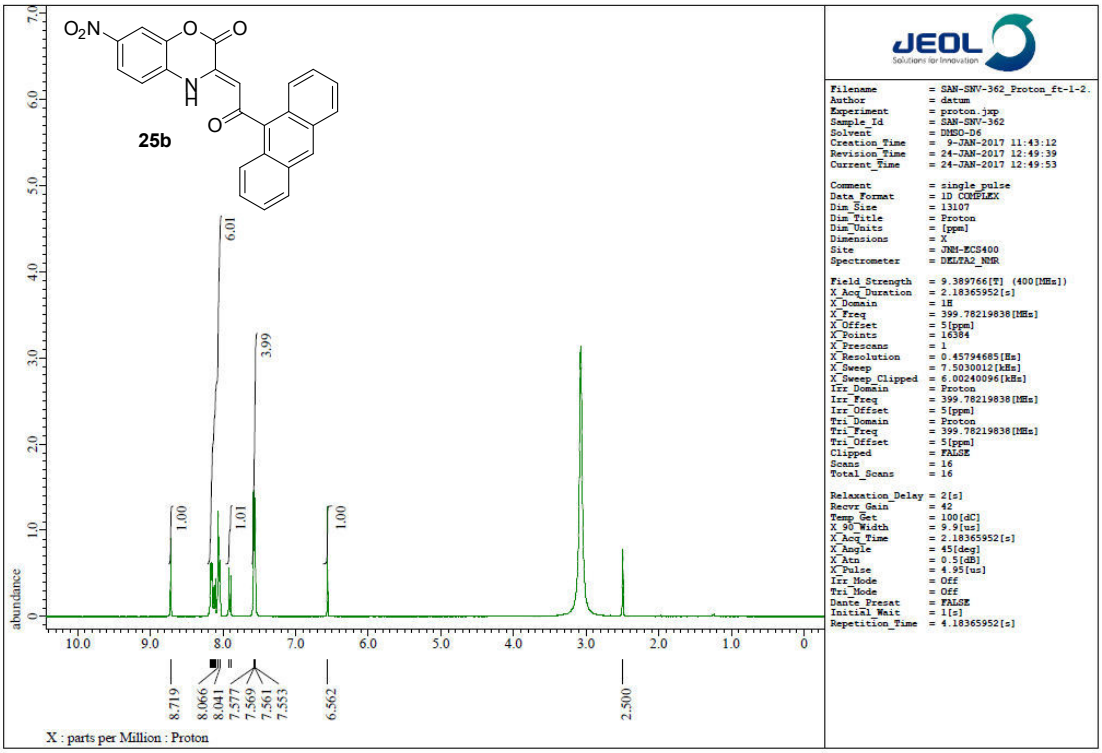


Figure 17. ^{1}H NMR Spectra of Compound **25b.**

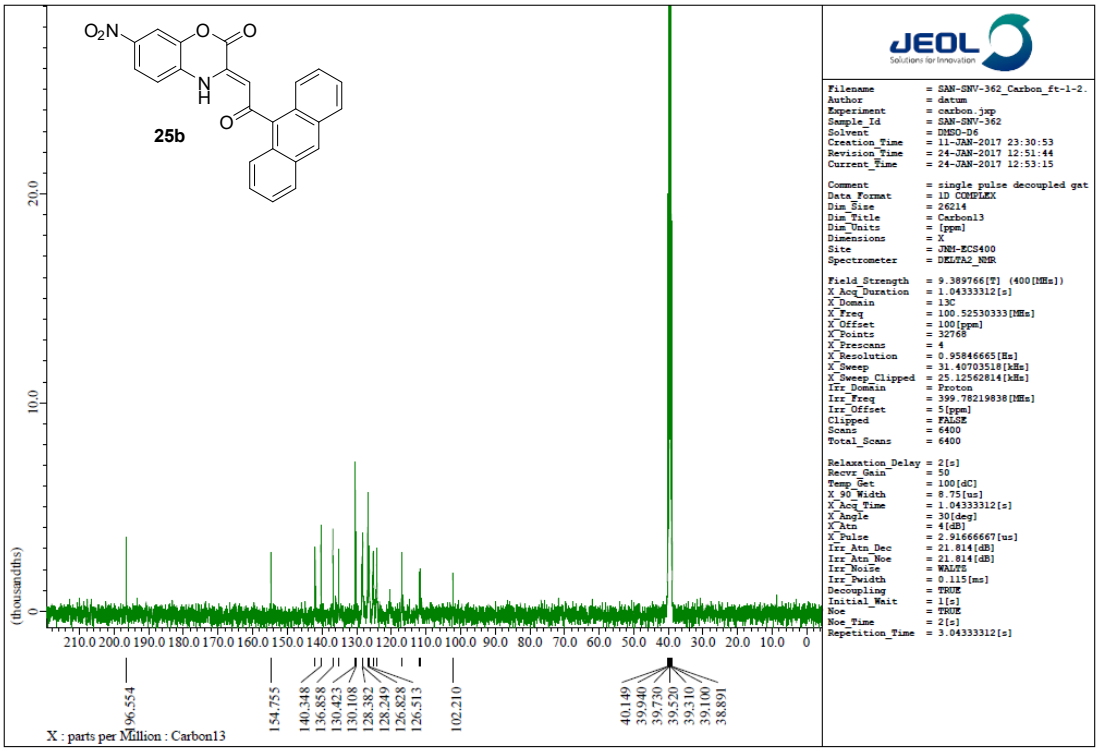


Figure 18. ^{13}C NMR Spectra of Compound **25b**.

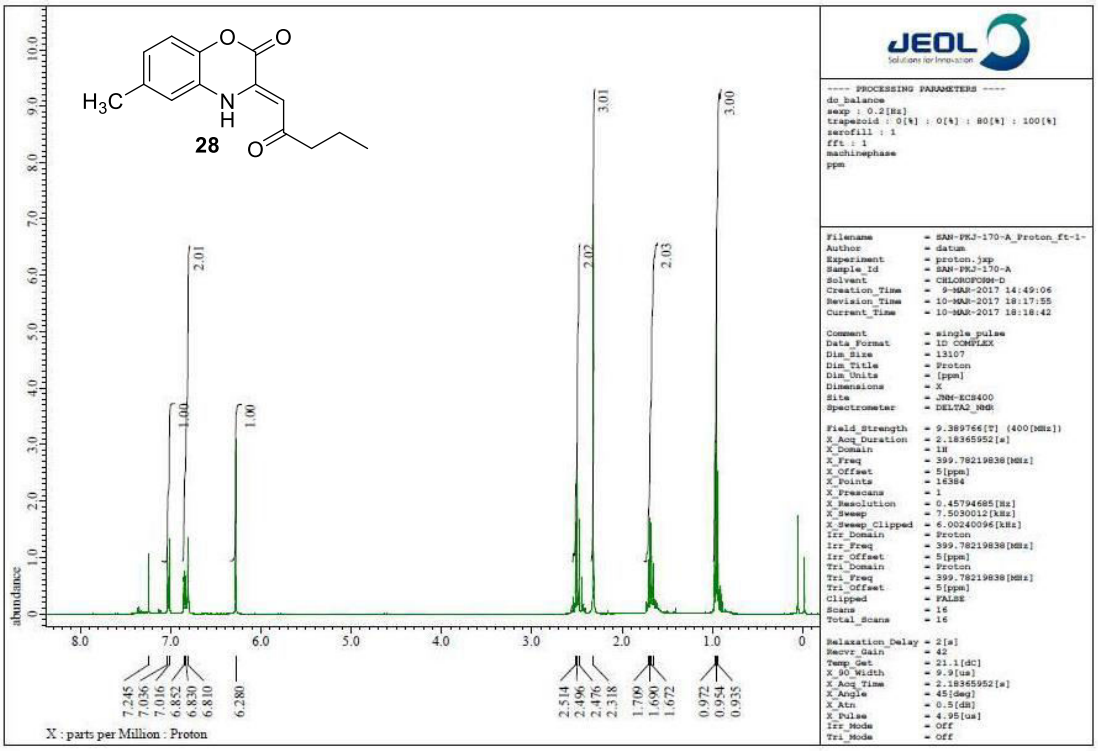


Figure 19.[1]H NMR Spectra of Compound **28**.

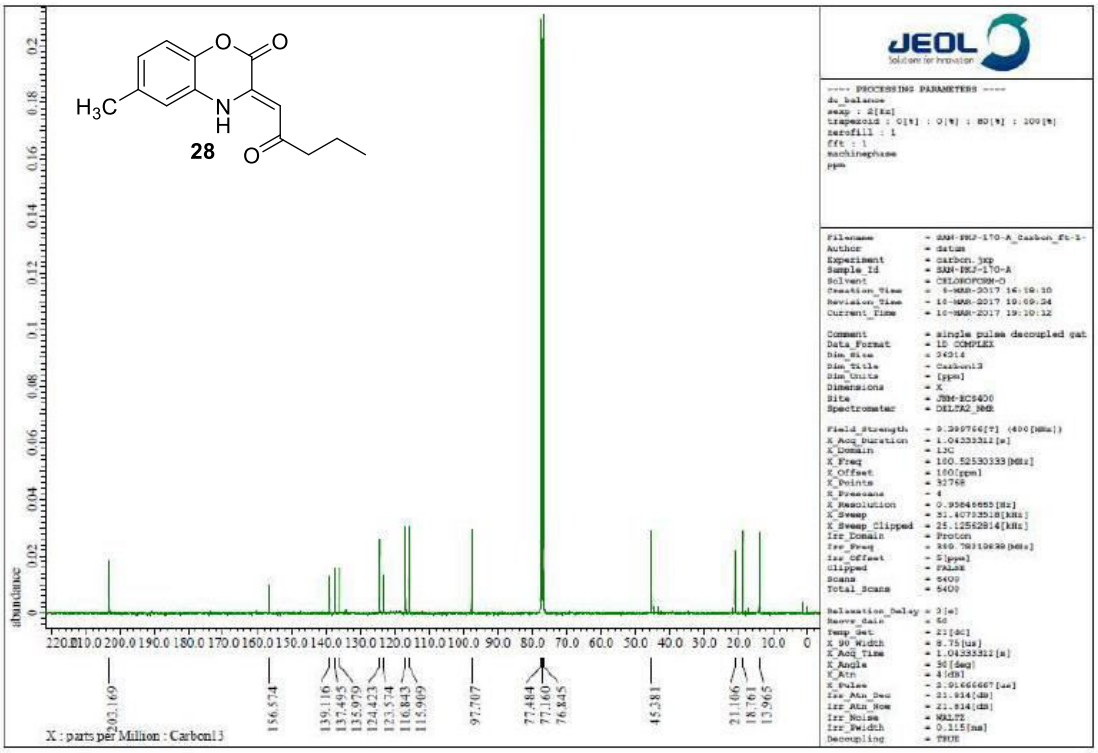


Figure 20. [13]C NMR Spectra of Compound **28**.

Section 2.3A

Rational Design, Synthesis, SAR and Molecular Docking simulation studies of C-3 tethered 2-oxo-benzyl-benzo[1,4]oxazines as potent antioxidants

2.3A.1 Introduction

"Antioxidant" are primarily reducing agents/compounds which refer to the activity of numerous vitamins, minerals and phytochemicals such as vitamin E, vitamin C, glutathione etc. by providing protection against the damage caused by reactive oxygen species (ROS).[1] It is known that ROS like superoxides (O_2^{2-}), peroxyls ($ROO^{\bullet}$), hydroxyls ($HO^{\bullet}$), alkoxyls ($RO^{\bullet}$), nitric oxides ($NO^{\bullet}$), play an important role in the development of dysfunction of body mechanism associated with several pathological metabolism, such as cardiovascular diseases, metabolic disorders, and even carcinogenesis.[2-4] Therefore, the human body is capable to neutralize ROS by antioxidant defence mechanisms by eradicating an excess of ROS from the cell.[5] An imbalances between the detoxification of ROS with respect to their production leads to a phenomena known as "oxidative stress (OS)" which is correlated to several diseases such as aging,[6] stroke,[7] myocardial infarction,[8] cancer,[9] Parkinson's disease[10] and Alzheimer's disease.[11] Therefore, the development of natural as well as synthetic antioxidants, which are able to scavenge ROS and keep cell integrity via prevention or reduction of the impact of OS on cells, is now currently an recognized area of research interest.

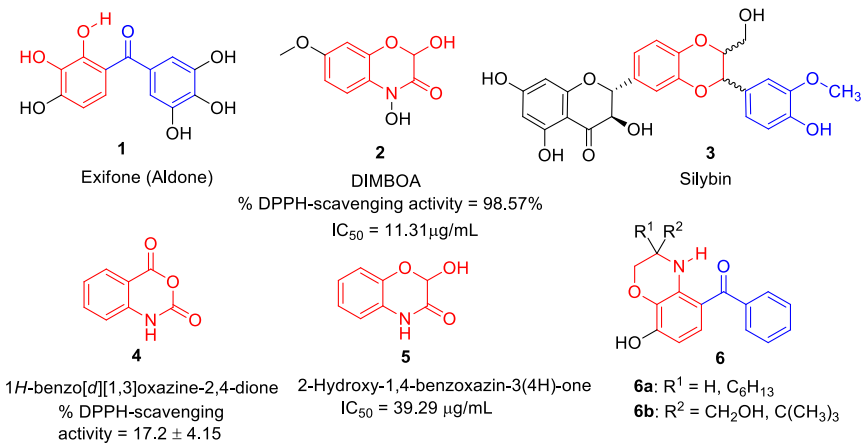


Figure 1. Structures of exifone (Aldone[R]) and natural as well as synthetic benzoxazines scaffold (**1-6**) having antioxidant activity.

Among natural as well as synthetic class of antioxidants; during the last decade, benzoxazines, benzodioxine and its derivatives have emerged as a possible antioxidants.[12-14] In this context, exifone **1**,[15] dimboa **2**,[16] sylbin **3**,[17] isobavachromene **4**,[18] **5**[19] and some synthetic analogues **6a-b**;[14] bearing benzoxazines as whole or as part in their structure, have been identified as good antioxidant agents in DPPH radical scavenging assay (Figure 1). This encourages us to synthesize non-naturally occurring benzo [1,4] oxazines analogues.

Moreover, several natural products like curcumine,[20] resveratrol,[21] quercetin,[22] Trolox,[23] Rosmarinic acid,[24a-b] Chalcones,[25a-c] Quinolines,[26a-c] Coumarines,[27a-b] etc. were also reported as antioxidants (Figure 2).

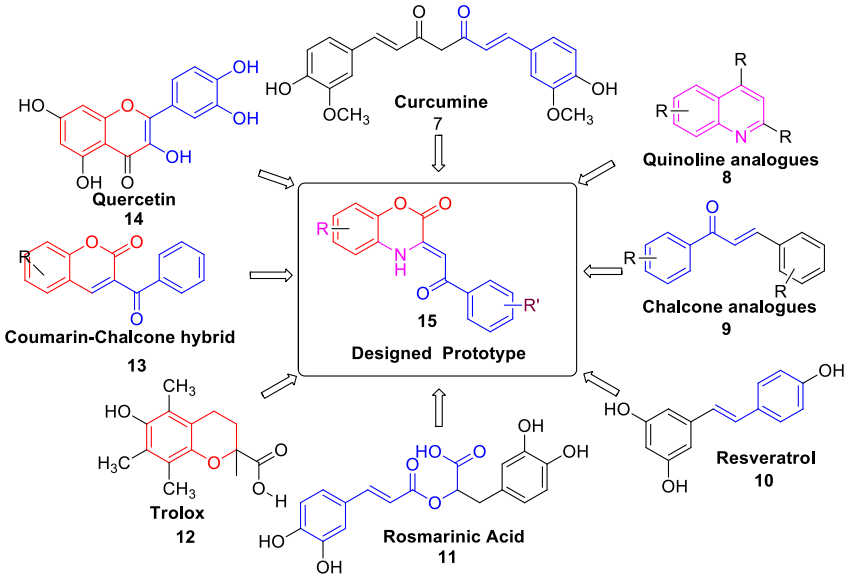


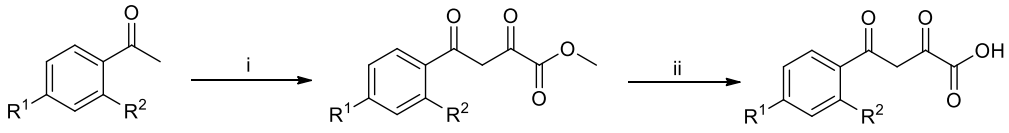
Figure 2: Design strategy for the target compound 2-oxo-benzo[1,4]oxazine **9a** as an antioxidant agent.

However, due to several drawbacks such as poor solubility, less abundance and severe toxicity; their antioxidant properties were found to be relatively lower. Thus, there is still an urgent need to develop a potent antioxidant by designing a new scaffold *via* structural modification and incorporation of functional group present in these antioxidants. Hence, based on above fact, we have designed **prototype 15** i.e., *C-3 tethered 2-oxo-benzo(1,4)oxazine*, incorporating similar sub-structural units assuming that the resulting structure will be a new class of potent antioxidant agent. (Figure 2)

In the continuation towards the search of new class of antioxidants; we were interested to explore the designed prototype **15,** which are already synthesized via ultrasound-assisted reaction conditions[28] (as given in Chapter 2 section 2b). Therefore herein, we report the *in vitro* antioxidant activity and SAR of a series of C-3 tethered 2-oxo-benzo[1,4]oxazine analogues **20a-20ab**. Although compounds **20a-i**, **20l**, **20t-**

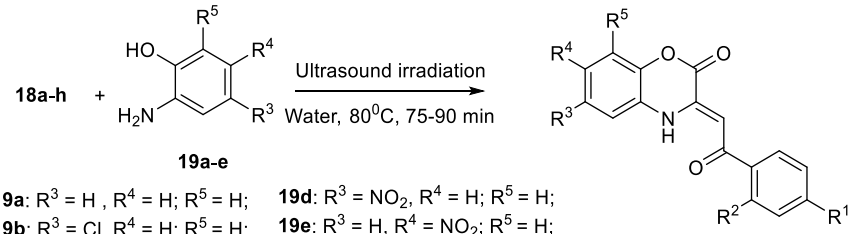
w, **20y** and **20aa-ab** have been earlier reported in the literature but were prepared by other routes.[29] Moreover, their antioxidant activities are also not reported so far in the literature. To the best of our knowledge, the antioxidant activities of all the synthesized compounds **20a-20ab**, were evaluated for the first time using DPPH radical scavenging assay taking ascorbic acid as standard reference and FRAP assay using BHT as standard reference. In addition, the cytotoxic studies of active compounds were also performed. Moreover, we also report the validation of our results via *in silico* molecular docking studies of compounds **20a**, **20b** and **20t** in comparison with standard reference ascorbic acid.

2.3A.2 Results and Discussion



16a: R^1 = H , R^2 = H; **17a**: R^1 = H , R^2 = H; **18a**: R^1 = H , R^2 = H;
16b: R^1 = F, R^2 = H; **17b**: R^1 = F, R^2 = H; **18b**: R^1 = F, R^2 = H;
16c: R^1 = Cl, R^2 = H; **17c**: R^1 = Cl, R^2 = H; **18c**: R^1 = Cl, R^2 = H;
16d: R^1 = Cl, R^2 = Cl; **17d**: R^1 = Cl, R^2 = Cl; **18d**: R^1 = Cl, R^2 = Cl;
16e: R^1 = Br, R^2 = H; **17e**: R^1 = Br, R^2 = H; **18e**: R^1 = Br, R^2 = H;
16f: R^1 = CH_3, R^2 = H; **17f**: R^1 = CH_3, R^2 = H; **18f**: R^1 = CH_3, R^2 = H;
16g: R^1 = OCH_3, R^2 = H; **17g**: R^1 = OCH_3, R^2 = H; **18g**: R^1 = OCH_3, R^2 = H;
16h: R^1 = NO_2, R^2 = H; **17h**: R^1 = NO_2, R^2 = H; **18h**: R^1 = NO_2, R^2 = H;

Scheme 1. Synthesis of starting substrate functionalized diketo-acid (**18a-h**).[a]
[a]*Reagents and conditions*: (i) NaH, dimethyl oxalate, toluene, 0°C to 80°C, 6h; (ii) LiOH.H_2O, MeOH:THF:H_2O (4:3:1), 0°C to rt, 4h.



19a: R^3 = H , R^4 = H; R^5 = H; **19d**: R^3 = NO_2, R^4 = H; R^5 = H;
19b: R^3 = Cl, R^4 = H; R^5 = H; **19e**: R^3 = H, R^4 = NO_2; R^5 = H;
19c: R^3 = CH_3, R^4 = H; R^5 = H; **19f**: R^3 = CH_3, R^4 = H; R^5 = Br.

Yield[b] = 74-98%

Scheme 2. Ultrasound-assisted one-pot green synthesis of C-3 tethered 2-oxo-benzo[1,4]oxazines analogues (**20a-20ab**).[a]
[a]*Reaction conditions*: **18a-h** (0.2 mmol), **19a-f** (0.2 mmol) in water (2.0 mL), 75-90 min at 80 °C under ultrasound irradiation. [b]Isolated yield after column chromatography/recrystalization.

The synthetic scheme for the synthesis of C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab** using our previously developed procedure[28], which is depicted in Scheme 1 and 2. The base-mediated reaction of acetophenone **16a-h** with dimethyl oxalate in toluene for 6h furnished the diketo-ester **17a-h** in 70-80% yields. Conversion of these diketoesters **17a-h** to 2, 4-dioxo-4-phenylbutanoic acid **18a-h** were achieved by hydrolysis with LiOH.H_2O in MeOH: THF: H_2O (4:3:1) solvent.

The reaction of nitro/alkyl/halide-substituted 2, 4-dioxo-4-phenylbutanoic acid **18a-h** with nitro/alkyl/halide-substituted 2-aminophenol **19a-f** in water furnished C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab** in 74-98% yields after purification either by flash column chromatography or by recrystallization method. All the synthesized compounds were well characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy, FTIR and HRMS analysis.

2.3A.3 Materials and Methods

2.3A.3.1 *In vitro* DPPH radical scavenging antioxidant activity and structure-activity relationship studies

All the synthesized C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab** were evaluated for *in vitro* antioxidant activities using DPPH radical scavenging assay compared with standard reference ascorbic acid. (Table 1) The choice of the reference compounds is based on hydrophilic nature of ascorbic acid and the maximum inhibition of the DPPH radical in IC_{50} value (µg/mL) by all the compounds **20a-20ab**. The DPPH radical scavenging assay is generally utilized as a quick and reliable parameter to investigate the antioxidant activities of diverse heterocycles.[30a] DPPH is a stable free radical, that can easily accept a hydrogen radical or an electron to become a stable molecule.[30b] In the methanolic medium, DPPH has odd electron configuration having a strong absorption band at 515 nm, whereas this absorption decreases slightly in the presence of free radical scavengers, and it results color change to yellow from deep purple.[30c-d] The radical trapping ability strongly depends on the structural availability of the radical trapping site. The steric hindrance as well as electron density plays a dynamic role in the antioxidant activity since they may prevent the test molecule from reaching reaching the radical site of DPPH and thus results in low activity. [30e]

Ariffin et. al. (2015) proposed two mechanisms involved in DPPH assay; first one is the hydrogen atom transfer (HAT) mechanism and the second one is the single electron transfer (SET) mechanism.[31] Similar to their interpretation, it can be speculated that, for DPPH assay, a dominant HAT mechanism is assumed and the favoured hydrogen abstraction sites are enamine –NH group, preferably with conjugation to the side chain of phenacyl group (-COPh), as the latter could stabilize by the resulting radical from additional resonance structures.

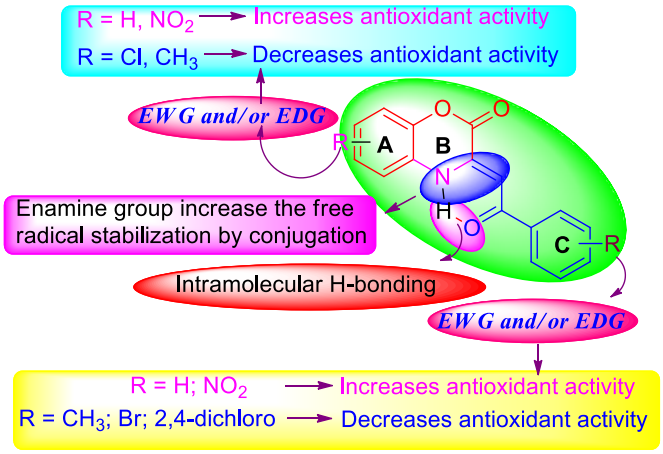


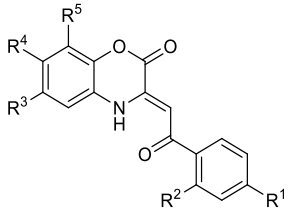
Figure 3: SAR analysis of C-3 tethered 2-oxo-benzo [1, 4]oxazines.

The generic scaffold of the newly synthesized C-3 tethered 2-oxo-benzo[1,4]oxazines, as illustrated in Figure 3, consists of a two fused cyclic ring A and B linked with ring C via α, β-unsaturated ketone having electron-withdrawing group (EWG) and/or electron-donating group (EDG) either at ring A or at C. The active group -CO-C=C-NH- enables resonance between the ring B and C, leading to multiple resonance structure, which may be further initiated by the attached substituents of ring A and C and in situ enhances the radical scavenging activity through the removal of hydrogen atom from NH of ring B via HAT mechanism. It has been found that electron withdrawing substituent NO_2 at ring A or C increase the antioxidant activity which may be due to resonance based stabilizing effects. Therefore, based on the structures and their antioxidant activities, it was found that the compounds have either no substitution at ring A and C or have EWG/EDG at ring A and C plays a very important role in deciding their DPPH radical scavenging activities. Hence, based on the substituent's (either EWG or EDG) at ring A and C of C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab**, and their antioxidant activities, their structure-activity relationship can be explained by grouping all compounds into two groups:

(i) **No substitution or EDG at ring A or ring C**: In the first group of compounds having no substitution at ring **A** and **C** i.e., the model compound **20a**, exhibited promising antioxidant activity (IC_{50} = 10.20 ± 0.08 µg/mL) in comparison with standard reference Ascorbic acid (IC_{50} = **4.57** µg/mL) [entry 1]. Then, by putting EDG (OMe group) at ring C, as in compound **20b**, further increases activity (IC_{50} = 6.89 ± 0.07 µg/mL) [entry 2]. Reversing the order i.e., halogen substitution at ring **A**

and no substitution at ring C do not cause any further increase in antioxidant activity as shown by compound **20c** (entry 3).

Table 1 Antioxidant activity of synthesized compounds **20a-ab** by DPPH radical scavenging assay and FRAP assay.



S. No.	Compound No.	R^1	R^2	R^3	R^4	R^5	Antioxidant activity	
							FRAP assay[a] ($C_{0.5FRAP}$ μM)	DPPH assay[b] IC_{50} (μg/mL)
1	**20a**	H	H	H	H	H	611.5 ± 23.2	10.20 ± 0.08
2	**20b**	OCH_3	H	H	H	H	686.4 ± 30.8	6.89 ± 0.07
3	**20c**	H	H	Cl	H	H	467.8 ± 22.4	28.80 ± 0.60
4	**20d**	F	H	Cl	H	H	732.7 ± 41.6	45.21 ± 0.92
5	**20e**	Cl	H	Cl	H	H	>1000	92.20 ± 1.54
6	**20f**	Cl	Cl	Cl	H	H	>1000	56.60 ± 1.12
7	**20g**	Br	H	Cl	H	H	798.6 ± 32.5	61.23 ± 1.23
8	**20h**	CH_3	H	Cl	H	H	821.9 ± 38.7	44.83 ± 0.81
9	**20i**	OCH_3	H	Cl	H	H	648.2 ± 29.5	34.94 ± 0.73
10	**20j**	F	H	CH_3	H	H	502.6 ± 18.2	34.41 ± 0.70
11	**20k**	Cl	Cl	CH_3	H	H	652.5 ± 30.6	43.58 ± 0.92
12	**20l**	OCH_3	H	CH_3	H	H	916.8 ± 21.4	43.80 ± 0.85
13	**20m**	H	H	CH_3	H	Br	536.7 ± 21.4	24.38 ± 0.46
14	**20n**	F	H	CH_3	H	Br	482.5 ± 35.5	16.86 ± 0.72
15	**20o**	Cl	Cl	CH_3	H	Br	641.6 ± 28.7	22.48 ± 0.64
16	**20p**	Br	H	CH_3	H	Br	>1000	44.32 ± 0.45
17	**20q**	CH_3	H	CH_3	H	Br	>1000	36.24 ± 0.27
18	**20r**	NO_2	H	CH_3	H	H	328.6 ± 25.8	12.23 ± 0.05
19	**20s**	NO_2	H	H	H	H	618.4 ± 23.9	21.27 ± 0.28
20	**20t**	H	H	NO_2	H	H	638.2 ± 32.6	4.74 ± 0.08
21	**20u**	F	H	NO_2	H	H	424.5 ± 19.7	32.11 ± 0.52
22	**20v**	OCH_3	H	NO_2	H	H	>1000	43.58 ± 0.92
23	**20w**	H	H	H	NO_2	H	597.4 ± 26.4	12.53 ± 0.09
24	**20x**	F	H	H	NO_2	H	628.6 ± 32.4	10.18 ± 0.10
25	**20y**	Cl	H	H	NO_2	H	708.2 ± 27.1	19.76 ± 0.35
26	**20z**	Cl	Cl	H	NO_2	H	437.6 ± 39.4	28.81 ± 0.67
27	**20aa**	Br	H	H	NO_2	H	518.6 ± 17.6	28.37 ± 0.16
28	**20ab**	OCH_3	H	H	NO_2	H	>1000	43.60 ± 0.74
29	**Ascorbic acid**	---	---	---	---	---	---	4.57
30	**BHT**	---	---	---	---	---	546.0 ± 13.6	---

*Results are expressed as a mean ± standard deviation (n = 3). [a]DPPH radical scavenging activities are expressed as IC_{50} concentrations of the compounds (μg/mL) required to inhibit 50% of the radicals and the maximum inhibition values and Positive control for DPPH assay = Ascorbic acid; [b] Positive control for FRAP assay = BHT.

Furthermore, when we incorporated halogen substituents (Cl, F, Br, 2,4-dichloro) or CH_3 substituent either at A or C as in the case of compounds **20c-l**; a decrease in the antioxidant activity was observed due to high electron density in compounds **20d-g, 20j** and **20k** (entry 4-12). Furthermore, It was observed that two EDG at ring A

(**20m-q**; entry 13-17) exhibited moderate antioxidant activity having IC_{50} value in the range of 18.86 ± 0.72 to 44.32 ± 0.45 μg/mL. In this series (**20m-q**; entry **13-17**), ring C having Fluorine substituent i.e. compound **20n** exhibited good antioxidant activity (IC_{50} = 16.86 ± 0.72 μg/mL) in comparison with other compounds (**20m** and **20o-q**).

To our surprise; when we incorporated EWG group i.e., NO_2 group at ring C and EDG group i.e., CH_3 group at ring A (compound **20r**); the antioxidant activity was regained and shows IC_{50} value of 12.23 ± 0.05 μg/mL nearly equivalent to **20a** (entry 18).

It is to be noted that EDG at ring A decreases antioxidant activity as shown in entries 3-17; so, we synthesized compound **20s** (having no any substitution at ring A and EWG i.e., NO_2 group at ring C), which, contrary to our expectations, displayed further decrease in antioxidant activity (IC_{50} = 21.27 ± 0.28 μg/mL) (entry 19).

(ii) **EWG (-NO_2 Group) at ring A**: Since **20r** having EWG (NO_2) at ring C showed promising antioxidant activity; inspired by this observation, we prepared **20t-v** having NO_2 group at C-4 position of ring A. 2-oxo-benzo[1,4]oxazine **20t** having no substitution at ring C, showed excellent antioxidant activity having IC_{50} value of 4.74 ± 0.08 μg/mL (entry 20). Since **20b** having OMe substituent at ring C, was also found to show excellent antioxidant activity; thus, we synthesized **20u** and **20v** having Fluoro as well as OMe substituent, respectively at ring C. Unfortunately, antioxidant activity diminishes (entry 21 and 22). In addition, we also prepared 2-oxo-benzo[1,4]oxazines **20w-20ab** having EWG group (NO_2) at C-5 position of ring A further to investigate SAR study. Compound **20w** having no substitution at ring C showed promising antioxidant activity having IC_{50} value of 12.53 ± 0.09 μg/mL (entry 23). On incorporating Fluoro group at ring C; activity of **20x** increases (IC_{50} = 10.18 ± 0.10 μg/mL, entry 24). Furthermore, when we incorporated halogen substituents (Cl, Br, 2,4-dichloro and OMe) at ring C as in the case of compounds **20y-20ab**; a decrease in the antioxidant activity was observed (entry 19-23).

Overall, we can interpret that no substitution or EWG at ring A or ring C enhances antioxidant activity of all the synthesized 2-oxo-benzo[1,4]oxazines **20a-20ab**. Whereas EDG either at ring A or C diminishes antioxidant activity. Our SAR results depict that **20b** and **20t**, the best compounds of the series, showed antioxidant activity comparable to standard reference Ascorbic acid.

2.3A.3.2 *In vitro* Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was deliberated using the method as illustrated by Benzie and Strain.[32] It reveals that the reducing potential of an antioxidant molecule, which reacts with a complex of ferric tripyridyltriazine [Fe^{3+}-TPTZ] and develops a colored ferrous tripyridyltriazine [Fe^{2+}-TPTZ]. The reducing nature of an antioxidant depends on their property to donate a hydrogen atom for the breaking of the free radical chain, which is responsible for oxidative stress etc.

All the synthesized C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab** were assessed for FRAP assay taking BHT as standard reference; as depicted in Table 2. In this study, the trend with respect to ferric ion reducing activities of all the screened compounds i.e. **20a-20ab** showed that eight compounds (**20c**, **20j**, **20m**, **20n**, **20r**, **20u**, **20z** and **20aa**) were found more potent than BHT ($C_{0.5FRAP}$ = 546.0 ± 13.6 µM).

In summary, all the compounds (**20a-20ab**) displayed good to moderate activity in comparison with BHT in the range of $C_{0.5FRAP}$ = 328.6 ± 25.8 µM to 916.8 ± 21.4 µM in *in-vitro* antioxidant FRAP assay except compounds **20e**, **20f**, **20l**, **20q, 20v** and **20ab** which showed $C_{0.5FRAP}$ greater than 1000 µM. Compounds having EWG i.e. NO_2 substituent at ring C (**20r** and **20s**) showed different potency than standard reference BHT. While **20r** having CH_3 at C-4 position of ring A displayed potent activity than BHT; **20s** was found to be less active. Anomaly was observed in the case of compounds having no substitution at ring C. While **20c** and **20m** showed greater potency; compounds **20a, 20t** and **20w** were found less active than BHT.

2.3A.4 Cell toxicity study

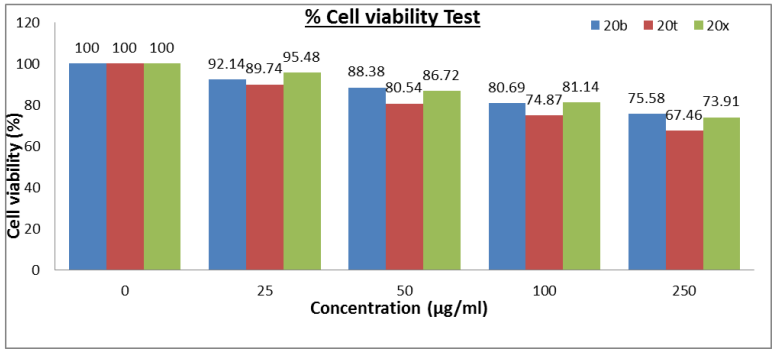


Figure 4. Percentage cell viability test.

Out of 28 compounds, three most active compounds i.e., **20b**, **20t** and **20x** were then selected for their cytotoxic studies. As depicted in Figure 4, compounds **20b**, **20t** and

20x were accessed for their cytotoxic study using $3T_3$ fibroblast cell lines in MTT assay.[33] The result shows that these compounds were non-toxic in nature even at 250 µg/mL concentration and therefore displays permissible values of cell viability.

2.3A.5 *Insilico* Molecular Docking Simulation Studies

Finally, the biological results were validated via *insilico* molecular docking studies of two most active compounds (**20b** and **20t**). Since **20b** has OMe group at ring C and **20t** has NO_2 group at ring A; it is worthwhile to compare the docking studies of active compounds with molecules having no substitution either at ring A or C. Therefore, we have also selected compound **20a** for our insilico molecular docking simulation studies.

The binding affinities and interactions of C-3 tethered 2-oxo-benzo[1,4]oxazines derivatives **20a**-**20ab** with the human antioxidant enzyme were investigated through molecular docking simulations. Binding affinities were predicted by the Sybyl docking total score upon docking with the Surflex-Dock program (Sybyl X 2.0).Compounds were docked into the active site of the known the human antioxidant enzyme target peroxiredoxins (Prxs) DTT complex (PDB ID: 3MNG) were taken from the Protein Data Bank (http://www.rcsb.org/pdb).[34-35]

Docking studies were carried out to evaluate the binding affinity and interactions with their target proteins. Hydrogen bonds (H-bonds, with a donor-receptor distance of 3Å) between the ligand and amino acids in the binding site of the protein were used for the ranking of compounds. The mode of interaction of the co-crystallized ligand dithiothreitol (DTT) within the crystal structure of enzyme in complex was used as a reference binding model. The root mean-square deviation (RMSD) of each docking pose was compared to the co-crystallised ligand and used for ranking and for RMSD calculation. The co-crystallized DTT molecule was re-docked onto the same binding site and the most probable binding mode was selected as that with the highest docking total score of 4.8921. An RMSD value 0.6772Å between the predicted and crystal binding mode indicates the high reliability of Surflex-Dock for this protein target.

On the other hand, docking results for **20a, 20b** and **20t** against the antioxidant target protein Prxs showed a high binding affinity docking score indicated by a total score of 3.8470 (Figure 5A), 3.6567 (Figure 5B) and 4.2709 (Figure 5C) forms a H-bond (NH_2...O) of length 1.8Å to the backbone of hydrophobic aliphatic residue that is, Glycine-46.

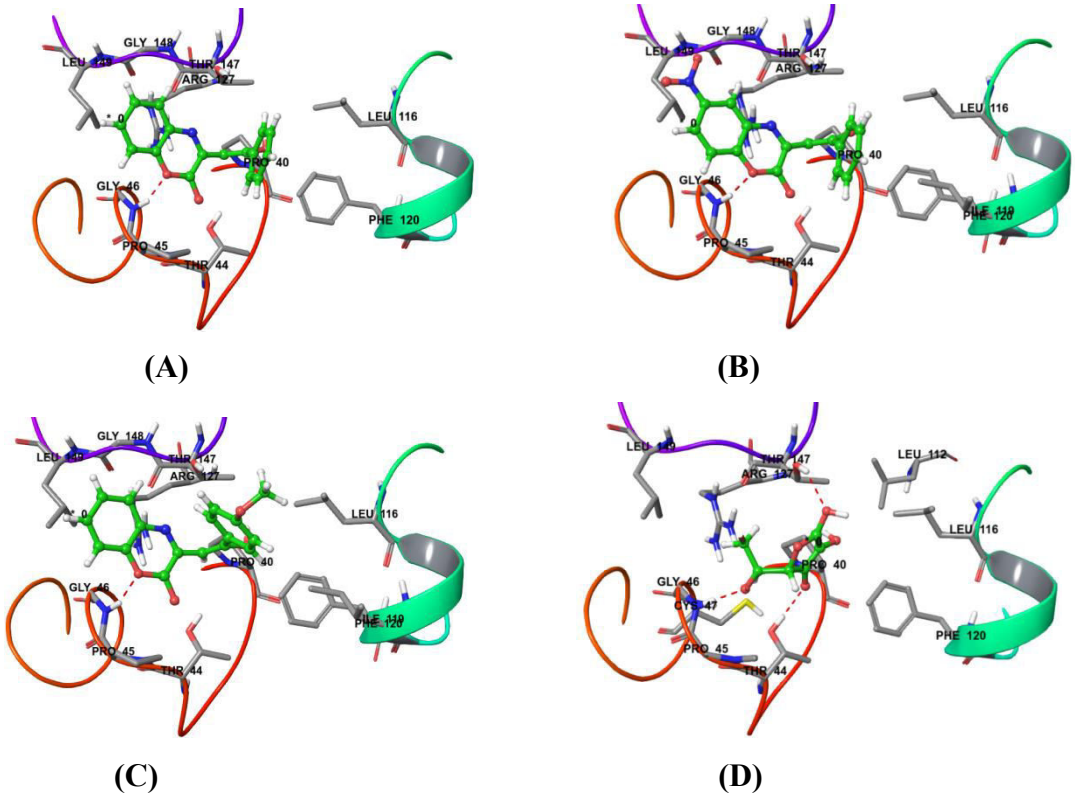


Figure 5 (A): Binding interactions of compound **20a** upon docking onto human antioxidant enzyme target peroxiredoxins (Prxs) (PDB ID: 3MNG). A top docking energy (total score) of 3.8470 was predicted. Formation of a H-bond of length 1.8Å to residue Gly-46 in the binding site was predicted. **(B):** Binding interactions of compound **20b** upon docking onto human antioxidant enzyme target peroxiredoxins (Prxs) (PDB ID: 3MNG). A top docking energy (total score) of 3.6567 was predicted. Formation of a H-bond of length 1.8Å to residue Gly-46 in the binding site was predicted. **(C):** Binding interactions of compound **20t** upon docking onto human antioxidant enzyme target peroxiredoxins (Prxs) (PDB ID: 3MNG). A top docking energy (total score) of 4.2709 was predicted. Formation of a H-bond of length 1.8Å to residue Gly-46 in the binding site was predicted. **(D):** Binding interactions of compound **Ascorbic acid** upon docking onto human antioxidant enzyme target peroxiredoxins (Prxs) (PDB ID: 3MNG). A top docking energy (total score) of 3.4829 was predicted. Formation of four H-bond of length 2.2, 1.8 and 2.1 Å to residue Thr-147, Gly-46 and Thr-44 in the binding site was predicted.

In the docking pose of the **20a, 20b** and **120t** and Prxs complex, the chemical nature of binding site residues within a radius of 3Å with diverse properties was aromatic (hydrophobic), for example, Phe-120, (Phenylalanine); hydrophobic, for example, Leu-116, Ile-119, Leu-149, Leu-112(Leucine), Gly-46, Gly-148(Glycine); (polar, hydrophobic, positive charged) residues, for example, Arg-127 (Arginine); nucleophilic (polar, hydrophobic), for example, Thr-147 and Thr-44 (Threonine), nucleophilic (polar uncharged), for example Cys-47 (Cysteine); and hydrophobic (polar,, uncharged) residues, for example, Pro-40 and Pro-45 (Proline) as a result, the bound compound showed a strong hydrophobic interaction with Prxs, thus leading to more stability and activity in this compound.

The docking results for the ascorbic acid (standard compound) with the antioxidant target protein Prxs showed a low binding affinity docking score, indicated by a low total score of 3.4829 with three H-bond (hydrogen bond) formation of length 2.2, 1.8 and 2.1Å to the Thr-147, Gly-46 and Thr44 (Figure 5D). The ascorbic acid-Prxc-docked complex also showed a similar type of binding site residues within a radius of 3Å of bound ligand such as Thr-147, Leu-116, Pro-40, Phe-120, Leu-112, Thr-44, Gly-46, Pro-45, Cys-47, Arg-127, Leu-149 shown in Figure 5D. Thus, the docking procedure of Surflex-dock software (Sybyl-X 1.3) in reproducing the experimental binding affinity seems reliable, and therefore predicted as true positive.

Thus, it can be inferred based on docking simulation studies that the most active compounds i.e. **20b** and **20t** having IC_{50} value of 6.89 ± 0.07 μg/mL and 4.74 ± 0.08 μg/mL, showed the binding affinity docking score of 3.6567 and 4.2709, respectively, which was found comparable to the binding affinity docking score of Ascorbic acid (3.4829). Thus, the *insilico* docking results of **20b** and **20t** successfully validated the *in vitro* experimental studies.

2.3A.6 Conclusion

In summary, we disclose C-3 tethered 2-oxo-benzo[1,4]oxazines **20a-20ab** as potent antioxidants. Compound **20b** and **20t,** the most active compounds of the series, showed promising antioxidant activity having IC_{50} value of 6.89 ± 0.07 μg/mL and 4.74 ± 0.08 μg/mL, respectively, in DPPH radical scavenging assay in comparison with ascorbic acid (IC_{50} = **4.57** μg/mL). Whereas in FRAP assay, eight compounds (**20c**, **20j**, **20m**, **20n**, **20r**, **20u**, **20z** and **20aa**) were found more potent than BHT ($\mathbf{C_{0.5FRAP}}$ = 546.0 ± 13.6 μM). The active compounds were also found non-toxic in $3T_3$ fibroblast cell lines in MTT assay. Our *insilico* molecular docking results reveal that **20b** and **20t** showed excellent docking total scores against human antioxidant enzyme target as compared to ascorbic acid. Thus, the *insilico* docking simulation studies effectively validates the *in vitro* experimental results.

2.3A.7 Experimental Details & Characterization Data

2.3A.7.1General experimental

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. Ultrasonic irradiation was performed in a Elmasonic S 30 (H) ultrasonic water bath cleaner and the reaction vessel was positioned in the maximum energy area in the

cleaner and the removal or addition of water was used to control the temperature of the water bath. 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 $CDCl_3$, $CD_3ODandCD_3SOCD_3$ as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S Q Tof (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.

2.3A.7.2 General Procedure for the synthesis of functionalized diketo-acid 18a-h:

Substituted acetophenone **16a-h** (2.00 mmol, 1eq.) were taken in toluene (50 ml) and NaH (2.20 mmol, 1.1 eq.) was added carefully. After stirring this reaction mixture at 0°C for 30 min dimethyl oxalate (2.20 mmol, 1.1 eq.) were added and reflux for 6h. The progresses of the reaction were monitored by TLC using 9:1 Hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was quenched with distilled water and extracted with ethyl acetate (3 × 50 ml); then with distilled water (2×10 mL) followed by brine solution (2×20 mL). The organic layer was combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified by recrystalization using EtOAc/Hexane (v/v = 20:80), which afforded the pure desired diketo-ester **17a-h** in 78-92% yields. Compounds **17a-h** was used for next step without any further purification.

To a solution of **17a-h** (1.00 mmol, 1eq.) in MeOH:THF: H_2O (10 ml, 7:2:1), added $LiOH.H_2O$ (1.20 mmol, 1.2eq) into the reaction mixture and stirred it for 3 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, it was quenched with 3N HCl solution and extracted with ethyl acetate (3×30 mL); then with distilled water (2×10 mL) followed by brine solution (2×20 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the corresponding crude product. These crude products were further purified by recrystallization with EtOAc/Hexane, which afforded diketo-acids

18a-h in excellent yields (up to 97%). Compounds **18a-h** were used for next step without any further purification.

2.3A.7.3 General Procedure for the synthesis of functionalized (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20a-ab):

To a solution of the compound **18a-h (**0.20 mmol; 1eq.) in water (2.0 mL) was added compound **19a-f (**0.20 mmol; 1eq.) and the reaction mixture was irradiated under ultrasonic sonicator at 80 ˚C temperature for about 75-90 min. (depending upon the substrate employed). The progress of the reaction was checked by TLC using 9:1 Hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was extracted with ethyl acetate (3 × 50 ml); then with distilled water (2×10 mL) followed by brine solution (2×20 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and the organic solvent was removed under reduced pressure to give the crude product. The crude products were purified either by recrystallization using Hexane/EtOAc (v/v = 90:10) or by flash column chromatography method over silica gel using 7.5:2.5 to 9:1 Hexane/ethyl acetate as an eluent which afforded the pure desired (Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]-oxazin-2-one **20a-ab** having good yields (74-98 %).

2.3A.7.4 Characterization data of 2-oxo-benzo[1,4]oxazin-2-one (20a-ab):

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20a)

Yield: 49.7 mg (94 %), spectral data can be seen at 2.1.7.4 (11a) or 2.2.5.4 (24a)

(Z)-3-[2-(4-Methoxy-phenyl)-2-oxo-ethylidene]-3,4-dihydro-benzo[1,4]oxazin-2-one (20b)

Yellowish solid; yield: 51.2 mg (88 %), R_f (EtOAc/Hexane; 20:80) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (8:2) as an eluent; m.p. 200-203 °C; FT-IR (KBr, νmax/cm-1) 3435, 1756, 1602, 1112; ^{1}H NMR (400 MHz) δ 7.99 (d, J = 8.8 Hz, 2H, Ar-H), 7.17 (t, J = 7.1 Hz, 2H, Ar-H), 7.09 – 7.05 (m, 2H, C=CH, Ar-H), 7.02 – 6.95 (m, 3H, Ar-H), 3.87 (s, 3H, O-CH_3); ^{13}C NMR (100 MHz) δ 190.5 (C=O), 163.5 (Ar-C-OCH_3), 156.6 (O=C-O), 141.2 (Ar-C-N), 138.6 (Ar-C-O), 131.2 (C=CH), 130.0 (Ar-C), 125.9 (Ar-C), 124.0 (Ar-C), 123.7 (Ar-C), 117.2 (Ar-C), 115.8 (Ar-C), 114.0 (Ar-C), 94.7 (C=CH), 55.6 (O-CH_3); HRMS (ESI) calcd. for $C_{17}H_{13}NO_4$ $[M+H]^+$: 296.0845; found 296.0849.

(Z)-6-chloro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20c)

Yield: 54.6 mg (91 %), spectral data can be seen at 2.2.5.4 (24d)

(Z)-6-chloro-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20d)

Yield: 62.1 mg (98 %); Yield: 54.6 mg (91 %), spectral data can be seen at 2.2.5.4 (24i)

(Z)-6-chloro-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20e)

Yield: 63.9 mg (96 %); spectral data can be seen at 2.2.5.4 (24g)

(Z)-6-chloro-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazine-2-one (20f)

Yield: 65.9 mg (89 %); spectral data can be seen at 2.2.5.4 (24h)

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-chloro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20g)

Yield: 70.7 mg (93 %); spectral data can be seen at 2.2.5.4 (24f)

(Z)-6-chloro-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20h)

Yield: 56.5 mg (90 %); spectral data can be seen at 2.2.5.4 (24j)

(Z)-6-chloro-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20i)

Yield: 56.6 mg (86 %); spectral data can be seen at 2.2.5.4 (24e)

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[1,4]oxazin-2-one (20j)

Yellowish solid; yield: 53.7 mg (90 %); R_f (EtOAc/Hexane; 20:80) = 0.85; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 145-147 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 2930, 1770, 1624, 1596, 1128; ^{1}H NMR (400 MHz) δ 8.02 (dd, *J* = 5.6, 8.8 Hz, 2H, Ar-H), 7.17 – 7.07 (m, 3H, Ar-H), 6.98 – 6.90 (m, 3H, C=C<u>H,</u> Ar-H), 2.36 (s, 1H, CH_3); ^{13}C NMR (100 MHz) δ 190.0 (C=O), 166.9 (Ar-C-F), 156.4 (O=C-O), 139.3 (Ar-<u>C</u>-CH_3), 136.1 (Ar-C-N), 134.7 (Ar-C-O), 130.3 (<u>C</u>=CH), 130.2 (Ar-C), 124.9 (Ar-C), 123.3 (Ar-C), 116.9 (Ar-C), 116.2 (Ar-C),

115.9 (Ar-C), 115.8 (Ar-C), 94.1 (C=CH), 21.1 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}FNO_3$ $[M+H]^+$: 298.0801; found 298.0807.

(Z)-3-[2-(2,4-Dichloro-phenyl)-2-oxo-ethylidene]-6-methyl-3,4-dihydro-benzo[1,4]oxazin-2-one (20k)

Yellowish solid; yield: 65.4 mg (94 %), R_f (EtOAc/Hexane; 20:80) = 0.80; Purification of crude product was done by recrystalization using Hexane/ethyl acetate; m.p. 142-145 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 2913, 1755, 1618, 1570, 1083; ^{1}H NMR (400 MHz) δ 7.53 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.45 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.33 – 7.30 (m, 1H, Ar-H), 7.10 – 7.08 (m, 1H, Ar-H), 6.94 – 6.92 (m, 2H, Ar-H), 6.73 (s, 1H, C=CH), 2.36 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 191.6 (C=O), 155.9 (O=C-O), 139.5 (Ar-C-CH_3), 139.2 (Ar-C-Cl), 137.5 (Ar-C-Cl), 137.2 (Ar-C-N), 136.2 (Ar-C-O), 132.5 (C=CH), 130.7 (Ar-C), 130.6 (Ar-C), 127.4 (Ar-C), 125.4 (Ar-C), 123.0 (Ar-C), 117.0 (Ar-C), 116.4 (Ar-C), 98.0 (C=CH), 21.1 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{11}Cl_2NO_3$ $[M+2]^+$: 349.0116; found 349.0112.

(Z)-3-[2-(4-Methoxy-phenyl)-2-oxo-ethylidene]-6-methyl-3,4-dihydro-benzo[1,4]oxazin-2-one (20l)

Yellowish solid; yield: 57.7 mg (93 %), R_f (EtOAc/Hexane; 20:80) = 0.75; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (8:2) as an eluent; m.p. 180-182 °C; FT-IR (KBr, νmax/cm^{-1}) 3452, 1755, 1625, 1581; ^{1}H NMR (400 MHz) δ 7.99 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.06 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.96 (d, *J* = 9.4 Hz, 2H, Ar-H), 6.88 - 6.86 (m, 2H, Ar-H, C=CH), 3.88 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 190.4 (C=O), 163.4 (Ar-C-OCH_3), 156.8 (O=C-O), 139.3 (Ar-C-CH_3), 138.7 (Ar-C-N), 136.0 (Ar-C-O), 131.3 (C=CH), 129.9 (Ar-C), 124.4 (Ar-C), 123.6 (Ar-C), 116.8 (Ar-C), 116.0 (Ar-C), 114.0 (Ar-C), 94.5 (C=CH), 55.6 (OCH_3), 21.1 (CH_3); HRMS (ESI) calcd. for $C_{18}H_{15}NO_4$ $[M+H]^+$: 310.1001; found 310.1009.

(Z)-8-bromo-6-methyl-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20m)

Yellowish solid; Yield: 65.7 mg (92 %); Rf (EtOAc/Hexane; 20:80) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (8.5:1.5) as an eluent; m.p. 190-192 °C; FT-IR (KBr, νmax/cm-1) 3417, 1767, 1626, 1583, 1282, 1177; ^{1}H NMR (400 MHz) δ 8.00 (d, J = 7.2 Hz, 2H, Ar-H), 7.58-7.47 (m, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 7.07 (s, 1H,

C=CH), 6.85 (s, 1H, Ar-H), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 191.7 (C=O), 155.7 (O=C-O), 138.6 (Ar-C-N), 138.1 (Ar-C-O), 136.9 (C=CH), 136.6 (Ar-C-CH_3), 132.9 (Ar-C), 128.9 (Ar-C), 128.2 (Ar-C), 127.8 (Ar-C), 124.7 (Ar-C), 115.5 (Ar-C), 110.2 (Ar-C-Br), 95.2 (C=CH), 20.9 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}BrNO_3$ [M+2]+: 359.0001; found 359.0008.

(Z)-8-bromo-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[b][1,4] oxazin-2-one (20n)

Yellowish solid; Yield: 67.6 mg (90 %); R_f (EtOAc/Hexane; 20:80) = 0.80; Purification of crude product was done by recrystallization using EtOAc/Hexane; m.p. 230-232 °C; FT-IR (KBr, νmax/cm-1) 3417, 1771, 1631, 1285, 1159; ^1H NMR (400 MHz) δ 8.03-7.99 (m, 2H, Ar-H), 7.17-7.13 (m, 3H, Ar-H), 6.99 (s, 1H, Ar-H), 6.84 (brs, 1H, C=CH), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 190.1 ((C=O), 167.0 (Ar-C-F), 164.5 (Ar-C-F), 155.6 (O=C-O), 138.7 (Ar-C-N), 136.9 (Ar-C-O), 136.6 (C=CH), 134.5 (Ar-C-CH_3), 134.4 (Ar-C), 130.4 (Ar-C), 130.3 (Ar-C), 128.3 (Ar-C), 124.6 (Ar-C-Br), 116.1 (Ar-C), 115.9 (Ar-C), 115.5 (Ar-C), 110.2 (Ar-C), 94.8 (C=CH), 20.9 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{11}BrFNO_3$ $[M+2]^+$: 376.9906; found 376.9909.

(Z)-8-bromo-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo [b][1,4]oxazin-2-one (20o)

Yellowish solid; Yield: 79.7 mg (94 %); R_f (EtOAc/Hexane; 20:80) = 0.85; Purification of crude product was done by recrystallization using Hexane/ethyl acetate; m.p. 224-226 °C; FT-IR (KBr, νmax/cm-1) 3417, 1763, 1626, 1561, 1294, 1127; ^1H NMR (400 MHz) δ 7.54 (d, J = 8.4 Hz, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.33 (d, J = 8.0 Hz, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 6.88 (s, 1H, C=CH), 6.79 (s, 1H, Ar-H), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 191.9 (C=O), 155.2 (O=C-O), 138.7 (Ar-C-Cl), 137.5 (Ar-C-N), 137.3(Ar-C-O), 137.0 (C=CH), 136.8 (Ar-C-CH_3), 132.7 (Ar-C-Cl), 130.8 (Ar-C), 130.7 (Ar-C), 128.8 (Ar-C), 127.5 (Ar-C), 124.3 (Ar-C), 115.7 (Ar-C), 110.3 (Ar-C), 98.8 (C=CH), 20.9 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{10}BrCl_2NO_3$ $[M+2]^+$: 426.9221; found 426.9227.

(Z)-8-bromo-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2H-benzo[b] [1,4]oxazin-2-one (20p)

Yellowish solid; Yield: 77.6 mg (89 %); R_f (EtOAc/Hexane; 20:80) = 0.90; Purification of crude product was done by flash column chromatography method over

silica gel using Hexane/ethyl acetate (9.5:0.5) as an eluent; m.p. 259-260 °C; FT-IR (KBr, νmax/cm-1) 3417, 1768, 1629, 1562, 1283, 1138; ^{1}H NMR (400 MHz) δ 7.86 (d, J = 8.8 Hz, 2H, Ar-H), 7.62 (d, J = 8.4 Hz, 2H, Ar-H), 7.16 (s, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.86 (s, 1H, C=C<u>H</u>), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 190.4 (C=O), 155.6 (O=C-O), 139.0 (Ar-C-N), 137.0 (Ar-C-O), 136.9 (<u>C</u>=CH), 136.7 (Ar-<u>C</u>-CH_3), 132.2 (Ar-C), 129.3 (Ar-C), 128.5 (Ar-C), 128.1 (Ar-C), 124.5 (Ar-C), 115.6 (Ar-C), 110.3 (Ar-C), 94.8 (C=<u>C</u>H), 20.9 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{11}Br_2NO_3$ $[M+2]^+$: 436.9106; found 436.9100.

(Z)-8-bromo-6-methyl-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20q)

Yellowish solid; Yield: 70.7 mg (95 %); R_f (EtOAc/Hexane; 20:80) = 0.90; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (8.5:1.5) as an eluent; m.p. 219-220 °C; FT-IR (KBr, νmax/cm-1) 3417, 1764, 1630, 1602, 1281, 1182; ^{1}H NMR (400 MHz) δ 8.13 (d, J = 8.0 Hz, 1H, Ar-H), 7.99 (d, J = 8.0 Hz, 2H, Ar-H), 7.69 (d, J = 7.6 Hz, 1H, Ar-H), 7.37 (d, J = 8.0 Hz, 2H, Ar-H), 7.27 (t, J = 8.8 Hz, 1H, Ar-H), 7.10 (s, 1H, C=C<u>H</u>), 2.39 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 191.4 (C=O), 155.8 (O=C-O), 143.9 (Ar-<u>C</u>-CH_3), 138.3 (Ar-C-N), 136.8 (Ar-C-O), 136.5 (<u>C</u>=CH), 135.6 (Ar-<u>C</u>-CH_3), 129.6 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 124.8 (Ar-C), 115.4 (Ar-C), 110.1 (Ar-C), 95.3 (C=<u>C</u>H), 21.8 (CH_3), 20.9 (CH_3); HRMS (ESI) calcd. for $C_{18}H_{14}BrNO_3$ $[M+2]^+$: 373.0157; found 373.0152.

(Z)-6-methyl-3-(2-(4-nitrophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[1,4]oxazin-2-one (20r)

Yield: 57.2 mg (82 %), R_f (EtOAc/Hexane; 20:80) = 0.75; Purification of crude product was done by recrystallization using Hexane/ethyl acetate; m.p. 211-213 °C; FT-IR (KBr, νmax/cm^{-1}) 3446, 3072, 1758, 1621, 1515, 1183; ^{1}H NMR (400 MHz) δ 8.32 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.13 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.12 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.01 – 6.96 (m, 3H, Ar-H, C=C<u>H</u>), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz) δ 188.8 (C=O), 155.9 (O=C-O), 149.9 (Ar-C-NO_2), 143.3 (<u>C</u>=CH), 140.5 (Ar-C-N), 139.7 (Ar-C-O), 136.4 (Ar-C), 128.6 (Ar-C), 125.8 (Ar-C), 124.0 (Ar-C), 123.9 (Ar-C), 117.2 (Ar-C), 116.7 (Ar-C), 94.1 (C=<u>C</u>H), 21.1 (CH_3); HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_5$ $[M+H]^+$: 325.0746; found 325.0748.

(Z)-3-(2-(4-nitrophenyl)-2-oxoethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20s)

Yellowish solid; yield: 52.2 mg (84 %); R_f (EtOAc/Hexane; 20:80) = 0.75; Purification of crude product was done by recrystallization using Hexane/ethyl acetate; m.p. 207-209 °C; FT-IR (KBr, νmax/cm-1) 3448, 3069, 1758, 1621, 1515; 1453; ^{1}H NMR (400 MHz) δ 8.32 (d, J = 8.8 Hz, 2H, Ar-H), 8.15 (d, J = 7.2 Hz, 2H, Ar-H), 7.26 – 7.17 (m, 4H, Ar-H), 7.04 (s, 1H, C=C<u>H</u>); ^{13}C NMR (100 MHz) δ 188.9 (C=O), 155.8 (O=C-O), 150.1 (Ar-C-NO_2), 143.3 (Ar-C-N), 141.7 (<u>C</u>=CH), 140.5 (Ar-C-O), 128.7 (Ar-C), 126.2 (Ar-C), 125.1 (Ar-C), 124.1 (Ar-C), 123.4 (Ar-C), 117.5 (Ar-C), 116.6 (Ar-C), 94.3 (C=<u>C</u>H); HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_5$ $[M+H]^+$: 311.0590; found 311.0596.

(Z)-6-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20t)

Yield: 52.2 mg (84 %); spectral data can be seen at 2.2.5.4 (24k)

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20u)

Yield: 52.7 mg (80 %); spectral data can be seen at 2.2.5.4 (24m)

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20v)

Yellowish solid; yield: 50.21 mg (74 %); R_f (EtOAc/Hexane; 20:80) = 0.75; Purification of crude product was done by flash column chromatography method over silica gel using Hexane/ethyl acetate (7:3) as an eluent; m.p. 195-197 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2926, 1599, 1758, 1633, 1594; ^{1}H NMR (400 MHz) δ 8.02 – 7.93 (m, 4H, Ar-H), 7.29 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.10 (s, 1H, C=C<u>H</u>), 6.98 (d, *J* = 8.7 Hz, 2H, Ar-H), 3.89 (s, 3H, OCH_3); ^{13}C NMR (100 MHz) δ 190.7 (C=O), 164.0 (Ar-<u>C</u>-OCH_3), 155.3 (O=C-O), 145.2 (Ar-<u>C</u>-NO_2), 144.9 (Ar-C-N), 137.0 (Ar-C-O), 130.7 (<u>C</u>=CH), 130.3 (Ar-C), 124.9 (Ar-C), 118.6 (Ar-C), 117.8 (Ar-C), 114.2 (Ar-C), 111.2 (Ar-C), 97.0 (C=<u>C</u>H), 55.7 (OCH_3); HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_6$ $[M+H]^+$: 341.0695; found 341.0692.

(Z)-7-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20w)

Yield: 53.4 mg (86 %); spectral data can be seen at 2.2.5.4 (24n)

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20x)

Yellowish solid; yield: 52.7 mg (80 %); R_f (EtOAc/Hexane; 20:80) = 0.70; Purification of crude product was done by recrystallization using Hexane/ethyl acetate; m.p. 235-237 °C; FT-IR (KBr, νmax/cm-1) 3411, 3090, 1773, 1626, 1516, 1473; ^{1}H NMR (400 MHz) δ 8.18 - 8.08 (m, 4H, Ar-H), 7.88 – 7.86 (m, 1H, Ar-H), 7.42 – 7.38 (m, 2H, Ar-H), 7.02 (s, 1H, C=C<u>H</u>); ^{13}C NMR (100 MHz) 188.7 (C=O), 163.7 (Ar-C-F), 155.5 (O=C-O), 141.8 (Ar-C-N), 140.6 (Ar-C-O), 138.7 (Ar-<u>C</u>-NO_2), 134.4 (<u>C</u>=CH), 130.7 (Ar-C), 130.6 (Ar-C), 130.5 (Ar-C), 120.9 (Ar-C), 116.9 (Ar-C), 116.2 (Ar-C), 115.9 (Ar-C), 112.0 (Ar-C), 95.3 (C=<u>C</u>H); HRMS (ESI) calcd. for $C_{16}H_9FN_2O_5$ $[M+H]^+$: 329.0495; found 329.0499.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20y)

Yield: 59.7 mg (87 %); spectral data can be seen at 2.2.5.4 (24q)

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20z)

Yellowish solid; yield: 64.6 mg (85 %); R_f (EtOAc/Hexane; 20:80) = 0.70; Purification of crude product was done by recrystallization using Hexane/ethyl acetate; m.p. 208-210 °C; FT-IR (KBr, νmax/cm-1) 3433, 3088, 1770, 1620, 1522, 1470, 1072; ^{1}H NMR (400 MHz) δ 12.39 (s, 1H, NH), 8.09 – 8.08 (m, 2H, Ar-H), 7.95 - 7.93 (s, 1H, Ar-H), 7.78 – 7.58 (m, 3H, Ar-H), 6.62 (s, 1H, C=C<u>H</u>); ^{13}C NMR (100 MHz) δ 190.3 (C=O), 155.3 (O=C-O), 142.2 (Ar-C-N), 140.8 (Ar-C-Cl), 138.5 (Ar-C-O), 137.7 (Ar-C-NO_2), 136.2 (<u>C</u>=CH), 131.2 (Ar-C-Cl), 131.0 (Ar-C), 130.5 (Ar-C), 130.1 (Ar-C), 127.9 (Ar-C), 120.8 (Ar-C), 117.3 (Ar-C), 112.1 (Ar-C), 98.8 (C=<u>C</u>H); HRMS (ESI) calcd. for $C_{16}H_8Cl_2N_2O_5$ $[M+H]^+$: 378.9810; found 378.9818.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one(20aa)

Yield: 69.5 mg (89 %); spectral data can be seen at 2.2.5.4 (24p)

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (20ab)

Yield: 55.4 mg (81 %); spectral data can be seen at 2.2.5.4 (24o)

2.3A.7.4 Pharmacological studies

2.3A.7.4.1 DPPH radical scavenging antioxidant assay[30-31]

Same as Section 2.1.3.1.

2.3A.7.4.2 Ferric Reducing Antioxidant Power (FRAP) Assay[32]

Same as section 2.1.3.2.

2.3A.7.4.3 Cell toxicity assay[33]

Cell toxicity of active analogues of 2-oxo-benzo [1, 4] oxazines were accessed using $3T_3$ fibroblast cell lines in MTT assay *via* reported method.

2.3A.7.4.4 *In silico* molecular docking studies[34-35]

Molecular modeling studies of functionalized 2-oxo-benzo [1, 4] oxazines derivatives **20a-ab** 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 human antioxidant enzyme in complex (PDB ID: 3MNG) (Hall et al. 2010; Bayoumi et al. 2012; Yapati et al. 2016). The Surflexdoc module in Sybyl was used to construct a 3D model of the structures.

To find the possible bioactive conformations of functionalized 2-oxo-benzo [1, 4] oxazines derivatives, molecular modeling studies were performed using the Sybyl X 2.0 interfaced for the synthesized compounds, which exhibited promising and lower antioxidant activity *in vitro* to find the preferred binding conformations in the receptor. The starting coordinates of the human antioxidant enzyme in complex with the competitive inhibitor DTT (PDB: 3MNG) were taken from the Protein Data Bank (http://www.rcsb.org/pdb). 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.

2.3A.8 References

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*2.3*A.9 Selected characterization spectra (^{1}H and ^{13}C NMR) of selected compounds:

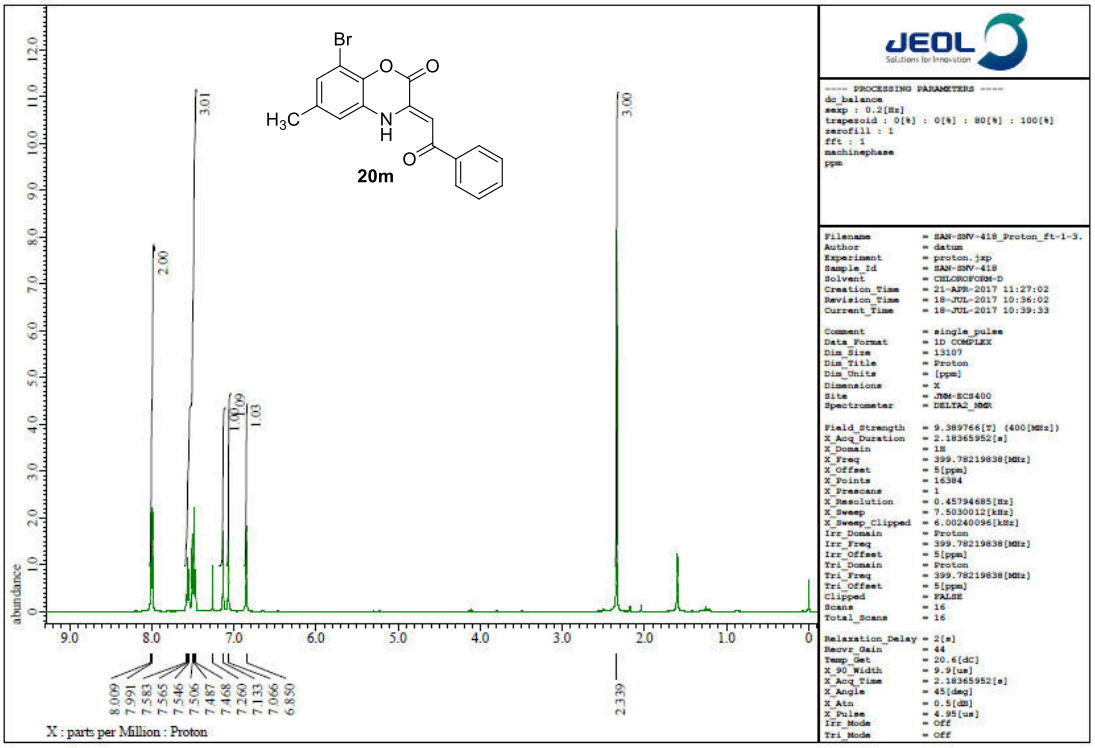


Figure 1. ^{1}H NMR Spectra of Compound **20m**.

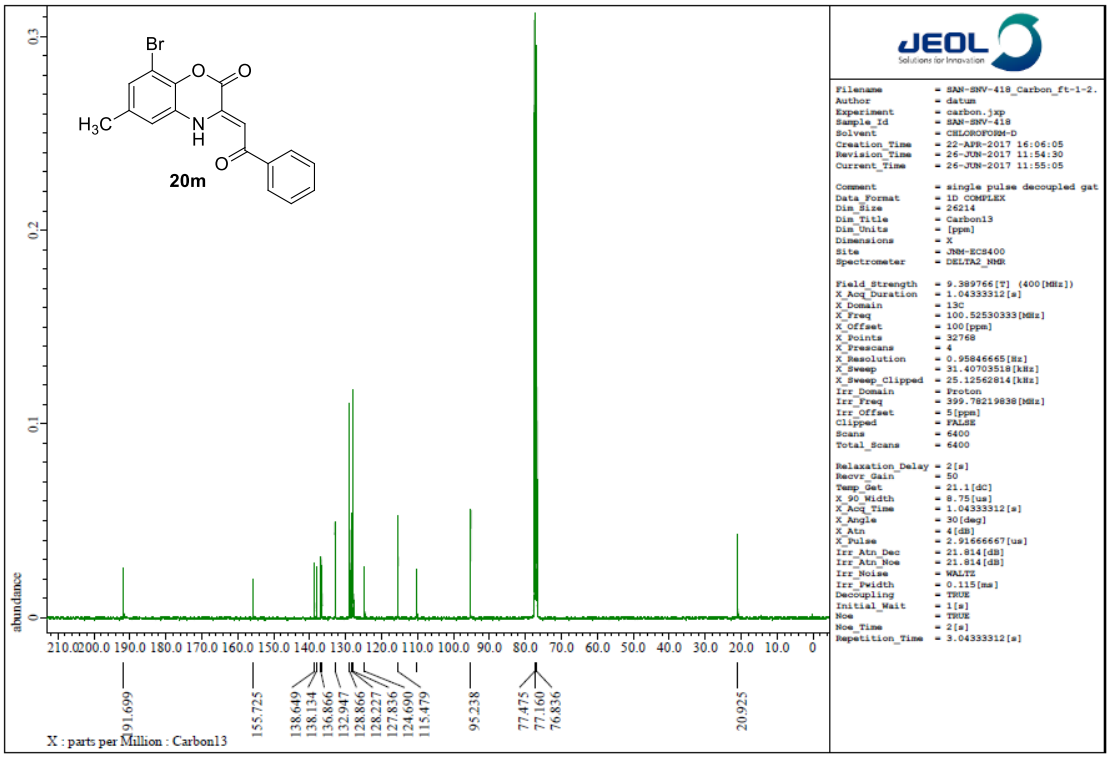


Figure 2. ^{13}C NMR Spectra of Compound **20m**.

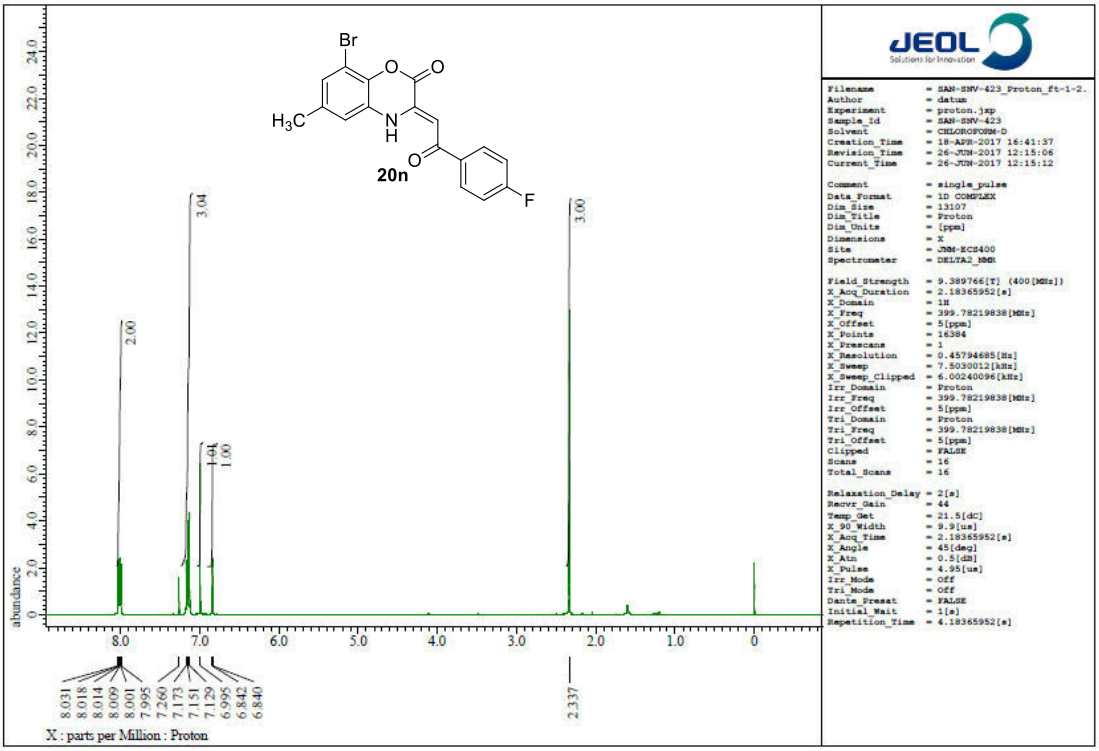


Figure 3. [1]H NMR Spectra of Compound **20n**.

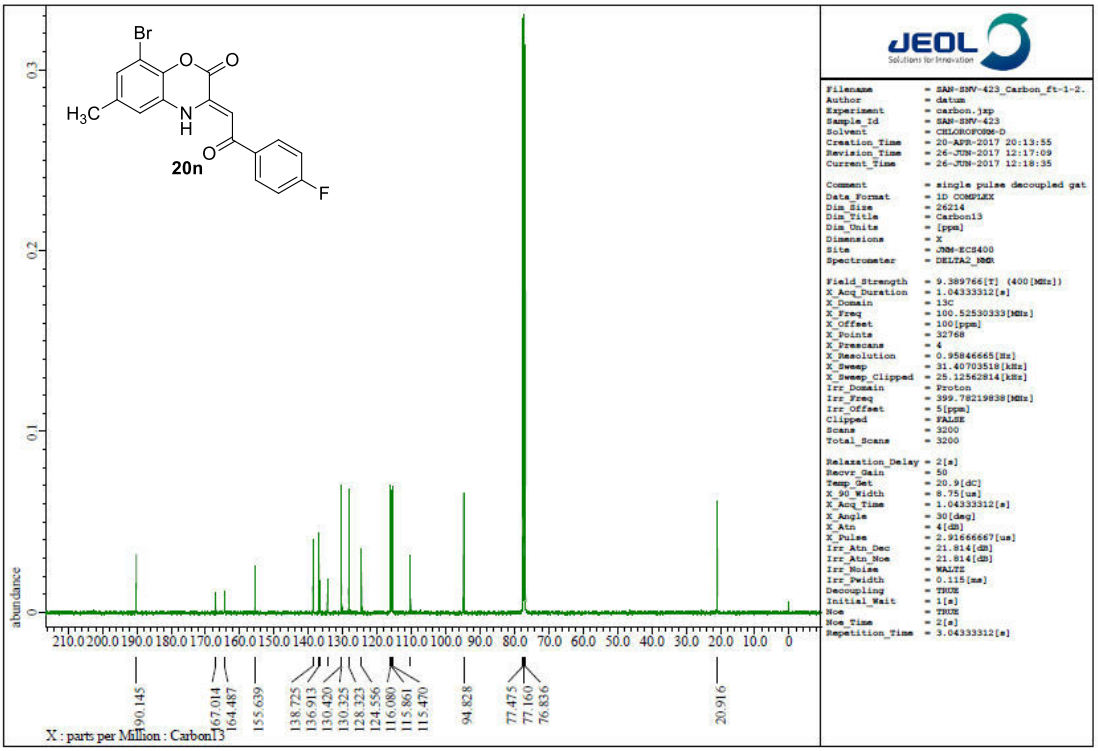


Figure 4. [13]C NMR Spectra of Compound **20n**.

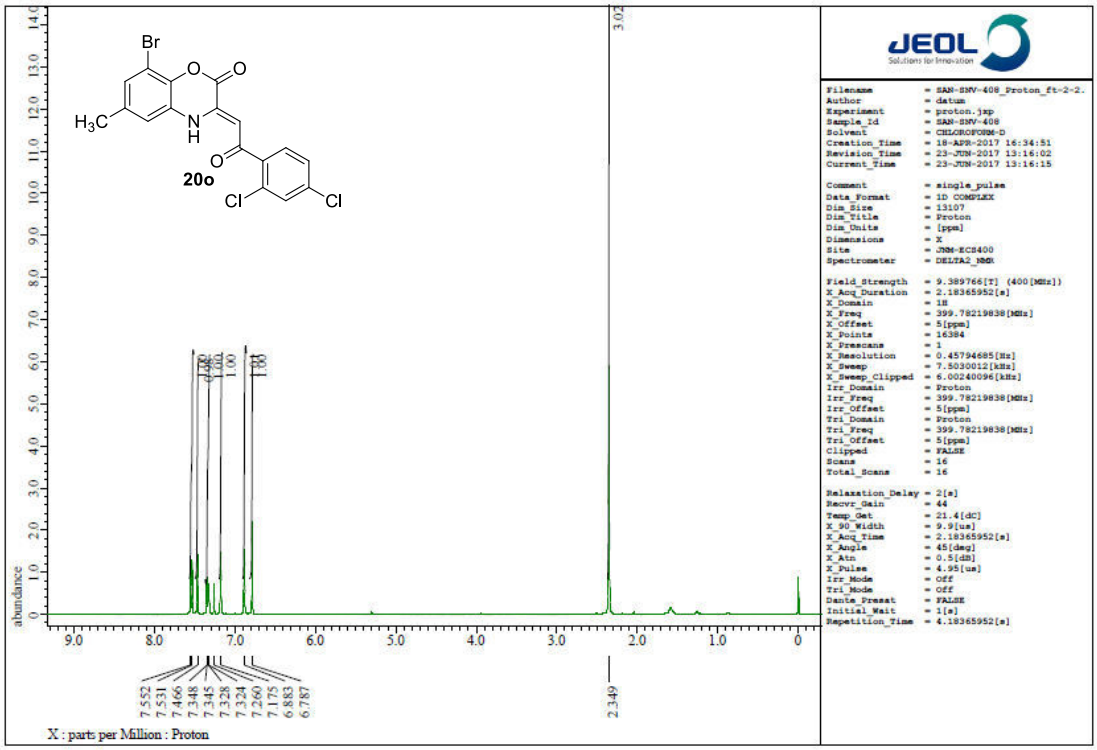


Figure 5. [1]H NMR Spectra of Compound **20o**.

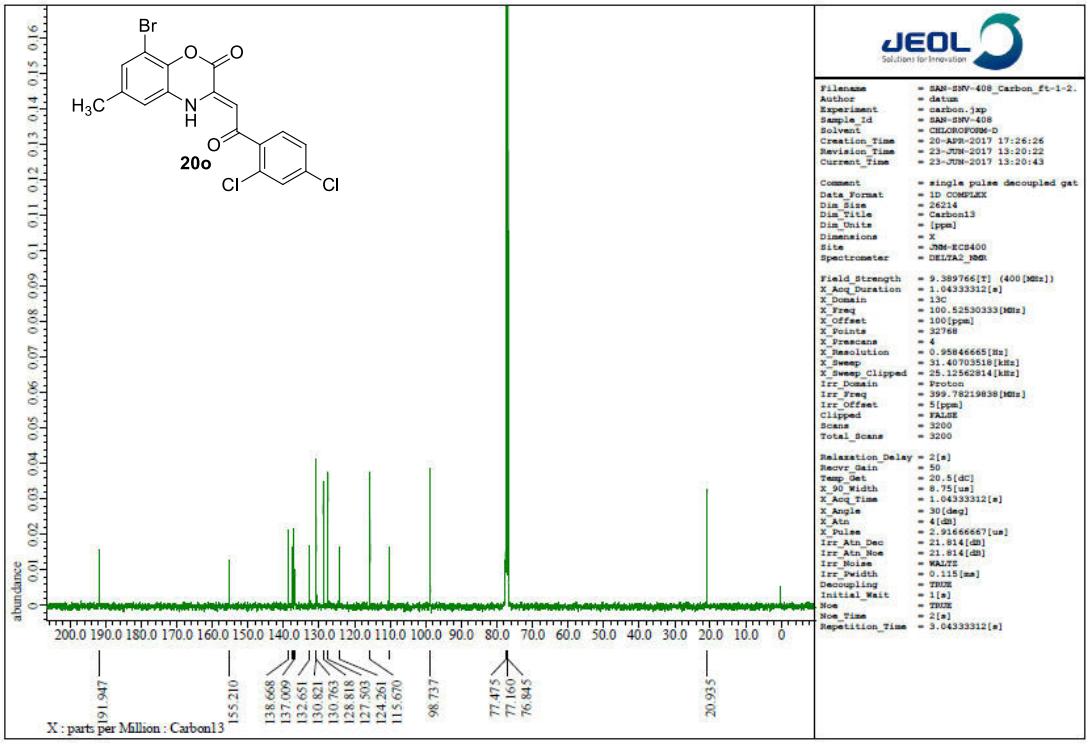


Figure 6. [13]C NMR Spectra of Compound **20o**.

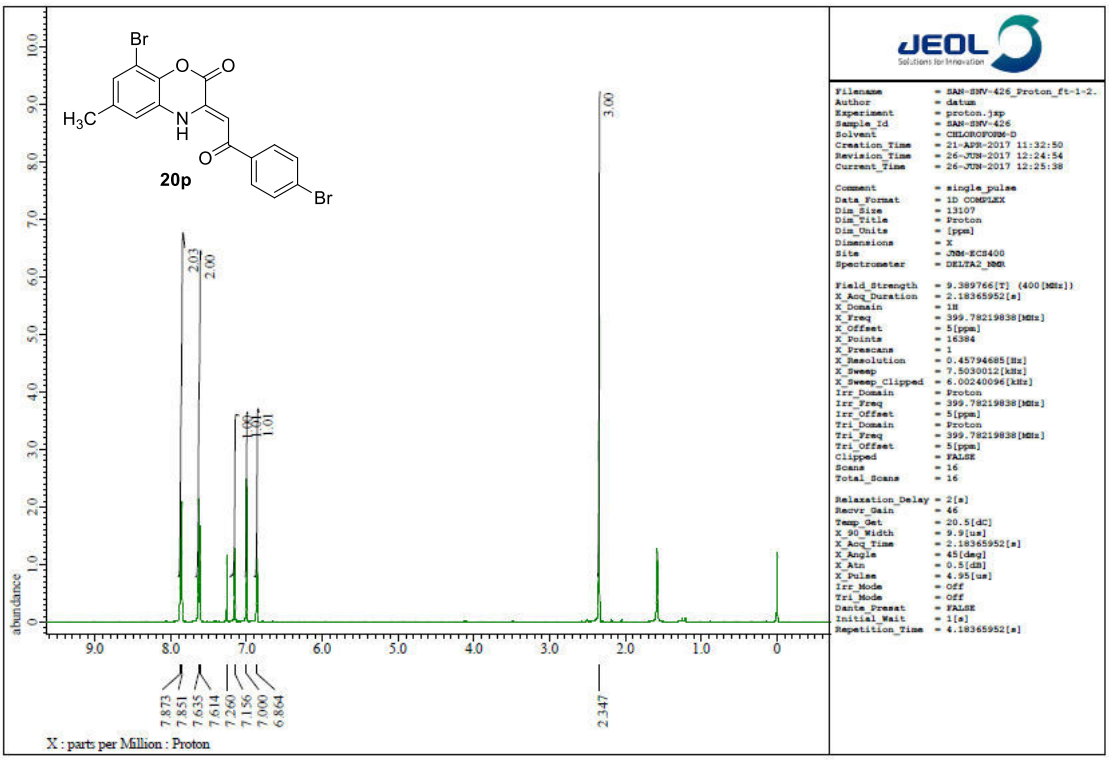


Figure 7. [1]H NMR Spectra of Compound **20p**.

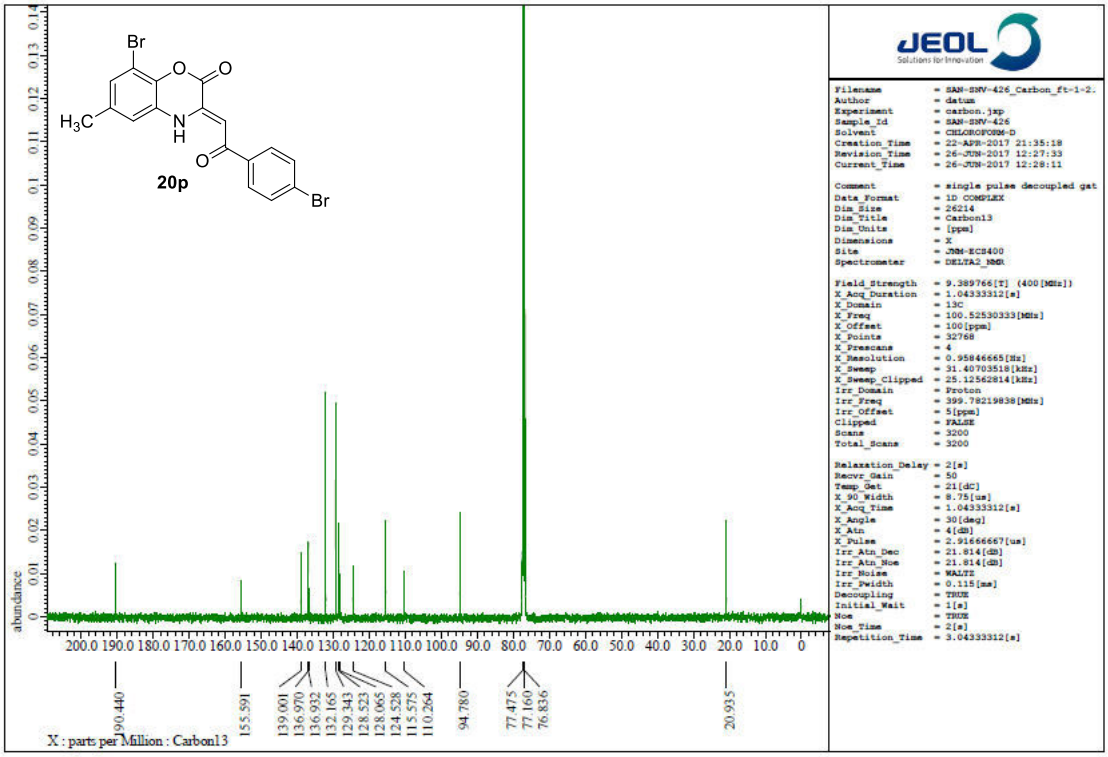


Figure 8. [13]C NMR Spectra of Compound **20p**.

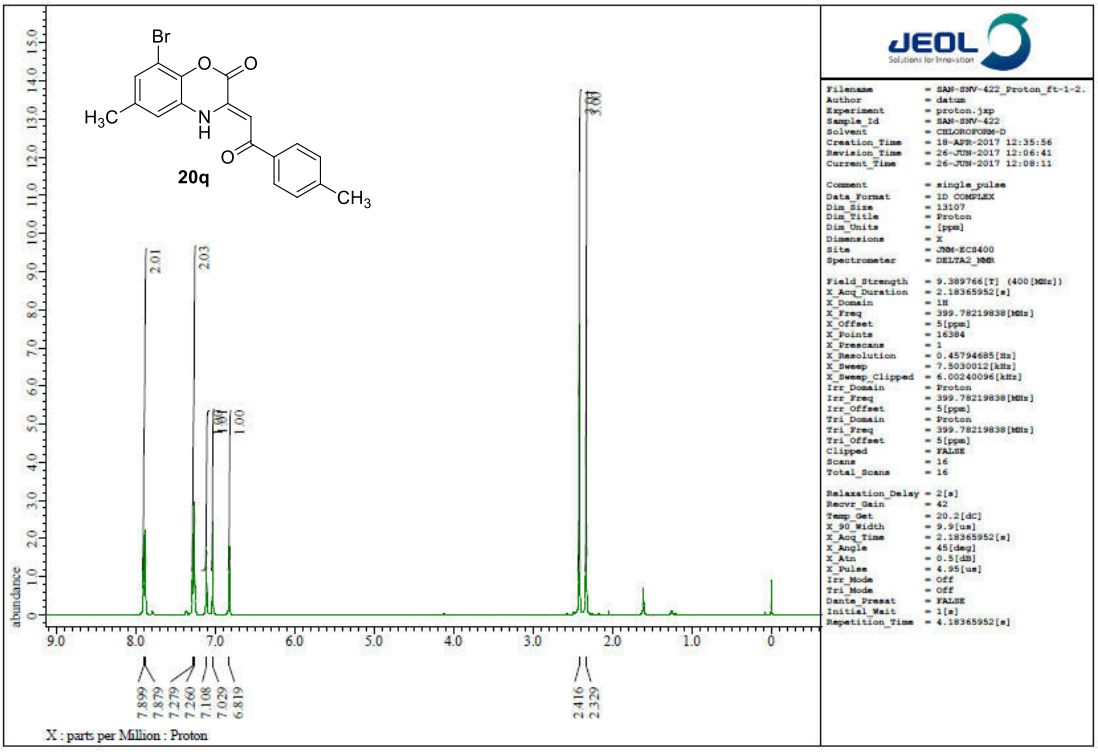


Figure 9. ^{1}H NMR Spectra of Compound **20q**.

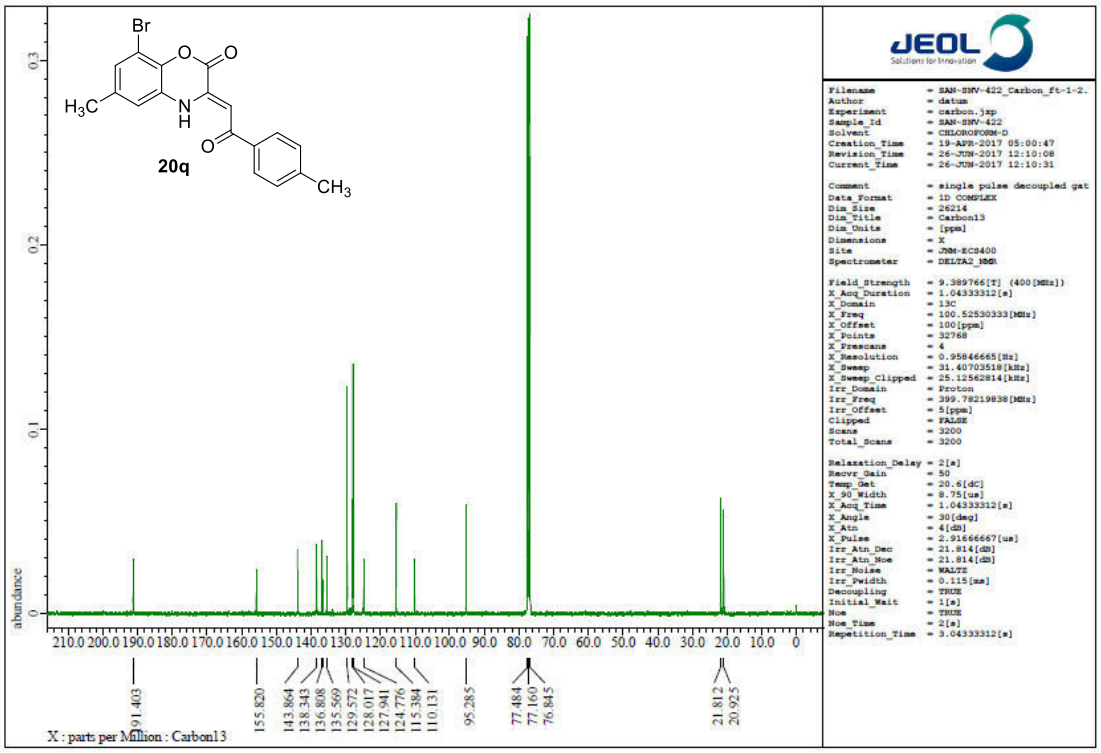


Figure 10. ^{13}C NMR Spectra of Compound **20q**.

Section 2.3B

Rational Design, Ultrasound-Assisted Synthesis SAR and Molecular Docking Simulation Studies towards the identification of 2-oxo-benzo[1,4]oxazines as Non-peptide based potential new class of platelet aggregation inhibitors.

2.3B.1 Introduction

Thrombosis represents one of the most important cause of cerebral stroke and arterial thromboembotic diseases, such as ischemic stroke, myocardial infarction, angina pectoris and other cardiovascular diseases, which are being caused by platelet aggregation; and are responsible for morbidity and mortality in developing countries.[1,2] Primarily, platelet aggregation is a vital process in haemostasis formation which is being activated by the enzyme thrombin and transforms the fibrinogen (insoluble) into fibrin (soluble). It was firstly reported by B.S. Coller (1960) that the platelet aggregation inhibitors might be the rationale for antithrombotic therapy.[3] Anti-thrombotic therapy, which represents the treatment of thrombin platelet and thrombosis, are divided into 3 categories: (1) anti-platelet drugs (such as aspirin, ticlopidine and indomethacin);[4] (2) Anti-coagulants or Thrombin inhibiting drugs (such as heparin and warfarin);[5] and (3) Thrombolytic drugs (such as fibrin receptor antagonists).[6] Based on the mode of action, type 1 category i.e., platelet aggregation inhibitors can be further sub-divided into 3 classes, such as (1) agents, which affect the metabolism of arachidonic acid; 2) platelet activation factor (PAF) receptor antagonists, and 3) agents which have an effect on the ADP dependent pathway of platelet aggregation.[7]

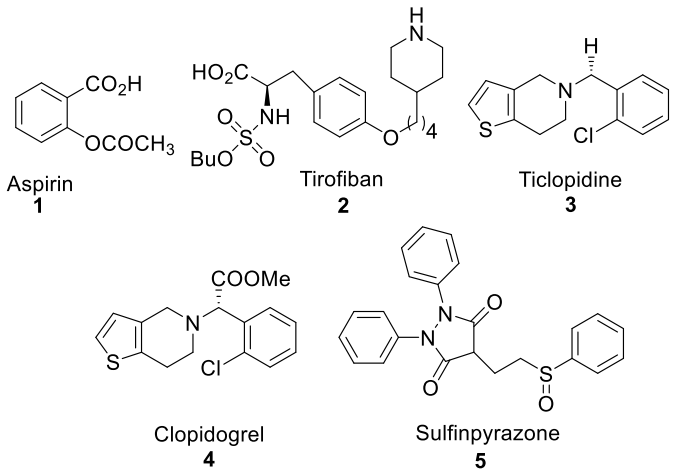


Figure. 1. Structures of some clinically used platelet aggregation inhibitors.

The most clinically used anti-platelet drugs are either phosphodiesterase inhibitor/ADP receptor antagonists, or cyclooxygenase (COX) inhibitor as well as GpIIb/IIIa receptor antagonist.[8] Arachidonic acid (AA) is a fatty acid, which is liberated from the activated platelets and is converted into a potent inducer of platelet aggregation by the enzyme cyclooxyenase.[9] On the other hand, AA is also a precursor for thromboxane A2 (TBXA2) synthesis, which can stimulate platelet aggregation after conversion to prostaglandin G1 and H2.[10] Thus, in the initial step, prostaglandin-endoperoxide synthase 1 (PTGS1; which is also known as cyclooxygenase-1) catalyses the transformation of AA into cyclic endoperoxide PG G2 and H2 and after that this PGG2 and PGH2 are converted into TBXA2 by TBXA synthase in platelets.[10] Therefore, the inhibition of cyclooxygenase-1 is an important target in the identification of novel platelet aggregation inhibitors.[11] As a result, several non-peptide mimics such as aspirin **1**, tirofiban **2**, ticlopidine **3**, Clopidogrel **4**, sulfinpyrazone **5** etc. have been developed as a potent inhibitors of platelet aggregation.[12] (Figure 1)

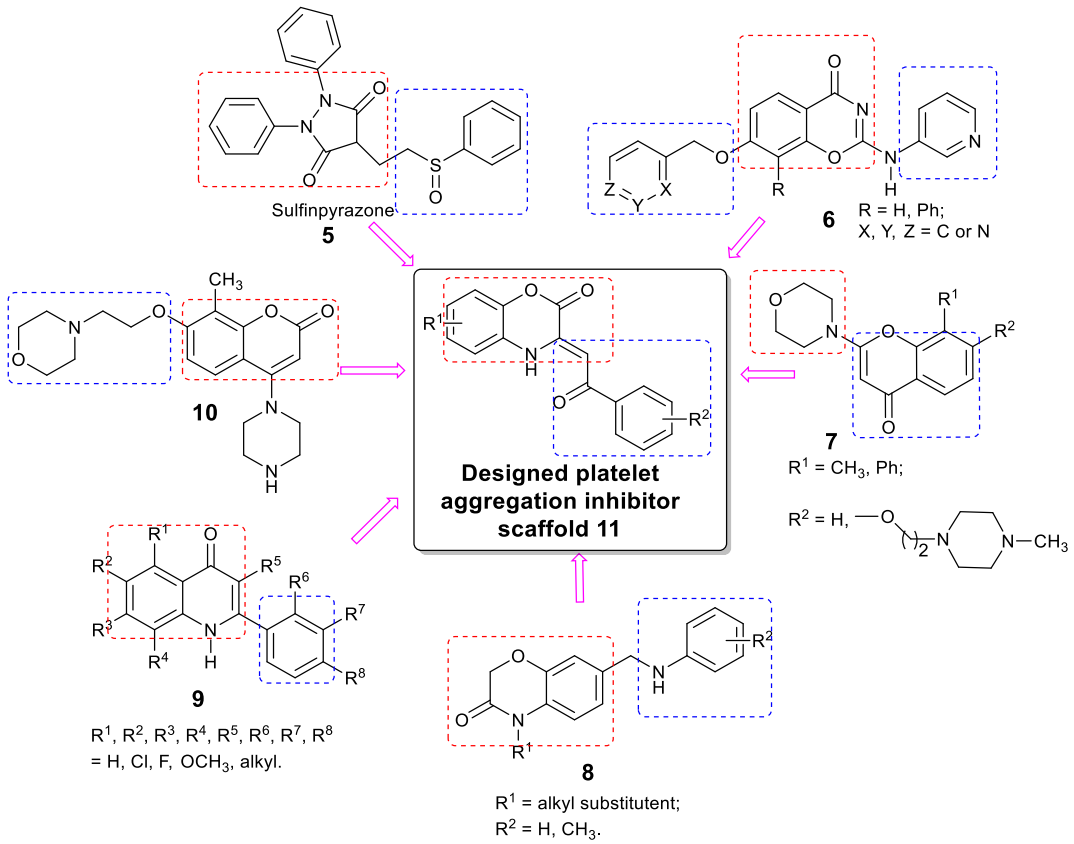


Figure 2. Design strategy for the target functionalized 3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one as a new class of potential platelet aggregation inhibitor.

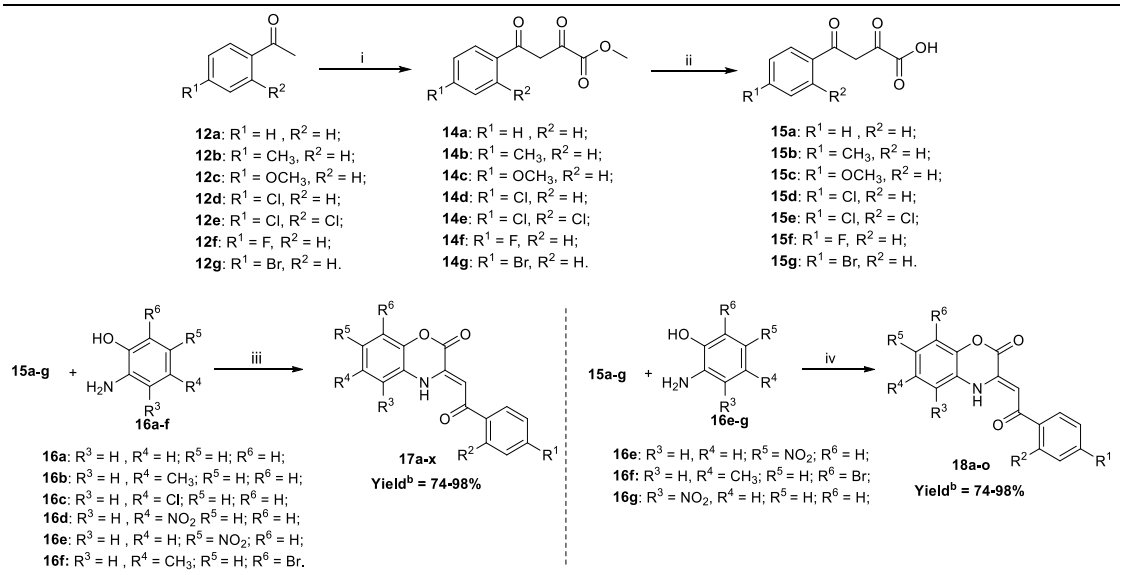
Inspite of the efficacy of platelet aggregation inhibitory effect, several antiplatelet agents have been withdrawn from the market as these are associated with serious drawbacks such as adverse side-effects, increased mortality rate, high toxicity etc.[13] Therefore, the identification of a potent and safe platelet aggregation inhibitors is of great interest to synthetic medicinal chemists.[14]

Benzoxazine class of compounds are endowed with a wide range of biological activities[15] such as anti-tumor, [15a] anti-inflammatory, [15b] anti-microbial,[15c] antifungal,[15d] COX-2 inhibitor,[15e] rennin inhibitor[15f] and non-steroidal progesterone receptor agonists.[15g] Recently, Zuo and his co-workers reported benz[1,4]oxazine-3-one derivatives as an efficient GPIIb/IIIa receptor antagonists and displayed promising platelet aggregation inhibiting activity.[8,16] Dudley *et al.* reported that benz[1,4]oxazine-3-one derivatives as remarkable inhibitor of Xa factor.[17] Jacobson *et al.* identified 2-aryl substituted benz[1,3]oxazine-4-one class of compounds as inhibitors of the tissue factor/factor VIIa-induced activation of factor Xa and *in-situ* activates the fibrin clot formation.[18] Moreover, Kikelj *et. al.*[19] and Ila`s *et. al.*[20] had also identified benz[1,4]oxazines as glycoprotein IIb/IIIa antagonists. Further SAR study on benz[1,4]oxazine-3-ones revealed it to show promising thrombin inhibitory and fibrinogen receptor antagonist activity.[21]

Recent literature revealed that, 2-phenyl-4-quinolones have been reported to show platelet aggregation inhibitory activity via COX-1 inhibitors. Moreover, several similar bioactive class of compounds were reported to show promising platelet aggregation inhibitory activity such as functionalized 1,3-benzoxazines **6**,[22a-c] 2-morpholino substituted benzoxazines **7**,[22d] benz[1,4]oxazine-3-ones **8**,[16] functionalized quinolin-4(1H)-ones **9**,[22e-f] substituted coumarins **10** etc.[22g] (Figure 2)

Relevant to the present study, 3-oxo-benz[1,4]oxazines analogues **8** displayed good ADP, collagen and PAF induced platelet aggregation inhibitory activity. Infact, the AA-induced platelet aggregation inhibiting activities of this class of compound were less explored. Therefore, in our endeavor in search for novel bioactive heterocycles as new antiplatelet agents, we designed ***prototype* 11** i.e., functionalized 2-oxo-benzo[1,4]oxazines incorporating subunits of **5**-**10** (Figure 2) and assessed their AA-induced platelet aggregation inhibiting activities with the anticipation that the 2-oxo-benz[1,4]oxazine class of compounds would also show promising inhibitory activity.

So far, literature report revealed that there is no report available showing 2-oxo-benz[1,4]oxazines as platelet aggregation inhibitory activity.



Scheme 1. Ultrasound-assisted one-pot green synthesis of 2-oxo-benzo [1, 4]oxazines analogues (**17a-x** and **18a-o**).[a]

[a]*Reagents and conditions*: (i) **12a-g** (1.0 mmol), NaH (1.2 mmol), dimethyl oxalate **13** (1.0 mmol), toluene, 0°C to 80°C, 6h; (ii) **14a-g** (1.0 mmol), $LiOH.H_2O$ (1.3 mmol), MeOH:THF:H_2O (15 ml; 7:2:1), 0°C to rt, 4h; (iii) **15a-g** (0.2 mmol), **16a-f** (0.2 mmol), water (2.0 mL), ultrasound irradiation, 80 °C, 75-90 min.; (iv) **15a-g** (0.2 mmol), **16e-g** (0.2 mmol), water (2.0 mL), ultrasound irradiation, 80 °C, 75-120 min. [b]Isolated yield after column chromatography/recrystallization.

Herein, we report the ultrasonic-assisted synthesis,[23] platelet aggregation inhibitory activity, structure-activity relationship and cytotoxic studies of a series of functionalized 2-oxo-benzo [1,4]oxazines **17a-x** and **18a-o**, respectively. We also report the validation of our activity results via *in silico* molecular docking simulation studies. Although compounds **17a-x**, **18a-b** and **18i** have been prepared earliar by other routes;[24] however, for the first time, compound **17b-c**, **17g**, **17q**, **17s-t**, **17v**, **18a-b**, and **18i** have been prepared via "on water" ultrasound-assisted methodology. Interestingly, the antiplatelet aggregation activities of all the synthesized compounds (**17a-x** and **18a-o**) have never been reported. To the best of our knowledge, for the first time, 2-oxo-benzo [1,4]oxazines, **17a-x** and **18a-o**, have been identified as new class of AA-induced platelet aggregation inhibitors. In this study, aspirin and indomethacin were used as standard reference. In addition, the cytotoxic studies of active compounds (**17i, 17x, 18f, 18g, 18h, 18i, 18l** and **18o**) using $3T_3$ fibroblast cell lines in MTT assay were also performed. We also report the validation of results via

in silico molecular docking simulation studies of active compounds **17i, 17x, 18f-i, 18l** and **18o**.

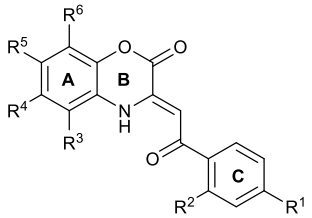
2.3B.2 Results and Discussion

Recently, we reported two highly efficient, one pot, green methodologies for the synthesis of functionalized 2-oxo-benzo[1,4]oxazines under mild conditions.[23] The "On water" ultrasonic-assisted[23b] methodology was utilized in the synthesis of a series of functionalized 2-oxo-benzo [1,4]oxazines **17a-x** and **18a-o** upto 98% yields. The required starting material for the synthesis of *prototype* **11** i.e., diketo-acids **15a-g** were synthesized in excellent yields (upto 92%) *via* base-catalyzed reaction of acetophenone **12a-g** with dimethyl oxalate **13** in toluene under refluxing condition for 6h followed by the hydrolysis of resulting diketoesters **14a-g** with $LiOH.H_2O$ in MeOH:THF:H_2O (7:2:1; v/v) as solvents. While exploring target prototype **11**, we had observed that, the reaction of nitro/alkyl/halide-substituted 2,4-dioxo-4-phenylbutanoic acid **15a-g** with nitro/alkyl/halide-substituted 2-aminophenol **16a-f** or **16e-g** in water furnished desired pure functionalized 2-oxo-benzo[1,4]oxazines **17a-x** and **18a-o** in excellent yields **(**up to 98%), respectively (Scheme 1). The structures of all the synthesized compounds were confirmed by their spectral analysis (^{1}H NMR, ^{13}C NMR, FT-IR and HRMS).

The generic structure of 2-oxo-benzo[1,4]oxazines is a bicyclic rings A & B having pendant substituted 2-oxo-phenylidene ring C (Table 1). Initially, we prepared twenty four 2-oxo-benzo[1,4]oxazines **17a-x** having various substituents at ring A and C, and evaluated for their arachidonic acid (AA) induced-antiplatelet aggregation inhibitory activities. In this study, the standard reference drug used were aspirin and indomethacin which showed IC_{50} values of 21.34±1.09 μg/mL and 0.27±0.04 μg/mL, respectively. As depicted from Table 1, out of all compounds, compounds such as **17i**, **17m**, **17s** and **17x** having halogen atom at ring C showed promising inhibitory activities in comparison to the standard reference except compound **17k**. The model compound **17a**; which has no substitutions at ring A or ring C; displayed more than two times lesser activity in comparison to the standard reference. Then, we synthesized compounds **17b-x** having various electron-withdrawing groups (EWG) as well as electron-donating groups (EDG) at ring A or ring C to interpret SAR studies with the anticipation that these substitutents might cause an increase in the platelet aggregation inhibitory activity.

Among all screened compounds, EDG (such as CH_3, Cl, Br etc) at ring A linked with EDG at ring C (compounds **17b-h** and **17j-o**; entry 2-8 and entry 10-15; Table 1) dislayed lesser activity than aspirin. Whereas, the compounds **17i** (IC_{50} = 22.87 ± 0.26 µg/ml; entry 9; Table 1), which has 3-methyl-6-bromo substitutent at ring A and bromo substitutent at ring B displayed comparable platelet aggregation inhibitory activity compared to aspirin.

Table 1: *In vitro* AA-induced platelet aggregation inhibitory activity[22f] of a series of functionalized 2-oxobenzo[1,4]oxazines **(17a-x).**



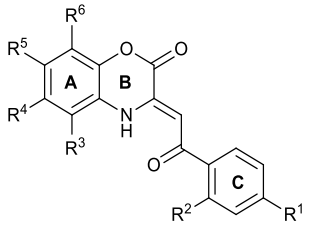
Generic Structure of 2-oxo-benzo[1,4]oxazines

S. No.	Comp. No.	R^1	R^2	R^3	R^4	R^5	R^6	Antiplatelet Activity [a,b] (IC_{50} in µg/ml)
1	**17a**	H	H	H	H	H	H	48.25±0.71
2	**17b**	H	H	H	CH_3	H	H	94.67±1.41
3	**17c**	CH_3	H	H	CH_3	H	H	85.21±1.16
4	**17d**	OCH_3	H	H	CH_3	H	H	525±3.41
5	**17e**	Cl	Cl	H	CH_3	H	H	95.4±1.43
6	**17f**	F	H	H	CH_3	H	H	93.37±1.38
7	**17g**	Br	H	H	CH_3	H	H	467±2.89
8	**17h**	H	H	H	CH_3	H	Br	87.32 ± 0.96
9	**17i**	Br	H	H	CH_3	H	Br	**22.87 ± 0.26**
10	**17j**	H	H	H	Cl	H	H	92.94±1.38
11	**17k**	CH_3	H	H	Cl	H	H	33.97±0.54
12	**17l**	Cl	H	H	Cl	H	H	531±3.44
13	**17m**	F	H	H	Cl	H	H	**30.11±0.37**
14	**17n**	Br	H	H	Cl	H	H	91.23±1.36
15	**17o**	OCH_3	H	H	Cl	H	H	473.45±2.47
16	**17p**	H	H	H	NO_2	H	H	455.38±2.28
17	**17q**	CH_3	H	H	NO_2	H	H	91.23±1.36
18	**17r**	OCH_3	H	H	NO_2	H	H	88.23±1.28
19	**17s**	Cl	H	H	NO_2	H	H	**28.12±0.22**
20	**17t**	Cl	Cl	H	NO_2	H	H	87.03±1.21
21	**17u**	F	H	H	NO_2	H	H	469.21±2.31
22	**17v**	Br	H	H	NO_2	H	H	89.11±1.27
23	**17w**	H	H	H	H	NO_2	H	84.21±1.13
24	**17x**	Br	H	H	H	NO_2	H	**19.74±0.21**
25	Aspirin	---	---	---	---	---	---	21.34±1.09
26	Indomethacin	---	---	---	---	---	---	0.27±0.04

[a]*Platelets were incubated along with either a tested compound or 0.5% DMSO at 37°C for 60 sec., then AA (100 µM) was added to accelerate the aggregation. Aspirin and indomethacin are positive controls. Values are expressed as mean_SE from three to three separations.* [b]The data represent mean of three independent determination.

The compounds, which have EWG on 4- or 5-position at ring A (**17p-w**; entry 16-23; Table-1) exhibited lesser inhibitory activity than both drugs aspirin, except compound **17x** (IC_{50} = 19.74 ± 0.21 µg/ml; entry 24; Table 1), which has NO_2 EWG on 5-position at ring A; displayed better platelet aggregation inhibitory activity than standard drug aspirin. However, all the compounds were found to be less active than indomethacin (IC_{50} = 0.27±0.04 µg/mL). The active compounds, **17i** and **17x**, found in our preliminary results prompted us to prepare further functionalized 2-oxo-benzo[1,4]oxazines having similar variations.

Table 2: *In vitro* AA-induced platelet aggregation inhibitory activity of a new series of functionalized 2-oxo-benzo[1,4]oxazines **(18a-o).**



S. No.	Comp. No.	R^1	R^2	R^3	R^4	R^5	R^6	Antiplatelet Activity [a, b] (IC_{50} in µg/ml)
1	**18a**	OCH_3	H	H	H	NO_2	H	82.35±1.11
2	**18b**	Cl	H	H	H	NO_2	H	28.12±0.22
3	**18c**	Cl	Cl	H	H	NO_2	H	98.75±1.46
4	**18d**	F	H	H	H	NO_2	H	41.11±0.47
5	**18e**	CH_3	H	H	CH_3	H	Br	45.97 ± 0.65
6	**18f**	OCH_3	H	H	CH_3	H	Br	**19.83 ± 0.22**
7	**18g**	Cl	Cl	H	CH_3	H	Br	**20.08 ± 0.24**
8	**18h**	F	H	H	CH_3	H	Br	**21.47 ± 0.24**
9	**18i**	H	H	NO_2	H	H	H	**16.96 ± 0.18**
10	**18j**	CH_3	H	NO_2	H	H	H	29.87 ± 0.30
11	**18k**	OCH_3	H	NO_2	H	H	H	36.54 ± 0.35
12	**18l**	Cl	H	NO_2	H	H	H	**19.19 ± 0.22**
13	**18m**	Cl	Cl	NO_2	H	H	H	41.35 ± 0.53
14	**18n**	F	H	NO_2	H	H	H	44.08 ± 0.64
15	**18o**	Br	H	NO_2	H	H	H	**17.96 ± 0.18**
16	Aspirin	---	---	---	---	---	---	21.34±1.09
17	Indomethacin	---	---	---	---	---	---	0.27±0.04

[a]*Platelets were incubated along with either a tested compound or 0.5% DMSO at 37°C for 60 sec., then AA (100 µM) was added to accelerate the aggregation. Aspirin and indomethacin are positive controls. Values are expressed as mean_SE from three to three separations.* [b]The data represent mean of three independent determination.

Based on the structure of active compounds, **17i** and **17x**, and the preliminary biological results, we observed that the compounds having bromo substitutent at ring C on 4-position and EDG (such as CH_3 and Br) on 3- and 6-position at ring A as well as EWG (such as NO_2) on 5-position at ring A were found to show potent platelet

aggregation inhibitory activity. Therefore, a new series of functionalized 2-oxo-benzo[1,4]oxazines (**18a-o**; Scheme 1) incorporating different EWG and EDG at ring A and C were synthesized and assessed for their inhibitory antiplatelet aggregation activity (Table 2).

As it can be seen from Table 2, all the fifteen compounds **18a-o** exhibited moderate to excellent anti-platelet aggregatory activities having IC_{50} values in the range of 17.96 ± 0.18 µg/mL to 98.75 ± 1.46 µg/mL in comparison to the reference drug aspirin. However, all the compounds **18a-o** showed lesser activity than indomethacin. Compounds having EWG such as NO_2 at 5-position on ring A (**18a-d**; entry 1-4) having halo or methoxy group on ring C displayed lesser platelet aggregation inhibitory activity than aspirin. Whereas 6-Br and 4-Me groups at ring A along with F-, Cl-, OMe or CH_3-group on ring C i.e. compound **18e-h** (entry 5-8) were found to show either comparable potent platelet aggregation inhibiting activity (**18g** and **18h**; entry 7-8) or greater inhibitory activity (**18f**; entry 6) than aspirin. Moreover, while the compounds having EWG such as NO_2 at 3-position of ring A along with either no substitution (**18i**; entry 9) or EDG (such as Cl or Br) at 4-position of ring C (**18l** and **18o**; entry 12 and 15) displayed greater inhibitory activity than aspirin; compounds having CH_3-, OCH_3- or F- groups exhibited lesser activity than aspirin. It is also noted that the compound having NO_2 EWG at ring A and 2,4-dichloro substitutent on ring C (**18m**; entry 13) further decreases the inhibitory activity in comparison to the monochloro-substitution at ring A (**18l**; entry 12).

The SAR study indicates that the compounds with two EDG (i.e. 3-methyl-6-bromo substituted) at ring A along with X- group (X = Br, Cl) or OMe- group at ring C (i.e. **17i** and **18f-h**) displayed either equal inhibitory activity (as in **17i** and **18g-h**) or greater (**18f**) aggregation inhibitory activity than aspirin. In addition, The NO_2 EWG at 3-position (**18i**, **18l** and **18o**) or 5-position (**17x**) on ring A along with either no substitution (**18i**) or X- group (X = Br, Cl) at 4-position on ring C (**18l** and **18o**) exhibited greater platelet aggregation inhibitory activity than aspirin.

2.3B.3 Cell Toxicity Study

Out of 39 compounds, eight most active compounds i.e., **17i, 17x, 18f, 18g, 18h, 18i, 18l** and **18o** were selected for their cytotoxic studies using $3T_3$ fibroblast cell lines in MTT assay.[25] The result showed that these compounds were non-toxic to $3T_3$

fibroblast cell lines even at 250 µg/mL concentration and consequently displayed permissible values of cell viability (Figure 3).

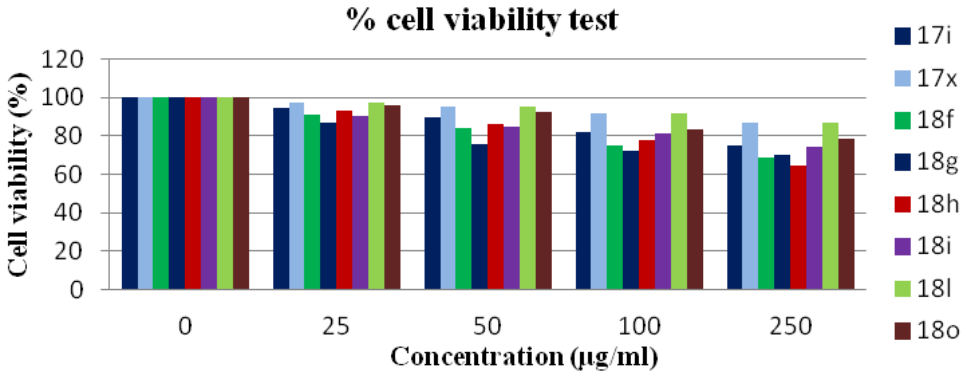


Figure 3. Percentage cell viability test.

2.3B.4 Insilico Molecular Docking Studies[27]

To study the binding modes of the active molecules (**17i, 17x, 18f, 18g, 18h, 18i, 18l** and **18o)** in the cyclooxygenase-1 (COX-1) enzyme, first we performed molecular docking study with reference standard compound aspirin and indomethacin on COX-1 domain (PDB ID: 2OYE) using Surflex–Dock. After running Surflex-Dock, the conformers were ranked in a molecular spread sheet based on their docking scores, and the best docked score conformers were selected, and speculated concerning the detailed binding mode in the active site.[27e-f] A docking score represent binding affinity, which include hydrophobic, repulsive, entropic, polar, and salvation. The docking results for **18i** and **18o** against the antiplatelet target COX-1 showed a high binding affinity docking score indicated by a total score of 5.5546 and 5.7941 respectively, forms a H-bond of length 1.7 and 2.3Å to the side chain of nucleophilic (polar, hydrophobic) residue i.e., Serine 530. In the docking pose of compound **18i** and **18o**-COX-1 complex, the chemical nature of binding site residues within a radius of 4Å was aromatic (hydrophobic), for example, Phe-381, Phe-518 (Phenylalanine), Trp-387 (Tryptophan) Tyr-385, Tyr-348(Tyrosine); nucleophilic (polar, hydrophobic) residue Ser-353(Serine); hydrophobic, for example, Val-349(Valine), Ala-527(Alanine), Gly-526 (Glycine), Ile-523(Isoleucine), Leu-352(leucine) and Met-522 (Methionine); as a result, the bound compound showed a strong hydrophobic interaction with COX-1, thus leading to more stability and activity in this compound (Figure **4A** and **4B**).

On the other hand, docking results for compound **18g** and **18l** against the target protein COX-1 showed a high binding affinity docking score indicated by a total score

of 4.5281 and 4.4766 respectively, forms a salt bridge of length 4.6Å to the polar, hydrophobic, positive charged residue Arginine-120. In the docking pose of the complex, the chemical nature of binding site residues within a radius of 4Å was acidic (polar, negative charged), for example, Glu-524 (Glutamic acid); hydrophobic, for example, Val-116, Val-349(Valine), Ala-527(Alanine), Ile-89, Ile-523 (Isoleucine), Leu-93, Leu-531, Leu-359, Leu-352 (Leucine) and Met-113 (Methionine); nucleophilic (polar, hydrophobic) residue Ser-530, Ser-353(Serine); and aromatic (hydrophobic), for example, Phe-518 (Phenylalanine), Tyr-355(Tyrosine); as a result, the bound compound showed a strong hydrophobic interaction with COX-1, thus leading to more stability and activity in this compound (Figure **4C** and **4D**).

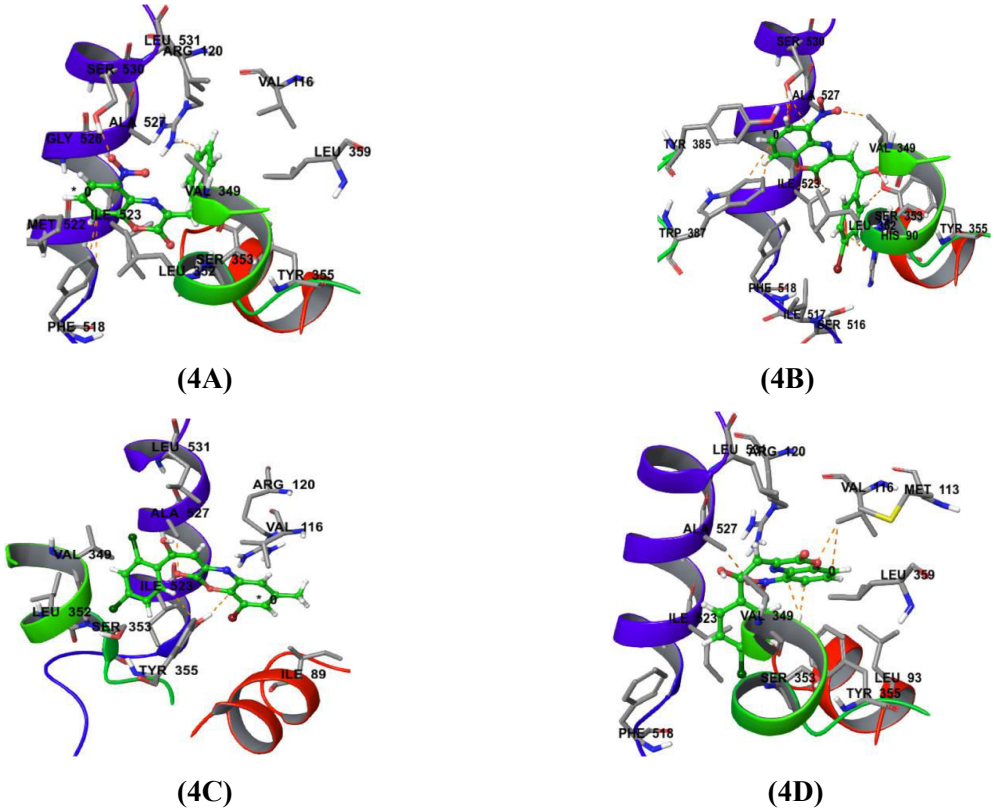


Figure 4 (A): Predicted interactions of **18i** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 5.5546, revealing a H-bonds of length 1.7, to the binding site pocket residues Serine-530. **(B)** Predicted interactions of **18o** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 5.7941, revealing a H-bonds of length 2.3, to the binding site pocket residues Serine-530. **(C)** Predicted interactions of **18g** with antiplateles target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.5281, revealing a salt bridge of length 4.6, to the binding site pocket residues Arginine-120. **(D)** Predicted interactions of **18l** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.4766, revealing a salt bridge of length 4.6, to the binding site pocket residues Arginine-120.

Likewise, docking results for compound **17i** and **17x** against the target protein COX-1 showed a high binding affinity docking score indicated by a total score of 4.9098 and 4.9098 respectively, forms a H-bond of length 1.8 and 2.7 Å to the side chain of hydrophobic aromatic residue that is, Phenylalanine-518 and nucleophilic (polar, hydrophobic) residue that is, Serine-553. On the other hand, docking results for **17x** formed a Pi-cation interaction with side chain of Tyr-385, $-NH^+$ atom of pyridine ring formed Pi-cation with -H atom of OH group of Tyr-385, (pyridine-NH^+.....OH, Tyr-385, 3.9Å).

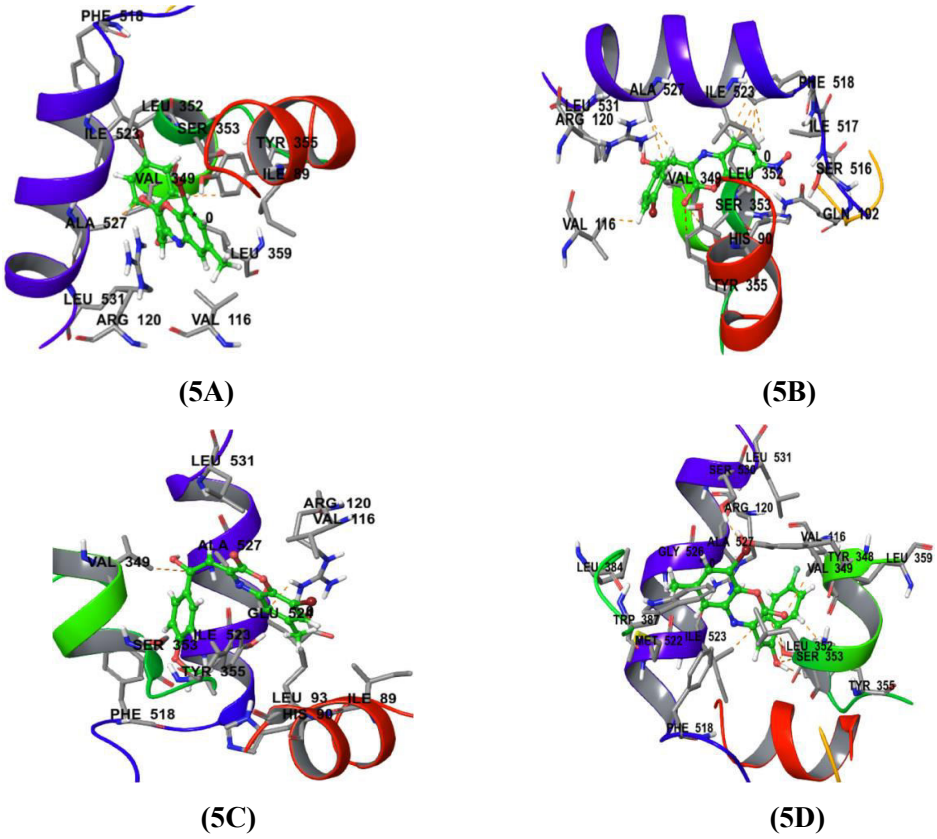
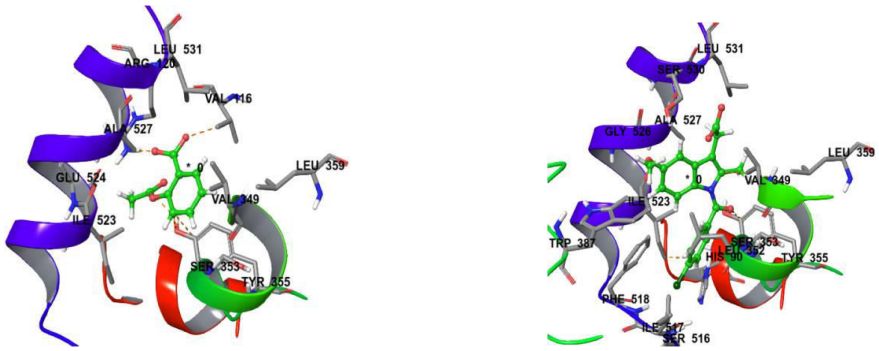


Figure 5 (A): Predicted interactions of **17i** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.9098, revealing a H-bonds of length 1.8, to the binding site pocket residues Phenylalanine-518. **(B)** Predicted interactions of **17x** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.9098, revealing a H-bonds of length 2.7, to the binding site pocket residues Serine-553. **(C)** Predicted interactions of **18f** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 5.6388, revealing a Pi-Pi stacking of length 5.3, to the binding site pocket residues Tyr-385. **(D)** Predicted interactions of **18h** with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.5653, revealing a Pi-Pi stacking of length 5.4, to the binding site pocket residues Tyr-355.

In the docking pose of the complex, the chemical nature of binding site residues within a radius of 4Å was basic (polar, hydrophobic, positive charged), for example,

Arg-120 (Arginine); acidic (polar, negative charged), for example, Glu-524 (Glutamic acid); aromatic (hydrophobic), for example, Tyr-355(Tyrosine); hydrophobic, for example, Leu-359, Leu-531, Leu-352, Leu-93 (Leucine), Met-113 (Methionine), Ala-527 (Alanine), Ile-523, Ile-89 (Isoleucine), Val-349, Val-116 (Alanine), as a result, the bound compound showed a strong hydrophobic interaction with COX-1, thus leading to more stability and activity in this compound (Figure **5A** and **5B**).

Moreover, the docking results for compound **18f** and **18h** against the target protein COX-1 showed a high binding affinity docking score indicated by a total score of 5.6388 and 4.5653 respectively. Compound **18f** formed a Pi-Pi stacking with of Tyr-385, -Cl atom of pyridine ring formed Pi-Pi stacking with -OH group of Tyr-385 (pyridine-Cl...OH, Tyr-385, 5.3 Å), and Tyr-355 -F atom of pyridine ring -OH group of Tyr-355 (pyridine-F...OH, Tyr-355, 5.4 Å). In the docking pose of the complex, the chemical nature of binding site residues within a radius of 4Å was hydrophobic, for example, Leu-352, Leu-359, Leu-531(Leucine), Ala-527(Alanine), Val-349, Val-116 (Alanine), Ile-523 (Isoleucine); polar amide type, for example, His-90 (Histidine); aromatic (hydrophobic), for example, Tyr-355(Tyrosine); nucleophilic (polar, hydrophobic) residue that is, Ser-353, Ser-530 (Serine); positive charged), for example, Arg-120 (Arginine); as a result, the bound compound showed a strong hydrophobic interaction with COX-1, thus leading to more stability and activity in this compound (Figure **5C** and **5D**).



(6A) **(6B)**

Figure 6 (A): (I) Predicted interactions of aspirin with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 4.4422, revealing a H-bonds of length 1.8, to the binding site pocket residues Arginine-120. **(B):** Predicted interactions of Indomethacin with antiplatelet target enzyme COX-1 (PDB: 2OYE) with a docking total score of 3.8227, revealing a H-bonds of length 1.9, to the binding site pocket residues Serine-530.

Furthermore, the docking results for the reference drug aspirin with the antiplatelet target protein COX-1 showed a low binding affinity docking score, indicated by a low

total score of 4.4422 and forms a H-bond of length 1.8Å to the side chain of basic (polar, hydrophobic, positive charged), for example, Arginine-120 (Figure **6A**) in comparison to the docking score of antiplatelet inhibitors such as Indomethacin, which showed a low total docking score of 3.8227 (Figure **6B**). 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.

2.3B.5 Pharmacokinetic and toxicity properties

These ADME descriptors were calculated for 2-oxo-2-phenylethylidene linked 2-oxo-benzo[1,4]oxazine analogues and compared with standard ranges. All the analogues possessed a good number of hydrogen bond donors and acceptors.[27c,d]

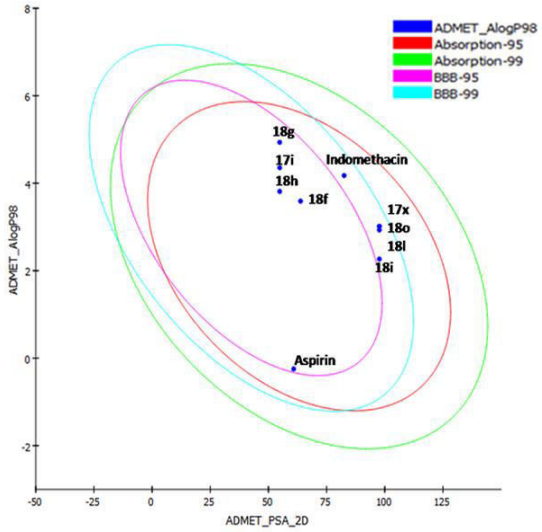


Figure 7: Plot of polar surface area (PSA) versus ALogP for 2-oxo-2-phenylethylidene linked 2-oxo-benzo [1,4]oxazine analogues **17i**, **17x**, **18f-i**, **18l** and **18o**. The 95% and 99% confidence limit ellipses corresponding to the blood brain barrier (BBB) and intestinal absorption are shown separately.

These derivatives were designed to increase the binding of the drug with the receptor through hydrogen bonding. These derivatives were found to follow Lipinski's rule of 5, affording drug likeness to the designed compounds. Polar surface area was calculated to estimate the ability of the compounds to permeate cell membranes. Lipophilicity was (ratio of octanol solubility to water solubility) measured through log P, which has been implicated in blood brain barrier penetration and permeability prediction. The excretion of drugs depends on molecular weight and log P values.[27e-h] Succinctly, the 2-oxo-2-phenylethylidene linked 2-oxo-benzo[1,4]oxazine analogues showed significant antiplatelet activity having some pharmacokinetic (PK) limitations.

The calculated TPSA (topological polar surface area) values of these compounds were within acceptable limits. The distribution of compounds in the human body was described by the predicted blood–brain barrier coefficient (logBB), apparent Caco-2 permeability, log Kp for skin permeability, volume of distribution and plasma protein binding (log Khsa for serum protein binding).[27f-h] All compounds show good aqueous solubility (Table 1 in Supplementary Information). Functionalized antiplatelet active analogue of 2-oxo-benzo [1,4]oxazines (i.e. **17i, 17x, 18f, 18g, 18h, 18i, 18l** and **18o)** were also analyzed for permeability compliance, a key determinant factor in ADMET studies or prior to clinical trials, with the help of human skin and human jejunal effective permeability parameters, along with apparent Madin-Darby Canine Kidney Cells-On-Sheet (MDCK COS) permeability, and permeability through rabbit cornea as well as permeability through human skin. MDCK permeability was in acceptable limit.

Moreover, ADMET results of predicted all analogues revealed liver high intrinsic passive uptake capacity, which is considered safe in sense of pharmacology studies. Also, calculated the brain/blood partition coefficient was detected (in logarithm), whereas the percent unbound to blood plasma proteins was detected under acceptable limit. The calculated values for these ADME parameters showed close similarity between the analogues and that of the reference drug Aspirin and Indomethacin and lie within the standard range of values exhibited by 95% of known drugs shown in Figure **7**.

2.3B.6 Conclusion

We have successfully designed and synthesized a series of functionalized 2-oxo-phenylidene linked functionalized 2-oxo-benz[1,4]oxazine analogues **17a-x** and **18a-o**, whose activities as platelet aggregation inhibitory activity as well as their SAR and cytotoxic studies followed by validation of results via *in silico* molecular docking simulation sudies, were further investigated. Compounds **17a-x** and **18a-o**, possessed moderate to good AA induced platelet aggregation inhibitory activities as compared to standard drug aspirin. Among all the tested compounds, three compounds (**17i**, **18g** and **18h**) exhibited comparable platelet aggregation inhibition activity to aspirin, whereas five compounds (**17x**, **18f**, **18i**, **18l** and **18o**) showed greater aggregation inhibitory activity than aspirin. The cytotoxicity of active compounds were found to be non-toxic in nature in 3T3 fibroblast cell line in MTT assay. Moreover, the *insilic*o

molecular docking simulation studies were also performed to validate the platelet aggregation inhibitory activity of active compounds. To the best of our knowledge, this is the first report of the identification, SAR and *insilico* molecular docking studies of 2-oxo-benz[1,4]oxazines as a novel AA-induced platelet aggregation inhibitors. These findings serve functionalized 2-oxo-benz[1,4]oxazines class of compounds as a promising scaffold to develop potent platelet aggregation inhibitors. These findings serve functionalized 2-oxo-benz[1,4]oxazines class of compounds as a promising scaffold to develop as hopeful inhibitors and aiming to improve the activity in order to get more potent and active platelet aggregation inhibitors. Further structural modifications are currently in progress in our laboratory and will be updated in other reports.

2.3B.7 Experimental section

2.3B.7.1 General information

All glass apparatus were completely oven dried prior to use. Melting points were taken in open capillaries using complab melting point apparatus and are presented uncorrected. Ultrasonic irradiation was performed in a Elmasonic S 30 (H) ultrasonic water bath cleaner and the reaction vessel was positioned in the maximum energy area in the cleaner and the removal or addition of water was used to control the temperature of the water bath. 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 $CDCl_3$, and CD_3SOCD_3 as well as mixture of ($CDCl_3$+CD_3SOCD_3) as solvent and tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI-MS) and HRMS were recorded on Xevo G2-S Q Tof (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.

2.3B.7.2 (a) General Procedure for the synthesis of starting compounds i.e. substituted diketo-acid (15a-g):

Substituted acetophenone **12a-g** (1.00 mmol, 1eq.) were taken in toluene (40 ml) and NaH (1.20 mmol, 1.2 eq.) was added cautiously. After stirring the reaction mixture at 0 °C for 30 min, dimethyl oxalate **13** (1.00 mmol, 1eq.) was added and heated at 80

°C for 5-6 h (depending upon the substrate used). The progresses of the reaction were monitored by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of the reaction, the reaction mixture was quenched with distilled water (2 ml) and 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 anhyd. Na_2SO_4 and concentrated under reduced pressure. The resultant crude products were purified by recrystalization using EtOAc/hexane (v/v = 20:80), which afforded the pure desired diketo-ester **14a-g** in 72-94% yields. Compounds **14a-g** were used for next step without any further purification.

To a solution of **14a-g** (1.00 mmol, 1eq.) in MeOH:THF:H_2O (15 ml, 7:2:1), added LiOH.H_2O (1.30 mmol, 1.3 eq.) at 0 °C, and stirred for 4-6 h at room temperature (depending upon the substrate used). The progresses of the reaction were monitored by TLC using hexane/ethyl acetate (30:70) as an eluent. After completion of the reaction, the reaction mixture was quenched by 3N HCl and 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 anhyd. Na_2SO_4 and concentrated under reduced pressure. The resultant crude product were further purified by recrystalization using EtOAc/hexane (v/v = 10:90), which furnished the pure diketo-acid **15a-g** in good yields (86-97%). Compounds **15a-g** were used for next step without any further purification.

(b) General Procedure for the synthesis of functionalized benzo[1,4]oxazin-2-one (17a-x and 18a-o):

To a solution of the compound **15a-g (**0.20 mmol; 1eq.) in water (2.0 mL) was added compound **16a-f (**0.20 mmol; 1eq.) or **16e-g (**0.20 mmol; 1eq.) and the reaction mixture was irradiated under ultrasonic sonicator at 80 ˚C temperature for 75-120 min. (depending upon the substrate employed). 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 × 10 mL); washed with distilled water (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 were further purified either by recrystallization using EtOAc/hexane (v/v = 10:90) or by flash column chromatography technique over silica gel (using 9:1 to 7:3 hexane/ethyl

acetate as an eluent), which furnished the 2-oxo-benzo[1,4]oxazine derivatives (**17a-x** and **18a-o**) in 74-98% yield range.

2.3B.7.3 **Characterization data of all the 2-oxo-benzo[1,4] oxazine analogues (17a-x and 18a-o):**

(Z)-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17a)

Yellowish solid; yield: 53.2 mg (98%), spectral data can be seen at 2.1.7.4 (11a) or 2.2.5.4 (24a), 2.3A.7.4 (20a).

(Z)-6-methyl-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17b)

Yellow solid; yield: 51.73 mg (93%), spectral data can be seen at 2.1.7.4 (11g).

(Z)-6-methyl-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17c)

Yellow solid; yield: 56.08 mg (96%), spectral data can be seen at 2.1.7.4 (11h).

(Z)-3-[2-(4-Methoxy-phenyl)-2-oxo-ethylidene]-6-methyl-3,4-dihydro-benzo[1,4]oxazin-2-one (17d)

Yellowish solid; yield: 57.7 mg (93%), spectral data can be seen at 2.3A.7.4 (20l).

(Z)-3-[2-(2,4-Dichloro-phenyl)-2-oxo-ethylidene]-6-methyl-3,4-dihydro-benzo [1,4] oxazin-2-one (17e)

Yellowish solid; yield: 65.4 mg (94%), %), spectral data can be seen at 2.3A.7.4 (20k).

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[1,4]oxazin-2-one (17f)

Yellowish solid; yield: 53.7 mg (90%); %), spectral data can be seen at 2.3A.7.4 (20j).

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17g)

Yellow solid; yield: 66.48 mg (93%); %), spectral data can be seen at 2.1.7.4 (11j).

(Z)-8-bromo-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (17h)

Yellowish solid; Yield: 67.6 mg (90%); spectral data can be seen at 2.3A.7.4 (20h).

(Z)-8-bromo-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (17i)

Yellowish solid; Yield: 77.6 mg (89%); spectral data can be seen at 2.3A.7.4 (20p).

(Z)-6-chloro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17j)

Yellowish solid; yield: 57.2 mg (95%), spectral data can be seen at 2.2.5.4 (24d) and 2.3A.7.4 (20c).

(Z)-6-chloro-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17k)

Yellowish solid; yield: 60.7 mg (95%); spectral data can be seen at 2.2.5.4 (24j) and 2.3A.7.4 (20h).

(Z)-6-chloro-3-(2-(4-chlorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17l)

Yellowish solid; yield: 65.2 mg (95%); spectral data can be seen at 2.2.5.4 (24g) and 2.3A.7.4 (20e).

(Z)-6-chloro-3-(2-(4-fluorophenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17m)

Yellowish solid; yield: 57.8 mg (93%); spectral data can be seen at 2.2.5.4 (24i) and 2.3A.7.4 (20d).

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-chloro-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17n)

Yellowish solid; yield: 71.5 mg (92%); spectral data can be seen at 2.2.5.4 (24f) and 2.3A.7.4 (20g).

(Z)-6-chloro-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17o)

Yellowish solid; yield: 62.6 mg (90%); spectral data can be seen at 2.2.5.4 (24e) and 2.3A.7.4 (20i).

(Z)-6-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17p)

Yellowish solid; yield: 57.2 mg (89%); spectral data can be seen at 2.2.5.4 (24k) and 2.3A.7.4 (20t).

(Z)-6-nitro-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17q)

Yellow solid; yield: 53.99 mg (83%); spectral data can be seen at 2.1.7.4 (11l).

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17r)

Yellowish solid; yield: 60.4 mg (86%); spectral data can be seen at 2.2.5.4 (24l) and 2.3A.7.4 (20v).

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17s)

Yellow solid; yield: 55.88 mg (81%); spectral data can be seen at 2.1.7.4 (11m).

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17t)

Yellow solid; yield: 64.05 mg (84%), spectral data can be seen at 2.1.7.4 (11n).

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (17u)

Yellowish solid; yield: 62.2 mg (94%); spectral data can be seen at 2.2.5.4 (24m) and 2.3A.7.4 (20u).

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-6-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17v)

Yellow solid; yield: 60.92 mg (78%); spectral data can be seen at 2.1.7.4 (11k).

(Z)-7-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17w)

Yellowish solid; yield: 57.5 mg (89%); spectral data can be seen at 2.2.5.4 (24n) and 2.3A.7.4 (20w).

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (17x)

Yellowish solid; yield: 69.6 mg (87%); spectral data can be seen at 2.2.5.4 (24p) and 2.3A.7.4 (20aa).

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (18a)

Yellowish solid; yield: 60.8 mg (92 %); spectral data can be seen at 2.2.5.4 (24o) and 2.3A.7.4 (20ab).

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18b)

Yellowish solid; yield: 63.2 mg (90%); spectral data can be seen at 2.2.5.4 (24q) and 2.3A.7.4 (20y).

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18c)

Yellowish solid; yield: 64.6 mg (85%); spectral data can be seen at 2.3A.7.4 (20z).

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-7-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18d)

Yellowish solid; yield: 52.7 mg (80%); spectral data can be seen at 2.3A.7.4 (20x).

(Z)-8-bromo-6-methyl-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (18e)

Yellowish solid; Yield: 70.7 mg (95%); spectral data can be seen at 2.3A.7.4 (20q).

(Z)-8-bromo-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo [*b*][1,4]oxazin-2-one (18f)

Yellowish solid; Yield: 70.5 mg (91%); R_f (EtOAc/hexane; 20:80) = 0.80; m.p. 189-190 °C; FT-IR (KBr, νmax/cm^{-1}) 3446, 2926, 1757, 1623, 1598, 1107; ^{1}H NMR (400 MHz) δ 7.92 (d, J = 8.0 Hz, 2H), 7.03 (s, 1H), 6.90 (m, 3H), 6.74 (s, 1H), 3.84 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz) δ 190.3, 163.5, 155.8, 137.9, 136.7, 136.4, 130.9, 130.0, 127.8, 124.8, 115.3, 114.0, 110.0, 95.1, 55.6, 20.9; HRMS (ESI) calcd. for $C_{18}H_{14}BrNO_4$ $[M+2]^+$: 389.0106; found 389.0109.

(Z)-8-bromo-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18g)

Yellowish solid; Yield: 79.7 mg (94%); spectral data can be seen at 2.3A.7.4 (20o).

(Z)-8-bromo-3-(2-(4-fluorophenyl)-2-oxoethylidene)-6-methyl-3,4-dihydro-2*H*-benzo[*b*] [1,4]oxazin-2-one (18h)

Yellowish solid; Yield: 67.6 mg (90%); spectral data can be seen at 2.3A.7.4 (20n).

(Z)-5-nitro-3-(2-oxo-2-phenylethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18i)

Yellowish solid; yield: 55.7 mg (88%); spectral data can be seen at 2.2.5.4 (27).

(Z)-5-nitro-3-(2-oxo-2-(p-tolyl)ethylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18j)

Brownish solid; yield: 51.5mg (79%); R_f (EtOAc/hexane; 20:80) = 0.70; m.p.= 230-232 °C; FT-IR (KBr, νmax/cm^{-1}) 3442, 2925, 1771, 1629, 1525, 1178; ^{1}H NMR (400 MHz) δ 8.13 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 2H), 7.67 (t, J = 8.4 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.27 (t, J = 8.8 Hz, 1H), 7.10 (s, 1H), 2.39 (s, 3H); ^{13}C NMR

(100 MHz) δ 189.9, 154.3, 143.2, 142.4, 136.5, 134.9, 133.2, 129.1, 127.3, 126.2, 122.3, 122.0, 121.3, 97.06, 96.9, 20.6; HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_5$ $[M+H]^+$: 325.0746; found 325.0742.

(Z)-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-5-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4] oxazin-2-one (18k)

Yellowish solid; yield: 55.3 mg (83%); R_f (EtOAc/hexane; 20:80) = 0.65; m.p.= 244-246 °C; FT-IR (KBr, νmax/cm^{-1}) 3417, 1764, 1631, 1598, 1524, 1257, 1168, 1319; ^{1}H NMR (400 MHz) δ 8.14 – 8.08 (m, 3H), 7.68 (d, J = 8.0 Hz, 1H), 7.25 (t, J = 8.4 Hz, 1H), 7.10 - 7.08 (m, 3H), 3.87 (s, 3H); ^{13}C NMR (100 MHz) δ 188.8, 163.1, 154.4, 142.3, 136.1, 133.1, 130.3, 129.6, 122.2, 121.1, 114.0, 113.8, 97.2, 96.9, 55.2; HRMS (ESI) calcd. for $C_{17}H_{12}N_2O_6$ $[M+H]^+$: 341.0695; found 341.0692.

(Z)-3-(2-(4-chlorophenyl)-2-oxoethylidene)-5-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18l)

Yellowish solid; yield: 59.4 mg (86%); R_f (EtOAc/hexane; 20:80) = 0.75; m.p.= 255-257 °C; FT-IR (KBr, νmax/cm^{-1}) 3416, 1767, 1629, 1588, 1316, 1258, 1092; ^{1}H NMR (400 MHz) δ 8.17 – 8.06 (m, 3H), 7.64 – 7.53 (m, 3H), 7.25 (t, J = 8.4 Hz, 1H), 7.10 (s, 1H); ^{13}C NMR (100 MHz) δ 188.9, 153.9, 142.2, 138.1, 136.3, 135.9, 133.3, 128.8, 128.3, 122.1 121.6, 121.4, 121.1, 97.2; HRMS (ESI) calcd. for $C_{16}H_9ClN_2O_5$ $[M+2]^+$: 346.0200; found 346.0204.

(Z)-3-(2-(2,4-dichlorophenyl)-2-oxoethylidene)-5-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18m)

Yellowish solid; yield: 61.7 mg (81%); R_f (EtOAc/hexane; 20:80) = 0.75; m.p.= 252-254 °C; FT-IR (KBr, νmax/cm^{-1}) 3417, 1762, 1630, 1518, 1384, 1285, 1116; ^{1}H NMR (400 MHz) δ 8.18 – 8.14 (m, 1H), 7.69 – 7.64 (m, 3H), 7.50 (d, J = 7.6 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 6.78 (s, 1H); ^{13}C NMR (100 MHz) δ 190.3, 153.7, 142.4, 136.9, 136.2, 133.4, 131.1, 130.4, 129.5, 129.4, 127.2, 127.1, 122.3, 121.7, 121.4, 100.4; HRMS (ESI) calcd. for $C_{16}H_8Cl_2N_2O_5$ $[M+2]^+$: 379.9810; found 379.9815.

(Z)-3-(2-(4-fluorophenyl)-2-oxoethylidene)-5-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18n)

Yellowish solid; yield: 58.6 mg (89%); R_f (EtOAc/hexane; 20:80) = 0.75; m.p.= 269-271 °C; FT-IR (KBr, νmax/cm^{-1}) 3417, 1760, 1628, 1595, 1514, 1279, 1256, 1237, 1159; ^{1}H NMR (400 MHz) δ 8.21 – 8.14 (m, 3H), 7.71 (d, J = 7.6 Hz, 1H), 7.41 – 7.37 (m, 2H), 7.31 – 7.27 (m, 1H), 7.11 (s, 1H); ^{13}C NMR (100 MHz) δ 189.8, 166.9,

164.4, 155.2, 143.4, 137.8, 135.1, 134.2, 131.2, 131.1, 123.4, 122.8, 122.3, 116.5, 97.73, 97.60; HRMS (ESI) calcd. for $C_{16}H_9FN_2O_5$ $[M+H]^+$: 329.0495; found 329.0498.

(Z)-3-(2-(4-bromophenyl)-2-oxoethylidene)-5-nitro-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (18o)

Yellowish solid; yield: 64.1 mg (82%); R_f (EtOAc/hexane; 20:80) = 0.75; m.p.= 232-234 °C; FT-IR (KBr, νmax/cm^{-1}) 3442, 3098, 1766, 1620, 1583, 1067; ^{1}H NMR (400 MHz) δ 8.13 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.6 Hz, 2H), 7.74 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.29 (t, J = 8.4 Hz, 1H), 7.09 (s, 1H); ^{13}C NMR (100 MHz) δ 189.1, 154.1, 142.5, 137.1, 136.4, 133.3, 131.5, 129.2, 126.7, 122.4, 121.8, 121.5, 121.4, 96.4; HRMS (ESI) calcd. for $C_{16}H_9BrN_2O_5$ $[M+2]^+$: 389.9695; found 389.9699.

Biological activity assays

2.3B.7.4 Platelet aggregation inhibitory activity evaluation

All synthesized 2-oxo-2-phenylethylidene linked 2-oxo-benzo[1,4]oxazineanalogues (17a–x and 18a–o)were dissolved in DMSO before testing. In order to eliminate the effects of the solvent on aggregation, the final concentration of DMSO was fixed at 0.5%. Arachidonic acid (AA), EDTA (disodium salt), bovine serum albumin, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. Blood was collected from the rabbit marginal ear vein (several studies established that rabbit platelets are surrogate to human platelets both in vitro as well as in vivo) and was mixed with EDTA to a final concentration of 6mM. It was centrifuged for 10min at 90g at room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at 500×g for 10min. The platelet pellets were washed with Tyrode's solution (Ca+2-free) containing 2mM EDTA, 0.1 and 3.5mg/mL bovine serum albumin, and centrifuged at 500×g for 10min. Then, the pellets were washed with Tyrode's solution without EDTA. After centrifugation under the same conditions, the platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), NaHCO3 (11.9), MgCl2 (2.1), NaH_2-PO_4 (0.33), CaCl2 (1.0), and glucose (11.2) containing bovine serum albumin (0.35%). Aggregation was measured by a turbidimetric method using a Lumi-aggregometer (Chrono-LogCorp., Havertown, PA). All glassware was siliconized. Three minutes before the addition of the aggregation inducer, the platelet

suspension was stirred at 1200rpm. The percentage of aggregation was calculated as follows (abs. =absorbance):

$$\%\text{Aggregation} = \frac{\text{Abs: of platelet suspension} - \text{Final abs: after aggregation}}{\text{Abs: of platelet suspension} - \text{abs: of Tyrode solution}} \times 100$$

Percent aggregation was expressed assuming the absorbance of platelet suspension as 0% aggregation and the absorbance of platelet free Tyrode's solution as 100% aggregation. For each compound IC50 values were calculated by Sigma Plot.

2.3B.7.5 Cytotoxicity MTT assay

The cytotoxic effect of the 17i, 17x, 18f–18i, 18l, and 18o on cells were detected in vitro using the mitochondrial cytotoxic test. 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 3.5×103 3T3 fibroblast cells per well. Samples were dissolved in DMSO (stock solution 10mM) and subsequently diluted in medium to the final concentration of 25–250μM (concentration of DMSO 0.5%) and after 24h they were added to the cells. Microplates were cultivated for 72h in thermostat at 37°C and 5% CO_2 atmosphere. After incubation thiazolyl blue tetrazolium bromide (3.33mg/mL phosphate buffered saline, pH=7.4) was pipetted to each well and left to incubate for further 2h. Then the medium with MTT solution was removed. Formazan crystals in viable cells were dissolved in the lysis solution (4mM HCl and 0.1% Nonidet P40 in ethanol). Microplates were shaken 15min at 1500rpm. Absorbance was measured at 540nm and reference wavelength at 740nm. Experiments were performed in triplicates and percent viability was calculated by dividing the OD obtained in treatment group by OD of the untreated cell control multiplied by hundred. Inhibition activity was expressed as percentages of control with DMSO.

2.3B.7.6 In silico molecular docking simulation studies

Molecular docking studies were performed via constructing 3D model of the structures using Surflex-Dock module in Sybyl-X 2.1 (Tripos International). The X-ray crystallographic structure of the ACC (PDB ID:2OYE) solved at 2.85Åre solution was retrieved from the PDB, and modified for docking calculations. Co-crystallized

ligand indomethacin was removed from the binding site, water molecules were removed, ─H atoms were added, and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field, and partial atomic charges were calculated by Gasteiger-Huckel method.

2.3B.7.7 Molecular docking

Molecular modeling studies of 2-oxo-2-phenylethylidene linked 2oxo-benzo[1,4]oxazine analogues 17i, 17x, 18f–i, 18l, and 18o were carried out using the molecular modeling software Sybyl-X 2.1 (Tripos International). Drawing of structures and simple geometry optimization were performed with ChemBio-Office suite Ultra v12.0 (2012) (Cambridge Soft Corp). The binding affinity of all compounds were predicted with the platelet aggregation inhibitor target enzyme cyclooxygenase-1 (COX-1). Energy minimization was done using the Tripos force field with a distance-dependent dielectric and the Powell gradient minimization algorithm, with a convergence criterion of 0.001kcal/mol for the determination of conformations with the most favorable (lowest energy). Aromatase at 3.5Å resolution (PDB: 2OYE) was selected. The crystal structure of COX-1 enzyme of sheep (it has been well established that COX-1 of sheep is almost similartothatofCOX-1ofhuman) in complex with indomethacin obtained from the PDB. Hydrogen atoms were added to the protein with the protonation 3D tool in Sybyl. Partial atomic charges were assigned using the Gasteiger-Hückel method in Sybyl. All 2D structures were converted to 3D structures using the program Concord v4.0 and the maximum number of iterations performed in the energy minimization was set to 2000. Further geometry optimization was done with the MOPAC-6 package using the semi empirical PM3 Hamiltonian method.

2.3B.7.8 Prediction of in silico pharmacokinetic and toxicity parameters

Pharmacokinetic (PK) properties depend on the chemical properties of drugs, which determine their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, which are the key descriptors for the human therapeutic use of any compound. Predictive ADMET mathematical models were derived with different PK parameters, namely, aqueous solubility, blood–brain barrier penetration, cytochromeP-4502D6 inhibition, hepatotoxicity, human intestinal absorption, and

plasma protein binding. Predictions from these models were contrasted with known rules for appropriate ADMET characteristics for 2-oxo-2-phenylethylidene linked 2-oxobenzo[1,4]oxazine analogues 17i, 17x, 18f–i, 18l, and 18o. Some properties correlate well with PK, e.g, primary determinant of fractional absorption can be represented by polar surface area (PSA) (cut-off≤140Å2) and low molecular weight (MW) for absorption. For secondary determination of fractional absorption (passive membrane transport), the sum of H-bond donors and acceptors (cut-off ≤12) was used. The number of rotatable bonds were used as a measure of flexibility (cut-off ≤10) and bioavailability. Drug distribution depends on a number off actors, such as permeability (indicated by apparent Caco-2 and MDCK permeability, log Kp for skin permeability), blood-brain barrier (logBB),the volume of distribution and plasma protein binding (log Khsa for serum protein binding). These ADME descriptors were calculated and compared with standard ranges. The octanol-water partition coefficient (logP) has been implicated in BB penetration and permeability studies. The excretion of drugs from the body depends on logP and MW. Likewise, rapid renal clearance is associated with hydrophilicity and small MW. In the liver, drug metabolism is associated with hydrophobicity and large MW. Higher lipophilicity leads to poor absorption and increase in metabolic processes. ADME descriptors for 90% of orally active compounds follow Lipinski's rule of 5. These ADME parameters were calculated through Qikprop v3.2 (Schrödinger, LLC, USA, 2015 and Discovery Studio 3.5). The recommended toxicity screening models for carcinogenicity are developmental toxicity, mutagenicity, and skin irritancy or sensitization, and these were calculated with the DSTOPKAT module. These predictions are useful for the optimization of therapeutic ratios of lead compounds and assessment of their potential safety. These predictions also help in evaluating intermediates, metabolites and pollutants, along with setting dose range for animal assays.

2.3B.7.9 References

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2.3B.10 Characterization spectral data (^{1}HNMR and ^{13}CNMR) of selected compounds:

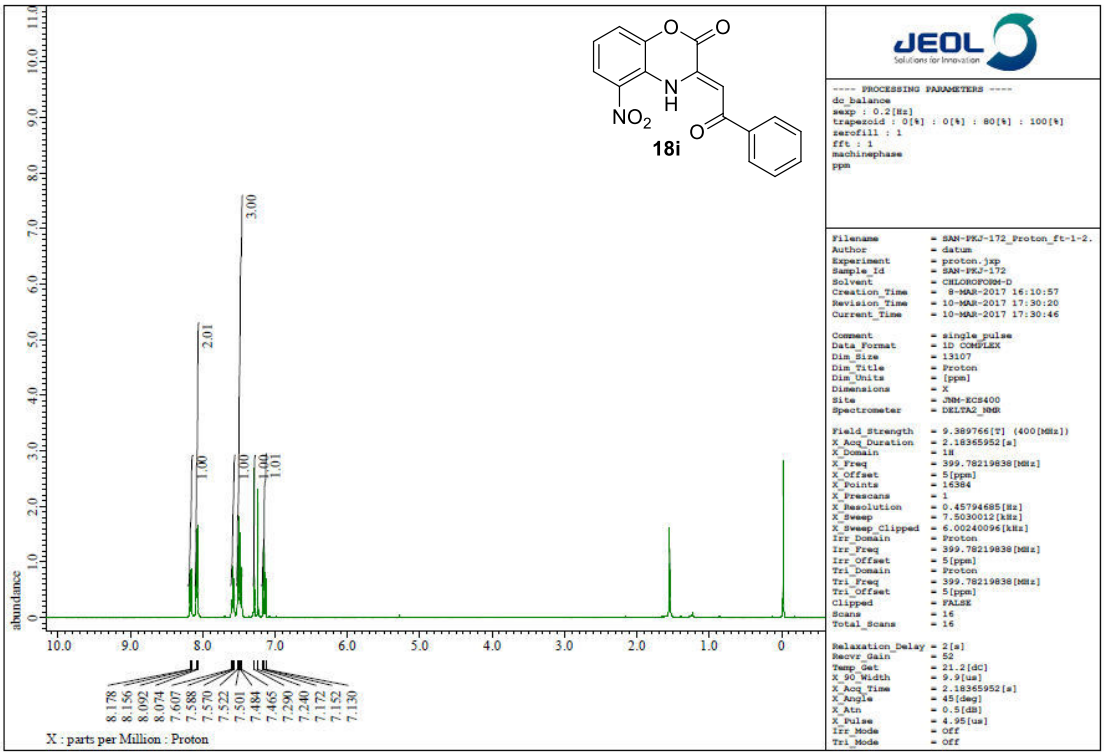


Figure 8.^{1}H NMR Spectra of Compound **18i**.

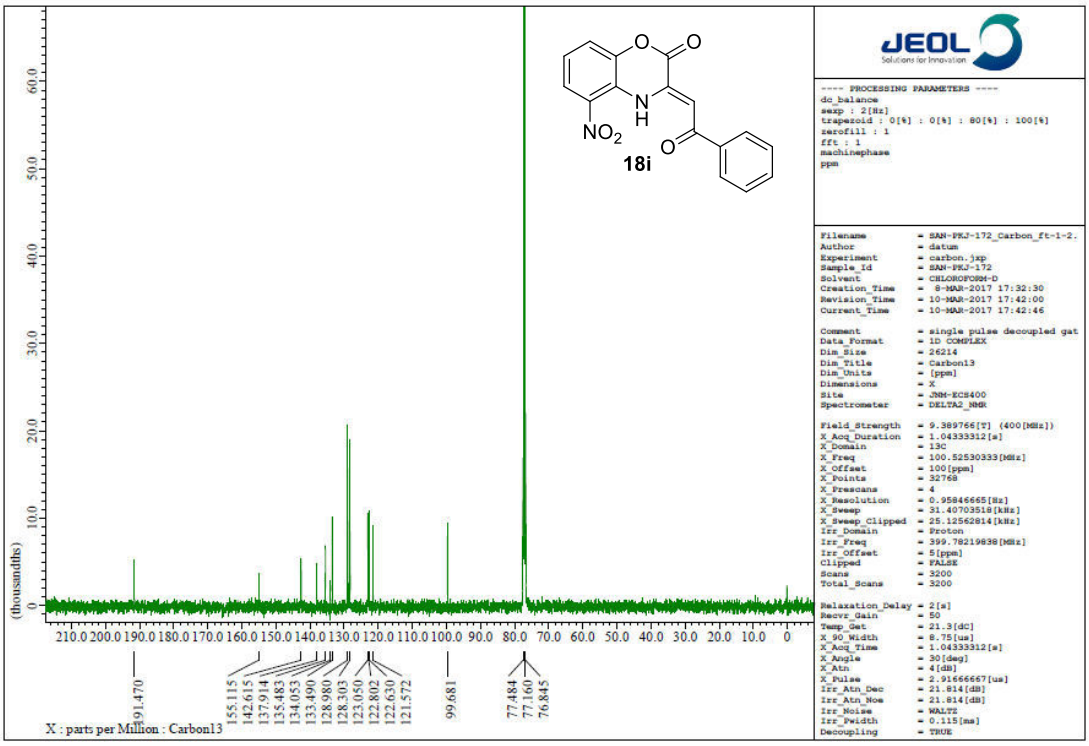


Figure 9.^{13}C NMR Spectra of Compound **18i**.

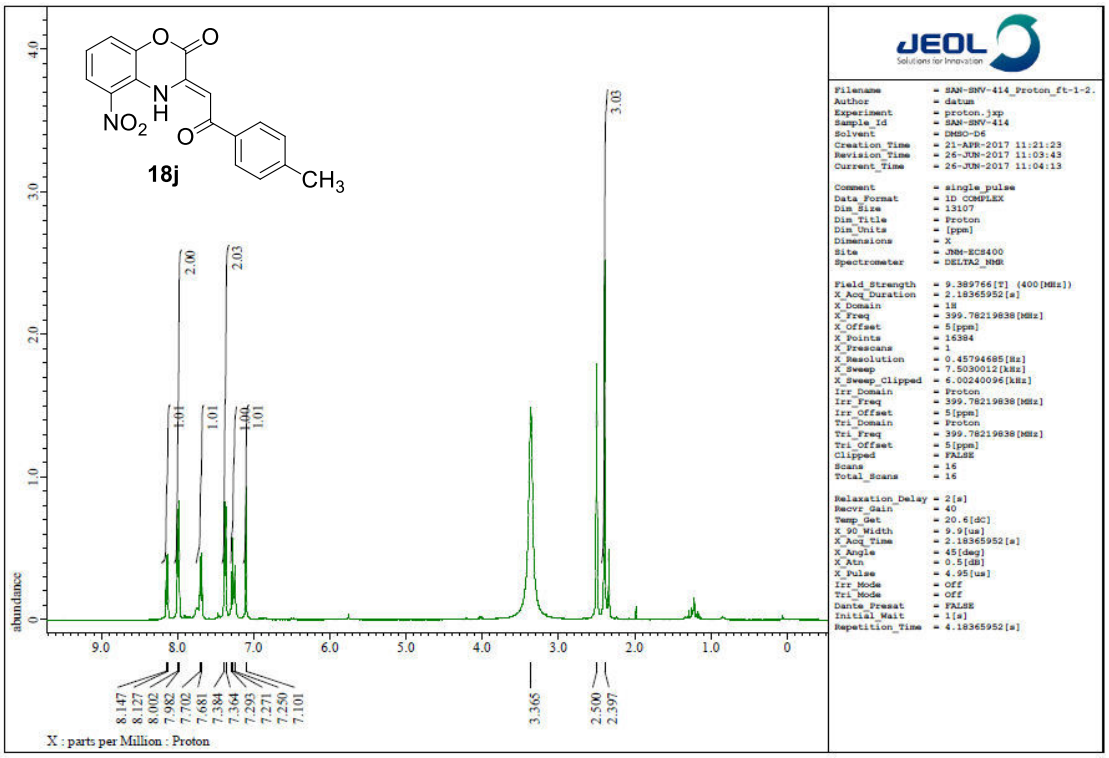


Figure 10. ^{1}H NMR Spectra of Compound **18j**.

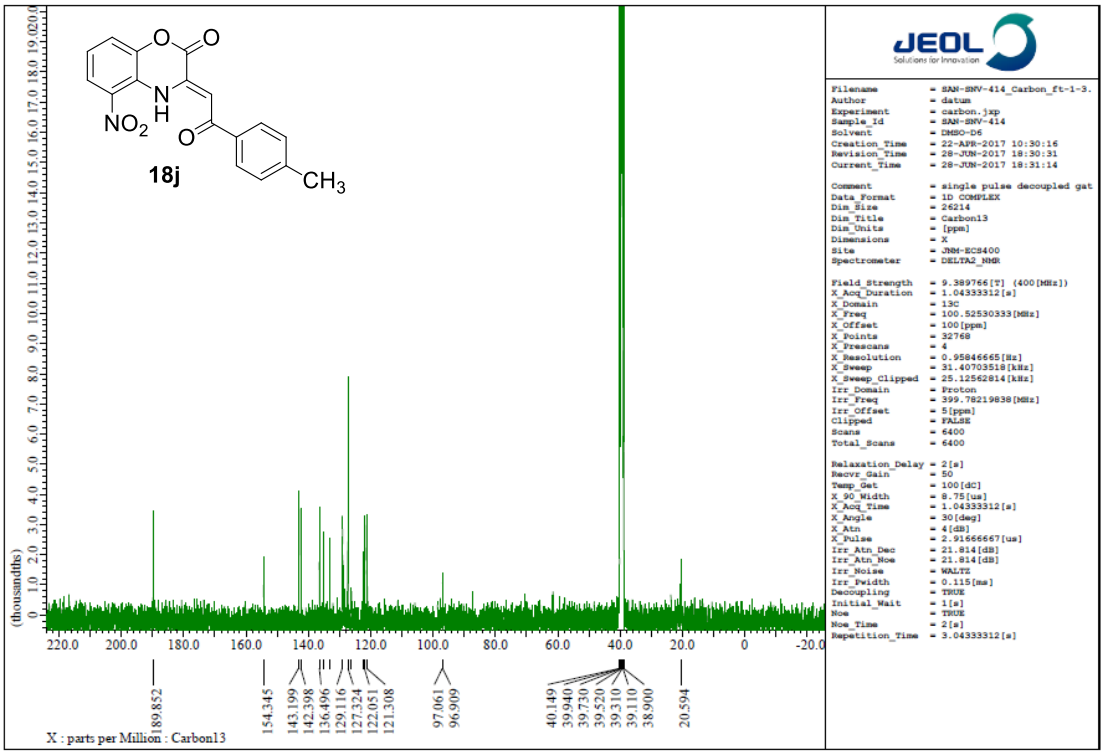


Figure 11.^{13}C NMR Spectra of Compound **18j**.

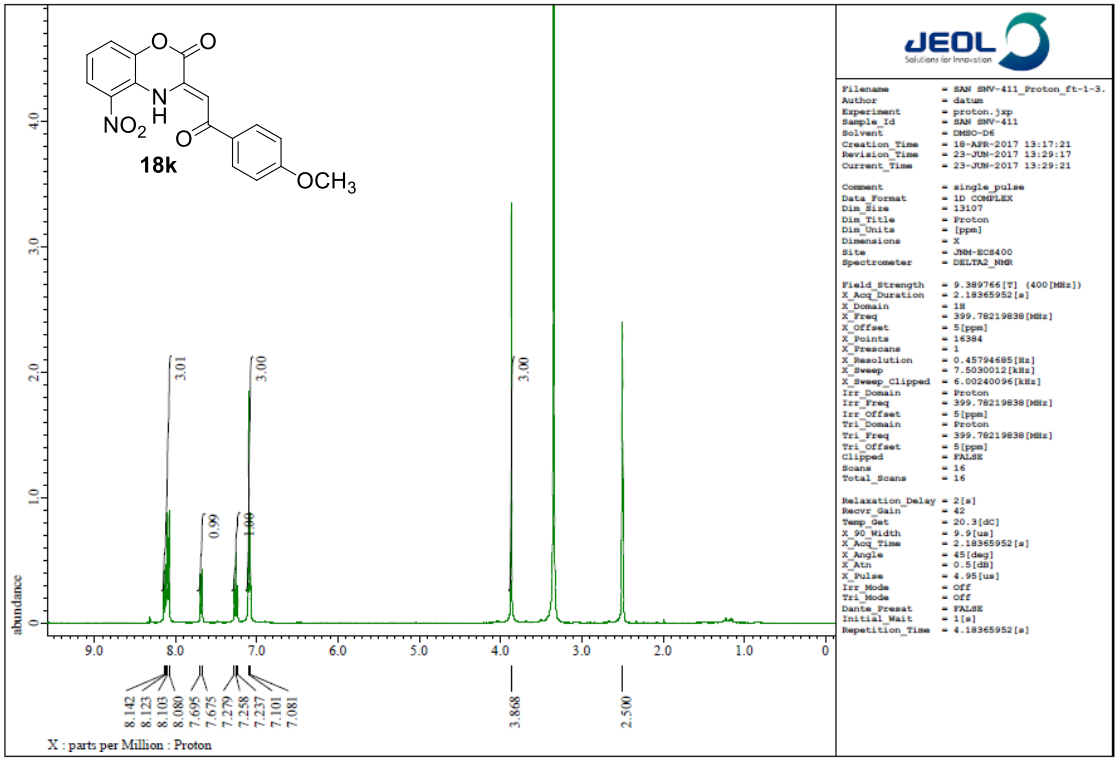


Figure 12. ^{1}H NMR Spectra of Compound **18k**.

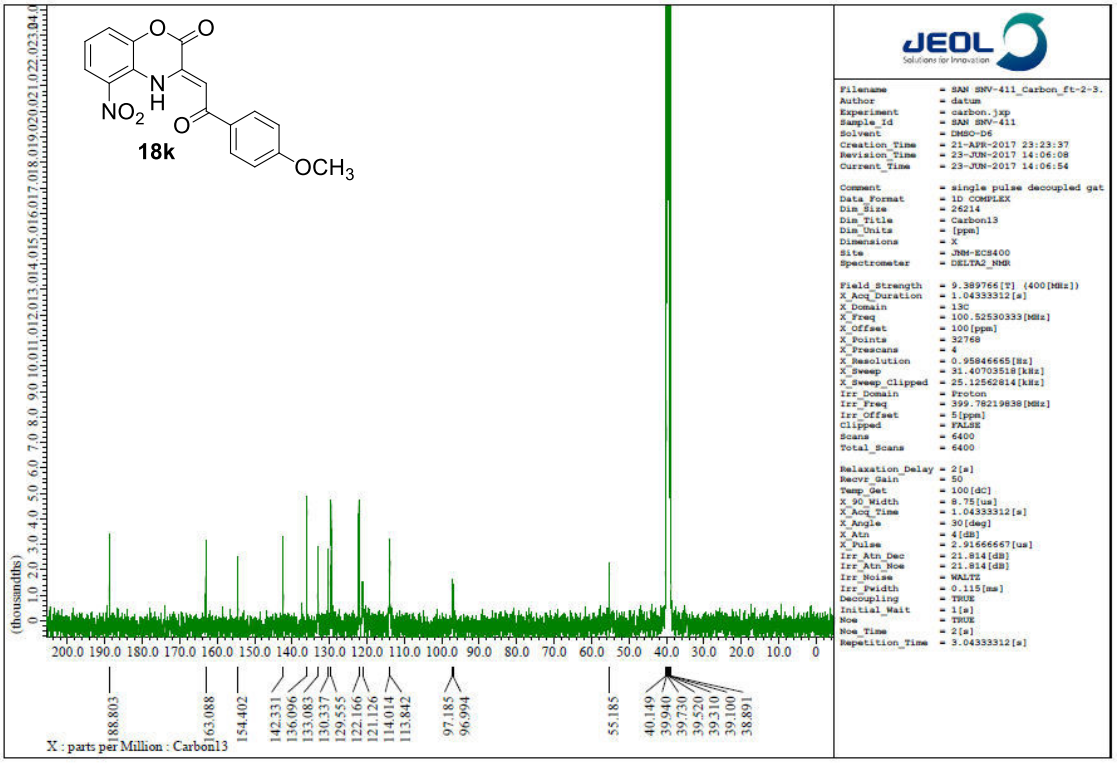


Figure 13.^{13}C NMR Spectra of Compound **18k**.

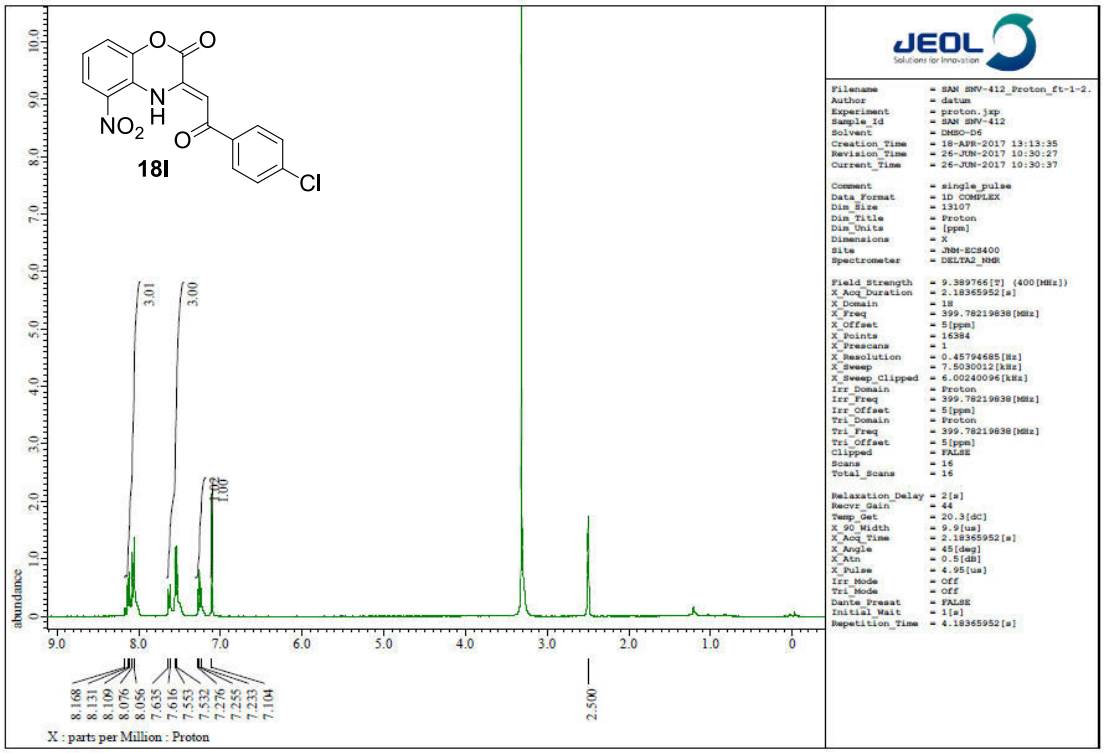


Figure 14. ^{1}H NMR Spectra of Compound **18l**.

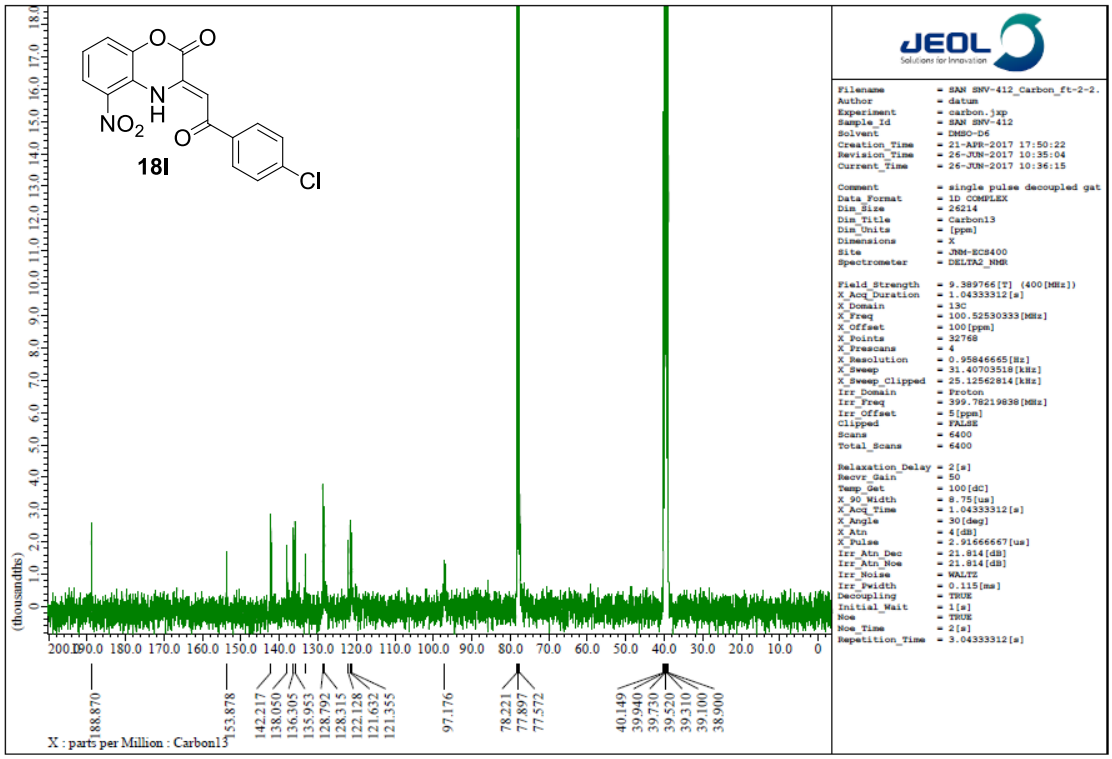


Figure 15. ^{13}C NMR Spectra of Compound **18l**.

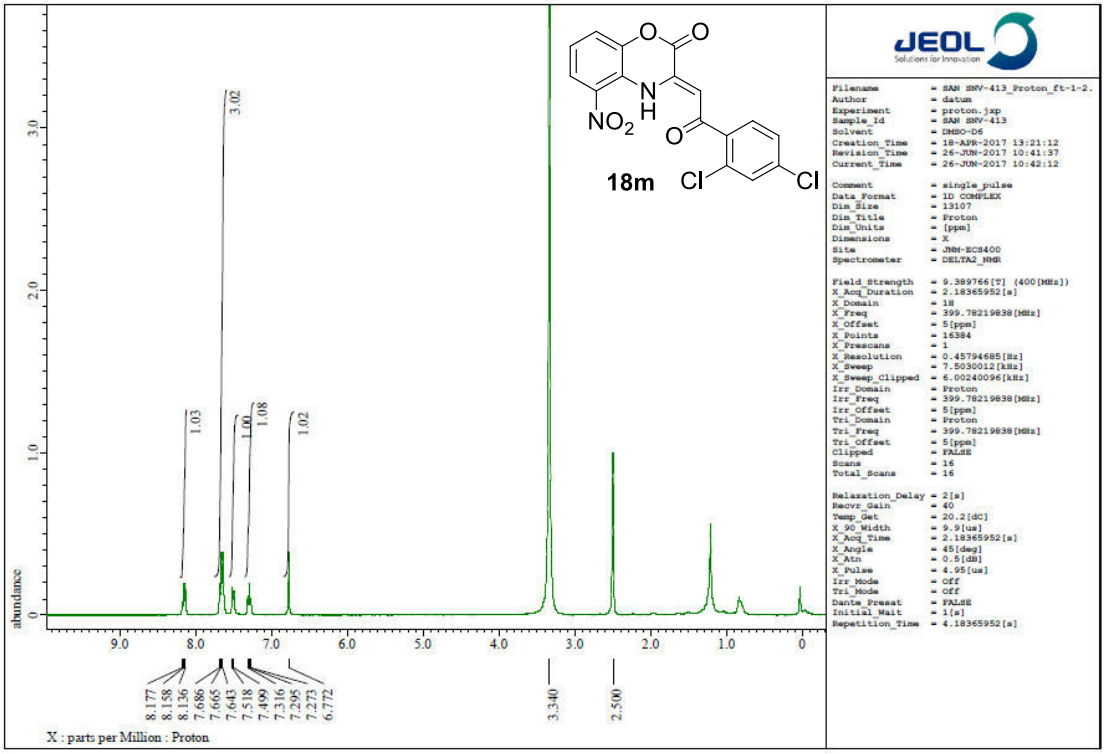


Figure 16. ^{1}H NMR Spectra of Compound **18m**.

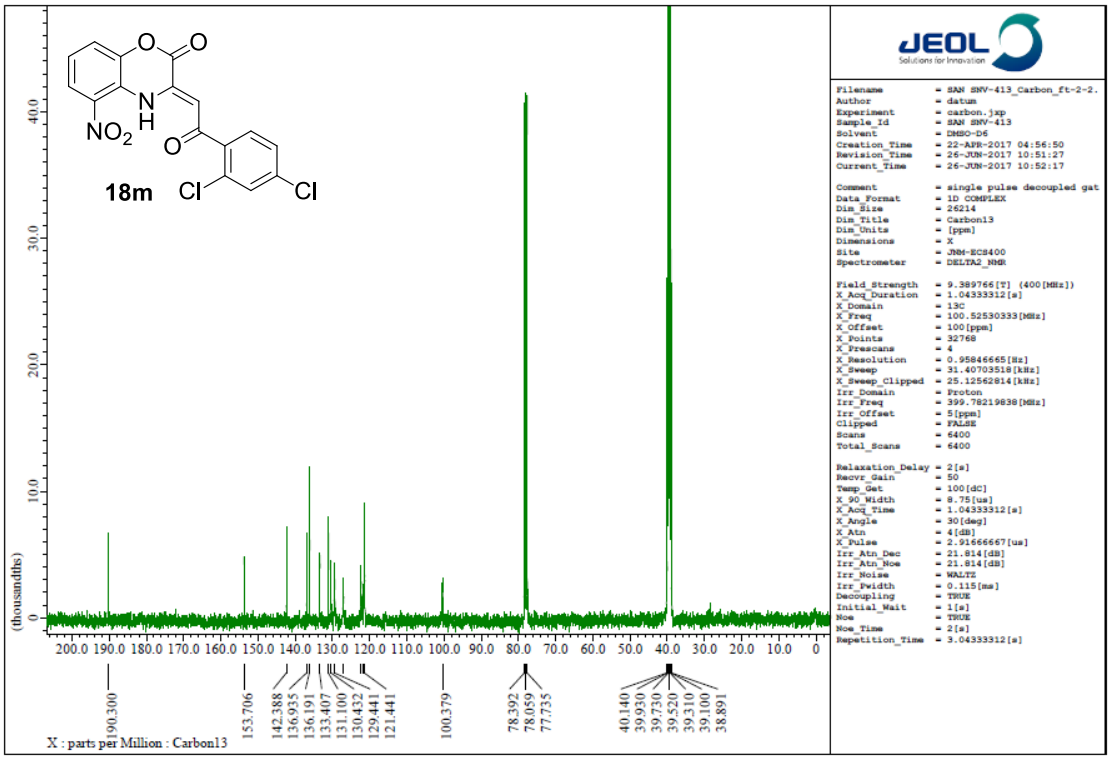


Figure 17.^{13}C NMR Spectra of Compound **18m.**

Chapter 3

"Organocatalytic modified Guareschi–Thorpe type Regioselective Synthesis of Novel Pyridine-Alicyclic Ring Fused Bioactive Heterocycles"

3.1 Introduction

Organocatalysis had received tremendous attention by synthetic chemist to catalyze transition-metal free reactions to construct various natural products, pharmaceuticals, and organic materials during the last two decades because of its several advantages such as selectivity, safety, ease of handle and economic perspectives.[1]

Pyridine nucleus constitutes the core of a large family of natural products, bioactive pharmaceuticals, vitamins, alkaloids, chiral ligands and functional materials.[2] Substituted alicyclic[*b*]-fused pyridines, as a part or as a whole, are present in several natural products,[3] and are endowed with wide range of biological activities such as antitubercular **1a**,[4a] vitamin B_6 **1b**,[4b] Sceletium alkaloids (+)-sceletium A-4, **1c**, (+)-tortuosamine **1da**, and (+)-N-formyltortuosamine **1db**,[4c] anti-Alzheimer (Tacrine) **1e**,[4d] thromboxane A2 synthese (P450 TxA2) or dual inhibitors of TxA2 **1f**,[4e] C5a receptor antagonists **1g**,[4f] Na^+/H^+ exchange inhibitors **1h**,[4g] anti-HIV **1i**,[4h] atypical antipsychotic **1j**,[4i] H1-antihistamine **1k**,[4j] cytotoxic **1l**,[4k] and antidepressant **1m**[4l] etc. (Figure 1).

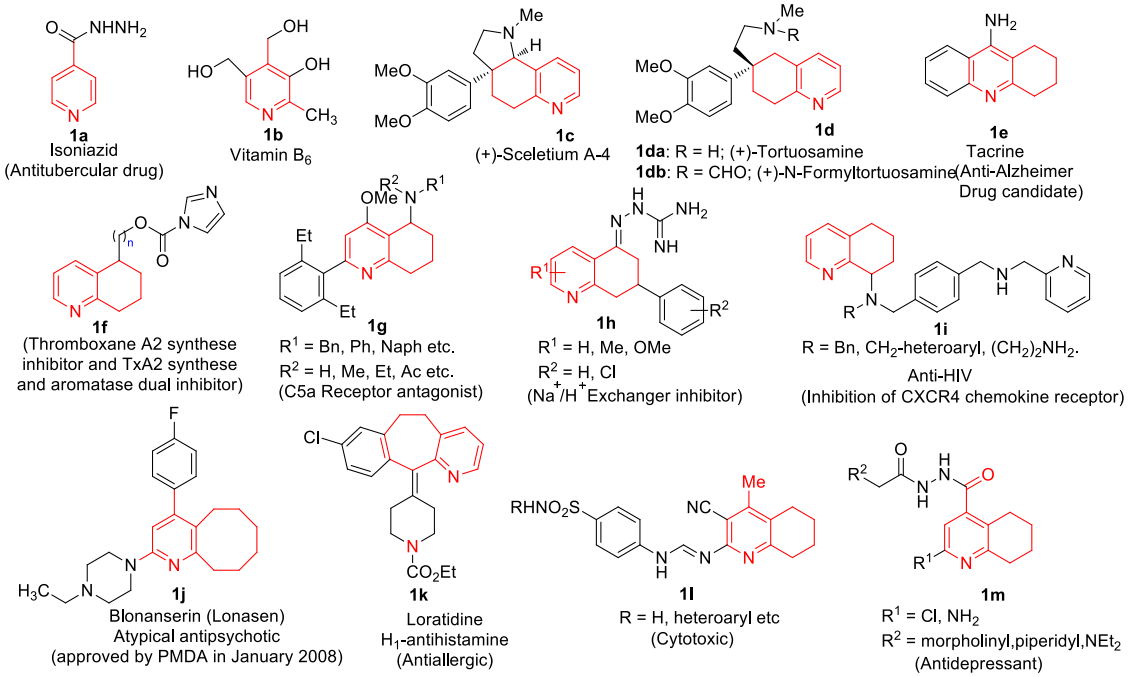


Figure 1. Selected examples of natural products and pharmaceutically privileged analogues 1a-m having functionalized alicyclic[*b*]-fused pyridines.

Several synthetic methodologies for the synthesis of functionalized alicyclic[*b*]-fused pyridines have been reported either *via* construction of the pyridine ring on alicyclic ring systems, or *via* construction of the alicyclic ring on pyridine ring. While the latter

route involves routine chemistry, whereas the former route is quite challenging as the construction of highly substituted pyridine is required to be built on alicyclic ring. Almost a century ago, Guareschi-Thorpe pyridine synthesis, which involves the condensation of cyanoacetic ester with acetoacetic ester in the presence of ammonia was the landmark reaction in the synthesis of functionalized pyridines [Figure 2. A].[5] Since then, several methodologies have been reported in the literature to construct the alicyclic[*b*]-fused pyridines using cyclic ketones or its analogues.[6]

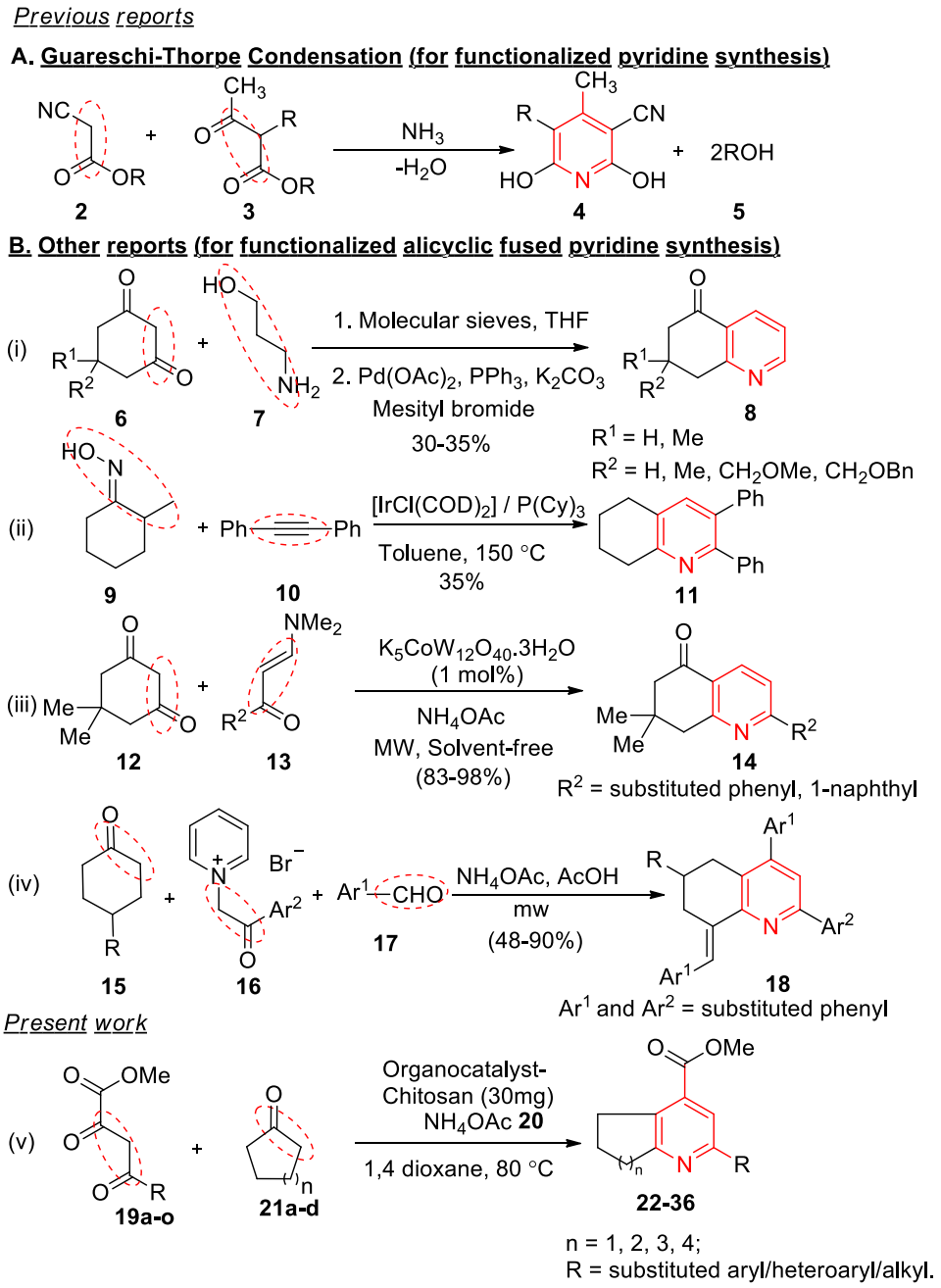


Figure 2. Some previous and present report for the synthesis of alicyclic[*b*]-fused pyridines.

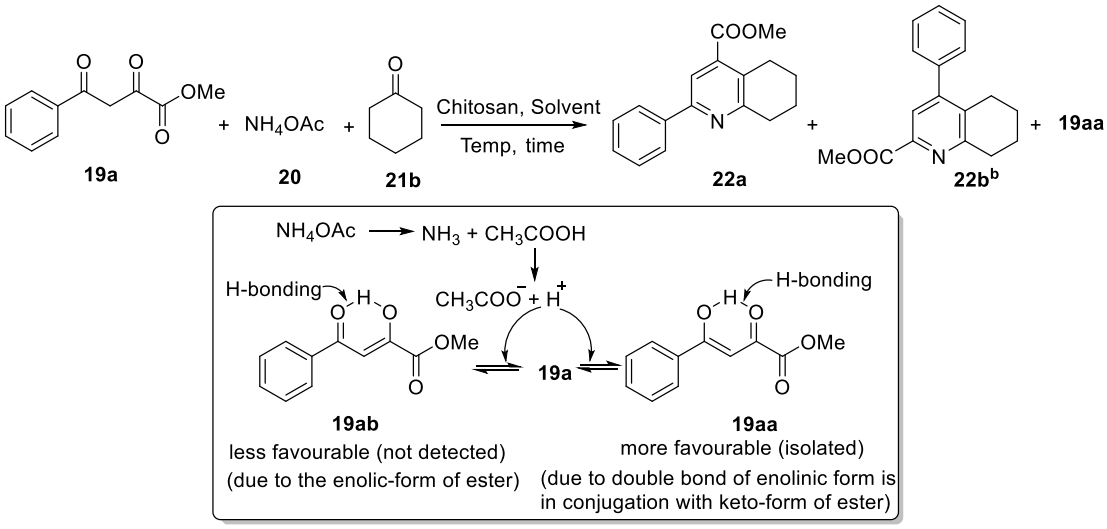
Earliar, the synthesis of alicyclic[*b*]-fused pyridines were carried out *via* Pd-catalyzed oxidation of hydroxyenaminones followed by subsequent cyclization/aromatization [Figure 2 B. (i)].[7a] Functionalized 5,6,7,8-tetrahydroquinoline, a cyclohexane[b]-fused pyridine, was obtained in lower yield when 2-methylcyclohexanone oxime was treated with diphenylacetylene in the presence of iridium catalyst [Figure 2 B. (ii)].[7b] The desired skeleton could also be constructed when cyclic/acyclic 1,3-dicarbonyl compounds reacts with activated α, β-unsaturated ketone in the presence of NH_4OAc using $K_5CoW_{12}O_{40}.3H_2O$ as catalyst [Figure 2 B. (iii)].[7c] A metal-free, MW-assisted four-component strategy using arylaldehydes, cyclohexanones, ammonium acetate and N-phenacylpyridinium bromide as a activated ketone, have been also utilized to construct alicyclic[*b*]-fused pyridines. [Figure 2 B. (iv)].[7d] However, all the previously reported protocols suffer from serious drawbacks, such as use of expensive and toxic transition metal catalysts, harsh reaction conditions, multi-step synthesis, activated carbonyl substrates, lower yields and limited substrate scope. Thus, the development of a more general, operationally simple, and environmentally benign method for the synthesis of alicyclic[*b*]-fused pyridine motifs is highly desirable.

In our endeavor to develop green methodologies for the synthesis of bioactive moieties, herein, we report the unprecedented identification of a commercially available heterogeneous organocatalyst i.e., chitosan, which catalyze the regioselective three-component condensation reaction of unactivated ketone, diketo-ester and NH_4OAc in 1,4-dioxane as solvent at 80 °C to furnish alicyclic[*b*]-fused pyridines **22-36**, with a broad range of functional groups tolerance and substrate compatibility [Figure 2 B. (v)]. To the best of our knowledge, this is the first report of organocatalyzed modified Guareschi-Thorpe type regioselective synthesis of novel alicyclic[*b*]-fused pyridines **22-33**. We also report the practicality of our methodology via the synthesis of novel classes of heterocycles **34-36**. For the first time, we also disclose the *in vitro* antifungal activity of 5,6,7,8-tetrahydroquinoline analogues i.e., **34b**, **35b** and **36b** against *Aspergillus Niger* and *Candida Albicans* fungal strains.

3.2 Result and discussion

We started our initial investigation on the model reaction using methyl 2,4-dioxo-4-phenylbutanoate **19a,** NH_4OAc **20** and cyclohexanone **21b** as an unactivated ketone, for the synthesis of methyl 2-phenyl-5,6,7,8-tetrahydroquinoline-4-carboxylate **22a** via screening of various catalysts and solvents (Table 1).

Table 1 Optimization study: Synthesis of 2-aryl alicyclic fused pyridine **22a** by the reaction of methyl 2,4-dioxo-4-phenylbutanoate **19a**, ammonium acetate **20**,and cyclohexanone **21b**.[a]



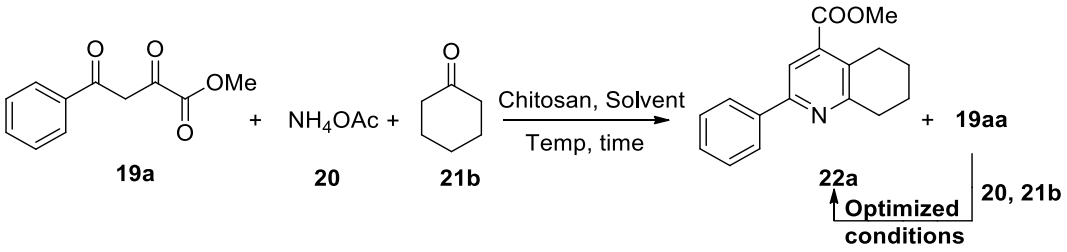
S. No.	Solvent	Catalyst	mol (%)	Temp (°C)	Time (h.)	Yield of 22a[c] (%)	Yield of 19aa[c] (%)
1	EtOH	----	----	80	4	trace	92
2	EtOH	DBU	30	80	4	46	48
3	EtOH	DABCO	30	80	4	37	52
4	EtOH	Et_2NH	30	80	4	32	53
5	EtOH	L-proline	30	80	4	34	57
6	EtOH	Piperidine	30	80	4	28	60
7	EtOH	DMAP	30	80	4	41	54
8	EtOH	Et_3N	30	80	4	21	68
9	EtOH	Pyrolidine	30	80	4	48	44
10	EtOH	Chitosan	30[d]	80	4	65	32

[a]*Reaction conditions*: **19a** (0.2 mmol), **20** (0.3 mmol), **21b** (0.3 mmol), and catalysts (mol %) were dissolved in EtOH and heated at 80 °C temperature under N_2 atm for 4 h. [b] Not isolated. [C]Isolated yield. [d]Performed with chitosan catalyst 60 mg (wt % w.r.t substrate **19a**).

As NH_3 was used as nitrogen source as well as a base in Guareschi-Thorpe pyridine synthesis, we also planned to perform our reaction using NH_4OAc which will provide basic medium and act also as nitrogen source. Unfortunately, it did not furnished the desired product **22a** (entry 1). **19a** exists in equilibrium with either **19aa** or **19ab** under reaction medium. While **19aa**, the unreacted form of **19a**, was isolated in our reaction conditions; **19ab** was not detected at all. This prompted us to investigate this

reaction using other various primary, sec- and tert-amines (Table 1; entry 2-9). A number of bases such as DBU, DABCO, Et_2NH, L-proline, Piperidine, DMAP, Et_3N, pyrolidine etc. were used in catalytic amount (30 mol %) under reflux conditions in EtOH which furnished **22a** in 21-52% yield range (Table 1, entry 2−9). Its other regio-isomer, **22b** was not detected at all. However, **19aa**, the unreacted form of **19a**, was isolated in 44-68% yields range. The structure of **22a** and **19aa** was fully characterized by ESI-MS, ^{1}H NMR, ^{13}C NMR, FT-IR, and HRMS data.

Table 2. Screening of different solvents, chitosan loading, temperature and time for the synthesis of **22a**.[a]



S. No.	Solvent	Amount (mg)	Temp (°C)	Time (h.)	Yield[b](%)
1	EtOH	60	80	4	61
2	$CHCl_3$	60	80	4	28
3	MeOH	60	80	4	63
4	DMF	60	80	4	52
5	DMSO	60	80	4	47
6	1,4-Dioxane	60	80	4	70
7	Toluene	60	80	4	20
8	H_2O	60	80	4	00
9	1,4-Dioxane	80	80	4	56
10	1,4-Dioxane	40	80	4	68
11	1,4-Dioxane	30	80	4	77
12	1,4-Dioxane	20	80	8	61
13	1,4-Dioxane	30	80	8	80
14	**1,4-Dioxane**	**30**	**80**	**10**	**87**
15	1,4-Dioxane	30	100	12	81
16	1,4-Dioxane	30	80	24	79

[a]*Reaction conditions*: **19a** (0.2 mmol), **20** (0.3 mmol), **21b** (0.3 mmol), and chitosan (given amount) were dissolved in given solvents and heated at the given temperature under N_2 atm at given time. bIsolated yields after two steps. In the second step, the intermediate **19aa** was again subjected in same reaction conditions along with **20** and **21**.

Then, it has been reported that bio-polymer chitosan (heterogeneous catalyst) had been utilized in pyridine synthesis.[8] Therefore, we also carried out our model reaction using chitosan as heterogeneous organocatalyst. To our surprise, **22a** was obtained in 65% yield (Table 1, entries 10). So, we planned to optimize the reaction efficacy using chitosan in different solvents (Table 2, entry 1−8). As it can be seen from Table 2; 1,4-dioxane was found to be the best solvent for this reaction which afforded **22a** in 70% yield (Table 2, entry 6). The reaction was also performed in H_2O, however, reaction didn't occur at all (Table 2, entry 8).

Catalyst loading study was analyzed on the model reaction from 80 mg to 30 mg chitosan (Table 2, entry 9-12). It was observed that an increase in chitosan loading (80 mg) decreases the yield of **22a** (Table 2, Entry9). Further decreasing the chitosan loading (40 mg) increases the product yield to 68% (Table 2, entry 10). Finally, 30% catalyst loading was found to be the optimum condition for obtaining the product in 77% yield (Table 2, Entry 11). Further decrease in chitosan loading do not have beneficial effects (Table 2, entry 12). Finally after further optimization of temperature and time (Table 2; entry 13-16); 80 °C temperature and 10 h time was found to be the best optimized conditions. Therefore, *catalyst loading of 30 mg chitosan in 1,4-dioxane solvent at 80 °C for 10h was found to be the best optimized reaction conditions for the synthesis of **22a*** (Table 2, entry 14).

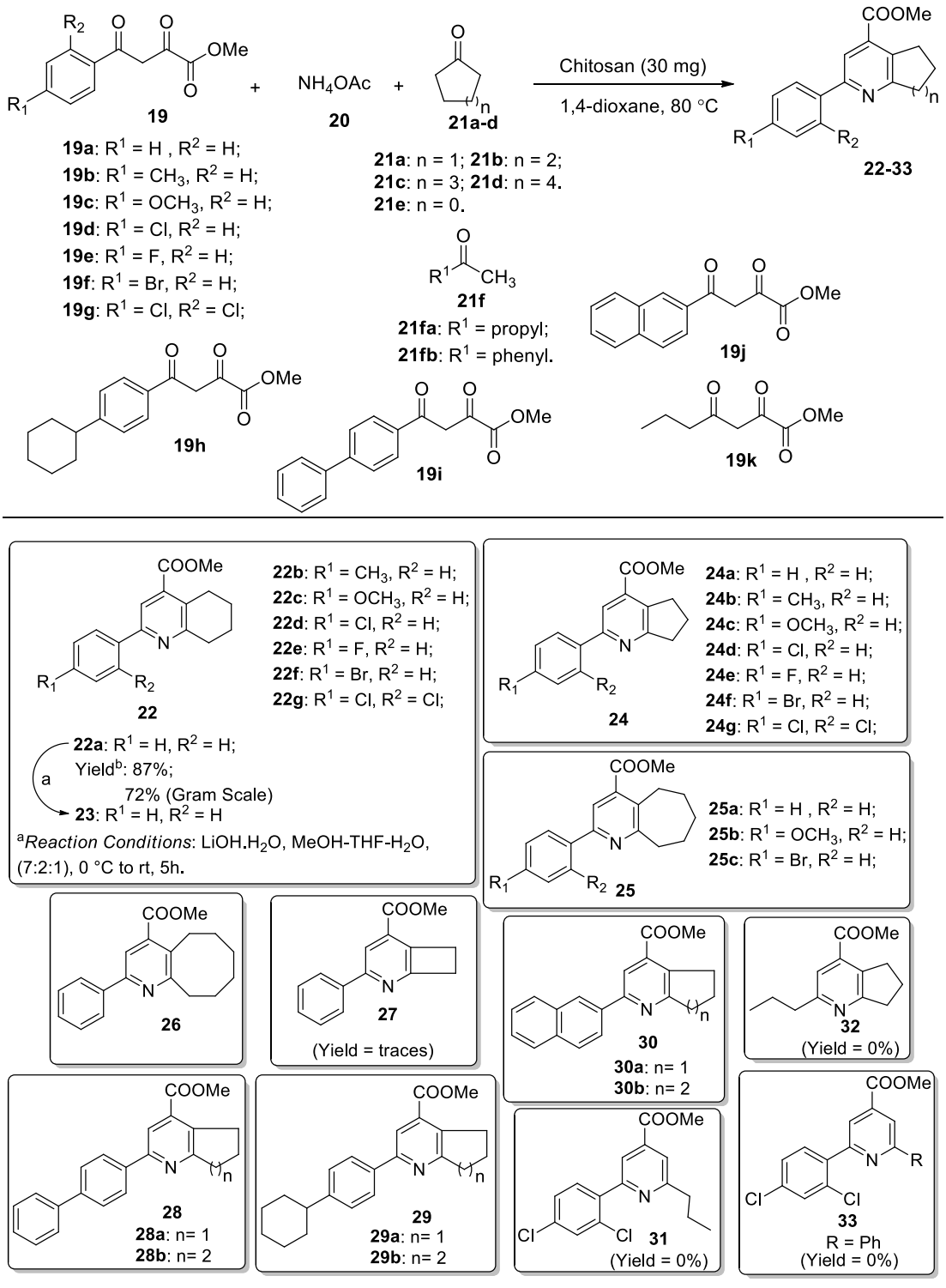
3.2.1 Substrate scope and versatility of the methodology

Various substituted aromatic **19a-19k** diketo-esters, different cyclic (4/5/6/7/8-memebered)/acyclic/aromatic carbonyl compounds **21a-f** along with **20** were subjected under the optimised reaction conditions which furnished the alicyclic[*b*]-fused pyridines **22-33** upto 89% yields (Scheme 1). The present methodology proceeds smoothly for the synthesis of a variety of functionalized alicyclic[*b*]-fused pyridines. Initial observation showed that **19a-j**, the diketo-ester having electron-donating or withdrawing groups, reacts with five-, six- or seven- and eight-membered cyclic ketone **21a-d** in presence of ammonium acetate **20** under optimized conditions afforded **22, 24-26** and **28-30** in good yields.

We also did reaction using highly constrained cyclobutanone **21e** along with **19a** and **20**; unfortunately, **27** was formed in traces but could not be isolated. This implies that the reaction proceeds smoothly with aromatic diketo esters **19a-j**. On contrary to this,

in the case of aliphatic diketoester **19k**, the reaction did not furnished alicyclic[*b*]-fused pyridine **32**.

Scheme 1. Substrate Scope[a]



[a]Reaction conditions: **19a-k** (0.2 mmol), **20** (0.3 mmol), **21a-e** or **21fa-fb** (0.3 mmol), and chitosan (15 wt% w.r.t. **19a-k,** respectively) were dissolved in 1,4-dioxane and heated at 80 °C temperature under N_2 atm at 10h. [b]Obtained isolated yields.

In addition, compound **31** was also not isolated when treated with **21fa**, **19g** and **20** under our optimized conditions but the ^{1}H NMR spectra showed an inseparable complex mixture. Moreover, the reaction of **19g** with **21fb** and **20** did not furnished **33** due to less reactivity of **21fb** in comparison with cyclic ketones **21a-d**. Overall, the reaction undergoes smoothly with cyclic ketones as compared to acyclic one. Aromatic diketoesters were found to be the most suitable substrate to generate a variety of 2-phenyl alicyclic [*b*]-fused pyridines.

Furthermore, we demonstrated its practicality by performing the model reaction via gram scale synthesis of **22a** in 72 % yield. Since isonicotinic acid moiety is present in large number of natural products and drug candidates;[4a,9] the base-catalyzed hydrolysis of **22a** to **23** i.e., alicyclic[*b*]-fused isonicotinic acid derivative, clearly indicates the versatility of our developed methodology (Scheme 2).

3.2.2 Reusability of organocatalyst

Chitosan Organocatalyst, being heterogeneous in nature, was further analyzed for its reusability (Table 3). Interestingly, as it can be seen from the Table 3; the catalyst can be used upto the fourth cycle with minor decrease in reaction yield from 87% to 77% which emphasize the catalytic efficiency of the chitosan organocatalyst.

Table 3. Reusability of chitosan catalyst.

Entry	Reusability	Time (h)	Yield (%)
1	Fresh Chitosan	10	87
2	First cycle	10	83
3	Second cycle	10	80
4	Third cycle	12	79
5	Fourth cycle	12	77

3.2.3 Plausible mechanism

A plausible mechanism for the synthesis of **22a** from **19a** catalysed by chitosan catalyst has been demonstrated in Figure 3. It can be anticipated that **19a** exists in equilibrium with either more stable **19aa** (isolated) or less stable **19ab** (not detected) under acidic medium of acetic acid generated from ammonium acetate.

The reaction starts with the activation of two carbonyl group of 1a with chitosan either via path A or path B to generate iminium ion intermediate **I** or **IA**, respectively. Then, the leftover carbonyl group will be immediately attacked by enamine **21aa**, which is formed *in-situ* from **21b** and NH_4OAc **20**; and form more favoured

intermediates **II** or less favoured **IIA**, respectively. Then, sequence of reactions i.e., removal of water, protonation or deprotonation of amines, abstraction of enolizable proton and cyclization furnished the desired product **22a** with the release of catalyst (path A). On the other hand, **22b** was not isolated in our reaction. It is anticipated that steric hindrance between cyclohexyl group and phenyl group destabilize the formation of transition state **IIA** (path B). Therefore, this reaction occur in regioselective manner via path A (Figure 3).

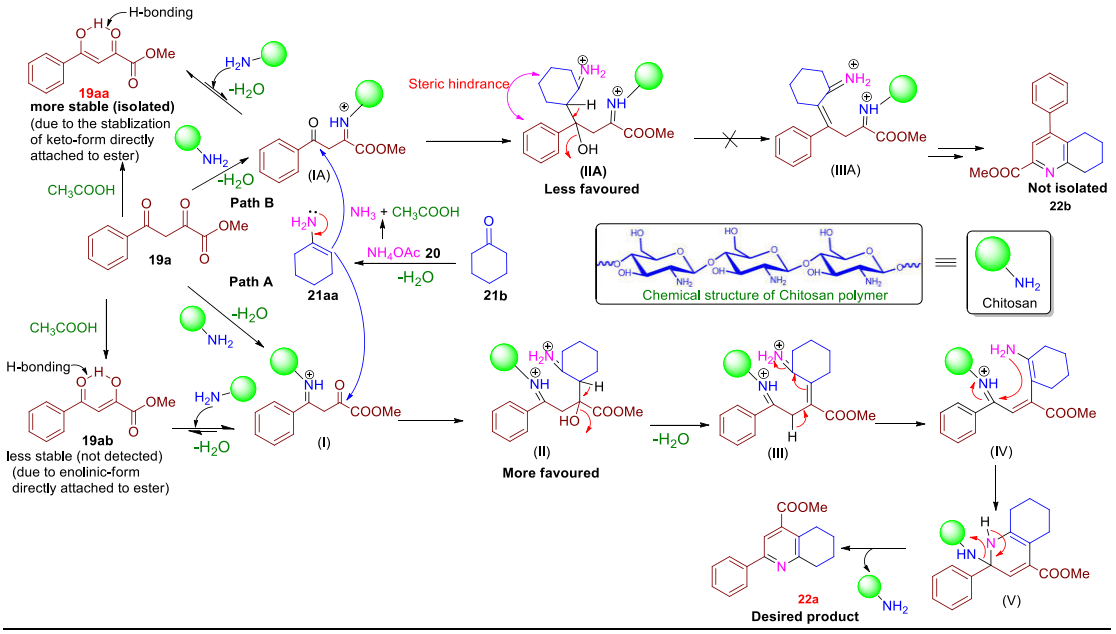
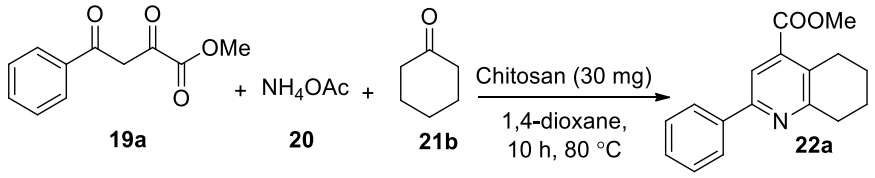


Figure 3. Plausible mechanism for the synthesis of **22a.**

Scheme 2. Control experiment



Catalyst	Yield of **22a** (%)
(i) Tempo (30 mol%)	81
(ii) Tempo (100 mol%)	74
(iii) BHT (30 mol%)	77

[a]Reaction conditions: **19a** (0.2 mmol), **20** (0.3 mmol), **21b** (0.3 mmol), and chitosan (15 wt% w.r.t. **19a-k,** respectively) were dissolved in 1,4-dioxane and heated at 80 °C temperature under N_2 atm at 10h. [b]Obtained isolated yields.

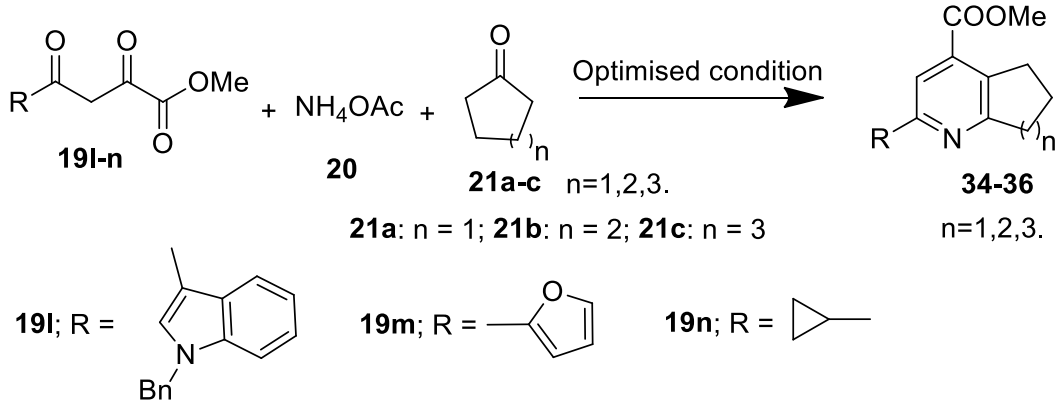
3.2.4 Control experiments

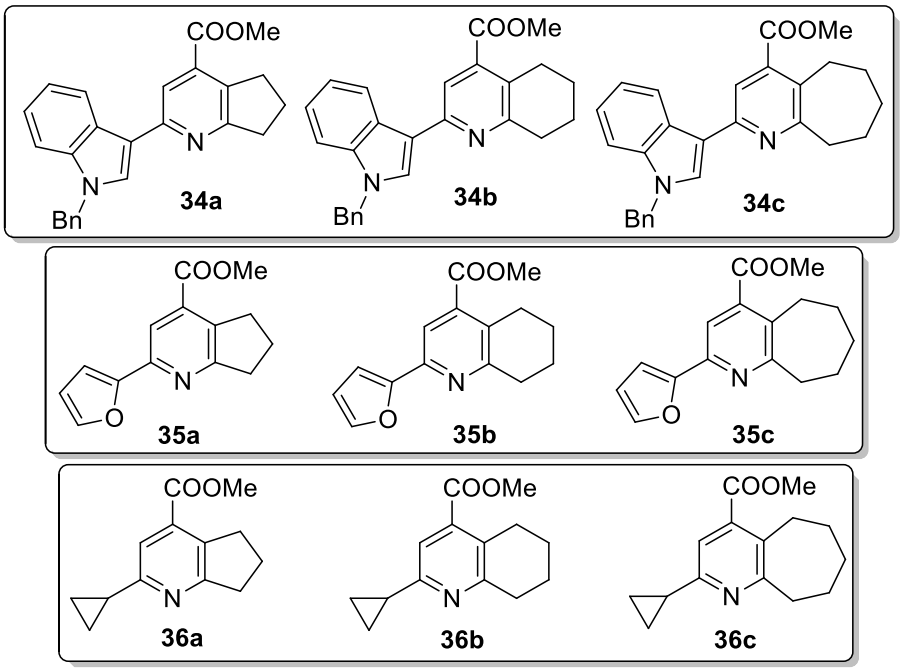
In order to illustrate the type of mechanism involved, control experiments were carried out under optimized reaction conditions in presence of free-radical scavengers

such as Tempo, BHT etc. which furnished **22a** in 81% and 77 % yields, respectively over two steps. This shows that reaction do not occur via free-radical mechanism but it undergoes via ionic pathways as illustrated in Scheme 2.

3.2.5 Synthetic applications

Scheme 3. Synthesis of hetero-[*b*]-fused pyridines and alicyclic-[*b*]-fused pyridines.[a]





[a]*Reaction conditions*: **19l-n** (0.2 mmol), **20** (0.3 mmol), **21a-c** (0.3 mmol), and chitosan (15 wt% w.r.t. **19l-n,** respectively) were dissolved in 1,4-dioxane and heated at 80 °C temperature under N_2 atm at 10h.

Novel classes of heterocycles **34-36** were prepared using our developed methodology. To the best of our knowledge, for the first time, 2-(3-indolyl)-[*b*]-fused pyridines **34**; 2-(furoyl)-[*b*]-fused pyridines **35** and 2-cyclopropyl-[*b*]-fused pyridines **36** were

synthesized via reaction of diketoesters **19l-n**, cyclic ketones **21a-c** along with ammonium acetate under our optimized conditions in 38-83% yield range (Scheme 3).

3.2.6 Antibacterial activity

The 5,6,7,8-tetrahydroquinoline analogues **34b**, **35b** and **36b** were assessed for their *in vitro* antifungal activity against *Aspergillus Niger* and *Candida Albicans* fungal strains.[10] While **34b** was found twice active to ketoconazole; **36b** was found equally active to ketoconazole in *Aspergillus Niger* fungal strain. However, **36b** was also found equally active to ketoconazole in *Candida albicans* fungal strain (Table 4).

Table 4. *In vitro* antifungal activities of **34b**, **35b** and **36b**.

S. No.	Compound name	[a]Fungal Strains (MIC in μg/mL)	
		AN[b]	***CA***[b]
1	**34b**	6.25	50
2	**35b**	25	100
3	**36b**	12.5	25
4	**KET**[c]	12.5	25

[a]MIC of all compounds was measured at the range from 6.25-100 μg/mL. [b]Fungi: AN, *Aspergillus* niger (ATCC 9029); CA, Candida Albicans (ATCC 10231); [c]KET: Ketoconazole.

3.3 Conclusion

In conclusion, our study uncovered the identification of chitosan as heterogeneous organocatalyst for the development of modified Guareschi-Thorpe type condensation reaction for the regioselective synthesis of structurally diverse alicyclic[*b*]-fused pyridines **22, 24-33**. With the operational simplicity, functional group compatibility, modularity, low catalyst loading and reusability of the catalyst; the present methodology has substantially expanded the scope of fused pyridine derivatives accessible from readily available cyclic ketones and diketoesters as starting substrates. The present methodology also enables for the direct functionalization of the fused pyridine core via the construction of a diverse array of heteroaryl (3-indolyl- and 2-furoyl-)/alicyclic (2-cyclopropyl-) substituted alicyclic[*b*]-fused pyridines **34-36**. In addition, the gram scale synthesis of **22a**, and its conversion to isonicotinic acid derivative **23** as well as the synthesis of 5,6,7,8-tetrahydroquinoline derivatives **22a-22g** emphasizes the practicality of this methodology. New 5,6,7,8-tetrahydroquinoline derivatives **34b**, **35b** and **36b,** for the first time, were found to be a new class of antifungal agents. This findings disclosed a new synthetic route for the green synthesis of structurally diverse alicyclic[*b*]-fused pyridine heterocycles.

3.4 Experimental Details & Characterization Data

3.4.1 General experimental

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 $CDCl_3$, 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 Q Tof (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.

3.4.2 General procedure for the Synthesis of 22a and 19aa (optimization study):

To a solution of the NH_4OAc **20** (0.3 mmol; 1.5 eq.) in EtOH (2.0 mL) was added freshly distilled cyclohexanone **21** (0.3 mmol; 1.5 eq.), and the reaction mixture was stirred under N_2 atmosphere at room temperature for 45 min. Methyl 2,4-dioxo-4-phenylbutanoate **19a (**0.20 mmol; 1eq.) and specified organocatalysts (30 mol%, entry 2-10; Table 1) was added and the reaction mixture was heated under N_2 atmosphere at 80 ˚C temperature for 4h. The progress of the reaction was checked by TLC using 9:1 hexane/ethyl acetate as an eluent. After completion of reaction, the reaction mixture was quenched with distilled water and evaporated under reduced pressure, which afforded the crude product. The crude product was dissolved in distilled water (10 ml) and extracted with ethyl acetate (3 × 10 ml). The organic layers were combined, dried over anhydrous Na_2SO_4 and removed under reduced pressure to give the crude product. The crude product were purified by using flash column chromatography method over silica gel using 9.5:0.5 to 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired methyl 2-phenyl-5,6,7,8-tetrahydroquinoline-4-carboxylate **22a** (21-65%) and **19aa** (32-92%) as shown in Table 1 (entry 1-10).

3.4.3 General procedure for the Synthesis of 22a (Table 2 study):

To a solution of the NH_4OAc **20** (0.3 mmol; 1.5 eq.) in EtOH (2.0 mL) was added freshly distilled cyclohexanone **21** (0.3 mmol; 1.5 eq.), and the reaction mixture was

stirred at room temperature under N_2 atmosphere for 45 min. Methyl 2,4-dioxo-4-phenylbutanoate **19a (**0.20 mmol; 1eq.) and chitosan (as given in entry 1-16, Table 2) was added and the reaction mixture was heated under N_2 atmosphere at given temperature for specified time (as shown in entry 1-16, Table 2). The progress of the reaction was checked by TLC using 9:1 hexane/ethyl acetate as an eluent. After that, the reaction mixture was quenched with distilled water and evaporated under reduced pressure, which afforded the crude product. The crude product was dissolved in distilled water (10 ml) and extracted with ethyl acetate (3 × 10 ml). The organic layers were combined, dried over anhydrous Na_2SO_4 and removed under reduced pressure to give the crude product. The crude product were purified by using flash column chromatography method over silica gel using 9.5:0.5 to 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired methyl 2-phenyl-5,6,7,8-tetrahydroquinoline-4-carboxylate **22a** and **19aa**. Further, the compound **19aa** (0.10 mmol; 1eq.) was again subjected using **20** (0.15 mmol; 1.5 eq.) and **21** (0.15 mmol; 1.5 eq.) under same reaction conditions and same protocol ware performed, which afforded pure intermediate **19aa** and desired **22a**. The combined yield (20-87%) of desired **22a** was shown after 2 steps in table 2.

3.4.5 General procedure for the Synthesis of pyridine fused alicyclic molecular frameworks 22-36

To a solution of the NH_4OAc **20** (0.3 mmol; 1.5 eq.) in 1,4-dioxane (2.0 mL) was added freshly distilled ketone **21a-21fb** (0.3 mmol; 1.5 eq.; as shown in scheme 2), **or 21a-c** (0.3 mmol; 1.5 eq.; as shown in scheme 4) and the reaction mixture was stirred at room temperature under N_2 atmosphere for 45 min. Substituted diketo-ester **19a-k (**0.20 mmol; 1eq.; as shown in scheme 2) or 19l-n (0.20 mmol; 1eq.; as shown in scheme 4) and chitosan (15 wt% with respect to 19a-k or 19l-n, respectively) was added and the reaction mixture was heated under N_2 atmosphere at given temperature for 8-16 h (as shown in Scheme 2 and 4). The progress of the reaction was checked by TLC using 9:1 hexane/ethyl acetate as an eluent. After that, the reaction mixture was quenched with distilled water and evaporated under reduced pressure, which afforded the crude product. The crude product was dissolved in distilled water (10 ml) and extracted with ethyl acetate (3 × 10 ml). The organic layers were combined, dried over anhydrous Na_2SO_4 and removed under reduced pressure to give the crude product. The crude product were purified by using flash column chromatography

method over silica gel using 9.5:0.5 to 9:1 hexane/ethyl acetate as an eluent which afforded the pure desired ate **22a-36** (38-89% after 2 consecutive steps, same as utilised in above mentioned table 2 study).

3.4.6 General procedure for the study of control experiment (22a as representative):

The free radical quencher TEMPO (30 and 100 mol%) and BHT (30 mol%) were used in 1,4-dioxane under same procedure, as mentioned in Table 2 study, which afforded **22a** (77-81%).

3.4.7 Characterization data of 2-aryl/heteroaryl/alicyclic substituted pyridine fused alicyclic molecular frameworks 22-36:

Methyl-2-phenyl-5,6,7,8-tetrahydroquinoline-4-carboxylate 22a

Solid; yield: 87 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 40-42 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 2920, 1732, 1590, 1437, 1247; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.97 (m, 2H), 7.88 (s, 1H), 7.51-7.37 (m, 3H), 3.94 (s, 3H), 3.07 (t, J = 6.0 Hz, 4H), 1.94 – 1.83 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 159.3, 154.8, 139.1, 138.2, 134.0, 128.9, 128.1, 126.9, 118.0, 52.5, 33.8, 29.8, 27.0, 22.8, 22.7; HRMS (ESI) calcd. for $C_{17}H_{17}NO_2$ $[M+H]^+$: 268.1332; found 268.1337.

2-phenyl-5,6,7,8-tetrahydroquinoline-4-carboxylic acid 23

Solid; yield: 94 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 180-182 °C; FT-IR (KBr, νmax/cm^{-1}) 3431, 2934, 1731, 1585, 1435, 1246; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.4 Hz, 2H), δ 7.90 (s, 1H) δ 7.49 – 7.39 (m, 3H), δ 3.82(s, 1H), δ 2.96 – 2.92 (m, 4H), δ 1.83 – 1.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 158.4, 153.2, 140.3, 138.1, 129.5, 129.2, 128.9, 126.5, 116.8, 33.1, 26.4, 22.3; HRMS (ESI) calcd. for $C_{16}H_{15}NO_2$ $[M+H]^+$: 254.1103; found 254.1107.

Methyl-2-(p-tolyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22b

Solid; yield: 81 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 55-60 °C; FT-IR (KBr, νmax/cm^{-1}) 3439, 2933, 1730, 1551, 1436, 1255; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (t, J = 8.4 Hz, 3H), 7.25 – 7.24

(m, 2H), 3.92 (s, 3H), 3.04 (t, J = 6.8 Hz, 4H), 2.39 (s, 3H), 1.92 – 1.81 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 159.2, 154.7, 138.9, 138.1, 136.3, 130.1, 129.6, 126.8, 117.6, 52.4, 33.8, 29.8, 26.9, 22.8, 22.7, 21.4; HRMS (ESI) calcd. for $C_{18}H_{19}NO_2$ $[M+H]^+$: 282.1489; found 282.1487.

Methyl-2-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22c

Solid; yield: 76 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 53-55 °C; FT-IR (KBr, νmax/cm^{-1}) 3431, 2929, 1729, 1606, 1550, 1436, 1250; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 – 7.94 (m, 2H), 7.82 (s, 1H), 6.99 – 6.97 (m, 2H), 3.93 (s, 3H), 3.86 (s, 3H), 3.06 – 3.03 (m, 4H), 1.92 – 1.83 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 160.5, 159.0, 154.4, 138.1, 131.8, 129.7, 128.2, 117.3, 114.2, 55.5, 52.4, 33.8, 29.8, 26.9, 22.9, 22.7; HRMS (ESI) calcd. for $C_{18}H_{19}NO_3$ $[M+H]^+$: $[M+H]^+$: 298.1438; found 298.1434.

Methyl-2-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22d

Solid; yield: 75 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 48-50 °C; FT-IR (KBr, νmax/cm^{-1}) 3407, 2925, 1711, 1545,1436, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.92 (m, 2H), 7.84 (s, 1H), 7.43 - 7.40 (m, 2H), 3.94 (s, 3H), 3.05 (q, J = 6.0 Hz, 4H), 1.94 – 1.80 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 159.5, 153.4, 138.2, 137.5, 135.1, 130.9, 129.0, 128.2, 117.7, 52.5, 33.8, 29.8, 27.0, 22.8; HRMS (ESI) calcd. for $C_{17}H_{16}ClNO_2$ $[M+H]^+$: 302.0942; found 302.0948.

Methyl-2-(4-fluorophenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22e

Solid; yield: 78 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 48-50 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 2929, 1711, 1603, 1548, 1403, 1232; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 7.95 (m, 2H), 7.83 (s, 1H), 7.16 – 7.11 (m, 2H), 3.94 (s, 3H), 3.05 – 3.04 (m, 4H), 1.93 – 1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 164.8, 162.4, 159.4, 153.7, 138.3, 135.2, 130.6, 128.8, 128.7, 117.7, 115.9, 115.6, 52.5, 33.8, 29.8, 26.9, 27.8, 22.7; HRMS (ESI) calcd. for $C_{17}H_{16}FNO_2$ [M+H]+: 286.1238; found 286.1234.

Methyl-2-(4-bromophenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22f

Solid; yield: 83 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 40-42 °C; FT-IR (KBr, νmax/cm^{-1}) 3438, 2926, 1731, 1590, 1550, 1444, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.85 (m, 3H), 7.59 – 7.56 (m, 2H), 3.94 (s, 3H), 3.06 – 3.03 (m, 4H), 1.92-1.84 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 159.5, 153.4, 138.3, 137.9, 131.9, 131.3, 128.5, 123.4, 117.7, 52.5, 33.8, 29.8, 27.1, 22.8, 22.7; HRMS (ESI) calcd. for $C_{17}H_{16}BrNO_2$ $[M+H]^+$: 346.0437; found 346.0443.

Methyl-2-(2,4-dichlorophenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 22g

Solid; yield: 80 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 72-74 °C; FT-IR (KBr, νmax/cm^{-1}) 3433, 2925, 1736, 1629, 1461, 1379, 1258; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.53 – 7.48 (m, 2H), 7.33 (d, J = 8.0 Hz, 1H), 3.92 (s, 3H), 3.11 – 3.03 (m, 4H), 1.93 – 1.85 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 159.5, 153.0, 137.5, 137.4, 134.9, 133.1, 132.5, 131.5, 129.9, 127.6, 122.0, 52.6, 33.6, 27.0, 22.7, 22.6; HRMS (ESI) calcd. for $C_{17}H_{15}Cl_2NO_2$ $[M+H]^+$: 336.0553; found 336.0559.

Methyl-2-phenyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24a

Solid; yield: 89 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 70-72 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2926, 1723, 1573,1432, 1384, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-7.99 (m, 3H), 7.49-7.38 (m, 3H), 3.96 (s, 3H), 3.31 (t, J = 8.0 Hz, 15.2 Hz, 2H), 3.13 (t, J = 8.0 Hz, 15.6 Hz, 2H), 2.22-2.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 166.8, 156.8, 139.3, 136.4, 134.1, 128.9, 128.8, 127.0, 117.8, 52.5, 34.5, 31.7, 22.9; HRMS (ESI) calcd. for $C_{16}H_{15}NO_2$ $[M+H]^+$: 254.1176; found 254.1171.

Methyl-2-(p-tolyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24b

Solid; yield: 86 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 80-81 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2948, 1788, 1573, 1435, 1384, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 3.96 (s, 3H), 3.29 (t, J = 7.2 Hz, 15.2 Hz, 2H), 3.12 (t, J = 8.0 Hz, 15.6 Hz, 2H), 2.40 (s, 3H), 2.22-2.14 (m, 2H); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 168.3, 166.9, 156.8, 138.9, 136.4, 136.0, 134.1, 129.6, 126.9, 117.4, 52.5, 34.5, 31.7, 22.9, 21.4; HRMS (ESI) calcd. for $C_{17}H_{18}NO_2$ $[M+H]^+$: 268.1332; found 268.1339.

Methyl-2-(4-methoxyphenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24c

Solid; yield: 81 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 103-105°C; FT-IR (KBr, νmax/cm^{-1}) 3424, 2961, 1717, 1607, 1568, 1362, 1270; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97-7.95 (m, 3H), 6.98 (d, J = 8.8 Hz, 2H), 3.95 (s, 3H), 3.86 (s, 3H), 3.28 (t, J = 7.6 Hz, 15.2 Hz, 2H), 3.11 (t, J = 7.6 Hz, 15.6 Hz, 2H), 2.21-2.13 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.9, 160.5, 156.5, 135.5, 134.1, 131.9, 128.3, 117.0, 114.2, 55.5, 52.4, 34.5, 31.7, 22.9; HRMS (ESI) calcd. for $C_{17}H_{18}NO_3$ $[M+H]^+$: 284.1281; found 284.1286.

Methyl-2-(4-chlorophenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24d

Solid; yield: 83 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 80-81 °C; FT-IR (KBr, νmax/cm^{-1}) 3422, 2945, 1718, 1614, 1576, 1493, 1397, 1270; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98-7.84 (m, 3H), 7.44-7.41 (m, 2H), 3.96 (s, 3H), 3.30 (t, J = 7.2 Hz, 14.8 Hz, 2H), 3.12 (t, J = 7.6 Hz, 15.6 Hz, 2H), 2.20-2.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 166.6, 155.5, 137.7, 136.8, 135.1, 134.2, 129.1, 128.3, 117.5, 52.5, 34.5, 31.7, 22.9 HRMS (ESI) calcd. for $C_{16}H_{15}ClNO_2$ $[M+H]^+$: 288.0786; found 288.0781.

Methyl-2-(4-fluorophenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24e

Solid; yield: 86 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 77-79 °C; FT-IR (KBr, νmax/cm^{-1}) 3429, 2922, 1728, 1513, 1383, 1240; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00-7.95 (m, 3H), 7.17-7.12 (m, 2H), 3.96 (s, 3H), 3.30 (t, J = 7.6 Hz, 15.2 Hz, 2H), 3.12 (t, J = 8.0 Hz, 16.0 Hz, 2H), 2.21-2.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 166.7, 155.8, 136.4, 134.2, 128.9, 128.8, 117.5, 115.9, 115.7, 52.5, 34.5, 31.7, 22.9; HRMS (ESI) calcd. for $C_{16}H_{15}FNO_2$ $[M+H]^+$: 272.1081; found 272.1087.

Methyl-2-(4-bromophenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24f

Solid; yield: 80 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 90-92 °C; FT-IR (KBr, νmax/cm^{-1}) 3411, 1716, 1574,1438, 1395, 1204; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 3.96 (s, 3H), 3.29 (t, J = 7.2 Hz, 15.2 Hz, 2H), 3.12 (t, J = 8.0 Hz, 15.6 Hz, 2H), 2.22-2.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7,166.6, 155.5, 138.1, 136.9, 134.2, 132.0, 128.6, 123.4, 117.5, 52.5, 34.5, 31.7, 22.9; HRMS (ESI) calcd. for $C_{16}H_{15}BrNO_2$ $[M+H]^+$: 332.0281; found 332.0289.

Methyl-2-(2,4-dichlorophenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 24g

Solid; yield: 77 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 83-85°C; FT-IR (KBr, νmax/cm^{-1}) 3441, 2918, 1715, 1611, 1590, 1434, 1364,1270; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.54-7.49 (m, 2H), 7.35-7.33 (m, 1H), 3.95 (s, 3H), 3.34 (t, J = 7.2 Hz, 15.2 Hz, 2H), 3.13 (t, J = 8.4 Hz, 16.0 Hz, 2H), 2.24-2.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 166.4, 154.7, 137.6, 137.3, 134.9, 133.5, 133.2, 132.5, 129.9, 127.5, 121.8, 52.6, 34.4, 31.8, 22.8; HRMS (ESI) calcd. for $C_{16}H_{14}Cl_2NO_2$ $[M+H]^+$: 322.0396; found 322.0391.

Methyl-2-phenyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 25a

Sticky solid; yield: 76 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; FT-IR (KBr, νmax/cm^{-1}) 3450, 2922, 1729, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01–7.99 (m, 2H), 7.73 (s, 1H), 7.47 – 7.36 (m, 3H), 3.94(s, 3H);, 3.22 – 3.19 (m, 2H);, 3.04 – 3.01 (m, 2H), 1.91– 1.87(m, 2H), 1.78–1.72(M, 4H); ^{13}C NMR (100 MHz, $CDCl_3$168.4, 165.4, 154.0, 139.0, 135.1, 128.9, 128.8, 126.9, 117.2, 52.6, 39.6, 32.3, 29.8, 27.4, 26.6.; HRMS (ESI) calcd. for $C_{18}H_{20}NO_2$ $[M+H]^+$: 282.1489; found 282.1497.

Methyl-2-(4-methoxyphenyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 25b

Sticky solid; yield: 71 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; FT-IR (KBr, νmax/cm^{-1}) 3445, 2924, 1704, 1580, 1426, 1235; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 2H), 7.66 (s, 1H), 6.97 (d, *J* = 9.2 Hz, 2H), 3.93 (s, 3H),, 3.84 (s, 3H),, 3.19 – 3.16 (m, 2H), 3.01 – 3.98 (m, 2H), 1.88 – 1.87 (m, 2H), 1.75 – 1.71 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.5, 165.2, 160.4, 153.7, 138.8, 134.3, 131.7, 128.1, 116.4, 114.2, 55.5, 52.6, 39.6, 32.3, 29.7, 27.5, 26.6; HRMS (ESI) calcd. for $C_{19}H_{22}NO_3$ $[M+H]^+$: 312.1594; found 312.1599.

Methyl-2-(4-bromophenyl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 25c

Solid; yield: 74 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 77-80 °C; FT-IR (KBr, νmax/cm^{-1}) 3416, 2926, 1735, 1593, 1493, , 1377, 1241; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.4 Hz, 2H), 7.70 (s, 1H), 7.57 (d, J = 8.8 Hz, 2H); 3.95 (s, 3H), 3.21 – 3.18 (m, 2H), 3.03 – 3.01 (m, 2H), 1.91–1.88 (m, 2H), 1.77– 1.72 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$) 168.3, 165.7, 152.7, 139.4, 138.9, 135.7, 131.9, 128.5, 116.9, 114.2, 52.7, 39.6, 32.1, 29.8,27.4, 26.6; HRMS (ESI) calcd. for $C_{18}H_{18}BrNO_2$ $[M+2]^+$: 361.0521; found 361.0526.

Methyl-2-phenyl-5,6,7,8,9,10-hexahydrocycloocta[*b*]pyridine-4-carboxylate 26

Solid; yield: 72 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 55-56 °C; FT-IR (KBr, νmax/cm^{-1}) 3437, 2925, 1727, 1581, 1494, 1331, 1230; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 8.00 (m, 2H), 7.85 (s, 1H), 7.48 – 7.45 (m, 2H), 7.41 – 7.38 (m, 1H), 3.95 (s, 3H), 3.15 – 3.07 (m, 4H), 1.88 – 1.79 (m, 4H), 1.50 – 1.34 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 163.6, 154.8, 139.1, 138.3, 133.3, 128.9, 128.8, 126.9, 118.1, 55.5, 35.8, 31.3, 31.1, 27.5, 26.7, 26.0; HRMS (ESI) calcd. for $C_{19}H_{22}NO_2$ $[M+H]^+$: 296.1645; found 296.1652.

Methyl-2-([1,1'-biphenyl]-4-yl)-6,7-dihydro-5H-cyclopenta[b]pyridine-4-carboxylate 28a

Solid; yield: 83 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl

acetate (9:1) as an eluent; m.p. 117-119 °C; FT-IR (KBr, νmax/cm^{-1}) 3428, 2953, 1604, 1727, 1573, 1487, 1244; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.07 (m, 3H), 7.72-7.65 (m, 4H), 7.46-7.35 (m, 3H), 3.97 (s, 3H), 3.32 (t, J = 7.6 Hz, 14.8 Hz, 2H), 3.17 (t, J = 8.0 Hz, 15.6 Hz, 2H), 2.24-2.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 166.8, 156.3, 141.7, 140.8, 138.2, 136.5, 134.2, 128.9, 127.6 (2-C), 127.4, 127.3, 117.7, 52.5, 34.6, 31.7, 22.9; HRMS (ESI) calcd. for $C_{22}H_{20}NO_2$ $[M+H]^+$: 330.1489; found 330.1482.

Methyl-2-([1,1'-biphenyl]-4-yl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 28b

Solid; yield: 85 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 65°C; FT-IR (KBr, νmax/cm^{-1}) 3423, 2945, 1720, 1570, 1352, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 8.4 Hz, 2H), 7.94 (s, 1H), 7.71-7.65 (m, 4H), 7.47 (t, J = 8.0 Hz, 15.2 Hz, 2H), 7.39-7.35 (m, 1H), 3.95 (s, 3H), 3.09 (t, J = 6.0 Hz, 12 Hz, 4H), 1.94-1.85 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 159.4, 154.2, 141.7, 140.8, 138.2, 137.9, 130.6, 128.9, 127.6, 127.5, 127.3, 127.2, 117.8, 52.5, 33.8, 27.0, 22.8, 22.7; HRMS (ESI) calcd. for $C_{23}H_{22}NO_2$ $[M+H]^+$: 344.1645; found 344.1651.

Methyl-2-(4-cyclohexylphenyl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 29a

Solid; yield: 79 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 80-82 °C; FT-IR (KBr, νmax/cm^{-1}) 3434, 2922, 1614, 1727, 1573, 1440, 1349, 1243; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.93-7.91 (m, 2H), 7.32-7.29 (m, 2H), 3.95 (s, 3H), 3.29 (t, J = 7.2 Hz, 15.2 Hz, 2H), 3.12 (t, J = 8.0 Hz, 15.6 Hz, 2H), 2.58-2.53 (s, 1H), 2.22-2.14 (m, 2H), 1.92-1.75 (m, 5H), 1.51-1.23 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 166.9, 156.9, 149.1, 136.9, 135.9, 134.0, 127.4, 126.9, 117.5, 52.4, 44.5, 34.5 (2-c), 31.7, 27.0, 26.3, 22.9; HRMS (ESI) calcd. for $C_{22}H_{26}NO_2$ $[M+H]^+$: 336.1958; found 336.1954.

Methyl-2-(4-cyclohexylphenyl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 29b

Solid; yield: 74 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 62 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2930, 1725, 1607, 1444, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.85 (m, 3H), 7.29 (d, J = 8.0

Hz, 2H), 3.93 (s, 3H), 3.07 – 3.03 (m, 4H), 2.58 – 2.55 (m, 1H), 1.90 – 1.74 (m, 9H), 1.50 – 1.26 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 159.2, 154.9, 149.1, 138.1, 136.8, 130.1, 127.4, 126.9, 117.7, 52.4, 44.5, 34.5, 33.8, 27.0, 26.3, 22.9, 22.7; HRMS (ESI) calcd. for $C_{23}H_{28}NO_2$ $[M+H]^+$: 350.2115; found 350.2120.

Methyl 2-(naphthalen-2-yl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 30a

Solid; yield: 81 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 93-95 °C; FT-IR (KBr, νmax/cm^{-1}) 3426, 2952, 1717, 1575, 1430, 1387, 1248; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.17-8.14(m, 2H), 7.95-7.86 (m, 3H), 7.51-7.49 (m, 2H), 3.99 (s, 3H), 3.33 (t, J = 7.6 Hz, 15.2 Hz, 2H), 3.18 (t, J = 7.6 Hz, 15.6 Hz, 2H), 2.25-2.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 166.8, 156.6, 136.5, 134.2, 133.6, 133.5, 128.8, 128.6, 127.8, 126.6, 126.4, 126.3, 124.7, 118.0, 52.5, 34.5, 31.7, 22.9; HRMS (ESI) calcd. for $C_{20}H_{18}NO_2$ $[M+H]^+$: 304.1332; found 304.1339.

Methyl-2-(naphthalen-2-yl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 30b

Solid; yield: 77 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 65 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2925, 1728, 1636, 1432, 1147; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.16 – 8.14 (m, 1H), 8.03 (s, 1H), 7.94 – 7.92 (m, 3H), 7.51 – 7.49 (m, 2H), 3.96 (s, 3H), 3.11 – 3.09 (m, 4H), 1.95 – 1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 159.4, 154.6, 138.3, 136.4, 133.8, 133.7, 130.7, 128.8, 128.6, 127.8, 126.6, 126.4, 126.2, 124.7, 118.2, 52.5, 33.9, 29.8, 27.1, 22.9, 22.8; HRMS (ESI) calcd. for $C_{21}H_{20}NO_2$ $[M+H]^+$: 318.1489; found 318.1480.

Methyl-2-(1-benzyl-1*H*-indol-2-yl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 34a

Solid; yield: 59 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 158°C; FT-IR (KBr, νmax/cm^{-1}) 3432, 2921, 1708, 1591, 1448, 1382, 1235; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37–8.35 (m, 1H), δ 8.00(s, 1H), δ 7.80 (s, 1H), δ 7.31 – 7.21 (m, 6H), δ 7.17 – 7.15 (m, 2H), δ 5.38 (s, 2H), δ 3.96(s, 3H), δ 3.28 (t, J = 8, 2H), δ 3.12(t, J= 8, 2H), δ 2.21–2.13(m, 2H); ^{13}C NMR

(100 MHz, $CDCl_3$) 167.1, 153.9, 137.5, 137.0, 133.9, 128.9, 128.6, 127.9, 126.9, 126.3, 122.5, 122.5, 121.4, 120.9, 117.4, 116.4, 110.2, 52.4, 50.5, 34.6, 31.7, 22.9; HRMS (ESI) calcd. for $C_{25}H_{22}N_2O_2$ $[M+H]^+$: 383.1681; found 383.1686.

Methyl-2-(1-benzyl-1*H*-indol-2-yl)-5,6,7,8-tetrahydroquinoline-4-carboxylate 34b

Solid; yield: 43 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 120-122 °C; FT-IR (KBr, νmax/cm^{-1}) 3436, 2935, 1704, 1590, 1448, 1380, 1231; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41– 8.39(m, 1H), 7.82 (s, 1H), 7.72 (s, 1H); δ 7.31– 7.19(m, 6H), δ 7.15–7.13 (m, 2H), δ 5.37 (S, 2H), δ 3.92 (s, 3H), δ 3.07– 3.01(m, 4H), δ 1.94–1.80 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$) 167.9, 158.9, 152.2, 137.8, 137.5, 137.1, 128.9, 128.3, 128.0, 127.9, 126.9, 126.4, 122.5, 121.7, 120.9, 117.7, 116.2, 110.1, 52.4, 50.4, 33.9, 26.9, 23.0, 22.8; HRMS (ESI) calcd. for $C_{26}H_{25}N_2O_2$ $[M+H]^+$: 397.1911; found 397.1918.

Methyl-2-(1-benzyl-1*H*-indol-2-yl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 34c

Solid; yield: 51 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 150-152 °C; FT-IR (KBr, νmax/cm^{-1}) 3447, 2923, 1725, 1586, 1436, 1386, 1235; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40–8.37 (m, 1H), δ 7.72 (s, 1H), δ 7.66 (s, 1H); δ 7.30 – 7.19 (m, 6H); δ 7.15–7.13 (m, 2H), δ 5.36(s, 2H), δ 3.93(s, 3H), 3.20–3.18 (m, 2H), 2.99–2.97 (m, 2H), δ 1.88–1.87 (m, 2H), δ 1.77–1.70 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$) 168.8, 165.1, 151.6, 137.5, 137.1, 132.7, 128.9, 128.3, 127.9, 126.9, 126.4, 122.5, 121.7, 120.9, 116.8, 116.2, 110.1, 52.6, 50.5, 39.7, 32.4, 29.8, 27.6, 26.7; HRMS (ESI) calcd. for $C_{27}H_{26}N_2O_2$ $[M+H]^+$: 411.1994; found 411.1999.

Methyl-2-(furan-2-yl)-6,7-dihydro-5*H*-cyclopenta[*b*]pyreidine-4-carboxylate 35a

Solid; yield: 38 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 120 °C; FT-IR (KBr, νmax/cm^{-1}) 3433,2924, 1711, 1607, 1576, 1495, 1344, 1245; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.52 (s, 1H), 7.03 (d, J = 3.2 Hz, 1H), 6.52 (q, J = 3.2 Hz, 1H), 3.94 (s, 3H), 3.27 (t, J = 7.6 Hz, 2H), 3.09 (t, J = 7.6 Hz, 2H), 2.15 (quintet, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 166.5, 153.4, 148.5, 143.4, 136.4, 134.1, 116.0, 112.1, 108.5, 52.5,

34.4, 31.7, 22.9; HRMS (ESI) calcd. for $C_{14}H_{13}NO_3$ $[M+H]^+$: 244.0895; found 244.0898.

Methyl-2-(furan-2-yl)-5, 6, 7, 8-tetrahydroquinoline-4-carboxylate 35b

Solid; yield: 41 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 60-62 °C; FT-IR (KBr, νmax/cm^{-1}) 3438, 2936, 1720, 1607, 1550, 1437,1327, 1242; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.52 (s, 1H), 7.01 (d, J = 3.2 Hz, 1H), 6.52 – 6.50 (m, 1H), 3.92 (s, 3H), 3.02 (q, J = 6.4 Hz, 4H), 1.91 – 1.80 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 159.3, 153.2, 146.7, 143.4, 138.0, 130.5, 116.3, 112.0, 108.5, 52.5, 33.7, 27.0, 22.7, 22.6; HRMS (ESI) calcd. for $C_{15}H_{15}NO_3$ $[M+H]^+$: 258.1052; found 258.1057.

Methyl-2-(furan-2-yl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 35c

Solid; yield: 47 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 53 °C; FT-IR (KBr, νmax/cm^{-1}) 3435, 2920, 1726, 1607, 1563, 1493,1389, 1237; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.51 (s, 1H), 7.00 (d, J = 3.2 Hz, 1H), 6.51 (q, J = 3.2 Hz, 1H), 3.93 (s, 3H), 3.17 – 3.14 (m, 2H), 3.00 – 2.98 (m, 2H), 1.89 – 1.84 (m, 2H), 1.75 – 1.67 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 165.6, 153.4, 146.1, 143.3, 138.7, 135.2, 115.5, 112.0, 108.5, 52.7, 39.4, 32.2, 29.8, 27.4, 26.5; HRMS (ESI) calcd. for $C_{16}H_{17}NO_3$ $[M+H]^+$: 272.1208; found 272.1203.

Methyl-2-cyclopropyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine-4-carboxylate 36a

Solid; yield: 80 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 73-75 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2924, 1725, 1590, 1432, 1374, 1284; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1H), δ 3.90 (s, 3H), δ 3.21 – 3.17 (m, 2H), δ 2.97 (t, J = 7.6Hz, 2H), 2.13–2.06 (m, 3H), .98 – .96 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 167.7, 166.9, 161.9, 134.3, 133.4, 117.6, 52.3, 34.4, 31.5, 22.9, 17.2, 9.8; HRMS (ESI) calcd. for $C_{13}H_{15}NO_2$ $[M+H]^+$: 218.1103; found 218.1107.

Methyl-2-cyclopropyl-5,6,7,8-tetrahydroquinoline-4-carboxylate 36b

Sticky solid; yield: 72 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. sticky solid; FT-IR (KBr, νmax/cm^{-1}) 3442, 2936, 1730, 1555, 1408, 1289; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (s, 1H), δ 3.87 (s, 3H), δ 2.94(t, J = 6.4Hz, 2H), δ 2.87 (t, J = 12.8Hz, 2H), δ2.03–1.97 (m, 1H), δ 1.85–1.73 (m, 4H); δ .97–.89 (m, 4H); δ ^{13}C NMR (100 MHz, $CDCl_3$) 167.7, 159.9, 158.4, 137.5, 128.3, 117.3, 52.3, 33.6, 26.7, 22.8, 22.7, 17.0, 14.2, 9.5; HRMS (ESI) calcd. for $C_{14}H_{17}NO_2$ $[M+H]^+$: 232.1259; found 232.1251.

Methyl-2-cyclopropyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*b*]pyridine-4-carboxylate 36c

Solid; yield: 83 %, R_f (EtOAc/hexane; 05:95) = 0.80; Purification of crude product was done by flash column chromatography method over silica gel using hexane/ethyl acetate (9:1) as an eluent; m.p. 77-78 °C; FT-IR (KBr, νmax/cm^{-1}) 3430, 2958, 1721, 1588, 1434, 1375, 1291; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (s, 1H), δ 3.89 (s, 3H), δ 3.04 – 2.88 (m, 4H); δ 2.04 – 1.97(m, 1H), δ 1.86 – 1.80 (m, 2H); δ 1.69–1.61 (m, 4H), δ .97– .93 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 168.7, 164.8, 159.3, 138.2, 132.9, 116.7, 52.5, 39.4, 32.3, 29.6, 27.5, 26.5, 16.9, 9.5; HRMS (ESI) calcd. for $C_{15}H_{19}NO_2$ $[M+H]^+$: 246.1416; found 246.1419.

3.5 Antifungal activity protocol

Antifungal activities of compounds **34b**, **35b** and **36b** were tested for their *in vitro* growth inhibitory activity against the pathogenic fungus, namely, *Aspergillus Niger* and *Candida Albicans* species cultured on potato dextrose agar medium (prepared by taking 11 ml of distilled water followed by the addition of following ingredients: mycological peptone (10 g), dextrose (30 g), and agar (12 g). The pH of the solution was maintained to 5.7 and boiling was continued until complete dissolution. After that, the solution was sterilized under autoclave at 15 lb pressure (120 °C for 20 min) by diffusion method and further incubated at 28 °C for 3 days. Test solutions of different concentrations (microgram per liter) were prepared in DMSO solution.

3.6 References

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3.7 Characterization spectra (^{1}H and ^{13}C NMR) of selected 2-aryl/heteroaryl/alicyclic substituted pyridine fused alicyclic molecular frameworks (22a, 24a, 25a, 34a, 35a and 36a):

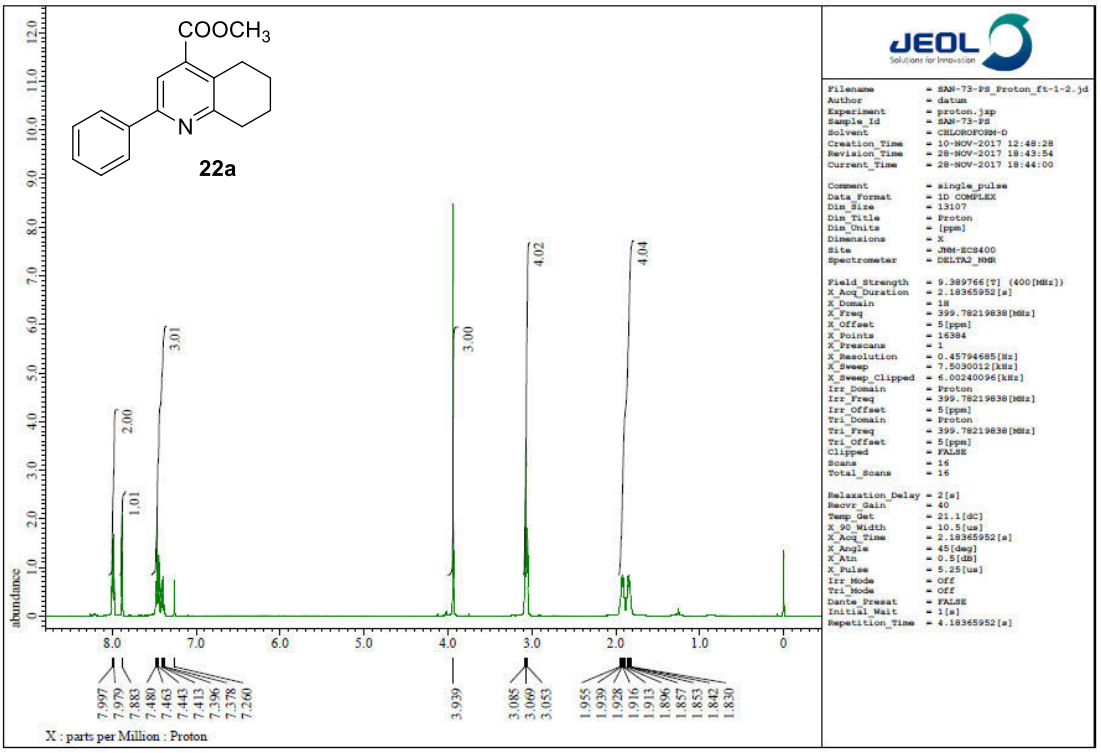


Figure 4. ^{1}H NMR Spectra of Compound **22a**.

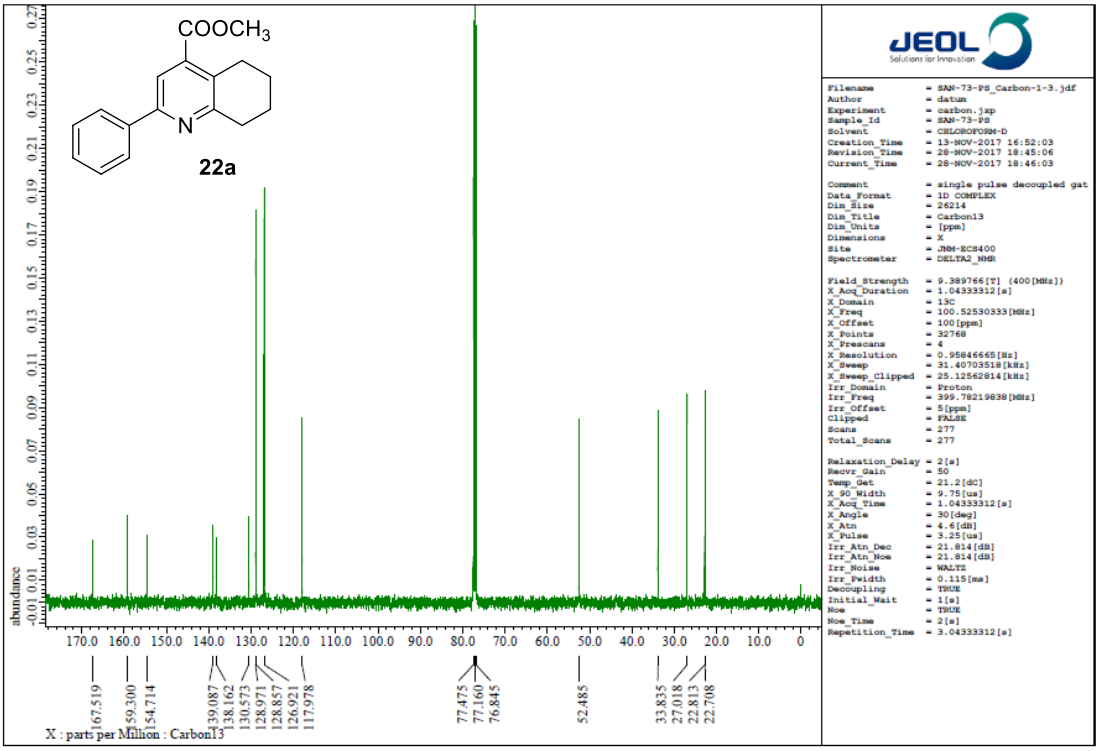


Figure 5. ^{13}C NMR Spectra of Compound **22a.**

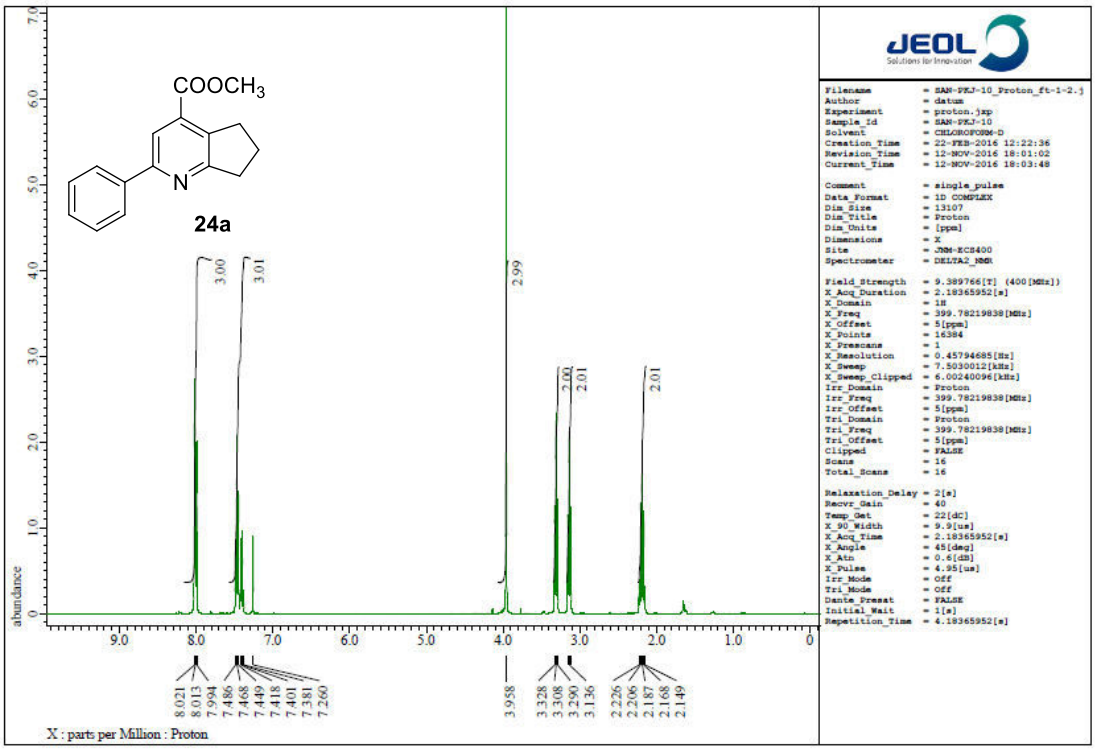


Figure 6. ^{1}H NMR Spectra of Compound **24a.**

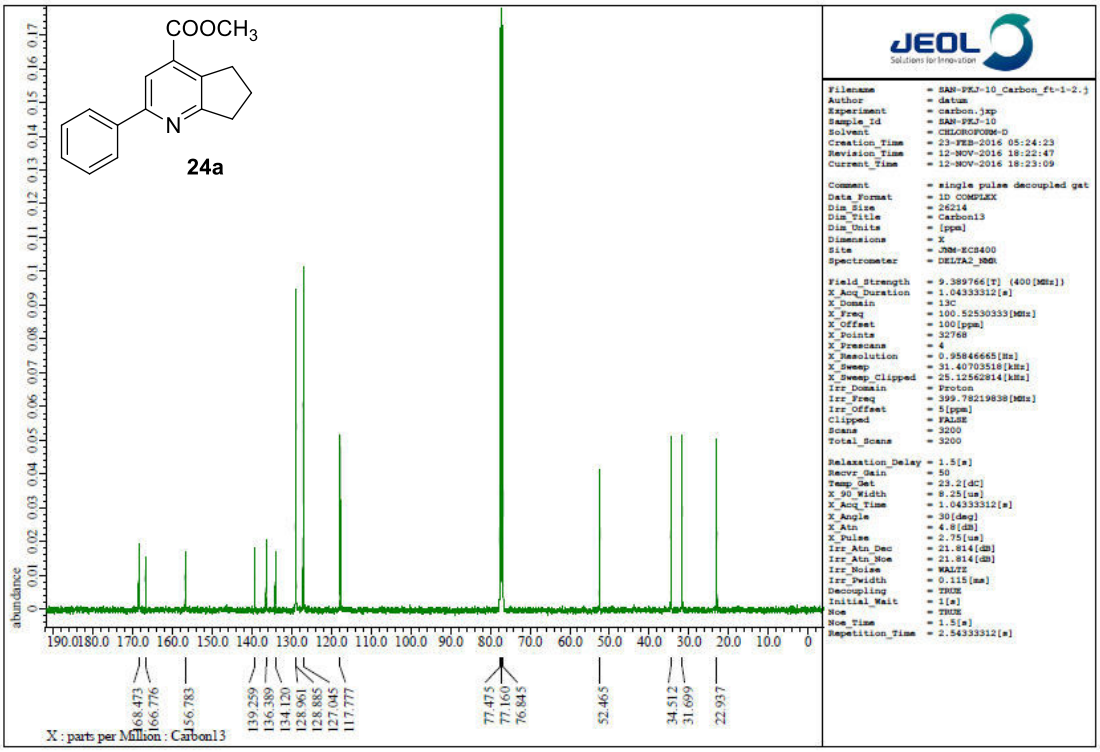


Figure 7. ^{13}C NMR Spectra of Compound **24a.**

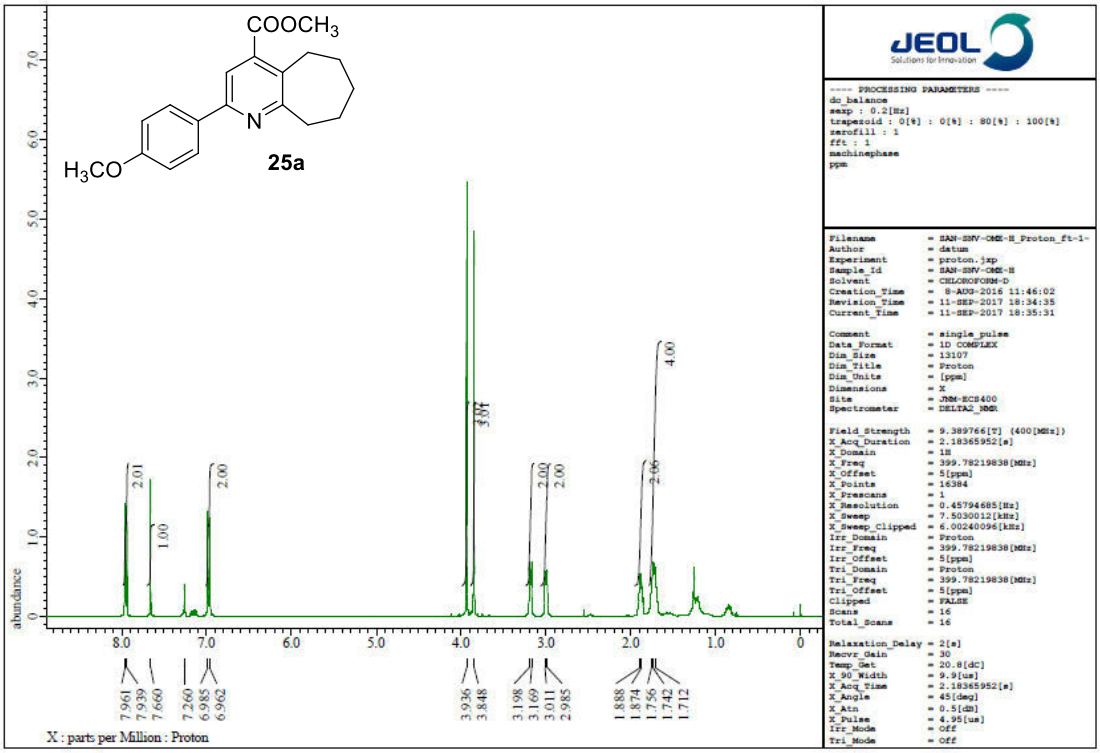


Figure 8. ^{1}H NMR Spectra of Compound **25a.**

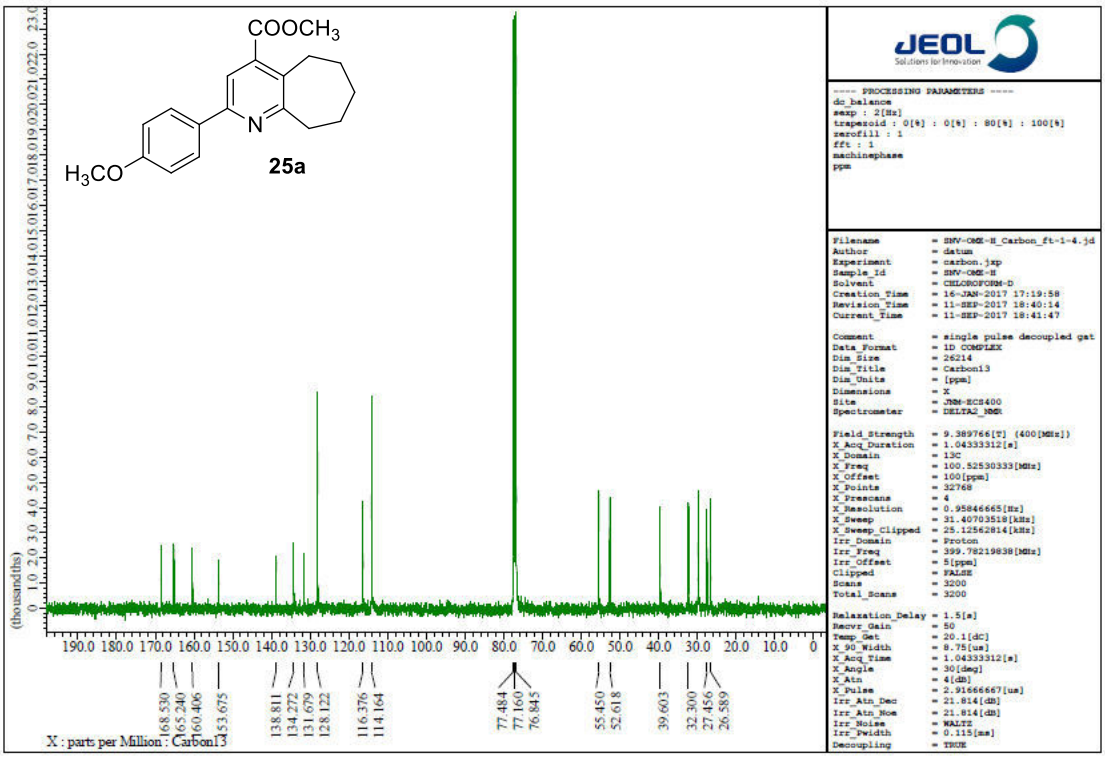


Figure 9. ^{13}C NMR Spectra of Compound **25a.**

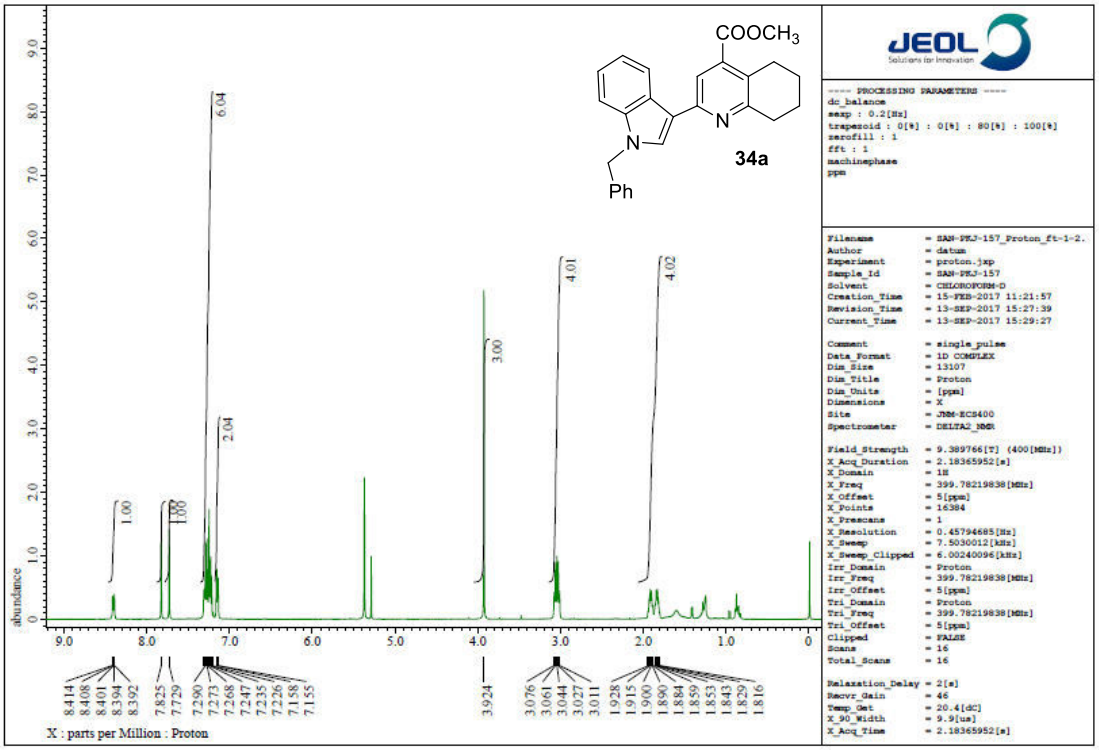


Figure 10. ^{1}H NMR Spectra of Compound **34a.**

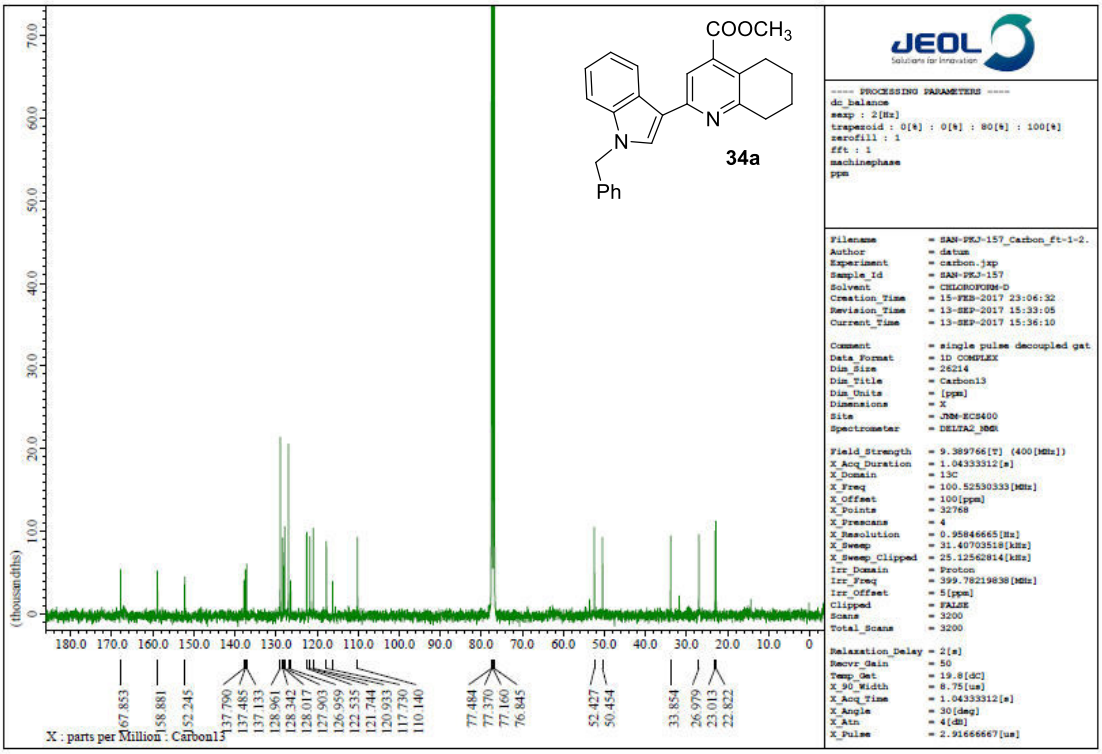


Figure 11. ^{13}C NMR Spectra of Compound **34a.**

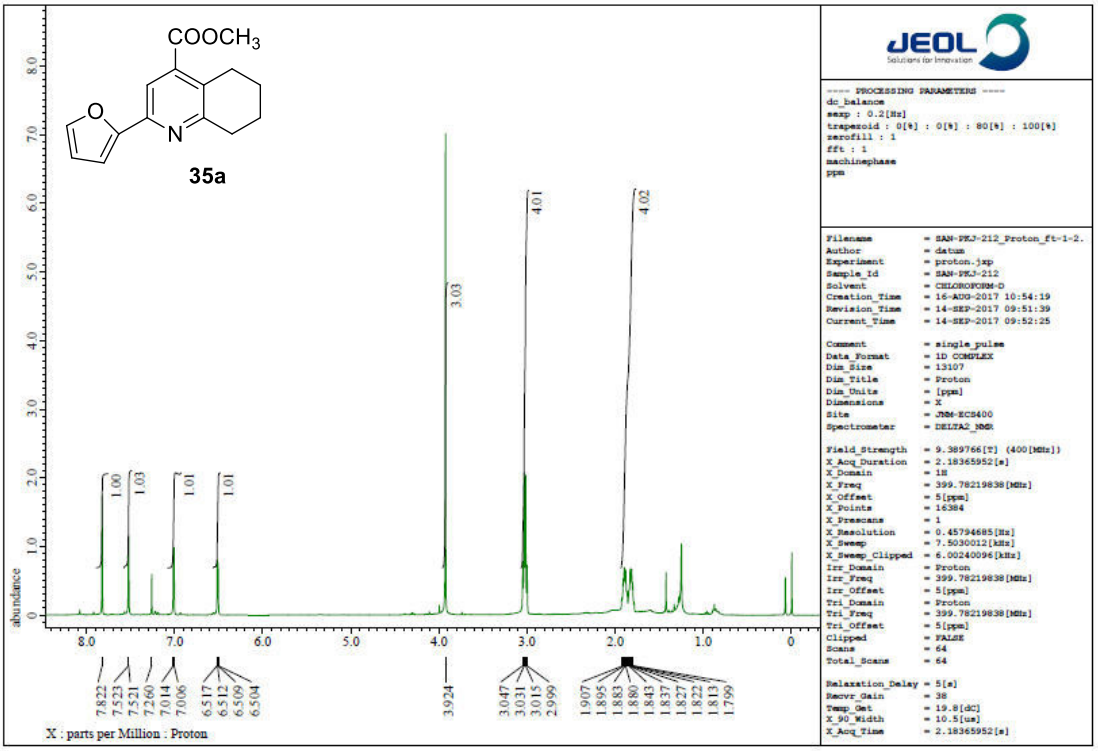


Figure 12. ^{1}H NMR Spectra of Compound **35a.**

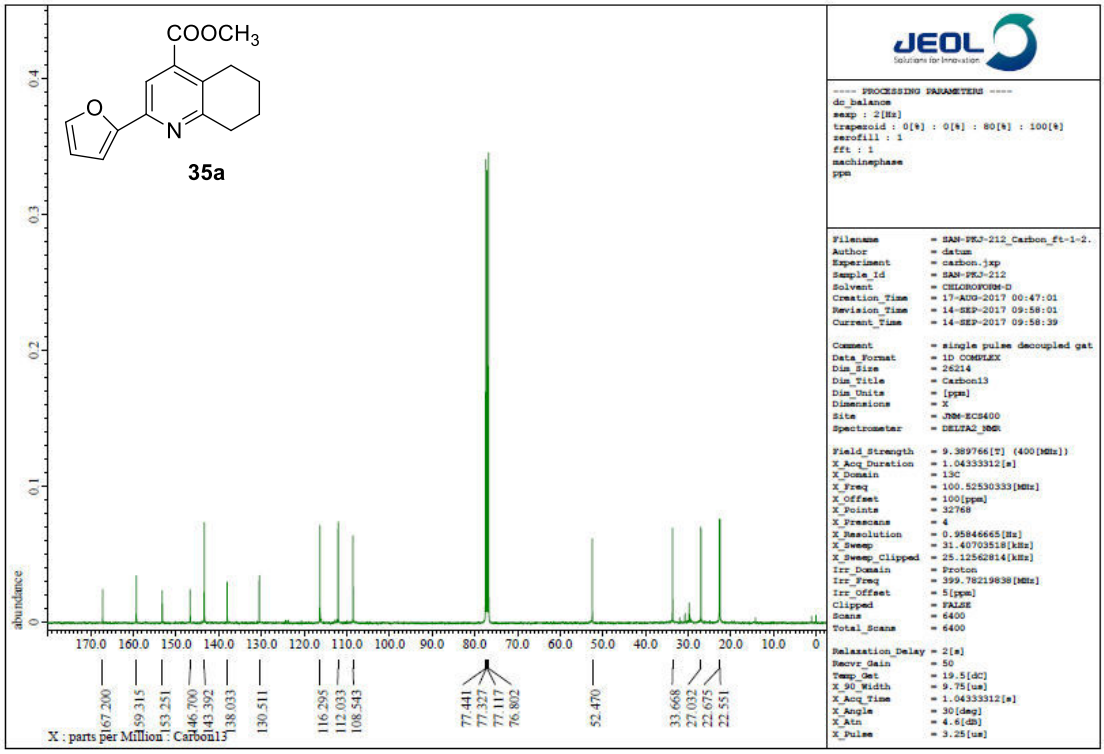


Figure 13. ^{13}C NMR Spectra of Compound **35a.**

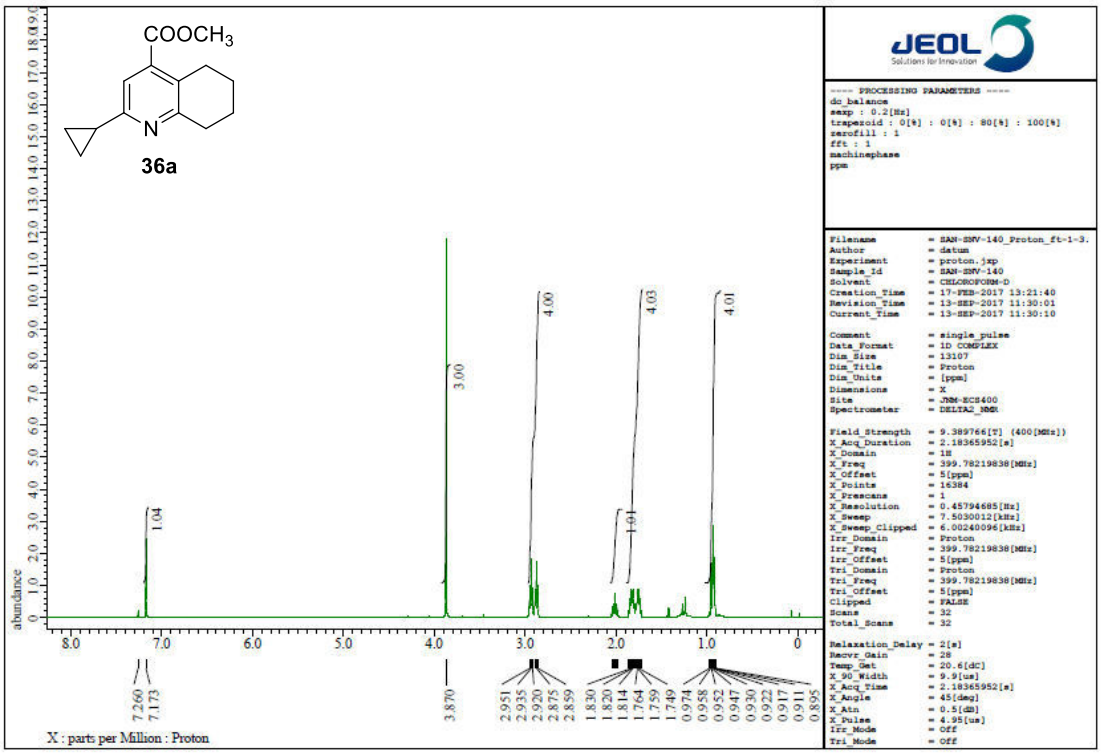


Figure 14. ^{1}H NMR Spectra of Compound **36a.**

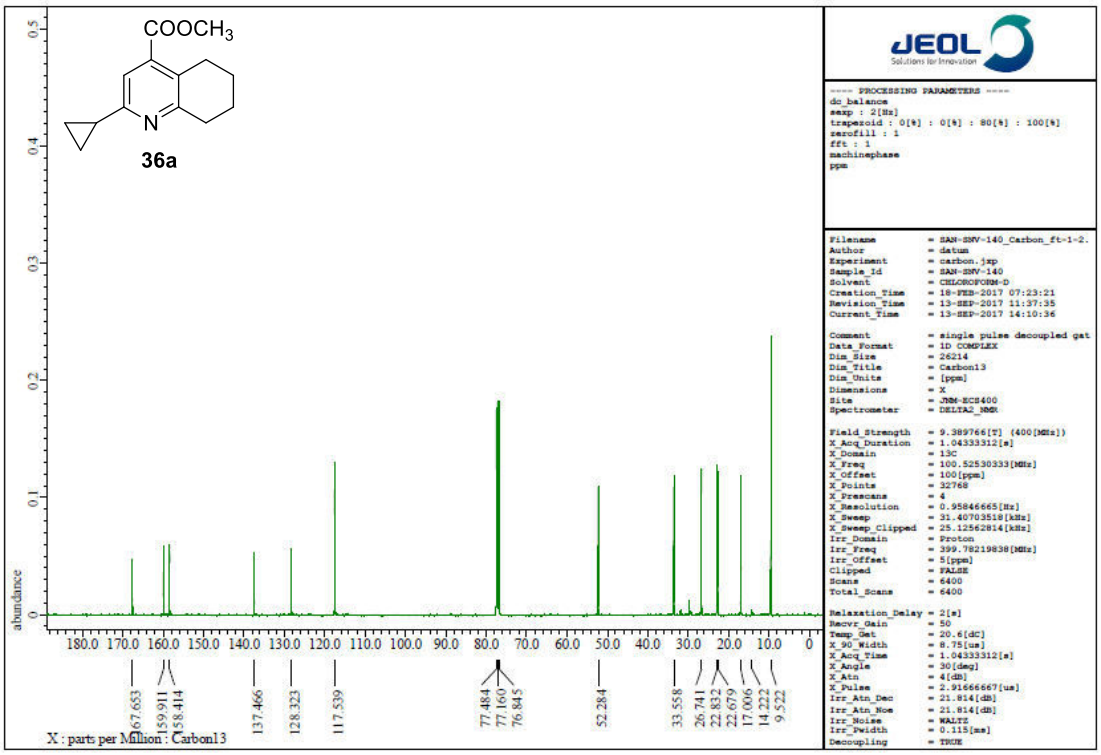


Figure 15. ^{13}C NMR Spectra of Compound **35a.**

Chapter 4

"*Novel Aporphine Alkaloids: Design, synthesis, SAR and biological evaluation*"

4.1 Introduction

Aporphine alkaloids consist of a tetracyclic framework having 1, 2, 3, 4-tetrahydroisoquinoline substructure. The basic structure of Aporphine is shown below.

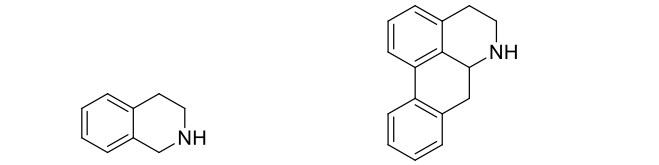


Figure 1: Basic structure of aporphine alkaloid

A large number of aporphine alkaloids have been isolated either from several plant species (such as Hernandiaceae, Lauraceae, Annonaceae, Menispermaceae, Monimiaceae etc.)[1] and many others have been synthesized also.

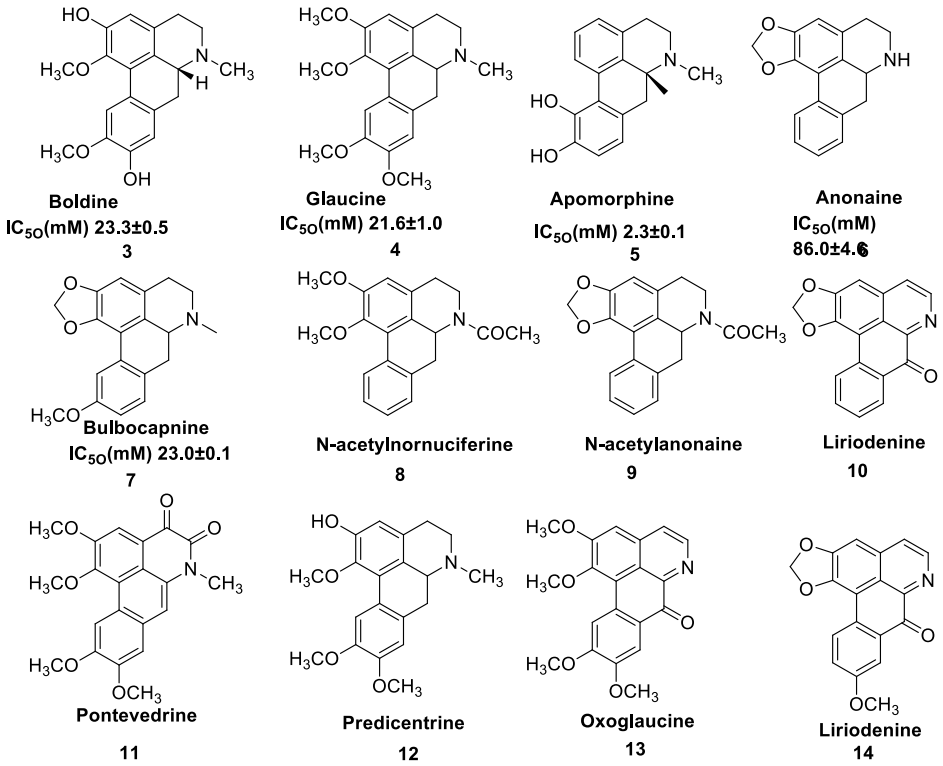


Figure 2: Aporphine alkaloid showing Antioxidant activity **3-7** and antiplatelet activity **8-14.**

Both natural as well as synthetic aporphine alkaloids displayed wide range of pharmacological activities and also serve as leads for the development of potential drug discovery scaffolds. For example, naturally/synthetic aporphines have been identified as antimicrobial,[2a] antiviral,[2b-c] acetylcholinesterase inhibitors,[2d] antimalarial,[2e-f] CNS receptor ligands,[2g-h] anti-Alzheimer[2d-i] and potent dopamine D1/D2 agonist.[2j] In addition, their characteristic tetracyclic motif with different levels

of oxidation on both aromatic rings also displayed a diverse range of interesting biological activities, including antimalarial[3a] serotonergic[3b] anticancer,[3c] and vasorelaxing activity[3d].

In our effort to develop novel bioactive molecules; several natural as well as semi synthetic analogues **1-14** were reported to show excellent antioxidant[4] as well as antiplatelet activities[5] (Figure 2).

On the other hand, several amides **15** as well as sulphonamide **16** analogues display promising antioxidant[6] (such as: cyclic amide Exifone, DIMBOA, benzo[1,3]oxazine, benzo[1,4]oxazine analogues etc.) as well as antiplatelet properties[7] (such as: Aspirin, tirofiban, sulfinpyrazone, clopidogrel etc.) (Figure 3).

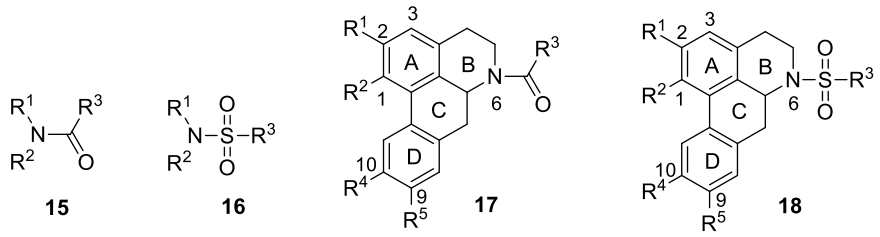


Figure 3: Structures of functionalized amide **15**, sulphonamide **16** and target prototype **17** and **18**.

Therefore, we plan to prepare the target aporphine prototypes **17** and **18,** amides as well as sulphonamides analogues of aporphine, with the anticipation that the analogues of **17** and **18** i.e. **34a-s** will show promising antioxidant as well as antiplatelet activities (Figure 3).

Till date, no systematic study of the structure–activity relationship (SAR) of synthetic aporphines as antiplatelet as well as antioxidant has been conducted. An understanding of SAR effects is a necessary aspect of any future undertaking to understand the role of different EWG (electron withdrawing group) and EDG (electron donating group) present at specific position in molecules as well as to exploit on their therapeutic potential. Herein, we present results from an SAR evaluation of the antiplatelet as well as antioxidant activity of a set of synthetic aporphine derivatives **34a-s**.

Based on the structural similarity of previously reported aporphine as a promising antiplatelet as well as antioxidant agent (Figure 2); we initially screened these compounds **34a-s** in AA-induced platelet aggregation inhibitory activity and DPPH free radical scavenging antioxidant activity assay taking ascorbic acid and aspirin as standard drug reference, respectively. To the best of our knowledge, it is the first

report of the synthesis, SAR, antiplatelet as well as antioxidant activity of novel aporphine analogues **34a-s**.

Thus, in this chapter, we have described the synthesis, bio-evaluation and SAR studies with respect to the effects of structural manipulations at C1 and C2 of ring A and N6 centres of the prototype **17** and **18** on antiplatelet as well as antioxidant activity.

4.2 Result and discussion

Chemistry

The retro-synthetic analysis for the synthesis of the novel aporphines analogues of prototype **17** and **18** is depicted in Figure 4.

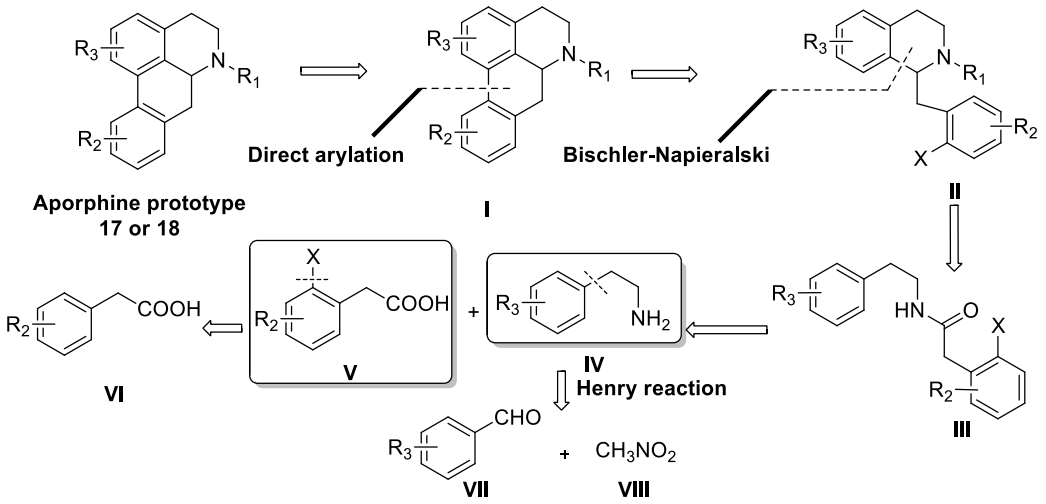
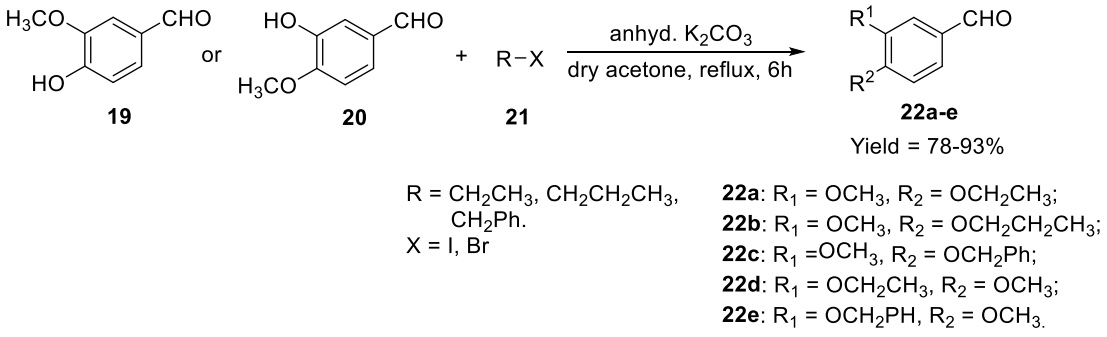


Figure 4: Retro-synthetic analysis.

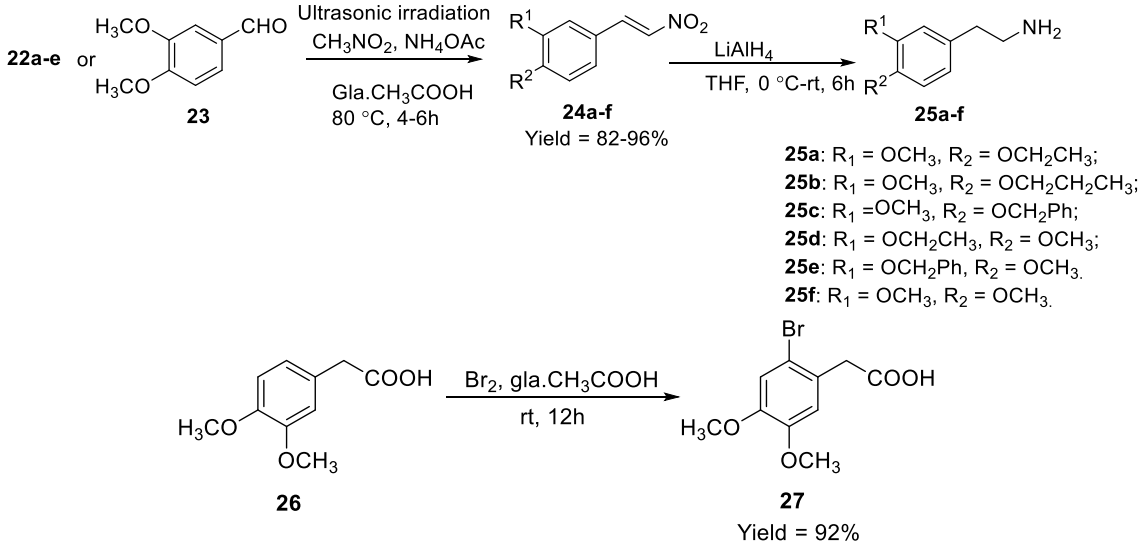
On the basis of retro-synthetic approach, the C1 and C2 of ring A and N-6 modified racemic analogues **34a-p** were prepared using reported procedures.[8] Compounds **22a-e** were prepared as outlined in Scheme 1.



Scheme 1: Synthesis of O-alkylated starting substrates **22a-e.**

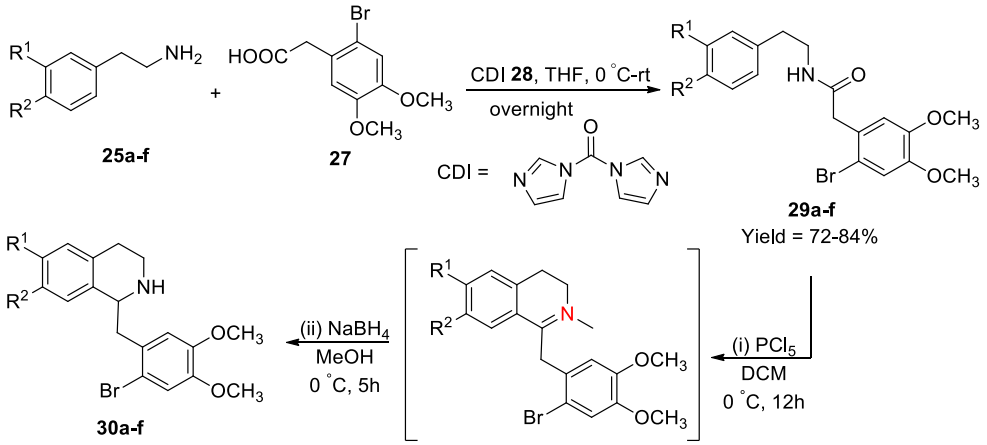
Commercially available aromatic aldehyde **19** and **20** were converted into O-alkylated nitrostyrene **24a-f** via sequential O-alkylation with respective alkyl bromides followed by nitro-aldol and dehydration reactions. The resulted O-alkylated at C1 and

C2 of ring A i.e. compounds **24a-f** converted into the key intermediate i.e. functionalized phenethylamine analogues **25a-f** after reduction. The second starting substrate **V** i.e. **27** was synthesized by the bromination of **26** (Scheme 2).



Scheme 2: Synthesis of starting substrates **25a-f** and **27.**

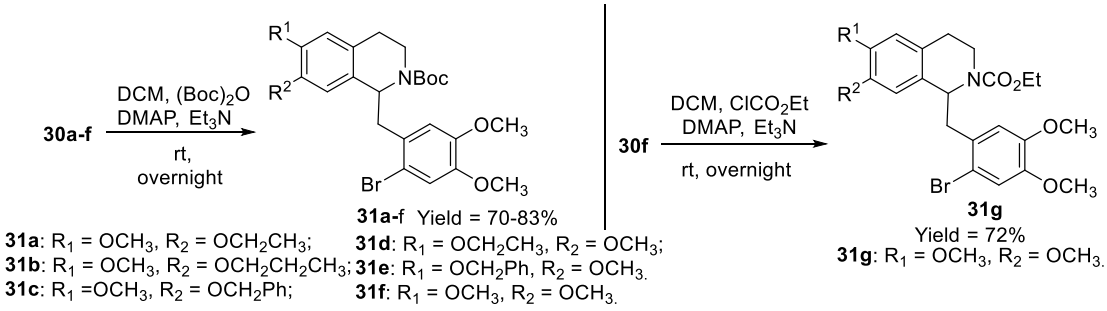
Finally, the coupling of functionalized phenethylamine analogues **25a-f** with bromomethylenedioxyphenylacetic acid **27** under standard peptide coupling conditions gave amide **29a-f**. The amide **29a-f** were subjected to Bischler–Napieralski reaction[9] which afforded a cyclized product, an *imine*, which on $NaBH_4$ reduction furnished crude *sec*-racemic amine **30a-f** (Scheme 3).



Scheme 3: Synthesis of analogues of aporphine precursor **30a-g**.

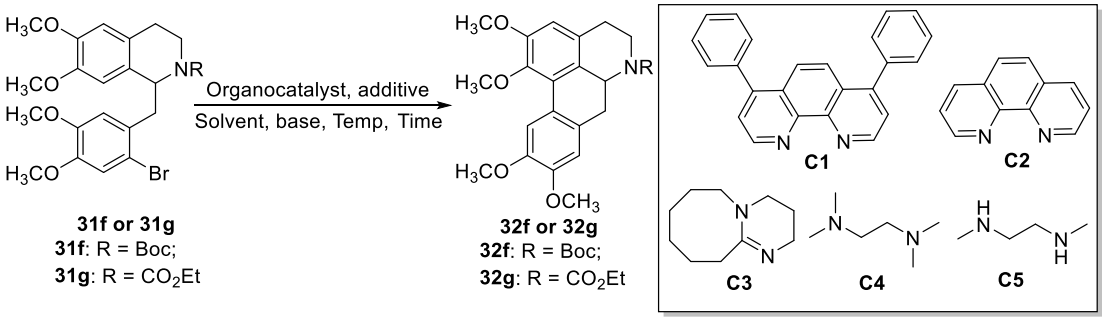
Sec-racemic amine **30a-f** on subjecting to protection with Boc anhydride and ethyl chloroformate furnished the C1 and C2 functionalized carbamates **31a-f** and **31g**, respectively (Scheme 4). It is to be noted that, there is no green protocol available in the literature to construct aporphine skeleton from its N-protected key bromo

precursor. Therefore, we decided to investigate this challenging task using organocatalysis.



Scheme 4: Synthesis of analogues of aporphine precursor **31a-g**.

Table 1. Optimization study: Synthesis of aporphine analogue **51a**.[a]



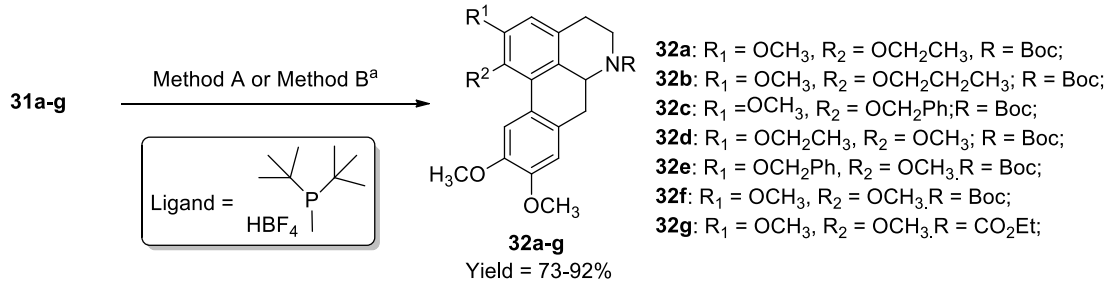
S. No.	R	Solvent	Organocatalyst (Mol %)	Additive (Mol %)	Base	Temp (°C)	Time (h)	Yield[b] (%)
1	Boc	Mesitylene	C1 (30)	---	KO^{t}Bu	135	18	0
2	Boc	Mesitylene	C1 (30)	---	KO^{t}Bu	135	36	0
3	Boc	DMSO	C1 (30)	PivOH (40)	KO^{t}Bu	135	18	<10
4	CO_2Et	DMSO	C1 (30)	PivOH (40)	KO^{t}Bu	135	18	traces
5	CO_2Et	DMSO	C2 (30)	PivOH (40)	KO^{t}Bu	125	18	traces
6	CO_2Et	DMSO	C3 (30)	PivOH (40)	KO^{t}Bu	125	18	traces
7	CO_2Et	DMSO	C4 (30)	---	KO^{t}Bu	125	18	60
8	CO_2Et	DMSO	C5 (30)	---	KO^{t}Bu	125	18	62
9	CO_2Et	DMSO	C4 (30)	PivOH (40)	KO^{t}Bu	125	18	67
10	CO_2Et	DMSO	C4 (30)	PivOH (40)	KO^{t}Bu	135	18	**74**
11	CO_2Et	DMSO	C4 (30)	PivOH (40)	KO^{t}Bu	135	24	72
12	CO_2Et	DMSO	C4 (30)	PivOH (40)	K_2CO_3	125	18	27
13	CO_2Et	DMSO	C4 (30)	PivOH (40)	KO^{t}Bu	125	36	52
14	CO_2Et	DMSO	C4 (30)	PivOH (20)	KO^{t}Bu	125	36	48
15	CO_2Et	DMSO	C4 (20)	PivOH (40)	KO^{t}Bu	125	36	45

[a]*Reaction conditions*: **31f-g** (0.3 mmol), catalysts **C1-C5** (mol %), additive (mol%), base (0.6 mmol) were dissolved in DMSO by rigorous purging with nitrogen and heated at specified temperature under N_2 atm for given time. [b]Isolated yield.

Boc-protected bromo precursor **31f** was taken as starting material to initially investigate this biaryl coupling using batho-phen as organocatalyst in the presence of potassium *tert*-butoxide in mesitylene at 135 °C for 18-36 h; unfortunately, reaction

did not occur, However, when pivalic acid was added; <10 % yield of the target aporphine **32f** was formed (entry 1-3; Table 1).

Earlier, we observed that palladium-catalysed biaryl coupling underwent in very high yields when coupling takes place with N-protection as carbamate (–COOEt) in key precursor i.e., **31g** in DMSO. Therefore, N-COOEt protected aporphine precursor i.e., **31g** was then subjected to various biaryl coupling conditions using C1-C5 organocatalyst, screening of base, temperature, time (entry 4-15, Table 1). Performing coupling reaction with organocatalyst C1-C3 furnished the desired product in traces (entry 4-6, Table 1). However, when C4 organocatalyst i.e., tetramethyl ethylenediamine (TMEDA) or Dimethyl ethylenediamine (DMEDA) were used without additive in DMSO at 125 °C for 18 h; the desired product was obtained in 60% and 62% yields, respectively (entry 7-8, Table 1). Thus, C4 organocatalyst and pivalic acid as additive was found to be the best for this biaryl coupling. Then, screening of base, temperature and time were performed and it was found that KO^{t}Bu, 135 °C temperature and 18h reaction time was found to be the best conditions as it furnished the desired product in 74% yields. To sum up, so far, tetramethyl ethylenediamine (TMEDA), pivalic acid in DMSO as solvent at 135 °C for 18h, was found to be the best optimised reaction condition for the synthesis of aporphine **32g** in 74% yield (entry 10, Table 1). The results are shown in table 1.

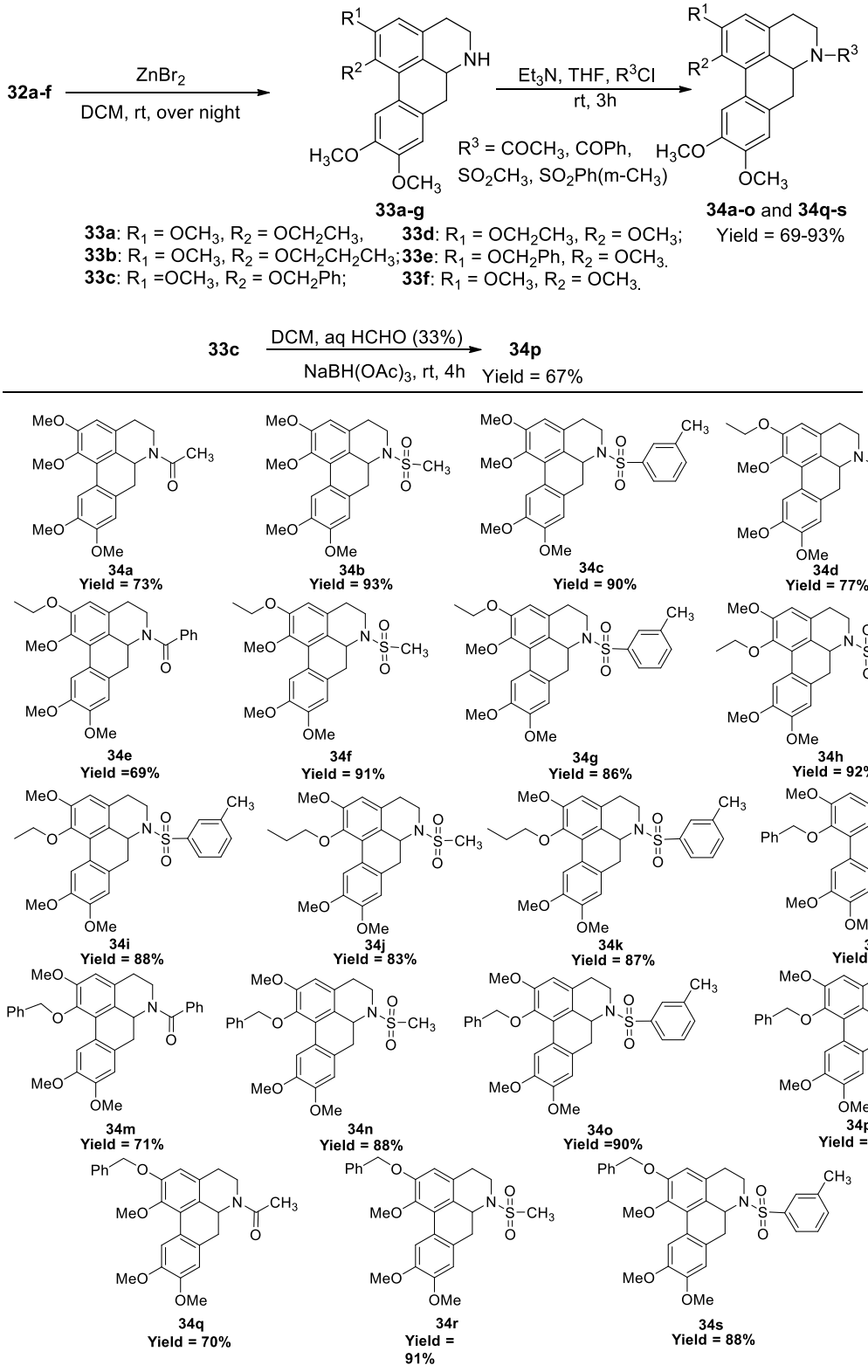


[a]Reaction conditions: Method A: $Pd(OAc)_2$, ligand, PivOH (40 mol%), K_2CO_3, DMSO, 130 °C, 18h,
Method B: TMEDA (30 mol%), PivOH (40 mol%), KOtBu, DMSO, 135 °C,18h.

Scheme 5. Synthesis of N-carbamate aporphine analogues **32a-g**.

After obtaining good yield under optimised conditions via organocatalysis; we plan to prepare aporphine analogues for their antiplatelet and antioxidant activity evaluation. We prepared N-carbamate aporphine analogues **32a-g** *via* Pd-catalyzed direct arylation strategy[10] as well as organocatalytic approach (Scheme 5). However, it was observed that, the low yields of aporphines **32a-g** were obtained (upto 74%) using organocatalysis as compared to that of using Pd-catalyzed direct arylation strategy (yield = upto 94%).

Scheme 6. Synthesis of aporphine analogues **34a-s**.



The N-carbamate (i.e. N-Boc or N-CO_2Et) aporphine analogue **32a-g** were used as the key precursor`s for the synthesis of novel aporphines analogues **34a-s** (Scheme 6). The synthesis of N-6 analogues were achieved via deprotection of the Boc/-COOEt

group[8b] with anhyd. $ZnBr_2$, subsequently, followed by N-alkylation of secondary (±)-amine with different alkyl/aryl halides (such as CH_3COCl, methanesulphonyl chloride, *m*-toluene sulphonyl chloride and C_6H_5COCl) which afforded the N-6 aporphine analogues **34a-o** and **34q-s** in racemic mixture**.**

Moreover, subsequent reductive amination of the secondary (±)-amine **33c** with formaldehyde afforded N-methylated aporphine analogue **34p** (Scheme 6). All compounds were characterized using ^1H NMR, ^{13}C NMR, FT-IR and HRMS spectroscopic analysis data. It is to be noted that, the NMR spectral data of N-acetyl analogues showed mixture of rotamers (see experimental section).

4.3 Pharmacological screening

Naturally occuring isolated aporphines have been reported in the literature to show excellent antioxidant and antiplatelet activities.[4-5] Thus, all the synthesized novel aporphines **34a-s** were evaluated for their AA-induced platelet aggregation inhibitory activity as well as DPPH radical scavanging antioxidant activity. Aspirin and ascorbic acid were taken as standard reference for antiplatelet and DPPH assay, respectively.

4.3.1 *In vitro* AA-induced platelet aggregation inhibitory activity and structure-activity relationship studies

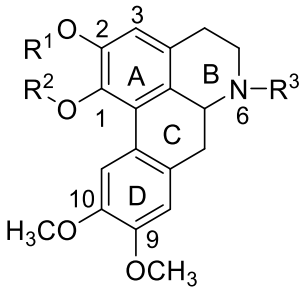
New aporphine analogues **34a-s** were prepared having structural modifications at C1/C2 of ring A and N-6 positions of ring B and evaluated initially for their arachidonic acid (AA) induced-antiplatelet aggregation inhibitory activities. The results are shown in Table 2.

As depicted from Table 2, initially, three compounds **34a-c** were prepared having OCH_3 substitutions at C1 and C2 of ring A and N-$COCH_3$, N-SO_2CH_3 and N- SO_2Ph (m-CH_3) substitutions, respectively; out of which compound **34a** (IC_{50} = **20.08±0.0.22** µg/mL) displayed excellent AA-induced platelet aggregation inhibitory activity more than aspirin (IC_{50} = 21.34±1.09 µg/mL; entry 1, Table 2). Changing from N-$COCH_3$ to N-SO_2CH_3 and N- SO_2Ph (*m*-CH_3) further decreases the activity (entries 2-3, Table 2). Then, higher homologues of **34a-c** i.e., **34d** (IC_{50} = 29.78±0.31 µg/mL), **34f-g** having O-ethyl group at C2 positions were assessed and we observed a similar decrease in antiplatelet activity when N-substitution changes from acetyl to either sulphonyl or *m*-methylsulphonyl group (entries 4 and 6-7, Table 2). While the N-benzoyl analogue **34e** (IC_{50} = 25.83±0.26 µg/mL) displayed better antiplatelet activity than **34d and 34f-g**; it showed comparable antiplatelet activity to aspirin (entry 5,

Table 2). Comparing **34a** and **34d** (IC_{50} = 29.78±0.31 µg/mL) having N-acetyl group; further homologation in ring A at C2 position decreases activity.

Compounds **34h** (IC_{50} = 101.83±1.08 µg/mL), **34i** (IC_{50} = 33.37±0.34 µg/mL), **34j** (IC_{50} = 37.71±0.38 µg/mL) and **34k** (IC_{50} = 40.00±0.48 µg/mL) having N-sulphonyl substitution and O-ethyl/propyl group at C2 position drastically decreases the platelet aggregation inhibitory activity when compared with parent analog having N-acetyl group **34a** (entry 8-11, Table 2).

Table 2. *In vitro* AA-induced platelet aggregation inhibitory activity of synthesized novel aporphine analogues (**34a-s)**.



34a-s

S. No.	Compound No.	R^1	R^2	R^3	AA-induced antiplatelet activity[a,b] (IC_{50} in µg/ml)
1	**34a**	CH_3	CH_3	$COCH_3$	**20.08±0.0.22**
2	34b	CH_3	CH_3	SO_2CH_3	31.19±0.29
3	34c	CH_3	CH_3	$SO_2Ph(m\text{-}CH_3)$	57.29±0.74
4	34d	CH_2CH_3	CH_3	$COCH_3$	29.78±0.31
5	34e	CH_2CH_3	CH_3	COPh	25.83±0.26
6	34f	CH_2CH_3	CH_3	SO_2CH_3	44.97±0.67
7	34g	CH_2CH_3	CH_3	$SO_2Ph(m\text{-}CH_3)$	103.64±1.01
8	34h	CH_3	CH_2CH_3	SO_2CH_3	101.83±1.08
9	34i	CH_3	CH_2CH_3	$SO_2Ph(m\text{-}CH_3)$	33.37±0.34
10	34j	CH_3	$CH_2CH_2CH_3$	SO_2CH_3	37.71±0.38
11	34k	CH_3	$CH_2CH_2CH_3$	$SO_2Ph(m\text{-}CH_3)$	40.00±0.48
12	34l	CH_3	CH_2Ph	$COCH_3$	28.81±0.37
13	34m	CH_3	CH_2Ph	COPh	58.13±0.77
14	34n	CH_3	CH_2Ph	SO_2CH_3	71.88±0.0.80
15	34o	CH_3	CH_2Ph	$SO_2Ph(m\text{-}CH_3)$	78.67±0.88
16	34p	CH_3	CH_2Ph	CH_3	**27.83±0.29**
17	**34q**	CH_2Ph	CH_3	$COCH_3$	**21.29±0.25**
18	34r	CH_2Ph	CH_3	SO_2CH_3	41.97±0.44
19	34s	CH_2Ph	CH_3	$SO_2Ph(m\text{-}CH_3)$	96.02±0.0.97
20	Aspirin	---	---	---	**21.34±1.09**

[a]Platelets were incubated along with either a tested compound or 0.5% DMSO at 37°C for 60 sec., then AA (100 µM) was added to accelerate the aggregation. Aspirin and indomethacin are positive controls. Values are expressed as mean_SE from three to three separations. [b]The data represent mean of three independent determination.

Since we do not observe noticable improvement in activity with increase in homologation in alkyl groups; we plan to introduce aromotic moieties at both C1 and C2 positions. So, we prepared **34l-p** having C1 O-benzyl group and **34q-s** having C2 O-benzyl group having N-acetyl, N-benzoyl, N-sulphonyl, N-(*m*-CH_3)sulphonyl and N-CH3 groups. As expected, while **34l** showed better AA-induced platelet aggregation inhibitory activity as compared to **34m-o**; **34p** having N-CH_3 group, the reduced form of **34l**, also have shown promising IC_{50} value of 27.83±0.29 μg/mL in comparison to the aspirin (entries 12-16, Table 2).

Thus it can be inferred that substituting N-acetyl group with N-benzoyl group do not have beneficial effect on antiplatelet activity. Analogue **34m** (IC_{50} = 58.13±0.77 μg/mL), having two phenyl moieties, exhibited lower activity than its acetyl analogue **34l**. Aporphine analogues **34q-s**, having O-benzyl group at C2 position and N-acetyl group, also follows the same trend i.e. **34q** (IC_{50} = 21.29±0.25 μg/mL) was found to be equally active to aspirin and more active than their N-sulphonyl counterparts (entries 17-18, Table 2).

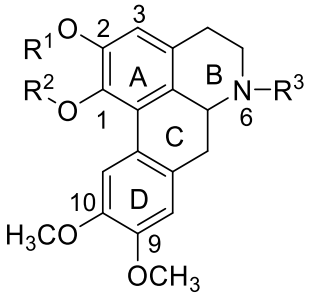
Overall, based on above SAR study, two compounds i.e. **34a** and **34q**, having OCH_3 or OCH_2Ph substitution at C1 as well as OCH_3 substitution at C2 position on ring A along with N-acetyl group, were found to be the most active compounds of the series and displayed greater antiplatelet activity in comparison to aspirin. It can be interpreted that N-acetyl protection on aporphines (as in compound **34a, 34d, 34l** and **34q**), enhances the platelet aggregation inhibitory activity in comparison to the N-sulphonamide analogues. However, the position of alkoxy substituent at C1 and C2 on ring A also efficiently responsible for the antiplatelet activity. Similarly, the N-methyl group on aporphine analogue (**34p**) further reduces the antiplatelet activity.

4.3.2 *In vitro* DPPH radical scavenging antioxidant activity and structure-activity relationship studies

DPPH is found as a stable free radical, which can easily convert into stable molecule after the acceptance of an electron or hydrogen radical. It is well documented that, the DPPH radical scavenging antioxidant activity assay works *via* a single electron transfer (SET) or a hydrogen atom transfer (HAT) mechanism.[11] DPPH molecule shows a strong absorption band at 515 nm in MeOH solution having the deep purple colour having odd electron configuration. The deep purple colour of MeOH medium containing DPPH changes to yellow in the presence of free radical scavengers.[11c-d]

The quenching of DPPH free radical is correlated with the structural architecture of the molecules, in which the steric hindrance, rigidity and the electron density is the key factors which may facilitate the molecule to reaching at the radical site of DPPH and thus enhances the antioxidant activity.[11e]

Table 3. *In vitro* DPPH radical scavenging antioxidant activity of synthesized novel aporphine analogues (**34a-s**)



34a-s

S. No.	Compound No.	R^1	R^2	R^3	Antioxidant Activity[a,b] (IC_{50} in µg/ml)
1	34a	CH_3	CH_3	$COCH_3$	70.69±0.68
2	**34b**	CH_3	CH_3	SO_2CH_3	**10.18±0.10**
3	34c	CH_3	CH_3	$SO_2Ph(m\text{-}CH_3)$	21.97±0.22
4	34d	CH_2CH_3	CH_3	$COCH_3$	30.08±0.34
5	34e	CH_2CH_3	CH_3	COPh	88.36±0.79
6	**34f**	CH_2CH_3	CH_3	SO_2CH_3	**5.87±0.07**
7	34g	CH_2CH_3	CH_3	$SO_2Ph(m\text{-}CH_3)$	103.11±1.01
8	**34h**	CH_3	CH_2CH_3	SO_2CH_3	**7.08±0.10**
9	34i	CH_3	CH_2CH_3	$SO_2Ph(m\text{-}CH_3)$	96.13±0.74
10	**34j**	CH_3	$CH_2CH_2CH_3$	SO_2CH_3	**5.13±0.07**
11	34k	CH_3	$CH_2CH_2CH_3$	$SO_2Ph(m\text{-}CH_3)$	29.31±0.30
12	34l	CH_3	CH_2Ph	$COCH_3$	**11.71±0.14**
13	34m	CH_3	CH_2Ph	COPh	23.14±0.29
14	**34n**	CH_3	CH_2Ph	SO_2CH_3	**4.36±0.09**
15	34o	CH_3	CH_2Ph	$SO_2Ph(m\text{-}CH_3)$	44.87±0.36
16	34p	CH_3	CH_2Ph	CH_3	19.63±0.22
17	34q	CH_2Ph	CH_3	$COCH_3$	41.58±0.39
18	34r	CH_2Ph	CH_3	SO_2CH_3	14.53±0.31
19	34s	CH_2Ph	CH_3	$SO_2Ph(m\text{-}CH_3)$	50.08±0.45
20	Ascorbic acid	---	---	---	4.57

[a]Results are expressed as a mean ± standard deviation (n = 3). [b]DPPH radical scavenging activities are expressed as IC_{50} concentrations of the compounds (µg/mL) required to inhibit 50% of the radicals and the maximum inhibition values and Positive control for DPPH assay = Ascorbic acid.

Initially, *in vitro* antioxidant screening of novel aporphine analogues **34a-c**, having OCH_3 substituents at C1/C2 position on ring A along with N-acetyl/sulphonyl/m-methylsulphonyl at N6 position, were assessed and it was observed that **34a (**IC_{50} value of 70.69±0.68 µg/mL), having OCH_3 substituent on both C1/C2 positions along

with N-acetyl group displayed lesser potency than ascorbic acid (IC_{50} = 4.57 µg/mL) (entry 1, Table 3). However, **34b** (IC_{50} = **10.18±0.10** µg/mL) having N-sulphonyl group significantly enhances the antioxidant activity compared to **34a** (entry 2, Table 3). Changing to N-sulphonylaryl group i.e. compound **34c** (IC_{50} = 21.97±0.22 µg/mL), further decreases the antioxidant activity (entry 3, Table 3).

Similarly, similar trends were observed when we analyzed **34d-k** having O-ethyl/propyl groups at C1/C2 position along with N-acetyl/benzoyl/sulphonylmethyl /sulphonylaryl groups at N6 position (entry 4-11, Table 3). While analogs **34f** (IC_{50} = **5.87±0.07** µg/mL), **34h** (IC_{50} = **7.08±0.10** µg/mL) and **34j** (IC_{50} = **5.13±0.07** µg/mL), displayed excellent antioxidant potency comparable to standard drug (entry 6, 8 and 10, Table 3); other analogues display poor potency as in compounds **34d** (IC_{50} = 30.08±0.34 µg/mL), **34e** (IC_{50} = 88.36±0.79 µg/mL), **34g** (IC_{50} = 103.11±1.01 µg/mL), **34i** (IC_{50} = 96.13±0.74 µg/mL) and **34k** (IC_{50} = 29.31±0.30 µg/mL) (entry 4-5, 7, 9 and 11, Table 3).

Then, we were also interested to study the effect of aromatic ring at C1/C2 position of ring A. Therefore, we prepared **34l-s** aporphine analogues having N-acetyl/benzoyl/sulphonylmethyl /sulphonylaryl groups at N6 position (entry 12-19, Table 3). Compound **34l** (IC_{50} = 11.71±0.14 µg/mL), having O-benzyl group at C2 position and N-acetyl group showed promising antioxidant activity. However, changing N-$COCH_3$ to N-SO_2CH_3 showed profound effect on antioxidant activity as observed in the case of **34n** (IC_{50} = 4.36±0.09 µg/mL), which showed activity more than ascorbic acid (IC_{50} = 4.57 µg/mL). Infact, **34n** was found to be the best compound of the series. The activity get further diminishes when N-Benzoyl or N-sulphonylaryl group were introduced at N6 position (Compound **34m**; IC_{50} = 23.14±0.29 µg/mL and **34o**; IC_{50} = 44.87±0.36 µg/mL; entries 13 and 15, Table 3). In addition, the tertiary amine analogue i.e. N-methyl aporphine **34p** (IC_{50} = 19.63±0.22 µg/mL), exhibited lesser antioxidant activity (entry 16, Table 3). We do not observe an incremental effect on activity when we reverse the position of aromatic substitution at C1/C2 position i.e., **34q-s** (entry 17-19, Table 3). However, **34r** (IC_{50} = 14.53±0.31 µg/mL) having N-SO_2CH_3 group showed promising antioxidant activity as compared to **34q** (IC_{50} = 41.58±0.39 µg/mL) and **34s** (IC_{50} = 50.08±0.45 µg/mL).

Based on above results; it was observed that the N-sulphonylmethyl substitution in aporphine analogues (**34b**, **34f**, **34h**, **34j** and **34n)** plays a crucial role in antioxidant activity and were found to be the most active compounds of the series. Other analogs

having N-substitution such as N-sulphonylaryl group (**34c**, **34g**, **34i**, **34k**, **34o** and **34s**) and N-acetyl/benzoyl aporphine analogues (**34a**, **34d**, **34e**, **34l**, **34m** and **34q)** displayed lesser antioxidant activity. Hence, it can be speculated that due to larger electronegativity of sulphur atom in N-sulphonylmethyl containing aporphine analogues, which accumulates the electron density (the N-sulphonylaryl aporphines have lower electron density due to the presence of electron-withdrawing aryl substituent in spite of electron-donating methyl substituent), restricts the delocalization of bonds due to which, the free electrons are not easily available for quenching of DPPH radical which might be the plausible reason for good antioxidant activity.

4.4 Conclusion

In this chapter, we disclose the identification of new aporphines as a potent antiplatelet as well as antioxidant agent via structural manipulation of the ring A and N6 position of aporphine skeleton. The results of our SAR study suggest that C1 and C2 alkoxy substituents along with N6 substituents may be modified to develop potent antiplatelet and antioxidant agents. In the case of AA-induced platelet aggregation inhibitory activity; aporphine analogues having N-acetyl group along with OCH_3/OCH_2Ph at C1 and OCH_3 at C2 position i.e., **34a** and **34q**, were found to be the best compounds of the series. However, in the case of antioxidant activity, aporphine analogues having N-sulphonylmethyl group along with OCH_3/OC_2H_5 at C1 and OCH_3/OC_3H_7/OCH_2Ph at C2 i.e., **34b**, **34f**, **34h**, **34j** and **34n** were found to be the best compounds of the series.. It is clear that the aporphine scaffold is sensitive to structural modifications and thus this template is amenable for the development of potent antiplatelet as well as antioxidant agents. Our result shows that the substitution at N6 position plays a very important role in antiplatelet as well as antioxidant activity. Interestingly, it is possible via structural modifications to synthesize potent aporphines showing both activities as displayed by one of the aporphine analogue **34l** in our study. Succinctly, these lead compounds were considered worthy of further structural optimization and development as potential antiplatelet as well as antioxidant agents.

4.5 Experimental Details & Characterization Data

4.5.1 General experimental

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 $CDCl_3$, 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 Q Tof (Waters, USA) Spectrometer. Microwave reactor (CEM Discover) was used for operation of reactions. Column chromatography was performed over Merck silica gel (particle size: 60-120 Mesh and 230-400 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.

4.5.2 General procedure for the Synthesis of starting substrate:

4.5.2.1 General procedure for the synthesis of O-alkylated aldehyde 22a-e

To a stirred solution of functionalized aldehydes **21a-e** (1 eq.) in acetone (20 mL) was added solid K_2CO_3 (2 eq.) and potassium iodide (2 eq.) at room temperature. The reaction mixture was stirred for 5 min and then alkyl halide i.e. RX (R = CH_3, C_2H_5, C_3H_7, CH_2Ph and X = I or Br; 1.5 equiv.) was added and refluxed for 7 h. The solvent was evaporated and the resulting solid dissolved in water and extracted with DCM (3 × 20 mL). The organic layer were combined and dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The crude products were purified by column chromatography using EtOAc/hexanes (10:90, v/v) as an eluent, which furnished the desired O-alkylated aldehydes **22a-e** in good yields (78-93%). The spectral data were well matched with reported data.

4.5.2.2 General procedure for the synthesis of functionalized nitrostyrenes 24a-f:

A mixture of O-alkylated aldehydes **22a-e** (10.0 mmol, 1eq.), ammonium acetate (10.0 mmol, 1eq.), nitromethane (50.0 mmol, 5eq.), and glacial AcOH (60 mL) was irradiated under ultrasonic irradiation at 50 °C for 3h. After cooling to room temperature, the product was filtered and recrystallized from EtOH, which afforded the functionalized nitrostyrenes analogue **24a-f** as a yellow solid in excellent yields (82-96%). The spectral data were well matched with reported data.

4.5.2.3 General procedure for the synthesis functionalized phenethylamine analogues 25a-f:

$LiAlH_4$ (10.6 mmol, 1.6 eq.) was added in anhyd THF (20 mL) under N_2-atmosphereand stirred at room temperature. Then, over a period of 5 min, a solution of nitrostyrene (10.0 mmol, 1 eq.) in anhyd THF (15 mL) was added and stirring of reaction mixture were continued for overnight at same temperature. The reaction mixture was quenched carefully with methanol (1 mL) at 0 °C. The solvent was removed under reduced pressure and the resulting crude residue was dissolved in 20% aq. KOH (15 mL), and extracted with DCM (3 × 20 mL). The combined extracts were dried on anhyd. Na_2SO_4 and then concentrated under reduced pressure which afforded the crude phenethylamine analogues **25a-f,** which was subsequently utilised in next step without further purification.

4.5.2.4 General procedure for the synthesis of 2-(2-bromophenyl)-N-phenethylacetamide analogues 29a-f:

A solution of 2-(2-bromo-4,5-dimethoxyphenyl)acetic acid **27**(10.0 mmol, 1 eq.) and 1,10-carbonyldiimidazole **28** (10.0 mmol, 1eq) in anhydrous THF (25 mL) was stirred at room temperature for 1h. The mixture was then cooled at 0 °C and phenethylamine analogues **25a-f** (10.0 mmol, 1 eq.) were added and the reaction mixture were stirred for 4 h at 0 °C and left to stir overnight at room temperature. The reaction mixture was evaporated under reduced pressure and the obtained residue was dissolved in EtOAc (20 mL) and washed sequentially with 1 N HCl (15 mL), water (2 × 25 mL), satd. $NaHCO_3$ solution (2 × 15 mL) and finally with brine (30 mL). The organic layer was dried over anhyd. Na_2SO_4 and evaporated under reduced pressure. The resultant crude product were purified by recrystalization using EtOAc/hexane (v/v = 20:80) or by flash column chromatography technique over silica gel (using 9:1 hexane/ethyl acetate as an eluent), which furnished the 2-(2-bromophenyl)-N-phenethylacetamide analogues **29a-f** in 72-84 % yields range.

4.5.2.5 General procedure for the synthesis of 1-(2-bromo-4,5-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline analogues 30a-f

To a stirred ice-cooled solution of 2-(2-bromophenyl)-N-phenethylacetamide analogues **29a-f** (5.00 mmol, 1eq.) in dry DCM (20 mL) was added solid PCl_5 (10.0 mmol, 2 eq.) in portions over 10 min. The reaction mixture was further stirred at 0 °C for 1 h and then left to stir at room temperature for 12h. The reaction mixture was poured onto a saturated solution of aq $NaHCO_3$ (30 mL) and stirred for 1h. The aqueous layer was extracted with DCM (3 × 20 mL) and the combined organic phase was sequentially washed with saturated $NaHCO_3$ solution (2 × 20 mL) and brine (30

mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure. The obtained crude imine was immediately taken for next step.

To a stirred ice-cooled solution of the crude imine i.e. dihydroisoquinoline (4.00 mmol, 1.0 eq.) in MeOH (15 mL) at 0°C, was added slowly sodium borohydride (5.20 mmol, 1.3 eq.) in three portions over 10 min. The reaction mixture was stirred at 0 °C for 1h and then, at room temperature for 2h. The reaction mixture was further cooled to 0°C, diluted with water (5 mL) and extracted with DCM (3 × 15 mL). The combined organic layer was washed with brine (25 mL), dried over anhyd. Na_2SO_4, and concentrated in reduced pressure, which afforded the crude product. The crude oily amine product **30a-f** were immediately utilised for the next step without further purification (Caution: *Do not store compound **30a-f** for long time as it was noticed that the **30a-f** gets decomposed if stored for more than 24h*).

4.5.2.6 General procedure for the synthesis of N-Boc-protected 1-(2-bromo-4,5-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline analogues 31a-f

To a stirred solution of crude oily amine **30a-f** (3.00 mmol, 1 eq.) in DCM (15 mL) was added DIPEA (6.00 mmol, 2 eq.), a catalytic amount of DMAP (0.01 g) followed by Boc-anhydride (3.6 mmol, 1.2 eq.) at room temperature and the resulting reaction mixture was stirred for 18 h. The reaction mixture was then quenched by aq NH_4Cl (2 mL) and extracted with DCM (3 × 10 mL) and water (20 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure. The resultant crude product was purified by flash column chromatography on deactivated 100-200 mesh silica gel using 100% EtOAc as an eluant, which furnished Boc-protected 1-(2-bromo-3,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline analogues **31a-f** as a white crystalline solid in good yields (70-83%).

4.5.2.7 Synthesis of ethyl 1-(2-bromo-4,5-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate 31g

The crude oily amine **30f** (3.00 mmol, 1 eq.) in THF (15 mL), ethyl chloroformate (3.30 mmol, 1.1 eq.) and triethylamine (7.50 mmol, 2.5 eq.) were added at room temperature and stirred for 18h. The progress of the reaction was checked by TLC. The removal of excess triethylamine and solvent under reduced pressure afforded a dark brown oily crude product, which was purified by flash column chromatography using MeOH/ DCM (1:99, v/v) as an eluent furnished the pure ethyl 1-(2-bromo-4,5-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate **31g** (72% from **29f**).

4.5.3.1 General procedure for the synthesis of N-Boc/ethylcarbamate protected 1,2,9,10-tetramethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline analogues 32f or 32g via organocatalytic coupling approach (optimization study: Table 1):

To a solution of the compound **31f** or **31g** (0.3 mmol; 1.5 eq.) in DMSO (10 mL) was added specified organocatalysts (30 mol%, entry 2-10; Table 1), additives (20 to 40 mol% entry 2-10; Table 1) by rigorous purging with nitrogen and the reaction mixture was heated at specified time (as given in table 1) temperature for specified time under N_2 atmosphere. The progress of the reaction was checked by TLC using 100% ethyl acetate as an eluent. The reaction mixture was then quenched with distilled water (0.2 ml) and extracted with EtOAc (3 × 10 mL) and water (10 mL). The organic layers were combined, dried over anhydrous Na_2SO_4 and removed under reduced pressure to give the crude product. The crude product were purified by using flash column chromatography deactivated silica gel column (100-200 mesh) using 35:65 hexane/ethyl acetate as an eluent which afforded the pure desired **8** (32-72%) as shown in Table 1 (entry 1-10).

4.5.3.2 General procedure for the synthesis of biaryl coupled N-protected 1,2,9,10-tetramethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline analogues 32a-g:

To a solution of compound **32a-g** (0.10 mmol, 1 eq.) in DMSO (1.0 mL), in microwave vial, were added $Pd(OAc)_2$ (0.02 mmol, 20 mol%), ligand A (0.04 mmol, 40 mol%), K_2CO_3 (0.3 mmol, 3 eq.), and Pivalic acid (0.04 mmol, 40 mol%) by rigorous purging with nitrogen and the reaction mixture was irradiated in microwave reactor in a sealed vial for 5 min at 135 °C. After cooling to room temperature, the reaction mixture was loaded on deactivated silica gel column (100-200 mesh) and eluted with EtOAc/hexanes (35:65, v/v), which afforded the desired N-protected 1,2,9,10-tetramethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline analogues **32a-g** as a white solid in excellent yields (upto 73-92%).

4.5.4.1 General procedure for the synthesis of 1,2,9,10-tetramethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline analogues 33a-g:

To a solution of compounds **32a-f** (1.00 mmol, 1 eq.) in dry DCM (10 mL) was added anhyd $ZnBr_2$ (4.00 mmol, 4 eq.) under N_2-atmosphere and stirred the reaction mixture at room temperature for 6 h. The reaction mixture was then quenched with a solution of saturated $NaHCO_3$ (10 mL) and extracted with DCM (3 × 20 mL). The combined organic layer was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure

afforded the crude amine **33a-g** as a yellow oil, which was immediately utilised for next step without any further purification. (***Caution:*** *compound was found to be unstable for long-term storage*).

4.5.4.2 Synthesis of 1-(benzyloxy)-2,9,10-trimethoxy-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline 34p

The crude amine **33c** (0.90 mmol, 1 eq.) and acetaldehyde solution (1.84 mmol, 2 eq.) was taken in anhydrous DCM (15 mL) and stirred at room temperature for 10 min. The sodium triacetoxy-borohydride (4.61 mmol, 5 eq.) was added into the reaction mixture and was allowed to stir at room temperature for overnight. The reaction mixture was quenched with $NaHCO_3$ (5% aq. solution, 10 mL), extracted with DCM (3 × 20 mL). The combined organic phase was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography using MeOH/DCM (1:99, v/v) as an eluent, which furnished **34p** (yield = 67%).

4.5.4.3 General procedure for the synthesis of N-acetyl/benzoyl/sulphonylmethyl/sulphonyltolyl(m-) protected 1,2,9,10-tetramethoxy-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline analogues 34a-o and 34q-s:

To a solution of crude amine **33a-g** (0.20 mmol, 1 eq.) in anhyd. DCM (20 mL) added triethylamine (0.50 mmol, 2.5 eq.) followed by acetyl chloride (0.24 mmol, 1.2 eq.) or methylsulfonyl chloride (0.24 mmol, 1.2 eq.) or *p*-toluenesulfonyl chloride (0.22 mmol, 1.1 eq.) or benzoyl chloride (0.22 mmol, 1.1 eq.) at room temperature and the reaction mixture was allowed to stir for 8 h under N_2 atmosphere. The reaction mixture was quenched with $NaHCO_3$ (5% aq. solution, 10 mL), extracted with DCM (3 × 20 mL). The combined organic phase was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography using MeOH/DCM (1:99, v/v) as an eluent which furnished **34a-o** and **34q-s** (yield = 69-93%) as a white solid.

4.5.5 Characterization data of precursors as well as final synthesized aporphine analogues 29-34:

2-(2-bromo-4,5-dimethoxyphenyl)-N-(4-ethoxy-3-methoxyphenethyl)acetamide 29a

White solid; yield: 75%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using

MeOH/DCM (2:98) as an eluent m.p. 119-121 °C; FT-IR (KBr, νmax/cm-1) 3297, 2930, 1636, 1551, 1464, 1337, 1213, 1164; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97(s, 1H), 6.76 (s, 1H), δ 6.71 (d, J = 7.6Hz 1H), δ 6.63-6.62(m, 1H), δ 6.56-6.53(m, 1H), δ 5.46 (s, 1H), δ 4.08- 4.03(m, 2H), δ 3.90-3.81 (m, 9H), δ 3.57(s, 2H), δ 3.45(q, J = 6.8Hz, 2H), δ 2.68 (t, J = 6.8Hz, 2H), δ 1.44 (t, J = 7.2Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.4, 149.0, 148.8, 147.0, 131.1, 126.7, 120.7, 115.6, 114.9, 113.8, 112.8, 112.1, 64.4, 56.3, 56.2, 55.9, 43.8, 40.9, 35.1, 14.9; HRMS (ESI) calcd. for $C_{21}H_{26}BrNO_5$ $[M+2]^+$: 453.0994; found 453.0999.

2-(2-bromo-4,5-dimethoxyphenyl)-N-(3-methoxy-4-propoxyphenethyl)acetamide 29b

White solid; yield: 72%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2.5:97.5) as an eluent; m.p.100-102 °C; FT-IR (KBr, νmax/cm-1) 3300, 2930, 1638, 1511, 1465, 1338, 1260, 1027; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98(s, 1H), δ 6.76(s, 1H),δ 6.72 (d, J = 8.0Hz 1H), δ 6.63-6.62(m, 1H), δ 6.56-6.54(m, 1H), δ 5.46 (s, 1H), δ 3.95-3.90(m, 2H), δ 3.87-3.77(m, 9H), 3.58(s, 2H), δ 3.45(q, J = 6.8Hz 2H), δ 2.68 (t, J = 6.8Hz, 2H), δ 1.84(q, J = 7.6Hz, 2H), δ 1.02 (t, J = 7.2Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.6, 149.1, 147.3, 131.1, 126.7, 120.7, 115.6, 114.9, 113.8, 113.1, 112.4, 70.7, 56.3, 56.2, 56.1, 43.8, 40.9, 35.1, 22.6, 10.6; HRMS (ESI) calcd. for $C_{22}H_{28}BrNO_5$ $[M+2]^+$: 467.1151; found 467.1156.

N-(4-(benzyloxy)-3-methoxyphenethyl)-2-(2-bromo-4,5-dimethoxyphenyl) acetamide 29c

White solid; yield: 78%, R_f (MeOH/DCM; 4:96) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p.125-127 °C; FT-IR (KBr, νmax/cm-1) 3387, 2900, 1635, 1510, 1463, 1384, 1265, 1030; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.42 (m, 2H), δ 7.38-7.27 (m, 2H), 6.96 (s, 1H), δ 6.82- 6.72(m, 3H) δ 6.65(s, 1H), δ 6.52-6.49 (m, 1H), δ 5.45(s, 1H), δ 5.12 (s, 2H), δ 3.87- 3.82 (m, 9H), δ 3.57 (s, 2H) δ 3.48- 3.42 (m, 2H) δ 2.74-2.67(m, 2H);^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.8, 149.0, 148.2, 146.9, 137.4, 131.8, 128.7, 127.9, 127.4, 126.7, 120.7, 115.6, 114.9, 114.2, 113.8, 112.4, 71.2, 56.3, 56.2, 56.0, 43.8, 40.8, 35.1; HRMS (ESI) calcd. for $C_{26}H_{28}BrNO_5$ $[M+2]^+$: 515.1151; found 515.1158.

2-(2-bromo-4,5-dimethoxyphenyl)-N-(3-ethoxy-4-methoxyphenethyl)acetamide 29d

White solid; yield: 84%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent m.p. 119-121 °C; FT-IR (KBr, νmax/cm-1) 3300, 2930, 1638, 1511, 1465, 1338, 1260, 1027; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97(s, 1H), δ 6.70 (d, J = 8.0Hz 1H), δ 6.64-6.63(m, 1H), δ 6.57-6.54(m, 1H), δ 5.45 (s, 1H), δ 4.03(q, J = 7.2Hz 2H), δ 3.86-3.82 (m, 9H), δ 3.57(s, 1H), δ 3.44(q, J = 6.4Hz, 2H), δ 2.67 (t, J = 6.8Hz, 2H), δ 1.44 (t, J = 7.2Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.0, 148.8, 148.5, 148.0, 131.1, 126.7, 120.7, 115.6, 114.9, 113.3, 111.6, 64.4, 56.3, 56.2, 56.1, 43.8, 40.9, 35.1, 14.9; HRMS (ESI) calcd. for $C_{21}H_{26}BrNO_5$ $[M+2]^+$: 453.0994; found 453.0998.

N-(3-(benzyloxy)-4-methoxyphenethyl)-2-(2-bromo-4,5-dimethoxyphenyl) acetamide 29e

White solid; yield: 79%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent m.p. 110-112 °C; FT-IR (KBr, νmax/cm-1) 3387, 2930, 1635, 1513, 1463, 1384, 1260, 1029; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.42 (m, 2H), δ 7.35 (t, J = 14.8Hz 2H), 7.31-7.29 (m, 1H), δ 6.97(s, 1H) δ 6.74- 6.67(m, 3H), δ 6.60-6.57 (m, 1H), δ 5.41(s, 1H), δ 4.15-4.10 (m, 12H), δ 3.86 (s, 2H), δ 3.74(q, J = 6.8Hz, 2H), δ 2.98 (t, J = 6.8Hz, 2H), ^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.0, 148.8, 148.5, 148.3, 137.2, 131.0, 128.7, 127.9, 127.5, 126.7, 121.4, 115.6, 114.9, 114.7, 113.8, 111.9, 71.1, 56.3, 56.2, 56.1, 43.9, 43.8, 40.8, 34.9; HRMS (ESI) calcd. for $C_{26}H_{28}BrNO_5$ $[M+2]^+$: 515.1151; found 515.1158.

2-(2-bromo-4,5-dimethoxyphenyl)-N-(3,4-dimethoxyphenethyl)acetamide 29f

White solid; yield: 81%, R_f (MeOH/DCM; 5:95) = 0.52; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 128-130 °C; FT-IR (KBr, νmax/cm-1) 3414, 2935, 1633, 1549, 1463, 1384, 1244, 1028; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), δ 6.98 (d, J = 8.4Hz 1H), δ 6.91(d, J = 1.6Hz, 1H) δ 6.85(dd, J =2.0, 10.4Hz, 1H), δ 5.75(s, 1H), δ 4.15-4.10 (m, 12H), δ 3.86 (s, 2H), δ 3.74 (q, J = 6.8Hz 2H), δ 2.98 (t, J = 6.8Hz 2H), ^{13}C NMR (100 MHz, $CDCl_3$ 169.9, 149.0, 148.8, 147.7, 131.1, 126.6, 120.7, 115.6, 114.8, 113.8, 111.8, 111.2, 56.3, 56.2, 56.2, 55.9, 43.7, 40.8, 35.0; HRMS (ESI) calcd. for $C_{20}H_{24}BrNO_5$[M+2]$^+$: 439.0838; found 439.0832.

***tert*-Butyl-1-(2-bromo-4, 5-dimethoxybenzyl)-7-ethoxy-6-methoxy- 3, 4-dihydroisoquinoline-2(1H)-carboxylate 31a**

White solid; yield: 78%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 110-112 °C; FT-IR (KBr, νmax/cm-1) 3419, 1687, 1510, 1417, 1383, 1257, 1162, 1097; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.03 and 6.96 (m, 1H, both rotamer), δ 6.77-6.51 (m, 3H, both rotamer), δ 5.36-5.21(m, 1H, both rotamer), δ 4.36-4.31and 4.09-4.03 (m, 1H, both rotamer), δ 3.91-3.79 (m, 11H, both rotamer), δ 3.28-3.17 (m, 2H, both rotamer), δ 3.02-2.54 (m, 2H, both rotamer), δ 1.48-1.32 (m, 6H, both rotamer), δ 1.24-1.16 (m, 6H, major rotamer) ; ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 154.3, 148.6, 148.4, 148.3, 146.8, 130.5, 128.9, 126.8, 115.5, 115.0, 114.4, 111.7, 111.7, 79.5, 64.7, 56.4, 56.3, 56.1, 54.4, 42.3, 36.6, 28.5, 28.3, 28.1; HRMS (ESI) calcd. for $C_{26}H_{34}BrNO_6$ $[M+2]^+$: 537.1570; found 537.1578.

***tert*-Butyl-1-(2-bromo-4, 5-dimethoxybenzyl)-6-methoxy-7-propoxy-3, 4-dihydroisoquinoline-2(1H)-carboxylate 31b**

White solid; yield: 70%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 120-122 °C; FT-IR (KBr, νmax/cm-1) 3416, 1685, 1512, 1415, 1385, 1260, 1164, 1098; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.03 and 6.96 (m, 1H, both rotamer), δ 6.75 (m, 1H, both rotamer), δ 6.63 and 6.61(m, 1H, both rotamer), δ 6.57 and 6.51 (m, 1H, both rotamer), δ 5.36-5.34 and 5.26-5.16 (m, 1H, both rotamer), δ 4.34-4.30 (m, 1H, both rotamer), δ 3.96-3.90 (m, 1H, both rotamer), δ 3.84-3.78(m, 9H, both rotamer),3.29-3.12 (m, 2H, both rotamer), δ 3.02-2.49 (m, 3H, both rotamer), δ 2.02-1.53 (m, 3H, both rotamer), δ 1.36 and1.15 (m, 7+2 9H, both rotamer), 1.06-0.94 (m, 3H, both rotamer); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 154.3, 148.5, 148.4, 148.0, 147.0, 130.4, 128.9, 126.7, 115.5, 115.2, 114.4, 111.9, 111.9, 79.5, 70.9, 56.4, 56.3, 56.2, 54.4, 42.3, 36.6, 28.5, 28.3, 28.1; HRMS (ESI) calcd. for $C_{27}H_{36}BrNO_6$ $[M+2]^+$: 551.1726; found 551.1722.

***tert*-Butyl-7-(benzyloxy)-1-(2-bromo-4,5-dimethoxybenzyl)-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate 31c**

White solid; yield: 83%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using

MeOH/DCM (3:97) as an eluent; m.p. 108-110 °C; FT-IR (KBr, νmax/cm-1) 3418, 1689, 1513, 1408, 1387, 1252, 1163, 1104; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.46-7.27 (m, 5H, both rotamer), δ 7.02 and 6.96 (m, 1H, both rotamer), 6.77 (s, 1H, major rotamer), δ 6.63-6.47(m, 2H, both rotamer), δ 5.31-4.95 (m, 3H, both rotamer), δ 4.36-4.32 (m, 1H, both rotamer), δ 3.91-3.76 (m, 9H, both rotamer), δ 3.35-3.13 and 3.06 -3.01(m, 2H, both rotamer),2.92- 2.71 and 2.63-2.50 (m, 3H, both rotamer), δ 1.36 and1.13 (m, (7+2) 9H, both rotamer; ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer)154.3, 148.6, 148.5, 148.4, 146.6, 137.2, 130.5, 128.7, 127.9, 127.4, 127.4, 115.4, 115.0, 114.3, 113.1, 111.9, 79.5, 71.5, 56.4, 56.2, 54.2, 42.2, 38.9, 36.4, 28.5, 28.1; HRMS (ESI) calcd. for $C_{31}H_{36}BrNO_6$[M+2]$^+$: 599.1726; found 599.1729.

***tert*-Butyl-1-(2-bromo-4, 5-dimethoxybenzyl)-6-ethoxy-7-methoxy-3, 4-dihydroisoquinoline-2(1H)-carboxylate 31d**

White solid; yield: 72%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 118-120°C; FT-IR (KBr, νmax/cm-1) 3415, 1687, 1503, 1384, 1033; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.03 and 6.96 (m, 1H, both rotamer), δ 6.73 and 6.65 (m, 1H, both rotamer), δ 6.61 and 6.57 (m, 1H, both rotamer), δ 6.52-6.47 (m, 1H, both rotamer), δ 5.38-5.35 and 5.25-5.22(m, 1H, both rotamer) δ 4.35-4.31 (m, 1H, both rotamer), δ 4.12-4.01 (m, 2H, both rotamer), δ 3.86-3.72 (m, 9H, both rotamer), 3.39-3.32 and 3.29- 3.18 (m, 2H, both rotamer), 3.05-3.00 and 2.95- 2.84 (m, 2H, both rotamer),δ 2.80-2.72 and 2.64-2.53 (m, 1H, both rotamer), 1.47-1.43(m, 3H, both rotamer), 1.38 and 1.17 (m, 2+7, 9H, both rotamer) ; ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 154.3, 148.6, 148.4, 147.8, 147.3, 130.5, 128.9, 126.7, 115.5, 115.1, 114.4, 112.9, 110.4, 79.5, 64.5 56.5, 56.3, 56.2, 42.3, 36.6, 28.5, 28.3, 28.1, 14.9; HRMS (ESI) calcd. for $C_{26}H_{34}BrNO_6$ [M+2]$^+$: 537.1570; found 537.1576.

***tert*-Butyl-6-(benzyloxy)-1-(2-bromo-4, 5-dimethoxybenzyl)-7-methoxy-3, 4-dihydroisoquinoline-2(1H)-carboxylate 31e**

White solid; yield: 74%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2.5:97.5) as an eluent; m.p. 149-150 °C; FT-IR (KBr, νmax/cm-1) 3418, 2929, 1687, 1512, 1461, 1252, 1163; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.45-7.28 (m, 5H, both rotamer), δ 7.04 and 6.97 (m, 1H, both rotamer),

6.76 (s, 1H, major rotamer), δ 6.65-6.61(m, 1H, both rotamer), δ 6.51-6.50 (m, 1H, both rotamer), δ 5.39-5.35 and 5.26-5.22 (m, 1H, both rotamer),5.12-5.11(m, 2H, both rotamer), δ 4.33-4.29 (m, 1H, both rotamer), δ 3.86-3.74 (m, 9H, both rotamer), δ 3.37-3.18(m, 2H, both rotamer),3.05-3.3.00 and 2.95-2.80 (m, 2H, both rotamer), δ 2.60-2.51 (m, 1H, both rotamer), δ 1.37 and1.17 (m, (7+2) 9H, both rotamer; ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 154.3, 148.6, 148.4, 148.2, 147.2, 137.2, 130.4, 129.5, 128.7, 127.9, 127.4, 126.7, 115.5, 115.0, 114.4, 114.1, 110.8, 79.5, 71.1, 56.5, 56.3, 56.1, 54.5, 42.3, 36.6, 28.5, 28.1; HRMS (ESI) calcd. for $C_{31}H_{36}BrNO_6$ $[M+2]^+$: 599.1726; found 599.1729.

***tert*-Butyl-1-(2-bromo-4, 5-dimethoxybenzyl)-6,7-dimethoxy-3, 4-dihydroiso - quinoline-2(1H)-carboxylate 31f**

White solid; yield: 76%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 123-125°C; FT-IR (KBr, νmax/cm-1) 3418, 1690, 1468, 1361, 1253, 1169, 1111; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.03 and 6.96 (m, 1H, both rotamer), δ 6.73 and 6.64 (m, 1H, both rotamer), δ 6.61 and 6.57 (m, 1H, both rotamer), δ 6.51-6.47 (m, 1H, both rotamer), δ 5.39-5.35 and 5.25- 5.22(m, 1H, both rotamer) δ 4.36-4.31 (m, 1H, both rotamer), δ 3.90-3.73 (m, 12H, both rotamer), 3.38-3.18 (m, 2H, both rotamer), 3.05-2.85 (m, 2H, both rotamer),δ 2.65-2.61 (m, 1H, both rotamer), 1.37 and 1.16 (m, 2+7, 9H, both rotamer) ; ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 154.3, 148.6, 148.4, 147.9, 147.5, 130.4, 128.9, 126.7, 115.5, 114.4, 111.5, 110.1, 79.5, 56.4, 56.3, 56.0, 54.4, 42.2, 36.6, 28.5, 28.3, 28.1; HRMS (ESI) calcd. for $C_{25}H_{32}BrNO_6$ $[M+2]^+$: 523.1413; found 523.1418.

Ethyl 1-(2-bromo-4, 5-dimethoxybenzyl)-6, 7-dimethoxy-3, 4-dihydroiso - quinoline-2(1H)-carboxylate 31g

White solid; yield: 72%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 128-130°C; FT-IR (KBr, νmax/cm-1) 3424, 2934, 1690, 1511, 1440, 1378, 1164; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 7.02 and 6.96 (m, 1H, both rotamer), δ 6.64 - 6.57 (m, 2H, both rotamer), δ 5.39-5.36 and 5.31-5.28 (m, 1H, both rotamer), δ 4.30-4.26 and 4.11-4.07 (m, 1H, both rotamer), δ 3.97-3.67 (m, 14H; 12H for OCH_3 and 2H for OCH_2 both rotamer) δ 3.46-3.19 (m, 2H, both rotamer), δ 3.10-2.59 (m, 3H, both rotamer), 1.24-1.19 (m, 1H,

minor rotamer), 1.02-0.94 (m, 2H, major rotamer); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 155.6, 148.4, 148.3, 148.0, 147.4, 130.1, 128.4, 126.5, 115.4, 115.4, 114.3, 111.4, 110.1, 61.3, 56.4, 56.2, 56.0, 56.0, 54.4, 42.3, 37.5, 28.2, 14.4; HRMS (ESI) calcd. for $C_{23}H_{28}BrNO_6$ $[M+2]^+$: 495.1100; found 495.1105.

***tert*-Butyl-1-ethoxy-2, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[de, g]quin -oline-6(5*H*)-carboxylate 32a**

White solid; yield: 79%, R_f (EtOAc/hexane; 40:60) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7:3) as an eluent; m.p. 113-115 °C; FT-IR (KBr, νmax/cm-1) 3417, 2934, 1685, 1515, 1463, 1391, 1252, 1018; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 6.75 (s, 1H), 6.61 (s, 1H), δ 4.63 (d, J = 12.4Hz 1H), δ 4.39(d, J = 8.0Hz, 1H) δ 3.94-3.91 (m, 6H), δ 3.87 (s, 3H), 3.68-3.60 (m, 1H), 2.96-2.73 (m, 4H),δ 2.62 (d, J = 14.8Hz 1H), 1.48(s, 9H), δ 1.31 (t, J = 7.2Hz 3H), ^{13}C NMR (100 MHz, $CDCl_3$ 154.8, 152.1, 148.2, 147.2, 143.8, 130.1, 130.1, 128.1, 125.9, 124.5, 111.9, 110.9, 110.9, 110.5, 79.9, 68.6, 55.9, 55.9, 52.0, 38.7, 35.0, 30.5, 29.8, 28.6, 16.0; HRMS (ESI) calcd. for $C_{26}H_{33}NO_6$ $[M+H]^+$: 456.2308; found 456.2303.

***tert*-Butyl-2, 9, 10-trimethoxy-1-propoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*] quinoline -6(5*H*)-carboxylate 32b**

White solid; yield: 84%, R_f (EtOAc/hexane; 40:60) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7:3) as an eluent; m.p. 95-97 °C; FT-IR (KBr, νmax/cm-1) 3417, 2933, 1691, 1512, 1462, 1391, 1247, 1045; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), δ 6.75 (s, 1H), δ 6.61 (s, 1H), δ 4.61 (s, 1H), δ 4.38(s, 1H) δ 3.91-3.85 (m, 9H), δ 3.81-3.75 (m, 2H), 3.55 (q, J = 8.4Hz 1H), 2.90-2.61 (m, 4H),δ 1.76-1.69 (m, 2H), 1.48-1.46(m, 9H), δ 0.92 (t, J = 7.6Hz 3H), ^{13}C NMR (100 MHz, $CDCl_3$ 154.8, 152.0, 148.2, 147.2, 144.0, 130.1, 129.9, 128.0, 125.9, 124.5, 112.1, 110.9, 110.6, 79.9, 74.9, 55.9, 55.9, 55.9, 52.0, 38.9, 35.2, 30.5, , 28.7, 23.8,10.5; HRMS (ESI) calcd. for $C_{27}H_{35}NO_6$ $[M+H]^+$: 470.2464; found 470.2468.

***tert*-Butyl-1-(benzyloxy)-2,9,10-trimethoxy-6a,7-dihydro-4*H*-dibenzo[*de,g*] quinoline-6(5*H*)-carboxylate 32c**

White solid; yield: 88%, R_f (EtOAc/hexane; 40:60) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7.5:2.5) as an eluent; m.p. 150-152 °C; FT-IR (KBr, νmax/cm-1) 3413, 2972, 1686, 1595, 1466, 1391, 1250, 1112; ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.07(s, 1H), δ 7.39-7.29 (m, 5H), δ 6.76 (s, 1H), δ 6.66 (s, 1H), δ 4.92(d, J = 10.0Hz, 1H), δ .031 (d, J = 10.0Hz, 1H), δ .027 (d, J = 10.8Hz, 1H), δ .027 (d, J = 8.4Hz, 1H),δ 3.92 (S, 6H), δ 3.51 (S, 3H), δ 2.98-2.64 (m, 5H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ 154.8, 152.2, 148.2, 147.2, 143.6, 137.4, 129.9, 128.8, 128.4, 128.2, 112.1, 110.9, 79.9, 74.7, 56.1, 55.9, 55.5, 51.9, 38.8, 35.2, 30.6, 28.7; HRMS (ESI) calcd. $C_{31}H_{35}NO_6$ $[M+H]^+$:518.2464 found 518.2469.

***tert*-Butyl-2-ethoxy-1, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinoline -6 (5*H*)-carboxylate 32d**

White solid; 90%, R_f (EtOAc/hexane; 40:60) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7:3) as an eluent; m.p. 101-103 °C; FT-IR (KBr, νmax/cm-1) 3417, 2930, 1681, 1514, 1412, 1380, 1250, 1105; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), δ 6.76 (s, 1H), δ 6.62 (s, 1H), δ 4.68-4.65 (m, 1H), δ 4.38(s, 1H) δ 4.12-4.07(m, 2H), δ 3.90 (d, J = 7.2 6H), 3.67 (s, 3H), 2.97-2.60 (m, 5H),δ 1.51-1.46 (m, 12H), ^{13}C NMR (100 MHz, $CDCl_3$ 154.8, 151.3, 148.3, 147.4, 144.9, 130.1, 127.8, 125.8, 124.4, 111.8, 111.6, 110.9, 79.9, 64.2, 60.0, 56.0, 55.9, 51.9, 38.8, 34.9, 30.5, 28.6, 15.5; HRMS (ESI) calcd.$C_{26}H_{33}NO_6$$[M+H]^+$: 456.2308; found 456.2305.

***tert*-Butyl -2-(benzyloxy)-1, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*] quinoline-6(5*H*)-carboxylate 32e**

White solid; yield: 73%, R_f (EtOAc/hexane; 40:60) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7.5:3.5) as an eluent; m.p. 143-145 °C; FT-IR (KBr, νmax/cm-1) 3416, 2930, 1690, 1513, 1460, 1380, 1252, 1027; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), δ 7.46-7.37 (m, 5H), δ 6.75 (s, 1H), δ 6.68 (s, 1H), δ 5.13 (q, J = 12Hz 2H), δ 4.67(s, 1H) δ 4.37(s, 1H), δ 3.91 (d, J = 2.4Hz 6H), 3.69 (s, 3H), 2.92-2.77 (m, 4H), δ 2.59 (d, 15.2Hz 1H), δ 1.48 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ 154.6, 151.0, 148.1, 147.2, 145.0, 136.9, 129.9, 128.5, 127.9, 127.8, 127.2, 126.2, 124.2, 112.2, 111.6, 110.8, 79.8, 70.7, 60.0, 55.9, 55.7, 51.8, 38.4, 34.8, 30.3, 28.5; HRMS (ESI) calcd. $C_{31}H_{35}NO_6$ $[M+H]^+$: 518.2464 found 518.2468.

***tert*-Butyl-1, 2, 9, 10-tetramethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinoline-6 (5*H*)-carboxylate 32f**

White solid; yield: 80%, R_f (EtOAc/hexane; 40:60) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7:3) as an eluent; m.p. 130-132 °C; FT-IR (KBr,

νmax/cm-1) 3416, 2941, 1632, 1518, 1465, 1328, 1253, 1199, 1023; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), δ 6.76 (s, 1H), δ 6.63 (s, 1H), δ 4.67-4.39 (m, 5H), δ 3.92-3.89 (m, 9H), δ 3.65 (S, 3H), δ 2.97-2.74 (m, 4H) δ 2.65-2.62 (m, 1H), 1.49 (s, 9H);^{13}C NMR (100 MHz, $CDCl_3$ 154.7, 152.0, 148.3, 147.4, 144.7, 130.2, 130.0, 128.2, 127.7, 125.9, 125.7, 124.2, 124.1, 111.7,110.9, 110.6,79.9, 60.1, 55.9, 55.9, 51.9, 51.8, 38.9, 34.8, 30.6, 29.7 28.6; HRMS(ESI) calcd. $C_{25}H_{31}NO_6$ $[M+H]^+$:442.2151 found 442.2156.

Ethyl 1, 2, 9, 10-tetramethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinoline-6(5*H*)-carboxylate 32g

White solid; yield: 73%, R_f (EtOAc/hexane; 40:60) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using hexane/ethyl acetate (7:3) as an eluent; m.p. 150-152 °C; FT-IR (KBr, νmax/cm-1) 3416, 2982, 1693, 1514, 1420, 1318, 1253, 1110, 1021; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15-8.14 (m, 1H), δ 6.77-6.61 (m, 2H), δ4.70 (s, 1H), δ 4.42 (s, 1H), δ 4.23-4.18 (m, 2H), δ 3.92-3.87 (m, 9H), δ 3.65-3.58 (m, 3H),δ 3.02-2.63 (m, 5H), δ 1.30-1.26 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ 155.6, 152.1, 148.4, 147.5, 144.8, 130.1, 129.9, 127.8, 125.6, 124.2, 111.8, 111.2, 110.6, 61.5, 60.1, 56.0, 55.9, 51.9, 39.0, 34.8, 30.5, 14.9; HRMS (ESI) calcd. $C_{23}H_{27}NO_6$ $[M+H]^+$: 414.1838 found 414.1835.

1-(1, 2, 9, 10-tetramethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinolin-6(5*H*)-yl)ethanone 34a

White solid; yield: 73%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 172-174 °C; FT-IR (KBr, νmax/cm-1) 2943, 2847, 1607, 1575, 1418, 1256, 1034; ^{1}H NMR (400 MHz, $CDCl_3$, both rotamer) δ 8.17–8.14 (m, 1H), δ 6.78–6.76 (m, 1H), δ 6.65–6.62 (m, 1H), δ 5.08–4.94(m, 1H), δ 4.00–3.83 (m, 10H), δ 3.65 (s, 3H), δ 3.33–3.27 (m, 1H), δ 3.08–2.63 (m, 5H), δ 2.21–2.18 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 169.2, 152.1, 148.4, 147.5, 145.0, 130.6, 129.9, 129.2, 128.0, 125.9, 124.0, 111.7, 110.9, 60.1, 56.0, 54.2, 50.8, 42.2, 36.2, 33.6, 30.9, 22.8; HRMS (ESI) calcd. for $C_{22}H_{25}NO_5$ $[M+H]^+$: 384.1733; found 384.1737.

1, 2, 9, 10-tetramethoxy-6-(methylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34b

White solid; yield: 93%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 258-260 °C; FT-IR (KBr, νmax/cm-1) 2964, 2846, 1632, 1595, 1458, 1329, 1254, 1106; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 6.78 (s, 1H), δ 6.63 (s, 1H), δ 4.49(t, J = 9.6Hz, 1H), δ 4.12 – 4.09(m, 1H), δ 3.92 – 3.89 (m, 9H), δ 3.65–3.64 (m, 3H), δ 3.30–3.23 (m, 1H), δ 2.99 (d, J = 10Hz, 2H), δ 2.94 – 2.86 (m, 4H), δ 2.69 (d, J = 15.6Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.5, 148.6, 147.7, 145.1, 129.5, 128.9, 128.2, 124.6, 123.8, 111.7, 111.2, 110.8, 60.1, 56.0, 55.9, 53.4, 40.7, 39.7, 37.0, 29.6; HRMS (ESI) calcd. For $C_{21}H_{25}NO_6S$ $[M+H]^+$:420.1403; found 420.1407.

1, 2, 9, 10-tetramethoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*] quinoline 34c

White solid; yield: 90%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 207-209 °C; FT-IR (KBr, νmax/cm-1) 2931, 2845, 1602, 1513, 1460, 1317, 1254, 1104; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), δ 7.62 – 7.59 (m, 2H), δ 7.31–7.26 (m, 2H), 6.81 (s, 1H), δ 6.48 (s, 1H), δ 4.59–4.55 (m, 1H), δ 4.10 (d, J = 15.2Hz, 1H), δ 3.94 (s, 3H), δ 3.90 (s, 3H), δ 3.83 (s, 3H), δ 3.62 (s, 3H), δ 3.29 (t, J = 12Hz, 1H), δ 3.13–3.09 (m, 1H), δ 3.03–2.96 (m, 1H), δ 2.46–2.36 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$)152.3, 148.5, 147.7, 144.9, 140.8, 139.5, 133.5, 129.6, 129.2, 128.1, 127.4, 127.4, 124.1, 123.9, 111.7, 111.3, 110.6, 60.1, 56.0, 55.9, 53.5, 41.2, 37.7, 29.8, 28.9, 21.5; HRMS (ESI) calcd. for $C_{27}H_{29}NO_6S$: $[M+H]^+$: 496.1716; found 496.1714.

1-(2-ethoxy-1, 9, 10-trimethoxy-6a, 7-dihydro-4H-dibenzo[*de, g*]quinolin-6(5*H*)-yl)ethanone 34d

White solid; yield: 77%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 219-221 °C; FT-IR (KBr, νmax/cm-1) 2931, 1639, 1512, 1449, 1353, 1255, 1099, ; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 8.18–8.15 (m, 1H), δ 6.77-6.76 (m, 1H), δ 6.64–6.61 (m, 1H), δ 5.08–4.93(m, 1H), δ 4.12–4.08 (m, 2H), δ 4.00–3.97 (m, 1H), δ 3.93–3.90 (m, 6H), δ 3.67–3.66 (m, 3H), δ 3.33–2.61 (m, 5H), δ 2.21–2.17 (m, 3H), δ 1.51–1.47 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 169.2, 151.4, 148.3, 147.4, 145.2, 130.5, 129.9, 129.1, 128.0, 127.6, 125.8, 124.1, 111.7, 111.4, 64.2, 60.0, 56.0, 54.2, 50.7,

42.2, 36.6, 33.6, 30.8, 22.8, 15.1; HRMS (ESI) calcd. for $C_{23}H_{27}NO_5$ $[M+H]^+$: 398.1889; found 398.1887.

(2-ethoxy-1, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinolin-6(5*H*)-yl) (phenyl)methanone 34e

White solid; yield: 69%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (1:99) as an eluent; m.p. 218-220°C; FT-IR (KBr, νmax/cm-1) 3292, 3053, 2921, 2852, 1713, 1604, 1428, 1151; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), δ 7.43 (S, 5H), δ 6.79 (s, 1H), δ 6.62(s, 1H), δ 4.13–4.07 (m, 3H), δ 3.91 (s, 6H), δ 3.69 (s, 3H), 3.27–3.21, (m, 3H), δ 2.89 (q, J = 13.2, 2H), δ 2.63–2.59 (m, 1H), δ 1.49 (t, J = 6.8, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.9, 151.5, 148.3, 147.5, 145.2, 136.9, 129.7, 129.3, 128.7, 128.0, 126.7, 125.4, 124.2, 111.8, 111.5, 111.3, 64.3, 60.0, 56.0, 55.9, 34.8, 29.8, 15.1; HRMS (ESI) calcd. for $C_{28}H_{29}NO_5$ $[M+H]^+$:460.2046; found 460.2049.

2-ethoxy-1, 9, 10-trimethoxy-6-(methylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34f

White solid; yield: 91%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 241-242 °C; FT-IR (KBr, νmax/cm-1) 2929, 2851, 1631, 1514, 1419, 1355, 1256, 1102; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 6.78 (s, 1H), δ 6.62 (s, 1H), δ 4.49(t, J = 9.2Hz, 1H), δ 4.12 – 4.07(m, 2H), δ 3.92 – 3.90 (m, 6H), δ 3.67 (s, 3H), δ 3.30 – 3.23 (m, 1H), δ 2.99 (d, J = 9.2Hz, 2H), δ 2.89 – 2.87 (m, 3H), δ 2.67 (d, J = 15.6Hz, 1H) δ 1.50 (t, J = 6.8Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 151.8, 148.5, 147.7, 145.3, 129.4, 128.8, 128.2, 124.4, 123.9, 111.8, 111.7, 111.2, 64.3, 60.1, 56.1, 55.9, 53.4, 40.7, 39.7, 37.1, 29.6, 15.1; HRMS (ESI) calcd. for $C_{22}H_{27}NO_6S$ $[M+H]^+$: 434.1559; found 433.1551.

2-ethoxy-1, 9, 10-trimethoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34g

White solid; yield: 86%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 196-197 °C; FT-IR (KBr, νmax/cm-1) 2927, 2849, 1735, 1632, 1515, 1467, 1336, 1252, 1099; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), δ 7.62 – 7.60 (m, 2H), δ 7.32–7.31 (m, 2H), 6.81 (s, 1H), δ 6.47 (s, 1H), δ 4.59–4.55 (m, 1H), δ 4.11–4.00 (m, 3H), δ 3.94 (s, 3H), δ 3.90 (s, 1H), δ 3.64

(s, 3H), δ 3.29 (t, J = 11.6, 1H), δ 3.13–2.96 (m, 2H), δ 2.45–2.33 (m, 5H), δ 1.46 (t, J = 7.2, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 151.6, 148.5, 147.6, 145.1, 140.8, 139.6, 133.5, 129.5, 129.3, 129.2, 128.1, 127.4, 124.5, 124.1, 124.0, 111.7, 111.5, 111.2, 64.2, 60.1, 56.1, 55.9. 53.5, 41.2, 37.7, 28.9, 21.5, 15.1; HRMS (ESI) calcd. for $C_{28}H_{31}NO_6S$: $[M+H]^+$: 510.1872; found 510.1879.

1-ethoxy-2, 9, 10-trimethoxy-6-(methylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34h

White solid; yield: 92%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 190-193 °C; FT-IR (KBr, νmax/cm-1) 2930, 2842, 1730, 1632, 1513, 1464, 1384, 1254, 1104; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 6.76 (s, 1H), δ 6.61 (s, 1H), δ 4.47– 4.42(m, 1H), δ 4.09 – 4.06 (m, 1H), δ 3.90 – 3.86 (m, 9H), δ 3.64 – 3.61 (m, 1H), δ 3.45 – 3.44 (m, 3H), δ 3.28 – 3.22 (m, 1H), δ 2.97 – 2.85 (m, 4H), δ 2.69 – 2.66 (m, 1H) δ 1.34 – 1.23 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.6, 148.4, 147.4, 144.1, 129.4, 128.7, 128.5, 124.6, 111.9, 111.1, 110.7, 68.7, 55.9, 55.9, 55.9, 53.4, 50.8, 40.7, 39.6, 37.1, 29.8, 15.9; HRMS (ESI) calcd. for $C_{22}H_{27}NO_6S$ $[M+H]^+$: 434.1559; found 433.1556.

1-ethoxy-2, 9, 10-trimethoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34i

White solid; yield: 88%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 230-231 °C; FT-IR (KBr, νmax/cm-1) 2924, 2830, 1631, 1513, 1464, 1340, 1254, 1105; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), δ 7.62 – 7.59 (m, 2H), δ 7.31 (s, 2H), 6.81 (s, 1H), δ 6.47 (s, 1H), δ 4.55–4.52 (m, 1H), δ 4.10–4.07 (m, 1H), δ 3.94– 3.91(m, 10H), δ 3.64–3.60 (m, 1H), δ 3.32–2.95 (m, 3H), δ 2.46–2.36 (m, 5H), δ 1.31–1.27 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.4, 148.4, 147.4, 143.9, 140.8, 139.5, 133.5, 129.5, 129.2, 129.1, 128.5, 127.4, 124.7, 124.2, 124.1, 111.9, 111.2, 110.5, 68.7, 55.9, 55.9, 53.6, 41.2, 37.8, 28.9, 21.5, 15.9; HRMS (ESI) calcd. for $C_{28}H_{31}NO_6S$ $[M+H]^+$: 510.1872; found 510.1875.

2, 9, 10-trimethoxy-6-(methylsulfonyl)-1-propoxy-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34j

White solid; yield: 83%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel

using MeOH/DCM (2:98) as an eluent; m.p. 185-186 °C; FT-IR (KBr, νmax/cm-1) 2925, 2849, 1631, 1514, 1462, 1386, 1254, 1077; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 6.78 (s, 1H), δ 6.62 (s, 1H), δ 4.45(t, J = 9.2Hz, 1H), δ 4.12 – 4.08(m, 1H), δ 3.92 – 3.88 (m, 9H), δ 3.78 (q, J = 8.4Hz, 1H), δ 3.55 (q, J = 8.0Hz, 1H), δ 3.26 (t, J = 12Hz, 1H), δ 2.99 – 2.97 (m, 2H), δ 2.89–2.87 (m, 4H), δ 2.71–2.67 (m, 1H), 1.77–1.68(m, 2H),), δ 0.92 (t, J = 8Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.5, 148.5, 147.5, 144.5, 129.4, 128.6, 128.5, 124.6, 124.1, 112.1, 111.1, 110.8, 74.9, 56.0, 55.9, 53.4, 40.7, 39.6, 37.2, 29.8, 29.8, 29.6, 23.8; HRMS (ESI) calcd. For $C_{23}H_{29}NO_6S$ $[M+H]^+$:448.1716; found 448.1719.

2, 9, 10-trimethoxy-1-propoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34k

White solid; yield: 87%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2.5:97.5) as an eluent; m.p. 265-267 °C; FT-IR (KBr, νmax/cm-1)2931, 1632, 1513, 1461, 1339, 1254, 1012; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), δ 7.62 – 7.59 (m, 2H), δ 7.34–7.31 (m, 2H), 6.81 (s, 1H), δ 6.47 (s, 1H), δ 4.54 (dd, J = 4.8,13.6 Hz 1H), δ 4.11–4.06 (m, 1H), δ 3.94–3.88 (m, 6H), δ 3.82 (s, 3H), δ 3.78–3.72 (m, 1H), δ 3.10 (dd, J = 3.6, 14.0 1H), δ 2.99 (t, J = 13.2 Hz, 1H), δ 2.36–2.34 (m, 5H), δ 1.77–1.65 (m, 2H), δ 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$)152.3, 148.4, 147.4, 144.2, 140.8, 139.5, 133.5, 129.5, 129.2, 128.9, 128.4, 127.4, 124.8, 124.2, 124.1, 112.0, 111.1, 110.6, 75.0, 55.9, 55.9, 55.6, 41.2, 37.8, 29.8, 28.8, 23.7, 21.5, 10.5; HRMS (ESI) calcd. for $C_{29}H_{33}NO_6S$: $[M+H]^+$: 524.2029; found 524.2023.

1-(1-(benzyloxy)-2, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinolin-6(5*H*)-yl) ethanone 34l

White solid; yield: 67%, R_f (MeOH/DCM; 5:95) = 0.55; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent; m.p. 165-167 °C; FT-IR (KBr, νmax/cm-1), 2929, 2840, 1737, 2852, 1621, 1510, 1432, 1392, 1221, 1027; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 8.09–8.06 (m, 1H), δ 7.37–7.29 (m, 5H), δ 6.78–6.76 (m, 1H), δ 6.69–6.65 (m, 1H), δ 5.08–5.05 (m, 1H), δ 4.98–;–3.93 (m, 1H), δ 4.02–3.90 (m, 6H), δ 3.49 (s, 3H), δ 3.32 (t, J = 12.4, 1H), δ 3.08–2.68 (m, 4H), δ 2.22–2.18 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 169.2, 152.2, 148.2, 147.2, 143.8, 137.4, 130.7, 129.7, 129.4, 128.9, 128.7, 128.4, 128.2, 126.0, 124.9,

124.1, 112.1, 111.3, 110.6, 74.6, 56.1, 55.8, 55.5, 54.2, 50.8, 42.2, 36.2, 34.7, 33.6, 31.7, 30.9, 30.9, 30.0, 29.8, 26.9, 25.4, 22.8, 21.8, 20.8. HRMS (ESI) calcd. for $C_{28}H_{29}NO_5$ $[M+H]^+$:460.2046; found 460.2042.

(1-(benzyloxy)-2, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinolin-6(*5H*)-yl) (phenyl)methanone 34m

White solid; yield: 71%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (1.5:98.5) as an eluent; m.p. 165-166 °C; FT-IR (KBr, νmax/cm-1) 2926, 2856, 1738, 1625, 1508, 1421, 1217, 1024; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), δ 7.44–7.38 (m, 7H), δ 7.35-7.28 (m, 3H), δ 6.79(s, 1H), δ 6.66 (s, 1H), δ 4.92 (d, J = 10.4, 1H), δ 4.55 (d, J = 10.8, 1H), δ 4.12–4.02 (m, 1H), δ 3.92–3.91 (m, 6H), δ 3.50 (s, 3H), δ 3.25–3.12 (m, 2H), δ 2.97–2.80 (m, 3H), δ 2.67–2.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.9, 152.4, 148.3, 147.3, 143.8, 137.4, 136.9, 129.7, 129.7, 128.8, 128.7, 128.6, 128., 128.2, 126.8, 125.7, 124.1, 112.1, 112.2, 110.7, 74.6, 56.1, 55.7, 55.6, 51.4, 34.03, 43.5, 30.9, 29.8; HRMS (ESI) calcd. for $C_{33}H_{31}NO_5$ $[M+H]^+$:522.2202; found 522.2208.

1-(benzyloxy)-2, 9, 10-trimethoxy-6-(methylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34n

White solid; yield: 88%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 178-179 °C; FT-IR (KBr, νmax/cm-1) 2932, 2838, 1738, 1581, 1510, 1448, 1383, 1011; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), δ 7.37–7.28 (m, 5H), δ 6.78 (s, 1H), δ 6.66 (s, 1H), δ 4.91 (d, J = 10, 1H), δ 4.52–4.46 (m, 2H), δ 4.14–4.10 (m, 1H), δ 3.92 (s, 6H), δ 3.50 (s, 3H), δ 3.32–3.24 (m, 1H), δ 3.15–3.13 (m, 1H), δ 3.02-2.87 (m, 5H), δ 2.79–2.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.6, 148.4, 147.4, 143.9, 137.2, 129.3, 129.1, 128.8, 128.7, 128.4, 128.3, 124.6, 123.9, 112.1, 111.1, 110.9, 74.7, 56.1, 55.9, 55.6, 53.4, 46.1, 39.6, 37.1, 29.6; HRMS (ESI) calcd. for $C_{27}H_{29}NO_6S$ $[M+H]^+$:496.1716; found 496.1711.

1-(benzyloxy)-2, 9, 10-trimethoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34o

White solid; yield: 90%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (1.5:98.5) as an eluent; m.p. 119-120 °C; FT-IR (KBr, νmax/cm-

1) 2929, 2856, 1737, 1591, 1449, 1339, 1249, 1001; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), δ 7.63–7.60 (m, 2H), δ 7.33–7.29 (m, 7H), δ 6.81 (s, 1H), δ 6.51 (s, 1H), δ 5.28(s, 2H), δ 4.89 (t, J = 5.2Hz, 1H), δ 4.58–4.45 (m, 2H), δ 4.10 (d, t, J = 14.0Hz, 1H), δ 3.94–3.86 (m, 6H), δ 3.51 (s, 3H), δ 3.33–3.26 (m, 1H), δ 3.14–3.95 (m, 1H), δ 2.51–2.28(m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 152.4, 148.4, 147.4, 143.7, 140.7, 139.5, 137.2, 133.5, 129.4, 128.8, 128.4, 128.3, 127.3, 124.1, 112.0, 111.1, 110.7, 74.7, 55.9, 55.9, 55.6, 53.5, 41.2, 32.0, 28.8, 26.9, 14.2. HRMS (ESI) calcd. for $C_{33}H_{33}NO_6S$ $[M+H]^+$:572.2029; found 572.2023.

1-(benzyloxy)-2, 9, 10-trimethoxy-6-methyl-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34p

Sticky solid; yield: 67%, R_f (MeOH/DCM; 5:95) = 0.50; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (3:97) as an eluent;; semi solid; FT-IR (KBr, νmax/cm-1) 2938, 2843, 1736, 1591, 1453, 1371, 1223, 1151; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), δ 7.33 – 7.23 (m, 5H), δ 6.75 (s, 1H), δ 6.59 (s, 1H), δ 4.88(d, J = 14.4Hz, 1H), δ 3.91 (s, 3H), δ 3.88 (s, 3H), δ 3.54 (s, 3H), δ 3.20 – 2.97 (m, 4H), δ 2.70 – 2.65 (m, 1H), δ 2.60 – 2.48 (m, 5H), ^{13}C NMR (100 MHz, $CDCl_3$ 152.2, 147.9, 147.3, 142.9, 137.3, 129.2, 129.1, 128.9, 128.3, 128.1, 127.7, 127.2, 124.6, 112.1, 110.7, 110.5, 74.9, 62.7, 55.9, 55.9, 55.7, 53.4, 44.1, 34.6, 29.3; HRMS (ESI) calcd. for $C_{27}H_{29}NO_4[M+H]^+$: 432.2097; found 432.2092.

1-(2-(benzyloxy)-1, 9, 10-trimethoxy-6a, 7-dihydro-4*H*-dibenzo[*de, g*]quinolin-6(5*H*)-yl) ethanone 34q

White solid; yield: 70%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 165-166 °C; FT-IR (KBr, νmax/cm-1) 2927, 2852, 1737, 1636, 1509, 1422, 1249, 1025; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamer) δ 8.19-8.16 (m, 1H), δ 7.49–7.32 (m, 5H), δ 6.78–6.67 (m, 2H), δ 5.17–4.93(m, 3H), δ 3.99–3.91 (m, 7H), δ 3.71 (s, 3H), δ 3.32–2.65 (m, 5H), δ 2.21–2.18 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) 169.3, 151.3, 148.4, 147.4, 145.5, 137.1, 129.9, 129.1, 128.7, 128.0, 127.4, 126.4, 125.3, 124.1, 112.3, 111.8, 111.4, 70.9, 60.2, 56.1, 50.8, 42.1, 31.7, 29.7, 22.8, 14.3; HRMS (ESI) calcd. for $C_{28}H_{29}NO_5$ $[M+H]^+$:460.2046; found 460.2049.

2-(benzyloxy)-1, 9, 10-trimethoxy-6-(methylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34r

White solid; yield: 91%, R_f (MeOH/DCM; 5:95) = 0.65; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 175-176 °C; FT-IR (KBr, νmax/cm^{-1}) 2998, 2844, 1738, 1592, 1459, 1322, 1250, 1018; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), δ 7.48 – 7.34 (m, 5H), δ 6.78 (s, 1H), δ 6.69 (s, 1H), δ 5.14(q, J = 12, 2H), δ 4.50 (t, J = 8.8, 1H), δ 4.12–4.07(m, 1H), δ 3.92–3.91(m, 6H), δ 3.71(s, 3H), δ 3.28–3.22(m, 1H), δ 2.99(d, J = 9.2, 2H) δ 2.90–2.83(m, 4H), δ 2.67–2.63(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 151.7, 148.6, 147.7, 145.6, 136.9, 129.5, 128.9, 128.8, 128.4, 128.2, 127.4, 124.9, 123.9, 112.5, 111.7, 111.2, 70.9, 60.2, 56.1, 55.9, 53.3, 40.6, 39.7, 36.9, 29.6; HRMS (ESI) calcd. for $C_{27}H_{29}NO_6S$ $[M+H]^+$: 496.1716; found 496.1713.

2-(benzyloxy)-1, 9, 10-trimethoxy-6-(m-tolylsulfonyl)-5, 6, 6a, 7-tetrahydro-4*H*-dibenzo[*de, g*]quinoline 34s

White solid; yield: 88%, R_f (MeOH/DCM; 5:95) = 0.60; Purification of crude product was done by flash column chromatography method over deactivated silica gel using MeOH/DCM (2:98) as an eluent; m.p. 125-126 °C; FT-IR (KBr, νmax/cm-1) 2929, 2840, 1735, 1587, 1457, 1333, 1250, 1017; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), δ 7.62 – 7.60 (m, 2H), δ 7.45–7.31 (m, 7H), 6.81 (s 1H), δ 6.54 (s, 1H), δ 5.11–5.04 (m, 2H), δ 4.61–4.56 (m, 1H), δ 4.10–4.06 (m, 1H), δ 3.94–3.91 (m, 6H), δ 3.72–3.68 (m, 3H), δ 3.31–3.24 (m, 1H), δ 3.13–2.96 (m, 2H), δ 2.43–2.36 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 151.5, 148.5, 147.6, 145.4, 140.8, 139.5, 136.9, 133.5, 129.6, 129.2, 129.2, 128.7, 128.1, 127.4, 125.1, 124.1, 123.9, 112.2, 111.7, 111.2, 70.8, 60.2, 56.1, 55.9, 53.5, 41.1, 37.6, 28.8, 21.5; HRMS (ESI) calcd. for $C_{33}H_{33}NO_6S$ $[M+H]^+$: 572.2029; found 572.2023.

4.5.6 Materials and Methods

4.5.6.1 Platelet aggregation inhibitory activity evaluation[12]

All synthesized novel aporphine analogues (**34a-s)** were dissolved in DMSO before testing. In order to eliminate the effects of the solvent on aggregation, the final concentration of DMSO was fixed at 0.5%. Arachidonic acid (AA), EDTA (disodium salt), bovine serum albumin and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co.

Platelet Aggregation inhibitory bioassay. Blood was collected from the rabbit marginal ear vein and was mixed with EDTA to a final concentration of 6 mM. It was centrifuged for 10 min at 90 g at room temperature, and the supernatant was obtained

as platelet-rich plasma. The latter was further centrifuged at 500 g for 10 min. The platelet pellets were washed with Tyrode's solution (Ca^{+2}-free) containing 2mM EDTA, 0.1 mg/mL and 3.5 mg/mL bovine serum albumin, and centrifuged at 500 g for 10 min. Then, the pellets were washed with Tyrode's solution without EDTA. After centrifugation under the same conditions, the platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2-PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2) containing bovine serum albumin (0.35%).

Aggregation was measured by a turbidimetric method using a Lumi-aggregometer (Chrono-Log Corp., Havertown, PA). All glassware was siliconized. Three minutes before the addition of the aggregation inducer, the platelet suspension was stirred at 1200 rpm. The percentage of aggregation was calculated as follows (abs. = absorbance):

$$\% \text{ Aggregation} = \frac{\text{Abs.of platelet suspension - Final abs. after aggregation}}{\text{Abs.of platelet suspension - abs. of Tyrode solution}} \text{ X } 100$$

Percent aggregation was expressed assuming the absorbance of platelet suspension as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation. For each compound IC_{50} values were calculated by SigmaPlot.

4.5.6.2 *In vitro* antioxidant DPPH radical scavenging activity[13a-e]

In DPPH radical scavenging method the sample at different concentrations ranging from 10 to 100 μg mL^{-1} was mixed with 1.5 mL of a DPPH methanolic solution (20 mg L^{-1}). Pure methanol was taken as control and ascorbic acid (vitamin C) was used as a reference compound. The percent of DPPH decoloration of the sample was calculated according to the formula.

Decoloration % = [1 − (Abs sample / Abs control)] × 100

The decoloration was plotted against the sample concentration and a logarithmic regression curve was established in order to calculate the IC_{50}. The results are expressed as antiradical efficiency (AE), which is 1000-fold inverse of the IC_{50} value AE=1000/ IC_{50}.

4.6 References

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4.7 Characterization spectra (^{1}H and ^{13}C NMR) of selected starting precursor's and aporphine analogues:

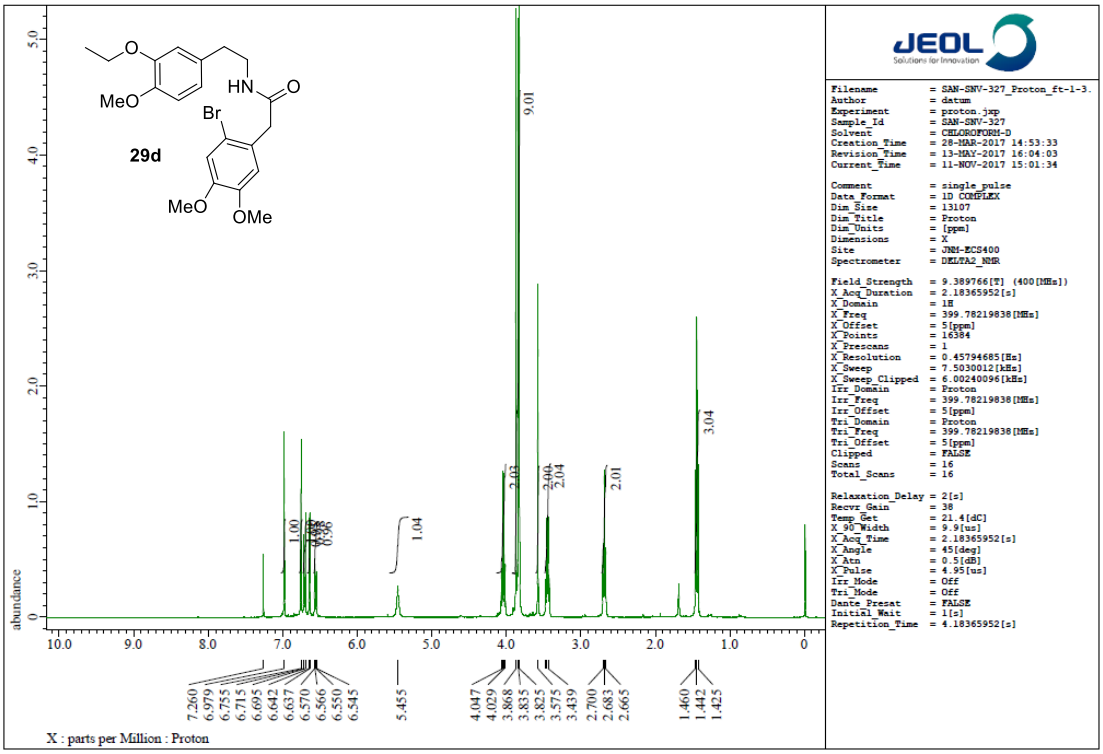


Figure 5.^{1}H NMR Spectra of Compound **29d.**

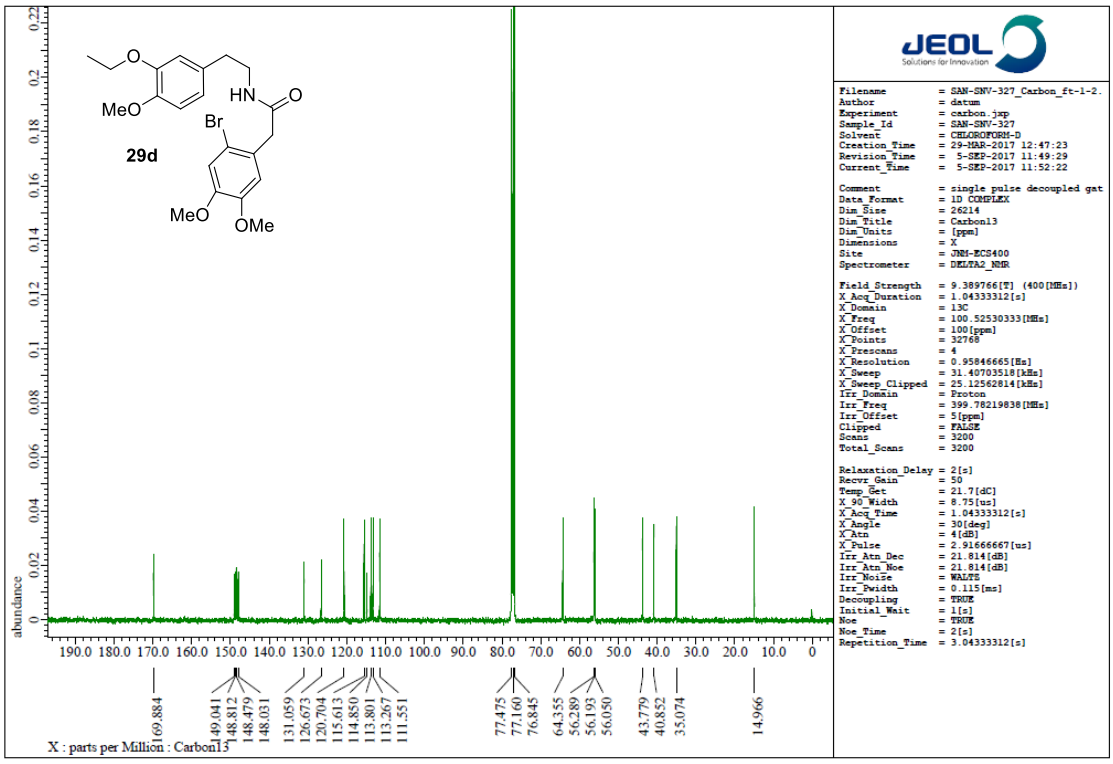


Figure 6.^{13}C NMR Spectra of Compound **29d.**

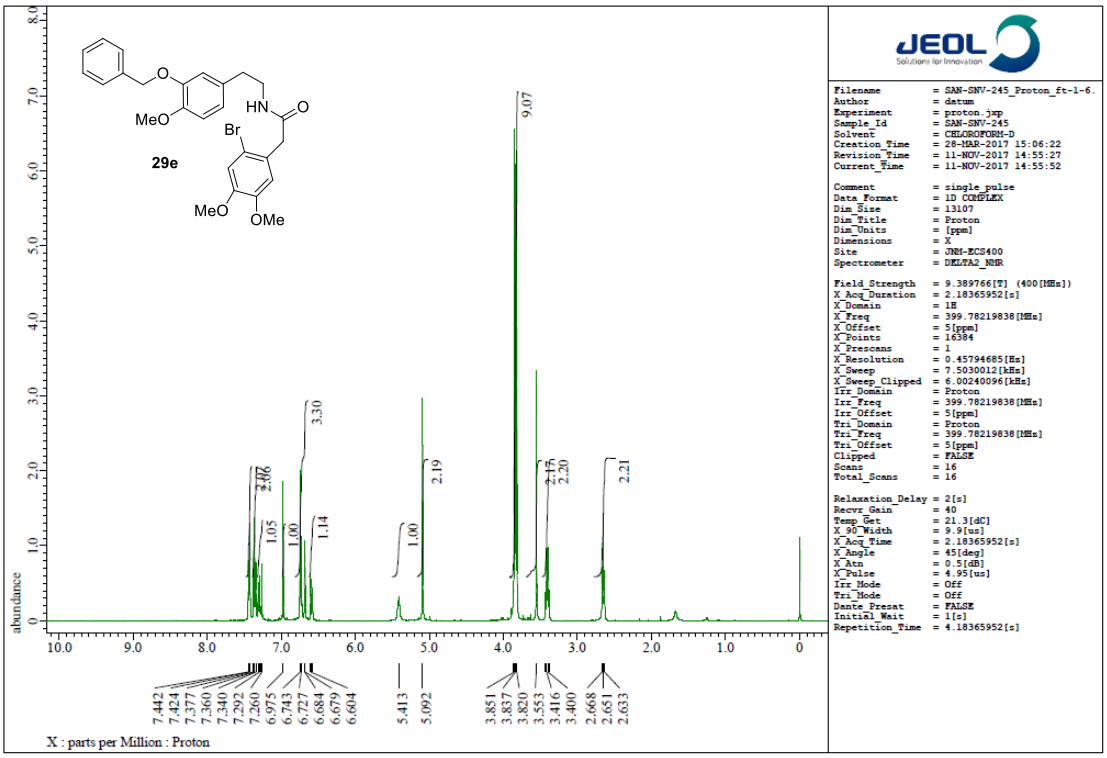


Figure 7.[1]H NMR Spectra of Compound **29e.**

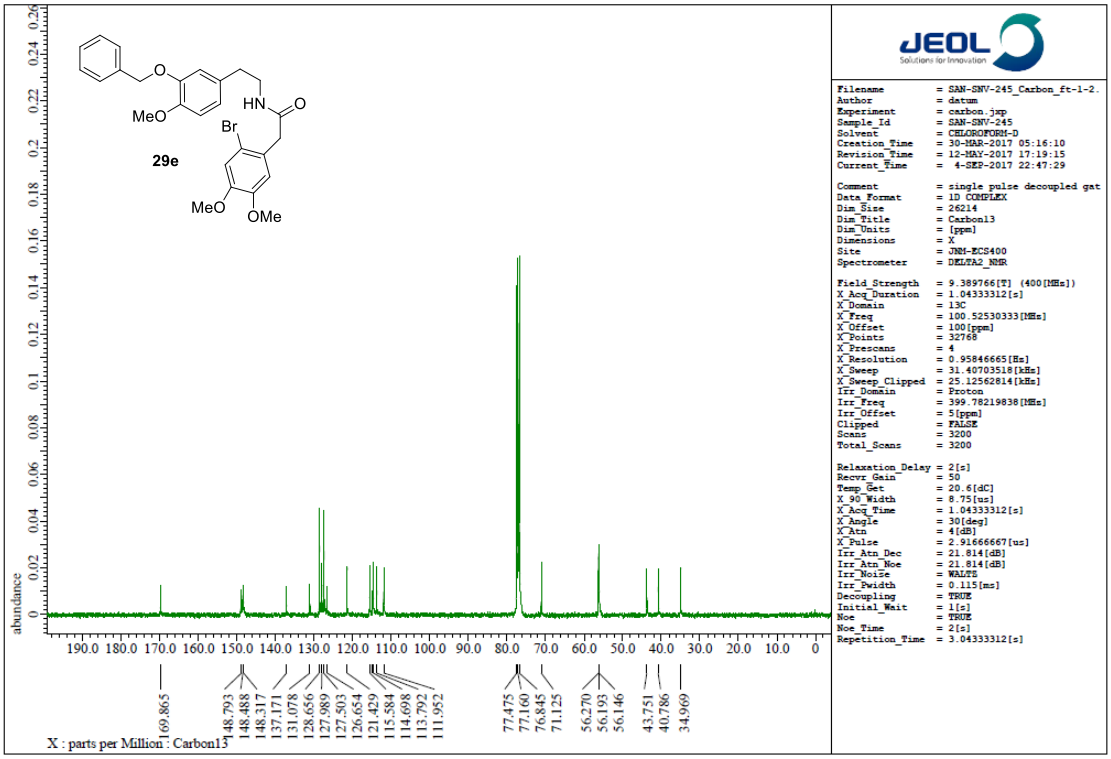


Figure 8.[13]C NMR Spectra of Compound **29e.**

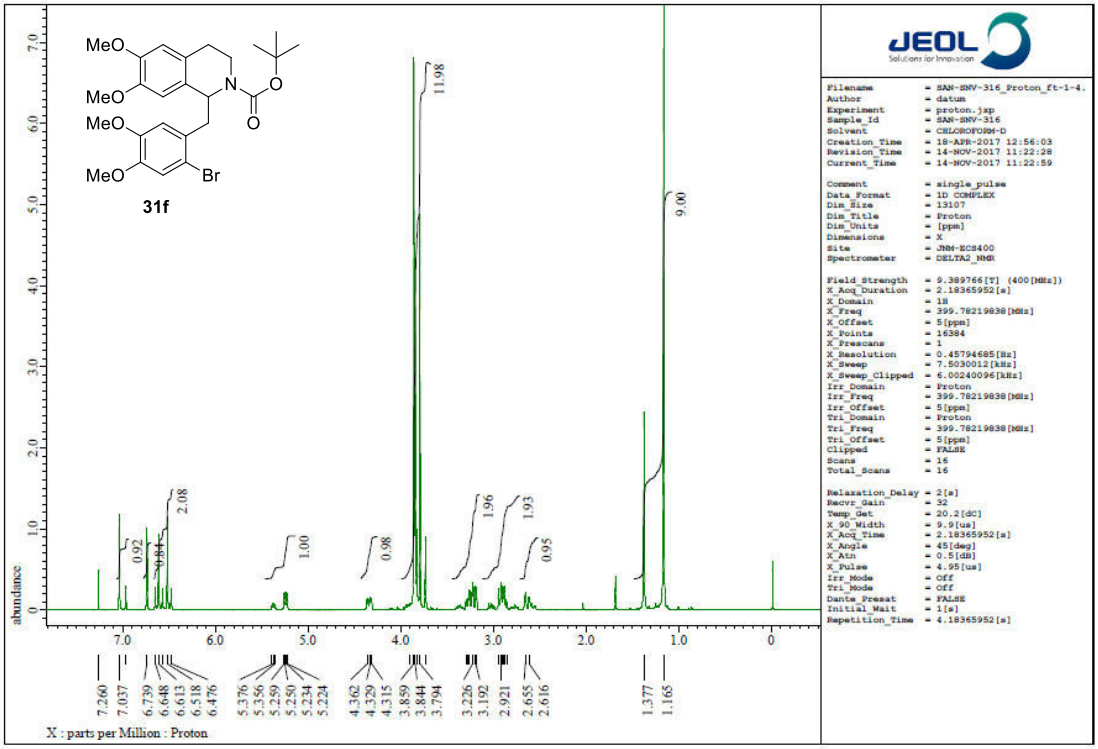


Figure: 9. ^{1}H NMR Spectra of compound **31f.**

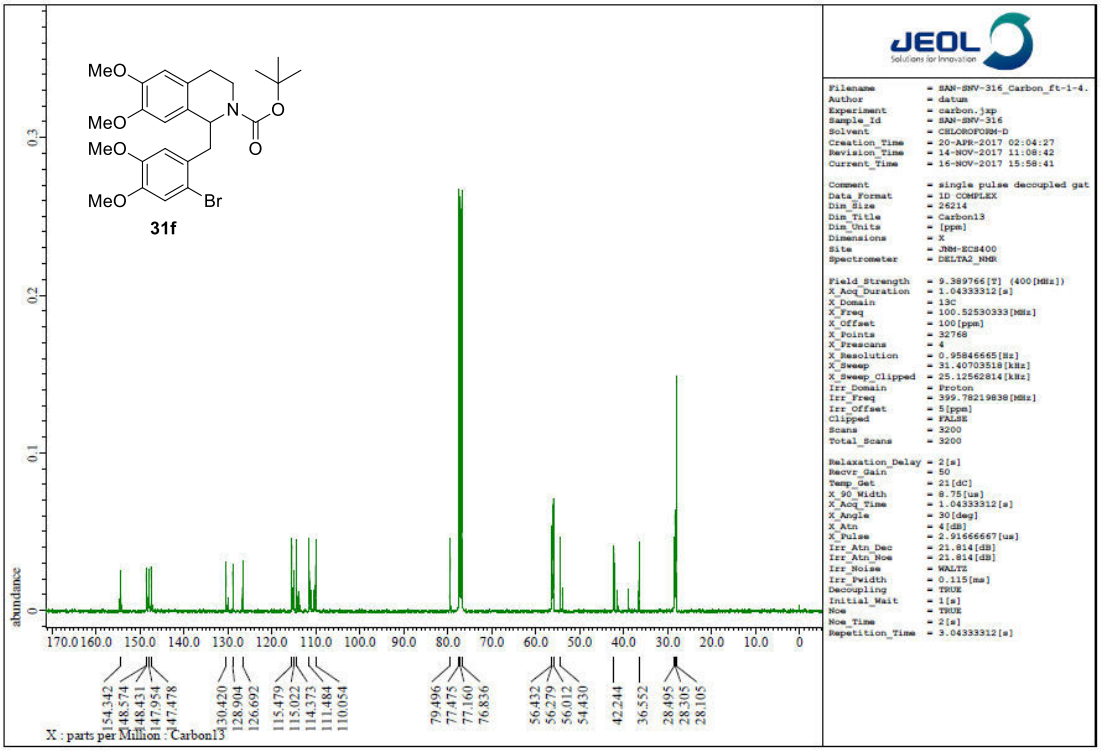


Figure 10. ^{13}C NMR Spectra of compound **31f.**

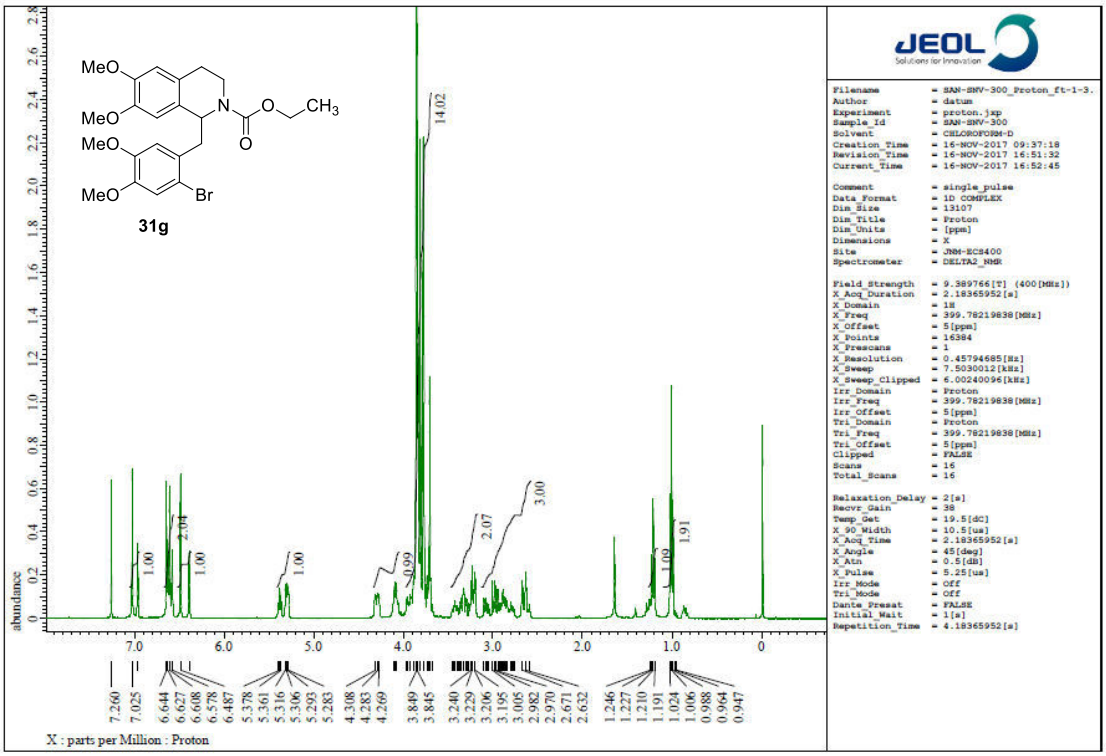


Figure 11. ^{1}H NMR Spectra of compound **31g.**

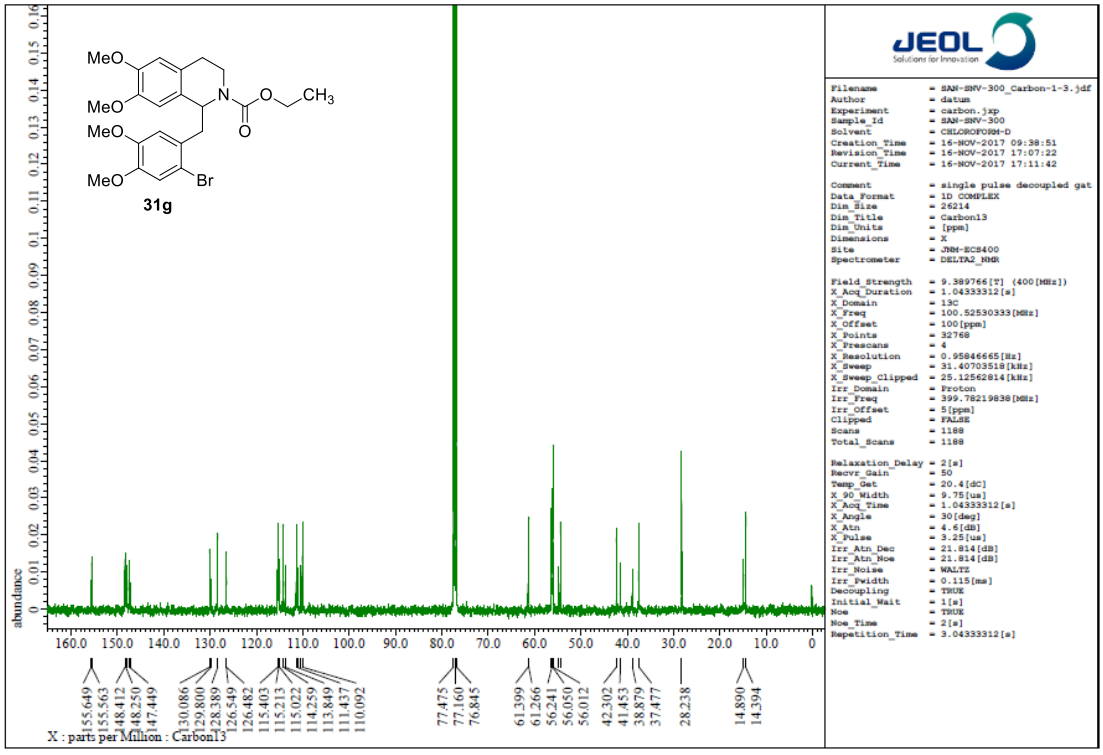


Figure12. ^{13}C NMR Spectra of compound **31g.**

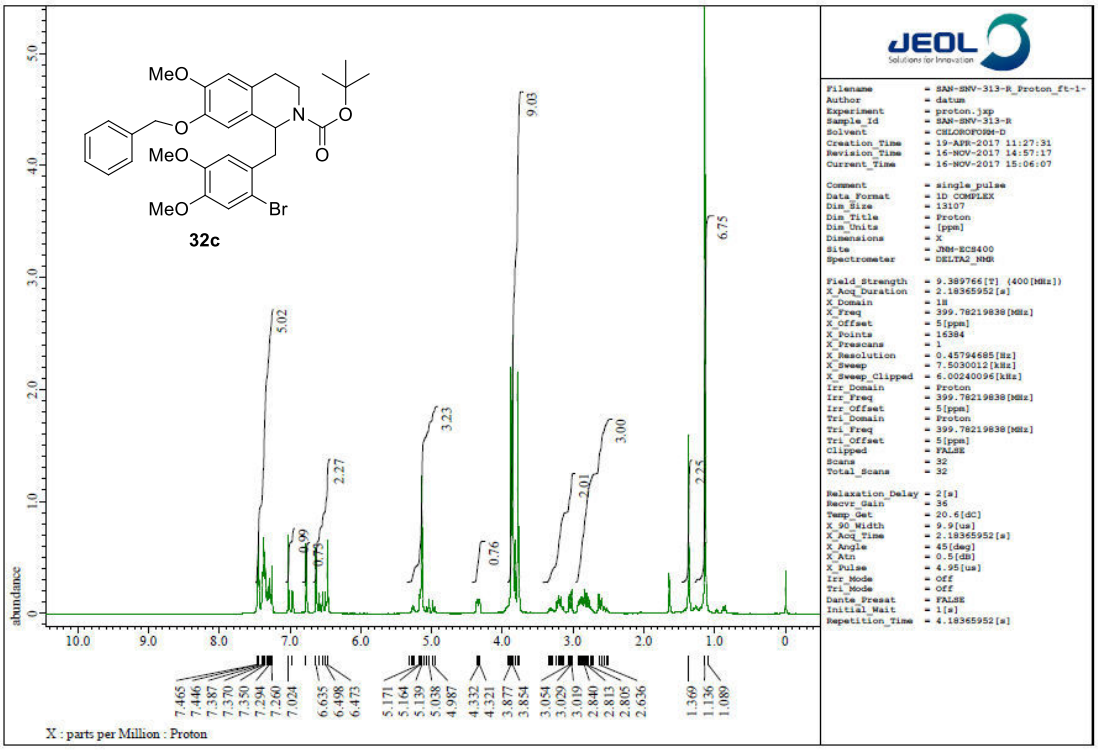


Figure 13. ^{1}H NMR Spectra of compound **32c.**

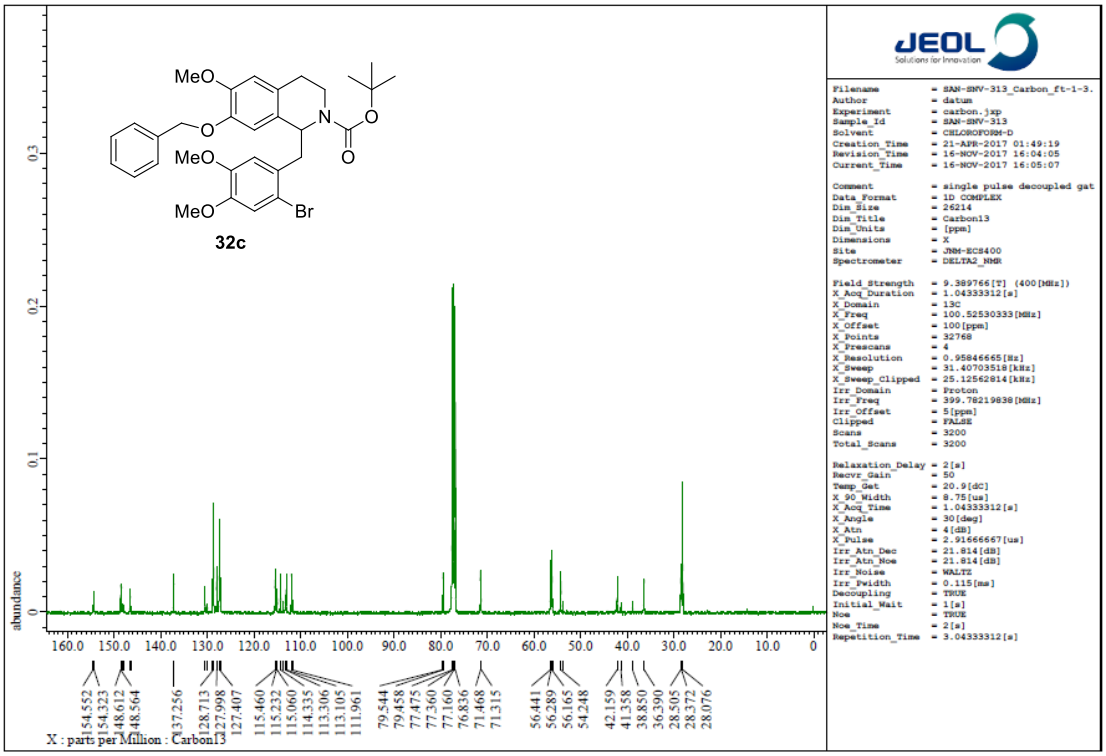


Figure 14. ^{13}C NMR Spectra of compound **32c.**

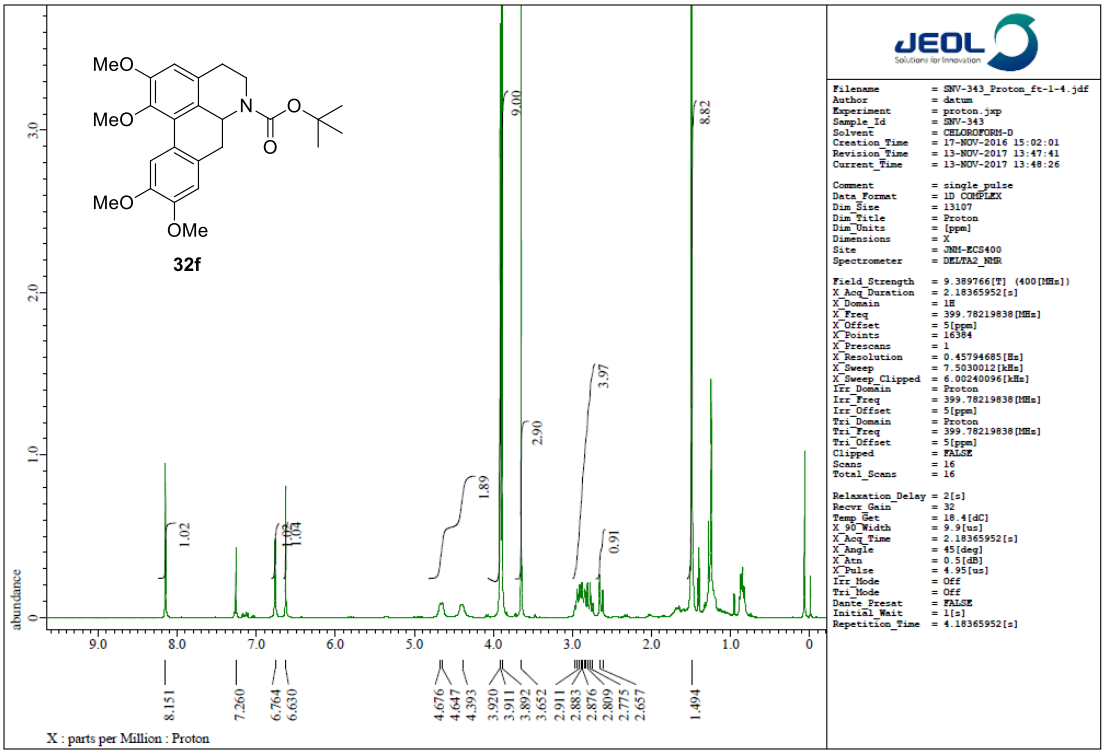


Figure 15. ^{1}H NMR Spectra of Compound **32f.**

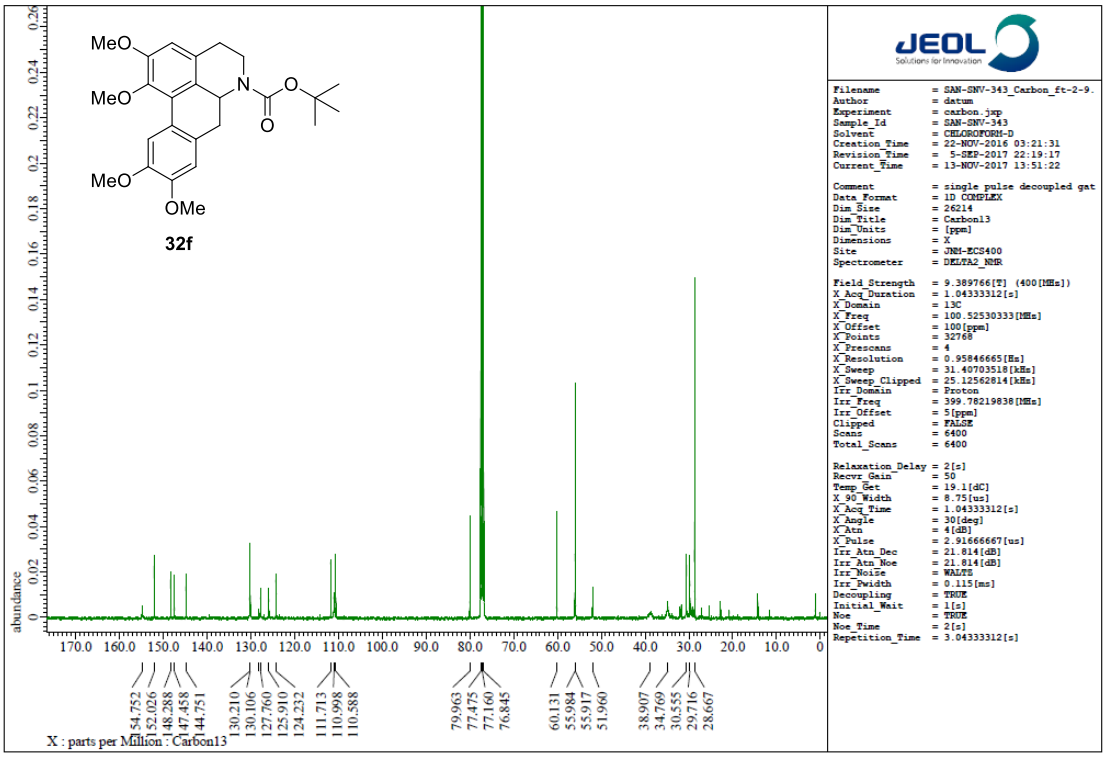


Figure 16. ^{13}C NMR Spectra of Compound **32f.**

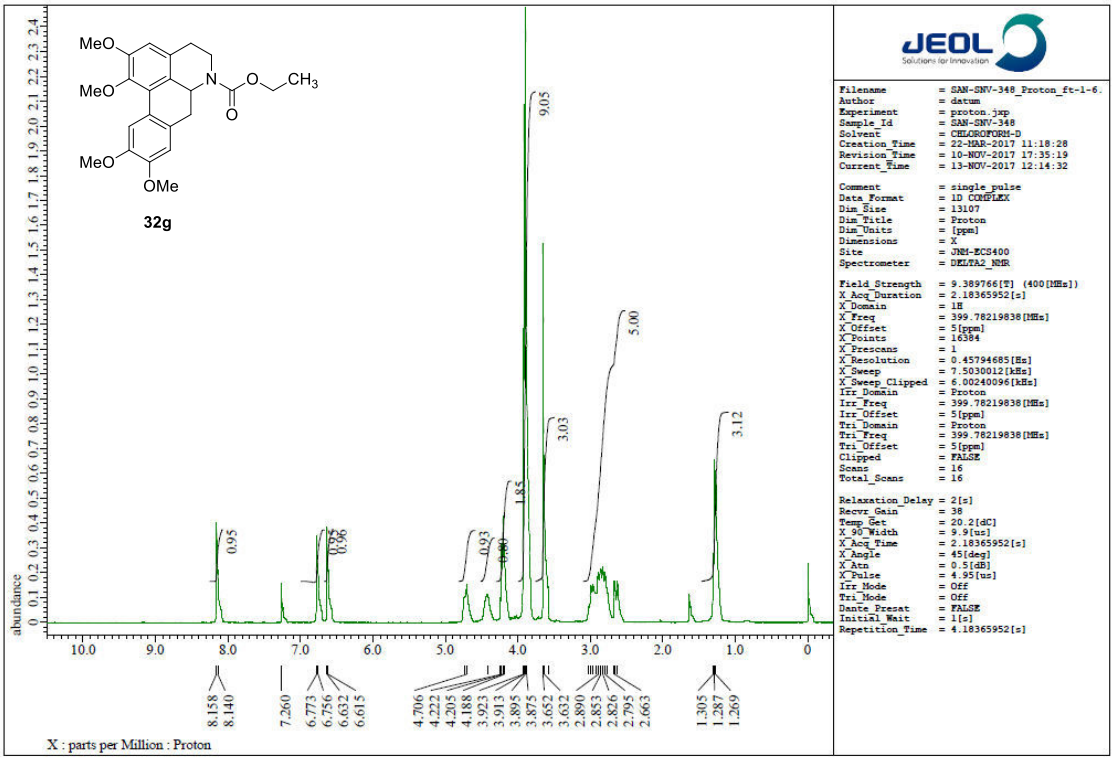


Figure 17. ^{1}H NMR Spectra of Compound **32g.**

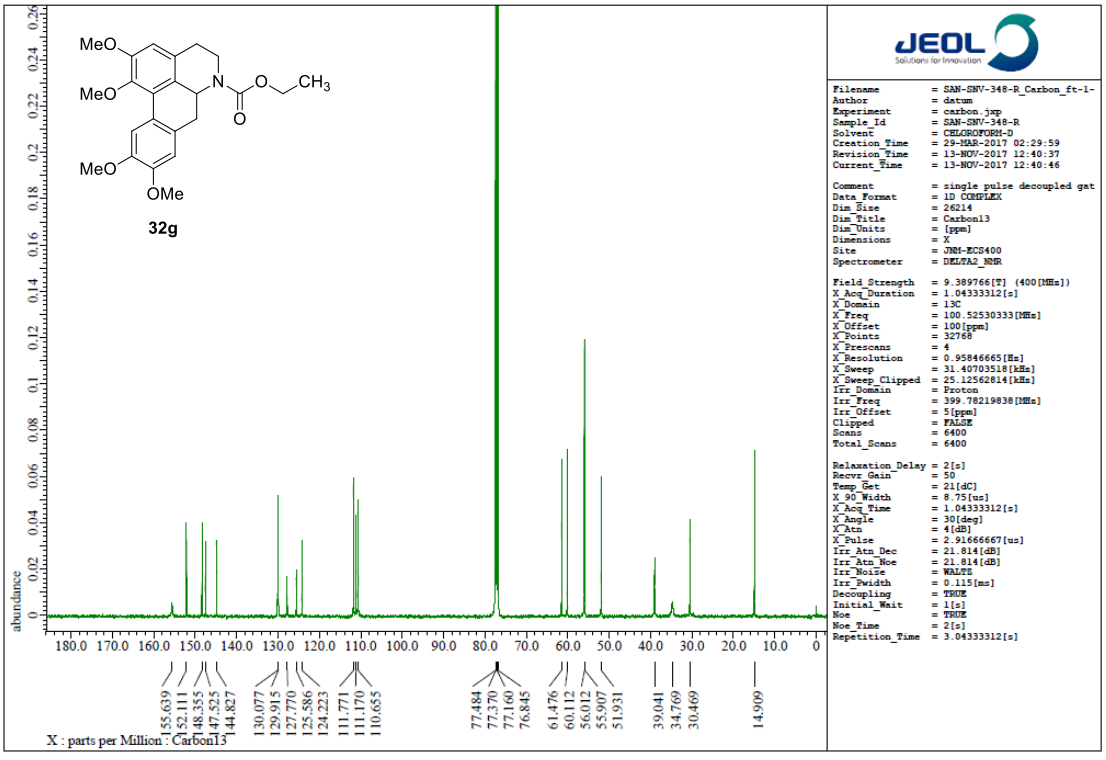


Figure 18.^{13}C NMR Spectra of Compound **32g.**

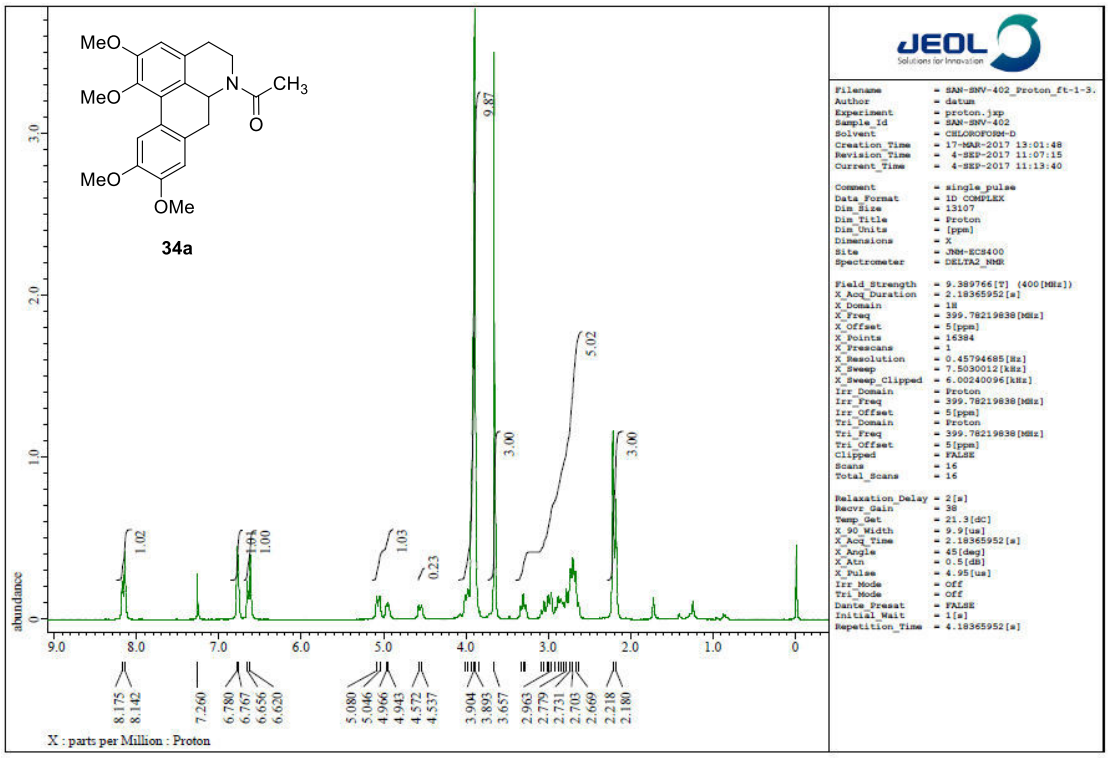


Figure 19.[1]H NMR Spectra of Compound **34a.**

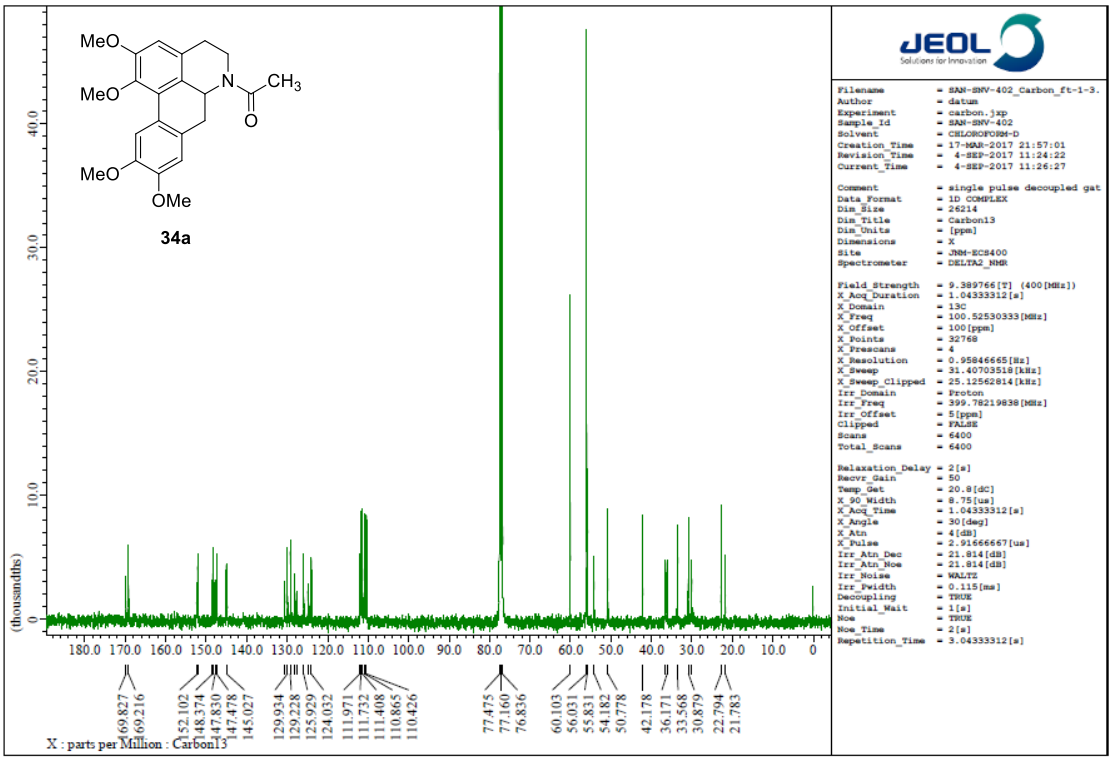


Figure 20.[13] C NMR Spectra of Compound **34a.**

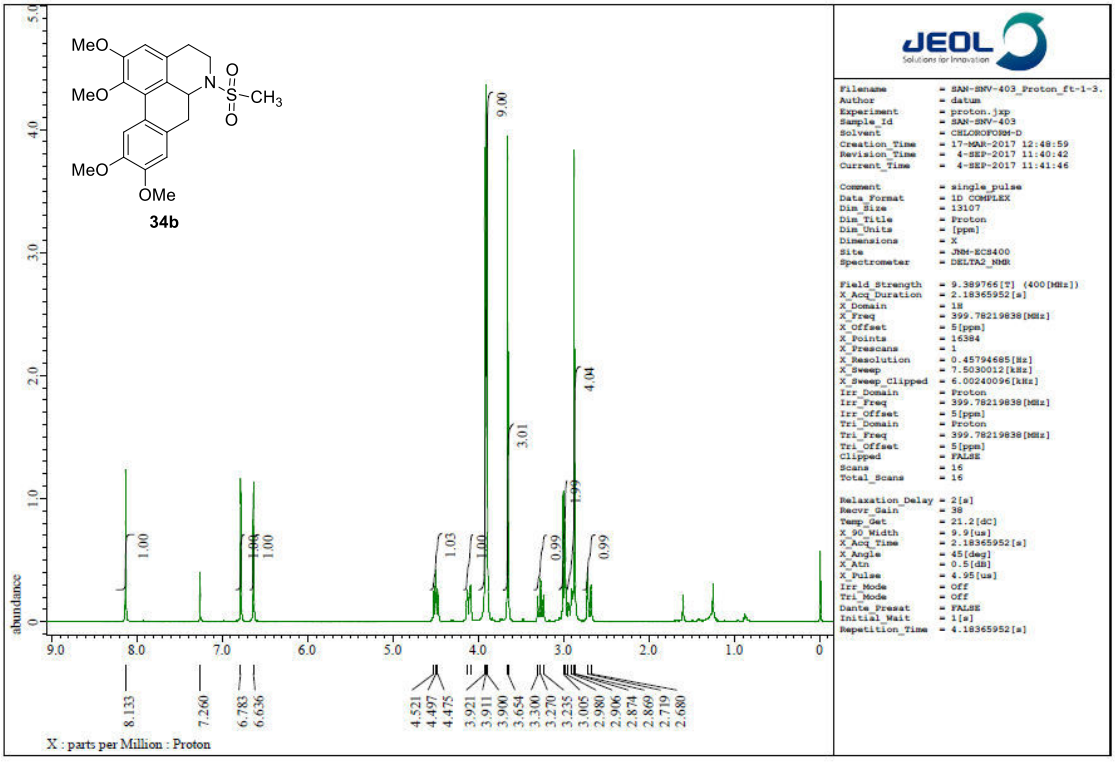


Figure 21.[1]H NMR Spectra of Compound **34b.**

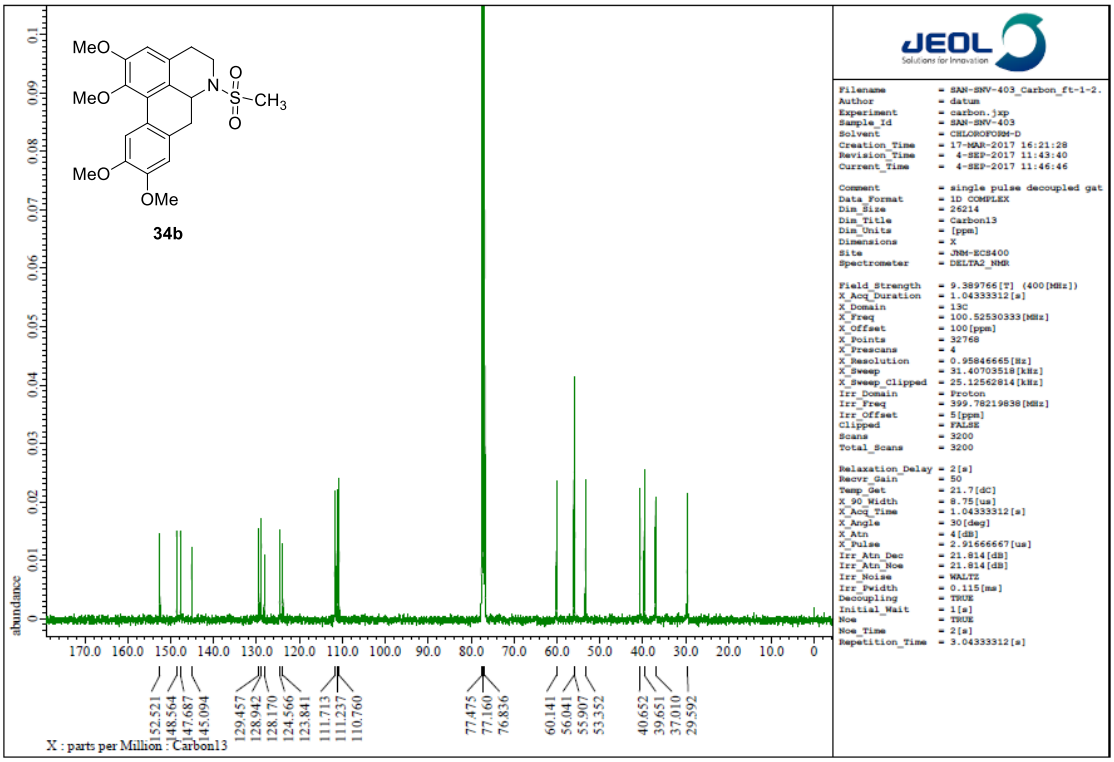


Figure 22.[13] C NMR Spectra of Compound **34b.**

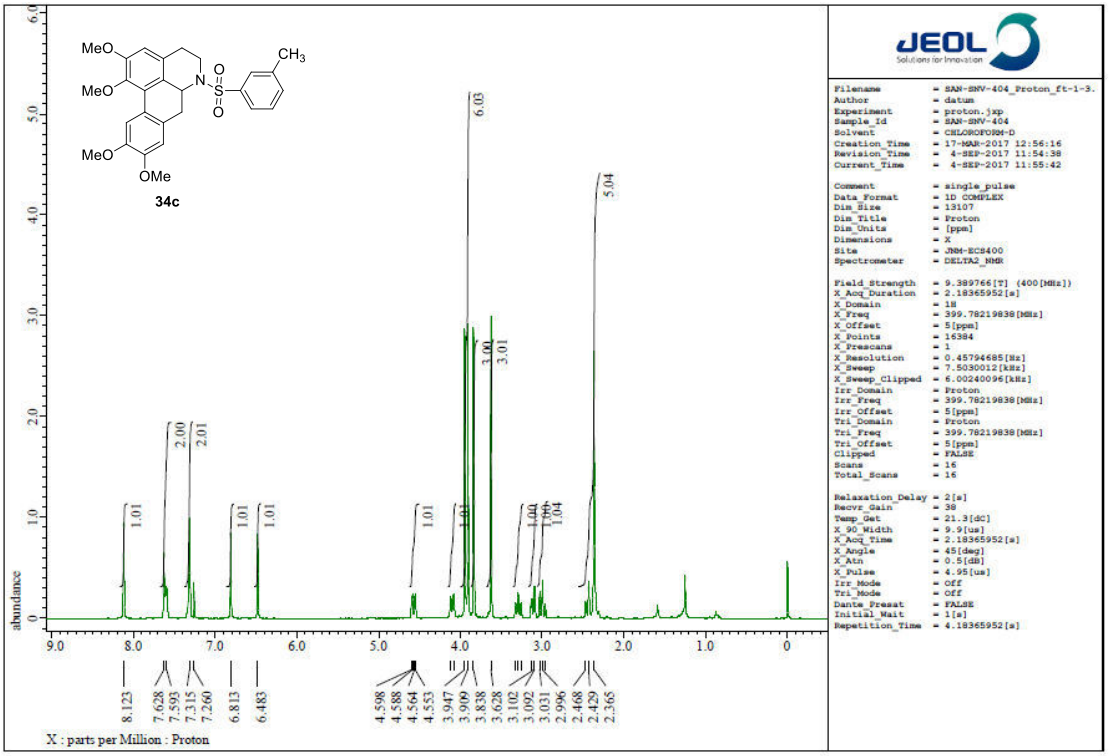


Figure 23.[1]H NMR Spectra of Compound **34c.**

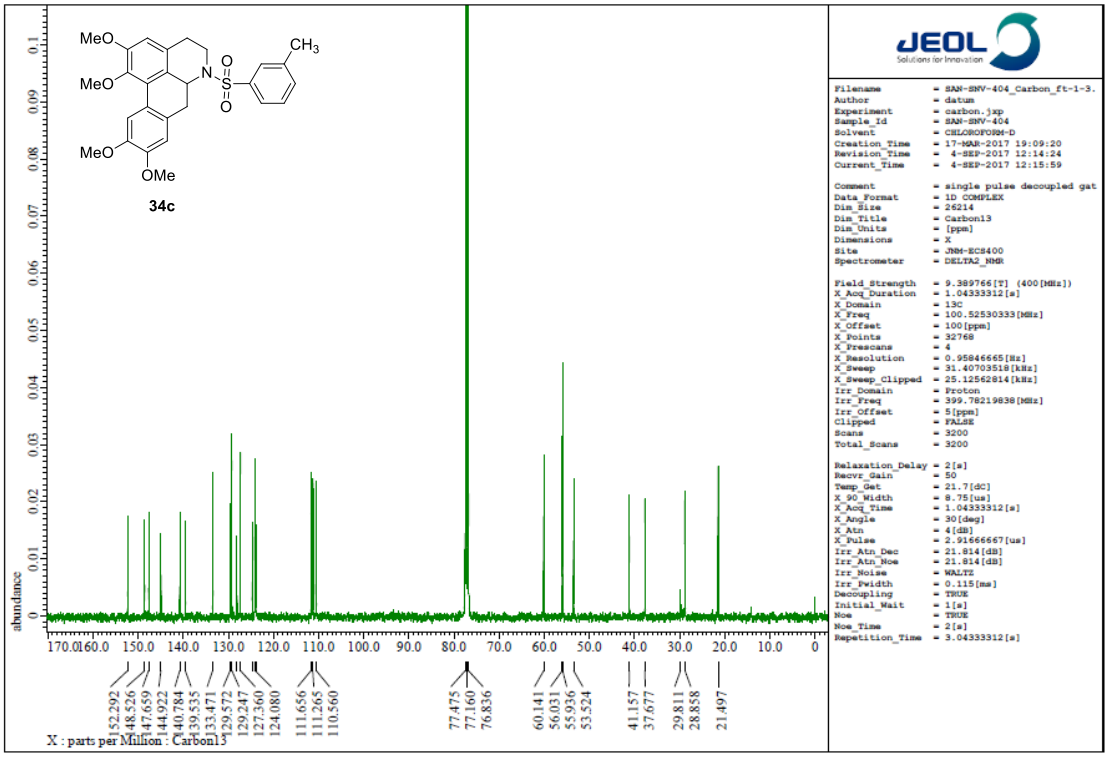


Figure 24.[13] CNMR Spectra of Compound **34c.**

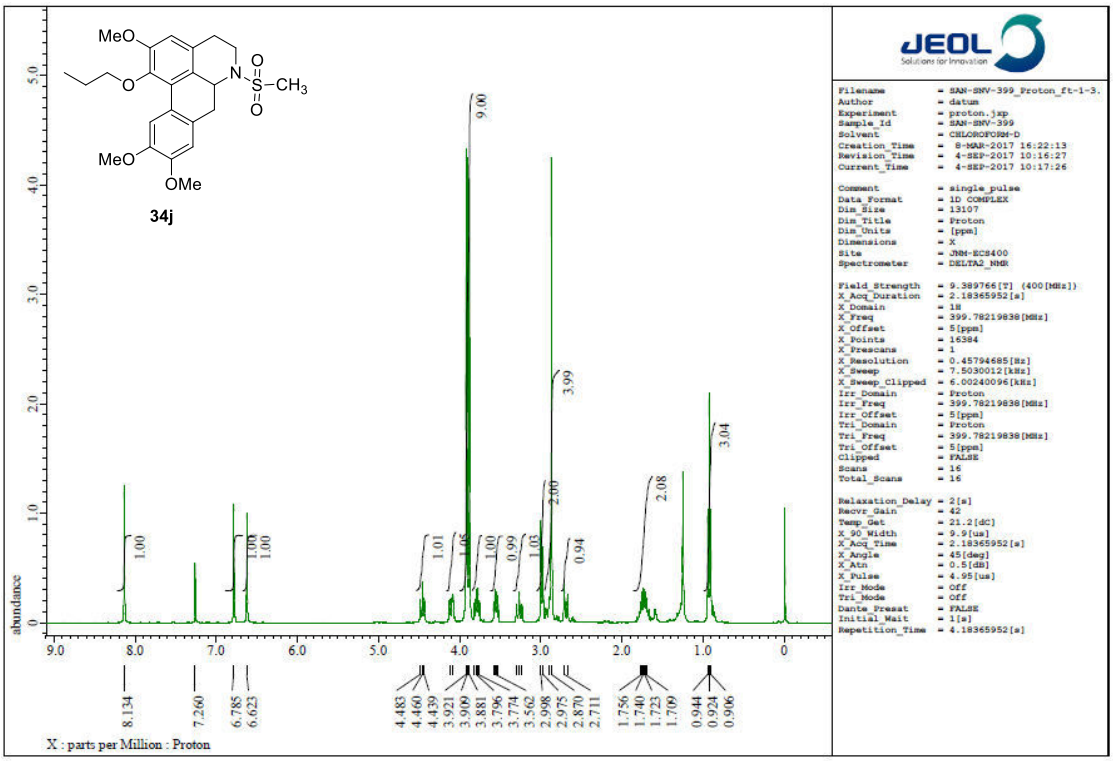


Figure 25.[1]H NMR Spectra of Compound **34j.**

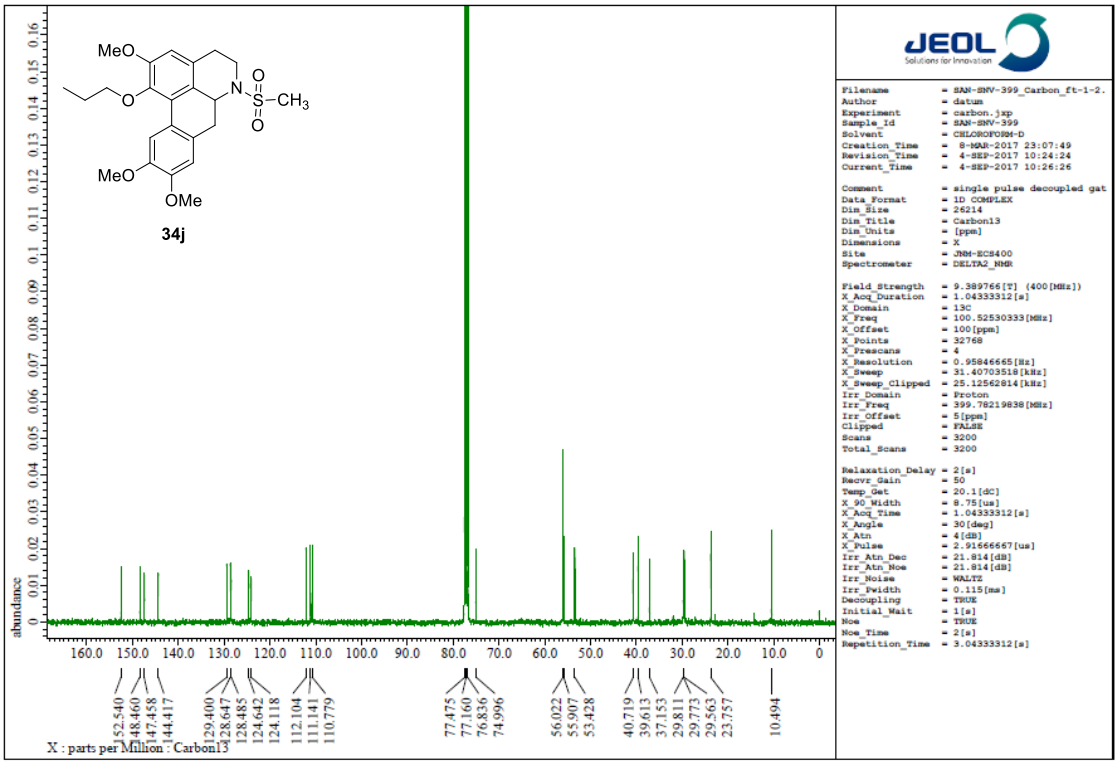


Figure 26.[13] C NMR Spectra of Compound **34j.**

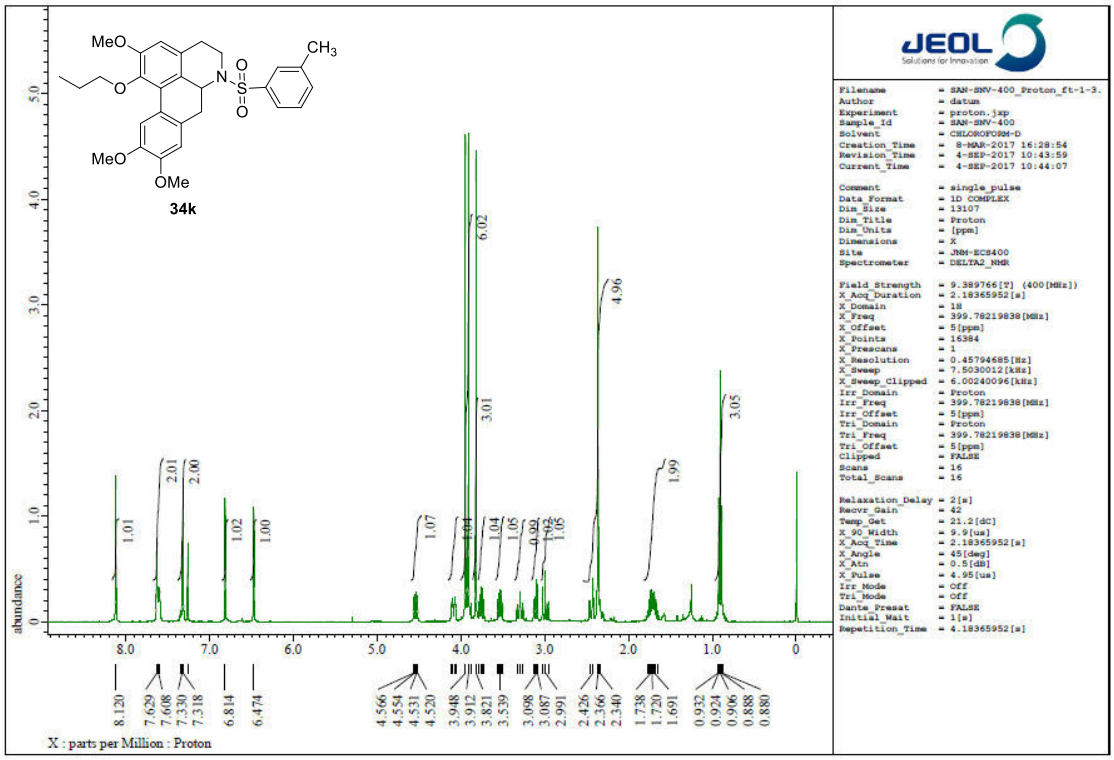


Figure 27.[1]H NMR Spectra of Compound **34k.**

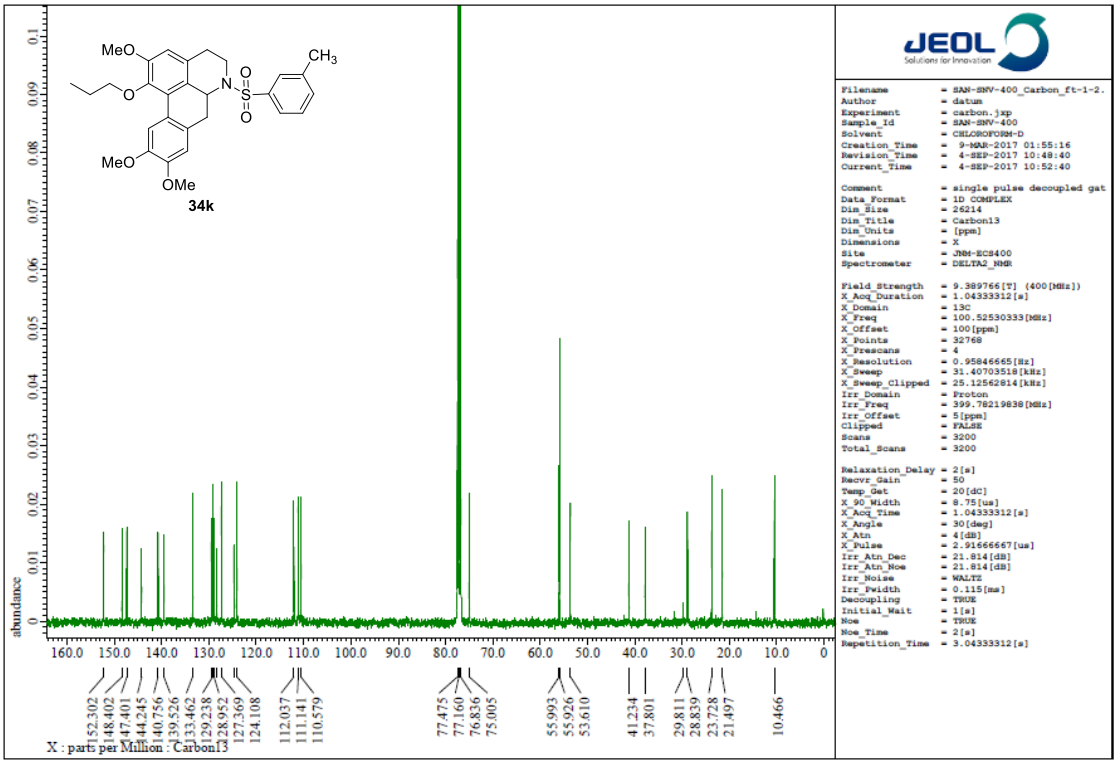


Figure 28.[13] C NMR Spectra of Compound **34k.**

Chapter 5

"*Novel Cephalandole A Analogues: Design, Synthesis, SAR and Biological Evaluation*"

5.1 Introduction

Cephalandole A [3-(1H-indol-3-yl)-2H-benzo[b][1,4]oxazin-2-one], a indole-benzo[1,4]oxazine hybrid, is a naturally occurring molecule which was isolated from the Taiwanese orchid *Cephalanceropsis gracilis* (Orchidaceae) in **2006.**[1]

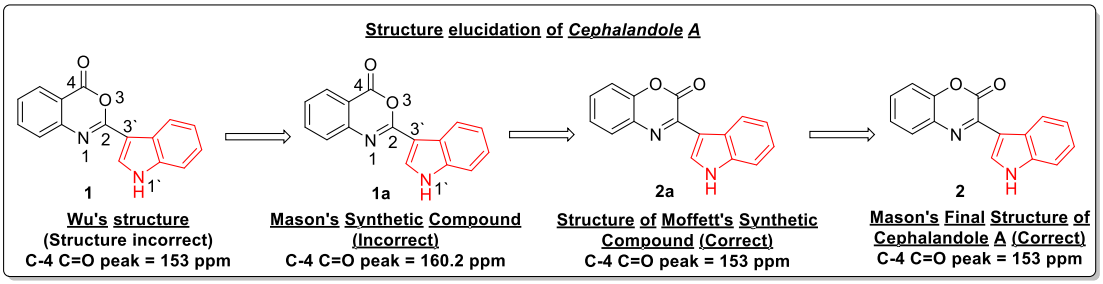


Figure 1. Structural revision of Cephalandole A natural product.

The initial structure of Cephalandole A was reported by Wu *et al*. in which C-4 carbonyl peak appeared at 153 ppm in ^{13}C NMR spectra of naturally isolated compound **1**.[1] Later on, Mason *et al.*[2] prepared synthetic compound **1a** in which the C-4 carbonyl value was at 160.2 ppm in ^{13}C NMR data. Moffett *et al.*[3] had already prepared Cephalandole A synthetically in which the ^{1}H and ^{13}C NMR spectrum of 2a was found exactly with **1**. Finally, Mason *et al.* proposed the compound **2** as the correct structure of Cephalandole A in which the C-4 carbonyl value was at 153 ppm in its ^{13}C NMR spectra which was in compliance with Wu's as well as Moffett's ^{13}C NMR spectral value of C-4 Carbonyl peak i.e. 153 ppm.

So far, only anticancer activity has been reported in this molecule. Its crude extract exhibited excellent activity against lung (NCI-H460; IC_{50} = 7.8 µM), breast (MCF-7; IC_{50} = 7.57 µM) and CNS (SF-268; IC_{50} = 12.2 µM) carcinoma cell lines. Since its discovery, no other biological activity has been reported yet.

Inspired by its unique hybrid structure of two bioactive moieties: *2-oxo-benzo[1,4]oxazine* as well as *indole*; it can be anticipated that this molecule might be able to show wide range of biological activities as it's individual moieties, itself, have several biological activities such as antibacterial, antiplatelet, antiulcerative, antimalarial, anticancer, antiviral, antileishmanial, antioxidant, antitubercular, immunomodulator, inhibition of chemical mediator's release and inhibition of leukotriene B_4 inhibition of tyrosinase inhibition of aldose - reductase activity.[4a-g]

Moreover, in chapter 2c of this thesis as well as other work from our laboratory, it has been revealed that, benzoxazines and its similar scaffold i.e. functionalized quinolones act as antimicrobial activity[5a-f] as well as promising thrombin inhibitory and

fibrinogen receptor antagonist activity[6] (figure 2). In addition, it is well documented that indoles also possesses good antimicrobial[7] as well as antiplatelet activities.[8]

The unique structural framework with bioactive moieties as subunits of **2** prompted us to study the medicinal chemistry of Cephalandole A in detail.

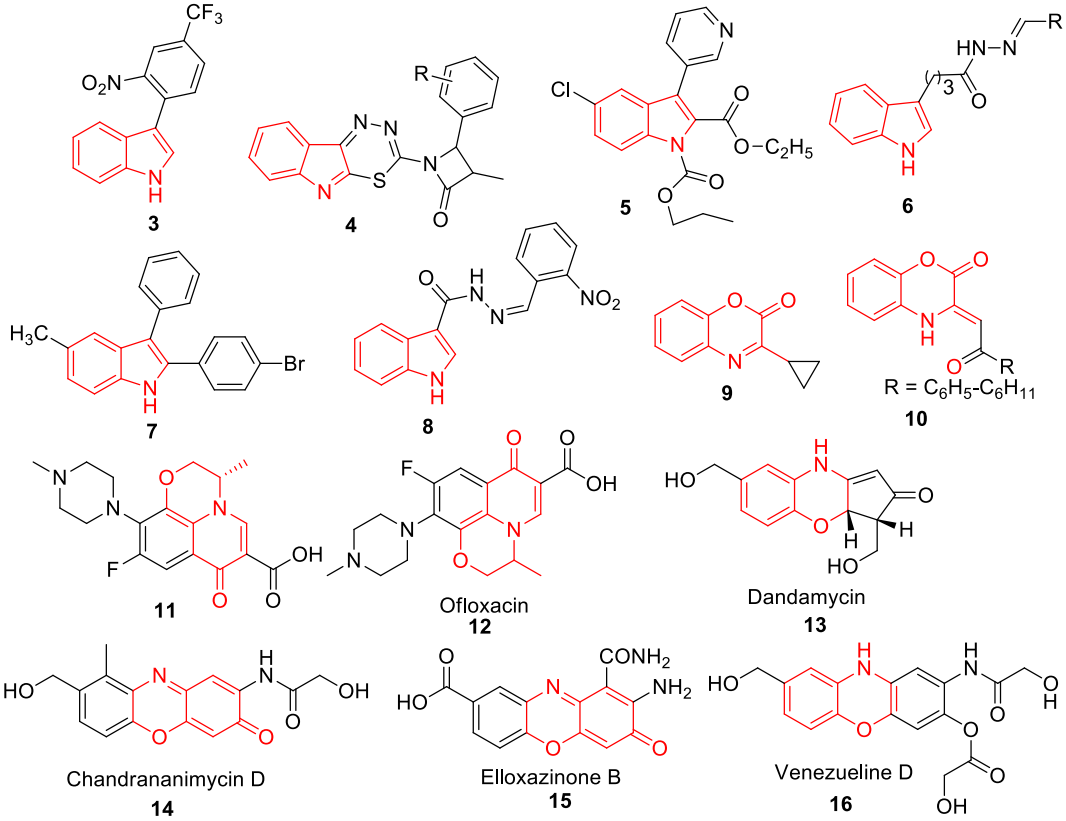


Figure 2. Few selected indoles and benzoxazines containing scaffolds as antibacterial and antiplatelet agent.

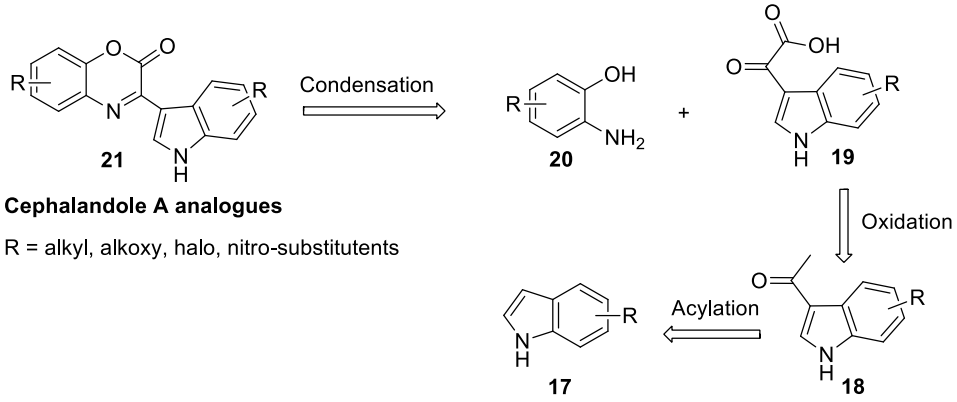
In our endeavour in search for novel bioactive heterocycles as new antimicrobial as well as antiplatelet agents, we have synthesized novel cephalandole A analogues **21a-p** and assessed them for their antimicrobial (antibacterial and antifungal) as well as AA-induced platelet aggregation inhibiting activities with the anticipation that these class of compounds would also show promising activity. So far, literature report revealed that there is no report available showing the antimicrobial as well as platelet aggregation inhibitory activity of the various analogues of Cephalandole A. To the best of our knowledge, for the first time, Cephalandole A analogues **21a-p**, have been identified as new class of antimicrobial as well as AA-induced platelet aggregation inhibitors.

5.2 Result and discussion

Chemistry

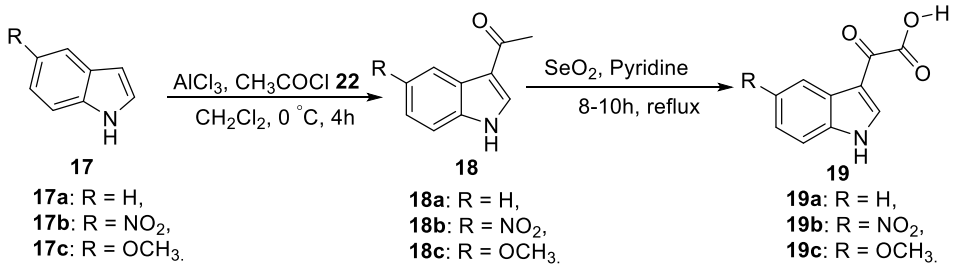
Few methodologies were reported in the literature for the synthesis of Cephalandole A **21a**.[9] Recently, we also have reported the environmentally benign green route for the synthesis of Cephalandole A under ultrasonic irradiation conditions in excellent yield (upto 81%).[10] However, we were unsuccessful in getting higher yields in the syntheses of the cephalandole A **21b-p** analogues using our protocol. Thus, still a modified method is needed to be developed for the synthesis of Cephalandole A analogues.

Its retro-synthetic analysis revealed that *Cephalandole A* analogues **21** can be constructed using substituted indoles **17** as starting material (Scheme 1).



Scheme 1: Retro-synthetic analysis.

The commercially available substituted indole **17a-c** were subjected to Friedel-Craft acylation reaction with acetyl chloride **22,** using reported protocol, furnished substituted 3-acetyl indole **18a-c** in excellent yields.[11] Substituted 3-acetyl indole **18a-c** were further converted into substituted 2-(1*H*-indol-3-yl)-2-oxoacetic acid **19a-c** by subsequent oxidation with SeO_2 via reported procedure.[12] (Scheme 2)

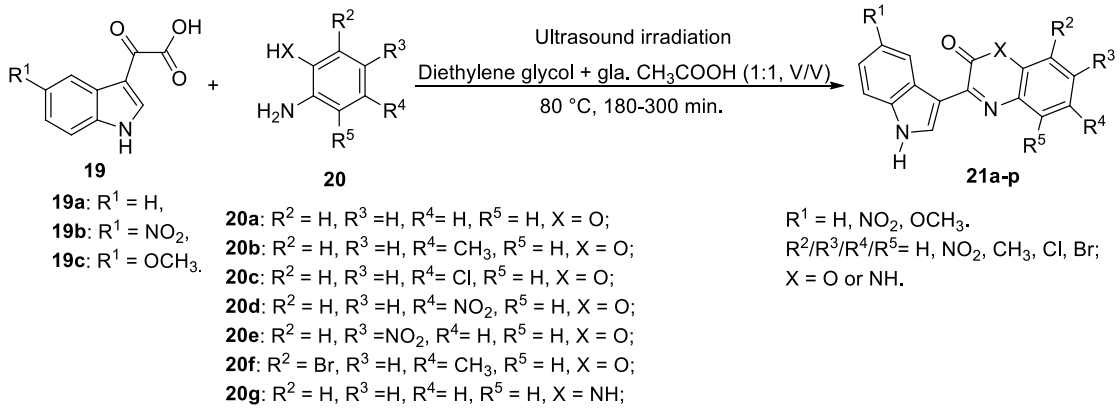


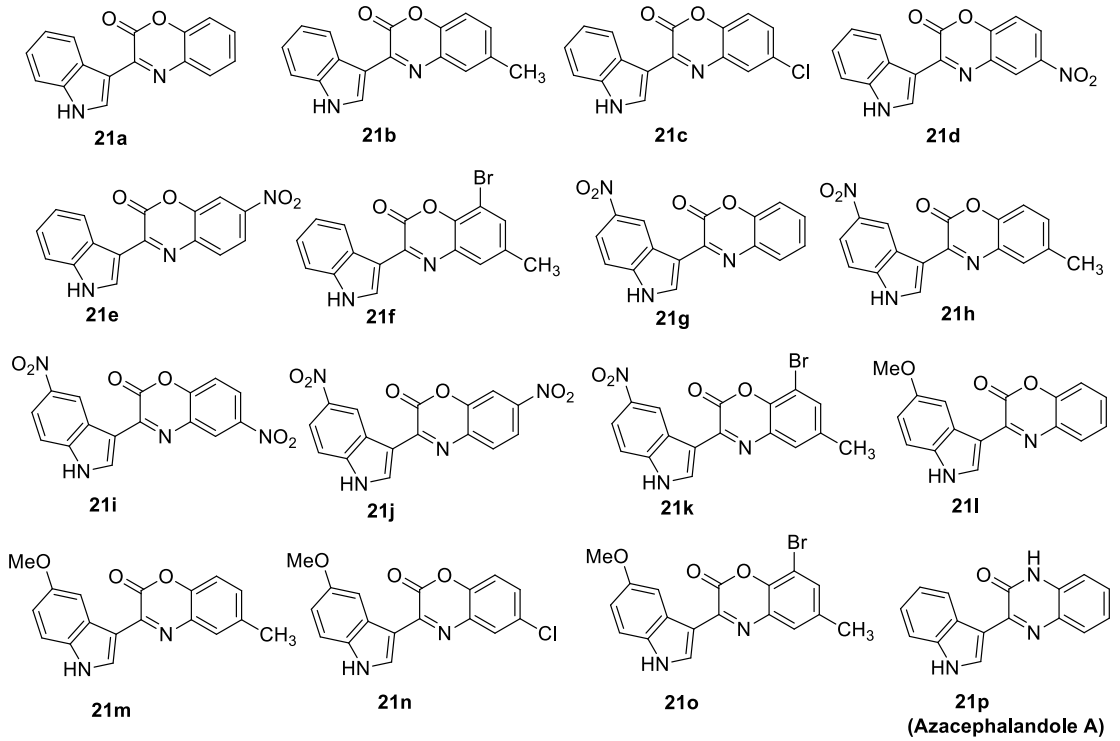
Scheme 2: Synthesis of substituted indole diketo-acid analogues **19a-c**.

The prepared 2-(1*H*-indol-3-yl)-2-oxoacetic acid **19a-c** were finally reacted with several substituted aminophenols **20a-f** and diaminobenzene **20g** under greener approach using different solvents or their combinations in order to optimize the protocol. After several permutations and combinations; diethylene glycol and gla.

AcOH (1:1, v/v) was found to be the best solvent system for the synthesis of Cephalandole A analogues **21a-p** in 58-79% yield range under ultrasound irradiation conditions (Scheme 3). The Cephalandole A analogues **21a-p** were purified either by flash column chromatography method or by recrystalization (see experimental section). The products obtained were fully characterized by its spectroscopic data (^{1}H and ^{13}C NMR, HRMS and IR).

Scheme 3. Synthesis of novel analogues of *Cephalandole A* (**21a-p**).





[a]Unless otherwise mentioned, all the reactions were carried out with substrates **19a-c** (0.2 mmol, 1eq) and **20a-g** (0.2 mmol, 1eq) in diethylene glycol and gla. AcOH (2.0 mL; 1:1, v/v) at 80 °C temperature for 180-300 min under ultrasound irradiation. [b]Isolated yield.

It has been observed that nitro-based cephalandole A analogues **21d-e** and **21g-k** were obtained in comparatively lesser yields. This is due to poor solubility in ethyl acetate, which makes the purification of these compounds via column chromatography very tedious and cumbersome (Scheme 3). In this study, the most characteristic feature observed was that a broad range of functional groups, like Cl, Br, OMe and NO_2 are well compatible in reaction conditions. Thus, these groups can further be manipulated to obtain new therapeutic molecules.

5.3 Biological evaluation

5.3.1 Antimicrobial Efficacy and Structure-Activity Relationship studies

Recently our group reported 2-oxo-benz[1,4]oxazines as promising antimicrobial agents.[5a] Since 2-oxo-benz[1,4]oxazine scaffolds is present in *Cephalandole A*; it is anticipated that *Cephalandole A* and its analogues may possesses antimicrobial agents.

Table 1. Minimum inhibitory concentration values for novel Cephalandole A (**21a-p**) and positive control drugs against bacteria and fungi[a]

S.No.	Compound Name	Bacterial Strains[b,c]				Fungal Strains[d]			
		***SG*[b]**	***SA*[b]**	***BS*[b]**	***EC*[c]**	***AN***	***PF***	***CA***	***TR***
1	**21a**	NA	NA	NA	NA	**100**	NA	**12.5**	NA
2	**21b**	**25**	NA	**50**	NA	**12.5**	NA	**50**	NA
3	21c	NA	NA	**12.5**	NA	NA	NA	**12.5**	NA
4	21d	NA	NA	NA	**100**	**12.5**	NA	NA	NA
5	21e	NA	**25**	NA	NA	**12.5**	NA	**12.5**	NA
6	21f	NA	NA	**100**	NA	NA	NA	**100**	NA
7	21g	NA	NA	**100**	NA	**100**	NA	NA	NA
8	21h	NA	NA	**100**	NA	**12.5**	NA	NA	NA
9	21i	**12.5**	NA	NA	NA	**25**	NA	**25**	NA
10	**21j**	**25**	NA	NA	NA	**12.5**	NA	**12.5**	NA
11	21k	**12.5**	NA	NA	NA	NA	NA	**100**	NA
12	21l	NA	**12.5**	NA	**50**	NA	NA	**100**	NA
13	**21m**	NA	**12.5**	NA	NA	**12.5**	NA	NA	NA
14	21n	NA	NA	NA	NA	**12.5**	NA	**100**	NA
15	21o	NA	NA	NA	NA	**12.5**	NA	NA	NA
16	**21p**	**12.5**	NA	**12.5**	NA	**12.5**	**100**	NA	NA
17	AMP[e]	12.5	12.5	25	12.5	------	-------	--------	-------
18	KET[f]	------	----	------	----	12.5	25	25	25

[a]MIC of all compounds was measured at the range from 6.25-100 µg/mL. [b]Gram-positive bacteria: SG, *Streptomyces griseus*; SA, *Staphylococcus aureus*; BS, *Bacillus subtilis*. [c]Gram-negative bacteria: EC, *Escherichia coli*, [d]Fungi: AN, *Aspergillus niger*; PF, *Penicillium funiculosum*; CA, *Candida albicans*; TR, *Trichoderma Reesei.* [e]AMP: Ampicillin. [f]KET: Ketoconazole. NA = Compounds which were found Not Active.

There is no report available in the literature showing antimicrobial activity of Cephalandole A and its analogues. Therefore, several novel analogues of *Cephalandole A* (**21a-p**) were prepared and screened, for the first time, for their *in vitro* antibacterial activity against gram-(+)ve and gram-(-)ve bacterial species (i.e. *S. griseus*, *S. aureus*, *B. subtilis* and *E. coli)* as well as *in vitro* antifungal activity against fungal species (i.e. *F. oxysporium*, *A. niger*, *P. funiculosum* and *T. reesei),* in comparison than standard drugs, ampicillin as well as ketoconazole, respectively. (Table 1)

5.3.1.1 Antibacterial Efficacy and Structure-Activity Relationship studies

Compounds (**21a-p**) 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), the antibiotic ampicillin was taken as positive control. Antibacterial screening of all the derivatives, **21a-p** as well as positive controls were performed at a concentration of 6.25 - 100 µg/mL.

The minimum inhibitory concentration (MIC) values for all the Cephalandole A analogues **21a-p** and the positive control drug ampicillin, were also determined against the four bacterial strains and the four fungal strains by the serial dilution method.[13a-f] In this study, several analogues exhibited superior antimicrobial activity compared to the positive control drugs, ampicillin and ketoconazole.

As it can be seen from Table 1; Compounds **21p** was identified as the most potent antibacterial agents of the series showing MIC value of 12.5 µg/mL against [BS] as well as [SG] strains respectively as it showed two times more activity than ampicillin against [BS] strain and equal potency than ampicillin against [SG] strains (Entry 16; Table 1). The next potent compound was **21c** (MIC = 12.5 µg/mL) which showed two times greater potency than ampicillin (MIC = 25 µg/mL) against [BS] strain. Furthermore, the next potent compounds found in this assay were **21i**, **21k**, **21l** and **21m** having MIC values of 12.5 µg/mL against [SG] and [SA] strains which showed equal potency than ampicillin. Compounds **21b**, **21e** and **21j** (MIC = 25 µg/mL) showed two times lesser potency than ampicillin against [SG] and [SA] strains. Finally, compounds **21b**, **21d**, **21f-h**, and **21l** which have shown (MIC = ≥25 µg/mL) in all strains were found to be either less active or no activity as compared to ampicillin. Overall, SAR study shows that introduction of nitrogen atom in

Cephalandole A i.e. **21p** increases its antibacterial potency. Lastly, it can be interpreted that substitution of electron-withdrawing group (EWG) i.e. NO_2 as well as halo group (-Cl, Br) at 2-oxo-benzo[1,4]oxazines moieties of Cephalandole A have significant beneficial effects on antibacterial activity than ampicillin except compound **21d**.

5.3.1.2 Antifungal Efficacy and Structure-Activity Relationship studies

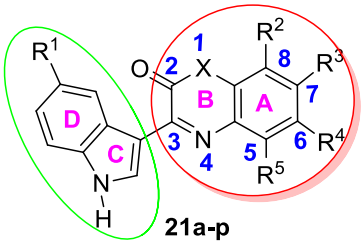
Compounds **21e** and **21j**, the most potent antifungal agents (MIC = 12.5 μg/mL) of the series, have shown comparable potency than ketoconazole (MIC = 12.5 μg/mL) in [AN] fungal strain and showed potency two times more than ketoconazole (MIC = 25 μg/mL) in [CA] fungal strain, respectively. In this study, it was observed that Cephalandole A analogues have shown antifungal potency, particularly to [AN] and [CA] strains. While compounds **21b**, **21d-e**, **21h**, **21j**, **21m-p** showed equal potency than ketoconazole (MIC = 12.5 μg/mL) in [AN] strain; compounds **21a**, **21c**, and **21j** showed two times greater potency than ketoconazole (MIC = 25 μg/mL) in [CA] strain. Compound **21i**, having nitro group at both indole and 2-oxo-benzo[1,4]oxazines moieties of Cephalandole A, showed equal potency in [CA] strain but showed less potency than ketoconazole in [AN] strain. All the compounds were found to be inactive in [PF] as well as [TR] strains except **21p** which showed MIC value of 100 μg/mL in [AN] strain. Finally, all those compounds which have shown (MIC = ≥50 μg/mL) in [AN], [PF], [CA] and [TR] strains were found to be either less active or no activity as compared to ketoconazole. Overall, SAR study shows that substitution of electron-donating group (EDG) i.e. OMe and CH_3 as well as halo group (-Cl, Br) either at indole moiety or at 2-oxo-benzo[1,4]oxazine moiety of Cephalandole A do not have significant beneficial effects on antifungal activity than ketoconazole. On the other hand, substitution of one electron-withdrawing group (EWG) i.e. NO_2 either at indole moiety or at 2-oxo-benzo[1,4]oxazine moiety of Cephalandole A also do not have significant beneficial effects on antifungal activity than ketoconazole. However, substitution of nitro group both at indole moiety and at 2-oxo-benzo[1,4]oxazine moiety of Cephalandole A showed improved activity. The probable reason for showing less activity or no activity at all in some of the fungal strains such as [PF] and [TR] strains could be because of their resistant nature. Structurally, ketoconazole have imidazole and piperazine moieties whereas cephalandole A have indole and 2-oxo-benzo[1,4]oxazine moieties. Hence, the probable reason for showing less or no activity in Cephalandole A could be due to

difference in their chemical structure. However, slight similarity in chemical structure could be the possible reason for activity in [AN] strain.

5.3.2 Platelet aggregation inhibitory activity evaluation and SAR studies

All the synthesized cephalandole A analogues **21a-o** along with aza-cephalandole A (**21p)** were screened for their arachidonic acid (AA) induced-platelet aggregation inhibitory activities using aspirin as the standard reference drug (IC_{50} = 21.34±1.09 µg/mL) and the results are shown in Table 2.

Table 2. AA-induced platelet aggregation inhibitory activity for novel Cephalandole A (**21a-p**).[a,b]



21a-p

S.No.	Compound Name	R^1	R^2	R^3	R^4	R^5	X	Antiplatelet Activity[a,b] (IC_{50} in µg/ml)
1	**21a**	H	H	H	H	H	O	**19.34±0.27**
2	21b	H	H	H	CH_3	H	O	31.56±0.88
3	21c	H	H	H	Cl	H	O	71.56±1.09
4	**21d**	H	H	H	NO_2	H	O	**20.21±0.28**
5	21e	H	H	NO_2	H	H	O	45.67±1.02
6	21f	H	Br	H	CH_3	H	O	44.32±0.98
7	**21g**	NO_2	H	H	H	H	O	**18.99**±0.21
8	21h	NO_2	H	H	CH_3	H	O	111.83±1.87
9	21i	NO_2	H	H	NO_2	H	O	30.18±0.72
10	**21j**	NO_2	H	NO_2	H	H	O	**21.59**±0.31
11	21k	NO_2	Br	H	CH_3	H	O	27.33±0.74
12	21l	OCH_3	H	H	H	H	O	52.46 ±1.06
13	21m	OCH_3	H	H	CH_3	H	O	56.81±1.11
14	21n	OCH_3	H	H	Cl	H	O	60.77±1.23
15	21o	OCH_3	Br	H	CH_3	H	O	54.54±1.08
16	21p	H	H	H	H	H	NH	104.45±1.63
17	Aspirin	---	---	---	---		---	**21.34±1.09**

[a] Platelets were incubated along with either a tested compound or 0.5% DMSO at 37°C for 60 sec., then AA (100 µM) was added to accelerate the aggregation. Aspirin and indomethacin are positive controls. Values are expressed as mean_SE from three to three separations. [b] The data represent mean of three independent determination.

For SAR study; the structure of cephalandole A i.e. **21a** can be interpreted as a 2-oxo-benzo[1,4]oxazine containing bicyclic rings A and B linked at C-3 position of

substituted indole rings C and D (Table 1). As depicted from Table 2, the natural product cephalandole A i.e., **21a** which has no substitutions on ring A or D; displayed greater AA-induced platelet aggregation inhibitory activity (IC_{50} = **19.34±0.27** μg/mL) in comparison to the standard reference drug aspirin (IC_{50} = **21.34±1.09** μg/mL). The promising antiplatelet activity of the natural product prompted us to prepare analogues of cephaalandole A and study their platelet aggregation inhibitory activities. Therefore, **21b-f** having various electron-withdrawing groups (EWG) as well as electron-donating groups (EDG) at ring A or ring D were prepared to study its SAR in detail. Compound **21d** having EWG i.e. NO_2 at C-6 position on ring A showed greater antiplatelet activity (IC_{50} = **20.21±0.28** μg/mL) than aspirin (entry 4; Table 2). Changing the position of NO_2 group diminishes the activity as in compound **21e** (IC_{50} = **45.67** μg/mL, entry 5; Table 2).

Further, putting EDG do not have substantial improvement in the antiplatelet activity which lead us to infer that electron-donating group do not have profound effect on antiplatelet activity whereas, EWG increases AA-inducted platelet aggregation inhibitory activity (entry 2-3 and 6; Table 2).

Since EWG inceases antiplatelet activity of Cephalandole A analogues; we were interested to study the effect of EWG on indole ring. Thus, we prepared **21g** and further substituted analogues at ring D i.e., **21h-k** and assessed for their antiplatelet activities. Compound **21g** showed high order of AA-induced platelet aggregation activity having IC_{50} value of **18.99 ±0.21** μg/mL and was found to be the active compound of the series (entry 7; Table 2). Then, electron-donating group i.e. CH_3 was introduced at C-4 position of ring A which lead to complete loss of antiplatelet activity (entry 8; Table 2). However, we were delighted when we observe the restoration of activity by the introduction of NO_2 group at C-3/4 position of ring A (entry 9-10; Table 2). The antiplatelet activity of compound **21j** (IC_{50} = **21.59±0.31** μg/mL)**,** was found slightly lesser than **21g** and exhibited comparable activity to aspirin (entry 10; Table 2). Similarly, **21i** (IC_{50} = **30.18±0.72** μg/mL, entry 9; Table 2), having NO_2 group at C-6 position of ring D, also displayed lesser activity than **21j**.

We were further interested to study the effect of EDG i.e., OMe group in indole moiety of cephalandole A. Thus, we prepared cephalandole A analogues **21l-o** and evaluated for their antiplatelet activity. Thus, no substitution in 2-oxo-benzo[1,4]oxazine moiety leads to substantial decrease in activity as in **21l** (IC_{50} = **52.46±1.06** μg/mL, entry 12; Table 2), Similarly, there is no further improvement in

antiplatelet activity was observed by substituting halo (Cl, Br) or CH_3 group (Compound **21n-o**; entry 14-15; Table 2).

In addition to the above; we also studied the effect of N-atom or O-atom in benzo[1,4]oxazine moiety of cephalandole A. Thus, we prepared the first aza analogues of cephalandole A i.e. **21p** through replacement of O-atom with N-atom, known as "*Azacephalandole A*". Unfortunately, it displayed very less platelet aggregation inhibitory activity with having IC_{50} value of **104.45±1.63** μg/mL (entry 16; Table 2) than aspirin (IC_{50} = **21.34±1.09** μg/mL) as well as the parent natural product cephalandole A **21a** (IC_{50} = **19.34±0.27** μg/mL).

Based on the above SAR study; it may be concluded that introduction of EWG or EDG have significant effect on antiplatelet activity. While EWG increases AA-induced platelet aggregation inhibitory activity, EDG leads to either decrease or complete loss of biological activity. Further, the positional effect of EWG i.e., NO_2 group was also interpreted. It was observed that by placing two nitro groups, one at indole ring and another at C-6 or C-7 position of 2-oxobenzo[1,4]oxazine moiety i.e., in compound **21i** and **21j**, respectively, resulted significant change in the AA-induced platelet aggregation inhibitory activity.

5.4 Conclusions

In summary, we have synthesized novel functionalized cephalandole A analogues **21a-p** under ultrasound irradiation conditions in an excellent yields (upto 79%). The synthetic methodology used tolerates a broad range of functional groups and their positions under simple reaction conditions, and also provides a straightforward access to a series of cephalandole A analogues **21a-p** from readily available starting substrates. To the best of our knowledge, this is the first report of ultrasound-assisted synthesis of various functionalized cephalandole A analogues (**21b-o**) and their screened for their *in vitro* antimicrobial as well as AA-induced platelet aggregation inhibitory activity. In addition, we also include, for the first time, the synthesis of azacephalandole A **21p** and its biological activity. Several derivatives were found to show promising *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,* better than standard drugs, (i.e., ampicillin as well as ketoconazole). Moreover, Compounds **21a, 21d, 21g and 21j**, exihibited excellent AA-induced platelet aggregation inhibitory activities, which were found to be comparable to that of

aspirin. The promising biological activity of cephalandole A analogues combined with the ease of preparation will found them as a promising scaffold worthy of further structural optimization to develop them as potent antimicrobial or antiplatelet agents.

5.5 Experimental Details & Characterization Data

5.5.1 General experimental

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. Ultrasonic irradiation was performed in a Elmasonic S 30 (H) ultrasonic water bath cleaner and the reaction vessel was positioned in the maximum energy area in the cleaner and the removal or addition of water was used to control the temperature of the water bath. 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 Q Tof (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.

5.5.2 General procedure for the Synthesis of functionalized 2-(1*H*-indol-3-yl)-2-oxoacetic acid 19a-c:

To a CH_2Cl_2 solution (15 mL) of indole **17a-c** (0.2 mmol, 1eq) was added anhyd. $AlCl_3$ (0.3 mmol, 1.5eq) at 0 °C. The mixture was stirred at 0 °C for 30 min. Then, to this solution was added dropwise acetyl chloride **22** (0.2 mmol, 1.1eq) dissolved in CH_2Cl_2 solution (2 mL) at 0 °C. The resulting solution was stirred at 0 °C for 4 h. After completion of the reaction, maintained at around pH 7, aqueous buffer was added to quench the reaction. After usual workup, the crude product was purified by silica gel using ethyl acetate: hexane as an eluant to furnish substituted 3-acetylindole **18a-c** in 72- 87% yields.

In the next step; substituted 3-acetylindole **18a-c** (0.2 mmol, 1eq) and selenium dioxide (0.3 mmol, 1.5 eq.) in pyridine (15 mL) were heated to 100 °C for 10 h. The selenium was filtered through celite pad and the orange solution was concentrated in

vacuo to approximately 5 mL in volume. The residue was dissolved in 100 mL of 5% NaOH and extracted with ethyl acetate (3 × 15 mL). The aqueous layer was acidified with 2N HCl solution (1-2 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with water (25 mL), then with brine (2 × 15 mL), dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure. The crude products were then purified either by recrystalization method or by flash column chromatography method over silica gel using MeOH/$CHCl_3$ (1:3, v/v) as an eluant which afforded the pure 2-(1*H*-indol-3-yl)-2-oxoacetic acid analogues **19a-c** in 62-82 % yields.

5.5.3 General procedure for the Synthesis of cephalandole A analogues 21a-p:

To a solution of **19a-c** (0.2 mmol, 1eq) in diethylene glycol and Gla. AcOH (1:1, v/v) was added **20a-g** (0.2 mmol, 1eq) and the reaction mixture was irradiated under ultrasonic sonicator at 80 ˚C temperature for about 120-300 min. (depending upon the substrate employed). The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was filtered off by passing through the celite pad, washed with distilled water (3 × 50 ml), and then with brine (2 × 30 ml), dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. The crude product were purified either by recrystallization using EtOAc/hexane; or by flash column chromatography method over silica gel using hexane/ethyl acetate as an eluent which afforded the pure cephalandole A analogues **21a-p** in good yields (58-79 %).

5.5.4 Characterization data of cephalandole analogues 21a-p:

3-(1H-indol-3-yl)-2H-benzo[b][1,4]oxazin-2-one (21a)

Yellowish solid; yield: 79%, m.p. 229-231 °C; FT-IR (KBr, νmax/cm-1) 3292, 3053, 2921, 2852, 1713, 1604, 1428, 1151; ^{1}H NMR (400 MHz) δ 11.99 (s, 1H), 8.77 – 8.75 (m, 1H), 8.70 (s, 1H), 7.84 (d, J = 6.4 Hz, 1H), 7.55 – 7.53 (m, 1H), 7.48 – 7.38 (m, 3H), 7.29 – 7.24 (m, 2H); ^{13}C NMR (100 MHz) δ 152.1, 147.9, 144.9, 136.6, 133.8, 131.9, 128.7, 127.7, 126.0, 125.3, 123.1, 122.9, 121.6, 115.9, 112.2, 110.6; HRMS (ESI) calcd. for $C_{16}H_{10}N_2O_2$ $[M+H]^+$: 263.0742; found 263.0744.

3-(1*H*-indol-3-yl)-6-methyl-2*H*-benzo[*b*][1,4]oxazin-2-one (21b)

Yellowish solid; yield: 77%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3411, 2923, 1714, 1629, 1529, 1444, 1063, 880; ^{1}H NMR (400 MHz, DMSO) δ 11.03 (s, 1H) δ 8.60-8.48 (m, 2H), δ 7.41 (s, 1H), δ 7.24 (s, 1H), δ 7.04-7.03 (m, 2H) δ 6.97-6.91 (m, 2H), δ 2.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 152.3, 147.2, 142.5, 136.4, 134.6,

133.2, 131.6, 129.1, 127.6, 125.8, 122.7, 122.7, 121.3, 114.9, 115.5, 110.8, 20.5; HRMS (ESI) calcd. For $C_{17}H_{12}N_2O_2$ $[M+H]^+$: 277.0899 ; found 277.0893.

6-chloro-3-(1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21c)

Yellowish solid; yield: 67%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3365, 2927, 1723, 1599, 1473, 1101, 799; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H) δ 8.67-8.63 (m, 2H), δ 7.78 (s, 1H), δ 7.52-7.50 (m, 1H), δ 7.42-7.39 (m, 1H), δ 7.33-7.31 (m, 1H), δ 7.27-7.21(m, 2H); ^{13}C NMR (100 MHz, DMSO) 151.5, 148.4, 143.6, 136.6, 134.1, 132.7, 128.9, 127.8, 126.5, 125.9, 123.0, 122.8, 121.6, 117.2, 112.0, 110.6; HRMS (ESI) calcd. For $C_{16}H_9ClN_2O_2$ $[M+2]^+$: 298.0353; found 298.0358.

3-(1*H*-indol-3-yl)-7-nitro-2*H*-benzo[*b*][1,4]oxazin-2-one (21d)

Yellowish solid; yield: 65%, m.p. > 300 °C; FT-IR (KBr, νmax/cm-1) 3373, , 2921, 1718, 1605, 1531, 1477, 1244, 1105, 941; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.67 (s, 1H), 8.70 (d, J = 8.4Hz, 1H), 8.63 (s, 1H), 7.81 (d, J = 8Hz, 1H), 7.53 – 7.33 (m, 3H), 7.28 – 7.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.9, 147.6, 144.7, 136.6, 133.5, 131.8, 128.6, 127.5,125.9, 125.2, 122.9, 122.5, 121.3, 115.6, 111.9, 110.6; HRMS (ESI) calcd. for $C_{16}H_9N_3O_4$ $[M+H]^+$: 308.0593; found 308.0596.

3-(1*H*-indol-3-yl)-6-nitro-2*H*-benzo[*b*][1,4]oxazin-2-one (21e)

Yellowish solid; yield: 62%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3414, 2922, , 1753, 1619, 1524, 1424, 1109, 877; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.89 (s, 1H), 8.76 – 8.70 (m, 2H), 8.56 (d, J = 2.8 Hz, 1H), 8.23 (dd, J = 2.0, 8.4 Hz, 1H), 7.57 – 7.53 (m, 2H), 7.29 – 7.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.6, 148.9, 144.4, 136.5, 134.5, 131.7, 125.7, 122.9, 122.6, 122.5, 122.1, 122.0, 121.7, 116.7, 111.9, 110.3; HRMS (ESI) calcd. for $C_{16}H_9N_3O_4$ $[M+H]^+$: 308.0593; found 308.0598.

8-bromo-3-(1*H*-indol-3-yl)-6-methyl-2*H*-benzo[*b*][1,4]oxazin-2-one (21f)

Yellowish solid; yield: 73%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3429, 2922, 1717, 1617, 1572, 1494, 1113, 751; ^{1}H NMR (400 MHz, DMSO) δ 11.51 (s, 1H) 8.66-8.63 (m, 2H), δ 7.50 (s, 1H), δ 7.41-7.40 (m, 1H), δ 7.32 (s, 1H), δ 7.18 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 151.1, 147.1, 139.2, 136.1, 135.1, 133.5, 132.1, 131.5, 126.7, 125.4, 122.5, 122.3, 121.1, 111.4, 110.0, 107.2, 19.8; HRMS (ESI) calcd. for $C_{17}H_{11}BrN_2O_2$ $[M+2]^+$: 356.0004; found 356.0009.

3-(5-nitro-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21g)

Yellowish solid; yield: 67%, m.p > 300 °C; FT-IR (KBr, νmax/cm-1) 3408, 2921, 1731, 1606, 1517, 1463, 1331, 1099, 780; ^{1}H NMR (400 MHz, DMSO) δ 12.09 (s,

1H) 9.41 (s, 1H), δ 8.61 (s, 1H), δ 7.97-7.94 (m, 1H), δ 7.72-7.69 (m, 1H), δ 7.41-7.33 (m, 3H) δ 7.17-7.15 (m, 1H); ^{13}C NMR (100 MHz, DMSO) 151.3, 146.4, 144.5, 142.2, 139.4, 135.7, 131.1, 128.9, 127.7, 124.9 (2C), 119.3, 117.8, 115.4, 111.9, 111.7; HRMS (ESI) calcd. for $C_{17}H_{10}BrN_3O_4$ $[M+H]^+$: 308.0593; found 308.0599.

6-methyl-3-(5-nitro-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21h)

Yellowish solid; yield: 64%, m.p. > 300 °C; FT-IR (KBr, νmax/cm-1) 3426, 2920, 2852, 1725, 1621, 1589, 1437, 1114, 808; ^{1}H NMR (400 MHz, DMSO) δ 12.4 (s, 1H), δ 9.33 (s, 1H) 8.58 (s, 1H), δ 7.98 (d, J = 10.8 Hz, 1H), δ 7.51 (d, J = 8.8 Hz 1H), δ 7. 40 (s, 1H), δ 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 151.8, 146.8, 142.8, 139.7, 136.1, 135.9, 135.2, 131.1, 130.4, 127.6, 125.2, 119.2, 118.2, 115.6, 112.5, 112.2, 20.2; HRMS (ESI) calcd. for $C_{18}H_{11}N_3O_4$ $[M+H]^+$: 322.0750; found 322.0755.

6-nitro-3-(5-nitro-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21i)

Yellowish solid; yield: 58%, m.p. > 300 °C; FT-IR (KBr, νmax/cm-1) 3376, 1759, 1631, 1574, 1419, 1308, 1229, 1112; ^{1}H NMR (400 MHz, DMSO) δ 9.02 (s, 1H) δ 8.73 (s, 1H), δ 8.34 (s , 1H), δ 8.04 (d, J = 8.8 Hz, 1H), δ 7.69-7.63 (m, 2H); δ 7.38-7.36 (m, 1H); HRMS (ESI) calcd. For $C_{16}H_8N_4O_6$ $[M+H]^+$: 353.0444 ; found 353.0449.

7-nitro-3-(5-nitro-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21j)

Yellowish solid; yield: 62%, m.p. > 300 °C; FT-IR (KBr, νmax/cm-1) 3376, 1760, 1630, 1576, 1422, 1303, 1233, 1116; ^{1}H NMR (400 MHz, DMSO) δ 9.06 (s, 1H) δ 8.39 (s, 1H), δ 8.05 – 8.02 (m , 1H), δ 7.68 – 7.51 (m, 3H), δ 6.63 (d, J = 8.8 Hz, 1H); HRMS (ESI) calcd. For $C_{16}H_8N_4O_6$ $[M+H]^+$: 353.0444 ; found 353.0443.

8-bromo-6-methyl-3-(5-nitro-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21k)

Yellowish solid; yield: 61%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3414, 2922, 1734, 1684, 1575, 1466, 1010, 830; ^{1}H NMR (400 MHz, DMSO) δ 9.37 (s, 1H) 8.66 (s, 1H), δ 8.03 (s, 2H), δ 7.59 (d, J = 8.8 Hz 1H), δ 7. 46 (s, 1H), δ 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 151.0, 147.3, 142.6, 139.8, 139.5, 136.5, 135.9, 132.6, 132.0, 127.0, 124.9, 118.7, 118.0, 112.5, 111.6, 107.3, 19.63; HRMS (ESI) calcd. for $C_{17}H_{10}BrN_3O_4$ $[M+2]^+$: 400.9855; found 400.9859.

3-(5-methoxy-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21l)

Yellowish solid; yield: 75%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3441, 2935, 1722, 1535, 1428, 1122, 588; ^{1}H NMR (400 MHz, DMSO) δ 11.63 (s, 1H) δ 8.60 (s, 1H), δ 8.29 (s, 1H), δ 7.81 (d, J = 7.6 MHz, 1H), δ 7.43-7.34 (m, 3H) δ 6.95-6.86 (m, 2H), δ 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 155.3, 151.8, 147.5, 144.6, 133.6,

131.7, 131.4, 128.2, 127.3, 126.6, 125.0, 118.9, 115.5, 112.3, 110.2, 105.5, 55.4; HRMS (ESI) calcd. For $C_{17}H_{12}N_2O_3$ $[M+H]^+$:293.0848 ; found 293.0842.

3-(5-methoxy-1*H*-indol-3-yl)-6-methyl-2*H*-benzo[*b*][1,4]oxazin-2-one (21m)

Yellowish solid; yield: 79%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3310, 2926, 2852, 1709, 1619, 1590, 1435, 1035, 874; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.39 (s, 1H), 8.58 (s, 1H), δ 8.27 (s, 1H), δ 7.50 (s, 1H), δ 7.30 (d, J = 8.8 Hz, 1H), δ 7.13 – 7.07 (m, 2H), δ 6.81 (d, J = 8.0 Hz, 1H), δ 3.86 (s, 3H), δ 2.38 (s, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) 155.2, 152.2, 147.2, 142.4, 134.6, 133.6, 131.5, 131.4, 128.9, 127.4, 126.6, 115.0, 112.3, 112.2, 110.4, 104.9, 55.2, 20.4; HRMS (ESI) calcd. for $C_{18}H_{14}N_2O_3$ $[M+H]^+$: 307.1004; found 307.1009.

6-chloro-3-(5-methoxy-1*H*-indol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-2-one (21n)

Yellowish solid; yield: 68%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3404, 2926, 1730, 1609, 1527, 1483, 1111, 801; ^{1}H NMR (400 MHz, DMSO) δ 11.73 (s, 1H) δ 8.62 (s, 1H), δ 8.23 (s, 1H), δ 7.81 (s, 1H), δ 7.44-7.35 (m, 3H) δ 6.93-6.90 (m, 1H), δ 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 156.3, 152.1, 149.2, 144.2, 135.3, 135.1, 133.5, 132.2, 129.6, 128.3, 127.4, 127.0, 117.9, 113.3, 110.9, 106.3, 56.3; HRMS (ESI) calcd. For $C_{17}H_{11}ClN_2O_3$ $[M+2]^+$: 328.0458 ; found 328.0452.

8-bromo-3-(5-methoxy-1*H*-indol-3-yl)-6-methyl-2*H*-benzo[*b*][1,4]oxazin-2-one (21o)

Yellowish solid; yield: 74%, m.p. > 200 °C; FT-IR (KBr, νmax/cm-1) 3411, 2933, 1729, 1615, 1587, 1477, 1105, 846; ^{1}H NMR (400 MHz, DMSO) δ 11.68 (s, 1H) δ 8.59 (s, 1H), δ 8.24 (s, 1H), δ 7.60 (s, 1H), δ 7.50 (s, 1H), δ 7.42 (d, J = 8.4Hz, 1H), δ 6.93-6.90 (m, 1H) δ 3.87 (s, 3H), δ 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO) 155.3, 151.2, 147.7, 142.5, 139.6, 135.6, 134.1, 132.5, 131.6, 131.4, 130.4, 126.7, 126.5, 121.0, 112.3, 109.9, 55.4, 19.6; HRMS (ESI) calcd. For $C_{18}H_{13}BrN_2O_3$ $[M+2]^+$: 386.0110 ; found 386.0116.

3-(1*H*-indol-3-yl)quinoxalin-2(1*H*)-one (21p)

Yellowish solid; yield: 71%, m.p. > 300 °C; IR (KBr, νmax/cm-1) 3421, 2921, 1713, 1660, 1580, 1420, 1018, 741; ^{1}H NMR (400 MHz, FT- DMSO) δ 8.89-8.84 (m, 2H) δ 7.85 (d, J = 8.8 Hz, 1H), δ 7.51 (d, J = 4.8, 1H), δ 7.44-7.30 (m, 3H), δ 7.25-7.23 (m, 2H); ^{13}C NMR (100 MHz, DMSO) 173.4, 154.4, 151.9, 136.4, 132.7, 130.1, 127.8, 127.4, 126.2, 123.2, 122.7, 122.4, 120.9, 114.9, 117.7, 111.4; HRMS (ESI) calcd. for $C_{16}H_{11}N_3O$ $[M+H]^+$: 262.0902; found 262.0907.

5.6 Material and Method

5.6.1 Determination of antimicrobial MIC value using serial dilution method [13]

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.

5.6.2. Platelet aggregation inhibitory activity evaluation[14]

All synthesized 2-oxo-2-phenylethylidene linked 2-oxo-benzo [1,4]oxazine analogues (**17a-x** and **18a-o)** were dissolved in DMSO before testing. In order to eliminate the effects of the solvent on aggregation, the final concentration of DMSO was fixed at 0.5%. Arachidonic acid (AA), EDTA (disodium salt), bovine serum albumin and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co.

Platelet Aggregation inhibitory bioassay[14a-c]

Blood was collected from the rabbit marginal ear vein and was mixed with EDTA to a final concentration of 6 mM. It was centrifuged for 10 min at 90 g at room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at 500 g for 10 min. The platelet pellets were washed with Tyrode's solution (Ca^{+2}-free) containing 2mM EDTA, 0.1 mg/mL and 3.5 mg/mL bovine serum albumin, and centrifuged at 500 g for 10 min. Then, the pellets were washed with Tyrode's solution without EDTA. After centrifugation under the same conditions, the platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2-PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2) containing bovine serum albumin (0.35%). Aggregation was measured by a turbidimetric method using a Lumi-aggregometer (Chrono-Log Corp., Havertown, PA). All glassware was siliconized. Three minutes before the addition of the aggregation inducer, the platelet suspension was stirred at 1200 rpm. The percentage of aggregation was calculated as follows (abs. = absorbance):

$$\text{Aggregation (\%)} = \frac{\text{Absorption of platelet suspension - Final absorption after aggregation (100)}}{\text{Absorption. of platelet suspension - Absorption of Tyrode solution}}$$

Percentage aggregation was expressed assuming the absorbance of platelet suspension

as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation. For each compound IC_{50} values were calculated by Sigma Plot.

5.7 References

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5.8 Selected characterization spectra (^{1}H and ^{13}C) of cephalandole analogues:

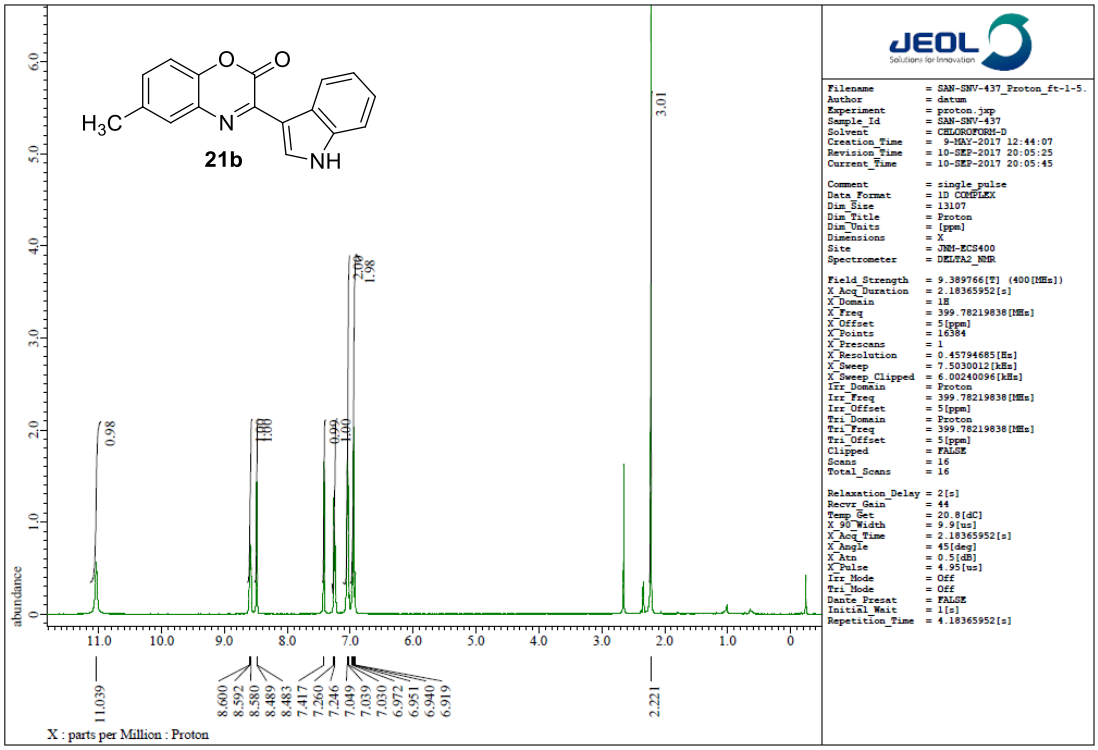

Figure 3. ^{1}H NMR Spectra of Compound **21b**.

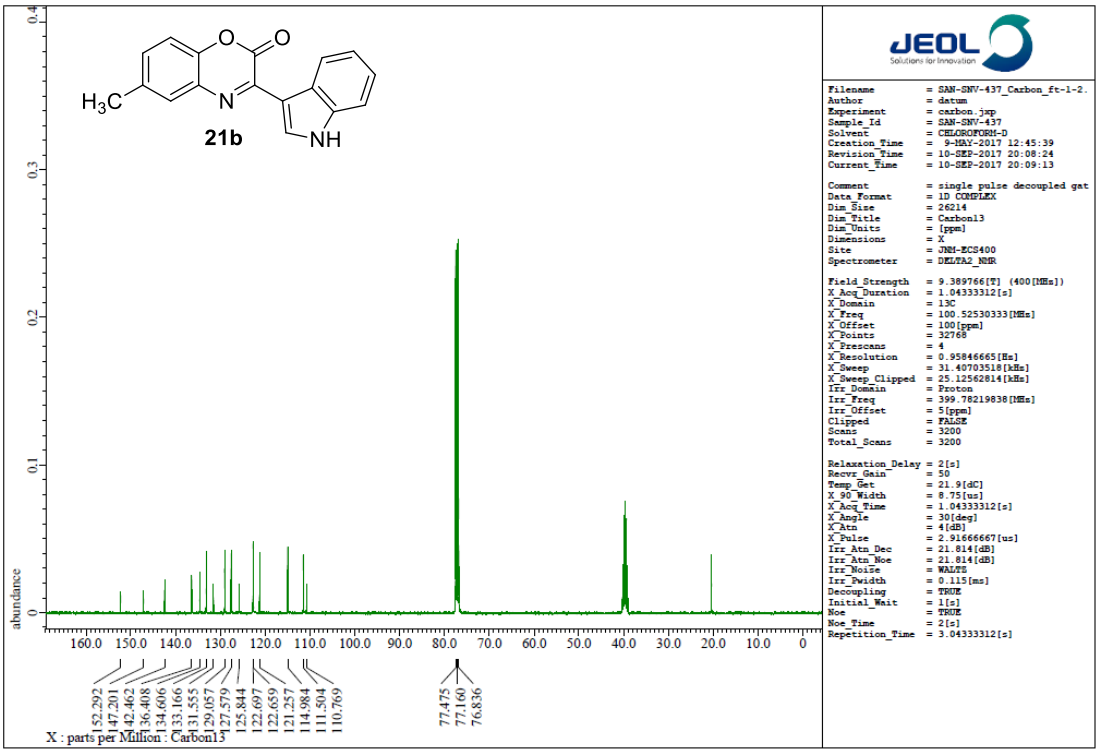

Figure 4. ^{13}C NMR Spectra of Compound **21b**.

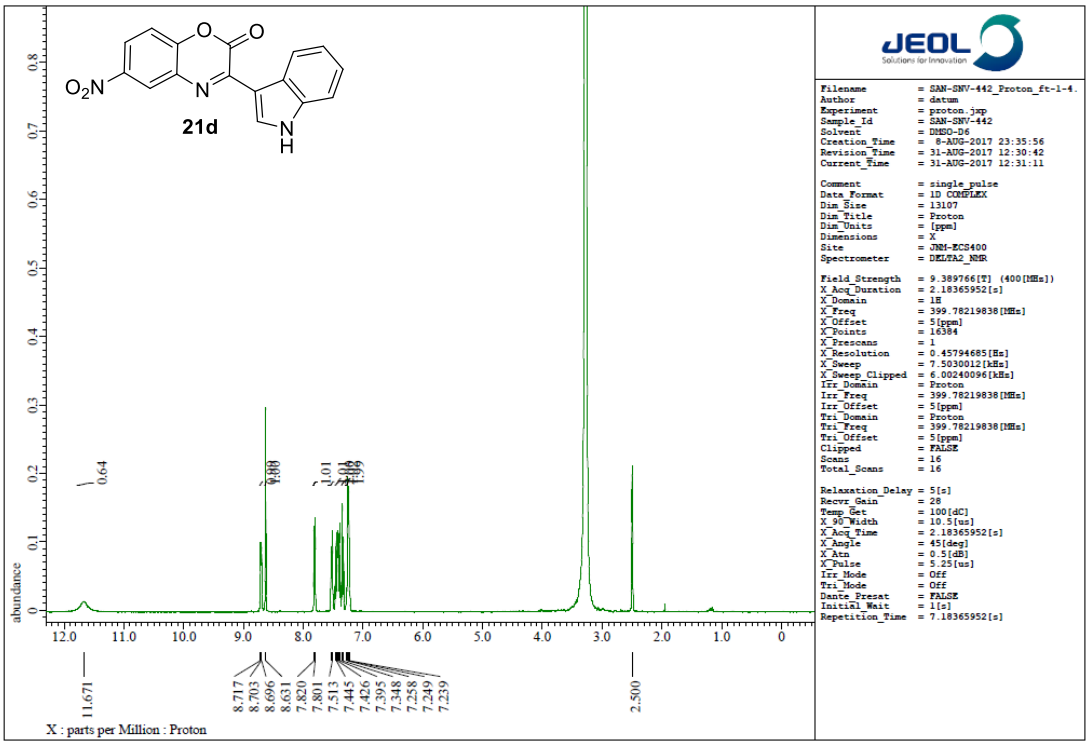

Figure 5. ^{1}H NMR Spectra of Compound **21d**.

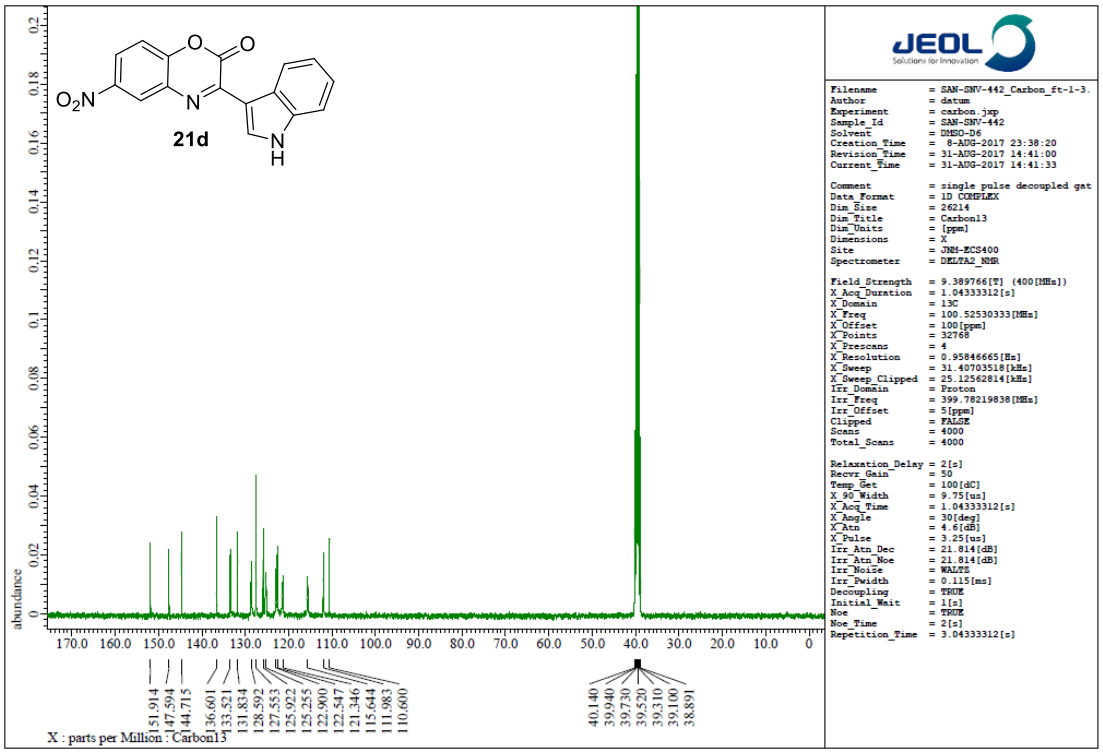

Figure 6. ^{13}C NMR Spectra of Compound **21d**.

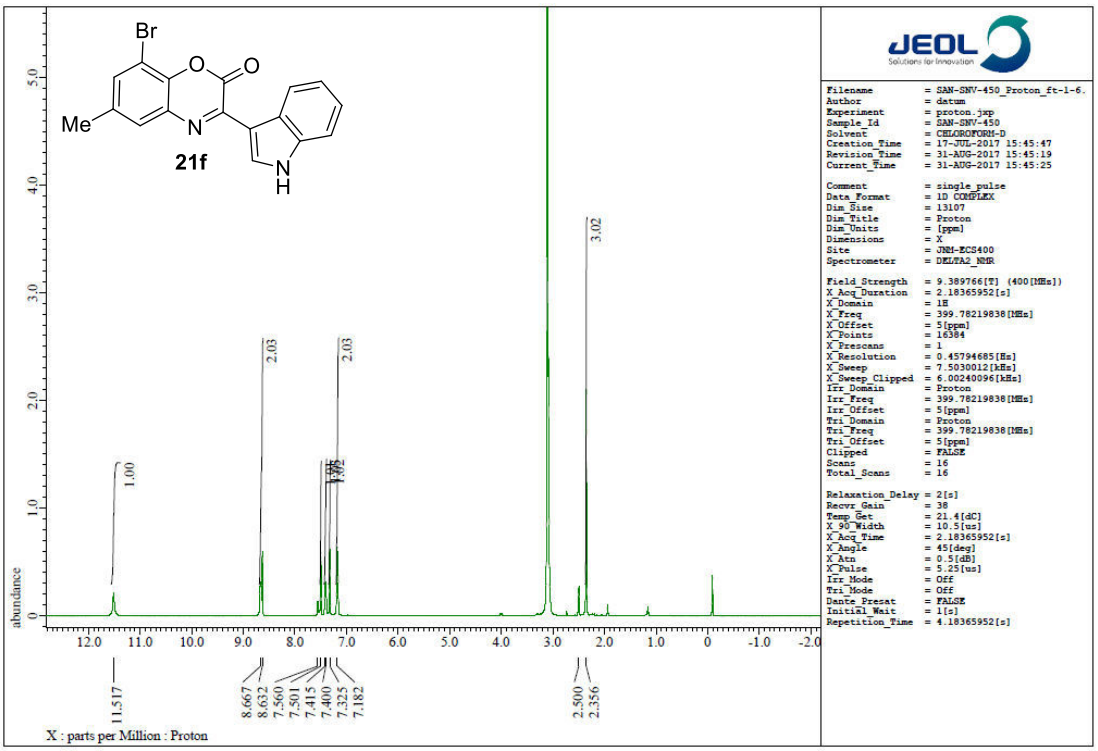

Figure 7. ^{1}H NMR Spectra of Compound **21f**.

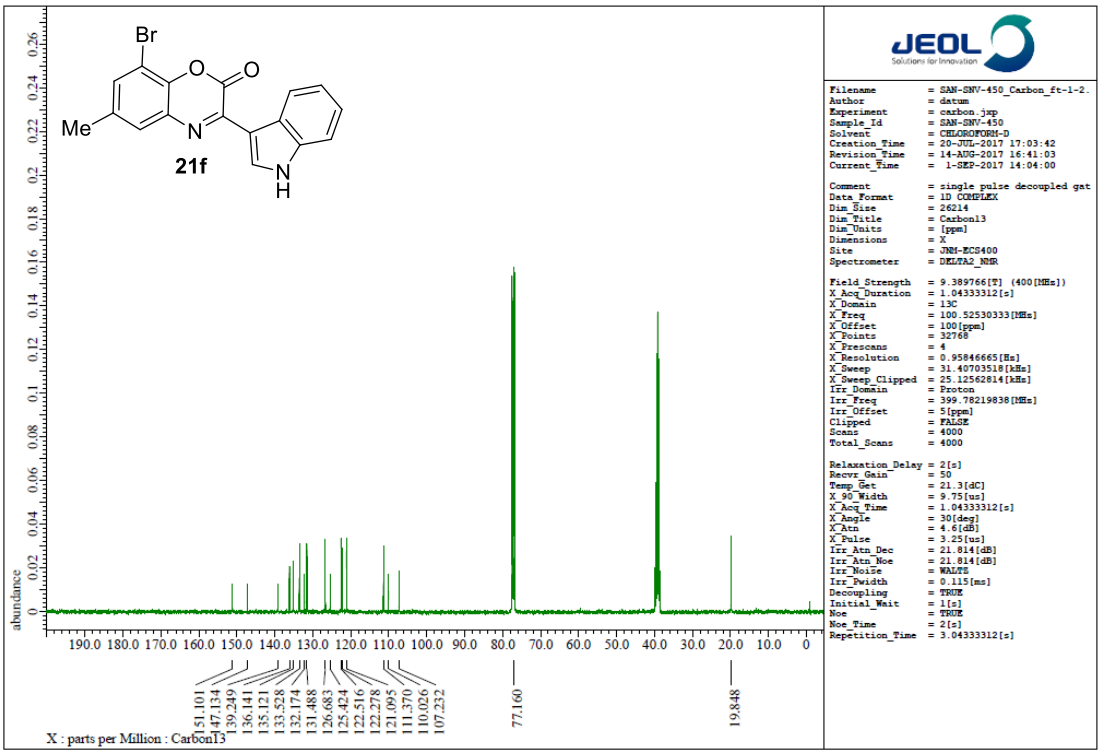

Figure 8. ^{13}C NMR Spectra of Compound **21f.**

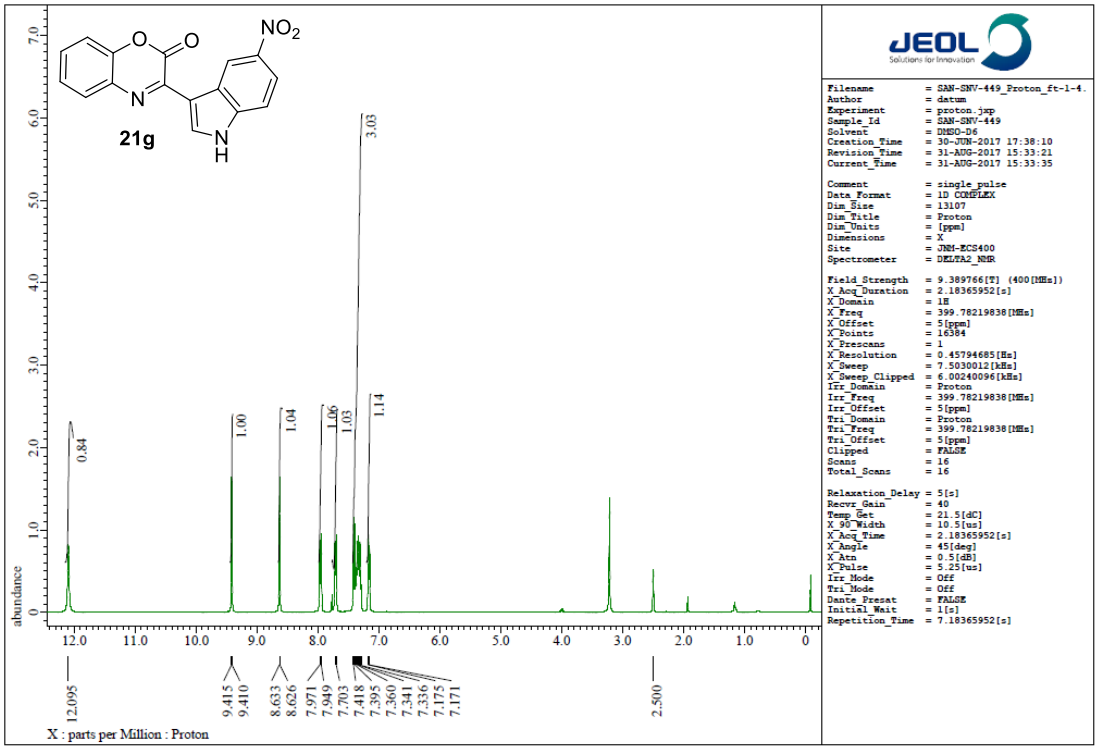

Figure 9. ^{1}H NMR Spectra of Compound **21g.**

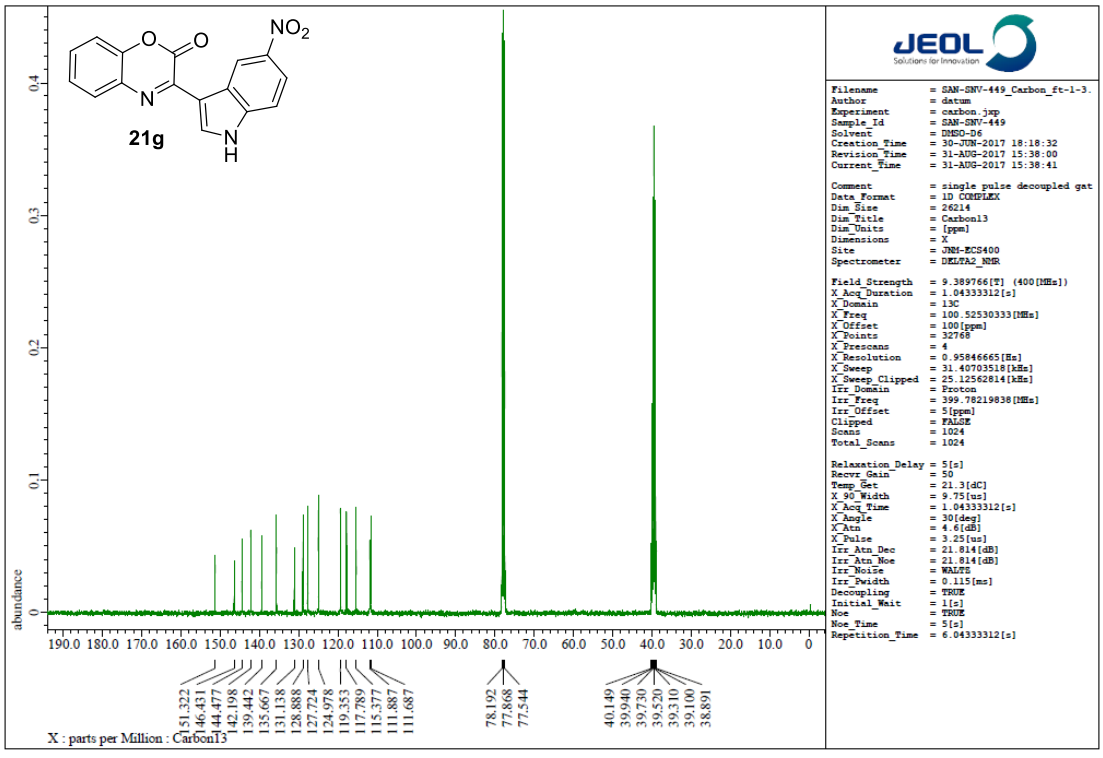

Figure 10. ^{13}C NMR Spectra of Compound **21g**.

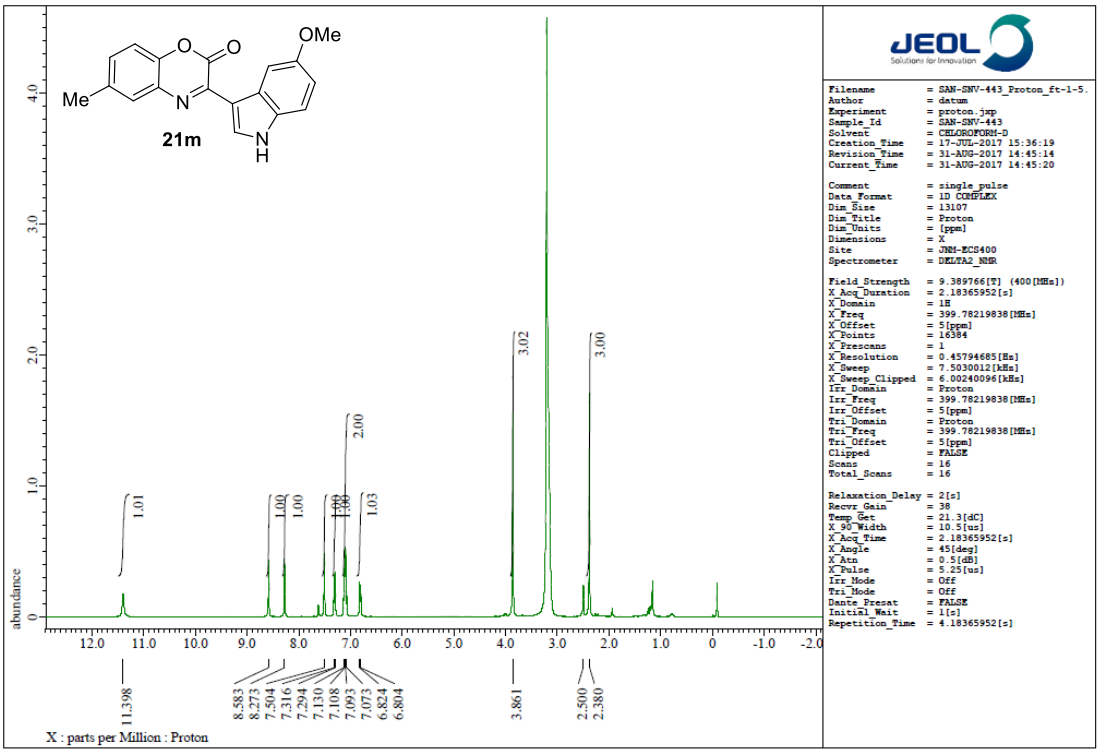

Figure 11. ^{1}H NMR Spectra of Compound **21m**.

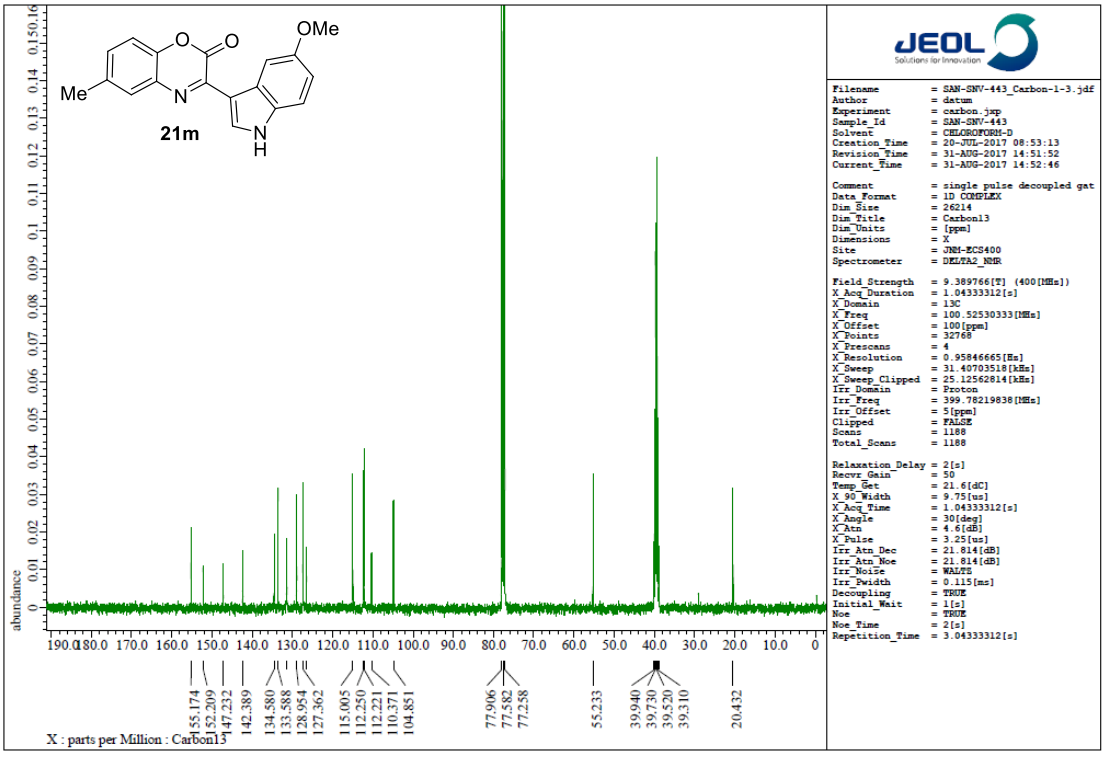

Figure 12. ^{13}C NMR Spectra of Compound **21m.**

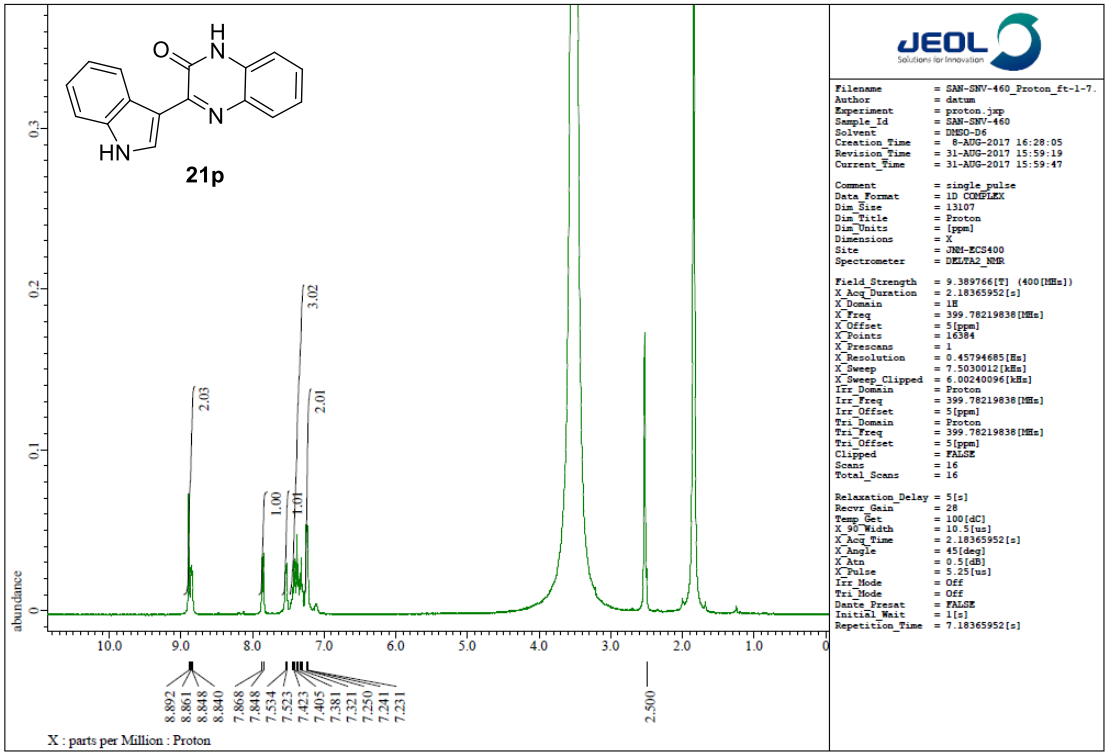

Figure 13. ^{1}H NMR Spectra of Compound **21p.**

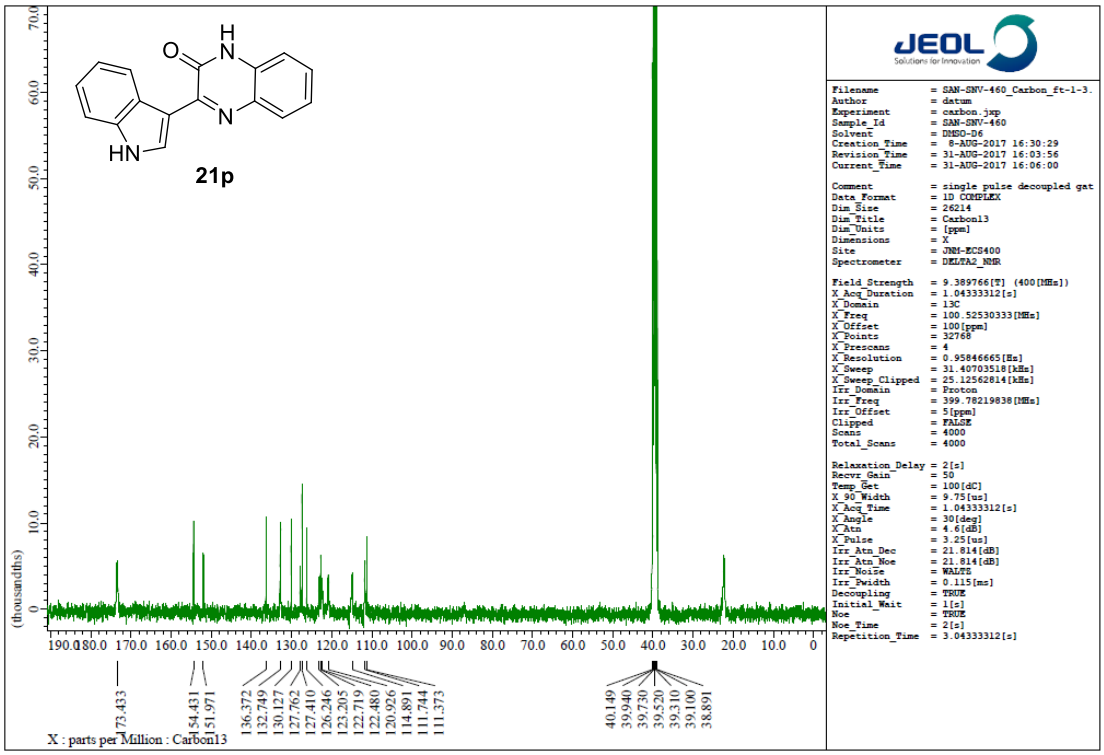

Figure 14. ^{13}C NMR Spectra of Compound **21p.**

Publications, Conferences/Seminars/Workshops

&

Curriculum Vitae

(Appendix I & II)

LIST OF PUBLICATION

1. Chaudhary, S.*; Sharma, V.; Jaiswal, P. K.; Gaikwad, A. N.; Sinha, S. K.; Puri, S. K.; Sharon, A.; Maulik, P. R.; Chaturvedi, V. "*Stable tricyclic antitubercular ozonides derived from Artemisinin*", *Org. Lett*., **2015**, *17 (20)*, 4948-4951. (IF: 6.492)
2. Singh, S.; Panwar, R.; Althagafi, I.; Sharma, V.; Chaudhary, S.; Pratap, R.* "*Base mediated regioselective synthesis of highly functionalized conjugated enones*", *Tetrahedron lett*., **2015**, *56(37)*, 5203–5208. (IF: 2.379)
3. Pradeep K. Jaiswal,† Vashundhra Sharma,† Jaroslav Prikhodko, Irina V. Mashevskaya, Sandeep Chaudhary, S.* *"On water" ultrasound-assisted one pot efficient synthesis of functionalized 2-oxo-benzo [1, 4] oxazines: First application to the synthesis of anticancer indole alkaloid, Cephalandole A*" *Tetrahedron Lett*., **2017**, *58(22)*, 2077-2083. (IF: 2.379)
4. Sharma, V.;† Jaiswal,; P. K.;† Yadav, D. K.; Saran, M.; Prikhodko, J.; Mathur, M.; Swami, A. K.; Mashevskaya, I. V.; Chaudhary, S.* "*Microwave-assisted one-pot efficient synthesis of functionalized 2-oxo-2-phenylethylidenes-linked 2-oxo-benzo[1,4]oxazines and 2-oxo-quino[1,4]oxalines: Synthetic Applications, Antioxidant activity, SAR and Cytotoxic Studies*" *Acta Chimica Slovenica*, **2017**, *67(4)*, 988-1004. (IF: 1.167)
5. Sharma, V.;† Jaiswal, P. K.;† Saran, M.; Yadav, D. K.; Saloni.; Mathur, M.; Swami, A. K.; Misra, S.; Kim, M.-H.; Chaudhary, S.* "*Discovery of functionalized 2-oxo-2-phenylethylidenes-linked 2-oxo-benzo [1, 4] oxazines as potent antioxidants: Bio-inspired based design, synthesis, biological evaluation, cytotoxic and Insilico molecular docking studies" Frontiers in Chemistry*, **2018**, *6(56)*, 1-17. (I.F = 3.994) [DOI: 10.3389/fchem.2018.00056]
6. Jaiswal, P. K.;† Sharma, V.;† Saloni; Mathur, M.; Swami, A. K.; Yadav, D. K.;* **Chaudhary, S.***; "N*on-peptide-based new class of platelet aggregation inhibitors: Design, synthesis, biological evaluation, SAR and in silico studies*" *Archiv Der Pharmazie*, **2018**, *351(3-4)*, 1-17. (I.F = 1.994) [DOI: 10.1002/ardp.201700349]
7. Jaiswal, P. K.; Sharma, V.; Mathur, M.; and **Chaudhary, S**.* "*Corrections to Organocatalytic Modified Guareschi−Thorpe Type Regioselective Synthesis: A Unified Direct Access to 5,6,7,8-tetrahydroquinolines and Other*

Alicyclic[b]-fused Pyridines" *Org. Lett.*, **2018**, *20 (19)*, 6059-6063. (I.F = 6.492) [DOI: 10.1021/acs.orglett.8b03587]

8. Jaiswal, P. K.; Sharma, V.; Mathur, M.; and **Chaudhary, S**.* "*Organocatalytic Modified Guareschi−Thorpe Type Regioselective Synthesis: A Unified Direct Access to 5,6,7,8-tetrahydroquinolines and Other Alicyclic[b]-fused Pyridines*" *Org. Lett.*, **2018**, *20 (19)*, 6059-6063. (I.F = 6.492) [DOI: 10.1021/acs.orglett.8b02132]
9. Sharma, V.;† Jaiswal, P. K.;† Kumar, S.; Mathur, M.; Swami, A. K.; Yadav, D. K.; **Chaudhary, S.*** "*Discovery of novel aporphine analogues as potential antiplatelet and antioxidant agents: Design, synthesis, SAR, biological evaluation and insilico molecular docking studies*" *ChemMedChem*, **2018**, *13*, 1-17. [DOI:10.1002/cmdc.201800318] **(I.F = 3.009)**
10. Sharma, V.;† Jaiswal,; P. K.;† Kumar, K.; Saran, M.; Mathur, M.; Swami, A. K.; **Chaudhary, S.*** "*An efficient synthesis and biological evaluation of novel analogues of natural product Cephalandole A: A new class of antimicrobial and antiplatelet agents" Fitoterapia*, **2018**, *129*, 13-19. **(I.F = 2.642)**

Paper presented in Conferences with proceedings (Abstract published)

1. Jaiswal, P. K.; **Sharma, V.**; Prikhodko, J. I.; Mashevskaya, I. V.; Chaudhary, S.; *"On water" ultrasound-assisted one pot efficient synthesis of functionalized 2-oxo-benzo [1, 4] oxazines: First application to the synthesis of anticancer indole alkaloid, Cephalandole A"* 15th-20th July, **2017** Abstracts of papers ISBN No. 978-5-7944-2925-1 (PP-01) International Conference on "*The study of the biological activities heterocycles with the purpose of creating innovative medicines*", Department of Organic Chemistry, Perm State University, Perm, Russia Federation.

2. **Sharma, V.**; Jaiswal, P. K.; Saran, M.; Prikhodko, J. I.; Mathur, M.; Swami, A. K.; Mashevskaya, I. V.; Chaudhary, S.; *An Efficient MW-Assisted, Green Synthesis of Functionalized 2-oxo-3,4-dihydro-2H-benzo[1,4]oxazines and 2-oxo-3,4-dihydroquinoxalines as Potential Antioxidant Agents"* 15th-20th July, **2017** Abstracts of papers ISBN No. 978-5-7944-2925-1 (PP-04) International Conference on "*The study of the biological activities heterocycles with the*

purpose of creating innovative medicines", Department of Organic Chemistry, Perm State University, Perm, Russia Federation.

3. Jaiswal, P. K.; **Sharma**, **V**.; Prikhodko, J. I.; Mashevskaya, I. V.; Chaudhary, S.; *"On water" ultrasound-assisted one pot efficient synthesis of functionalized 2-oxo-benzo [1, 4] oxazines: First application to the synthesis of anticancer indole alkaloid, Cephalandole A*" **15th-20th July**, **2015** Abstracts of papers ISBN No. 978-5-7944-2925-1 (PP-01) International Conference on "The study of the biological activities heterocycles with the purpose of creating innovative medicines", Department of Organic Chemistry, Perm State University, Perm, Russia Federation.
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5. Jaiswal, P. K.; **Sharma**, **V**. and Chaudhary, S. (**Nov, 2015**), "*Development of highly efficient one pot green synthetic protocol for the construction of benzo [1, 4] oxazin-2-one incorporated novel antiplatelet agents*" **23rd-25th Nov**, **2015** Abstracts of papers ISBN No. 978-93-84869-93-9 (PP-128) International Conference on "Current Challenges in Drug Discovery Research CCDDR-2015", MNIT Jaipur-302017, Rajasthan, India.
6. Jaiswal, P. K.; **Sharma**, **V**.; Gaikwad, A. N.; Sinha, S. K.; Puri, S. K.; Sharon, A.; Maulik, P. R.; Chaturvedi, V. and Chaudhary, S. (**Nov**, **2015**), "*An expedient synthesis of stable tricyclic antitubercular ozonides derived from artemisinin*" **23rd-25th Nov, 2015** Abstracts of papers ISBN No. 978-93-84869-93-9 (PP-129) International Conference on "Current Challenges in Drug Discovery Research CCDDR-2015", MNIT Jaipur-302017, Rajasthan, India.
7. **Sharma**, **V**.; Jaiswal, P. K. and Chaudhary, S. (**Nov**, **2015**), "*Studies towards Benzo (1, 4) oxazine-2-one based novel antifungal agents: Design, synthesis and their in vitro antifungal activities*" **23rd-25th Nov, 2015** Abstracts of

papers ISBN No. 978-93-84869-93-9 (PP-181) International Conference on "Current Challenges in Drug Discovery Research CCDDR-2015", MNIT Jaipur-302017, Rajasthan, India.

8. **Sharma**, **V**.; Jaiswal, P. K. and Chaudhary, S. (**Nov, 2015**), "*Microwave-assisted green synthesis of novel functionalized Benzo[1, 4]oxazine analogues and their anti-oxidant activity*" **23r^{d}-25th Nov, 2015** Abstracts of papers ISBN No. 978-93-84869-93-9 (PP-182) International Conference on "Current Challenges in Drug Discovery Research CCDDR-2015", MNIT Jaipur-302017, Rajasthan, India.

Curriculum Vitae

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Department of Chemistry
Malaviya National Institute of Technology
Jawaharlal Nehru Marg, Jaipur-302017
Rajasthan, India.
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Educational Qualifications

Ph.D Chemistry, (4th Jan, 2013- 4th Dec, 2017) Malaviya National Institute of Technology (MNIT) Jaipur, India.
Ph.D. thesis title: **Synthetic Studies towards Coupling Reactions: Chemistry and Biology of Bio-Active Alkaloids. (**Supervisor: **Dr. Sandeep Chaudhary, MNIT, Jaipur, Rajasthan)**
M.Sc. Chemistry (2011): St. Stani Memorial P. G. College, (Affiliated to University of Rajasthan, Jaipur), Jaipur, India.
B.Sc. (2009): Mahatma Jyoti Rao Phoole Mahila Mahavidyalaya, Jaipur, (Affiliated to University of Rajasthan, Jaipur), Jaipur, 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, **2012**.

Research experience

Pre-Ph.D Courses Work
- **Course 1**: *Heterocyclic Chemistry*
- **Course 2**: *Basics of Electrochemistry*
- **Course 3**: *Research Methodology and Design of Experiment*
- **Course 4:** *Bioinorganic Chemistry*
- **Course 5:** *Symmetry and Group theory*

Other research experiences
- Handling of Organic Peroxides such as H_2O_2etc.
- 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 I

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 Bruker Topspin 1.3 and Jeol NMR 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.
- Stereoselective synthesis: Development of new synthetic methodologies.
- Drug Development-Lead generation and lead optimization, applying different approaches towards drug development.

Seminars Delivered

- *Transition metal- Catalysed C-H Bond activation: Application to the synthesis of biologically active natural Products*, Departmental Seminar, **01-06-2013**, Venue – Seminar room, Department of Chemistry, MNIT Jaipur, India.

Profile

- Strong background in basic organic chemistry including structural elucidation and spectroscopic interpretation of organic molecules.
- 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.
- Capable of working individually as well as part of team and ready to accept a challenging task. Gained little experience in managing research projects from conception to completion.

References

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