

DESIGN, SYNTHESIS, AND APPLICATIONS OF INDOLE AND ISOINDOLE DERIVATIVES

**A Thesis submitted
in Partial Fulfillment of the Requirements for
the Degree of**

DOCTOR OF PHILOSOPHY

by

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(2K21/PHDAC/02)**

Under supervision of
Professor Ram Singh



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***Dedicated
To My Parents***



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ABSTRACT

The present work entitled “Design, Synthesis, and Applications of Indole and Isoindole Derivatives” has been divided into six chapters.

Chapter 1 covers the general description of various synthetic methodologies of indole and isoindolin-1-one heterocyclic compounds and their biological applications.

Chapter 2 covers the *in-silico* studies of 3-acyl-3-hydroxyisoindolin-1-one derivatives as potential MAO-B inhibitors for neurodegenerative diseases focusing on PD. A large library of 279 derivatives was created and molecular docking of the complete library was performed. Then, the top two docked scored compounds were further investigated using other parameters such as MDS, MM-GBSA binding free energy, ADME, and DFT studies. Two ligands, 3-hydroxy-2-(4-isopropylphenyl)-3-(4-(trifluoromethyl)benzoyl)isoindolin-1-one (**90**) and 2-(3,4-dichlorophenyl)-3-hydroxy-3-(4-(trifluoromethyl)benzoyl)isoindolin-1-one (**173**) displayed superior docking score values of -12.088 kcal/mol and -12.085 kcal/mol, respectively. MDS over 500 ns demonstrated the stability of both the ligands **90** and **173** with RMSD and RMSF values below 2 Å. ADME profiling of both ligands indicated favourable pharmacokinetic parameters, while DFT analysis showed high chemical reactivity and low kinetic stability. This suggested that ligands **90** and **173** exhibit strong potential as promising lead candidates for MAO-B inhibition.

Chapter 3 discusses a new Selectfluor-mediated synthetic route for the regioselective synthesis of 3-acyl-3-hydroxyisoindolin-1-ones from N-substituted-2-(1-alkynyl)benzamides with sodium bicarbonate as mild base in acetonitrile solvent. Water was used as the source for oxidation and hydroxylation. All the derivatives of 3-acyl-3-hydroxyisoindolin-1-ones were synthesized and characterized with 73-85% isolated yield. Control experiments revealed that this new synthetic method followed free radical pathway. Anti-oxidant activities of synthesized compounds are also discussed in this chapter.

Chapter 4 covers the *in-silico* studies of N-alkenylindole derivatives to evaluate their inhibitory activity against MAO-B. A library of 147 N-alkenylindole ligands was made and molecular docking of the complete library with the MAO-B protein target was performed. Further, the top two docked scored ligands were investigated using other *in-silico* tools which were MDS, MM-GBSA binding free energy, ADME, and DFT studies. Two ligands, 3-(1-(1,2,3,6-tetrahydro-[1,1'-biphenyl]-4-yl)-1H-indol-2-yl)phenol (**66**) and 2-(4-fluorophenyl)-1-(1,2,3,6-tetrahydro-[1,1'-biphenyl]-4-yl)-1H-indole (**63**) showed superior docking scores of -11.8 kcal/mol and -11.4 kcal/mol, respectively in their library. MDS performed over 500 ns

demonstrated lower RMSD and RMSF values for ligand **66** compared to ligand **63**, indicating greater ligand **66**-protein docked complex stability. Post-MDS MM-GBSA calculations depicting energetically favourable interactions. ADME profiling of both ligands indicated favourable drug-likeness and physicochemical properties. Whereas DFT analysis showed higher chemical reactivity and low kinetic stability of ligand **66** compared to ligand **63**.

Chapter 5 covers a new metal-free synthetic method for the regioselective synthesis of N-alkenyloindoles from 2-(1-alkynyl)anilines and cyclic ketones catalyzed by molecular iodine in toluene:DMF (20:1, v/v) solvent ratio. This developed method facilitated the synthesis of 19 derivatives with yields ranging from 56 to 73 % yield, of which 18 are new derivatives. This developed method was well tolerated the presence of both electron-donating and electron-withdrawing groups including hetero-aromatic rings. According to control experiment, this new synthetic route follows an ionic pathway. Moreover, the antioxidant activities of the synthesized N-alkenyloindole compounds were evaluated and discussion in this chapter.

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List of Important Abbreviations

S. No.	Abbreviations	Full Name
1	SAR	Structure Activity Relationship
2	PD	Parkinson's Disease
3	MAO-B	Monoamine Oxidase-B
4	SMILES	Simplified Molecular Input Line Entry System
5	MDS	Molecular Dynamics Simulation
6	SPC	Simple Point Charge
7	RMSD	Root Mean Square Deviation
8	RMSF	Root Mean Square Fluctuation
9	MM-GBSA	Molecular Mechanics/Generalized Born Surface Area
10	ADME	Absorption, Distribution, Metabolism, Excretion, and Toxicity
11	DFT	Density Functional Theory
12	HOMO	Highest Occupied Molecular Orbital
13	LUMO	Lowest Unoccupied Molecular Orbital
14	MESP	Molecular Electrostatic Potential
15	TLC	Thin Layer Chromatography
16	DCM	Dichloromethane
17	DMF	Dimethylformamide
18	MeCN	Acetonitrile
19	THF	Tetrahydrofuran
20	DMSO	Dimethyl Sulfoxide
21	FT-IR	Fourier Transform Infrared
22	NMR	Nuclear Magnetic Resonance
23	UV-Vis	Ultraviolet-Visible
24	HRMS	High Resolution Mass Spectrometry
25	TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl
26	DPPH	2,2-Diphenyl-1-picrylhydrazyl
27	AA	Antioxidant Activity

CHAPTER 1

Introduction: General aspects of Indole and Isoindole Derivatives

1.1 Introduction

Heterocyclic compounds displayed an important class of cyclic organic compounds in which one or more atoms other than carbon are present [1]. These compounds hold significant applications due to their diverse physical and chemical properties [2]. They are widely present in natural products and exhibited crucial role in biochemistry, pharmaceuticals, agrochemicals, and materials science [3]. Heterocyclic compounds also find applications in the production of sanitizers, antioxidants, and copolymers [4]. In addition, several alkaloids like morphine and reserprine, and anitbiotics like penicillin and cephalosporin contain heterocyclic scaffolds [5].

Studies have reported that heterocyclic moieties are present in more than 85% of physiologically active compounds. This makes them significant scaffolds in drug discovery [6-8]. Nitrogen (N)-containing heterocyclic compounds demonstrate a large class of heterocycles among biologically active molecules [9]. Indoles, Isoindolin-1-ones, pyridines, pyrroles, imidazoles, quinolones, benzimidazoles, quinazolines, triazoles, and piperidines etc are examples of N-containing heterocyclic compounds (**Figure 1.1**). These compounds possess a broad range of pharmacological activities [10]. Consequently, the synthesis of N-containing heterocycles has drawn much attention towards the organic synthetic chemists [11,12]. Over the past decades, several synthetic strategies have been developed for their preparation [13].

Indoles are fused heterocyclic ring systems consisting of a six-membered benzene ring with a five-membered ring containing one nitrogen atom [14]. These compounds act as important scaffolds in biological and medicinal chemistry because of their structural and electronic features [15]. These compounds exhibited several biological properties, such as anti-cancer [16,17], anti-viral [18,19], anti-bacterial [20,21], against neurodegenerative diseases [22,23], anti-inflammatory [24,25], anti-diabetic [26,27], and anti-fungal [28,29]. Indoles are also present in several natural products [30-32]. Previously, several researchers reported the synthesis of different indole moieties through acid and base-promoted, metal-catalyzed, photo- and electro-catalyzed cyclization reactions [33]. Thus, there has been a continuous interest in the synthesis of indole substituted compounds due to their widespread biological activities.

Furthermore, isoindolin-1-one (2,3-dihydro-1H-isoindole, C_8H_7NO) has also emerged as a N-containing heterocyclic molecule present in biologically active synthetically prepared and naturally occurring compounds. To explore the potentiality of isoindolin-1-one

derivatives, various groups have synthesized a library of this molecule and have evaluated them for diseases like cancer [34-36], bacterial [37], parasitic [38], psychotic [39,40], hypertension [41], inflammatory [42], fungal [43], viral [44,45], and diabetes [46]. Various isoindolin-1-ones have been isolated from plants of the Chilean *Berberis* species, creeper *Fumaria parviflora* Lam (Fumariaceae) [47]. Aristolactams are phenanthrene lactam alkaloids that exist naturally and have an isoindolin-1-one ring. They have been isolated from Aristolochiaceae, Annonaceae, Piperaceae, and Saururaceae plant families [48]. Owing to their broad pharmacological significance, various synthetic methods have been developed for the synthesis of isoindolin-1-one compounds, including electrochemical, transition metal-catalyzed, radical and oxidant-driven, acid-catalyzed, ultrasound-assisted, and ring opening reactions [49]. This chapter discusses the various synthetic methodologies previously reported for the synthesis of indoles and isoindolin-1-ones, along with their biological applications.

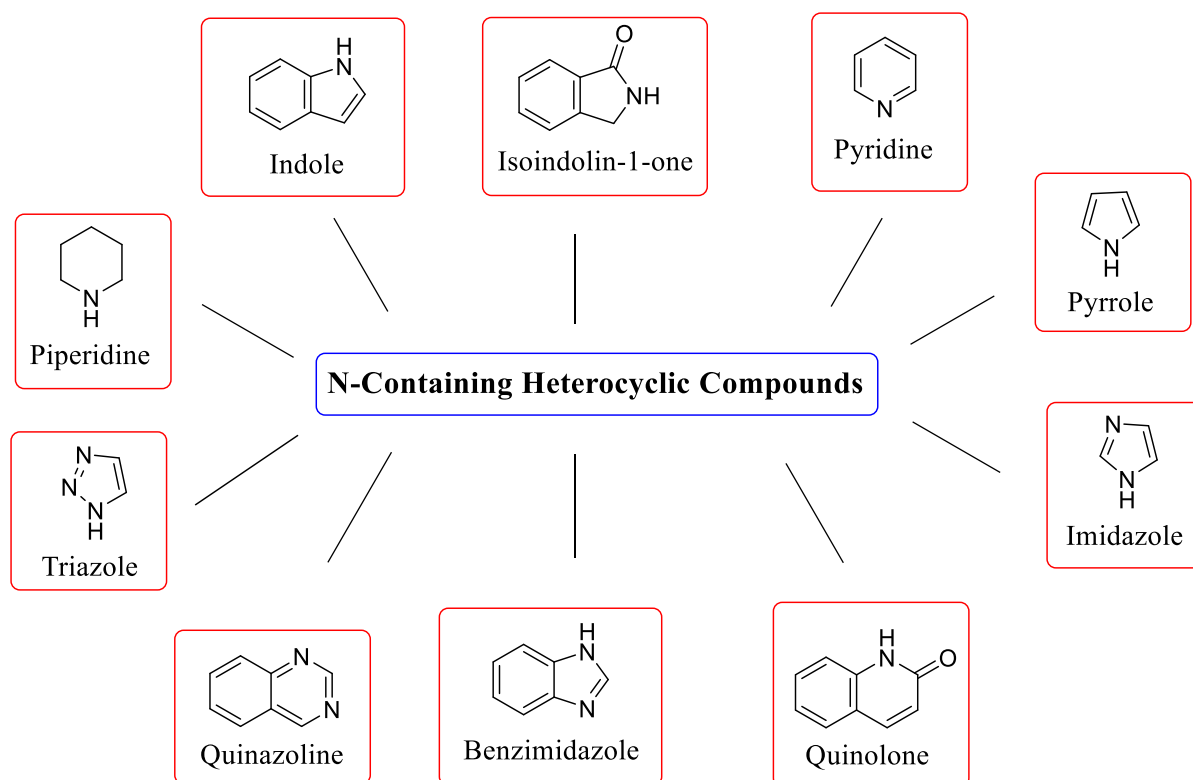


Figure 1.1 Examples of N-containing heterocyclic compounds.

1.2 Synthetic Methods of Indoles

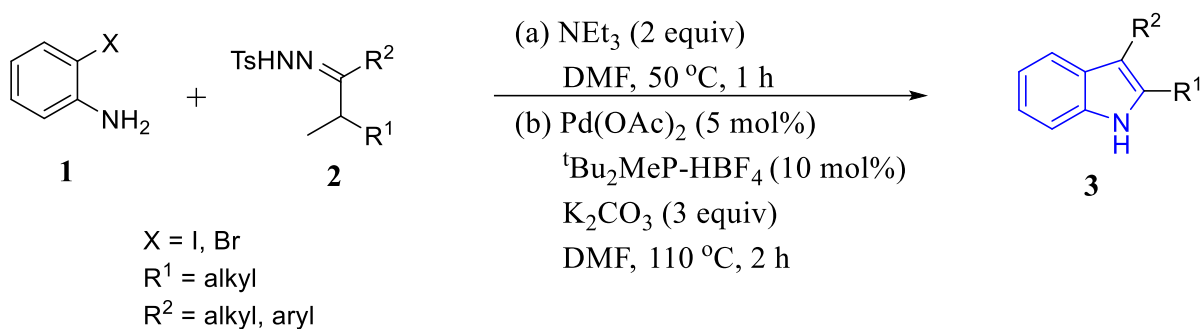
This section summarizes the methodologies reported from 2017 to 2025 for the synthesis of indoles using metal-catalysed (palladium, lead, ruthenium, copper, platinum), acid-mediated, base-mediated, oxidant-mediated, electrochemical, and UV-visible light mediated protocols.

1.2.1 Palladium-catalyzed Synthesis of Indoles

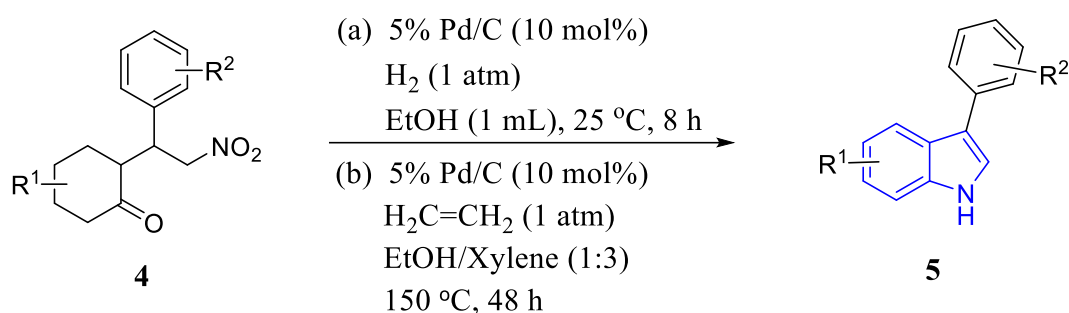
Chebieb and Cha group synthesized indoles from o-haloanilines **1** and α -ketol-derived N-tosylhydrazones **2** through an intramolecular Barluenga cross coupling reaction mediated by palladium acetate (**Scheme 1.1**). Reaction started with 1,4-elimination of **2** in the presence of triethylamine gave sulfonylazoalkene intermediate which further underwent aza Michael addition reaction with o-haloanilines **1** in the presence of palladium acetate and potassium carbonate, followed by Barluenga cross coupling to generate indole product **3** [50].

Kuang et al. reported the synthesis of 3-substituted indoles **5** using 2-(2-nitro-1-phenylethyl)cyclohexanone **4** starting materials with Pd/C as catalyst. Reaction proceeds with the treatment of **4** with 1 atm pressure of hydrogen (H₂) in ethanol solvent using Pd/C catalyst (10 mol%). Furthermore, ethene was used in place of hydrogen using same catalyst to give the desired product **5** (**Scheme 1.2**). According to their proposed mechanism, initially nitro group of **4** underwent hydrogenation using H₂ followed by cyclization to generate cyclized intermediate, which further underwent dehydrogenation using ethene to get 3-substituted indole product **5** [51].

Hsia et al. described the synthesis of 2,3-disubstituted indoles **8** through electrophilic cyclization of N,N-dimethyl-o-alkynylanilines **6** with arylsiloxanes **7** in the presence of 5 mol% of palladium acetate as catalyst, while 2 equiv of silver oxide was used as the oxidant. Reaction was stirred for 12 h in DMF solvent at 100 °C temperature under air (**Scheme 1.3**). According to control experiments, presence of 2 equiv of both silver oxide and silver fluoride is necessary for the reaction to occur. Their plausible mechanism suggested that palladium acetate converted **6** into an aminopalladation intermediate, which upon reaction with aryl siloxane **7** silver complex (generated using silver fluoride), afforded a σ -aryl- σ -indolylpalladium intermediate. Further, reductive elimination of this intermediate made the desired 2,3-disubstituted indole **8** [52].

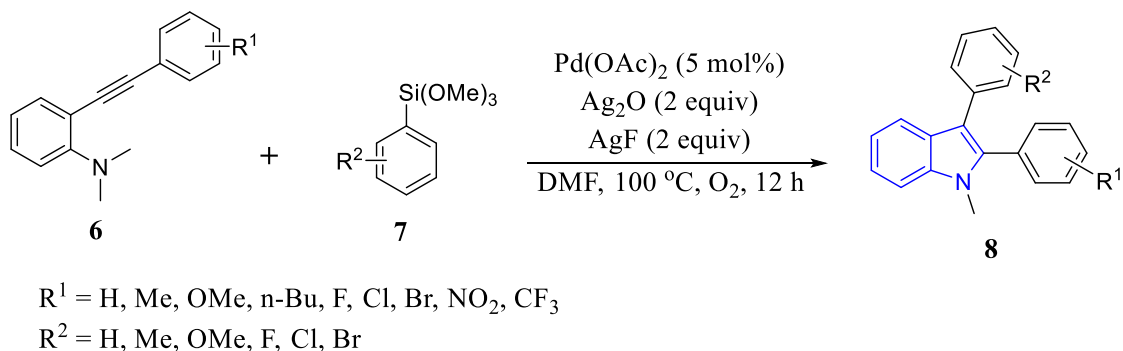


Scheme 1.1 Palladium-catalysed synthesis of indoles from o-haloanilines



$\text{R}^1 = \text{H, methyl, ethyl, t-Bu, C}_6\text{H}_5, 4\text{-OHC}_6\text{H}_4, \text{COOC}_2\text{H}_5, \text{NHCOCH}_3$
 $\text{R}^2 = \text{methyl, OH, OMe, F, 2-furyl}$

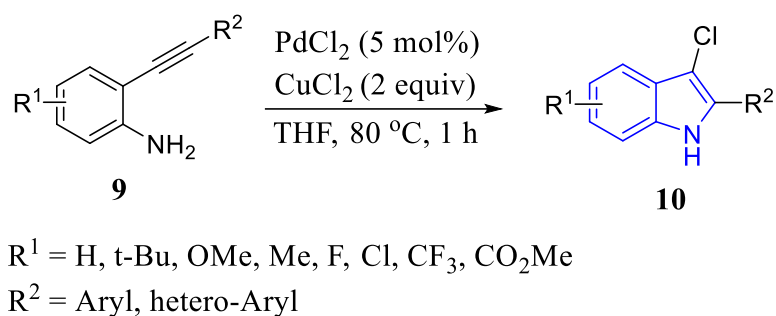
Scheme 1.2 Palladium-catalyzed synthesis of 3-substituted indoles from 2-(2-nitro-1-phenylethyl)cyclohexanones



Scheme 1.3 Palladium-acetate catalyzed synthesis of 2,3-disubstituted indoles using N,N-dimethyl-o-alkynylanilines and arylsiloxanes

Zheng et al. reported the synthesis of 3-chloroindoles **10** from 2-(1-alkynyl)aniline **9** mediated by palladium chloride (5 mol%). The source of chlorine was from the copper chloride (2 equiv). The reaction of **9** was performed in THF solvent at 80 °C for 1 h (**Scheme**

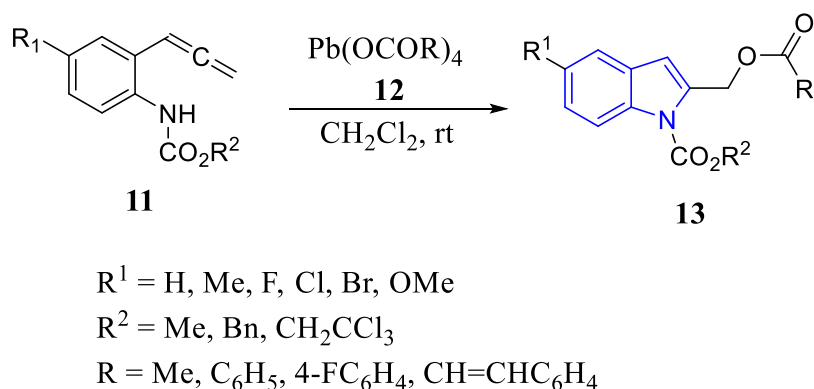
1.4). Reaction was well tolerated with various electron-donating, electron-withdrawing groups, including heteroaryl groups present on substrates. According to their proposed mechanism, palladium chloride was used for the triple bond activation and cyclization of **9** and then copper chloride was used for the insertion of Cl atom on 3rd position to cyclized intermediate to get final 3-chloroindole product **10** [53].



Scheme 1.4 Palladium-chloride catalyzed synthesis of 3-chloroindoles

1.2.2 Lead-catalyzed Synthesis of Indoles

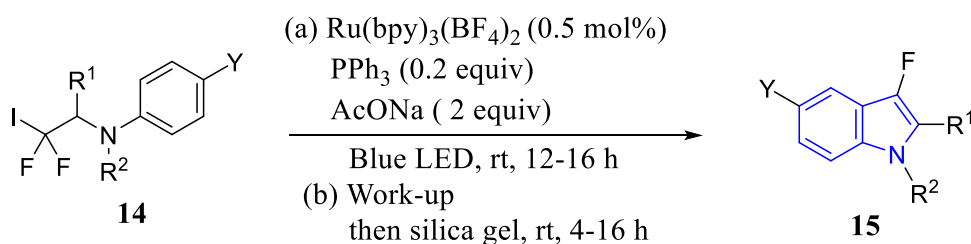
In year 2021, Lauterbach's group reported the synthesis of indoles using lead(IV) carboxylate and o-allyl anilines as starting materials. Intramolecular oxidative amino-hydroxylation of carbamate-protected o-allyl anilines **11** using lead(IV) carboxylate **12** in dichloromethane solvent at room temperature resulted in the formation of both C-N and C-O bonds, affording substituted indoles **13** (**Scheme 1.5**). The reaction was proposed to proceed initially through a de-aromatization step, followed by cyclization and subsequent re-aromatization [54].



Scheme 1.5 Lead(IV) carboxylate-mediated synthesis of substituted indole

1.2.3 Ruthenium-catalyzed Synthesis of Indoles

Panferova et al. developed a ruthenium-catalyzed method for the synthesis of 3-fluoroindoles. The reaction was carried out from N-arylamines starting materials **14** using 0.5 mol% of the ruthenium catalyst ($\text{Ru}(\text{bpy})_3(\text{BF}_4)_2$) and 20 mol% of triphenyl phosphine in the presence of blue LED to get 3-fluoroindoles **15** (**Scheme 1.6**). According to the authors, substrate is firstly converted into difluorinated cyclized intermediate through intramolecular cyclization. Then, cleavage of C-F bond followed by elimination of proton gave the final indole product **15** [55].

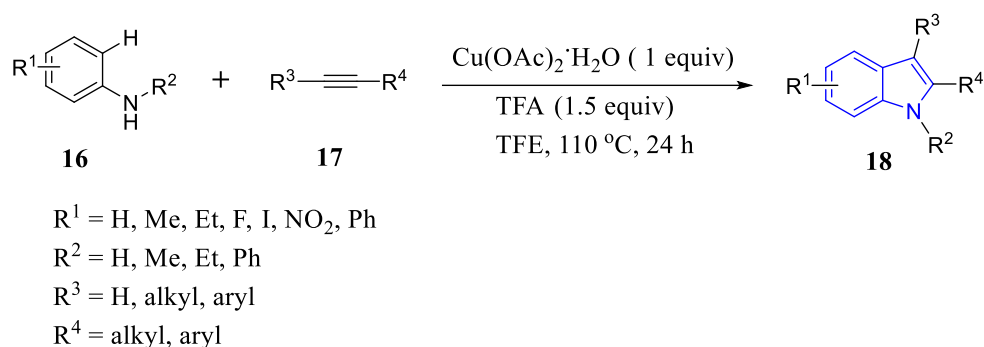


$\text{R}^1 = \text{C}_6\text{H}_5$, 4- ClC_6H_4 , 4- OMeC_6H_4 , 3,4,5- $(\text{OMe})_3\text{C}_6\text{H}_2$, 1-naphthyl, 2-thiophenyl, 4- MeC_6H_4 , 4- BrC_6H_4 , iso-butyl
 $\text{R}^2 = \text{Me}$, propyl, iso-butyl, $\text{CH}_2\text{CO}_2\text{Me}$
 $\text{Y} = \text{H}$, OMe , Br

Scheme 1.6 Ruthenium-catalyzed synthesis of 3-fluoroindoles

1.2.4 Copper-catalyzed Synthesis of Indoles

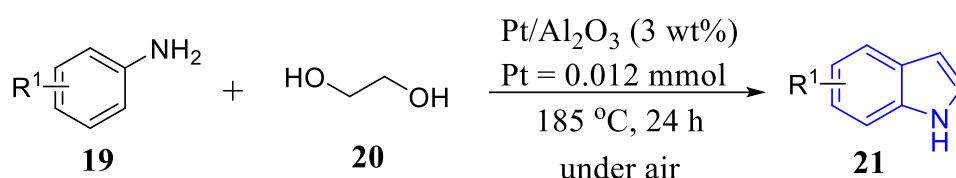
Jagtap et al. showed the synthesis of 2,3-disubstituted indoles **18** mediated by copper acetate (1 equiv) from commercially available N-substituted anilines **16** and internal alkynes **17** with 1.5 equivalent of trifluoroacetic acid (TFA) was used for the reaction in 2,2,2-trifluoroethanol (TFE) solvent and the reaction was stirred at 110 °C for 24 h (**Scheme 1.7**). The reaction was well tolerated with different electron-donating and electron-withdrawing groups present on internal alkynes and also well tolerated with different primary and secondary anilines. Proposed reaction mechanism suggested that triple bond of **17** was activated by copper catalyst, which underwent hydroamination with **16** to get imine intermediate, which further underwent C-H annulation and reductive elimination in the presence of copper catalyst to get final 2,3-disubstituted indole product **18** [56].



Scheme 1.7 Copper-catalyzed synthesis of indoles from N-substituted anilines and internal alkynes

1.2.5 Platinum-catalyzed Synthesis of Indoles

Wei et al. displayed a green and inexpensive method for the synthesis of indoles **21** from anilines **19** and ethylene glycol **20** using 3 wt% of Pt/Al₂O₃ as catalyst. The reaction was stirred at 185 °C for 24 h under air atmosphere (**Scheme 1.8**). Nine derivatives of indoles were synthesized with moderate to good yields. According to them, reaction mechanism involved the formation of N-(2-hydroxyethyl) aniline intermediate, formed from the dehydrogenated intermediate of ethylene glycol and aniline. N-(2-hydroxyethyl) aniline intermediate further underwent cyclization to generate final indole product **21** with the removal of water molecule [57].

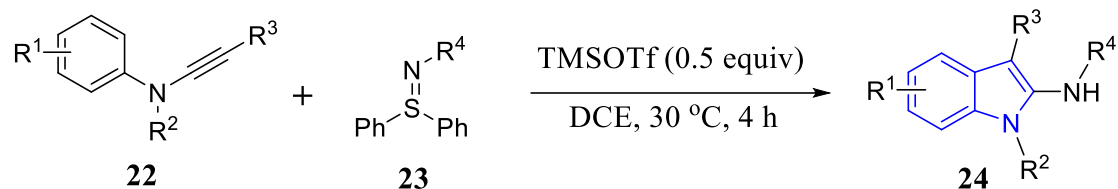


Scheme 1.8 Pt/Al₂O₃-mediated synthesis of indoles using ethylene glycol and anilines

1.2.6 Acid-mediated Synthesis of Indoles

Wang et al. reported the synthesis of 2-aminoindoles **24** mediated by TMSOTf from aryl-terminated N-arylnamides **22** and sulfilimines **23** starting materials. For the synthesis, 0.5 eq of TMSOTf was used in DCE solvent and the reaction was performed at 30 °C for 4 h (**Scheme 1.9**). Different types of electron donating and electron withdrawing groups present on starting materials were well tolerated. According to their proposed mechanism, reaction is initiated by the coordination of TMSOTf with **22**, forming a silicon π -alkyne intermediate,

which further underwent nucleophilic attack by **23** and N-S bond cleavage to give carbocation intermediate. Intramolecular cyclization of carbocation intermediate followed by aromatization generated the final 2-aminoindole product **24** [58].



$R^1 = \text{H, Me, OMe, F, Cl, Br}$

$R^2 = \text{Ts, Cs, Ns, Mbs, Ms, Boc, SO}_2\text{Ph}$

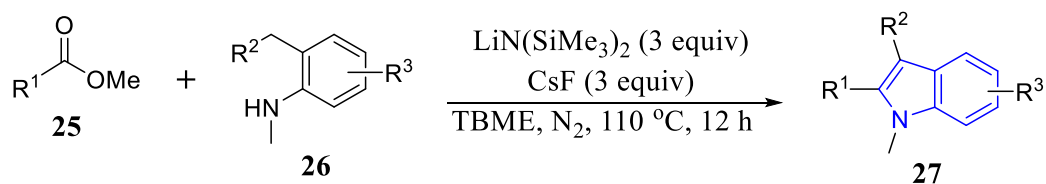
$R^3 = \text{aryl, hetero-aryl, Tol, PMP}$

$R^4 = \text{Ms, Ts, SO}_2\text{Ph, Mbs, Cs, Ns}$

Scheme 1.9 TMSOTf mediated synthesis of 2-aminoindoles

1.2.7 Base-mediated Synthesis of Indoles

Ma and co-workers showed a base system ($\text{LiN}(\text{SiMe}_3)_2\text{-CsF}$) mediated tandem Madelung indole **27** synthesis from methyl benzoate **25** and N-methyl-o-toluidine **26**. A total of thirty-one derivatives of indoles were synthesized with 50 to 90% yields. 3 equiv of both $\text{LiN}(\text{SiMe}_3)_2$ and CsF were required for the synthesis. The reaction was reported in TBME solvent under an inert atmosphere and stirred at 110 °C for 12 h (**Scheme 1.10**).



$R^1 = \text{aryl, hetero-aryl}$

$R^2 = \text{H, Me, Ph, 3-FPh, 1-naphthyl, 2-thiophenyl}$

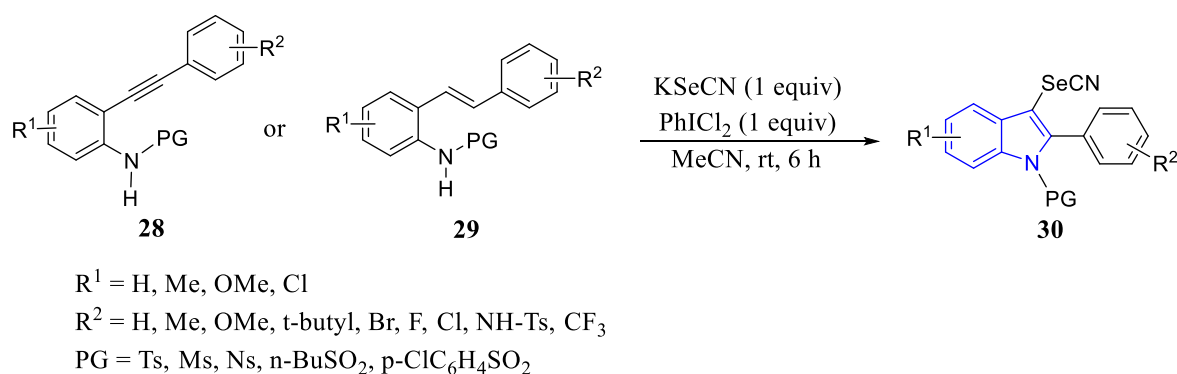
$R^3 = \text{H, Me, OMe, OCF}_3, \text{Cl, Br}$

Scheme 1.10 Base system ($\text{LiN}(\text{SiMe}_3)_2\text{-CsF}$) mediated synthesis of indoles

Their plausible mechanism indicated that the reaction of **25** with **26** initially generated an amide intermediate, which upon further deprotonation by the $\text{LiN}(\text{SiMe}_3)_2\text{-CsF}$ base system, formed a benzylic anion intermediate. Further, intramolecular cyclization of this intermediate afforded the final product **27** [59].

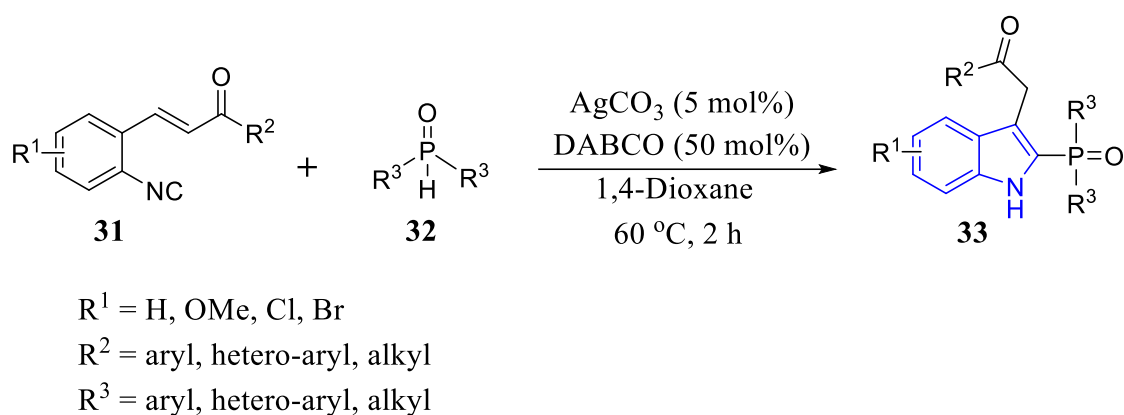
1.2.8 Oxidant-mediated Synthesis of Indoles

Xu et al. reported 3-selenocyanato-substituted indoles synthesis from N-protected 2-alkynylanilines **28** or 2-alkenylanilines **29** and potassium selenocyanate (KSeCN) compounds. The transformation was mediated by 1 equivalent of (dichloriodo)benzene (PhICl₂) and was carried out in acetonitrile solvent at room temperature for 6 h (**Scheme 1.11**). The reaction mechanism suggested that PhICl₂ combined with KSeCN to form a reactive selenocyanogen chloride species. Subsequent attack of the alkyne or alkene moiety of the substrates on this electrophilic species, followed by intramolecular cyclization, generated the corresponding 3-selenocyanato-substituted indoles **30** [60].



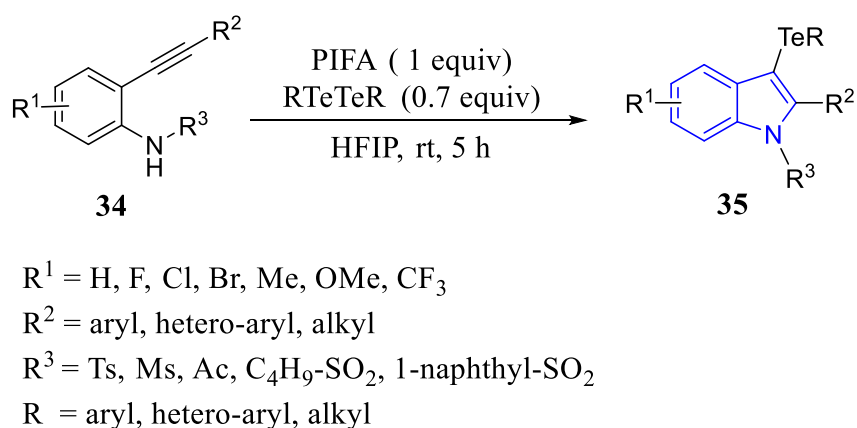
Scheme 1.11 PhICl₂ mediated synthesis of 3-selenocyanato-substituted indoles

Liu et al. displayed a silver catalyzed synthesis of 2-phosphinoylindoles **33** via isocyanides **31** and phosphine oxides **32**. 5 mol% of silver catalyst (AgCO₃) with 50 mol% of DABCO was used in the reaction and reaction was stirred at 60 °C for 2 h in 1,4-dioxane solvent (**Scheme 1.12**). Reaction is comfortable with various aryl, hetero-aryl or alkyl groups of substrates. According to their control experiments, the reaction mechanism did not involve formation of free radicals and instead proceeded through an ionic pathway. The electrophilic character of isocyano part of **31** was increased by its coordination with silver salt which further underwent nucleophilic addition with the phosphine oxide anion. The resulting intermediate subsequently underwent cyclization to get the desired indole product **33** [61].



Scheme 1.12 Silver-catalysed synthesis of 2-phosphinoylindoles from isocyanides and phosphine oxides

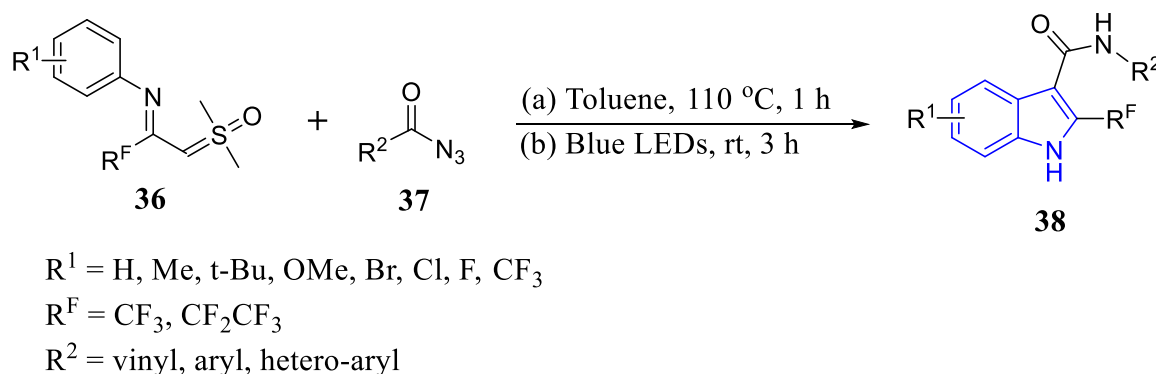
Zhang et al. reported the hypervalent iodine reagent (PIFA) mediated synthesis of 3-aryltelluroindoles **35** from o-alkynylanilines **34** and diarylditellurides. The reaction showed broad substrate scope with certain electron-donating, electron-withdrawing, and hetero-aryl groups present in the substrates. The reaction was performed in hexafluoroisopropanol (HFIP) solvent at room temperature for 5 h (**Scheme 1.13**). According to them, reaction mechanism involved ionic pathway. Initially, diarylditellurides and PIFA combined to form reactive PhTeOCOCF_3 species. Then, **34** underwent intramolecular cyclization using PhTeOCOCF_3 species with the generation of C-Te bond to make final 3-aryltelluroindole product **35** [62].



Scheme 1.13 Synthesis of 3-aryltelluroindoles from o-alkynylanilines and diarylditellurides

1.2.9 Visible Light-mediated Synthesis of Indoles

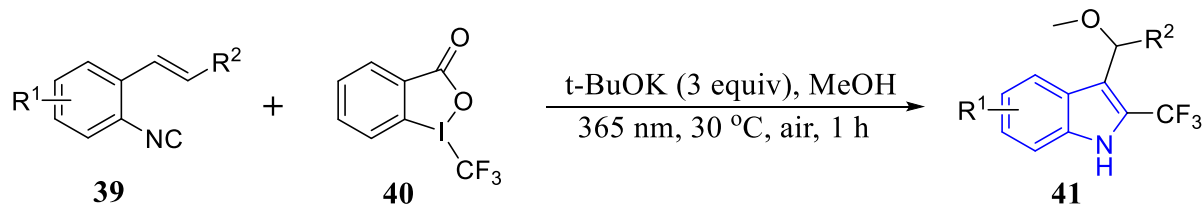
Cui et al. reported visible light-mediated synthesis of 2-trifluoromethyl indoles **38** from the intramolecular cyclization of sulfoxonium ylides **36** and azides **37**. Firstly, azide **37** was taken in toluene solvent. Reaction mixture was then stirred at 110 °C for 1 h. Afterwards, reaction mixture was cooled to room temperature and then **36** was added. The reaction mixture was then irradiated with blue LED for 3 h at room temperature (**Scheme 1.14**). The reaction was well tolerated with different types of electron-donating and electron-withdrawing substituents attached to substrates. Their plausible mechanism suggested that initially **36** underwent nucleophilic attack to isocyanate formed from azide **37** to generate intermediate, which further underwent intramolecular cyclization via blue LED irradiation and hydrogen atom transfer to afford the desired 2-trifluoromethyl indole product **38** [63].



Scheme 1.14 Visible light-mediated synthesis of 2-trifluoromethyl indoles

1.2.10 UV Light-mediated Synthesis of Indoles

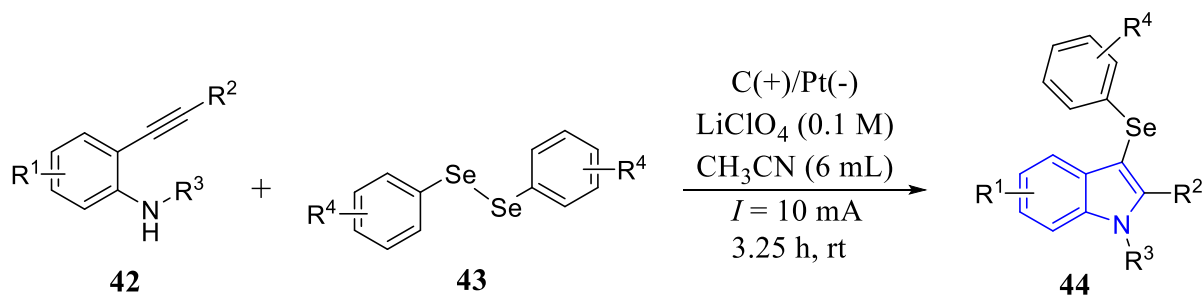
Wang and co-workers reported the synthesis of indoles **41** via β -substituted *o*-vinylphenylisocyanides **39** and Togni's reagent **40**. The reaction was performed using 3 equivalents of potassium tertiary butoxide (t-BuOK) in methanol solvent at 30 °C for 1 h under irradiation with a 365 nm light source (UV-A light) (**Scheme 1.15**). The reaction was tested with different types of electron-donating and electron-withdrawing groups present on **39**, synthesizing the corresponding products with moderate yields. According to them, the reaction mechanism followed free radical pathway and formation of an electron donor-acceptor (EDA) complex formed from t-BuOK and Togni's reagent. Afterwards, light irradiation of EDA complex generated a CF_3 radical. Then, **39** on attack by CF_3 radical followed by intramolecular cyclization and oxidation generated the carbocation intermediate. Finally, nucleophilic attack by methanol generated the desired product **41** [64].



Scheme 1.15 Synthesis of indoles from β -substituted o-vinylphenylisocyanides and Togni's reagent

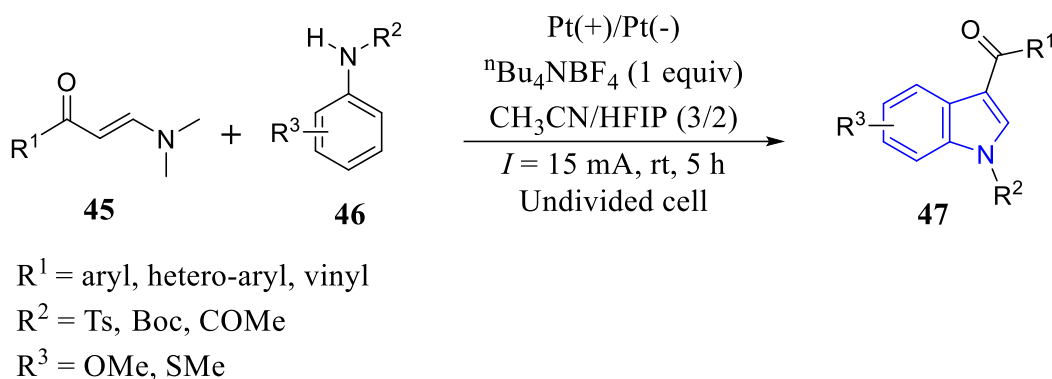
1.2.11 Electrochemical Methods Synthesis of Indoles

Dapkekar and Satyanarayana displayed the electrochemical method of synthesis of 3-selenylindoles **44** from N-protected o-alkynylanilines **42** and diselenides **43**. The reaction was done in undivided cell with graphite (C) as working electrode and platinum as counter electrode in the presence of 0.1 M of lithium perchlorate (LiClO_4) as electrolyte in acetonitrile solvent with a constant current (I) of 10 mA (**Scheme 1.16**). This methodology showed a broad substrate scope by synthesizing derivatives upto 95 % yield. Control experiment suggested that reaction mechanism done through ionic pathway. In the presence of electrochemical environment, **43** converted to reactive seleno species which was used for the activation of triple bond and further cyclization of **42** to generate the final 3-selenylindole product **44** [65].



Scheme 1.16 Electrochemical method for the synthesis of 3-selenylindoles

Furthermore, Zhong et al. displayed the electrochemical approach for the regioselective synthesis of 3-acylindoles **47**, synthesized from enaminones **45** and N-protected anilines **46**. Reaction was performed in an undivided cell with platinum present on both cathode and anode in a solvent ratio of CH₃CN:HFIP (3/2) at room temperature for 5 h under a constant current (I) of 15 mA. 1 equivalent of tetrabutylammonium tetrafluoroborate (nBu₄NBF₄) was also used for the reaction (**Scheme 1.17**). Different types of electron-donating and electron-withdrawing aryl groups, including heteroaryl groups present on substrates were well tolerated in the reaction. Their proposed mechanism suggested that the anodic oxidation of **45** generated an N-cation radical intermediate, which reacted with **46**, followed by intramolecular cyclization and amine elimination to get the desired 3-acylindole product **47** [66].



Scheme 1.17 Electrochemical method for the synthesis of 3-acylindoles

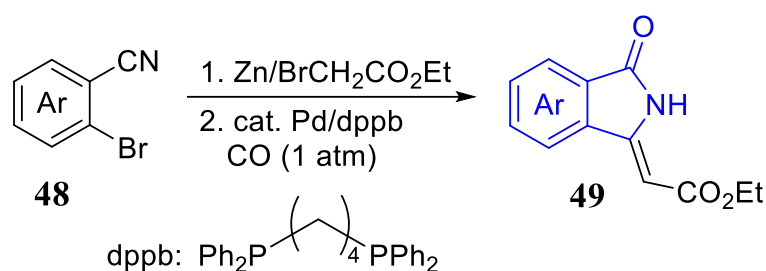
1.3 Synthetic Methods of Isoindolin-1-ones

This section highlights several synthetic methods reported between 2013 and 2025 for the synthesis of isoindolin-1-ones, through metal-catalyzed (palladium, rhodium, ruthenium), acid-mediated, oxidant-mediated, electrochemical, and ultrasound-assisted methods.

1.3.1 Palladium-catalyzed Synthesis of Isoindolin-1-ones

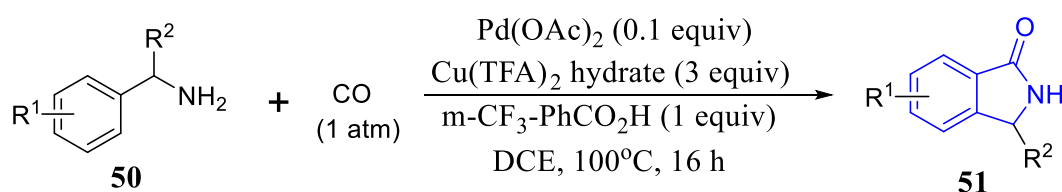
Xuan et al. demonstrated the intramolecular aminocarbonylation of 2-bromo-aryl nitriles **48** through palladium catalyst and carbon monoxide (CO) to give 3-methyleneisoindolin-1-ones **49** scaffold (**Scheme 1.18**). Initially, 2-bromoaryl nitriles **48** underwent Reformatsky reaction at 80 °C using zinc dust and ethyl bromoacetate followed by palladium-catalyzed aminocarbonylation at 120 °C for 5 h using dppb as a ligand to provide

49 with excellent yields. According to their proposed mechanism, initially, an intermediate was formed from **48** with the help of Zn dust and ethyl bromoacetate. The Zn-complex then underwent oxidative addition of Pd⁰ species to generate nitrogen-coordinated intermediate. This intermediate then underwent CO insertion, nucleophilic substitution of triethylamine, and reductive elimination to give 3-methyleneisoidolin-1-one **49** as the final product [67].



Scheme 1.18 Synthesis of 3-methyleneisoidolin-1-ones from 2-bromo-aryl nitriles

Zhang et al. reported the C-H carbonylation of primary benzyl amines **50** at 100 °C for 12 h using palladium acetate and copper(II) trifluoroacetate hydrate as catalyst and oxidant respectively, producing 3-substituted isoindolin-1-ones **51** with 96% maximum yield (**Scheme 1.19**). The plausible mechanism of this reaction showed benzylamine **50** underwent chelation-assisted *ortho*-C-H bond activation with palladium acetate to give five-membered palladacycle intermediate, which coordinated with CO to generate another intermediate, which further underwent insertion of CO into the Pd-C bond to made acyl Pd(II) intermediate. Then, deprotonation and C-N reductive elimination gave the final product **51** [68].



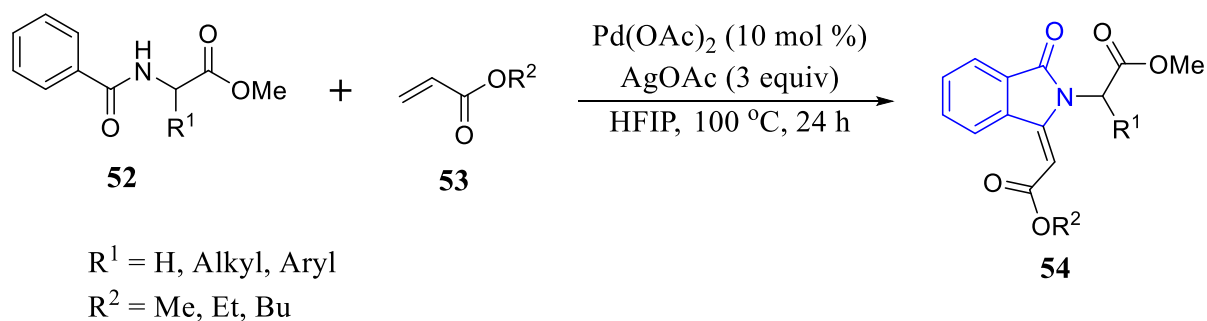
$\text{R}^1 = \text{H, OMe, Me, Cl, Br, F, CO}_2\text{Et}$

$\text{R}^2 = \text{H, Ph, Me}$

Scheme 1.19 Synthesis of 3-substituted isoindolin-1-ones from primary benzyl amines

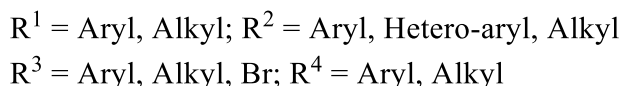
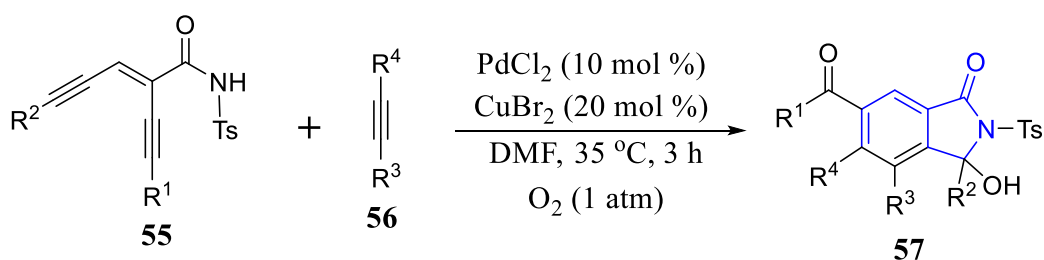
Gupta et al. in 2021 displayed the synthesis of N-alkyl-3-methenyl isoindolin-1-one compounds **54** catalyzed by palladium acetate (10 mol%). The synthesis was done from aryl

amides of L-amino acids **52** and substituted alkenes **53** in HFIP solvent with 3 equivalents of AgOAc and the reaction was done at 100 °C for 24 h (**Scheme 1.20**). 18 derivatives were synthesized with maximum yield of upto 81%. According to their plausible mechanism, initially **52** underwent C-H bond activation using Pd catalyst, followed by insertion of **53** to generate Pd complex intermediate. This further underwent 1,2-migration and β -hydride elimination to form *ortho*-olefinated aryl amide intermediate. Finally, C-H activation and reductive elimination of this product generated the final N-alkyl-3-methenyl isoindolin-1-one product **54** [69].



Scheme 1.20 Palladium-catalyzed synthesis of N-alkyl-3-methenyl isoindolin-1-ones

Ling et al. synthesized substituted 3-hydroxyisoindolin-1-ones **57** catalysed by palladium chloride with copper bromide used as additive. They synthesized using enediynes **55** and internal alkynes **56** starting materials. The reaction was stirred in DMF solvent at 35 °C for 3 h under air (**Scheme 1.21**).



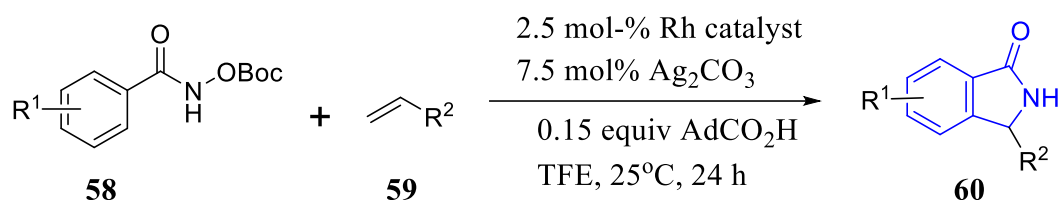
Scheme 1.21 Palladium chloride-mediated synthesis of 3-hydroxyisoindolin-1-ones

High yields were obtained when aryl groups were present on R^1 and R^2 positions of **55**. Proposed reaction mechanism suggested that reaction was done through 5-endo

cyclization of **55** using palladium catalyst, followed by syn carbopalladation onto **56** to generate palladium complex intermediate. This intermediate underwent nucleophilic attack by H₂O followed by oxidation with O₂ to afford the final 3-hydroxyisoindolin-1-one product **57** [70].

1.3.2 Rhodium-catalyzed Synthesis of Isoindolin-1-ones

Cui et al. synthesized isoindolin-1-ones **60** via N-protected benzamide **58** and alkene **59** enantioselective [4+1] annulation process utilizing Rhodium (Cp^mRh(III)) as a catalyst. 1-Adamantanecarboxylic acid (AdCO₂H) and silver carbonate (Ag₂CO₃) were used as additives at 25°C (**Scheme 1.22**). The reaction was completed in 24 h producing a yield to a maximum of 94%. According to their plausible mechanism, the reaction initially started by the generation of Cp^{*}Rh(OCOAd)₂ from p^{*}RhCl₂ formed by reacting with AdCOOH and Ag₂CO₃. Then, N-protected benzamide **58** underwent ligand exchange followed by *ortho* C-H bond activation to give 5-membered rhodacycle intermediate which further coordinated with **59** and underwent migratory insertion to form 7-membered rhodacycle intermediate. *Syn* β-H elimination and second migratory insertion gave 6-membered rhodacycle intermediate which further underwent C-N bond reductive elimination and N-O bond oxidative addition to give isoindolin-1-one final product **60** [71].



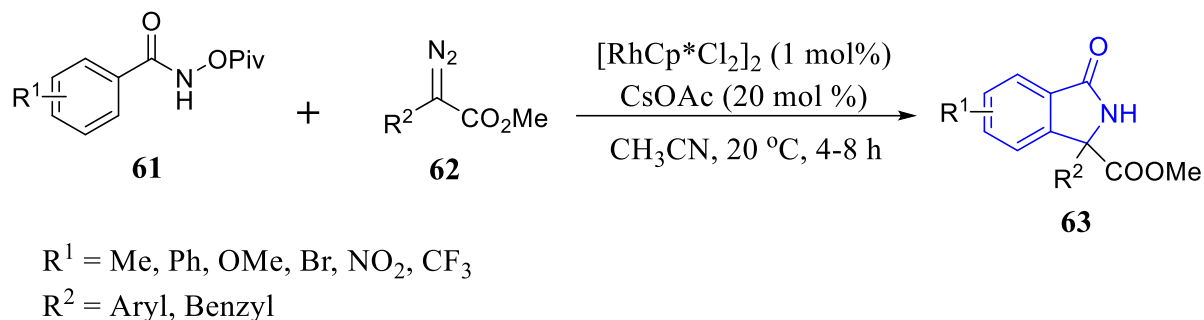
R¹ = H, Me, OMe, OPh, t-Bu, NO₂, CF₃, Ph, F, Cl, Br, I

R² = Aryl

Scheme 1.22 Rhodium-catalysed synthesis of isoindolin-1-ones from N-protected benzamides and alkenes

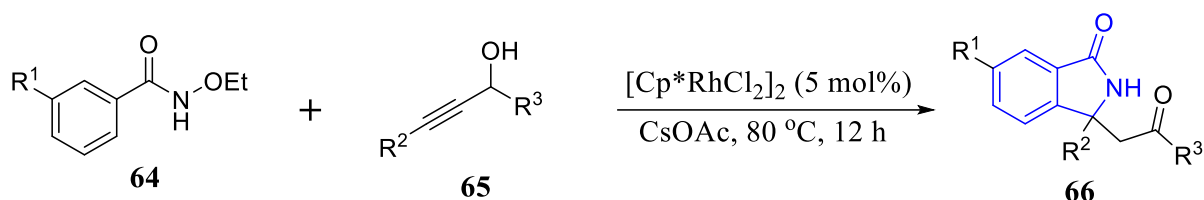
Hyster et al. reported the rhodium catalyzed synthesis of isoindolin-1-ones **63** from the coupling reaction of O-pivaloyl benzhydroxamic acids **61** with diazo molecules **62** in the presence of 20 mol% of cesium acetate (CsOAc). The reaction was stirred at 20 °C for 4-8 h in acetonitrile solvent (**Scheme 1.23**). 32 derivatives were synthesized with maximum yield of upto 99%. According to their plausible mechanism, initially rhodium catalyst combined

with **61** to get Rh-N complex intermediate, which further reacted with **62**, followed by migratory insertion, cyclization, and C—O bond cleavage to get the final isoindolin-1-one product **63** [72].



Scheme 1.23 Rhodium-catalyzed synthesis of isoindolin-1-ones from O-pivaloyl benzhydroxamic acids and diazo compounds

Wu et al. reported the synthesis of 3-substituted isoindolin-1-ones with quaternary carbon **66**. The synthesis was done from N-acetylbenzamides **64** and propargyl alcohols **65** via C-H activation and further [4+1] cyclization. The reaction was stirred at 80 °C for 12 h in anhydrous DCE solvent with 1 equivalent of CsOAc (**Scheme 1.24**). The reaction displayed a broad substrate scope and synthesized derivatives with moderate to good yields. Proposed reaction mechanism suggested that initially active Rh(III) catalyst was formed from anion exchange with CsOAc. Then, Rh(III) catalyst coordinated with **64** and promoted C-H activation to generate a five-membered rhodacycle intermediate, which further reacted with **65** to get a seven-membered rhodacyclic intermediate. Subsequently, allylic proton abstraction, reductive elimination, and keto-enol tautomerization gave to another intermediate, which further underwent intramolecular N—O bond oxidation to afford the final 3-substituted isoindolin-1-one product **66** [73].

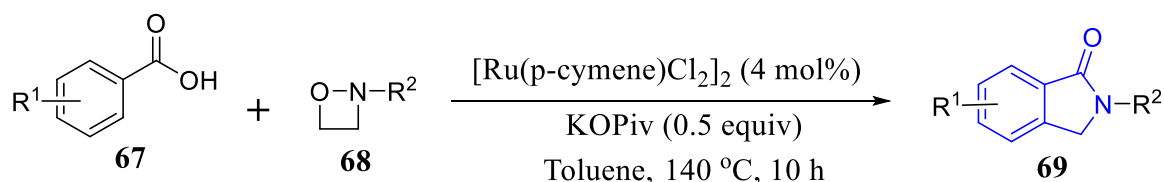


$\text{R}^1 = \text{H, Me, OMe, Cl, Br, NO}_2, \text{COOMe, CF}_3, \text{CN, Ph}$
 $\text{R}^2 = \text{Alkyl; R}^3 = \text{Aryl, Hetero-aryl, Benzyl}$

Scheme 1.24 Rhodium-catalyzed synthesis of 3-substituted isoindolin-1-ones from N-acetylbenzamides and propargyl alcohols

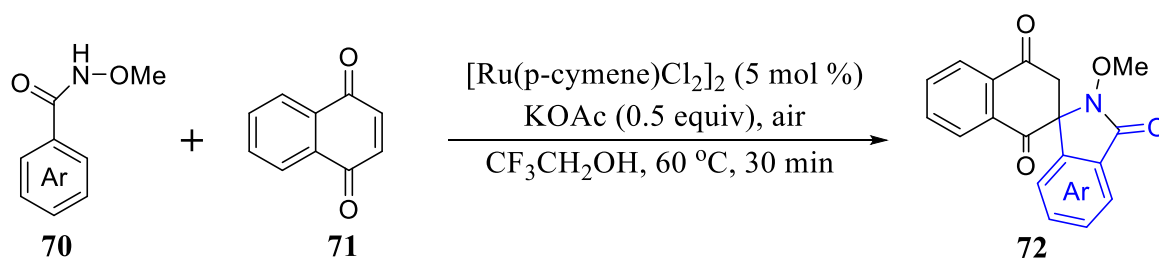
1.3.3 Ruthenium-catalyzed Synthesis of Isoindolin-1-ones

Hu et al. synthesized N-substituted isoindolin-1-ones **69** by reacting benzoic acids **67** with 1,2-oxazetidines **68** with the help of dichloro(p-cymene)ruthenium(II) dimer, $[\{\text{Ru}(\text{p-cymene})\text{Cl}_2\}]_2$ catalyst and potassium pivalate, KOPiv as a base at 140 °C in toluene solvent producing maximum yield upto 96% (**Scheme 1.25**). According to their plausible mechanism, initially, **67** was deprotonated and converted to 2-methylbenzoate using KOPiv base, which further underwent C-H activation to generate ruthenacycle intermediate. This intermediate then reacted with **68**, followed by β -carbon elimination and migratory insertion to generate a Ru-complex intermediate. Finally, intramolecular cyclization followed by dehydration generated the final isoindolin-1-one product **69** [74]. Baidiya et al. reported ruthenium catalyzed synthesis of spiro isoindolin-1-ones **72** from N-methoxy benzamides **70** and naphthoquinones **71** in the presence of KOAc base. Reaction was stirred at 60 °C in 2,2,2-trifluoroethanol ($\text{CF}_3\text{CH}_2\text{OH}$) solvent for 30 minutes (**Scheme 1.26**). 23 derivatives were synthesized with maximum yield of upto 86 %. Plausible reaction mechanism indicated that initially **70** underwent ortho-C-H metalation with Ru catalyst to generate metallacycle intermediate, which combined with **71**, followed by migratory insertion and hydroxylation to get amide intermediate. This intermediate then underwent oxidation followed by 5-exo-trig cyclization to generate the final spiro isoindolin-1-one product **72** [75].



$\text{R}^1 = \text{Me, Et, Ph, Bn, CH}_2\text{CH}_2\text{Ph, Cl, N(Me)}_2, \text{Br, n-butyl, I, OMe}$
 $\text{R}^2 = \text{Ts, 4-OMePh-SO}_2, \text{4-ClPh-SO}_2, \text{4-CF}_3\text{Ph-SO}_2, \text{4-BrPh-SO}_2, \text{4-FPh-SO}_2, \text{2,4,6-(Me)}_3\text{Ph-SO}_2$

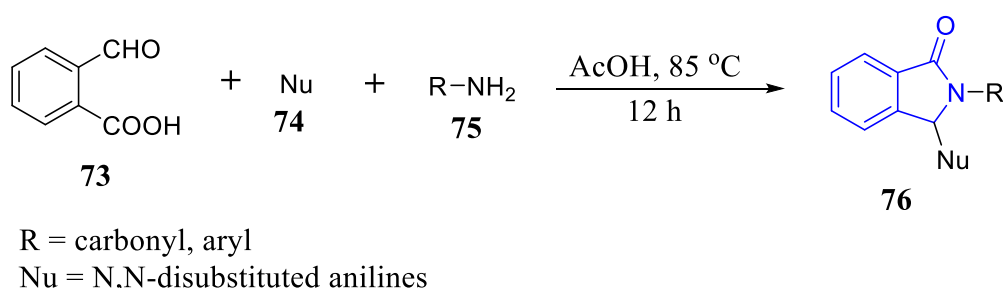
Scheme 1.25 Ruthenium-catalyzed synthesis of N-substituted isoindolin-1-ones



Scheme 1.26 Ruthenium-catalyzed synthesis of spiro isoindolin-1-ones

1.3.4 Acid-Mediated Synthesis of Isoindolin-1-ones

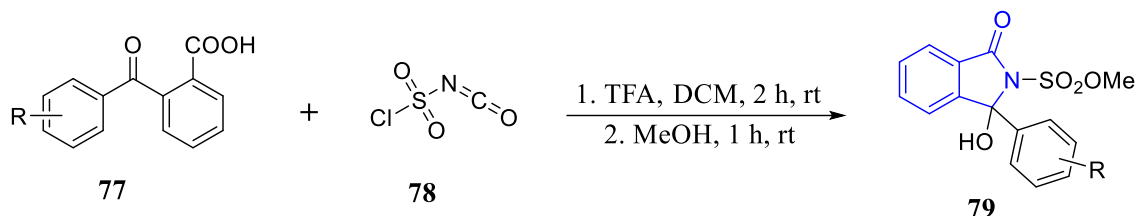
Karu et al. in 2025 reported a one-pot, acid-mediated synthesis of N-substituted isoindolin-1-one compounds **76** from 2-carboxybenzaldehyde **73**, nucleophile **74**, and anilines **75**. Reaction was carried out at 85 °C for 12 h (**Scheme 1.27**). Derivatives of N-substituted isoindolin-1-ones were synthesized in good to excellent yields. According to them, firstly the carbonyl group of **73** is protonated using AcOH acid, which on further nucleophilic attack by **75**, rearomatization, and dehydration generated a carbocation intermediate. This intermediate on further reaction with **74** and intramolecular cyclization made the desired N-substituted isoindolin-1-one product **76** [76].



Scheme 1.27 Acid-mediated synthesis of N-substituted isoindolin-1-ones.

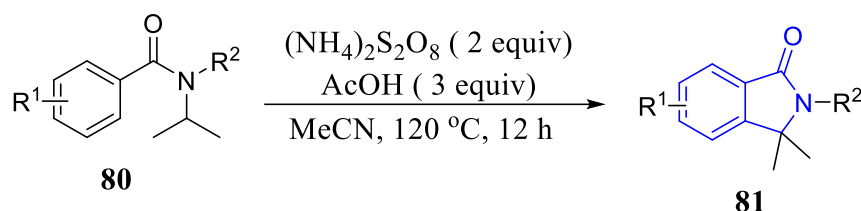
Atmaca et al. displayed synthetic methodology for isoindolin-1-ones **79** from 2-benzoylbenzoic acids **77** and chlorosulfonyl isocyanates **78** mediated by trifluoroacetic acid (TFA). Firstly, the reaction was stirred in DCM solvent at room temperature for 2 h. After 2 h, reaction was stirred at room temperature for one h in methanol solvent (**Scheme 1.28**). Twelve derivatives were synthesized with yields in range of 70-96% containing both electron-donating and electron-withdrawing groups. According to their plausible mechanism, initially, carbonyl carbon of **78** underwent nucleophilic attack by COOH group of **77**, followed by decarboxylation and protonation using TFA to generate carbamate intermediate. This intermediate then on intramolecular cyclization followed by addition of methanol to afford the final product **79** [77]. Han et al. reported the synthesis of substituted isoindolin-1-ones **81** from N,N-dialkyl benzamides **80** using 2 equiv of ammonium persulfate as an oxidant and 3 equiv of acetic acid (AcOH). The reaction was completed in 12 h at 120 °C using acetonitrile (MeCN) as solvent providing final products with the highest yield of up to 81% (**Scheme 1.29**). The authors proposed a mechanism in which initially per sulfate was converted to sulfate radical anion upon heating. Then, **80** underwent hydrogen atom transfer (HAT) using sulfate radical anion to generate radical intermediate, which further underwent

intramolecular cyclization followed by single-electron transfer (SET) using sulfate radical anion and finally deprotonation to generate the desired substituted isoindolin-1-one product **81** [78].



R = H, Me, Et, propyl, iso-butyl, F, Cl, Br

Scheme 1.28 TFA-mediated synthesis of isoindolin-1-ones from 2-benzoylbenzoic acids and chlorosulfonyl isocyanates

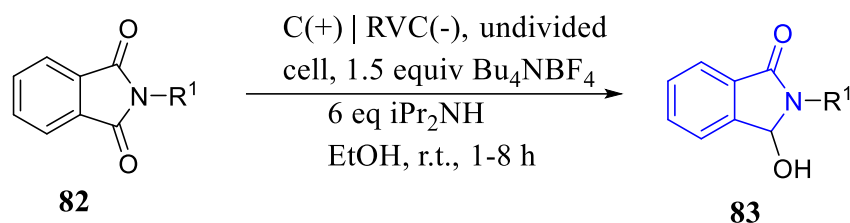


R¹ = H, Me, OMe, Et, n-Pr, i-Pr, n-Bu, t-Bu, n-pentyl, F, Cl, Br, I, Ph
 R² = t-Bu, cyclohexyl

Scheme 1.29 Acid-mediated synthesis of substituted isoindolin-1-one compounds from N,N-dialkyl benzamides

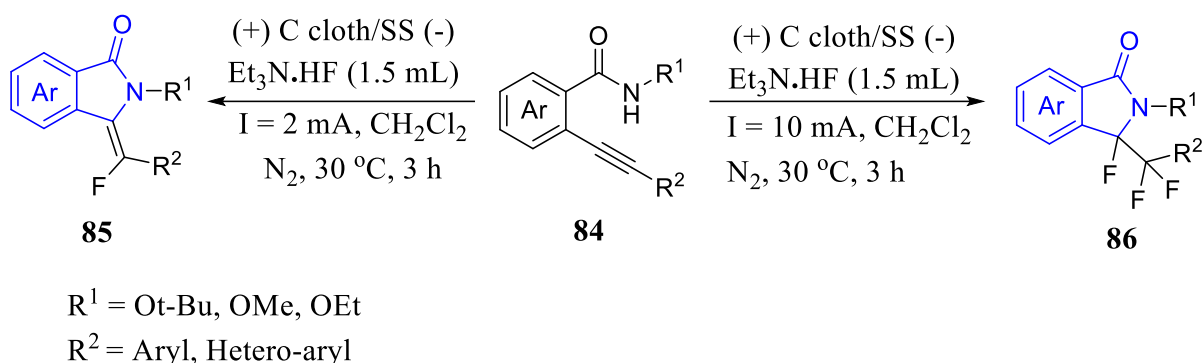
1.3.5 Electrochemical Methods for Synthesis of Isoindolin-1-ones

Bai et al. synthesized 3-hydroxyisoindolin-1-ones **83** by electrochemical reduction of cyclic imidates **82** with constant-current electrolysis having a current value of 20 mA in the presence of diisopropylamine (iPr₂NH) and ethanol (EtOH) providing a highest yield of about 95% at room temperature (**Scheme 1.30**). Control experiments suggested that the reaction followed a free radical pathway. According to their plausible mechanism, diisopropylamine underwent proton exchange with ethanol to form α-aminoalkyl radical by losing proton and electron at the anode. Then, cyclic imide **82** was converted to radical intermediate with the gain of a proton and an electron at the cathode. The radical then finally underwent C-H abstraction using α-aminoalkyl radical to give the final 3-hydroxyisoindolin-1-one product **83** [79].



Scheme 1.30 Electrochemical reduction of cyclic imide to give 3-hydroxyisoindolin-1-one

Ding et al. reported the synthesis of monofluorinated and trifluorinated isoindolin-1-one compounds (**85** and **86**) from electrochemical intramolecular 5-exo-dig aza-cyclization of 2-alkynylbenzamides **84** and further nucleophilic fluorination. Carbon cloth (30 mm × 30 mm × 0.1 mm) was used at the anode and stainless steel (30 mm × 30 mm × 0.1 mm) was used at the cathode. Triethylamine trihydrofluoride (Et₃N·3HF) was used as the source of fluorine atom. Monofluorinated isoindolin-1-one derivatives **85** were synthesized by using a constant electric current of 2 mA while trifluorinated isoindolin-1-one derivatives **86** were made with a constant electric current of 10 mA (**Scheme 1.31**) [80].

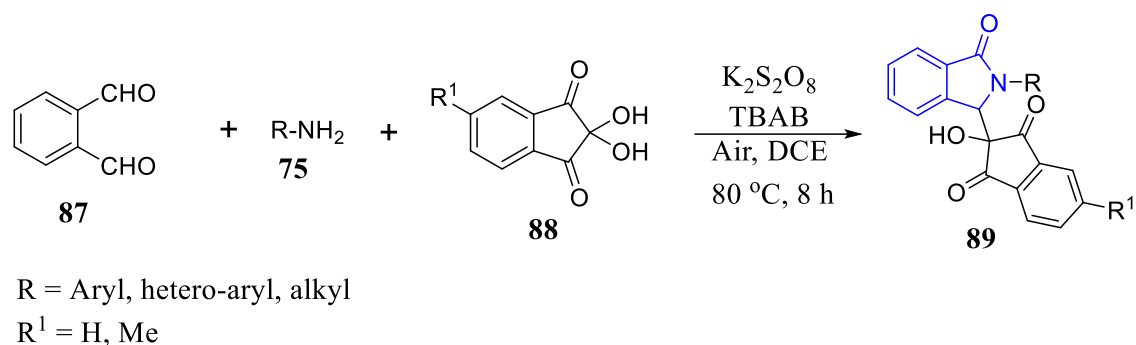


Scheme 1.31 Electrochemical method of synthesis of monofluorinated and trifluorinated isoindolin-1-one compounds from 2-alkynylbenzamides

1.3.6 Oxidant-mediated Synthesis of Isoindolin-1-ones

Rana et al. synthesized 2-hydroxy-2-[N-(alkyl/aryl/heteroaryl)-3-oxoisoindolin-1-yl]-1H-indene-1,3(2H)-diones **89** with highest yield of up to 94% from phthalaldehyde **87**, amine **75**, and 2,2-dihydroxyindane-1,3-dione **88** mediated by potassium persulfate (K₂S₂O₈) oxidant and tetrabutyl ammonium bromide (TBAB) (**Scheme 1.32**). The reaction was performed in air using DCE solvent at 80 °C for 8 h. Derivatives were synthesized with yields

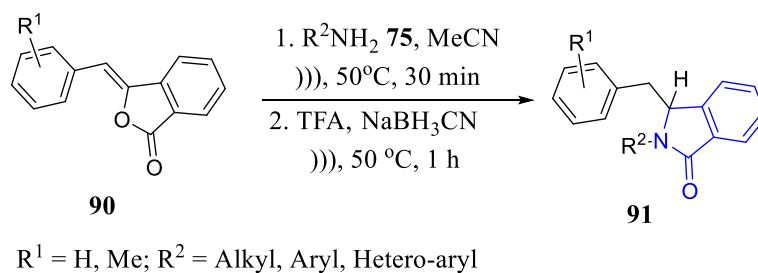
of upto 75-94% containing aryl, alkyl, and hetero-aryl groups. Control experiments suggested that the reaction mechanism followed generation of free radicals [81].



Scheme 1.32 Oxidant-mediated synthesis of 2-hydroxy-2-[N-(alkyl/aryl/heteroaryl)-3-oxoisindolin-1-yl]-1H-indene-1,3(2H)-diones

1.3.7 Ultrasound-assisted Synthesis of Isoindolin-1-ones

Mardjan et al. reported the ultrasound-assisted one-pot synthesis of 3-substituted isoindolin-1-ones **91** with the highest yield of up to 93% from (Z)-3-benzylideneisobenzofuran-1(3H)-ones **90** and primary amines **75**. Their synthetic procedure started with dissolving of (Z)-3-benzylideneisobenzofuran-1(3H)-one **90** and primary amine **75** in 1 mL of acetonitrile and subjected to ultrasonic irradiation at 50°C for 30 min. Then, after cooling in an ice bath, NaBH_3CN and trifluoroacetic acid (TFA) were added, and the reaction continued under ultrasonic irradiation at 50°C for 60 min (**Scheme 1.33**). According to their proposed mechanism, firstly **90** underwent nucleophilic attack by **75** under ultrasonic irradiation to generate an enol intermediate, which subsequently tautomerized to its keto form. The keto form of intermediate then underwent intramolecular N-cyclization, followed by dehydration using TFA and finally, reduction with NaBH_3CN to generate the final 3-substituted isoindolin-1-one product **91** [82].



Scheme 1.33 Ultrasound-assisted synthesis of 3-substituted isoindolin-1-ones from (Z)-3-benzylideneisobenzofuran-1(3H)-ones

1.4 Biological activities of indoles and isoindolin-1-ones against neurodegenerative diseases

Neurodegenerative diseases are brought on by abnormalities and disorders of the brain that impair mental and physical functioning by causing a gradual and selective loss of neurons [83]. These diseases are associated with overexpression or dysregulation of different types of protein targets or receptors, which includes butyrylcholinesterase (BChE), monoamine oxidase - B (MAO-B), 5-hydroxytryptamine 2A (5-HT_{2A}) and 5-hydroxytryptamine 6 (5-HT₆) receptors, as well as metabotropic glutamate receptors (mGluR1 and mGluR2) [22,23,40,84-86]. Thus, inhibiting these targets are significant approach for the treatment of neurodegenerative diseases. In this section, the inhibitory activities of indoles and isoindolin-1-one substituted compounds against these targets are mentioned, including their structure-activity relationship studies.

Purgatorio et al. displayed the butyrylcholinesterase (BChE)-selective inhibition studies of 1,2,3,4,5,6-hexahydroazepino [4,3-b] indole compounds. Compound **92** exhibited highest potency in *in-vitro* BChE enzyme inhibition assay with IC₅₀ value of 0.17 μ M among its library. The reference standards, Galantamine and Donepezil showed IC₅₀ values of 12.0 μ M and 2.30 μ M, respectively. Butylbenzene group attached to N-atom in **92** enhances its BChE inhibition according to SAR (**Figure 1.2**). In addition, compound **92** displayed neuroprotective activity in SH-SY5Y cell line against cytotoxicity persuaded by A β 42 peptide and oxidative stress. According to molecular docking studies of **92** with BChE protein, **92** formed conventional hydrogen bonding interactions with Thr120 and His438 residues of protein [22]. Furthermore, Kondej et al. checked the 5-HT_{2A} inhibition potential of 5-substituted-3-(1-arylmethyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-indole compounds for the treatment of schizophrenia disease. Out of these compounds, compound **93** displayed potent 5-HT_{2A} inhibition in *in-vitro* CHO-K1 cell line assay with pIC₅₀ of 5.62 $\pm$ 0.20 as compared to standard risperidone (pIC₅₀ = 9.15 $\pm$ 0.04). Molecular docking of **93** with 5-HT_{2A} showed that H-atom of NH of indole ring formed hydrogen bonding with Cys227 residue of protein. This explained the importance of indole moiety and presence of thiophene ring further increased the inhibitory activity [23].

Elkamhawy et al. reported indole substituted compound **94** as a potent and selective hMAO-B inhibitor for the treatment of Parkinson's disease. Compound **94** showed high hMAO-B inhibition potency having IC₅₀ value of 42 nM and high selectivity index of greater than 2375. The presence of 3,4-dichlorobenzamide group is responsible for increased hMAO-

B inhibition (**Figure 1.2**). Furthermore, the kinetic studies showed that inhibition mode of **94** is reversible and competitive inhibition. Also, compound **94** displayed high CNS bioavailability and low cytotoxicity profile. Molecular docking studies of **94** with MAO-B protein showed high docking score of **94** (-11.23 kcal/mol) and π - π stacking interaction of indole ring with Tyr398 amino acid residue of MAO-B protein [84]. Moreover, Yi et al. in 2022 displayed the inhibition potential of 1-(phenylsulfonyl)-4-(piperazin-1-yl)-1H-indole compounds as 5-HT₆ receptor antagonist. Among these compounds, compound **95** exhibited superior potency with high binding affinity with 5-HT₆ receptor having K_i of 0.52 ± 0.08 nM as compared to Idalopirdine reference standard (K_i = 0.83 nM). The presence of fluorine atom and difluoromethyl group in **95** further increased its biological activity (**Figure 1.2**). Compound **95** also showed good pharmacokinetic profile and good oral bioavailability. It also displayed favourable brain penetration [85].

Sasidharan et al. evaluated the inhibition potential of indole-based chalcone compounds against human MAO-B enzyme. Compound **96** showed high hMAO-B inhibition with K_i value of 0.01 ± 0.005 μ M and selectivity index value of 120 in its library (**Figure 1.3**). SAR studies indicated that the presence of bromine atom is helpful in increasing its biological activity. Compound **96** was found to non-toxic. In the molecular docking of **96** with hMAO-B enzyme, it was found that **96** displayed hydrogen bonding with Tyr325 residue and π - π stacking interaction with Tyr435 residue [86]. Furthermore, Nam et al. checked indole-benzothioazole hybrids as hMAO-B inhibitors. Among them, compound **97** exhibited high in-vitro hMAO-B inhibition with IC₅₀ value of 29 nM as compared to safinamide reference standard (IC₅₀ = 51 nM). According to their SAR studies, methyl group attached to nitrogen atom of indole ring enhanced the hMAO-B inhibition (**Figure 1.3**). Compound **97** displayed no significant inhibition of CYP isozymes, indicating a low potential for drug–drug interactions. In addition, oral administration of **97** indicated neuroprotective effects and improved motor function in an MPTP-induced Parkinson's disease mouse model [87]. Moreover, Chirkova et al. checked the MAO-A and MAO-B inhibition potential of indole-5,6-dicarbonitrile substituted compounds, out of which compound **98** showed superior potency with IC₅₀ values of 0.014 and 0.017 μ M against MAO-A and MAO-B, respectively. Further studies indicated that **98** acted as reversible and competitive inhibitor. The presence of methyl group in **98** is responsible for its potent inhibition according to SAR studies [88].

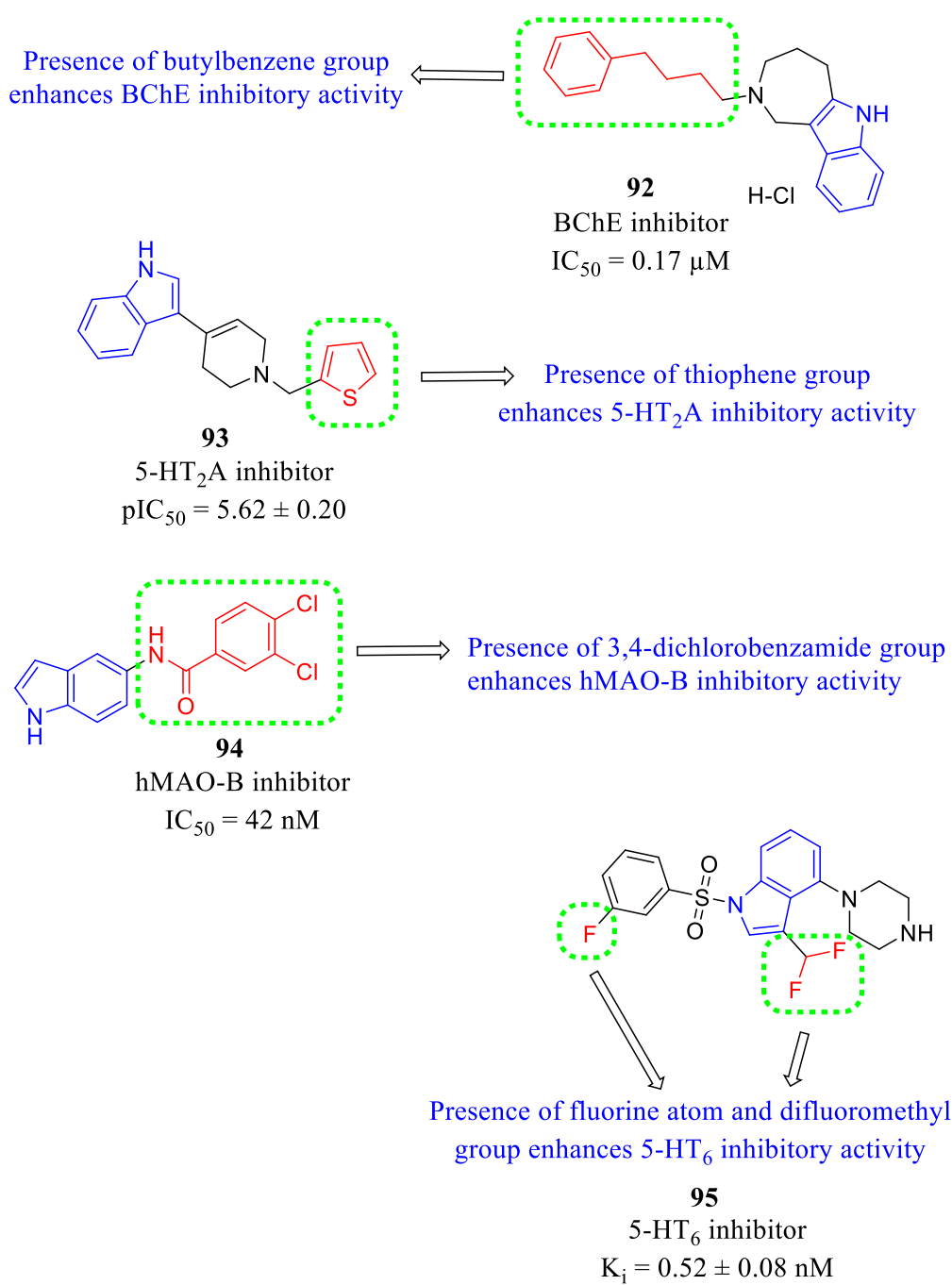


Figure 1.2 SAR of substituted indoles **92**, **93**, **94**, and **95** exhibiting potent activity against neurodegenerative diseases

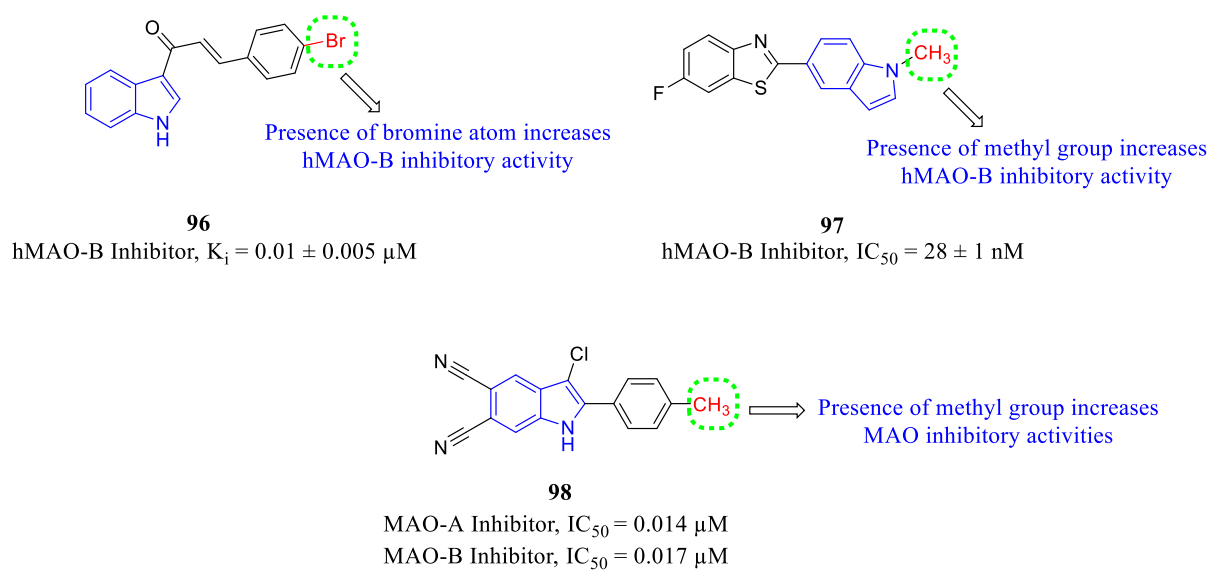


Figure 1.3 SAR of substituted indoles **96**, **97**, and **98** exhibiting potent activity against neurodegenerative diseases

Examples of isoindolin-1-one derivatives with potential therapeutic applications against neurodegenerative diseases are discussed here including SAR studies. Among these, Ito et al. screened derivatives of 5-(1-aryl-3-methyl-1,2,3-triazole-4-yl) isoindolin-1-one as antagonists of metabotropic glutamate receptor 1 (mGluR1). The compound with 2,4-difluorobenzene substituent **99** showed high potency with IC_{50} of 4.3 nM in mGluR1 antagonist activity for humans and IC_{50} value of 3.6 nM for rats (**Figure 1.4**). This compound **99** displayed good selectivity of mGluR1 over other mGluRs. *In-vivo* studies of **99** suggested that **99** achieved satisfactory brain and plasma concentration values in mice which were 0.17 μM and 0.45 nmol/g at 30 min post-dose, respectively after oral administration of 1 mg/Kg and persisted 46% oral bioavailability in rats [89]. Furthermore, Sidique et al. investigated the inhibitory activity of isoindolin-1-one derivatives against mGlu₂ receptor-positive allosteric modulators (PAMs). Compound **100** with cyclopentane group attached to N-atom (**Figure 1.4**) showed superior potency and selectivity in *in-vitro* assay and *in-vivo* pharmacokinetic (PK) properties. Compound **100** displayed an EC_{50} value of $0.05 \pm 0.02 \mu\text{M}$ in an *in-vitro* mGlu₂ receptor PAM assay. **100** displayed excellent oral bioavailability value of $71.4 \pm 2.3 \%$, adequate brain penetration, and reduced nicotine infusions by 40% having a dose of 20 mg/Kg po in a rat model [40]. Furthermore, Kamauchi et al. displayed the MAO inhibition potential of isoindolin-1-one derivatives against MAO-A and MAO-B enzymes. Among these, derivative **101** showed high potency with IC_{50} values of 17.8 and 15.8 μM in *in-vitro* MAO-A and MAO-B enzyme inhibition assays, respectively (**Figure 1.4**) [90]. Moreover,

Atmaca et al. evaluated the BChE inhibition of isoindolin-1-one compounds. Compound **102** exhibited the potent K_i value of $1.45 \pm 0.08 \mu\text{M}$ in BChE inhibition assay. In addition, molecular docking studies of **102** with BChE suggested high docking score value of -8.1 kcal/mol and conventional hydrogen bonding interactions with Thr120 residue of protein [91].

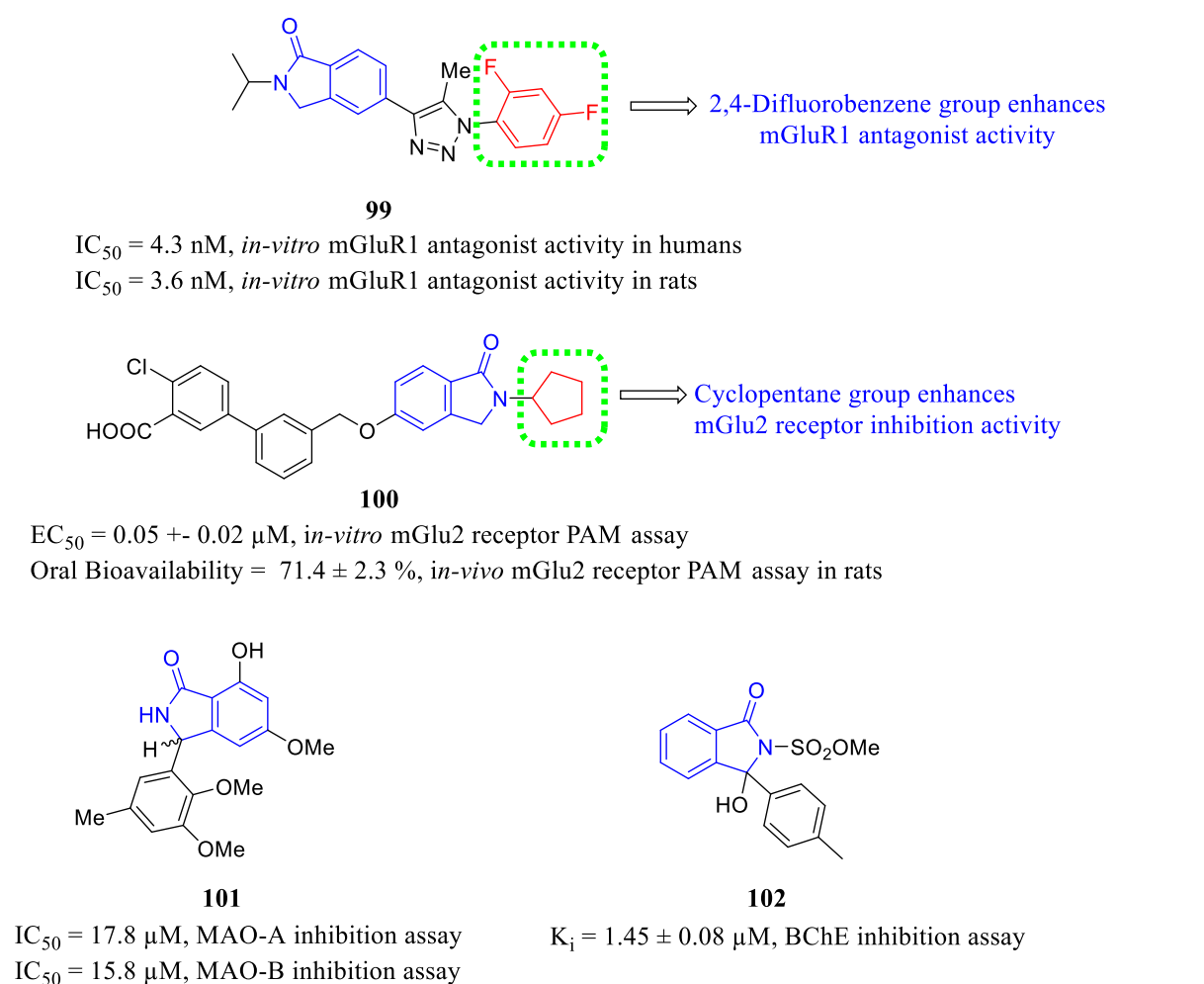


Figure 1.4 Structures of substituted isoindolin-1-ones **99**, **100**, **101**, and **102** exhibiting potent biological activity against neurodegenerative diseases

1.5 Limitations and Research Gaps

Several methods of the synthesis of indoles and isoindolin-1-ones and neuroprotective activities of these moieties is discussed in this chapter. Many of them exhibit certain limitations, which includes high catalyst loading, limited substrate scope, long reaction time, requirement of metal-based catalyst, harsh reaction conditions. These drawbacks reduce the sustainability, practicality, and economic feasibility of the reported protocols. The limitations

of existing synthetic methods highlight the need for the development of facile, metal-free, mild conditions, and environmentally friendly methods for the synthesis of functionalized indole and isoindolin-1-one derivatives. Moreover, biological investigations for neurodegenerative diseases like Parkinson's Disease have not been explored for various substituted indole and isoindolin-1-one derivatives.

To overcome some of the limitations, and research gaps, the thesis has been divided into six chapters:

- Chapter 1:** Introduction: General aspects of Indole and Isoindole Derivatives
- Chapter 2:** *In-silico* studies of 3-acyl-3-hydroxyisoindolin-1-one derivatives as potential MAO-B inhibitors
- Chapter 3:** Selectfluor mediated synthesis of 3-acyl-3-hydroxyisoindolin-1-ones and their antioxidant studies
- Chapter 4:** *In-silico* studies of N-alkenylindole derivatives as potential MAO-B inhibitors
- Chapter 5:** Iodine catalyzed synthesis of N-alkenylindoles and their antioxidant studies
- Chapter 6:** Summary, Future prospects, and Social Impact

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CHAPTER 2

In-silico studies of 3-acyl-3-hydroxy
isoindolin-1-one derivatives as
potential MAO-B inhibitors

2.1 Introduction

Neurodegeneration is a group of disorders caused by neuronal dysfunction that pose a serious long-term risk to human health [1,2]. Older people are mostly affected by neurodegenerative disorders (ND), which can be recognized by their prevalent incidence as well as intricate pathophysiological mechanisms [3]. Several neurodegenerative diseases are brought on by abnormalities and disorders of the brain that impair mental and physical functioning by causing a gradual and selective loss of neurons [4]. Various factors contribute to ND including oxidative stress, decreased neurotransmitter levels, neuro-inflammation, mitochondrial disorder, proteolytic stress, and neuron death [5]. Parkinson's disease (PD) is the most common neurodegenerative disease, which is associated with motor dysfunction [6,7]. Dopamine (DA) levels in the striatum decrease due to the loss of dopaminergic neurons in the *substantia nigra*. Other non-motor symptoms of PD are depression, cognitive decline, and sleep disorders [5]. It is estimated that 6 million people worldwide suffer from PD, which has an incidence rate of 150 per 100,000. By 2030, the number is expected to double or quadruple [8].

Monoamine oxidase (MAO, EC 1.4.3.4, amine-oxygen oxidoreductase) is a flavoenzyme that contains flavin adenine dinucleotide (FAD) and is found near the outer mitochondrial membrane of neurons, glial cells, and other mammalian cells, particularly in the liver and brain [9-13]. The oxidative deamination of xenobiotic amines, monoamine neurotransmitters, and hormones that consist of dopamine, serotonin, and norepinephrine in the brain and other tissues is catalyzed by the MAO enzyme [14,15]. There are two isoforms of MAO: MAO-A and MAO-B. Both isoforms are encoded by genes derived from the X chromosome and exhibit 70% genetic similarity. MAO-A is mainly located in neurons, whereas MAO-B is within astrocytes' outer mitochondrial membrane [16,17]. The difference in both isoforms is due to their substrate specificity and inhibitor selectivity [18]. Serotonin is typically metabolized by MAO-A, whereas 2-phenylethylamine and benzylamine are mostly deaminated by MAO-B. Dopamine, norepinephrine, and epinephrine metabolism are mediated by both MAO isoforms [19]. High levels of MAO-B are mainly seen in the 50-60 age group of people [20]. Overexpression levels of the MAO-B isoform are responsible for promoting oxidative stress in the neuro-system due to the generation of hydrogen peroxide, which is produced from a deamination reaction catalyzed by MAO-B [21]. MAO-A inhibitors are employed in the treatment of anxiety and depression, while MAO-B inhibitors are used for treating Alzheimer's disease (AD) and PD [22-24]. Therefore, the development

of effective and selective MAO-B inhibitors is necessary for the treatment of Parkinson's disease and other neurodegenerative illnesses [25-27]. Several MAO-B inhibitors have been reported previously, with the most commonly known being selegiline, rasagiline, and safinamide [28-33]. Selegiline and rasagiline act as irreversible inhibitors. While effective, they can inhibit MAO-A at higher doses, leading to potential side effects and dietary restrictions [34,35]. In addition, long-term use of selegiline, rasagiline, or safinamide may lead to insomnia, nausea, or gastrointestinal issues [36,37]. Thus, the development of highly selective, reversible MAO-B inhibitors with reduced side effects is still a challenge.

Isoindolin-1-ones are a significant class of heterocyclic compounds that come from both natural and synthetic sources and display various types of biological activities like anti-bacterial, anti-cancer, anti-psychotic, anti-viral, anti-hypertensive, and against neurological disorders [38-47]. Previous studies showed that the presence of the isoindolin-1-one ring is helpful in the inhibition of several neurological disorders like AD and PD [48-51]. When going through the literature search, we found that the synthesis of 3-acyl-3-hydroxyisoindolin-1-one compounds has been reported previously [52-54]. Still, their *in-silico* investigations on neurodegenerative diseases have not been explored to date to the best of our knowledge. This gap in the literature presents an opportunity to investigate the computational aspects of these compounds as MAO-B inhibitors.

In this chapter, we examined the *in-silico* studies of a library of 279 3-acyl-3-hydroxyisoindolin-1-one derivatives. We performed the molecular docking of the complete library with the MAO-B protein target (PDB ID:6FWC). We also validated the docking protocol with the help of the co-crystal ligand of the MAO-B protein (a known inhibitor of MAO-B) [55]. Then the top two docked scored compounds were further investigated using other *in-silico* tools which were ADME, MDS, MM-GBSA, and DFT. The Schrodinger suite 2022.4 was employed for all the *in-silico* studies [56]. In addition, the Desmond module gives robust and efficient long-timescale MDS for understanding protein-ligand dynamics, often surpassing conventional MD engines in speed and reliability [57]. Moreover, Prime's MM-GBSA, QikProp, and Jaguar modules of Schrodinger suite 2022.4 have demonstrated greater accuracy than several alternative tools for binding free energy estimation, ADME prediction, and quantum mechanical DFT calculations, respectively, thus ensuring the validity and reproducibility of this work [58-59].

2.2 Materials and Methods

2.2.1 Molecular Docking

3-D crystal structure of MAO-B protein in protein data bank (pdb) format with PDB ID of 6FWC was downloaded from the protein data bank website (<https://www.rcsb.org/>) [60] having a high resolution of 1.70 Å. We have prepared protein using the Protein Preparation Wizard, which is available in the Schrodinger suite 2022.4 [61]. Initially, water molecules and chain B of the MAO-B protein present in the pdb file were removed, and then missing side chains of protein and polar hydrogen atoms were added at the physiological pH of 7.0 ± 2.0 during the pre-processing step. The protonation states of ionizable residues were determined using Epik [62]. Afterward, optimization of the protein was performed in which hydrogen bonds in the protein were accurately assigned and checked for potential errors in hydrogen bond placements considering, the protonation state of amino acid residues of the protein. Then, the protein underwent restrained energy minimization using the OPLS_2005 force field (a comprehensive atom-level force field used to represent biomolecular conformations and non-bonded interactions) [63] with heavy atoms convergence RMSD set to 0.3 Å.

For ligand preparation, initially, 279 structures of 3-acyl-3-hydroxyisoindolin-1-one substituted compounds were drawn in ChemDraw Professional 15.0 [64] and their SMILES (Simplified Molecular Input Line Entry System (SMILES)) were generated. SMILES is a text-based notation that encodes the structure of a compound into a string of characters, making it easy to store, share, and process chemical structures for computational studies [65]. These SMILES were taken and inserted in the LigPrep module of Schrodinger suite 2022.4 [66]. 3-D structure generation and energy minimization were done at pH 7.0 ± 2.0 using OPLS_2005 force field [63]. A grid box defines a 3-D region of the protein where the ligand is allowed to bind. So, the grid box for docking was generated using the Glide Module of Schrodinger suite 2022.4 [67,68] with the position of the grid box set at the centroid of the co-crystal ligand of 6FWC. The Vander Waals radius scaling factor taken for grid box generation was 1.0 with a partial charge cutoff value of 0.25. Default parameters were used for other settings. Finally, protein-ligand docking was done with the help of the Glide Module of Schrodinger suite 2022.4 in Extra Precision (XP) mode to perform more accurate molecular docking [69]. The receptor was kept rigid while all the 279 ligands were kept as flexible molecules throughout the docking process. Glide scores and protein-ligand interactions were observed using Maestro tools of Schrodinger suite 2022.4 [70]. The docking protocol was validated by

redocking the co-crystal ligand of 6FWC protein using the exact same docking procedure (same grid and docking parameters) as followed for the 3-acyl-3-hydroxyisoindolin-1-one substituted compounds library, and then superimposing the redocked pose with the experimental pose of MAO-B protein, and then the RMSD between both the poses was calculated. Molecular docking was also performed with selegiline, rasagiline, and safinamide known inhibitors for comparative analysis.

2.2.2 Molecular Dynamics Simulation (MDS)

The top two docked scored protein-ligand complexes were further analyzed for their stability by a 500 ns molecular dynamics simulation. The Linux operating system was used for simulation using an AMD Ryzen 7 3.2 GHz processor with 16 GB of RAM and NVidia GeForce RTX3060-6 GB Graphics Card. Initially, the simulation system was set up using the system builder function present in the Desmond Module of Schrodinger Suite 2022.4 [71]. The Simple Point Charge (SPC) solvent model [72] was used for the solvation of the system. The boundary box shape size was set to orthorhombic with a size of 10 Å in all x, y, and z axes. Counter Na⁺ ions were added to neutralize the system. OPLS_2005 force field [63] was used for dynamics system minimization. Additionally, salt with a concentration of 0.15 M was applied to the system. Then, the prepared system was loaded in the molecular dynamics toolbar of the Desmond Module of Schrodinger suite 2022.4. The short-range van der Waals and Coulomb interactions cut-off was set at a radius of 9 Å. The simulation was run under an isothermal and isobaric ensemble (NPT) having a temperature of 300 K and a pressure of 1.01325 bar. Noose-Hoover chain Thermostat [73] and Martyna-Tobias-Klein Barostat [74] methods were used for maintaining temperature and pressure with a relaxation time of 0.1 ns for both methods. In the RESPA integrator tab, the time steps for bonded, near, and far were 0.000002 ns, 0.000002 ns, and 0.000006 ns, respectively [75]. Finally, a 500 ns molecular dynamics simulation was run, and the simulation trajectory was recorded after every 0.5 ns interval. The simulation Interactions Diagram option of Desmond was used to analyze the complete simulation trajectory. Analysis included Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and protein-ligand interactions. In addition, 500 ns MDS of the co-crystal ligand of MAO-B was also performed to do comparison with these two top docked scored protein-ligand complexes.

2.2.3 MM-GBSA Binding Free Energy Calculations

Prime MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) is helpful in estimating the average binding free energy of protein-ligand complexes after their MDS studies [76]. In this study, a 500 ns simulation trajectory was utilized to calculate the average binding free energy of the top two docked scored MAO-B protein-ligand complexes. Additionally, the co-crystal bound MAO-B protein complex was included for comparative analysis. The MM-GBSA calculations were carried out using the `thermal_mmgsa.py` script of Schrodinger suite 2022.4 [77], which is specifically designed for post-MD trajectory-based binding free energy analysis.

2.2.4 ADME Prediction

The Qikprop module of Schrodinger suite 2022.4 [78] was used to calculate the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME) properties of ligands that give significant information regarding the molecules. Qikprop gives rapid, structure-based prediction of key physicochemical and pharmacokinetic properties of a molecule. SMILES of the top two docked scored ligands were used as input for calculating their ADME properties using Qikprop. Qikprop predicts several important descriptors, including molecular weight, aqueous solubility, blood-brain barrier penetration, human oral absorption, number of rotatable bonds, hydrogen bond donors and acceptors, Lipinski's Rule of Five, and Jorgensen's Rule of Three violations. Each estimated property was compared against Qikprop's recommended optimal ranges to evaluate drug-likeness and pharmacokinetic suitability of the ligands.

2.2.5 Density Functional Theory (DFT)

DFT calculations of top docked scored ligands were performed with the help of the Jaguar Module of Schrodinger suite 2022.4 [79]. 3D structures of these two ligands were geometry optimized in the gas phase using the 6-31G** basis set and B3LYP method. The B3LYP method is a widely used hybrid functional in DFT that balances accuracy and efficiency by mixing exact exchange with density-based correlation [80], while the 6-31G** basis set is a split-valence set with added polarization functions that provides a flexible and realistic description of electron orbitals [81]. Geometry optimization employed the Self-Consistent Field (SCF) procedure [82], where the electron density is iteratively refined until a stable solution is reached. It is similar to repeatedly adjusting values until input and output

match. The SCF convergence criteria for geometry optimization included a maximum of 48 iterations, an energy change threshold of 5×10^{-5} hartree, and an RMS density matrix change of 5×10^{-6} . Then, geometry-optimized ligands were investigated for their frontier Highest Occupied Molecular Orbital (HOMO) - Lowest Unoccupied Molecular Orbital (LUMO) and Molecular Electrostatic Potential (MESP) studies using the same basis set, 6-31G** and B3LYP method. Visualization of their HOMO-LUMO and MESP figures was done using the Maestro graphical interface.

2.3 Results and Discussion

The sequential representation of the work carried out is shown in **Figure 2.1**.

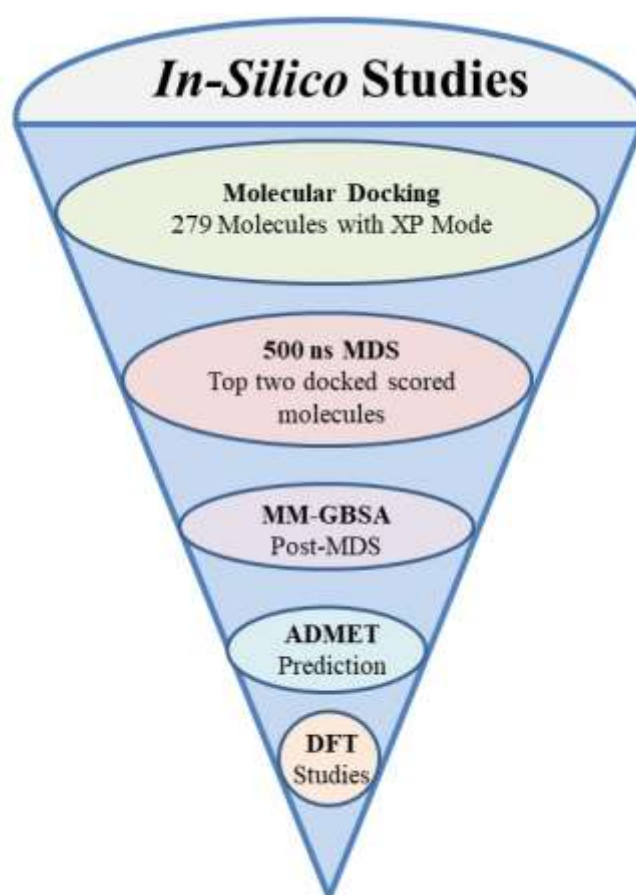


Figure 2.1 Schematic representation of the work carried out

2.3.1 Molecular Docking

The molecular docking technique is widely used in drug discovery. This provides information regarding how the ligand fits into the protein's binding site by exploring several

orientations and positions of the ligand, identifying the best binding pose, and predicting the binding affinity of the ligand with the protein [83]. In the present study, for molecular docking, MAO-B was selected as the target protein for its crucial role in the pathophysiology of neurotransmitters like dopamine, which is highly relevant to PD. Inhibiting MAO-B can enhance dopamine levels in the brain, thereby providing a therapeutic effect, which makes it a significant drug target [25-27]. Additionally, MAO-B has a well-characterized active site with available high-resolution crystal structures, facilitating accurate molecular docking and interaction analysis [84]. By focusing on MAO-B, this study aims to identify compounds with strong binding affinity and favorable interactions, supporting the rational design of novel inhibitors with potential neuroprotective properties.

Effective drug design requires that the three-dimensional structure of the target protein be accurate, high-quality, and reliable. Ramachandran plot is a graphical representation to visualize the most favored, allowed, and disallowed regions for backbone dihedrals, phi (ϕ) and psi (ψ) of protein amino acid residues [85]. The plot helps us to estimate protein structure quality by showing whether the backbone dihedral angles of residues fall within energetically favorable regions or not. PROCHECK online web server [86] was used to form a Ramachandran plot of the chosen protein target, MAO-B (PDB ID: 6FWC) (**Figure 2.2**). **Table 2.1** shows the Ramachandran plot statistics of the MAO-B protein. According to **Table 2.1** and **Figure 2.2**, 92.6 % of MAO-B protein residues lied in the most favored regions, and only 0.5% of residues lied in the disallowed regions. This reflected the high stereochemical quality and reliability of the MAO-B protein structure for *in-silico* studies.

Table 2.1 Ramachandran plot validation statistics of the MAO-B protein.

Properties	Residues	Percentage (%)
Residues in most favored regions	789	92.6 %
Residues in additionally allowed regions	57	6.7 %
Residues in generously allowed regions	2	0.2 %
Residues in disallowed regions	4	0.5 %

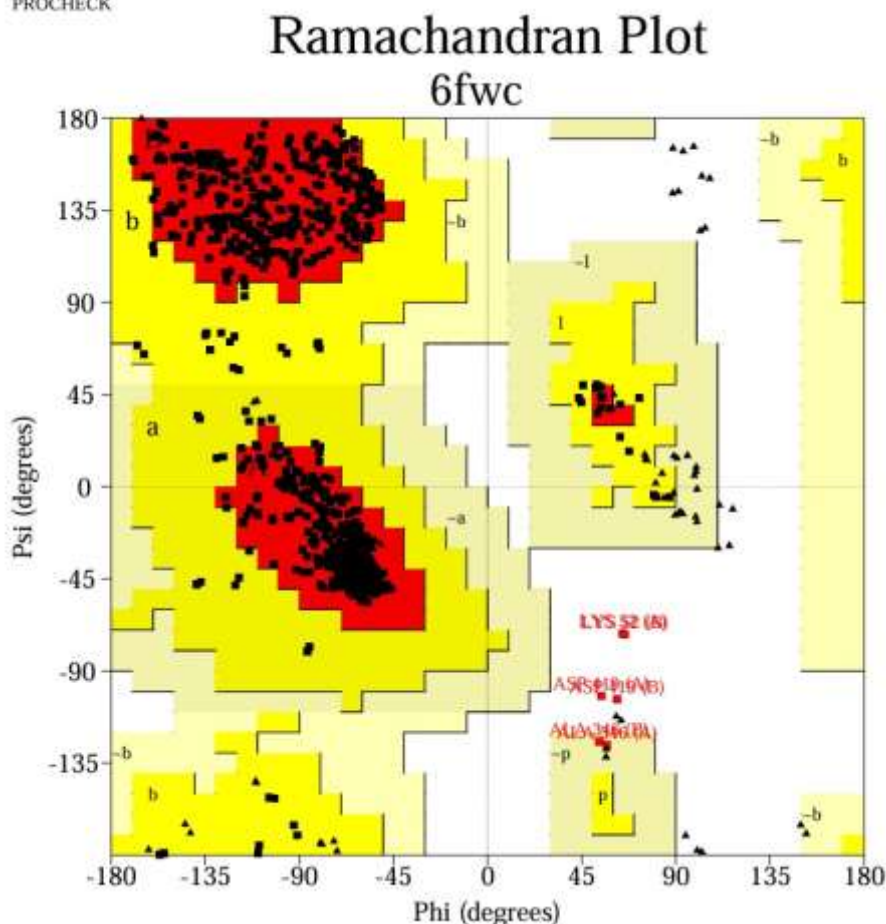
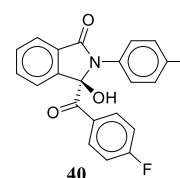
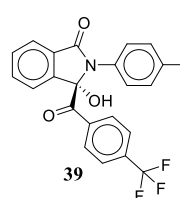
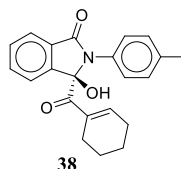
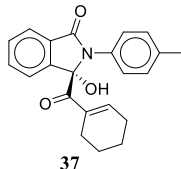
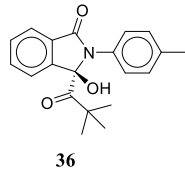
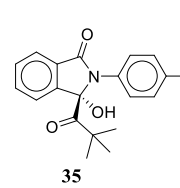
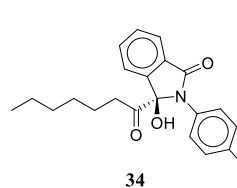
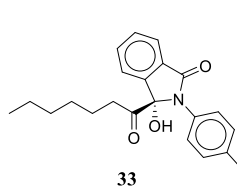
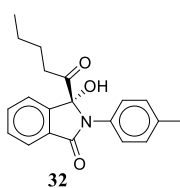
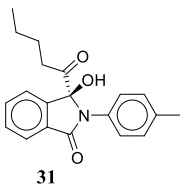
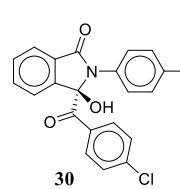
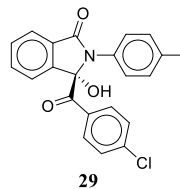
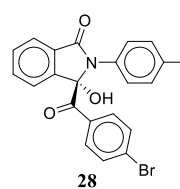
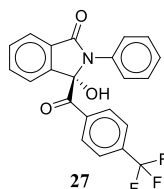
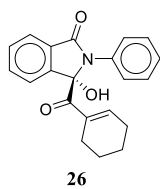
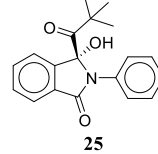
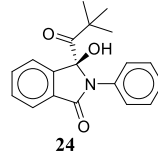
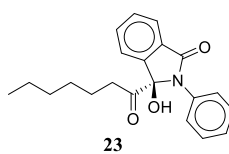
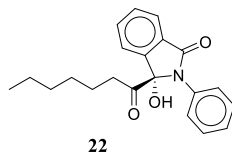
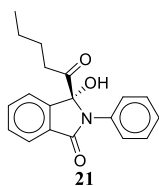
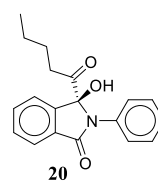
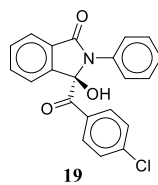
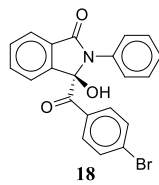
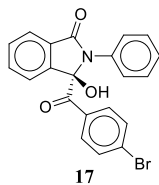
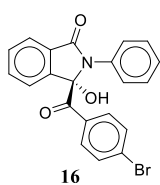
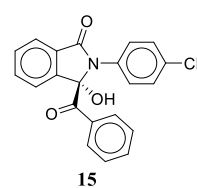
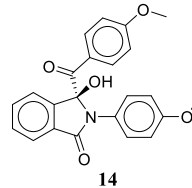
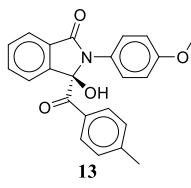
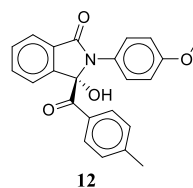
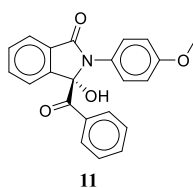
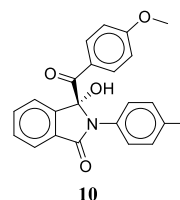
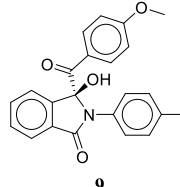
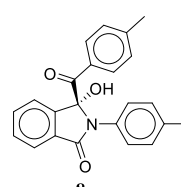
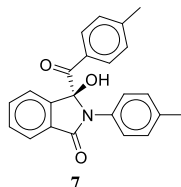
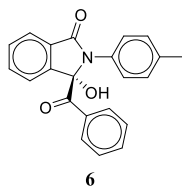
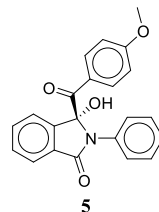
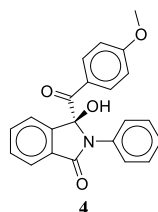
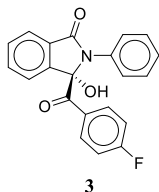
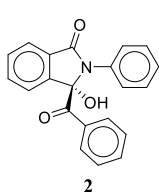
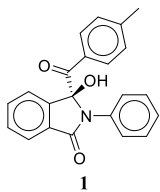


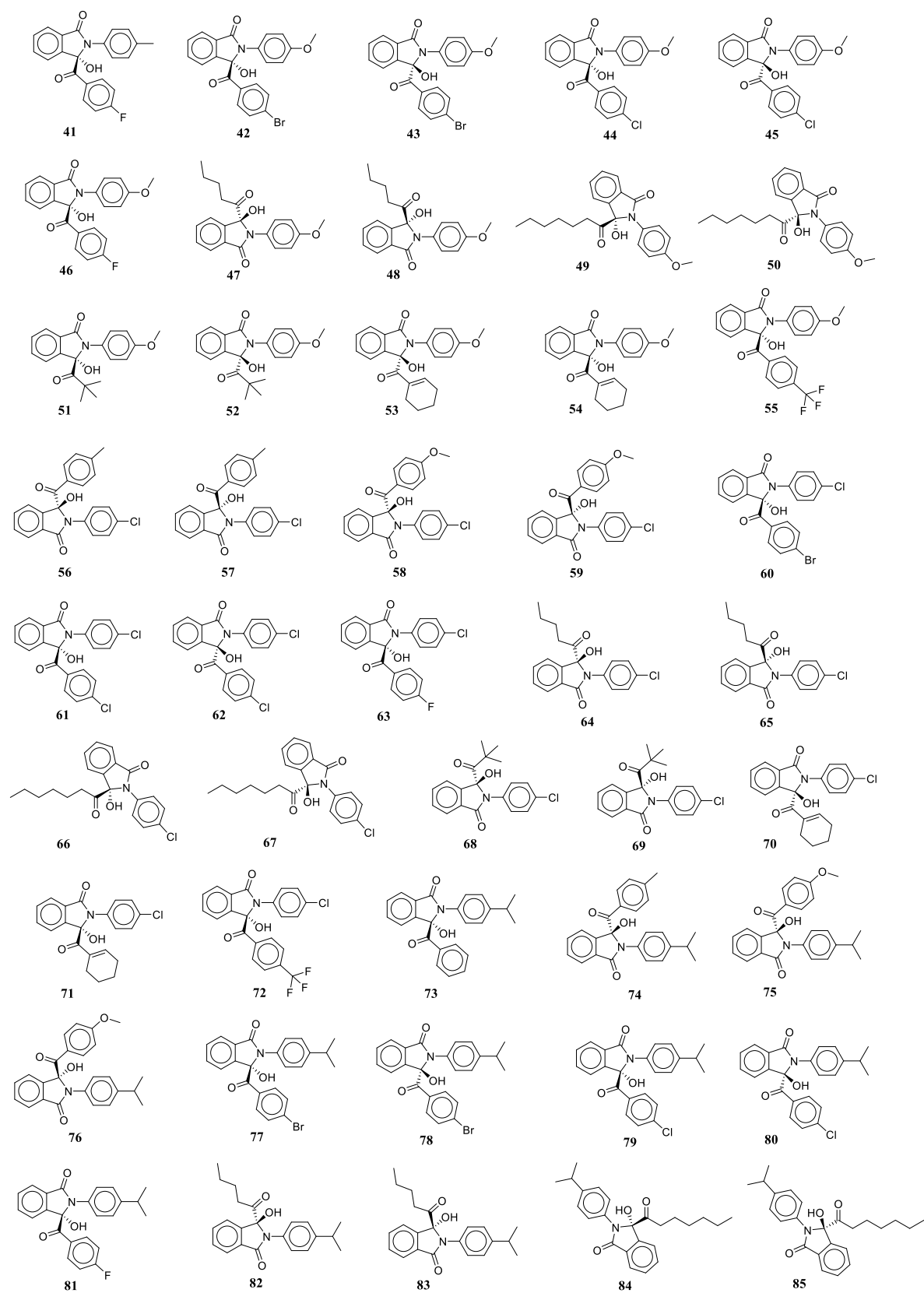
Figure 2.2 Ramachandran plot showing the distribution of MAO-B protein residues in most favored, allowed, and disallowed regions

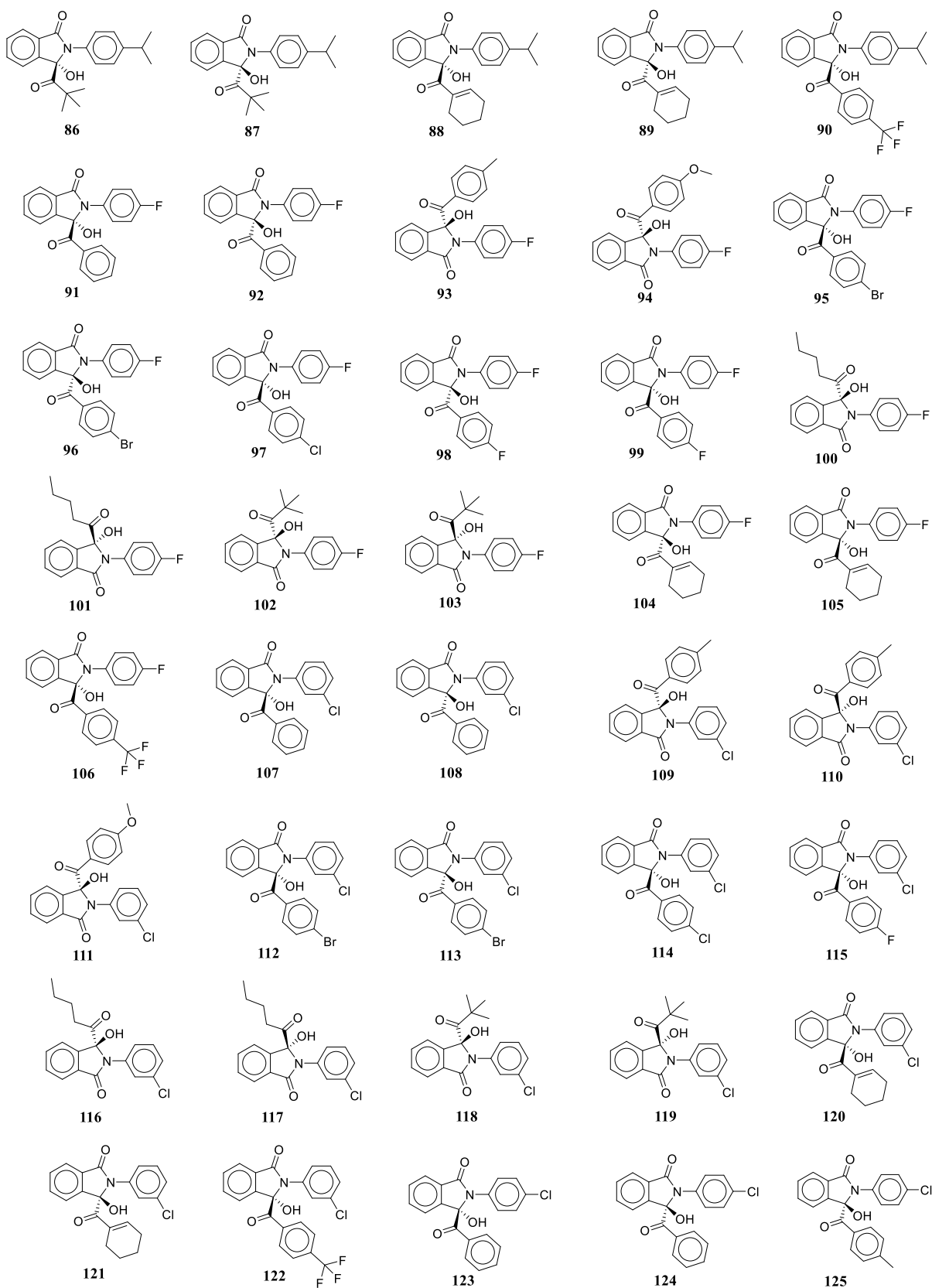
The glide score (gscore), or we can say the docking score of ligands, was observed after molecular docking through XP mode in the range from -2.360 kcal/mol to -12.088 kcal/mol. The structures of all the 3-acyl-3-hydroxyisoindolin-1-one ligands were shown in **Figure 2.3**. Fifty-three molecules displayed a glide score higher than the co-crystal ligand of 6FWC (-10.645 kcal/mol). **Table 2.2** showed the ligand numbers, and glide score values of all the 3-acyl-3-hydroxyisoindolin-1-one ligands, including co-crystal ligand, safinamide, rasagiline, and saligiline. This has been studied that the more negative the glide (docking) score, the higher the binding affinity between protein and ligand [87]. Out of the 279 docked ligands, the ligands **90** and **173** showed superior glide score values of -12.088 kcal/mol and -12.085 kcal/mol, respectively, compared to rasagiline (-7.396 kcal/mol), saligiline (-6.094 kcal/mol), and co-crystal ligand (10.645 kcal/mol). Their glide scores were also close to safinamide (-12.368 kcal/mol). This indicated that ligands **90** and **173** exhibit strong potential as promising lead candidates.

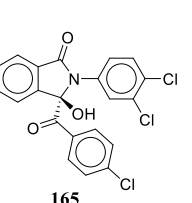
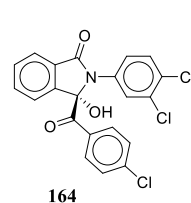
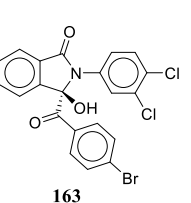
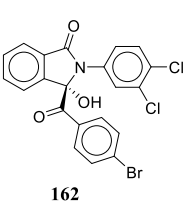
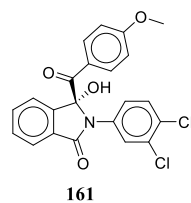
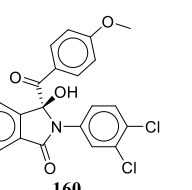
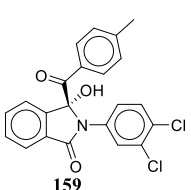
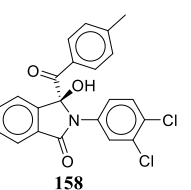
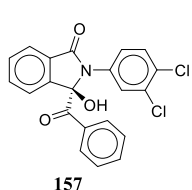
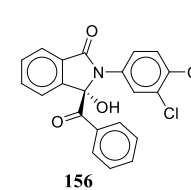
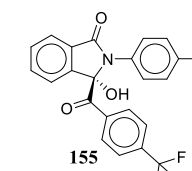
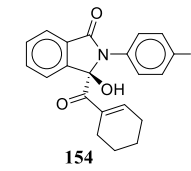
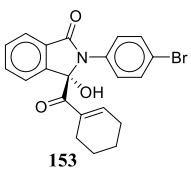
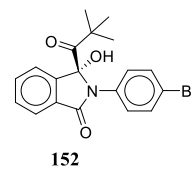
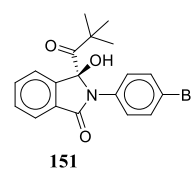
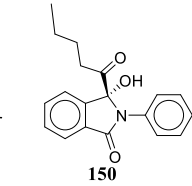
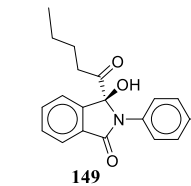
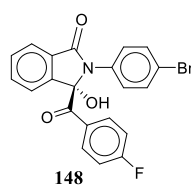
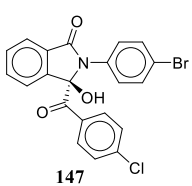
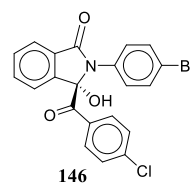
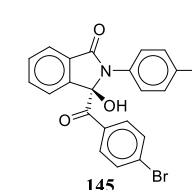
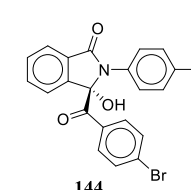
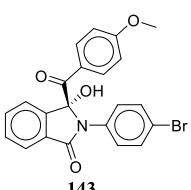
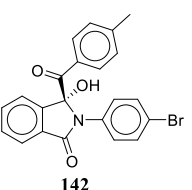
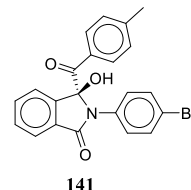
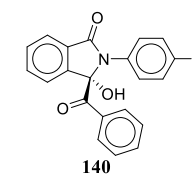
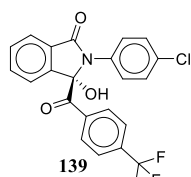
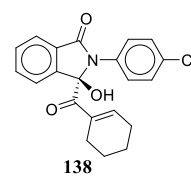
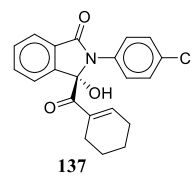
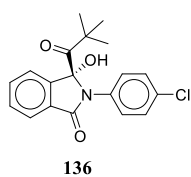
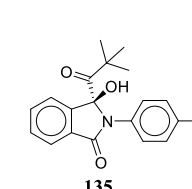
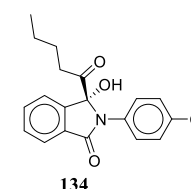
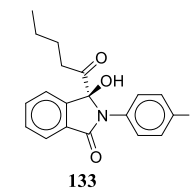
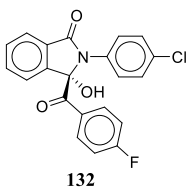
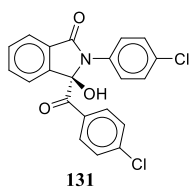
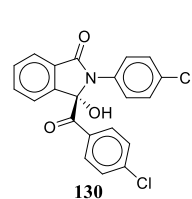
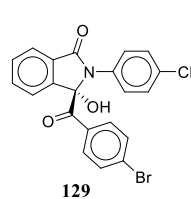
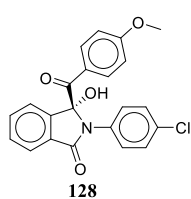
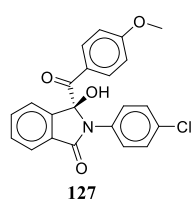
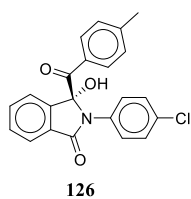
Binding interactions between ligands **90** and **173** and the MAO-B protein were investigated. **Figures 2.4** and **2.5** displays the 2-D and 3-D binding interactions of these ligands, **90** and **173**, respectively, with MAO-B. It was observed that ligand **90** showed hydrogen bonding interactions of the oxygen atom of the carbonyl group with Ser59 and Tyr60 amino acid residues of the MAO-B protein. Similarly, ligand **173** displayed hydrogen bonding interactions between carbonyl oxygen and Ser59 and Tyr60 residues. Moreover, the Phe343 residue underwent π - π stacking interaction with the phenyl ring of ligand **173**. Thus, the greatest glide scores and conventional hydrogen bonding interactions of ligands **90** and **173** concluded high and effective binding between these ligands and active amino acid residues of MAO-B, and due to this, we took these two compounds for further *in-silico* studies, which included MDS, MM-GBSA free energy, ADME, and DFT.

In addition, we validated the docking protocol by the superimposition method. We redocked the co-crystal ligand of MAO-B protein, {N}-(3-fluorophenyl)-4-oxidanylidene-chromene-3-carboxamide, and then superimposed the redocked pose with the crystal pose of the co-crystal ligand of MAO-B protein taken for docking. **Figure 2.6** displays the superimposition image in which the redocked and crystal poses were highlighted in green and red colors, respectively. The RMSD value between both the poses was observed to be 0.1405 Å, which was significantly lower than 2 Å. Thus, the superimposition image and very low RMSD value suggested an accurate and efficient docking protocol applied for molecular docking between 3-acyl-3-hydroxy isoindolin-1-one molecules and MAO-B protein.

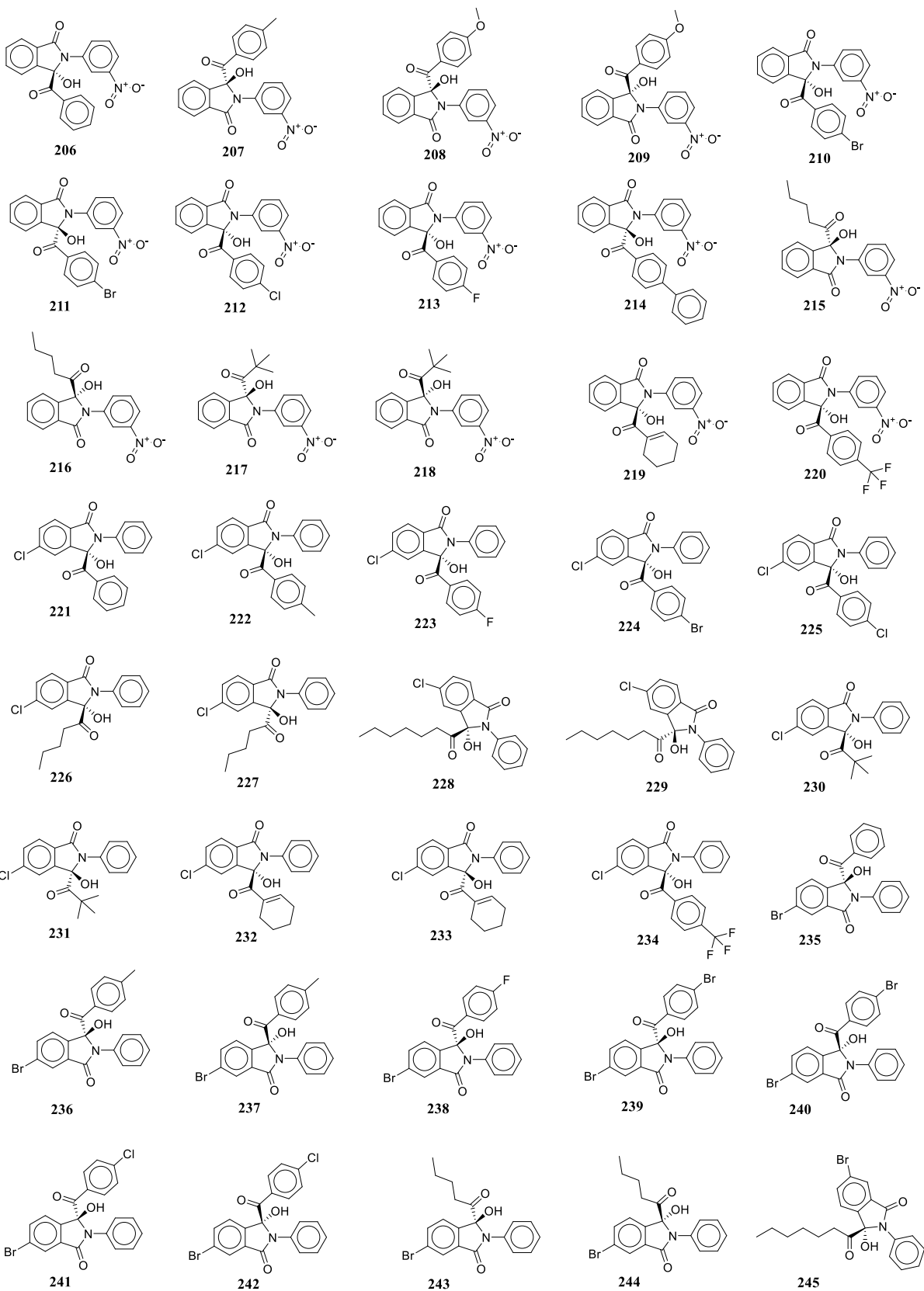












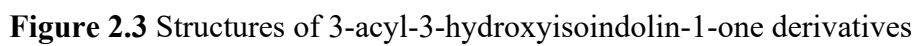


Table 2.2 Ligand no. and GLIDE scores (docking scores) of all docked molecules of 3-acyl-3-hydroxyisoindolin-1-ones with MAO-B protein

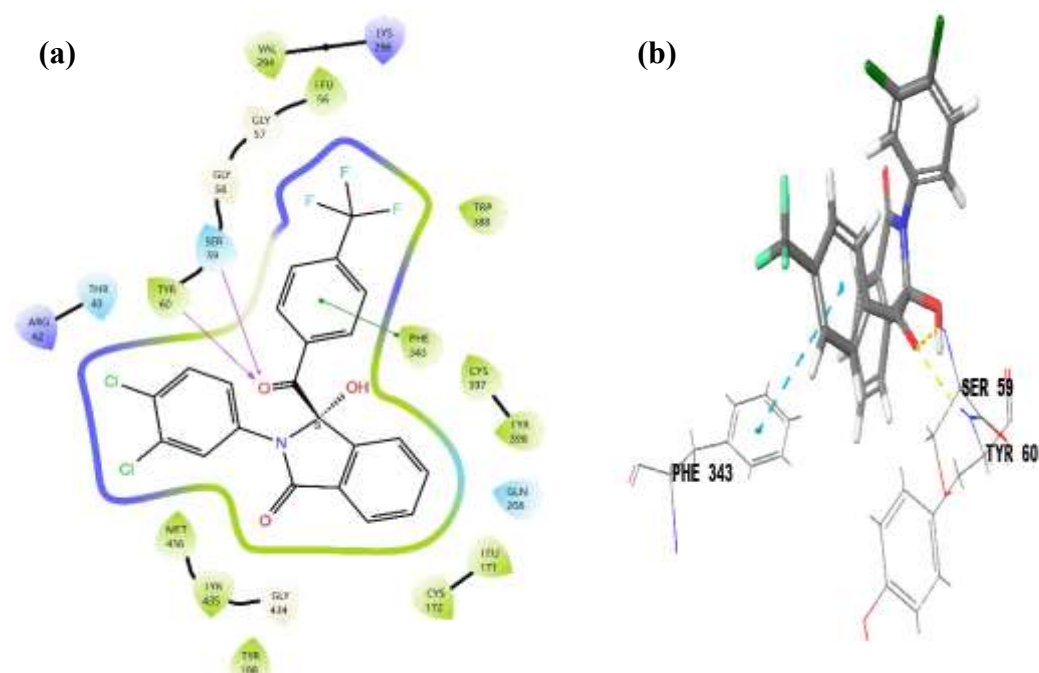
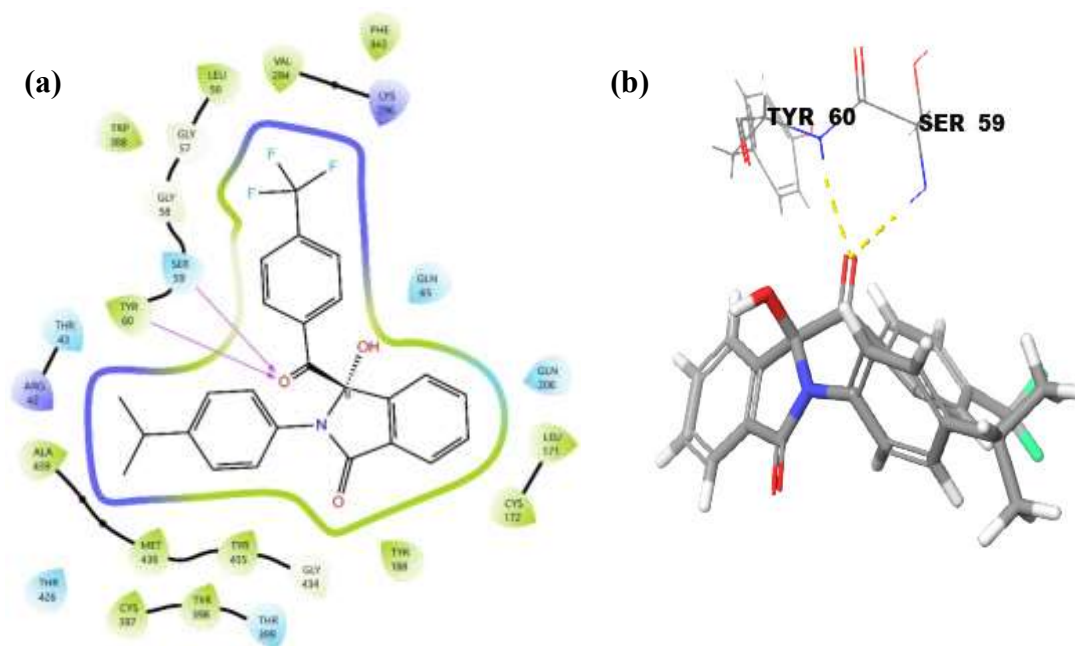
Ligand No.	GLIDE Score (Docking Score)	Ligand No.	GLIDE Score (Docking Score)
1	-9.142	29	-10.749
2	-9.537	30	-9.100
3	-9.781	31	-10.138
4	-9.601	32	-8.117
5	-8.415	33	-11.303
6	-9.365	34	-11.707
7	-9.017	35	-10.648
8	-9.442	36	-8.883
9	-9.908	37	-9.825
10	-9.091	38	-8.961
11	-9.809	39	-10.754
12	-10.402	40	-9.825
13	-9.297	41	-9.596
14	-9.006	42	-10.332
15	-9.850	43	-9.235
16	-8.888	44	-10.163
17	-8.922	45	-9.368
18	-9.966	46	-10.424
19	-8.654	47	-10.039
20	-9.900	48	-7.522
21	-8.039	49	-11.400
22	-10.294	50	-8.942
23	-7.770	51	-10.635
24	-9.302	52	-9.229
25	-9.718	53	-9.538
26	-9.453	54	-6.781
27	-11.591	55	-10.765
28	-10.443	56	-8.507

57	-9.279	89	-9.702
58	-9.735	90	-12.088
59	-9.281	91	-9.862
60	-10.453	92	-8.904
61	-9.068	93	-10.332
62	-9.559	94	-9.981
63	-10.378	95	-10.325
64	-10.047	96	-9.188
65	-7.901	97	-10.404
66	-11.097	98	-9.672
67	-10.486	99	-10.424
68	-10.576	100	-10.148
69	-4.912	101	-6.913
70	-9.288	102	-10.625
71	-8.982	103	-8.998
72	-10.918	104	-7.561
73	-10.023	105	-9.442
74	-11.110	106	-10.761
75	-10.860	107	-9.466
76	-10.023	108	-9.325
77	-11.008	109	-10.822
78	-10.258	110	-9.487
79	-10.445	111	-10.418
80	-10.342	112	-10.558
81	-10.226	113	-9.635
82	-10.465	114	-10.514
83	-8.544	115	-10.873
84	-11.296	116	-11.281
85	-10.761	117	-7.021
86	-10.309	118	-10.048
87	-10.214	119	-9.888
88	-5.963	120	-7.344

121	-9.873	153	-5.791
122	-11.589	154	-8.975
123	-9.422	155	-11.621
124	-9.022	156	-10.188
125	-8.507	157	-9.993
126	-9.279	158	-11.187
127	-9.735	159	-10.272
128	-9.281	160	-9.906
129	-10.453	161	-9.896
130	-9.068	162	-11.290
131	-9.559	163	-10.323
132	-10.378	164	-11.179
133	-10.047	165	-10.315
134	-7.901	166	-10.408
135	-10.576	167	-11.271
136	-4.912	168	-10.047
137	-9.319	169	-10.904
138	-9.525	170	-10.544
139	-10.918	171	-8.803
140	-9.520	172	-10.329
141	-10.015	173	-12.085
142	-9.323	174	-10.880
143	-9.104	175	-2.360
144	-10.354	176	-11.176
145	-9.452	177	-10.124
146	-9.745	178	-10.607
147	-9.485	179	-9.878
148	-10.079	180	-11.203
149	-10.757	181	-10.136
150	-7.549	182	-11.172
151	-10.525	183	-10.327
152	-9.796	184	-9.712

185	-10.598	217	-8.801
186	-8.158	218	-8.419
187	-10.521	219	-9.124
188	-6.827	220	-10.935
189	-9.475	221	-9.639
190	-10.365	222	-10.028
191	-11.814	223	-9.847
192	-5.835	224	-9.985
193	-11.113	225	-9.836
194	-9.912	226	-10.655
195	-11.313	227	-8.545
196	-11.451	228	-10.825
197	-9.881	229	-6.930
198	-9.174	230	-10.890
199	-10.618	231	-4.821
200	-8.196	232	-9.580
201	-9.896	233	-6.837
202	-10.133	234	-11.917
203	-5.530	235	-10.147
204	-7.599	236	-10.292
205	-11.991	237	-9.001
206	-8.849	238	-10.320
207	-10.186	239	-10.431
208	-9.738	240	-9.238
209	-8.634	241	-10.137
210	-10.244	242	-9.123
211	-8.806	243	-10.457
212	-10.373	244	-10.503
213	-10.289	245	-11.570
214	-10.332	246	-10.805
215	-9.287	247	-10.515
216	-6.950	248	-9.966

249	-8.959	267	-10.231
250	-8.737	268	-2.433
251	-10.873	269	-11.333
252	-8.914	270	-10.499
253	-10.446	271	-10.567
254	-9.261	272	-8.454
255	-11.303	273	-11.119
256	-10.182	274	-10.594
257	-9.563	275	-10.181
258	-9.034	276	-8.810
259	-11.169	277	-9.953
260	-7.983	278	-3.263
261	-10.225	279	-11.645
262	-7.828	Co-Crystal Ligand	-10.645
263	-7.085	Safinamide	-12.368
264	-11.722	Rasagiline	-7.396
265	-10.044	Saligiline	-6.094
266	-10.419		



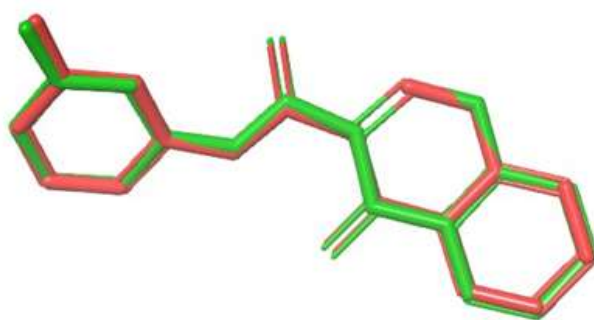


Figure 2.6 Representation of superimposition of redocked pose of co-crystal ligand of MAO-B (shown in green color) with the crystal pose of co-crystal ligand of MAO-B (shown in red color)

2.3.2 Molecular Dynamics Simulation (MDS)

The stability of the binding docked complexes of ligands **90** and **173** was investigated with the help of MDS for complete 500 ns simulation time. The simulation interaction diagram of Desmond was used to analyse the RMSD and RMSF of docked poses of ligands **90** and **173** throughout the complete simulation. In addition, protein-ligand interaction fraction and summary of protein-ligand contacts were also analysed.

Root Mean Square Deviation (RMSD) is used to find the stability of the docked pose of the molecule in the binding region of the protein. A high RMSD value leads to less stability of the docked pose and high chances of diffusion of the docked pose position from its initial binding region, while a low RMSD value results in high stability of the docked pose and less deviation of the docked pose position from the binding pocket [88]. **Figure 2.7** shows the RMSD plot of heavy atoms of ligands **90**, **173**, and the co-crystal ligand. For ligand **90**, the average RMSD observed for the complete 500ns simulation trajectory with respect to the protein was 1.57 Å, indicating that it maintained a highly stable binding conformation within the protein binding pocket throughout the simulation. Ligand **173** exhibited an average heavy atoms RMSD value of 1.63 Å, suggesting stable binding with only minor conformational fluctuations relative to its initial docked pose. Whereas the co-crystal ligand showed a higher average ligand RMSD value of 4.3 Å, indicating greater conformational flexibility and larger positional deviations from its initial binding orientation.

Root Mean Square Fluctuation (RMSF) is a measure of fluctuations of the molecule atoms with respect to the protein. It depicts how much atoms of the molecule change their positions during the complete simulation time. A low RMSF value corresponds to lower

fluctuations of the atoms of the molecule, while a high RMSF value implies larger fluctuations and less stability of the docked pose [89]. RMSF plots of ligands **90**, **173**, and the co-crystal ligand during the simulation are shown in **Figure 2.8**. The X-axis demonstrated the atom index number of the ligand, which is simply the serial numbering of atoms in the ligand, while the Y-axis showed the average RMSF values of these atoms throughout the simulation. RMSF values of both the ligands **90** and **173** were less than 1.6 Å, while the co-crystal ligand showed the highest RMSF value of 3 Å, indicating reduced atomic fluctuations for ligands **90** and **173** throughout the complete 500 ns simulation time compared to the co-crystal ligand. Hence, low RMSD and RMSF values of both ligands **90** and **173** demonstrated high docked poses stability in the MAO-B binding region, exhibiting minimal positional deviations and low atomic fluctuations. In contrast, the co-crystal ligand of MAO-B showed higher RMSD and RMSF values, which suggests reduced stability within the binding site. Overall, these RMSD and RMSF results correspond to stronger conformational stability of ligands **90** and **173** throughout the simulation compared to the co-crystal ligand.

Protein-ligand interactions profile throughout the complete simulation is another important factor in depicting the stability of the docked poses of ligands [90]. **Figure 2.9** displays the 2-D bar graph representation of the interaction fraction of amino acid residues of the MAO-B protein with ligands **90**, **173**, and the co-crystal ligand, while **Figure 2.10** shows the summary of protein-ligand contacts in terms of interaction percentage of residues throughout the simulation. In the case of ligand **90**, it was shown that the hydrogen bonding interaction fraction of Tyr60 residue was 1.685, and for Met436, it was 0.945 (**Figure 2.9**). The oxygen atom of the carbonyl group of ligand **90** lost a hydrogen bonding interaction with the Ser59 residue during the MD simulation that was observed above in XP-molecular docking. Hydrogen bonding between Tyr60 and the oxygen atom of the carbonyl group was preserved for up to 96% of simulation time. Two new hydrogen bonds were observed during the MDS between the hydrogen atom of the hydroxyl group and Tyr60 and between the oxygen atom of the hydroxyl group and Met436, which were retained for about 71% and 94% of simulation time, respectively. Additionally, Tyr60 formed a hydrophobic interaction, specifically a π - π stacking interaction with the phenyl ring of the molecule for up to 62% of the simulation time (**Figure 2.10**).

Similarly, ligand **173** exhibited hydrogen bonding interaction fractions of Tyr60 and Met436 residues of MAO-B protein with 1.955 and 0.449, respectively, as shown in **Figure 2.9**. The hydrogen bonding interaction between Ser59 and carbonyl oxygen was lost, while the hydrogen bonding interaction between Tyr60 and carbonyl oxygen atom was retained for

97% of the simulation time. In addition, new hydrogen bonds between the hydrogen atom of the hydroxyl group and Tyr60 and between the oxygen atom of hydroxyl group and Met436 showed consistency for 97% and 44% of simulation time, respectively (**Figure 2.10**). Thus, these MDS results concluded high stability of docked poses of ligands **90** and **173**. In contrast, the co-crystal ligand showed no consistent hydrogen bonding with MAO-B protein residues and displayed only π - π stacking interaction with Tyr326 and Phe343 amino acid residues, which retained for only 32% and 38% of simulation time, respectively. This strongly indicated that ligands **90** and **173** formed more consistent and stable binding interactions with the MAO-B binding pocket compared to the co-crystal ligand.

2.3.3 MM-GBSA Binding Free Energy Calculations

To further evaluate the stability and strength of ligand binding with the protein, post-simulation binding free energy calculations were performed. The post-simulation average binding free energies of ligand **90**, ligand **173**, and co-crystal bound MAO-B protein complexes were estimated with the help of Prime MM-GBSA module of the Schrodinger Suite 2022.4 [77] based on trajectories obtained from MDS. The co-crystal ligand bound MAO-B complex showed average binding free energy value of -47.892 kcal/mol. Ligand **90** bound MAO-B complex displayed a significantly lower binding free energy value of -85.465 kcal/mol whereas complex with ligand **173** exhibited a value of -78.840 kcal/mol. These findings indicated that ligand **90** maintained a highly stable and energetically favourable interaction with the MAO-B active site throughout the simulation. Although ligand **173** demonstrated a slightly less favourable interaction than ligand **90**, it still showed markedly improved binding compared to the co-crystallized ligand, which showed the weakest binding. Overall, the binding free energy values of ligand **90** and **173** reflect the strong and stable interactions with MAO-B, highlighting ligand **90** as the most promising candidate for further drug development, with ligand **173** also showing considerable potential.

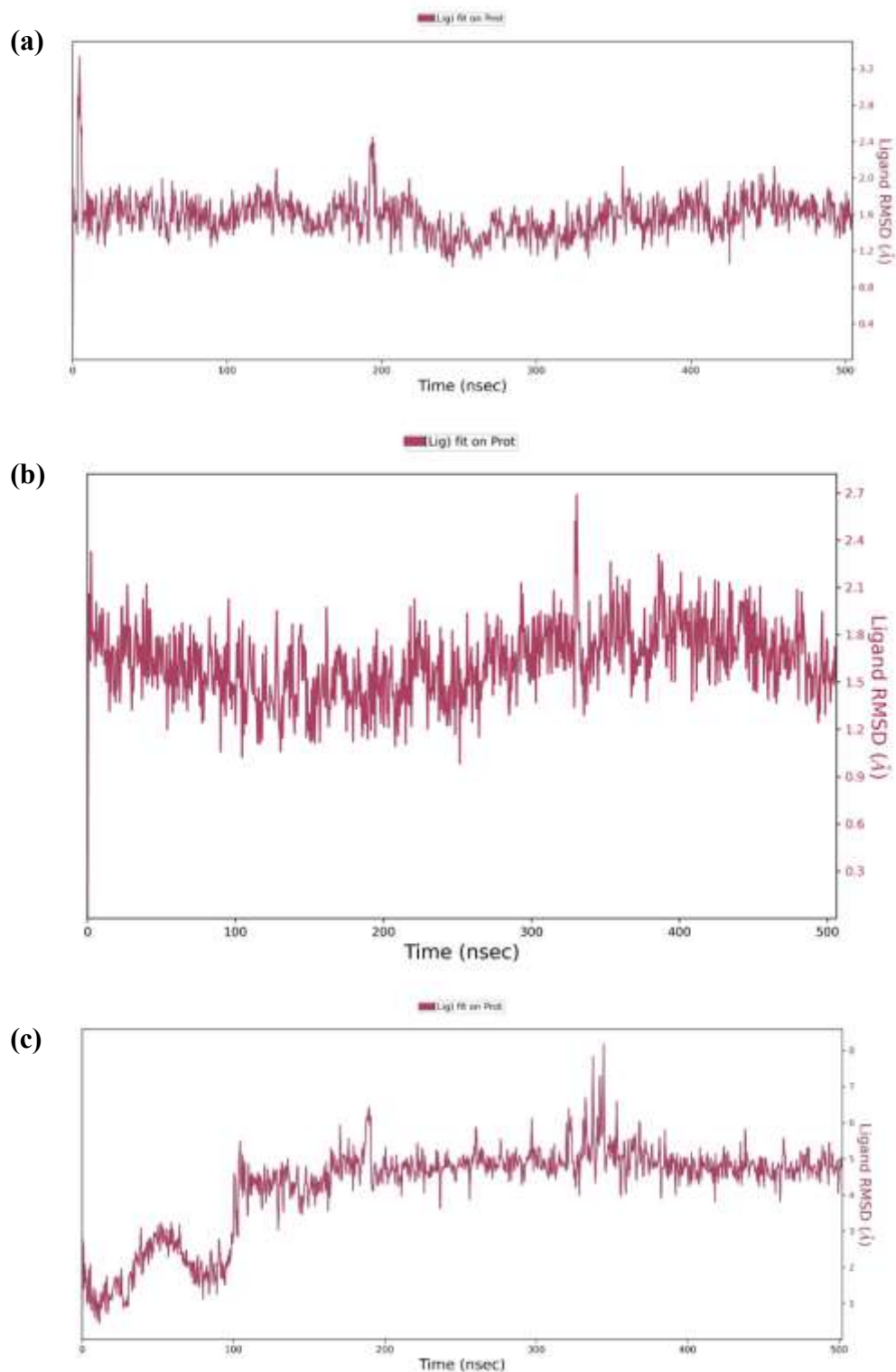


Figure 2.7 RMSD plots of heavy atoms of **(a)** ligand 90, **(b)** ligand 173, and **(c)** co-crystal ligand, all calculated with respect to protein. The Y-axis represented the RMSD values, while the X-axis represented the simulation time

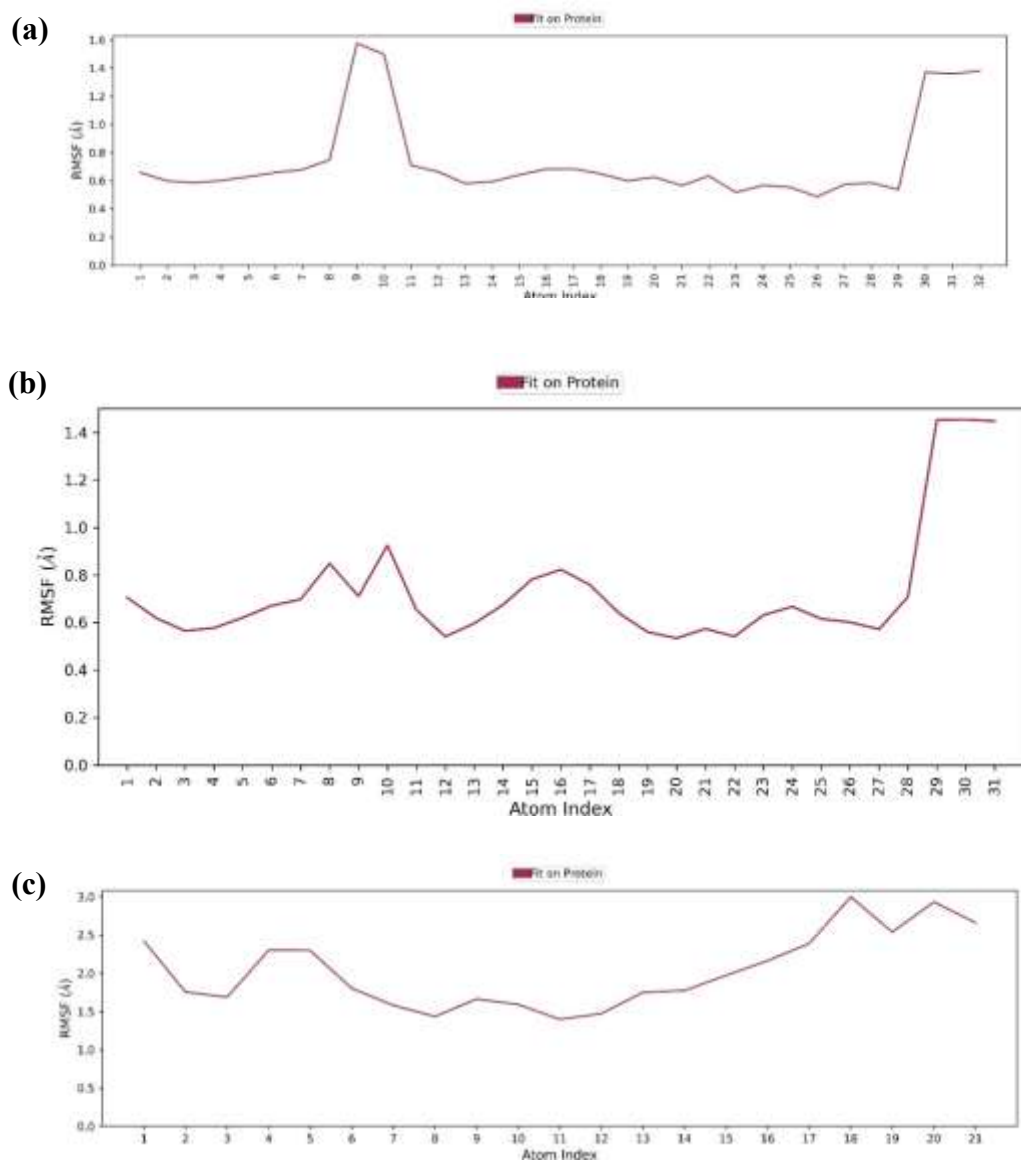


Figure 2.8 RMSF plots of (a) ligand 90, (b) ligand 173, and (c) co-crystal ligand, all calculated with respect to protein. The Y-axis illustrated the RMSF values, while the X-axis demonstrated the atom index of the ligand

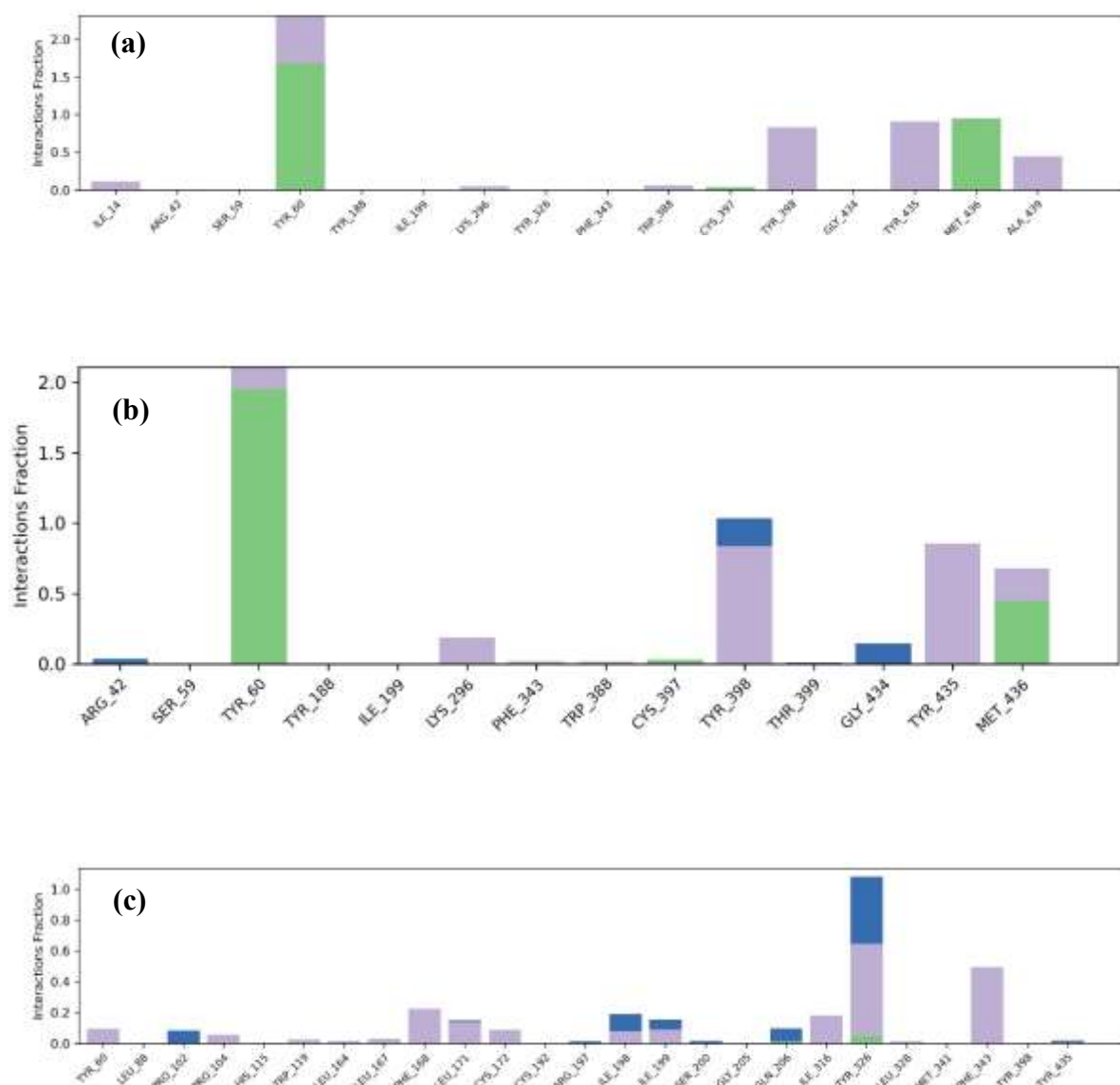


Figure 2.9 2-D bar graph representation of protein-ligand interactions showing the interaction fractions of amino acid residues of MAO-B protein with **(a)** ligand **90**, **(b)** ligand **173**, and **(c)** co-crystal ligand. Hydrogen bonds are shown in green colour, while hydrophobic interactions are displayed in violet colour

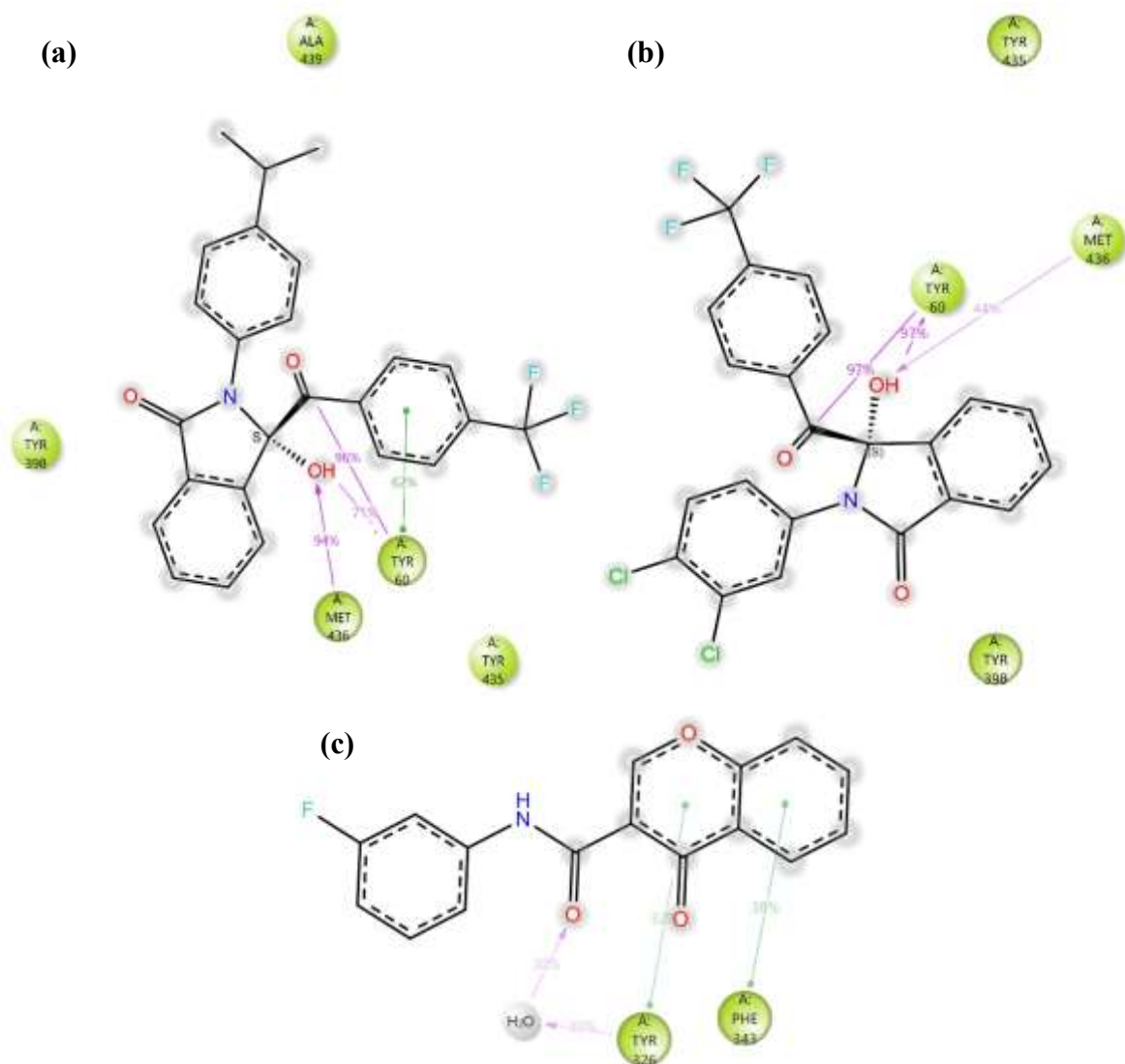


Figure 2.10 Summary of protein-ligand interactions showing interaction percentage of amino acid residues of MAO-B protein with **(a)** ligand **90**, **(b)** ligand **173**, and **(c)** co-crystal ligand. Hydrogen bonding interactions are displayed in pink colour, while π - π stacking interactions are displayed in green colour

2.3.4 ADME Prediction

Ligands must possess appropriate physicochemical and pharmacokinetic properties to be considered as a potential drug. Prediction of Absorption, Distribution, Metabolism, and Excretion (ADME) properties is an efficient way to increase the success rate of potential ligands [91]. ADME properties of ligands **90** and **173** were predicted using Qikprop, and the optimum range of properties was summarized in **Table 2.3**. The total solvent accessible surface area (SASA) values of ligands **90** and **173** were $683.874\text{\AA}^2$ and $655.07\text{\AA}^2$, respectively, both of which fall in the optimum range of $300\text{-}1000\text{\AA}^2$. The fraction of oral

absorption (FOSA), fraction of intestinal solubility (FISA), predicted intestinal solubility average (PISA), and water partition solubility average (WPSA) values of both ligands were in the standard ranges except for WPSA of ligand **173**. The predicted values of FOSA, FISA, PISA, and WPSA for ligand **90** were 161.268 Å², 100.985 Å², 303.672 Å², and 117.949 Å², respectively, while for ligand **173** were 0.448 Å², 100.283 Å², 329.954 Å², and 224.386 Å², respectively. The volume of ligand **90** depicted was 1263.157 Å³ whereas the volume of ligand **173** observed was 1194.311 Å³. The number of rotatable bonds (RB), donor hydrogen bonds (DHB), and acceptor hydrogen bonds (AHB) of ligand **90** observed were 4, 1, and 6, respectively, whereas in the case of ligand **173**, the values were 3, 1, and 6, respectively.

The Polarizability (Z-parameter) (QPpolrz), which is a measure of the polarizability of the molecule, was found to be 44.729 for ligand **90** and 42.867 for ligand **173**, and these values fell in the standard range of QPpolrz. The predicted partition coefficient of ligand between octanol and water, QPlogPo/w values for ligands **90** and **173** were depicted to be 4.915 and 4.924, which lie in the acceptable range of -2 to 6.5. The aqueous solubility of a ligand was estimated using the QPlogS parameter; ligand **90** was under the acceptable range of QPlogS (-6.5 to 0.5), showing a value of -6.468, while the value of QPlogS for ligand **173** was slightly higher (-6.586).

QPlogHERG is the logarithmic prediction of the inhibiting potential of ligands for the human Ether-à-go-go-Related Gene (hERG) potassium channel. The acceptable range for QPlogHERG is above -5. Values of QPlogHERG for ligands **90** and **173** predicted were -5.79 and -5.839, which were slightly higher than the optimum range. The permeability of ligands across a Caco-2 cell monolayer (model for intestinal absorption) was predicted using QPPCaco. A high value of QPPCaco indicates better permeability. Thus, both ligands **90** and **173** showed better permeability with QPPCaco values of 1092.084 nm/sec and 1108.961 nm/sec, respectively. The predicted blood-brain partition coefficient (QPlogBB), predicted permeability of ligand across an MDCK (Madin-Darby Canine Kidney) cell monolayer (QPPMDCK), skin permeability prediction (QPlogKp), and prediction of partition coefficient of ligand in human serum albumin (QPlogKhsa) values of ligand **90** were -0.374, 2408.941 nm/sec, -1.927, 0.757, respectively, and for ligand **173**, these values were -0.035, 9377.419 nm/sec, -1.917, 0.616, respectively. For both the ligands, QPlogBB, QPPMDCK, QPlogKp, and QPlogKhsa predicted were in the satisfactory range. The predicted human oral absorption of both ligands was 100% and the number of likely metabolic reactions (metab) of ligand **90** was 2, and **173** was 1, and these were in the acceptable range of metab (1-8).

Table 2.3 ADME properties of ligands **90** and **173** predicted using Qikprop.

ADME Property	Ligand 90	Ligand 173	Optimum Range
molMW (g/mol)	439.433	466.243	130-725
Dipole	2.201	7.535	1-12.5
SASA (Å ²)	683.874	655.07	300-1000
FOSA (Å ²)	161.268	0.448	0-750
FISA (Å ²)	100.985	100.283	7-330
PISA (Å ²)	303.672	329.954	0-450
WPSA (Å ²)	117.949	224.386	0-175
Volume (Å ³)	1263.157	1194.311	500-2000
Rotatable bonds (RB)	4	3	0-15
Donor HB	1	1	0-6
Acceptor HB	6	6	2-20
QPpolrz	44.729	42.867	13-70
QPlogPC16	12.264	12.597	4-18
QPlogPoct	20.035	20.578	8-35
QPlogPw	10.405	10.767	4-45
QPlogPo/w	4.915	4.924	-2 to 6.5
QPlogS (mol dm ⁻³)	-6.468	-6.586	-6.5 to 0.5
QPlogHERG	-5.79	-5.839	Concern below -5
QPPCaco (nm/sec)	1092.084	1108.961	<25 poor, >500 great
QPlogBB	-0.374	-0.035	-3 to 1.2
QPPMDCK (nm/sec)	2408.941	9377.419	<25 poor, >500 great
QPlogKp	-1.927	-1.917	-8 to -1
QPlogKhsa	0.757	0.616	-1.5 to 1.5
Human Oral Absorption (%)	100	100	>80%(high), <25%(poor)
metab	2	1	1-8
RO5 number of violations of Lipinski's rule of five	0	0	maximum is 4
R03 number of violations of Jorgensen's rule of three	1	1	maximum is 3

The drug-likeness of ligands was predicted using Lipinski's rule of five and Jorgensen's rule of three. According to Lipinski's rule of five, a ligand with good oral bioavailability must not have more than one violation of the following conditions, which are: molecular weight (MW) < 500 Da, octanol-water partition coefficient (logP) < 5, donor hydrogen bond ≤ 5 , acceptor hydrogen bond ≤ 10 [92]. According to Jorgensen's rule of three, an orally bioavailable ligand follows some or all the rules, which are: QPPCaco > 22 nm/sec, QPlogS > -5.7, and no. of primary metabolites < 7 [93]. Both the ligands showed 0 violations in Lipinski's rule of five and 1 violation in Jorgensen's rule of three. Thus, the ADME properties of both ligands **90** and **173** almost came under the acceptable range. These results suggested that both ligands exhibited favorable physicochemical and pharmacokinetic properties, including good oral bioavailability and blood-brain barrier penetration, which are essential for effective MAO-B inhibition in the central nervous system. This indicates their suitability as prospective MAO-B inhibitors for treating neurodegenerative diseases.

2.3.5 Density Functional Theory (DFT)

2.3.5.1 Frontier HOMO-LUMO

We performed the DFT calculations of ligands **90** and **173** to study their molecular properties. Initially, ligands **90** and **173** were geometry optimized using the B3LYP/6-31G** method. Then, frontier HOMO-LUMO and MESP studies of these ligands were done using their optimized structures.

The highest occupied molecular orbital (HOMO) refers to the ability of the ligand to donate an electron, while lowest unoccupied molecular orbital (LUMO) stands for the ligand's tendency to accept an electron. The energy value of HOMO (E_{HOMO}), the energy value of LUMO (E_{LUMO}), and the energy gap value between HOMO and LUMO (E_{GAP}) are useful for studying the reactivity and kinetic stability of ligands. High E_{GAP} shows high kinetic stability and low chemical reactivity of molecules, whereas low E_{GAP} indicates low kinetic stability and high chemical reactivity [94]. Visual 3-D representations of HOMO and LUMO of ligands **90** and **173**, including their E_{HOMO} , E_{LUMO} , and E_{GAP} values, were shown in **Figures 2.11** and **2.12**, respectively, in which blue color represented the electron-deficient region while red color represented the electron-denser region. E_{HOMO} and E_{LUMO} values of ligand **90** predicted were -5.978791 eV and -2.132524 eV, and the value of E_{GAP} was found to be 3.846267 eV. In the case of ligand **173**, the values of E_{HOMO} , E_{LUMO} , and E_{GAP} predicted were -6.406689 eV, -2.732888 eV, and 3.673801 eV, respectively. Thus, **Figures 2.11** and **2.12** and the energy values concluded that there is charge transfer in both ligands by the

conjugated pathway. In addition, the low value of E_{GAP} suggested high chemical reactivity and low kinetic stability of both ligands.

Table 2.4 demonstrates the global reactivity parameters of both ligands, which include ionization potential, electron affinity, electronegativity, chemical potential, global hardness, global softness, and electrophilicity index estimated with the help of E_{HOMO} and E_{LUMO} values. On a comparison basis of both ligands, ligand **173** had a slightly lower E_{GAP} than ligand **90**, indicating more reactivity of ligand **173** than ligand **90**. Ionization potential, electron affinity, electronegativity, and chemical potential values of ligand **173** were higher than those of ligand **90**. However, the high value of global hardness and low value of global softness of ligand **173** compared to ligand **90** suggest high stability and less reactivity of ligand **90** compared to ligand **173**. Additionally, the high electrophilicity index of ligand **173** as compared to ligand **90** makes it more electrophilic in nature and more susceptible to nucleophilic species. Overall, these global reactivity parameters suggested that ligand **173** was found to be more reactive and less stable in comparison to ligand **90**.

Table 2.4 Global Reactivity Parameters calculated for ligands **90** and **173**, providing insights into their chemical stability and reactivity.

Parameters	Ligand 90	Ligand 173
HOMO Energy (in eV)	-5.978791	-6.406689
LUMO Energy (in eV)	-2.132524	-2.732888
E_{GAP} (in eV)	3.846267	3.673801
Ionization Potential (IP) (in eV)	5.978791	6.406689
Electron Affinity (A) (in eV)	2.132524	2.732888
Electronegativity (χ) (in eV)	4.0556575	4.5697885
Chemical Potential (μ) (in eV)	-4.0556575	-4.5697885
Chemical Hardness (η) (in eV)	1.9231335	1.8369005
Chemical Softness (S) (in eV ⁻¹)	0.259992351	0.27219765
Electrophilicity Index (u) (in eV)	4.276447204	5.684294532

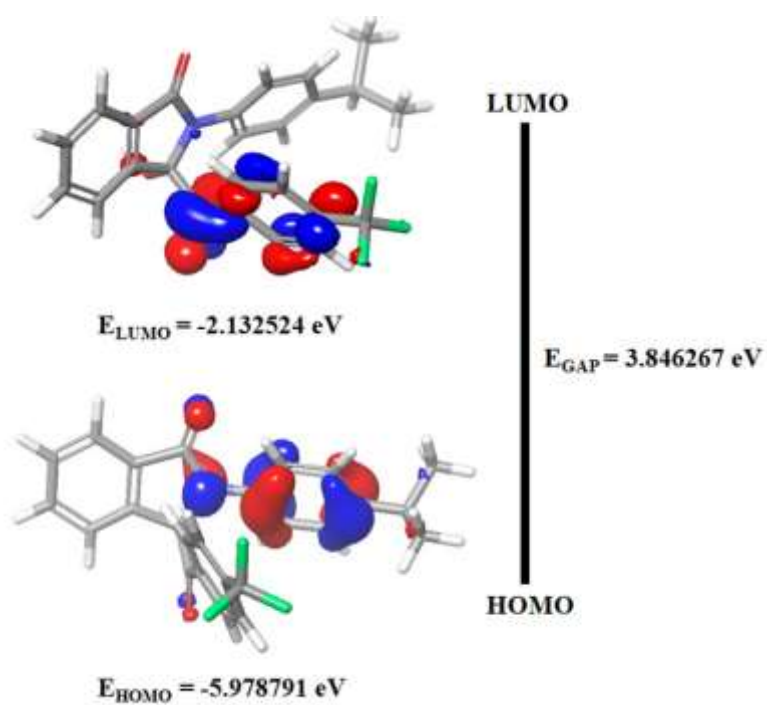


Figure 2.11 Visual 3-D representation of Frontier HOMO and LUMO of ligand **90**, including their E_{HOMO} , E_{LUMO} , and E_{GAP} values

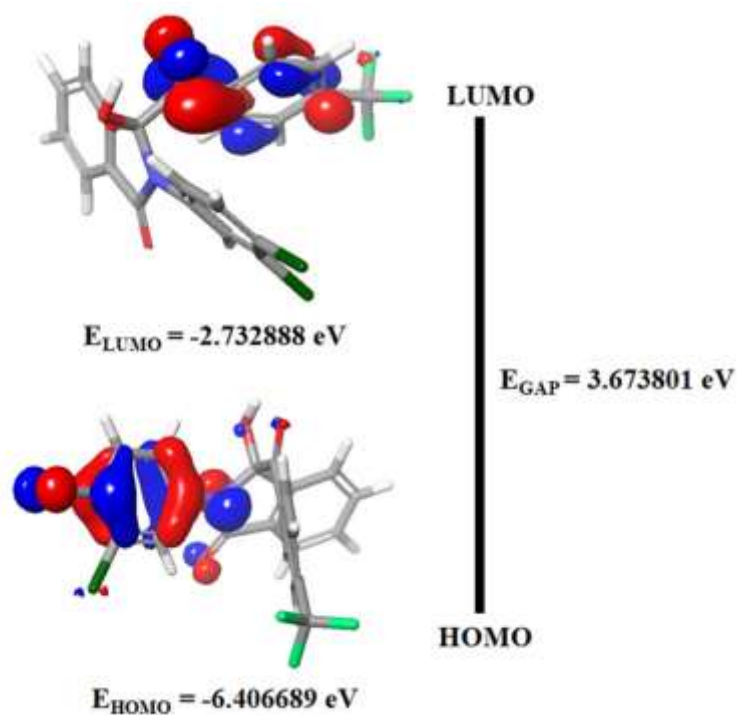


Figure 2.12 Visual 3-D representation of Frontier HOMO and LUMO of ligand **173**, including their E_{HOMO} , E_{LUMO} , and E_{GAP} values

2.3.5.2 Molecular Electrostatic Potential (MESP)

MESP showed the distribution of electron density around the ligand and is also helpful in the prediction of the chemical reactivity of the ligand [95]. In the MESP surface, the positive region (highlighted in light blue color) is the electrophilic region and susceptible to nucleophilic attack, while the negative region (highlighted in red color) is the nucleophilic region and susceptible to electrophilic attack [96]. **Figures 2.13** and **2.14** displayed the 3-D MESP images of ligands **90** and **173**, respectively. In **Figure 2.13**, the positive region was localized mainly on the nitrogen and hydrogen atoms of ligand **90**, whereas the negative region was localized over the oxygen atoms of the carbonyl groups, the oxygen atom of the hydroxyl group, the fluorine atoms, and the phenyl ring. These findings were correlated with the binding interactions between MAO-B protein and ligand **90** as reported above in the molecular docking and MDS section, in which the nucleophilic carbonyl oxygen atom showed hydrogen bonding with the electrophilic part of Tyr60 amino acid residue of MAO-B. Additionally, hydrogen bonding between the hydrogen atom of the hydroxyl group and Tyr60, and between the oxygen atom of the hydroxyl group and Met436 residue, were also well correlated with the ligand **90** MESP results. Similar findings were observed in the case of ligand **173**, the positive (electrophilic) region was present mainly at the nitrogen and hydrogen atoms, and the negative (nucleophilic) region was localized over the carbonyl oxygen atoms, the oxygen atom of the hydroxyl group, fluorine, and chlorine atoms. In addition, hydrogen bonding interactions shown above in molecular docking and MDS results: between the electrophilic part of Tyr60 and the nucleophilic part of the carbonyl oxygen atom, between the nucleophilic part of Tyr60 and proton (electrophilic part) of the hydroxyl group, and between the electrophilic part of Met436 and oxygen atom (nucleophilic part) of hydroxyl group was well correlated with the MESP studies of ligand **173**. Overall, the MESP analysis supports that the distribution of the electron density of both ligands **90** and **173** aligned well with the observed binding interactions with the MAO-B protein, pinpointing the nucleophilic and electrophilic regions involved in hydrogen bonding with the active site residues.

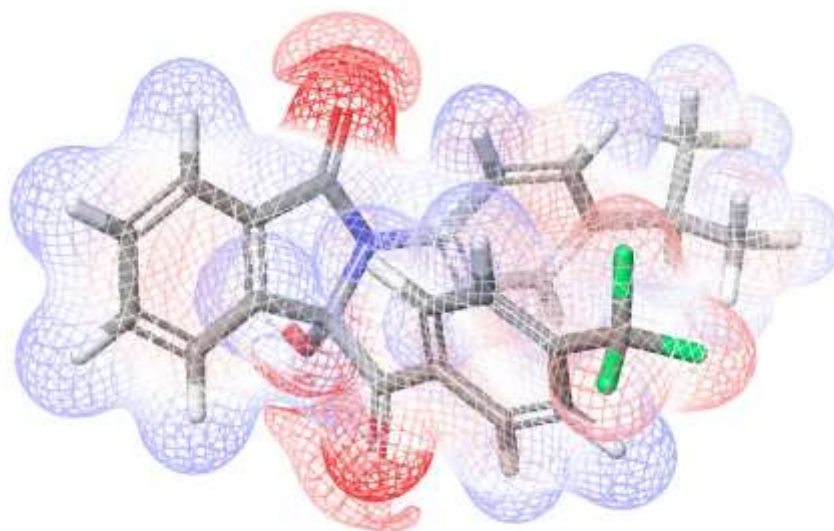


Figure 2.13 Molecular Electrostatic Potential Surface (MESP) of ligand **90**. Red colour corresponded to the nucleophilic region, while blue colour indicated the electrophilic region of the ligand

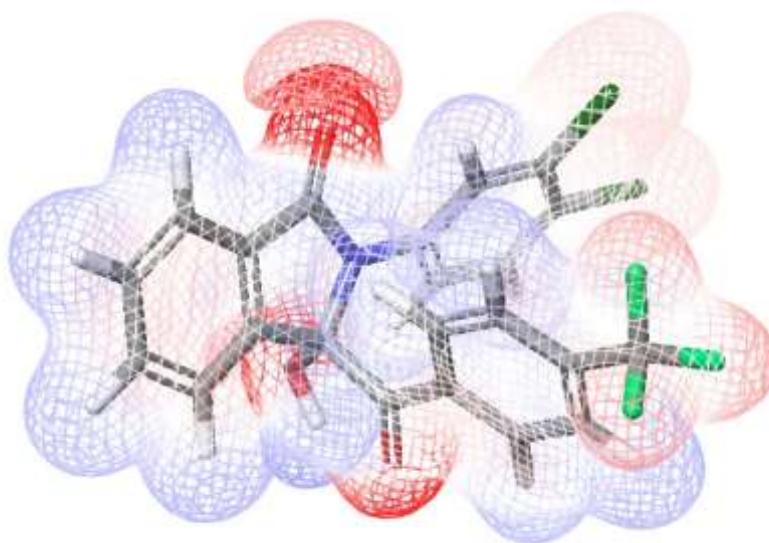


Figure 2.14 Molecular Electrostatic Potential Surface (MESP) of ligand **173**. Red colour represented the nucleophilic region, while blue colour indicated the electrophilic region of the ligand

2.4 Conclusion

In this chapter, we performed *in-silico* investigations on a library of 279 3-acyl-3-hydroxy isoindolin-1-one compounds as potential inhibitors of MAO-B. Molecular docking studies identified ligands **90** and **173** as the most promising candidates, with docking scores of -12.088 kcal/mol and -12.085 kcal/mol, respectively, and the key hydrogen bond interactions with Ser59 and Tyr60 residues of the MAO-B protein. Molecular dynamics simulations over 500 ns demonstrated the stability of both the ligands **90** and **173** with RMSD and RMSF values below 2 Å and consistent hydrogen bonding with Tyr60 residue for up to 96% of the simulation time. Post-MDS MM-GBSA calculations revealed average binding free energies of -85.465 kcal/mol and -78.840 kcal/mol for ligands **90** and **173**, respectively, indicating energetically favourable interactions. ADME profiling of both ligands indicated favourable pharmacokinetic parameters, while DFT analysis showed high chemical reactivity and low kinetic stability. In addition, 3D MESP maps highlighted the nucleophilic character of the carbonyl oxygen atoms, aligning with docking and MDS studies. Overall, these computational studies suggested that ligands **90** and **173** formed highly stable and energetically favourable complexes with MAO-B protein, providing a strong foundation for future experimental validation and the development of novel therapeutic agents for Parkinson's disease (PD).

2.5 References

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CHAPTER 3

Selectfluor-mediated synthesis of 3-acyl-3-hydroxyisoindolin-1-ones and their antioxidant studies

3.1 Introduction

Isoindolin-1-ones are an important class of heterocyclic compounds. They showed diverse biological and medicinal properties such as anti-HIV [1], anti-cancer [2], anti-microbial [3], anti-psychotic [4], anti-hypertensive [5], anti-diabetic [6], etc. Pagoclone [7], JM-1232 [8], and DN-2327 [9] are examples of FDA approved drugs containing an isoindolin-1-one scaffold (**Figure 3.1**). In addition, isoindolin-1-ones are present in several natural products [10,11]. Due to their diverse medicinal importance and presence in natural products [12-14], there has been a continuous interest in the synthesis of isoindolin-1-ones with different synthetic pathways. Various alkyne annulation reactions have been utilized for its synthesis, which involves base-mediated, electrochemical-mediated and metal-catalyzed annulation reactions [15-21].

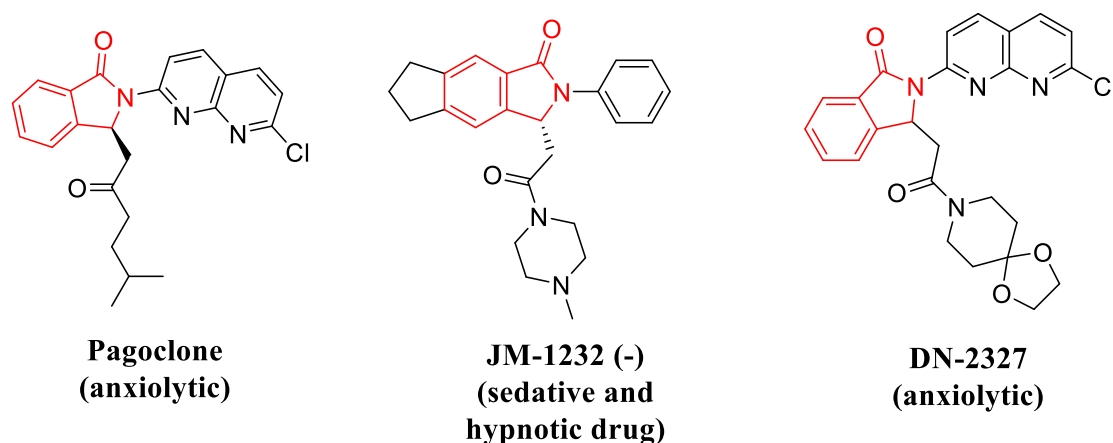
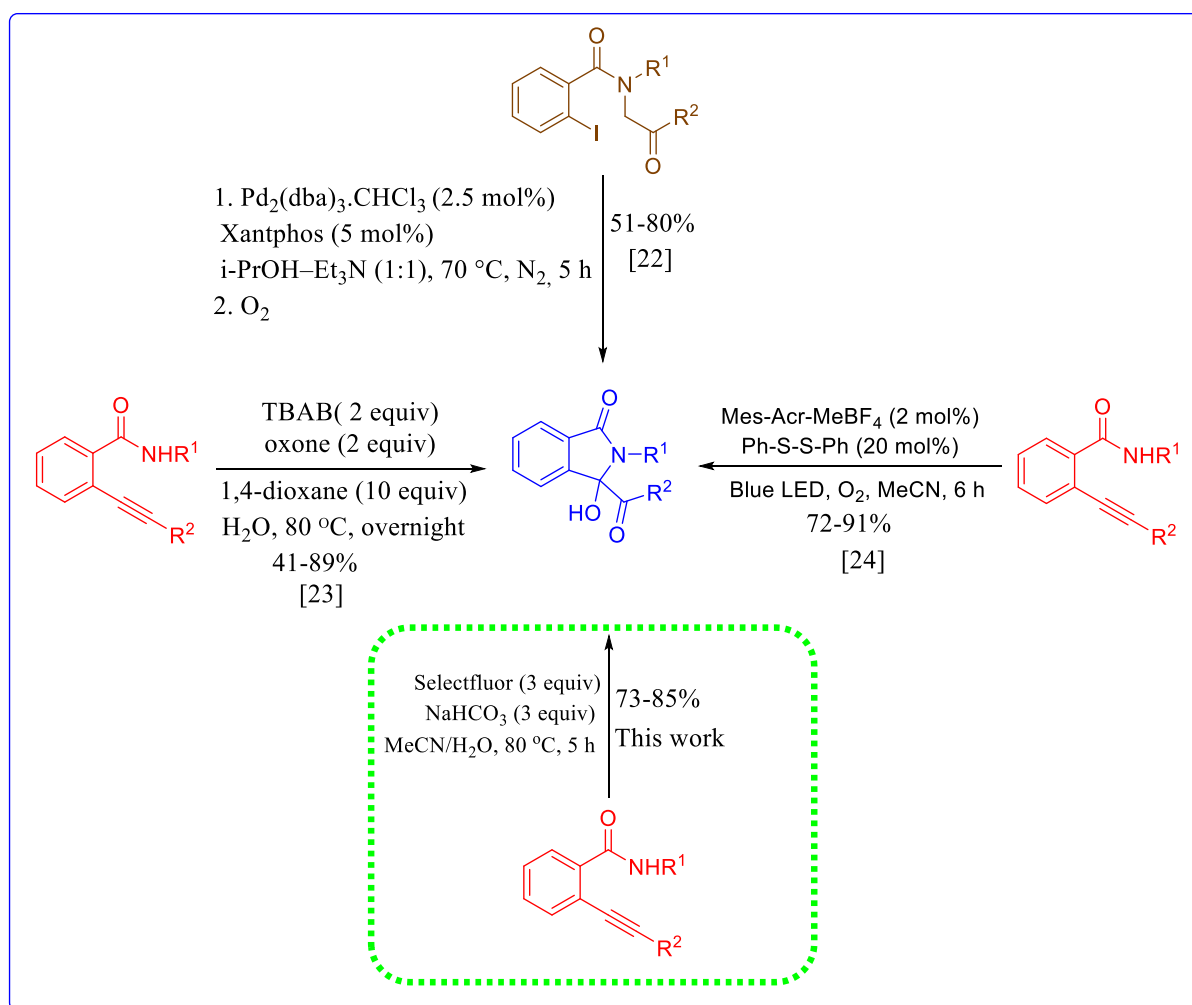


Figure 3.1 Selected biologically active compounds with isoindolin-1-one scaffold

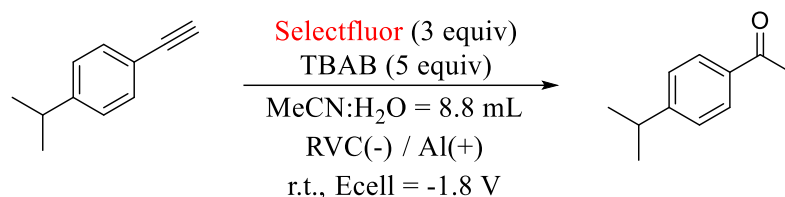
Over the past years, different research groups reported the synthesis of 3-hydroxy-3-acylisoindolin-1-ones. In 2013, Chen et al. synthesized 3-acylisoindolin-1-ones from 2-iodobenzamides catalyzed by palladium ($\text{Pd}_2(\text{dba})_3 \cdot \text{CHCl}_3$) and Xantphos [22]. They used triethylamine as a base and isopropanol as a solvent for synthesis under the N_2 atmosphere. After that, they converted 3-acylisoindolin-1-ones to 3-acyl-3-hydroxyisoindolin-1-ones by stirring the reaction under an O_2 balloon for 24 h [22]. Liu et al. in 2020 reported the synthetic route of 3-hydroxy-3-acylisoindolin-1-ones from 2-(1-alkynyl)benzamides utilizing n-tetrabutyl ammonium bromide (TBAB) as bromine source and oxone, 1,4-dioxane as additives [23]. Reddy et al. also synthesized 3-hydroxy-3-acylisoindolin-1-ones from 2-(1-alkynyl)benzamides in 2022 by using diphenyl disulfide, 9-mesityl-10-methylacridinium tetrafluoroborate, and Blue LED light [24] (**Scheme 3.1**).



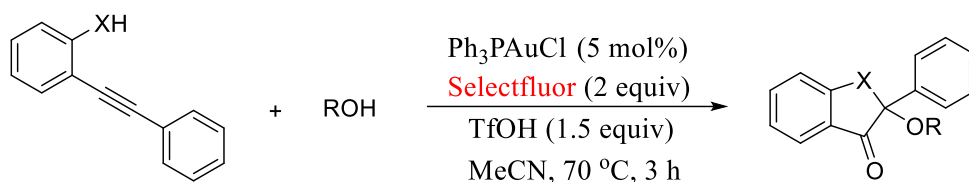
Scheme 3.1 Synthetic methodologies of 3-acyl-3-hydroxyisoindolin-1-ones

In the present work, we developed a new synthetic route for the synthesis of 3-hydroxy-3-acylisoindolin-1-ones in less reaction time. According to previous literature, Selectfluor was used in various cyclization and oxidation reactions for synthesis of organic compounds (**Scheme 3.2**) [25-28]. Thus, with the help of Selectfluor reagent and mild base (NaHCO_3), N-cyclization of N-substituted-2-(1-alkynyl)benzamides was performed followed by hydroxylation and oxidation, all in a single step to give 3-acyl-3-hydroxyisoindolin-1-ones as the desired product. Some control experiments were conducted to explore the mechanistic pathway of this reaction and to propose the reaction mechanism. We have also done the anti-oxidant studies of the synthesized compounds.

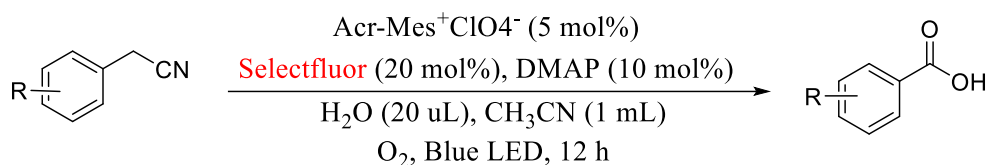
Yuan et. al. work [25]:



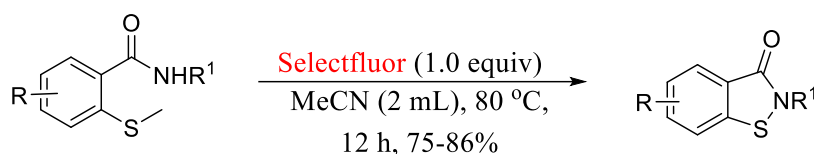
Du et. al. work [26]:



Borthakur et. al. work [27]:



Yang et. al. work [28]:



Scheme 3.2 Some examples of reported Selectfluor mediated oxidation and cyclization reactions

3.2 Experimental Section

3.2.1 General Information

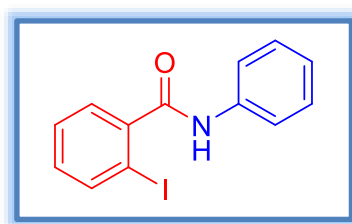
Unless otherwise stated, commercially available reagents and solvents were used for synthesis of starting materials and final products without purification. Oven-dried glasswares were used for the reactions. The progress of the reaction was monitored by thin layer chromatography (TLC). TLC was performed on silica gel 60 F₂₅₄ 0.25 mm precoated aluminum foil plates and visualization of TLC plate was done with short wavelength UV light (254 nm) chamber. Purification of starting materials and final products were performed with the help of column chromatography using commercially available silica with a mesh size of

100-200 and using ethyl acetate-petroleum ether as solvent. Melting points were determined on laboratory open-glass melting apparatus and were uncorrected. FTIR spectras were recorded in KBr on a Perkim-Elmer Spectrum II spectrophotometer. ^1H -NMR, ^{13}C -NMR, and ^{19}F -NMR spectras were obtained at 400, 100, and 377 MHz, respectively on Jeol JNM-ECZ 400S spectrometer model with tetramethylsilane (TMS) used as internal standard, deuterated chloroform (CDCl_3) as the solvent, and chemical shift values were reported in ppm. HRMS data was recorded on an Agilent G6530AA (LC-HRMS-Q-TOF) mass spectrometer model with having a HPLC pressure of 600 bar and a MS pressure of $10^{-4} - 10^{-5}$ Torr.

3.2.2 Procedure for the synthesis of N-substituted-2-iodobenzamides (2a-2d):

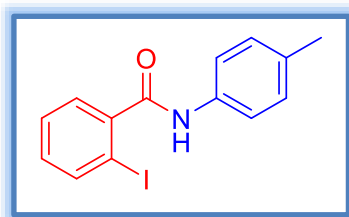
Commercially available 2-iodobenzoic acids **1** (1 equiv) were taken in DCM solvent. Then, 1.5 equiv of oxalyl chloride and 2 drops of DMF were added. Stirring of reaction mixture was continued till the solid disappeared. The volatile solvent was removed under reduced pressure. Then DCM was added followed by addition of 1 equiv of substituted aniline and 1equiv of triethylamine. After being stirred at room temperature for another one hour, the mixture was quenched with water and the aqueous phase was extracted with ethyl acetate (EtOAc). The combined organic phase was washed with 10% HCl (aq.) and saturated NaHCO_3 (aq.) and brine, successively, and dried over Na_2SO_4 . Evaporation of the organic layer afforded 2-iodobenzamides **2**.

2-Iodo-N-phenylbenzamide (2a)



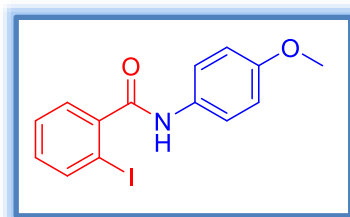
Yield: 90%; light brown solid; mp. 142-144 °C (lit. mp. 143-144 °C^[29]); FTIR (KBr, cm^{-1}): 3232, 3030, 2914, 1648, 1582, 1546, 1316, 1268, 1173, 1106, 1015, 783, 737, 695; ^1H NMR (400 MHz, CDCl_3) δ : 7.87-7.82 (m, 2H, NH and ArH), 7.60 (d, $J = 8$ Hz, 2H, ArH), 7.41 (dd, $J = 7.6, 1.6$ Hz, 1H, ArH), 7.33 (t, 3H, ArH), 7.14 (t, 1H, ArH), 7.08 (t, 1H, ArH).

2-Iodo-N-(p-tolyl)benzamide (2b)



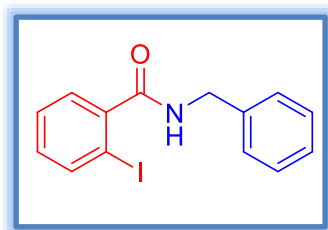
Yield: 96%; off white solid; mp. 170-172 °C (lit. mp. 171-173 °C^[29]); FTIR (KBr, cm⁻¹): 3246, 3064, 2920, 1652, 1588, 1547, 1430, 1315, 1266, 1171, 1107, 1016, 781, 736, 694; ¹H NMR (400 MHz, CDCl₃) δ: 7.90 (d, *J* = 8.0 Hz, 1H, ArH), 7.51-7.49 (m, 3H, NH and ArH), 7.44-7.39 (m, 2H, ArH), 7.28-7.24 (m, 1H, ArH), 7.14 (t, 1H, ArH), 6.99 (d, *J* = 8 Hz, 1H, ArH), 2.38 (s, 3H, CH₃).

2-Iodo-N-(4-methoxyphenyl)benzamide (2c)



Yield: 97%; off white solid; mp. 176-178 °C (lit. mp. 174-176 °C^[29]); FTIR (KBr, cm⁻¹): 3310, 3052, 2932, 1652, 1598, 1510, 1464, 1412, 1318, 1244, 1178, 1112, 1017, 902, 824, 742, 682; ¹H NMR (400 MHz, CDCl₃) δ: 7.88 (d, *J* = 8 Hz, 1H, ArH), 7.55-7.50 (m, 3H, NH and ArH), 7.49 (dd, *J* = 8.0, 1.6 Hz, 1H, ArH), 7.40 (t, 1H, ArH), 7.13 (t, 1H, ArH), 6.90 (d, *J* = 12 Hz, 2H, ArH), 3.81 (s, 3H, OCH₃).

N-Benzyl-2-iodobenzamide (2d)

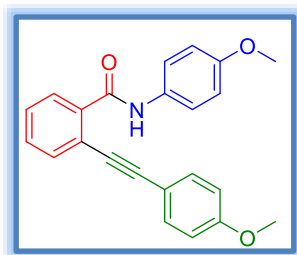


Yield: 92%; off white solid; mp. 122-124 °C (lit. mp. 124-125 °C^[29]); FTIR (KBr, cm⁻¹): 3278, 3056, 2941, 2851, 1642, 1588, 1546, 1452, 1316, 1267, 1172, 1108, 1016, 758, 695; ¹H NMR (400 MHz, CDCl₃) δ: 7.85 (d, *J* = 8 Hz, 1H, ArH), 7.43-7.39 (m, 4H, ArH), 7.38-7.29 (m, 4H, ArH), 6.15 (br s, 1H, NH), 4.64 (d, *J* = 8 Hz, 2H, CH₂).

3.2.3 Procedure to synthesize N-substituted-2-(1-alkynyl)benzamides (3a-3n)

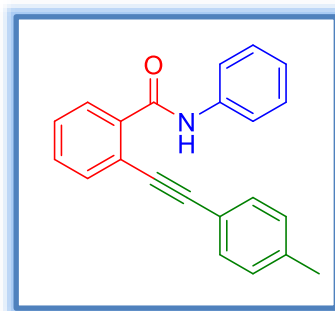
2-Iodobenzamide **2** (1 equiv) was placed in DMF in an oven-dried vial. Pd(PPh₃)₂Cl₂ (0.04 equiv) and copper iodide (0.02 equiv) were added to the vial, and the reaction mixture was placed under an inert atmosphere. The reaction mixture was stirred (5 min) at room temperature, and triethylamine (4.0 equiv) was added by syringe. The temperature was then increased to 70 °C. The terminal alkyne (1.2 equiv) dissolved in DMF was added dropwise over 5 min. The reaction mixture was allowed to stir at 70 °C for 2 h, and the reaction was monitored by TLC to establish completion of the reaction. After the reaction was over, it was allowed to cool and was quenched with aqueous NH₄Cl (10 mL) and water (10 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine solution, was dried using anhydrous Na₂SO₄. This was evaporated under reduced pressure. The crude product thus obtained was purified through column chromatography with the help of petroleum ether–ethyl acetate as eluent.

N-(4-Methoxyphenyl)-2-((4-methoxyphenyl)ethynyl)benzamide (**3a**)



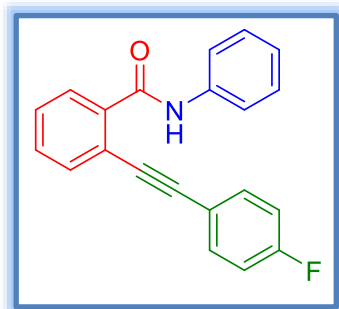
Yield: 92%; light yellow solid; mp. 136-138 °C (lit. mp. 135-137 °C^[29]); FTIR (KBr, cm⁻¹): 3362, 3036, 2956, 2836, 2206, 1660, 1600, 1538, 1410, 1238, 1174, 1020, 822, 786, 756; ¹H NMR (400 MHz, CDCl₃) δ: 9.25 (br, s, 1H, NH), 8.17-8.13 (m, 1H, ArH), 7.65-7.56 (m, 3H, ArH), 7.50-7.42 (m, 4H, ArH), 6.90-6.86 (m, 4H, ArH), 3.84 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃).

N-Phenyl-2-(*p*-tolylethynyl)benzamide (**3b**)



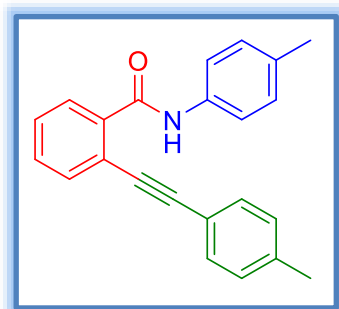
Yield: 87%; light yellow solid; mp. 158-160 °C (lit. mp. 156-158 °C^[30]); FTIR (KBr, cm⁻¹): 3272, 3058, 2918, 2218, 1654, 1594, 1524, 1440, 1324, 1256, 1176, 816, 752, 692; ¹H NMR (400 MHz, CDCl₃) δ: 9.29 (br, s, 1H, NH), 8.21-8.09 (m, 1H, ArH), 7.77 – 7.58 (m, 3H, ArH), 7.55-7.45 (m, 2H, ArH), 7.41-7.34 (m, 4H, ArH), 7.20-7.10 (m, 3H, ArH), 2.38 (s, 3H, CH₃).

2-((4-Fluorophenyl)ethynyl)-N-phenylbenzamide (3c)



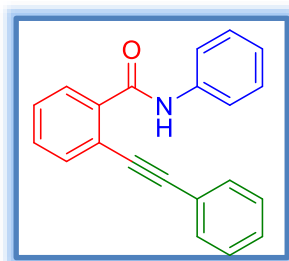
Yield: 84%; light yellow solid; mp. 154-156 °C (lit. mp. 153-155 °C^[31]); FTIR (KBr, cm⁻¹): 3262, 3038, 2922, 2222, 1655, 1592, 1526, 1442, 1320, 1220, 1168, 1152, 948, 836, 754, 692; ¹H NMR (400 MHz, CDCl₃) δ: 9.05 (br, s, 1H, NH), 8.12-8.09 (m, 1H, ArH), 7.66-7.64 (m, 3H, ArH), 7.51-7.46 (m, 4H, ArH), 7.35 (t, 2H, ArH), 7.15 (t, 1H, ArH), 7.05 (t, 2H, ArH).

N-(p-Tolyl)-2-(p-tolyethynyl)benzamide (3d)



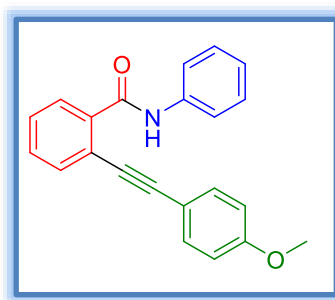
Yield: 88%; light yellow solid; mp. 124-126 °C (lit. mp. 125-127 °C^[31]); FTIR (KBr, cm⁻¹): 3254, 3050, 2918, 2215, 1654, 1592, 1526, 1430, 1310, 1164, 950, 816, 776, 752, 688; ¹H NMR (400 MHz, CDCl₃) δ: 9.27 (br, s, 1H, NH) 8.15-8.13 (m, 1H, ArH), 7.64-7.62 (m, 1H, ArH), 7.48-7.44 (m, 4H, ArH), 7.42-7.38 (m, 3H, ArH), 7.16 (d, *J* = 8 Hz, 2H, ArH), 6.95 (d, *J* = 4 Hz, 1H, ArH), 2.37 (s, 3H, CH₃), 2.31 (s, 3H, CH₃).

N-Phenyl-2-(phenylethynyl)benzamide (**3e**)



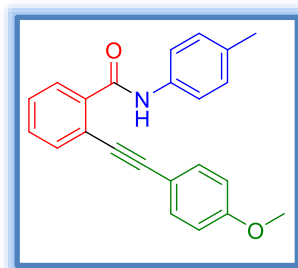
Yield: 83%; light yellow solid; mp. 154-156 °C (lit. mp. 153-154 °C^[20]); FTIR (KBr, cm⁻¹): 3282, 3056, 2921, 2216, 1657, 1596, 1524, 1440, 1322, 1258, 1178, 1014, 916, 888, 754, 689; ¹H NMR (400 MHz, CDCl₃) δ: 9.21 (br, s, 1H, NH), 8.16-8.13 (m, 1H, ArH), 7.68-7.65 (m, 4H, ArH), 7.51-7.50 (m, 2H, ArH), 7.50-7.49 (m, 2H, ArH), 7.38-7.35 (m, 4H, ArH), 7.14 (t, 1H, ArH).

2-((4-Methoxyphenyl)ethynyl)-*N*-phenylbenzamide (**3f**)



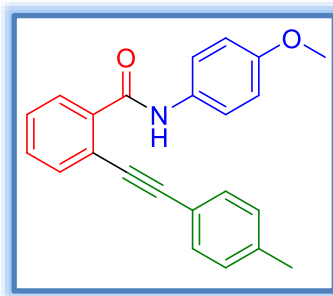
Yield: 89%; light yellow solid; mp. 110-112 °C (lit. mp. 109-111 °C^[32]) ; FTIR (KBr, cm⁻¹): 3299, 3061, 2933, 2830, 2218, 1648, 1597, 1545, 1416, 1242, 1178, 1025, 825, 789, 754; ¹H NMR (400 MHz, CDCl₃) δ: 9.32 (br, s, 1H, NH), 8.17-8.15 (m, 1H, ArH), 7.67-7.63 (m, 4H, ArH), 7.49-7.48 (m, 2H, ArH), 7.45-7.43 (m, 2H, ArH), 7.16-7.12 (m, 2H, ArH), 6.88 (d, *J* = 8 Hz, 2H, ArH), 3.84 (s, 3H, OCH₃).

2-((4-Methoxyphenyl)ethynyl)-*N*-(*p*-tolyl)benzamide (**3g**)



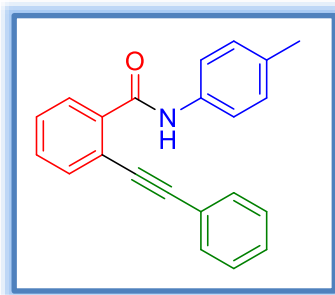
Yield: 91%; light yellow solid; mp. 106-108 °C (lit. mp. 105-107 °C^[31]); FTIR (KBr, cm⁻¹): 3283, 3068, 2934, 2836, 2214, 1645, 1600, 1536, 1510, 1302, 1284, 1246, 1174, 1028, 828, 776, 756, 690; ¹H NMR (400 MHz, CDCl₃) δ: 9.32 (br, s, 1H, NH), 8.18-8.14 (m, 1H, ArH), 7.66-7.61 (m, 1H, ArH), 7.53-7.48 (m, 2H, ArH), 7.48-7.43 (m, 4H, ArH), 7.22 (t, 1H, ArH), 6.95 (d, *J* = 8 Hz, 1H, ArH), 6.91-6.86 (m, 2H, ArH), 3.84 (s, 3H, OCH₃), 2.32 (s, 3H, CH₃).

N-(4-Methoxyphenyl)-2-(*p*-tolylethynyl)benzamide (**3h**)



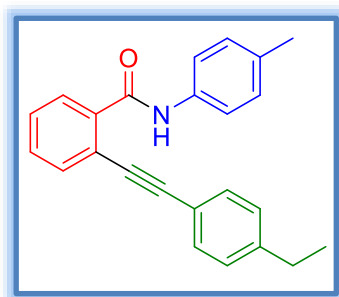
Yield: 86%; light yellow solid; mp. 144-146 °C (lit. mp. 145-146 °C^[30]) ; FTIR (KBr, cm⁻¹): 3295, 3071, 2940, 2850, 2216, 1644, 1598, 1539, 1450, 1375, 1240, 1178, 1023, 824, 789, 759; ¹H NMR (400 MHz, CDCl₃) δ: 9.12 (br, s, 1H, NH), 8.24-8.07 (m, 1H, ArH), 7.62-7.32 (m, 6H, ArH), 7.28-7.10 (m, 3H, ArH), 6.99-6.77 (m, 2H, ArH), 3.83 (s, 3H, OCH₃), 2.40 (s, 3H, CH₃).

2-(Phenylethynyl)-*N*-(*p*-tolyl)benzamide (**3i**)



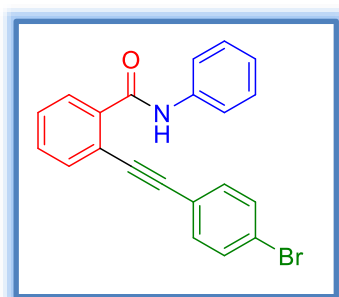
Yield: 84%; light yellow solid; mp. 134-136 °C; FTIR (KBr, cm⁻¹): 3362, 3038, 2954, 2924, 2854, 2208, 1658, 1602, 1540, 1454, 1378, 1162, 825, 788, 757; ¹H NMR (400 MHz, CDCl₃) δ: 9.32 (br, s, 1H, NH), 8.22-8.16 (m, 1H, ArH), 7.70-7.65 (m, 1H, ArH), 7.55-7.49 (m, 4H, ArH), 7.48-7.42 (m, 3H, ArH), 7.24-7.16 (m, 3H, ArH), 7.00 (d, *J* = 4 Hz, 1H, ArH), 2.42 (s, 3H, CH₃).

2-((4-Ethylphenyl)ethynyl)-N-phenylbenzamide (3j)



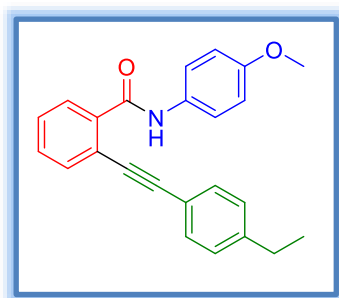
Yield: 87%; light yellow solid; mp. 164-166 °C; FTIR (KBr, cm^{-1}): 3259, 2968, 2920, 2847, 2214, 1654, 1595, 1512, 1438, 1322, 1258, 1175, 1070, 920, 891, 829, 748, 689; ^1H NMR (400 MHz, CDCl_3) δ : 9.29 (br, s, 1H, NH), 8.19-8.14 (m, 1H, ArH), 7.70-7.62 (m, 3H, ArH), 7.52-7.47 (m, 2H, ArH), 7.44-7.40 (m, 2H, ArH), 7.35 (t, 2H, ArH), 7.22-7.17 (m, 2H, ArH), 7.17-7.12 (m, 1H, ArH), 2.68 (q, 2H, CH_2), 1.25 (t, 3H, CH_3).

2-((4-Bromophenyl)ethynyl)-N-phenylbenzamide (3k)



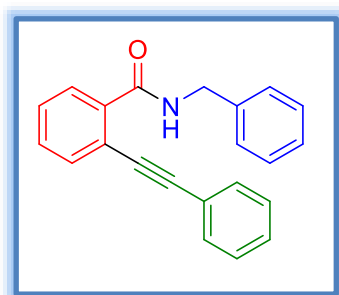
Yield: 79%; light yellow solid; mp. 152-154 °C; FTIR (KBr, cm^{-1}): 3263, 3037, 2914, 2216, 1656, 1593, 1513, 1324, 1259, 1172, 1072, 923, 893, 749, 693; ^1H NMR (400 MHz, CDCl_3) δ : 8.93 (br, s, 1H, NH), 8.13-8.07 (m, 1H, ArH), 7.67-7.62 (m, 3H, ArH), 7.52-7.46 (m, 4H, ArH), 7.38-7.31 (m, 4H, ArH), 7.15 (t, 1H, ArH).

2-((4-Ethylphenyl)ethynyl)-N-(4-methoxyphenyl)benzamide (3l)



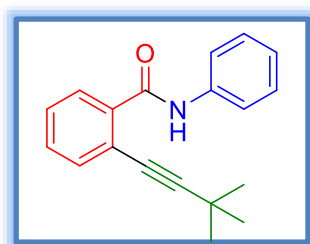
Yield: 80%; light yellow solid; mp. 144-146 °C; FTIR (KBr, cm^{-1}): 3250, 3061, 3006, 2962, 2932, 2218, 1648, 1591, 1512, 1408, 1338, 1246, 1172. 1107, 1033, 927, 908, 825, 754, 692; ^1H NMR (400 MHz, CDCl_3) δ : 9.20 (br, s, 1H, NH), 8.19-8.13 (m, 1H, ArH), 7.66-7.62 (m, 1H, ArH), 7.61-7.56 (m, 2H, ArH), 7.51-7.46 (m, 2H, ArH), 7.44-7.40 (m, 2H, ArH), 7.22-7.17 (m, 2H, ArH), 6.91-6.86 (m, 2H, ArH), 3.81 (s, 3H, OCH_3), 2.68 (q, 2H, CH_2), 1.25 (t, 3H, CH_3).

N-Benzyl-2-(phenylethynyl)benzamide (**3m**)



Yield: 84%; light yellow solid; mp. 100-102 °C (lit. mp. 99-101 °C^[16]); FTIR (KBr, cm^{-1}): 3277, 3056, 2916, 2848, 2218, 1644, 1531, 1493, 1313, 1238, 1153, 1098, 1026, 752, 728, 682, 605; ^1H NMR (400 MHz, CDCl_3) δ : 8.18-8.13 (m, 1H, ArH), 7.80 (br, s, 1H, NH), 7.62-7.57 (m, 1H, ArH), 7.48-7.44 (m, 2H, ArH), 7.40-7.35 (m, 2H, ArH), 7.35-7.31 (m, 1H, ArH), 7.28-7.24 (m, 5H, ArH), 7.16-7.11 (m, 2H, ArH), 4.71 (d, $J = 4$ Hz, 2H, CH_2).

2-(3,3-Dimethylbut-1-yn-1-yl)-*N*-phenylbenzamide (**3n**)

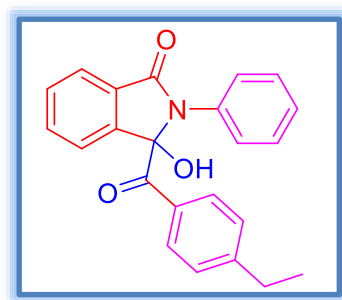


Yield: 72%; light yellow solid; mp. 94-96 °C; FTIR (KBr, cm^{-1}): 3250, 3134, 3072, 2964, 2862, 2247, 1650, 1597, 1540, 1488, 1439, 1326, 1279, 1254, 1200, 1132, 950, 927, 890, 754, 696, 588; ^1H NMR (400 MHz, CDCl_3) δ : 9.14 (br, s, 1H, NH), 8.12-8.07 (m, 1H, ArH), 7.71-7.64 (m, 2H, ArH), 7.53-7.48 (m, 1H, ArH), 7.44-7.35 (m, 4H, ArH), 7.15 (t, 1H, ArH), 1.31 (s, 9H, CH_3).

3.2.4 Procedure for the synthesis of 3-acyl-3-hydroxyisoindolin-1-ones (4a-4l) and difluoro-3-hydroxyisoindolin-1-one (5a)

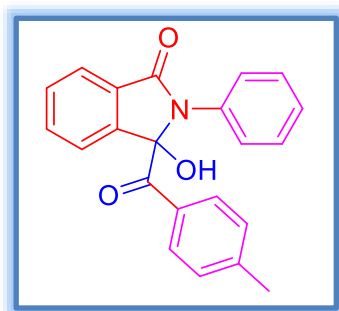
To an oven dried vial, 1 equiv of starting material (N-substituted-2-(1-alkynyl)benzamides) (**3**) was added followed by addition of 3 equiv of selectfluor and 3 equiv of sodium bicarbonate. The mixture was then purged with inert atmosphere. Now, acetonitrile (MeCN) solvent was added using syringe followed by addition of 2-3 drops of water (H₂O) under inert atmosphere. The reaction mixture was then stirred at 80°C for 5 hours. After the completion of the reaction, product was extracted with ethyl acetate and washed with brine solution. The combined organic layer was dried using anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography (using petroleum ether–ethyl acetate as eluent) to give the pure final product (**4**).

3-(4-Ethylbenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (4a)



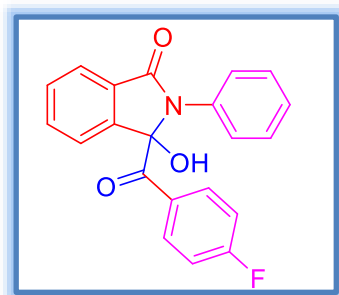
Yield: 79%; white solid; mp. 158-160 °C; FTIR (KBr, cm⁻¹): 3307, 2968, 2922, 2848, 1699, 1672, 1602, 1491, 1361, 1313, 1253, 1223, 1173, 1140, 1109, 1058, 997, 912, 871, 756, 700, 598; ¹H NMR (400 MHz, CDCl₃) δ: 8.07-8.04 (m, 1H, ArH), 7.65-7.62 (m, 2H, ArH), 7.44-7.42 (m, 2H, ArH), 7.39-7.36 (m, 1H, ArH), 7.28-7.24 (m, 3H, ArH), 7.19-7.16 (m, 2H, ArH), 7.09 (d, *J* = 8 Hz, 2H, ArH), 6.02 (br, s, 1H, OH), 2.61 (q, 2H, CH₂), 1.18 (t, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 195.1, 168.0, 151.9, 144.6, 134.8, 133.7, 131.5, 130.8, 129.9, 129.2, 128.5, 127.9, 127.1, 124.9, 122.8, 91.7, 29.0, 14.8; HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₃H₁₉NNaO₃ 380.1263; Found 380.1270.

3-Hydroxy-3-(4-methylbenzoyl)-2-phenylisoindolin-1-one (4b)



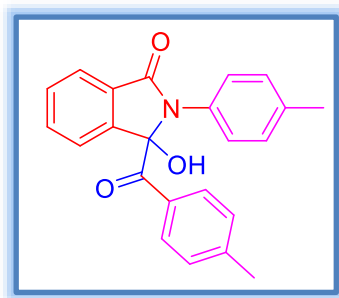
Yield: 83%; white solid; mp. 158-160 °C (lit. mp. 156-158 °C^[24]); FTIR (KBr, cm⁻¹): 3338, 2923, 2853, 1697, 1675, 1604, 1492, 1365, 1260, 1217, 1173, 1111, 1060, 1000, 914, 871, 812, 756, 692; ¹H NMR (400 MHz, CDCl₃) δ: 8.08-8.02 (m, 1H, ArH), 7.67-7.59 (m, 2H, ArH), 7.45-7.39 (m, 2H, ArH), 7.39-7.35 (m, 1H, ArH), 7.31-7.25 (m, 3H, ArH), 7.18 (d, *J* = 8 Hz, 2H, ArH), 7.07 (d, *J* = 8 Hz, 2H, ArH), 6.02 (br, s, 1H, OH), 2.32 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 194.9, 168.0, 145.8, 144.6, 134.8, 133.7, 131.5, 130.8, 129.8, 129.7, 129.6, 129.2, 127.9, 127.1, 124.9, 122.8, 91.7, 21.8; HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₂H₁₇NNaO₃ 366.1106; Found 366.1096.

3-(4-Fluorobenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (4c)



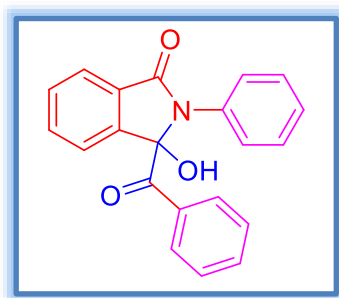
Yield: 73%; white solid; mp. 132-134 °C (lit. mp. 130-132 °C^[22]); FTIR (KBr, cm⁻¹): 3241, 2919, 2851, 1685, 1653, 1504, 1493, 1466, 1368, 1236, 1157, 1113, 1045, 1003, 874, 837, 755, 693; ¹H NMR (400 MHz, CDCl₃) δ: 8.08-8.06 (m, 1H, ArH), 7.67-7.64 (m, 2H, ArH), 7.56-7.52 (m, 2H, ArH), 7.40-7.37 (m, 1H, ArH), 7.31-7.27 (m, 3H, ArH), 7.20-7.15 (m, 2H, ArH), 6.98-6.93 (m, 2H, ArH), 5.93 (br, s, 1H, OH); ¹³C NMR (100 MHz, CDCl₃) δ: 194.1, 167.8, 167.5, 164.9, 144.1, 134.6, 133.8, 132.5, 132.4, 131.3, 131.0, 130.5, 130.0, 129.4, 129.2, 128.9, 127.9, 126.8, 125.0, 122.7, 116.4, 116.2, 91.7.

3-Hydroxy-3-(4-methylbenzoyl)-2-(p-tolyl)isoindolin-1-one (4d)



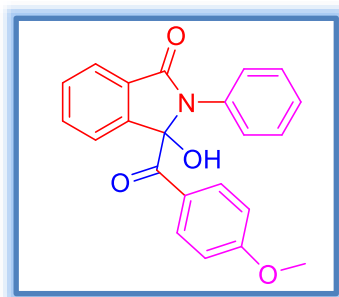
Yield: 80%; white solid; mp. 126-128 °C; FTIR (KBr, cm^{-1}): 3259, 2946, 2842, 1703, 1687, 1604, 1496, 1454, 1379, 1263, 1219, 1180, 1104, 1066, 1005, 920, 879, 810, 762, 696; ^1H NMR (400 MHz, CDCl_3) δ : 8.06-8.04 (m, 1H, ArH), 7.67-7.61 (m, 3H, ArH), 7.43-7.35 (m, 4H, ArH), 7.12-7.04 (m, 4H, ArH), 5.99 (br, s, 1H, OH), 2.33 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 195.0, 168.0, 145.7, 144.5, 139.0, 134.7, 133.7, 133.6, 130.7, 129.7, 128.9, 128.8, 128.0, 124.8, 124.1, 122.7, 115.7, 115.5, 91.6, 21.7, 21.3; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{23}\text{H}_{20}\text{NO}_3$ 358.1443; Found 358.1422.

3-Benzoyl-3-hydroxy-2-phenylisoindolin-1-one (4e)



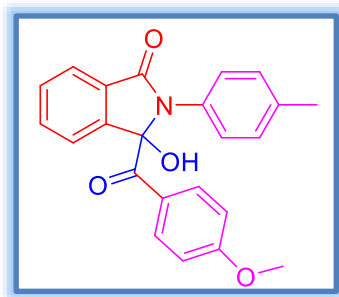
Yield: 79%; white solid; mp. 120-122 °C (lit. mp. 118-120 °C^[24]); FTIR (KBr, cm^{-1}): 3261, 2958, 2846, 1708, 1683, 1602, 1494, 1455, 1263, 1220, 1170, 1114, 1065, 1004, 918, 875, 820, 760, 694; ^1H NMR (400 MHz, CDCl_3) δ : 8.09-8.05 (m, 1H, ArH), 7.69-7.63 (m, 2H, ArH), 7.53-7.43 (m, 4H, ArH), 7.43-7.38 (m, 1H, ArH), 7.30-7.27 (m, 3H, ArH), 7.26-7.24 (m, 1H, ArH), 7.18-7.14 (m, 2H, ArH), 5.96 (br, s, 1H, OH); ^{13}C NMR (100 MHz, CDCl_3) δ : 195.7, 167.9, 144.2, 134.6, 134.4, 133.7, 132.3, 131.4, 130.9, 129.4, 129.1, 128.9, 127.8, 127.0, 124.9, 122.7, 91.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{16}\text{NO}_3$ 330.1130; Found 330.1142.

3-Hydroxy-3-(4-methoxybenzoyl)-2-phenylisoindolin-1-one (4f)



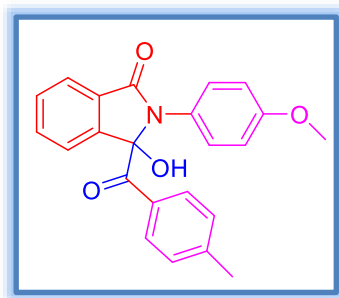
Yield: 81%; white solid; mp. 114-116 °C (lit. mp. 116-118 °C^[24]); FTIR (KBr, cm⁻¹): 3258, 3048, 2937, 2833, 1699, 1686, 1599, 1498, 1241, 1223, 1178, 1114, 1066, 1001, 919, 878, 819, 759, 691; ¹H NMR (400 MHz, CDCl₃) δ: 8.05-8.03 (m, 1H, ArH), 7.63-7.59 (m, 2H, ArH), 7.56 (d, *J* = 8 Hz, 2H, ArH), 7.38-7.34 (m, 1H, ArH), 7.28-7.18 (m, 5H, ArH), 6.73 (d, *J* = 8 Hz, 2H, ArH), 6.15 (br, s, 1H, OH), 3.77 (s, 3H, OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 193.3, 168.1, 164.6, 145.0, 134.9, 133.8, 132.9, 132.4, 131.3, 130.8, 129.2, 127.8, 127.1, 124.9, 124.7, 122.8, 114.3, 91.6, 55.6; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₂H₁₈NO₄ 360.1236; Found 360.1236.

3-Hydroxy-3-(4-methoxybenzoyl)-2-(p-tolyl)isoindolin-1-one (4g)



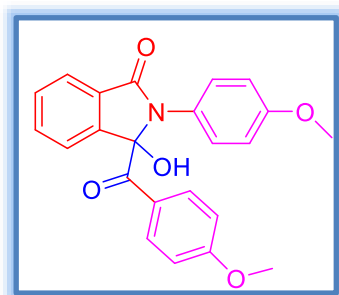
Yield: 83%; white solid; mp. 126-128 °C; FTIR (KBr, cm⁻¹): 3261, 3056, 2943, 2858, 1694, 1689, 1606, 1502, 1494, 1376, 1243, 1218, 1171, 1112, 1064, 1005, 919, 878, 823, 764, 691; ¹H NMR (400 MHz, CDCl₃) δ: 8.05-8.03 (m, 1H, ArH), 7.63-7.60 (m, 2H, ArH), 7.57-7.53 (m, 2H, ArH), 7.38-7.34 (m, 1H, ArH), 7.15 (t, 1H, ArH), 7.05 (d, *J* = 12 Hz, 2H, ArH), 6.93 (d, *J* = 8 Hz, 1H, ArH), 6.77-6.71 (m, 2H, ArH), 6.10 (br, s, 1H, OH), 3.78 (s, 3H, OCH₃), 2.25 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 193.2, 168.0, 164.4, 144.8, 138.9, 134.5, 133.5, 132.2, 131.3, 130.6, 128.8, 128.6, 127.8, 124.7, 124.6, 123.9, 122.7, 114.1, 91.4, 55.5, 21.3; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₃H₂₀NO₄ 374.1392; Found 374.1394.

3-Hydroxy-2-(4-methoxyphenyl)-3-(4-methylbenzoyl)isoindolin-1-one (4h)



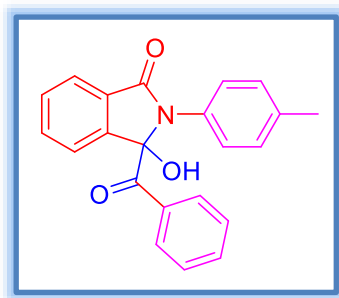
Yield: 80%; white solid; mp. 134-136 °C; FTIR (KBr, cm^{-1}): 3271, 3061, 2985, 2840, 1703, 1690, 1608, 1504, 1495, 1457, 1371, 1252, 1214, 1176, 1117, 1067, 1004, 913, 871, 814, 762, 692; ^1H NMR (400 MHz, CDCl_3) δ : 8.05-8.03 (m, 1H, ArH), 7.64-7.58 (m, 2H, ArH), 7.41 (d, J = 8Hz, 2H, ArH), 7.38-7.34 (m, 1H, ArH), 7.10-7.02 (m, 4H, ArH), 6.82-6.76 (m, 2H, ArH), 6.00 (br, s, 1H, OH), 3.75 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.9, 168.3, 159.2, 145.8, 144.6, 133.6, 131.7, 130.8, 129.9, 129.8, 129.7, 129.1, 127.0, 124.9, 122.9, 114.5, 91.5, 55.4, 21.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{23}\text{H}_{20}\text{NO}_4$ 374.1392; Found 374.1392.

3-Hydroxy-3-(4-methoxybenzoyl)-2-(4-methoxyphenyl)isoindolin-1-one (4i)



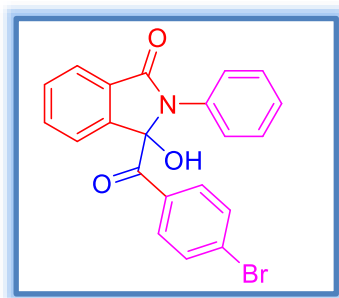
Yield: 85%; white solid; mp. 142-144 °C; FTIR (KBr, cm^{-1}): 3253, 3054, 2919, 2844, 1706, 1683, 1608, 1499, 1253, 1218, 1174, 1116, 1066, 1008, 926, 879, 828, 769, 692; ^1H NMR (400 MHz, CDCl_3) δ : 8.05-8.03 (m, 1H, ArH), 7.64-7.59 (m, 2H, ArH), 7.57-7.53 (m, 2H, ArH), 7.38-7.34 (m, 1H, ArH), 7.08-7.03 (m, 2H, ArH), 6.82-6.77 (m, 2H, ArH), 6.75 (d, J = 12 Hz, 2H, ArH), 6.09 (br, s, 1H, OH), 3.79 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 193.3, 168.3, 164.5, 159.2, 145.0, 133.6, 132.4, 131.5, 130.7, 129.0, 127.1, 124.9, 124.7, 122.9, 114.5, 114.3, 91.4, 55.6, 55.4; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{23}\text{H}_{20}\text{NO}_5$ 390.1341; Found 390.1320.

3-Benzoyl-3-hydroxy-2-(p-tolyl)isoindolin-1-one (4j)



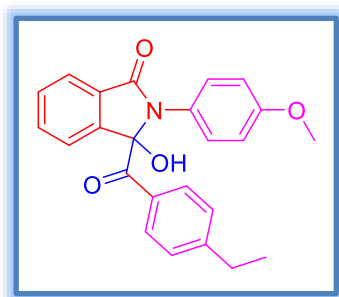
Yield: 81%; white solid; mp. 138-140 °C (lit. mp. 136-138 °C^[24]); FTIR (KBr, cm⁻¹): 3264, 2928, 2856, 1707, 1691, 1607, 1493, 1453, 1376, 1261, 1221, 1174, 1107, 1063, 1003, 918, 876, 814, 760, 694; ¹H NMR (400 MHz, CDCl₃) δ: 8.01-7.98 (m, 1H, ArH), 7.61-7.55 (m, 3H, ArH), 7.38-7.29 (m, 4H, ArH), 7.10 (t, 1H, ArH), 7.05-6.99 (m, 4H, ArH), 5.94 (br, s, 1H, OH), 2.27 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 195.3, 168.3, 146.0, 144.8, 139.3, 134.7, 134.0, 133.9, 131.0, 130.0, 129.2, 129.1, 128.3, 125.1, 124.4, 123.0, 116.0, 115.8, 91.9, 22.0.

3-(4-Bromobenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (4k)



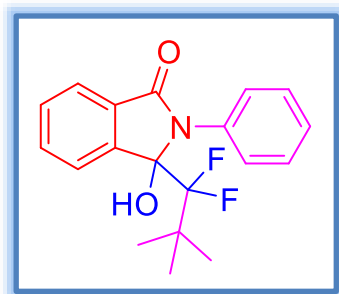
Yield: 75%; white solid; mp. 164-166 °C (lit. mp. 165-167 °C^[24]); FTIR (KBr, cm⁻¹): 3262, 2920, 2847, 1682, 1650, 1578, 1490, 1362, 1308, 1276, 1252, 1221, 1188, 1173, 1111, 1072, 998, 911, 872, 767, 754, 698, 593; ¹H NMR (400 MHz, CDCl₃) δ: 8.07-8.03 (m, 1H, ArH), 7.66-7.63 (m, 2H, ArH), 7.45-7.40 (m, 3H, ArH), 7.36-7.32 (m, 2H, ArH), 7.31-7.27 (m, 3H, ArH), 7.20-7.16 (m, 2H, ArH), 5.89 (br, s, 1H, OH); ¹³C NMR (100 MHz, CDCl₃) δ: 195.0, 167.8, 144.0, 134.6, 133.9, 132.5, 132.4, 132.2, 131.4, 131.1, 130.9, 130.1, 129.3, 128.0, 126.9, 125.1, 122.8, 91.8; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₁H₁₅BrNO₃ 408.0235; Found 408.0238.

3-(4-Ethylbenzoyl)-3-hydroxy-2-(4-methoxyphenyl)isoindolin-1-one (4l)



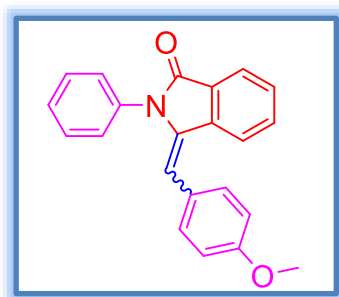
Yield: 78%; white solid; mp. 150-152 °C; FTIR (KBr, cm^{-1}): 3358, 3055, 2920, 2847, 1717, 1664, 1604, 1511, 1465, 1346, 1242, 1178, 1143, 1115, 1069, 1026, 993, 912, 831, 763, 696, 624, 595; ^1H NMR (400 MHz, CDCl_3) δ : 8.07-8.03 (m, 1H, ArH), 7.66-7.61 (m, 2H, ArH), 7.46-7.41 (m, 2H, ArH), 7.40-7.35 (m, 1H, ArH), 7.13-7.07 (m, 2H, ArH), 7.06-7.00 (m, 2H, ArH), 6.83-6.77 (m, 2H, ArH), 5.98 (br, s, 1H, OH), 3.76 (s, 3H, OCH_3), 2.62 (q, 2H, CH_2), 1.19 (t, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 195.0, 168.3, 159.2, 151.9, 144.6, 133.6, 131.7, 130.8, 130.0, 129.9, 129.1, 128.6, 128.5, 127.0, 124.9, 122.9, 114.5, 91.5, 55.5, 29.0, 14.9; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{24}\text{H}_{22}\text{NO}_4$ 388.1549; Found 388.1549.

3-(1,1-Difluoro-2,2-dimethylpropyl)-3-hydroxy-2-phenylisoindolin-1-one (5a)



Yield: 68%; white solid; mp. 130-132 °C; FTIR (KBr, cm^{-1}): 3328, 2992, 2874, 1685, 1590, 1490, 1467, 1367, 1318, 1138, 1105, 1074, 1047, 1010, 924, 858, 756, 700; ^1H NMR (400 MHz, CDCl_3) δ : 7.86-7.73 (m, 2H, ArH), 7.68-7.55 (m, 4H, ArH), 7.48-7.38 (m, 2H, ArH), 7.34-7.29 (m, 1H, ArH), 4.17 (br, s, 1H, OH), 0.79 (s, 9H, CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 167.8, 144.1, 143.4, 136.2, 132.9, 132.5, 130.9, 130.7, 128.5, 127.5, 127.2, 127.0, 124.7, 124.6, 124.3, 117.2, 92.2 (t, $J = 30$ Hz), 39.0 (t, $J = 30$ Hz), 26.0; ^{19}F NMR (377 MHz, CDCl_3) δ : -106.23 (d, $J = 263.90$ Hz, 1F), -111.61 (d, $J = 263.90$ Hz, 1F); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{19}\text{H}_{19}\text{F}_2\text{NNaO}_2$ 354.1282; Found 354.1295.

3-(4-Methoxybenzylidene)-2-phenylisoindolin-1-one (V)



Yield: 30%; yellow solid; mp. 154-156 °C (lit. mp. 152-154 °C^[33]); FTIR (KBr, cm⁻¹): 3271, 3032, 1712, 1648, 1602, 1515, 1476, 1320, 1121, 1030, 758; ¹H NMR (400 MHz, CDCl₃) δ: 7.93 (d, *J* = 8 Hz, 1H, ArH), 7.82 (d, *J* = 8 Hz, 1H, ArH), 7.64 (t, 1H, ArH), 7.52 (t, 1H, ArH), 6.99-6.91 (m, 5H, ArH), 6.88-6.83 (m, 2H, ArH), 6.80 (s, 1H, AlkeneH), 6.63-6.58 (m, 2H, ArH), 3.69 (s, 3H, OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ: 168.2, 158.4, 138.6, 134.7, 133.7, 132.4, 129.3, 128.4, 127.3, 126.6, 123.9, 119.5, 113.7, 107.5, 55.6.

3.2.5 Antioxidant studies of synthesized final products

Synthesized final compounds **4** and **5** were evaluated for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [34]. Firstly, stock solutions of compounds were prepared in methanol solvent. Then, 1 mL of each solution was mixed with 3 mL of freshly prepared DPPH solution (4 mg in 100 mL of methanol). DPPH solution was allowed to react with solutions for 30 minutes in dark at room temperature. After 30 minutes, absorbance values were measured on PerkinElmer UV-Visible spectrophotometer at 517 nm. DPPH solution in methanol was used as blank. Gallic acid solution in methanol was used as reference standard. Percentage of anti-oxidant activity, AA (%) of all the compounds including gallic acid were calculated using this formula:

$$AA (\%) = [(Ab - As)/As] * 100$$

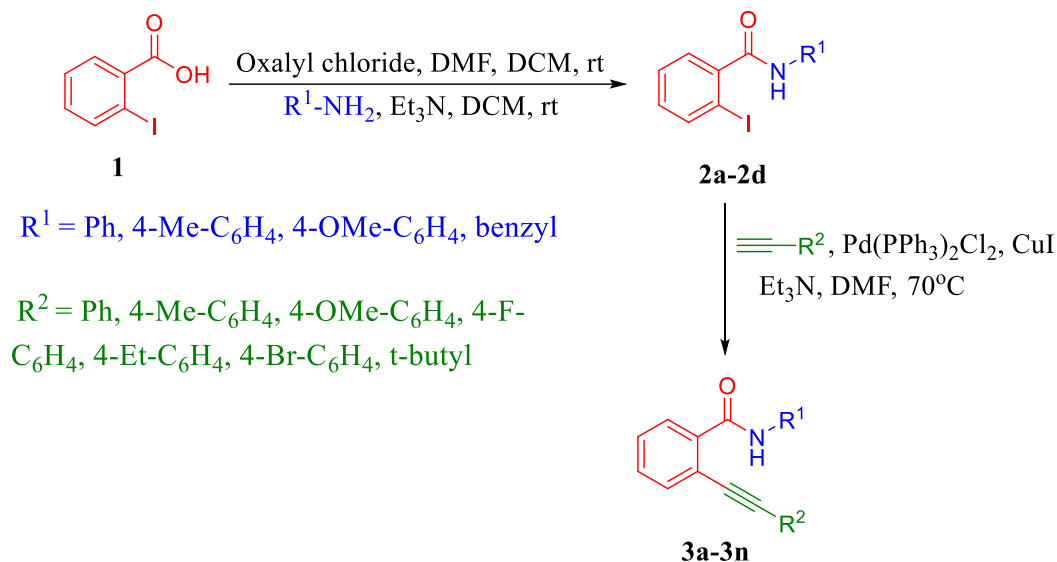
Where Ab = Absorbance of blank; As = Absorbance of sample

3.3 Results and Discussion

3.3.1 Synthesis of N-substituted-2-(1-alkynyl)benzamides

Our work started with the synthesis of N-substituted-2-iodobenzamides **2a-2d** in 90-97% yields. Initially, 2-iodobenzoic acids **1** underwent reaction with oxalyl chloride, followed by reaction with N-substituted amines to give N-substituted-2-iodobenzamides **2a-**

2d [30]. N-substituted-2-iodobenzamides further underwent Sonogashira coupling reaction with terminal alkynes using Pd and Cu catalysts to generate corresponding N-substituted-2-(1-alkynyl)benzamides **3a-3n** (Scheme 3.3) [15].



Scheme 3.3 Synthetic methodology for the synthesis of N-substituted-2-(1-alkynyl)benzamides (**3**)

All the synthesized starting materials were characterized using melting points, FT-IR, and ^1H NMR spectroscopic techniques. The FT-IR spectrum of **3a** exhibits a broad absorption band at 3362 cm^{-1} due to the N-H stretching vibration of the amide group (**Figure 3.2**). A characteristic peak at 1660 cm^{-1} corresponds to the carbonyl ($C=O$) stretching vibration of the amide group, confirming the presence of amide group in the molecule. While the characteristic alkyne ($C\equiv C$) stretching vibration is displayed at 2206 cm^{-1} , suggesting the presence of the ethynyl moiety. In addition, the peaks at 2956 and 2836 cm^{-1} correspond to C-H stretching vibrations. The observed vibrational stretching frequencies are in accordance with the functional groups expected for the compound **3a**. Thus, the FT-IR spectra of **3a** confirms the presence of both amide and alkyne groups in the molecule.

The ^1H NMR spectrum of N-(4-methoxyphenyl)-2-((4-methoxyphenyl)ethynyl)benzamide (**3a**) displays characteristic resonance peaks due to aromatic, aliphatic, and NH protons. A characteristic singlet peak for the NH proton is shown at 9.25 ppm . This downfield chemical shift value is attributed to the deshielding effect of the adjacent carbonyl group. The aromatic protons of the phenyl rings show chemical shift values between 8.17 and 6.86 ppm as multiplets. The two methoxy group protons present in **3a**

display singlet peaks at 3.84 and 3.81 ppm, each with integration value of three, confirming the presence of two methoxy groups in the molecule. The total integration value of all protons is found to be 19, which is in accordance with the total number of protons in the structure of **3a** (Figure 3.3). These chemical shift values are in consistent with the structure. Similarly, the characterization of other synthesized starting materials has been done (Experimental Section 3.2.3).

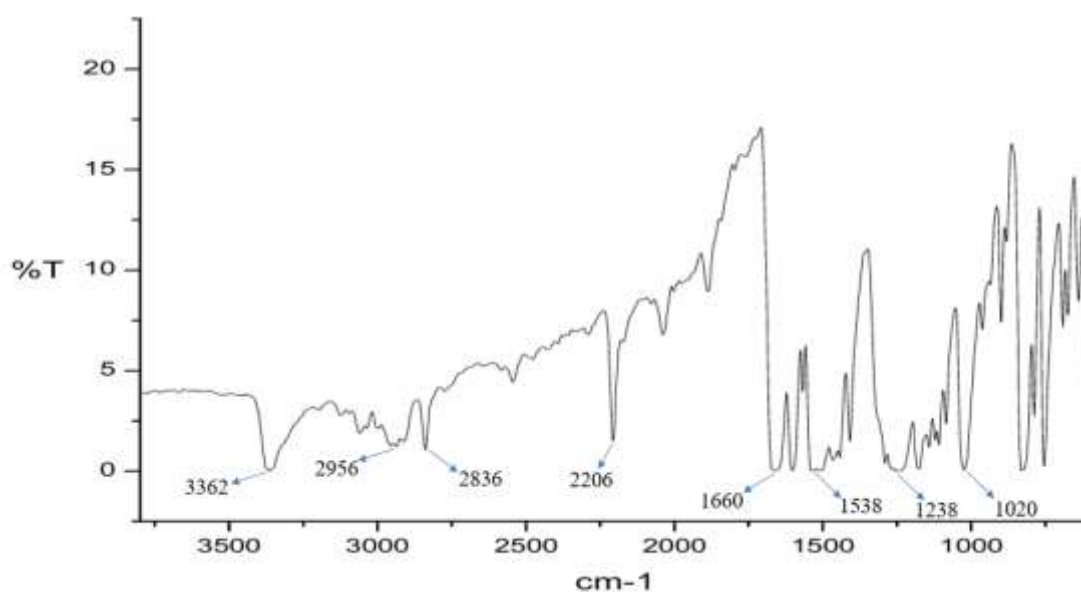


Figure 3.2 FT-IR of N-(4-methoxyphenyl)-2-((4-methoxyphenyl)ethynyl)benzamide (**3a**)

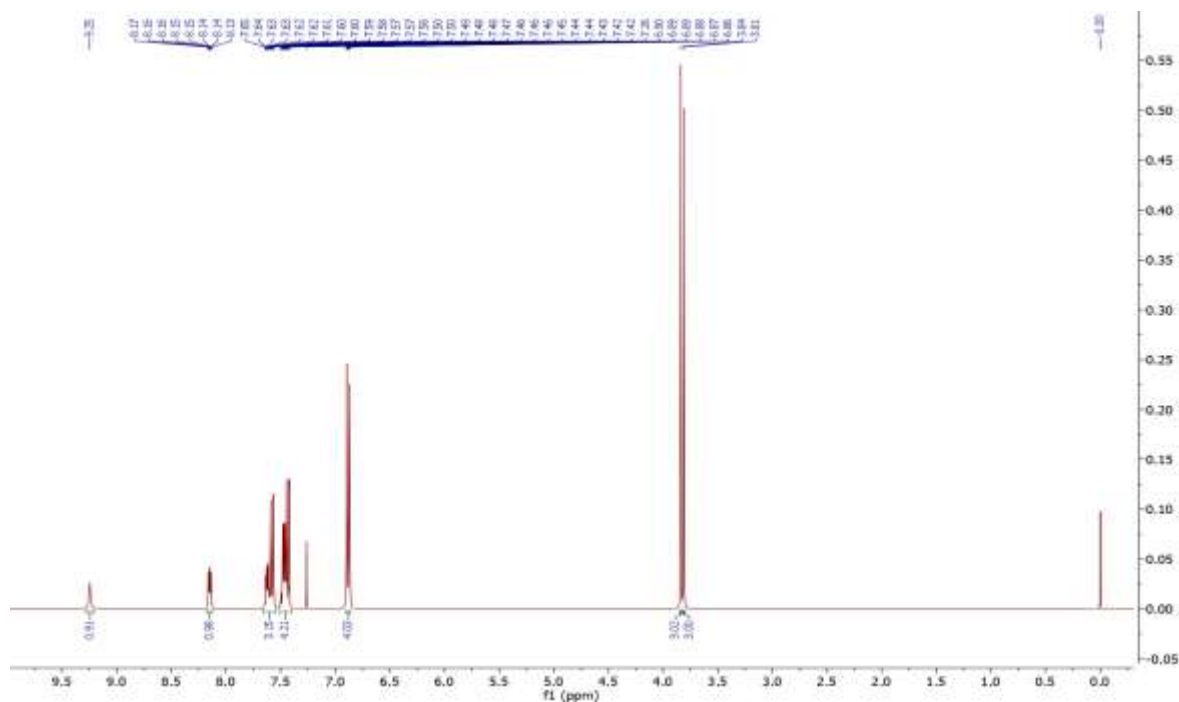
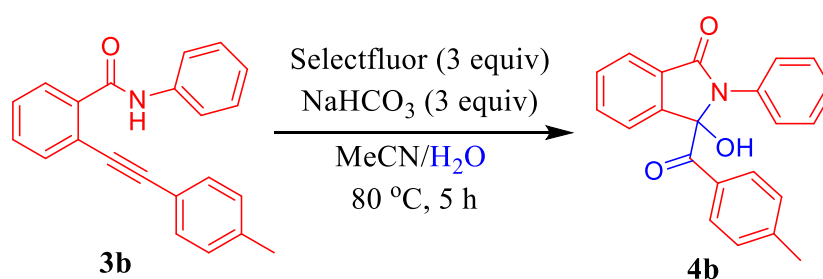


Figure 3.3 ^1H NMR of N-(4-methoxyphenyl)-2-((4-methoxyphenyl)ethynyl)benzamide (**3a**)

3.3.2 Synthesis of 3-acyl-3-hydroxyisoindolin-1-ones **4**

The 3-acyl-3-hydroxyisoindolin-1-one derivatives **4** have been synthesized using Selectfluor under basic conditions from 2-(1-alkynyl)benzamides **3**. The first reaction was performed from N-phenyl-2-(p-tolyethynyl)benzamide **3b** to obtain 3-hydroxy-3-(4-methylbenzoyl)-2-phenylisoindolin-1-one **4b** using Selectfluor and sodium bicarbonate (NaHCO_3) with acetonitrile (MeCN) used as solvent and H_2O as source of O and OH for oxidation and hydroxylation of terminal alkyne carbon atoms at 80°C (**Scheme 3.4**). Cyclization, oxidation, and hydroxylation, all were completed in a single step in 5 h while previously reported schemes of this product 3-hydroxy-3-(4-methylbenzoyl)-2-phenylisoindolin-1-one **4b** [22-24] required reaction time larger than 5 h (**Scheme 3.1**).



Scheme 3.4 Reaction of N-phenyl-2-(p-tolyethynyl)benzamide (**3b**) to give 3-hydroxy-3-(4-methylbenzoyl)-2-phenylisoindolin-1-one (**4b**)

To optimize the reaction conditions, different amounts of Selectfluor and NaHCO_3 have been tried in different solvent systems with **3b** (**Table 3.1**). The reaction was started with 1 equiv each of NaHCO_3 and Selectfluor in acetonitrile (MeCN) with 2 drops of water. The reaction was performed from room temperature to 80°C without getting product **4b** in isolated amount. A faint spot on TLC was observed (Entry 1, **Table 3.1**). Increasing the equiv of NaHCO_3 from 1 equiv to 2 equiv, while keeping Selectfluor constant at 1 equiv also gave trace amount of product (Entry 3, **Table 3.1**). However, with 1 equiv of NaHCO_3 and 2 equiv of Selectfluor, product was formed with 23% yield (Entry 2, **Table 3.1**), suggesting that the Selectfluor plays a significant role in initiating the reaction. Additionally, improvement was achieved by increasing the amount of NaHCO_3 to 3 equiv in the presence of 2 equiv of Selectfluor, affording product **4b** in 37% yield at 80°C of temperature (Entry 4, **Table 3.1**). The optimum conditions for the synthesis of **4b** have been achieved using 3 equiv of NaHCO_3 and 3 equiv of Selectfluor in acetonitrile solvent at 80°C of temperature in the presence of 2 drops of water, which provided **4b** with 83% yield (Entry 7, **Table 3.1**). Further

increasing the equiv of NaHCO₃ and Selectfluor from 3 equiv to 4 equiv decreased the yield of **4b** from 83 to 57% (Entry 8, **Table 3.1**), possibly due to competitive side reactions or over-oxidation of substrate.

The influence of temperature conditions on reaction was also investigated. Performing the reaction at room temperature and at 50°C exhibited 20% and 52% yield of **4b** respectively (Entries 5 and 6, **Table 3.1**), suggesting that 80°C temperature is essential to get the product with highest possible yield. The use of NaHCO₃ helped in the formation of hydroxyl ion. In its absence, the reaction afforded **4b** in 35% yield using 3 equiv of Selectfluor (Entry 13, **Table 3.1**), indicating that although the reaction can proceed without NaHCO₃, its presence significantly improves the reaction efficiency.

The effect of solvents other than MeCN on the reaction was also investigated. The other solvents tried for the reaction included tetrahydrofuran (THF), dimethylsulfoxide (DMSO), dimethylformamide (DMF), and toluene. The product **4b** did not form under THF and DMSO solvents (Entries 9 and 11, **Table 3.1**) and only trace amount of **4b** was formed under DMF and toluene solvents (Entries 10 and 12, **Table 3.1**), highlighting that MeCN is the preferred solvent for this transformation. Furthermore, performing the reaction under air without inert atmosphere decreased the product's yield, suggesting that an inert atmosphere is essential for achieving the highest possible product yield.

Table 3.1. Optimization studies for the synthesis of **4b**

Entry	NaHCO ₃ (equiv)	Selectfluor (equiv)	Solvent	Temp. (°C)	Yield of 4b (%)
1	1	1	MeCN	80	trace
2	1	2	MeCN	80	23
3	2	1	MeCN	80	trace
4	3	2	MeCN	80	37
5	3	3	MeCN	rt	20
6	3	3	MeCN	50	52
7	3	3	MeCN	80	83
8	4	4	MeCN	80	57
9	3	3	THF	60	NR
10	3	3	DMF	80	trace
11	3	3	DMSO	80	NR
12	3	3	Toluene	80	trace
13	0	3	MeCN	80	35

After optimizing the reaction conditions, twelve derivatives of 3-acyl-3-hydroxyisoindolin-1-ones **4** were synthesized (**Scheme 3.5**). All these derivatives were achieved in good yields ranging from 73-85%. It was found that the starting materials containing electron donating groups were more compatible for this reaction than with electron withdrawing groups. The compound 3-(4-fluorobenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (**4c**) containing electron withdrawing group (4-F-C₆H₄) on R² position gave less yield of 73% while 3-hydroxy-3-(4-methoxybenzoyl)-2-(4-methoxyphenyl)isoindolin-1-one (**4i**) containing electron donating group (4-OMe-C₆H₄) on both R¹ and R² positions gave high yield of 85%. Reaction with N-benzyl-2-(phenylethynyl)benzamide (**3m**) substrate does not give any product and starting material remained intact. This indicated that the presence of aryl group on N atom of 2-(1-alkynyl)benzamides is necessary for the reaction to occur.

All the synthesized derivatives of 3-acyl-3-hydroxyisoindolin-1-ones (**4**) were characterized by melting point, FT-IR, ¹H NMR, ¹³C NMR, and HRMS. In the FT-IR spectrum of **4a** (**Figure 3.4**), a broad peak at 3307 cm⁻¹ is attributed to the O-H vibrational stretching frequency of the hydroxyl group. Vibrational stretching frequencies at 1699 cm⁻¹ and 1672 cm⁻¹ correspond to the carbonyl (C=O) stretching vibrations of the ketone and amide groups, respectively. The ketone carbonyl group absorbs at a relatively higher wavenumber than the amide carbonyl group as the amide carbonyl group is involved in resonance with the adjacent nitrogen atom, that decreases the double-bond character of the C=O bond and thus lowers its vibrational stretching frequency. The peaks at 2968, 2922, and 2848 cm⁻¹ are assigned to C-H stretching vibrations. Therefore, the presence of amide, ketone, and hydroxyl groups is confirmed by FT-IR spectrum.

The ¹H NMR spectrum of 3-(4-ethylbenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (**4a**) displays a characteristic broad singlet corresponding to the O-H proton at chemical shift value of 6.02 ppm. The downfield chemical shift of the hydroxyl proton is attributed to the deshielding effect of the electronegative oxygen atom. All aromatic protons of the phenyl rings exhibit chemical shift values in the range of 8.07-7.16 ppm as multiplets and 7.09 ppm as doublet. The CH₂ protons of the ethyl group attached to the phenyl ring in **4a** appear as a quartet at 2.61 ppm. This relatively downfield chemical shift results from de-shielding effect of conjugated aromatic ring. Whereas, the CH₃ protons of the ethyl group display a triplet at 1.18 ppm, these protons are relatively shielded as they are far from the conjugated aromatic

ring. The total integration value of protons was found to be 19, which is in accordance with the total number of protons present in the structure of **4a** (Figure 3.5).

In addition, the ^{13}C NMR spectrum of **4a** shows a peak at 195.1 ppm, corresponding to the carbonyl carbon of the acyl group, this highly downfield chemical shift is due to the deshielding effect of the carbonyl oxygen. Another peak at 168.0 ppm is assigned to the amide carbonyl carbon of the isoindolin-1-one ring, which appears comparatively upfield relative to the acyl carbonyl carbon. The carbon atom attached to the hydroxyl group shows a chemical shift value at 91.7 ppm. This downfield chemical shift is due to deshielding effect of the directly attached electronegative oxygen atom. The aliphatic CH_2 and CH_3 carbons of the ethyl group attached to the phenyl ring show chemical shift values at 29.0 and 14.8 ppm, respectively. The CH_2 carbon appears relatively downfield due to the deshielding nature of the adjacent aromatic ring and the conjugated benzoyl system, while CH_3 carbon is relatively shielded as it is far from the aromatic and conjugated benzoyl system. The remaining aromatic carbons of phenyl rings exhibit chemical shift values in the range of 151.9-122.8 ppm (Figure 3.6).

In the HRMS spectrum of **4a**, the peak at m/z 380.1270 corresponds to $[\text{M}+\text{H}]^+$ which is matched upto 2 decimal places with the calculated $[\text{M}+\text{H}]^+$ value of **4a** (380.1263) (Figure 3.7). Similarly, the characterization of other derivatives of 3-acyl-3-hydroxyisoindolin-1-ones is done (Experimental Section 3.2.4).

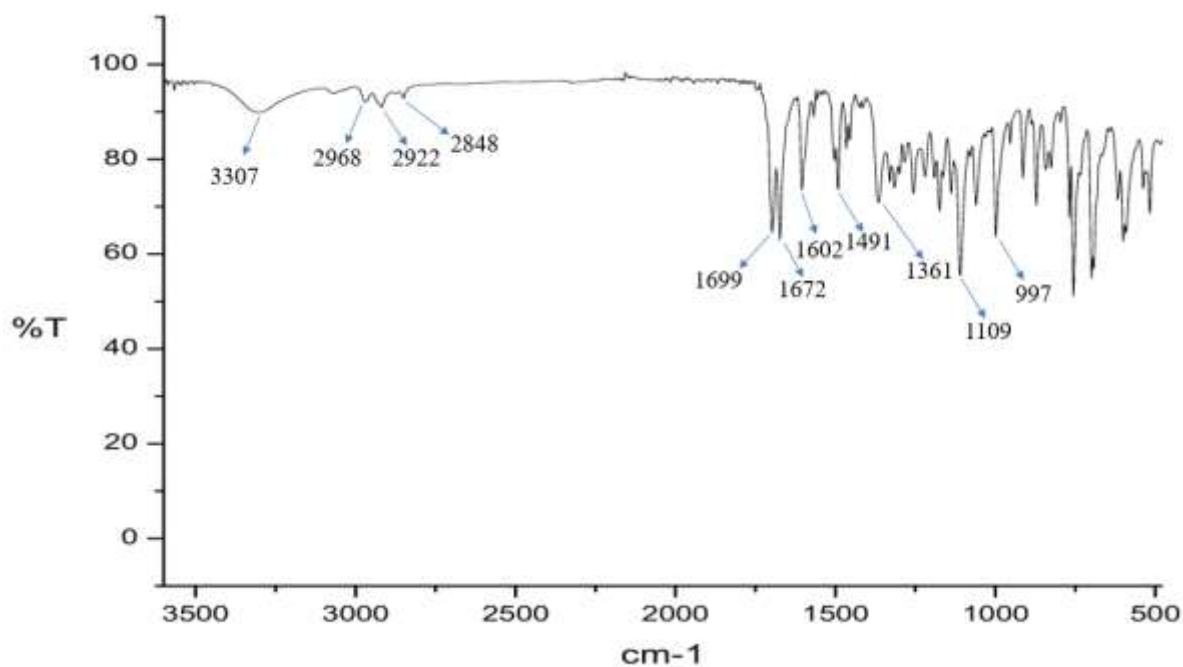
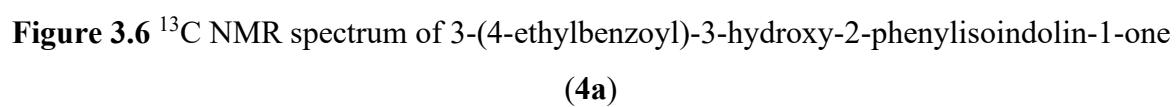
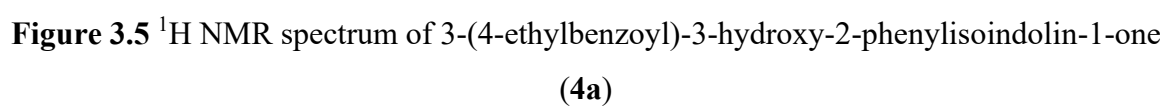
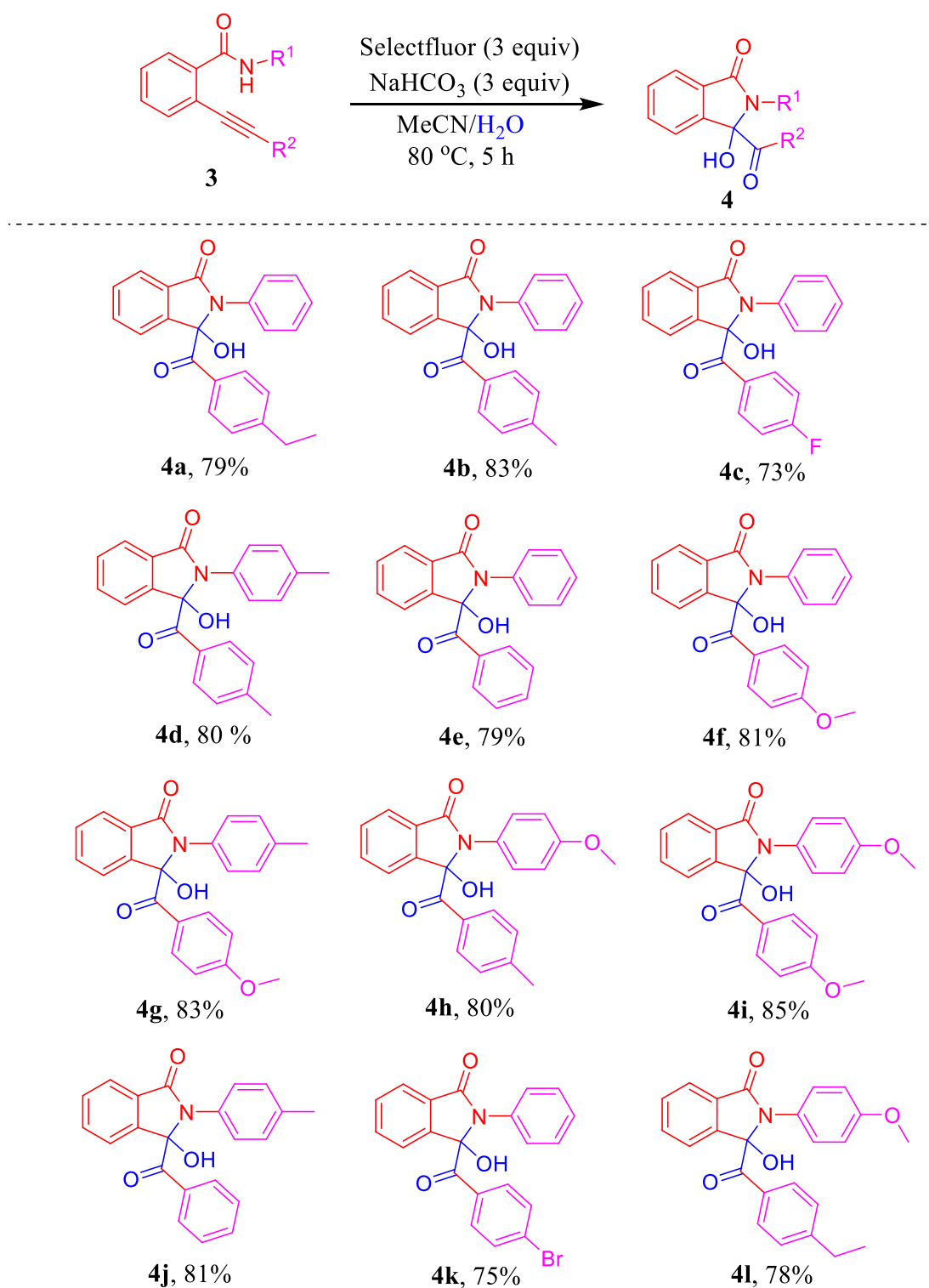


Figure 3.4 FT-IR spectrum of 3-(4-ethylbenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (**4a**)





Scheme 3.5 Derivatives of 3-acyl-3-hydroxyisoindolin-1-ones **4**

To understand the mechanism of the synthetic pathway, control experiments have been performed (**Scheme 3.6**). Performing the reaction of 2-((4-methoxyphenyl)ethynyl)-N-phenylbenzamide (**3f**) in molecular sieves 4Å (dry conditions) did not give the product 3-hydroxy-3-(4-methoxybenzoyl)-2-phenylisoindolin-1-one (**4f**) (**3f** remained intact), which

confirmed that the source of oxygen and hydrogen atoms for oxidation and hydroxylation were from H₂O.

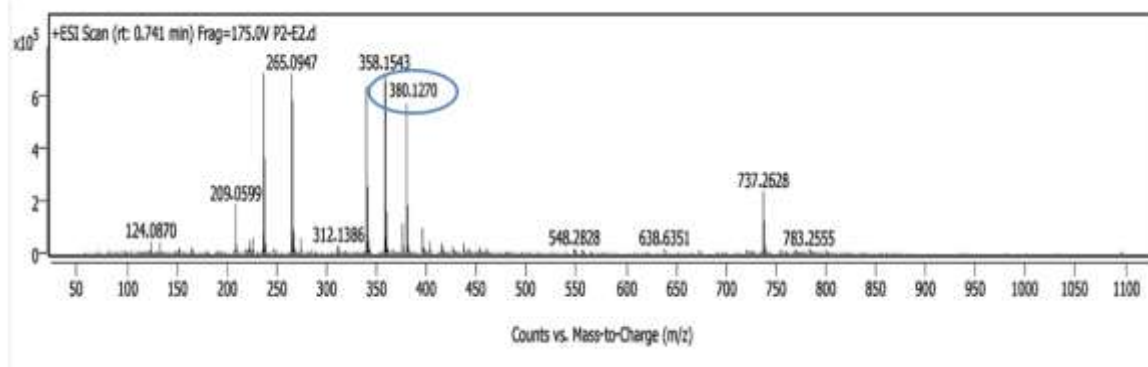
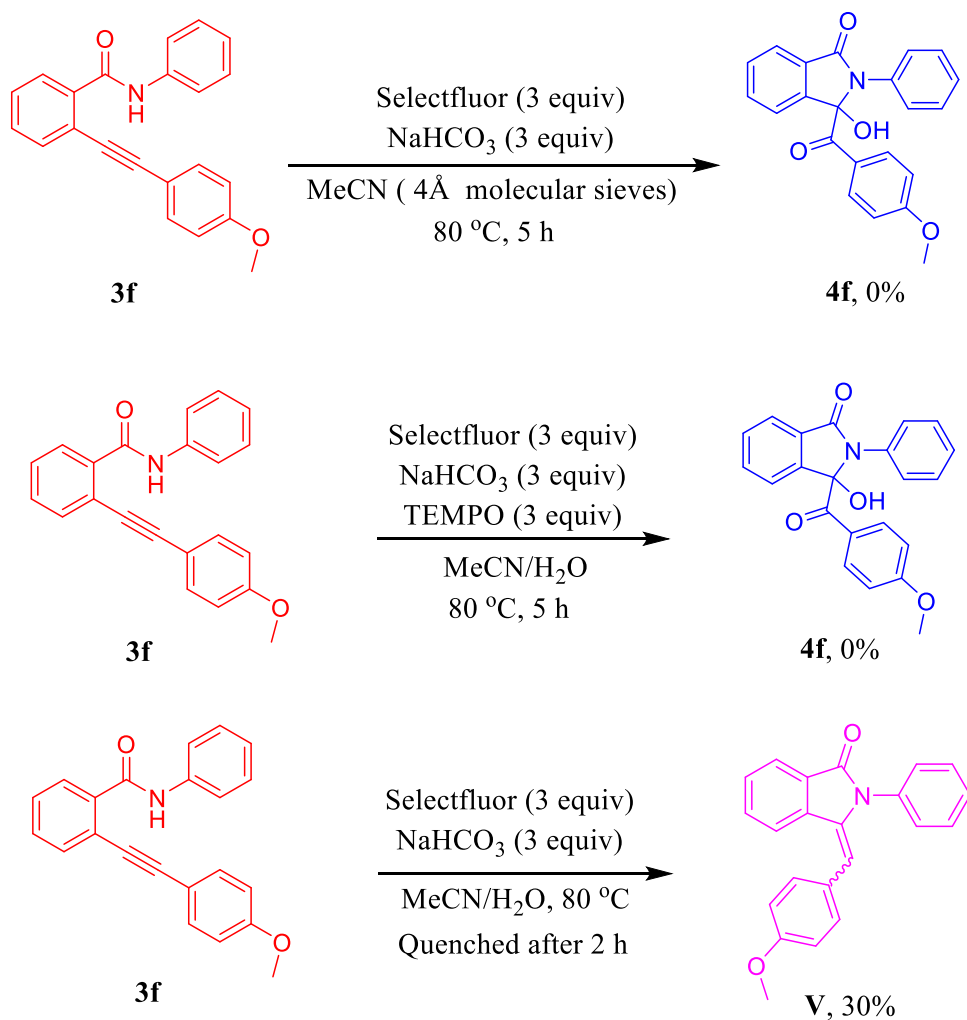
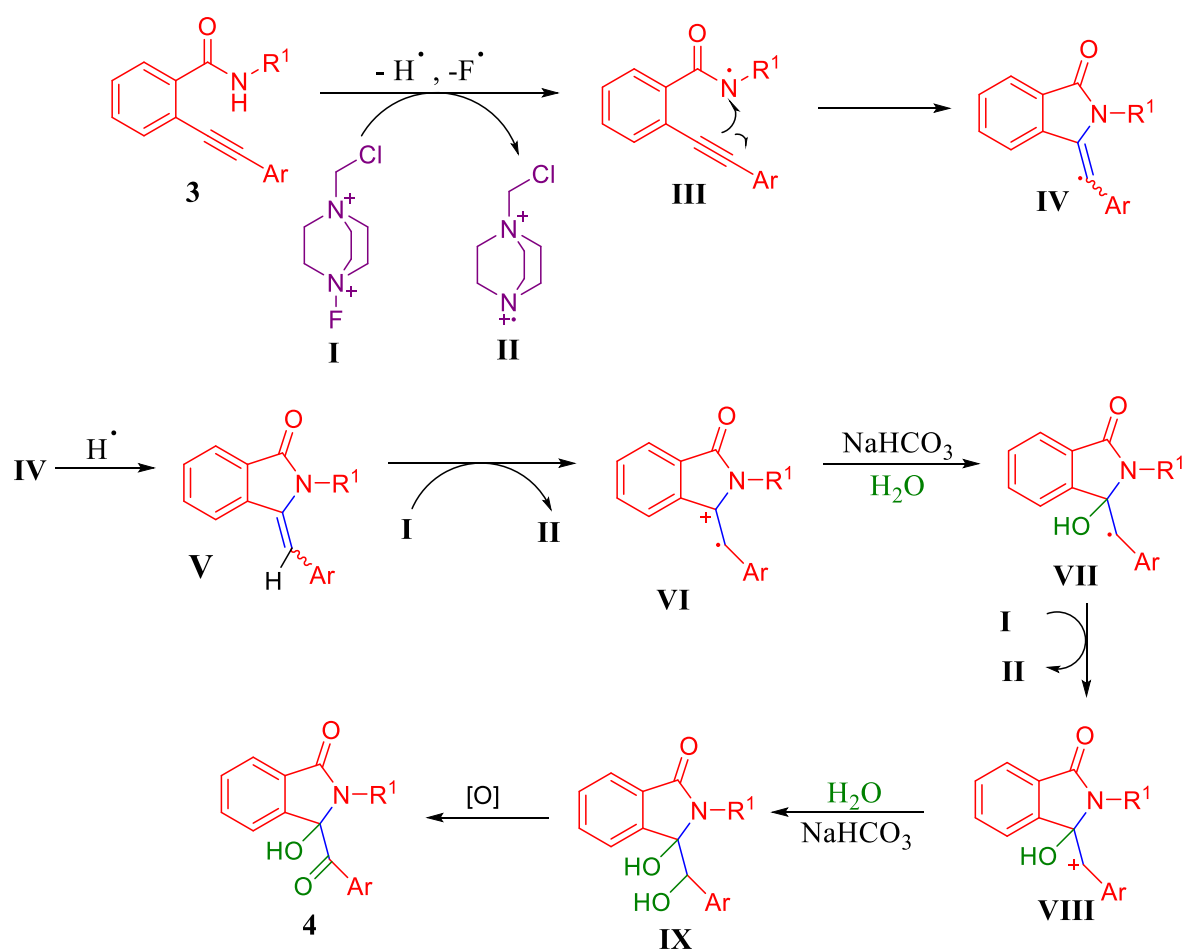


Figure 3.7 HRMS spectrum of 3-(4-ethylbenzoyl)-3-hydroxy-2-phenylisoindolin-1-one (**4a**)



Scheme 3.6 Control experiments of 2-((4-methoxyphenyl)ethynyl)-N-phenylbenzamide (**3f**)

Additionally, to find whether the reaction followed free radical pathway or ionic pathway, free radical scavenger (TEMPO), was used. In the presence of 3 equiv of TEMPO, product **4f** was not formed with starting material remained intact which indicated that this new synthetic pathway included the formation of free radicals. Intermediate **V** (3-(4-methoxybenzylidene)-2-phenylisoindolin-1-one) was isolated by quenching the reaction of **3f** after 2 h with 30 % yield which was confirmed by ^1H and ^{13}C NMR spectroscopy (**Scheme 3.6**). Based on these control experiments and reported literatures [23,35-37], reaction mechanism was proposed (**Scheme 3.7**).



Scheme 3.7 Proposed reaction mechanism for the synthesis of 3-acyl-3-hydroxy isoindolin-1-ones (**4**)

Initially, 2-(1-alkynyl)benzamide **3** reacted with Selectfluor **I** to generate radical cation intermediate **II** and N-radical intermediate **III** with the release of hydride radical and fluorine radical. **III** through homolytic cleavage converted to N-cyclized radical intermediate **IV**. Furthermore, hydride radical combined with **IV** to generate N-cyclized intermediate **V**. Then, **V** was converted to radical cation intermediate **VI** with the help of **I**. Hydroxylation of

VI using H₂O in the presence of NaHCO₃ gave intermediate **VII**. Again, Selectfluor **I** was used for the conversion of **VII** to cation intermediate **VIII**. **VIII** further underwent hydroxylation in the presence of H₂O to generate intermediate **IX**. Finally, **IX** was converted to 3-acyl-3-hydroxyisoindolin-1-one product **4** using NaHCO₃.

3.3.3 Synthesis of gem-difluoro-3-hydroxyisoindolin-1-one

Interestingly, the reaction performed with an aliphatic acetylene substituent in 2-(1-alkynyl)benzamide, under the same reaction conditions, yielded a gem-difluoro-3-hydroxyisoindolin-1-one derivative instead of the expected 3-acyl-3-hydroxyisoindolin-1-one. The reaction of 2-(3,3-dimethylbut-1-yn-1-yl)-N-phenylbenzamide (**3n**) afforded 3-(1,1-difluoro-2,2-dimethylpropyl)-3-hydroxy-2-phenylisoindolin-1-one (**5a**) with 68% yield. Previously, only Munoz et al. reported the synthesis of gem-difluoro-3-hydroxyisoindolin-1-one compounds using N-substituted-3-benzylideneisoindolin-1-one as the starting materials. They used 3 equiv of selectfluor and 10 equiv of trifluoroacetic acid (TFA) and performed the reaction in a MeCN/H₂O solvent system (**Scheme 3.8**) [38].

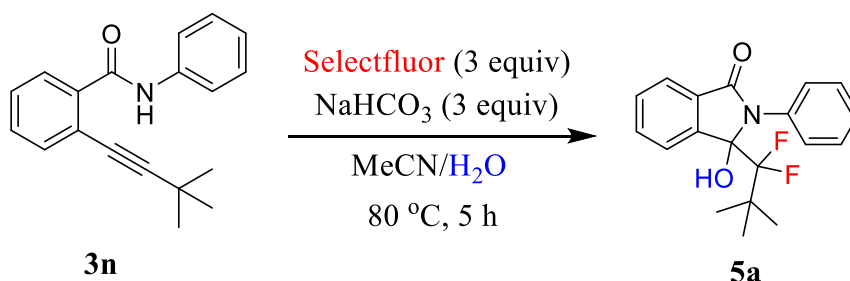
Compound **5a** was characterized using FT-IR, ¹H NMR, ¹³C NMR, ¹⁹F NMR, and HRMS techniques (experimental section 3.2.4). In the ¹H NMR spectrum of **5a**, a broad singlet corresponding to the hydroxyl (O-H) proton which shows at chemical shift value of 4.17 ppm (**Figure 3.8**). The downfield chemical shift of the hydroxyl proton is attributed to the de-shielding effect of the electronegative oxygen atom. The nine protons of the tert-butyl group show chemical shift value at 0.79 ppm, corresponding to the three equivalent methyl groups of tert-butyl. The upfield chemical shift of these methyl protons is attributed to their highly shielded environment as they are attached to an electron-donating alkyl carbon. All the aromatic protons display chemical shift in the range of 7.86-7.29 ppm as multiplets. Furthermore, the total integration value was found to be 19, which matches with the total number of protons present in the structure of **5a**.

In the ¹³C NMR spectrum of **5a**, the signal at 167.8 ppm is due to the amide carbonyl carbon of the isoindolin-1-one ring. This downfield value arises due to the deshielding effect of the carbonyl oxygen. The triplet peak at 92.2 ppm corresponds to the carbon atom attached to hydroxyl group. This downfield chemical shift is due to the deshielding effect of the directly attached electronegative oxygen atom. The quaternary carbon of the tert-butyl group shows triplet peak at 39.0 ppm, whereas the carbon atoms of methyl groups attached to quaternary carbon exhibit peak at 26.0 ppm. The quaternary carbon shows relatively downfield as it is directly attached to the hydroxyl-bearing carbon atom, whereas the methyl

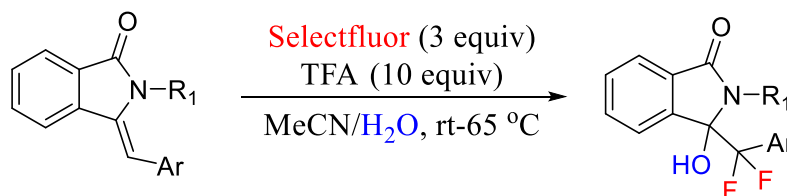
carbons are comparatively shielded due to their electron-rich alkyl environment. All the remaining aromatic carbons exhibit chemical shift values in the range of 144.1-117.2 ppm (**Figure 3.9**). Furthermore, the incorporation of two fluorine atoms in **5a** was confirmed by ^{19}F NMR spectroscopy. The ^{19}F NMR spectrum shows two doublets at -106.23 ppm and -111.61 ppm, showing less difference in both chemical shift values, thus, indicating that both fluorine atoms are attached to the same carbon atom, confirming that **5a** is a gem-difluorinated compound (**Figure 3.10**).

Proposed mechanism for gem-difluoro-3-hydroxyisoindolin-1-one compound (**5a**) is shown in **Scheme 3.9**. Initially, 2-(3,3-dimethylbut-1-yn-1-yl)-N-phenylbenzamide **3n** reacted with Selectfluor **I** to generate radical cation intermediate **II** and N-radical intermediate **X** with the removal of fluorine radicals. Intermediate **X** was converted to intermediate **XI** through homolytic cleavage. Then, two fluorine radicals combined with **XI** to generate radical intermediate **XII** which was converted to cation intermediate **XIII** with the help of Selectfluor **I**. Finally, intermediate **XIII** underwent hydroxylation using H_2O in the presence of NaHCO_3 to give final 3-(1,1-difluoro-2,2-dimethylpropyl)-3-hydroxy-2-phenylisoindolin-1-one product (**5a**).

Our Work:



Munoz et al. Work:



Scheme 3.8 Reaction of 2-(3,3-dimethylbut-1-yn-1-yl)-N-phenylbenzamide (**3n**) to give 3-(1,1-difluoro-2,2-dimethylpropyl)-3-hydroxy-2-phenylisoindolin-1-one (**5a**)

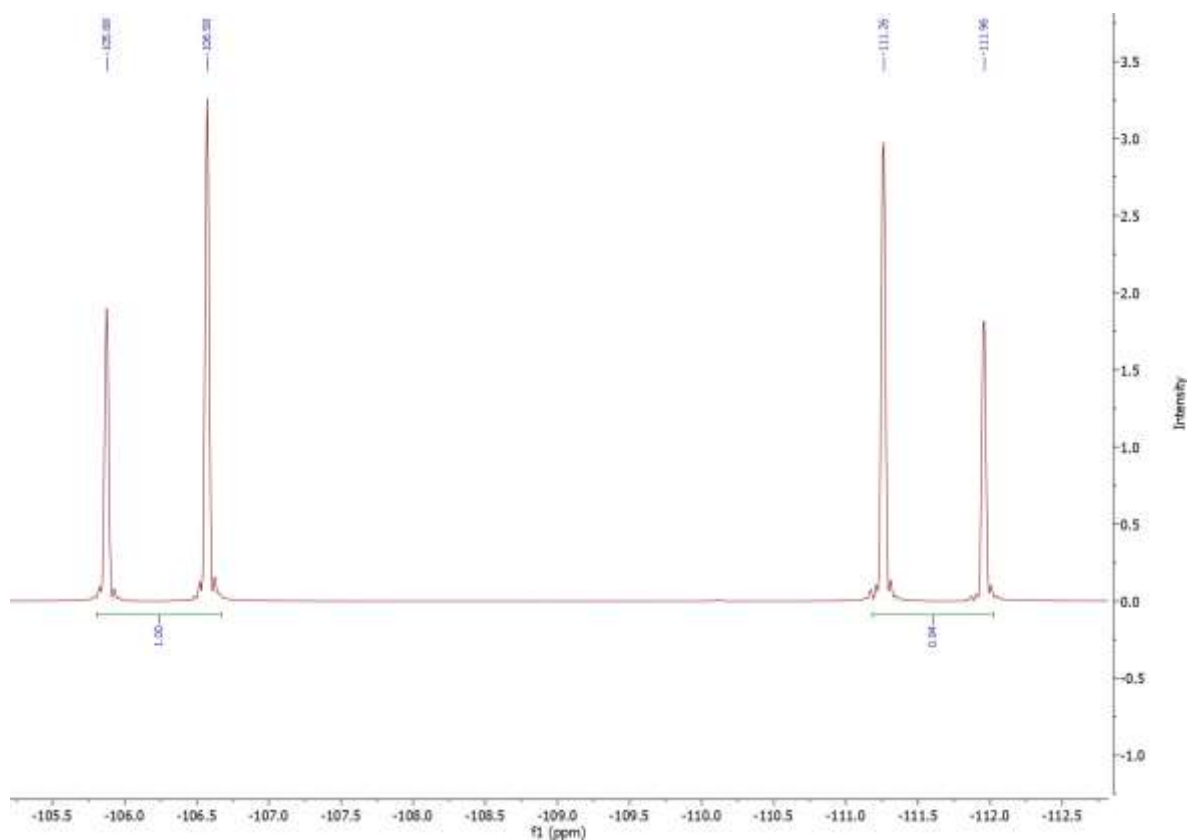
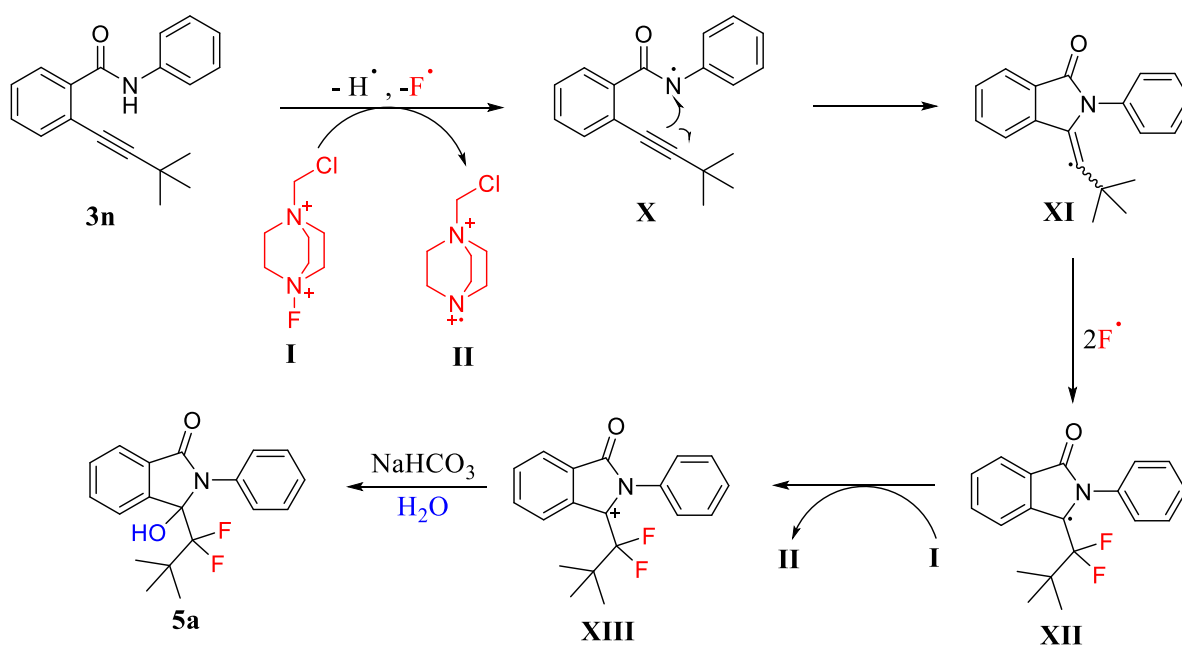


Figure 3.10 ^{19}F NMR spectrum of 3-(1,1-difluoro-2,2-dimethylpropyl)-3-hydroxy-2-phenylisoindolin-1-one (**5a**)



Scheme 3.9 Proposed reaction mechanism for the synthesis of difluoro-3-hydroxyisoindolin-1-one (**5a**)

3.3.4 Antioxidant studies of synthesized final products

The antioxidant activities of all the synthesized final compounds **4,5** were evaluated using the DPPH radical scavenging assay. DPPH is a stable free radical. In the presence of an antioxidant, DPPH becomes paired off in the presence of hydrogen donor and thus reduced to the DPPH-H. As a result, the dark violet color of DPPH changes to yellow color upon conversion to DPPH-H, leading to a decrease in the initial absorbance of the DPPH radical. Higher the antioxidant activity, more decrease in absorbance value observed [39]. The antioxidant activities of all the synthesized compounds **4a-4l** and **5a** fall within the range of 4.68-26.37 %, whereas gallic acid (reference standard) shows an antioxidant activity of 94.4 % (Table 3.2).

Table 3.2 Percentage of anti-oxidant activity, AA (%) of all the synthesized compounds with gallic acid as reference standard

Compound No.	Percentage of anti-oxidant activity, AA (%)
4a	16.90
4b	4.68
4c	22.23
4d	9.12
4e	10.18
4f	11.57
4g	12.05
4h	18.00
4i	14.19
4j	26.37
4k	18.15
4l	21.02
5a	21.73
Gallic Acid	94.4

Compounds **4c** and **5a** containing electron-withdrawing fluorine groups display antioxidant activity more than 20%. Additionally, some compounds with electron-donating groups

exhibit antioxidant activity above 20%. Overall, these compounds did not demonstrate significant antioxidant activity.

3.4 Conclusion

In conclusion, a new synthetic route for the regioselective synthesis of 3-acyl-3-hydroxyisoindolin-1-ones from N-substituted-2-(1-alkynyl)benzamides mediated by Selectfluor was developed. Water was used as the source for oxidation and hydroxylation with sodium bicarbonate used as a mild base. All the derivatives of 3-acyl-3-hydroxyisoindolin-1-ones were synthesized with 73-85% yield. Interesting result was obtained with aliphatic acetylene as the substituent in 2-(1-alkynyl)benzamide which afforded gem-difluoro-3-hydroxyisoindolin-1-one derivative instead of the expected 3-acyl-3-hydroxyisoindolin-1-one. Control experiments revealed that this new synthetic pathway followed free radical pathway. A plausible mechanism was made on the basis of control experiments and reported literature. In addition, antioxidant studies of these compounds did not show considerable results.

3.5 References

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CHAPTER 4

In-silico studies of N-alkenylindole
derivatives as potential MAO-B
inhibitors

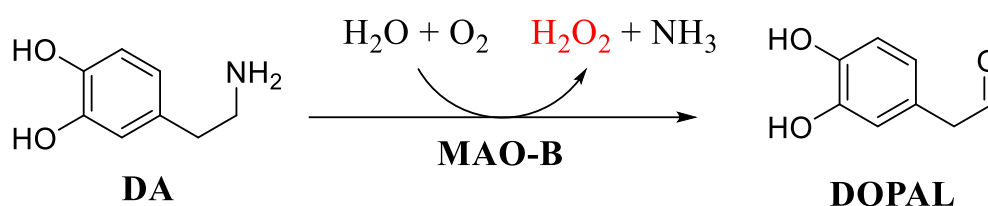
4.1 Introduction

Indoles display an important class of biologically active heterocyclic compounds. They are part of the nitrogen-containing fused heterocycles consisting of a six-membered benzene ring with a five-membered pyrrole ring containing one nitrogen atom [1]. The lone pair of electrons present on the nitrogen atom of the pyrrole ring participates in the delocalized six pi-electron aromatic system of the ring, thus, adding to the aromaticity and stability of the indole moiety. In addition, the fused benzene ring further increases the resonance stabilization and conjugation of the system. Due to these structural features, indoles show a high electron-rich character. They act as important scaffolds in biological and medicinal chemistry because of their structural and electronic features [2].

Indoles exhibited several biological properties, such as anti-cancer [3,4], anti-viral [5,6], anti-bacterial [7,8], against neurodegenerative diseases [9,10], anti-inflammatory [11,12], anti-diabetic [13,14], and anti-fungal [15,16]. Lurbinectedin, Osimertinib, Etrasimod, and Flortaucipir F-18 are examples of some FDA-approved drugs containing this moiety [17,18]. Their presence in the natural products also make them significant molecules [19-21]. Thus, there has been a continuous interest in the synthesis of indole substituted compounds. Previously, several researchers reported the synthesis of different indole moieties through acid and base-promoted, metal-catalyzed, photo- and electro-catalyzed reactions [22]. When going through the literature, it was found that N-alkenylindoles have been synthesized through cyclization reaction using different metals [23-25]. However, *in-silico* studies on these N-alkenylindole ligands on neurodegenerative diseases have not been reported to date. This gives an opportunity to perform *in-silico* investigations on these ligands.

Neurodegenerative diseases are the abnormalities and disorders of the brain that impair mental and physical functioning by causing a gradual and selective loss of neurons [26]. Parkinson's disease (PD) is one of the neurodegenerative diseases, which is associated with motor dysfunction [27]. The pathophysiology of PD suggests the progressive degeneration of neurotransmitter dopamine producing neurons [28]. Dopamine (DA) levels in the striatum decrease due to the loss of dopaminergic neurons in the *substantia nigra*. Other non-motor symptoms of PD are depression, cognitive decline, and sleep disorders [29]. MAO is one of the targets to treat PD [30]. Monoamine oxidase (MAO, EC 1.4.3.4, amine-oxygen oxidoreductase) is a flavoenzyme that contains flavin adenine dinucleotide (FAD), plays a key role in the oxidative deamination of xenobiotic amines, monoamine neurotransmitters, and hormones that consist of dopamine, serotonin, and norepinephrine in the brain and other

tissues [31,32]. MAO exist in two isoforms: MAO-A and MAO-B. Both isoforms differ in their substrate specificity and inhibitor selectivity [33]. High levels of MAO-B are mainly seen in the 50-60 age group of people [34]. Overexpression levels of the MAO-B isoform are responsible for promoting oxidative stress in the neurosystem due to the generation of hydrogen peroxide (H_2O_2), which is produced from a deamination reaction of DA catalyzed by **MAO-B** (**Scheme 4.1**) [35]. Thus, there is need for the development of effective and selective MAO-B inhibitors for the treatment of PD.



Scheme 4.1 MAO-B-catalyzed deamination reaction of dopamine (DA).

In this chapter, we performed *in-silico* studies of N-alkenylindole ligands to evaluate their inhibitory activity against MAO-B. Initially, a library of 147 N-alkenylindole molecules was made and molecular docking of the complete library with the MAO-B protein target was performed. Validation of the docking protocol was performed with the help of the co-crystal ligand of the MAO-B protein. Further, the top two docked scored ligands were investigated using other *in-silico* tools which were molecular dynamics simulation (MDS), Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) binding free energy, Absorption, Distribution, Metabolism, and Excretion (ADME), and Density Functional Theory (DFT).

4.2 Materials and Methods

4.2.1 Molecular Docking

For molecular docking, the same 3-D crystal structure of the MAO-B protein (PDB ID: 6FWC) used in Chapter 2 was taken. Molecular docking was done with the help of AutoDock Vina [36]. For protein preparation, firstly, hetatoms, water molecules, and the co-crystal ligand of MAO-B were removed using BIOVIA Discovery Studio [37]. Subsequently, missing atoms of amino acid residues, polar hydrogens, and Kollman charges were added using AutoDockTools-1.5.7 software [38], and the protein was saved in .pdbqt format.

All the 2-D structures of *N*-alkenylindoles, including the co-crystal ligand of MAO-B and known inhibitors of MAO-B, were drawn using Chemdraw Professional 15.0 [39]. Their SMILES were converted into 3-D geometry optimized structures using Open Babel 2.4.1 software [40]. The MMFF94 force field was applied during the conversion, and ligand files were saved in .pdb format. These .pdb files were then converted into .pdbqt format using the same Open Babel software. The grid box coordinates were set to $x = 50.739$, $y = 155.602$, and $z = 27.372$ with a grid box size of 40 Å in the x, y, and z directions. The exhaustiveness value was set as default (8). After completion of the molecular docking procedure, the binding affinities or we can say that docking scores of all the ligands were analyzed. Furthermore, protein-ligand binding interactions were visualized using BIOVIA Discovery Studio.

Validation of the molecular docking protocol is essential to ensure its accuracy and reliability. To validate the docking protocol, the co-crystal ligand of the MAO-B protein was extracted and redocked using the same grid box and docking parameters, that means, with the same docking procedure as applied for *N*-alkenylindoles. The crystalline pose and the redocked pose were then superimposed, and the root mean square deviation (RMSD) between both the poses was estimated.

4.2.2 Molecular Dynamics Simulation (MDS)

Methodology for MDS is same as in **Chapter 2 (Section 2.2.2)**

4.2.3 MM-GBSA Binding Free Energy Analysis

Methodology for MM-GBSA binding free energy analysis is same as in **Chapter 2 (Section 2.2.3)**

4.2.4 ADME Studies

Absorption, Distribution, Metabolism, and Excretion (ADME) studies is one of the most critical tasks during the early phases of drug discovery [41]. The SMILES of the top two docked-scored ligands were used for ADME analysis using the SwissADME online web server [42]. These ADME studies predicted the drug-likeness, physicochemical properties, and pharmacokinetics profiles of the ligands.

4.2.5 DFT Studies

Density Functional Theory (DFT) studies of the top two docked-scored ligands were performed using ORCA software [43]. Avogadro software [44] was used to prepare the input files for the molecules. Firstly, the ligands were geometry optimized using the B3LYP method with the def2-TZVP basis set. Subsequently, the geometry-optimized ligands were estimated for their frontier molecular orbitals (HOMO-LUMO) and MESP studies. Visualization of the HOMO, LUMO, and MESP was performed using Avagadro and VESTA softwares [45].

4.3 Results and Discussion

The expected outcome of this study is to identify N-alkenylindole ligands with high predicted binding affinity and selectivity toward MAO-B based on molecular docking studies. Furthermore, MDS and MM-GBSA binding energy studies provide insights into the stability of the docked complexes. Moreover, ADME studies give insight into the pharmacokinetic and drug-likeness profiles of the ligands. Whereas, DFT studies reveal the chemical reactivity and electronic properties of the ligands. Therefore, these studies are anticipated to provide computational insights into their interaction with the MAO-B enzyme and to identify promising candidates for future experimental validation, thereby supporting the development of safer and more effective MAO-B inhibitors for the treatment of PD. **Figure 4.1** displayed the sequential pictorial representation of the methodology adopted for the *in-silico* studies.

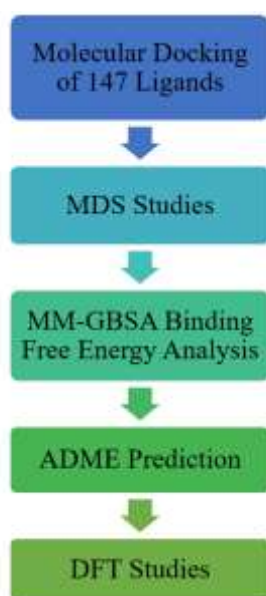


Figure 4.1 Pictorial representation of the full methodology adopted for the *in-silico* studies of N-alkenylindoles

4.3.1 Molecular Docking

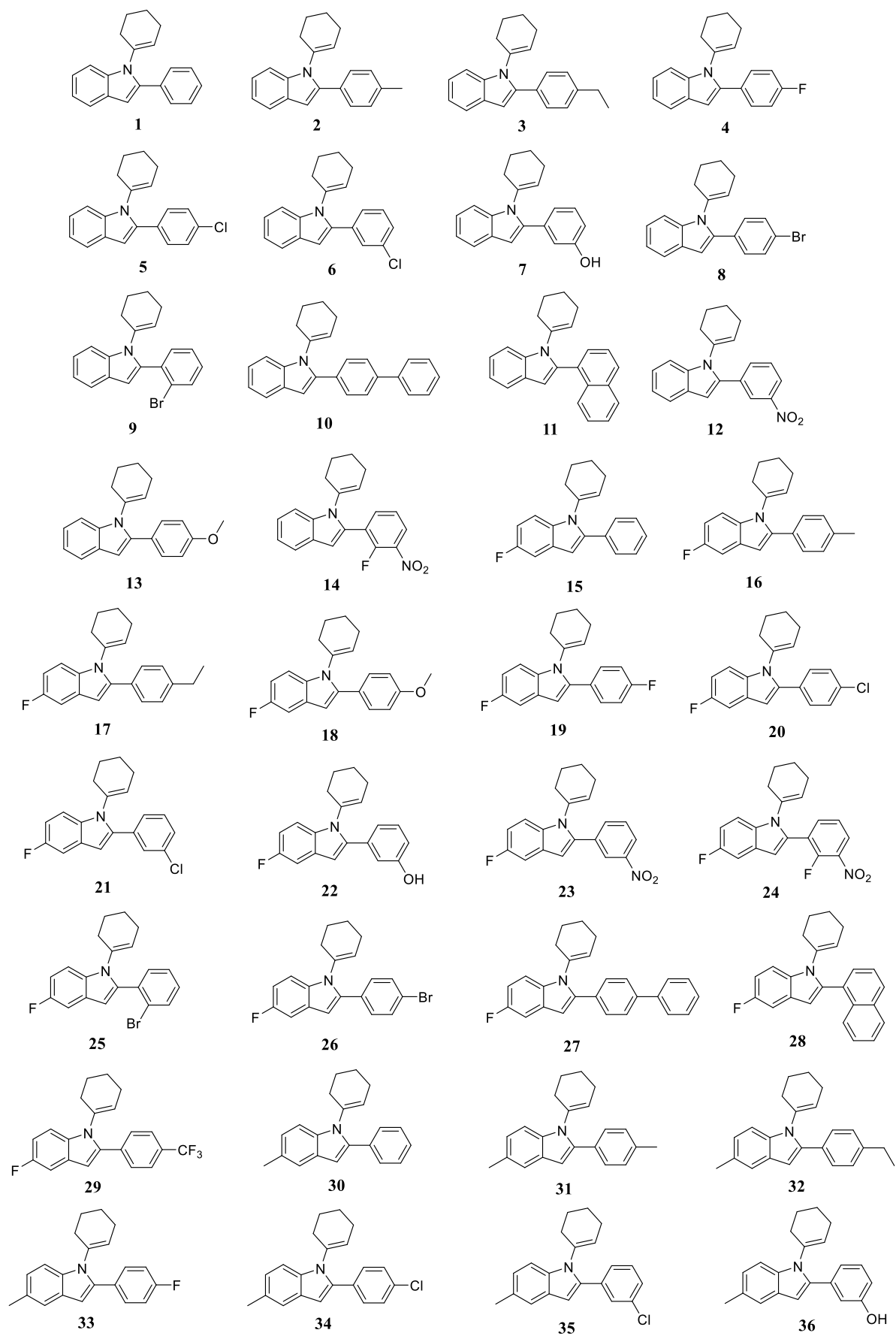
The molecular docking technique is widely used in drug discovery. This provides information regarding how the ligand fits into the protein's binding site by exploring several orientations and positions of the ligand, identifying the best binding pose, and predicting the binding affinity of the ligand with the protein [46]. In this chapter, for molecular docking, MAO-B was selected as the target protein for its crucial role in the pathophysiology of neurotransmitters like dopamine, which is highly relevant to PD. Inhibiting MAO-B can enhance dopamine levels in the brain, thereby providing a therapeutic effect, which makes it a significant drug target. Additionally, MAO-B has a well-characterized active site with available high-resolution crystal structures, facilitating accurate molecular docking and interaction analysis [47]. By focusing on MAO-B, this study aims to identify ligands with strong binding affinity and favorable interactions, supporting the rational design of novel inhibitors with potential neuroprotective properties.

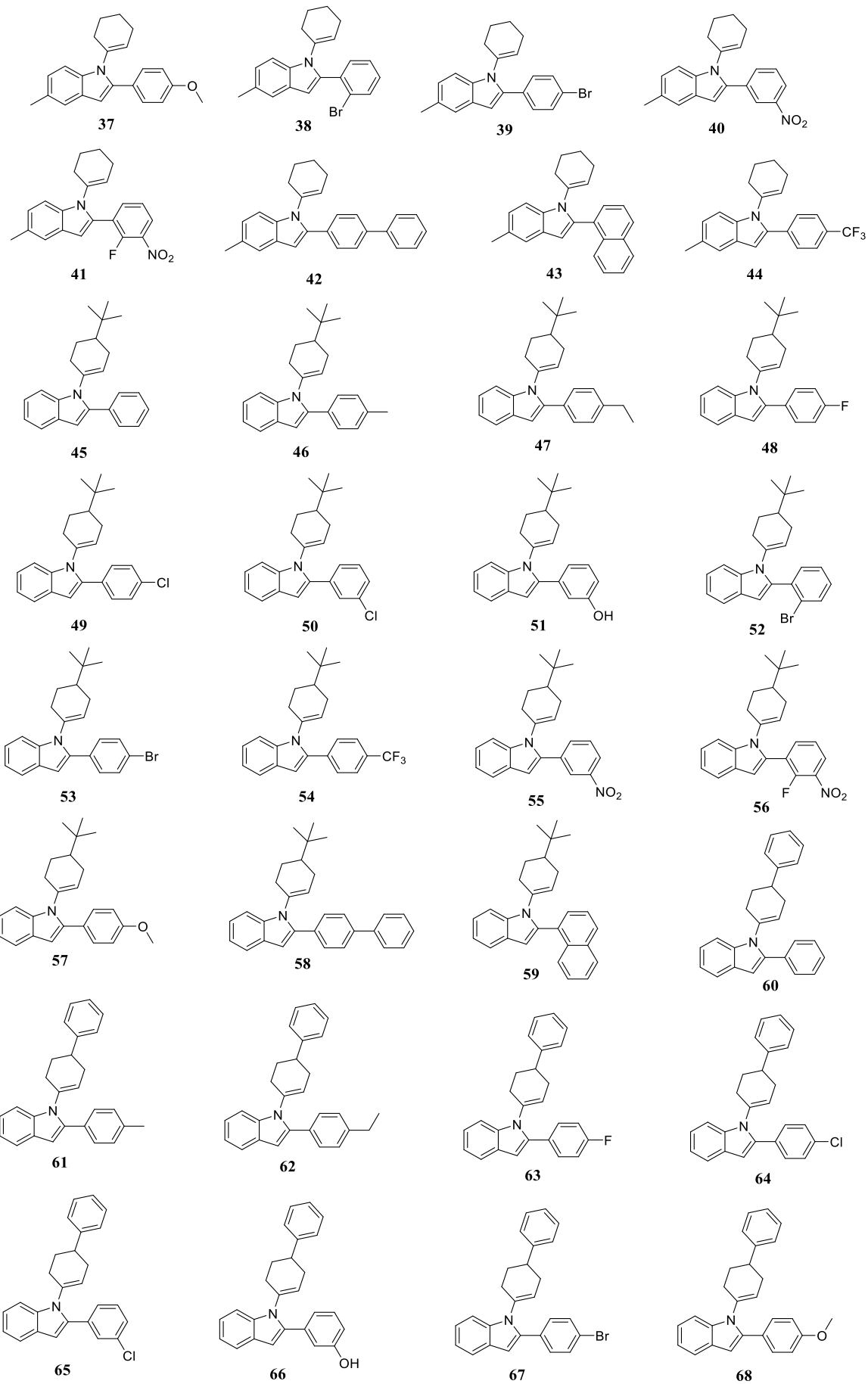
Effective drug design requires that the three-dimensional structure of the target protein be accurate, high-quality, and reliable. The chosen protein target, MAO-B (PDB ID:6FWC), was previously validated for its structural accuracy and reliability in **Chapter 2 (Section 2.3.1)** using a Ramachandran plot generated with the PROCHECK online web server. The structures of all the N-alkenylindole ligands on which the docking was performed is shown in **Figure 4.2**. Binding affinities between protein and ligands were estimated quantitatively using docking scores (binding scores). **Table 4.1** showed the ligand numbers, and docking scores of all the N-alkenylindole ligands, including co-crystal ligand, safinamide, rasagiline, and saligiline. This has been studied that the more negative the docking score, the higher the binding affinity between protein and ligand [48]. N-alkenylindole ligands exhibited docking scores in the range of -8.0 to -11.8 kcal/mol. The results showed that 21 ligands displayed docking scores higher than the co-crystal ligand of MAO-B protein (-10.6 kcal/mol). Out of 147 docked ligands, the ligands **66** and **63** showed superior docking scores of -11.8 kcal/mol and -11.4 kcal/mol, respectively. The known inhibitors rasagiline, selegiline, and safinamide displayed docking scores of -7.8, -6.9, and -10.0 kcal/mol, respectively. This indicated that ligands **66** and **63** exhibit strong potential as promising lead candidates to inhibit MAO-B compared to the co-crystal ligand and known inhibitors.

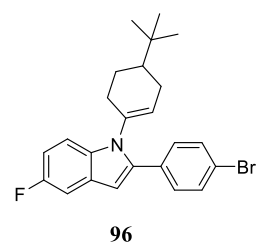
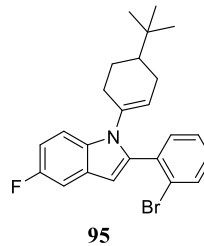
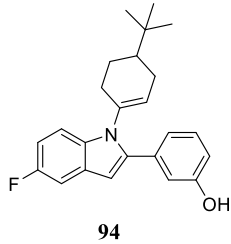
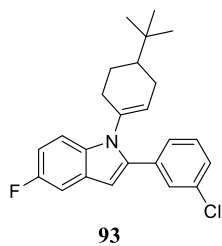
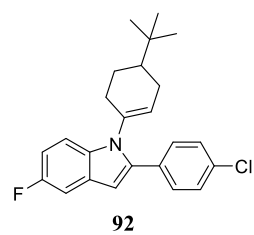
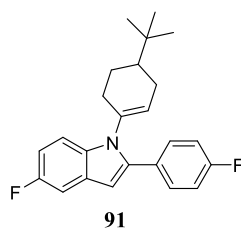
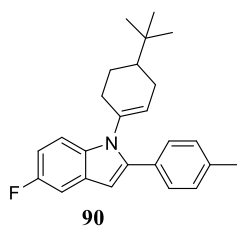
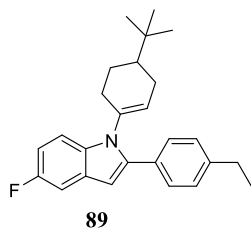
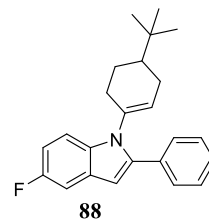
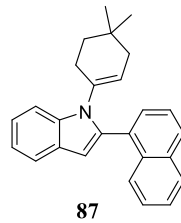
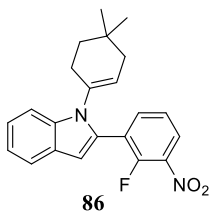
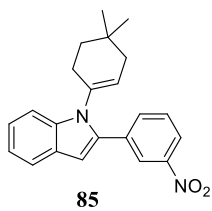
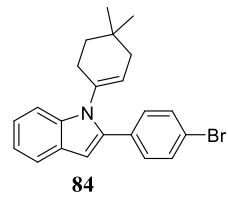
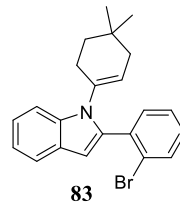
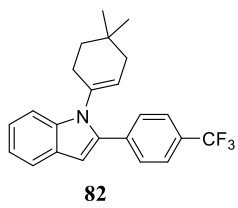
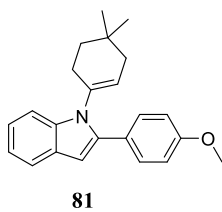
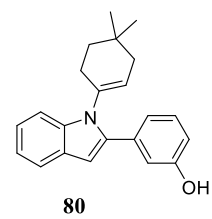
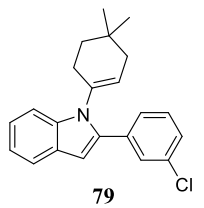
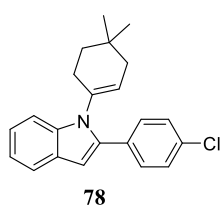
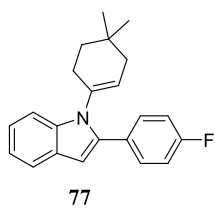
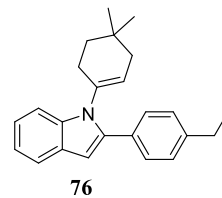
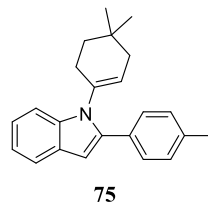
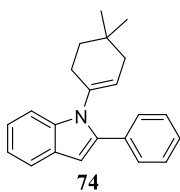
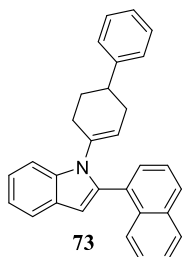
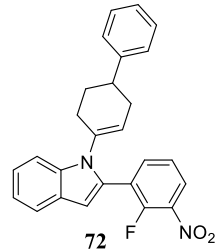
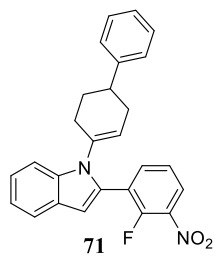
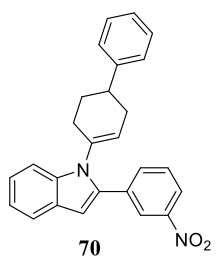
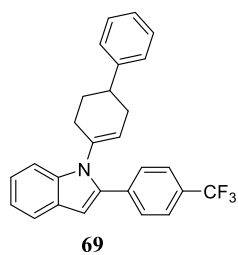
Furthermore, binding interactions between ligands **66** and **63** and the MAO-B protein have been investigated. **Figures 4.3** and **4.4** displays the 2-D and 3-D binding interactions of these ligands, **66** and **63**, respectively, with MAO-B. Conventional hydrogen bonding

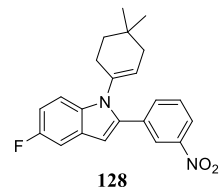
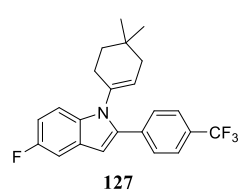
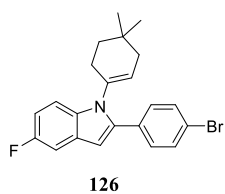
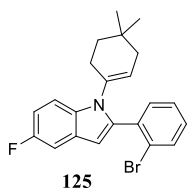
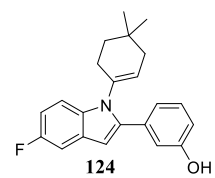
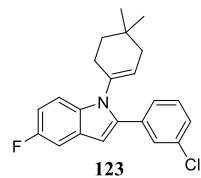
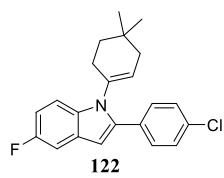
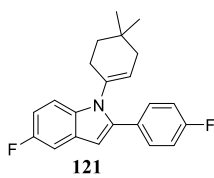
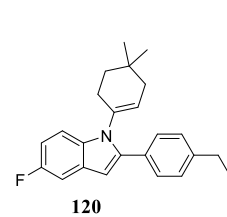
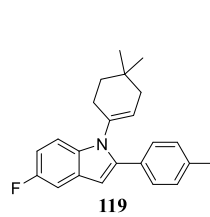
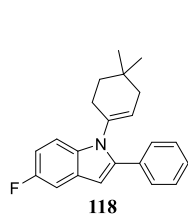
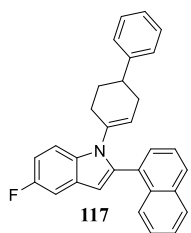
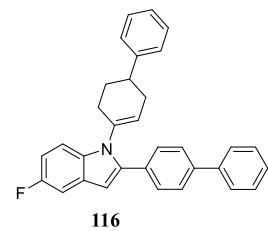
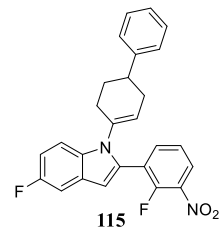
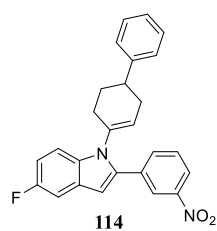
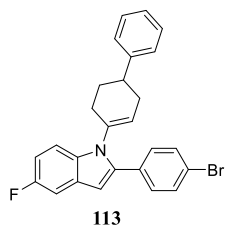
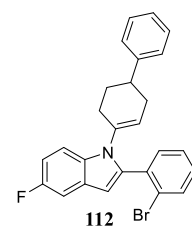
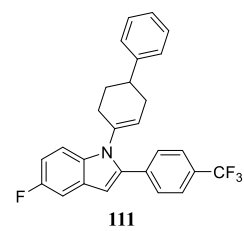
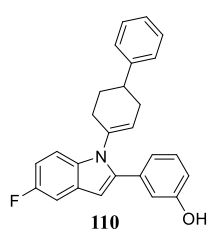
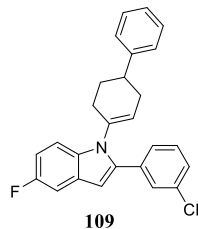
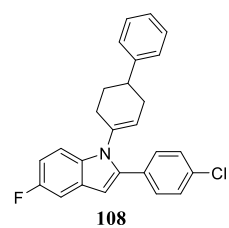
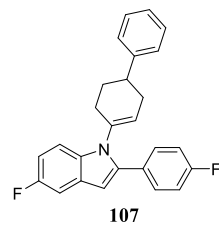
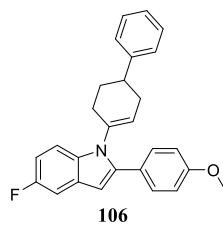
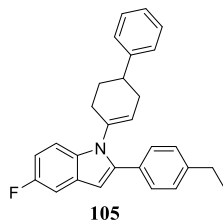
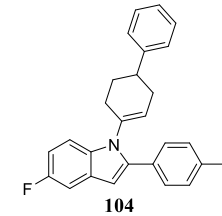
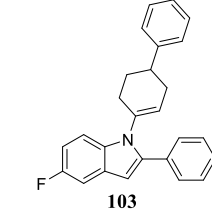
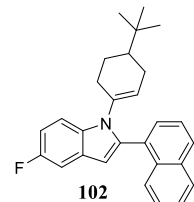
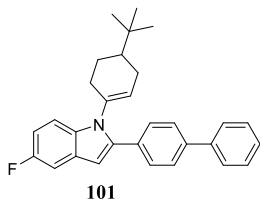
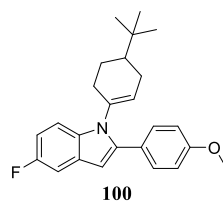
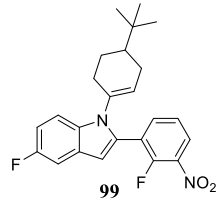
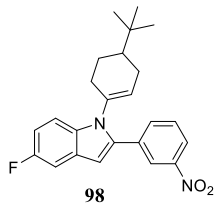
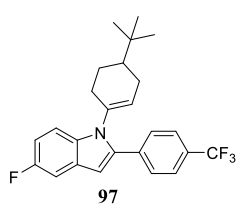
interactions are shown in green color. It was found that hydrogen atom of hydroxyl group of ligand **66** showed hydrogen bonding with Gly434 amino acid residue of MAO-B and oxygen atom of hydroxyl group exhibited hydrogen bonding with Arg42 residue. Other binding interactions of **66** which included pi-pi T shaped, pi-donor hydrogen bond, pi-sulfur, pi-pi stacked, amide-pi stacked, and pi-alkyl shown with Met436, Cys397, Gly57, Gly58, Lys296, Trp388, Val294, Tyr398, Tyr435 residues. In contrary, ligand **63** showed no hydrogen bonding with any residue of MAO-B. Additionally, ligand **63** displayed halogen (fluorine), pi-donor hydrogen bond, pi-sulfur, pi-pi stacked, pi-pi T-shaped, amide-pi stacked, and pi-alkyl interactions with Gly58, Gly57, Trp388, Lys296, Val294, Cys397, Tyr398, Arg42, Tyr435 residues. Thus, the highest docking scores and binding interactions of both ligands **66** and **63** concluded the high and effective binding with the protein. Based on the above results, these two ligands were taken for further *in-silico* studies, which included MDS, MM-GBSA binding free energy, ADME, and DFT studies.

The docking protocol was validated by the superimposition method. The co-crystal ligand of MAO-B protein, {N}-(3-fluorophenyl)-4-oxidanylidene-chromene-3-carboxamide was redocked, and then the redocked pose was superimposed with the crystal pose of the co-crystal ligand of MAO-B protein. **Figure 4.5** displays the superimposition image in which the redocked and crystal poses were highlighted in green and yellow colors, respectively. The RMSD value between both the poses was observed to be 0.3 Å, which was significantly lower than 2 Å. Thus, the superimposition image and very low RMSD value suggested an accurate and efficient docking protocol applied for molecular docking between N-alkenylindole ligands and MAO-B protein.









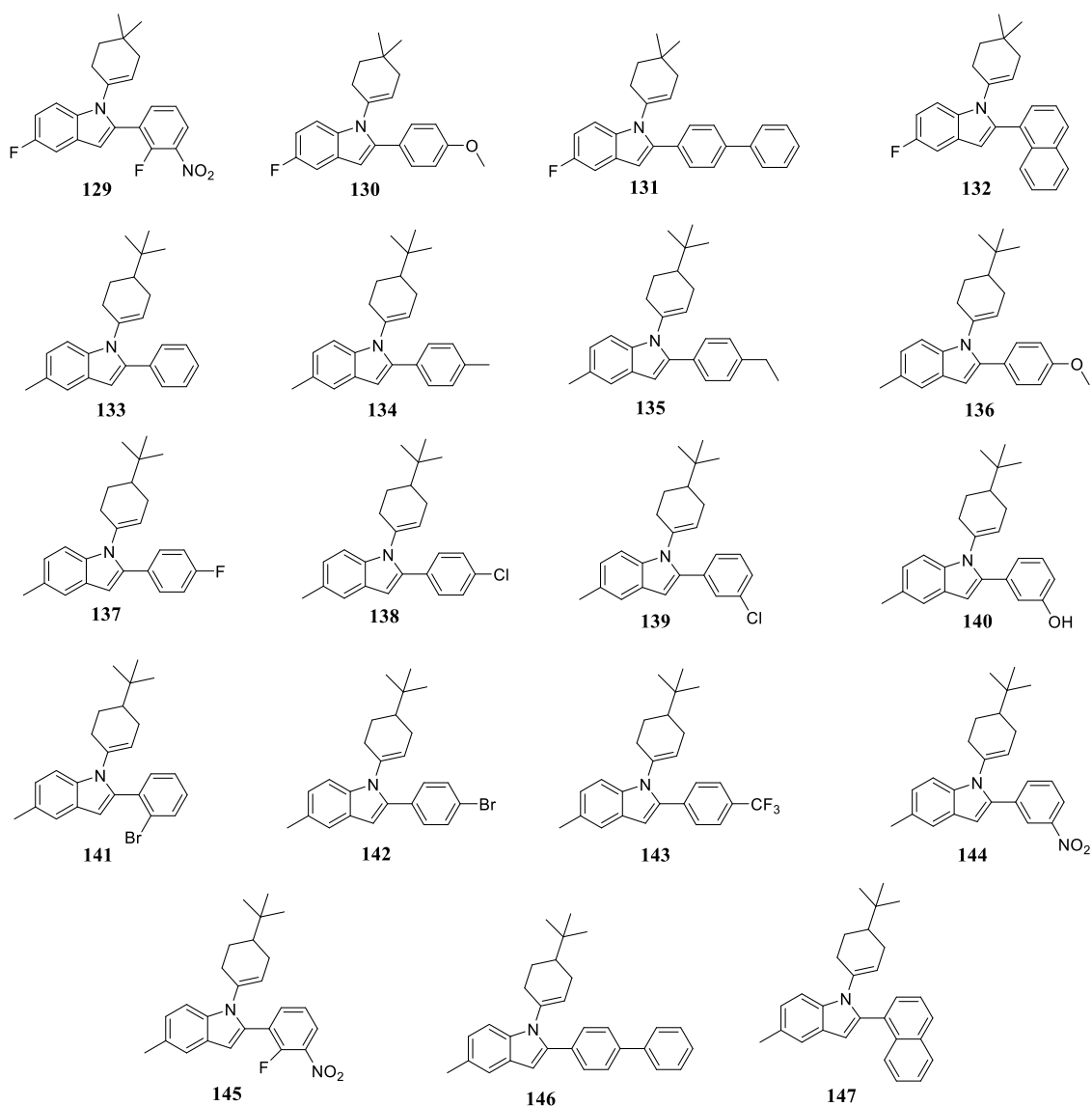


Figure 4.2 Structures of N-alkenylindole derivatives.

Table 4.1 Ligand numbers and docking scores of all the N-alkenylindoles docked with the MAO-B protein

Ligand No.	Docking Score (Kcal/mol)	Ligand No.	Docking Score (Kcal/mol)
1	-8.4	6	-9.6
2	-9.4	7	-10
3	-11.1	8	-10.9
4	-10.6	9	-8.4
5	-10.7	10	-10.2

11	-9.3	43	-10.7
12	-9.9	44	-10.5
13	-10.7	45	-9.1
14	-8.1	46	-9.3
15	-9.5	47	-9.1
16	-9.7	48	-9.1
17	-9.7	49	-9.1
18	-9.3	50	-9.7
19	-10.9	51	-9.5
20	-11	52	-8
21	-9.1	53	-9.2
22	-9.1	54	-10.3
23	-10.3	55	-8.8
24	-9.1	56	-8.4
25	-8.3	57	-8.9
26	-9.6	58	-9.9
27	-10.2	59	-9.9
28	-9.3	60	-10.3
29	-10.3	61	-11.2
30	-9	62	-9.3
31	-9.9	63	-11.4
32	-9.6	64	-11.1
33	-10.1	65	-10.2
34	-9.8	66	-11.8
35	-8.8	67	-11.1
36	-9.3	68	-10.2
37	-9.6	69	-9.3
38	-8.4	70	-10.4
39	-9.9	71	-10.2
40	-9.5	72	-10.7
41	-8.5	73	-9.1
42	-11.1	74	-9.4

75	-9.9	107	-10.8
76	-9.6	108	-9.8
77	-9.9	109	-11.2
78	-9.7	110	-9.5
79	-9.9	111	-10
80	-9.7	112	-9.8
81	-9.7	113	-10.9
82	-10.5	114	-9.7
83	-8.1	115	-10
84	-9.4	116	-11.4
85	-9.2	117	-10.6
86	-9.6	118	-9.1
87	-8.6	119	-10.2
88	-9.8	120	-10.1
89	-9.2	121	-10
90	-9.3	122	-10
91	-9.6	123	-10.1
92	-9.6	124	-9.8
93	-9.7	125	-9
94	-9.2	126	-10.1
95	-9.4	127	-10.7
96	-9.3	128	-9.1
97	-10.2	129	-9.6
98	-9.2	130	-9.6
99	-9.3	131	-10.8
100	-9.1	132	-10.4
101	-10.5	133	-9.7
102	-10.1	134	-9.6
103	-11.1	135	-9.3
104	-9.2	136	-9.3
105	-8.9	137	-9.7
106	-9.4	138	-9.4

139	-9.7	146	-10.4
140	-9.8	147	-10.1
141	-8.7	Co-Crystal Ligand	-10.6
142	-9.3	Rasagiline	-7.8
143	-10.1	Selegiline	-6.9
144	-9.2	Safinamide	-10.0
145	-9.1		

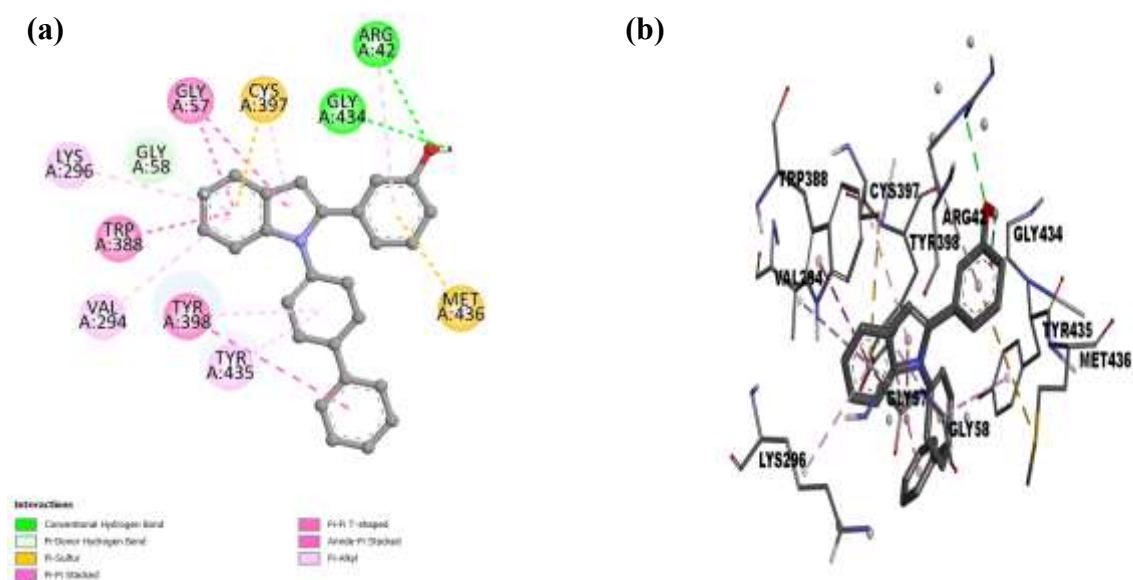


Figure 4.3 (a) 2-D representation of binding interactions of ligand **66** with MAO-B protein
(b) 3-D representation of binding interactions of ligand **66** with MAO-B protein.

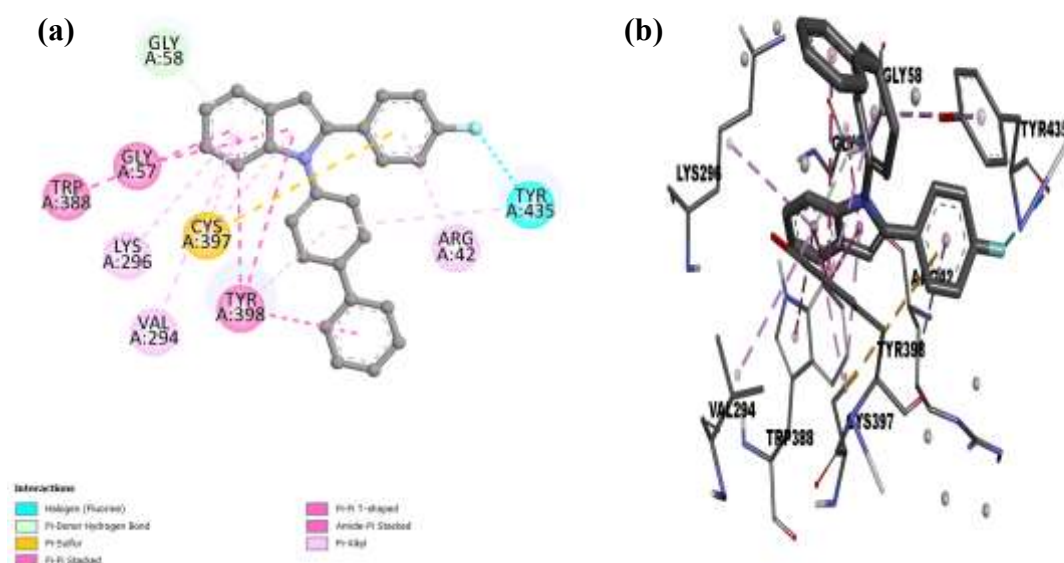


Figure 4.4 (a) 2-D representation of binding interactions of ligand **63** with MAO-B protein
(b) 3-D representation of binding interactions of ligand **63** with MAO-B protein.

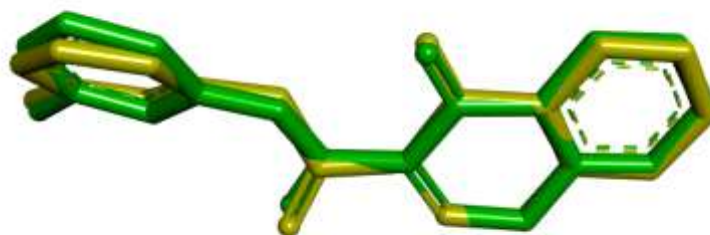


Figure 4.5 Representation of superimposition of redocked pose of co-crystal ligand of MAO-B (shown in green color) with the crystal pose of co-crystal ligand of MAO-B (shown in yellow color).

4.3.2 Molecular Dynamics Simulation (MDS)

The stability of the binding docked complexes of ligands **66** and **63** was investigated with the help of MDS for a complete 500 ns simulation time. The simulation interaction diagram of Desmond was used to analyze the RMSD and RMSF of docked poses of ligands **66** and **63** throughout the complete simulation. In addition, protein-ligand contacts throughout the simulation time were also analyzed.

Root Mean Square Deviation (RMSD) is used to find the stability of the docked pose of the molecule in the binding region of the protein. A high RMSD value leads to less

stability of the docked pose and high chances of diffusion of the docked pose position from its initial binding region, while a low RMSD value results in high stability of the docked pose and less deviation of the docked pose position from the binding pocket [49]. **Figure 4.6** shows the RMSD plot of heavy atoms of ligands **66** and **63** with respect to protein. For ligand **66**, the average RMSD observed for the complete 500 ns simulation trajectory with respect to the protein was 1.83 Å, indicating that it maintained a stable binding conformation within the protein binding pocket. Whereas, Ligand **63** exhibited a higher average heavy atoms RMSD value of 3.77 Å, demonstrating high conformational flexibility and larger positional deviations with respect to its initial binding pose during the simulation time.

Root Mean Square Fluctuation (RMSF) is a measure of fluctuations of the molecule atoms with respect to the protein. It depicts how much atoms of the molecule change their positions during the complete simulation time. A low RMSF value corresponds to lower fluctuations of the atoms of the molecule, while a high RMSF value implies larger fluctuations and less stability of the docked pose [50]. RMSF plots of ligands **66** and **63** throughout the simulation are shown in **Figure 4.7**. The X-axis exhibited the atom index number of the ligand, which is simply the serial numbering of atoms in the ligand, while the Y-axis showed the average RMSF values of these atoms throughout the simulation. RMSF values of both the ligands **66** and **63** were 1.37 and 2.01 Å, respectively. The lower RMSF observed for ligand **66** compared to ligand **63** suggested reduced atomic fluctuations and greater conformational stability throughout the simulation. Thus, ligand **66** having low RMSD and RMSF values suggested stronger docked pose stability compared to ligand **63** in the MAO-B binding pocket.

Protein-ligand interactions profile throughout the complete simulation is another important factor in depicting the stability of the docked poses of ligands [51]. **Figure 4.8** displays the 2-D bar graph representation of the interaction fraction of amino acid residues of the MAO-B protein with ligands **66** and **63**, while **Figure 4.9** shows the timeline representation of interactions between MAO-B protein and ligands **66** and **63**. In **Figure 4.9**, the top panel displays the total number of specific contacts of protein with ligands throughout the simulation trajectory, whereas the bottom panel shows which amino acid residues of protein interact with ligands in each trajectory frame. For ligand **66**, multiple residues of MAO-B contribute to the binding interactions. Tyr398 and Gly434 residues showed hydrogen bonding interactions with interaction fractions of 0.33 and 0.67 respectively. Other residues displayed hydrophobic interactions with ligand **66** which are Tyr60, Ile199, Lys296, Trp388, Tyr398, Tyr435, and Met436, with interaction fractions of more than 0.2. Highest

hydrophobic interaction fraction was observed with Tyr398 residue (**Figure 4.8**). The total number of contacts MAO-B protein residues make with ligand **66** lied between 3 and 9 throughout the simulation time. Notably, Tyr398 exhibited consistently high contact intensity during the simulation. Additionally, other residues Tyr60, Lys296, Trp388, Gly434, and Tyr435 maintained stable interactions over longer time (**Figure 4.9**). In case of ligand **63**, all the residues of MAO-B showed only hydrophobic interactions and no hydrogen bonding. Residues Lys296, Phe343, and Tyr398 displayed interaction fraction of more than 1. While, Val294, Tyr326, and Tyr435 residues exhibited interaction fraction values of 0.23, 0.26, and 0.73, respectively (**Figure 4.8**). The timeline representation plot for ligand **63**-protein complex indicated that the total number of contacts between ligand **63** and protein residues stayed between 0 and 8 over the simulation time. Specifically, Lys296, Phe343, Tyr398, and Tyr435 residues of MAO-B protein maintained stable interactions for almost complete simulation time (**Figure 4.9**). This strongly indicated that ligand **66** formed more consistent and stable binding interactions with the MAO-B binding pocket compared to ligand **63**.

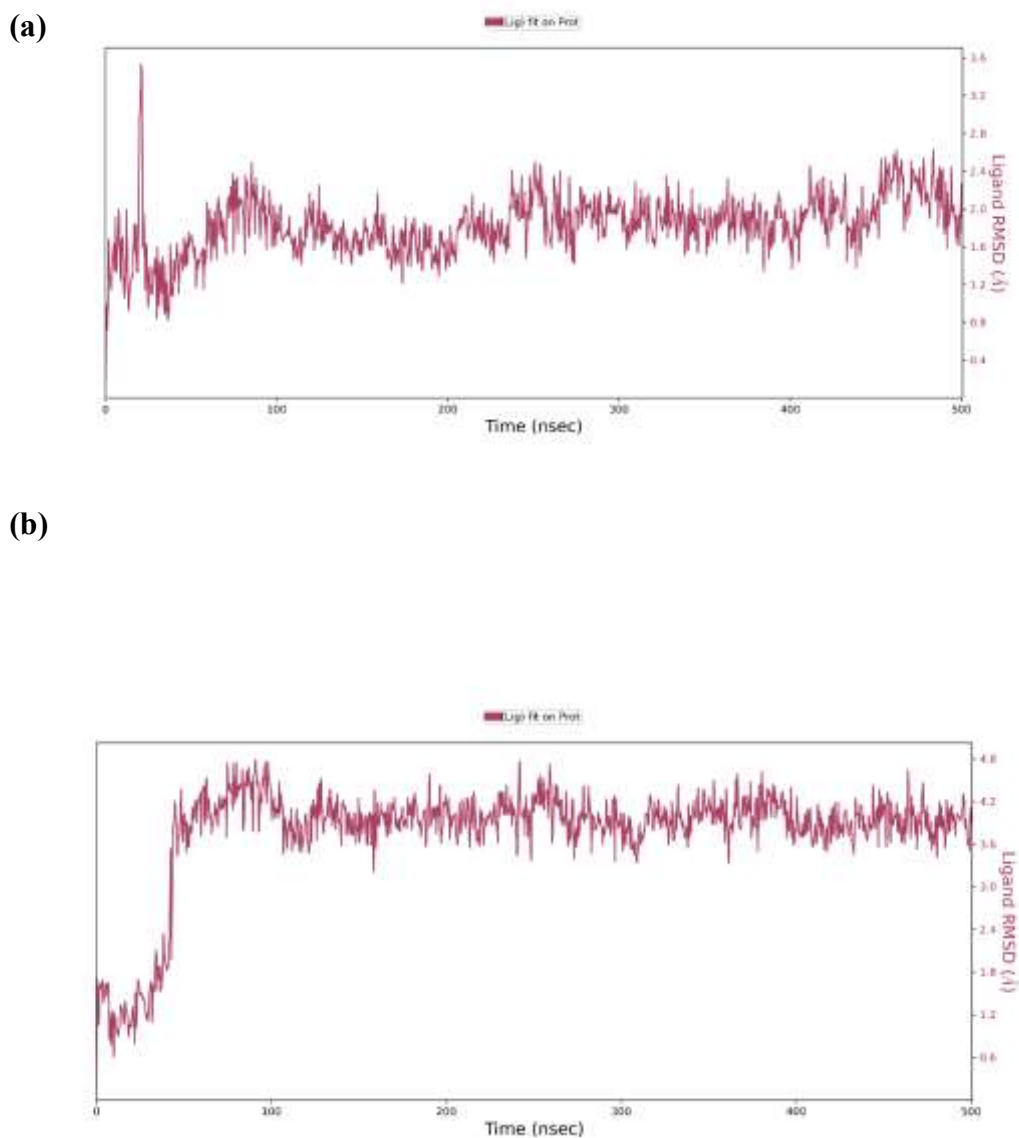


Figure 4.6 RMSD plots of heavy atoms of (a) ligand **66** (b) ligand **63**, all calculated with respect to protein. The Y-axis represented the RMSD values, while the X-axis represented the simulation time.

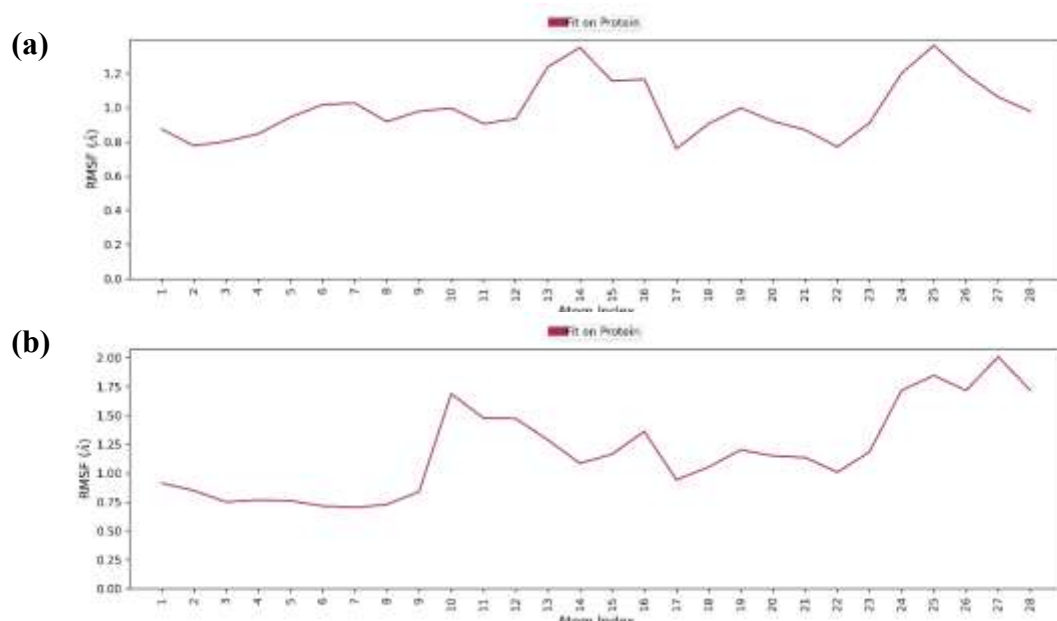


Figure 4.7 RMSF plots of (a) ligand **66** (b) ligand **63**, all calculated with respect to protein. The Y-axis illustrated the RMSF values, while the X-axis demonstrated the atom index of the ligand.

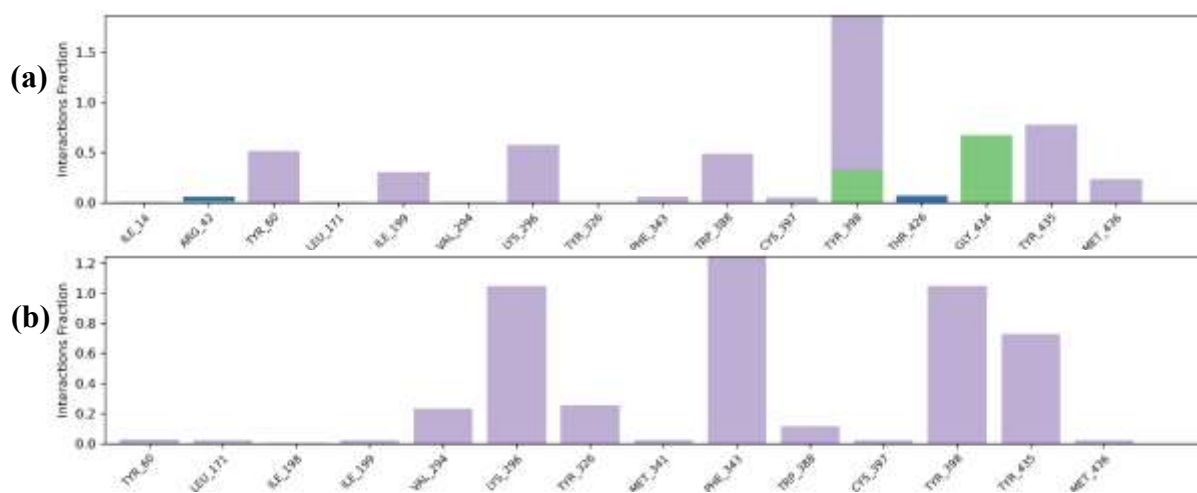


Figure 4.8 2-D bar graph representation of protein-ligand interactions showing the interaction fractions of amino acid residues of MAO-B protein with (a) ligand **66** (b) ligand **63**. Hydrogen bonds are shown in green colour, while hydrophobic interactions are displayed in violet colour.

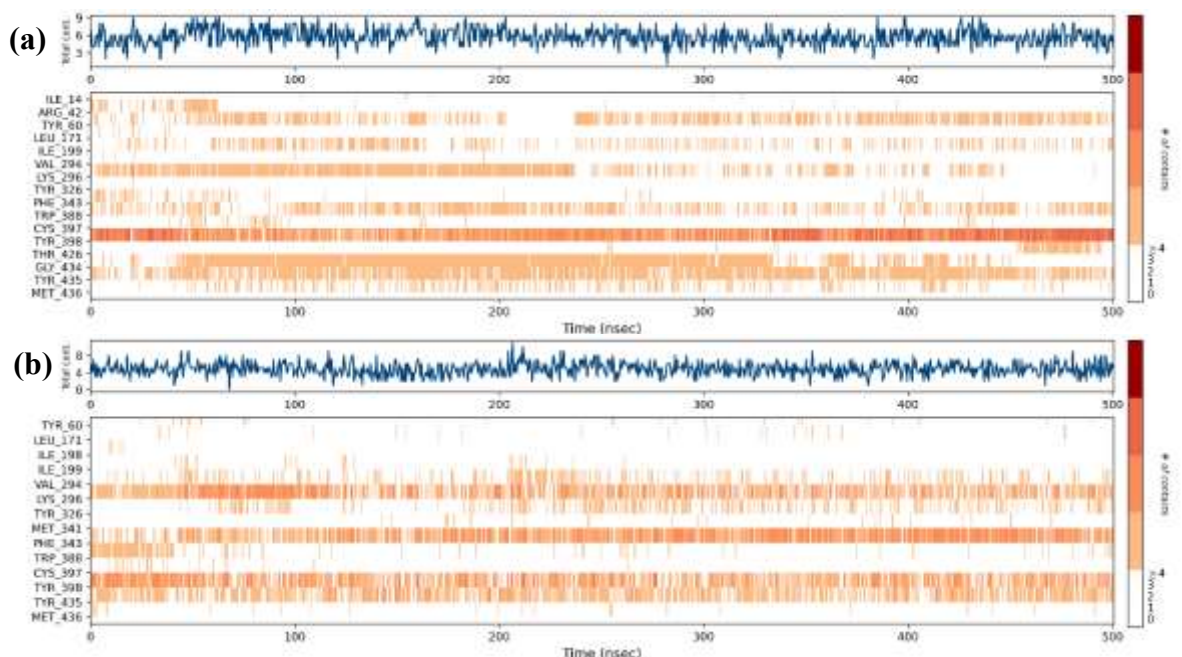


Figure 4.9 Timeline representation of interactions between MAO-B protein and **(a)** ligand **66** **(b)** ligand **63**. Top panel displays the total number of specific contacts of protein with ligands throughout the simulation trajectory. Bottom panel shows which amino acid residues of protein interact with ligands in each trajectory frame.

4.3.3 MM-GBSA Binding Free Energy Analysis

Following the completion of MDS, the MM-GBSA binding free energies of top two docked protein-ligand complexes were estimated to evaluate the stability of ligand binding with the protein throughout the complete simulation time [52]. The binding free energies were calculated at every 50th frame, starting from the first frame, among all the frames extracted from the complete 500 ns simulation trajectory. The analysis was performed for the top two docked complexes containing ligands **66** and **63**. **Table 4.2** displayed the calculated binding free energy values for all the selected frames.

Both the docked complexes containing ligands **66** and **63** maintained high negative binding free energy values, indicating stable protein-ligand binding. The ligand **66**–protein complex showed binding free energy values ranging from -63.542 to -79.339 kcal/mol. The most negative binding free energy was observed at the 200th frame (-79.339 kcal/mol), while the lowest negative binding free energy was shown at the 450th frame (-63.542 kcal/mol), with an average binding free energy value of -71.235 kcal/mol. In contrast, the ligand **63**–protein complex displayed binding free energy values ranging from -58.142 to -75.157 kcal/mol, with the strongest interaction was observed at the 500th frame (-75.157 kcal/mol)

and the weakest was at the 1st frame (-58.142 kcal/mol), with an average value of -65.578 kcal/mol. Thus, these results indicated that the ligand **66**–protein complex showed more negative binding free energy and therefore, a stronger binding affinity with the MAO-B protein compared to the ligand **63**–protein complex.

Table 4.2 MM-GBSA binding free energy of complexes of ligands **66** and **63** with MAO-B protein after MDS.

Frame No.	Ligand 66-Protein Complex	Ligand 63-Protein Complex
1st	-64.389	-58.142
50th	-67.608	-68.543
100th	-79.022	-66.338
150th	-72.986	-61.797
200th	-79.339	-58.642
250th	-73.560	-68.015
300th	-76.314	-66.716
350th	-70.117	-60.597
400th	-72.450	-68.465
450th	-63.542	-66.108
500th	-76.246	-75.157
550th	-74.182	-63.358
600th	-68.369	-70.324
650th	-72.001	-62.925
700th	-71.297	-69.736
750th	-68.462	-64.793
800th	-76.588	-67.500
850th	-70.500	-66.146
900th	-65.638	-60.386
950th	-69.107	-69.198
1000th	-64.222	-64.258
Average	-71.235	-65.578

4.3.4 ADME Studies

For the successful discovery of drugs, ADME is a critical component [53]. Thus, it is crucial to check the physicochemical properties, pharmacokinetics, and drug-likeness profiles of the top docked ligands **66** and **63**. The SwissADME web tool calculated the ADME properties of both the ligands **66** and **63** which is shown in **Table 4.3**.

Both the ligands showed similar physicochemical properties, with molecular weights of 365.47 and 367.46, respectively, with same number of heavy atoms (28), number of aromatic heavy atoms (21), and fraction Csp³ (0.15). Both ligands have same number of rotatable bonds (3) and number of hydrogen bond acceptors (1). In addition, ligand **66** has 1 hydrogen bond donor, whereas ligand **63** has none. Ligand **66** displayed high topological polar surface area (TPSA) value of 25.16 Å² while ligand **63** exhibited TPSA value of 4.93 Å² indicating that ligand **66** is somewhat more polar than **63**. Lipophilicity was depicted by applying the average of physics Log Po/w (iLOGP), topological Log Po/w(MLOGP), atomistic Log Po/w (WLOGP), atomistic and knowledge Log Po/w(XLOGP3), and hybrid fragment/topological Log Po/w (SILICOS-IT) which is called as the consensus Log Po/w [42]. Both the ligands **66** and **63** showed high consensus Log Po/w values of 5.38 and 6.13, respectively, demonstrating high lipophilicity. The logarithmic solubility, or Log S (ESOL), was used to estimate the water solubility. Ligands **66** and **63** exhibited low ESOL Log S values of -6.4 and -6.7, respectively and classified as poorly soluble ligands.

Both ligands are predicted as substrates of P-glycoprotein (P-gp), indicating reduced circulation of ligands into the bloodstream and target site. Ligand **66** was predicted to inhibit cytochrome P450 enzymes CYP1A2, CYP2C19, and CYP2D6, while it does not inhibit CYP2C9 or CYP3A4. In contrast, ligand **63** was predicted to inhibit only CYP1A2 and CYP2C19, and does not inhibit CYP2C9, CYP2D6, or CYP3A4. Thus, ligand **66** showed a broader cytochrome P450 inhibition profile, that will enhance the metabolic stability but also increase the risk of the drug-drug interactions. While ligand **63** showed more selective inhibition, indicating safer interaction profile with slower metabolic clearance.

Skin permeability is a measure of how well a ligand can penetrate the skin which is expressed in log Kp. Both the ligands **66** and **63** exhibited low log Kp values of -4.09 and -3.78 cm/s, respectively indicating both the ligands do not easily pass through the skin barrier. Both the ligands showed bioavailability scores of 0.55 with no PAINS or Brenk alerts. According to Lipinski's rule of five, an orally bioactive drug must adhere to the following criteria: molecular weight < 500 Da, Log Po/w ≤ 5, number of hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5 [54].

Table 4.3 Predicted ADME Properties of ligands **66** and **63** estimated using SwissADME.

ADME Property	Ligand 66	Ligand 63
Molecular Weight	365.47	367.46
No. of heavy atoms	28	28
No. of aromatic heavy atoms	21	21
Fraction Csp ³	0.15	0.15
No. of rotatable bonds	3	3
No. of H-bond acceptors	1	1
No. of H-bond donors	1	0
MR	117.39	115.32
TPSA	25.16	4.93
Consensus Log P	5.38	6.13
ESOL Log S	-6.4	-6.7
ESOL Solubility (mg/ml)	1.45E-04	7.40E-05
ESOL Class	Poorly soluble	Poorly soluble
GI absorption	Low	Low
BBB permeant	No	No
Pgp substrate	Yes	Yes
CYP1A2 inhibitor	Yes	Yes
CYP2C19 inhibitor	Yes	Yes
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	Yes	No
CYP3A4 inhibitor	No	No
log Kp (cm/s)	-4.09	-3.78
Lipinski violations	1	1
Veber violations	0	0
Bioavailability Score	0.55	0.55
PAINS alerts	0	0
Brenk alerts	0	0

According to Veber's rule, an orally bioavailable drug must meet two conditions: number of rotatable bonds ≤ 10 and polar surface area $\leq 140 \text{ \AA}^2$, or alternatively, total number of hydrogen bond donors and acceptors ≤ 12 [55]. Both the ligands showed only one violation in Lipinski's rule of five and zero violation in Veber's rule, indicating favourable drug-likeness properties. Overall, both the ligands displayed favourable physicochemical and drug-likeness properties. However, their high lipophilicity, poor solubility, and low GI absorption highlighted pharmacokinetic limitations that require further structure optimization of ligands.

4.3.5 DFT Studies

Frontier HOMO-LUMO

Furthermore, the DFT calculations of ligands **66** and **63** were performed to study their molecular properties. Initially, ligands **66** and **63** were geometry optimized using the B3LYP/def2-TZVP method. Afterwards, frontier HOMO-LUMO and MESP studies of these ligands were performed using their optimized structures.

The highest occupied molecular orbital (HOMO) refers to the ability of the ligand to donate an electron, while lowest unoccupied molecular orbital (LUMO) stands for the ligand's tendency to accept an electron. The energy value of HOMO (E_{HOMO}), the energy value of LUMO (E_{LUMO}), and the energy gap value between HOMO and LUMO (E_{GAP}) are useful for studying the reactivity and kinetic stability of ligands. High E_{GAP} shows high kinetic stability and low chemical reactivity of molecules, whereas low E_{GAP} indicates low kinetic stability and high chemical reactivity [56]. Visual 3-D representations of HOMO and LUMO of ligands **66** and **63**, including their E_{HOMO} , E_{LUMO} , and E_{GAP} values, were shown in **Figures 4.10(a)** and **4.10(b)**, respectively, in which blue color represented the electron-deficient region while red color represented the electron-denser region.

For ligand **66**, the HOMO is mainly localized over the indole ring and 3-hydroxyphenyl group attached to 2nd position of indole ring and somewhat distributed over the cyclohex-1-ene group attached to *N*-atom of indole ring. Whereas, the LUMO is mainly distributed over the indole ring and 3-hydroxyphenyl group. For ligand **63**, the HOMO is mostly located over the indole ring and 4-fluorophenyl group, with slight delocalization on the cyclohex-1-ene group. The LUMO is mainly concentrated on the indole ring and 4-fluorophenyl group. The predicted E_{HOMO} , E_{LUMO} , and E_{GAP} values for ligand **66** were -5.375, -1.011, and 4.364 eV, respectively. For ligand **63**, the corresponding values were -5.513, -

1.052, and 4.461 eV, respectively. Both the ligands exhibited low values of E_{GAP} with ligand **66** showed a slightly lower E_{GAP} value than ligand **63**, suggesting slightly lower kinetic stability and higher chemical reactivity than ligand **63**.

Global reactivity parameters, including ionization potential (IP), electron affinity (A), electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S), and electrophilicity index (ω), were calculated for both ligands **66** and **63** using values of E_{HOMO} and E_{LUMO} (Table 4.4). Ligand **66** showed a slightly lower value of ionization potential than ligand **63**. In addition, ligand **66** displayed slightly lower values of electron affinity, electronegativity, and chemical potential than ligand **63**. Furthermore, ligand **66** displayed a slightly higher value of global softness and a slightly lower value of global hardness, indicating that ligand **66** is slightly more chemically reactive than ligand **63**. The electrophilicity index measures the electrophilic nature of a ligand. Ligand **66** displayed slightly lower electrophilicity index value than ligand **63** making ligand **63** slightly more electrophilic than ligand **66**. Thus, the HOMO-LUMO analysis and global reactivity parameters indicated that ligand **66** has the tendency for high chemical reactivity compared to ligand **63**.

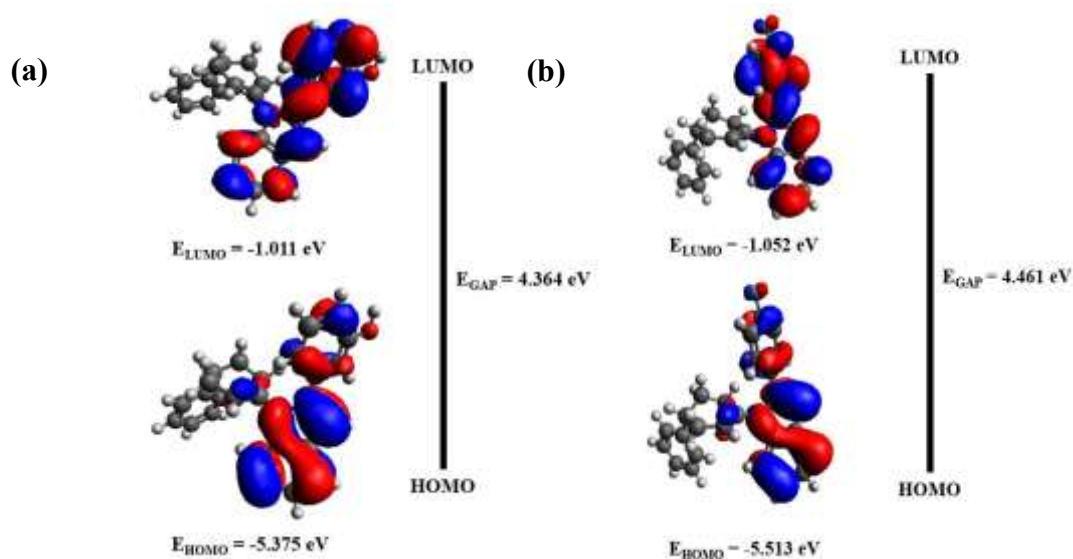


Figure 4.10 Visual 3-D representation of Frontier HOMO and LUMO orbitals of (a) ligand **66** (b) ligand **63**, including their E_{HOMO} , E_{LUMO} , and E_{GAP} values.

Table 4.4 Global Reactivity Parameters calculated for ligands **66** and **63**, providing insights into their chemical stability and reactivity.

Parameters	Ligand 66	Ligand 63
HOMO Energy (in eV)	-5.375	-5.513
LUMO Energy (in eV)	-1.011	-1.052
E_{GAP} (in eV)	4.364	4.461
Ionization Potential (IP) (in eV)	5.375	5.513
Electron Affinity (A) (in eV)	1.011	1.052
Electronegativity (χ) (in eV)	3.193	3.283
Chemical Potential (μ) (in eV)	-3.193	-3.283
Chemical Hardness (η) (in eV)	2.182	2.231
Chemical Softness (S) (in eV ⁻¹)	0.229	0.224
Electrophilicity Index (ω) (in eV)	2.336	2.415

Molecular Electrostatic Potential (MESP)

MESP displayed the distribution of electron density around the ligand and is also helpful in the prediction of the chemical reactivity of the ligand [57]. In the MESP surface, the most positive region (highlighted in blue color) is the electrophilic region and susceptible to nucleophilic attack, while the most negative region (highlighted in red color) is the nucleophilic region and susceptible to electrophilic attack. Whereas, green color represents nearly neutral regions. The overall color progression in a MESP from most positive region to most negative region follows the order: Blue > Green > Yellow > Orange > Red [58]. **Figures 4.11** and **4.12** displayed the 3-D MESP images of ligands **66** and **63**, respectively.

In **Figure 4.11**, the yellow-orange to red region was localized on the oxygen atom of hydroxyl group attached to phenyl ring of ligand **66** indicating that oxygen atom was acting as nucleophile and susceptible for electrophilic attack. Whereas, blue color was mostly observed on the hydrogen atoms and thus these hydrogens atoms are susceptible for nucleophilic attack. Additionally, aromatic and aliphatic carbon atoms cover the green region. These findings were well correlated with the binding interactions between MAO-B protein and ligand **66** as reported above in the molecular docking and MDS sections. Hydrogen atom of hydroxyl group attached to phenyl ring of ligand **66** showed conventional hydrogen bonding with Gly434 amino acid residue of MAO-B and oxygen atom of hydroxyl group exhibited hydrogen bonding with Arg42 residue in the docking studies. Additionally, in the

MDS studies, Gly434 residue showed hydrogen bonding interaction with interaction fraction of 0.67 throughout the complete simulation time. Similarly, in the case of MESP of ligand **63** (**Figure 4.12**), the yellow-red region was mainly localized on the fluorine atom attached to phenyl ring indicating electron-rich area with strong negative electrostatic potential. Blue color was mostly covered by hydrogen atoms, indicating strong positive electrostatic potential area, and thus these hydrogen atoms are susceptible for nucleophilic attack. The aromatic and aliphatic carbon atoms predominantly exhibited green to light blue color. Overall, the MESP analysis of ligands **66** and **63** demonstrated the distribution of electron density across the molecules.

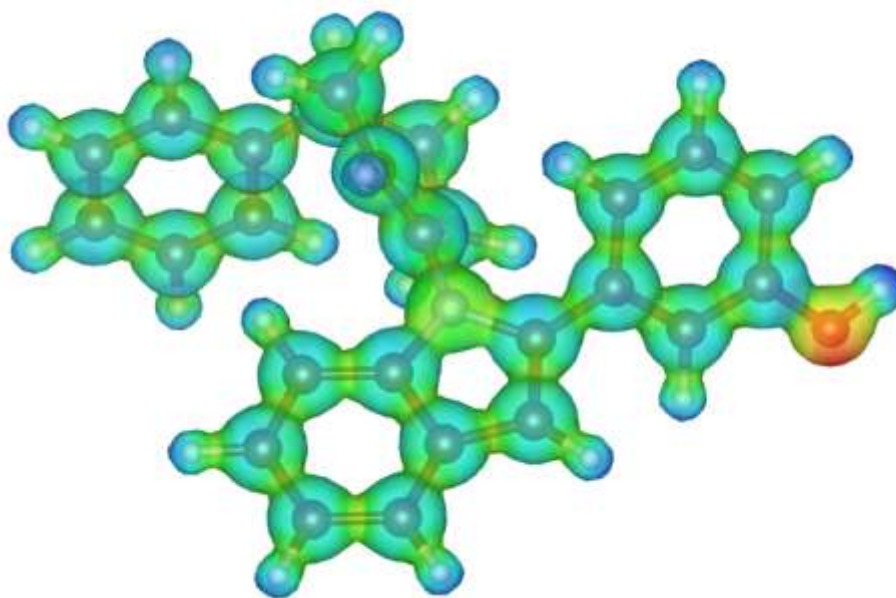


Figure 4.11 Molecular Electrostatic Potential Surface (MESP) of ligand **66**. Red colour corresponded to the nucleophilic region, while blue colour indicated the electrophilic region of the ligand.

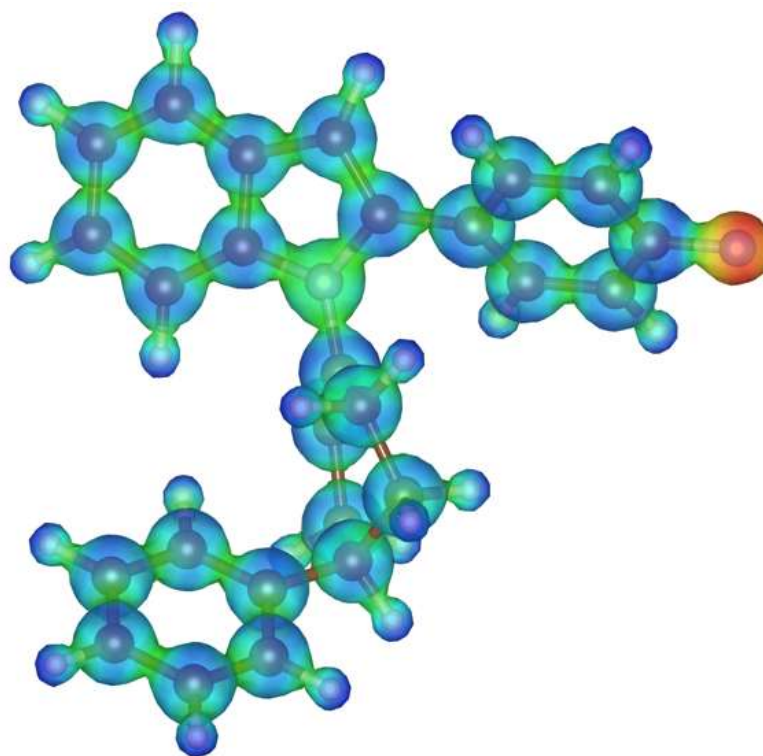


Figure 4.12 Molecular Electrostatic Potential Surface (MESP) of ligand **63**. Red colour corresponded to the nucleophilic region, while blue colour indicated the electrophilic region of the ligand.

4.4 Conclusion

In this chapter, *in-silico* investigations on a library of 147 N-alkenylindoles as potential inhibitors of MAO-B were carried out. Molecular docking studies identified ligands **66** and **63** as the most promising candidates, with high docking scores of -11.8 kcal/mol and -11.4 kcal/mol, respectively compared to the co-crystal ligand of MAO-B (-10.6 kcal/mol). The hydroxyl group of ligand **66** exhibited hydrogen bonding with Gly434 amino acid residue of MAO-B and oxygen atom of hydroxyl group of **66** displayed hydrogen bonding with Arg42 residue. However, ligand **63** showed halogen (fluorine atom) interaction with Tyr435 residue and no hydrogen bonding. Molecular dynamics simulations performed over 500 ns demonstrated lower RMSD and RMSF values for ligand **66** compared to ligand **63**, indicating greater stability of the docked complex of ligand **66**-protein. For ligand **66**, the Gly434 residue showed a conventional hydrogen-bonding interaction with an interaction fraction of 0.67 throughout the entire simulation time. Whereas for ligand **63**, residues of MAO-B displayed no hydrogen bonding interaction but Lys296, Phe343, and Tyr398 residues showed hydrophobic interaction fraction of more than 1 during the simulation. Post-

MDS MM-GBSA calculations revealed average binding free energies of -71.235 kcal/mol and -65.578 kcal/mol for ligands **66** and **63**, respectively, indicating energetically favourable interactions. ADME profiling of both ligands indicated favourable drug-likeness and physicochemical properties; however, their high lipophilicity and poor solubility may require further structural optimization. DFT analysis showed higher chemical reactivity and low kinetic stability of ligand **66** compared to ligand **63**. In addition, the 3D MESP of ligand **66** highlighted the nucleophilic character of the oxygen atom of hydroxyl group, which was consistent with its docking and MDS studies. Overall, these computational studies suggested that ligand **66** formed a highly stable and energetically favourable complex with the MAO-B protein, providing a strong foundation for future experimental validation and the development of novel therapeutic agents for Parkinson's disease.

Both the ligands demonstrated low gastrointestinal (GI) absorption and are not permeant to the blood-brain barrier (BBB), limiting their potential for central nervous system activity. This conveys that further functionalization/modification in structure is required to enhance the GI and BBB.

4.5 References

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CHAPTER 5

Iodine-catalyzed synthesis of *N*-alkenylindoles and their antioxidant studies

5.1 Introduction

Indole represents an important class of *N*-heterocyclic compounds represented by a bicyclic structure in which a six-membered benzene ring is fused to a five-membered, nitrogen-containing pyrrole ring [1]. This moiety is the core structure of many molecules that exhibit a diverse spectrum of biological activities [2-15], including anticancer [2,3], antiviral [4,5], antibacterial [6,7], anti-inflammatory [10,11], antidiabetic [12,13], and antifungal [14,15], as well as therapeutic potential against neurodegenerative diseases [8,9]. The clinical significance of indole core structure is proved by its presence in several FDA-approved drugs, such as Lurbinectedin, Osimertinib, Etrasimod, and Flortaucipir F-18 (**Figure 5.1**) [16,17]. Additionally, indole substituted with alkyl substituents, have been found to protect DNA and erythrocytes from radical-induced oxidative damage [18]. Beyond synthetic pharmaceuticals, the indole frameworks are also present in various natural compounds such as Caulerpin alkaloid and Topsentin alkaloid [19-21] (**Figure 5.1**). Several indole alkaloids exhibited a range of biological activities [22]. In addition, indole substituted compounds have the ability to mimic peptide structures and bind reversibly to enzymes [23,24].

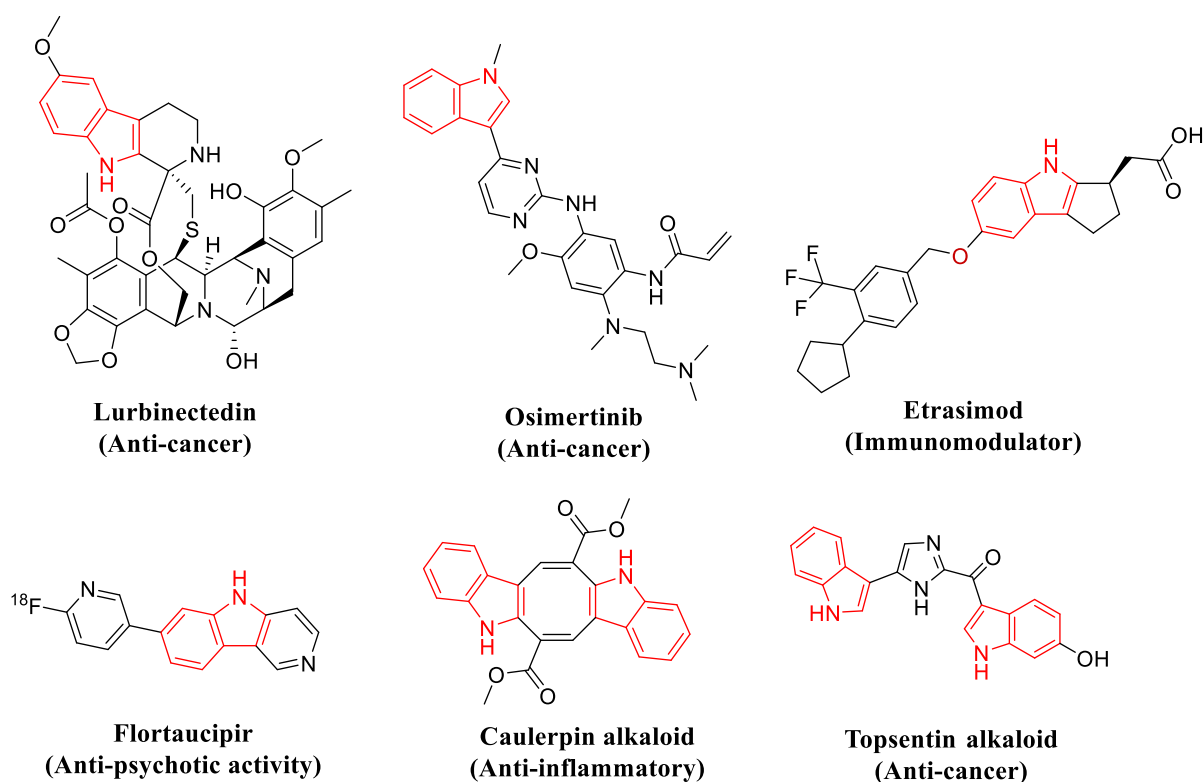


Figure 5.1 Selected FDA-approved drugs and natural products containing indole ring

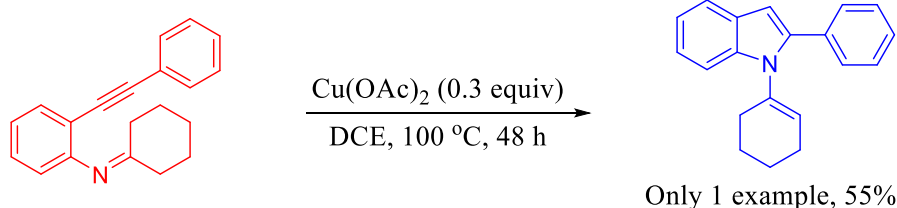
Furthermore, *N*-substituted indoles displayed diverse medicinal applications. The introduction of benzyl, benzoyl, sulphonyl benzyl chloride, furyl, triazolyl, amide, and thiourea groups has been shown to enhance the biological activities of *N*-substituted indole compounds [25]. Previously, three research groups have reported the synthesis of *N*-alkenylindoles, a subclass of *N*-substituted indoles. Wang et al. (2015) synthesized the *N*-alkenylindole derivative 1-(cyclohex-1-en-1-yl)-2-phenyl-1H-indole compound in 55% yield [26]. The reaction was carried out at 100 °C for 48 h using *N*-(2-(phenylethynyl)phenyl)cyclohexanimine as the starting material and copper acetate as the catalyst in dichloroethane (DCE) solvent (**Scheme 5.1**). According to authors, copper acetate was used to activate the triple bond of the starting material. Subsequently, the lone pair of the nitrogen atom attacked the carbon atom of activated triple bond to generate an *N*-cyclized intermediate, which further underwent deprotonation to give the *N*-alkenylindole product [26]. Subsequently, in 2018, Dhandabani et al. reported the palladium catalyzed synthesis of same 1-(cyclohex-1-en-1-yl)-2-phenyl-1H-indole compound from 2-(phenylethynyl)aniline and cyclohexanone as starting materials achieving 59% yield in dimethyl sulfoxide (DMSO) solvent at 110 °C for 3 h [27]. Here also, palladium catalyst was used for triple bond activation of 2-(phenylethynyl)aniline [27]. Furthermore, Marsicano et al. (2021) reported a zinc bromide catalyzed synthesis of four *N*-alkenylindole derivatives [28]. The reaction was performed using 2-(1-alkynyl)anilines and cyclic ketones in toluene solvent at 110 °C for 2-6 h, affording products in yields ranging from 60 to 77 % (**Scheme 5.1**). Zinc bromide facilitates the triple bond activation of 2-(1-alkynyl)anilines. According to their plausible mechanism, the *N*-alkenylindoles were synthesized through a tandem amination process, followed by *5-endo dig* intramolecular hydroamination of 2-(1-alkynyl)anilines with cyclic ketones [28].

All of these methods required the use of metals and substrate scope is limited. From the previous literatures, it was found that molecular iodine (I₂) was used in the triple bond activation or in cyclization of substrates (**Scheme 5.2**). The molecular iodine was used to cyclize *N*-aryl-2-alkynylanilines through iodo-cyclization reaction, followed by proto-deiodination to afford *N*-arylindole derivatives [29-32]. Thus, to improve the reaction conditions from a metal-free perspective, herein we reported the metal-free synthesis of *N*-alkenylindoles, expanding the scope to 19 compounds with 18 new derivatives. We developed a strategy utilizing molecular iodine for cyclization of 2-(1-alkynyl)anilines to synthesize *N*-attached cyclohex-1-ene or cyclopent-1-ene indoles in the presence of

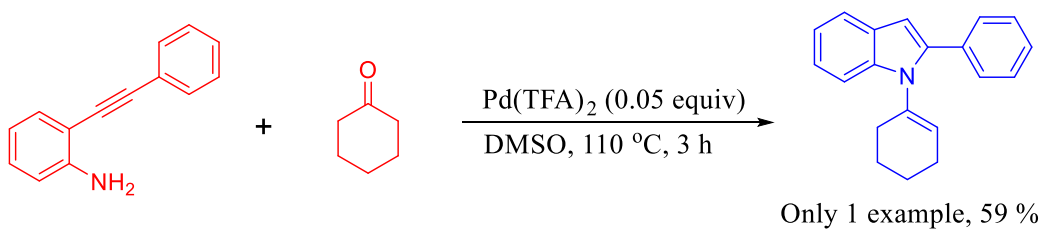
cyclohexanone and cyclopentanone respectively. Additionally, control experiments were performed to understand the mechanistic pathway.

Antioxidant molecules are biologically important as they protect cellular components from oxidative stress by scavenging free radicals and the reactive oxygen species, which aids in prevent cellular damage associated with certain diseases, including neurodegenerative diseases [33]. To date, the antioxidant activities of *N*-alkenylindoles has not been explored. The prepared compounds have been evaluated for their antioxidant activity.

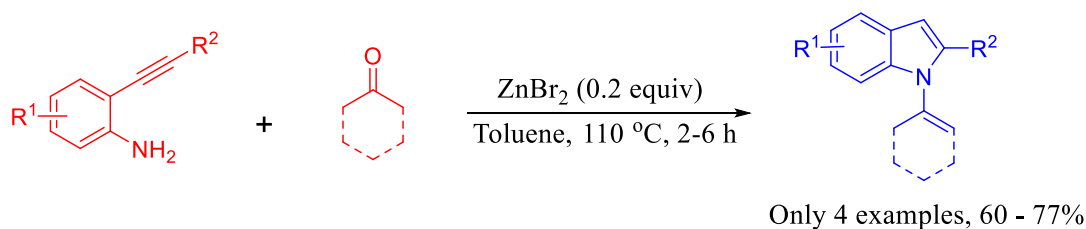
(a) Wang et al. work (2015) [26]



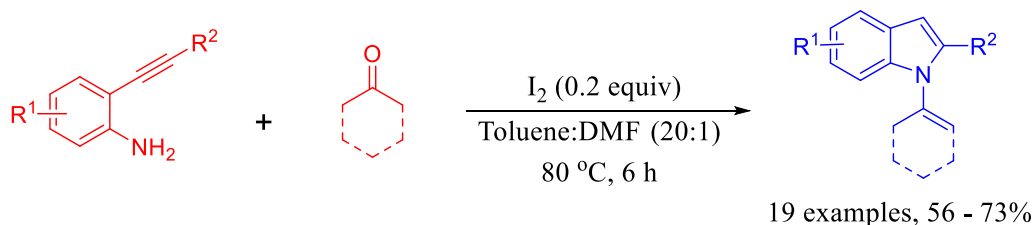
(b) Dhandabani et al. work (2018) [27]



(c) Marsicano et al. work (2021) [28]

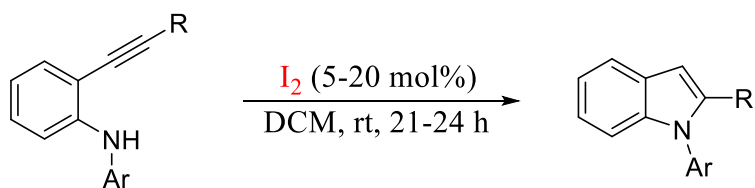


(d) This Work

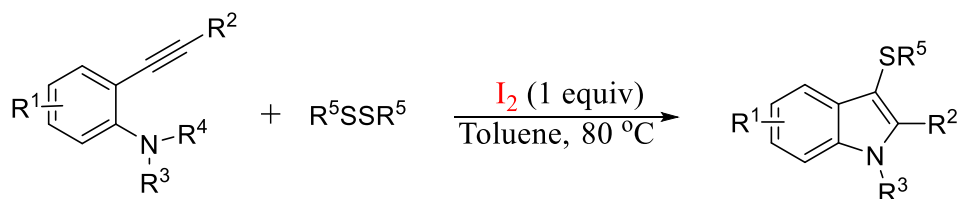


Scheme 5.1 Background work and this work on synthesis of *N*-alkenylindoles

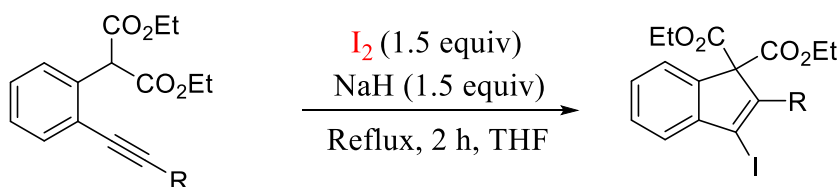
Takeda et al. work [29]:



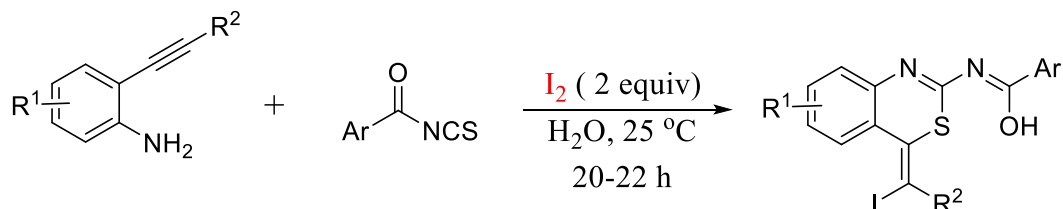
Tao et al. work [30]:



Khan and Wirth work [31]:



Saini et al. work [32]:



Scheme 5.2 Some representative examples where molecular iodine used for triple bond activation and cyclization

5.2 Experimental Section

5.2.1 General Information

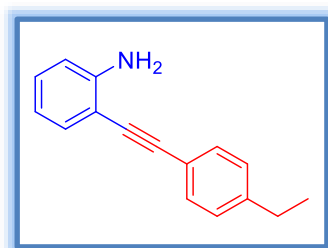
Unless otherwise stated, commercially available reagents and solvents were used for synthesis of starting materials and final products without purification. Oven-dried glasswares were used for the reactions. The progress of the reaction was monitored by thin layer chromatography (TLC). TLC was performed on silica gel 60 F₂₅₄ 0.25 mm precoated aluminum foil plates and visualization of TLC plate was done with short wavelength UV

light (254 nm) chamber. Purification of starting materials and final products were performed with the help of column chromatography using commercially available silica with a mesh size of 60-120 and using petroleum ether as solvent. Melting points were determined on laboratory open-glass melting apparatus and were uncorrected. FTIR spectras were recorded in KBr on a Perkim-Elmer Spectrum II spectrophotometer. ^1H -NMR, and ^{13}C -NMR were obtained at 400, and 100 MHz respectively on Jeol JNM-ECZ 400S spectrometer model with tetramethylsilane (TMS) used as internal standard, deuterated chloroform (CDCl_3) as the solvent, and chemical shift values were reported in ppm. HRMS data was recorded on a Synapt XS HD QTOF mass spectrometer model.

5.2.2 Procedure for the synthesis of 2-(1-alkynyl)anilines (3a-3r)

2-Iodoaniline **1** (1 equiv) was placed in DMF in an oven-dried vial. $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.04 equiv) and copper iodide (0.02 equiv) were added to the vial. The reaction mixture was placed under an inert atmosphere. This was stirred (5 min) at room temperature, and triethylamine (4.0 equiv) was added by syringe. The temperature was then increased to 70 °C. Then terminal alkyne **2** (1.2 equiv) dissolved in DMF was added dropwise over 5 min. The reaction mixture was allowed to stir at 70 °C for 2 h, and the reaction was monitored by TLC to establish completion. After the reaction was over, it was allowed to cool and was quenched with aqueous NH_4Cl (10 mL) and water (10 mL). The aqueous layer was extracted with EtOAc (3×20 mL). The combined organic layer was washed with brine solution, was dried using anhydrous Na_2SO_4 , and was evaporated under reduced pressure. The crude product thus obtained was purified through column chromatography with the help of petroleum ether as eluent.

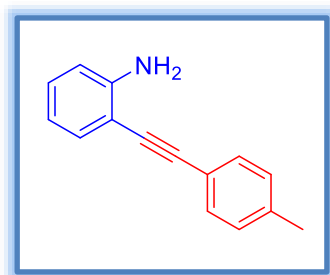
2-((4-Ethylphenyl)ethynyl)aniline (**3a**)



Yield: 88%; yellow solid; mp. 112.0-114.0 °C; FTIR (KBr, cm^{-1}): 3472, 3370, 2953, 2926, 2867, 2203, 1605, 1480, 1448, 1311, 1244, 1158, 934, 828, 741; ^1H NMR (400 MHz, CDCl_3) δ : 7.45-7.43 (m, 2H, ArH), 7.35 (dd, J = 7.6, 1.6 Hz, 1H, ArH), 7.15 (d, J = 8 Hz, 2H), 7.14-

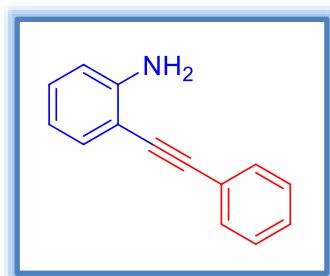
7.09 (m, 1H, ArH), 6.72-6.68 (m, 2H, ArH), 4.25 (br, s, 2H, ArH), 2.65 (q, 2H, CH₂), 1.23 (t, 3H, CH₃).

2-(p-Tolylethynyl)aniline (3b)



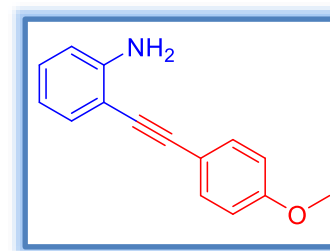
Yield: 90%; yellow solid; mp. 100.0-102.0 °C (lit. mp. 99.1-100.0 °C^[34]); FTIR (KBr, cm⁻¹): 3479, 3370, 3028, 2929, 2212, 1615, 1492, 1315, 1247, 1162, 945, 831, 748; ¹H NMR (400 MHz, CDCl₃) δ: 7.42 (d, *J* = 8 Hz, 2H, ArH), 7.35 (d, *J* = 8 Hz, 1H, ArH), 7.16-7.10 (m, 3H, ArH), 6.73-6.69 (m, 2H, ArH), 4.26 (br, s, 2H, NH), 2.37 (s, 3H, CH₃).

2-(Phenylethynyl)aniline (3c)



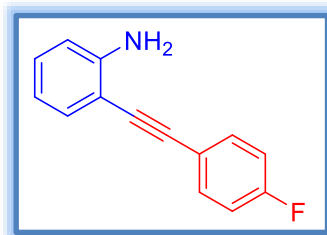
Yield: 89%; light yellow solid; mp. 82.0-84.0 °C (lit. mp. 82.2-83.5 °C^[34]); FTIR (KBr, cm⁻¹): 3475, 3370, 2954, 2921, 2205, 1615, 1499, 1308, 1246, 1167, 939, 824, 748; ¹H NMR (400 MHz, CDCl₃) δ: 7.54-7.51 (m, 2H, ArH), 7.37-7.33 (m, 4H, ArH), 7.14 (t, 1H, ArH), 6.73-6.70 (m, 2H, ArH), 4.27 (br, s, 2H, NH).

2-((4-Methoxyphenyl)ethynyl)aniline (3d)



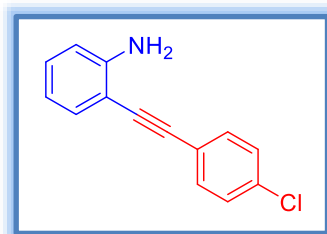
Yield: 86%; yellow solid; mp. 244.0-246.0 °C (lit. mp. 242.3-244.0 °C^[34]); FTIR (KBr, cm⁻¹): 3468, 3389, 2939, 2915, 2216, 1608, 1485, 1314, 1246, 1167, 938, 834, 764; ¹H NMR (400 MHz, CDCl₃) δ: 7.46 (d, *J* = 8 Hz, 2H), 7.35-7.33 (m, 1H, ArH), 7.14-7.10 (m, 1H, ArH), 6.87 (d, *J* = 8 Hz, 2H, ArH), 6.72-6.69 (m, 2H, ArH), 4.25 (br, s, 2H, ArH), 3.82 (s, 3H, OCH₃).

2-((4-Fluorophenyl)ethynyl)aniline (3e)



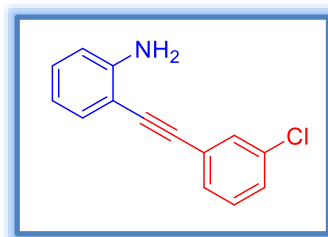
Yield: 83%; yellow solid; mp. 108.0-110.0 °C (lit. mp. 107.1-108.2 °C^[34]); FTIR (KBr, cm⁻¹): 3482, 3371, 3046, 2925, 2213, 1610, 1488, 1311, 1224, 1169, 945, 826, 752; ¹H NMR (400 MHz, CDCl₃) δ: 7.50-7.47 (m, 2H, ArH), 7.36-7.33 (m, 1H, ArH), 7.15-7.11 (m, 1H, ArH), 7.05-7.01 (m, 2H, ArH), 6.73-6.69 (m, 2H, ArH), 4.24 (br, s, 2H, NH).

2-((4-Chlorophenyl)ethynyl)aniline (3f)



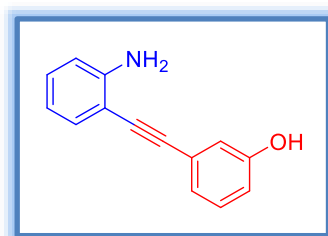
Yield: 84%; yellow solid; mp. 98.0-100.0 °C (lit. mp. 96.4-98.2 °C^[34]); FTIR (KBr, cm⁻¹): 3476, 3367, 3056, 3031, 2209, 1613, 1483, 1452, 1394, 1306, 1256, 1138, 1079, 1008, 827, 741; ¹H NMR (400 MHz, CDCl₃) δ: 7.44-7.42 (m, 2H, ArH), 7.35-7.29 (m, 3H, ArH), 7.15-7.11 (m, 1H, ArH), 6.72-6.69 (m, 2H, ArH), 4.24 (br, s, 2H, NH).

2-((3-Chlorophenyl)ethynyl)aniline (3g)



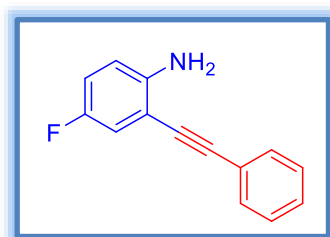
Yield: 85%; yellow solid; mp. 60.0-62.0 °C (lit. mp. 59.9-63.0 °C^[34]); FTIR (KBr, cm⁻¹): 3448, 3358, 3063, 3028, 2214, 2187, 1605, 1488, 1317, 1249, 1150, 1068, 937, 825, 783, 742; ¹H NMR (400 MHz, CDCl₃) δ: 7.51-7.50 (m, 1H, ArH), 7.40-7.34 (m, 2H, ArH), 7.31-7.24 (m, 2H, ArH), 7.17-7.13 (m, 1H, ArH), 6.73-6.69 (m, 2H, ArH), 4.25 (br, s, 2H, NH).

3-((2-Aminophenyl)ethynyl)phenol (3h)



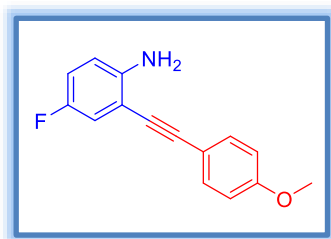
Yield: 87%; brown solid; mp. 182.0-184.0 °C; FTIR (KBr, cm⁻¹): 3512, 3477, 3373, 3057, 2923, 2212, 1612, 1495, 1310, 1249, 1169, 943, 828, 752; ¹H NMR (400 MHz, CDCl₃) δ: 7.35 (dd, *J* = 7.6, 1.6 Hz, 1H, ArH), 7.21-7.07 (m, 3H, ArH), 6.97-6.96 (m, 1H, ArH), 6.81-6.78 (m, 1H, ArH), 6.74-6.71 (m, 2H, ArH).

4-Fluoro-2-(phenylethynyl)aniline (3i)



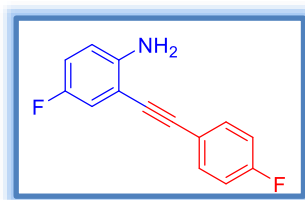
Yield: 88%; yellow solid; mp. 74.0-76.0 °C (lit. mp. 72.4-74.4 °C^[34]); FTIR (KBr, cm⁻¹): 3464, 3379, 3070, 3045, 2202, 1599, 1485, 1429, 1313, 1248, 1192, 1136, 948, 887, 814, 756, 688; ¹H NMR (400 MHz, CDCl₃) δ: 7.53-7.50 (m, 2H, ArH), 7.35-7.33 (m, 3H, ArH), 7.06 (dd, *J* = 8, 4 Hz, 1H, ArH), 6.89-6.84 (m, 1H, ArH), 6.65-6.62 (m, 1H, ArH), 4.13 (br, s, 2H, NH).

4-Fluoro-2-((4-methoxyphenyl)ethynyl)aniline (3j)



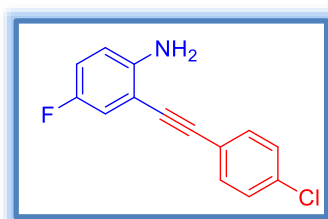
Yield: 89%; yellow solid; mp. 208.0-210.0 °C; FTIR (KBr, cm^{-1}): 3487, 3363, 3064, 2941, 2845, 2201, 1594, 1492, 1433, 1249, 1167, 1108, 1023, 879, 832, 817; ^1H NMR (400 MHz, CDCl_3) δ : 7.47-7.44 (m, 2H, ArH), 7.05 (dd, $J = 7.6, 1.6$ Hz, 1H, ArH), 6.89-6.82 (m, 3H, ArH), 6.66-6.62 (m, 1H, ArH), 4.12 (br, s, 2H, NH), 3.82 (s, 3H, OCH_3).

4-Fluoro-2-((4-fluorophenyl)ethynyl)aniline (3k)



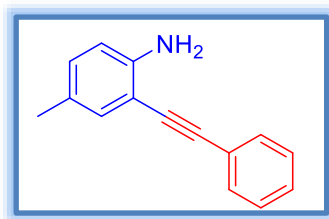
Yield: 82%; yellow solid; mp. 124.0-126.0 °C; FTIR (KBr, cm^{-1}): 3470, 3378, 3075, 2926, 2207, 1593, 1488, 1428, 1220, 1192, 1153, 1094, 882, 835, 815, 745; ^1H NMR (400 MHz, CDCl_3) δ : 7.52-7.47 (m, 2H, ArH), 7.07-7.02 (m, 3H, ArH), 6.90-6.85 (m, 1H, ArH), 6.67-6.63 (m, 1H, ArH), 4.12 (br, s, 2H, NH).

2-((4-Chlorophenyl)ethynyl)-4-fluoroaniline (3l)



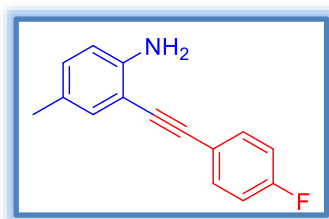
Yield: 83%; yellow solid; mp. 116.0-118.0 °C; FTIR (KBr, cm^{-1}): 3464, 3370, 3067, 3032, 2203, 1621, 1488, 1428, 1244, 1189, 1138, 1083, 1008, 878, 831, 807, 773; ^1H NMR (400 MHz, CDCl_3) δ : 7.46-7.43 (m, 2H, ArH), 7.35-7.32 (m, 2H, ArH), 7.06 (dd, $J = 7.6, 1.6$ Hz, 1H, ArH), 6.91-6.86 (m, 1H, ArH), 6.68-6.65 (m, 1H, ArH), 4.13 (br, s, 2H, NH).

4-Methyl-2-(phenylethynyl)aniline (3m)



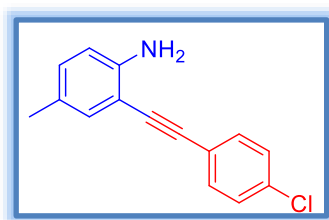
Yield: 87%; yellow solid; mp. 126.0-128.0 °C (lit. mp. 126.2-127.8 °C^[34]); FTIR (KBr, cm⁻¹): 3478, 3386, 3071, 2926, 2208, 1599, 1503, 1494, 1313, 1256, 1156, 814, 758, 693; ¹H NMR (400 MHz, CDCl₃) δ: 7.52-7.50 (m, 2H, ArH), 7.35-7.30 (m, 3H, ArH), 7.18 (d, *J* = 1.2 Hz, 1H, ArH), 6.95-6.93 (m, 1H, ArH), 6.63 (d, *J* = 8 Hz, 1H, ArH), 4.12 (br, s, 2H, NH), 2.22 (s, 3H, CH₃).

2-((4-Fluorophenyl)ethynyl)-4-methylaniline (3n)



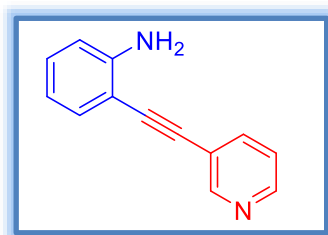
Yield: 85%; yellow solid; mp. 142.0-144.0 °C; FTIR (KBr, cm⁻¹): 3486, 3376, 3049, 2928, 2210, 1613, 1492, 1313, 1228, 1173, 949, 830, 756; ¹H NMR (400 MHz, CDCl₃) δ: 7.49-7.44 (m, 2H, ArH), 7.16 (d, *J* = 1.2 Hz, 1H, ArH), 7.05-6.99 (m, 2H, ArH), 6.95-6.93 (m, 1H, ArH), 6.62 (d, *J* = 8 Hz, 1H, ArH), 4.11 (br, s, 2H, NH), 2.21 (s, 3H, CH₃).

2-((4-Chlorophenyl)ethynyl)-4-methylaniline (3o)



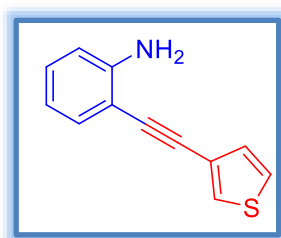
Yield: 87%; yellow solid; mp. 128.0-130.0 °C (lit. mp. 127.0-129.0 °C^[35]); FTIR (KBr, cm⁻¹): 3479, 3373, 3058, 3036, 2215, 1615, 1486, 1416, 1398, 1302, 1250, 1139, 1065, 820 746; ¹H NMR (400 MHz, CDCl₃) δ: 7.44-7.41 (m, 2H, ArH), 7.32-7.29 (m, 2H, ArH), 7.17 (d, *J* = 1.2 Hz, 1H, ArH), 6.97-6.94 (m, 1H, ArH), 6.63 (d, *J* = 12 Hz, 1H, ArH), 4.11 (br, s, 2H, NH), 2.22 (s, 3H, CH₃).

2-(Pyridin-3-ylethynyl)aniline (3p)



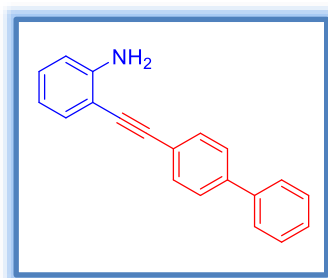
Yield: 84%; yellow solid; mp. 104.0-106.0 °C (lit. mp. 102.3-104.1 °C^[34]); FTIR (KBr, cm⁻¹): 3449, 3319, 3060, 3024, 2207, 1626, 1488, 1451, 1407, 1314, 1260, 1155, 1118, 1019, 806, 754, 699; ¹H NMR (400 MHz, CDCl₃) δ: 8.76-8.75 (m, 1H, ArH), 8.54 (dd, *J* = 5.2, 1.6 Hz, 1H, ArH), 7.80-7.77 (m, 1H, ArH), 7.38-7.36 (m, 1H, ArH), 7.29-7.25 (m, 1H, ArH), 7.18-7.14 (m, 1H, ArH), 6.74-6.70 (m, 2H, ArH), 4.29 (br, s, 2H, NH).

2-(Thiophen-3-ylethynyl)aniline (3q)



Yield: 86%; yellow solid; mp. 168.0-170.0 °C (lit. mp. 166.9-168.2 °C^[34]); FTIR (KBr, cm⁻¹): 3440, 3376, 3108, 2968, 2205, 1614, 1497, 1310, 1244, 1169, 943, 827, 747; ¹H NMR (400 MHz, CDCl₃) δ: 7.51-7.50 (m, 1H, ArH), 7.36-7.34 (m, 1H, ArH), 7.32-7.30 (m, 1H, ArH), 7.20-7.19 (m, 1H, ArH), 7.16-7.12 (m, 1H, ArH), 6.73-6.69 (m, 2H, ArH), 4.26 (br, s, 2H, NH).

2-([1,1'-Biphenyl]-4-ylethynyl)aniline (3r)



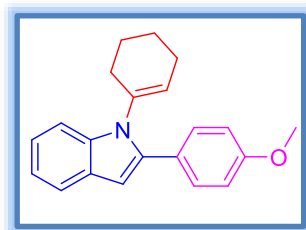
Yield: 87%; yellow solid; mp. 132.0-134.0 °C; FTIR (KBr, cm⁻¹): 3478, 3379, 3028, 2918, 2844, 2215, 1606, 1480, 1444, 1311, 1256, 1158, 1001, 832, 757, 694; ¹H NMR (400 MHz,

CDCl₃) δ : 7.62-7.59 (m, 6H, ArH), 7.45 (t, 2H, ArH), 7.39-7.34 (m, 2H, ArH), 7.16-7.12 (m, 1H, ArH), 6.74-6.70 (m, 2H, ArH), 4.28 (br, s, 2H, NH).

5.2.3 Procedure for the synthesis of N-alkenyndole derivatives (5a-5s)

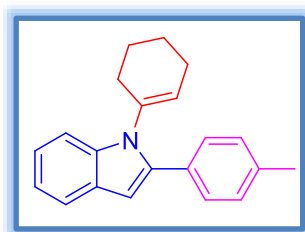
2-(1-Alkynyl)aniline **3** (1 equiv), cyclic ketone **4** (1 equiv), and iodine (0.2 equiv) were placed in toluene:DMF (20:1 v/v) solvent ratio in an oven-dried vial. The reaction mixture was then stirred at 80 °C for 6 h. After the reaction was over, it was allowed to cool and was quenched with aqueous sodium thiosulfate solution (20 mL). The aqueous layer was extracted with ethyl acetate (3 $\times$ 20 mL). The combined organic layer was washed with brine solution, was dried using anhydrous Na₂SO₄, and was evaporated under reduced pressure. The crude product thus obtained was purified through column chromatography with the help of petroleum ether as eluent.

1-(Cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (5a)



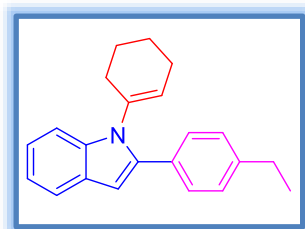
Yield: 71%; white solid; mp. 114.0-116.0 °C; FTIR (KBr, cm⁻¹): 3052, 2997, 2929, 2828, 1664, 1601, 1531, 1496, 1448, 1370, 1342, 1306, 1244, 1173, 1023, 839, 780, 729; ¹H NMR (400 MHz, CDCl₃) δ : 7.60-7.55 (m, 3H, ArH), 7.37 (d, *J* = 8 Hz, 1H, ArH), 7.18-7.09 (m, 2H, ArH), 6.95-6.91 (m, 2H, ArH), 6.59 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 3.84 (s, 3H, OCH₃), 2.36-2.26 (m, 2H, Cyclohexenyl-CH₂), 1.98-1.90 (m, 2H, Cyclohexenyl-CH₂), 1.67-1.65 (m, 4H, Cyclohexenyl-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ : 159.3, 139.8, 138.2, 136.1, 129.3, 128.3, 127.8, 126.2, 121.6, 120.2, 113.9, 110.6, 101.7, 55.4, 29.3, 25.1, 23.0, 21.9; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₁H₂₂NO 304.1701; Found 304.1581.

1-(Cyclohex-1-en-1-yl)-2-(p-tolyl)-1H-indole (5b)



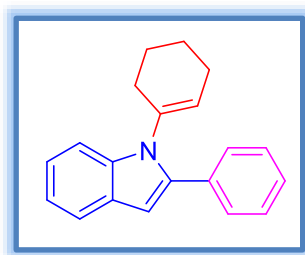
Yield: 73%; white solid; mp. 106.0-108.0 °C; FTIR (KBr, cm^{-1}): 3018, 2955, 2914, 2833, 1666, 1499, 1444, 1370, 1340, 1308, 1178, 1065, 1023, 919, 824, 780, 735; ^1H NMR (400 MHz, CDCl_3) δ : 7.61-7.58 (m, 1H, ArH), 7.54-7.51 (m, 2H, ArH), 7.38-7.36 (m, 1H, ArH), 7.20-7.17 (m, 3H, ArH), 7.15-7.09 (m, 1H, ArH), 6.63 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 2.38 (s, 3H, CH_3), 2.34-2.27 (m, 2H, Cyclohexenyl- CH_2), 1.99-1.91 (m, 2H, Cyclohexenyl- CH_2), 1.67-1.65 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 140.0, 138.4, 137.4, 136.1, 130.7, 129.2, 128.3, 128.0, 127.7, 121.7, 120.3, 120.2, 110.7, 102.2, 29.4, 25.1, 23.0, 21.9, 21.4; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{22}\text{N}$ 288.1752; Found 288.1603.

1-(Cyclohex-1-en-1-yl)-2-(4-ethylphenyl)-1H-indole (5c)



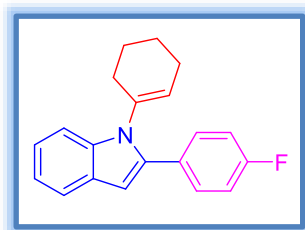
Yield: 72%; yellow liquid; FTIR (KBr, cm^{-1}): 3054, 2991, 2932, 2827, 1661, 1605, 1533, 1498, 1449, 1372, 1340, 1305, 1246, 1176, 1021, 842, 781, 731; ^1H NMR (400 MHz, CDCl_3) δ : 7.61-7.54 (m, 3H, ArH), 7.37 (d, $J = 8$ Hz, 1H, ArH), 7.22-7.09 (m, 4H, ArH), 6.64 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 2.68 (q, 2H, CH_2), 2.36-2.24 (m, 2H, Cyclohexenyl- CH_2), 2.00-1.90 (m, 2H, Cyclohexenyl- CH_2), 1.66-1.65 (m, 4H, Cyclohexenyl- CH_2), 1.27 (t, 3H, CH_3); ^{13}C NMR (100 MHz, CDCl_3) δ : 143.7, 140.0, 138.4, 136.1, 130.9, 128.3, 128.0, 127.7, 121.7, 120.4, 120.2, 110.7, 102.2, 29.4, 28.8, 25.1, 23.0, 22.0, 15.5.

1-(Cyclohex-1-en-1-yl)-2-phenyl-1H-indole (5d)



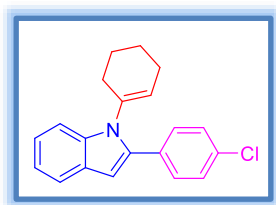
Yield: 70%; white solid; mp. 104.0-106.0 °C (lit. mp. 104.2-105.9 °C^[26]); FTIR (KBr, cm⁻¹): 3021, 2953, 2917, 2831, 1669, 1492, 1448, 1365, 1301, 1170, 1021, 920, 827, 784, 738; ¹H NMR (400 MHz, CDCl₃) δ: 7.65-7.61 (m, 3H, ArH), 7.42-7.38 (m, 3H, ArH), 7.35-7.31 (m, 1H, ArH), 7.21-7.17 (m, 1H, ArH), 7.14-7.11 (m, 1H, ArH), 6.68 (s, 1H, AlkenylH), 6.06-6.04 (m, 1H, Cyclohexenyl-CH), 2.38-2.25 (m, 2H, Cyclohexenyl-CH₂), 2.04-1.87 (m, 2H, Cyclohexenyl-CH₂), 1.67-1.66 (m, 4H, Cyclohexenyl-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 139.8, 138.4, 135.9, 133.6, 128.4, 128.2, 128.1, 127.8, 127.6, 121.9, 120.4, 120.2, 110.7, 102.6, 29.4, 25.0, 22.9, 21.9; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₀H₂₀N 274.1596; Found 274.1406.

1-(Cyclohex-1-en-1-yl)-2-(4-fluorophenyl)-1H-indole (5e)



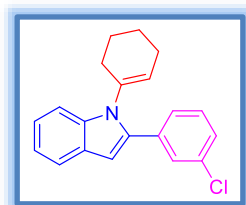
Yield: 64%; white solid; mp. 136.0-138.0 °C; FTIR (KBr, cm⁻¹): 3052, 2946, 2919, 2836, 1665, 1590, 1492, 1452, 1370, 1339, 1315, 1217, 1153, 925, 839, 784, 737; ¹H NMR (400 MHz, CDCl₃) δ: 7.61-7.55 (m, 3H, ArH), 7.38-7.36 (m, 1H, ArH), 7.21-7.17 (m, 1H, ArH), 7.14-7.05 (m, 3H, ArH), 6.62 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 2.39-2.22 (m, 2H, Cyclohexenyl-CH₂), 2.00-1.89 (m, 2H, Cyclohexenyl-CH₂), 1.66-1.65 (m, 4H, Cyclohexenyl-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 163.7, 161.3, 138.8, 138.3, 135.8, 129.8, 128.2, 122.0, 120.4, 115.6, 115.4, 110.7, 102.5, 29.4, 25.0, 22.9, 21.9; HRMS (ESI-TOF) *m/z*: [M + H]⁺ Calcd for C₂₀H₁₉FN 292.1502; Found 292.1358.

2-(4-Chlorophenyl)-1-(cyclohex-1-en-1-yl)-1H-indole (5f)



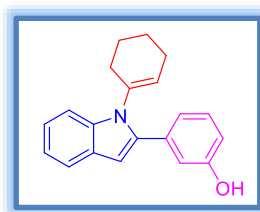
Yield: 67%; white solid; mp. 128.0-130.0 °C; FTIR (KBr, cm^{-1}): 3062, 3053, 2927, 2837, 1664, 1585, 1486, 1449, 1367, 1341, 1310, 1173, 1087, 1016, 833, 785, 733; ^1H NMR (400 MHz, CDCl_3) δ : 7.62-7.59 (m, 1H, ArH), 7.58-7.54 (m, 2H, ArH), 7.38-7.34 (m, 3H, ArH), 7.22-7.17 (m, 1H, ArH), 7.14-7.10 (m, 1H, ArH), 6.66 (s, 1H, AlkenylH), 6.05-6.02 (m, 1H, Cyclohexenyl-CH), 2.39-2.22 (m, 2H, Cyclohexenyl- CH_2), 2.00-1.89 (m, 2H, Cyclohexenyl- CH_2), 1.68-1.66 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 138.5, 135.8, 133.6, 132.1, 129.2, 128.7, 128.2, 128.1, 122.2, 120.5, 120.4, 110.8, 102.9, 29.4, 25.0, 22.9, 21.9; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{20}\text{H}_{19}\text{ClN}$ 308.1206; Found 308.1063.

2-(3-Chlorophenyl)-1-(cyclohex-1-en-1-yl)-1H-indole (5g)



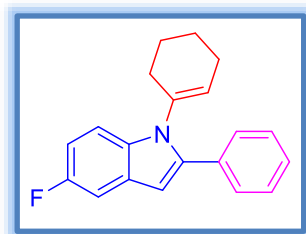
Yield: 63%; yellow liquid; FTIR (KBr, cm^{-1}): 3067, 3055, 2935, 2843, 1669, 1590, 1489, 1450, 1369, 1321, 1302, 1164, 1081, 1010, 831, 778, 728; ^1H NMR (400 MHz, CDCl_3) δ : 7.64-7.59 (m, 2H, ArH), 7.50-7.47 (m, 1H, ArH), 7.38-7.36 (m, 1H, ArH), 7.26-7.25 (m, 2H, ArH), 7.21-7.17 (m, 1H, ArH), 7.13-7.09 (m, 1H, ArH), 6.67 (s, 1H, AlkenylH), 6.01-5.99 (m, 1H, Cyclohexenyl-CH), 2.31-2.25 (m, 2H, Cyclohexenyl- CH_2), 2.02-1.87 (m, 2H, Cyclohexenyl- CH_2), 1.66-1.64 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 138.4, 137.9, 135.5, 135.1, 134.2, 129.5, 128.2, 127.9, 127.7, 127.3, 125.9, 122.2, 120.5, 120.3, 110.6, 103.1, 29.3, 24.9, 22.7, 21.7.

3-(1-(Cyclohex-1-en-1-yl)-1H-indol-2-yl)phenol (5h)



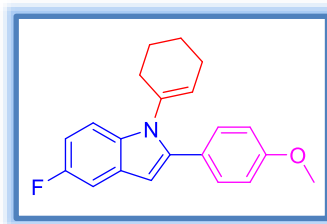
Yield: 69%; brown liquid; FTIR (KBr, cm^{-1}): 3251, 3060, 3045, 2930, 2855, 1658, 1602, 1587, 1488, 1452, 1368, 1345, 1255, 1172, 1090, 1020, 845, 785, 731; ^1H NMR (400 MHz, CDCl_3) δ : 7.58 (d, $J = 8$ Hz, 1H, ArH), 7.37 (d, $J = 8$ Hz, 1H, ArH), 7.21-7.15 (m, 3H, ArH), 7.12-7.08 (m, 2H, ArH), 6.76-6.73 (m, 1H, ArH), 6.62 (s, 1H, AlkenylH), 5.99-5.97 (m, 1H, Cyclohexenyl-CH), 2.30-2.18 (m, 2H, Cyclohexenyl- CH_2), 2.02-1.83 (m, 2H, Cyclohexenyl- CH_2), 1.62-1.60 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 155.6, 139.3, 138.3, 135.7, 134.8, 129.4, 128.0, 127.5, 121.8, 120.3, 120.2, 120.1, 114.8, 114.5, 110.6, 102.5, 29.1, 24.8, 22.7, 21.7.

1-(Cyclohex-1-en-1-yl)-5-fluoro-2-phenyl-1H-indole (5i)



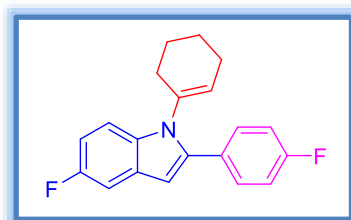
Yield: 61%; white solid; mp. 116.0-118.0 $^{\circ}\text{C}$; FTIR (KBr, cm^{-1}): 3041, 2922, 2881, 2854, 1665, 1616, 1579, 1464, 1441, 1374, 1348, 1200, 1126, 1076, 1026, 919, 850, 795, 753, 733, 691; ^1H NMR (400 MHz, CDCl_3) δ : 7.62-7.59 (m, 2H, ArH), 7.41-7.36 (m, 2H, ArH), 7.34-7.32 (m, 1H, ArH), 7.31-7.23 (m, 2H, ArH), 6.92 (tt, 1H, ArH), 6.61 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 2.37-2.22 (m, 2H, Cyclohexenyl- CH_2), 1.99-1.83 (m, 2H, Cyclohexenyl- CH_2), 1.65-1.63 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 159.5, 157.2, 141.3, 136.0, 135.0, 133.3, 128.5, 128.1, 127.9, 111.4, 111.3, 110.3, 110.0, 105.2, 104.9, 102.5, 102.4, 29.3, 25.0, 22.9, 21.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{20}\text{H}_{19}\text{FN}$ 292.1502; Found 292.1358.

1-(Cyclohex-1-en-1-yl)-5-fluoro-2-(4-methoxyphenyl)-1H-indole (5j)



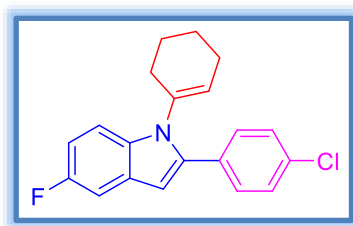
Yield: 65%; white solid; mp. 102.0-104.0 °C; FTIR (KBr, cm^{-1}): 3000, 2949, 2907, 2832, 1665, 1603, 1573, 1536, 1499, 1462, 1372, 1343, 1243, 1171, 1106, 1024, 952, 841, 786, 759; ^1H NMR (400 MHz, CDCl_3) δ : 7.56-7.52 (m, 2H, ArH), 7.28-7.21 (m, 2H, ArH), 6.95-6.87 (m, 3H, ArH), 6.54 (s, 1H, AlkenylH), 6.04-6.02 (m, 1H, Cyclohexenyl-CH), 3.84 (s, 3H, OCH_3), 2.35-2.26 (m, 2H, Cyclohexenyl- CH_2), 1.96-1.86 (m, 2H, Cyclohexenyl- CH_2), 1.66-1.65 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 159.4, 157.1, 141.3, 136.1, 134.8, 129.3, 128.5, 128.4, 128.0, 125.8, 113.9, 111.3, 111.2, 109.8, 109.6, 104.9, 104.7, 101.5, 55.4, 29.2, 25.0, 22.9, 21.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{21}\text{H}_{21}\text{FNO}$ 322.1607; Found 322.1443.

1-(Cyclohex-1-en-1-yl)-5-fluoro-2-(4-fluorophenyl)-1H-indole (5k)



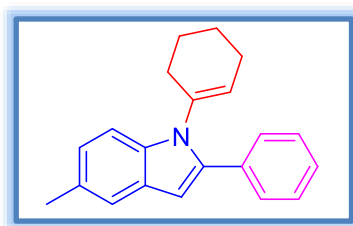
Yield: 60%; white solid; mp. 106.0-108.0 °C; FTIR (KBr, cm^{-1}): 2933, 2893, 2846, 1666, 1621, 1583, 1540, 1497, 1466, 1444, 1411, 1375, 1340, 1222, 1182, 1160, 1099, 840, 798, 769; ^1H NMR (400 MHz, CDCl_3) δ : 7.60-7.55 (m, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 7.13-7.07 (m, 2H, ArH), 6.92 (tt, 1H, ArH), 6.57 (s, 1H, AlkenylH), 6.05-6.03 (m, 1H, Cyclohexenyl-CH), 2.39-2.23 (m, 2H, Cyclohexenyl- CH_2), 1.98-1.82 (m, 2H, Cyclohexenyl- CH_2), 1.66-1.65 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 163.8, 161.4, 159.5, 157.2, 140.3, 135.8, 134.9, 129.8, 129.7, 129.4, 128.4, 128.3, 128.2, 115.6, 115.4, 111.4, 111.3, 110.4, 110.1, 105.2, 104.9, 102.4, 102.3, 29.3, 25.0, 22.8, 21.8; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{20}\text{H}_{18}\text{F}_2\text{N}$ 310.1407; Found 310.1209.

2-(4-Chlorophenyl)-1-(cyclohex-1-en-1-yl)-5-fluoro-1H-indole (5l)



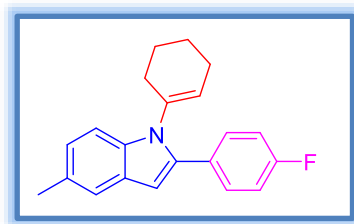
Yield: 62%; white solid; mp. 122.0-124.0 °C; FTIR (KBr, cm^{-1}): 2963, 2903, 2865, 2832, 1665, 1608, 1575, 1529, 1465, 1443, 1409, 1375, 1342, 1199, 1186, 1124, 1081, 1009, 835, 777, 732; ^1H NMR (400 MHz, CDCl_3) δ : 7.56-7.53 (m, 2H, ArH), 7.39-7.35 (m, 2H, ArH), 7.29-7.22 (m, 2H, ArH), 6.93 (tt, 1H, ArH), 6.60 (s, 1H, AlkenylH), 6.05-6.03 (m, 1H, Cyclohexenyl-CH), 2.37-2.23 (m, 2H, Cyclohexenyl- CH_2), 1.98-1.84 (m, 2H, Cyclohexenyl- CH_2), 1.67-1.66 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 159.5, 157.2, 140.0, 135.8, 135.1, 133.8, 131.7, 129.2, 128.8, 128.5, 128.2, 128.1, 111.5, 111.4, 110.6, 110.4, 105.2, 105.0, 102.8, 102.7, 29.3, 25.0, 22.9, 21.8.

1-(Cyclohex-1-en-1-yl)-5-methyl-2-phenyl-1H-indole (5m)



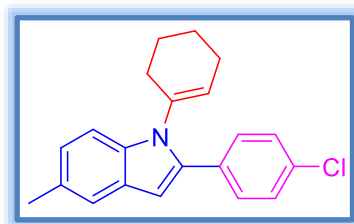
Yield: 72%; white solid; mp. 102.0-104.0 °C; FTIR (KBr, cm^{-1}): 3021, 2937, 2924, 2849, 1669, 1597, 1471, 1441, 1376, 1323, 1304, 1205, 1180, 1138, 1074, 914, 869, 794, 757, 696; ^1H NMR (400 MHz, CDCl_3) δ : 7.63-7.61 (m, 2H, ArH), 7.39-7.36 (m, 3H, ArH), 7.32-7.26 (m, 2H, ArH), 7.02-7.00 (m, 1H, ArH), 6.59 (s, 1H, AlkenylH), 6.03-6.01 (m, 1H, Cyclohexenyl-CH), 2.45 (s, 3H, CH_3), 2.33-2.26 (m, 2H, Cyclohexenyl- CH_2), 1.97-1.90 (m, 2H, Cyclohexenyl- CH_2), 1.65-1.64 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 139.9, 136.9, 136.1, 133.7, 129.5, 128.5, 128.4, 128.0, 127.5, 123.5, 120.1, 110.4, 102.2, 29.3, 25.0, 23.0, 21.9, 21.5.

1-(Cyclohex-1-en-1-yl)-2-(4-fluorophenyl)-5-methyl-1H-indole (5n)



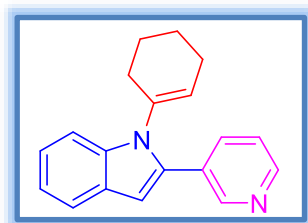
Yield: 66%; white solid; mp. 110.0-112.0 °C; FTIR (KBr, cm^{-1}): 3024, 2923, 2865, 2836, 1667, 1544, 1499, 1471, 1443, 1371, 1320, 1214, 1149, 1091, 917, 870, 839, 791, 743; ^1H NMR (400 MHz, CDCl_3) δ : 7.59-7.55 (m, 2H, ArH), 7.39-7.38 (m, 1H, ArH), 7.26-7.23 (m, 1H, ArH), 7.10-7.04 (m, 2H, ArH), 7.02-7.00 (m, 1H, ArH), 6.54 (s, 1H, AlkenylH), 6.02-6.00 (m, 1H, Cyclohexenyl-CH), 2.45 (s, 3H, CH_3), 2.32-2.27 (m, 2H, Cyclohexenyl- CH_2), 1.97-1.86 (m, 2H, Cyclohexenyl- CH_2), 1.66-1.64 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 163.6, 161.2, 138.9, 136.8, 136.0, 129.8, 129.7, 129.6, 128.4, 127.9, 123.6, 120.1, 115.5, 115.3, 110.4, 102.1, 29.3, 25.0, 22.9, 21.9, 21.5.

2-(4-Chlorophenyl)-1-(cyclohex-1-en-1-yl)-5-methyl-1H-indole (5o)



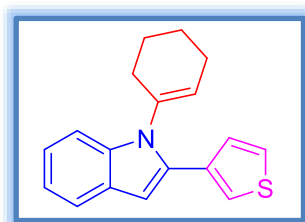
Yield: 68%; white solid; mp. 118.0-120.0 °C; FTIR (KBr, cm^{-1}): 3024, 2941, 2915, 2832, 1668, 1541, 1471, 1444, 1412, 1375, 1321, 1205, 1172, 1084, 1014, 919, 870, 836, 791, 744; ^1H NMR (400 MHz, CDCl_3) δ : 7.56-7.53 (m, 2H, ArH), 7.39-7.33 (m, 3H, ArH), 7.27-7.24 (m, 1H, ArH), 7.03-7.01 (m, 1H, ArH), 6.57 (s, 1H, AlkenylH), 6.03-6.01 (m, 1H, Cyclohexenyl-CH), 2.45 (s, 3H, CH_3), 2.33-2.27 (m, 2H, Cyclohexenyl- CH_2), 2.00-1.85 (m, 2H, Cyclohexenyl- CH_2), 1.67-1.65 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 138.5, 137.0, 135.9, 133.4, 132.2, 129.7, 129.1, 128.6, 128.3, 127.9, 123.8, 120.1, 110.4, 102.5, 29.3, 25.0, 22.9, 21.9, 21.5.

1-(Cyclohex-1-en-1-yl)-2-(pyridin-3-yl)-1H-indole (5p)



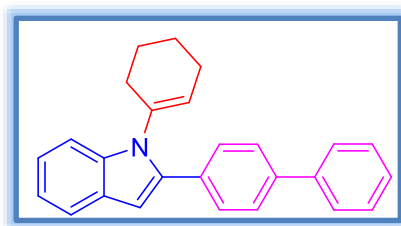
Yield: 58%; white solid; mp. 114.0-116.0 °C; FTIR (KBr, cm^{-1}): 3026, 2935, 2912, 2850, 1662, 1588, 1542, 1470, 1410, 1352, 1315, 1202, 1175, 1088, 1018, 910, 868, 812, 770; ^1H NMR (400 MHz, CDCl_3) δ : 8.90-8.89 (m, 1H, ArH), 8.57-8.56 (m, 1H, ArH), 7.93-7.90 (m, 1H, ArH), 7.64 (d, $J = 8$ Hz, 1H, ArH), 7.40-7.37 (m, 1H, ArH), 7.35-7.32 (m, 1H, ArH), 7.25-7.21 (m, 1H, ArH), 7.17-7.13 (m, 1H, ArH), 6.74 (s, 1H, AlkenylH), 6.07-6.05 (m, 1H, Cyclohexenyl-CH), 2.35-2.32 (m, 4H, Cyclohexenyl- CH_2), 2.04-1.96 (m, 2H, Cyclohexenyl- CH_2), 1.89-1.83 (m, 2H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 148.9, 148.6, 138.6, 136.2, 135.5, 135.0, 129.5, 129.0, 128.0, 123.3, 122.5, 120.7, 120.5, 110.8, 103.6, 29.5, 25.0, 22.8, 21.8.

1-(Cyclohex-1-en-1-yl)-2-(thiophen-3-yl)-1H-indole (5q)



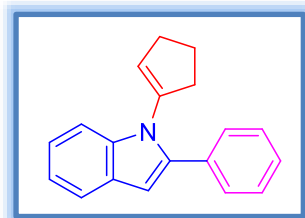
Yield: 56%; yellow liquid; FTIR (KBr, cm^{-1}): 3096, 2940, 2906, 2835, 1665, 1510, 1457, 1412, 1354, 1334, 1320, 1211, 1188, 1164, 1082, 1018, 945, 920, 800, 772, 749; ^1H NMR (400 MHz, CDCl_3) δ : 7.59 (d, $J = 8$ Hz, 1H, ArH), 7.52-7.51 (m, 1H, ArH), 7.40-7.39 (m, 1H, ArH), 7.34-7.31 (m, 2H, ArH), 7.19-7.15 (m, 1H, ArH), 7.12-7.08 (m, 1H, ArH), 6.69 (s, 1H, AlkenylH), 6.08-6.06 (m, 1H, Cyclohexenyl-CH), 2.38-2.30 (m, 2H, Cyclohexenyl- CH_2), 2.10-2.02 (m, 2H, Cyclohexenyl- CH_2), 1.75-1.73 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 138.2, 136.2, 135.1, 134.0, 128.5, 128.1, 127.7, 125.4, 121.9, 121.2, 120.4, 120.2, 110.4, 101.8, 29.1, 25.1, 23.0, 21.9; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{18}\text{H}_{18}\text{NS}$ 280.1160; Found 280.1129.

2-([1,1'-Biphenyl]-4-yl)-1-(cyclohex-1-en-1-yl)-1H-indole (5r)



Yield: 60%; white solid; mp. 122.0-124.0 °C; FTIR (KBr, cm^{-1}): 3028, 2929, 2905, 2852, 2830, 1673, 1600, 1483, 1448, 1374, 1340, 1316, 1189, 1071, 1005, 922, 844, 785, 762, 748, 699; ^1H NMR (400 MHz, CDCl_3) δ : 7.73-7.71 (m, 2H, ArH), 7.66-7.61 (m, 5H, ArH), 7.47-7.33 (m, 4H, ArH), 7.22-7.11 (m, 2H, ArH), 6.73 (s, 1H, AlkenylH), 6.09-6.07 (m, 1H, Cyclohexenyl-CH), 2.37-2.30 (m, 2H, Cyclohexenyl- CH_2), 2.06-1.94 (m, 2H, Cyclohexenyl- CH_2), 1.69-1.68 (m, 4H, Cyclohexenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 140.7, 140.2, 139.4, 138.6, 136.1, 132.5, 129.0, 128.3, 127.9, 127.5, 127.1, 122.0, 120.5, 120.3, 110.7, 102.8, 29.4, 25.1, 23.0, 21.9; HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{24}\text{N}$ 350.1909; Found 350.1779.

1-(Cyclopent-1-en-1-yl)-2-phenyl-1H-indole (5s)



Yield: 58%; white solid; mp. 102.0-104.0 °C; FTIR (KBr, cm^{-1}): 3022, 2951, 2919, 2850, 1663, 1491, 1446, 1362, 1298, 1168, 1020, 922, 824, 781, 736; ^1H NMR (400 MHz, CDCl_3) δ : 7.50-7.45 (m, 3H, ArH), 7.31 (d, $J = 8$ Hz, 1H, ArH), 7.25-7.15 (m, 3H, ArH), 7.10-6.99 (m, 2H, ArH), 6.54 (s, 1H, AlkenylH), 5.82-5.74 (m, 1H, Cyclopentenyl-CH), 2.41-2.37 (m, 2H, Cyclopentenyl- CH_2), 2.12-2.08 (m, 2H, Cyclopentenyl- CH_2), 1.81-1.73 (m, 2H, Cyclopentenyl- CH_2); ^{13}C NMR (100 MHz, CDCl_3) δ : 139.0, 138.8, 137.1, 132.4, 127.3, 127.2, 126.9, 126.5, 125.1, 121.0, 119.3, 109.8, 102.1, 32.9, 29.5, 21.5.

5.2.4 Antioxidant studies of *N*-alkenylindole derivatives

Synthesized *N*-alkenylindole compounds **5** were estimated for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [36]. Firstly, stock solutions of compounds were prepared in methanol solvent. Then, 1 mL of each

solution was mixed with 3 mL of freshly prepared DPPH solution (4 mg in 100 mL of methanol). DPPH solution was allowed to react with solutions for 30 minutes in dark at room temperature. After 30 minutes, absorbance values were measured on PerkinElmer UV-Visible spectrophotometer at 517 nm. DPPH solution in methanol was used as blank. Gallic acid solution in methanol was used as reference standard. Percentage of antioxidant activity, AA (%) of all the compounds including gallic acid were calculated using this formula:

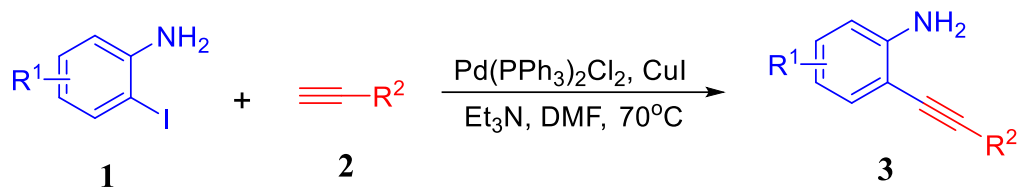
$$AA (\%) = [(Ab - As)/As] * 100$$

Where Ab = Absorbance of blank; As = Absorbance of sample

5.3 Results and Discussion

5.3.1 Synthesis of 2-(1-alkynyl)anilines (3)

Firstly, we synthesized starting materials 2-(1-alkynyl)anilines using Sonogashira coupling reaction in 82-90% yields. Substituted 2-iodoanilines **1** underwent Sonogashira coupling reaction with terminal alkynes **2** with the help of Pd(PPh₃)₂Cl₂ and CuI catalysts to give 2-(1-alkynyl)anilines **3** in the presence of triethylamine (Et₃N) as a base [37] (**Scheme 5.3**). The prepared starting materials were characterized with melting point, FTIR, and ¹H NMR and their comparison with literature. The FT-IR spectrum of 2-((4-ethylphenyl)ethynyl)aniline (**3a**) exhibits two N-H stretching vibrations at 3472 and 3370 cm⁻¹ due to asymmetrical and symmetrical N-H stretchings for primary amine group, respectively. The peaks at 2953, 2926, and 2867 cm⁻¹ correspond to C-H stretching vibrations. A characteristic peak at 2203 cm⁻¹ corresponds to the alkyne (C≡C) stretching vibration (**Figure 5.2**). Thus, the FT-IR spectrum of **3a** confirms the presence of primary amine and alkyne groups in the molecule. The ¹H NMR spectrum of **3a** (**Figure 5.3**) displays peaks due to NH₂, aromatic, and aliphatic protons. A characteristic broad singlet at 4.25 ppm corresponds to the NH₂ protons. The aromatic protons of the phenyl rings show chemical shift values between 7.45-6.68 ppm. Aliphatic CH₂ protons of ethyl group attached to phenyl group exhibit quartet at 2.65 ppm, whereas aliphatic CH₃ protons of ethyl group display triplet at 1.23 ppm. The total integration value of all protons is 15, which matches with the total number of protons in the structure of **3a**. In a similar manner, the characterization of other synthesized 2-(1-alkynyl)anilines starting materials has been done (Experimental Section 5.2.2).



$\text{R}^1 = \text{H, F, CH}_3$;

$\text{R}^2 = \text{C}_6\text{H}_5, 4\text{-Me-C}_6\text{H}_4, 4\text{-Et-C}_6\text{H}_4, 4\text{-OMe-C}_6\text{H}_4, 4\text{-F-C}_6\text{H}_4, 4\text{-Cl-C}_6\text{H}_4, 3\text{-Cl-C}_6\text{H}_4, 3\text{-OH-C}_6\text{H}_4, \text{pyridin-3-yl, thiophenyl-3-yl, biphenyl-3-yl}$

Scheme 5.3 Synthetic methodology for 2-(1-alkynyl)aniline derivatives (**3**)

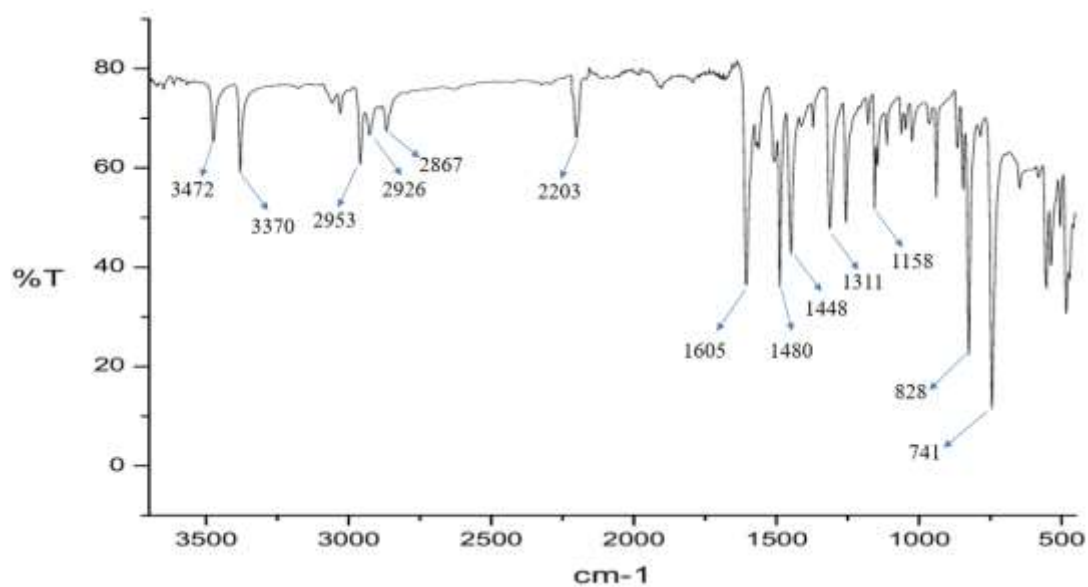
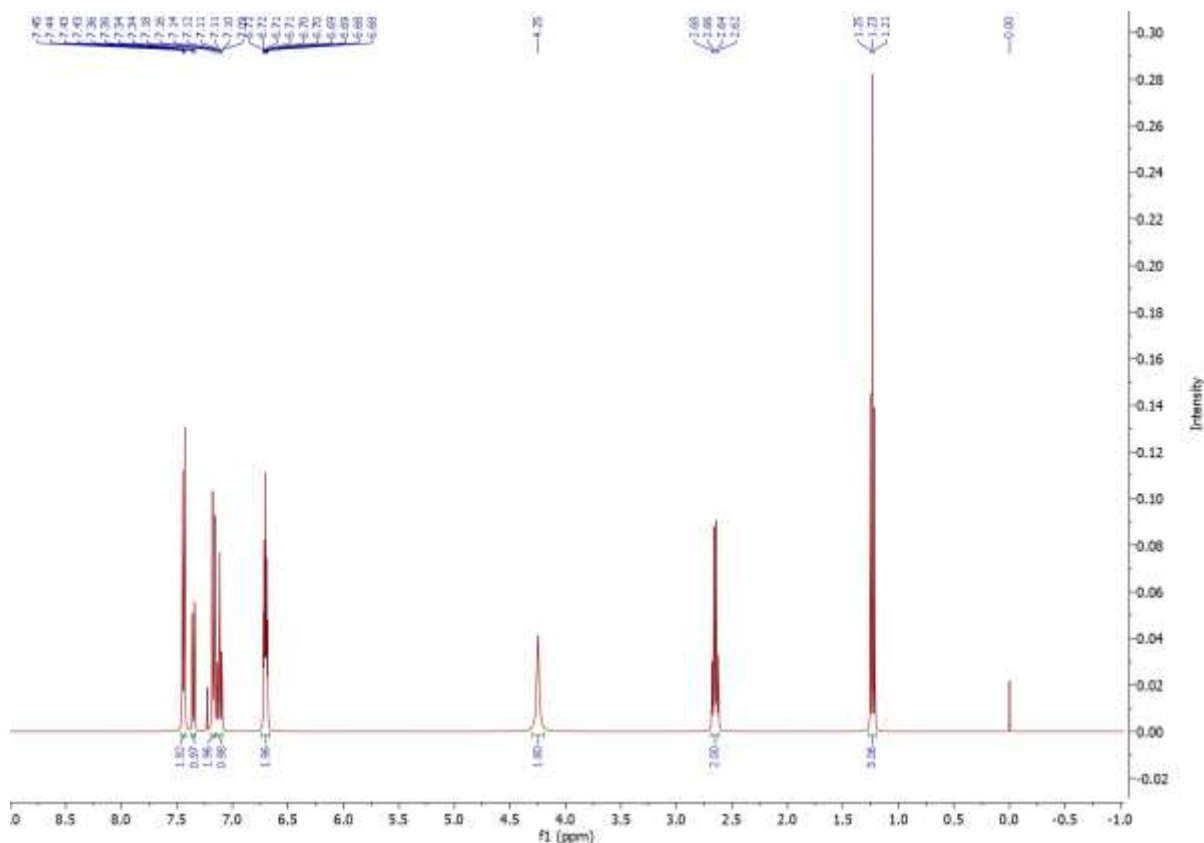


Figure 5.2 FT-IR spectrum of 2-((4-ethylphenyl)ethynyl)aniline (**3a**)



(**Entry 10, Table 5.1**) while at 60 °C temperature, **5d** was formed with 38 % yield (**Entry 11, Table 5.1**). Increasing the temperature from 80 °C to 100 °C did not change the % yield appreciably (**Entry 12, Table 5.1**). Furthermore, we changed the equiv of iodine from 0.2 equiv to 0.1 equiv and 0.4 equiv. With 0.1 equiv of iodine, **5d** was obtained with 42% yield (**Entry 13, Table 5.1**) whereas with 0.4 equiv of iodine, **5d** was attained with 54% yield (**Entry 14, Table 5.1**). Thus, the optimized conditions achieved for the reaction include 0.2 equiv of molecular iodine in solvent toluene:DMF (20:1 v/v), and at temperature 80 °C.

Table 5.1 Optimization studies for the synthesis of **5d** using **3c** and **4**

Entry	I ₂ (equiv)	Solvent	Temperature (°C)	Yield of 5d (%)
1	0.2	Toluene	80	60
2	0.2	DMF	80	NR
3	0.2	1,4-Dioxane	80	Trace
4	0.2	DMSO	80	NR
5	0.2	Chloroform	60	Trace
6	0.2	Ethanol	78	NR
7	0.2	THF	60	Trace
8	0.2	MeCN	80	NR
9	0.2	Toluene:DMF (20:1)	80	70
10	0.2	Toluene:DMF (20:1)	rt	20
11	0.2	Toluene:DMF (20:1)	60	38
12	0.2	Toluene:DMF (20:1)	100	67
13	0.1	Toluene:DMF (20:1)	80	42
14	0.4	Toluene:DMF (20:1)	80	54

The scope of the reaction was explored after optimizing the reaction conditions. A total of 19 derivatives of *N*-alkenylindoles were synthesized (**Scheme 5.4**). These derivatives were obtained in good to moderate yields (56-73%). The reaction was well tolerated with both electron-donating and electron-withdrawing substituents. The reaction was also compatible with substituents having hetero-aromatic rings, pyridine and thiophene which gave products **5p** and **5q** in yields of 58% and 56%, respectively. In addition, biphenyl-substituted substrate produced product **5r** in a 60% yield. To check the applicability of another cyclic ketone, cyclopentanone was used as substrate to get the product **5s** in 58% yield. In contrast, no product was formed in the case of the acyclic ketone, and the starting materials remained intact. This indicated that cyclic ketone is necessary for the reaction to occur. Additionally, when 2-(1-alkynyl)aniline substrate with aliphatic substituent attached to alkyne was used, again, no product formation was observed, and the substrates remained intact. Thus, these results indicated that the reaction is well compatible with 2-(1-alkynyl)aniline substrates bearing aromatic alkynes with both electron-donating and electron-withdrawing groups, alkynes with heteroaromatic rings and other cyclic ketone substrates.

All the synthesized *N*-alkenylindoles were characterized by melting point, FT-IR, ¹H NMR, ¹³C NMR, and HRMS characterization techniques (Experimental Section **5.2.3**). In the FT-IR spectrum of 1-(cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (**5a**), vibrational stretching frequencies at 3052, 2997, 2929, and 2828 cm⁻¹ correspond to C-H stretching vibrations. A characteristic peak at 1664 cm⁻¹ is attributed to C=C stretching vibration, indicating the presence of the cyclohex-1-enyl ring in compound **5a** (**Figure 5.4**).

The ¹H NMR spectrum of **5a** displays a singlet at 6.59 ppm corresponding to the indole C-3 proton. A multiplet integrating for one proton at 6.04-6.02 ppm is attributed to the vinylic proton of the cyclohex-1-enyl ring. The multiplets corresponding to the aliphatic CH₂ protons of the cyclohex-1-enyl ring appear in the range of 2.36–1.65 ppm. The methoxy protons (OCH₃) attached to the phenyl ring display a singlet at 3.84 ppm with integration value of 3. All the aromatic protons of phenyl rings lie in the range of 7.60-6.91 ppm. The total integration value of all the protons is 21, which matches with the total number of protons of **5a** (**Figure 5.5**).

Furthermore, the ¹³C NMR spectrum of **5a** shows a characteristic peak at 159.3 ppm corresponding to the aromatic carbon atom attached to the methoxy group (**Figure 5.6**). The methoxy carbon exhibits chemical shift at 55.4 ppm. The aliphatic saturated carbons of the cyclohex-1-enyl ring display chemical shift in the range of 29.3-21.9 ppm. The vinylic carbon of the cyclohex-1-enyl ring shows a signal at 110.6 ppm. The C-3 carbon of the indole ring

exhibit peak at 101.7 ppm. The remaining carbon atoms (aromatic carbon atoms) display chemical shift in the range of 139.8-113.9 ppm. In the HRMS spectrum of **5a**, the peak at m/z 304.1701 corresponds to $[M+H]^+$ which is matched with the calculated $[M+H]^+$ value of **5a** (304.1581) (**Figure 5.7**). In a similar way, the characterization of other derivatives of *N*-alkenylindoles is done (Experimental Section 5.2.3).

To find out whether developed synthetic pathway followed free radical pathway or ionic pathway, control experiment was conducted. In the control experiment, reaction of **3c** in the presence of 3 equiv of free radical scavenger, TEMPO was done with same reaction conditions. Product **5d** was formed with almost same yield of 68%, which rule out a radical chain mechanism. (**Scheme 5.5**). In developed reaction, nitrogen atom of starting material **3** attacked on the carbonyl carbon **4** to generate intermediate **A** (**Scheme 5.5**). Then, oxygen atom abstracted proton from amine group to give intermediate **B**. Further, oxygen atom abstracted another proton from amine group in **B** to give **C** which on removal of water molecule generated intermediate **D** and further isomerization to **E**. Upon treatment with molecular iodine, the alkyne moiety in **E** was activated through formation of a cyclic iodonium ion, giving intermediate **F** which undergoes iodocyclization to generate **G**. Finally, **G** underwent proto-deiodination to generate the final product **5** with the regeneration of molecular iodine. The iodo-cyclization of alkynes using iodine and proto-deiodination by acids have been studied earlier [38-40].

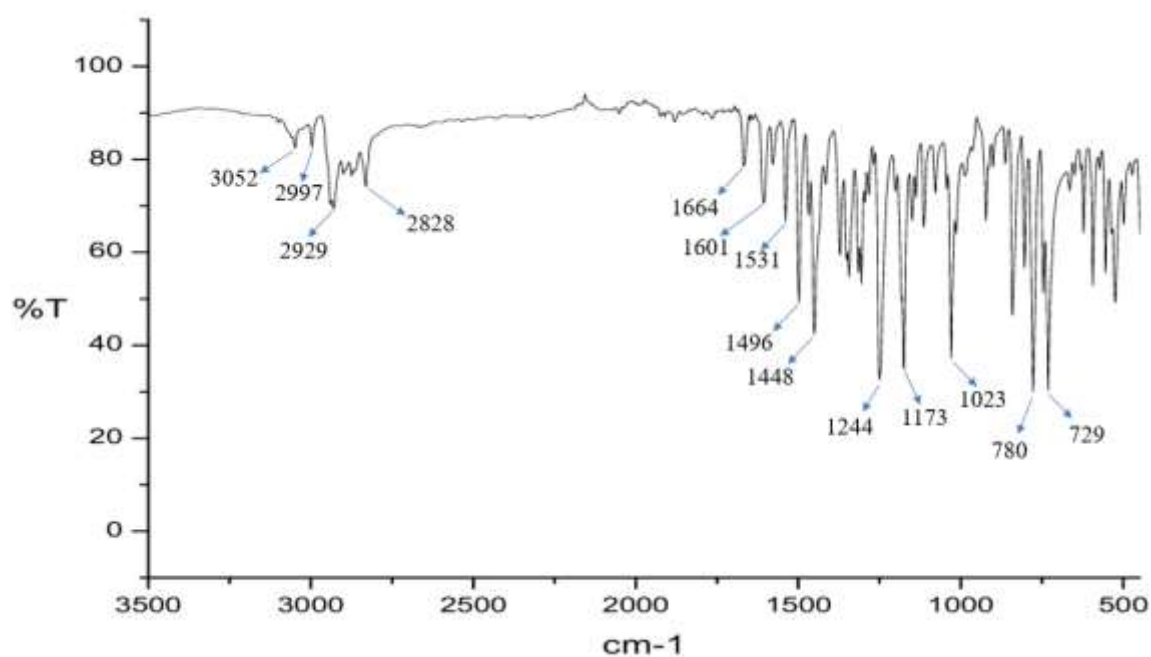


Figure 5.4 FT-IR spectrum of 1-(cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (**5a**)

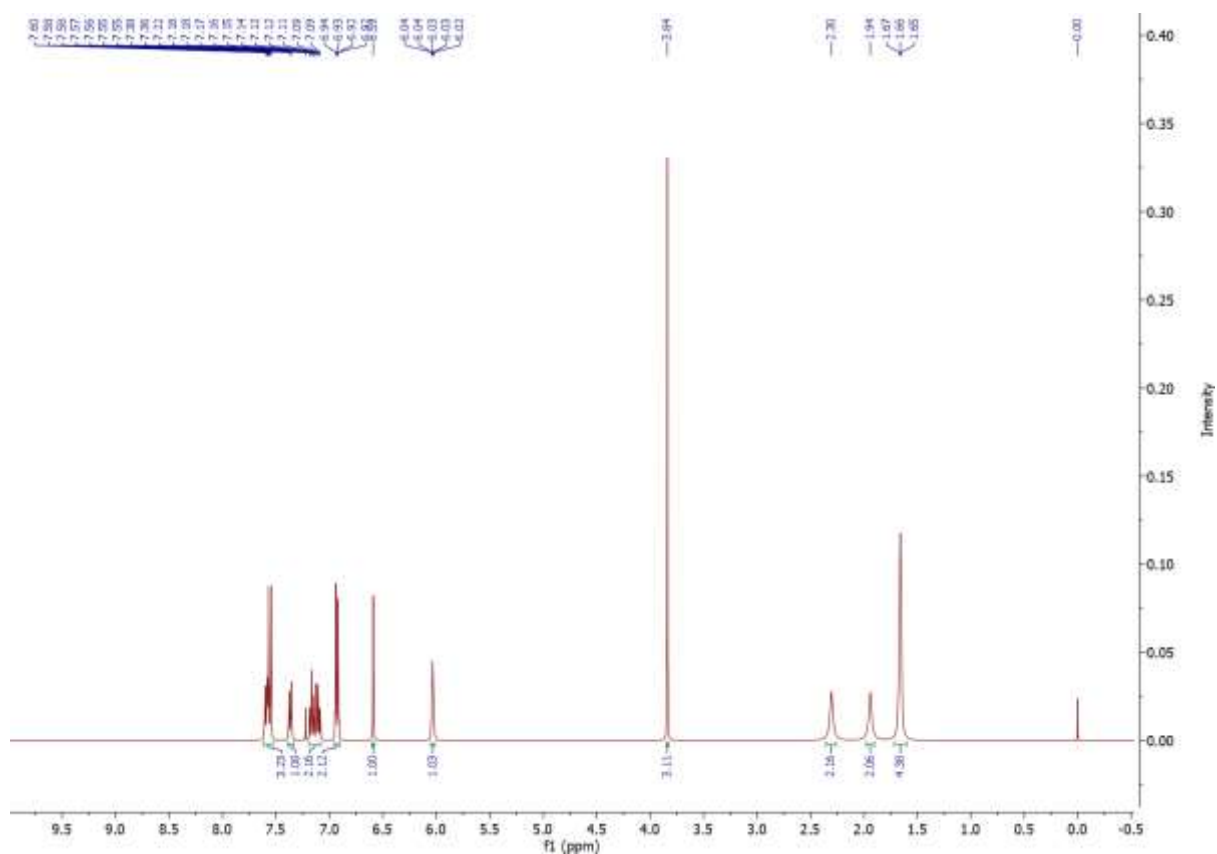


Figure 5.5 ^1H NMR spectrum of 1-(cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (5a)

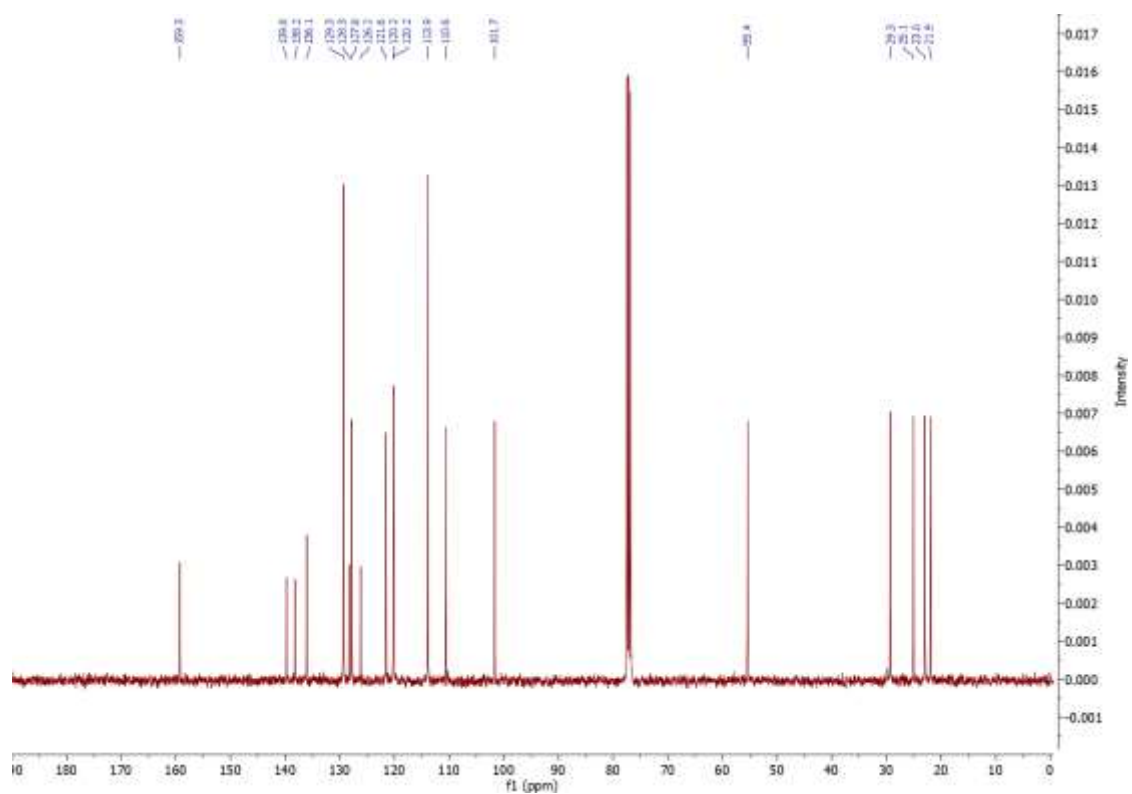


Figure 5.6 ^{13}C NMR spectrum of 1-(cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (5a)

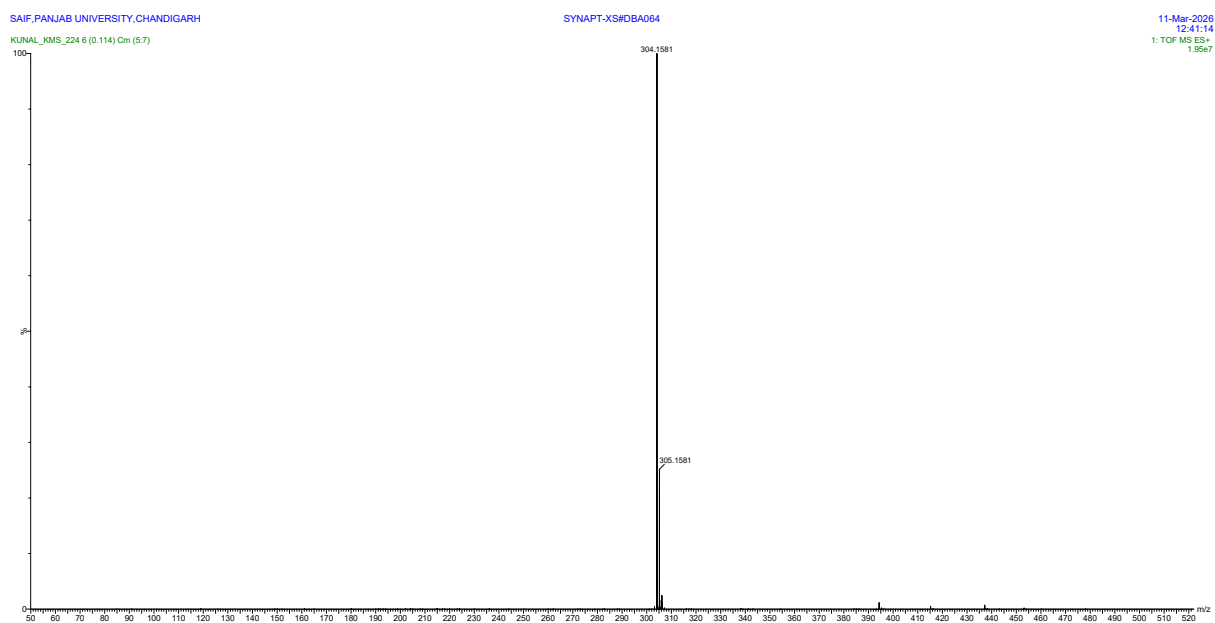
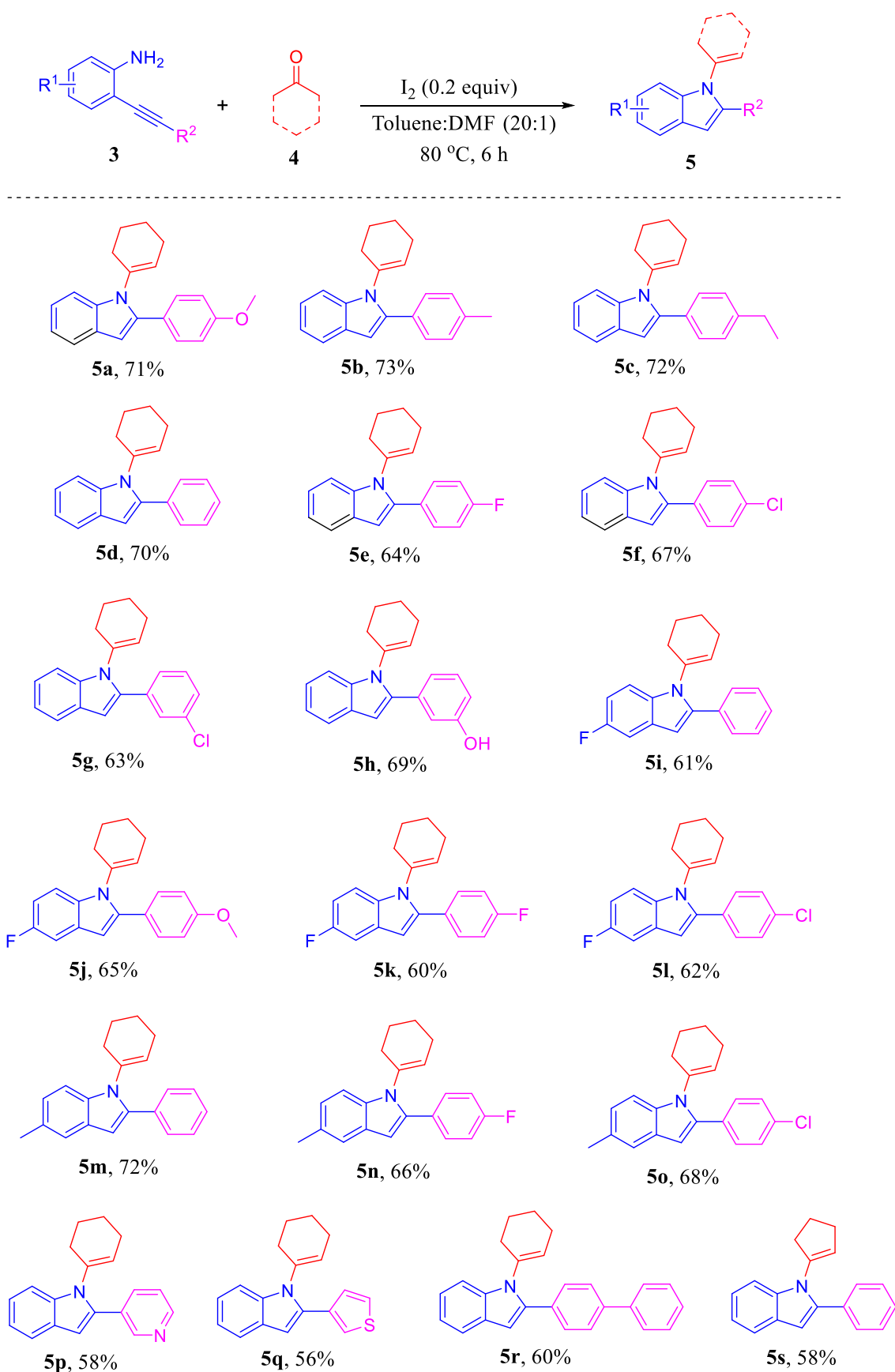
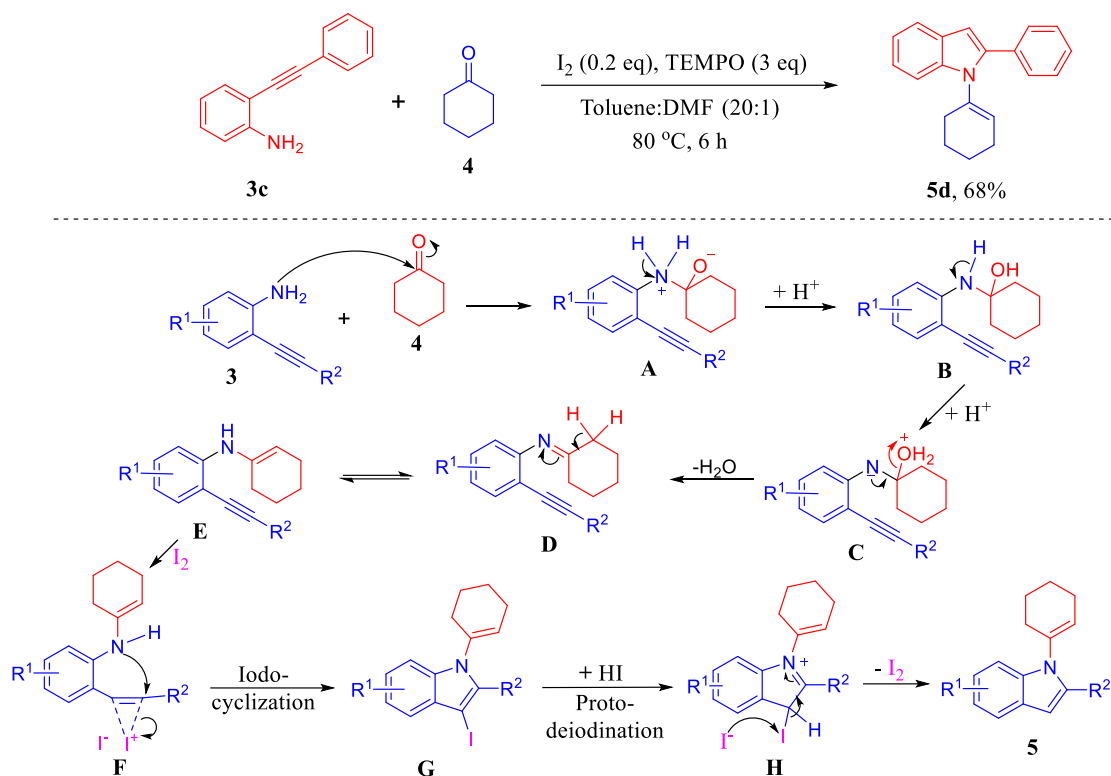


Figure 5.7 HRMS spectrum of 1-(cyclohex-1-en-1-yl)-2-(4-methoxyphenyl)-1H-indole (**5a**)



Scheme 5.4 Scope of reaction with substrates containing electron-donating, electron-withdrawing, and hetero-aromatic rings



Scheme 5.5 Control experiment and proposed reaction mechanism

5.3.3 Antioxidant Studies of *N*-alkenylindole Derivatives (5)

The antioxidant activities of all the synthesized compounds (**5**) were evaluated using the DPPH radical scavenging assay. DPPH is a stable free radical. In the presence of an antioxidant, DPPH becomes paired off in the presence of hydrogen donor and thus reduced to the DPPH-H. As a result, the dark violet color of DPPH changes to yellow color upon conversion to DPPH-H, leading to a decrease in the initial absorbance of the DPPH radical. Higher the antioxidant activity, more decrease in absorbance value is observed [41]. The antioxidant activities of all the synthesized *N*-alkenylindole compounds **5a-5r** fall within the range of 5.99-28.4%, whereas gallic acid (reference standard) shows an antioxidant activity of 92.28% (**Table 5.2**). Compound **5a** having methoxy group attached to phenyl ring display antioxidant activity more than 25% (28.4%). The antioxidant activities of compounds containing electron-donating and electron-withdrawing groups come almost in the same range. Overall, these compounds did not demonstrate significant antioxidant activity.

Table 5.2 Percentage of antioxidant activity, AA (%) of all the synthesized *N*-alkenylindole compounds with gallic acid as reference standard

Compound No.	Percentage of antioxidant activity, AA (%)
5a	28.47
5b	16.27
5c	15.87
5d	23.06
5e	5.99
5f	16.41
5g	15.19
5h	14.53
5i	13.56
5j	8.59
5k	10.17
5l	11.36
5m	12.59
5n	20.45
5o	16.93
5p	18.43
5q	18.92
5r	17.81
Gallic Acid	92.28

5.4. Conclusion

In conclusion, a new metal-free synthetic method for the regioselective synthesis of *N*-alkenylindoles from 2-(1-alkynyl)anilines and cyclic ketones catalyzed by molecular iodine was reported. The starting materials 2-(1-alkynyl)anilines were synthesized in 82-90% yields using Sonogashira coupling reaction with the help of Pd(PPh₃)₂Cl₂ and CuI catalysts and were characterized using FT-IR and ¹H NMR techniques. 19 derivatives of *N*-alkenylindoles were reported with yields ranging from 56 to 73% yield. Optimization studies suggested that the highest possible yield of *N*-alkenylindole was obtained when 0.2 equiv of iodine is used in toluene:DMF (20:1 v/v) solvent ratio at 80 °C temperature for 1 equiv each

of 2-(1-alkynyl)aniline and cyclic ketone. *N*-alkenylindole derivatives were characterized using FT-IR, ¹H NMR, ¹³C NMR, and HRMS techniques. This developed method was well tolerated the presence of both electron-donating and electron-withdrawing groups including hetero-aromatic rings. Control experiment suggested ionic pathway of the reaction mechanism. The reaction mechanism was proposed to be underwent through iodo-cyclization followed by proto-deiodination. In addition, antioxidant studies of these *N*-alkenylindole compounds did not show considerable results.

5.5. References

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CHAPTER 6

Summary, Future prospects, and Social Impact

6.1 Summary

Heterocyclic compounds are an important class of cyclic organic compounds containing one or more heteroatoms such as oxygen, nitrogen, or sulphur within the ring system. These compounds possess significant medicinal and industrial importance due to their wide occurrence in natural products and their applications in biochemistry, pharmaceuticals, agrochemicals, materials science, sanitizers, antioxidants, and copolymers.

Indoles and isoindolin-1-ones are important class of nitrogen-containing heterocyclic compounds with significant biological and medicinal applications. Indole and isoindolin-1-one derivatives are fused heterocyclic systems consisting of a benzene ring fused with a pyrrole ring, whose aromatic and electron-rich nature makes them privileged scaffolds in medicinal chemistry. Therefore, both indoles and isoindolin-1-ones continue to attract considerable interest in synthetic and medicinal chemistry.

This work entitled “Design, Synthesis, and Applications of Indole and Isoindole Derivatives” has been divided into six chapters:

- Chapter 1:** Introduction: General aspects of Indole and Isoindole Derivatives
- Chapter 2:** *In-silico* studies of 3-acyl-3-hydroxyisoindolin-1-one derivatives as potential MAO-B inhibitors
- Chapter 3:** Selectfluor mediated synthesis of 3-acyl-3-hydroxyisoindolin-1-ones and their antioxidant studies
- Chapter 4:** *In-silico* studies of *N*-alkenylindole derivatives as potential MAO-B inhibitors
- Chapter 5:** Iodine-catalyzed synthesis of *N*-alkenylindoles and their antioxidant studies
- Chapter 6:** Summary, Future prospects and Social Impact

Chapter 1 covered the general description of indole and isoindolin-1-one heterocyclic compounds, their different synthetic methodologies, and their biological and medicinal applications. The literature given in this chapter discussed the general aspects of these scaffolds, research gaps, and listed chapters under which work has been carried out.

Chapter 2 discussed the *in-silico* studies of 3-acyl-3-hydroxyisoindolin-1-one compounds as potential MAO-B inhibitors for neurodegenerative diseases focusing on Parkinson's disease (PD). PD is a prevalent neurological disorder, and the enzyme monoamine oxidase-B (MAO-B), which contributes to neurodegeneration via oxidative stress, is a significant therapeutic target. In this chapter, a large library of 279 ligands was created and molecular docking of the complete library was performed with the MAO-B protein target (PDB ID:6FWC).

Validation of the docking protocol was done with the help of the co-crystal ligand of the MAO-B protein (a known inhibitor of MAO-B). Then the top two docked scored compounds were further investigated using other *in-silico* studies such as molecular dynamics simulation (MDS), Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) binding free energy, Absorption, Distribution, Metabolism, and Excretion (ADME), and Density Functional Theory (DFT) studies.

Among the library, two ligands 3-hydroxy-2-(4-isopropylphenyl)-3-(4-(trifluoromethyl)benzoyl)isoindolin-1-one (**90**) and 2-(3,4-dichlorophenyl)-3-hydroxy-3-(4-(trifluoromethyl)benzoyl)isoindolin-1-one (**173**) (**Figure 6.1**) showed superior glide score values of -12.088 kcal/mol and -12.085 kcal/mol, respectively, and conventional hydrogen bonding interactions with MAO-B protein. MDS over 500 ns demonstrated the stability of both the ligands **90** and **173** with RMSD and RMSF values below 2 Å and consistent hydrogen bonding with Tyr60 residue for up to 96% of the simulation time. ADME profiling of both ligands indicated favourable pharmacokinetic parameters, while DFT analysis showed high chemical reactivity and low kinetic stability. This indicated that ligands **90** and **173** exhibit strong potential as promising lead candidates for MAO-B inhibition.

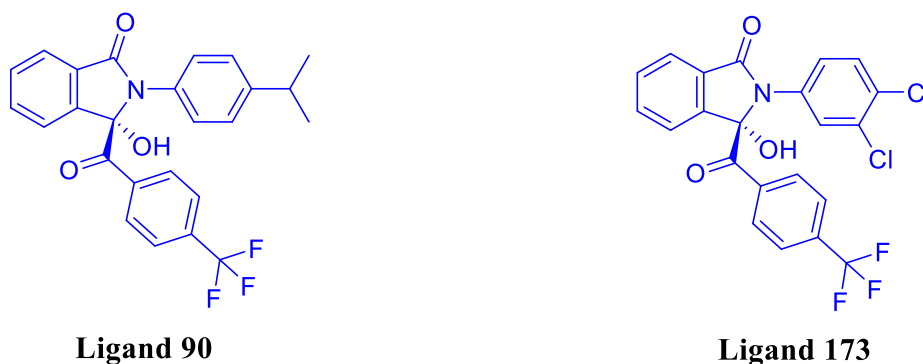


Figure 6.1 Chemical structures of ligands **90** and **173**

Figures 6.2 and **6.3** displays the 2-D and 3-D binding interactions of these ligands, **90** and **173**, respectively, with MAO-B. It was observed that ligand **90** showed hydrogen bonding interactions of the oxygen atom of the carbonyl group with Ser59 and Tyr60 amino acid residues of the MAO-B protein. Similarly, ligand **173** displayed hydrogen bonding interactions between carbonyl oxygen and Ser59 and Tyr60 residues. In addition, 3D MESP maps highlighted the nucleophilic character of the carbonyl oxygen atoms, aligning with docking and MDS studies (**Figure 6.4**).

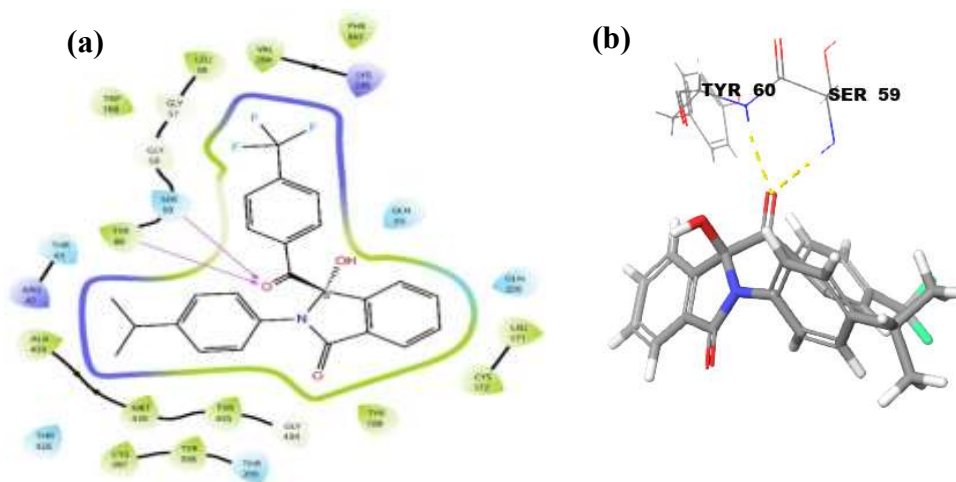


Figure 6.2 (a) 2-D representation of binding interactions of ligand **90** with MAO-B protein residues. (b) 3-D representation of binding interactions of ligand **90** with MAO-B protein residues.

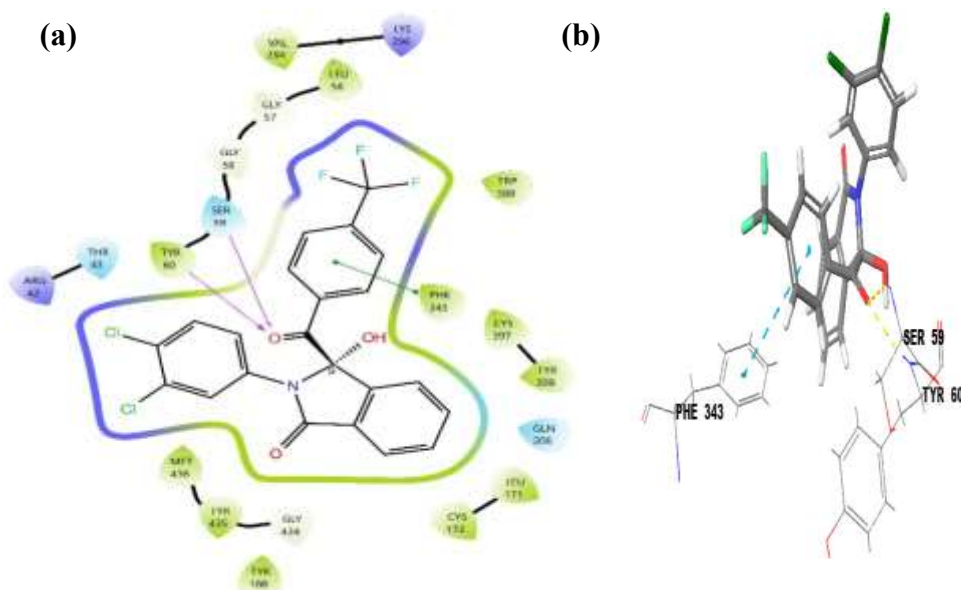


Figure 6.3 (a) 2-D representation of binding interactions of ligand **173** with MAO-B protein residues. (b) 3-D representation of the binding interactions of the same complex.

Chapter 3 covered a new synthetic route for the regioselective synthesis of 3-acyl-3-hydroxyisoindolin-1-ones from N-substituted-2-(1-alkynyl)benzamides mediated by Selectfluor. Firstly, the N-substituted-2-(1-alkynyl)benzamides starting materials were synthesized by known methods. Initially, 2-iodobenzoic acids **1** underwent reaction with oxalyl chloride, followed by reaction with N-substituted amines to give N-substituted-2-

iodobenzamides **2**. N-substituted-2-iodobenzamides further underwent Sonogashira coupling reaction with terminal alkynes using Pd and Cu catalysts to generate corresponding N-substituted-2-(1-alkynyl)benzamides **3** (**Scheme 6.1**).

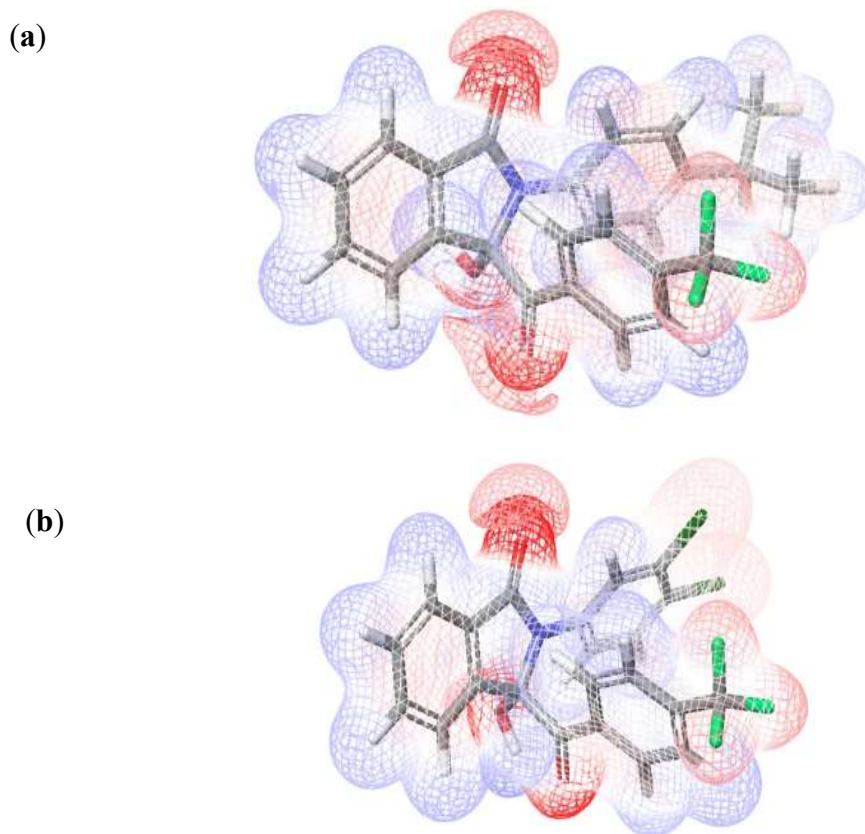
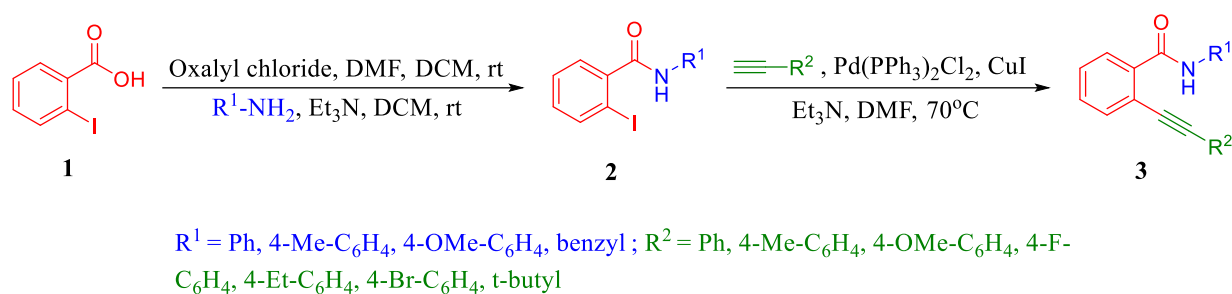
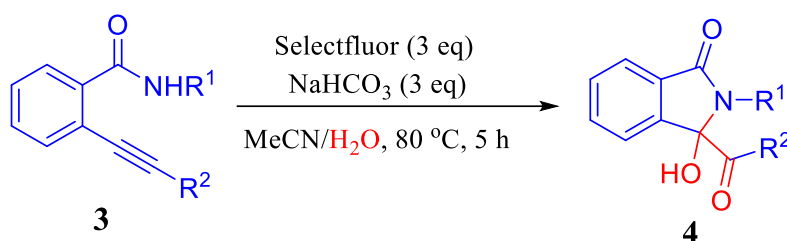


Figure 6.4 Molecular Electrostatic Potential Surface (MESP) of (a) ligand **90** (b) ligand **173**

The 3-acyl-3-hydroxyisoindolin-1-one derivatives **4** have been synthesized using Selectfluor from N-substituted-2-(1-alkynyl)benzamides **3** with sodium bicarbonate (NaHCO_3) as mild base in acetonitrile (MeCN) solvent. Water (H_2O) was used as the source for oxidation and hydroxylation (**Scheme 6.2**). This developed method performed the synthesis under mild conditions, required fewer reagents, and reduced the reaction time. Cyclization, oxidation, and hydroxylation were all completed in a single step in 5 hours. All the derivatives of 3-acyl-3-hydroxyisoindolin-1-ones were synthesized with 73-85% yield. Control experiments revealed that this new synthetic pathway followed free radical pathway. A plausible mechanism was made on the basis of control experiments.



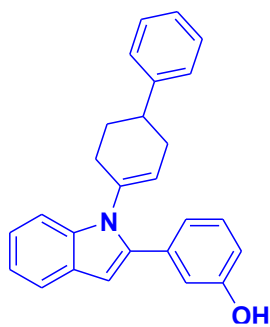
Scheme 6.1 Synthetic methodology for the synthesis of N-substituted-2-(1-alkynyl)benzamides (**3**)



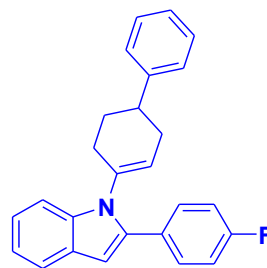
Scheme 6.2 Synthesis of 3-acyl-3-hydroxyisoindolin-1-ones (**4**)

Chapter 4 discussed the *in-silico* studies of *N*-alkenylindole ligands to evaluate their inhibitory activity against MAO-B. Initially, a library of 147 *N*-alkenylindole ligands was made and molecular docking of the complete library with the MAO-B protein target was performed. Validation of the docking protocol was performed with the help of the co-crystal ligand of the MAO-B protein. Further, the top two docked scored ligands were investigated using other *in-silico* tools which were MDS, MM-GBSA binding free energy, ADME, and DFT studies.

N-Alkenylindole ligands exhibited docking scores in the range of -8.0 to -11.8 kcal/mol. 21 ligands displayed docking scores higher than the co-crystal ligand of MAO-B protein (-10.6 kcal/mol). Two ligands, 3-(1-(1,2,3,6-tetrahydro-[1,1'-biphenyl]-4-yl)-1H-indol-2-yl)phenol (**66**) and 2-(4-fluorophenyl)-1-(1,2,3,6-tetrahydro-[1,1'-biphenyl]-4-yl)-1H-indole (**63**) showed superior docking scores of -11.8 kcal/mol and -11.4 kcal/mol, respectively in their library (**Figure 6.5**). **Figures 6.6** and **6.7** displays the 2-D and 3-D binding interactions of these ligands, **66** and **63**, respectively, with MAO-B. It was found that hydrogen atom of hydroxyl group of ligand **66** showed hydrogen bonding with Gly434 amino acid residue of MAO-B and oxygen atom of hydroxyl group exhibited hydrogen bonding with Arg42 residue.



Ligand 66



Ligand 63

Figure 6.5 Chemical structures of ligands **66** and **63**

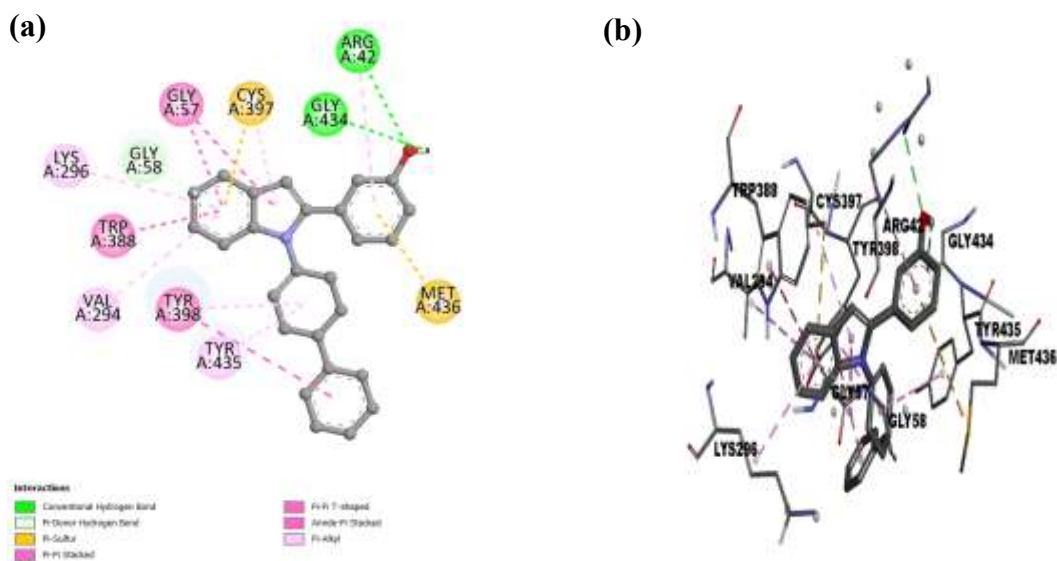
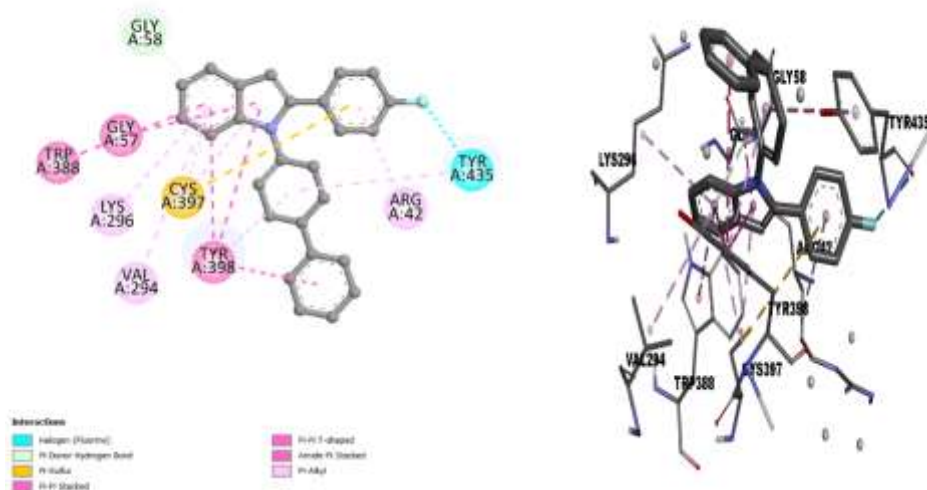


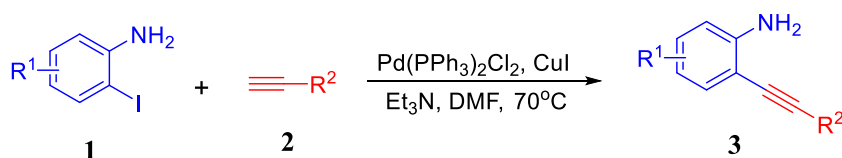
Figure 6.6 (a) 2-D representation of binding interactions of ligand **66** with MAO-B protein
(b) 3-D representation of binding interactions of ligand **66** with MAO-B protein

Chapter 5 discussed a new metal-free synthetic method for the regioselective synthesis of *N*-alkenylindoles from 2-(1-alkynyl)anilines catalyzed by molecular iodine. Firstly, starting materials 2-(1-alkynyl)anilines were synthesized using Sonogashira coupling reaction in 82-90% yields. Substituted 2-iodoanilines **1** underwent Sonogashira coupling reaction with terminal alkynes **2** with the help of $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ and CuI catalysts to give 2-(1-alkynyl)anilines **3** in the presence of triethylamine (Et_3N) as a base (**Scheme 6.3**). The synthesis of *N*-alkenylindoles **5** was performed with 1 equiv each of 2-(1-alkynyl)aniline **3** and cyclic ketone **4** using 0.2 equiv of molecular iodine (I_2) in toluene:DMF (20:1) solvent ratio at 80 °C for 6 hours (**Scheme 6.4**). This developed method facilitated the synthesis of 18 new derivatives under mild and metal-free conditions. This developed method was well

(b)



(b) 3-D representation of binding interactions of ligand **63** with MAO-B protein



R² = C₆H₅, 4-Me-C₆H₄, 4-Et-C₆H₄, 4-OMe-C₆H₄, 4-F-C₆H₄, 4-Cl-C₆H₄, 3-Cl-C₆H₄, 3-OH-C₆H₄, pyridin-3-yl, thiophenyl-3-yl, biphenyl-3-yl

Reaction scheme showing the synthesis of indole 5 from substituted aniline 3 and cyclic ketone 4.

Starting materials:

- 3: Substituted aniline derivative (R¹ and R² substituents).
- 4: Cyclic ketone derivative (dashed red structure).

Reaction conditions:

- I₂ (0.2 equiv)
- Toluene:DMF (20:1)
- 80 °C, 6 h

Product:

- 5: Indole derivative (R¹ and R² substituents).

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6.2 Future Prospects

- The scope of the presented methodologies for synthesis of N-alkenylindoles and 3-acyl-3-hydroxyisoindolin-1-ones can be further explored using different types of starting materials to expand its versatility and applicability.
- All the presented methodologies avoid the use of expensive catalysts. Based on this, more efficient synthetic routes for the construction of more challenging molecules could be developed.
- Both the N-alkenylindole ligands (**66** and **63**) demonstrated low gastrointestinal (GI) absorption and are not permeant to the blood-brain barrier (BBB), limiting their potential for central nervous system activity. This conveys that further functionalization/modification in structure is required to enhance the GI and BBB.
- The synthesized compounds can be further evaluated against other PD targets through *in-silico*, *in-vitro* and *in-vivo* studies.
- The synthesized compounds can be also explored for other biological activities like anti-cancer, anti-viral, anti-inflammatory, and anti-microbial.

6.3 Social Impact

- The metal-free nature of the reactions reduces toxic waste and heavy metal contamination, contributing to greener and safer chemical processes.
- In addition, avoiding expensive catalysts and using faster reaction conditions, lower production costs, making advanced pharmaceuticals more affordable and accessible.
- Moreover, *in-silico* evaluation of the synthesized compounds against PD may facilitate the development of novel therapeutic agents for its treatment.

List of Publications

Publications Included in the Thesis

1. **Kunal Madaan**, and Ram Singh; Progress in the chemistry and pharmacology of isoindolin-1-one scaffolds, *ChemMedChem*, **2025**, 20(17), e202500420, <https://doi.org/10.1002/cmdc.202500420>
2. **Kunal Madaan**, and Ram Singh; Selectfluor-Mediated Regioselective Synthesis of 3-Hydroxy-3-acylisoindolin-1-ones from 2-(1-alkynyl)benzamides; *Tetrahedron Letters*, Volume 175, **2026**, 155927; <https://doi.org/10.1016/j.tetlet.2025.155927>.
3. **Kunal Madaan**, and Ram Singh; Molecular Docking, Molecular Dynamics simulation, DFT and ADMET prediction of 3-carbonyl-3-hydroxyl-isoindolin-1-onescaffolds revealing potential inhibitors of MAO-B, *Current Computer-Aided Drug Design*, In-press **2026**. <https://doi.org/10.2174/0115734099417682251124070748>
4. **Kunal Madaan**, and Ram Singh; Molecular Iodine-catalyzed Metal-free Regioselective Synthesis of N-Alkenylindoles from 2-(1-Alkynyl)anilines, *Synlett (Under revision)*
5. **Kunal Madaan**, and Ram Singh; Exploring N-Alkenylindoles for MAO-B Inhibition via Computational Approaches, *Biointerface Research in Applied Chemistry (Submitted)*

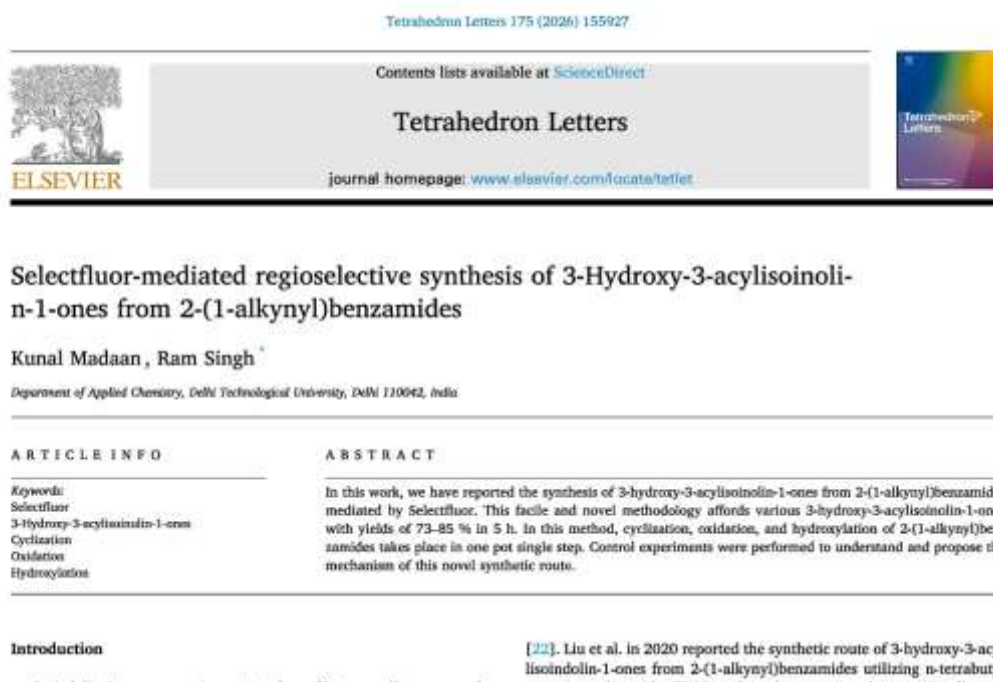
Other Publications

1. Chahat Arora, **Kunal Madaan**, Saurabh Mehta, and Ram Singh; Exploring Isoindolin-1-ones as Potential CDK7 Inhibitors using Cheminformatic Tools; *In Silico Pharmacology*, Volume 12, article number 51, **2024**. <https://doi.org/10.1007/s40203-024-00225-0>
2. **Kunal Madaan**, Saurabh Mehta, and Ram Singh; Structure-Based Virtual Screening, ADMET prediction and Molecular Dynamics simulation of isoindolin-1-one scaffolds as potential inhibitors of MNK2; *Biomedical and Therapeutics Letters*; 11 (2), 910-910, **2024**.

List of Conferences

1. Chandra Prakash, **Kunal Madaan**, Anvita Chaudhary, Richa Srivastava, and Ram Singh; Fluorinated Heterocycles using Microwave Irradiation (PP43); International Conference CARBO XXXVII: Basic, Analytical and Allied Sciences at the Interface of Carbohydrates and Biomass Valorisation” organized by Department of Applied Chemistry, Delhi Technological University, 30 Nov – 2 Dec **2023**.
2. **Kunal Madaan** and Ram Singh; CADDTS-2025: Exploring Potential Inhibition of Isoindolin-1-one Compounds Against MNK2 Protein through In-silico Studies at International Conference on Current Advances in Drug Discovery and Translational Science, February 26-28, **2025** organized by Jamia Millia Islamia, Delhi.

3. Sushmita Bhatia, **Kunal Madaan**, and Ram Singh; In-Silico Insights into the MAO-B Inhibitory Potential of Isoindole Derivatives; Indian Analytical Congress (IAC 2025), An International Analytical Conference and Exhibition, November 20-22, **2025** (22nd Nov 2025), jointly organized by ISAS Delhi Chapter and MDU Rohtak (IAC-25-238).
4. **Kunal Madaan** and Ram Singh; IAC-25-242: Integrated Computational Approach for Identifying MAO-B Inhibitors from Isoindole Frameworks at Indian Analytical Congress (IAC 2025) International Analytical Conference and Exhibition on Analytical Sciences-Shaping the Future November 20-22, **2025** (22nd November 2025), organized by MDU University, Rohtak, Haryana.



RESEARCH ARTICLE

Molecular Docking, Molecular Dynamics Simulation, DFT, and ADMET Prediction of 3-Carbonyl-3-Hydroxyl-Isoindolin-1-ones, Revealing Potential Inhibitors of MAO-B

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Abstract: **Introduction:** Parkinson's Disease (PD) is the most common neurodegenerative disease worldwide. Monoamine Oxidase B (MAO-B), which is associated with oxidative stress, is a suitable target for PD treatment. This study aimed to explore the inhibitory potential of 3-carbonyl-3-hydroxyl-isoindolin-1-one compounds against MAO-B through various *in-silico* studies.

Methods: A library of 279 isoindolin-1-one compounds was screened against the MAO-B protein using molecular docking with the GLIDE module of Schrödinger suite 2022.4. The top two candidates were further evaluated using 500 ns molecular dynamics simulations (MDS) with the Desmond module, MM-GBSA binding free energy calculations with Prime, ADMET analysis with the QikProp module, and density functional theory (DFT) calculations with the Jaguar module of Schrödinger suite 2022.4.

Results and Discussion: Ligands 90 and 173 exhibited the highest docking scores of -12.088 kcal/mol and -12.085 kcal/mol, respectively, and formed hydrogen bonds with MAO-B residues. 500 ns MDS studies confirmed the stability of the docked poses of ligands 90 and 173 with RMSD values below 2 Å and stable hydrogen bonding interactions with the protein. MM-GBSA analysis indicated that the MAO-B complexes of ligands 90 and 173 displayed significantly lower binding free energy values of -85.465 kcal/mol and -78.840 kcal/mol, respectively. ADMET predictions of ligands 90 and 173 were observed to be within the satisfactory range.

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Progress In the Chemistry and Pharmacology of Isoindolin-1-One Scaffolds

Kunal Madaan and Ram Singh*

Isoindolin-1-one scaffolds have emerged as privileged structures in medicinal and synthetic chemistry due to their diverse biological activities and synthetic accessibility. This review comprehensively highlights the recent advancements in the development of synthetic methodologies for constructing isoindolin-1-one cores, encompassing both metal-catalyzed and metal-free approaches. Key strategies include the electrochemical method, transition metal-catalyzed synthesis, radical and oxidant-driven metal-free method, acid-catalyzed and multicomponent method, ring opening method, and ultrasound-assisted method of synthesis. The flexibility of these methods arises from the wide range of starting materials, such as benzamides, imidates, nitriles, and aziridines, and the effective use of catalysts and additives, including

Rh(III), Pd(II), Ru(II), Ag₂CO₃, CO, and various oxidants. This review also explores the structure-activity relationships of isoindolin-1-one derivatives in diverse therapeutic areas. Specific functional groups and substituents have been shown to enhance anticancer, antiviral, antipsychotic, antimicrobial, cardiovascular, anti-inflammatory, and antidiabetic activities. Modifications including lipophilic, electron-withdrawing, polar, and heterocyclic moieties have significantly improved biological efficacy, target specificity, and pharmacokinetics. The integration of these structural variations underscores the scaffold's versatility and its potential in drug discovery. This review aims to provide a consolidated foundation for future research on isoindolin-1-one based therapeutics and their strategic synthesis.

1. Introduction

Isoindolin-1-one (2,3-dihydro-1H-isoindole, C₈H₇NO, **1**, Figure 1) is a nitrogen (N)-containing heterocyclic molecule present in biologically active synthetically prepared and naturally occurring compounds. The approved synthetic drugs like CC-90009 (cereblon E3 ligase modulator), Iberdomide (cereblon-modulating agent), and lenalidomide (anticancer drug) along with others possess isoindolin-1-one motif.^[1] To explore the potentiality of isoindolin-1-one derivatives, various groups have synthesized a

the natural isoindolin-1-one derivatives have also been obtained from other sources.^[1a,1b]

The present review article covers some of the important synthetic pathways of isoindolin-1-one derivatives along with their mechanism. This article further covers the pharmacological activities of isoindolin-1-one derivatives. The evaluation against diseases like cardiovascular, autoimmune, and diabetes has also been covered. This article shall be useful to all the stakeholders working in the field of medicinal chemistry.

Structure-Based Virtual Screening, ADMET prediction and Molecular Dynamics simulation of isoindolin-1-one scaffolds as potential inhibitors of MNK2

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Article

ABSTRACT

Mitogen-activated protein kinase 2 (MNK2) is a promising target for various types of cancers like breast, prostate, colorectal, glioblastoma lung, and melanoma cancers. It activates the oncogene eukaryotic initiation factor 4E (eIF4E) by phosphorylation. Isoindolin-1-one scaffolds displayed significant anti-cancer activity as reported previously in research papers. In this study, we explored potential inhibition of various substituted isoindolin-1-one compounds against MNK2 through *in-silico* studies. Structure-based virtual screening of library of 180 compounds having isoindolin-1-one scaffold was done followed by fragment molecular orbital (FMO) calculations and molecular dynamics simulation (MDS) of the best two scored docked poses (compounds **132** and **160**). ADMET prediction of compounds **132** and **160** were performed. Sixteen compounds showed docking scores higher than or equal to co-crystal ligand of MNK2 protein and hydrogen bonding with MET162 residue. 100 ns molecular dynamics simulation of compound **160** displayed excellent 95% hydrogen bond occupancy with MET162 residue of MNK2 protein showing remarkable docked pose stability throughout the simulation. Further, the ADMET prediction of compound **160** falls under the satisfactory range. Compound **160** exhibited potential inhibition against MNK2 protein. Thus, *in-silico* studies of these isoindolin-1-ones will be helpful in the development of highly potent and selective MNK2 inhibitors.

Keywords: Cancer; MNK2; ADMET; virtual screening; MDS



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ORIGINAL RESEARCH



Exploring isoindolin-1-ones as potential CDK7 inhibitors using cheminformatic tools

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Abstract

In women who die from cancer, breast cancer is the most common cause of death. The development of small molecular scaffolds as specific Cyclin-dependent kinase (CDK) inhibitors is a promising strategy in the discovery of anti-breast cancer drugs. Isoindolin-1-ones are heterocyclic compounds with useful therapeutic properties. In this study, a library of 48 isoindolinones has been virtually screened by molecular docking that showed high binding affinity up to -10.1 kcal/mol and conventional hydrogen bonding interactions with active amino acid residues of CDK7. The molecular dynamics simulation (MDS), fragment molecular orbital (FMO), density functional theory (DFT), and pharmacokinetics studies of the best two docked scored ligands **7** and **14** have been studied. Examining the ligand root mean square deviation and hydrogen bonding occupancy of the 100 ns MDS trajectory, both ligands **7** and **14** showed docked pose stability. FMO calculations displayed that LYS139 and LYS41 are majorly contributing to the binding interactions with ligands **7** and **14** in the docked poses. DFT studies of ligands **7** and **14** showed high values of global softness and low values of global hardness and chemical potential thus displaying chemically reactive soft molecules and this influences their anti-cancer activity. Our hits exhibited superior qualities to known CDK7 inhibitors, according to the comprehensive pharmacokinetic parameters that were predicted. The



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