Synthesis and Anticancer Activity Studies of Some Novel Indolyl Heterocycles

THESIS

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of the requirements for the degree of
DOCTOR OF PHILOSOPHY

by

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Under the supervision of

Prof. Dalip Kumar

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CERTIFICATE

This is to certify that the thesis entitled **Synthesis and Anticancer Activity Studies of Some Novel Indolyl Heterocycles** and submitted by **Mr. Maruthi Kumar Narayanam** ID No **2007PHXF003P** for award of Ph.D. Degree of the Institute embodies the original work done by him under my supervision.

Signature in full of the Supervisor:
Name in capital block letters: **Dr. DALIP KUMAR**
Designation: **Associate Professor**

Date:

Dedicated to My Family and Teachers

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ABSTRACT

Cancer is reported as the second most likely cause of death world-wide, killing millions of people. Development of potent and selective anticancer agents with reduced side-effects gives great relief for millions of cancer patients. This thesis explores the synthesis and anticancer activity studies of some novel indolyl heterocycles.

The first chapter gives an introduction to the indole chemistry and its significant role in the diverse therapeutic areas. Classification of anticancer drugs and role of natural and synthetic indoles in anticancer research have been elaborated with examples. Diverse structural classes of indoles and rational approaches for designing novel indole-based anticancer agents have also been discussed in this chapter.

Rational design, synthesis and *in-vitro* anticancer activities of three series of novel indolylazoles are discussed in the second chapter. In part-A of this chapter, a microwave assisted solvent-free synthesis and anticancer activities of 4-(3′-indolyl)oxazoles is reported. The most potent indolyloxazole exhibited significant anticancer activity against MCF-7 breast cancer cell line (IC_{50} = 14.1 μM). Part-B explains the synthesis and anticancer activities of 5-(3′-indolyl)-1,3,4-thiadiazoles which led to a potent compound with an IC_{50} value of 1.5 μM against PaCa2, pancreatic cancer cell line. Part-C of this chapter discloses a facile synthesis of indolyl-1,2,4-triazoles and their cytotoxicity. The most cytotoxic 1,2,4-triazole has an IC_{50} value of 0.8 μM against PaCa2 cancer cell line. The preliminary mechanistic studies suggests that indolyl-1,2,4-triazoles may interfere with tubulin depolymerisation.

Part A of third chapter discloses the synthesis of bis(indolyl)-1,2,4-thiadiazoles by iodobenzene diacetate mediated oxidative-dimerization of indole-2(3)-thiocarboxamides Some of the bis(indolyl)-1,2,4-thiadiazoles exhibited selective cytotoxicity against LnCap cancer cell line (IC_{50} = 14.6 μM). In part B, bis(indolyl)hydrazide-hydrazones were identified as potent and selectively cytotoxic against MDA-MB-23 breast cancer cell line (IC_{50} = 0.7 μM).

The fourth chapter describes synthesis of indolyl chalcones and their *in-vitro* cytotoxicity. Some of the synthesized indolyl chalcones displayed the potent cytotoxicity against PaCa2 cancer cell line (IC_{50} = 30 nM).

Synthesis and anticancer activity of novel 2-arylamino-indolyl-1,3,4-thiadiazoles are reported in the chapter five of thesis. Most of the synthesized 2-arylamino-1,3,4-thiadiazoles showed selective cytotoxicity against breast cancer cell line (MDA-MB-231), whereas the compound with 3,4,5-trimethoxyphenylamino substituent displayed high cytotoxicity against LnCap cancer cell line (IC_{50} = 0.15 μM).

In the sixth chapter, a facile and short synthesis of indoloquinoline alkaloid isocryptolepine and its analogues is achieved from the reaction of readily available *N*-methyl-1,2,3,4-tetrahydroquinoles with arylhydrazines in presence of *p*-toluenesulfonic acid under solvent-free condition.

Finally, overall thesis work is summarized in the chapter seven. The future scope of the research work undertaken is also highlighted in this chapter.

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation/Symbol	Description
%	Percentage
A°	Angstrom
Ac_2O	Acetic anhydride
ATP	Adenosine triphosphate
Bn	Benzyl
CA	Carbonic anhydrase
°C	Centigrade
CA4	Combretastatin A4
Calcd	Calculated
$CDCl_3$	Deuterated chloroform
d	Doublet
dd	Doublet of doublet
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMF-DMA	*N,N*-Dimethylformamide dimethyl acetal
DMSO-d_6	Deuterated dimethylsulfoxide
EC_{50}	Maximal effective concentration
ED_{50}	Effective Dose 50%
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EI	Electron ionization
ESI	Electrospray ionization
FAS	Ferric ammonium sulfate
GPCR	G-protein coupled receptors
H	Hours
HCN	Hydrogen cyanide
HRMS	High resolution mass spectra
5-HT	5-Hydroxytryptamine
HTS	High-throughput screening
IC_{50}	half maximal inhibitory concentration

IR	Infrared
J	Coupling constant
Lit.	Literature
m	Multiplet
Mp	Melting point
m-CPBA	*m*-Choroperbenzoic acid
mg	Milligram
MES	Maximum Electric Shock
MHz	Mega hertz
MIC	Minimum inhibitory concentration
min	Minutes
mL	Milliliter
mmol	Millimole
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NCI	National Cancer Institute
NMDA	*N*-methyl-D-aspartic acid
NMR	Nuclear magnetic resonance
NMP	*N*-Methyl pyrrolidine
PDE	Phosphodiesterase
PEG	Polyethylene glycol
PPA	Polyphosphoric acid
PPAR	Peroxisome proliferator-activated receptor
PTK	Protein tyrosine kinase
ppm	Parts per million
p-TsOH	*p*-Toluene sulfonic acid
s	Singlet
SAR	Structure-activity relationship
t	Triplet
t-Bu	tertiary butyl
TCPTP	T-Cell Protein Tyrosine Phosphatase
TEBAC	Triethylbenzylammonium chloride
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane

TMSCl	Trimethylsilyl chloride
TNF α	Tumor necrosis factor α
TOSMIC	Tosylmethyl isocyanide
TMOF	Trimethyl orthoformate
UV	Ultraviolet
XRD	X-ray diffraction
μM	Micromolar

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Chapter 1

1.1 Introduction-Indole

The indole ring system is most privileged heterocycle in nature. The indole nucleus is embedded in many biological systems including the essential amino acid tryptophan, the neurotransmitter serotonin, and the mammalian hormone melatonin. Tryptophan is a structural constituent of many proteins as well as the biosynthetic precursor of serotonin, which in turn serves as the precursor of melatonin. Serotonin plays a critical role in neuronal cell formation and maintenance, sleep, cognition, appetite, and mood, while melatonin is a natural bio regulator that induces and maintains sleep. Due to the great structural diversity of biologically active indole containing compounds, it has become an important structural component in many pharmaceutical agents. Indole nucleus plays a vital role in anticancer drug research, starting from vinca alkaloids to recently emerging anticancer synthetic agents indole-3-carbinol and indibulin which displayed prominent anticancer activity. Years of efforts from pharmaceutical and academic researchers' to identify indole-based new chemical entities (NCE's) led to many potent molecules in clinical trials. Despite the presence of marketed drugs targeting cancer chemotherapy, still appearance of large number of cancer related research papers, patents and magnitude of research work being carried out in academia and pharmaceutical industries reflect its potential as therapeutic area of paramount importance.

1.2 Chemical reactivity

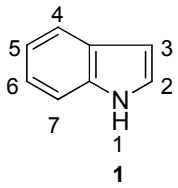

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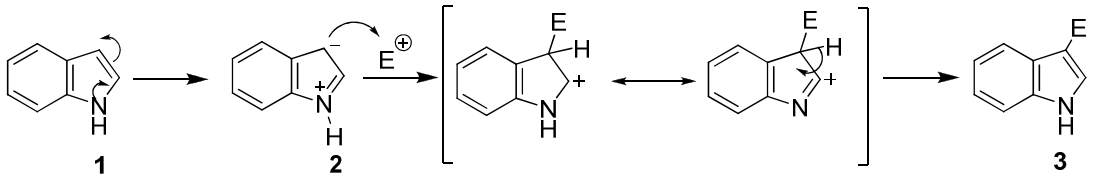
Figure 1.1 Indole

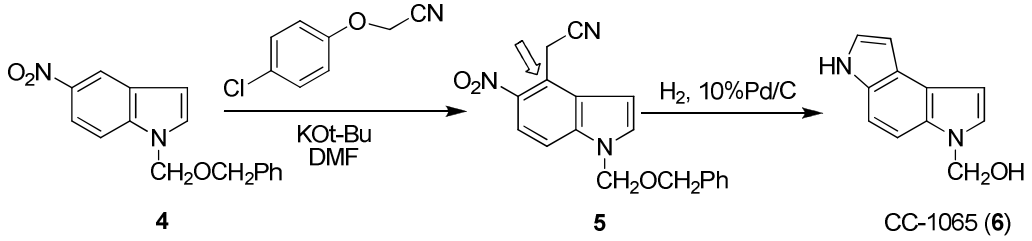
Indole and substituted indoles have diverse variety of chemical and physical properties. The basic component indole is known as benzo[*b*]pyrrole (figure 1.1). Many commercially available indoles are colourless crystalline solids with a pronounced smell and stable in air. Indole is classified as π-excessive aromatic compound, which is a very weak base and its conjugate acid is estimated to have pK_a = -3.5. The protonation occurs

at C-3 rather than at nitrogen due to this nature, it is quite reactive towards strong acids. As an electron rich heteroaromatic, indole has a relatively high-energy HOMO and is subject to oxidative processes, including photosensitized electron transfer. Many indoles are sensitive towards atmospheric oxygen, acids and light. The reactivity of indole is more dominated by electrophilic substitution reactions and is highly reactive towards classical electrophilic substitution reactions such as protonation, halogenation, alkylation and acylation.[1] The C-3 position of indole is highly electron rich and typically functions as the primary nucleophilic site that reacts with a large array of electrophiles, thereby leading to various functionalized indoles (scheme 1.1). The electrophiles attack at C-3 position and is followed by the N-1 and C-2 positions.[2]



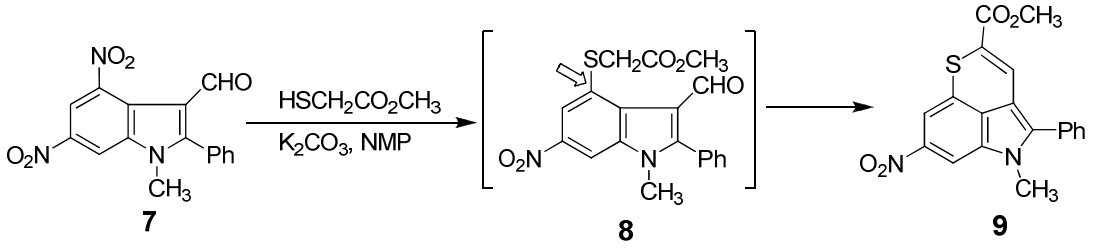
Scheme 1.1 Intermediate for β-attack by electrophile

Despite the π-excessive tendency of indole, there are numerous examples of nucleophilic addition and substitution reactions are reported on indole.[2] Electron-withdrawing groups, mostly nitro group at C-2, C-3 and/or N-1 of indole or several instances where placement of the electron-withdrawing group on the benzene ring of indole results in nucleophilic addition or substitution at C-4, C-5, C-6, and C-7 positions.[3] α-Functionalization of nitroindoles was achieved by vicarious nucleophilic substitution (VNS) reaction. VNS was a key step in the first synthesis of antitumor antibiotic CC-1065 (pyrrolo[3,2-e]indole). α-Cyano side chain is installed at the C-4 position of *N*-(benzyloxymethyl)-5-nitroindole (**4**) by VNS reaction with 2-(4-chlorophenoxy) acetonitrile, so formed intermediate **5** is reductively cyclized to give CC-1065 (**6**) as shown in scheme 1.2.[4]



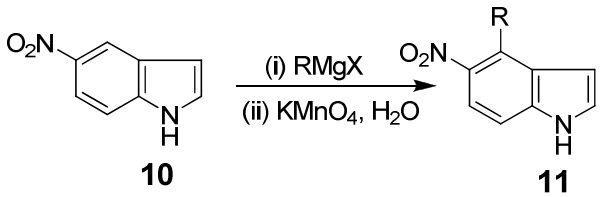
Scheme 1.2 Synthesis of CC-1065 (**6**) *via* vicarious nucleophilic substitution

Ipso-substitution was observed in 2-aryl-4,6-dinitroindoles. Natural antibiotic Chuansinmycin (**9**) was obtained in good yields from the reaction of 4,6-dinitroindole (**7**) with methyl thioglycolate followed by cyclization of *ipso*-substituted intermediate **8** (scheme 1.3).[5]



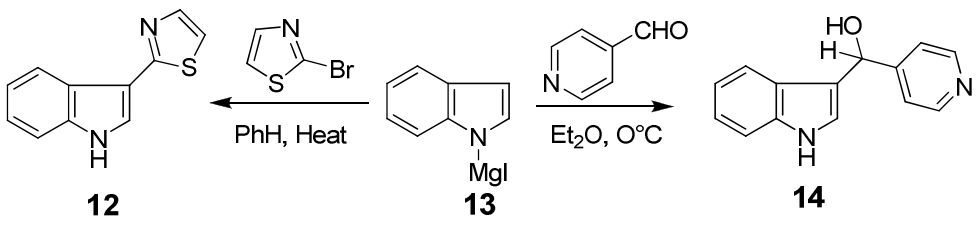
Scheme 1.3 Synthesis of Chuansinmycin (**9**)

The various nucleophilic addition reactions have been reported on indole, for example, the Grignard reaction of 5-nitroindole (**10**) and followed by treatment of the resulting mixture with aqueous potassium permanganate led to the formation of alkylated indole (**11**) (scheme 1.4).[3]



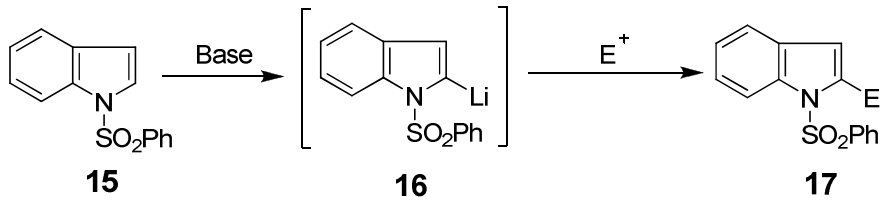
Scheme 1.4 Reaction of 5-nitroindole **10** with Grignard reagent

The reaction with strong base will effect complete conversion of an *N*-unsubstituted indole into the corresponding indolyl anion, amongst the most convenient being sodium hydride, *n*-butyllithium or an alkyl Grignard reagent. Indolyl *N*-Grignard's (**13**), undergoes reactions predominantly at C-3 with a variety of carbon electrophiles such as aldehydes, ketones and acid halides, or reactive heteroaryl halides. Scheme 1.5 describes the application of indolyl *N*-Grignard's for the synthesis of natural product Camalexin (**12**).[6]

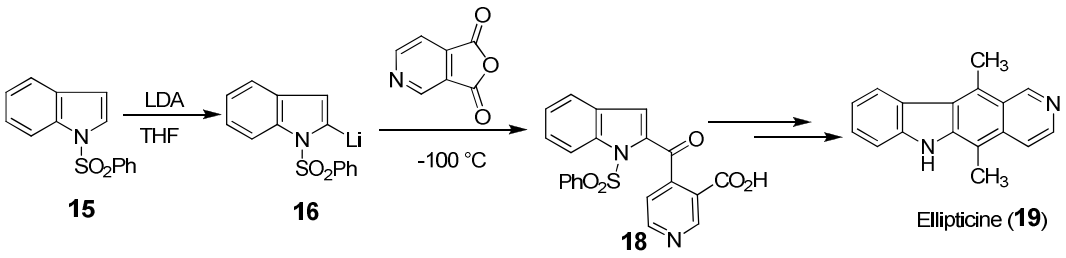


Scheme 1.5 Reactions of indolyl *N*-Grignard (**13**)

Metalation reactions, either by proton abstraction or directed *ortho* metalation or metal-halogen reactions have significant role in the construction of complex indoles from simple indole. Reaction of *N*-substituted indoles with strong bases leads to selective deprotonation at C-2, C-3 and C-7 depending on nature of protecting group. The scheme 1.6 describes the metalation at C-2 conducted in the absence of acidic hydrogen at *N*-1. The C-2 lithiated intermediate (**16**) can be quenched with variety of electrophiles to get 2-substituted indoles (**17**).[7]

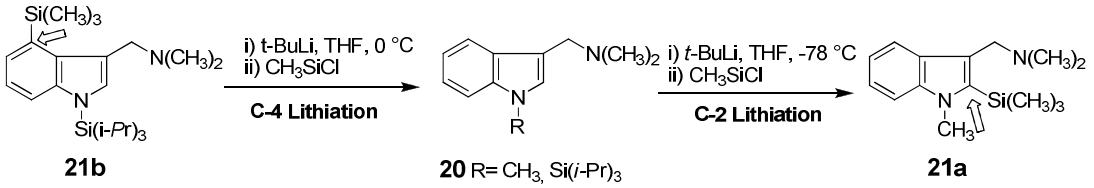


Scheme 1.6 Formation of 2-substituted indoles (**17**)



Scheme 1.7 Synthesis of Ellipticine (**19**)

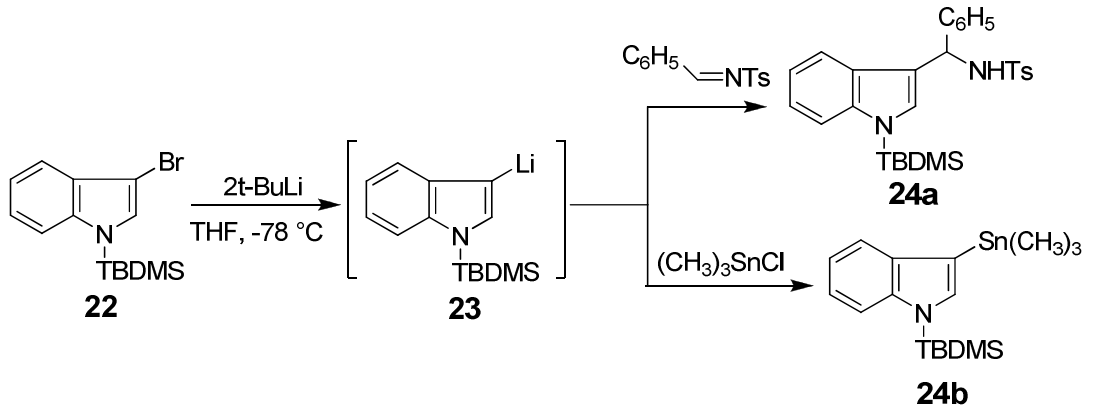
Several indole containing natural products such as Minovine, Ellipticine, Aristoteline, Sempervirine, Clavicipitic acid and Hippadine are synthesized, using the metalation reactions. For example, Gribble *et al.* reported antitumor alkaloid, Ellipticine (**19**) *via* C-2 metalation reaction (scheme 1.7).[8] Directed ortho metalation (DOM) is another class of metalation reaction. The substituents such as ethers, alkoxides, halogens, carboxylates, carboxamides, sulfonamides, pyridines and oxazolines direct metalation (metal–hydrogen exchange) to *ortho* positions to them. The scheme 1.8 describes the C-2 and C-4 metalations directed by dimethylamino group in Gramine (**20**). Depending on the nitrogen substituents, lithiation can occur at C-2 or C-4. Simple substituents such as methyl or methoxy direct lithiation to C-2 position to afford **21a** upon quenching with trimethylsilyl chloride. On the other hand, lithiation occurs preferentially at C-4 giving **21b** (scheme 1.8).[9]



21b **20** R= CH_3, $Si(i\text{-}Pr)_3$ **21a**

Scheme 1.8 Regioselective lithiation of Gramine (**20**)

Halogen-metal exchange is another useful strategy to synthesize diverse functionalized indoles. Halogen-lithium exchanges at C-2 or C-3 or on benzenoid ring of indole are widely reported protocols. Scheme 1.9 describes the metal-halogen exchange at C-3 of indole. The intermediate (**23**) is generated from *N*-protected 3-bromoindole (**22**) by reacting with strong base *t*-butyl lithium, which upon reaction with electrophiles led to the substituted indoles (**24a** or **24b**).



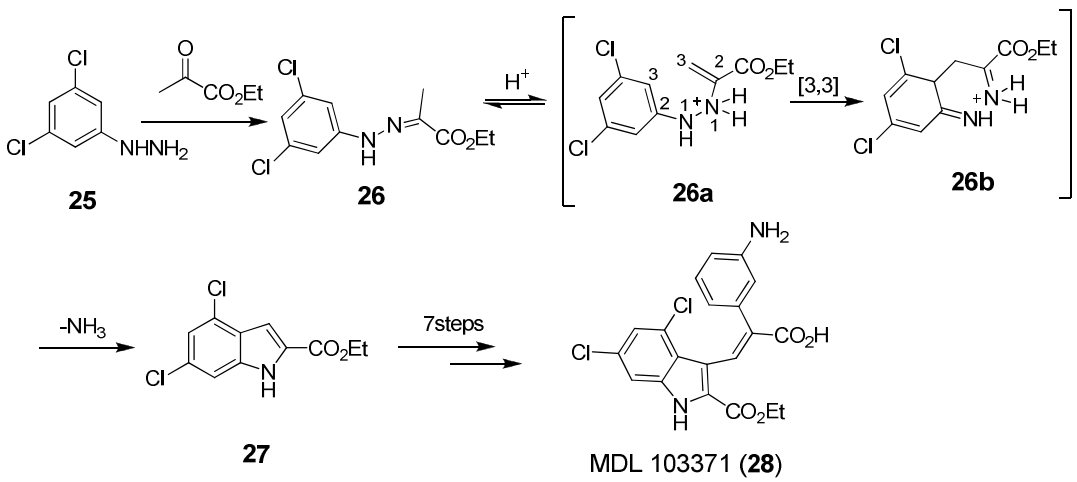
22 **23** **24a** **24b**

Scheme 1.9 Halogen–metal exchange involving 3-haloindole (**22**)

1.3 Some important synthetic methods for indole ring construction

The indole ring is one of the most widely distributed heterocycle in nature. Several structurally diverse natural or synthetic indoles found to have significant biological activities; as a result indole becomes a subunit in numerous pharmaceuticals. Therefore, synthesis of diverse variety of indoles has drawn the attention of the organic chemists to develop novel synthetic routes utilizing simple and readily available starting materials. In the past, a large number of synthetic protocols have been reported for the construction of indole scaffolds and many researchers have highlighted these efforts in the form of reviews.[10-12] This section mainly focuses on some important synthetic protocols used for the construction of indole ring and also discussed their utility as intermediates for large-scale preparations of biologically active molecules.

(i) Fischer Indole Synthesis: Indole and its derivatives are usually prepared from non-heterocyclic precursors by cyclization reactions on suitably substituted benzenes. Fischer synthesis is the most popular and oldest methodology for indole ring construction.[13] The Fischer reaction often provides a simple and efficient method for the transformation of enolizable *N*-arylhydrazones into indoles in presence of acid or acid catalysts.[13] The mechanism of the Fischer indole cyclization is thought to involve a [3,3]-sigmatropic rearrangement of an ene-hydrazine tautomer (**26a**) to a bis iminobenzyl ketone (**26b**) and later cyclization and aromatization with the loss of NH_3 affords indole **27**.[14-15] Synthesis of key intermediate (**27**), required for the preparation of *N*-methyl-D-aspartate (NMDA)-type glycine receptor antagonist,MDL 103371 (**28**), was carried out using Fischer indole strategy. The reaction of 3,5-dichlorophenylhydrazine (**25**) and ethyl pyruvate gave hydrazone (**26**) which was then cyclized in presence of PPA to produce **27** as out lined in scheme 1.10.[16] The compound **28** was prepared from **27** in seven steps..

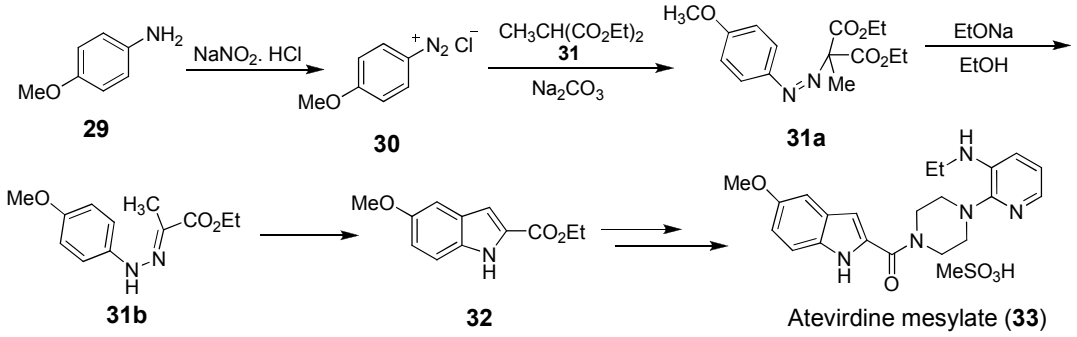


Scheme 1.10 Fischer indole synthesis of MDL 103371 (**28**)

Fischer synthesis has shown industrial applications for the construction of indole ring in the generic drugs such as Almotriptan, Sumatriptan, Avitriptan, Indomethacin and Nosiheptide.[17]

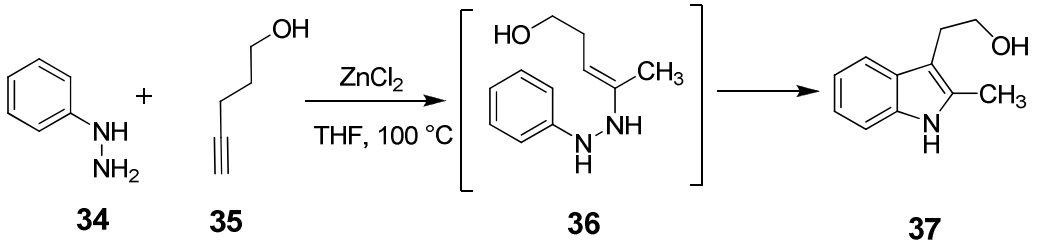
(ii) Via Japp-Klingemann Reaction: This reaction provides an alternative approach to prepare arylhydrazones employed in the Fisher cyclization. Diazotization of *p*-anisidine **29** using classical conditions afforded the diazonium salt **30** which was directly treated with β-ketoesters **31** to obtain azo intermediate **31a**. Treatment of **31a** with catalytic sodium ethoxide followed by Fischer cyclization provided indole-2-caboxylic ester **32**. Utilizing extremely useful intermediate indole-2-caboxylic acid, Bessard and co-workers

developed a production-scale synthesis of non-nucleoside reverse transcriptase inhibitor, Atevirdine mesylate (U-87201E; **33**) from diazonium salt **30** (scheme 1.11).[18]



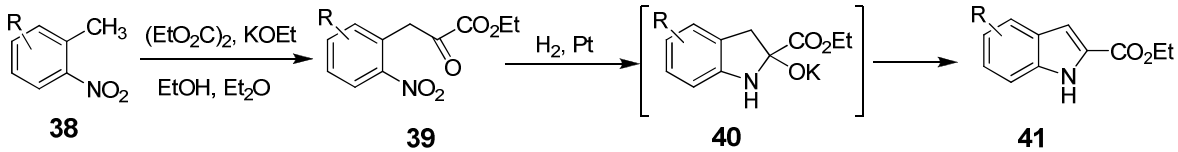
Scheme 1.11 Japp-Klingemann synthesis of Atevirdine mesylate (**33**)

Beller and co-workers reported zinc-mediated hydroamination reaction of phenylhydrazine (**34**) with terminal alkyne (**35**) to afford hydroxyl-functionalized indole **37**. The reaction proceeds *via* intermediate **36** which followed by Fischer cyclization (scheme 1.12).[19]



Scheme 1.12 Zinc-mediated synthesis of indole **37**

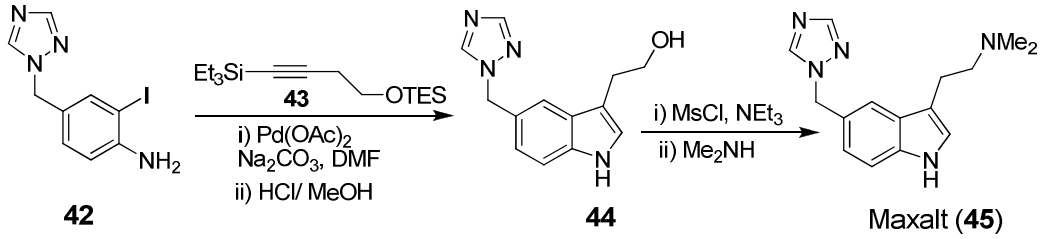
(iii) Reissert Indole Synthesis: The classical Reissert indole synthesis involves the reaction of *o*-nitrotoluene (**38**) with diethyloxalate in presence of strong base to produce *o*-nitrobenzylcarbonyl compounds (**39**), which upon reductive cyclization via intermediate (**40**) undergoes spontaneous loss of water to afford C-2 substituted indoles (**41**) (scheme 1.13).



Scheme 1.13 Reissert synthesis of indole-2-carboxylates (**41**)

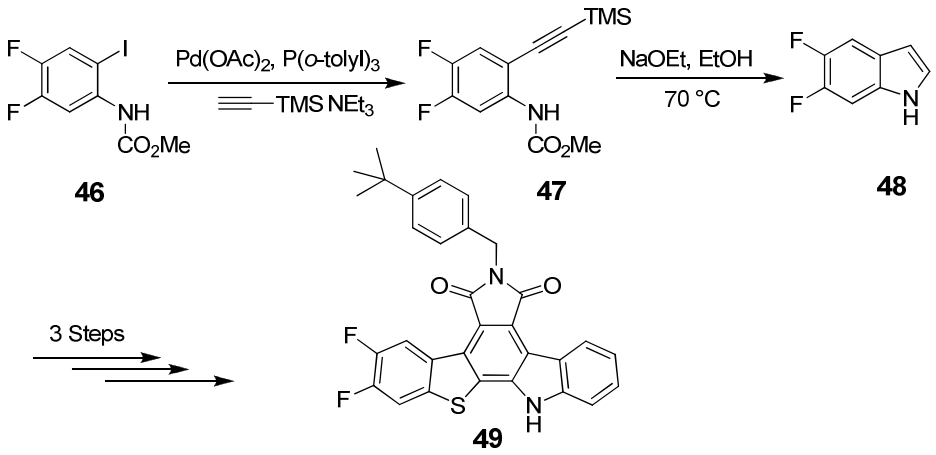
(iv) Via Larock Heteroannulation: The Larock heteroannulation is one of the attractive methods for the synthesis of 2,3-disubstituted indoles. The protocol involves the

palladium-catalyzed reaction of internal alkyne with *o*-iodoaniline.[11] Chen and co-workers utilized this methodology for the synthesis of antimigrane drug, Maxalt (**45**). Heteroannulation reaction of *o*-iodoaniline (**42**) and alkyne (**43**) in presence of palladium acetate led to indole **44**, which was further treated with dimethylamine to give desired drug Maxalt (**45**) as illustrated in scheme 1.14.[20]



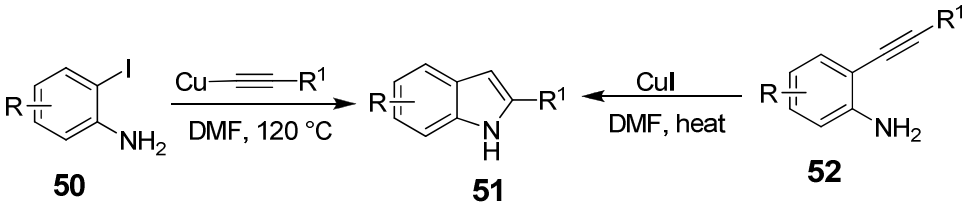
Scheme 1.14 Synthesis of Maxalt (**45**) *via* heteroannulation

The alkoxide mediated cyclization of 2-alkynylanilines is a valuable synthetic method for the construction of 2,3-unsubstituted and 2-substituted indoles. Wang and co-workers utilized this methodology for the synthesis of 5,6-difluoroindole (**48**), which was needed in large quantities for the preparation of the Rebeccamycin analogue **49**. Preparation of indole (**48**) involves initial palladium-catalyzed coupling of carbamate **46** with trimethylsilylacetylene in the presence of $Pd(OAc)_2$ and $P(o\text{-tolyl})_3$ to afford trimethylsilyl acetylenide **47**. The reaction of **47** with sodium ethoxide in ethanol resulted in cyclization, desilylation, and deacylation to provide **48** in 82% yield. Further elaboration of indole (**48**) to (**49**) was completed in three additional steps (scheme 1.15).[21]



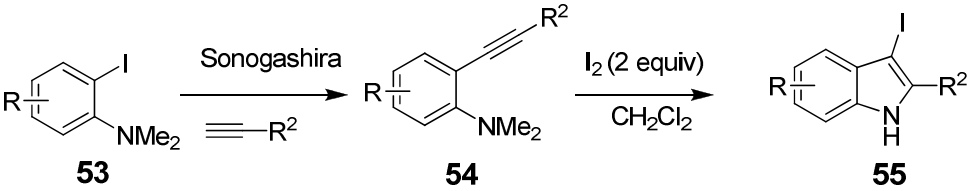
Scheme 1.15 Synthesis of rebeccamycin analogue **49**

(v) Castro Indole Synthesis: The Castro indole synthesis formally involves cyclization of either *o*-iodoaniline derivatives (**50**) with cuprous acetylides or 2-alkynylanilines (**52**) with copper (I) iodide (scheme 1.16).[17]



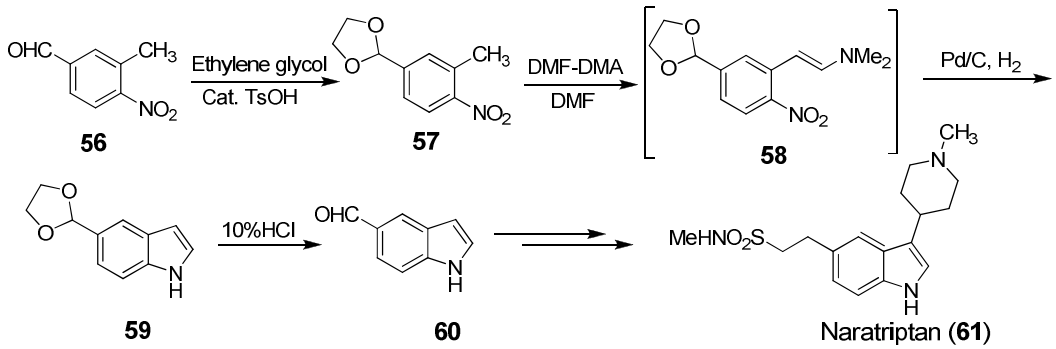
Scheme 1.16 Copper-catalyzed Castro indole synthesis **51**

Yue and Larock have also demonstrated the iodine catalyzed electrophilic cyclization of 2-alkynyldimethylanilines **54** to afford 3-iodoindole (**55**). The Sonogashira coupling of 2-iodo-*N,N′*-dimethylanilines **53** with various alkynes furnished **54** which upon reaction with molecular iodine afforded 3-iodoindoles **55** (scheme 1.17).[17]



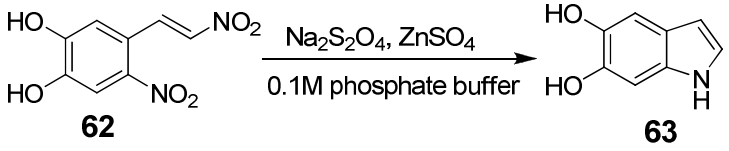
Scheme 1.17 Iodine-promoted synthesis of 3-iodoindoles (**55**)

(vi) Leimgruber-Batcho Indole Synthesis: The reductive cyclization of aromatic nitro compounds is an extremely powerful method for the preparation of the indole ring. Leimgruber-Batcho is one of the most important and commonly used methods for the preparation of 2,3-unsubstituted indoles.[22-23] The Leimgruber-Batcho indole synthesis involves the condensation of an appropriately substituted *o*-nitrotoluenes (**56**) with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) to give intermediate *β*-(dimethylamino)-2-nitrostyrene (**57**).[24-26] Reductive cyclizations of **57** *via* the intermediacy of **58** led to substituted indole (**59**). The reaction is capable of tolerating a large range of substituents present on benzene ring and has been extensively useful for the construction of both natural products and pharmaceutically important compounds. Further, Simig and co-workers have demonstrated the utility of indole **59** in the practical synthesis of 5-formylindole (**60**) to develop a new manufacturing route for the antimigrane drug, Naratriptan (**61**) (scheme 1.18).[27]



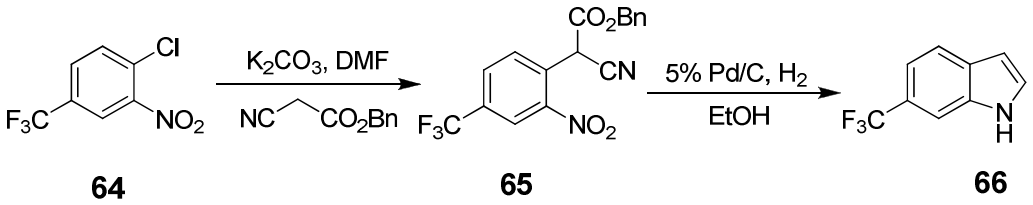
Scheme 1.18 Leimgruber-Batcho synthesis of Naratriptan (**61**)

Reductive cyclization of *β*-nitrostyrenes **62** is an effective method for the construction of indoles. Prota and co-workers have reported 5,6-dihydroxyindole (**63**) *via* reductive cyclization of *β*-nitrostyrenes (**62**) with $Na_2S_2O_4/ZnSO_4$ in 0.1 M phosphate buffer at pH 4 (scheme 1.19).[28]



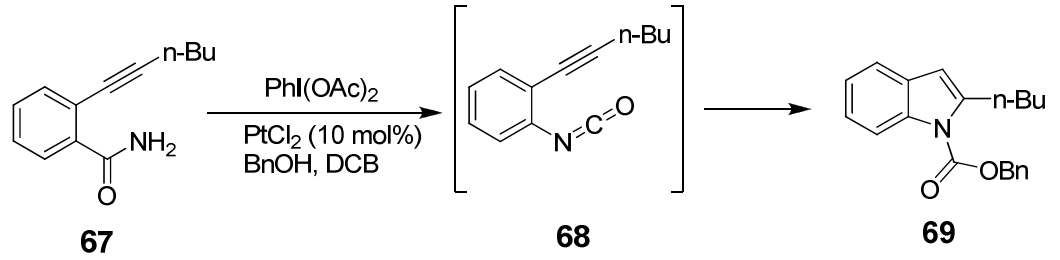
Scheme 1.19 Synthesis of 5,6-dihydroxyindole (**63**)

Reductive cyclization of *o*-nitrophenylacetonitrile (**65**) is an useful strategy for the preparation of 2,3-unsubstituted indoles. Walkington and co-workers utilized this process for the preparation of >100 kg of 6-(trifluoromethyl)indole (**66**). Nucleophilic aromatic substitution of 4-chloro-3-nitrobenzotrifluoride (**64**) with benzyl cyanoacetate gave compound (**65**) in good yields. Hydrogenation of crude (**65**) with 5% Pd/C in ethanol afforded the desired indole **66** (scheme 1.20).



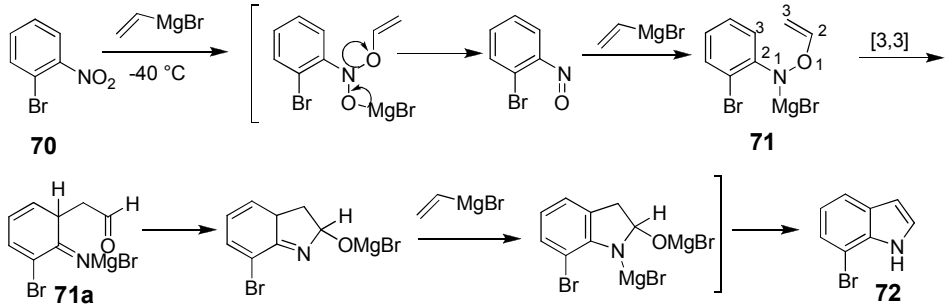
Scheme 1.20 Synthesis of 6-(trifluoromethyl)indole (**66**)

Yanada reported a concise synthesis of *N*-carboxyl indoles **69** from 2-alkynylbenzamides (**67**). Iodobenzenediacetate-promoted a Hofmann rearrangement of benzamide (**67**) led to isocyanate (**68**) which upon platinum-catalyzed cyclization and esterification, efficiently afforded indole (**69**) (scheme 1.21).[29]



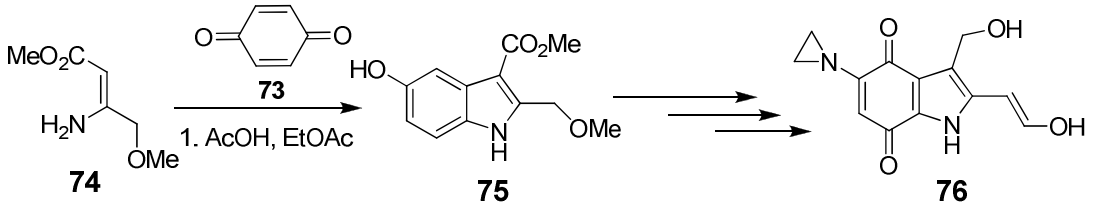
Scheme 1.21 Indole synthesis *via* Hofmann rearrangement of **67**

(vii) Bartoli Indole Synthesis: This protocol involves the reaction of *o*-substituted nitrobenzene (**70**) with three mole equivalents of vinylmagnesium bromide to produce 7-bromoindole (**72**). Reaction involves the [3,3]-sigmatropic rearrangement, much like that involved in the Fischer cyclization, and finally hetero ring closure of intermediate **71a** occurs to produce **72** (scheme 1.22).[30]



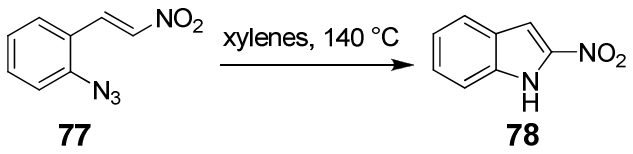
Scheme 1.22 Bartoli synthesis of 7-bromoindole (**72**)

(viii) Nenitzescu Indole Synthesis: The classical Nenitzescu indole synthesis involves the condensation of *p*-benzoquinones (**73**) with *β*-aminocrotonic ester (**74**) for the regioselective formation of 1,2,3-trisubstituted-5-hydroxyindole (**75**). Kasai *et al.* utilized Nenitzescu method for the synthesis of indole (**75**) to develop a large scale synthesis of antitumour indole quinine, EO9 (**76**) (scheme 1.23).[31]



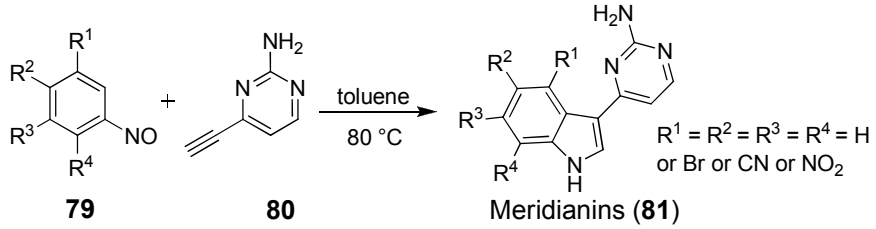
Scheme 1.23 Nenitzescu synthesis of antitumour indole quinine, EO9 (**76**)

(ix) Sundberg Indole Synthesis: The Sundberg indole synthesis proceeds *via* cyclization of 2-azidonitrostyrene **77** to give 2-nitroindoles **78** in good yields (scheme 1.24).[32]



Scheme 1.24 Synthesis of 2-nitroindole (**78**)

(x) Penoni Indole Synthesis: This approach involves the cycloaddition reaction of nitroso arenes with alkynes to accomplish the 3-substituted indoles. Most recently, Penoni *et al.* used this strategy for one-pot synthesis of biologically active marine indole alkaloids, Meridianin and its analogues **81**. Reaction involves the thermal annulation of nitroso arenes (**79**) and 2-amino-4-ethynylpyrimidine (**80**) in toluene at 80 °C to produce Meridianins (**81**) in good yields (scheme 1.25).[33-34]



Scheme 1.25 Synthesis of Meridianins (**81**)

1.4 Indole scaffold in drug discovery

Indole is a privileged structure due to its significance in chemical and pharmaceutical fields. Diverse varieties of indole containing new chemical entities have been emerging as clinical candidates for several diseases. Several academic and industrial laboratories are working on indole-based scaffolds in diverse therapeutic areas. Many of designer molecules in medicinal chemistry have their roots from natural products. Naturally occurring, several alkaloids contain indole nucleus, which motivated researchers to pay attention towards synthetic indoles and their biological evaluation in various therapeutic areas.[35-36] The significance of indole nucleus is well recognized in medicinal chemistry due to its wide spectrum of biological activities (figure 1.2). A brief discussion on some classes of indole-based drugs is described in the following sections.

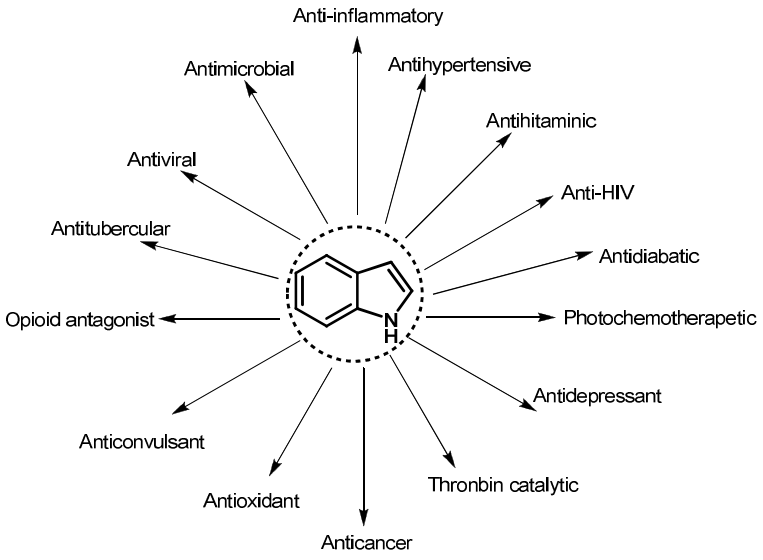
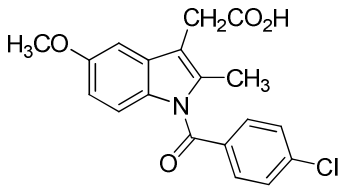


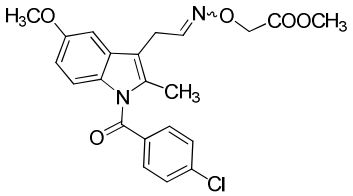
Figure 1.2 Biological significance of indole nucleus

1.4.1 Indole-based antiinflammatory agents

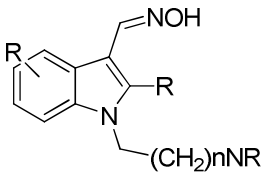
Indole derivatives are reported for their antiinflammatory activities. The drug indomethacin (**82a**) is one of the first non-steroidal antiinflammatory agents. Series of papers were devoted to the study of carboxylates of indole oximes (**82b**) and (**82c**) as inhibitors of leucotriene or prostaglandin biosynthesis. l,8-Diethyl-l,3,4,9-tetrahydropyrano[3,4-b]indole-l-acetic acid (etodolic acid, USAN) (**82d**) is a potent antiinflammatory agent, particularly active against a chronic rat model of inflammation (ED_{50} 0.7± 0.1 mg/kg).[37] Radwan *et al.* reported 3-(3′-indolyl)thiophene derivative (**82e**) as a potent antiinflammatory compound, whereas thiazolidine-4-one derivative (**82f**) exhibited analgesic activity.[38] 2-Phenyl-3-sulfonylphenyl-indole derivatives (**82g**) possess higher COX-2 inhibitory activity than the drug Celecoxib in cellular assay.[39] The drug Tadalafil (**82h**) and *N*-benzylindole derivative AWD 12281 (**82i**) are selective PDE5 and PDE4 inhibitors (figure 1.3).[40-41]



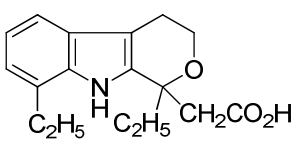
Indomethacin (**82a**)

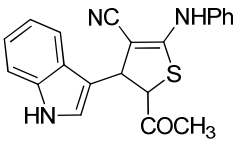


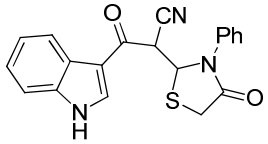
Indole oxime (**82b**)

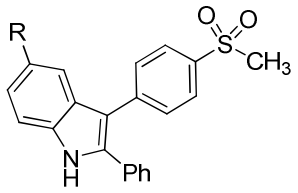


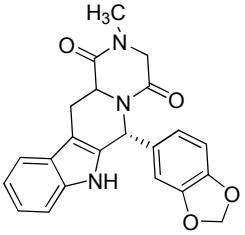
1-Aminoalkylindoles (**82c**)


Etodolic acid (**82d**)


Indolylthiophene (**82e**)


Thiazolidine-4-one (**82f**)


COX-2 inhibitor (**82g**)


Tadalafil (**82h**)

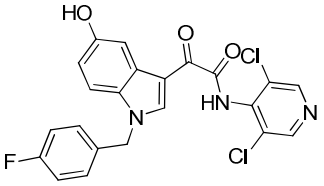
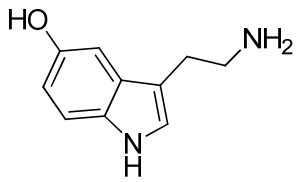
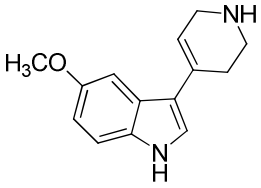

AWD 12-281 (**82i**)

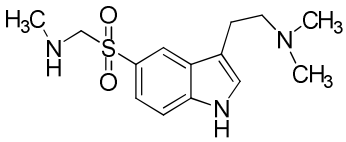
Figure 1.3 Indole containing antiinflammatory agents (**82a-i**)

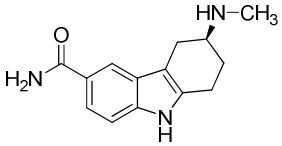
1.4.2 Indoles as serotonin receptor agonists

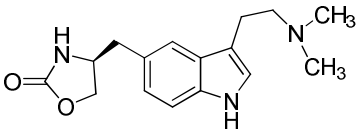
Serotonin (5-hydroxytryptamine) (**83a**) is the endogenous agonist of 5-HT receptors. It plays a vital role in normal brain functions including modulation of mood states, hunger, sex, sleep, memory, emotion, anxiety and endocrine effects. Serotonin activates at least seven distinct receptors (5-HT_1 to 5-HT_7) in the central and peripheral nervous systems to produce important modulatory effects.

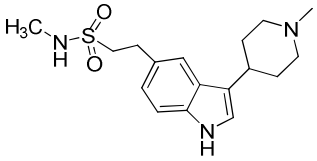

Serotonin (**83a**)

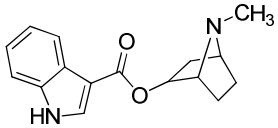

RU-24969 (**83b**)

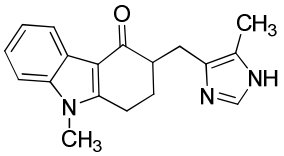

Sumatriptan (**83c**)


Frovatriptan (**83d**)


Zolmitriptan (**83e**)


Naratriptan (**83f**)


Tropisetron (**83g**)


Ondansetron (**83h**)

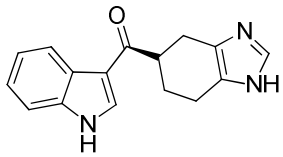
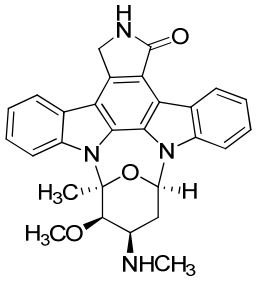

Ramosetron (**83i**)

Figure 1.4 Serotonin receptor agonists (**83a-i**)

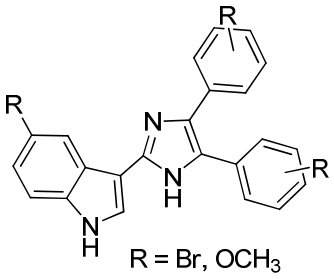
The 5-HT_{1A} generally involved in psychiatric disorders such as anxiety and depression. Indolyl alkylamine RU-24969 (**83b**) is a selective and potent 5-HT_{1A} and 5-HT_{1B} receptor agonist. Most of these classes of drugs are agonists of migraine associated with $5HT_{1B}$ and $5HT_{1D}$ serotonin receptors. Glaxo's Sumatriptan (**83c**) used for treatment of migraine,[42] Frovatriptan (FROVA) (**83d**) developed by Vernalis for the treatment of menstruation associated headaches,[43] the drugs Zolmitriptan (**83e**) (AstraZeneca) and Naratriptan (**83f**) (GlaxoSmithKline's) are used to treat acute migraine attacks and cluster headaches (figure 1.4).[44-45] The 5-HT_3 receptor antagonists, Tropisetron (**83g**), Ondansetron (**83h**) and Ramosetron (**83i**) are used for the treatment of chemotherapy-induced or radiation-induced nausea and vomiting and there are indications that they may be effective in the treatment of migraine or the pain associated with it.[46]

1.4.3 Indole-based antimicrobial, antifungal and antiviral agents

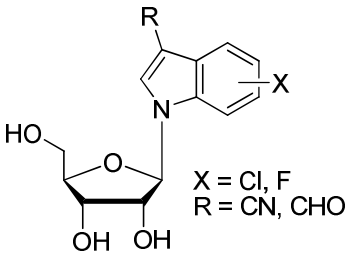
Indole derivative, Staurosporine (**84a**) was found to be an antifungal agent. The [3-(4,5-diaryl-imidazol-2-yl)-indoles] (**84b**) were found to exhibit potent antimicrobial activity against strains of *Staphylococcus aureus*, including methicillin-resistant strains (MRSA).[47] N-1 sugar substituted indoles (**84c**) were reported for their antiviral activity (figure 1.5).[48]



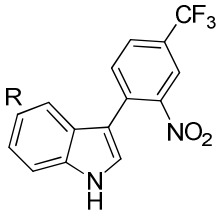
Staurosporine (**84a**)



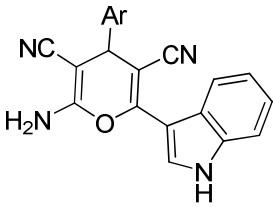
Indolyimidazoles (**84b**)



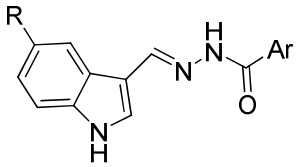
Antiviral agents (**84c**)



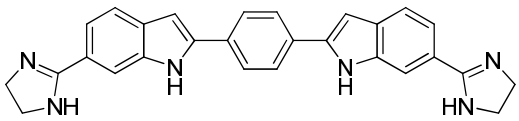
Arylindoles (**84d**)



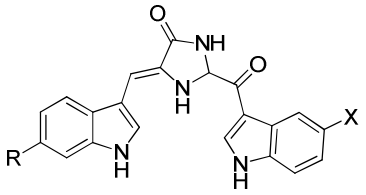
3-Pyranylindoles (**84e**)



Indolylhydrazones (**84f**)



Bis(indole) (**84g**)



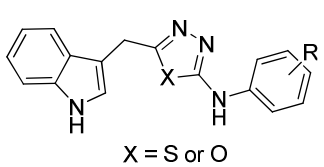
Rhopaladins C (**84h**)

Figure 1.5 Indoles as antibacterial and antifungal agents' (**84a-h**)

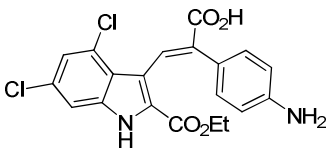
Panwar *et al.* reported the aryl substituted indoles (**84d**) for their antibacterial activity against *Escherichia coli and Staphylococcus aureus* exhibiting MIC value 7 µg/mL.[49] Perumal *et al.* reported the antimicrobial and antioxidant properties of-pyranylindoles (**84e**).[50] 3Indolylhydrazide and hydrazones (**84f**) were evaluated for their *in-vitro* antimicrobial activities using the 2-fold serial dilution technique against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.[51] Novel bis(indoles) (**84g**) were reported for their *in-vitro* antibacterial study against Gram-Positive and Gram-negative bacteria.[52] Indole alkaloid Rhopaladins C (**84h**) from marine tunicate *Rhopalaea sp* showed antibacterial activity against *Sarcina lutea* and *Corynebacterium xerosis* (MIC = 16 µg/mL, each).[53]

1.4.4 Indole-based molecules for diverse therapeutic areas

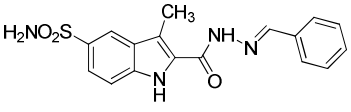
Siddiqu *et al.* synthesized a diverse series of indolylthiadiazoles and oxadiazoles (**85**a) and evaluated for their anticonvulsant activities.[54] The 4,6-dichloro-indole-2-carboxylic acids (**85b**) were reported as selective *N*-methyl-D-aspartate (NMDA) receptor antagonists.[55] 3-Phenyl-5-sulfonamidoindole-2-carboxylic acid hydrazide-hydrazones (**85c**) were reported as antidepressants.[56] Indole alkaloid, cryptolepines (**85d**) exhibited antimalarial activity,[57] oxindole derivatives (**85e**) acts as antituberculosis agents and compounds (**85f**) and (**85g**) were reported for their antidiabetic and antioxidant properties (figure 1.6).[58]



Indolylazoles (**85a**)



Indole-2-carboxylic acid (**85b**)



Indolylhydrazide (**85c**)

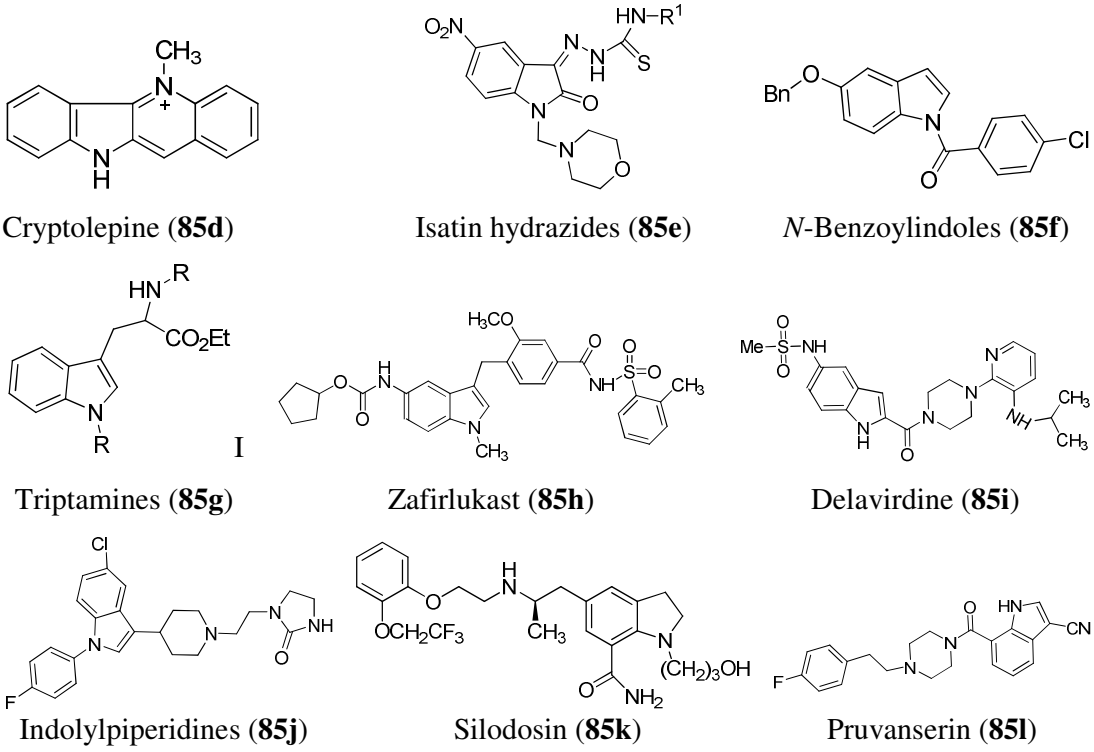


Figure 1.6 Indole containing compounds (**85a-l**) with broad therapeutic potential

AstraZeneca's' anti asthmatic drug, Zafirlukast (**85h**) is an oral leukotriene receptor antagonist often used as an inhaled steroid and/or long-acting bronchodilator.[59] Delavirdine (**85i**) is a non nucleoside reverse transcriptase inhibitor used for antiretroviral therapy. Antipsychotic drug Sertindole (**85j**) used for the treatment of schizophrenia, Silodosin (**85k**) has been launched recently for the treatment of urinary dysfunction associated with benign prostatic hyperplasia, Pruvanserin (**85l**) was identified as a highly specific $5HT_2$-receptor antagonist which in phase II studies for the treatment of insomnia.[60]

1.5 Cancer and its treatments

It is observed that cancer is a major life threatening disease in the past several decades. It is second largest death causing disease on earth. Cancer remains prime challenge for the researchers since 20th century. The simplest definition of cancer is uncontrolled (abnormal) growth of cells. In detail cancer arises from an accumulation of cells by mutations in oncogenes, tumor suppressor genes and genes that maintain the genomic integrity of the cell. Oncogenes lead to increase the net growth rate of the cells, when

activated by point mutations, amplifications or over expressions. The major differences in benign and malignant cell are, benign cells growth is slow, non-invasive and no metastasis where as in malignant cells growth is rapid, invasive and potential for metastasis. Some types of cancers, not all, are genetic but the majority of mutations that lead to cancer are somatic (figure 1.7).

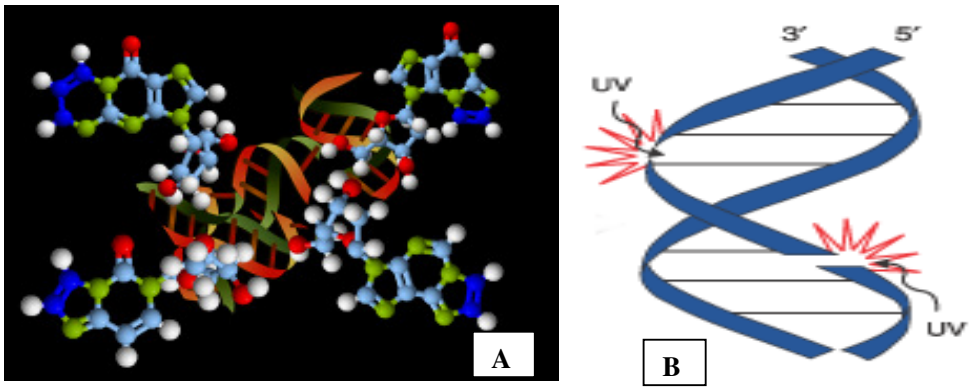


Figure 1.7 Mutations due to chemicals (A) and UV radiations (B)

Viruses and bacteria are sources of some types of cancers which cause mutations through insertional mutagenesis (cervical cancer). Chemicals (through food intake or pollution) cause mutations by binding to DNA forming DNA adducts (lung cancer). UV and ionizing radiation cause mutations in single and double strand DNA breaks (skin cancer). The major phenotypes of cancer cells are having characters such as self-sufficient growth signals, resistance to anti-growth signals (inactivate the cell cycle check points), adopt immortality (inactivated cell death pathways). Cancer cells are resistance to cell death, sustained angiogenesis and lose cell-to-cell interactions.

The majority of the cancer treatments involve surgery, radiation, chemotherapy and biological therapy. Surgery and radiation remains mainstay for the accessible tumors. In both the cases there are limitations of removing or destroying the bulk of the tumor mass and leaving behind residual tumor cells in the vicinity of the main tumor mass. Thus, these treatments are often followed by chemotherapy. Targeted therapies work by influencing the processes that control growth, division, and spread of cancer cells, as well as the signals that cause cancer cells to die naturally. Targeted therapies are suitable only for small subgroups of patients.

1.5.1 Cancer chemotherapy

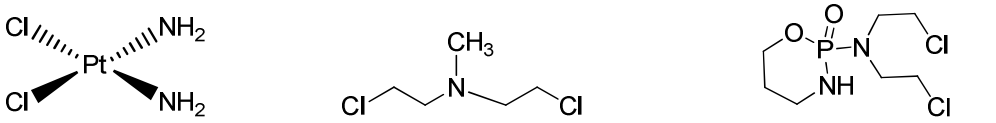
Cancer Chemotherapy involves the usage of chemical agents to stop or kill the cancer cell growth. The aim of most cancer chemotherapeutic drugs currently in clinical use is to kill malignant tumor cells by inhibiting some of the mechanisms implied in cellular division. Accordingly, the antitumor compounds developed through this approach are cytostatic or cytotoxic. These chemical agents mostly identified from libraries of naturally derived compounds or of chemically synthesized and evaluated their cytotoxicity to identify the promising lead molecules. Marketed anticancer drugs do not particularly target the cancer cells exclusively and can equally be cytotoxic to normally dividing cells of the healthy tissues, but fortunately have greater effect on cancer cells. Several years of cancer research across the globe dealt few medical approaches to cure the cancer to some extent and increase life span of cancer patients. But till date there is no drug invented that can cure cancer completely.

1.5.2 Classification of anticancer drugs

Anticancer drugs are classified according to chemical structure or mechanism of action and according to the cycle, phase specificity of the drug. The main classes of anticancer drugs are discussed in the following sections.

1.5.2.1 Alkylating agents

Alkylating agents are considered as cell cycle nonspecific agents (CCNSA) which remains active throughout the cell cycle. Alkylating agents are one of the earliest and commonly used chemotherapeutics. Alkylating agents have ability to alkylate any nucleophilic functional group under *in-vivo* conditions present in the cells. These drugs are effective during all phases of cell cycle. Therefore, they are used to treat a large number of cancers. However, they are more effective in treating slow-growing cancers such as solid tumors and leukemia. Some drugs like cis-platin complex (**86**) reacts *in-vivo*, binding to DNA and causing crosslinking of DNA, which ultimately triggers apoptosis. Methylchlorethamine (**87**) and Cyclophosphamide (**88**) are used for the treatment of prostate and leukemia cancers respectively (figure 1.8).



Cis-platin **86** Methylchlormethine **87** Cyclophosphamide **88**

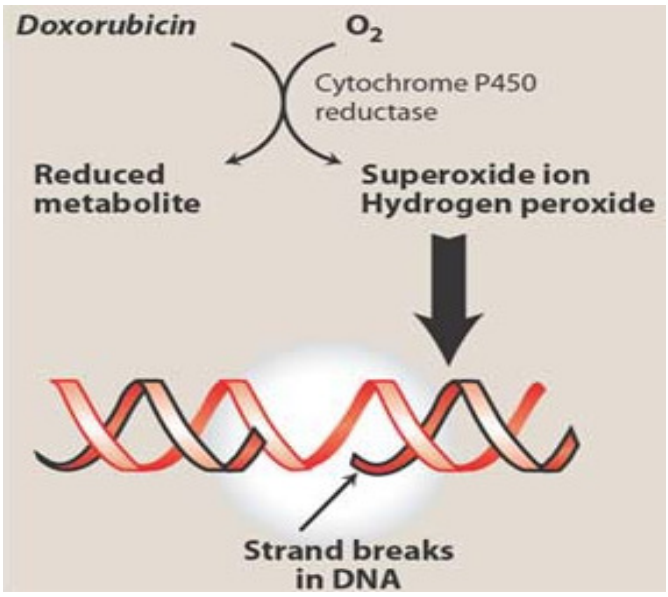
Figure 1.8 Alkylating agents (**86-88**) as anticancer drugs

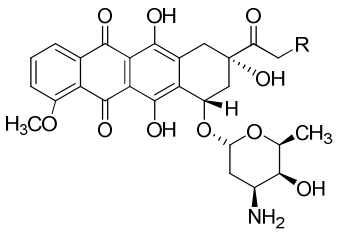
Alkylating agents inhibit cell reproduction by binding irreversibly with the nucleic acids after alkylation, DNA is unable to replicate and therefore can no longer synthesize proteins and other essential cell metabolites. Consequently, cell reproduction is inhibited and the cell eventually dies from the inability to maintain its metabolic functions.

1.5.2.2 Anticancer antibiotics

Anthracyclines (Doxorubicin, Daunorubicin), Bleomysin, Dactinomycin and Mitomycin are the classes of drugs come under this category. Clinically useful anticancer antibiotics are derived from *Streptomyces*. These drugs act *via* DNA intercalation and blocking DNA and RNA synthesis. Actinomycin D, intercalates DNA and thereby prevents DNA transcription and the drug is limited to the treatment of trophoblastic (gestational) tumors and the treatment of pediatric tumors.[61]

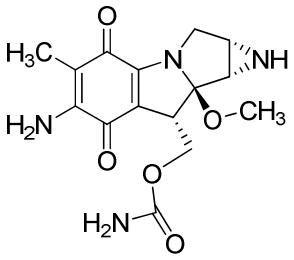
Mechanism action of Doxorubicin





R= OH Doxorubicin (**89a**)

R= H Daunorubicin (**89b**)



Mitomycin C (**90**)

Figure 1.9 Antibiotics (**89-90**) as anticancer agents

Tetracycline rings (Doxorubicin **89a** and Daunorubicin **89b**) are DNA intercalating agents and inhibit the progression of topoisomerase II and block the synthesis of DNA

and RNA (figure 1.9). These drugs are used in acute leukemias, lymphoma and a number of solid tumors.[62] Mitomycin C (**90**) is aziridine containing natural product used to treat upper gastro-intestinal (e.g. esophageal carcinoma), anal and breast cancers.[63]

1.5.3 Cell Cycle Specific Agents

Drugs that act during a specific phase of the cell cycle are classified under this category. The cell cycle consists of M (Mitosis) phase, G1 (Gap1, period before S) phase, S (DNA synthesis) phase G2 (Gap2, period after S) phase. The following figure 1.10 depicts the class of drugs in different phases of the cell cycle.

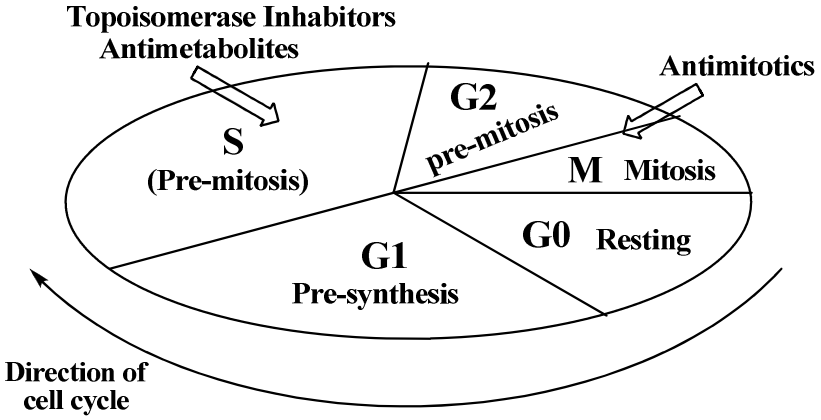


Figure 1.10 Cell cycle specific anticancer drugs

1.5.3.1 Antimetabolites

Antimetabolites interfere with nucleic acid synthesis or nucleotide synthesis. These are S phase-specific drugs that are structural analogues of essential metabolites and that interfere with DNA synthesis.

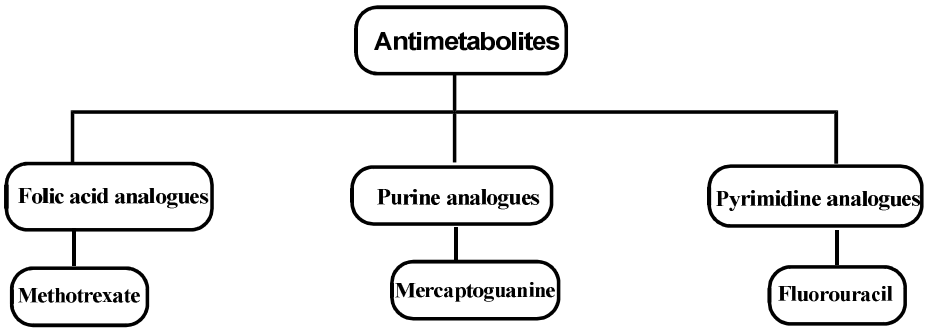


Figure 1.11 Classification of antimetabolite drugs

Structure-based classification of antimetabolites is shown in the figure 1.11. For example, Methotrexate (MTX) (**91**) is widely used for breast, bladder, lung, osteosarcoma, head

and neck cancers. Mechanism of action involves inhibition of dihydrofolate reductase enzyme (the enzyme that normally converts dietary folate to tetrahydrofolate form ,which is required for thymidine and purine synthesis).[64] Other class of drugs purine and pyrimidine antagonists such as mercaptopurine (**92**) and 5-fluorouracil (**93**) are used exclusively for acute myelogenous leukemia. Fluorouracil is an analogue of thymine in which the methyl group is replaced by a fluorine atom. It has two active metabolites. 5-FdUMP (5-fluorodeoxyuridine monophosphate) inhibits thymidylate synthetases and prevents the synthesis of thymidine, a major building block of DNA. 5-FdUTP (5-fluorodeoxyuridine triphosphate) is incorporated into RNA by RNA polymerase and interferes with RNA function (figure 1.12).

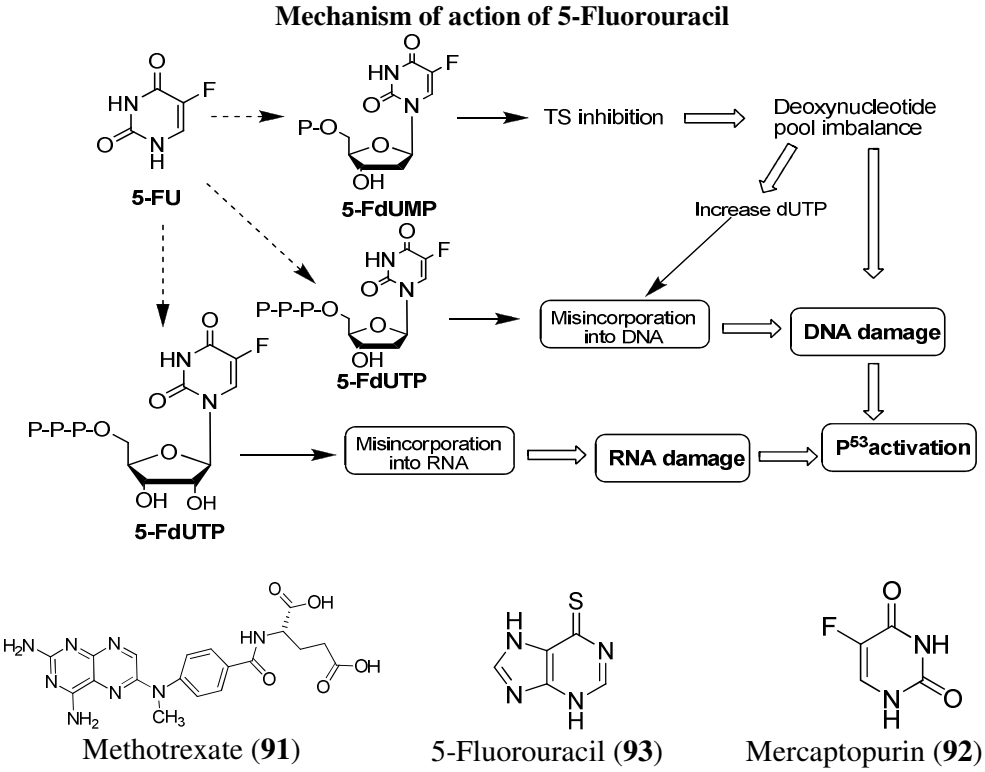
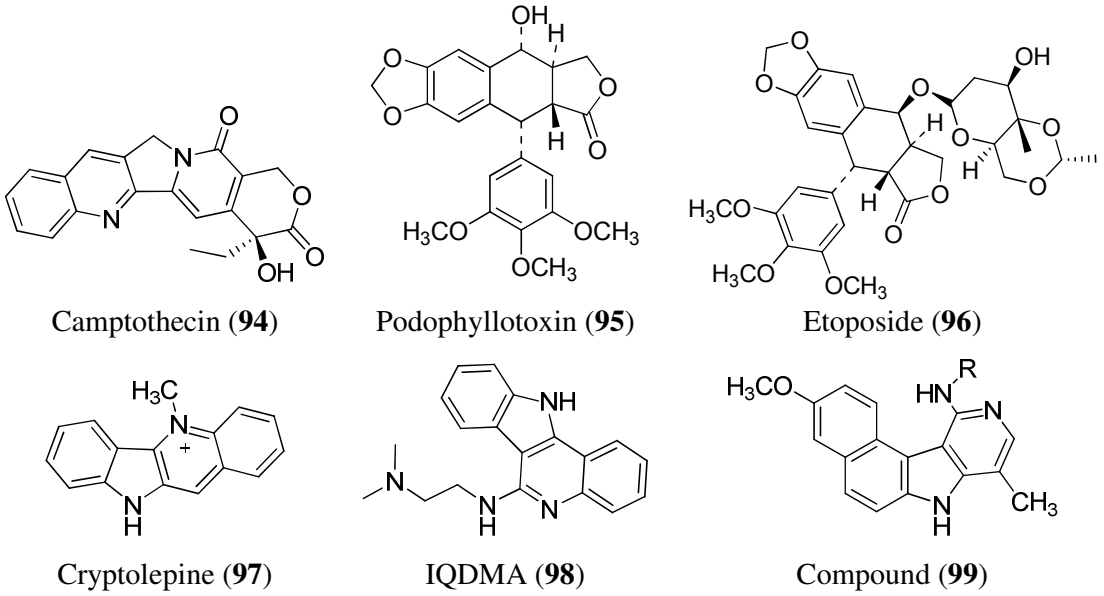


Figure 1.12 Antimetabolites (**91-93**) as anticancer agents

1.5.3.2 Topoisomerase Inhibitors

Topoisomerase inhibition is one of the most important targets in anticancer research. These drugs interfere with the action of topoisomerase enzymes (topoisomerases I and II). For example, natural product Camptothecin (**94**) inhibits Topoisomerase I by trapping Topcc,[65] Podophyllotoxin (**95**), Etoposide (**96**) and its derivatives show anticancer activity against Ewing's sarcoma, lung cancer, testicular cancer, lymphoma, non-

lymphocytic leukemia, and glioblastoma. Indoloquinolines Cryptolepine (**97**) and its synthetic analogues **98** and **99** showed prominent anticancer activity via DNA intercalation (figure 1.13).[66-68]



Camptothecin (**94**) Podophyllotoxin (**95**) Etoposide (**96**)

Cryptolepine (**97**) IQDMA (**98**) Compound (**99**)

Figure 1.13 Topoisomerase inhibitors (**94-99**) as anticancer agents

1.5.4 Antimitotic agents

Microtubules are the basic components of cell structure, which take part in a wide number of pivotal cellular functions, such as motility, division, shape maintenance, and intracellular transport.[69] Microtubules are made up of heterodimeric α and β-tubulin polymers characterized by a highly dynamic behavior; it was established that this dynamicity is responsible for functions of microtubules (figure 1.14). Drugs interfering with microtubules/tubulin dynamic equilibrium are called antimitotic agents.[70] These drugs cause the appearance of typical features at the level of chromosomes, nuclear membrane, mitotic spindle and G2/M phase and cause the mitotic arrest. Dynamic equilibrium of tubulin-microtubule is among the popular targets for anticancer therapy. Based on the mechanism of action of alternation of microtubule dynamics, drugs can be classified into two categories. One group of drugs, like Paciltaxel and Docetaxel, stabilizes microtubule polymer and the other group, like Vinblastine and Vincristine, inhibits (destabilizes) tubulin polymerization into microtubule.

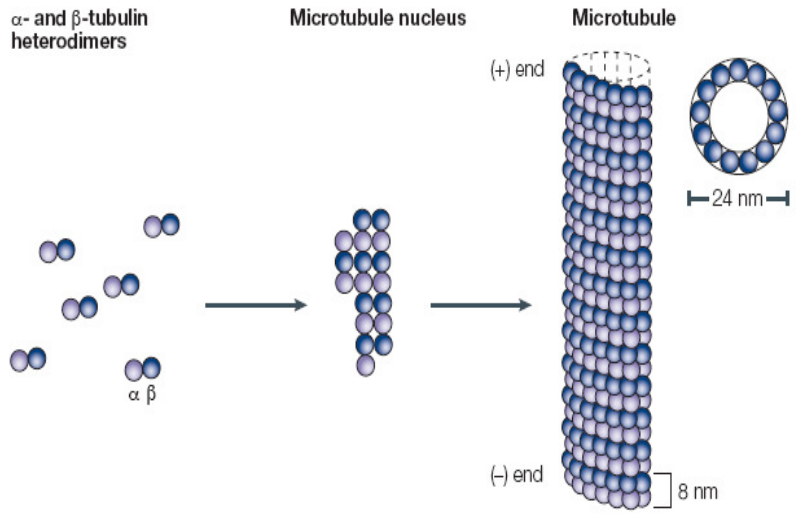


Figure 1.14 Microtubule formation[71]

Colchicine is one of the newly emerging clinical agents for anticancer research, molecules like combretastatin and its analogues bind the microtubule assembly distinct to vinca alkaloids and taxanes. These drugs bind to the microtubule assembly in diverse sites. Figure 1.15 shows the affinity of the antimitotic drugs to microtubule assembly.

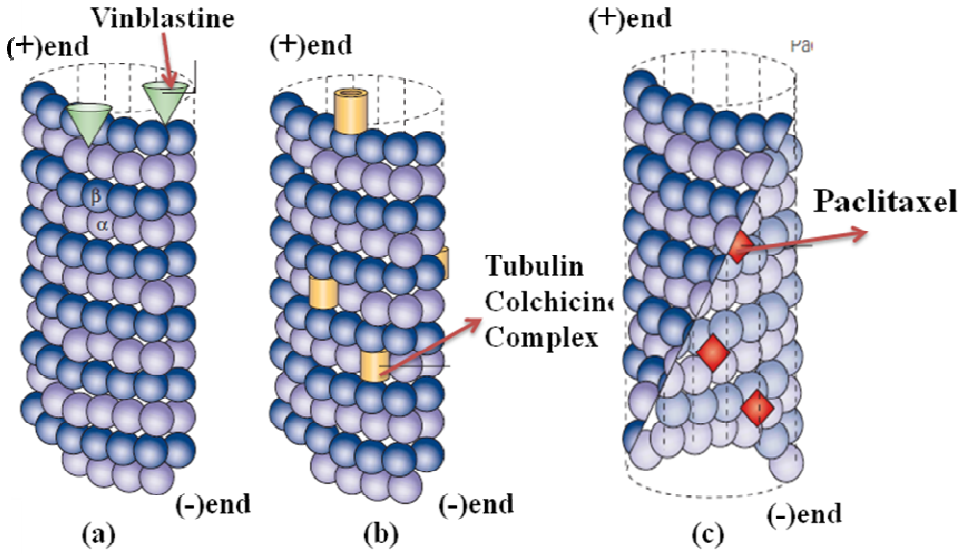
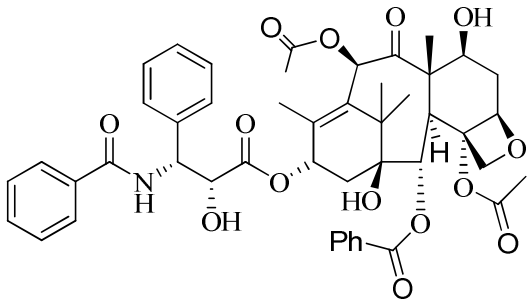


Figure 1.15 Microtubules binding interactions of antimitotic drugs[71]

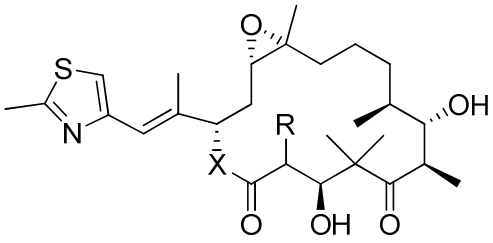
In figure 1.15 (a) represents few molecules of vinblastine bound to high-affinity sites at the microtubule plus end suffice to suppress microtubule dynamics, figure 1.15 (b) Colchicine forms complexes with tubulin dimers and copolymerizes into the microtubule

lattice, suppressing microtubule dynamics and figure 1.15 (c) A microtubule cut away to view the interior surface is shown. Paclitaxel binds along the interior surface of the microtubule, suppressing its dynamics.

Some of the microtubule destabilizing and stabilizing agents are shown in the figure 1.16. Paclitaxel (**100**) and Ixabepilone analogues (**101**) are microtubule stabilizing agents, approved for broad spectrum of caner chemotherapy.[72]



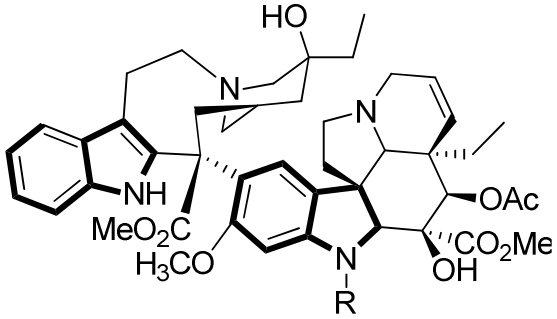
Paclitaxel (**100**)



Ixabepilone (X = NH_2, R = H) (**101a**)

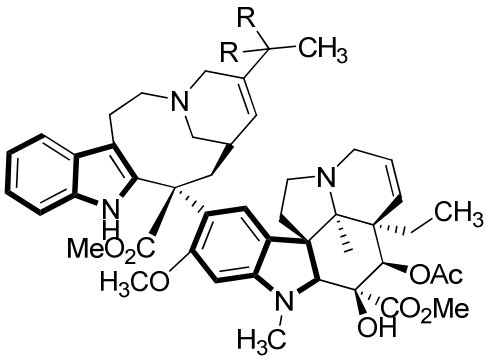
Patupilone (X = O, R = H) (**101b**)

Sagopilone (X = O, R = $(CH_2)CH_3$(**101c**)



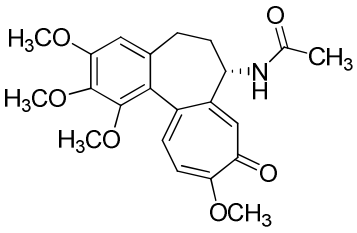
Vinblastine (R = CH_3) (**102a**)

Vicristine (R = CHO) (**102b**)

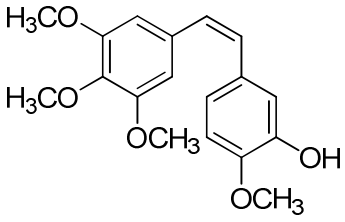


Vinorelbine (R = H) (**103a**)

Vinflunine (R = F) (**103b**)



Colchicine (**104**)



Combretastatin A-4 (CA4) (**105**)

Figure 1.16 Tubulin polymerization inhibitors (**100-105**)

Vinca alkaloids (**102**) and semi-synthetic derivatives (**103**) have shown tubulin depolymerization mechanism, whereas, colchicine (**104**) and combretastatin (**105**) follows the depolymerization and leads to disruption of dynamic equilibrium needed for the formation of microtubules from α and β- tubulin heterodimer, leading to formation of abnormal mitotic spindles. Diverse varieties of new chemical entities were designed as combretastatin analogues and evaluated for their tubulin binding capacities. Several authors have demonstrated the effect of structural modification to combretastatin A-4 (CA-4) in the form of review articles.[73-77]

1.6 Indole nucleus in anticancer research

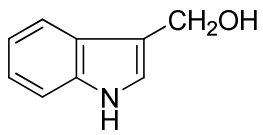
Indole nucleus plays a vital role in anticancer research. Many of the natural and synthetic indole analogues have shown remarkable anticancer activities. The development of indole-based anticancer new chemical entities is motivated from well known anticancer drugs vinblastine, vincristine (vinca alkaloids). Indole-based anticancer molecules have shown diverse mechanism of actions to kill the cancer cells. Majority of the synthetic indoles have structural analogy to anticancer natural products (vinca alkaloids or combretastatin) and exhibited anticancer activity through tubulin interactions. Natural and synthetic indole-based anticancer agents may be divided into three groups based on their structural features.

(a) Functionalized indoles as anticancer agents
(b) Indolylazoles and indolocarbazoles as anticancer agents
(c) Bis(indoles) as anticancer agents

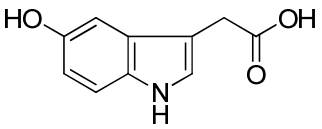
1.6.1 Functionalized indoles as anticancer agents

Diverse functional groups substituted indoles are described under this category (figure 1.17). The indole-3-carbinol (**106**) is one of the naturally occurring simple indoles isolated from cruciferous vegetables, such as broccoli, cabbage, and cauliflower has shown prominent anticancer activity and is in developmental clinical trials for regression of cervical and vulvar intraepithelial neoplasia (VIN) cancers. The compound (**106**) prevents chemically induced and spontaneous tumorigenesis in several animal studies[78] has potential therapeutic applications for treatment of metastatic breast cancers.[79-80] Indole-3-carbinol (**106**) induces a G1 growth arrest of human reproductive cancer cells (LnCaP) by induced production of the activated phosphorylated forms of p53, which

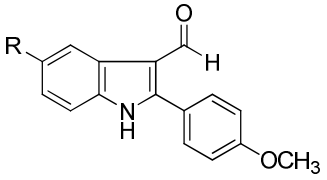
stimulate transcription of the CDK2 inhibitor p21.[81] Marconett *et al.* showed that indole-3-carbinol is most effective and tissue specific in disrupting the estrogen-dependent growth of human cancer cells, such as breast cancer, that coexpress ERα, GATA3, AhR and therefore will have a significantly reduced systemic side effects such as in heart and bone.[82]



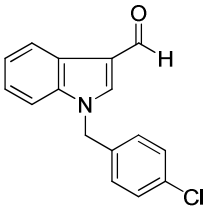
Indole-3-carbinol (**106**)



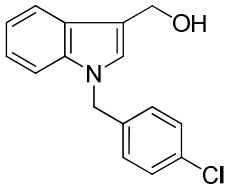
5-Hydroxyindole-3-acetic acid (5-IHAA) (**107**)



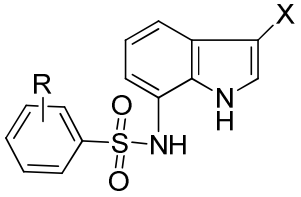
Indole-2-phenyl-3-carboxaldehyde (**108**)



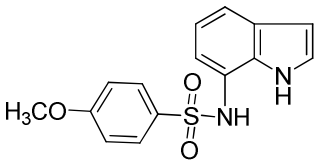
Oncrasin-1(**109**)



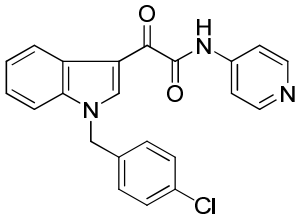
NSC-741909 (**110**)



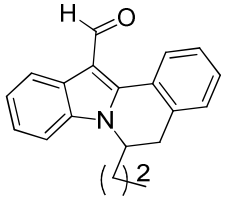
R = 4-SO_2NH_2 X = Cl (E7070) (**111**)
R = 4-CN, X = CN (ER68487) (**112**)



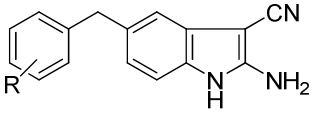
Indole-7-sulfonamide (**113**)



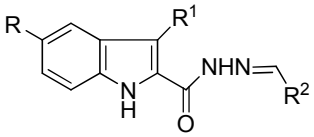
D-24851 (**114**)



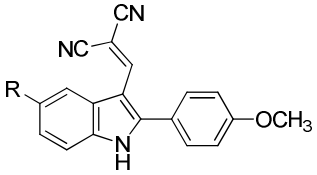
Indoloisoquinoline (**115**)



2-Amino-3-cyanoindole (**116**)



Indolylhydrazide (**117**)



2-Arylindoles (**118**)

Figure 1.17 Functionalized indoles (**106-118**) as anticancer agents

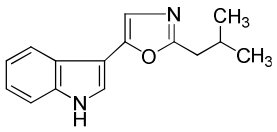
Ultra-violet B activated 5-hydroxyindole-3-acetic acid (5-$HIAA^{UVB}$) (**107**) induces apoptosis in prostate and bladder cancer cells through the stress signaling and apoptotic pathways and 5-$HIAA^{UVB}$ markedly increased the sub-G0/G1phase and resulted in cell cycle disruption.[83] Erwin von Angerer *et al.* reported simple indole derivatives 3-formyl-2-phenylindoles (**108**) as tubulin polymerization inhibitors. The compound **108** Inhibit the polymerization of tubulin to functional microtubules by binding to the colchicine binding

site and cell cycle arrest in the G2/M phase and probably leads to an apoptotic cell death. The indole aldehyde Oncrasin-1 (**109**) effectively kills K-Ras mutant cancer cells.[84-85] Shuhong *et al.* have recently reported Oncrasin-1 analogues as inhibitors of the C-terminal domain of RNA polymerase II and their antitumor activities revealed analogues are less cytotoxic to normal cells.[86] Induced anticancer activity of simple molecule NSC-741909 (**110**) is associated with sustained Jun N-terminal kinase (JNK) activation, resulting from suppression of JNK dephosphorylation associated with decreased protein levels of MAPK phosphatase-1.[87-88] Sulphonamide derived novel indole compound E7070 (**111**), ER-68487 (**112**) and oxindole ER-67865 have excreted anticancer activity at multiple points of cell cycle G1/S or G2/M phases,[89-91] The indole-7-sulfonamide (**113**) induces G2/M phase arrest in P388 cells, suggesting that the methoxy group plays a crucial role in binding to tubulin.[92] The compound D-24851 (**114**) exhibited excellent *in-vitro* and *in-viv*o antitumor properties and is in advanced pre-clinical evaluation. Compound **114** has a mechanism of action similar to that of vincristine and paclitaxel.[93-96] The dihydroindoloisoquinolines **115** showed inhibition of tubulin polymerization by binding to colchicine site.[97] Recently, Indole derivatives **116** (form AstraZeneca) and indolyl-2-hydrazide-hydrazones (**117**) (Abbott Lab) have been approved as angiogenesis inhibitors that cause selective destruction of tumor vasculature.[92] Indole methylene propanedinitriles (**118**) inhibited the growth of MDA-MB-231 and MCF-7 breast cancer cells with IC_{50} values below 100 nM.[98]

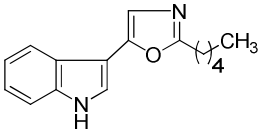
1.6.2 Indolylazoles and indolocarbazoles as anticancer agents

Indoles attached to any nitrogen containing heterocycle are classified under this category. The following discussion describes anticancer activity of natural and synthetic indolylazoles. Indolyloxazoles Labradorin 1 and Labradorin 2 (**119**) isolated of from *Pseudomonas syringae* pv. *Coronafaciens* found to be cytotoxic against NCI–H 460 (lung-NSC) human cancer cell lines with GI_{50} values of 9.8 µg/mL and 9.6 µg/mL, respectively.[99] Indolylthiazole, Camalexine (**120**), isolated from phytoalexin of *Arabidopsis thaliana* induces apoptosis in T-leukemia Jurkat cells.[100] The most recently, Fuchun Xie *et al.* reported the pyrimidine substituted indole (**121**) for their tubulin binding ability (IC_{50} = 0.79 µM) and **121** displayed high antiproliferative activities against several cancer cell lines with IC_{50} values ranging from 16 to 62 nM.[101] Calothrixins (**122**) exert *in-vitro* growth inhibitory in human malaria parasite and human cancer cells.

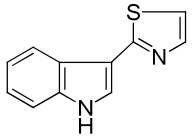
Some fused indole alkaloids Ervatamine (**123**) showed cytotoxicity against P-388 murine leukemia and A549 human lung cancer cell lines.[102] Duocarmycin SA (**124**) exhibits cytotoxicity through covalent reaction with adenine N-3 in the minor groove of AT-rich sequences.[103] Pyrimidine class of marine alkaloids indolylpyrimidines (Meridianins A-E) (**125**) isolated from the tunicate *Aplidium meridianum* displayed cytotoxicity towards murine tumor cell lines and are known as kinase inhibitors.[104-105] Aplycianins (**126**) are cytotoxic to the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma) and HT-29 (colorectal carcinoma).[106] Fused ring systems Variolin A-D (**127**) and Discodermindole (**128**) exhibit potent cytotoxicity against P388 murine leukemia cell lines and also being effective against Herpes simplex type I.[107-108] Didemnimides (**129**) and Granulatimides (**130**) having maleimide at C-3 of indole ring acts as G2 specific cell cycle checkpoint inhibitors.[109]



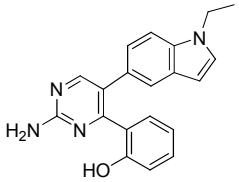
Labradorins 1(**119a**)



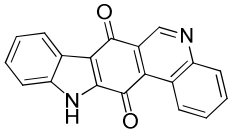
Labradorins 2 (**119b**)



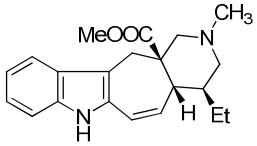
Camalexine(**120**)



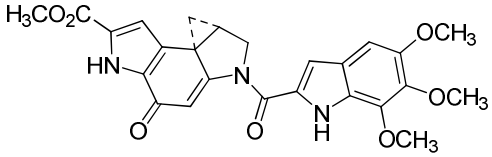
Indolylpyrimidine (**121**)



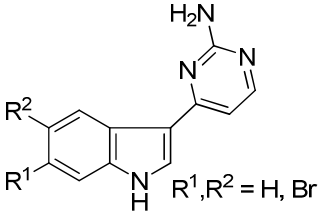
Calothrixins (**122**)



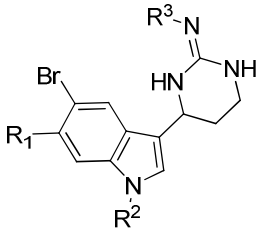
Ervatamine (**123**)



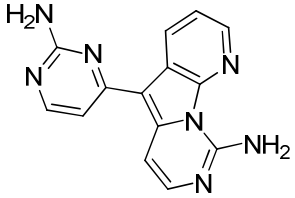
Duocarmycin SA (**124**)



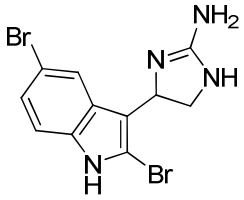
Meridianin A-E (**125**)



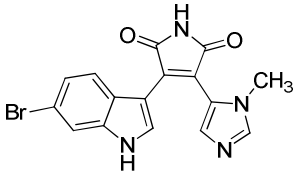
Aplycianins (**126**)



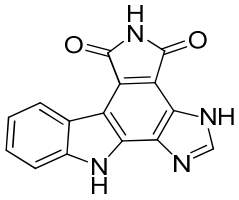
Variolin A-D (**127**)



Discodermindole (**128**)



Didemnimides (**129**)



Granulatimides (**130**)

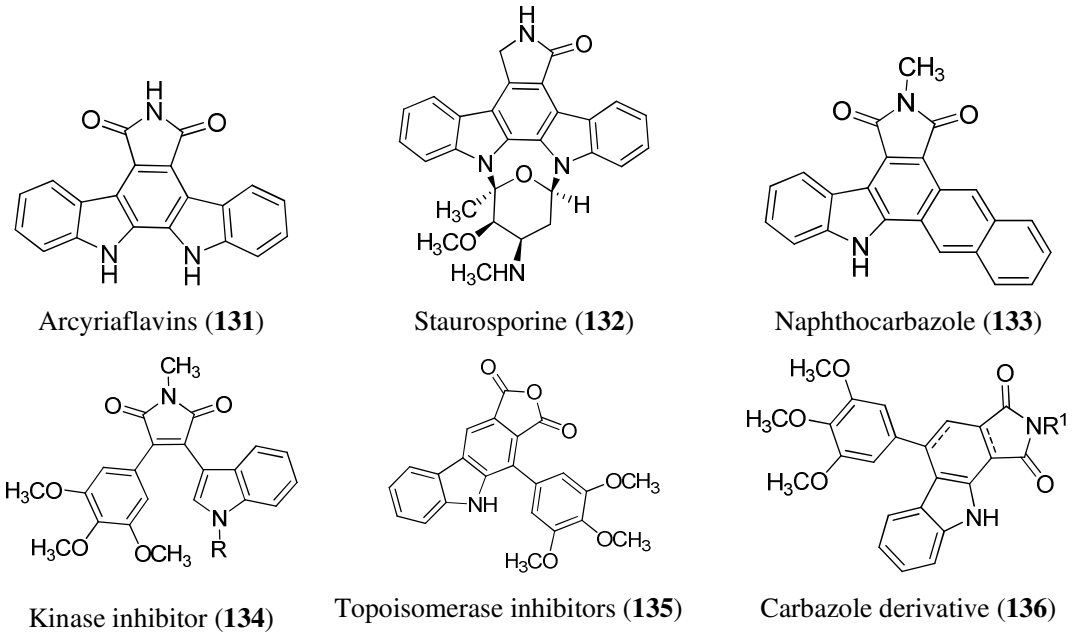


Figure 1.18 Indolylazoles and indolocarbazoles (**119-136**) as anticancer agents

Maleimido-indolocarbazole class of alkaloids is known for their cytotoxic properties. Recently, Arcyriaflavins (**131**) have been identified as CDK1 and CDK2 inhibitors,[110] The staurosporine (**132**) and its analogues (**133**) were found to be nonselective ATP-competitive kinase inhibitors of different Protein kinase C, cyclin-dependent kinases (CDKs) and tyrosine kinases which are involved in cell proliferation.[111-112] Another staurosporine analogue (**134**) with trimethoxyphenyl as one of the substituent have been reported as a selective VEGF-R2/3 kinase inhibitor and shown anticancer activity.[113] The carbazole derivative (**135**) reported as topoisomerase inhibitors with significant cytotoxicity.[114] The carbazole derivative (**136**) displayed cytotoxicity against murine B16 melanoma cell and acts as anti-vascular agents (figure 1.18).[115]

1.6.2.1 Synthetic indole-based combretastatin 4 (CA-4) analogues

Presence of indole nucleus in many of the natural products which are used for cancer chemotherapy had provoked the researcher to work on indole-based new chemical entities. Varieties of functional groups are tailored at C-2 and C-3 positions of the indole ring and evaluated for their anticancer activity; these structural modifications are mostly rational to the anticancer natural products. More over investigation of small molecules for cancer therapy is advantageous in terms of synthetic efforts, cost and environmental hazards. Synthesis of novel indole molecules involves either construction of indole ring

itself or derivatization of indole at different positions. Following discussion is mainly based on reported indoles whose anticancer activities are well documented over the past several years. Majority of indole-based drugs are able to modulate the microtubule assembly either by inhibition of tubulin polymerization or by blocking microtubule disassembly.

Diverse structural modifications were made around the indole nucleus and evaluated for their tubulin binding properties. Most of the synthesized molecules mimic the structural features of Combretastatin A-4 (CA-4) (**105**). The trimethoxyphenyl group ('A' ring) and "*cis*" conformation in the CA-4 are retained in the synthetic analogues. Rational for the structural modifications of **150** is shown in the figure 1.19. The 3,4,5-trimethoxybenzoyl substitution at C-3 of indole ring leads to an potent antimitotic agent BPR0L075 (**137**). BPR0L075 inhibits tubulin polymerization and induces mitochondrial-dependent apoptosis in various human cancer cells and effective in suppressing cell growth of both MDR-positive and negative tumor cells both *in-vitro* and *in-vivo*.[116-117] The compound BPR0L081 (**138**) showed anticancer activity against human NUGC3 stomach, MKN45 stomach, MESSA uterine, A-549 lung, and MCF-7 breast carcinoma cell lines in nano molar concentrations and also revealed the importance of trimethoxy phenyl group.[118] The indole derivative D64131 (**139**) also exhibited the tubulin polymerization with good activity against the human HeLa/KB cervical, SK-OV-3 ovarian, and U373 astrocytoma carcinoma cell lines (IC_{50} = 20-75 nM) and Further studies suggested that 2-aroylindoles (**139)** competitively binds with [^{3}H]colchicine to α,β-tubulin and inhibiting microtubule formation in the G2/M phase of the cell division cycle.[119-120] Medardea and co-workers reported 3(2)-aroylindoles (**140**) for the tubulin polymerization inhibition.[121-122] Effect of trimethoxyphenyl substituent was further evaluated by synthesizing 7-(3′,4′,5′-trimethoxybenzoyl)indoles (**141**) with improved inhibition of tubulin polymerization. In a process to modify substituents on indole the keto group of **137** is replaced by ether, thioethers and their sulphoxides (**142**, **143** and **144**). The compound **144** was found to be 1.6 times more active than colchicine and inhibited the growth of MCF7 human breast carcinoma cells with IC_{50} value 13 nM.[123-125] The ester group in **143** bioisosterically replaced with 5-memberd heterocycle to get a series of compounds (**144**) which improved its anticancer activity.[126]

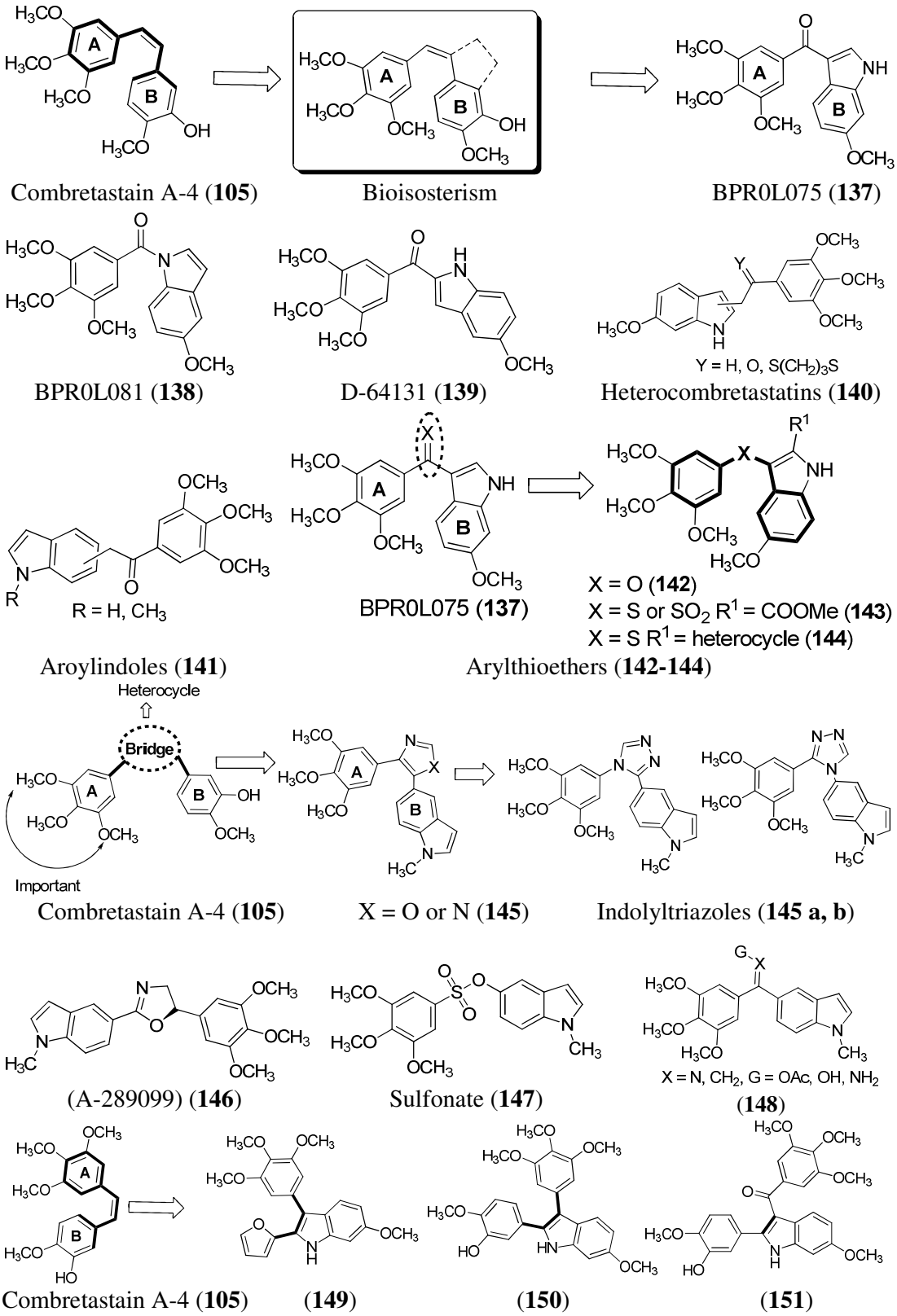


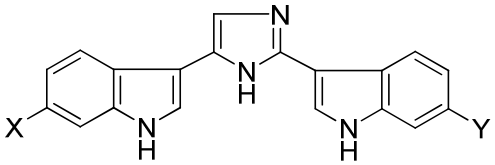
Figure 1.19 Indole-based combretastatin derivatives (**137-151**) as tubulin inhibitors

The *cis* double bond in CA-4 can be replaced by a 1,2-disubstituted five-membered heterocycle such as imidazole, oxazole and the 3-hydroxy-4-methoxyphenyl in CA-4 was substituted by an *N*-methyl-5-indolyl group. The compounds (**145**) showed potent antitubulin and cytotoxic activity.[127] Zhang and co-workers have reported triazole substituted indoles (**145a**) as potent anticancer agents. The 1,2,4-Triazole ring in **145a** retained the *cis* configuration required for bioactivity. These compounds exhibited potent tubulin polymerization inhibitory activity and cytotoxicity against a variety of cancer cells including MDR cancer cell lines.[128] Compounds with 3,4,5-Trimethoxyphenyl substituent at C-5 of indole ring *via* oxazoline[129] (A-289099) (**146**) or sulphonate[130] (**147**) or one carbon linkers (**148**) exhibited significant tubulin binding activity and cytotoxicity against wide range of cancer cell lines. The substituents on both C-2 and C-3 positions of indole ring led to another class of combretastatin analogues. The *cis* restricted five or six membered fused heteroatomic bridgehead analogues with two or three atom distance are reported with improved anticancer activity. Medarde *et al.* reported diarylindoles with trimethoxyphenyl at C-3 and aryl/heteroaryl at C-2 of indole ring. The furan substituted compound (**149**) was found to be best in inhibiting cancer cell lines and displayed a remarkable cytostatic activity with logIC_{50} values ranging from -7.48- to -7.64 for T-47D breast cancer cells to NB-10CNS cancer cells.[131] Compound **150** showed moderate activity as tubulin polymerization inhibitors. Insertion of keto functionality led to compound (**151**) with 1.3 times more active than CA-4 as a tubulin polymerization inhibitor The compounds (**149-151**) were less potent than CA-4 as inhibitors of [^{3}H]colchicine binding to tubulin and are cytotoxic against the MCF-7 breast carcinoma cells. The most potent indole **151** was 4.1 times less active than combretastatin A-4 (**105**) on the MCF-7 cells.[76, 132]

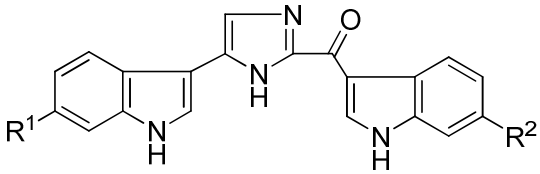
1.6.3 Bis(indoles) as anticancer agents

Bis(indoles) are one of the important class of indole containing natural products, in which two indole nuclei are separated by a heterocyclic ring spacer or any functional group. Bis(indole) alkaloids showed significant role in anticancer research, For example Nortopsentins (**152**) and Topsentins (**153**) were isolated from marine sponge *spongsorites ruetzleri*.[133] Nortopsentins A-D exhibited *in-vitro* cytotoxicity against P388 cancer cells (IC_{50} of Nortopsentin A-C are: 7.6;7.8;1.7 µg/ml), and antifungal activity against *candida albicans.*[134-135] Topsentins (**153**) inhibit the growth of P388 mouse leukemia cells and

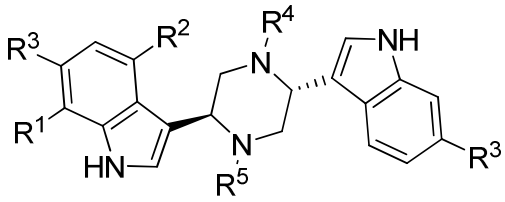
Herpes simplex virus, type 1 (HSV-1).[136] Piperazine containing bis(indole) Dragmacidin (**154**) from a deep water marine sponge *Dragmacidin* sp. exhibited *in-vitro* cytotoxicity with IC_{50} values of 15 µg/mL against P-388 cell lines and 1-10 µg/mL against A-549 (human lung), HCT-8 (human colon) and MDA-MB-231 (human mammary) cancer cell lines (figure 1.20). [137] Dragmacidin D and E (**159d** and **159e**)[138] have been identified as potent inhibitors of serine–threonine protein phosphatases.[139] Hyrtinadine A (**155**) exhibited *in-vitro* cytotoxicity against murine leukemia L-1210 and human epidermoid carcinoma KB cells (IC_{50} = 1.0 and 3 μg/mL, respectively).[140] Rhopaladins A-D (**156**) from Okinawan marine tunicate *Rhopa/aea sp* exhibited inhibitory activity against cyclin dependent kinase 4 (CDK4) and *c-erbB-2* kinase (IC_{50} = 12.5 and 7.4 µg/mL, respectively).[53]



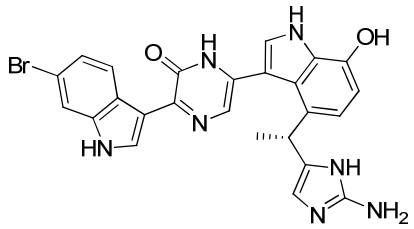
Nortopsentin A (X = Y = H) (**152a**)
Nortopsentin B (X = H, Y = Br) (**152b**)
Nortopsentin C (X = Br, Y = H) (**152c**)
Nortopsentin D (X = Y = Br) (**152d**)



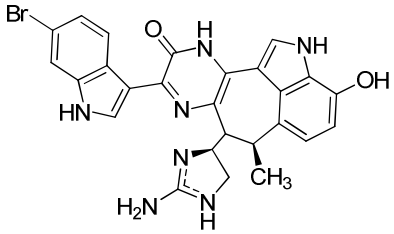
Topsentin (R^1 = H, R^2 = OH) (**153a**)
Bromotopsentin (R^1 = Br, R^2 = OH) (**153b**)
Isotopsentin (R^1 = OH, R^2 = H) (**153c**)
Deoxytopsentin (R^1 = R^2 = H) (**153d**)



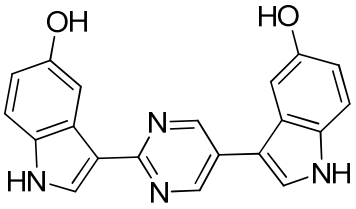
R^1 = OH, R^2 = R^3 = Br, R^4 = Me, R^5 = H
(Dragmacidin A) (**154a**)
R^1 = H, R^2 = R^3 = Br, R^4 = Me, R^5 = H
(Dragmacidin B) (**154b**)
R^1 = H, R^2 = R^3 = Br, R^4 = Me, R^5 = Me
(Dragmacidin C) (**154c**)



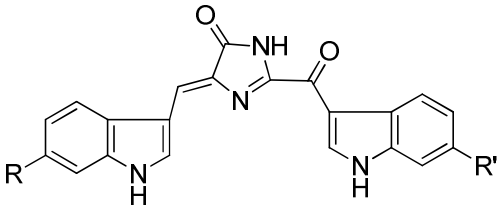
Dragmacidin D (**154d**)



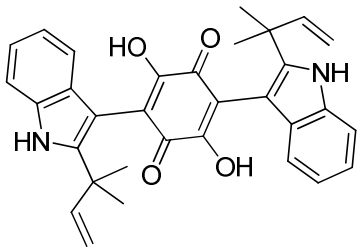
Dragmacidin E (**154e**)



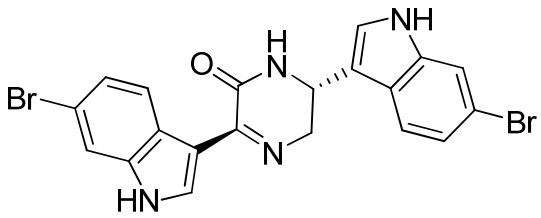
Hyrtinadine A (**155**)



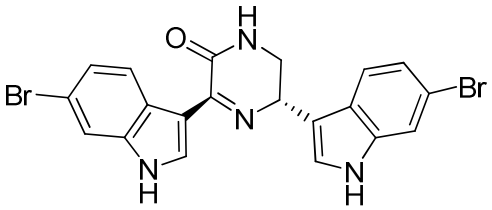
Rhopaladins A-D (**156**) R = H, OH, R^1 = H, Br



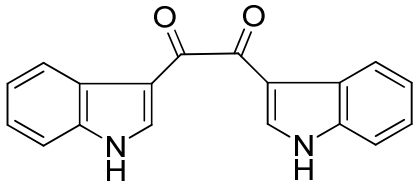
Asterriquinone (**157**)



Hamacanthin A (**158a**)



Hamacanthin B (**158b**)



Hyrtiosins B (**159**)

Figure 1.20 Bis(indolyl) alkaloids (**152-159**) as anticancer agents

Asterriquinone (**157**) is an indolylbenzoquinone exhibit wide range of biological activities, including antitumor properties and inhibition of HIV reverse transcriptase. Asterriquinone inhibit the interaction between the SH2 (Src homology 2) domains of tyrosine kinase receptor and their adapter protein Grb2.[141-142] Hamacanthin A and B (**158a,b**) isolated from the marine sponge *Spongosorites sp.* has cytotoxic activities.[143] Hyrtiosins B (**159**) exhibited cytotoxic activity against human epidermoid carcinoma KB cells *in-vitro* (IC_{50} = 4.3 μg/mL).[144]

1.6.3.1 Nortopsentin analogues as anticancer agents

Bis-indoles are one of the major classes of indoles reported for anticancer activity. In the previous section naturally occurring bis(indoles) and their biological significance was discussed. Among the several isolated natural bis(indoles) nortopsentin A-D showed significant anticancer activity, for this reason several analogues of nortopsentin were synthesized by replacing central imidazole ring with diverse heterocycles.

The 2,4-bis(3′-indolyl)imidazole (**152**) exhibited *in-vitro* cytotoxicity against P388 cells (IC_{50} = 4.5–20.7 μM). Synthetic analogues of nortopsentins are being focused area of research for many researchers. The imidazole heterocyclic ring spacer between the indole rings of nortopsentin was replaced with wide variety of 5 or 6-mememered ring spacers and evaluated for their anticancer activity (figure 1.21). The synthetic analogues include bis(indolyl)thiazoles (**160**) have shown anticancer activity against a panel of NCI-60 human cancer cell lines in sub micromolar concentrations. The same authors have reported bis(indolyl)pyrazines (**161**), bis(indolyl)pyrazinones (**162**) and found that 6-membered spacers exhibited excellent *in-vitro* cytotoxicity against multiple cancer cell lines.[145-146] Bis(indolyl)pyrimidines (**163**) with *N*-tosyl indoles as substituents have exhibited significant inhibitory activities against leukemia SR, CNS Cancer SF-539 and breast cancer MDA-MB-435 cell lines with the GI_{50} value of 0.22, 0.16 and 0.22 μM, respectively. 3,5-Bis(indolyl)pyrazine (**164**) demonstrated good inhibitory effects against a variety of tumor cell lines with the GI_{50} values less than 10 μM.[147] Bis(indolyl)-4-trifluoromethylpyridines (**165**) exhibited weak cytotoxicity towards murine leukemia cells (P388), and some compounds in this series displayed moderate inhibitory activity against human lung cancer cells (A-549).[148]

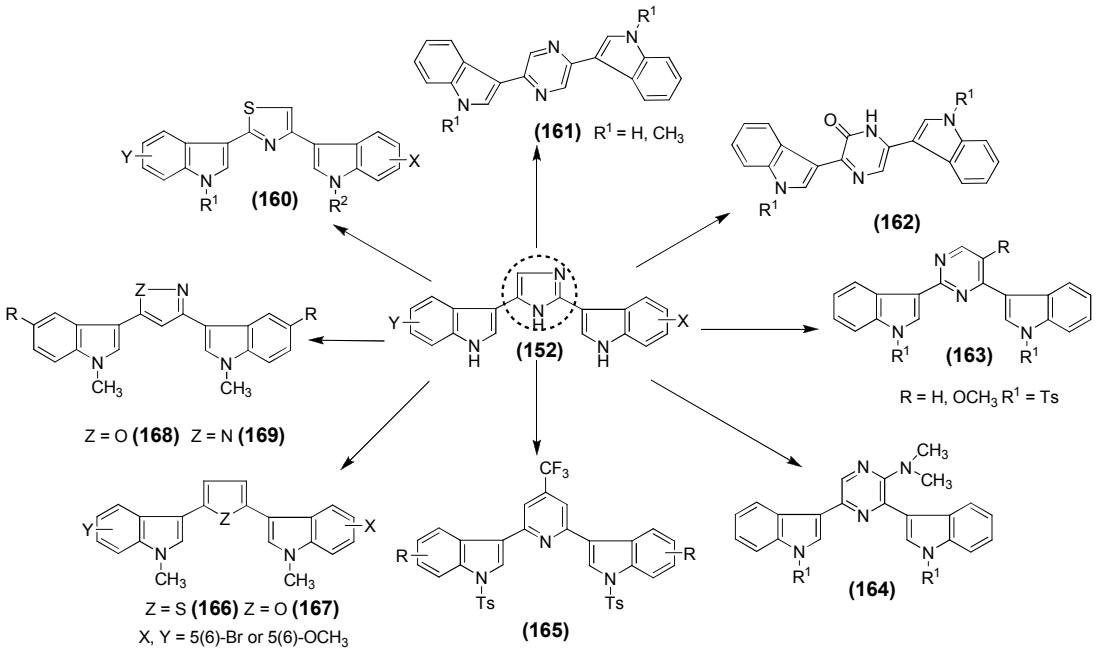


Figure 1.21 Nortopsentin analogues (**160**-**169**) as anticancer agents

Diana and co-workers reported bis(indolyl)thiophenes (**166**) as effective against the leukemia sub-panel of cancer cell lines having GI_{50} in the range 0.34-3.54 μM.[149] Bis(indolyl)furans (**167**) with C-5 methoxy and *N*-methyl substituted indoles have shown selectivity towards multiple cancer lines in a panel of NCI-60 human cancer cell lines. The compounds (**166**) and (**167**) revealed methoxy group at C-5 position of indole is critical for anti-cancer activity. Heterocyclic spacers pyrazol & isoxazole give analogues bis(indolyl)pyrazoles (**169**) and bis(indolyl)isoxazoles (**168**) and found to be cytotoxic against NCI-60 human cancer cell lines. Pyrazole compounds (**169**) were evaluated for DNA-intercalating assay. Assays revealed that DNA cannot be main cause for cell death and anticancer activity is due to some other mechanism (figure1.22).[150-151]

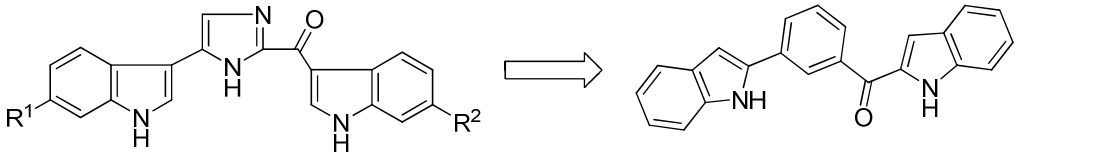


Figure 1.22 Topsentin analogue (**170**) as anticancer agent

Most recently, Sunjoo *et al.* reported the topsentin analogue 3-(2′-indolyl)phenyl methanone (**170**) as micro-tubule destabilizing agent (figure 1.22). The compound **170** inhibits tubulin action and exhibits potent antitumor activity in various preclinical models. Nanomolar concentrations of the compound caused down-regulation of bcl-2, induced PARP cleavage, and induced apoptosis in both LnCaP and PC-3 prostate cancer cells. Bis(indole) (**170**) inhibited polymerization of purified tubulin and induced a strong and concentration-dependent G2/M arrest in PC-3 cells.[152]

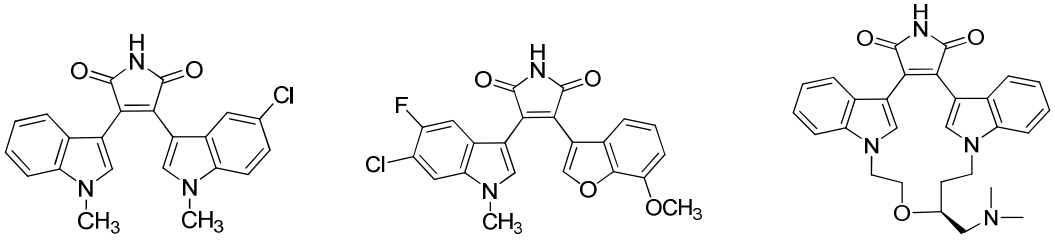


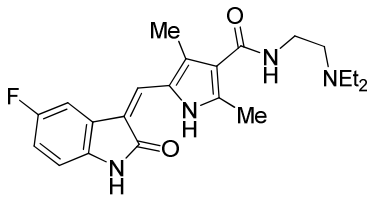
Figure 1.23 Staurosporine analogues (**171-173**) as anticancer agents

Staurosporine (**132**) analogues of bis(indolyl)maleimide compounds (**171**) found to have inhibition for Protein Kinase C (IC_{50} = 0.11 μM) implicated in unabated growth responses

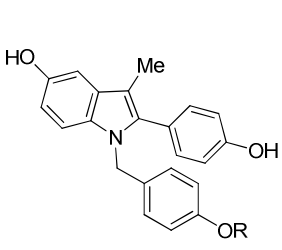
reported in several malignancies *in-vivo* (figure 1.23).[153] Replacement of an indole ring with a benzofuran moiety in (**172**) C-3 has been reported to afford selective and potent GSK-3β inhibition.[154] Bisindolylmaleimide, ruboxistaurin mesylate (Arxxant®) **173** developed by Eli-Lilly had approved as a drug and shows discrete enzyme selectivity with IC_{50} values for PKC-β1 and β2 (4.7 and 5.9 nM, respectively).[155]

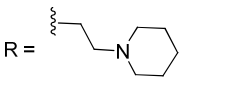
1.7 Indole containing anticancer clinical candidates

Numerous indole-based compounds are currently undergoing clinical studies; some of the representative investigational drugs are presented in the following discussion. Sunitinib (**174**) is an orally bioavailable, multi-targeted receptor tyrosine kinase inhibitor and was approved by the US-FDA for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor in 2006.[156-157] 2-Phenylindole analogues, Bazedoxifene (**175**) and Pipendoxifene (**176**) selective estrogen receptor modulators, or SERMs, selectively stimulate or inhibit the estrogen receptors of different target tissues and can be used for the treatment of both breast cancer and osteoporosis[158-160] Indole-based Histone deacetylase (HDAC) inhibitors Dacinostat (**177**) and Panobinostat (**178**), results in accumulation of acetylated histones, thereby causing cell cycle arrest and apoptosis. Panobinostat (**178**) is currently in phase II/III studies for the treatment of hematological cancers.[161-162] BMS-540215 (**179**) is a member of angiokinase inhibitors with excellent kinase selectivity but has poor solubility, hence (**179**) was modified by attaching L-alanine to make prodrug Brivanib (**180**) and has good solubility. Brivanib is currently undergoing phase III clinical studies for various cancer treatments.[163-164] Cediranib (**181**) is orally bioavailable tyrosine kinase inhibitor of all three VEGF receptors (VEGFR1-3), PDGF receptor b (PDGFRB) and c-kit. Cediranib (**181**) is currently in Phase II/III development for advanced non-small cell lung cancer and advanced colorectal cancer (scheme 1.24).[165-167]

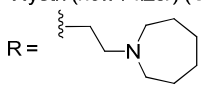


Sunitinib (Sutent™) Pfizer- (**174**)

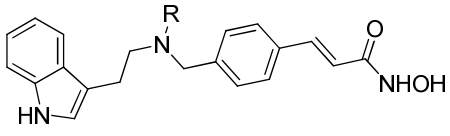




Bazedoxifene (ViviantTM)
Wyeth (now Pfizer) (**175**)

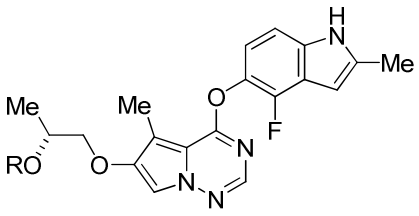


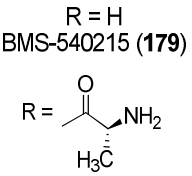
Pipendoxifene Phase II
Wyeth (now Pfizer (**176**)



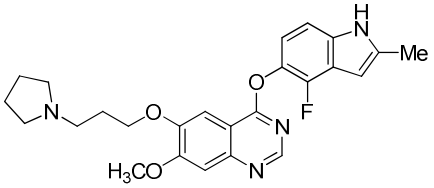
Dacinostat R= $(CH_2)_2OH$ (**177**)

Panobinostat (Novartis) R = H (**178**)



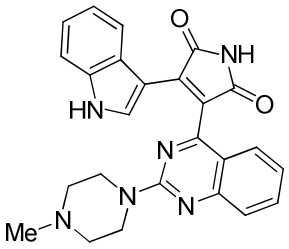


Brivanib (**180**)



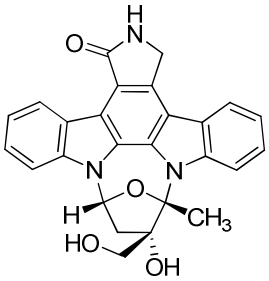
Cediranib (Recentin™)

Astra-Zeneca (Phase III) **(181)**



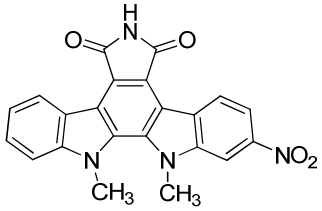
Sotrastaurin (Novartis)

(Phase II) **(182)**



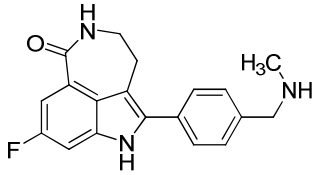
Lestaurtinib (Cephalon)

(Phase III) **(183)**



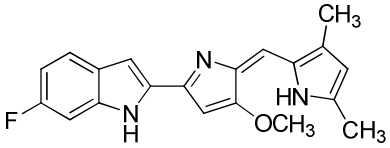
MKC-1 (Ro-31-7453)

(Roche) **(184)**



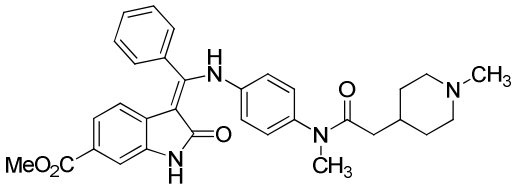
AG-14699 (Pfizer)

(Phase II) **(185)**



Obatoclax (Gemin X Biotech.)

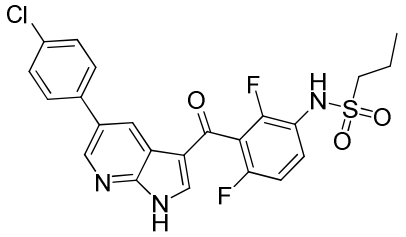
(Phase II) **(186)**



Intedanib (Vargatef™)

(Boehringer Ingelheim)

(Phase III) **(187)**



Vemurafenib (Zelboraf™)

(Roach) Approved drug **(188)**

Figure 1.24 Indole-based clinical candidates (**174-188**) for cancer treatment

Sotrastaurin (**182**) protein kinase C inhibitor is being developed for the potential oral prevention of organ transplant rejection and also for the potential treatment of psoriasis, uveitis and ulcerative colitis.[168-170] Lestaurtinib (**183**) is an orally active multiple tyrosine kinase inhibitor with specificity for the tropomyosin receptor kinases TrkA, TrkB and TrKC. Lestaurtinib (**183**) is in phase III trials for the treatment of various cancers either as a mono-therapy or in combination with other chemotherapeutic agents.[171-172]

MKC-1 (**184**) a bis(indolyl)maleimide compound was identified as an oral cell cycle inhibitor that induces apoptosis in cancer cells by targeting tubulin and is in clinical trials for the potential treatment of breast cancer, non-small-cell lung cancer, leukemia and ovarian cancer, but it was suspended after phase II studies for toxicity.[173-176] Pfizer's Poly(ADP-ribose) polymerase (PARP) inhibitor AG-14699 (**185**) is being evaluated in phase II studies for the potential treatment of cancer including melanoma, breast and ovarian cancers.[177-178] Gemin X Biotechnologies introduced Obatoclax (**186**), Bcl-2 protein inhibitors entered in to phase II clinical trials for the treatment of Hodgkin's lymphoma, myelodysplastic/myeloproliferative disorders and follicular lymphoma.[179] An oxindole Intedanib (**187**) is a triple angiokinase inhibitor that targets three growth factor receptors simultaneously: VEGFR, PDGFR and FGFR and used for the treatment of non-small-cell lung cancer, ovarian, prostate, and colorectal cancers.[180-181] Roche's aza-indole-based drug molecule Vemurafenib (Zelboraf™) (**188**) is approved in year 2011 as a selective inhibitor of the activated BRAFV600E gene, a gene found in 70% of malignant melanomas and a significant percentage of other cancers.[182]

Since several years of medicinal chemistry research has generated numerous investigational drugs bearing indole, indoline and oxindole nucleus, and these indole-based compounds showed great promise in curing various types of cancer diseases. As the indole scaffold has become one of the most important structural subunits in drug discovery, more indole-containing drugs will be unearthed in the future.

1.8 Conclusions and present work

In spite of several natural and synthetic molecules invented for cancer chemotherapy, a huge demand for the new chemical entities in the area of anticancer research for the development of potent anticancer drugs. The major obstacles and disadvantages associated with the marketed drugs for the successful treatment of cancer are drug resistance and drug toxicity. The drug resistance is mainly includes de nova resistance (drugs are unable to reach the target cells because of permeability barriers such as the blood-brain barrier), acquired resistance (result from genomic mutations, such as the induction or deletion of enzymes involved in drug inactivation or drug activation, respectively) and finally multidrug resistance (P-glycoprotein transports many naturally occurring drugs out of neoplastic cells, and its induction may lead to multidrug resistance). Another major drawback with cancer drug is toxicity. The most common

toxicities of antineoplastic drugs result from inhibition of cell replication in the bone marrow, gastrointestinal epithelium, and hair follicles. Many antineoplastic drugs also stimulate the chemoreceptor trigger zone in the medulla and thereby elicit nausea and vomiting. To over-come these problems and scientific understanding of the mechanisms of drug resistance may emerge new treatments may be developed to counteract resistance and decrease the toxicity.

The present work mainly focuses on the synthesis of novel indole-based chemical entities for anticancer activity. Design and synthesis of various series of indolyl heterocycles is based on the naturally occurring bioactive compounds. The exploration of heterocycles and functional groups at C-3 position of the indole ring for cytotoxic studies against various human cancer cell lines gave insight about required structural modifications. The preliminary anticancer activity results of indolyl heterocycles encouraged us to study further structure-activity relationship and detailed mechanism of actions of the highly potent molecules.

1.9 Conspectus of the thesis

The thesis is segregated into seven chapters including a brief introduction to indole chemistry and its role in diverse therapeutic areas, including detailed discussion on anticancer research and conclusion of the work. The first chapter introduces the indole chemistry and clearly elaborates importance of indole scaffold in drug discovery as various classes of inhibitors. A brief discussion was made on classification of anticancer drugs and rational approaches for the design and synthesis of indole-based anticancer agents.

The second chapter describes the syntheses and *in-vitro* anticancer activities of three series of indolyl heterocycles, 4-(3′-indolyl)oxazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles and indolyl-1,2,4-triazoles. The key-step in the synthesis of 4-(3′-Indolyl)oxazoles involves the greener and solvent-free microwave-assisted cyclization of 3-tosyloxyacetyl-1-benzenesulfonylindole with appropriate amides. The most potent 4-(3′-indolyl)oxazole exhibited significant anticancer activity against MCF-7 breast cancer cell lines (IC_{50} = 14.1 μM). In part-B of this chapter a series of 5-(3′-indolyl)-1,3,4-thiadiazoles were synthesized by thionation, followed by cyclization of intermediate *N,N*′-diacylhydrazines. The most cytotoxic compound suppressed the growth of PaCa2 cancer cells with IC_{50} value 1.5 μM. A series of indolyl-1,2,4-triazoles is reported in part-C of this chapter.

The synthesis of indolyl-1,2,4-triazoles involves the reaction of 3-cyanoindoles with aryl or heteroaryl hydrazides in basic medium afford the desired triazoles. The most active compound in the series has IC_{50} value 0.8 μM against PaCa2 cancer cell lines. Initial molecular target studies of the active compounds in three series indicated that the compounds kill the cancer cells via tubulin depolymerization mechanism.

The third chapter discloses the synthesis of two series of novel bis(indole) derivatives 3,5-bis(indolyl)-1,2,4-thiadiazoles and bis(indolyl)hydrazide-hydrazones. Synthesis of 3,5-bis(indolyl)-1,2,4-thiadiazoles involve simple oxidative-dimerization of indole-3-thiocarboxamides in presence of iodobenzene diacetate. The anticancer activities of synthesized 3,5-bis(indolyl)-1,2,4-thiadiazoles reveal that replacement of imidazole ring of nortopsentin by 1,2,4-thiadiazole reduces activity but showed selective cytotoxicity against prostate cancer cell lines (LnCaP) with an IC_{50} value of 14.6 μM. Replacement of central heterocycle in bis(indole) with hydrazide-hydrazone functionality led to a series of bis(indolyl)hydrazide-hydrazones. The preliminary cytotoxicity results showed that hydrazide-hydrazones are highly selective against MDA-MB-231 breast cancer cell line (IC_{50} = 0.7 μM) and induces apoptosis.

The fourth chapter deals the synthesis of a series of indolyl chalcones. The series includes two types of indolyl chalcones, one set is prepared from the reaction of indole-3-carboxaldehyde with variety of aromatic acetophenones and the other set is prepared from the reaction of 3-acetylindole with various aromatic aldehydes in basic condition to afford the desired chalcones. The *in-vitro* anticancer activity results of these chalcones are excellent and the best compound showed IC_{50} values 0.3 μM.

The fifth chapter discloses the design and synthesis of 2-arylamino-1,3,4-indolylthiadiazoles and their anticancer activity. The synthetic protocol involves the initial preparation of substituted thiosemicarbazides from the reaction of indole-3-carbohydrazide with various phenyl isothiocyanates. The intermediate thiosemicarbazides were cyclized in acidic medium to afford a series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles in good yields. Anticancer activity results showed that all the synthesized 2-arylamino-1,3,4-indolylthiadiazoles are selective against breast cancer cell line (IC_{50}<1 μM against MDA-MB-231). The most active compound exhibits cytotoxicity and selectivity against prostate LnCaP cancer cell lines (IC_{50} = 150 nM).

A short and facile synthesis of an indoloquinoline alkaloid cryptosanguinolentine and its analogues is described in the sixth chapter. The synthesis engages the solvent-free

eco-friendly simple heating of quinolone and arylhydrazines in presence of *p*-toluene sulfonic acid under solvent-free conditions. Generality of the protocol was demonstrated by preparing a series of analogues of cryptosanguinolentines. The seventh chapter gives a comprehensive overview and concluding remarks about the thesis.

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Chapter 2

2.1 Introduction

The indole ring is one of the most widely distributed heterocycles in nature and indole alkaloids and its synthetic analogues have spread over diverse applications in medicinal chemistry. Therefore, the synthesis and biological evaluation of indoles have been the focus of researchers over the years. An "azole" is a class of five-membered nitrogen containing heterocycles ring with at least one other non carbon atom such as nitrogen, oxygen or sulfur. Indolylazoles include the compounds possessing an indole ring attached to the five-membered heterocyclic ring such as oxazole, thiazole and imidazole, isoxazole, pyrazole and isothiazole, oxadiazole, thiadiazole and triazole (figure 2.1.1). For example, 5-(3′-indolyl)oxazoles having 2,5-disubstituted oxazole ring which is linked to indole ring at C-3 position. Similarly, 3,5-disubstituted isoxazole and 2,5-disubstituted oxadiazole rings are linked to indole at C-3 position in case if 5-(3′-indolyl)isoxazole and 5-(3′-indolyl)oxadiazole, respectively (figure 2.1.1).

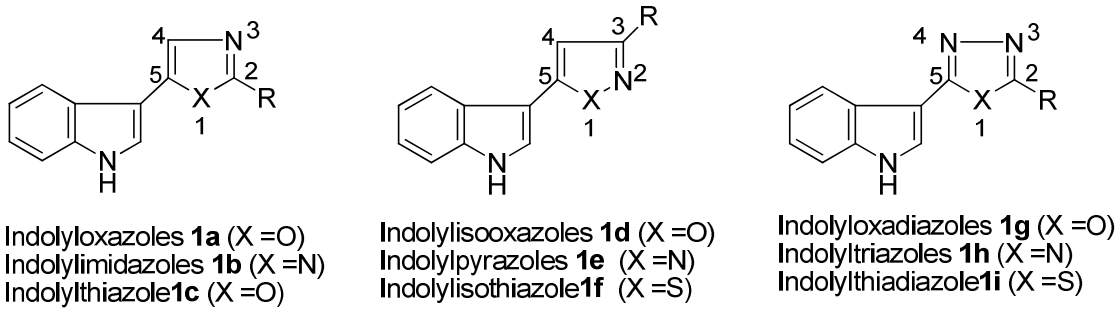


Figure 2.1.1 Structures of 5-(3′-indolyl)azoles (**1a-i**)

The different classes of indole alkaloids include spiroindoles, tryptamine-based metabolites and bis(indole) alkaloids. 3-Substituted indoles structurally represent a major class of marine invertebrates. These alkaloids contain the true indole nucleus or dihydroindole, pseudoindoxyl or oxoindole units as core scaffolds. The first indole alkaloid, 3-indolylimidazol-4-one (**2**) was isolated from the tunicate *dendrodoa grossularia.*[1] Nortopsetin (**3**), is a bis(indole) alkaloid isolated from the marine sponge *genera Spongosorites* (figure 2.1.2).[2]

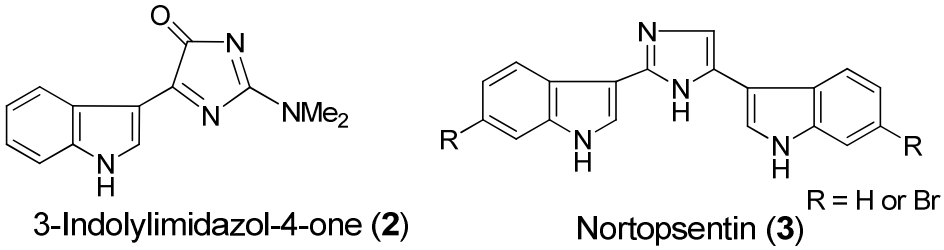


Figure 2.1.2 Indole alkaloids (**2**) and (**3**)

Indole alkaloids bearing a 2,5-disubstituted oxazole moiety occur in small number of natural products and many of them are found in *red alga* of the coast of Senegal.[3] Many oxazole containing natural products and their synthetic analogues are known to exhibit interesting biological activities.[4-5] For example, the pimprinine[6] family comprises of oxazole unit at C-3 position of the indole ring is known for its antioxidant properties (figure 2.1.3).

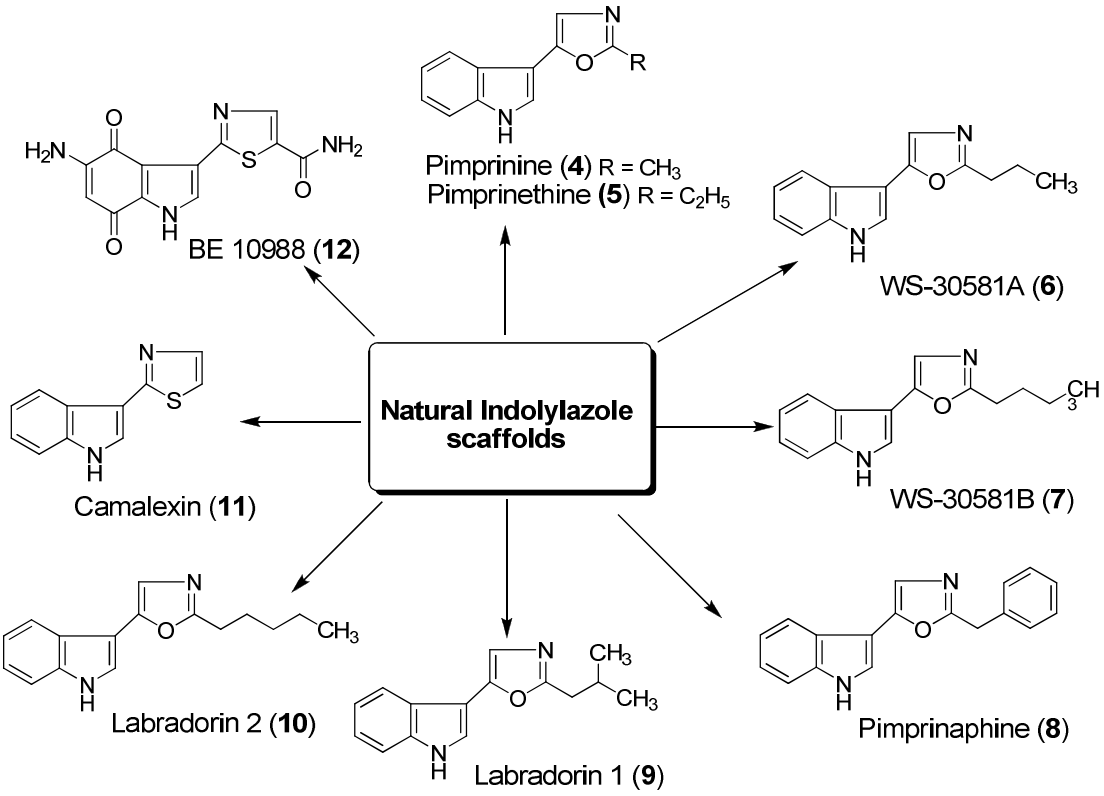


Figure 2.1.3 Naturally occurring 5-(3′-indolyl)azoles (**4**-**12**)

This family includes Pimprinine (**4**), Pimprinethine (**5**) WS-30581 A and B (**6** and **7**) and pimprinaphine (**8**) isolated from *Streptomyces pimprina*,[7] *Streptomyces cinnamoneus*,[8] *Streptoverticillium waksmanii,*[9] and *Streptoverticillium olivoreticuli*, respectively. Exploration of cancer cell growth inhibitory constituents of *Pseudomonas syringae* pv. *Coronafaciens* led to the isolation of Labradorin 1 (**9**) and Labradorin 2 (**10**).[10]

Camalexin (**11**), is the characteristic phytoalexin of *Arabidopsis thaliana* which is induced by a great variety of plant pathogens.[11] Another thiazole derivative BE 10988 (**12**) found to be DNA topoisomerase II inhibitor. [12-13]

A complex indolyloxazole alkaloid Martefragin A (**13a**) was isolated from *red alga Martensia fragillis.*, possess an indole and oxazole moiety showed lipid peroxidation inhibition activity (figure 2.1.4).[3,14] Another indolyloxazole, Almazole D (**13b**) mostly found in red seaweed showed antibacterial activity against gram-negative bacteria.[15] Almazole A-D have a 2,5-disubstituted oxazole ring inserted between indole and *N,N*-dimethyl-L-phenyl alaninamide moieties.[16]

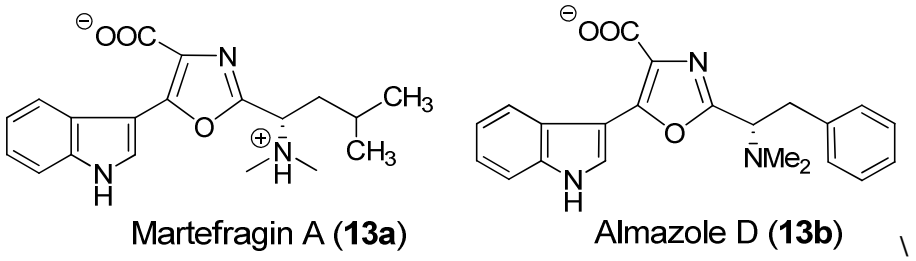


Figure 2.1.4 Complex indolyloxazoles (**13**)

Bis(indole)alkaloids are a versatile class of indole alkaloid family having two indole units separated by 5/6-membered heterocycles. Bis(indole)alkaloids, Nortopsentin A-D (**14**) and Topsentin (**15**), isolated from marine sponge *Topsentina genitrix* have an imidazole ring as a spacer[17] and are known to possess a wide range of biological activities. Due to encouraging anticancer activity results of Nortopsentin, many diversified analogues of Nortopsentin were synthesized (**16** and **17**) and evaluated for their anticancer activities (figure 2.1.5).[18-19]

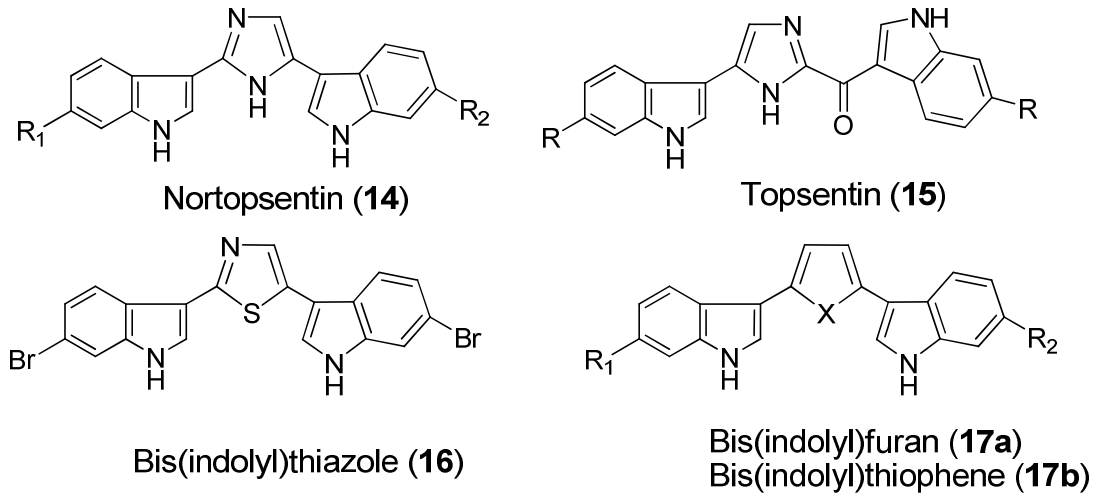


Figure 2.1.5 Natural and synthetic bis(indoles) (**14-17**)

2.1.1 Indolylazoles in drug discovery

The Pimprinine (**4**) was first isolated by Bhate *et al*. and chemically synthesised by Joshi *et al.*[7] exhibits antiepileptic and monoamine oxidase inhibitory activities.[20] Recently, the strain *Streptomyces* CDRIL-312 produced Pimprinine extracellularly showed promising anticonvulsant activity in both minimum and maximum electric seizure threshold test in mice,[21] which is comparable to that of phenyl hydantion sodium. The WS-30581 A (**6**) and WS-30581 B (**7**), were reported to have potent inhibitory effects on platelet aggregation.[22-23] Labradorin 1 (**9**) showed good anticancer activity with GI_{50} values of 9.8 and 6.2 µg/mL against the human cancer cell lines (lung-NSC) and BXPC-3, respectively. The Labradorin 2 (**10**) displayed GI_{50} value of 9.6 µg/mL against both the human cancer cell lines, NCI-H460 (lung-NSC) and BXPC-3.[10] Martefragin A (**12**) is a strong inhibitor of lipid peroxidation than drug α-Tocopherol.[24] Indolyloxazole, Diazonamide A, a halogenated cyclic peptide isolated from the colonial ascidian *Diazona chinensis* exhibits *in-vitro* cytotoxicity against human tumour cell lines (HCT-116 IC_{50} < 15 µg/mL).[25] Synthetic analogues of naturally occurring indolylthiazole, BE 10988 (**12**) showed cytotoxicity against SKBr3 cancer cells. Bis(indolyl)imidazole, Nortopsentin (**14**) skeleton (figure 2.1.5), exhibited *in-vitro* cytotoxicity against P388 cells (IC_{50} = 4.5-20.7 µM). *N*-Methylated derivatives of Nortopsentin (**14**) showed significant improvement in antitumor activity against P388 murine cancer cells (IC_{50} = 0.8-2.1 µM). [17]

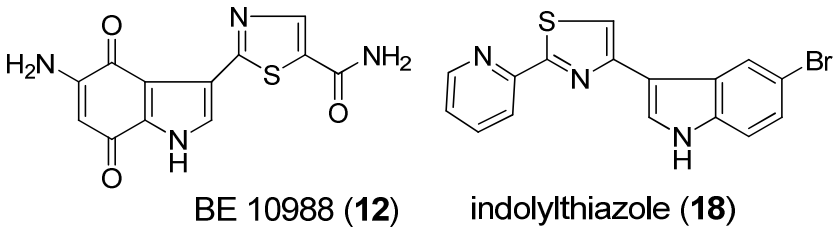


Figure 2.1.6 Antitumor indolylthiazoles (**12**) and (**18**)

Nortopsentin analogues with thiazole, furan and thiophene, as spacers have been studied extensively for their cytotoxicities.[19, 26-28] Bis(indolyl)thiazoles (**16**) were effectively inhibited MCF-7 (breast) cancer cells with GI_{50} value 0.888 µM.[18, 29] The indolylthiazole (**18**) with a pyridyl substituent inhibited cancer cells promiscuously. The GI_{50} values against leukemia HL-60, CNS cancer SF-295 and renal cancer RXF 393 are 1.96, 2.57 and 2.31 µM, respectively. Thiazole substituted indolequinole, BE 10988 (**12**) was identified as topoisomerase inihibitor.[29] Topsentin (**15**) and five related bis(indole) marine

sponge metabolites were shown to displace ligand binding to α_{1a} and α_{1b} adrenergic receptors with Ki values for the α_{1b} receptor ranging from 0.08 to 1.15 μM.[30] Several patents have been published on indolylazoles to reveal the potency of the scaffolds. Indolylisoxazoles (**19**) were reported as GABA inhibitors and used in Alzheimer's disease.[31] Pfizer Inc., US reported 5-heteroyl indole derivatives (**20**) for treating migraine and other disorders. The synthesis of a plethora of compounds with diverse substitutions was described. The indolylthiazoles (**20**) were indicated as therapeutic probable's for migraine.[32] Pharmacia (Italy) patented indolylpyrazole (**21**) as potent kinase inhibitors.[33]

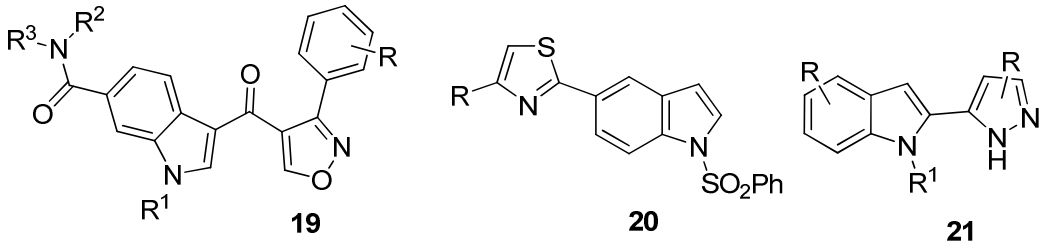


Figure 2.1.7 Indole-based heterocycles (**19-21**) and their therapeutic uses

Swain *et al*. reported several indolyl-1,2,4-oxadiazoles (**22**) as 5-HT_3 antagonists.[34] Ziedan *et al.* showed indolyl-1,2,4-oxadiazoles (**23**) as pro-apoptotic antitumour agents.[35] Kiyoi *et al.* reported novel 3-(3′-indolyl)-1,2,4-oxadiazoles and thiadiazoles (**24**) as potent CB1 cannabinoid receptor agonists.[36] Narayana *et al.* showed that the nitro substituted indolyl-1,3,4-oxadiazoles (**25**) as potent antiinflammatory agents (figure 2.1.8).[37] With the encouraging results of indolylazoles our research group have reported a series of indolyl-1,3,4-oxazdiazoles and studied their cytotoxicity against various cancer cell lines.[38]

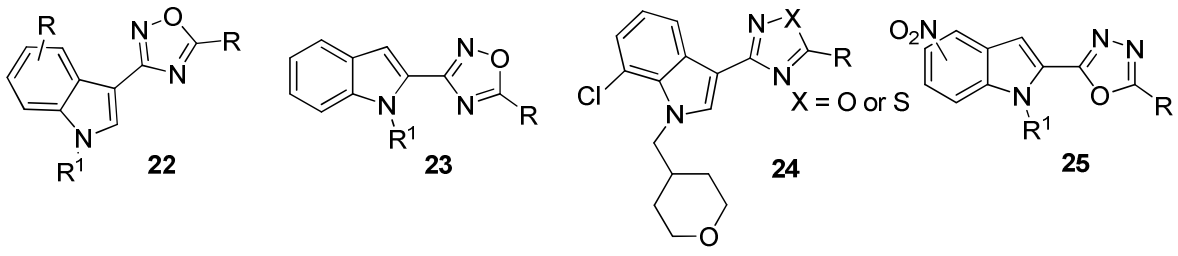


Figure 2.1.8 Indolyloxadiazoles (**22-25**) in drug discovery

Indolyl-1,3,4-oxazdiazoles (**26a-c**) were found to be cytotoxic against cancer cell lines. In addition, compound **26c** was selectively cytotoxic towards PaCa2 cell line with an IC_{50} value of 1.4 μM (figure 2.1.9).

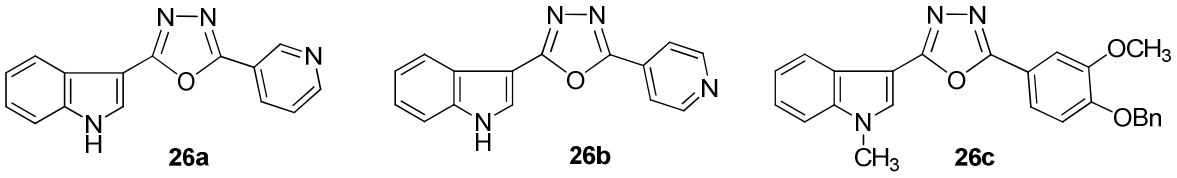


Figure 2.1.9 Indolyl-1,3,4-oxadiazoles (**26a-c**) as potent anticancer agents

The structure-activity relationship study showed that 1,3,4-oxadiazoles with *N*-methylated indole at C-5 and pyridyl or 4-benzyloxy-3-methoxyphenyl substituents at C-2 position showed better anticancer activity and selectivity.

2.1.2 Bioisosterism: A strategy in molecular modification of indolylazoles

Bioisosterism is a strategy of medicinal chemistry for the rational design of new drugs, applied with a lead compound for molecular modification.

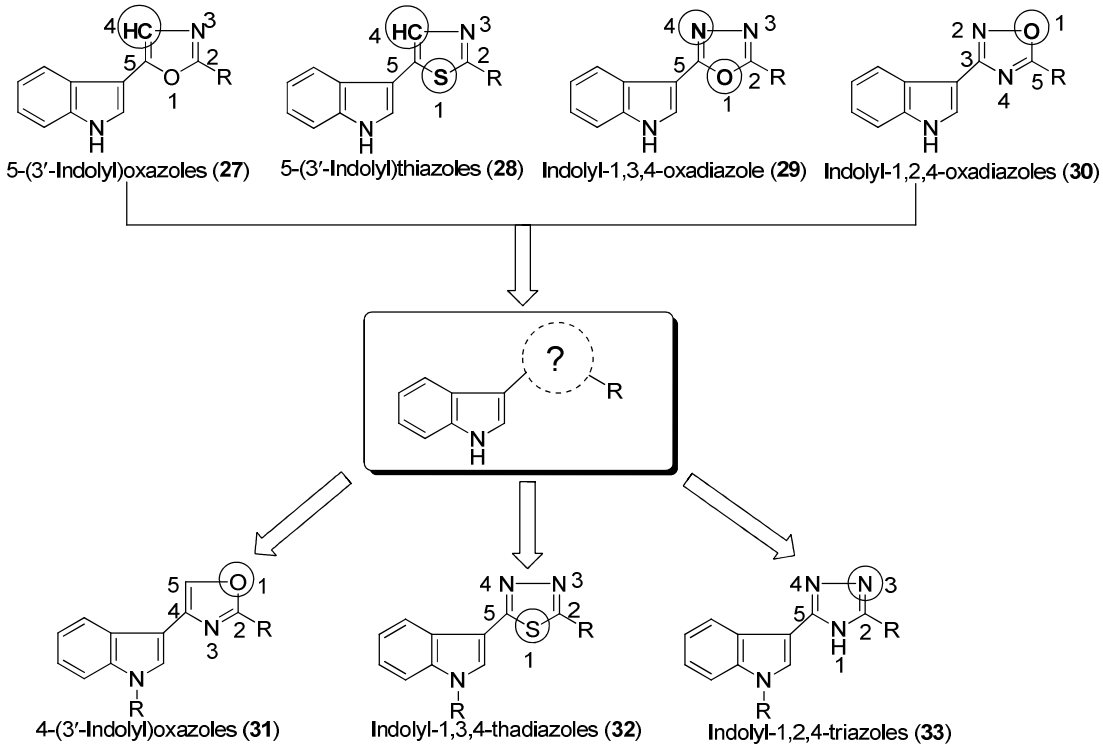


Figure 2.1.10 Design of novel indolylazoles (**31-33**)

The correct use of this strategy allocates the identification of new classes of lead compounds with attractive therapeutic activity, simplifying the synthetic route and consequently maximizing the chances for success in discovering medications. The role of bioisosterism in rational drug design as well as in the molecular modification and optimization process aims to improve pharmacodynamic and pharmacokinetic properties of lead compound. There are innumerous reasons for the use of bioisosterism to design new drugs, including the necessity to improve pharmacological activity, gain selectivity for a determined receptor or enzymatic isoform subtype with simultaneous reduction of certain adverse effects. According to Alfred Burger the bioisosteric replacements of atoms, subunits and functional groups of same valance and ring equivalents are categorized into classical bioisosters, whereas non-classical bioisosters were those which practically did not fit the definitions of the first class.

Indolylazoles attracted both organic and medicinal chemists, due to their wide range of chemical and pharmacological applications. Structural modifications, design and development of new chemical entities is a routine task for the drug discovery research. Synthesis of natural and synthetic indolylazoles is always an interesting research area due to vast scope to develop the therapeutics for medicinal purpose. Keeping in view of biological significance of natural and synthetic 5-(3′-indolyl)oxazoles, indolylthiazoles, bis(indole) derivatives and recently reported indolyl-1.3.4-oxadiazoles, we have synthesized novel indolylazoles by varying the five-membered heterocyclic ring attached to C-3 position of the indole (figure 2.1.10). In present work we have prepared three series of indolylazoles; 4-(3′indolyl)oxazoles (**31**), indolyl-1,3,4-thiadiazoles (**32**) and indolyl-1,2,4-traizoles (**33**) and evaluated for their anticancer activities.

2.2 Part A: Novel 4-(3′-indolyl)oxazoles as anticancer agents

2.2.1 2,4-Disubstituted oxazoles in drug discovery

Oxazoles are five-membered nitrogen and oxygen containing heterocycle, which gained much importance in the medicinal chemistry. Oxazole ring system is a basic building block of several biologically interesting compounds.[39] Oxazoles are part of many of the naturally occurring macro molecules which usually possess complex molecular architectures and intriguing biological activities.[40] The isomeric 2,4-disubstituted oxazole represent a common structural moiety of many biologically potent natural products such as Phenoxan (anti HIV agent **34**), Calyculins and Rhizoxin (anti mitotic agents).[41-42] Few examples of simple and complex oxazole containing drug leads have shown in the figure 2.2.1. Phorboxazole A and B are natural marine macrolides have been found to exhibit significant cytostatic activity at the S phase with a mean GI_{50} < 1.58 nM against a broad spectrum of human cancer cell lines.

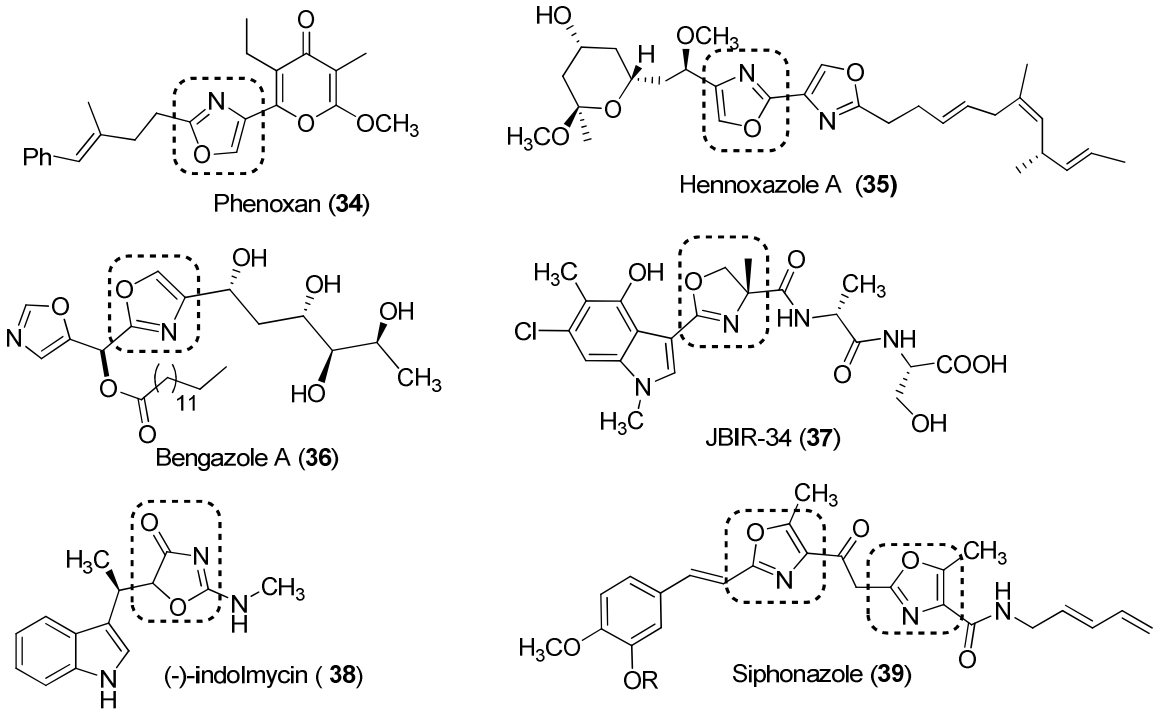


Figure 2.2.1 Naturally occurring 2,4-disubstituted oxazoles (**34-39**) as drug leads

This extraordinary cytotoxic activity and complex molecular structures of oxazoles have made them as fascinating synthetic targets.[43-44] Macrolides, Hennoxazole A (**35**) and Bengazole A (**36**) showed antiviral activity against Herpes Simplex and antifungal activity respectively.[45-46] Tetrapeptides, JBIR-34 (**37**) isolated from a marine sponge-derived *Actinomycete*, *Streptomyces sp*. Sp080513GE-23 showed diverse clinical

properties.[39] Indolmycin (**38**) is a highly selective antibiotic which acts as a tryptophan anti-metabolite. Recent research has shown that indolmycin is active against *Mycobacteria* and *Helicobacter pylori* and can stimulate transcription in *Escherichia coli.*[47] Structurally unusual bis-oxazole Siphonazole (**39**) has significant chemical applications (figure 2.1.2).[48] 5-(3′-Indolyl)oxazoles, regioisomers of 4-(3′-indolyl) oxazoles have unprecedented biological applications (described in section 2.1.1), oxazole containing natural products such as balsoxin, texaline and annuloline have shown wide range of chemical and biological applications.[49]

2.2.2 Rational design

The oxazole unit is an active pharmacophore in many of the anticancer lead molecules. Oxazole derivatives (**40**) synthesized as combretastatin A-4 analogues have shown significant cytotoxicity and specificity towards human HL-60 leukemia, 518A2 melanoma and colon carcinomas HCT-116 cell lines.[50] The oxazole derivative (**41**) showed an IC_{50} of 46 nM against MCF-7 human breast tumor cells.[51]

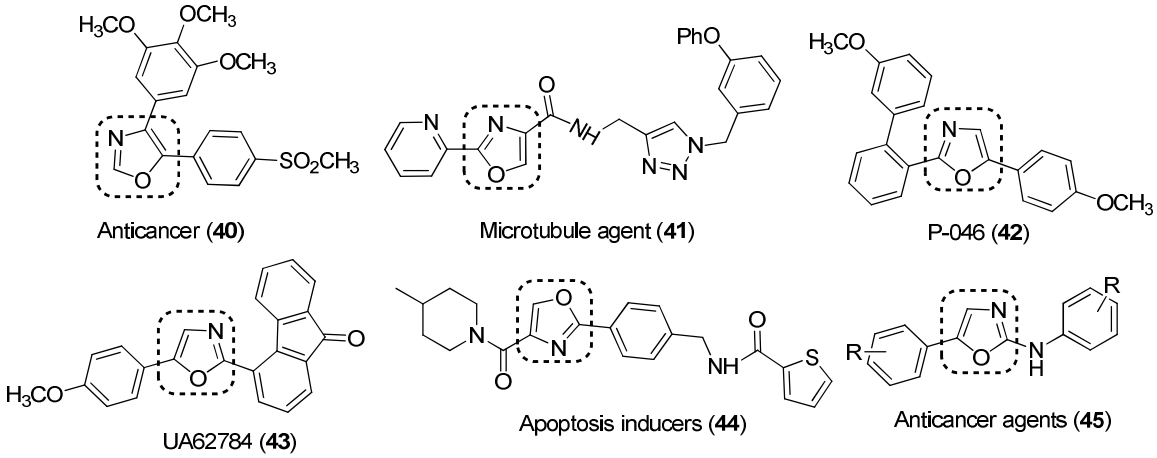


Figure 2.2.2 Oxazole containing anticancer agents (**40-45**)

Novel diaryl oxazole-based compounds (**42** and **43**) were reported for the treatment of pancreatic cancer. Compound, P-046 (**42**) has demonstrated the high efficacy *in-vivo* in a human pancreatic tumor model in SCID mice.[52] The oxazole, UA62784 (**43**) interacts with tubulin dimers ten times more potent than colchicine, vinblastine, or nocodazole. Nanomolar doses of UA62784 promote the accumulation of mammalian cells in mitosis, due to aberrant mitotic spindles, as shown by immune fluorescence and live cell imaging.[53-54] The 2-phenyloxazole-4-carboxamide (**44**) scaffold was identified as a potent

apoptosis inducers.[55] Smith Kline Beecham patented the amino-oxazoles (**45**) for their anticancer activity (figure 2.2.2).

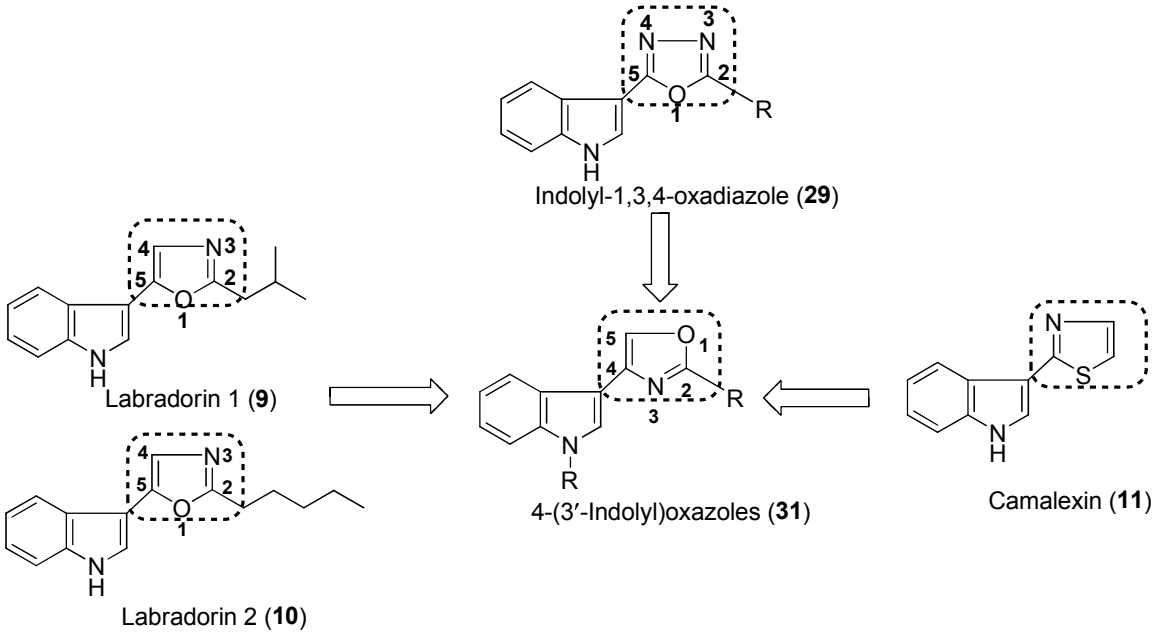


Figure 2.2.3 Rational approach for the synthesis of 4-(3′-indolyl)oxazoles (**31**)

Rational approach to design novel indolylazoles was previously shown in the figure 2.1.10. Our rationality to synthesize a series of 4-(3′-indolyl)oxazoles is based on the encouraging anticancer activity results of indolylazoles such as Labradorins 1 & 2, Camalexin and synthetic anticancer agents, indolyl-1,3,4-oxadiazoles. It would be interesting to synthesize 4-(3′-indolyl)oxazoles in order to find the effect of geometrical change that would occur by shifting indole ring from C-5 to C-4 of oxazole may lead improvement in the anticancer activity of the scaffold (**31**).

2.2.3 Results and discussion

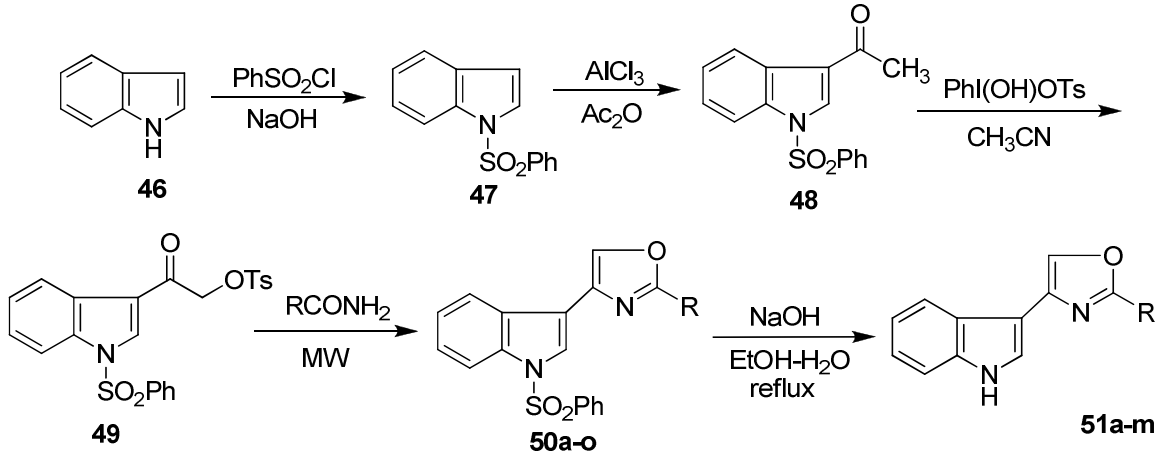
2.2.3.1 Chemistry

A number of synthetic strategies have been evolved for the construction and incorporation of the 2,4-disubstituted oxazole moiety into complex synthetic targets. There are very few reports on direct conversion of ketones to 2,4-disubstituted oxazoles. Some of the indirect methods for formation of oxazole ring include cyclodehydration of β-ketoamides,[56] dehydrogenation of oxazolines[57] *via* aza-Wittig reactions,[58] Schmidt rearrangements,[59] use of isocyanides,[60] aryl substituted tosylmethyl isocyanate (TosMIC) reagents[61] and intramolecular alkyne additions.[62] Despite the literature mentioned protocols, there is a

need for simple, environmental friendly and inexpensive synthetic methodology for convenient and rapid synthesis of 2,4-disubstituted oxazoles which are utilized as building blocks in construction of natural products. Over the past three decades, microwave-assisted chemistry has evolved as an established field of science. Microwave technology has an edge over conventional heating methods for conducting chemical reactions, and is preferred for performing chemical synthesis relating to lead development in pharmaceutical companies. In both lead identification and lead optimization processes, there is an acute need for new small organic molecules. Conventional methods of organic synthesis are too slow to satisfy the demand for generation of such compounds. There are many examples for the application of microwave-assisted chemistry to organic synthesis such as the use of benign reaction media, solvent-free conditions and the use of solid-supported, reusable catalysts.[63-64] Microwave irradiation has proved to be a highly effective heating source in chemical reactions. It can accelerate the reaction rate, provide better yields and uniform and selective heating, achieve greater reproducibility of reactions, and help in developing cleaner and greener synthetic routes.[65]

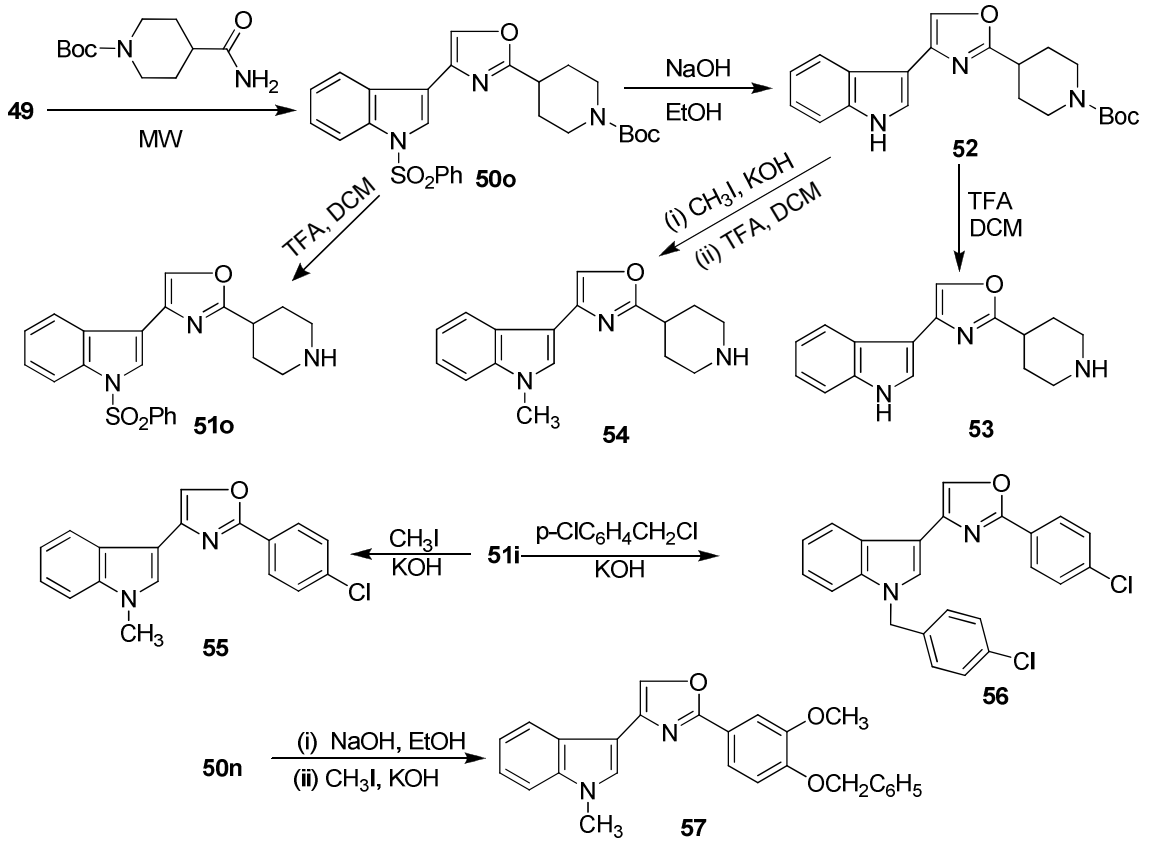
Our synthesis of 4-(3′-indolyl)oxazoles was initiated from commercially available indole. The synthetic protocol starts from *N*-protection of indole (**46**) using benzenesulfonyl chloride to afford 1-benzenesulfonylindole (**47**). Acylation of **47** with acetic anhydride in the presence of $AlCl_3$ afforded 3-acetyl-1-benzenesulfonylindole (**48**).[66] Preparation of 3-tosyloxyacetyl-1′-benzenesulfonyl indole (**49**)[67] followed by reaction with appropriate amide. The α-tosyloxy ketones are very useful starting materials to synthesize various heterocyclic compounds and can be easily prepared from the reaction of enolizable ketones with [hydroxy(tosyloxy)iodo]benzene (HTIB). Further, α-tosyloxy ketones are good replacement for lachrymatory α-halo ketones in synthetic organic chemistry. Reaction of 3-acetyl-1-benzenesulfonylindole (**48**) with HTIB in acetonitrile at 45 °C afforded pure **49** in good yield. For the successful synthesis of **49**, protection of indole nitrogen was essential as the reaction of 3-acetylindole with HTIB led to a complex mixture. Use of 3-bromoacetyl-1′-benzenesulfonylindole is disadvantageous as the bromination of **48** always produces minor dibromo derivative which requires further purification. Our model reaction of **49** with amides under conventional condition produced moderate yield of product and required longer reaction time (8-10 h). In view of demonstrated advantages of microwave-accelerated reactions under solvent-free conditions, we explored the reaction of **49** with amides under

microwave irradiation to prepare a series of 4-(1′-benzenesulfonylindol-3′-yl)-2-substituted oxazoles (**50a-o**) (scheme 2.2.1).



Scheme 2.2.1 Synthesis of novel 4-(3′-indolyl)oxazoles (**51a-m**)

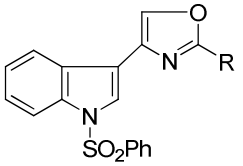
After several attempts under varying reaction conditions it was realized that the neat reaction of **49** with amides resulted in the exclusive formation of **50** within short time (6-15 min).



Scheme 2.2.2 Synthetic route for novel 4-(3′-indolyl)oxazoles (**53-57**)

Under similar reaction conditions analogues of 4-(1′-benzene-sulfonylindol-3′-yl)-2-substituted oxazoles (**50**) were prepared. Finally, treatment of **50** with aqueous sodium hydroxide under refluxing conditions afforded pure 4-(3′-indolyl)-2-substituted oxazoles (**51**) in excellent yield. The oxazole derivatives (**53-57**) were prepared according to scheme 2.2.1. The reaction of **49** with *N*-Boc-isonipecotinamide resulted in the formation of indolyl oxazole (**50o**) which upon deprotection of benzenesulfonyl and *N*-Boc moieties produced **53**. Methylation of indolyloxazole (**52**) followed by *N*-Boc deprotection yielded compound **54**. Compounds **55** and **56** were prepared by the *N*-alkylation of **51i** with methyl iodide and *p*-chlorobenzyl chloride, respectively. Deprotection of benzenesulfonyl group of **50n** followed by reaction with methyl iodide produced compound **57** in good yield. All the 4-(1′-benzenesulfonylindol-3′-yl)-2-substituted oxazoles (**50a-o**) and 4-(3′-indolyl)-2-substitutedoxazoles (**51a-o**) and (**53-57**) were characterized by their NMR and mass spectral data.

Table 2.2.1 Synthesis of 4-(3′-indolyl)oxazoles (**50a-o**)



Compound[a]	R	Time (min)	Yield (%)[b]	mp (^{o}C)
50a	C_6H_5	15	79	145
50b	CH_3	8	83	154
50c	3,4-$(CH_3O)_2C_6H_3$	15	86	136
50d	4-FC_6H_4	10	77	182
50e	4-HOC_6H_4	8	78	128
50f	$CH{=}CHC_6H_5$	8	79	156-159
50g	4-pyridyl	12	73	142
50h	$CH_2C_6H_5$	12	81	172-175
50i	4-ClC_6H_4	10	80	136
50j	4-$CH_3OC_6H_4$	10	75	176-179
50k	H	6	80	126
50l	$CH{=}CH_2$	10	78	138
50m	4-OH-3-$CH_3OC_6H_3$	15	82	192-194
50n	4-$(OCH_2C_6H_5)$-3-$(OCH_3)C_6H_3$	14	60	142-144
50o	N-Boc-piperidin-4-yl	8	50	124-128

[a]Compounds were characterized by their spectral data (^{1}H NMR and Mass). [b]Yields refer to pure isolated products.

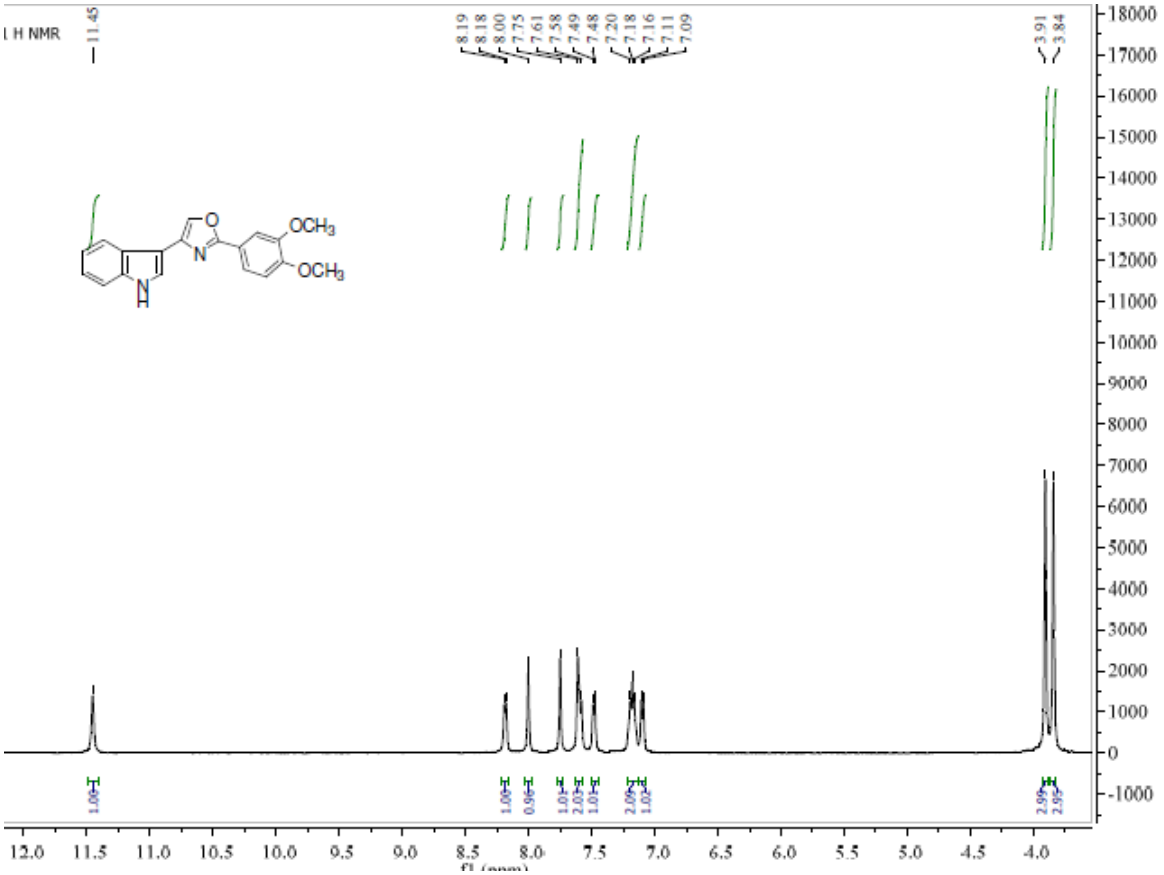


Figure 2.2.4 ^{1}H NMR spectrum of the compound **51c**

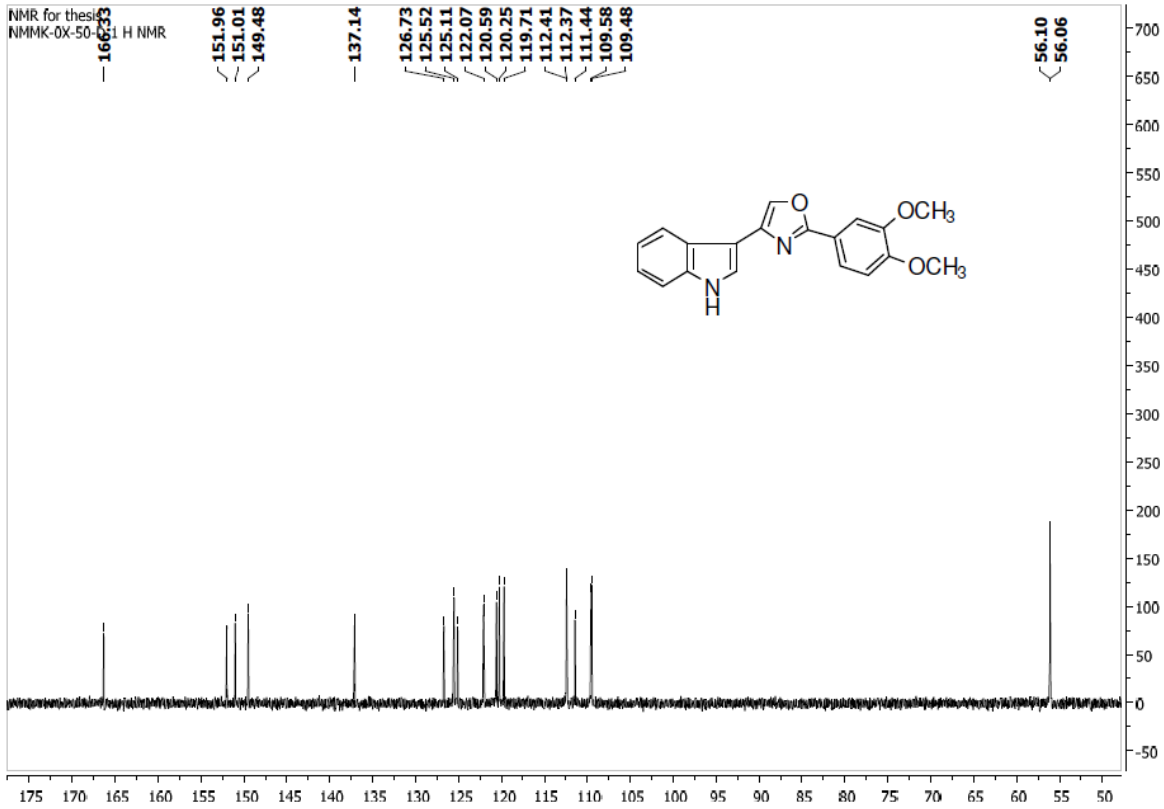
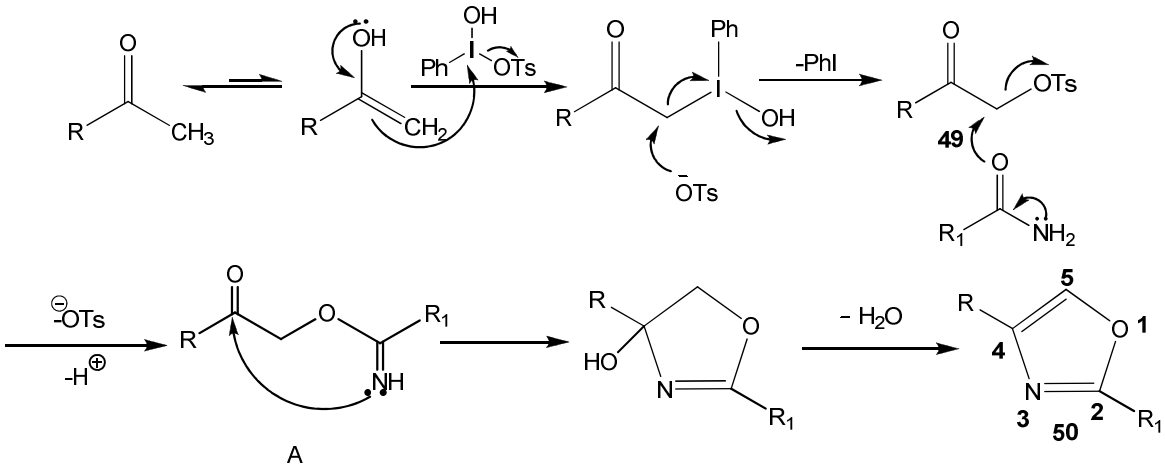


Figure 2.2.5 ^{13}C NMR spectrum of the compound **51c**

The ^{1}H and ^{13}C spectra of the synthesized 2-(3′,4′-dimethoxyphenyl)-4-(3′-indolyl) oxazole (**51c**) showed in the figures 2.2.4 and 2.2.5.

2.2.3.2 Probable mechanism for the formation of 2,4-disubstituted oxazoles

The proposed mechanism for synthesis of oxazole heterocyclic ring from ketones is shown in scheme 2.2.3. The reaction of HTIB with enolizable ketone leads to respective α-tosyloxyketone **49**. Subsequent cyclization of intermediate (**A**) by removal of water resulted in the formation of oxazole ring **50**.



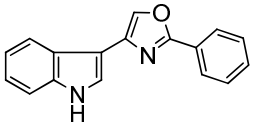
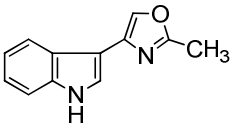
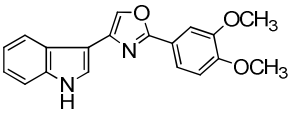
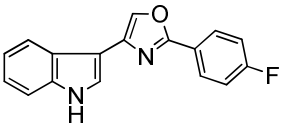
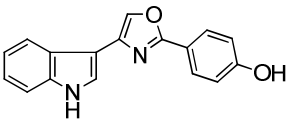
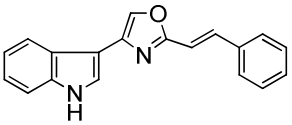
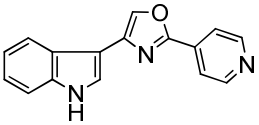
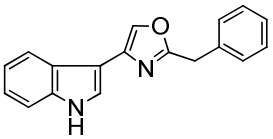
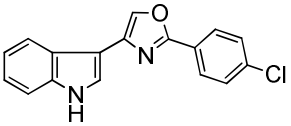
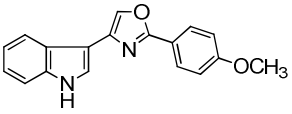
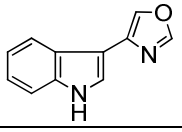
Scheme 2.2.3 Probable mechanism for the formation of 2,4-disubstituted oxazoles (**50)**

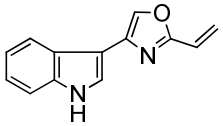
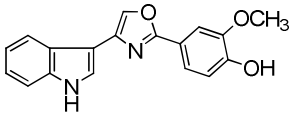
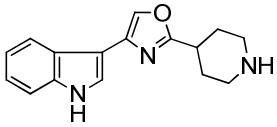
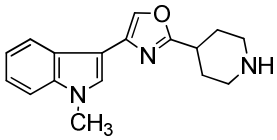
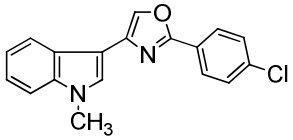
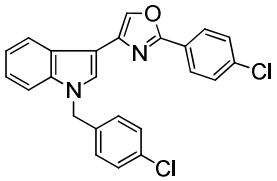
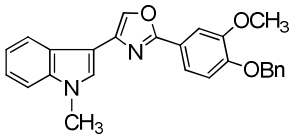
2.2.4 Anticancer activity

Synthesized 4-(3′-indolyl)oxazoles (**51a-m**) and (**53-57**) were tested *in-vitro* for their anticancer potential in various human cancer cell lines, prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreatic (PaCa2). All compounds decreased cell viability as determined by colorimetric MTT assay with IC_{50} values ranging from nanomolar to greater than 1mM. As shown in the table 2.2.2, compounds **51d**, **51g**, **51j**, **51l** and **56** are the active molecules against various cell lines. The compound **51d** is active against all the cancer cell lines, suggesting that *p*-fluorophenyl at C-2 position is beneficial for the activity. Alkylation of indole nitrogen improved the activity; however, *p*-chlorobenzyl as an alkylating group is preferred over methyl (compound **55** *vs* **56**). The compound **56** with *N*-(*p*-chlorobenzyl) and C-2, *p*-chlorophenyl moiety is potent and showed selective cytotoxicity against MCF-7 (14.1 μM) and PaCa2 (26 μM) cell lines. Substitution at C-2 position of oxazole ring is

important for the activity as compound **51k** without any substituent exhibited very poor activity.

Table 2.2.2 Cytotoxicity profile of 4-(3′-indolyl)oxazoles (**51a-m**) and (**53-57**)

4-(3′-Indolyl)oxazoles		Cytotoxicity IC_{50} (μM)[a]					
		PC3	DU145	LaCaP	MCF-7	PaCa2	MDA-MB-231
	**(51a)**	170.1	167.2	460.7	131.3	112.6	171.1
	**(51b)**	336.7	243.5	316.1	801.8	688.5	10^3
	**(51c)**	358.6	299.9	277.9	758.7	10^3	10^3
	**(51d)**	**42.8**	**31.8**	59.8	**28**	**40.6**	90.4
	**(51e)**	381.6	246.8	448.6	579.7	10^3	10^3
	**(51f)**	221.2	134.9	182.8	354.7	872.5	10^3
	**(51g)**	298.3	178.2	238	54.9	**38.4**	105
	**(51h)**	368.1	348.6	376.6	10^3	10^3	10^3
	**(51i)**	150.6	205.5	505.1	410.4	325.5	607.8
	**(51j)**	133.1	164.5	507.3	**43.8**	**35**	108.4
	**(51k)**	321.2	93.5	381.9	10^3	10^3	10^3

	**(51l)**	426.2	59.8	529.5	10^3	**37.6**	10^3
	**(51m)**	302.9	174.6	445.1	10^3	10^3	10^3
	**(53)**	406.8	381.1	476.3	294.5	230.2	527.5
	**(54)**	577.8	375.6	400.1	10^3	758.9	10^3
	**(55)**	302	412.1	378	637.1	89.2	789.8
	**(56)**	349.8	80.5	181.6	**14.1**	**26**	216.3
	**(57)**	450.1	382.5	367.6	197.6	317.5	10^3

[a]These experiments were conducted in triplicates at three independent times. IC_{50} values were obtained using a dose response curve by nonlinear regression using a curve fitting program, GraphPad Prism 5.0. Bold values show IC_{50} of less than 50 μM.

In general, an aromatic/heteroaromatic ring with an electronegative atom at C-2 position is beneficial for the activity. The compound **51l** with C-2 ethenyl moiety was found to be active against DU145 (59.8 μM) and PaCa2 (37.6 μM) cell lines. Introduction of a *p*-methoxyphenyl group at C-2 position (compound **51j**) showed selective cytotoxicity against MCF-7 (43.8 μM) and PaCa2 (35 μM) cell lines. However, an additional electron-donating group at the *meta* position in C-2 aryl ring (compounds **51c, 51m** and **57**) is detrimental for the activity. Compounds **53** and **54** with a piperidinyl ring at C-2 also exhibited poor activity. The most active compound in the series is **56** having the highest activity against breast cancer cell lines (MCF-7) with an IC_{50} value of 14.1 μM (figure 2.2.6).

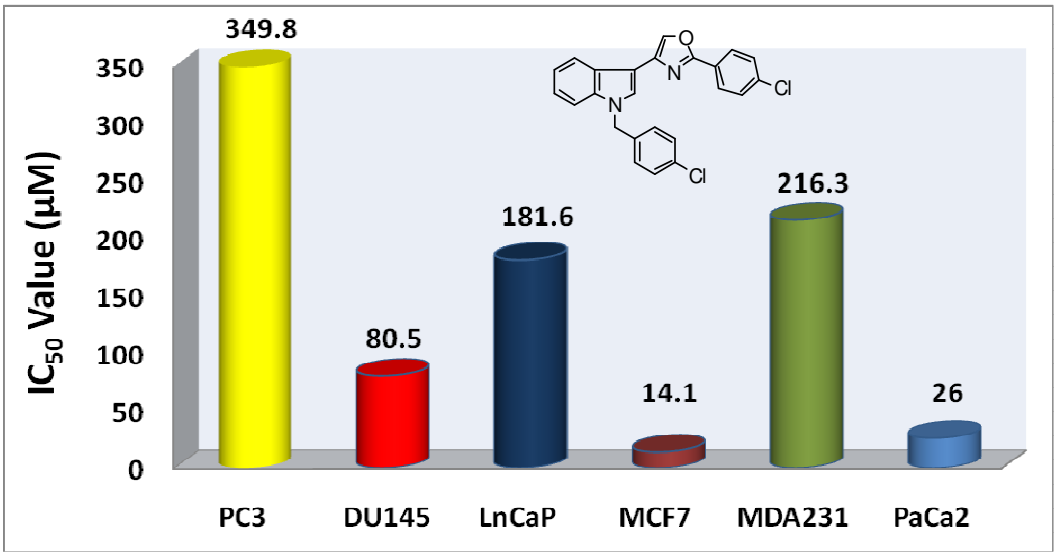


Figure 2.2.6 Activity profile of the most active 4-(3′-indolyl)oxazole (**56**)

The most active compound in the series is **56** having the highest activity against breast cancer cell lines (MCF-7) with an IC_{50} value of 14.1 µM (figure 2.2.6).

2.2.5 Experimental procedures

General: All the reagents were purchased from Aldrich and Spectrochem chemicals. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Column chromatography was performed using 100-200 mesh silica gel and appropriate mixture of hexane and ethyl acetate for elution. The solvents were evaporated using Buchi rotary evaporator. Melting points (mp) were determined with electrothermal capillary melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. Mass spectra was obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972.

2.2.5.1 Synthesis of 4-(3′-indolyl)oxazoles (51-57)

[Hydroxy(tosyloxy)iodo]benzene (HTIB)

A solution of *p*-toluenesulfonic acid monohydrate (761 mg, 40 mmol) in acetonitrile (30 mL) was added to a suspension of iodobenzene diacetate (644 mg, 20 mmol) in acetonitrile (45 mL) at room temperature. A clear solution was obtained, which was allowed to stand at room temperature for one hour, white needles with a yellowish hue separated out. The crude product was washed with acetone and diethylether, dried to

obtain pure HTIB as yellow needles (7.3 g, Yield 93%); mp. 134-137 °C (lit.[30] 135-138 °C).

1-Benzenesulfonylindole (47)

To a mixture of indole (**46**, 11.7 g, 0.1 mol), 50% sodium hydroxide (35 mL), tetrabutylammonium bromide (TBAB) (0.03g, 0.001 mol), and water (20 mL) was added a solution of benzenesulfonyl chloride (21.12 g, 1.2 mmol) in toluene dropwise with continuous stirring. After the completion of reaction, toluene layer was separated and washed with saturated sodium bicarbonate, water and brine solutions (100 mL each). The organic layer was extracted and dried on anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain off-white solid **47** (22.6 g, Yield 88%), mp 195 °C (lit.[66] 196-197 °C).

3-Acetyl-1-benzenesulfonylindole (48)

To a magnetically stirred solution of $AlCl_3$ (24 g, 0.15 mol) in dichloromethane (100 mL) was added acetic anhydride (9.2 g, 0.09 mol). A solution of **47** (7.7 g, 0.03 mol) in dichloromethane (25 mL) was added to the above solution and stirred for 2 h. After completion of the reaction product was extracted by using dichloromethane (500 mL) and was washed with brine solution (100 mL). The organic layer was dried over anhydrous sodium sulfate and reduced under pressure to obtain an off-white colored solid which upon recrystallization using methanol afforded pure **48** (8.2 g, Yield 92%), mp 158 °C (lit.[66] 159-160 °C).

3-Tosyloxyacetyl-1-benzenesulfonylindole (49)

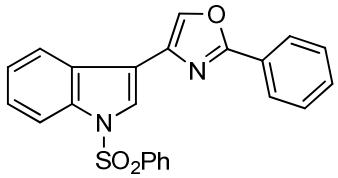
A mixture of 3-acetyl-1′-benzenesulfonyl indole (**48**, 4.0 g, 13.36 mmol) and HTIB (6.44 g, 16.04 mmol) in acetonitrile (30 mL) were stirred at 45 °C for 8 h. Reaction mixture was concentrated under reduced pressure and washed with petroleum ether (20 mL). The residue so obtained was recrystallized from methanol to afford **49** (5.5 g, Yield 88 %), mp 137-140 °C (lit.[67] 138-140 °C).

4-(1′-Benzenesulfonylindol-3′-yl)-2-susbstitutedoxazoles (50)

A neat mixture of 3-tosyloxyacetyl-1′-benzene-sulfonylindole (**49**, 1 mmol) and appropriate amide (1 mmol) was irradiated in a microwave oven for appropriate time (6-15 min) with 1 min heating and 30 sec cooling. After completion of reaction as indicated by TLC, the contents were taken into water (20 mL) and extracted with

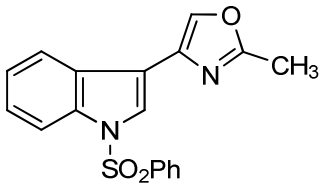
dichloromethane (2 × 10 mL). Organic phase was separated and dried over anhydrous sodium sulfate. Excess of dichloromethane was removed and the residue so obtained was percolated through a bed of silica-gel using column chromatography (hexane: ethyl acetate, 9:1) to afford **50** in good yield.

4-(1′-Benzenesulfonylindol-3-yl)-2-phenyloxazole (50a)



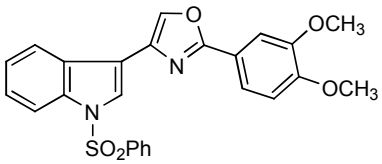
Yield 79%; mp 145 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.32 (m, 5H, Ar-H), 7.39-7.50 (m, 5H, Ar-H), 7.62 (s, 1H, Ar-H), 7.86 (s, 1H, Ar-H), 7.91 (d, 2H, *J* = 7.94 Hz, Ar-H), 7.97-8.01 (m, 2H, Ar-H); HRMS: *m/z* calcd. for $C_{23}H_{16}N_2O_3S$:: 400.0882, found: 401.1105 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-methyloxazoles (50b)



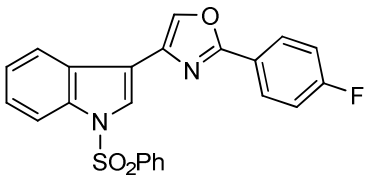
Yield 83%; mp 154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.79 (s, 3H, CH_3), 7.32-7.44 (m, 5H, Ar-H), 7.50-7.52 (m, 1H, Ar-H), 7.62 (s, 1H, Ar-H), 7.91-7.94 (m, 2H, Ar-H), 7.98 (dd, 1H, *J* = 7.12, 1.12 Hz, Ar-H), 8.07 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{18}H_{14}N_2O_3S$: 338.0725, found: 339.1200 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(3,4-dimeth-oxyphenyl)oxazoles (50c)



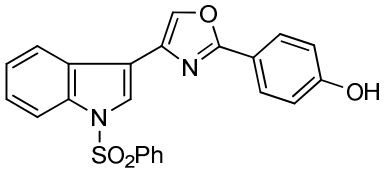
Yield 86%; mp 136 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.58 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 7.32-7.36 (m, 3H, Ar-H), 7.39-7.41 (m, 2H, Ar-H), 7.44-7.49 (m, 5H, Ar-H), 7.58 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 7.95-7.97 (m, 2H, Ar-H); HRMS: *m/z* calcd. for $C_{25}H_{20}N_2O_5S$: 460.1171, found: 461.1202 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-fluoro-phenyl)oxazole (50d)



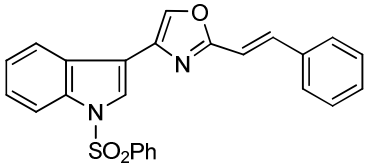
Yield 77%; mp 182 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 7.26-7.28 (m, 4H, Ar-H), 7.35-7.38 (m, 5H, Ar-H), 7.69 (s, 1H, Ar-H), 7.84-7.87 (m, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 8.12-8.16 (m, 2H, Ar-H). HRMS: *m/z* calcd. For $C_{23}H_{15}FN_2O_3S$: 418.0787, found: 418.1012 $(M)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-hydroxy-phenyl)oxazole (50e)



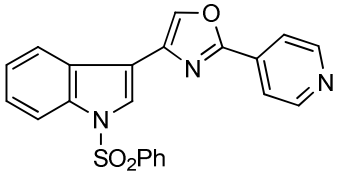
Yield 78%; mp 128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (s, 1H, OH), 7.32-7.36 (m, 5H, Ar-H), 7.39- 7.42 (m, 4H, Ar-H), 7.69 (s, 1H, Ar-H), 7.72 (s, 1H, Ar- H), 7.86 (dd, 2H, *J* = 6.56, 1.76 Hz, Ar-H), 7.91-7.95 (m, 2H, Ar-H); HRMS: *m/z* calcd. For $C_{23}H_{16}N_2O_4S$: 416.0831, found: 417.1024 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(styryl)oxazole (50f)



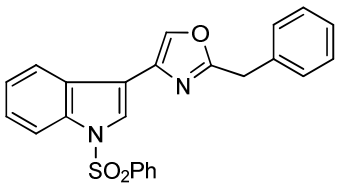
Yield 79%; mp 156-159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.79 (m, 2H, CH), 7.20-7.24 (m, 5H, Ar-H), 7.33-7.36 (m, 2H, Ar-H), 7.38-7.42 (m, 5H, Ar-H), 7.58 (s, 1H, Ar-H), 7.62-7.64 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{25}H_{18}N_2O_3S$: calcd: 426.1038, found: 426.1101 $(M)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-pyridyl)oxazole (50g)



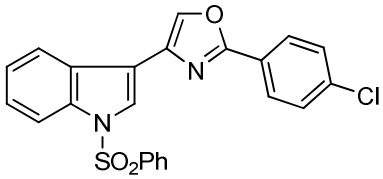
Yield 73%; mp 142 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 7.19-7.21 (m, 2H, Ar-H), 7.29-7.32 (m, 5H, Ar-H), 7.49-7.51 (m, 2H, Ar-H), 7.54 (d, 1H, *J* = 2.56 Hz, Ar-H), 7.61-7.62 (m, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 7.90-7.93 (m, 2H, Ar-H); HRMS: *m/z* calcd. for $C_{22}H_{15}N_3O_3S$: 401.0834, found: 402.1011 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(benzyl)oxazole (50h)



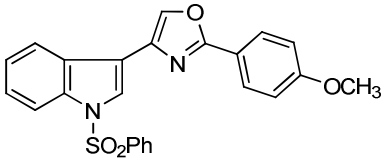
Yield 81%; mp 172-175 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 4.37 (s, 2H, CH_2), 7.28-7.33 (m, 10H, Ar-H), 7.34-7.38 (m, 2H, Ar-H), 7.62 (s, 1H, Ar-H), 7.72 (m, 2H, Ar- H), 7.81 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{24}H_{18}N_2O_3S$: 415.1116, found: 415.1201 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-chloro-phenyl)oxazole (50i)



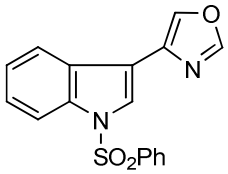
Yield 80%; mp 136 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 7.21-7.28 (m, 4H, Ar-H), 7.36-7.39 (m, 2H, Ar-H), 7.42-7.47 (m, 5H, Ar-H), 7.62 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 7.86-7.89 (m, 2H, ArH); HRMS: *m/z* calcd. for $C_{23}H_{15}ClN_2O_3S$: 434.0592: found: 435.0721 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-methoxyphenyl)oxazole (50j)



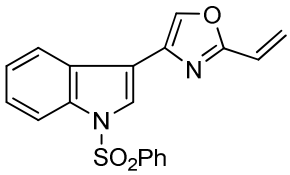
Yield 75%; mp 176-179 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 3.41 (s, 3H, OCH_3), 7.35-7.42 (m, 4H, Ar-H), 7.45-7.47 (m, 5H, Ar-H), 7.49-7.52 (m, 4H, Ar- H), 7.58 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{24}H_{18}N_2O_4S$: 430.0987, found: 431.1132 $(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)oxazole (50k)



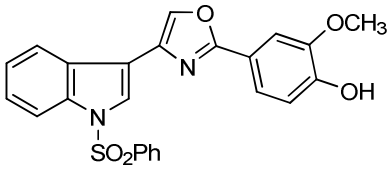
Yield 80%; mp 126 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 7.21-7.24 (m, 4H, Ar-H), 7.31-7.34 (m, 5H, Ar-H), 7.53 (d, 1H, *J* = 2.55 Hz, Ar-H), 7.69 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{17}H_{12}N_2O_3S$: 324.0569, found: 325.0901$(M+H)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(ethenyl)oxazole (50l)



Yield 78%; mp 138 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 5.19 (d, 2H, *J* = 3.6 Hz, CH_2), 6.12 (t, 1H, *J* = 2.6 Hz, CH), 7.35-7.38 (m, 5H, Ar-H), 7.40-7.43 (m, 2H,Ar- H), 7.52 (s, 1H, Ar-H), 7.55-7.58 (m, 2H, Ar-H), 7.79 (s, 1H, Ar-H). HRMS: *m/z* calcd. for$C_{19}H_{14}N_2O_3S$: 350.0725, found: 351.1015 $(M+H)^+$.

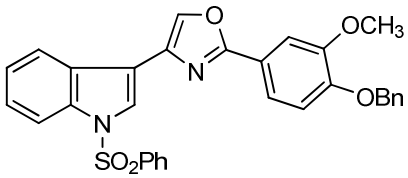
4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-hydroxy-3′-methoxyphenyl)oxazole (50m)



Yield 82%; mp 192-194 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 3.61 (s, 3H, OCH_3), 5.37 (s, 1H, OH), 7.32-7.36 (m, 5H, ArH), 7.41-7.45 (m, 3H, Ar-H), 7.51-7.54 (m, 2H, Ar-H), 7.55 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H),

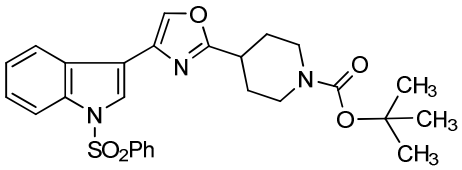
7.92-7.95 (m, 2H, Ar-H); HRMS: *m/z* calcd. for $C_{24}H_{18}N_2O_5S$: 446.0936, found: 446.1101 $(M)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-benzyloxy-3′-methoxyphenyl)oxazole (50n)



Yield 70%; mp 142-144 ^oC; ^1H NMR (400 MHz, $CDCl_3$,): δ 3.44 (s, 3H, OCH_3), 5.02 (s, 2H, CH_2, Ar-H), 7.29-7.41 (m, 5H, Ar-H), 7.49-7.55 (m, 3H, Ar-H), 7.6-7.64 (m, 2H, Ar-H), 7.83 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.93-7.98 (m, 4H, Ar-H), 7.99 (s, 1H, Ar-H), 8.21 (d, *J* = 8.1 Hz, 1H, Ar-H), 8.36 (s, 1H, Ar-H). IR (KBr, *v* cm^{-1}): 3360, 3169, 1633, 1454, 1126, 750; HRMS: *m/z* calcd. for $C_{31}H_{24}N_2O_5S$: 536.14, found: 536.15 $(M)^+$.

4-(1′-Benzenesulfonylindol-3′-yl)-2-(4′-N-Boc-piperidinyl)oxazole (50o)

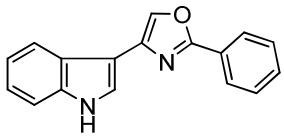


Yield 50%; mp 124-128^{o}C: ^1H NMR (400 MHz, $CDCl_3$,): δ 1.49 (s, 9H), 2.1-2.4 (m, 4H), 2.6-2.8 (m, 4H, CH_2), 2.9 (m, 1H, CH), 7.43-7.52 (m, 5H, Ar-H), 7.68(s, 1H, Ar-H), 7.83-7.88 (m, 4H, Ar-H), 7.9 (s, 1H, Ar-H); HRMS: *m/z* calcd. for $C_{27}H_{29}N_3O_5S$: 507.1828, found: 508.1401 $(M+H)^+$.

General procedure for the synthesis of 4-(3′-indolyl)-2-substituted oxazole (51)

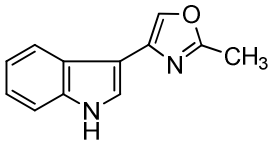
A stirred solution of **50** (1 mmol) and sodium hydroxide (3 mmol) in ethanol (10 mL) and water (3 mL) was refluxed till the completion of the reaction. The ethanol was evaporated under vacuum, and the remaining aqueous solution was extracted with dichloromethane (3 × 10 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum to afford **51** in good yield.

4-(3′-Indolyl)-2-phenyloxazole (51a)



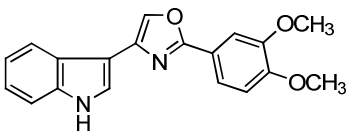
Yield 82%; mp 172 °C; ^1H NMR (400 MHz, $CDCl_3$,): δ 7.28-7.32 (m, 2H, Ar-H), 7.39-7.50 (m, 5H, Ar-H), 7.94 (d, 1H, *J* = 7.92 Hz, Ar-H), 7.97-8.01 (m, 2H, Ar-H), 8.03 (s, 1H, Ar-H), 8.49 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6,): δ 162.54, 153.07, 147.00, 137.52, 136.97, 131.87, 129.22,128.63, 127.90, 126.01, 121.93, 119.90, 116.12, 112.12, 107.03 (Ar-C); HRMS: *m/z* calcd. for $C_{17}H_{12}N_2O$: 260.095, found: 261.1800 $(M+H)^+$.

4-(3′-Indolyl)-2-methyloxazole (51b)



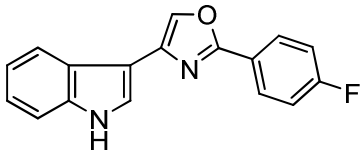
Yield 80%; mp 163 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ2.75 (s, 3H, CH_3), 7.21-7.30 (m, 2H, Ar-H), 7.39-7.44 (m, 2H, Ar-H), 7.79 (s, 1H, Ar-H), 7.87 (d, 1H, *J* = 7.84 Hz, Ar-H), 8.36 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6,): δ165.0, 150.30, 136.56, 125,10, 123.72, 122.49, 120.57, 120.02, 112.80, 111.47, 109.62 (Ar-C), 19.25 (CH_3). HRMS: *m/z* calcd. for$C_{12}H_{10}N_2O$ $(M + H)^+$ 199.0871, found: 199.0932.

2-(3′,4′-Dimethoxyphenyl)-4-(3′-indolyl)oxazole (51c)



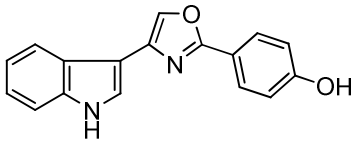
Yield 83%; mp182-185 °C; ^{1}H NMR (500 MHz, DMSO d_6): δ 3.84 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 7.10 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.22–7.13 (m, 2H, Ar-H), 7.48 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.61-7.58 (m, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 8.19 (d, *J* = 7.0 Hz, 1H, Ar-H), 11.45 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 166.33, 151.96, 151.01, 149.48, 137.14, 126.73, 125.52, 125.11, 122.07, 120.59, 120.25, 119.71, 112.41, 112.37, 111.44, 109.58, 109.48, 56.10, 56.06; HRMS: *m/z* calcd. for $C_{19}H_{16}N_2O_3$: 320.1161, found: 321.1252 $(M+H)^+$.

2-(4′-Fluorophenyl)-4-(3′-indolyl)oxazole (51d)



Yield 81%; mp 145-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ7.22- 7.29 (m, 1H, Ar-H), 7.42-7.51 (m, 4H, Ar-H), 7.78 (d, 1H, *J* = 2.52 Hz, Ar-H), 7.84-7.87 (m, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.16-8.19 (m, 2H, Ar-H), 8.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ165.71, 162.82,145.58, 137.39, 130.59, 130.39, 130.30, 129.69, 124.86, 122.90, 122.36, 120.76, 115.38, 112.05, 111.97 (Ar-C); HRMS calcd. for $C_{17}H_{11}FN_2O$: 278.0855, found: 279.1013 $(M+H)^+$.

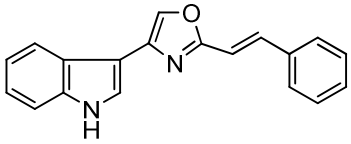
2-(4′-Hydroxyphenyl)-4-(3′-indolyl)-oxazole (51e)



Yield 84%; mp 138 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36 (s, 1H, OH), 7.22-7.29 (m, 1H, Ar-H), 7.42-7.51 (m, 4H, Ar-H), 7.77 (d, 1H, *J* = 2.52 Hz, Ar-H), 7.86 (dd, 1H, *J* = 6.56, 1.76 Hz, Ar-H), 8.01 (s, 1H, Ar-H), 8.11-8.16 (m, 2H, Ar-H), 8.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.78, 162.58, 145.61,

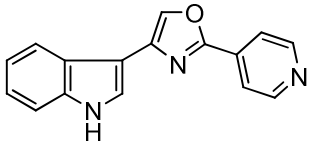
140.79, 137.21, 136.18, 133.51, 132.64, 131.14, 129.56, 128.64, 126.47, 125.62, 124.45, 13.84 (Ar-C); HRMS: *m/z* calcd. for $C_{17}H_{12}N_2O_2$: 277.0899, found: 277.0982 $(M+H)^+$.

4-(3′-Indolyl)-2-(styryl)oxazole (51f)



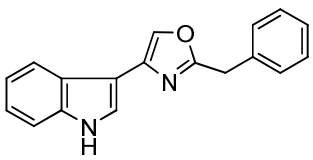
Yield 85%; mp 162 °C; ^{1}H NMR (400 MHz, $CDCl_3$,): δ 6.82 (m, 2H, C-H), 7.12-7.23 (m, 5H, Ar-H), 7.31-7.33 (m, 2H, Ar-H), 7.56 (d, 1H, *J* = 2.53 Hz, Ar-H), 7.58-7.60 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 8.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6,): δ 160.72, 137.52, 136.95, 135.52 (C=C),131.50, 129.23, 127.90,127.21, 126.00 (C=C), 124.72, 123.64, 121.95, 119.92, 119.70, 114.02, 112.10, 107.09 (Ar-C); HRMS: *m/z* calcd. for $C_{19}H_{14}N_2O$: 286.1, found: 286.1 $(M)^+$.

4-(3′-Indolyl)-2-(4′-pyridyl)oxazole (51g)



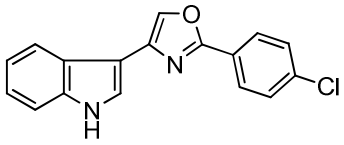
Yield 85%; mp 122 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15-7.19 (m, 2H, Ar-H), 7.52-7.55 (m, 2H, Ar-H), 7.57 (d, 1H, *J* = 2.53 Hz, Ar-H), 7.62-7.64 (m, 2H, Ar-H), 7.78 (s, 1H, Ar-H), 7.93-7.96 (m, 2H, Ar-H), 8.42 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 159.50, 148.63, 133.17, 130.99,129.66, 128.95,128.14, 127.43, 126.46, 125.95, 124.41, 122.18, 120.06, 112.40 (Ar-C); HRMS: *m/z* calcd. for $C_{16}H_{11}N_3O$: 261.0920, found: 262.1056 $(M+H)^+$.

2-(Benzyl)4-(3′-indolyl)oxazole (51h)



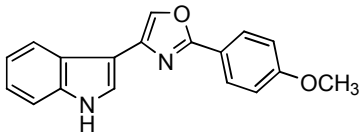
Yield 80%; mp 138 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.39 (s, 2H, CH_2). 7.15-7.40 (m, 9H, Ar-H), 7.69 (d, 1H, *J* = 2.54 Hz, Ar-H), 8.00 (s, 1H, Ar-H), 8.61 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 166.58, 143.66, 140.26, 137.84, 136.96,132.96, 129.58, 128.46, 126.75, 125.30, 123.06, 122.15, 121.04, 111.36, 109.88 (Ar-C), 33.19 (CH_2); HRMS: *m/z* calcd. for $C_{18}H_{14}N_2O$: 274.1106, found: 275.2200 $(M+H)^+$.

2-(4′-Chlorophenyl)4-(3′-indolyl)oxazole (51i)



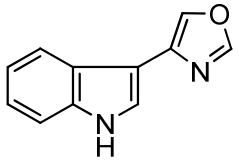
Yield 84%; mp 158-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25-7.32 (m, 4H, Ar-H), 7.39-7.41 (m, 2H, Ar-H), 7.59 (d, 1H, *J* = 2.54 Hz, Ar-H), 7.78 (s, 1H, Ar-H), 7.93-8.03 (m, 2H, Ar-H), 8.41 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.70, 148.31, 137.68, 135.64, 133.45, 131.39, 129.82, 128.20, 127.65, 126.27, 124.78, 122.21, 120.30, 112.44, 106.75 (Ar-C); HRMS: *m/z* calcd. for $C_{17}H_{11}ClN_2O$: 294.056, found: 295.0739 $(M+H)^+$.

4-(3′-Indolyl)-2-(4′-methoxyphenyl)oxazole (51j)



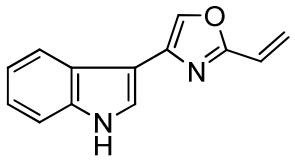
Yield 86%; mp 164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.39 (s, 3H, OCH_3), 7.39-7.43 (m, 4H, Ar-H), 7.54 (d, 1H, *J* = 2.54 Hz, Ar-H), 7.65 (d, 2H, *J* = 8 Hz, Ar-H), 7.79 (s, 1H, Ar-H), 7.96 (d, 2H, *J* = 5.96 Hz, Ar-H), 8.20 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ162.44, 162.16, 144.87, 137.49, 136.23, 130.56, 129.78, 126.55, 124.83, 123.06, 122.52, 120.80, 114.10, 112.57, 112.29 (Ar-C), 55.86 (OCH_3); HRMS: *m/z* calcd. for $C_{18}H_{14}N_2O_2$: 290.1, found: 291.1 $(M+H)^+$.

4-(3′-Indolyl)oxazole (51k)



Yield 83%; mp 110 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22-7.30 (m, 4H, Ar-H), 7.56 (d, 1H, *J* = 2.55 Hz, Ar-H), 7.71 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.35 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ152.26, 148.60, 128.96, 128.14, 125.95, 124.64, 124.40, 123.50, 121.45, 120.50, 119.50 (Ar-C); HRMS: *m/z* calcd. for $C_{11}H_8N_2O$: 184.0637, found: 185.0813 $(M+H)^+$.

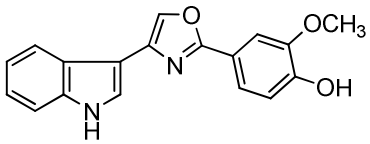
2-(Ethenyl)-4-(3′-indolyl)oxazole (51l)



Yield 79%; mp 140-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (d, 2H, *J* = 3.7 Hz, CH_2), 6.14 (t, 1H, *J* = 2.6 Hz, CH), 7.39-7.42 (m, 2H, Ar-H), 7.54 (d, 1H, *J* = 2.54 Hz, Ar-H), 7.56-7.59 (m, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 8.34 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.21, 145.08, 137.85,

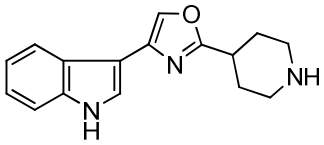
136.98,133.40 (C=C), 132.84, 129.53, 128.56, 125.33, 123.10 (C=C), 121.04, 111.38, 109.91 (Ar-C); HRMS: *m/z* calcd. for $C_{13}H_{10}N_2O$: 210.0793, found: 211.1023 $(M+H)^+$.

2-(4′-Hydroxy-3′-methoxyphenyl)-4-(3′-indolyl)oxazole (51m)



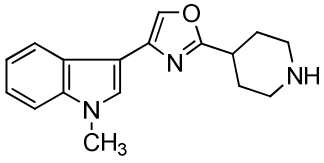
Yield 88%; mp 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.59 (s, 3H, CH_3), 5.39 (s, 1H, OH), 7.39-7.43 (m, 3H, Ar-H), 7.48-7.53 (m, 2H, Ar-H), 7.55 (d, 1H, *J* = 2.57 Hz, Ar-H), 7.81 (s, 1H, Ar-H), 7.95-7.97 (m, 2H, Ar-H), 8.32(s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 165.29, 162.81, 160.49, 137.17, 136.36, 135.35, 130.63, 129.28, 127.30, 126.03, 124.13, 123.68, 121.90, 116.97, 116. 75, 114.38, 113.86 (Ar-C), 57.80 (OCH_3); HRMS: *m/z* calcd. for $C_{18}H_{14}N_2O_3$: 307.1083, found: 307.1092 $(M+H)^+$.

4-(3′-Indolyl) 2-(piperidinyl)oxazole (53)



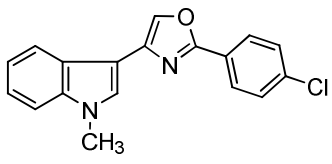
Yield 40%; mp 118-119^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85–1.77 (m, 3H), 2.11–2.04 (m, 2H), 2.74-2.72(m, 2H), 2.87–2.82 (m, 2H), 2.96-2.94 (m, 1H, NH), 7.39–7.34 (m, 2H, Ar-H), 7.75–7.71 (m, 1H, Ar-H), 7.84-7.80 (m, 2H, Ar-H), 7.93 (s, 1H, Ar-H), 8.32 (s, 1H, NH); HRMS: *m/z* calcd. for $C_{16}H_{17}N_3O$: 268.14, found: 268.15 $(M+H)^+$.

4-(1-Methyl-3′-indolyl)-2-(piperidinyl)oxazole (54)



Yield 50%; mp 108-109^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81-1.77 (m, 2H), 2.54-2.48 (m, 4H), 2.83-2.80 (m, 3H), 2.94-2.92 (m, 1H), 3.75 (s, 3H), 7.47–7.43 (m, 2H), 7.68 (s, 1H), 7.89–7.84 (m, 1H), 7.97 (s, 1H), 7.91 (dt, *J* = 8.0, 3.1 Hz, 1H); HRMS: *m/z* calcd. for $C_{17}H_{19}N_3O$: 281.15, found: 282.15 $(M+H)^+$.

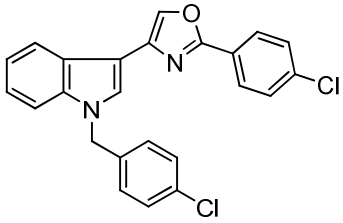
2-(4′-Chlorophenyl)-4-(N-methyl-3′-indolyl)oxazole (55)



Yield 70%; mp 133-135^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (s, 3H, NCH_3), 7.24-7.49 (m, 5H, Ar-H), 7.65 (s, 1H, Ar-H), 7.83 (d, *J* = 8.8 Hz, 1H, Ar-H) 7.99 (s, 1H, Ar-H), 8.08 (d, *J* = 8.8 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz,

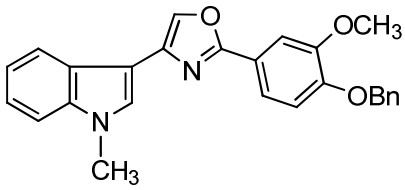
DMSO-d_6): δ 159.72, 137.49, 137.34, 135.66, 133.44, 129.80, 128.67, 128.20, 126.27, 125.08, 122.31, 120.51, 120.28, 110.69, 106.00 (Ar-C), 33.09 (N-CH_3); HRMS: *m/z* calcd. for $C_{18}H_{13}ClN_2O$: 308.0716, found: 308.0962 $(M)^+$.

4-(*N*-(4-Chlorobenzyl)-2-(4′-chlorophenyl)-3′-indolyl)oxazole (56)



Yield 75%; mp 181-183 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36 (s, 2H, CH_2), 7.12 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.25-7.32 (m, 6H, Ar-H), 7.47 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.71 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.06-8.09 (m, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.79, 137.44, 137.12, 136.79, 135.72, 133.77, 132.55, 129.82, 129.52, 129.07, 128.24, 126.00, 125.35, 122.62, 120.73, 120.59, 111.14, 106.92 (Ar-C), 48.89 (CH_2); HRMS: *m/z* calcd. for $C_{24}H_{16}Cl_2N_2O$: 418.0648, found: 419.0709 $(M+H)^+$.

2-(4′-Benzyloxy-3-hydroxyphenyl)-4-(N-methyl-3′-indolyl)oxazole (57)



Yield 60%; mp 142-144 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, OCH_3), 4.02 (s, 3H, NCH_3), 5.24 (s, 2H, CH_2), 6.98 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.22-7.48 (m, 9H, Ar-H), 7.66 (s, 1H, Ar-H), 7.02 (d, *J* =2 Hz, 1H, Ar-H), 7.82 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.95 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.03, 155.14, 150.50, 148.97, 138.19, 137.23, 135.82, 128.92, 128.42, 128.35, 127.23, 126.29, 125.60, 123.19, 122.71, 121.29, 120.44, 112.91, 111.55, 110.96, 110.80 (Ar-C), 70.23 (CH_2), 56.02 (OCH_3), 33.32 (NCH_3); HRMS: *m/z* calcd. for $C_{26}H_{22}N_2O_3$: 410.1, found: 411.1 $(M+H)^+$.

2.2.5.2 MTT assay

A series of indolyl oxazoles **(51a-m, 53-57)** were screened against prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA231) and pancreatic (PaCa2) cancer cell lines. The human cancer cell lines screened were cultured in RPMI 1640 medium containing 5% fetal bovine serum. Cells were seeded in 96-well microtiter plate, at an expected target cell density of 5000-10,000 cells per well, based on cell growth. Inoculates were allowed to pre-incubate for 12 h at 37 °C for stabilization. Test compounds were evaluated at different concentrations ranging from 100 nM to 1000 μM. Incubation lasted for 48 h in

5% CO_2 atmosphere. The anticancer activity was determined for each cell line using formazan dye (MTT) conversion assay.

2.3 Part B: Novel 5-(3′-indolyl)-1,3,4-thiadiazoles as anticancer agents

2.3.1 1,3,4-Thiadiazoles in drug discovery

1,3,4-Thiadiazoles are five-membered nitrogen and sulfur containing heterocycle which has been commonly used as privileged scaffold to produce various novel therapeutic molecules. The 2,5-disubstituted-1,3,4-thiadiazoles have attained significance in the design of novel therapeutics for a wide range of diseases. The 1,3,4-thiadiazoles are much explored for their broad spectrum of biological activities including antiinflammatory (**58a**),[68] antihypertensive (**58b**),[69] antibacterial (**58c**),[70] anticonvulsant (**58d**),[71] antimicrobial (**58e**),[72] antifungal (**58f**),[73] antileishmanial (**58g**),[74] and thiadiazoles (**58h**) were studied for their protein kinase activity (figure 2.3.1).

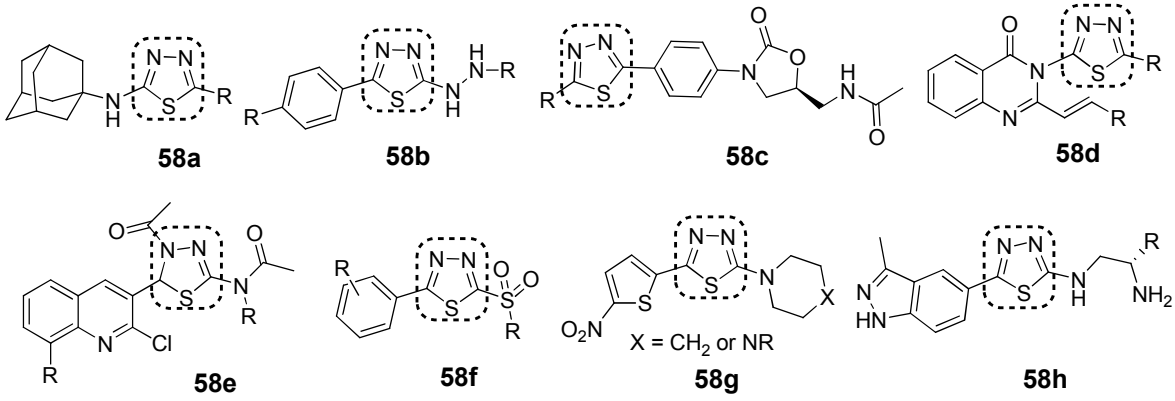


Figure 2.3.1 1,3,4-Thiadiazoles (**58a-h**) in drug discovery

Simple 2-amino-1,3,4-thiadiazole (**59**) (ATDA) and its derivatives are well known as compounds of a wide range of anticancer activity,[75] including *in-vivo* conditions.

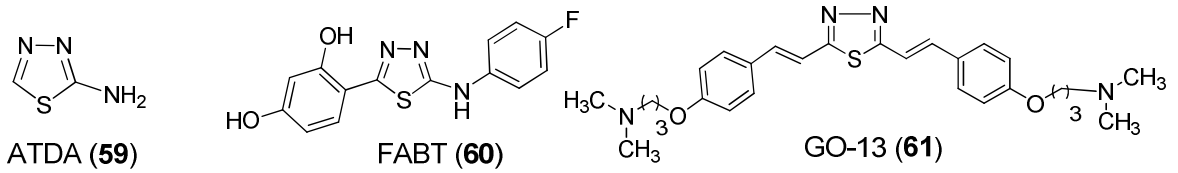


Figure 2.3.2 1,3,4-Thiadiazole containing anticancer agents (**59-61**)

Furthermore, widely explored 2-aminothiadiazoles are in clinical trials for the treatment of patients with different tumors. 2-(4-Fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) (**60**), a most promising thiadiazole containing anticancer compound in malign tumors of nervous system, inhibits proliferation in tumor cells by decreasing cell division and inhibiting metastasis. Diverse variety of 1,3,4-thiadiazoles

were tested for their anticancer activity.[76-78] The compound GO-13 (**61**) was studied for their anticancer activity against A549 lung cancer cell lines (figure 2.3.2).[79]

2.3.2 Rational approach

The design of indolyl-1,3,4-thiadiazoles follows the rational approach based on the diverse applications of indolylazoles. The rational approach includes the anticancer activities of naturally occurring indolyloxazoles **9** and **10** (Labradorins 1 and 2) and their synthetic analogues, indolylthiazoles (Camalexin) **1c** and indole-based synthetic anticancer agents (**62-64**) and indolyl-1,3,4-oxadiazole (**29**). In continuation to explore the anticancer activity of indolylazoles and interesting results of indolyl-1,3,4-oxadiazoles from our research group motivated us to explore indolyl-1,3,4-thiadiazoles (**65**) as potential anticancer agents (figure 2.3.3).

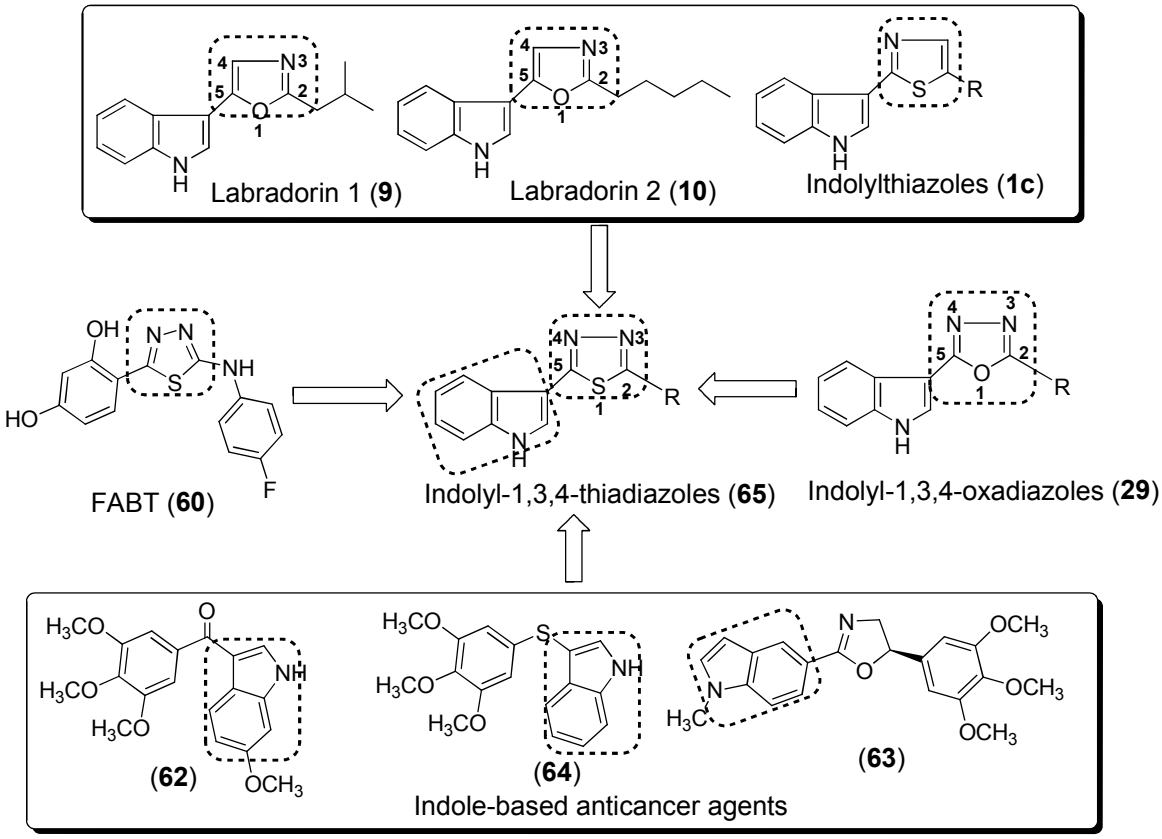


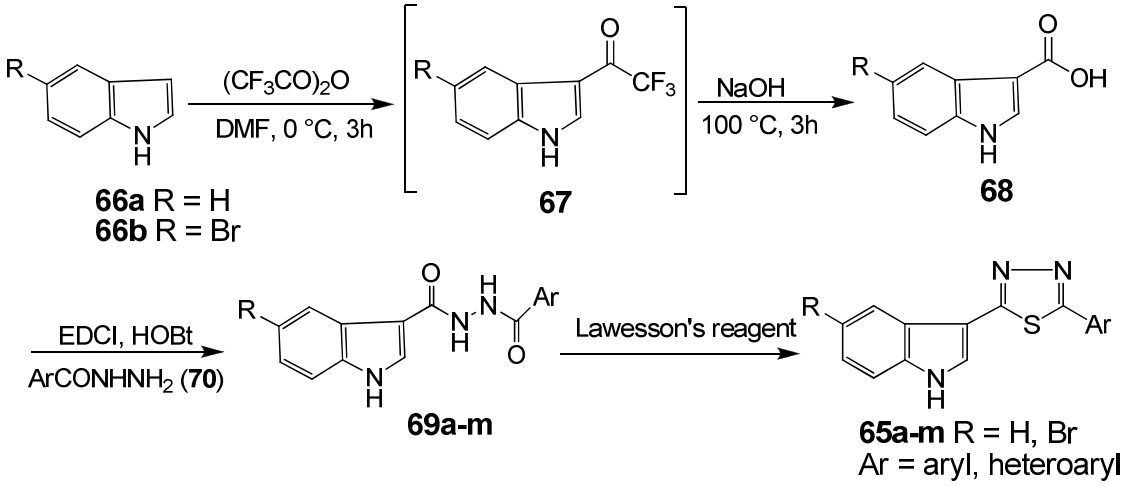
Figure 2.3.3 Rational for the synthesis of novel indolyl-1,3,4-thiadiazoles (**65**)

2.3.3 Results and discussion

2.3.3.1 Chemistry

The common method for the preparation of 1,3,4-thiadiazoles involves the reaction of aldehydes, hydrazine hydrate and elemental sulfur under conventional and microwave conditions.[80-81] Thionation of dibenzoylhydrazines using Lawesson's reagent or phosphorus pentasulfide followed by cyclization and dehydrosulfurization is reported to produce 1,3,4-thiadiazoles in good yields.[63, 80, 82] Verma *et al.* reported microwave accelerated solvent-free synthesis of 1,3,4-thiadiazoles from the reaction of acid hydrazides and triethylorthoalkanates in presence of phosphorus pentasulfide in alumina.[83-84]

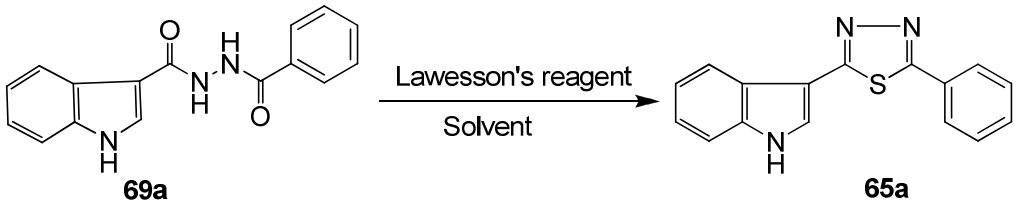
Synthesis of 5-(3′-indolyl)thiadiazoles (**65a-m**) was achieved following a convenient three-step procedure starting from commercially available indole (**66**) as outlined in the scheme 2.3.1. The indole-3-carboxylic acids (**68**) were prepared from the reaction of **66** with trifluoroacetic anhydride initially to give intermediate **67** and followed by hydrolysis with sodium hydroxide. Reaction of **68** with arylhydrazides in the presence of versatile coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 1-hydroxy benzotriazole (HOBt) in dry tetrahydrofuran afforded diacylhydrazines **69**. Thionation of diacylhydrazine **24** with Lawesson's reagent followed by cyclization in dry THF led to the indolyl-1,3,4-thiadiazoles (**65**) in good yields.



Scheme 2.3.1 Synthesis of 5-(3′-indolyl)-2-aryl-1,3,4-thiadiazoles (**65a-m**)

Synthesis of indolyl-1,3,4-thiadiazoles was investigated in different organic solvents such as toluene, dioxane and xylene but resulted in poor yields when compared to tetrahydrofuran.

In a model reaction the compound **69a** was treated with Lawesson's reagent in various solvents such as toluene, dioxane and xylene (scheme 2.3.2) and found the yields are poor and unreacted starting material was remained in the reaction mixture. Solvent-free reaction of compound **69a** with Lawesson's reagent under microwave irradiation led to the mixture of by-products along with desired indolyl-1,3,4-thiadiazole (**65a**).



69a **65a**

Scheme 2.3.2 Synthesis of 5-(3′-indolyl)-2-phenyl-1,3,4-thiadiazole (**65a**)

. **Table 2.3.1** Synthesis of 2-phenyl-5-indolyl-1,3,4-thiadiazole (**65a**) in various solvents

Entry	Solvent	Isolated yields (%)
1.	Toluene	20
2.	Dioxane	15
3.	Xylene	30
4.	**Tetrahydrofuran**	**65**
5.	Solvent-free	10

Finally, the desired 1,3,4-thiadiazole **65a** was successfully achieved in good yield using tetrahydrofuran as a solvent. The optimized protocol was utilized to synthesize a series of indolyl-1,3,4-thiadiazoles **65a-m** and characterized by their IR, ^{1}H NMR and Mass spectral data. The ^{1}H NMR and Mass spectra of compound **65l** are shown in the figures 2.3.4 and 2.3.5.

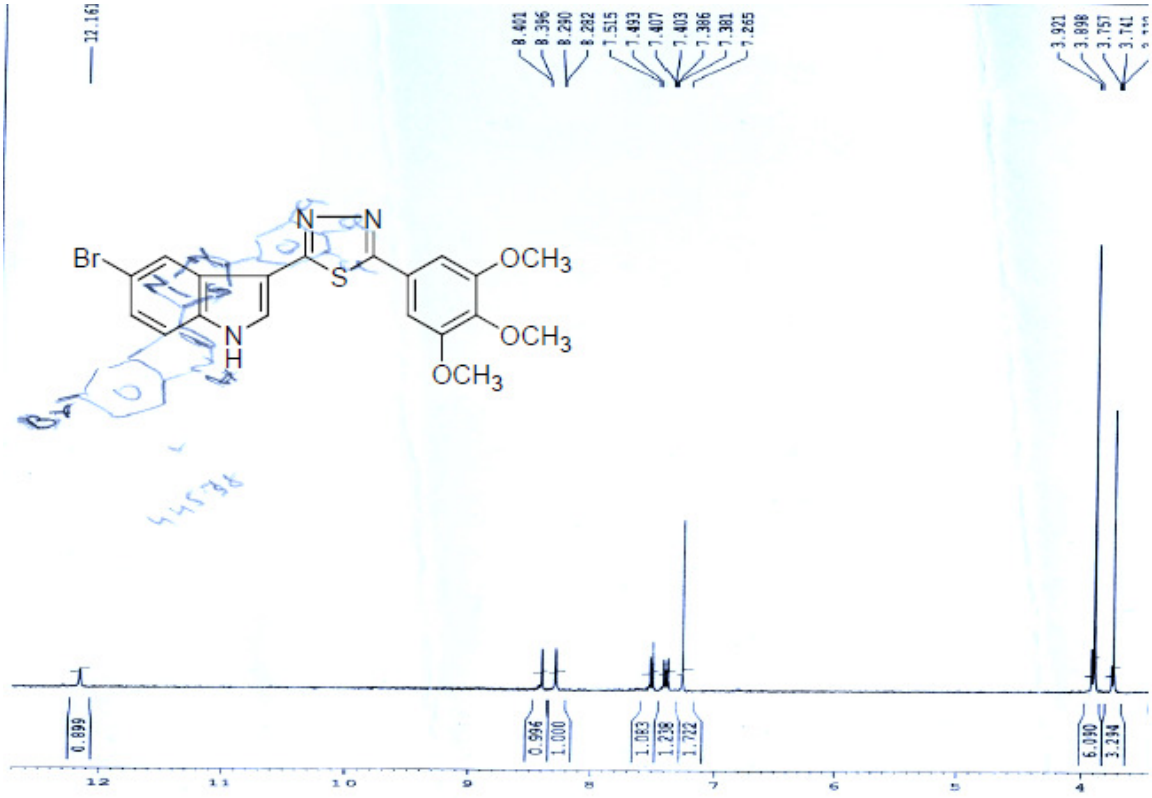


Figure 2.3.4 ^{1}H NMR spectrum of indolyl-1,3,4-thiadiazole **65l**

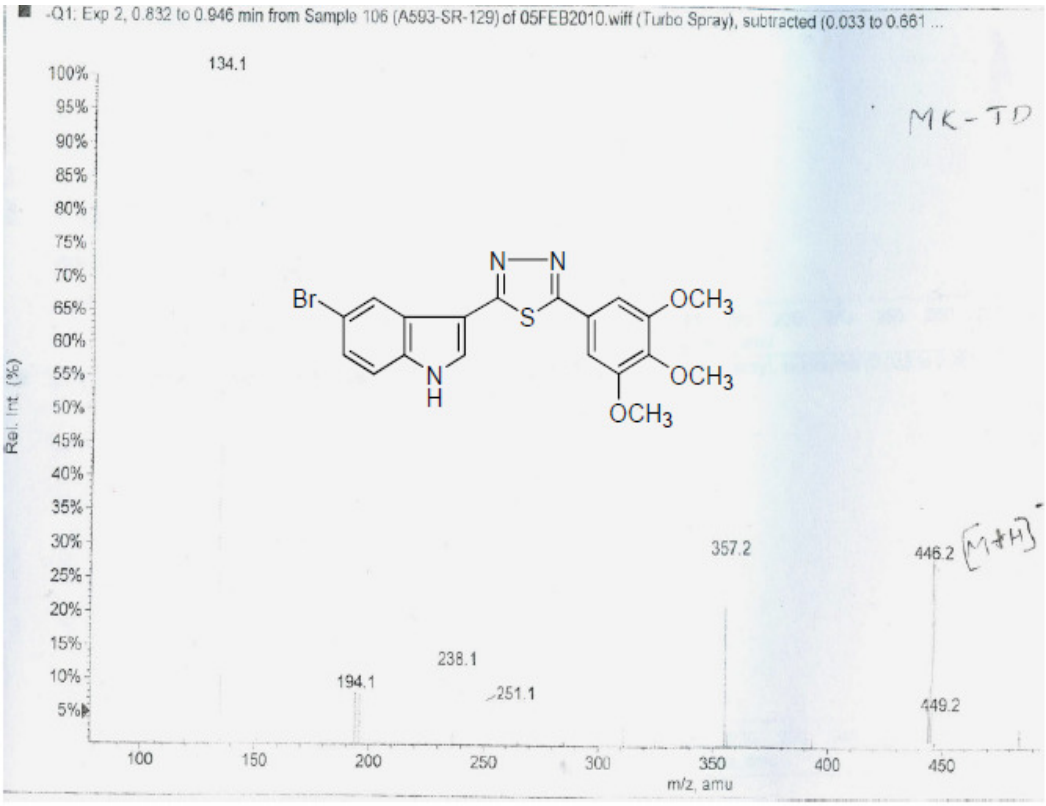
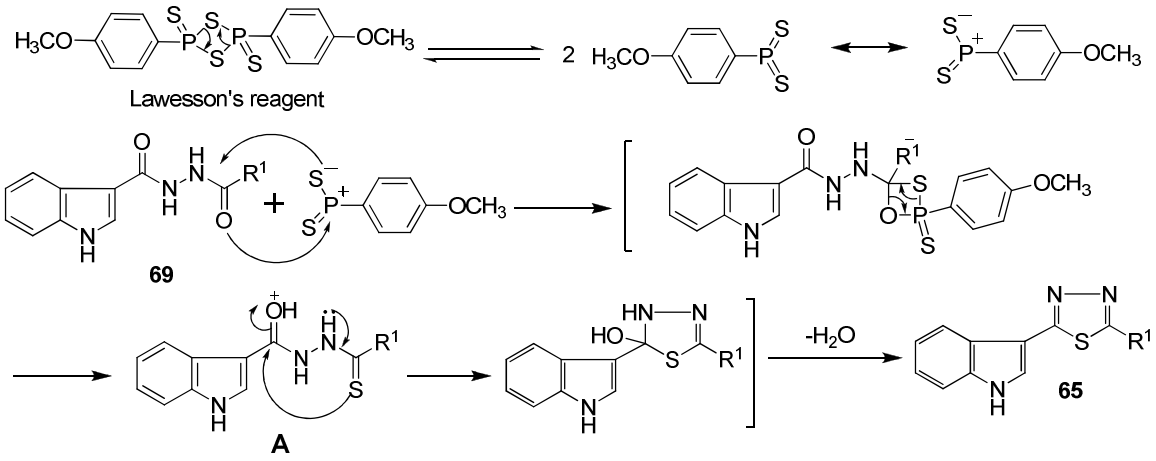


Figure 2.3.5 Mass spectrum of indolyl-1,3,4-thiadiazole **65l**

2.3.3.2 Plausible mechanism for the formation of 1,3,4-thiadiazoles 65

The reaction of intermediate diacylhydrazine **69** with Lawesson's reagent follows the mechanism as shown in the scheme 2.3.3 to give respective indolyl-1,3,4-thaidiazoles. Initially the intermediate **A** might have formed by thionation of diacylhydrazine and then followed by cyclization and aromatization affords the thiadiazoles **65**.

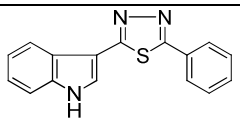
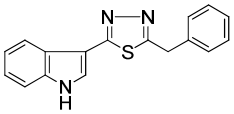
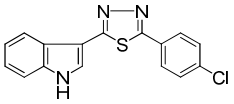
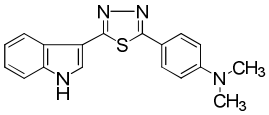
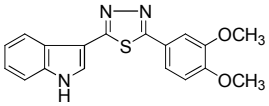
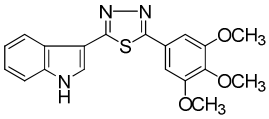
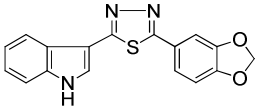
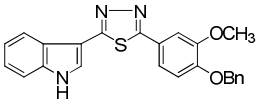
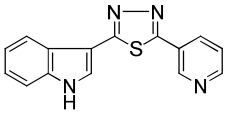
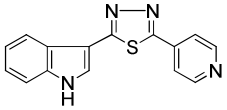
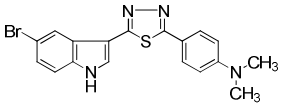
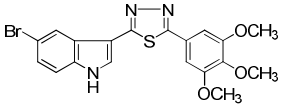
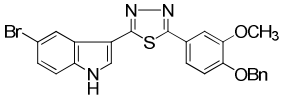


Scheme 2.3.3 Plausible mechanism for the formation of indolyl-1,3,4-thiadiazoles (**65**)

2.3.3.3 Anticancer activity

Synthesized 5-(3′-indolyl)-2-substituted-1,3,4-thiadiazoles (**65a-m**) were screened against prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreatic (PaCa2) cancer cell lines. Many of the compounds have shown the anticancer activity as determined by the cell viability assay. The activity results mentioned in table 2.3.2 show the cytotoxic effects of 5-(3′-indolyl)-2-substituted 1,3,4-thiadiazoles (**65a-m**) against various cancer cell lines. Most of the compounds showed significant cytotoxic effect. The bold values in the table indicate the IC_{50} values less than 20 μM. The structure-activity relationship (SAR) study revealed that the substituents at C-2 position of the 1,3,4-thiadiazole ring plays an important role in imparting the cytotoxic activity to the compound. The compound **65a** possessing a phenyl ring at C-2 position was found to be selectively cytotoxic against PaCa2 cell line (IC_{50} = 41.7 μM).

Table 2.3.2 *In-vitro* cytotoxicity 5-(3′-indolyl)-1,3,4-thiadiazoles (**65a-m**) IC_{50} (μM)

5-(3′-Indolyl)-1,3,4-thiadiazoles		LnCaP	DU145	PC3	MCF-7	MDA-MB-231	PaCa2
	**(65a)**	264.9	261.5	366.7	280.5	445.7	**41.7**
	**(65b)**	**13.9**	**17**	29	**13.5**	27.8	**13**
	**(65c)**	53.1	79.6	99.2	149.5	54.7	$> 10^3$
	**(65d)**	23	35.6	704.9	**12.3**	124.1	188.1
	**(65e)**	21	**10.7**	**11.9**	**6.8**	24	**13.8**
	**(65f)**	40	168.5	29.3	144.9	54.5	**14.2**
	**(65g)**	799	162	157.9	$> 10^3$	$> 10^3$	231.1
	**(65h)**	**19.2**	**9.1**	22.3	**6.5**	26.2	28.9
	**(65i)**	151.7	170	37.2	91.6	96.3	$> 10^3$
	**(65j)**	95	77	39.2	67	53.8	451.3
	**(65k)**	31.3	22	26.6	28.7	**12.7**	130.5
	**(65l)**	66.4	58.4	95.4	594.7	**19.1**	$> 10^3$
	**(65m)**	**8.9**	**3.6**	**7.5**	**8.3**	**6.2**	**1.5**

Replacement of phenyl ring with benzyl moiety led to the compound **65b** with increased cytotoxicity against all cancer cell lines, the IC_{50} values are nearly uniform against LnCaP, MCF-7 and PaCa2 cell lines and below 30 μM in PC3, DU145 and MDA-MB-231 cell lines. Next, we investigated the effect of electron with-drawing and electron-donating groups by introducing chloro and *N,N′*-dimethylamino substituents at the para position of C-2 aryl ring. It was observed that compound **65c** with *p*-chlorophenyl is moderately active against four cancer cell lines (< 100 μM) whereas compound **65d** with *N,N′*-dimethylaminophenyl showed an apparent increase in activity against three cancer cell lines LnCaP (23 μM), DU145 (35.6 μM), MCF-7 (12.3 μM) and selective cytotoxicity against MCF-7 (IC_{50} = 12.3 μM). Introducing 3,4-dimethoxyphenyl group at C-2 resulted in compound **65e** with significantly increased activity against all the cancer cell lines especially against MCF-7 with an IC_{50} value of 6.8 μM. Interestingly, a 3,4-methylenedioxy moiety (compound **65g**) in the C-2 aryl ring drastically reduces the activity. Introduction of third methoxy group on phenyl ring (compound **65f**) also reduced the activity but enhanced the selectivity against PaCa2 cell line (IC_{50} = 14.2 μM). Increasing the size of 4-methoxy group by replacing it with 4-benzyloxy group (compound **65h**) led to substantial gain in activity and selectivity against MCF-7 (IC_{50} = 6.5 μM) and DU145 (IC_{50} = 9.1 μM) cell lines.

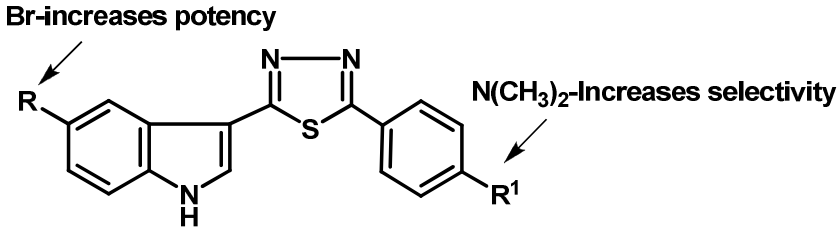


Figure 2.3.6 Structure-activity relationship of indolyl-1,3,4-thiadiazoles (**65a-m**)

Compound with C-2 pyridyl substituent (compound **65j**) displayed improved activity and selectivity against PC3 cell line (IC_{50} < 50 μM) when compared with **65a**. Shifting the position of heteroatom in the ring led to 4-pyridyl derivative **65i** with further improved activity. The compound **65k** with C-2 dimethylaminophenyl and 5-bromo indole exhibited reduced activity except selective cytotoxicity against MDA-MB-231 cell line with an IC_{50} value of 12.7 μM**.** Replacing *N,N′*-dimethylphenyl of **65k** with trimethoxyphenyl resulted in **65l** with reduced activity, whereas 4-benzyloxy-3-methoxyphenyl group led to compound **65m** with significant improved activity and selectivity against PaCa2 (IC_{50} = 1.5 μM) cancer cell line and found to be series most

active compound. The overall structure-activity relationship results were summarized in the figure 2.3.7. SAR shows the bromo substituent at C-5 of indole induces better activity and *N,N'*-dimethylphenyl at C-2 of 1,3,4-thiadiazole bring selectivity to the compounds.

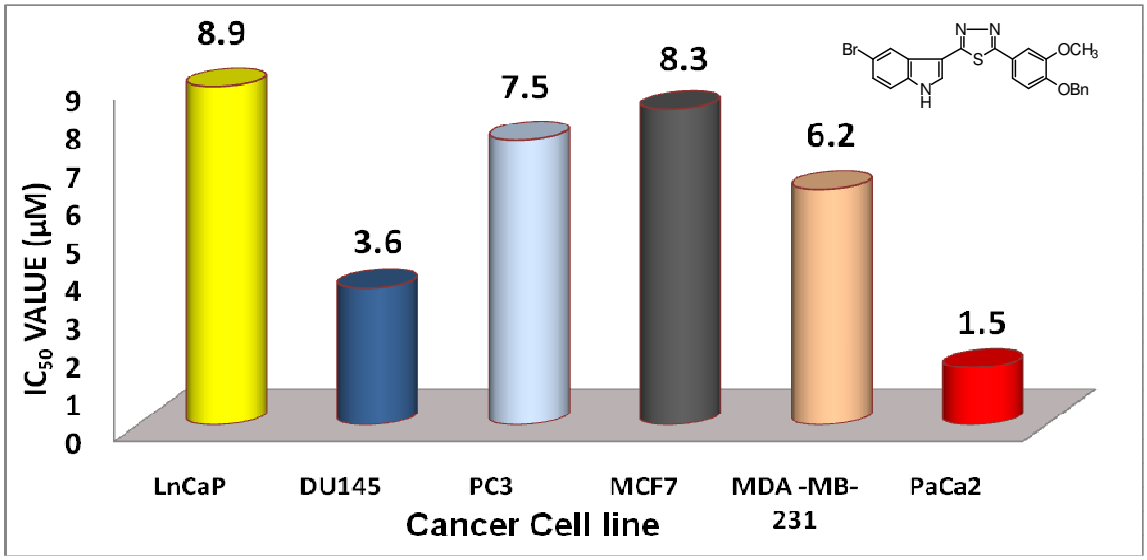


Figure 2.3.7 Anticancer activity profile of the most active compound **65m**

The anticancer activity of most active compound **65m** in the series has shown in the figure 2.3.7. The best activity was shown against PC3 (prostrate) cancer cell lines with IC_{50} 1.5 µM (figure 2.3.8).

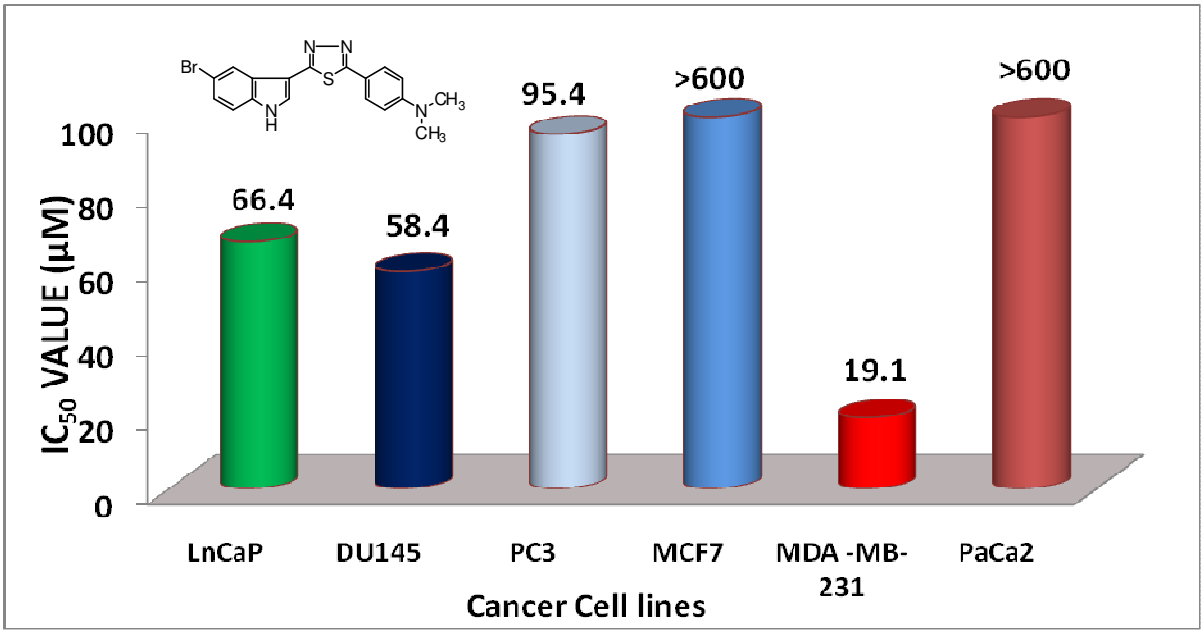


Figure 2.3.8 Anticancer activity profile of the most selective compound **65k**

The compound **65k** has shown the selective cytotoxicity towards breast cancer cell lines with an IC_{50} value 19.1 μM (figure 2.3.8).

2.3.4 Experimental Procedures

General: All the laboratory grade reagents were obtained commercially. The reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Column chromatography was performed using 100-200 mesh silica gel and appropriate mixture of hexane and ethyl acetate for elution. The solvents were evaporated using Buchi rotary evaporator. Melting points were determined with electrothermal capillary melting point apparatus (*E-Z* melting). ^{1}H NMR spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. Mass spectra were obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972.

2.3.4.1 Synthesis of 5-(3′-indolyl)-2-substituted-1,3,4-thiadiazoles (65a-m)

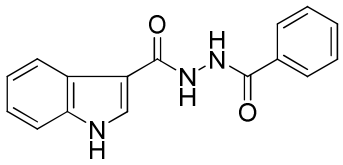
Indole-3-carboxylic acid (68)

To a stirred solution of indole/ 5-bromoindole (**66**, 38 mmol) in dimethylformamide (50 mL), trifluoroacetic anhydride (44 mmol) was added dropwise at 0 °C. After 3 h the reaction mixture was poured into water (200 mL) and the product **67** was isolated by filtration. The residue was washed with water (3 × 50 mL). Crude was suspended in 20% aqueous NaOH (200 mL) and heated at reflux 6 h. The mixture was cooled, washed with CH_2Cl_2 (2 × 100 mL) and acidified. The precipitate was isolated by filtration and dried to get pure indole-3-carboxylic acid **68**. (**68a**, yield 80%); mp 230-235 °C (Lit.[34] 229-234°C), similar method was applied to synthesize 5-bromoindole-3-carboxylic acid (**68b**, yield 78%), mp 221-224 °C (Lit.[34] 221-225).

Synthesis of 1,2-diacylhydrazines (69a-m)

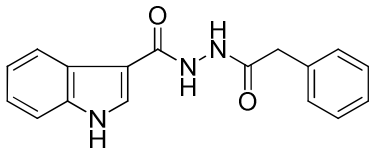
A mixture of indole-3-carboxylic acid (**68**, 1 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1 mmol) and 1-hydroxy-benzotriazole (1 mmol) in dry tetrahydrofuran (10 mL) was stirred at room temperature for 15 min. To this reaction mixture, appropriate aryl hydrazide (**80**, 1 mmol) was added and continued stirring at room temperature for 6 h. The reaction contents were concentrated at reduced pressure and the solid 1,2-diacylhydrazine **69** was filtered off, washed with water (20 mL), dried and used as such for the next step.

_N_-Benzoyl-3-indolylcarbohydrazide (69a)



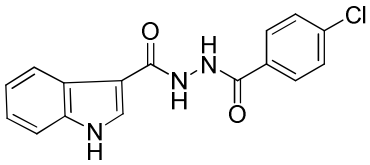
Yield 75%; White solid; mp 188-189 ^{o}C; IR (KBr, ν cm^{-1}): 3379, 3225, 1668, 1618, 1496, 1271, 784, 696. MS (ESI): *m/z* calcd. for $C_{16}H_{13}N_3O_2$: 279.10, found: 279.33 $(M)^+$.

_N_-(2′-Phenylacetyl)-3-indolylcarbohydrazide (69b)



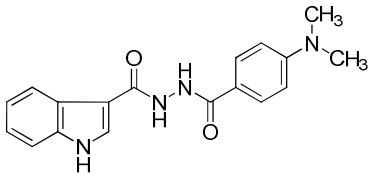
Yield 65%; White solid; mp 230-232 ^{o}C; IR (KBr, ν cm^{-1}): 3349, 3255, 1665, 1610, 1503, 1234, 774, 690; MS (ESI): Calcd. for $C_{17}H_{15}N_3O_2$: 293.1, found: 294.3 $(M+H)^+$.

_N_-(4′-Chlorobenzoyl)-3-indolylcarbohydrazide (69c)



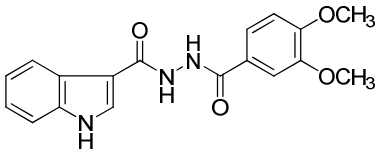
Yield 70%; Off-white solid; mp 229-232 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.67 (s, 1H), 10.44 (s, 1H), 9.94 (s, 1H), 8.13 (d, J = 6.6 Hz, 2H), 7.96 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 7.3 Hz, 1H), 7.21-7.10 (m, 2H); IR (KBr, ν cm^{-1}): 3349, 3255, 1665, 1610, 1503, 1234, 774, 690; MS (ESI): *m/z* calcd. for $C_{16}H_{12}ClN_3O_2$: 313.1, found: 312.2 $(M-H)^+$.

_N_-(4′-(Dimethylamino)benzoyl)-3-indolylcarbohydrazide (69d)



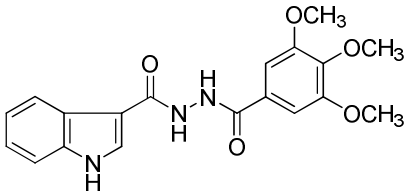
Yield 67%; Brown solid; mp 202-203 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.63 (s, 1H, NH), 9.97 (s, 1H, NH), 9.75 (s, 1H, NH), 8.14 (d, J = 5.1 Hz, 2H, Ar-H), 7.82 (d, J = 8.9 Hz, 2H, ArH), 7.46 (d, J = 8.0 Hz, 1H, Ar- H), 7.20 – 7.09 (m, 2H, Ar-H), 6.75 (d, J = 9.0 Hz, 2H, Ar-H), 3.00 (s, 6H, 2 × CH_3); IR (KBr, ν cm^{-1}): 3319, 3271, 1720, 1620, 1513, 1487, 1263, 794, 742; MS (ESI): *m/z* calcd. for $C_{18}H_{18}N_4O_2$: 322.2, found: 321.4 $(M-H)^+$.

***N*-(3′,4′-Dimethoxybenzoyl)-3-indolylcarbohydrazide (69e)**



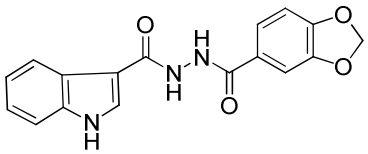
Yield 75%; Pale yellow solid; mp 140-143 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.65 (s, 1H, NH), 10.21 (s, 1H, NH), 9.85 (s, 1H, NH), 8.14 (d, J = 7.2 Hz, 2H, Ar-H), 7.61 – 7.53 (m, 2H, Ar-H), 7.47 (d, J = 7.5 Hz, 1H, Ar-H), 7.21 – 7.06 (m, 3H, Ar-H),3.84 (s, 6H, 2 × OCH_3); IR (KBr, ν cm^{-1}): 3375, 3211, 1630, 1604, 1531, 1454, 1244, 833, 744; MS (ESI): *m/z* calcd. for $C_{18}H_{17}N_3O_4$: 339.2, found: 338.3 $(M-H)^+$.

***N*-(3′,4′,5′-Trimethoxybenzoyl)-3-indolylcarbohydrazide (69f)**



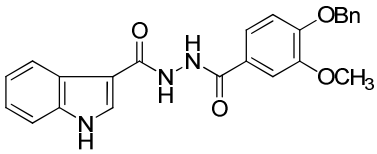
Yield 65%; White solid; mp 196-198 ^{o}C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.66 (s, 1H, NH), 10.30 (s, 1H, NH), 9.90 (s, 1H, NH), 8.14 (d, J = 6.8 Hz, 2H, Ar-H), 7.47 (d, J = 7.7 Hz, 1H, Ar-H), 7.29 (s, 2H, Ar-H), 7.21-7.10 (m, 2H, Ar-H), 3.86 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3); IR (KBr, ν cm^{-1}): 3479, 3217, 1680, 1614, 1504, 1440, 1271, 835, 750; MS (ESI): *m/z* calcd. for $C_{19}H_{19}N_3O_5$: 369.23, found: 368.4 $(M-H)^+$.

***N*-(3′,4′-Methylenedioxybenzoyl)-3-indolylcarbohydrazide (69g)**



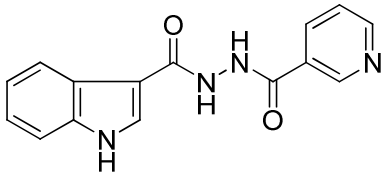
Yield 70%; Dark brown solid; mp 190-192 ^{o}C; IR (KBr, ν cm^{-1}): 3207, 3178, 1660, 1554, 1487, 1263, 841, 740; MS (ESI): *m/z* calcd. for $C_{17}H_{13}N_3O_4$: 323.09, found: 322.2 $(M-H)^+$.

***N*-(4′-(Benzyloxy)-3′-methoxybenzoyl)-3-indolylcarbohydrazide (69h)**



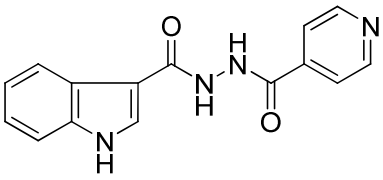
Yield 65%; Off-white solid; mp 196-198 ^{o}C; IR (KBr, ν cm^{-1}): 3379, 3196, 1668, 1618, 1496, 1431, 1271, 742; MS (ESI): *m/z* calcd. for $C_{24}H_{21}N_3O_4$: 415.15, found: 415.2 $(M)^+$.

***N*-(Nicotinyl)-3-indolylcarbohydrazide (69i)**



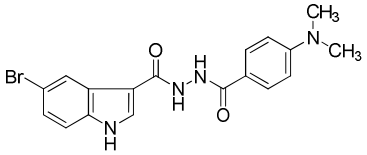
Yield 60%; Off-white solid; mp 167-168 ^{o}C; IR (KBr, ν cm^{-1}): 3352, 3196, 1651, 1556, 1496, 1424, 1269, 748; MS (ESI): *m/z* calcd. for $C_{15}H_{12}N_4O_2$: 280.09, found: 280.0 $(M)^+$.

_N_-(Isonicotinyl)-3-indolylcarbohydrazide (69j)



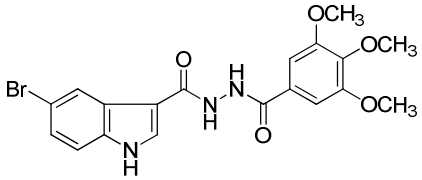
Yield 70%; Pale yellow solid; mp 190-192 °C; IR (KBr, ν cm^{-1}): 3332, 3256, 1672, 1610, 1584, 1465, 1414, 1243, 740; MS (ESI): *m/z* calcd. for $C_{15}H_{12}N_4O_2$: 280.9, found: 281.1 $(M+H)^+$.

5-Bromo-_N_-(4′-(dimethylamino)benzoyl)-3-indolylcarbohydrazide (69k)



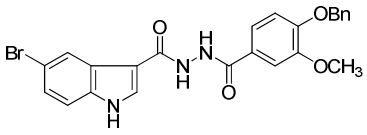
Yield 76%; Brown solid; mp 167-168 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.83 (s, 1H, NH), 9.99 (s, 1H, NH), 9.85 (s, 1H, NH), 8.29 (d, *J* = 1.9 Hz, 1H, Ar- H), 8.18 (d, *J* = 2.6 Hz,1H, Ar-H), 7.81 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.46 (dd, *J* = 7.5, 4.1 Hz, 1H, Ar-H), 7.30 (dd, *J* = 8.6, 2.0 Hz, 1H, Ar-H), 6.75 (d, *J* = 9.0 Hz, 2H, Ar-H), 3.00 (s, 6H, 2 × N-CH_3); IR (KBr, ν cm^{-1}): 3345, 3215, 1707, 1681, 1636, 1519, 1446, 1206, 773, 761. MS (ESI): *m/z* calcd. for$C_{18}H_{17}BrN_4O_2$: 400.1, found: 400.1 $(M)^+$ and 402.1 $(M+2)^+$.

5-Bromo-_N_-(3′,4′,5′-trimethoxybenzoyl)-3-indolylcarbohydrazide (69l)



Yield 75%; White solid; mp 138-142 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.87 (s, 1H, NH), 10.33 (s, 1H, NH), 10.01 (s, 1H, NH), 8.28 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.92 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.64 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.5 Hz, 2H, Ar-H), 3.86 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3); IR (KBr, ν cm^{-1}): 3323, 3197, 1683, 1662, 1534, 1484, 1226, 734, 690; MS (ESI): *m/z* calcd. for $C_{19}H_{18}BrN_3O_5$: 446.2, found: 446.2 $(M-H)^+$.

***N*-(4′-(Benzyloxy)-3′-methoxybenzoyl)-5-bromo-3-indolylcarbohydrazide (69m)**



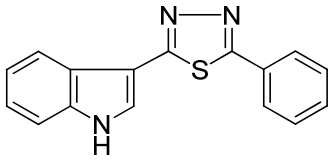
Yield: 80%; Off-white solid; mp 170-172 °C; IR (KBr, ν cm^{-1}): 3292, 3174, 1690, 1610, 1556, 1434, 1269, 1147, 796, 742; MS (ESI): *m/z* calcd. for $C_{24}H_{20}BrN_3O_4$: 493.3, found: 494.4 $(M+H)^+$.

General procedure for the synthesis of indolyl-1,3,4-thiadiazole (65a-m)

A mixture of 1,2-diacylhydrazines (**69**, 1 mmol) and Lawesson's reagent (0.44 g, 1.1 mmol) in tetrahydrofuran (10 mL) was refluxed at 80 °C for 5 h. After completion of the reaction as monitored by TLC, the crude product was adsorbed over silica-gel and

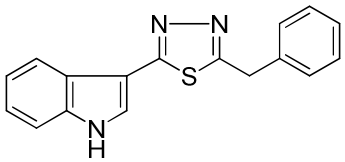
purified by column chromatography using ethyl acetate: hexane (7:3; v/v) as eluent to afford pure product **65**.

5-(3′-Indolyl)-2-phenyl-1,3,4-thiadiazole (65a)



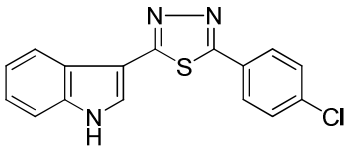
Yield 65%; mp 187-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, NH), 8.14-8.10 (m, 1H, Ar-H), 7.94 (d, 1H, *J* = 2.92 Hz, Ar-H), 7.49-7.47 (m, 2H, Ar-H), 7.40-7.36 (m, 3H, Ar-H), 7.33-7.27 (m, 1H, Ar-H), 7.24-7.20 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 3271, 1624, 1604, 1552, 1454, 1126, 736, 684; MS (ESI): *m/z* calcd. for $C_{16}H_{11}N_3S$: 277.1, found: 278.2 $(M+H)^+$.

2-Benzyl-5-(3′-indolyl)-1,3,4-thiadiazole (65b)



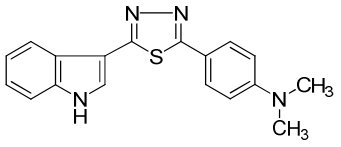
Yield 65%; mp 189-192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (s, 1H, NH), 8.18-8.15 (m, 1H, Ar- H), 7.86-7.81 (m, 2H, Ar-H), 7.49-7.46 (m, 1H, Ar-H), 7.39-7.33 (m, 3H, Ar-H), 7.31-7.27 (m, 1H, Ar-H), 7.25-7.17 (m, 2H, Ar-H). 4.44 (s, 2H, CH_2); IR (KBr, ν cm^{-1}): 3190, 2981, 1624, 1604, 1556, 1454, 1159, 794, 744; MS (ESI): *m/z* calcd. for $C_{17}H_{13}N_3S$: 291.03, found: 292.3 $(M+H)^+$.

2-(4′-Chlorophenyl)-5-(3′-indolyl)-1,3,4-thiadiazole (65c)



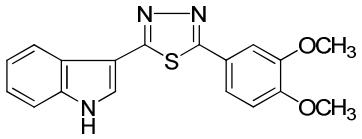
Yield 75%; mp 231-234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H, NH), 8.32-8.22 (m, 2H,. Ar-H), 8.14 (d, 1H, *J* = 8.48 Hz, Ar-H), 8.03 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.72-7.64 (m, 2H, Ar-H), 7.55 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.30-7.27 (m, 2H, Ar-H); IR (KBr, ν cm^{-1}): 3273, 1602, 1548, 1454, 1246, 1124, 774, 738; MS (ESI): *m/z* calcd. for $C_{16}H_{10}ClN_3S$: 311.0, found: 312.0 $(M+H)^+$.

5-(3′-Indolyl)-2-(4′-N,N'-dimethylaminophenyl) 1,3,4-thiadiazole (65d)



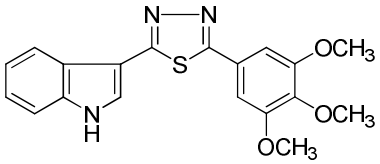
Yield 72%; mp 195-199 °C; ^{1}H NMR (400 MHz, DMSO-d_6,): δ 11.89 (s, 1H, NH), 8.23-8.20 (m, 1H, Ar-H), 7.99 (d, 2H, *J* = 8.8 Hz), 7.62-7.49 (m, 3H, Ar-H), 7.03-6.97(m, 2H, Ar-H), 6.87-6.81 (m, 1H), 2.98 (s, 6H, NCH_3); IR (KBr, ν cm^{-1}): 3270, 1606, 1556, 1427, 1294, 1193, 800, 738; MS (ESI): *m/z* calcd. for $C_{18}H_{16}N_4S$: 320.1, found: 321.34 $(M+H)^+$.

2-(3′,4′-Dimethoxyphenyl)-5-(3′-indolyl)-1,3,4-thiadiazole (65e)



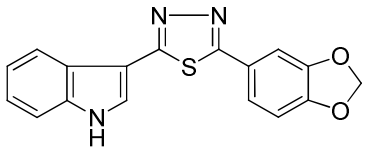
Yield 72%; mp 173-177 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, NH), 8.32-8.17 (m, 2H, Ar-H), 7.72-7.07 (m, 6H, Ar-H), 3.90 (s, 6H, OCH_3); IR (KBr, ν cm^{-1}): 3198, 1602, 1548, 1433, 1244, 1134, 800, 736; MS (ESI): *m/z* calcd. for $C_{18}H_{15}N_3O_2S$: 337.08, found: 338.20 $(M+H)^+$.

2-(3′-Indolyl)-5-(3′,4′,5′-trimethoxyphenyl)-1,3,4-thiadiazole (65f)



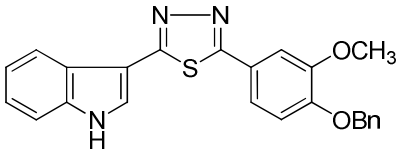
Yield 75%; mp 220-221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11 (s, 1H, NH), 8.35-8.21 (m, 2H, Ar-H), 8.14 (s, 1H, Ar-H), 7.71-6.97 (m, 4H, Ar-H), 3.90 (s, 6H, OCH_3), 3.76 (s, 3H, OCH_3). IR (KBr, ν cm^{-1}): 3230, 1600, 1546, 1496, 1244, 1136, 833, 732; MS (ESI): *m/z* calcd. for $C_{19}H_{17}N_3O_3S$: 367.1, found: 368.3 $(M+H)^+$.

2-(3′,4′-Methylenedioxyphenyl)-5-(3′-indolyl)-1,3,4-thiadiazole (65g)



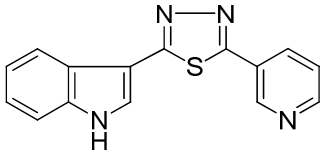
Yield 66%; mp 218-220. ^{1} H NMR (400 MHz, DMSO-d_6): δ 11.66 (s, 1H, NH), 8.24 (d, 2H, *J* = 8.32 Hz, Ar-H), 7.94-7.89 (m, 3H, Ar-H), 7.44(d, 2H, *J* = 9.32 Hz, Ar-H), 6.95 (d, 1H, *J* = 8.08 Hz, Ar-H), 6.10 (s, 2H, CH_2), IR (KBr, ν cm^{-1}): 3273, 2902, 1610, 1556, 1504, 1261, 1124, 802, 746; MS (ESI): *m/z* calcd. for $C_{17}H_{11}N_3O_2S$: 321.05, found: 322.18 $(M+H)^+$.

2-(4′-(Benzyloxy)-3′-methoxyphenyl)-5-(3′-indolyl)-1,3,4-thiadiazole (65h)



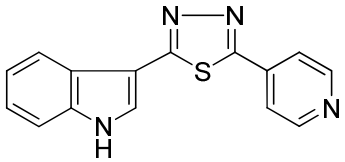
Yield 55%; mp 240-245 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H, NH), 8.30 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.22-8.19 (m, 1H, Ar-H), 7.73-7.62 (m, 1H, Ar-H), 7.50-7.46 (m, 4H, Ar-H), 7.43-7.32(m, 3H, Ar-H), 7.29-7.20 (m, 3H, Ar-H), 5.18 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3); IR (KBr, ν cm^{-1}): 3172, 2931, 1600, 1550, 1508, 1269, 1176, 752, 698; MS (ESI): *m/z* calcd. for $C_{24}H_{19}N_3O_2S$: 413.11, found: 415.20 $(M+2)^+$.

2-(3′-Indolyl)-5-(3′-pyridyl)-1,3,4-thiadiazole (65i)



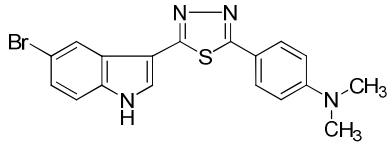
Yield 64%; mp 221-224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.01 (s, 1H, NH), 8.56 (d, 2H, *J* = 8.0 Hz, Ar- H), 8.26 (d, 1H, *J* = 3.6 Hz, Ar-H), 8.21 (s, 1H, Ar- H), 7.94 (d, 2H, *J* = 5.6 Hz, Ar-H), 7.54-7.47 (m, 3H, Ar-H); IR (KBr, ν cm^{-1}):3192, 2985, 1658, 1625, 1546, 1454, 1226, 750, 696; MS (ESI): *m/z* calcd. for $C_{15}H_{10}N_4S$: 278.06, found: 279.09 $(M+H)^+$.

2-(3′-Indolyl)-5-(4′-pyridyl)-1,3,4-thiadiazole (65j)



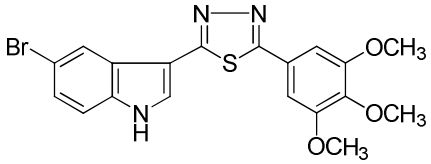
Yield 65%; mp 260-263 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, NH), 8.77 (d, 2H, *J* = 5.6 Hz, Ar-H), 8.32 (d, 1H, *J* = 1.2 Hz, Ar-H), 8.23 (t, 1H, *J* = 8.8 Hz, Ar-H), 7.94 (d, 2H, *J* = 5.6 Hz, Ar-H), 7.54 (t, 1H, *J* = 8.8 Hz, Ar-H), 7.28-7.26 (m, 2H, Ar-H); IR (KBr, ν cm^{-1}): 3138, 1685, 1597, 1460, 1242, 1139, 744, 698; MS (ESI): *m/z* calcd. for $C_{15}H_{10}N_4S$: 278.06, found: 279.10 $(M+H)^+$.

5-(5′-Bromo-3′-indolyl)-2-(4′-*N,N*-dimethylaminophenyl)-1,3,4-thiadiazole (65k)



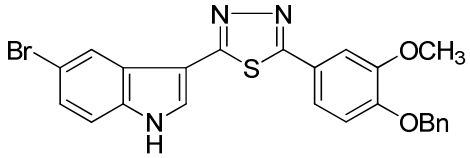
Yield 60%; mp 217-219 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.92 (s, 1H, NH), 8.38 (d, 2H, *J* = 8.12 Hz, Ar-H), 8.24 (d, 2H, *J* = 8.2 Hz,A r-H), 7.50 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.42-7.22 (m, 2H, Ar-H), 7.18 (s, 1H, Ar-H), 3.08 (s, 6H, CH_3); IR (KBr, ν cm^{-1}): 3311, 1606, 1537, 1462, 1234, 1130, 717, 659; MS (ESI): *m/z* calcd. for $C_{18}H_{15}BrN_4S$: 398.02, found: 398.1 $(M)^+$ and 400.1 $(M+2)^+$.

2-(5′-Bromo-3′-indolyl)-5-(3′,4′,5′-trimethoxyphenyl)-1,3,4-thiadiazole (65l)



Yield 68%; mp 291-294 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H, NH), 8.39 (d, 1H, *J* = 2 Hz, Ar-H), 8.29 (d, 1H, *J* = 3.2 Hz, Ar-H), 7.50 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.40-7.38 (m, 2H, Ar-H), 7.26 (s, 1H, Ar-H), 3.92 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3); IR (KBr, ν cm^{-1}): 3172, 1600, 1550, 1469, 1236, 1130, 788, 650; MS (ESI): *m/z* calcd. for $C_{19}H_{16}BrN_3O_3S$: 445.0, found: 446. 2 $(M+H)^+$.

2-(4′-(Benzyloxy)-3′-methoxyphenyl)-5-(5′-bromo-3′-indolyl)-1,3,4-thiadiazole (65m)



Yield 75%; mp 252-254 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.14 (s, 1H, NH), 8.40 (d, 1H, *J* = 1.6 Hz, Ar-H), 8.26 (d, 1H, *J* = 2.8 Hz, Ar-H), 7.50 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.50-7.46 (m, 4H, Ar-H), 7.42-7.22 (m, 4H, Ar-H), 7.20 (s, 1H, Ar-H), 5.18 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3); IR (KBr, ν cm^{-1}): 3197, 2902, 1600, 1543, 1467, 1274, 1141, 736, 692; MS (ESI): *m/z* calcd. for $C_{24}H_{18}BrN_3O_2S$: 491.03, found: 491. 03, $(M)^+$ and 493.49 $(M+2)^+$.

2.3.4.2 MTT assay

Six human cancer cell lines (LnCap, DU145, PC3, MCF-7, MDA-MB-231, and PaCa2) were cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 4×10^3 cells per well for 12 h. Cells were incubated with various concentrations of the compounds ranging from 10 nM to 1 mM. After 48 h, MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetra-zoliumbromide) was added to the final concentration of 0.2 mg/mL and incubated for 30 min. The cells were washed twice with PBS and lysed in 100 µL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.

2.4 Part C: Novel indolyl-1,2,4-triazoles as anticancer agents

2.4.1 1,2,4-Triazoles in drug discovery

Nitrogen containing five-membered heterocycles play a vital role in drug discovery to identify novel chemical entities as potential therapeutic agents. Triazoles are the most privileged structures which are widely explored for their broad range of pharmacological properties. Amitrole (3-amino-1,2,4-triazole) (**71a**) is used as a herbicide and also to defoliate cotton plants before mechanical harvesting (figure 2.4.1).

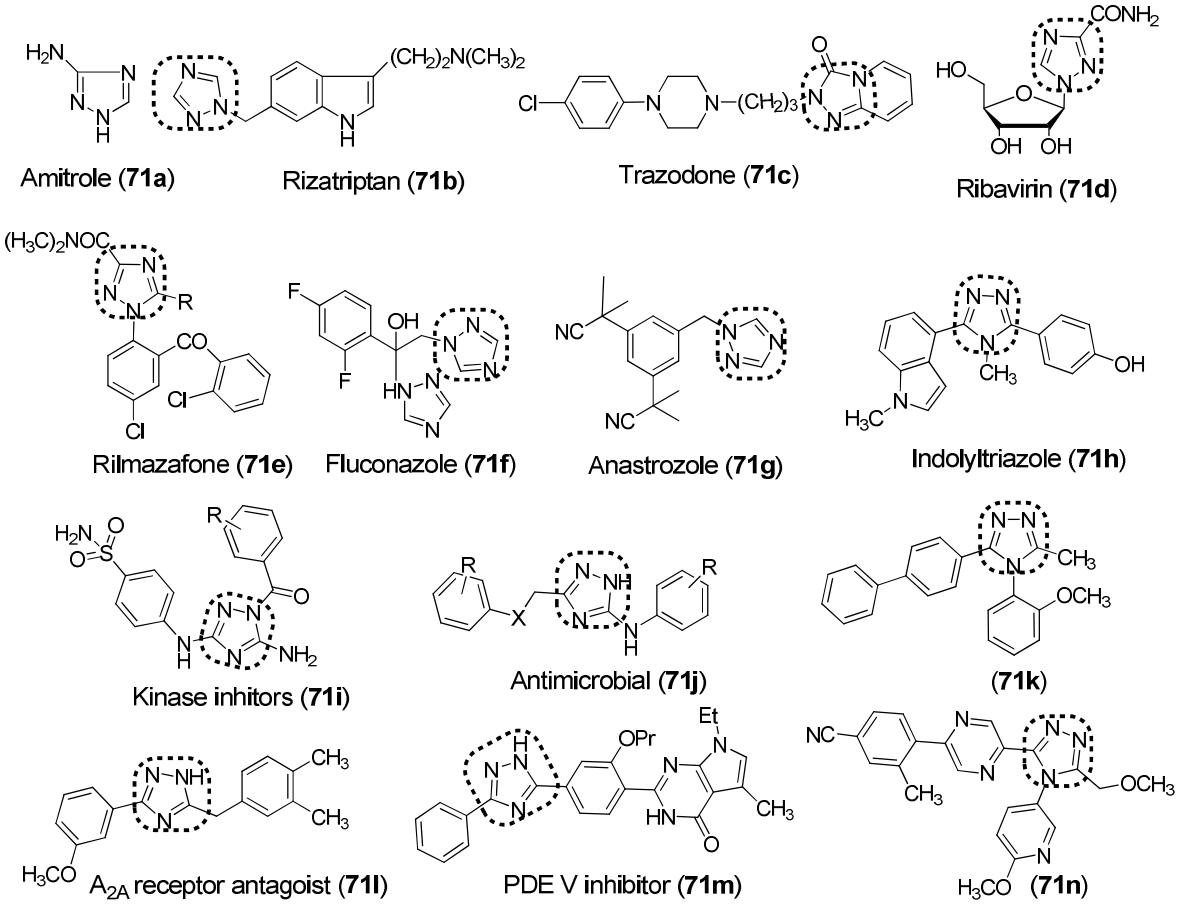


Figure 2.4.1 1,2,4-Triazoles (**71a-n**) as potential therapeutic agents

The 1,2,4-Triazole derivatives have therapeutic importance which include the drug candidates Rizatriptan (**71b**) (antimigrane), Trazodone (**71c**) (antidepressant), Ribavirin (**71d**) (antiviral), Rilmazafone (**71e**) (sedative and hypnotic). Due to immense biological significance of 1,2,4-triazole scaffold, the researchers paid attention to develop novel chemical entities framing triazole as key pharmacophore, thus the outcome of recent medicinal chemistry research is the marketed antifungal drugs Fluconazole (**71f**) and Itraconazole, Ravuconazole, Voriconazole, ICI 153066 and Posaconazole. The potent

antiestrogens inhibitor, Anastrozole (**71g**) consists 1,2,4-triazoles as an active pharmacophore. The drugs Vorozole and Letrozole used for the treatment of breast cancer after surgery.[85] It is known that 1,2,4-triazole moieties interact strongly with heme iron, and aromatic substituents on the triazoles are very effective for interacting with the active site of aromatase.[86] Apart from the marketed drugs, recent academic research has produced vast number of lead molecules. Indole containing 1,2,4-triazole **71h** acts as selective inhibitor of 11β-hydroxysteroid dehydrogenase type 1 and compound **71i** reported as kinase inhibitor.[87] 3-Arylamino-5-aryloxymethyl[1,2,4]triazoles (**71j**) known for their antimicrobial activity[88] and diaryl-1,2,4-triazoles **71k** are selective antagonists for the human V_{1A} receptor.[89] 1,2,4-Triazole derivatives **71l-n** were investigated as A_{2A} receptor antagonists,[90] phosphodiesterase V inhibitors and oxytocin antagonists respectively.[91] 1,2,4-Triazole scaffolds play a vital role in anticancer research. A series of pyridinyl-1,2,4-triazoles (**72a**) have been discovered as tubulin polymerization inhibitors and binds to the colchicine site of tubulin and cause G2/M arrest in tumor cells.[92] 1,2,4-Triazoles with 3,4,5-trimethoxyphenyl ring (**72b-d**) inhibit tubulin polymerization and interact with colchicine binding site. 1,2,4-Triazole **72b** has an IC_{50} value 29.6 nM (HeLa cancer cell lines) and indolyltriazoles **72c** and **72d** exhibited potent cytotoxicity (21 and 32 nM, respectively) against positive multi-drug resistance cancer cell line KB-V (figure 2.4.2).[93]

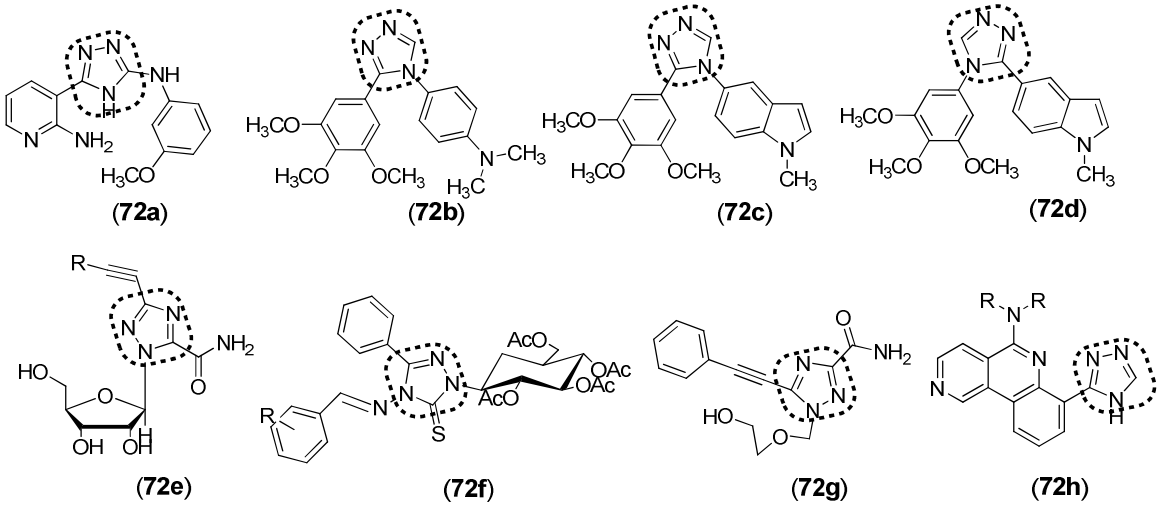


Figure 2.4.2 1,2,4-Triazoles (**72a-h**) as anticancer agents

3-Arylethynyltriazolyl ribonucleosides (**72e**) were evaluated for their anticancer activity on the drug-resistant pancreatic cancer cell line MiaPaCa-2 and found to exhibit more potent antiproliferative effects than the marketed drug Gemcitabine.[94]

D-Glucopyranosyl-1,2,4-triazole-3-thiones (**72f**) have shown cytotoxic activity against human MCF-7 and Bel-7402 malignant cell lines.[95] Novel arylethynyltriazole acyclonucleosides (**72g**) were synthesized and assessed for their anticancer activity on drug-resistant pancreatic cancer cells.[96] 7-(4*H*-1,2,4-Triazol-3-yl) benzo[*c*] [2,6]naphthyridines (**72h**) had potent antiproliferative effects on the AML cell line MV-4-11 (IC_{50} <30 nM).

2.4.2 Rational approach

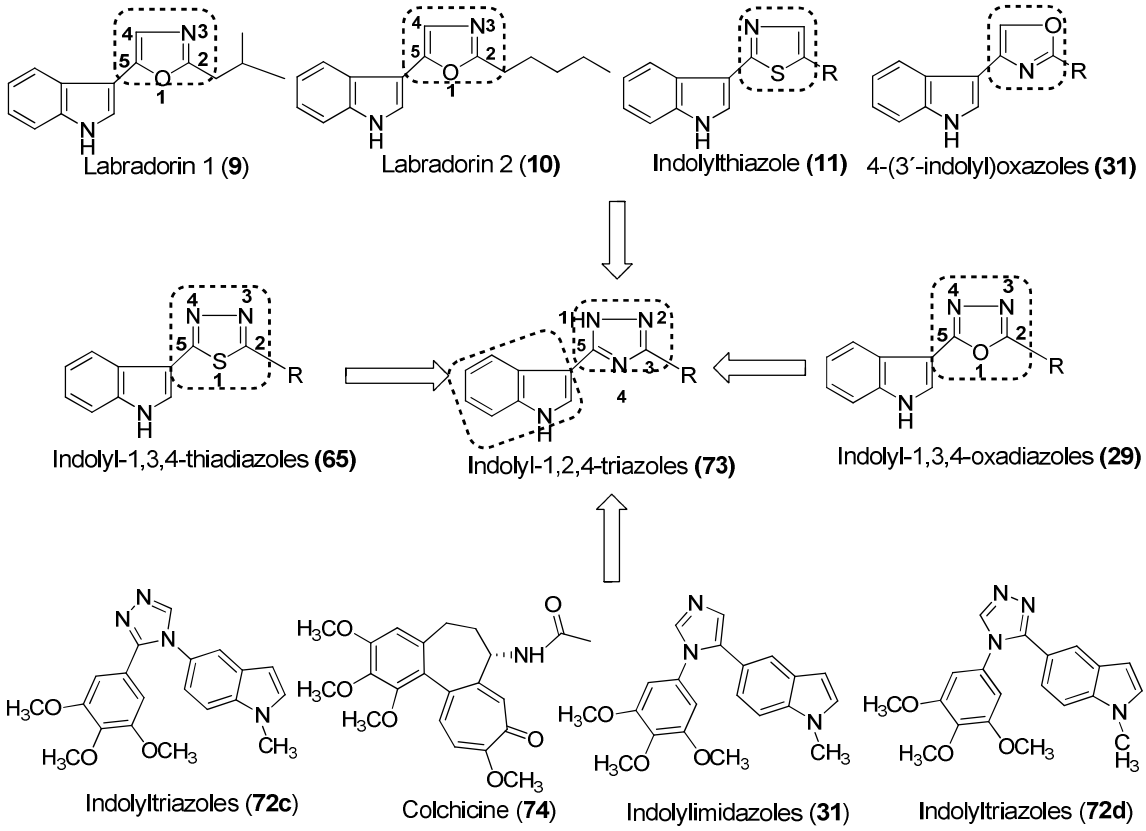


Figure 2.4.3 Rational approach for the synthesis of indolyl-1,2,4-triazoles (**73**)

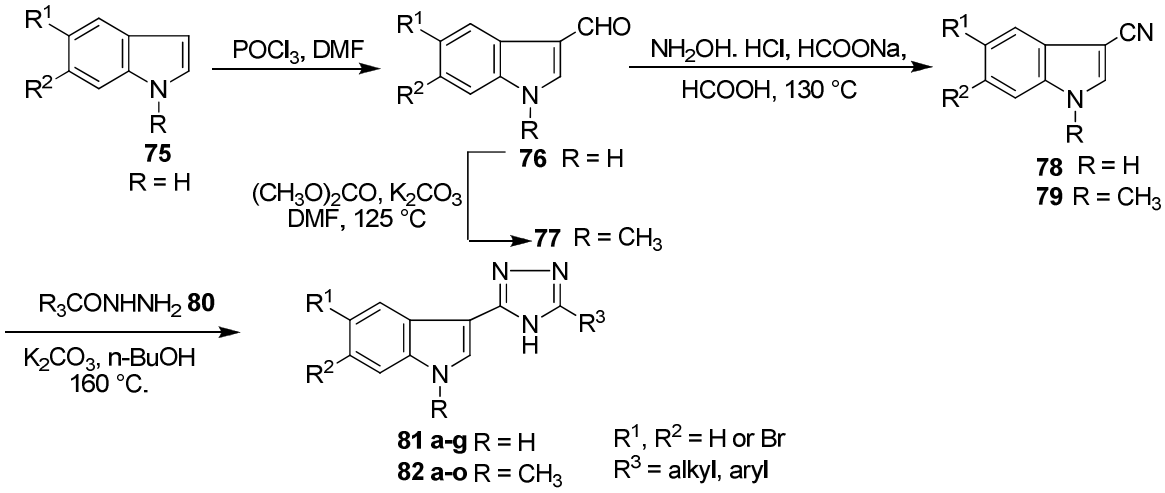
The synthesis of novel indolyl-1,2,4-triazole follows the rational approach, based on the various biological applications of indolylazoles (figure 2.4.3). The rational includes the remarkable anticancer activities of naturally occurring indolyloxazoles Labradorins 1 & 2 (**9** & **10**) and their synthetic analogues indolylthiazoles (**11**). In continuation our efforts to improve the anticancer activity of our previously reported indolylazoles; 4-(3′-indolyl)oxazoles (**53**), 1,3,4-oxadiazoles (**29**) and indolyl-1,3,4-thiadiazoles (**65**) we have made structural modification in the five-membered heterocyclic ring of indolylazoles to

prepare novel indolyl-1,2,4-triazoles. Further, indole being a privileged scaffold has shown important role as anticancer agents via inhibition of tubulin polymerization and the combination of indole ring and 1,2,4-triazole in a single molecule may result potent anticancer lead molecules.

2.4.3 Results and discussion

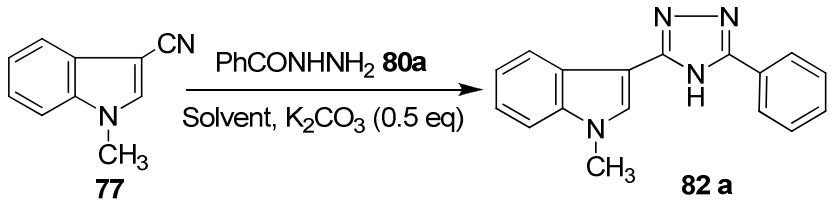
2.4.3.1 Chemistry

The general synthesis of 1,2,4-triazole involves the condensation of hydrazides with nitriles or thioamides at elevated temperatures.[92] Recently, *N*-substituted triazoles have been prepared in good yields using one-pot reaction of arylamines, *N,N*-dimethylformamide dimethyl acetal and acylhydrazide in acetonitrile.[97] A solid-phase synthesis of 3-alkylamino-1,2,4-triazoles involves the initial preparation of immobilized *N*-acyl-benzotriazoles followed by reaction with hydrazine.[98] Another efficient route uses the reaction of nitriles with sodium methoxide to generate methyl imidate ester which upon treatment with aryl hydrazides at high temperature produced 1,2,4-triazoles in good yields.[70, 99-101] Recently, Al-masoudi *et al.* have reviewed most of the synthetic routes adopted for the efficient preparation of 1,2,4-triazoles.[102] In view of broad spectrum applications of 1,2,4-traizoles in medicinal chemistry a novel series of 5-(3′-indolyl)-3-substituted-1,2,4-triazoles (**81a-g**) and (**82a-o**) were prepared as described in the (scheme 2.4.1). The indole-3-carboxaldedyde (**76**) and *N*-methylindole-3-carboxaldehyde (**77**) were prepared according to the literature procedures.[103-104] Indole-3-carbonitriles (**78** and **79**) were synthesized in good yields from the corresponding indole-3-carboxaldehydes (**76** and **77**) by reacting with hydroxylamine hydrochloride in presence of sodium formate and formic acid at reflux temperature. Reaction of indole-3-cabrbonitriles (**78** or **79**, 2 mmol) with appropriate acid hydrazides (**80**, 1 mmol) in presence of potassium carbonate (0.5 mmol) in *n*-butanol afforded the desired product indolyl-1,2,4-triazole **81** and **82** in good yields.



Scheme 2.4.1 Synthesis of 5-(3′-indolyl)-3-substituted-1,2,4-triazoles (**81a-g**) and (**82a-o**)

A model reaction was carried out using *N*-methyl-indole-3-carbonitrile (**77**) and phenyl hydrazide using different solvents in presence of potassium carbonate (scheme 2.4.2).



Scheme 2.4.2 Synthesis of 3-phenyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (**82a**)

Table 2.4.1 Synthesis of indoly-1,2,4-triazoles (**82a**) in various solvents

Solvent	Temp (°C)	Time (h)	Yield (%)
Ethanol	80	48	10
Isopropanol	90	24	15
N,N-Dimethylformamide	130	24	5
Chlorobenzene	130	24	Nil
Polyethylene glycol	150	15	10
n-Butanol	80	15	30
n-Butanol	120	10	45
***n*-Butanol**	**160**	**8**	**65**

Key step was attempted in polar solvents, such as ethanol, *iso*-propanol, *N*,*N*-dimethylformamide and polyethylene glycol at high temperatures which resulted in poor yields. Finally, reaction of 3-cyanoindole (**77**) and phenylhydrazide (**80a**) in *n*-butanol at 160 °C afforded the desired product (**82a**) in good yield (table 2.4.1).

Following these reaction conditions a series of novel indolyl-1,2,4-triazoles were prepared. All the synthesized 5-(3′-indolyl)-3-substituted-1,2,4-triazoles were characterized by using NMR and Mass spectral data. ^{1}H NMR & ^{13}C NMR spectra of compound **82m** are provided in figures 2.4.4. and 2.4.5.

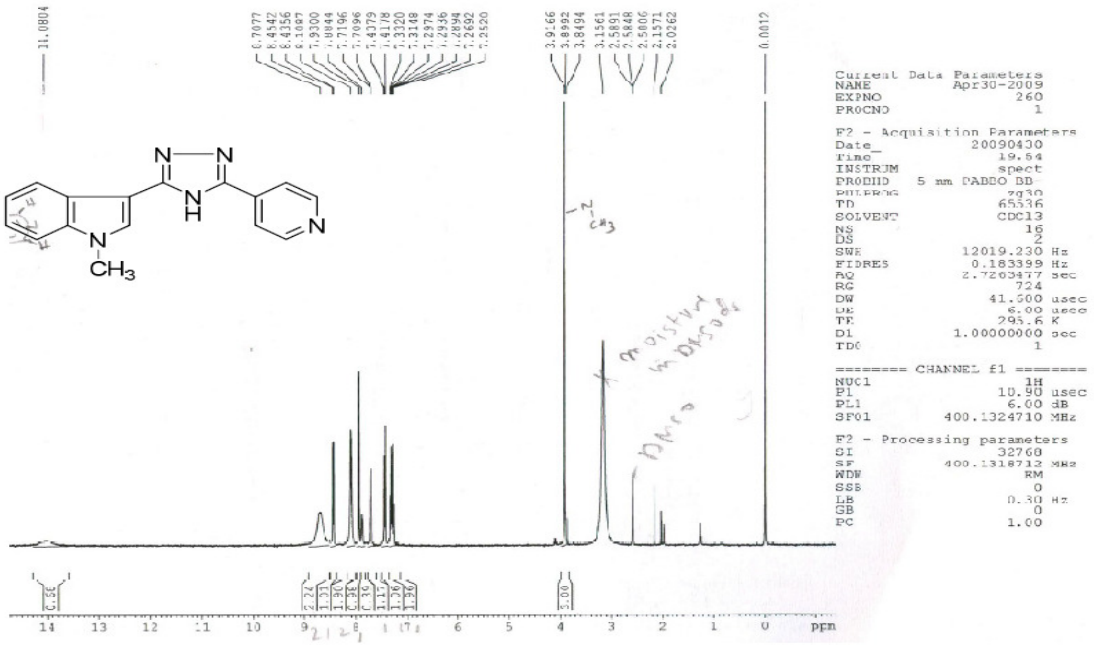


Figure 2.4.4 ^{1}H NMR spectrum of **82m**

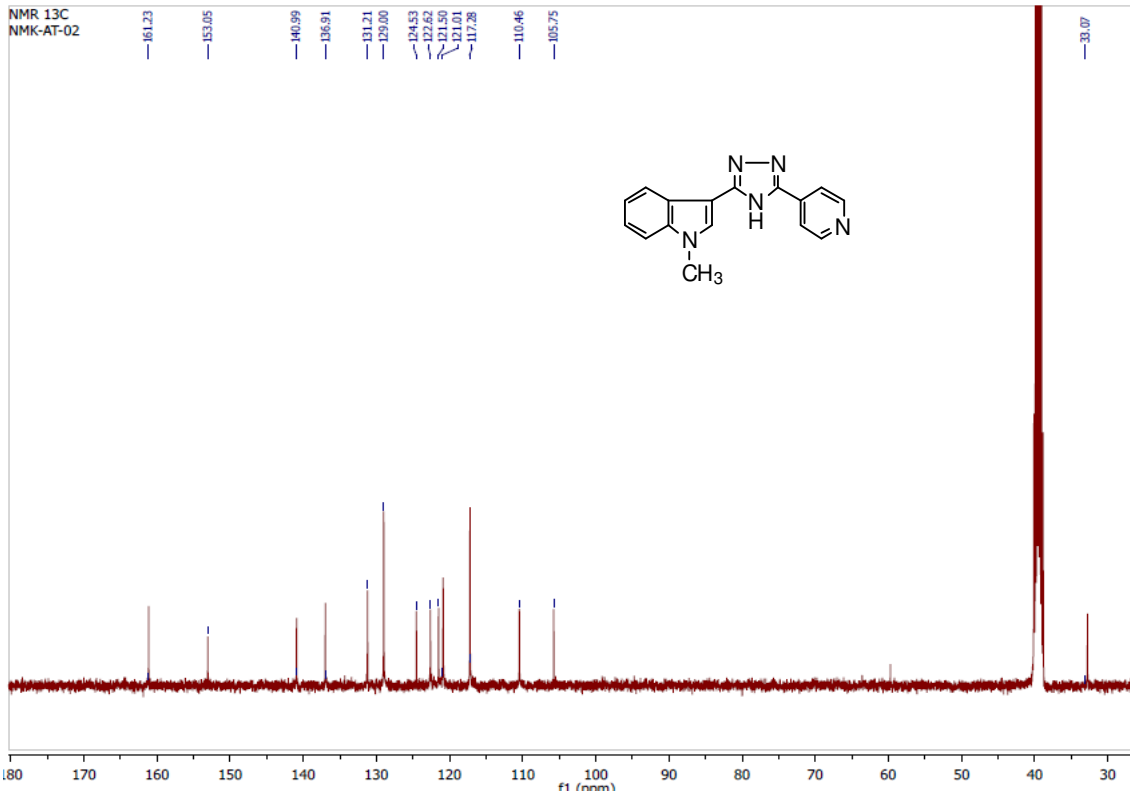
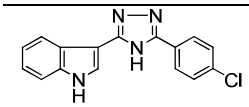
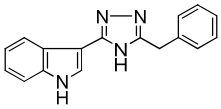
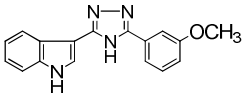
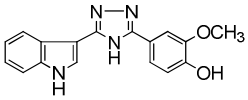
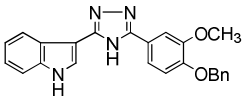
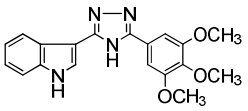
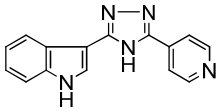
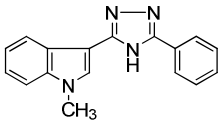
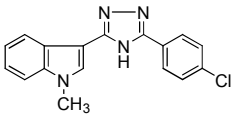
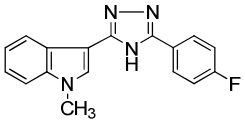
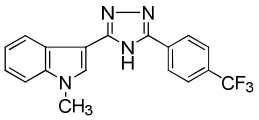
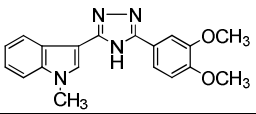


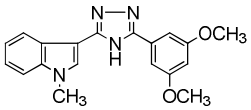
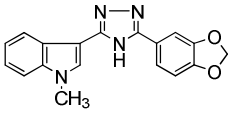
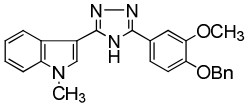
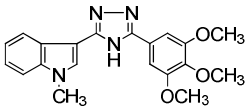
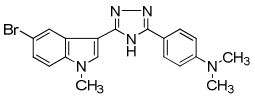
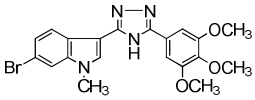
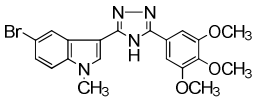
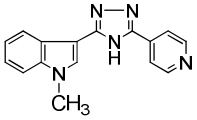
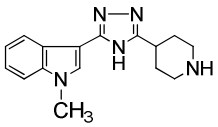
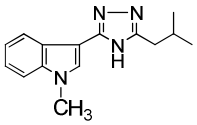
Figure 2.4.5 ^{13}C NMR spectrum of **82m**

2.4.3.2 Anticancer activity

Synthesized 5-(3′-indolyl)-3-substituted-1,2,4-triazoles (**81a-g**) and (**82a-o**) were screened against prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreatic (PaCa2) cancer cell lines (table 2.4.2). Some of the compounds have shown significant anticancer activity with IC_{50} values ranging from 1 µM to 1mM concentration. The compound **81a** with 4-chlorophenyl was inactive up to IC_{50} value of 581 µM. Compounds **81b** and **81c** with benzyl and methoxyphenyl groups at C-3 position of 1,2,4-triazole showed selective cytotoxicity against DU145 (IC_{50} 57 µM) and PC3 (IC_{50} 58 µM) cell lines, respectively. Introduction of 4-hydroxy group in C-3 phenyl ring of **81c** led to 4-hydroxy-3-methoxyphenyl analog **81d** with improved activity. Further introduction of a bulkier 4-benzyloxy-3-methoxyphenyl led to analog **81e** that was highly potent with IC_{50} values 8.5 µM (LnCaP), 8.9 µM (DU145) and 7.1 µM (MCF-7) which suggests that a large group is tolerable in the C-3 phenyl ring. The 3,4,5-trimethoxyphenyl analog **81f** was moderately selective cytotoxic against PaCa2 cell line (IC_{50} = 83.3 µM). Replacement of the C-3 aryl moiety with a heteroaryl ring led to an inactive compound **81g** up to 142.4 µM (MCF-7).

Table 2.4.2 Anticancer activity of indolyl-1,2,4-triazoles (**81a-g**) and (**82a-o**)

Indolyl-1,2,4-triazoles		IC_{50} (μM)[a]					
		LnCaP	DU145	PC3	MCF-7	MDA-MB-231	PaCa2
	**(81a)**	> 10^3	670.6	> 10^3	903.2	> 10^3	581.7
	**(81b)**	354.8	57	> 10^3	182.9	789.3	282.6
	**(81c)**	154.2	231.8	58	181.8	169.8	183.3
	**(81d)**	27.7	30.6	252.5	32.6	135.8	39.6
	**(81e)**	**8.5**	**8.9**	41.8	**7.1**	22.5	13.3
	**(81f)**	558.8	145	901.4	319.7	484.2	83.3
	**(81g)**	> 10^3	449.1	389.9	142.4	> 10^3	> 10^3
	**(82a)**	150.4	191.1	331.3	64.3	282.3	231.5
	**(82b)**	231	155.8	145.8	204.3	178.6	405.5
	**(82c)**	72	128	**4**[b]	41.7	25.7	90.5
	**(82d)**	188	> 10^3	> 10^3	539	631.8	> 10^3
	**(82e)**	12.2	**5.8**	46.4	**8.1**	16.4	14.1

	**(82f)**	133.8	163.8	95.5	64	28.2	713.5
	**(82g)**	22.7	23.8	43.5	**4.8**	16.1	22.1
	**(82h)**	30.4	23.5	**4.2**	20.1	19.6	41.5
	**(82i)**	**6.9**	**10.2**	**2.3**	**1.4**	**4.7**	**0.8**
	**(82j)**	75.6	55.2	91	125.8	575.5	286
	**(82k)**	**6.3**	16	**8.6**	**10**	13.7	20.5
	**(82l)**	101.4	51.8	14.1	16	20.5	153
	**(82m)**	204.2	725.6	91.5	350	226	$> 10^3$
	**(82n)**	**6.9**	**6**	**3.2**	**1.6**	**2.6**	**3.2**
	**(82o)**	188.2	198.5	149	$> 10^3$	76.5	$> 10^3$

[a]Values are reported as IC_{50} in micromolar concentration of the compound required to effect 50% inhibition of the net tumor cell growth. GraphPad Prism 5.0, a curve fitting programme was used to plot dose response curve by nonlinear regression analysis to calculate IC_{50} values. [b]The IC_{50} values less than 10 μM are indicated in bold font

We have synthesized a series of indolyl-1,2,4-triazoles (**82**) bearing *N*-methylindole moiety. Compounds **82a** and **82b** with phenyl and 4-chlorophenyl substituents were moderately active. The 4-fluorophenyl derivative **82c** displayed selective cytotoxicity

against PC3 cell line with IC_{50} 4.0 μM. Compound **82d** having stronger electron-withdrawing group in the C-3 aryl ring resulted in complete loss of activity. Introduction of 3,4-dimethoxyphenyl ring at C-3 led to **82e** with enhanced activity against all the cancer cell lines with best results against DU145 and MCF-7 with IC_{50} 5.8 and 8.1 μM respectively. However, 3,5-dimethoxyphenyl analogue **82f** was inactive up to 28 μM indicating that *para* methoxy substituent is important for the activity. The 3,4-methylenedioxy analog **82g** was found to be less potent than **82e** but showed improved activity and selectivity against MCF-7 (IC_{50} = 4.8 μM) cell lines. *N*-Methylation of 4-benzyloxy-3-methoxyphenyl analog **81e** led to compound **82h** with improved activity and selectivity against PC3 cell line with IC_{50} value of 4.2 μM but reduced activity was observed against other tested cell lines. Similarly *N*-methylation of compound **81f** led to 3,4,5-trimethoxyphenyl analog **82i** with remarkable improvement in activity against all the tested cell lines. Introduction of bromine atom on indole moiety at C-5 or C-6 positions resulted the compounds **82k** and **82l** with lower activity relative to parent analog **82i**. Surprisingly, introduction of lipophilic cyclic amine moiety (piperidinyl) led to compound **82n** which displayed excellent cytotoxicity against all the tested cell lines with two-fold selectivity against MCF-7 (IC_{50} = 1.6 μM) cell line. However, 4-pyridyl analog **82m** displayed diminished activity. Compound **82o** closely related to known anti-cancer agent Labradorin 1 with an isobutyl group also exhibited poor activity. The overall structure activity relation of the series can be represented in a pictorial diagram as shown in the (figure 2.4.6).

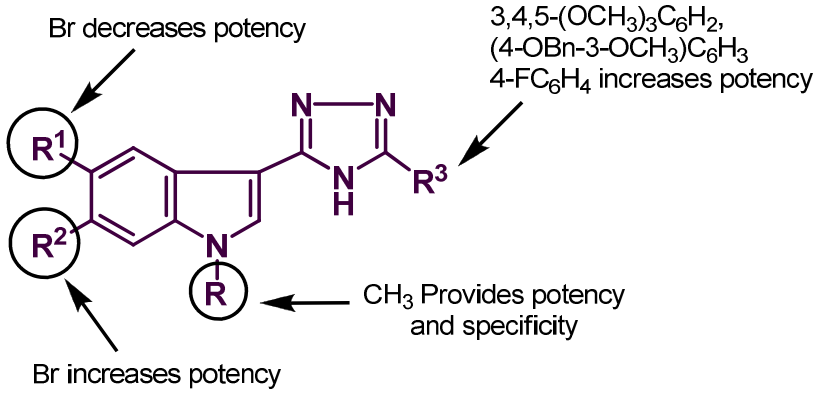


Figure 2.4.6 Structure-activity relationship of indolyl-1,2,4-triazoles (**81, 82**)

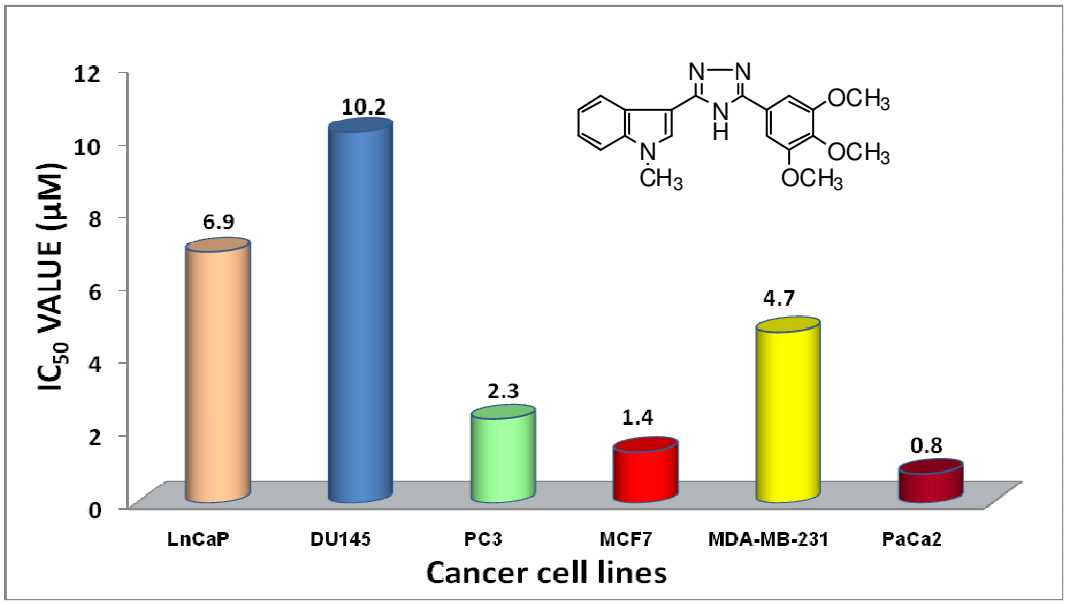


Figure 2.4.7 The most active indolyl-1,2,4-triazole **82i**

The figure 2.4.7 depicts the most active triazole in the series is **82i** with *N*-methyl indole and 3,4,5-trimethoxyphenyl substituents at C-3 and C-5 positions. The figure 2.4.8 demonstrates the IC_{50} values of the most selective compound **82c** with *N*-methyl indole and 4-fluorophenyl substituents and the compound is most selective towards PC3 cancer cell line (IC_{50} = 4 µM).

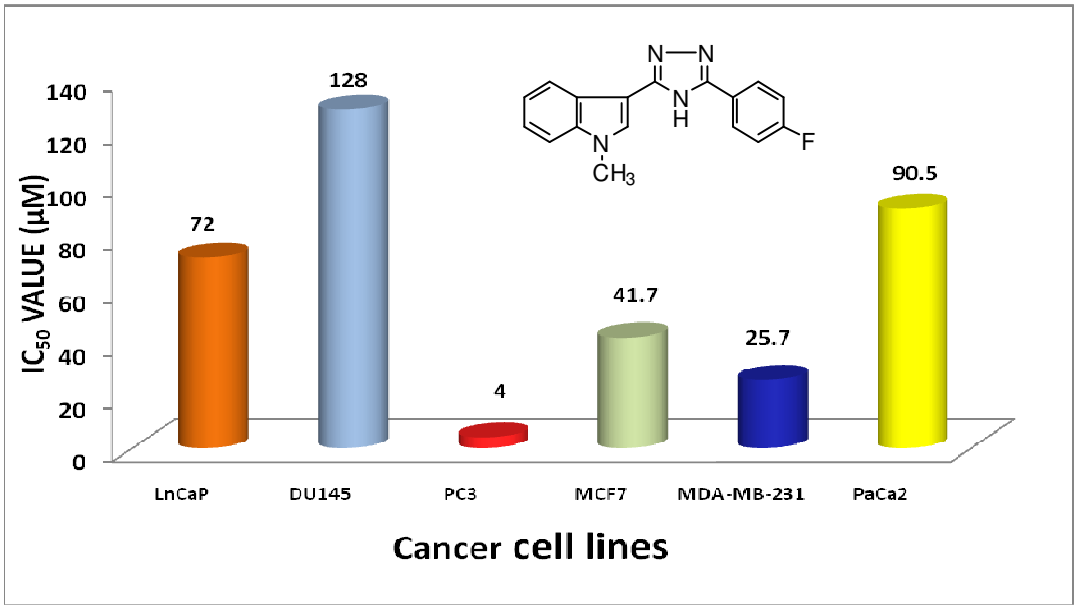


Figure 2.4.8 The most selective indolyl-1,2,4-triazole **82c**

Tubulin polymerization activity

Microtubules are the components of cell structure, which take part in diverse crucial cellular functions. Microtubules play a pivotal role in mitosis and cell division process and hence are recognized as an important target in cancer therapy.[105] Drugs interfering with microtubules/ tubulin dynamic equilibrium are called antimitotic agents. These drugs cause the appearance of typical hallmarks at the level of chromosomes, nuclear membrane, mitotic spindle, and G2/M cells as a consequence of the mitotic arrest.[106] The anticancer research for novel drugs able to modulate the microtubule assembly either by inhibition of tubulin polymerization or by blocking microtubule disassembly, gives a great boost to the progress of anticancer therapy. Since the many years the indole nucleus has been explored for its anticancer chemotherapy. Indole containing natural and synthetic analogues has shown anticancer activity *via* tubulin polymerization or depolymerization mechanism. A diverse variety of indole derivatives such as aroyl indoles, indolyl heterocycles and fused molecules were screened for their tubulin polymerization activity.[107] Recently, Qiang Zhang *et al.* reported 1,2,4-triazole containing analogues for their tubulin binding activity.[93] Our efforts to identify most potent anticancer agents, indolyl-1,2,4-triazoles **82i** and **82k** were taken up for their tubulin activity.

Inhibition of microtubule polymerization by menadione in-vitro: We have examined the effect of **82i** on microtubule polymerization using purified tubulin. We first analyzed the ability of **82i** to inhibit polymerization of purified tubulin into microtubules *in vitro* by the light scattering assay at 350 nm. Purified tubulin was polymerized in the absence or presence of different concentrations of **82i**, **82k** and the results are shown in figure 2.4.9.

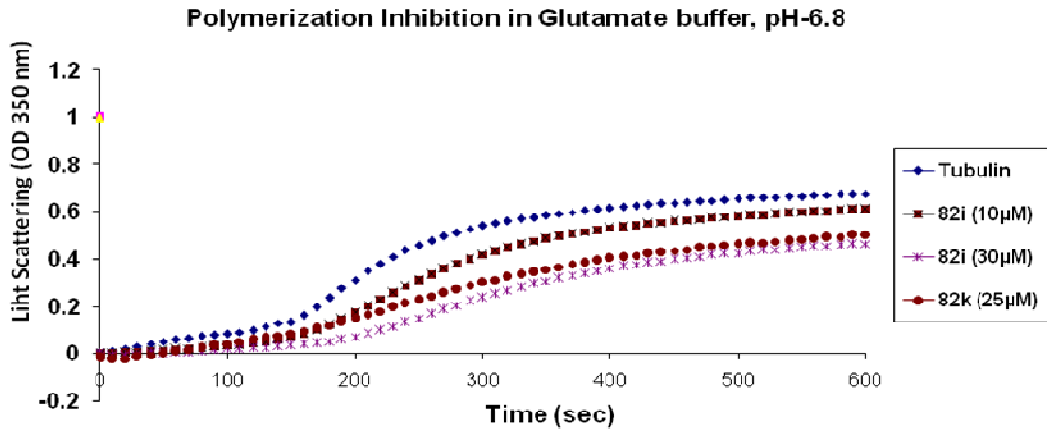


Figure 2.4.9 Effect of indolyl-1,2,4-triazoles (**82i**) and (**82k**) on microtubule polymerization kinetics assessed by monitoring the increase in light scattering at 350 nm

The triazoles **82i** and **82k** inhibited the rate and extent of tubulin polymerization in a concentration-dependent manner. The fluorescence assay was carried out at various concentrations of compound **82i** the plot A in the figure 2.4.10 shows the inhibition of tubulin assembly at different concentrations. The percentage inhibition of microtubule polymerization was calculated using the absorbance readings in the absence and presence of different concentrations of **82i** (plot B of figure 2.4.10). For example, the level of inhibition of polymerization was 78% when 75μM of **82i** was added and 50% inhibition of microtubule polymerization (IC_{50}) occurred at a concentration of 47.0 ± 0.89 μM.

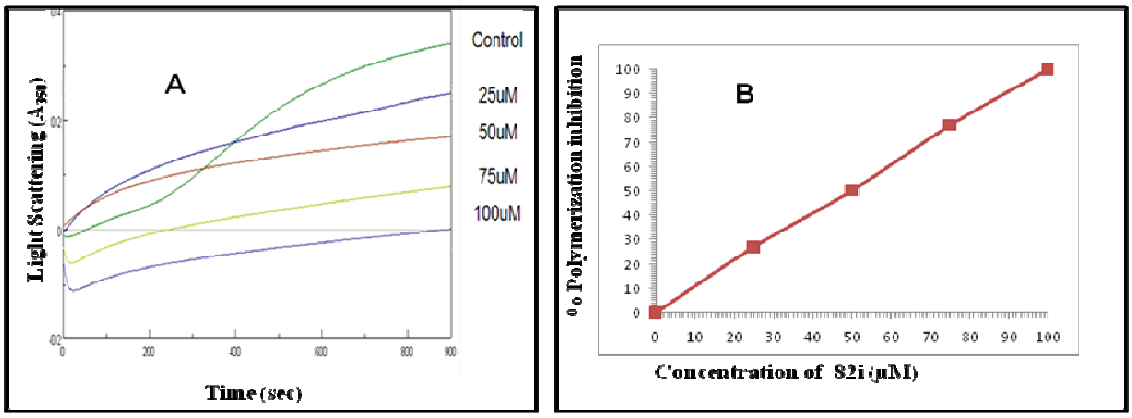


Figure 2.4.10 Inhibition of microtubule assembly by indolyl-1.2.4-triazole (**82i**). (A) Effect of **82i** on microtubule polymerization kinetics assessed by monitoring the increase in light scattering at 350 nm: control and 25, 50, 75 and 100 μM. (B) Inhibition of tubulin polymerization plotted as a function of triazole concentration

The effect of compound **82i** on phase of microtubule of A549 cells was shown in the figure 2.4.11. The compound **82i** was taken in different concentrations 5, 8 and 10 µM. The cells were incubated with above concentration of the drug for 48 hr. Microtubule tagged with rhodamine (red) and nucleus with DAPI (blue) were visualized with a confocal microscope.

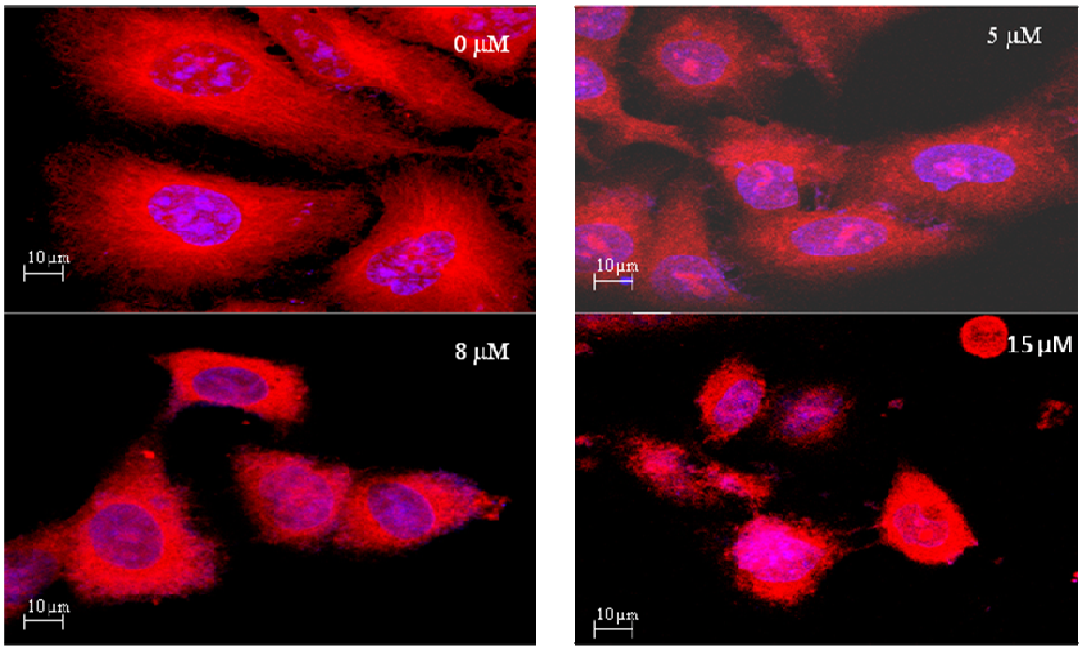


Figure 2.4.11 Effect of indolyl-1,2,4-triazole (**82i**) on the interphase microtubules of A549 cells

A substantial reduction in the number of microtubules at the periphery of the cells is apparent, and the central networks are disorganized at concentration of 5µM and above at 48 h of treatment. The compound **82i** has affected microtubule network in lung cancer cells by depolymerization of microtubules network. The cytotoxicity of **82i** may be through microtubule depolymerization. Further studies are in progress.

2.4.4 Experimental Procedures

General: The reactions were monitored by thin layer chromatography (TLC) by using Merck silica gel plate (60 F_{254}, 0.25 mm) and TLC plates were visualized by fluorescence quenching under UV light (254 nm). ^{1}H NMR spectra were recorded using Bruker Avance (400 MHz) spectrometer. Compounds were taken in DMSO-d_6 and $CDCl_3$ for recording the spectra using tetramethylsilane (TMS) as an internal standard, chemical shifts (δ) were reported in ppm and coupling constant *J* values were expressed in Hz. Mass spectra were recorded using Hewlett-Packard' HP GS/MS 5890/5972. Melting points were determined using electrothermal capillary melting point apparatus and are

uncorrected. Büchi rotavapor was used to distill off the solvents. All commercially available reagents and solvents were purchased from Merck and Aldrich and used as such without further purification. Compounds were purified by column chromatography using 100-200 mesh silica gel and hexane and ethyl acetate as eluent.

2.4.4.1 Synthesis of indolyl-1,2,4-triazoles (81) and (82)

Indole-3-carboxaldehyde (76)

A round-bottomed flask containing 28.8 mL (27.4 g, 370 mmol) of freshly distilled dimethylformamide (DMF) was cooled in an ice-salt bath for about 0.5 h and 8.6 mL (14.4 g, 90 mmol) of freshly distilled phosphorus oxychloride was subsequently added with stirring to the DMF over a period of 0.5 h. A solution of indole (**75**, 10 g, 85.47 mmol) in DMF (9.5 g, 130 mmol) was added to the yellow solution over a period of 1 h. The solution was stirred at 35 °C to become a yellow paste. At the end of the reaction 30 g of crushed ice was added to the paste with stirring which becomes a clear cherry-red aqueous solution. A solution of sodium hydroxide (37.5 g, 94 mmol) in 100 mL of water was added dropwise with stirring. The resulting suspension was heated rapidly to 90 °C and allowed to cool to room temperature, after which it was placed in refrigerator overnight. The product was filtered, washed with water (2 ×100 mL) and air-dried to afford the 12 g of pure indole-3-carboxaldehyde (**76**). Yield 90%; Pale yellow solid; mp 194-196 °C (Lit.[103] 196-197 °C).

1-Methylindole-3-carboxaldehyde (77)

Indole-3-carboxaldehyde (**6a**, 3 g, 20.68 mmol), potassium carbonate (1.5 g), of dimethyl formamide (20 mL) and dimethyl carbonate (5.2 mL, 61 mmol) were mixed together and heated to reflux at 130 °C. After competition of the reaction the mixture was cooled to about 3 °C, and 60 mL of ice-cold water was slowly added. The product precipitated as dark oily suspension was extracted with 60 mL of diethylether and washed with water (2 × 50 mL). The removal of excess solvent led to the product 1-methylindole-3-carboxalehyde (**77**) off-white solid in yield 85%, mp 68-70 °C (Lit.[104] 69-73 °C)

General procedure for the synthesis of indole-3-carbonitriles 78 and 79

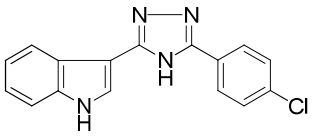
To a stirred solution of indole-3-carboxaldehyde (**76** or **77**, 0.01 mol) in formic acid (10 mL) was added sodium formate (0.02 mol) and hydroxylamine hydrochloride (0.01 mol). The reaction mixture was refluxed for 3 h at 130 °C. After completion of the reaction as monitored by TLC, the reaction mixture was cooled to room temperature and

poured into ice-cold water (100 mL) and extracted with dichloromethane (2 × 30 mL). The combined extracts were washed with saturated sodium bicarbonate solution (30 mL) and then with brine solution (30 mL). Organic layer was separated, dried over sodium sulfate, and solvent was evaporated under vacuum, the residue so obtained was subjected to a silica gel column chromatography (hexane and ethyl acetate as eluent) to afford pure indole-3-carbonitriles (**78**) or (**79**). The melting points of the products were in agreement with the reported in literature.[108]

General procedure for the synthesis of indolyl-1,2,4-triazoles (81) and (82)

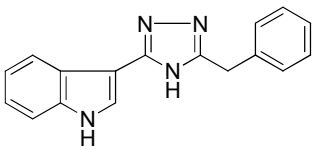
To a mixture of indole-3-carbonitrile (**78** or **79**, 2 mmol) and potassium carbonate (0.5 mmol) in *n*-BuOH (3 mL) was added acid hydrazide (**80**, 1 mmol) and the reaction mixture was stirred at 160 °C for 8 h. The progress of the reaction was monitored by TLC After completion of reaction, the solvent was removed under reduced pressure and the residue so obtained was purified by column chromatography on silica gel with hexane and ethyl acetate as eluent to obtain pure indolyl-1,2,4-triazoles **81** and **82**.

3-(4′-Chlorophenyl)-5-(3′-indolyl)-1,2,4-triazole (81a)



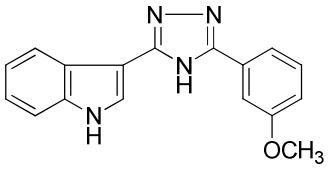
Yield 65%; White solid; mp 263-265 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1H, NH), 9.32 (s, 1H, NH), 8.03 (d, 2H, J = 8.12 Hz, Ar-H), 7.82 (s, 1H, Ar-H), 7.41 (d, 2H, J = 8.20 Hz, Ar- H), 7.32-7.20 (m, 3H, Ar-H), 6.92-6.90 (m, 1H, Ar-H); MS (ESI): *m/z* calcd. for $C_{16}H_{11}ClN_4$ 294.06, found: 295.1 $(M+H)^+$.

3-(4′-Benzyl)-5-(3′-indolyl)-1,2,4-triazole (81b)



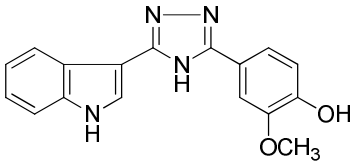
Yield 72%; white solid; mp 170-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (s, 1H, NH), 9.89 (s, 1H, NH), 8.18-8.15 (m, 1H, Ar-H), 7.86-7.87 (m, 2H, Ar-H), 7.49-7.46 (m, 1H, Ar-H), .39-7.33 (m, 3H, Ar-H), 77.31-7.27 (m, 1H, Ar-H), 7.25-7.17 (m, 2H, Ar-H) 4.76 (s, 2H, CH_2); MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4$, 274.13 found: 274.13 $(M)^+$.

3-(3′-Methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (81c)



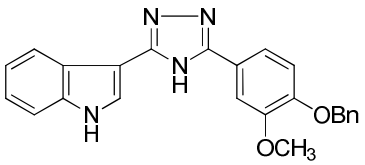
Yield 68%; White solid; mp 165-167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.08 (s, 1H, NH), 9.88 (s, 1H, NH), 8.32-8.17 (m, 2H, Ar-H), 7.72 (s, 1H, Ar-H), 7.27-7.30 (m, 6H, Ar-H), 3.90 (s, 3H, OCH_3); MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4O$ 290.11 found: 291.14 $(M+H)^+$.

3-(4′-Hydroxy-3′-methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (81d)



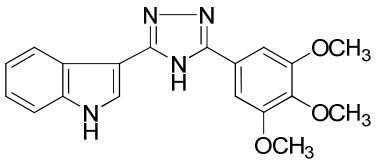
Yield 65%; White solid; mp 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1H, NH), 9.79 (s, 1H, NH), 8.30 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.22-8.19 (m, 2H, Ar-H), 7.50-7.46 (m, 2H, Ar-H), 7.29-7.20 (m, 3H, Ar-H), 4.90 (s, 1H, CH_2), 3.79 (s, 3H, OCH_3); MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4O_2$: 306.11 found 306.12 $(M)^+$

3-(4′-Benzyloxy-3′-methoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (81e)



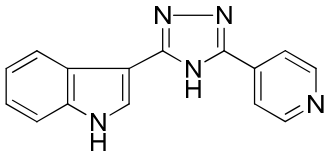
Yield 65%; Off-white solid; mp 225 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H, NH), 9.89 (s, 1H, NH), 8.26 (d, 1H, *J* = 7.84 Hz, Ar-H), 7.69-7.64 (m, 1H, Ar-H), 7.41-7.39 (m, 2H, Ar-H), 7.35-7.23 (m, 5H, Ar- H), 7.28-7.21 (m, 2H, Ar-H), 7.03 (d, 2H, *J* = 1.9 Hz, Ar-H), 5.21 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3); MS (ESI): *m/z* calcd. for $C_{24}H_{20}N_4O_2$: , 396.11 found 397.11 $(M+H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(3′-indolyl)-1,2,4-triazole (81f)



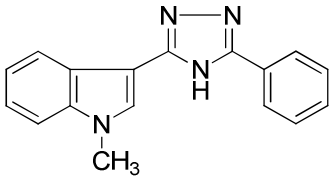
Yield 74%; White solid; mp 245-247 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.57 (s, 1H, NH), 9.87 (s, 1H, NH), 8.37-8.17 (m, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7,55-7.24 (m, 3H, Ar-H), 6.90 (d, 2H, *J* = 2.16 Hz, Ar-H), 3.93 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3); MS (ESI): *m/z* calcd. for $C_{19}H_{18}N_4O_3$: 351.2 found: 374.24 $(M+Na)^+$.

3-(4′-Pyridyl)-5-(3′-indolyl)-1,2,4-triazole (81g)



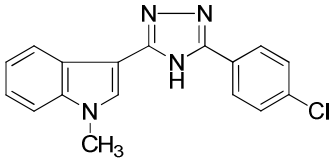
Yield 62%; Pale yellow solid; mp 190-193 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (s, 1H, NH), 9.87 (s, 1H, NH), 8.77 (d, 2H, *J* = 5.6 Hz, Ar-H), 8.32 (d, 1H, *J* = 1.2 Hz, Ar-H), 8.23 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.94 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.54 (d, 1H, *J* = 8.8 Hz, Ar-H), 7.28-7.26 (m, 2H, Ar-H); MS (ESI): *m/z* calcd. for $C_{15}H_{11}N_5$: 261.1, found: 261.1 $(M)^+$.

3-Phenyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82a)



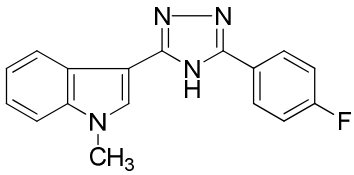
Yield 65%; Off-white solid; mp 180-182 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H, NH), 8.13 (d, 2H, *J* = 7.80 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 7.48 (d, 1H, *J* = 7.48 Hz, Ar-H), 7.24-7.14 (m, 5H, Ar-H), 7.12-7.10 (m, 1H, Ar-H), 3.81 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4$: 274.12, found: 275.2 $(M+H)^+$.

3-(4′-Chlorophenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82b)



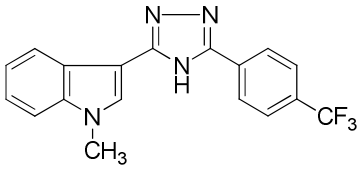
Yield 62%; Pale yellow, mp 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6,): δ 13.72 (s, 1H, NH), 8.42 (d, 1H, *J* = 7.72 Hz, Ar-H), 8.16 (d, 2H, *J* = 7.72 Hz, Ar-H), 7.98-7.88 (m, 3H, Ar-H), 7.67 (d, 1H, *J* = 7.12 Hz, Ar-H), 7.30-7.25 (m, 2H, Ar-H), 3.91 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{17}H_{13}ClN_4$: 308.1, found: 308.1 $(M)^+$.

3-(4′-Fluorophenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82c)



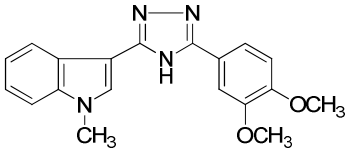
Yield 65%; Off-white, mp 202-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H, NH), 9.80 (s, 1H, Ar-H), 8.19 (d, 2H, *J* = 7.82 Hz, Ar-H), 7.92-7.89 (m, 1H, Ar-H), 7.35-7.25 (m, 2H, Ar-H), 7.23-7.05 (m, 3H, Ar-H), 3.77 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{17}H_{13}FN_4$: 292.11, found: 293.23 $(M+H)^+$.

3-(4′-Trifluoromethylphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82d)



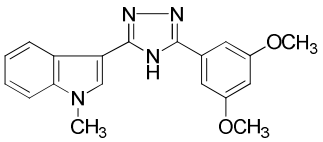
Yield 62%; Yellow solid. mp 197-199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.86 (s, 1H, NH), 8.43 (d, 1H, *J* = 7.56 Hz, Ar-H), 8.38 (d, 2H, *J* = 6.84 Hz, Ar-H), 7.95 (s, 1H, Ar-H), 7.72 (d, 2H, *J* = 6.64 Hz, Ar-H), 7.41 (d, 1H, *J* = 7.36 Hz, Ar-H), 7.34-7.28 (m, 2H, Ar-H), 3.90 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{18}H_{13}F_3N_4$: 342.11, found: 343.12 $(M+H)^+$.

3-(3′,4′-Dimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82e)



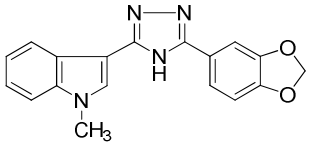
Yield 63%; Off-white solid; mp 200-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.10 (s, 1H, NH), 8.27 (d, 1H, *J* = 7.87 Hz, Ar-H), 7.71-7.64 (m, 3H, Ar-H), 7.27-7.23 (m, 2H, Ar-H), 7.18-7.14 (m, 1H, Ar-H), 7.06 (d, 1H, *J* = 8.00 Hz, Ar-H), 3.86 (s, 6H, OCH_3). 3.68 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{19}H_{18}N_4O_2$: 334.2, found: 335.2 $(M+H)^+$.

3-(3′,5′-Dimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82f)



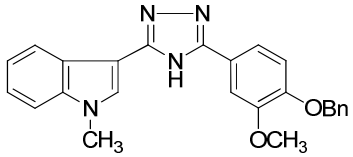
Yield 65%; Off-white solid; mp 183-185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H, NH), 8.36 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.48-7.39 (m, 1H, Ar-H), 7.10 (d, 2H, *J* = 8.2 Hz, Ar-H), 6.90 (d, 1H, *J* = 7.56 Hz, Ar-H), 6.61-6.48 (m, 2H, Ar-H), 3.90 (s, 6H, OCH_3). 3.83 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{19}H_{18}N_4O_2$: 334.15, found: 335.15 $(M+H)^+$.

3-(3′,4′-Methylenedioxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82g)



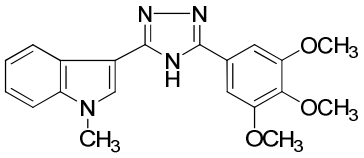
Yield 68%; Brown solid; mp 252-254 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 13.89 (s, 1H, NH), 8.29 (d, 1H, *J* = 7.56 Hz, Ar-H), 7.97 (s, 1H, Ar-H), 7.67 (d, 1H, *J* = 7.48 Hz, Ar-H), 7.58-7.54 (m, 2H, Ar-H), 7.32-7.24 (m, 2H, Ar-H), 7.06 (t, 1H, *J* = 7.92 Hz, Ar-H), 6.11 (s, 2H, CH_2), 3.88 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. $C_{18}H_{14}N_4O_2$: 318.11, found: 319.13 $(M+H)^+$.

3-(4′-Benzyloxy-3′-methoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82h)



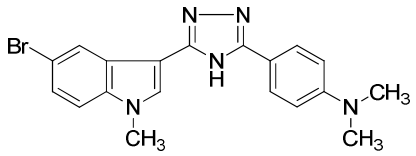
Yield 58%; Brown solid; mp 182-184 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (s, 1H, NH), 8.26 (d, 1H, *J* = 7.84 Hz, Ar-H), 7.63-7.54 (m, 3H, Ar-H), 7.41-7.39 (m, 2H, Ar-H), 7.35-7.23 (m, 5H, Ar-H), 7.15-6.77 (m, 2H, Ar-H), 5.08 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.58 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{25}H_{22}N_4O_2$: 410.16, found: 411.16 $(M+H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82i)



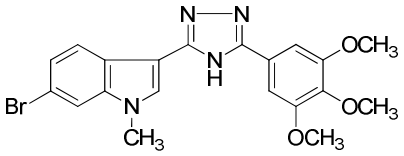
Yield 70%; Yellow solid; mp 146-148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1H, NH), 8.57 (d, 1H, *J* = 2.32 Hz, Ar-H), 8.23 (d, 1H, *J* = 1.8 Hz, Ar-H), 8.00 (s, 1H, Ar- H), 7.41-7.23 (m, 4H, Ar-H), 3.90 (s, 9H, OCH_3), 3.73 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{20}H_{20}N_4O_3$: 364.15, found: 365.16 $(M+H)^+$.

3-(4′-*N*,*N*-Dimethylaminophenyl)-5-(5′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (82j)



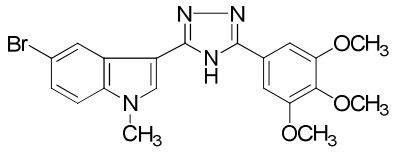
Yield 70%; Off-white solid; mp 226-228 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (s, 1H, NH), 8.40 (d, 1H, *J* = 1.80 Hz, Ar-H), 7.91 (s, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.33-7.25 (m, 3H, Ar-H), 6.73-6.68 (m, 2H, Ar-H), 3.83 (s, 3H, NCH_3). 3.13 (s, 6H, NCH_3); MS (ESI): *m/z* calcd. for $C_{19}H_{18}BrN_5$: 395.1 found: 396.12 $(M+H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(6′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (82k)



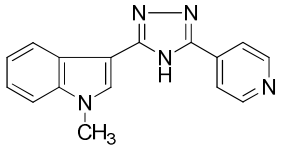
Yield 72%; White solid; mp 160-163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.57 (s, 1H, NH), 8.37 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.10 (d, 1H, *J* = 2.20 Hz, Ar-H), 6.90 (d, 1H, *J* = 2.16 Hz, Ar-H), 6.62-6.47 (m, 2H, Ar-H), 3.94 (s, 9H, OCH_3). 3.83 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{20}H_{19}BrN_4O_3$: 442.06, found: 443.11 $(M+H)^+$.

3-(3′,4′,5′-Trimethoxyphenyl)-5-(5′-bromo-*N*-methyl-3′-indolyl)-1,2,4-triazole (82l)



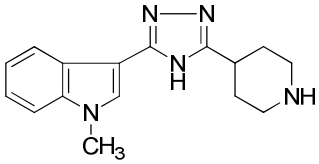
Yield 68%; White solid; mp 209-211 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.6 (s, 1H, NH), 8.37 (d, 1H, *J* = 1.76 Hz, Ar-H), 7.86 (s, 1H, Ar-H), 7.47-7.34 (m, 2H, Ar-H), 7.29-7.14 (m, 2H, Ar-H), 3.94 (s, 9H, OCH_3), 3.83 (s, 3H, NCH_3); MS (ESI): *m/z* calcd. for $C_{20}H_{19}BrN_4O_3$: 442.06 found: 443.08 $(M+H)^+$.

5-(*N*-Methyl-3′-indolyl)-3-(4′-pyridyl)-1,2,4-triazole (82m)



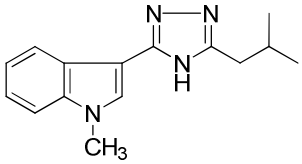
Yield 65%; Pale yellow, mp 214-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.08 (s, 1H, NH), 8.70-8.69 (m, 1H, Ar-H), 8.44 (d, 1H, *J* = 7.44 Hz, Ar-H), 8.10 (s, 1H, Ar-H), 7.93-7.88 (m, 2H, Ar- H), 7.70 (d, 1H, *J* = 7.40 Hz, Ar-H), 7.42 (d, 1H, *J* =7.12 Hz, Ar-H), 7.33-7.25 (m, 2H, Ar-H), 3.89 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.23, 153.05, 140.99, 136.91, 131.21, 129.00, 124.53, 122.62, 121.50, 121.01, 117.28, 110.46, 105.75, 33.07; MS (ESI): *m/z* calcd. for $C_{16}H_{13}N_5$: 275.11 found 276.21 $(M+H)^+$.

3-(4′-Piperidinyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (82n)



Yield 65%; Brown solid; mp 147-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (s, 1H, NH), 7.74-7.71 (m, 2H, Ar-H), 7.50 (d, 1H, *J* = 7.64 Hz, Ar-H), 7.30-7.20 (m, 2H, Ar-H), 3.94 (s, 3H, NCH_3). 3.41-3.39 (m, 1H CH), 3.19-3.12 (m, 4H, CH_2), 2.59 (s, 1H, NH), 1.15-1.12 (m, 4H, CH_2); MS (ESI): *m/z* calcd. for $C_{16}H_{19}N_5$: 281.2 found: 281.2 $(M)^+$.

3-(Isobutyl)-5-(*N*-methyl-3′-indoly)-1,2,4-triazole (82o)



Yield 58%; Brown solid; mp 130-133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H, NH), 8.16 (d, 1H, *J* = 8.88 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 7.28-7.24 (m, 1H, Ar-H), 7.21-7.19 (m, 2H, Ar-H), 3.86 (s, 3H, NCH_3). 2.68 (d, 2H, *J* = 7.2 Hz, CH_2), 1.27-1.23 (m, 1H, CH), 1.15 (d, *J* = 6.6 Hz, 6H, CH_3); MS (ESI): *m/z* calcd. for $C_{15}H_{18}N_4$: 254.15, found: 255.12 $(M+H)^+$.

2.4.4.2 MTT assay

The human cancer cell lines, prostate (PC3, DU145 and LnCaP), breast (MCF7 and MDA-MB-231) and pancreatic (PaCa2) were obtained from ATCC. Cytotoxic effects were examined in six human cancer cell lines (LnCaP, DU145, PC3, MCF-7, MDA-MB-231, and PaCa2) using MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium-bromide) assay. Cells were cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 4×10^3 cells per well for 12 h. Cells were incubated with varying concentrations (10 nM-1 mM) of the compounds for 48 h at 37 ^oC. MTT was added to the final concentration of 0.2 mg/mL and incubated for 30 min. The cells were washed twice with PBS and lysed in 100 µL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus. IC_{50} values were determined by a nonlinear regression analysis with a curve fitting programme, Graph Pad Prism 5.0.

2.5 Conclusions

Three series of novel indolylazoles, 4-(3′-indolyl)oxazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles and indolyl-1,2,4-triazoles were prepared and their anticancer activities against various human cancer cell lines were evaluated. The section 2.2 describes short and solvent-free synthesis of 4-(3′-indolyl)oxazoles. The *in-vitro* cytotoxic effects of 4-(3′-indolyl)oxazoles on six human cancer cell lines revealed that the compounds 2-(4′-fluorophenyl)-4-(3′-indolyl)oxazole (**51d**) and 4-(*N*-(4-chlorobenzyl)-2-(4′-chlorophenyl)-3′-indolyl)oxazole (**56**) found to be most active and selective towards breast cancer cell lines (MCF-7 IC_{50} = 28 and 14.1 µM respectively).

The section 2.3 illustrates the synthesis and *in-vitro* anticancer activity of 5-(3′-indolyl)-1,3,4-thiadiazoles. The anticancer activity results revealed that the 1,3,4-thiadiazole ring at C-3 of indole is more potent in suppressing the cancer cell growth than the previously discussed 2,4-disubstituted oxazole. The 2-(4′-(benzyloxy)-3′-methoxyphenyl)-5-(5′-bromo-3′-indolyl)-1,3,4-thiadiazole (**65m**) is the most potent compound of this series (PaCa2 IC_{50} = 1.5 µM). Compounds **65e** and **65h** have also shown good cytotoxicity toward prostate cancer cell lines (PC3, IC_{50} = 6.8 and 6.5 µM respectively).

The section 2.4 includes the synthesis and anticancer activity of novel indolyl-1,2,4-triazoles. The cytotoxicity results showed that the compounds 3-(3′,4′,5′-trimethoxyphenyl)-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (**82i**) (PaCa2, IC_{50} = 0.8 µM)

and 3-(4′-piperidinyl-5-(*N*-methyl-3′-indolyl)-1,2,4-triazole (**82n**) (MCF-7, IC_{50} = 1.6 μM) showed significant inhibitory effects against cancer cell lines. The substituents such as 4-fluorophenyl, 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 4-benzyloxy-3-methoxyphenyl, 4-piperidinyl and *N*-methylindole are beneficial for the activity of indolyl-1,2,4-triazoles. Among the synthesized indolylazoles, indolyl-1,2,4-triazoles were found to be relatively most active compounds. To identify the mechanism for the cytotoxicity, indolyltriazoles **82i** and **82k** were evaluated for their tubulin activity. Preliminary results showed that the compound **82i** has the ability to depolymerize the microtubule network which indicates that tubulin may be the probable target for these compounds. The overall study gives an idea that five-membered heterocycle ring present at C-3 of indole can play a vital role in altering the anticancer activity of indolylazoles.

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Chapter 3

3.1 Part A: 3,5-Bis(indolyl)-1,2,4-thiadiazoles as anticancer agents

3.1.1 Introduction

Indole alkaloids isolated from the plants and marine organisms have been widely recognized for their diversity in terms of chemistry and biology. Particularly, bis(indole) alkaloids have received a great attention due to their wide range of biological applications.[1-3] This class of molecules are having indole rings connected *via* a 5/6-membered heterocyclic ring and are abundant in marine organisms. Among marine organisms, sponges appear to be one of the richest phyla in toxicogenic species.[4-6] They are also remarkable for their ability to synthesize a wide variety of secondary metabolites and specified for their defense mechanisms.[7-8] In literature, isolation of variety of bis(indole) alkaloids is reported from different marine sponges. For example the topsentins (*Topsentia genitrix*) having a ketone and imidazole spacer, nortopsentins (*Spongosorites sp*) with imidazole as spacer exhibit good anticancer properties. Dragmacidins (*Dragmacidon sp* and *Hexadella sp*.) with piperazine spacer associated with diverse biological applications such as antibacterial, antiinflammatrory and cytotoxic activities. Asterriquinones isolated from *Aspergillus terreus* having benzoquinone as linker showed cytotoxic and antifungal properties (figure 3.1.1).[5-6, 9-13]

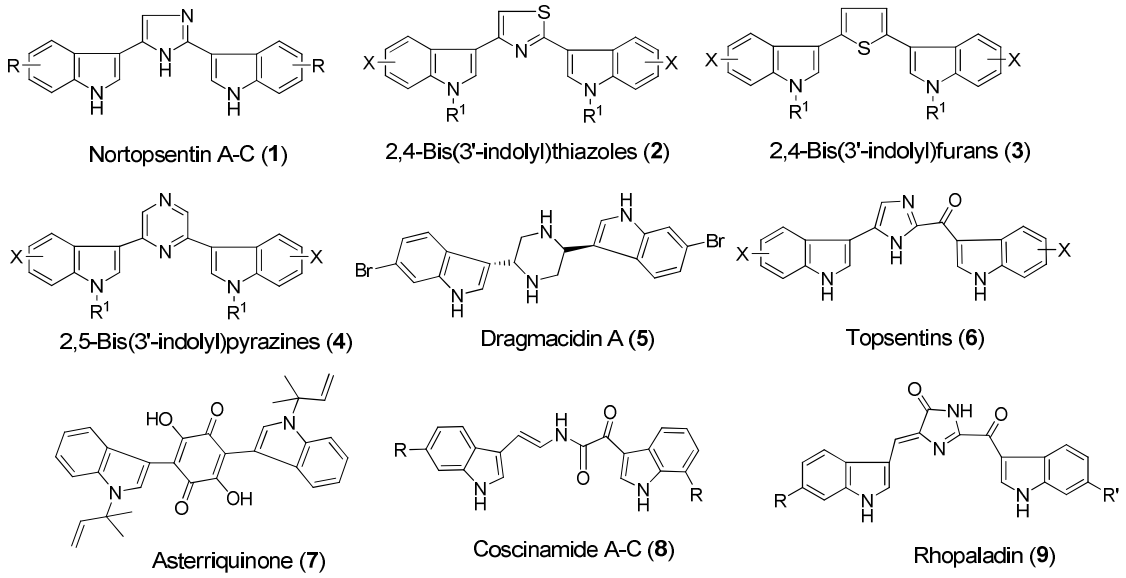
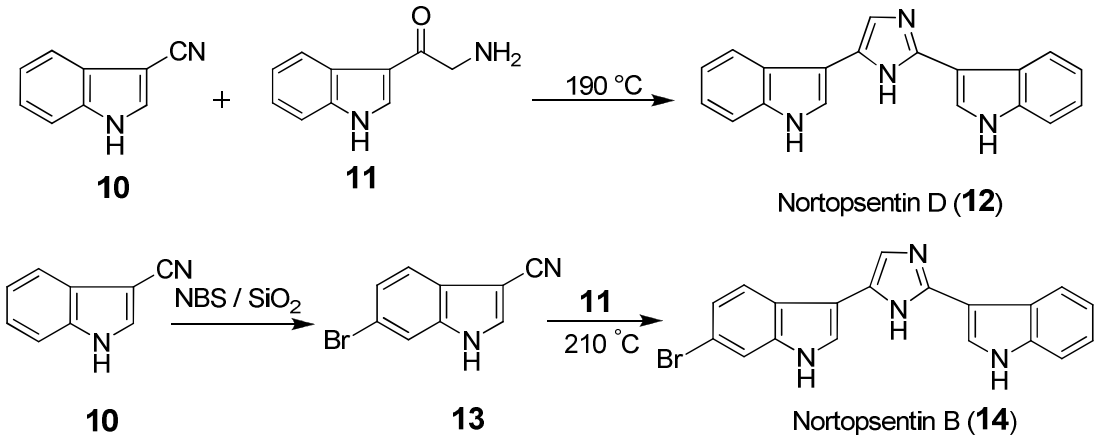


Figure 3.1.1 Natural and synthetic bis(indolyl)heterocycles (**1-9**)

All the isolated bis(indole) alkaloids have shown broad range of biological activities such as antiviral, antimicrobial, antiinflammatory and anticancer.[14-16] Most of the natural bis(indoles) found to be cytotoxic against various cancer cell lines.[17-20] In view of immense biological significance, many researchers paid attention on synthetic analogues of natural bis(indole) alkaloids and several 5/6-membered heterocyclic ring spacers such as thiazole,[21] furan,[17] thiophene,[18] pyrimidine[22] and pyridine[23] were incorporated between the indole rings and evaluated for their biological activities (figure 3.1.1). In this chapter we have synthesized two series of bis(indoles), bis(indolyl)-1,2,4-thiadiazoles and bis(indolyl)hydrazide-hydrazones and evaluated their anticancer activity against wide range of human cancer cell lines.

3.1.2 Synthetic routes and biological significance of bis(indoles)

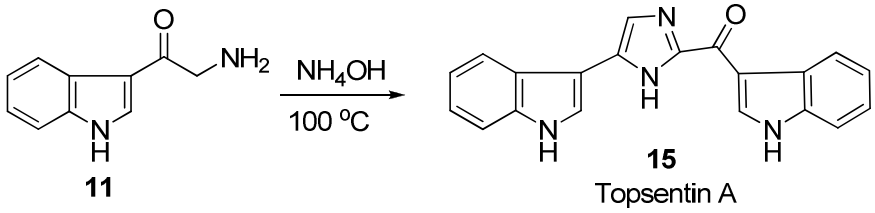
Synthetic and natural bis(indole) alkaloids have exhibited a broad range of biological applications. Natural products, Nortopsentins A-C, with its 2,4-bis(3′-indolyl)imidazole skeleton exhibited *in-vitro* cytotoxicity against P388 cells and antifungal activity against *Candida albicans*.[14, 16, 20, 24] A concise synthesis of two bis(indole)alkaloids, Nortopsentin D (**12**) and Nortopsentin B (**14**) was achieved by using α-aminoketone (**11**) as a key intermediate (scheme 3.1.1). The condensation of 3-cyanoindole (**10**) with **11** produced Nortopsentin D. Bromination of **10** using *N*-bromosuccinimide afforded 6-bromo-3-cyanoindole **13**, which upon condensation with **11** produced Nortopsentin B (**14**).[25]



Scheme 3.1.1 Synthesis of Nortopsentins B (**12** and **14**)

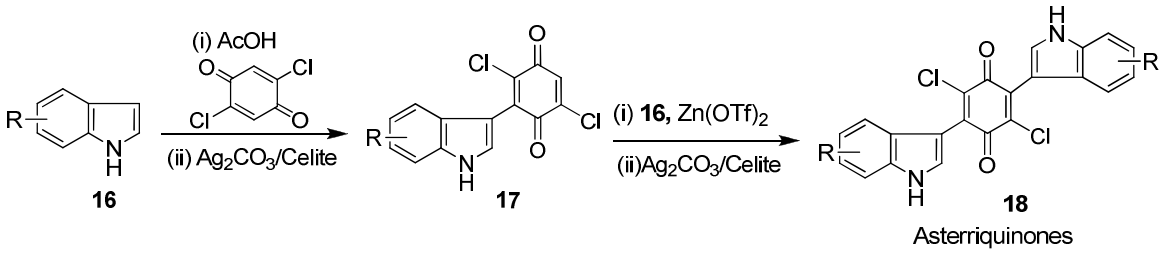
N-Methylated derivatives of Nortopsentin showed improved P388 activity when compared with parent compounds.[15] Another imidazole containing bis(indole) alkaloid,

Topsentin A (**15**) was prepared by oxidative dimerization of α-amino ketone **11** (scheme 3.1.2).[25]



Scheme 3.1.2 Preparation of Topsentin A (**15**)

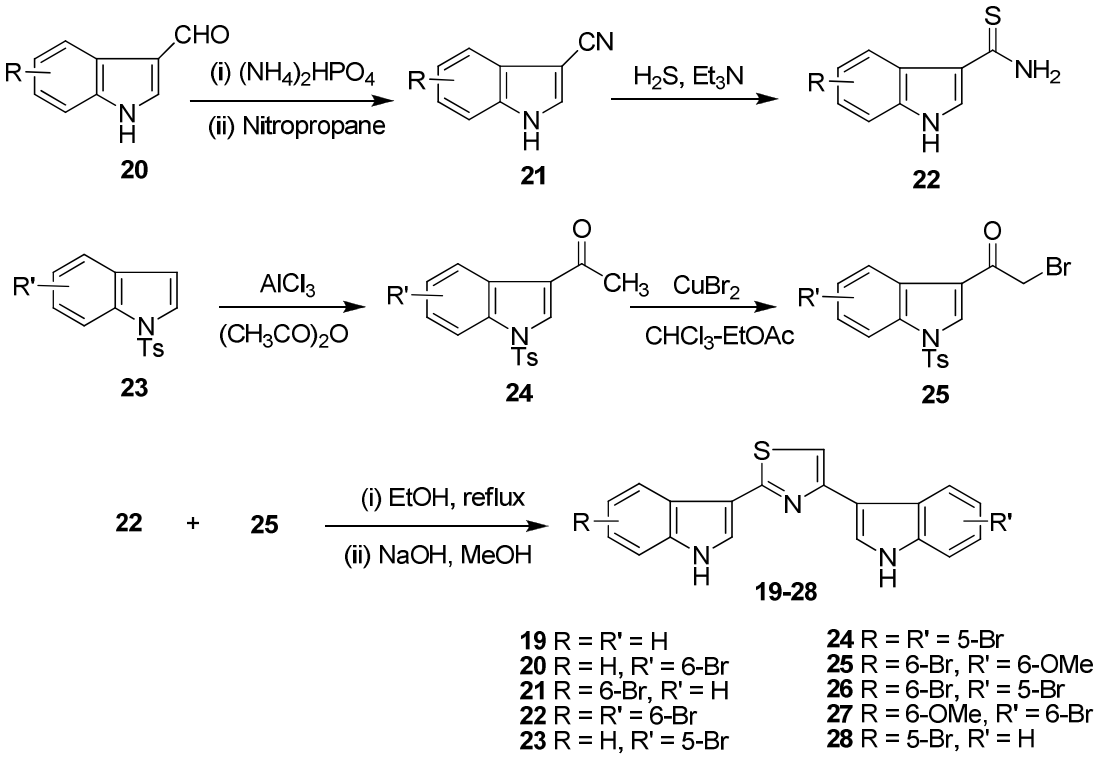
Topsentin A (**15**), inhibited the proliferation of cultured human and murine tumor cells at micromolar concentrations (IC_{50} = 4-40 μM). Fluorescence spectral changes and competitive binding experiments with ethidium bromide indicated that topsentin binds in the minor groove of DNA.[26] Asterriquinones (**18**), a family of naturally occurring bis(indolyl) dihydroxyquinones was isolated from *Aspergillus terreus* and reported to inhibit the interaction between adaptor proteins and protein tyrosine kinase directly.[27-28] Synthesis of asterriquinones (**18**) was carried out by Bronsted acid-catalyzed condensation of indole **16** with dichlorobenzoquinone, and followed by reaction of resulting indolylquinones **17** with indole(scheme 3.1.3).[29]



Scheme 3.1.3 Synthesis of indolylquinones- Asterriquinones (**18**)

Bis(indolyl)thiazoles (**19-28**) were prepared in analogy to Nortopsentins and evaluated for their cytotoxicity against a panel of 60 human tumour cell lines derived from leukaemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. Jiang *et al.* described a convergent procedure for the synthesis of bis(indolyl)thiazoles (**19-28**) by utilizing the reaction of indol-3-thioamide (**22**) with α-bromo ketone (**25**) and followed by removal of tosyl protection.[30] All 2,4-bis(indolyl)thiazoles (**19**–**28**) exhibited cytotoxic activities against a variety of human cancer cell lines. The compound **19** exhibited significant *in-vitro* cytotoxicity against leukaemia and ovarian cancer cell lines. Incorporation of methoxy or bromo substituents in the C-5/C-6 position of indole ring resulted increase in potency and

showed broad effects on leukaemia, colon, CNS and breast cancer cell lines while unsubstituted counterpart **19** brought out highly selective cytotoxicity against leukaemia and IGROV1 ovarian cancer cell lines.



Scheme 3.1.4 Synthesis of 2,4-bis(indolyl)thiazoles (**19-28**)

The dibromo derivative **26** effectively inhibited MCF-7 cell line (GI_{50} = 0.888 μM). The 3,5-bis(3′-indolyl)-2(*1H*)pyrazine **29** with 6-membered ring spacer exhibited remarkable cytotoxicity against various cancer cell lines with GI_{50} values ranging from 0.05 - 1μM. The 3,6-bis[3′-(*N*-methyl-indolyl)]pyrazinone **30** also demonstrated strong inhibitory effect against MCF-7 breast cancer cell lines with GI_{50} value of 6.67 μM (figure 3.1.2).

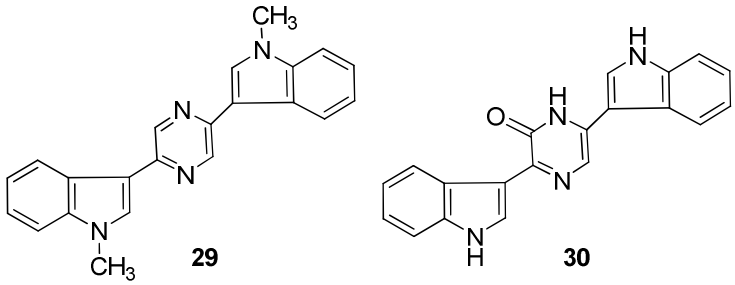
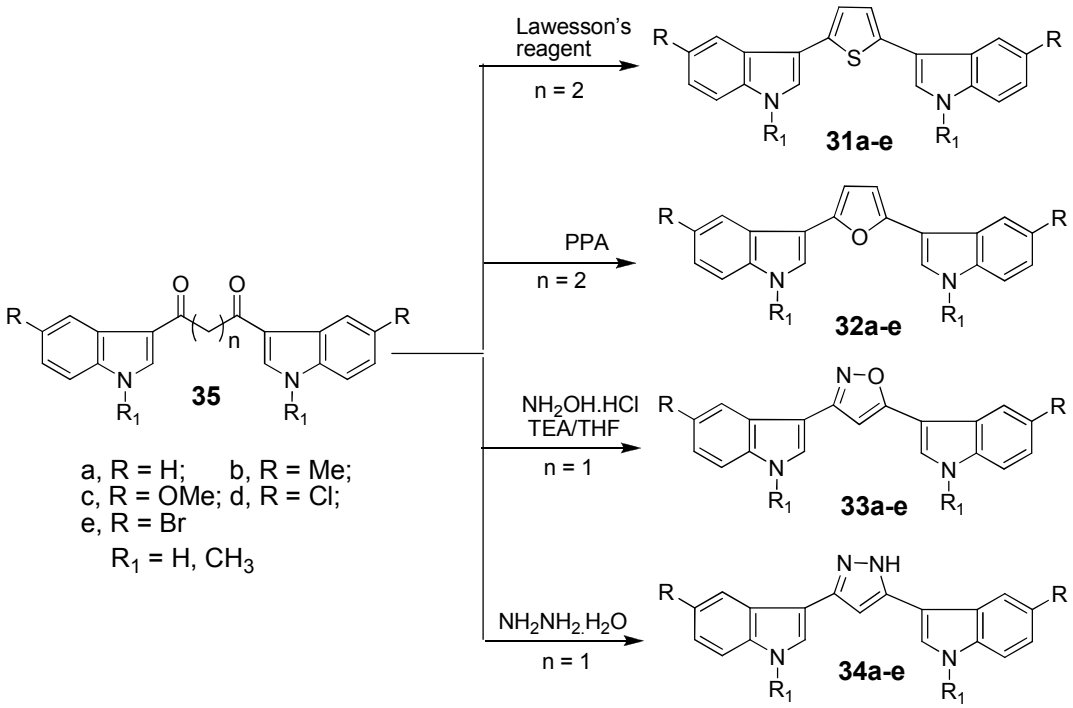


Figure 3.1.2 Antitumor bis(indolyl)pyrazine (**29**) and pyrazinone (**30**)

Diana. P *et al.* reported series of 2,5-bis(3′-indolyl)thiophenes(**31a-e**),[18] 2,5-bis(3′-indolyl)furans (**32a-e**),[17] 3,5-bis(3′-indolyl)isoxazoles (**33a-e**)[17] and

3,5-bis(3′-indolyl) pyrazoles (**34a-e**)[31] in analogy to Nortopsentin and evaluated their cytotoxicity over NCI panel of 60 human cancer cell lines. The syntheses of above bis(indoles) (**31-34**) were carried out by using common intermediate **35** as shown in scheme 3.1.5 and exhibited significant anticancer activity when compared to Nortopsentins. Bis(indolyl)thiazole **31c** was effective against the leukemia cells (GI_{50} = 0.34-3.54 μM). Furan and isoxazole analogues **32** and **33** were found to be cytotoxic by exhibiting mean IC_{50} values of 17.4 μg/mL and 20.5 μg /mL, respectively. Compound **32c** showed high selectivity with respect to the SK-MEL-2 (GI_{50} = 2.13 μM), OVCAR-4 (GI_{50} = 2.06 μM) and OVCAR-5 (GI_{50} = 2.79 μM) cell lines. It also showed selective cytotoxicity with respect to NCI/ADR-RES (GI_{50} = 2.37 μM), T-47D (GI_{50} = 2.72 μM). and SF-539 (GI_{50} = 2.47 μM) cancer cell lines. Bis(indolyl)pyrazole **34d** exhibited significant cytotoxicity against a wide range of cancer cell lines (GI_{50} = 1.63–9.64 μM) and also **34d** has positive TGI (14.5 μM) and LC_{50} (58.9 μM) values against all tested cancer cell lines.



Scheme 3.1.5 Synthesis of bis(indolyl)heterocycles (**31-34**) as anticancer agents

Bis(indole) alkaloids, Coscinamides **35 A-C** from the marine sponge *Coscinoderma sp* have shown cyclo protection against HIV in the NCI assay. Recently Chauhan *et al.* have synthesized 8,9-dihydrocoscinamide B and its analogues (**36** and **37**) and screened for

their *in-vitro* antileishmanial activity. The compounds **36** and **37** showed strong inhibition against promastigote and amastigotes models, respectively (figure 3.1.3).[32-33]

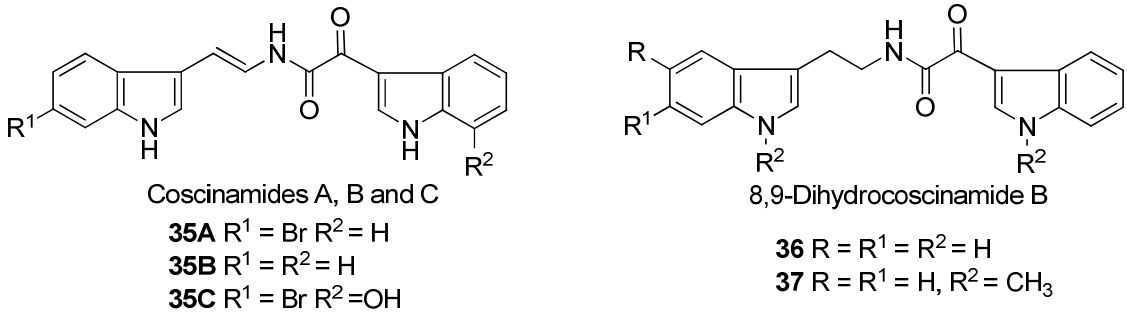
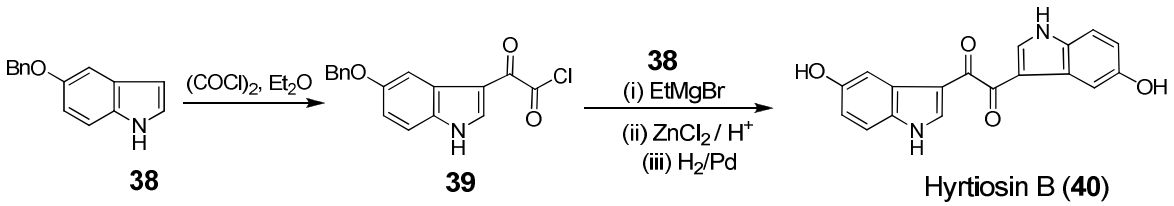


Figure 3.1.3 Natural and synthetic Coscinamides (**35-37**)

Bis(indole) alkaloid, Hyrtiosin B (**40**) [34] was isolated from the Okinawan marine sponge *Hyrtios erecta* and later Bergman *et al.* reported its short syntheses *via* indole-3-glyoxylyl chloride (**39**) prepared from indole (**38**) as out lined in the scheme 3.1.6.[35] Hyrtiosin B has displayed a potent inhibitory activity against isocitrate lyase (ICL) of *Candida albicans* (an opportunistic fungal pathogen) with an IC_{50} value of 89.0 µM.[36] Hyrtiosin B demonstrated to possess *in-vitro* cytotoxic activity against human epidermoid carcinoma KB cells. [34]



Scheme 3.1.6 Short and facile synthesis of Hyrtiosin B (**40**)

3.1.3 Rationale design

Various natural and synthetic bis(indoles) are reported for their potent anticancer activity. Natural products, Nortopsentins A-C having imidazole as a spacer found to be cytotoxic against P388 murine cancer cell lines. In analogy to Nortopsentin, various bis(indoles) were reported with mono and di hetero atomic ring spacers including thiophene, furan, pyridine, pyrazole, thiazole, oxazole, pyrazine and piperazine.[21-23] From these reports it has been found that central heterocyclic ring plays a crucial role in altering the anticancer activity of bis(indoles). In order to design novel bis(indolyl) heterocycles with improved anticancer activity and selectivity, we have prepared a series of bis(indolyl)-1,2,4-

thiadiazoles (**45**) by replacing imidazole ring in Nortopsentin with 1,2,4-thiadiazole. (figure 3.1.4).

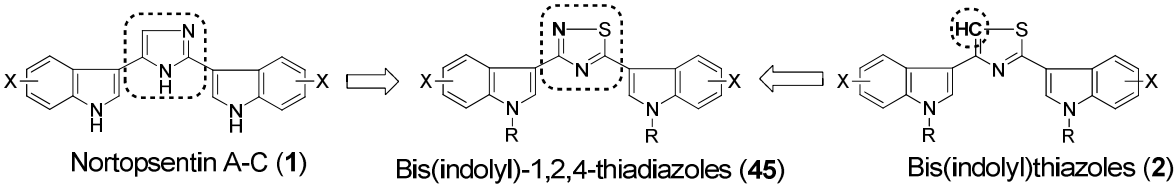


Figure 3.1.4 Rational for bis(indolyl)-1,2,4-thiadiazoles (**45**)

3.1.4 Significance of 1,2,4-thiadioazoles in drug discovery

The choice of 1,2,4-thiadiazole ring was due to its great importance in medicinal chemistry as antimicrobials,[19] antiinflammatory agents,[37] cardiovascular,[38] PPARα,-agents,[39] PDE7 and capthepsin B inhibitors,[40] acts against neurological disorders[41] and cannabinoid CB1 receptor agonists.[42] Apart from therapeutics, 1,2,4-thiadiazoles displayed their role as pesticides[43] and herbicides (figure 3.1.5).[44]

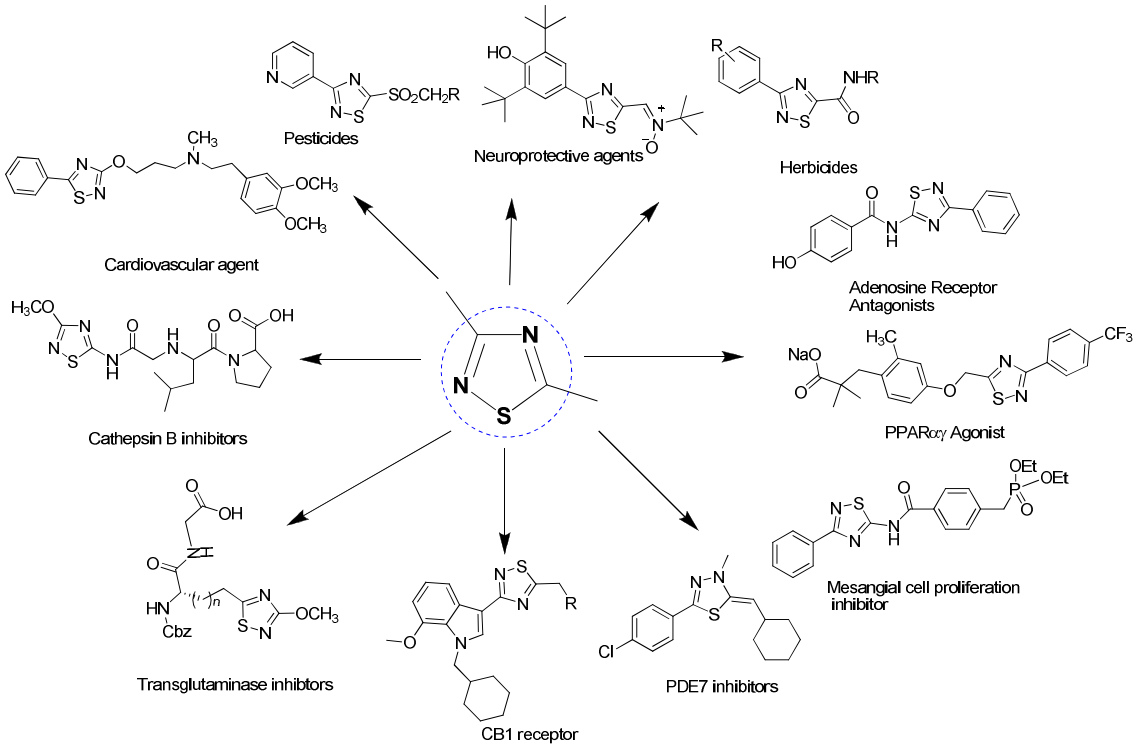


Figure 3.1.5 Biological significance of 1,2,4-thiadiazoles

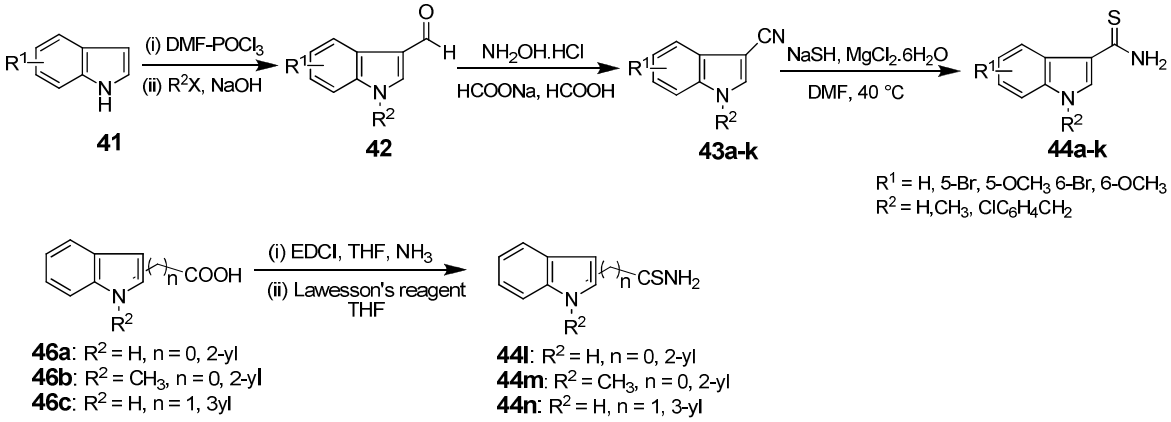
In view of biological importance of 1,2,4-thiadiazoles and bis(indolyl)heterocycles, we synthesized a diverse series of 3,5-bis(indolyl)-1,2,4-thiadiazoles and evaluated for their anticancer activity against selected human cancer cell lines.

3.1.5 Results and discussion

3.1.5.1 Chemistry

In our present work, synthesis of 3,5-bis(indolyl)-1,2,4-thiadiazoles was accomplished by oxidative dimerization of indole-3-thiocarboxamide using relatively benign and non-toxic hypervalent iodine reagent, iodobenzene diacetate (IBD). Due to the facile preparation and low toxicity when compared with other transition-metal based oxidants, hypervalent iodine reagents have been extensively used in the construction of bioactive heterocycles. In general, synthesis of 1,2,4-thiadiazoles was achieved using intermolecular cyclization,[45] intramolecular cyclization[46] and several oxidants were explored to achieve oxidative dimerization from corresponding thioamides.[47-52] In the process to find the synthetic applications of hypervalent iodine reagents iodobenzene diacetate (IBD)[53] and *o*-iodoxybenzoic acid with phase transfer catalyst tetraethylammonium bromide were also utilized to prepare 1,2,4-thiadiazoles.[54]

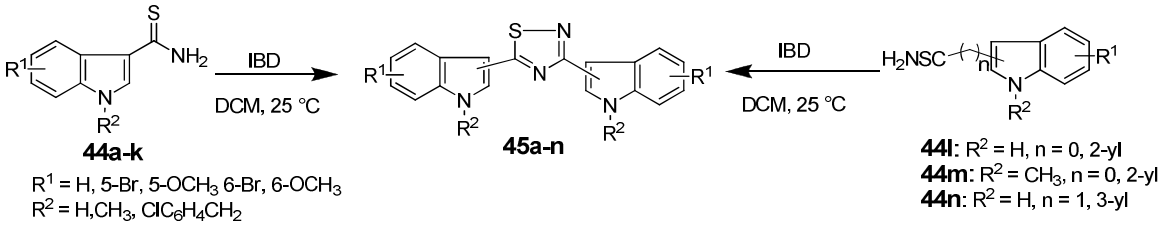
Synthesis of 3,5-bis(indolyl)-1,2,4-thiadiazoles was achieved as outlined in the schemes 3.1.7 and 3.1.8. Initially, we have synthesized indole-3-carboxaldehydes and their *N*-alkylated derivatives (**42**) from corresponding indoles (**41**). The indole-3-carbonitriles (**43**) were prepared in good yields by reacting indole-3-carboxaldehydes (**42**) with hydroxylamine hydrochloride and formic acid. The reaction of indole-3-carbonitriles (**43**) with sodium hydrosulfide and magnesium chloride in presence of dimethylformamide led to the formation of indole-3-thiocarboxamides (**44a-k**) as illustrated in scheme 3.1.7.



Scheme 3.1.7 Syntheses of indole(2)3-thiocarboxamides (**44a-k**)

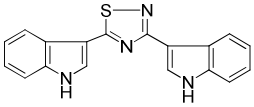
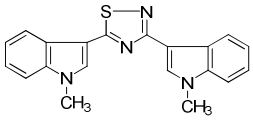
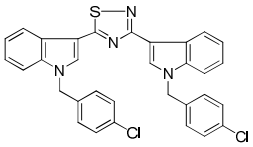
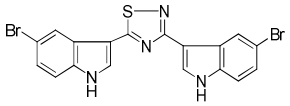
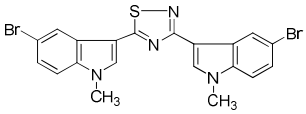
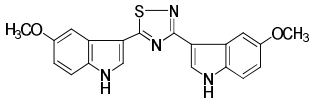
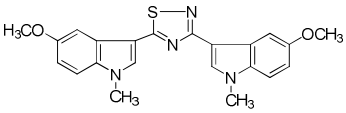
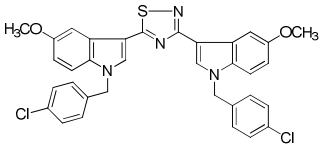
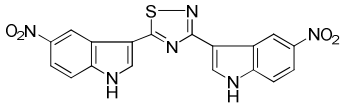
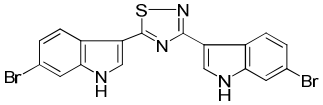
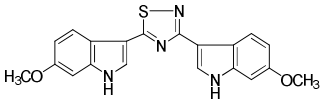
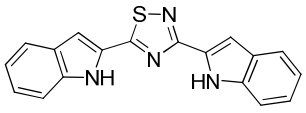
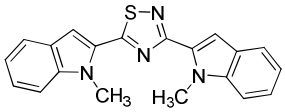
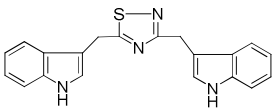
Intermediates indole-(2)3-thiocarboxamides (**44l-m**) were prepared by reacting indole-carboxylic acids (**46a-c**) with ammonia in presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Synthesized thiocarboxamides (**44a-k**) were used for further construction

of bis(indolyl)-1,2,4-thiadiazoles (**45**). The key-step of the protocol involves oxidative-dimerization of indole-thiocarboxamides (**44a-n**) and it was carried out using relatively benign hypervalent iodine reagent iodobenzene diacetate (IBD). We have also studied the role of solvent and temperature to accomplish the oxidative-dimerization of indole-3-thiocarboxamides using IBD. Several polar and non-polar solvents, such as acetonitrile, tetrahydrofuran, dioxane, dichloromethane and toluene were screened at various temperatures. We found the reaction in dichloromethane at 25 °C temperature resulted in 1,2,4-thiadiazoles (**45**) with lower impurity profiles. With the optimized experimental conditions, a series of diverse 3,5-bis(indolyl)-1,2,4-thiadiazoles (**45**) were synthesized in good yields (50-70%) by reacting indole-thiocarboxamides (**44a-n**) with iodobenzene diacetate in anhydrous dichloromethane (scheme 3.1.8).



Scheme 3.1.8 Synthesis of 3,5-bis(indole)-1,2,4-thiadiazoles (**45a-n**)

Table 3.1.1 Synthesis of 3,5-bis(indolyl)-1,2,4-thiadiazoles (**45**)

1,2,4-Thiadiazoles (**45**)		Time (min)	Yield (%)
	**(45a)**	12	65
	**(45b)**	15	60
	**(45c)**	10	72
	**(45d)**	14	55
	**(45e)**	13	60
	**(45f)**	15	70
	**(45g)**	10	65
	**(45h)**	16	60
	**(45i)**	20	52
	**(45j)**	15	52
	**(45k)**	8	70
	**(45l)**	10	62
	**(45m)**	12	68
	**(45n)**	14	55

All the synthesized compounds were well characterized by IR, ^{1}H NMR and Mass spectral data. ^{1}H NMR spectrum of compound **45c** is displayed in the figure 3.1.6. In figure 3.1.7 the mass spectrum of the compound **45c** indicate the molecular ion peak at *m/z* value 565.8 $(M+H)^{+}$ which is in agreement with the calculated value (564.7).

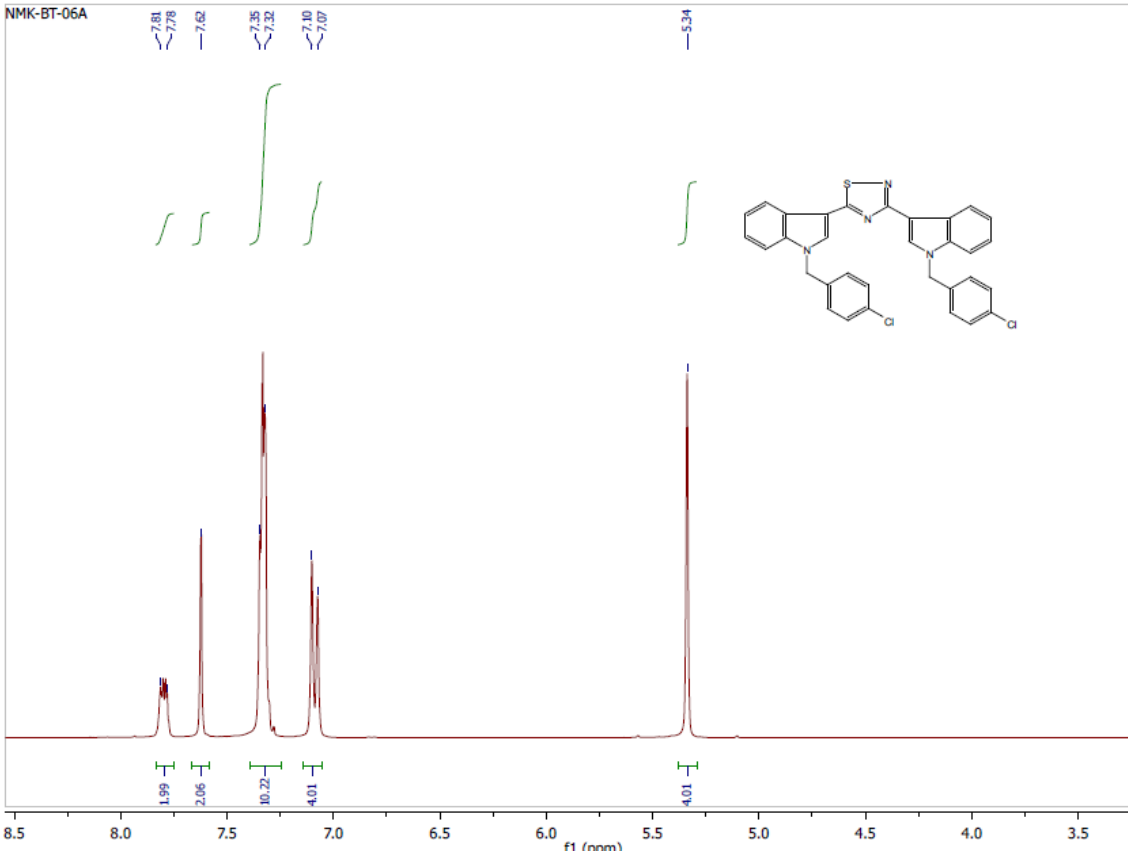


Figure 3.1.6 ^{1}H NMR spectrum of bis(indolyl)-1,2,4-thiadiazole **45c**

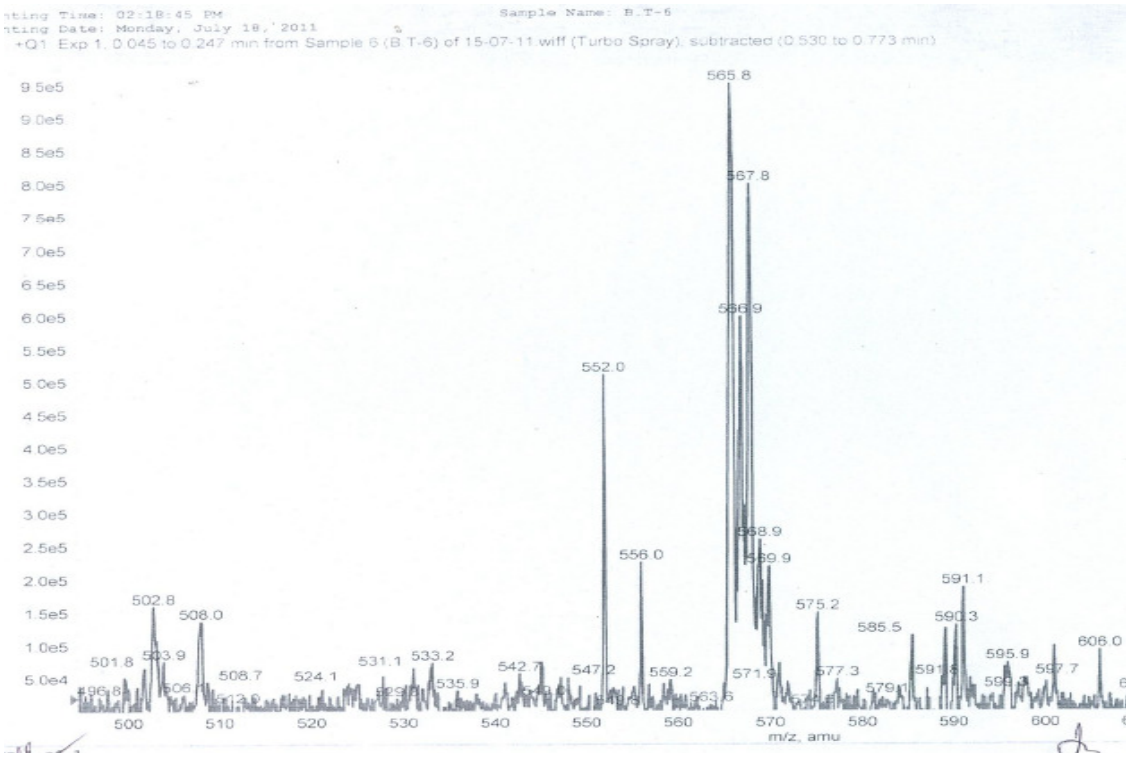
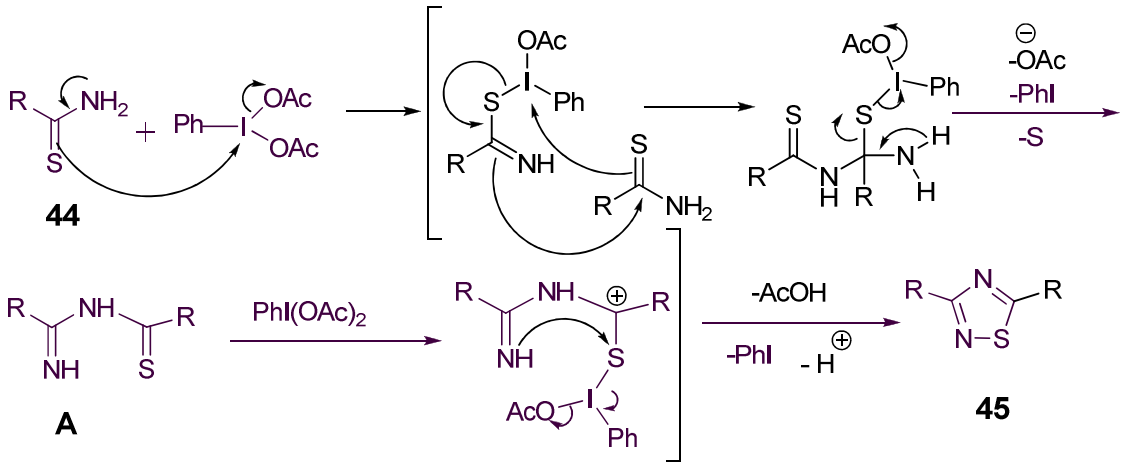


Figure 3.1.7 Mass spectrum of bis(indolyl)-1,2,4-thiadiazole (**45c**)

3.1.5.2 Plausible Mechanism

A plausible mechanism for the formation of 3,5-bis(indolyl)-1,2,4-thiadiazoles (**45a-n**) is mentioned in the scheme 3.1.9. Initial reaction of thioamide (**44**) with IBD generates iminothioamide **A** by elimination of iodobenzene and sulfur. The intermediate **A** which upon reaction with IBD undergoes intramolecular cyclization to produce 1,2,4-thiadiazole (**45**).

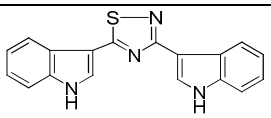
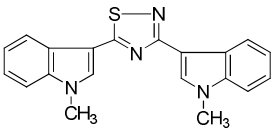
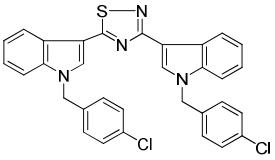
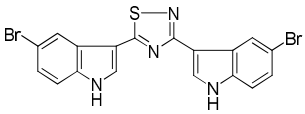
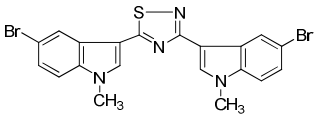
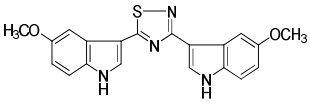
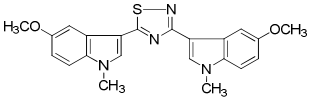
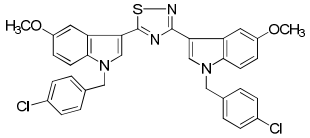
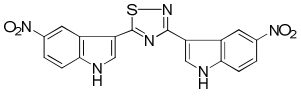


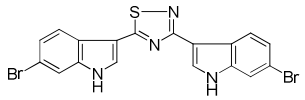
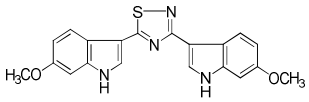
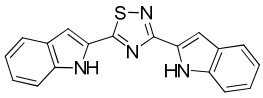
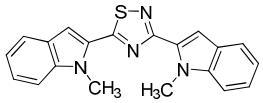
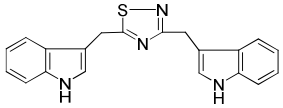
Scheme 3.1.9 Plausible mechanism for the formation of 1,2,4-thiadiazoles (**45**)

3.1.5.3 *In-vitro* anticancer activity

The synthesized 3,5-bis(indolyl)-1,2,4-thiadiazoles (**45**) were screened *in-vitro* for their anticancer activity against human cancer cell lines: prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA-MB-231) and pancreatic (PaCa2). Anticancer activities of bis(indolyl)-1,2,4-thiadiazoles **45** against cancer cell lines are expressed in terms of IC_{50} values (Table 3.1.2).

Table 3.1.2 Anticancer activity of bis(indolyl)-1,2,4-thiadiazoles (**45a-n**)

Bis(indolyl)-1,2,4-thiadiazoles (**45**)		IC_{50} (μM)					
		MDA-MB-231	MCF-7	LnCaP	DU145	PC3	PaCa2
	**(45a)**	701.5	143.2	$>10^3$	248.4	$>10^3$	$>10^3$
	**(45b)**	236.3	177.4	226.3	64	92.3	185.2
	**(45c)**	$>10^3$	$>10^3$	413.3	$>10^3$	$>10^3$	100.6
	**(45d)**	100.3	**31.3**	159.2	56.7	162.2	124.3
	**(45e)**	$>10^3$	63.6	$>10^3$	$>10^3$	$>10^3$	$>10^3$
	**(45f)**	$>10^3$	423.5	295.1	206.2	224	45.8
	**(45g)**	95.7	79.9	111.3	137.1	269.8	177
	**(45h)**	67.9	32.1	**14.6**	369.8	21.4	21.2
	**(45i)**	98.6	83.5	176.6	324.9	679.6	187.3

	**(45j)**	38.3	39.2	80.1	**30.2**	132.5	83
	**(45k)**	44.3	35.3	35.8	42.3	76.1	95.3
	**(45l)**	$>10^3$	162.3	246.8	453	326.2	102.3
	**(45m)**	$>10^3$	66.4	206.8	$>10^3$	432.1	260.2
	**(45n)**	65	40.5	43.3	51.2	44.2	**31.3**

Most of the compounds have shown significant to moderate cancer cell growth inhibition. The effect of various substitutions on indole ring was examined. The structure-activity relationship (SAR) study revealed that the substituents at *N*-1 and C-6 positions of indole ring are crucial for inducing cytotoxicity and selectivity against particular cancer cell line. Bis(indolyl)-1,2,4-thiadiazole **45a** showed modest selective cytotoxicity against MCF-7 breast cancer cell line (IC_{50} = 143.2 µM). Methyl and 4-chlorobenzyl substituents at the *N*-1 position of indole ring led to compounds **45b** and **45c** with improved activity and selectivity against DU145 (IC_{50} = 64 µM) and PaCa2 (IC_{50} = 100.6 µM) cancer cell lines, respectively. Introduction of bromo substituent at C-5 position of the indole ring (compound **45d**) increases the activity and showed selective inhibition against MCF-7 (IC_{50} = 31.3 µM) cell lines. Its *N*-methyl derivative **45e** is exclusively selective cytotoxic against MCF-7 cancer cell line (IC_{50} = 63.6 µM). It was observed that compound **45f** with 5-methoxyindole was successful to induce selectivity against PaCa2 cancer cell line (IC_{50} = 45.8 µM) but failed to improve the activity against other cell lines. The N-methylation of **45f** led to the compound **45g** with improved activity except against PaCa2 cell line. The *N*-chlorobenzyl derivative **45h** demonstrated good inhibitory effects against all the tested cancer cell lines, especially against LnCap (IC_{50} = 14.6 µM). Introduction of 5-nitro group moderately improved the activity (compounds **45i** *vs* **45a**) against breast cancer cell lines. The compounds **45j** and **45k** with bromo or methoxy group at C-6 position of indole ring showed better cytotoxicity when compared with its regioisomers **45d** and **45f** having similar substituent at C-5 position. Shifting the connectivity of 1,2,4-thiadiazole with indole from C-3 to C-2 position led to the

compounds **45l** and **45m** with reduced activity except for compound **45m** which displayed moderate selective cytotoxicity against MCF-7 (IC_{50} = 66.4 μM). Further, introduction of a methylene unit between indole and 1,2,4-thiadiazole ring resulted in compound **45n** with broad-spectrum of improved activity in all the cell lines employed (IC_{50} = 31.3 μM). By altering the central heterocyclic ring, anticancer activities of bis(indolyl)heterocycles varies against different cancer cell lines. For example, inhibitory activity against MCF-7 cells of 3,5-bis(indolyl)-1,2,4-thiadiazole **45a** is better than reported 2,4-bis(3-indolyl)thiazole (**2**). The 3,5-bis(6-bromo-3-indolyl)-1,2,4-thiadiazole **45j** is less potent towards MCF-7 cells than the reported 2,4-bis(6-bromo-3-indolyl)thiazole but more selective. The 3,5-bis(5-methoxy-3-indolyl)-1,2,4-thiadiazole **45f** showed poor activity against MCF-7 cell line when compared with 2,5-bis(3-indolyl)thiophene (**3**). For exact comparison and to identify the role of heterocyclic ring in various bis(indolyl) heterocycles more analogues of 3,5-bis(indolyl)-1,2,4-thiadiazoles need to be synthesized.

3.1.6 Conclusions

We have reported a facile synthesis of novel 3,5-bis(indolyl)-1,2,4-thiadiazoles (**45a-n**) by iodobenzene diacetate-mediated oxidative-dimerization of indole-3-thiocarboxamides (**44a-n**). Most of the 3,5-bis(indolyl)-1,2,4-thiadiazoles exhibited pronounced antitumor activity against a panel of cancer cell lines. In particular, the indoly-1,2,4-thiadiazole **45h** was identified as the most potent compound in this series with IC_{50} values of 14.6, 21.4 and 21.4 μM against cancer cell lines LnCaP, PC3 and PaCa2, respectively. When compared with reported bis(indolyl) heterocycles, the present series of 3,5-bis(indolyl)-1,2,4-thiadiazoles showed comparable anticancer activity.

3.1.7 Experimental procedures

General: All reagents such as indole, iodobenzene diacetate were purchased from Aldrich chemicals. The reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized under UV light (254 nm). Column chromatography was performed using 100-200 mesh silica gel and appropriate mixture of hexane and ethyl acetate for elution. Melting points (mp) were determined with *E-Z*-Melt automated melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker Avance II spectrophotometer. Mass spectra were obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972.

3.1.7.1 Synthetic procedures for 3,5-bis(indolyl)-1,2,4-thiadiazoles (45a-n)

Indole-3-carboxaldehyde (**42**)

A round-bottomed flask containing 28.8 mL (27.4 g, 370 mmol) of freshly distilled dimethylformamide (DMF) was cooled in an ice-salt bath for about 0.5 h and 8.6 mL (14.4 g, 90 mmol) of freshly distilled phosphorus oxychloride was subsequently added with stirring to the DMF over a period of 0.5 h. A solution of indole (**41**, 10 g, 85.47 mmol) in DMF (9.5 g, 130 mmol) was added to the yellow solution over a period of 1 h. The solution was stirred at 35 °C to become a yellow paste. At the end of the reaction 30 g of crushed ice was added to the paste with stirring which becomes a clear cherry-red aqueous solution. A solution of sodium hydroxide (37.5 g, 94 mmol) in 100 mL of water was added dropwise with stirring. The resulting suspension was heated rapidly to 90 °C and allowed to cool to room temperature, after which it was placed in refrigerator overnight. The product was filtered, washed with water (2 × 100 mL) and air-dried to afford the 12 g of pure indole-3-carboxaldehyde (**42**). Yield 90%; Pale yellow solid; mp 194-196 °C (Lit.[55] 196-197 °C). Similar procedure was used to prepare 5-bromo, 5(6)-methoxyindole-3-carboxaldehyde and their melting points are in agreement with commercial available materials.

Alkylation of indole-3-carboxaldehydes was achieved by reacting indole-3-carboxaldehydes (5g, 34 mmol), 50% aqueous sodium hydroxide (40 mL), water (60 mL), and tetrabutylammonium bromide (0.1 g, 3.4 mmol) was added methyl iodide (14.5 g, 103.4 mmol) or chloro benzylchloride (11.0 g, 68 mmol) in toluene (30 mL). After the completion of reaction, organic phase was washed twice with aqueous $NaHCO_3$ (50 mL), water and saturated brine (100 mL), and then dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and residue was washed with anhydrous ether to give crude **42**. Recrystallization from ethyl acetate and hexane led to a colorless crystalline product **42**. 1-Methylindole-3-carboxaldehyde-(14 g, Yield 87%), mp 68-70 °C (Lit[56]. 67-72 °C) 1-(4-Chlorbenzyl)-indole-3-carboxaldehyde[57] (7.8 g, 85%) mp. 124-126 °C

Synthesis of indole-3-carbonitrile (**43**)

To a stirred solution of Indole-3-carboxaldehyde (**42**, 3.0 g, 0.02 mol) in formic acid (25 mL) was added sodium formate 2.80 g (0.04 mol) and hydroxylamine hydrochloride 1.38 g (0.01 mol). The reaction mixture was refluxed for 3 h at 130 °C. After completion of the reaction as monitored by TLC, cooled the reaction mixture to room temperature

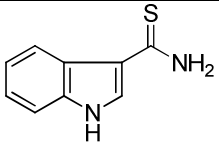
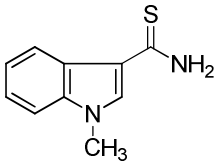
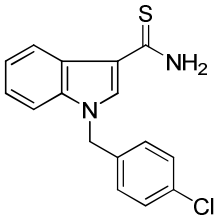
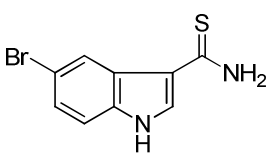
and poured into ice-cold water (100 mL) and extracted with dichloromethane (2×30 mL). The combined extracts were washed with saturated sodium bicarbonate solution (30 mL) and then with brine solution (30 mL). Organic phase was separated, dried over sodium sulfate and excess of solvent was distilled off. The residue so obtained was subjected to a silica gel column chromatography (hexane & ethyl acetate as eluent) to afford pure indole-3-carbonitrile (**43a**) mp.179-180 °C (Lit[30] 178-180 °C). Similar procedure was used to synthesize series of nitriles **43b-k**.[30] The melting points of the products **43a-k** were in agreement with the reported in literature.[58]

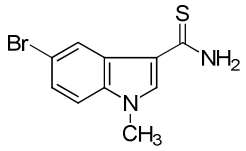
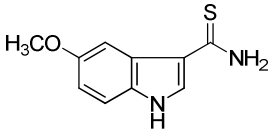
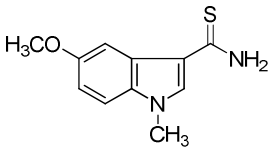
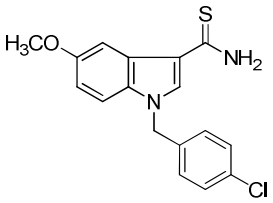
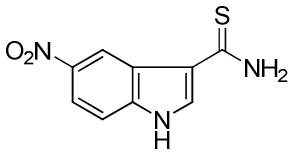
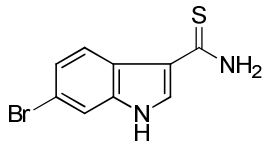
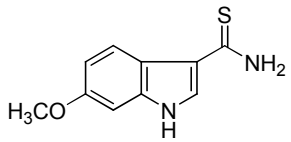
Indole-2(3)-thiocarboxamide (**44a-k**)

Indole-3-thiocarboxamide (**44**)

A mixture of indole-3-carbonitrile (**43**, 0.02 mol), sodium hydrosulfide (0.04 mol) and magnesium chloride hexahydrate (0.02 mol) were taken in a round bottomed flask and stirred for 10 h at 40 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, contents were poured into cold water (50 mL) and solid obtained was filtered. Solid was re suspended in HCl solution (3N) and stirred for 10 min and then filtered-off the solid to obtain indole-3-thiocarboxamide (**44**)

Physical data for indole-2(3)-thiocarboxamides (**44**)

Indolethiocarboxamides (**44**)		Analytical Data
	(**44a**)	Yield 75%; mp 149-151 °C (Lit[30] 148-152 °C); IR (KBr, *v* cm^{-1}): 3386, 3276, 3180, 1623, 1529, 1442, 1365, 1155, 765.
	(**44b**)	Yield 60 %; mp 179-181 °C (Lit[59] 178-182 °C); IR (KBr, *v* cm^{-1}): 2966, 1627, 1533, 1433, 1228, 794.
	(**44c**)	Yield 63%; mp 137-139 °C; IR (KBr, *v* cm^{-1}): 2945, 1645, 1565, 1475, 1442, 1223, 786.
	(**44d**)	Yield 57 %; mp 241-242 °C (Lit[30] 240-242 °C); IR (KBr, *v* cm^{-1}): 3381, 3190, 1623, 1525, 1435, 1295, 1191, 794.

	(**44e**)	Yield 57 %; mp 180-182 °C; IR (KBr, *v* cm^{-1}): 2943, 1618, 1586, 1438, 1223, 1145, 765.
	(**44f**)	Yield 57 %; mp 225-227 °C (Lit[30] 226-228 °C); IR (KBr, *v* cm^{-1}): 3200, 2837, 1630, 1510, 1435, 1234, 790;
	(**44g**)	Yield; 65%; mp 142-145 °C; IR (KBr, *v* cm^{-1}): 2837, 1626, 1567, 1435, 1235, 1154, 754.
	(**44h**)	Yield 60%; White solid; mp 195-197 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 2H), 8.20 (d, *J* = 7.8 Hz, 1H), 7.79 (s, 1H), 7.68 (s, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 7.8 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 5.58 (s, 2H), 3.83 (s, 3H); IR (KBr, *v* cm^{-1}): 2966, 1672, 1537, 1492, 1462, 1228, 794; MS FAB: *m/z* calcd. for $C_{17}H_{15}ClN_2OS$ 330.05, found: 330.1$(M)^+$.
	(**44i**)	Yield 55%; yellow solid; mp 189-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 11.34 (s, 1H), 8.74 (s, 1H), 8.62 (s, 2H), 8.10 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 8.0Hz, 1H), 7.47 (s, 1H); IR (KBr, *v* cm^{-1}): 3245, 3176, 1627,1447, 1408, 1365, 1204, 1147, 794; MS (FAB): *m/z* calcd. for $C_9H_7N_3OS$: 221.03, found: 221.0 $(M)^+$.
	(**44j**)	Yield 64 %; mp 192-194 °C (Lit[21] 193-196 °C); IR (KBr, *v* cm^{-1}): 3234, 3176, 1630,1434, 1365, 1204, 1147, 794.
	(**44k**)	Yield 70 %; mp 238-241 °C (Lit[30] 240 °C); IR (KBr, *v* cm^{-1}): 3235, 2985, 1633,1495, 1390, 1233, 1147, 794.

Indole-2(3)-thiocarboxamides (44l-n)

To a stirred solution of indole-2-carboxylic acid **46l** or 1-methylindole-2-carboxylic acid **46m** or indole-3-aceticacid **46n** (0.01 mol) in 15 mL of THF was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (0.01 mol) and 1-hydroxybenzotriazole (0.001 mol) at 20 °C temperature. Reaction mixture was stirred at this temperature for 20 min and cooled the contents to 0 °C and 25% ammonia solution (1 mL) was added and stirred for 4 h. After completion of the reaction as monitored by TLC poured in to ice

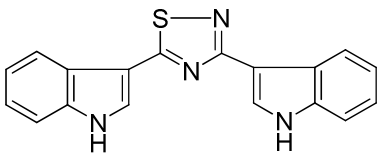
cold water (30 mL) and neutralized with citric acid solution. Solid obtained was filtered and purified by recrystallization from ethanol to get pure indole-2(3)-carboxamides (Yield 60-70%).

To above obtained indole-2(3)-carboxamides (0.01mol) in THF (20 mL) was added Lawesson's reagent (0.01 mol) and stirred the reaction mixture for 2 h at 60 °C. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and the residue was subjected to silica gel column chromatography and eluted with ethyl acetate: hexane (1:1) to obtain indole-2(3)-thiocarboxamide (**44l-n**) in good yields (70-75%).

Synthesis of 3,5-bis(indolyl)-1,2,4-thiadiazoles (45a-n)

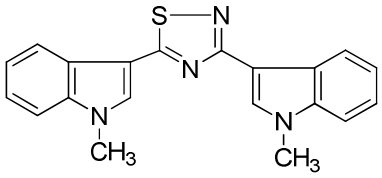
Iodobenzene diacetate (1 mmol) was added to a stirred solution of indole-2(3)-thiocarboxamide (**44a-n**, 1 mmol) in dichloromethane (5 mL) at 25 °C. Progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, the precipitated sulfur was removed and washed the resulting solution with saturated sodium bicarbonate (25 mL) and brine (25 mL) solution. Organic phase was dried over anhydrous sodium sulfate and distilled off at reduced pressure. The crude product obtained was recrystallized from chloroform-methanol. The compounds **45d**, **45i**, **45J** and **45l** were purified by column chromatography on silica gel (100-200 mesh) using ethyl acetate and hexane as eluent.

3,5-Bis(3′-indolyl)-1,2,4-thiadiazole (45a)



Yield 65%; Pale yellow solid; mp 168–170 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.68 (s, 2H), 7.78 (d, 2H, J = 2.92 Hz), 7.68 (d, 2H, J = 7.5 Hz), 7.50 (d, 2H, J = 7.8 Hz), 7.29-7.21 (m, 4H); IR (KBr, ν cm^{-1}): 3109, 1660, 1583, 1523, 1433, 1240, 736; MS (FAB): *m/z* calcd. for $C_{18}H_{12}N_4S$: 316.07, found: 317.08 $(M+H)^+$.

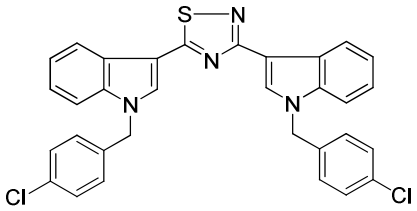
3,5-Bis(3′-(1-methylindolyl))-1,2,4-thiadiazole(45b)



Yield 60%; Pale brown solid; mp 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.33-8.30 (m, 2H), 7.67 (s, 2H), 7.38-7.33 (m, 6H), 3.87 (s, 6H); IR (KBr, ν cm^{-1}): 1640, 1566, 1556, 1477, 1440, 1240, 792; MS (FAB)

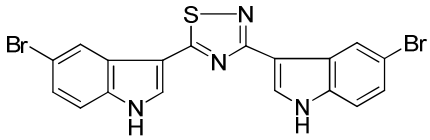
m/z calcd. for $C_{20}H_{16}N_4S$: 344.10, found: 345.1$(M+H)^+$.

3,5-Bis(3′-(1-(4-chlorobenzyl)lindolyl))-1,2,4-thiadiazole (45c)



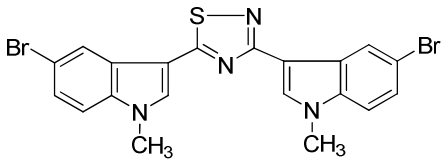
Yield 72%; Brown solid; mp 146–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81-7.78 (m, 2H), 7.62 (s, 2H), 7.35-7.32 (m, 10H), 7.10-7.07 (m, 4H), 5.34 (s, 4H); IR (KBr, *v* cm^{-1}): 3099, 2926, 1597, 1562, 1492, 1246, 858, 744; MS (FAB): *m/z* calcd. for $C_{32}H_{22}C_{l2}N_4S$: 564.7, found: 565.8 $(M+H)^+$.

3,5-Bis(3′-(5′-bromoindolyl))-1,2,4-thiadiazole(45d)



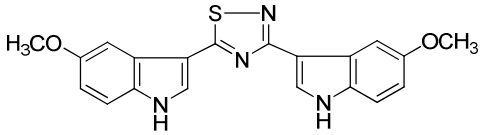
Yield 55%; Brown solid; mp 163–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 2H), 8.68 (s, 2H), 8.05 (d, *J* = 2.6 Hz, 2H), 7.39-7.25 (m, 4H); IR (KBr, *v* cm^{-1}): 3124, 2926, 1730, 1579. 1566, 1514, 1469, 1278, 794; MS (FAB): *m/z* calcd. for $C_{18}H_{10}Br_2N_4S$: 471.1, found: 489.1 $(M+NH_4)^+$.

3,5-Bis(3′-(1-methyl-5′-bromoindolyl))-1,2,4-thiadiazole (45e)



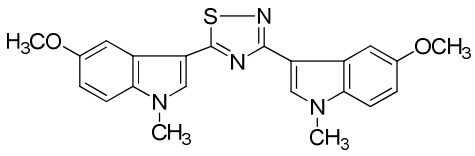
Yield 60%; Brown solid; mp 160–161 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (s, 2H), 8.03 (d, 2H, *J* = 2.8 Hz), 7.34-7.30 (m, 4H), 3.81 (s, 6H); IR (KBr, *v* cm^{-1}): 3265, 1606, 1546, 1485, 1479, 1280, 792; MS (FAB): *m/z* calcd. for $C_{20}H_{14}Br_2N_4S$: 499.9 found: 499.9 $(M)^+$and 539.0 $(M+K)^+$.

3,5-Bis(3′-(5′-methoxyindolyl))-1,2,4-thiadiazole (45f)



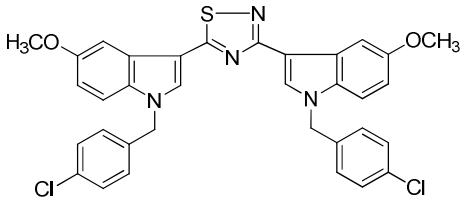
Yield 70%; White solid; mp 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (s, 2H), 7.68 (d, *J* = 3.0 Hz, 2H), 7.34 (d, 2H, *J* = 8.84 Hz), 7.18 (d, *J* = 2.4 Hz, 2H), 6.97 (dd, *J* = 8.84, 2.4 Hz, 2H), 3.88 (s, 6H); IR (KBr, *v* cm^{-1}): 3155, 2998, 1678, 1585. 1558, 1520, 1455, 1245, 1187, 796; MS (FAB): *m/z* calcd. for $C_{20}H_{16}N_4O_2S$: 376.1, found: 399.08 $(M+Na)^+$.

3,5-Bis(3′-(1-methyl-5′-methoxyindolyl))-1,2,4-thiadiazole (45g)



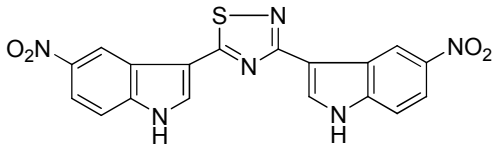
Yield 65%; White solid; mp 142–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 7.74 (d, 2H, J = 2.8 Hz), 7.43 (d, 2H, J = 8.0 Hz), 7.29 (d, 2H, J = 3.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 3.86 (s, 6H), 3.77 (s, 6H); IR (KBr, v cm^{-1}): 2966, 1672, 1537, 1492, 1462, 1228, 794; MS (FAB): *m/z* calcd. for $C_{22}H_{20}N_4O_2S$: 404.10, found: 405.0 $(M+H)^+$.

3,5-Bis(3′-(1-(4-chlorobenzyl)-5′-methoxyindolyl))-1,2,4-thiadiazole (45h)



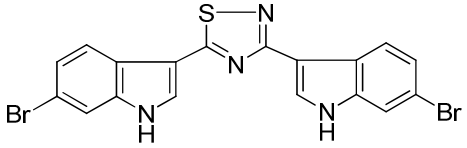
Yield 60%; Brown solid; mp 137–139 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.49 (s, 2H), 7.25-7.22 (m, 4H), 7.11-7.08 (m, 4H), 6.98 (d, 4H, J = 8.0 Hz), 6.87-6.84 (m, 2H), 5.21 (s, 4H), 3.79 (s, 6H); IR (KBr, v cm^{-1}): 2947, 1580, 1529, 1480, 1445, 1228, 796; MS (FAB): *m/z* calcd. for $C_{34}H_{26}Cl_2N_4O_2S$: 624.1, found: 625.0 $(M+H)^+$.

3,5-Bis(3′-(5′-nitroindolyl))-1,2,4-thiadiazole (45i)



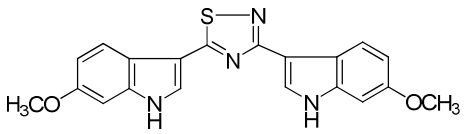
Yield 52%; Brown solid; mp 178–179 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.45 (s, 2H), 8.45 (d, 2H, J = 8.0 Hz), 7.95 (s, 2H), 6.89-6.75 (m, 4H); IR (KBr, v cm^{-1}): 3356, 3134, 1622, 1585, 1471, 1240, 736; MS (FAB): *m/z* calcd. for $C_{18}H_{12}N_7O_4S$: 406.05, found: 407.06 $(M+H)^+$.

3,5-Bis(3′-(5′-bromoindolyl))-1,2,4-thiadiazole (45j)



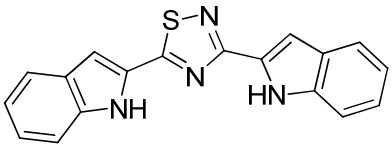
Yield 52%; Brown solid; mp 190–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 2H), 8.25 (s, 2H), 8.12 (d, J = 2.9 Hz, 2H), 7.42-7.38 (m, 4H); IR (KBr, v cm^{-1}): 3169, 2895, 1612, 1523, 1469, 1240, 796; MS (FAB): *m/z* calcd. for $C_{18}H_{10}Br_2N_4S$: 471.9, found: 472.9 $(M+H)^+$.

3,5-Bis(3′-(6′-methoxyindolyl))-1,2,4-thiadiazole (45k)



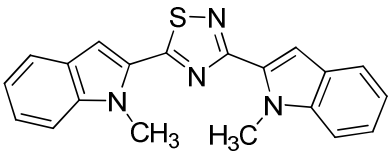
Yield 70%; Brown solid; mp 180–181 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 2H), 7.53-7.50 (m, 4H), 6.7-6.82 (m, 4H), 3.77 (s, 6H); IR (KBr, *v* cm^{-1}): 3219, 1631, 1585, 1469, 1265, 808; MS (FAB): *m/z* calcd. for $C_{20}H_{16}N_4O_2S$: 376.1, found: 377.1 $(M+H)^+$.

3,5-Bis(2′-indolyl)-1,2,4-thiadiazole (45l)



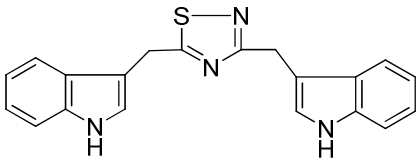
Yield 62%; Brown solid; mp 183–185. °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.94 (s, 2H), 7.33-7.27 (m, 4H), 7.09-7.04 (m, 2H), 6.83-6.80 (m, 4H); IR (KBr, *v* cm^{-1}): 3053, 2972, 1614, 1579, 1556, 1514, 1454, 1261, 804, 736; MS (FAB): *m/z* calcd. for $C_{18}H_{12}N_4S$: 316.07, found: 317.60 $(M+H)^+$.

3,5-Bis(2′-(1-methylindolyl))-1,2,4-thiadiazole (45m)



Yield 68%; Brown solid; mp 132–133 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68-7.65 (m, 2H), 7.44 (s, 2H), 7.30-7.19 (m, 6H), 3.75 (s, 6H); IR (KBr, *v* cm^{-1}): 3026, 1554, 1531, 1469, 1421, 1265, 788; MS (FAB): *m/z* calcd. for $C_{20}H_{16}N_4S$: 344.1, found: 344.1 $(M)^+$ and 383.1 $(M+K)^+$.

3,5-bis((3′-indolyl)methyl)-1,2,4-thiadiazole (45n)

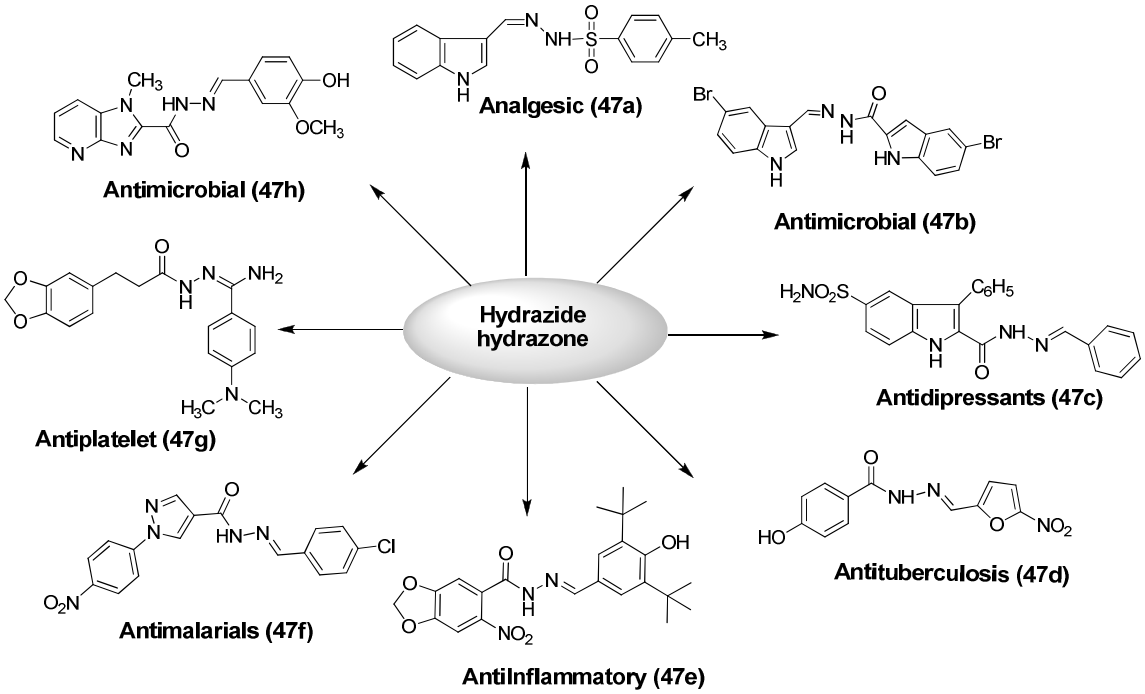


Yield 52%; Brown solid; mp 170–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.39-7.33 (m, 4H), 6.67-6.58 (m, 4H), 3.75 (s, 4H); IR (KBr, *v* cm^{-1}): 3245, 2928, 1573, 1460, 1232, 767; MS (FAB): *m/z* calcd. for $C_{20}H_{16}N_4S$: 344.1, found: 345.1 $(M+H)^+$.

3.2 .Part B: Bis(indolyl)hydrazides-hydrazone as anticancer agents

3.2.1 Significance of hydrazide-hydrazones in drug discovery

Hydrazide-hydrazones are of wide interest because of their diverse pharmacological and chemical applications. A large variety of hydrazide-hydrazones have been synthesized and screened for their various biological activities such as anticonvulsant,[60] antidepressant (**47c**),[61] analgesic (**47a**),[62] antiinflammatory (**47e**),[63] antiplatelet,[64] antimalarial (**47f**),[65] antimicrobial (**47h**),[66] antimycobacterial (**47b**),[67] antitumor (**47g**)[68] and antituberculosis (**47d**).[69] Hydrazone-hydrazides possessing an azomethine (-NHN=CH-) group constitute an important class of compounds that have been used to identify novel hydrazide-hydrazones with significant biological activities (figure 3.2.1).



Check structure 47g

Figure 3.2.1 Biological significance of hydrazide-hydrazones (**47a-h**)

The extensive study on hydrazones and hydrazides resulted in well known drugs Isoniazide, Nifuroxazide and several other drug candidates which are in various phases of the clinical studies. Hydrazones of isoniazide (INH) reported by Sah *et al.* showed prominent antituberculosis activity against *M. tuberculosis* and have lower toxicity than the parent isoniazide.[70] The reduction products of INH analogues such as iproniazide and isocarboxazide were developed for the treatment of tuberculosis.[71] Antiepileptic activity

of hydrazones of (2-oxobenzoxazoline-3-yl)acetohydrazide (**48a**) have been tested in scPTZ and the compound with fluoro substituent was found to be potent. Hydrazones of principal inhibitory neurotransmitter in the mammalian brain, GABA, were evaluated for their anticonvulsant properties in different animal models of epilepsy.[60] The *N*-acylarylhydrazones synthesized from natural safrole were more potent than marketed drugs Dipyrone and Indomethacin.[72] Duarte *et al*. have described *N*′-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-6-nitro-1,3-benzodioxole-5-carbohydrazine (**48c**) as a novel antiinflammatory compound.[73] In another work, Sunidhi *et al.* synthesized a number of indole substituted hydrazides as an antiinflammatory agents.[63] 3-Phenyl-5-sulfonamidoindole-2-carboxylic acid hydrazides reported as anti-depressant agents at 100 mg/kg.[61] In the search for novel chemical entities, hydrazide-hydrazones were emerged as highly potent anticancer agents. Vicini *et al.* reported variety of benzo[*d*]isothiazole-3-carboxylic acid hydrazides (**48b**) for their antiproliferative activity against several leukaemia and solid tumor cell lines and suggested the importance of hydrazide functionality for intermolecular hydrogen bonding.[74] Recently, Zheng *et al.* reported novel pyrazole derivatives (**48c**) as apoptosis inducers of A549 lung cancer cell lines (IC_{50} = 0.28 μM).[75] Karalı *et al.* synthesized quinoline derivatives **48e** with potent activity against set of renal cancer cell lines. Song *et al.* reported 3,4,5-trimethoxybenzohydrazide-hydrazones (**48a**) for their anticancer activity against PC3 cancer cell lines (IC_{50} = 0.2 μM).[76] The 5-chloro-3-methyl-indole-2-carboxylic acid benzylidene hydrazides (**48h**) were identified as potent apoptosis inducers by inhibiting tubulin polymerization in G2/M phase against breast cancer cell lines T47D (EC_{50} = 2.0 μM).[77] Most recently, Vogel *et al.* reported aroyl hydrazones of 2-phenylindole-3-carboxaldehydes (**48g**) with excellent anticancer activity by blocking of cell cycle in G_2/M phase and drive the tumor cells into apoptosis as demonstrated by the strong increase of caspase-3 activity against MCF-7 breast cancer cells (IC_{50} = 20–30 nM).[78] Sirisoma *et al.* studied the hydrazides of oxindoles (**48d**) as potent inhibitors of tubulin polymerization (IC_{50} = 0.19-0.97μM).[79]

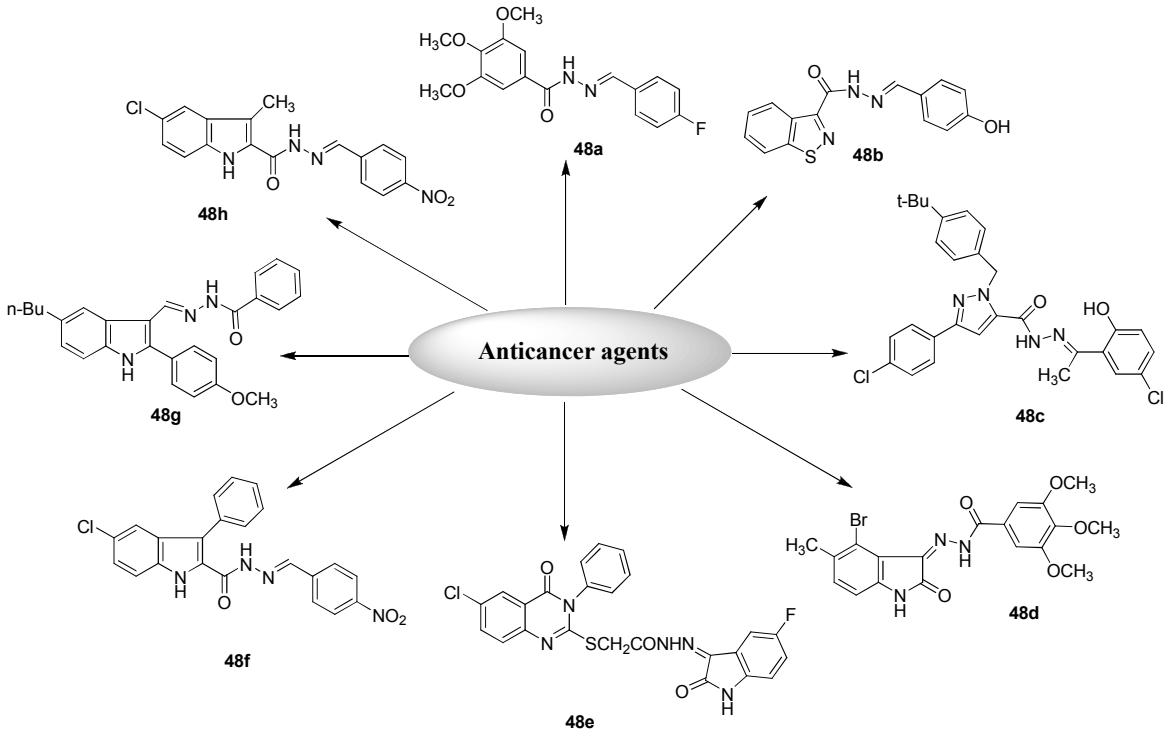


Figure 3.2.2 Hydrazide-hydrazones as potent anticancer agents (**48a-h**)

3.2.2 Rational design

Natural and synthetic bis(indolyl)heterocycles are known for their wide range of biological activitiesIn bis(indoles), various spacers such as heterocyclic rings, linear functional groups like diketo and diamides, hydrazides have been evaluated for their biological activities. In literature, it is well documented that central spacers in bis(indole) analogues play a crucial role in altering the anticancer activity. In a way to find potent anticancer agents and interesting biological features of bis(indoles) and hydrazide-hydrazones, we have prepared a novel series of bis(indolyl)hydrazide-hydrazones by replacing the central heterocyclic ring spacer of bis(indole) alkaloids with an active pharmacophore hydrazide-hydrazone (–CO-NH-N=CH-) . Our rational approach includes the substitution of heterocyclic spacer as well as incorporation of key structural features of indole-based anticancer drug candidate (figure 3.9).[80]

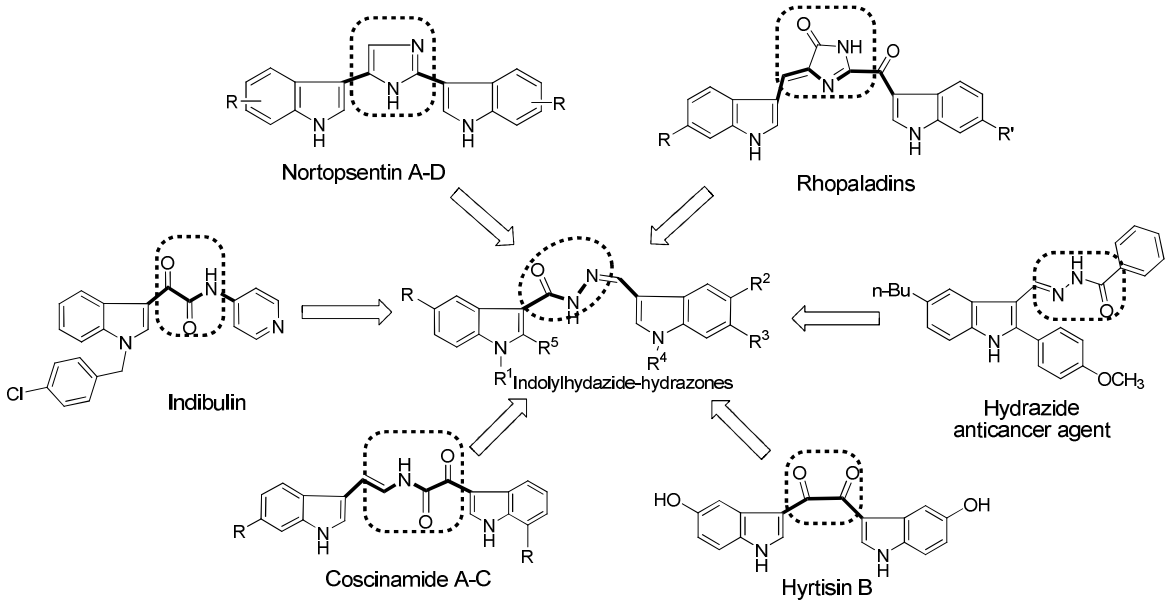
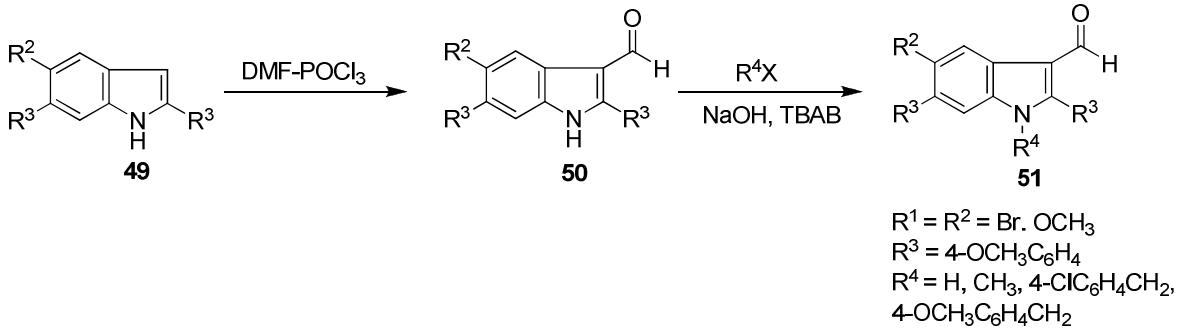


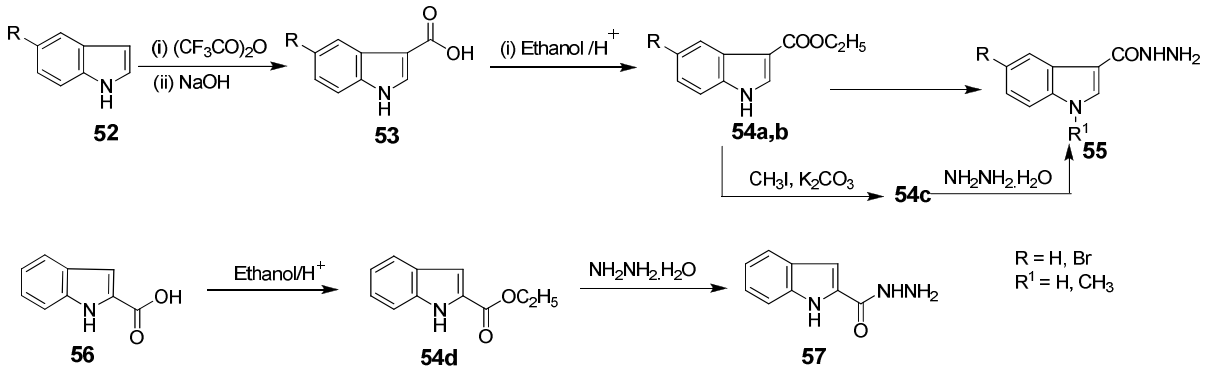
Figure 3.2.3 Rational design for bis(indolyl)hydrazide-hydrazones (**58**)

3.2.3 Results and discussion

3.2.3.1 Chemistry

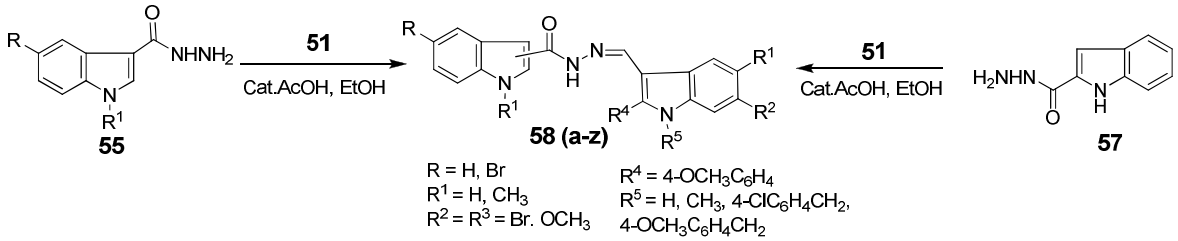
Synthesis of bis(indolyl)hydrazide-hydrazones involves initial preparation of intermediates indole-3-carboxaldehydes (**50** and **51**) and indole-2(3)-carbohydrazides (**55** and **57**). Several of indole-3-carboxaldehydes (**50**) were synthesized through Vilsmeier-Haack formylation of corresponding indoles **49**. Further, alkylation leads to the compounds **51**. On the other hand, indole-3-carboxylic acids (**53**) were prepared by reacting indoles with trifluoroacetic anhydride and followed by hydrolysis. Synthesized indole-3-carboxylic acids were esterified (**54**) and later reacted with hydrazine hydrate to afford corresponding indole-3-carbohydrazides (**55**). Indole-2-carbohydrazide (**57**) was prepared form commercially available indole-2-caboxylic acid (**56**) *via* preparation of ester **54d** (Scheme 3.2.1).





Scheme 3.2.1 Synthesis of indole-3-carboxaldehydes (**50** and **51**) and indole-2(3)-carbohydrazides **55** and **57**

Synthesis of targeted bis(indole)hydrazide-hydrazones **58a-z** were achieved by reacting indole-3-carboxaldehydes (**51**) with indole-2(3)-carbohydrazides (**55/57**) in presence of catalytic amount of acetic acid as illustrated in scheme 3.2.2.



Scheme 3.2.2 Synthesis of bis(indole)hydrazide-hydrazones (**58a-z**)

Various indole-carboxaldehydes and indole-2(3)-carbohydrazides were used to synthesize a series of bis(indolyl) hydrazide-hydrazones (**58a-z**) in good yields (75-85%). All the synthesized bis(indolyl) hydrazide-hydrazones (**58a-z**) were well characterized by their IR, NMR and Mass spectral data. In FT-IR spectra of **58a-z** exhibited hydrazone N-H and C=O stretching bands in the range of 3300-3200 and 1680-1630 cm^{-1}, respectively and azomethine (-CH=N-) group at 1580-1600 cm^{-1}. ^1H NMR spectra of hydrazide-hydrazones (**58a-z**) showed two characteristic singlet's at about 8.5 ppm and 11.00 ppm (broad) due to the azomethine (–CH=N-) and hydrazide (-CO-NH=N-) protons, respectively. The other protons of **58a-n** appeared at the expected chemical shifts and integral values. Mass spectral data of all the compounds **58a-n** are in agreement with their molecular ion peaks. The ^1H NMR and mass spectra of **58f** are displayed in the figures 3.2.4 and 3.2.5.

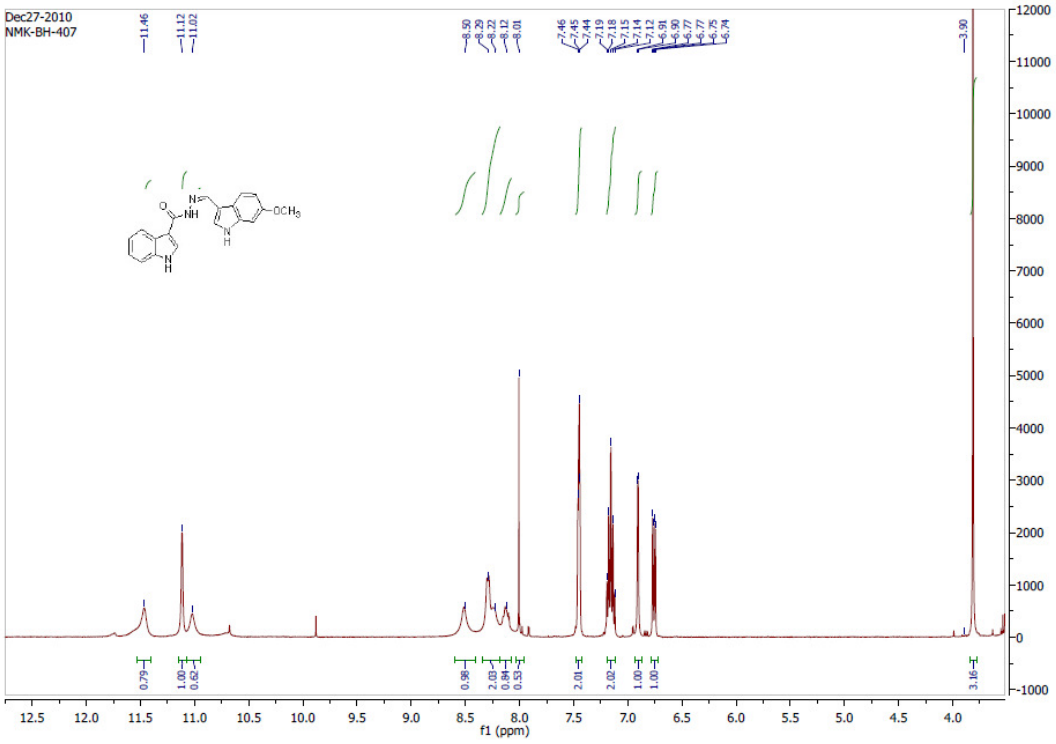


Figure 3.2.4 ^{1}H NMR spectrum of **58f**

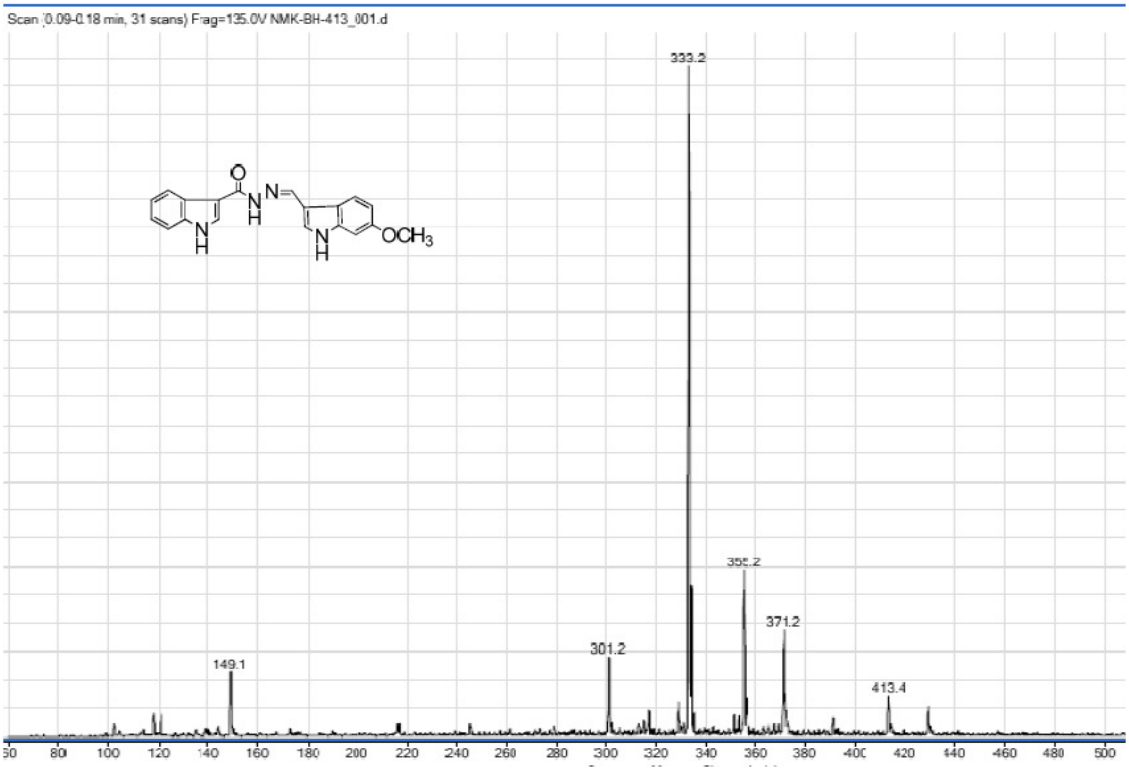
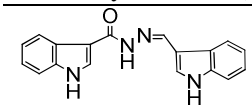
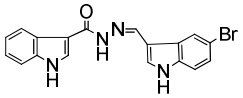
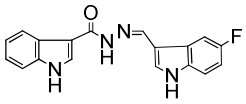
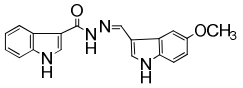
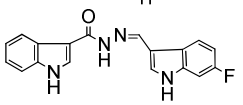
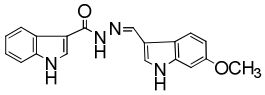
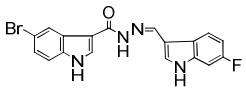
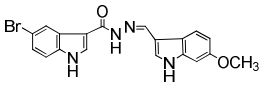
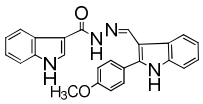
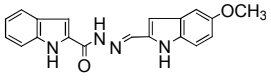
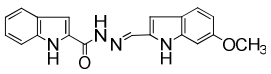
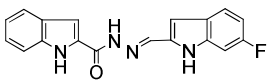
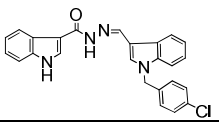


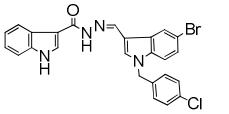
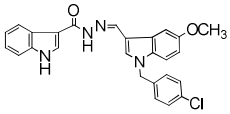
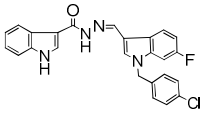
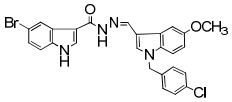
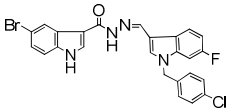
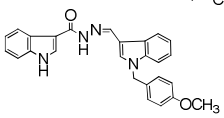
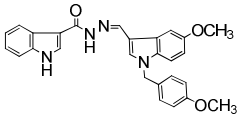
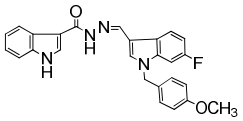
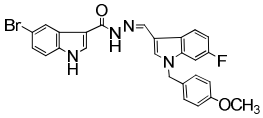
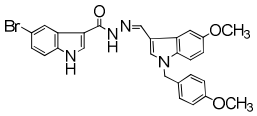
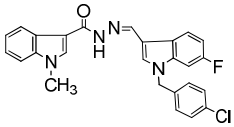
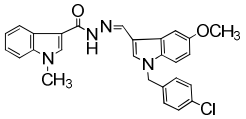
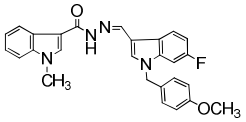
Figure 3.2.5 Mass spectrum of **58f**

3.2.3.2 *In-vitro* anticancer activity of bis(indolyl)hydrazide-hydrazones (58a-z)

All the synthesized compounds **58a-z** were screened for their *in-vitro* cytotoxicity against six human cancer cell lines: prostate (PC3, DU145 and LnCaP), breast (MCF-7 and MDA -MB-231) and pancreatic (PaCa2). Cytotoxicity was expressed in terms of IC_{50} in μM.

Table 3.2.1 *In-vitro* anticancer activity of bis(indolyl)hydrazide-hydrazones (**58a-z**)

Bis(indolyl)hydrazide-hydrazones (**58**)		IC_{50} (μM)					
		LnCaP	MCF-7	MDAMB-231	DU145	PaCa2	PC3
	**(58a)**	35.1	32.6	4.9	37.9	53.9	14.8
	**(58b)**	**3.4**	**2.5**	**1.0**	5.7	8.9	14.5
	**(58c)**	31.3	13.4	5.2	17.2	13.1	28.1
	**(58d)**	28.6	16.1	**3.3**	16.4	12.6	14.4
	**(58e)**	14.3	**3.4**	**1.6**	32.5	11.7	15.1
	**(58f)**	35.8	12.1	**2.3**	12.6	18.3	13.2
	**(58g)**	**3.7**	21.9	**1.3**	**2.7**	14.0	57.0
	**(58h)**	8.8	**1.8**	**1.3**	14.3	15.3	164.1
	**(58i)**	**3.9**	**3.1**	**1.3**	9.0	8.9	15.7
	**(58j)**	**3.1**	6.1	**1.8**	6.3	9.9	14.0
	**(58k)**	**5.0**	**2.0**	**0.7**	**1.1**	6.8	7.1
	**(58l)**	8.8	9.6	**1.0**	**4.4**	9.8	19.0
	**(58m)**	749.3	96.7	66.4	52.1	44.3	16.2

	**(58n)**	**3.7**	**3.2**	**1.0**	**3.6**	8.7	12.4
	**(58o)**	210.2	8.7	82.7	50.0	26.7	30.3
	**(58p)**	55.2	6.0	5.3	18.6	18.0	19.5
	**(58q)**	241.6	29.1	**5.0**	57.0	26.3	15.9
	**(58r)**	24.8	8.3	5.1	12.5	10.6	12.4
	**(58s)**	31.9	12.4	15.1	43.9	41.6	14.6
	**(58t)**	243.0	15.0	9.8	19.1	30.5	13.6
	**(58u)**	446.4	13.1	71.2	16.2	43.3	18.4
	**(58v)**	194.8	31.7	99.8	67.5	45.5	21.8
	**(58w)**	765.7	**3.1**	61.2	84.4	44.5	28.0
	**(58x)**	156.5	90.6	170.7	84.2	50.4	22.1
	**(58y)**	387.9	99.1	194.8	93.0	56.4	21.2
	**(58z)**	9.6	22.6	17.7	15.2	55.8	12.8
Doxorubicin		5.23	11.67	3.07	15.65	18.23	62.26

The activity results mentioned in the table 3.2.1 shows the significant cytotoxic effects of bis(indole)hydrazide-hydrazones **58** against tested cancer cell lines. The structure-activity relationship (SAR) study revealed that the bromo and methoxy substituents at

C-5/C-6 position of indole ring plays an important role in imparting the activity to bis(indolyl)hydrazide-hydrazones **58**. The compound **58a** was found to be selectively cytotoxic towards MDA-MB-231 cell line (IC_{50} = 4.9 μM). Introduction of bromo, fluoro and methoxy substituents in the indole ring was found to be beneficial for the activity. Activity results of bis(indolyl)hydrazide-hydrazones (**58**) revealed that substituents on both the indole rings play a key role in altering their cytotoxicity. The compound **58b** with 5-bromoindole increased the cytotoxicity against all the tested cancer cell line having IC_{50} values 3.4 μM (LnCaP),2.5 μM (MCF-7) and 1.0 μM (MDA-MB-231). Fluoro and methoxy substituents at C-5/C-6 of indole ring led to compounds **58c, 58d, 58e** and **58f** with improved activity against tested cancer cell lines when compared with compound **58a**. Further introduction of C-5 bromo substituent in other indole ring of compounds **58e** and **58f** resulted in analogues **58g** and **58h**, respectively, with improved activity against prostate and breast cancer cell lines. The methoxy/ fluoro group is preferred at C-6 instead C-5 position of indole ring (**58d** *vs* **58f**) for better activity and selectivity. As reported in the literature introduction of 4-methoxyphenyl group enhances the activity of indolyl hydrazides, we prepared compound **58i** with improved activity when compared with parent compound **58a** (IC_{50} = 1.3 μM, MDA-MB-231). It is interesting to note that activity against all the tested cancer cell lines remarkably improved by changing linkage of hydrazide-hydrazone functionality from C-3 to C-2 of indole ring (compounds **58j**, **58k** and **58l**). The compound **58k** with C-6 methoxy substituent in the indole ring is highly cytotoxic against MDA-MB-231 (IC_{50} = 0.7 μM) and found to be the most potent compound (figure 3.2.6). Anticancer activity *N*-alkylated indoles were also studied by introducing methyl, *p*-chlorobenzyl and *p*-methoxybenzyl groups at *N*-1 position of indole ring. Introduction of *p*-chlorobenzyl in **58a**,**b**,**d**,**e** and **g** led to the compounds **58m-r** with reduced cytotoxicity, but for bromo derivative **58n** activity was retained against all the tested cancer cell lines with best result against MDA-MB-231 cancer cell line (IC_{50} = 1.0 μM).

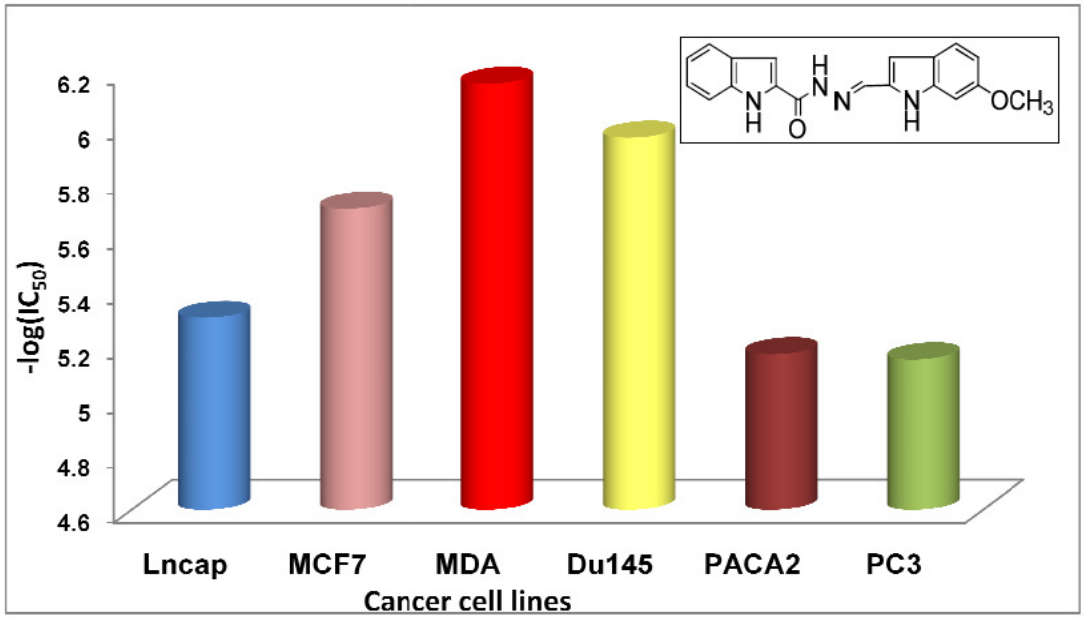


Figure 3.2.6 The most active bis(indole)hydrazide-hydrazone (**58k**)

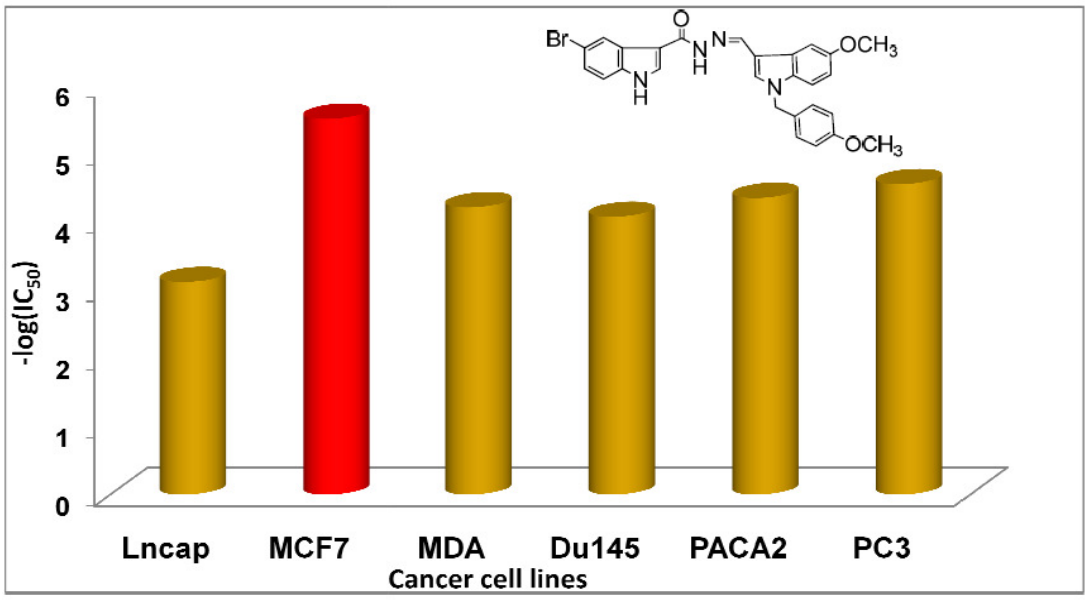


Figure 3.2.7 The most selective bis(indolyl)hydrazide-hydrazone (**58w**)

Compound **58q** (bromo analogue **58o**) has shown selectivity against breast cancer cell lines MDA-MB-231 (IC_{50} = 5 µM). Introduction of 4-methoxybenzyl in place of 4-chlorobenzyl have not shown any significant improvement in cytotoxicity. Compound **58w** showed high selectivity towards MCF-7 (IC_{50} = 3.1 µM; figure 3.2.7). Introduction of methyl group gave mixed results as cytotoxicity was decreased in case of compounds

58x and **58y** and selectivity was seen in case of **58z** against LnCap cancer cell line (IC_{50} = 9.6 µM) with 40 folds selectivity than parent compound **58u**.

3.2.3.3 Bis(indolyl)hydrazide-hydrazones induced apoptosis in MDA-MB-231 cells

Apoptosis, or programmed cell death, plays a vital role in normal embryonic development as well as adult life, such as elimination of dispensable or excess cells. It has been known that defects of apoptosis pathways and the ability to evade cell death is one of the hallmarks of cancers, which results in uncontrollable tumor cell growth, as well as tumor resistance to chemotherapeutic treatment.[78] Therefore, the development of new apoptosis inducers as chemotherapeutic agents is a promising approach. The present series of compounds has been identified as a new class of potent apoptosis inducers. we examined the integrity of nuclei using propidium iodide staining in MDA-MB-231 cells treated with either DMSO or 10 µM concentration of **58a-l**. The MDA-MB-231 cells were chosen as they were found to be most sensitive to bis(indolyl)hydrazide-hydrazones and effectively induced toxicity in cancer cells (table 3.2.1). As shown in figure 3.2.8, treatment of bis(indolyl)hydrazide-hydrazones (**58a-l**) resulted in the formation of condensed nuclei, suggesting that the cell death induced by these compounds is predominantly apoptotic.

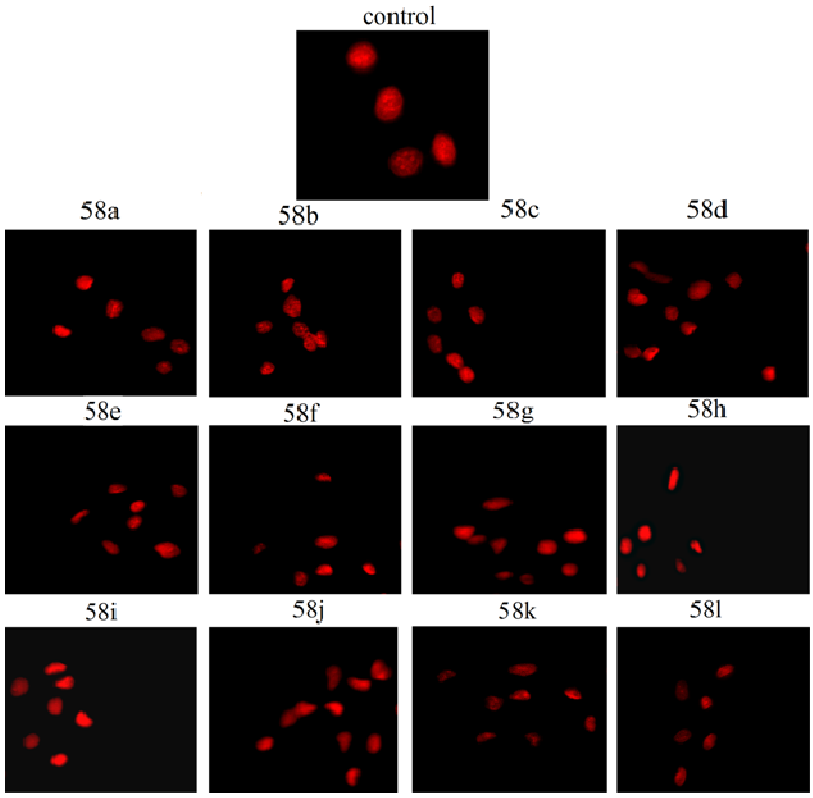


Figure 3.2.8 Bis(indolyl)hydrazide-hydrazones (**58a-l**) induced apoptosis in MDA-MB-231 cells

3.2.4 Conclusions

We have synthesized a novel series of bis(indolyl)hydrazide-hydrazones (**58a-z**). Most of the compounds displayed potent anticancer activity against various human cancer cell lines. The cytotoxicity results of **58** revealed the importance of bromo, fluoro and methoxy substituents in the indole ring. The synthesized hydrazide-hydrazones **58k** and **58w** were identified as the most potent (IC_{50} = 0.7 μM) and selective (MCF-7 , IC_{50} = 3.7 μM) compounds in the series. Many of the hydrazide-hydrazones (**58a-z**) exhibited good cytotoxicity (IC_{50} less than 5 μM) and are selective towards breast cancer cell line (MDA-MB-231). The activity results revealed that bis(indolyl)hydrazide-hydrazones are more potent than analogues of bis(indolyl)heterocycles and replacement of central heterocycles with hydrazide-hydrazone is beneficial for the activity. Due to diverse structural relationship of these compounds, they are interesting candidates to identify novel and selective anticancer agents.

3.2.5 Experimental procedures

General: NMR (^{1}H & ^{13}C) spectra were obtained on Bruker Avance II (400 MHz) spectrometers in DMSO-d_6. The chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972 mass spectrometer. IR spectra were recorded Shimadzu IR-Prestige-21 FTIR spectrophotometer. Melting points were determined on *EZ*-Melt (Stanford Research Systems, USA) automated melting point apparatus and are uncorrected. All the laboratory grade reagents and solvents were obtained commercially. All the reactions were monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel 60 F_{254}, 0.25 mm) and visualized by fluorescence quenching under UV light (254 nm).

3.2.5.1 Synthesis of bis(indolyl)hydrazide-hydrazones (58a-z)

3.2.5.1.1 Indole-3-carboxaldehyde (51)

A round-bottomed flask containing 28.8 mL (27.4 g, 370 mmol) of freshly distilled dimethylformamide (DMF) was cooled in an ice-salt bath for about 0.5 h and 8.6 mL (14.4 g, 90 mmol) of freshly distilled phosphorus oxychloride was subsequently added with stirring to the DMF over a period of 0.5 h. A solution of indole (**49**, 10 g, 85.47 mmol) in DMF (9.5 g, 130 mmol) was added to the yellow solution over a period of 1 h. The solution was stirred at 35 °C till it become a yellow paste. At the end of the reaction,

30 g of crushed ice was added to the paste with stirring which becomes a clear cherry-red aqueous solution. A solution of sodium hydroxide (37.5 g, 94 mmol) in 100 mL of water was added dropwise with stirring. The resulting suspension was heated rapidly to 90 °C and allowed to cool to room temperature, after which it was placed in refrigerator overnight. The product was filtered, washed with water (2 × 100 mL) and air-dried to afford the 12 g of pure indole-3-carboxaldehyde (**50**). Yield 97%; Pale yellow solid; mp 194-196 °C (Lit.[55] 196-197 °C). Similarly 5-bromo, 5(6)-methoxyindole-3-carboxaldehyde and 2-(4-methoxyphenyl)-indole-3-carboxaldehyde were prepared.

Alkylation of indole-3-carboxaldehydes: To a stirred mixture of indole-3-carboxaldehyde **50** (5g, 34 mmol) in 50% aqueous NaOH (40 mL), water (60 mL) and tetrabutylammonium bromide (0.1 g, 3.4 mmol) was added methyl iodide or 4-chlorobenzylchloride or 4-methoxybenzylchloride (34 mmol) in toluene (30 mL). After the completion of reaction, organic phase was washed twice with aqueous $NaHCO_3$ (50 mL), water and saturated brine (100 mL), and then dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and residue was washed with anhydrous ether to give crude **51** which upon recrystallization with ethylacetate and hexane led to pure product.

1-(4-Chlorobenzyl)-indole-3-carboxaldehyde (yield 85%) mp 123-124 °C

1-(4-Methoxybenzyl)-indole-3-carboxaldehyde (yield 80%) mp 126-128 °C

5-Bromo-1-(4-chlorobenzyl)-indole-3-carboxaldehyde (yield 80%) mp 193-196

5-Methoxy-1-(4-chlorbenzyl)-indole-3-carboxaldehyde (yield 75%) mp 135-137 °C

6-Fluoro-1-(4-chlorobenzyl)-indole-3-carboxaldehyde (yield 80%) mp 140-142 °C

5-Methoxy-1-(4-methoxybenzyl)-indole-3-carboxaldehyde (yield 78%) mp 85-88 °C

6-Fluoro-1-(4-metnoxybenzyl)-indole-3-carboxaldehyde (yield 72%) mp 146-148 °C

3.2.5.1.2 Indole-3-carbohydrazide (55) and (57)

Synthesis of indole-3-carboxylicacid (53): To a stirred solution of indole or 5-bromoindole (**52**, 38 mmol) in dimethylformamide (50 mL), trifluoroacetic anhydride (44 mmol) was added dropwise at 0 °C. After 3 h the mixture was poured into water (200 mL) and the product was isolated by filtration. The residue was washed with water (3 × 50 mL). The crude so obtained was suspended in 20% aqueous NaOH (200 mL) and refluxed for 6 h. The mixture was cooled, washed with CH_2Cl_2 (2 × 100 mL) and

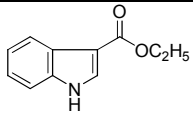
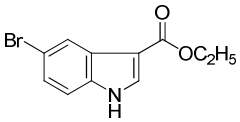
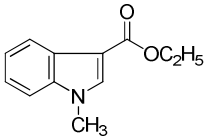
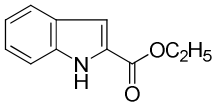
acidified. The precipitate was isolated by filtration and dried to get pure indole-3-carboxylic acid **53**. Indole-3-carboxylic acid (80%) mp. 230-235 °C (Lit.[59] 229-234 °C) 5-Bromoindole-3-carboxylic acid (yield 78%) mp. 221-224 °C (Lit.[81] 221-225 °C)

Synthesis of ethylester of indole-2(3)-carboxylate (54)

Indole-3-carboxylic acid **53** or indole-2-carboxylic acid **56** (10 mmol) was dissolved in 50 mL of ethanol to this solution 0.5 mL of conc.H_2SO_4 was added and refluxed for 20 h. After competition of the reaction, ethanol was evaporated and extracted the ester with ethyl acetate (50 mL) and washed with brine solution (25 mL) and water (25 mL). The organic phase was dried over anhyd. Na_2SO_4 and concentrated the solvent to afford pure ethyl ester of indole-2(3)-carboxylate in good yields (80-90%).

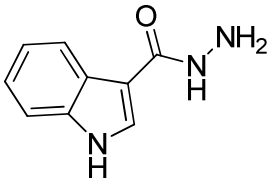
Ethyl-(1-methyl)-indole-3-carboxylate (54c): The compound **54c** was prepared from the reaction of **54a** (10 mmol) with methyliodide (20 mmol) in presence of potassium carbonate (20 mmol) in acetone under refluxing condition (6 h).

Physical data for ethyl indole-2(3)-carboxylate (**54**)

Ethyl indole-2(3)-carboxylate (**54**)		mp (°C)	Yield (%)
	(**54a**)	120-123 (Lit[82] 120-124)	80
	(**54b**)	180-182 (Lit[82] 180-184)	85
	(**54c**)	80- 83 (Lit.[81] 81-84)	80
	(**54d**)	195-196 (Lit [83]194-196)	90

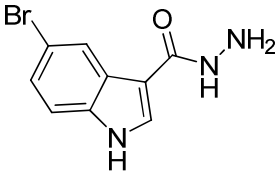
Synthesis of indole-2(3)-carbohydrazide 55 and 57:To a stirred solution of esters (**54**, 10 mmol) in 25 mL of ethanol was added 2 mL of hydrazine hydrate and heated to reflux for 24 h. After competition of the reaction, the contents were cooled and solid so obtained was filtered off to get pure indole-2(3)-carbohydrazides (**55** and **57**).

Indole-3-carbohydrazide (55a)



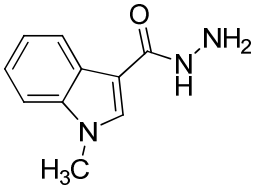
Yield 85%; White solid; mp 232-234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 9.14 (s, 1H), 8.17 (d, J = 7.10 Hz, 1H), 7.95 (d, J = 2.80 Hz, 1H), 7.41 (d, J = 8.10 Hz, 1H), 7.20 –6.99 (m, 2H), 3.95 (s, 2H); IR (KBr, v cm^{-1}) : 3256, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736; MS (ESI): *m/z* calcd. for $C_9H_9N_3O$: 175.07, found: 175.2 $(M)^+$.

5-Bromoindole-3-carbohydrazide (55b)



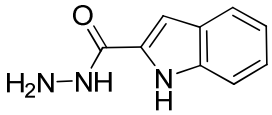
Yield 90%; White solid; mp 255-257 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.68 (s, 1H), 9.78 (s, 1H), 7.62 (s, 1H), 7.52 (s, 1H), 7.46 (d, J = 8.00 Hz, 2H), 4.23 (s, 2H); IR (KBr, ν cm^{-1}): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724; MS (ESI): *m/z* calcd. for $C_9H_8BrN_3O$: 253.0, found: 253.0 $(M)^+$.

(1-Methyl)-indole-3-carbohydrazide (55c)



Yield 80%; White solid; mp 149-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (s, 1H), 8.14 (d, J = 7.80 Hz, 1H), 7.92 (d, J = 7.80 Hz, 1H), 7.37 (d, J = 8.10 Hz, 1H), 7.20–6.99 (m, 2H), 4.03 (s, 2H), 3.65 (s, 3H). IR (KBr, v cm^{-1}): 3212, 3098,1658, 1604, 1582, 1520, 1465, 1235, 740. MS (ESI): *m/z* calcd. for $C_{10}H_{11}N_3O$: 189.09, found: 189.1$(M)^+$.

Indole-2-carbohydrazide (57)



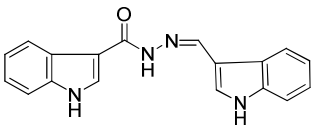
Yield 90%; White solid; mp 247-248 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.70 (s, 1H), 9.85 (s, 1H), 7.80-7.10 (m, 5H), 4.2 (s, 2H); IR (KBr, ν cm^{-1}): 3356 3217, 3080, 2900, 1640, 1615, 1550, 780, 740; MS (ESI): *m/z* calcd. for $C_9H_9N_3O$: 175.07, found: 176.2 $(M+H)^+$.

General synthesis of bis(indole)hydrazide-hydrazone (58a-z)

To a mixture of indole-3-carboxylic acid hydrazide **55** or indole-2-carboxylic acid hydrazide **57** (10 mmol) and indole-3-carboxaldehydes (**51**, 10 mmol) in absolute ethanol (20 mL) was added catalytic amount of glacial acetic acid and allowed the reaction

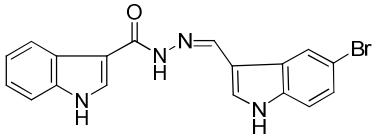
contents to reflux at 80 °C for 5h. The reaction mixture was cooled and the solid separated out was filtered and recrystallized from ethanol to afford desired product **58**.

Indole-3-carboxylic acid-(indole-3-methylene)-hydrazide (58a)



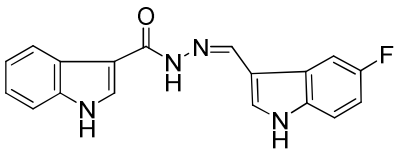
Yield 78%; Off-white solid; mp 263-264 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 11.10 (s, 1H), 10.88 (s, 1H), 8.57 (s, 1H), 8.33-8.11 (m, 3H), 7.52 (s, 1H), 7.45-7.42 (m, 2H), 7.22-7.14 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.57, 142.27, 137.00, 136.11, 129.52, 127.78, 126.41, 123.88, 124.31, 122.49, 122.05, 121.19, 121.09, 120.53, 120.21, 111.86; IR (KBr, ν cm^{-1}): 3326, 3224 (N-H), 3109, 2986, 1660 (C=O), 1583, 1523, 1433, 1240, 736; MS (ESI): *m/z* calcd. for $C_{18}H_{14}N_4O$: 302.12, found: 303.1 $(M+H)^+$.

Indole-3-carboxylic acid-(5-bromoindole-3-methylene)-hydrazide (58b)



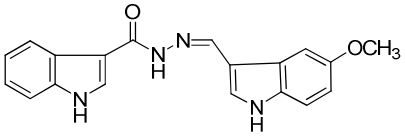
Yield 80%; Pale brown solid; mp 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 2H), 10.95 (s, 1H), 8.49 (s, 1H), 8.31 (d, J = 4.2 Hz, 1H), 7.73 (s, 1H), 7.57–7.46 (m, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.19 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 164.71, 141.69, 136.29, 135.67, 132.45, 130.84, 127.91, 125.95, 125.56, 124.92, 124.04, 122.08, 121.10, 120.55, 113.82, 112.91, 112.32, 111.89; IR (KBr, ν cm^{-1}): 3342, 3217 (N-H), 3110, 2975, 1707 (C=O), 1614, 1537, 1433, 1199, 746; MS (ESI): *m/z* calcd. for $C_{18}H_{13}BrN_4O$: 380.1, found: 381.4 $(M+H)^+$.

Indole-3-carboxylic acid-(5-fluoroindole-3-methylene)-hydrazide (58c)



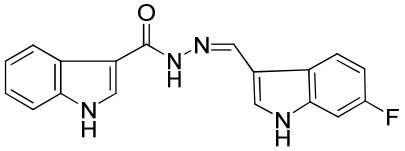
Yield 82%; White solid; mp 266-267 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ11.40 (s, 1H), 11.33 (s, 1H), 11.01 (s, 1H), 8.55 (s, 1H), 8.30 (d, J = 5.9 Hz, 1H), 8.14-8.04 (m, 1H), 7.91 (s, 1H), 7.59 (s, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.40-7.37 (m, 1H), 7.20-7.13 (m, 2H), 6.98-6.93 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 164.60, 142.56, 136.40, 135.53, 132.13, 131.75, 128.75, 126.78, 125.26, 124.22, 123.14, 122.18, 121.15, 120.45, 112.42, 112.81, 112.02, 111.69; IR (KBr, ν cm^{-1}): 3332, 3265 (N-H), 3120, 2927, 1678 (C=O), 1531, 1433, 1369, 740; MS (ESI): *m/z* calcd. for $C_{18}H_{13}FN_4O$: 320.1, found: 321.1 $(M+H)^+$.

Indole-3-carboxylic acid-(5-methoxyindole-3-methylene)-hydrazide (58d)



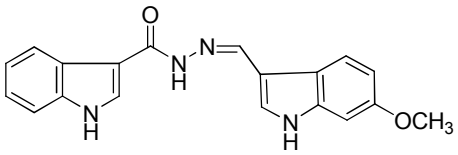
Yield 78%; White solid; mp 243-244 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 11.09 (s, 1H), 10.51 (s, 1H), 8.58 (s, 1H), 8.13-8.03 (m, 2H), 7.90 (s, 1H), 7.50-7.44 (m, 2H), 7.32 (d, J = 8.7 Hz, 1H), 7.18-7.12 (m, 2H), 6.83 (d, J = 8.0 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): 167.83, 154.33, 142.47, 140.10, 136.08, 131.96, 129.82, 127.76, 126.45, 122.03, 120.98, 120.70, 114.09, 112.67, 111.82, 108.25, 109/45, 104.13, 55.10; IR (KBr, ν cm^{-1}): 3324, 3206 (N-H),2933, 1645 (C=O), 1537, 1507, 1435, 1220, 746; MS (ESI): *m/z* calcd. for $C_{19}H_{16}N_4O_2$: 332.12, found: 333.14 $(M+H)^+$.

Indole-3-carboxylic acid-(6-fluoroindole-3-methylene)-hydrazide (58e)



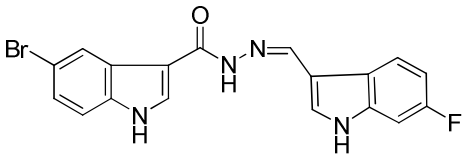
Yield 70%; Off-white solid; mp 279-280 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.40 (s, 1H), 11.27 (s, 1H), 11.03 (s, 1H), 8.53 (s, 1H), 8.31 (d, J = 8.0 Hz, 2H), 7.87 (s, 1H), 7.52 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.19-7.11 (m, 3H), 6.83 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.54, 158.07, 141.66, 137.00, 136.92, 135.92, 130.08, 127.77, 126.53, 123.58, 122.06, 120.98, 120.54, 112.20, 111.82, 108.67, 108.43, 98.03; IR (KBr, ν cm^{-1}): 3246, 3124(N-H), 2927, 1674 (C=O), 1556, 1503, 1444, 1209, 752; MS (ESI): *m/z* calcd. for $C_{18}H_{13}FN_4O$: 320.1, found: 321.2 $(M+H)^+$.

Indole-3-carboxylic acid-(6-methoxyindole-3-methylene)-hydrazide (58f)



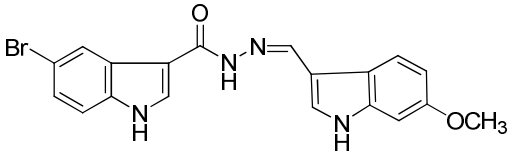
Yield 85%; Pale yellow solid; mp 226-227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.46 (s, 1H), 11.12 (s, 1H), 11.02 (s, 1H), 8.50 (s, 1H), 8.29-8.22 (m, 2H), 8.12 (s, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.18-7.14 (m, 2H), 6.90 (d, J = 2 Hz, 1H), 6.76 (dd, J = 8.80 Hz, 2.40 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.13, 156.28, 142.24, 137.58, 136.01, 128.39, 127.84, 126.53, 122.69, 122.04, 121.05, 120.51, 118.38, 112.14, 111.85, 110.12, 109.23, 94.78, 55.25; IR (KBr, ν cm^{-1}): 3340, 3256 (N-H), 2970, 1640 (C=O), 1537, 1446, 1240, 759; MS (ESI): *m/z* calcd. for $C_{19}H_{16}N_4O_2$: 333.14, found: 333.2 $(M+H)^+$.

5-Bromoindole-3-carboxylic acid-(6-fluoroindole -3-methylene)-hydrazide (58g)



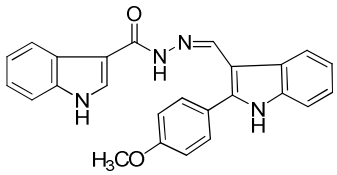
Yield 76%; Off-white solid; mp 284-285 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 11.02 (s, 2H), 8.53 (s, 1H), 8.46 (s, 1H), 8.36 (s, 1H), 8.15 (s, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.26 (dd, *J* = 8.40 Hz, 1.60 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.93-6.88 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.87, 153.34, 151.28, 142.60, 137.89, 135.61, 130.10, 129.42, 125.60, 124.56, 122.70, 120.34, 118.48, 113.93, 113.36, 111.97, 110.35, 98.80; IR (KBr, ν cm^{-1}): 3277, 3122 (N-H), 2926, 1645 (C=O), 1537, 1503, 1444, 1236, 748; MS (ESI): *m/z* calcd. for $C_{18}H_{12}BrFN_4O$: 398.5, found: 399.6 $(M+H)^+$.

5-Bromoindole-3-carboxylic acid-(6-methoxy indole-3-methylene)-hydrazide (58h)



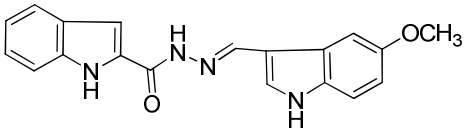
Yield 90%; Pale brown solid; mp 285-286 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 1H), 11.03 (s, 2H), 8.44 (s, 2H), 8.20 (s, 1H), 8.10 (s, 1H), 7.39 (d, *J* = 2.4 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.20 (d, *J* = 2.0 Hz, 1H), 6.86 (s, 1H), 6.72 (d, *J* = 8.8 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.87, 156.29, 154.45, 142.60, 137.89, 134.71, 133.36, 129.10, 128.52, 124.60, 123.26, 122.70, 118.48, 113.93, 113.36, 111.97, 110.35, 94.86, 55.25; IR (KBr, ν cm^{-1}): 3340, 3286 (N-H), 2926, 1647 (C=O), 1558, 1510, 1416, 1242, 731; MS (ESI): *m/z* calcd. for $C_{19}H_{15}BrN_4O_2$: 410.3, found: 411.5 $(M+H)^+$.

Indole-3-carboxylic acid(2-(4-methoxy phenyl)- indole-3-methylene)-hydrazide (58i)



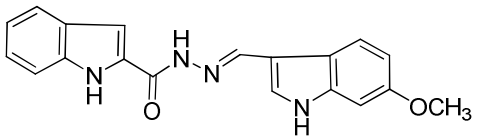
Yield 75%; White solid; mp 251-252 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11. 40 (s, 2H), 10.98 (s, 1H), 8.65 (s, 1H), 8.56 (s, 1H), 8.32 (d, *J* = 5.60 Hz, 1H), 8.16 (d, *J* = 8.00 Hz, 1H), 7.92 (s, 1H), 7.61 (d, *J* = 8.80 Hz, 2H), 7.43 (t, *J* = 8.00 Hz, 2H), 7.21-7.14 (m, 3H), 7.09 (d, *J* = 8.80 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.49, 160.10, 147.48, 143.27, 136.59, 136.34, 132.93, 130.15, 128.41, 126.78, 125.79, 123.94, 123.06, 122.64, 121.78, 121.67, 120.35, 119.63, 118.71, 114.63, 114.33, 111.98, 110.85, 105.52, 56.04; IR (KBr, ν cm^{-1}): 3315 3245 (N-H), 2936, 1664 (C=O), 1614, 1537, 1464, 1247, 845, 796, 748; MS (ESI): *m/z* calcd. for $C_{25}H_{20}N_4O_2$; 408.17, found: 409.3 $(M+H)^+$.

Indole-2-carboxylic acid-(5-methoxyindole-3-methylene)-hydrazide (58j)



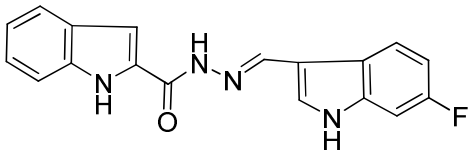
Yield 80%; Pale yellow solid; mp 147-148 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (s, 1H), 11.44 (s, 1H), 11.21 (s, 1H), 8.64 (s, 1H), 8.34 (s, 1H), 7.93 (s, 1H), 7.52 (d, *J* = 2.80 Hz, 1H), 7.51 (d, *J* = 2.80 Hz, 1H), 7.33 (d, *J* = 8.80 Hz, 1H), 7.27 (s, 1H), 7.22-7.18 (m, 1H), 7.06 (t, *J* = 7.60 Hz, 1H), 6.84 (d, *J* = 8.40 Hz, 1H), 3.89 (s, 3H).); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.88, 157.05, 154.38, 144.42, 136.62, 131.99, 130.77, 130.50, 124.99, 124.91, 123.50, 121.64, 119.72, 113.10, 112.15, 111.47, 104.16, 102.74, 55.96; IR (KBr, ν cm^{-1}): 3321, 3265 (N-H), 2944, 1634 (C=O), 1571, 1487, 1257, 748; MS (ESI): *m/z* calcd. for $C_{19}H_{16}N_4O_2$: 332.13, found: 333.2 $(M+H)^+$.

Indole-2-carboxylic acid-(6-methoxyindole-3-methylene)-hydrazide (58k)



Yield 88%; White solid; mp 235-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 2H), 11.08 (s, 1H), 8.60 (s, 1H), 8.23 (d, *J* = 8.40 Hz, 1H), 7.83 (s, 1H), 7.63 (d, *J* = 7.60 Hz, 1H), 7.53 (d, *J* = 8.00 Hz, 1H), 7.48 (s, 1H), 7.21 (t, *J* = 8.00 Hz,1H), 7.09-7.05 (m, 1H), 6.91 (s, 1H), 6.89 (d, *J* = 8.40 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.83, 157.04, 156.36, 144.43, 137.98, 136.57, 130.71, 129.37, 127.00, 123.50, 122.57, 121.55, 119.90, 118.41, 112.32, 111.73, 110.25, 102.84, 55.08; IR (KBr, ν cm^{-1}): 3373, 3258 (N-H), 2938, 1649 (C=O), 1606, 1531, 1454, 1246, 806, 742; MS (ESI): *m/z* calcd. for $C_{19}H_{16}N_4O_2$: 332.12, found: 332.3 $(M)^+$.

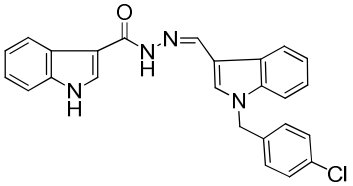
Indole-2-carboxylic acid-(6-fluoroindole-3-methylene)-hydrazide (58l)



Yield 82%; Off-white solid; mp 281-282 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 11.48 (s, 1H), 11.42 (s, 1H), 8.62 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.99 (s, 1H), 7.63 (d, *J* = 2.80 Hz, 1H), 7.51 (d, *J* = 2.80 Hz, 1H), 7.27 (s, 1H), 7.22 (t, *J* = 7.80 Hz, 1H), 7.14 (d, *J* = 7.60 Hz, 1H), 7.06 (t, *J* = 7.60 Hz, 1H), 6.93 (t, *J* = 9.20 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.55, 158.23, 157.12, 143.86, 136.99, 136.65, 130.62, 127.13, 123.50, 123.03, 121.61, 121.05, 119.85, 112.34, 111.88, 108.99, 108.65, 102.78;

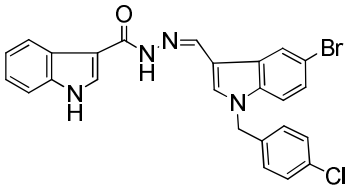
IR (KBr, v cm^{-1}): 3338 (N-H), 3062, 2934, 1650 (C=O), 1617, 1537, 1448, 1232, 839, 746; MS (ESI): *m/z* calcd. for $C_{18}H_{13}FN_4O$: 321.15, found: 321.2 $(M+H)^+$.

Indole-3-carboxylic acid-N'-((1-(4-chlorobenzyl)-indole)-3-methylene)hydrazide (58m)



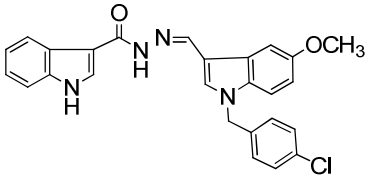
Yield 92%; White solid; mp 240-241 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.40 (s, 1H), 11.07 (s, 1H), 8.57 (s, 1H), 8.38-8.14 (m, 3H), 7.85 (s, 1H), 7.59 (s, 1H), 7.45 (d, *J* = 7.60 Hz, 1H), 7.38 (d, *J* = 8.00 Hz, 1H), 7.20-7.15 (m, 5H), 6.84 (d, *J* = 8.40 Hz, 2H), 5.23 (s, 2H); IR (KBr, *v* cm^{-1}): 3390, 3144 (N-H), 2954, 1656 (C=O), 1546, 1513, 1452, 1242, 1139, 846, 804, 750; MS (ESI): *m/z* calcd. for $C_{25}H_{19}ClN_4O$: 426.12, found: 427.13 $(M+H)^+$ and 428.1 $(M+2)^+$.

Indole-3-carboxylic acid-*N*'-((1-(4-chlorobenzyl)-5-bromoindole)-3-methylene)-hydrazide (58n)



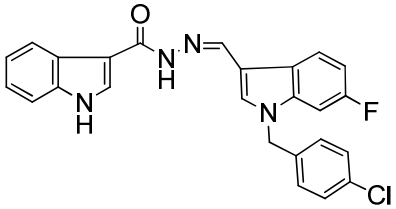
Yield 90%; Off-white solid; mp 170-172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 11.05 (s, 1H), 8.50 (s, 1H), 8.20-8.10 (m, 2H), 7.90 (s, 1H), 7.48 (s, 1H), 7.43 (d, *J* = 8.00 Hz, 2H), 7.35 (d, *J* = 8.00 Hz, 1H), 7.22-7.18 (m, 4H), 6.88 (d, *J* = 8.00 Hz, 2H), 5.28 (s, 2H); IR (KBr, *v* cm^{-1}): 3390, 3145 (N-H), 2893, 1633 (C=O), 1614, 1573, 1377, 1238, 1203, 798, 748; MS (ESI): *m/z* calcd. for $C_{25}H_{18}BrClN_4O$: 504.1, found: 504.2 $(M)^+$. ·.

Indole-3-carboxylic acid-*N*'-((1-(4-chlorobenzyl)-5-methoxyindole)-3-methylene)-hydrazide (58o)



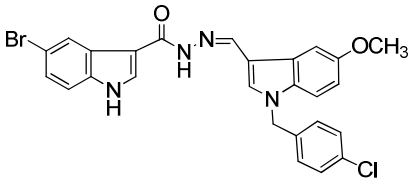
Yield 85%; White solid; mp 152-153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 11.67 (s, 1H), 11.11 (s, 1H), 8.52 (s, 1H), 8.20-8.14 (m, 2H), 7.91 (s, 1H), 7.88 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.39 (dd, *J* = 8.4 Hz, 2.4 Hz, 3H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.19-7.11(m, 2H), 6.84 (d, *J* = 8.0 Hz, 1H), 5.41 (s, 2H), 3.79 (s, 3H); IR (KBr, *v* cm^{-1}): 3226 (N-H), 3024, 2970, 1660 (C=O), 1602, 1537, 1484, 1229, 1178, 794, 752; MS (ESI): *m/z* calcd. for $C_{26}H_{21}ClN_4O_2$; 456.14, found: 457.2 $(M+H)^+$.

Indole-3-carboxylic acid-*N*'-((1-(4-chlorobenzyl)-5-fluoroindole)-3-methylene)-hydrazide (58p)



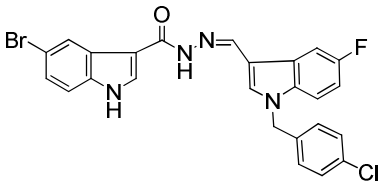
Yield 73%; Pale yellow solid; mp 163-164 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 11.10 (s, 1H), 8.48 (s, 1H), 8.35 (s, 1H), 8.23 (d, *J* = 8.0 Hz, 2H), 8.09 (s, 1H), 7.75 (s, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.23-7.20 (m, 2H), 7.17-7.09 (m, 2H), 6.95-690 (m, 1H), 5.38 (s, 2H); IR (KBr, *v* cm^{-1}): 3223 (N-H), 3120, 2913, 1661 (C=O), 1614, 1529, 1454, 1225, 862, 813, 752; MS (ESI): *m/z* calcd. for $C_{25}H_{18}ClFN_4O$: 444.12, found: 445.2 $(M+H)^+$.

5-Bromoindole-3-carboxylic acid-*N*'-((1-(4-chlorobenzyl)-5-methoxyindole)-3-methyl ene)-hydrazide (58q)



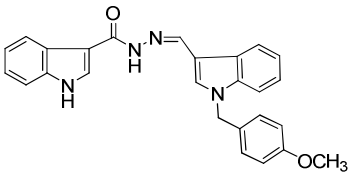
Yield 85%; Light brown solid; mp 150-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, 1H), 11.16 (s, 1H), 8.51 (s, 1H), 8.37 (s, 1H), 8.20 (s, 1H), 7.92 (s, 1H), 7.58 (s, 1H), 7.45 (d, *J* = 8.00 Hz, 1H), 7.39-7.36 (m, 3H), 7.30 (dd, *J* = 8.40, 2.00 Hz, 1H), 7.24 (d, *J* = 8.40 Hz, 2H), 6.85 (d, *J* = 7.60 Hz, 1H), 5.41 (s, 2H), 3.79 (s, 3H); IR (KBr, *v* cm^{-1}): 3236 (N-H), 3106, 2933, 1664 (C=O), 1612, 1552, 1484, 1257, 889, 796, 752; MS (ESI): *m/z* calcd. for $C_{26}H_{20}BrClN_4O_2$: 534.1, found:, 534.1 $(M)^+$ and 536.6 $(M+2)^+$.

5-Bromoindole-3-carboxylic acid-*N*'-((1-(4-chlorobenzyl)-5-fluoroindole)-3-methyle ne)-hydrazide (58r)



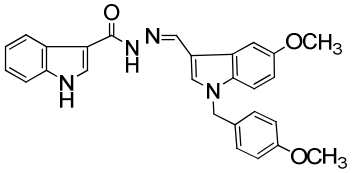
Yield 78%; Pale yellow solid; mp 261-262 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.65 (s, 1H), 11.15 (s, 1H), 8.48 (s, 1H), 8.42-8.36 (m, 2H), 8.12 (s, 1H), 8.00 (s, 1H), 7.69 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H),7.23 (d, *J* = 8.0 Hz, 1H), 7.19-7.13 (m, 2H), 6.93-688 (m, 1H), 5.35 (s, 2H); IR (KBr, *v* cm^{-1}): 3238, 3045 (N-H), 2913, 1674 (C=O), 1605, 1537, 1454, 1220, 810, 771, 734; MS (ESI): *m/z* calcd. for $C_{25}H_{17}BrClFN_4O$: 522.03, found: 523.06 $(M+H)^+$.

Indole-3-carboxylic acid-*N*'-((1-(4-methoxybenzyl)-indole)-3-methylene)-hydrazide (58s)



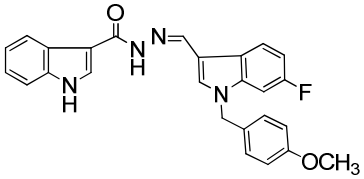
Yield 80%; white solid; mp 212-213 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 8.63 (s, 1H), 8.46 (s, 1H), 8.33 (d, *J* = 8.0 Hz, 2H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.76 (s, 1H), 7.47 (d,*J* = 8.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.38-7.29 (m, 6H), 7.11 (d, *J* = 8.0 Hz, 2H), 5.35 (s, 2H), 3.83 (s, 3H); IR (KBr, *v* cm^{-1}): 3236 (N-H), 3011, 2924, 1654 (C=O), 1572, 1537, 1436, 1214, 864, 769, 746; MS (ESI): *m/z* calcd. for $C_{26}H_{22}N_4O_2$: 422.2, found: 423.3 $(M+H)^+$.

Indole-3-carboxylic acid-*N*'-((1-(4-methoxybenzyl)-5-methoxyindole)-3-methylene)-hydazide (58t)



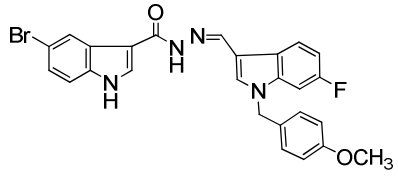
Yield 85%; White solid; mp 150-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11. 97 (s, 1H), 11.15 (s, 1H), 8.51 (s, 1H), 8.35-8.15 (m, 2H), 8.00 (s, 2H), 7.64 (s, 1H), 7.46-7.40 (m, 2H), 7.30 (d, *J* = 8.40 Hz, 1H), 7.23-7.21 (m, 2H), 6.86 (d, *J* = 8.40 Hz, 3H), 5.31 (s, 2H), 3.78 (s, 3H), 3.69 (s, 3H); IR (KBr, *v* cm^{-1}): 3233 (NH's), 3012, 2964, 1654 (C=O), 1610, 1532, 1484, 1246, 859, 794, 752; MS (ESI): *m/z* calcd. for $C_{27}H_{24}N_4O_3$: 452.2, found: 452.2 $(M)^+$.

Indole-3-carboxylic acid-N'-((1-(4-methoxybenzyl)-6-fluoroindole)-3-methylene)-hydrazide (58u)



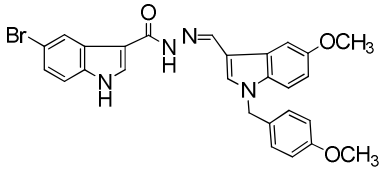
Yield 75%; Light yellow solid; mp 193-195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H), 11.07 (s, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 8.11 (s, 1H), 7.72 (s,1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.25-7.16 (m, 3H), 7.15-7.09 (m, 2H), 6.92 (t, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 2H), 5.29 (s, 2H), 3.72 (s, 3H); IR (KBr, *v* cm^{-1}): 3243, 3038(N-H), 2926, 1654 (C=O), 1603, 1537, 1434, 1246, 845, 819, 754; MS (ESI): *m/z* calcd. for $C_{26}H_{21}FN_4O_2$: 440.2, found: 441.2 $(M+H)^+$.

5-Bromoindole-3-carboxylic acid-N'-((1-(4-methoxybenzyl)-6-fluoroindole)-3-methyl -ene)-hydrazide (58v)



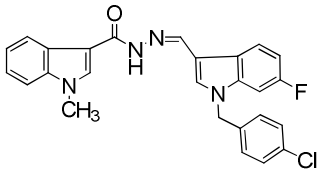
Yield 78%; Pale brown solid; mp 248-249 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.62 (s, 1H), 11.11 (s, 1H), 8.46 (s, 1H), 8.41 (s, 1H), 8.34 (s, 1H), 8.02 (s, 1H), 7.65 (s, 1H), 7.35 (d, *J*= 8.4 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 3H), 6.89 (t, *J* = 8.0 Hz , 1H), 6.82 (d, *J* = 8.4 Hz, 2H), 5.26 (s, 2H), 3.71 (s, 3H); IR (KBr, *v* cm^{-1}): 3232, 3018 (N-H), 2958, 1664 (C=O), 1537, 1454, 1249, 825, 794, 751; MS (ESI): *m/z* calcd. for $C_{26}H_{20}BrFN_4O_2$: 518.2, found: 519.3 $(M+H)^+$.

5-Bromoindole-3-carboxylic acid-N'-((1-(4-methoxybenzyl)-5-methoxyindole)-3-methylene)-hydrazide (58w)



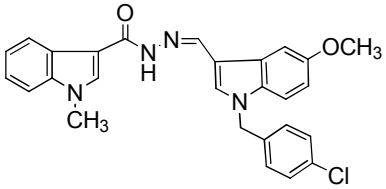
Yield 78%; Pale yellow solid; mp 260-261 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (s, 2H), 11.40 (s, 1H), 8.54 (s, 1H), 8.21 (s, 1H), 8.13-8.07 (m, 2H), 7.86 (s, 1H), 7.47-7.40 (m, 3H), 7.22 (d, *J* = 8.00 Hz, 2H), 7.03-6.97 (m, 2H), 5.31 (s, 2H), 3.79 (s, 3H), 3.69 (s, 3H); IR (KBr, *v* cm^{-1}): 3213, 3076 (N-H), 2934, 1655 (C=O), 1612, 1537, 1444, 1247, 887, 794; MS (ESI): *m/z* calcd. for, $C_{27}H_{23}BrN_4O_3$: 530.1, found: 531.2 $(M+H)^+$.

1-Methyl-indole-3-carboxylic acid-N'-((1-(4-chlorobenzyl)-6-fluoroindole)-3-methylene)-hydrazide (58x)



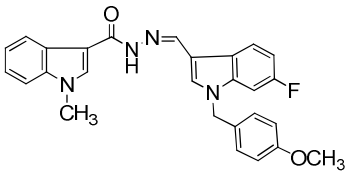
Yield 88%; Off-white solid; mp 235-236 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11. 00 (s, 1H), 8.49 (s, 1H), 8.39 (s, 1H), 7.83 (s, 1H), 7.54 (s, 1H), 7.41 (d, *J* = 8.00 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 8.00 Hz, 1H), 7.23-7.15 (m, 3H), 7.10 (d, *J* = 9.20 Hz, 1H), 6.95-6.90 (m, 1H), 6.86 (d, *J* = 8.80 Hz, 2H), 5.26 (s, 2H), 3.90 (s, 3H); IR (KBr, *v* cm^{-1}): 3219 (N-H), 2904, 1644 (C=O), 1536, 1424, 1256, 831, 795, 738; MS (ESI): *m/z* calcd. for $C_{26}H_{20}ClFN_4O$: 458.2, found: 458.2 $(M)^+$, 460.2 $(M+2)^+$.

1-Methyl-indole-3-carboxylic acid-N'-((1-(4-chlorobenzyl)-5-methoxyindole)-3-methyl ene)-hydrazide (58y)



Yield 90%; White solid; mp 211-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11. 00 (s, 1H), 8.54 (s, 1H), 8.29 (s, 1H), 7.96 (s, 1H), 7.65 (s, 1H), 7.43 (d, J = 8.00 Hz, 1H), 7.30 (d, J = 8.40 Hz, 2H), 7.23-7.21 (m, 2H), 7.18-7.13 (m, 4H), 6.85 (d, J = 8.00 Hz, 1H), 5.37 (s, 2H), 3.90 (s, 3H), 3.73 (s, 3H); IR (KBr, v cm^{-1}): 3217 (N-H), 2936, 1644 (C=O), 1602, 1537, 1469, 1236, 852, 796, 742; MS (ESI): *m/z* calcd. for $C_{27}H_{23}ClN_4O_2$: 470.2, found: 470.2 $(M)^+$ and 472.2 $(M+2)^+$.

1-Methyl-indole-3-carboxylic acid-N'-((1-(4-methoxybenzyl)-6-fluoroindole)-3-methylene)-hydrazide (58z)



Yield 85%; Off-white solid; mp 281-283 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11. 16 (s, 1H), 8.46 (s, 1H), 8.31 (s, 1H), 8.22-8.13 (m, 2H), 7.96 (s, 1H), 7.52 (d, J = 8.40 Hz, 1H), 7.45-7.36 (m, 3H), 7.29 (d, J = 8.40 Hz, 2H), 7.25-7.16 (m, 2H), 7.06 (d, J = 8.00 Hz, 1H), 5.43 (s, 2H), 3.87 (s, 3H), 3.70 (s, 3H); IR (KBr, v cm^{-1}): 3243 (N-H), 3020, 2904, 1649 (C=O), 1601, 1537, 1458, 1242, 836, 800, 736; MS (ESI): *m/z* calcd. for $C_{27}H_{23}FN_4O_2$: 454.2, found: 454.2 $(M)^+$.

3.2.5.2 MTT Assay

Six human cancer cell lines (LnCaP, DU145, PC3, MCF-7, MDA-MB-231 and PaCa2) were cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 4 x 10^3 cells per well for 12 h. Cells were incubated with various concentrations of the compounds (**58a-z**) ranging from 10 nM–1 mM. After 48 h, MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was added to the final concentration of 0.2 mg/ml and incubated for 30 min. The cells were washed twice with PBS and lyses in 100 µL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.

3.2.5.3 Nuclear staining using propidium iodide:

MDA-MB-231cells were treated with 10 µM of **58a-l** and incubated for 48 h. After the treatment, cells were fixed with cold methanol for 5 min, followed by rehydration in PBS and permeabilization using 0.1% Triton X-100 in PBS plus 2% BSA. Cells were treated with 0.1 µg/mL RNaseA in PBS for 1h, rinsed, and stained with 2.5 µg/ml propidium iodide in PBS for 1 h. Before mounting with Mowiol, coverslips were washed twice with PBS and once with H_2O.

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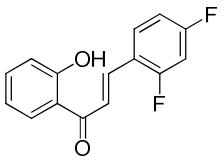
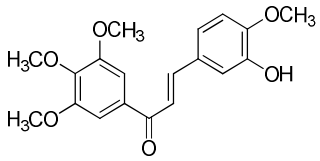
Chapter 4

4.1 Introduction

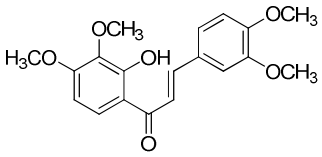
Chalcones **1** are 1,3-diarylpropen-1-ones in which two aromatic rings are linked by a three-carbon α,β-enone system. A large number of naturally occurring chalcones are phenolic compounds with immense potential in the design of novel therapeutics in drug discovery. Chalcones are one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuffs have been recently subjects of great interest for their remarkable pharmacological activities.[1-2] They are also valuable precursors for the preparation of their cyclic analogues 2-aryl-2,3-dihydro-quinolin-4(1*H*)-ones and 2-aryl-2,3-dihydro-4*H*-chromen-4-ones.[3]



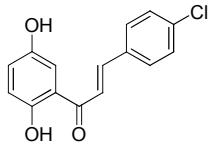
Antioxidant agent (**1a**)



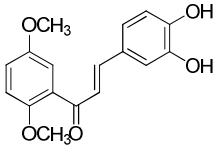
Anticancer agent (**1b**)



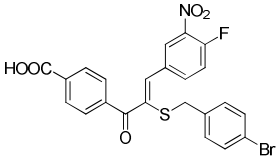
LTB4 production inhibitor (**1c**)



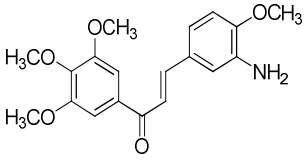
Antiplatelet agent (**1d**)



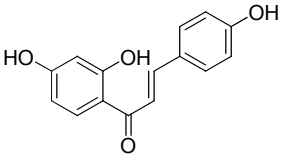
COX inhibitor (**1e**)



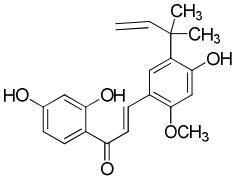
BCR-ABL kinase inhibitor (**1f**)



ACE inhibitors (**1g**)



Cytotoxic agent (**1h**)



Antimicrobial (**1i**)

Figure 4.1 Biological activities of chalcones (**1a-i**)

Because of simple structural features and significant biological properties, chalcones have attracted attention of chemists and biologists. Chalcones exhibited a wide range of biological activities such as antiinflammatory,[4] antioxidant,[5] antimitotic,[6] antiplatelet agents,[7] calcium channel blockers.[8] The natural chalcone, Isoliquiritigenin (**1h**) is currently in use for the treatment of cardiovascular diseases[9] and also acts as an anticancer agent,[10] The licochalcone (**1i**) has shown potent antibacterial activity

especially to *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* and also reported for antifungal and insecticidal activities (figure 4.1).[11]

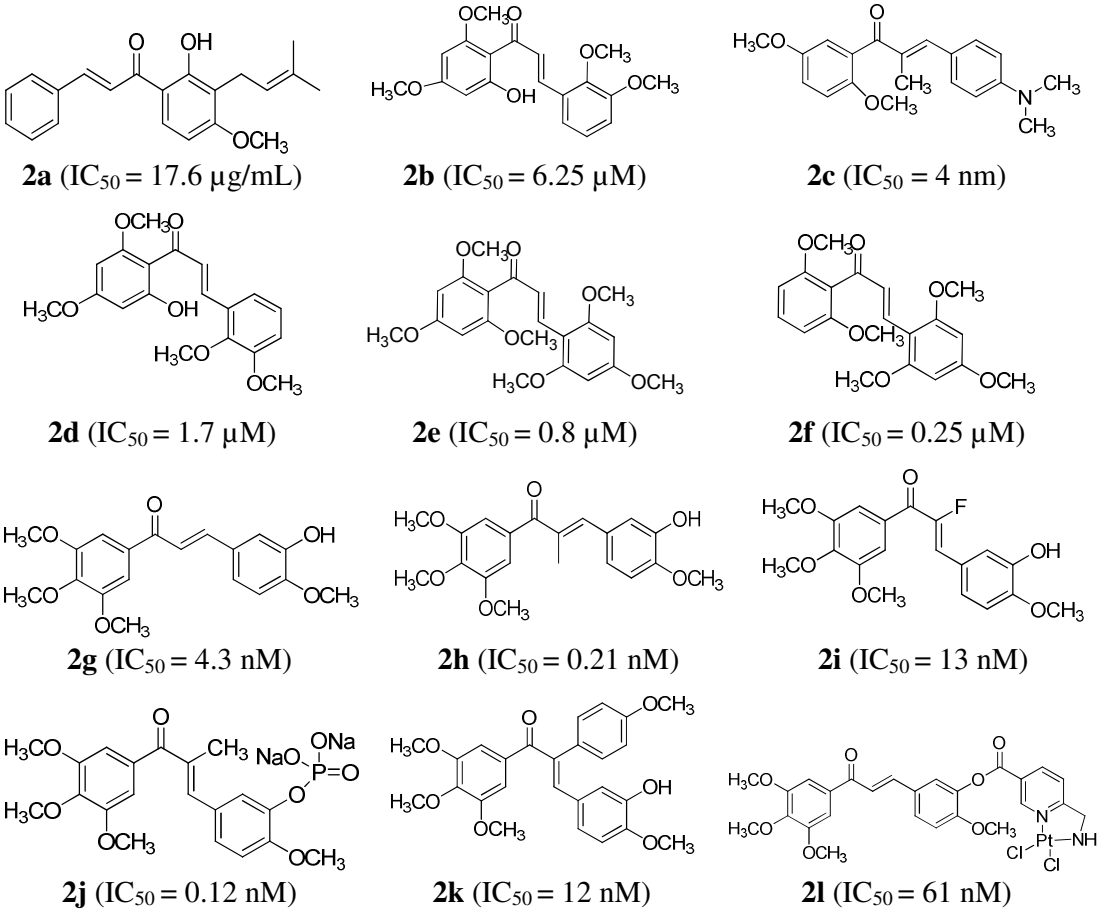


Figure 4.2 Chalcones (**2a-l**) as anticancer agents

Cancer remains one of the most life threatening diseases in the world which is responsible for most number of deaths in developed countries. To address the challenges associated with several types of cancers, diverse new chemical entities have been developed as novel anticancer agents. Chalcones are advantageous molecular scaffold which are free from the problems of mutagenicity and carcinogenicity that are associated with many alkylating agents used in cancer chemotherapy. Wide varieties of chalcones are known for their anticancer activities through different mechanism of actions. The naturally occurring derricin chalcone (**2a**) reportedly disrupt the cell cycle by interfering with microtubule assembly.[12] Yew-Min Tzeng *et al.* reported the anticancer activity of tetramethoxy chalcone (**2b**) through the inhibition of telomerase enzyme in cancer cells[13] and also showed that it induces G1 phase arrest through p53-dependent pathway in A549 cells.[14] Edward *et al.* reported antimitotic α-substituted chalcones (**2c**) which induces apoptosis

by interfering with microtubule assembly.[15-16] A series of oxygenated chalcones (**2d**) were studied against Jurkat cancer cells to show the significance of substitution pattern in chalcones.[17] Chalcones **2e** and **2f** exhibited cytotoxicity by cell cycle arrest at G2/M phase.[18] Duke *et al.* reported the multi-methoxy substituted chalcones (**2g**) for their capacity to bind with microtubule assembly and behaving like combretastatin A-4 and further demonstrated, α-substitutions on chalcones (**2h-j**) improved the anticancer activity. α-Substitution imparts s-*trans* conformation to the chalcone instead of s-*cis* and contributes to its remarkable anticancer activity. Disodium phosphate salts (**2j**) and (**2k**) were proved to be potential anticancer agents.[19-20] Platinum complex adduct (**2l**) found to influence the multidrug resistance cancer cells (figure 4.2).[21]

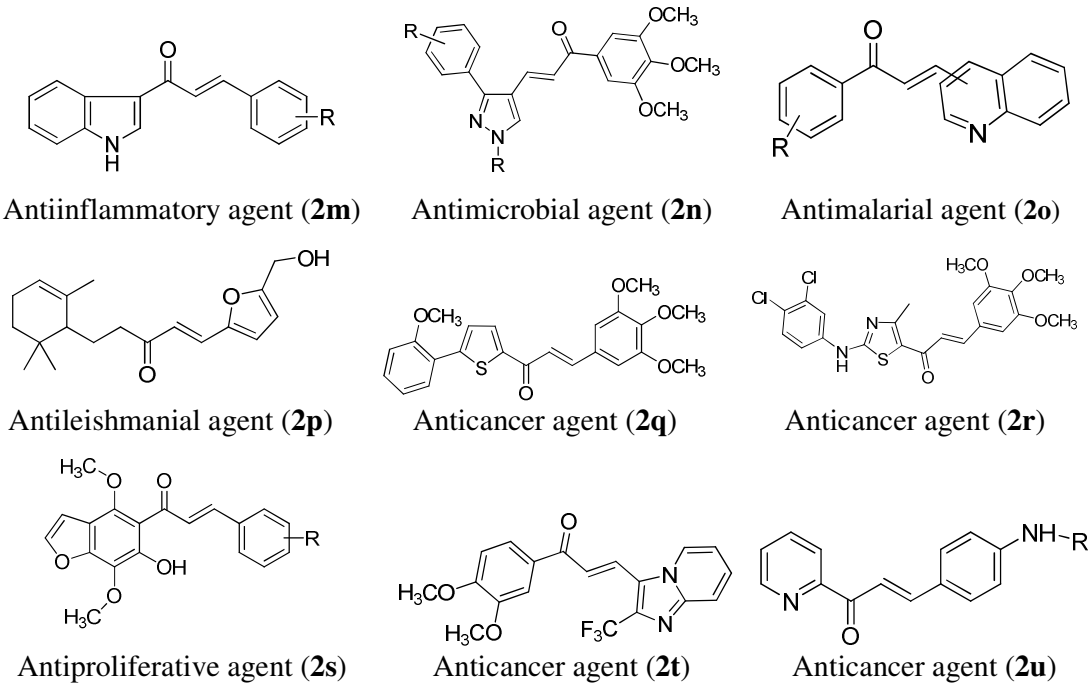


Figure 4.3 Heterocyclyl chalcones (**2m-u**) in drug-discovery

In the recent past, several heteroaryl chalcones have been reported for their diverse biological properties. For instance, indolyl chalcones (**2m**) were identified as antiinflammatory agents against carrageenan induced oedema in albino rats.[22] Pyrazolyl chalcones (**2n**) were reported for their antimicrobial and antioxidant activities.[23] Quinoline containing chalcones (**2o**) exhibited good antimalarial activity against *Plasmodium falciparum* strain *in-vitro*.[24] Several thiophene and furan containing chalcones (**2p** and **2q**) displayed significant anticancer [25] and antileishmanial activities.[26] Shi *et al.* reported novel *in-vitro* and *in-vivo* anticancer activity of thiazolyl chalcones (**2r**)

with IC_{50} below 10 μM against various human cacner cell lines.[27] Baell *et al.* patented novel benzofuran chalcones (**2s**) for their antiproliferative activity.[28] Ahmed Kamal *et al.* recently reported the imidazopyridine/pyrimidine/pyridine chalcones (**2t-u**) as potent anticancer agents. [29]-[30] Indolyl chalcones were also used as precursors for the synthesis of indolylpyrazoles, indolylpyridines and indolylpyrimidines, however, their anticancer potential remains largely unexplored.[22, 31-34]

4.2 Rational design

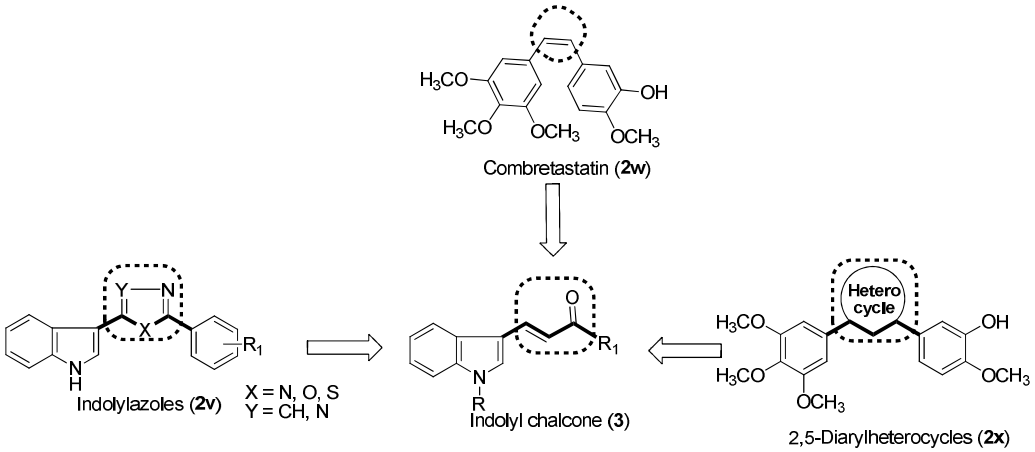


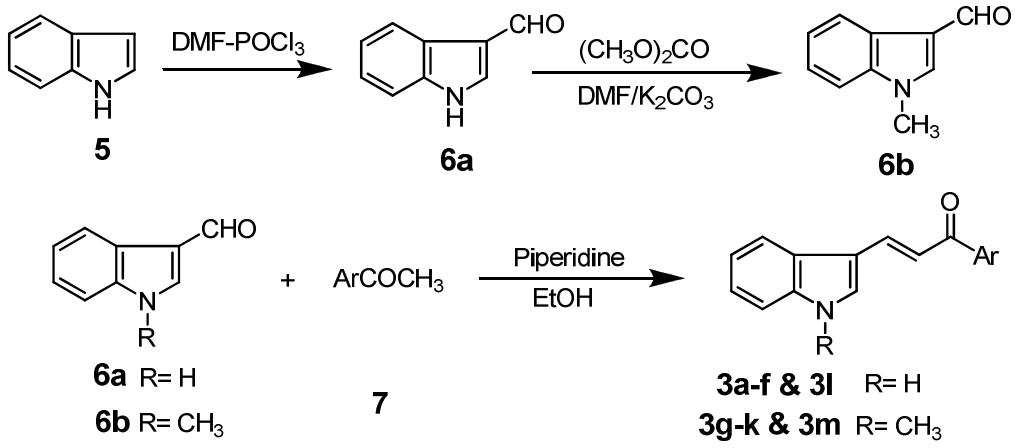
Figure 4.4 Rational design for the synthesis of indolyl chalcones (**3**)

Due to the structural simplicity and diversity, chalcones have emerged as an interesting family of molecules to be investigated for their therapeutic potential. Wide variety of 2,5-diarylheterocycles (**2x**) having central five/six-membered heterocyclic ring with two aryl rings separated by three atom bond distance were reported as potential anticancer agents.[35-39] Diverse varieties of chalcones were designed in analogy to combretastatin A-4 (**2w**) and evaluated for their anticancer activity.[14, 19-21, 40] Recent efforts to identify novel anticancer agents involve structural modification to chalcones to improve their anticancer activity and to study the role of various aryl or heteroaryl ring substituents around the enone funtionality.[41] Recently a large number of indolylazoles (**2v**) such as 5-(3-indolyl)oxazoles and indolyloxadiazoles have been studied for their anticancer potential.[42-44] Our rational design to synthesize present series of indolyl chalcones, retain the three atom bond distance which is observed in anticancer agents indolylazoles and 2,5-diarylheterocycles and these structural modification may lead to improve the anticancer activity of indolyl chalcones.

4.3 Results and discussion

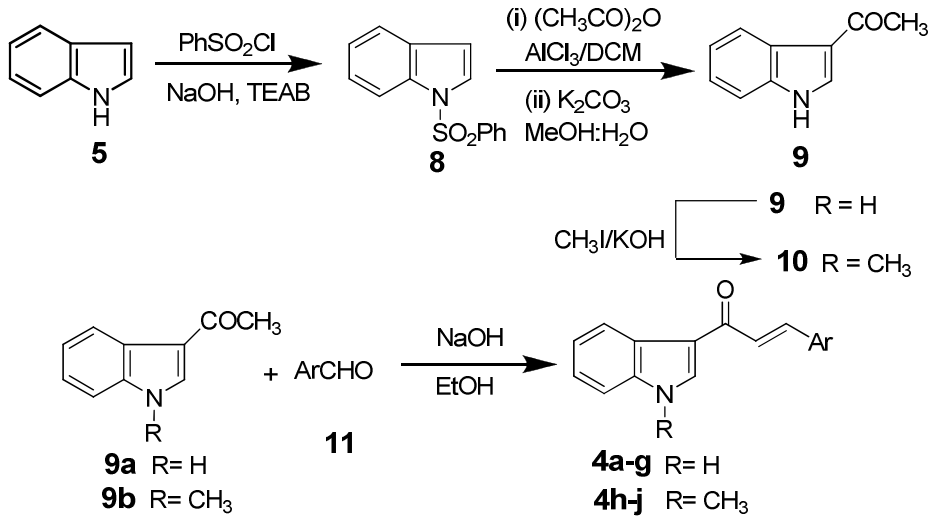
4.3.1 Chemistry

In our present work, a series of indolyl chalcones (**3**) and (**4**) were synthesized by the base catalyzed Claisen–Schmidt reaction. In general, the synthesis of chalcones involve the reaction of arylmethyl ketones and aldehydes in presence of acid-catalysts[45-46] or base-catalysts[32-34, 47-48] or solvent-free conditions.[49] Our syntheses of indolyl chalcones **3** involve the initial preparation of indole-3-carboxaldehyde (**6a**) from indole (**5**) by Vilsmeier-Haack formylation and followed by its alkylation in presence of dimethyl carbonate and potassium carbonate Finally, indolyl chalcones (**3**) were prepared by the reaction of indole-3-carboxaldehyde (**6**) with appropriate substituted acetophenones **7** in presence of piperidine under refluxing conditions (scheme 4.1). [50]



Scheme 4.1 Synthesis of indolyl chalcones (**3a-m**)

Indolyl chalcones (**4**) were prepared from 3-acetylindole (**9**), which was obtained by initial synthesis of 1-benzenesulfonyl indole (**8**), followed by acylation and deprotection of benzenesulfonyl group. Further, reaction of 3-acetylindole (**9**) with methyl iodide yielded 1-methyl-3-acetylindole (**10**). Finally, the reaction of acetylindole **9/10** with appropriate aromatic/heterocyclic aldehydes (**11**) in presence of sodium hydroxide under refluxing conditions afforded the chalcones (**4a-j**) in good yields (scheme 4.2).



Scheme 4.2 Synthesis of indolyl chalcones (**4a-j**)

All synthesized compounds were well characterized by IR, ^{1}H NMR and Mass spectral data. The ^{1}H NMR spectrum of indolyl chalcone (**3b**) is displayed in the figure 4.5.

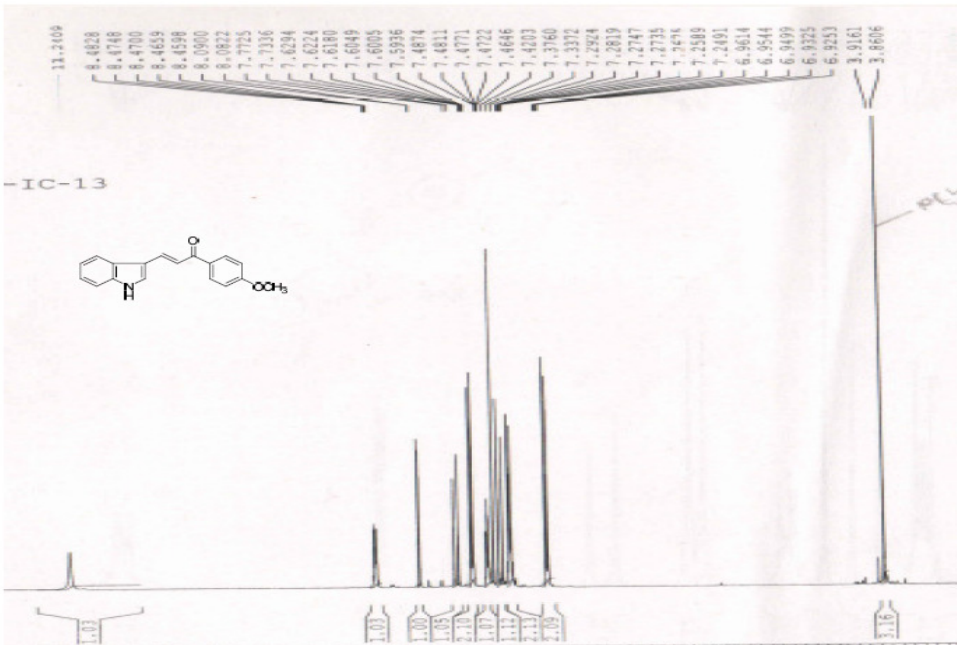


Figure 4.5 ^{1}H NMR spectrum of indolyl chalcone **3b**

The mass spectrum of indolyl chalcone **3b** showed the molecular ion peak *m/z* = 278 $(M+H)^+$ which is in agreement with the calculated value (figure 4.6.)

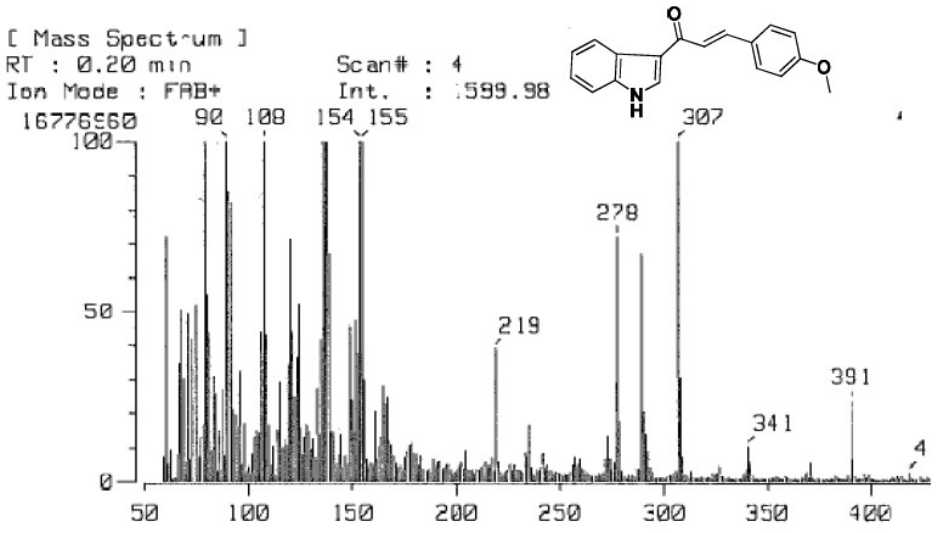


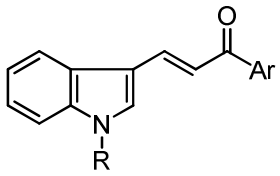
Figure 4.6 Mass spectrum of indolyl chalcone **3b**

4.3.2 Anticancer activity

Synthesized indolyl chalcones (**3**, **4**) were tested against three different human cancer cell lines including the epithelial lung cancer (A549), pancreatic carcinoma (PaCa-2) and androgen-independent human prostatic adenocarcinoma (PC-3)The tested compounds have shown significant anticancer activity which is expressed in the terms of IC_{50} in micromolar concentrations. Anticancer activity resultsof indolyl chalcones (**3a-m**) are shown in table 4.1. Structure-activity relationship was drawn by taking variety of acetophenones with methoxy and multi-methoxy substitutions. The present series of indolyl chalcones (**3a-m**) suggests that introduction of multiple methoxy groups on phenyl ring enhances their activities. The compound **3b** with *p*-methoxy group is moderately cytotoxic against all the three cell lines without any selectivity. Introduction of second methoxy group in the aryl ring is beneficial for the activity (compounds **3c** *vs* **3b**). The compound **3c** exhibited improved cytotoxicity against the tested cancer cell lines when compared to the compound **3b**. However, replacement of 3,4-dimethoxy groups with methylenedioxy moiety led to the compound **3d** with reduced activity and showed the free methoxy groups are better when compared to cyclic system. Further, compounds **3g** and **3m** bearing 3,4,5-trimethoxy groups in the aromatic ring are the most active compound in this series, the compound **3g** is selectively cytotoxic against PaCa-2 cancer cell line with an IC_{50} value of 0.03 μM. *N*-methylation of indolyl chalcones **3b, 3c, 3d, 3e**

and **3g** led to the compounds **3j, 3i, 3k, 3h** and **3m**. *N*-Methylation imparts reduction in anticancer activity to the compounds **3i, 3k** and **3m**. Indolyl chalcone **3i** showed 10 times reduction in anticancer activity against tested cancer cell lines than the parent compound **3c**. *N*-methyl derivative of compound **3g** with 3,4,5-trimethoxyphenyl substituent (compound **3m**) showed 3 times less potency against PaCa-2 (IC_{50} = 0.09 μM). Where as in case of *N*-methylated derivatives **3h** and **3j** with 4-flurophenyl and 4-methoxyphenyl substituents, increase in anticancer activity was observed when compared their parent compound **3b** and **3e**.

Table 4.1 *In-vitro* cytotoxicity of indolyl chalcones (**3a-m**)



Compound	R	Ar	IC_{50} (μM) [a]					
			A549		PaCa-2		PC-3	
			24h	48h	24h	48h	24h	48h
3a	H	4-OHC_6H_4	ND	ND	>100	ND	ND	ND
3b	H	4-$OCH_3C_6H_4$	58.2	41.5	48.0	46.5	ND	56.2
3c	H	3,4-$(OCH_3)_2C_6H_3$	9.5	4.9	7.6	4.5	50.6	27.9
3d	H	3,4-$(OCH_2O)C_6H_3$	15.2	10.8	15.1	7.9	>100	34.2
3e	H	4-FC_6H_4	ND	ND	ND	ND	ND	ND
3f	H	2-C_5H_4N	ND	ND	ND	ND	ND	ND
3g	H	3,4,5-$(OCH_3)_3C_6H_2$	**0.20**	**0.10**	ND	**0.03**	ND	**0.10**
3h	CH_3	4-FC_6H_4	8.5	8.3	8.9	3.9	ND	34.3
3i	CH_3	3,4-$(OCH_3)_2C_6H_3$	58.4	39.7	56.8	40.3	>100	66.6
3j	CH_3	4-$OCH_3C_6H_4$	21.6	16.2	24.8	13.4	>100	>100
3k	CH_3	3,4-$(OCH_2O)C_6H_3$	92.8	37.1	83.7	36.1	ND	65.6
3l	CH_3	4- OHC_6H_4	ND	ND	>100	ND	ND	ND
3m	CH_3	3,4,5-$(OCH_3)_3C_6H_2$	**0.21**	**0.15**	**0.28**	**0.09**	ND	**0.15**

[a]Data expressed in terms of IC_{50} values were obtained by dose dependent response; ND: not determined;

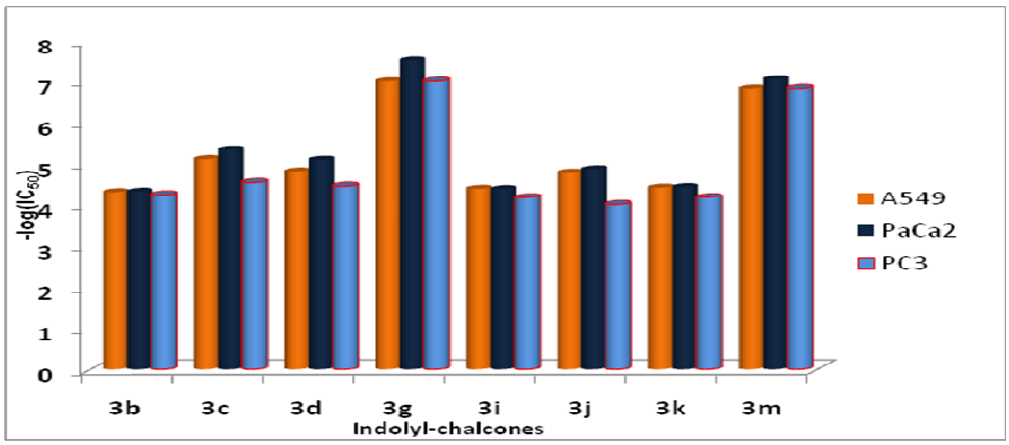
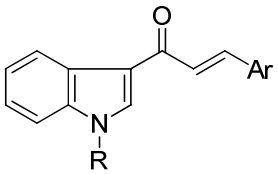


Figure 4.7 Anticancer activity of indolyl chalcones (**3b-d, 3g, 3i-k** and **3m**)

The anticancer activity results of methoxyphenyl substituted indolyl chalcones **3** are shown in figure 4.7. The compounds **3g** and **3m** showed the high cytotoxicity against tested cancer cell lines.

Table 4.2 *In-vitro* cytotoxicity of indolyl chalcones **(4a-j)**



Compound	R	Ar	IC_{50} (μM) [a]					
			A549		PaCa-2		PC-3	
			24h	48h	24h	48h	24h	48h
4a	H	4-OHC_6H_4	-----	-----	-----	-----	-----	-----
4b	H	4-$OCH_3C_6H_4$	ND	ND	ND	ND	ND	ND
4c	H	3,4-$(OCH_3)_2C_6H_3$	-----	-----	-----	-----	-----	-----
4d	H	3,4-$(OCH_2O)C_6H_3$	ND	ND	ND	ND	ND	ND
4e	H	4-$N(CH_3)_2C_6H_4$	ND	ND	ND	ND	ND	ND
4f	H	3-indolyl	ND	ND	ND	ND	ND	ND
4g	H	4-C_5H_4N	12.9	6.0	7.2	4.4	19.1	9.0
4h	CH_3	4-$OCH_3C_6H_4$	-----	-----	-----	-----	-----	-----
4i	CH_3	3,4-$(OCH_3)_2C_6H_3$	-----	-----	-----	-----	-----	-----
4j	CH_3	4-$N(CH_3)_2C_6H_4$	19.5	8.7	17.1	15.0	ND	ND

[a]Data expressed in terms of IC_{50} values were obtained by dose dependent response; ND: not determined; -----: not soluble in buffer.

In another series of indolyl chalcones (**4a-j**), the connectivity of enone moiety with indole and aromatic ring was reversed. This series of chalcones showed inferior activities when compared with indolyl chalcones (**3a-m**). *In-vitro* cytotoxicity results of chalcones (**4a-j**) are shown in table 4.2. Of the synthesized indolyl chalcones, the compounds **4g** and **4j** displayed good activity against A549 and PaCa-2 cancer cell lines. The compound **4g** has displayed significant cytotoxicity against PaCa-2 with an IC_{50} value of 4.4 μM. Indolyl chalcone **4j** with a *N,N*-dimethylaminophenyl substituent was moderately active and selective against A549 with an IC_{50} value of 8.7 μM.

4.4 Conclusions

In summary, we have synthesized two diverse series of indolyl chalcones (**3** and **4**) and evaluated for their cytotoxic activity. We observed indolyl chalcones in which the double bond is directly attached to indole ring (**3a-m**) are found to be more potent than the series where keto is linked to indole ring (**4a-j**). Compounds **3c**, **3h**, **3g**, and **3m** have significant cytotoxicity against all the tested cancer cell lines with selectivity towards PaCa-2. In another series, compounds **4g** and **4j** exhibited good cytotoxicity. The preliminary anticancer activity study of indolyl chalcones revealed that 3,4,5-trimethoxyphenyl, 4-pyridyl and *N,N*-dimethylphenyl moieties are beneficial for activity as well as selectivity.

4.5 Experimental Procedures

General: All reagents such as indole, aromatic aldehydes and ketones were purchased from Spectrochem and Aldrich, India. The reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized under UV light (254 nm). Column chromatography was performed using 100-200 mesh silica gel and appropriate mixture of hexane and ethylacetate for elution. Melting points (mp) were determined with *EZ*-Melt automated melting point apparatus. FT-IR spectra were recorded on Shimadzu spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance II spectrophotometer. Mass spectra were obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972.

4.5.1 Synthesis of indolyl chalcones 3 and 4

Indole-3-carboxaldehyde (6a)

A round-bottomed flask containing 28.8 mL (27.4 g, 370 mmol) of freshly distilled dimethylformamide (DMF) was cooled in an ice-salt bath for about 0.5 h and 8.6 mL (14.4 g, 90 mmol) of freshly distilled phosphorus oxychloride was subsequently added with stirring to the DMF over a period of 0.5 h. A solution of indole (**5**, 10 g, 85.47 mmol) in DMF (9.5 g, 130 mmol) was added to the yellow solution over a period of 1 h. The solution was stirred at 35 °C to become a yellow paste. At the end of the reaction 30 g of crushed ice was added to the paste with stirring which becomes a clear cherry-red aqueous solution. A solution of sodium hydroxide (37.5 g, 94 mmol) in 100 mL of water was added dropwise with stirring. The resulting suspension was heated rapidly to 90 °C and allowed to cool to room temperature, after which it was placed in refrigerator overnight. The product was filtered, washed with water (2 × 100 mL) and air-dried to afford the 12 g of pure indole-3-carboxaldehyde (**6a**). Yield 97%; Pale yellow solid, mp 194-196 °C (Lit.[51] mp 196-197 °C)

1-Methylindole-3-carboxaldehyde (6b)

Indole-3-carboxaldehyde (**6a**, 3 g, 20.68 mmol), potassium carbonate (1.5 g), dimethyl formamide (20 mL) and dimethyl carbonate (5.2 mL, 61 mmol) were mixed together and heated to reflux at 130 °C. After competition of the reaction, the contents were cooled to about 3 °C, and 60 mL of ice-cold water was slowly added. The product precipitated as dark oily suspension was extracted with 60 mL of diethylether and washed with water (2 × 50 mL). The removal of excess solvent led to the 1-methylindole-3-carboxalehyde (**6b**) as off-white solid in 85% yield; mp 68-70 °C (Lit.[50] mp 69-73 °C).

1-Benzenesulfonylindole (8)

To a mixture of indole (**5**, 11.7 g, 0.1 mol), 50% sodium hydroxide (35 mL), tetrabutylammonium bromide (TBAB) (0.03g, 0.001 mol), and water (20 mL) was added a solution of benzenesulfonyl chloride (21.12 g, 1.2 mmol) in toluene dropwise with continuous stirring. After the completion of reaction, toluene layer was separated and washed with saturated sodium bicarbonate, water and brine solutions (100 mL each). The organic layer was extracted and dried on anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain off-white solid **8** (22.6 g, Yield 88%), mp 195 °C (Lit.[52] 196-197 °C).

3-Acetylindole (9)

(i) To a stirred solution of $AlCl_3$ (24 g, 0.15 mol) in dichloromethane (100 mL) was added acetic anhydride (9.2 g, 0.09 mol). A solution of **8** (7.7 g, 0.03 mol) in dichloromethane (25 mL) was added to the above solution and stirred for 2 h. After completion of the reaction product was extracted by using dichloromethane (500 mL) and was washed with brine solution (100 mL). The organic layer was dried over anhydrous sodium sulfate and reduced under pressure to obtain an off-white colored solid which upon recrystallization using methanol afforded pure 3-acetyl-1-benzensulfonylindole (8.2 g, Yield 92%), mp 158 °C (Lit.[52] 159-160 °C).

(ii) To a stirred solution of above obtained 3-acetyl-1-benzensulfonylindole (7.00 g, 0.023 mol), K_2CO_3 (8.30 g, 0.06 mol), MeOH (400 mL) and H_2O (100 mL) was refluxed 2 h. The methanol was evaporated and the aqueous residue was thoroughly extracted with CH_2C1_2. The organic extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo to give **9** as a white solid (3.57 g, Yield 96%), mp 192°C (Lit.[52] 191-193 °C).

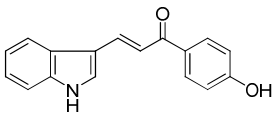
1-Methyl-3-acetylindole (10)

To a solution of 3-acetylindole (**9**, 1.56 g, 0.01mol) in dimethysulfoxide (8 mL), KOH pellets were added (1.12g, 0.02 mol) and the mixture was stirred at 10 °C for 5 min. to this methyliodide (2.81g, 0.02 mol) was added slowly. After completion of the reaction, contents ware poured into ice-cold water, solid so obtained was filtered and dried to get pure 1-methyl-3-acetylindole **10** (1,38 g, Yield 88 %), mp 106-107 °C (Lit.[53] 107 °C).

General procedure for the synthesis of indolyl chalcones (3a-m)

A mixture of indol-3-carboxaldehyde (**6**, 1mmol) and appropriate acetophenone (**7**, 1 mmol) in absolute ethanol (30 mL) was refluxed in presence of piperidine (0.5 mL) for 20 h. The reaction mixture was poured onto crushed ice, neutralized with acetic acid to afford solid compound was filtered crude, which was purified by recrystallization from ethanol to produce pure indolyl chalcone **3**.

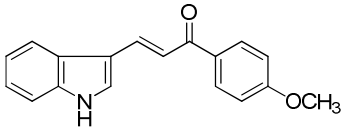
1-(4-Hydroxyphenyl)-3-(indol-3-yl)prop-2-en-1-one (3a)



Yield 65%; Dark brown solid; mp 147-148 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.10 (br, s, 1H, OH), 6.54 (d, 1H, *J* = 8.76 Hz, Ar-H), 7.20-7.25 (m, 2H, Ar-H), 7.49 (d, 1H, *J* = 8.32 Hz, Ar-H), , 7.59 (d, 1H, *J* = 14.64 Hz, H_α), 7.64 (m, 1H, Ar-H), 8.06-8.08 (m, 3H, Ar-H), 8.09 (d, 1H, *J* = 14.48 Hz, H_β). 8.37 (d, 1H, *J* =3.32 Hz, Ar-H), 9.92 (s, 1H, NH); IR (KBr, *v* cm^{-1}):

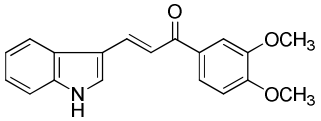
3275-3197 (OH), 3178 (NH), 1658 (C=O), 1577 (C=C), 1520, 1487, 972, 837, 752; HRMS (FAB^+): *m/z* calcd. for $C_{17}H_{13}NO_2$: 263.0960, found: 263.0940 $(M)^+$.

3-(Indol-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (3b)



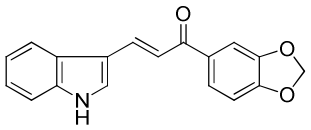
Yield 70%; Yellow solid; mp 169-171 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 3H, OCH_3), 6.92-6.96 (m, 2H, Ar-H), 7.24-7.29 (m, 2H, Ar-H), 7.35 (d, 1H, *J* = 15.52 Hz, H_α), 7.46-7.48 (m, 1H, Ar-H), 7.58-7.62 (m, 2H, Ar-H), 8.75 (d, 1H, *J* = 15.56 Hz, H_β), 8.08 (d, 1H, *J* = 4.4 Hz, Ar-H), 8.45-8.48 (m, 1H, Ar-H), 11.24 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3387 (NH), 1653 (C=O), 1600 (C=C), 1585, 1523, 1448, 1261 (O-C), 829, 740; HRMS (FAB^+): *m/z* calcd. for $C_{18}H_{15}NO_2$: 277.1103, found: 278.1178 $(M+H)^+$.

3-(Indol-3-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (3c)



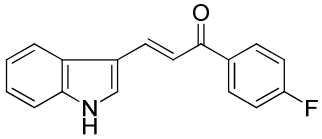
Yield 75%; Yellow solid; mp 189-191 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 3.93 (s, 6H, OCH_3), 6.94(d, 1H, *J* = 8.56 Hz, Ar-H), 7.28 (d, 1H, *J* = 14.16 Hz, H_α), 7.43-7.59 (m, 3H, Ar-H), 7.72 (s, 1H, Ar-H), 7.84 (d, 1H, *J* = 8.24 Hz, Ar-H), 8.08(d, 1H, *J* = 15.36 Hz, H_β). 8.30 (d, 1H, *J* = 4.4 Hz, Ar-H), 8.55(s, 1H, Ar-H), 10.08 (s, 1H, NH). IR (KBr, *v* cm^{-1}): 3219 (NH), 1645 (C=O), 1593 (C=C), 1554, 1516, 1417, 1228 (O-C), 800, 740; HRMS (FAB^+): *m/z* calcd. for $C_{19}H_{17}NO_3$: 307.1208, found: 308.1292 $(M+H)^+$.

1-(3,4-Methylenedioxyphenyl)-3-(indol-3-yl)prop-2-en-1-one (3d)



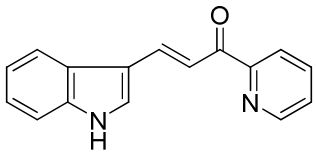
Yield 65%; Pale yellow solid; mp 184-187 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 6.08 (s, 2H, CH_2), 6.93 (d, 1H, *J* = 8.12 Hz, Ar-H), 7.24-7.28 (m, 2H, Ar-H), 7.48 (d, 2H, *J* = 9.52 Hz, Ar-H), 7.53 (d, 1H, *J* = 15.84 Hz, H_α), 7.66-7.70 (m, 2H, Ar-H), 7.96-7.98 (m, 1H, Ar-H), 8.08 (d, 1H, *J* =15.40 Hz, H_β). 11.24 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3190 (NH), 1650 (C=O), 1585 (C=C), 1558, 1527, 1445, 1248 (C-O), 830,735; HRMS (FAB^+): *m/z* calcd. for $C_{18}H_{13}NO_3$: 291.0950, found: 292.0970 $(M+H)^+$.

1-(4-Fluorophenyl)-3-(indol-3-yl)prop-2-en-1-one (3e)



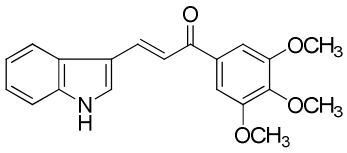
Yield 70%; Off-white solid; mp185-188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16-7.21 (m, 2H, Ar-H), 7.31-7.33 (m, 2H, Ar-H), 7.45-7.47 (m, 1H, Ar-H), 7.56 (d, 1H, *J* = 15.52 Hz, H_α), 7.63 (d, 1H, *J* = 2.68 Hz), 8.01-8.03 (m, 1H, Ar-H), 8.09 (d, 1H, *J* = 15.40 Hz, H_β). 8.10-8.13 (m, 2H, Ar-H), 10.24 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3242 (NH), 1649 (C=O), 1593 (C=C), 1595, 1537, 1433, 815, 740; HRMS (FAB^+): *m/z* calcd. for $C_{17}H_{12}FNO$: 265.0976, found: 266.0981 $(M+H)^+$.

3-(Indol-3-yl)-1-(2-pyridinyl)prop-2-en-1-one (3f)



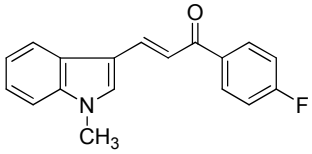
Yield 65%; Dark brown solid; mp 216-218 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15-7.22 (m, 2H, Ar-H), 7.30-7.35 (m, 2H, Ar-H), 7.48 (d, 1H, *J* = 15.60 Hz, H_α), 7.60 (d, 2H, *J* = 8.22 Hz), 7.85-7.92 (m, 1H, Ar-H), 8.10 (d, 1H, *J* = 15.44 Hz, H_β), 8.10-8.13 (m, 2H, Ar-H), 10.24 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3240 (NH), 1697 (C=O), 1593 (C=C), 1541, 1528, 1433, 794, 744; HRMS (FAB^+): *m/z* calcd. for $C_{16}H_{12}N_2O$: 248.0950, found: 249.1924 $(M+H)^+$.

3-(Indol-3-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3g)



Yield 75%; Pale yellow solid; mp 183-185 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 6H, OCH_3), 3.90 (s, 3H, OCH_3), 7.24-7.32 (m, 5H, Ar-H), 7.47 (d, 1H, *J* = 16.25 Hz, H_α), 7.53 (s, 1H, Ar-H), 7.98 (d, 1H, *J* = 9.04 Hz, Ar-H), 8.05 (d, 1H, *J* = 15.58 Hz, H_β), 10.05 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3190 (NH), 1643(C=O), 1587 (C=C), 1552, 1504, 1456 (indole ring), 1246 (C-O), 792, 725 (aromatic C=C); HRMS (FAB^+): *m/z* calcd. for $C_{20}H_{19}NO_4$: 337.1314, found: 338.1348 $(M+H)^+$.

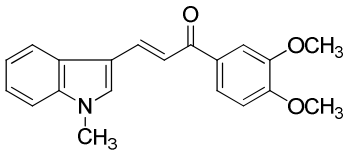
1-(4-Fluorophenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3h)



Yield 70%; Pale yellow solid; mp 147-149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (s, 3H, NCH_3), 7.14 (d, 1H, *J* =8.52 Hz, Ar-H), 7.30-7.36 (m, 3H, Ar-H),7.47 (d, 1H, *J* = 4.88 Hz, Ar-H), 7.50 (d, 1H, *J* = 15.64 Hz, H_α), 7.98 (d, 2H, *J* = 8.24 Hz, Ar-H), 7.95 (d, 2H, *J* = 7.62 Hz, Ar-H), 8.07 (d, 1H, *J* = 15.61 Hz, H_β); IR (KBr, *v* cm^{-1}): 3050 (C-H), 1649 (C=O), 1597 (C=C), 1562, 1529, 1408, 813, 732; HRMS (FAB^+): *m/z*

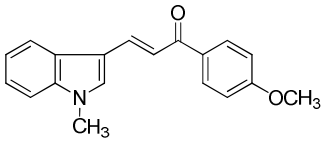
calcd. for $C_{18}H_{14}FNO$: 280.1138, found: 280.1141 $(M+H)^+$.

1-(3,4-Dimethoxyphenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3i)



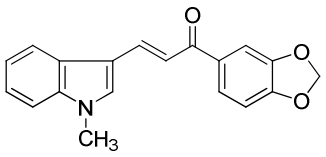
Yield 80%; Yellow solid; mp 124-125 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.82 (s, 3H, NCH_3), 3.90 (s, 6H, OCH_3), 6.89 (d, 1H, *J* = 8.28 Hz, Ar-H), 7.23-7.33 (m, 3H, Ar-H),7.41 (s, 1H, Ar-H), 7.51 (d, 1H, *J* = 15.6 Hz, H_α), 7.60 (d, 1H, *J* = 1.96 Hz, Ar-H), 7.65 (d, 1H, *J* = 8.36 Hz, Ar-H), 7.95 (d, 1H, *J* = 8.36 Hz, Ar-H) 8.01 (d, 1H, *J* = 15.36 Hz, H_β). IR (KBr, *v* cm^{-1}): 3005 (C-H), 1651 (C=O), 1593 (C=C), 1558, 1532, 1416, 813. HRMS (FAB^+): *m/z* calcd. for $C_{20}H_{19}NO_3$: 321.1365, found: 322.1432 $(M+H)^+$.

1-(4-Methoxyphenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3j)



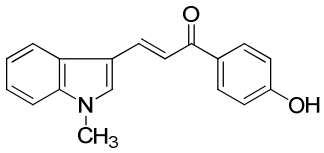
Yield 85%; yellow solid; mp 130-133 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.83 (s, 3H, NCH_3), 3.88 (s, 3H, OCH_3), 6.96 (d, 2H, *J* = 8.76 Hz, Ar-H), 7.27-7.35 (m, 3H, Ar-H), 7.45 (s, 1H, Ar-H), 7.55 (d, 1H, *J* = 15.64 Hz, H_α), 8.00 (d, 1H, *J* = 8.36 Hz, Ar-H), 8.02 (d, 2H, *J* = 3.17 Hz, Ar-H) 8.06 (d, 1H, *J* = 15.10 Hz, H_β). IR (KBr, *v* cm^{-1}): 3045 (C-H), 1651 (C=O), 1600 (C=C), 1564, 1531, 1462, 1219 (C-O), 812, 736; HRMS (FAB^+): *m/z* calcd. for $C_{19}H_{17}NO_2$: 291.1259, found: 292.1334 $(M+H)^+$.

1-(3,4-Methylenedioxyphenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3k)



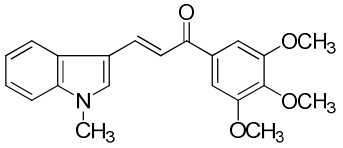
Yield 82%; Pale yellow solid; mp 165-169 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H, NCH_3), 6.04 (s, 2H, CH_2), 6.89 (d, 1H, *J* = 8.04 Hz, Ar-H), 7.27-7.36 (m, 3H, Ar-H), 7.46 (d, 1H, *J* = 9.52 Hz, Ar-H), 7.53 (d, 1H, *J* = 15.84 Hz, H_α), 7.55 (s, 1H, Ar-H), 7.67 (d, 1H, *J* = 8.08 Hz, Ar-H), 7.99 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.05 (d, 1H, *J* = 15.60 Hz, H_β); IR (KBr, *v* cm^{-1}): 3043 (C-H), 1651 (C=O), 1614 (C=C), 1556, 1531, 1487, 1211 (C-O), 813, 752; HRMS (FAB^+): *m/z* calcd. for $C_{19}H_{15}NO_3$: 305.1130, found: 306.1131 $(M+H)^+$.

1-(4-Hydroxyphenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3l)



Yield 65%, Dark brown solid; mp 175-177 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H, NCH_3), 4.3 (br, s, 1H, OH), 6.73 (d, 2H, *J* = 8.6 Hz, H_α), 7.21 (d, 1H, *J* = 8.4 Hz, 7.32-7.39 (m, 3H, Ar-H), 7.47 (d, 1H, *J* = 16.76 Hz, H_α), 7.57 (d, 1H, *J* = 15.48 Hz, H_β), 7.97 (d, 1H, *J* = 8.64 Hz, Ar-H), 8.01-8.05 (m, 2H, Ar-H); IR (KBr, *v* cm^{-1}): 3464 (O-H), 1620 (C=O), 1598 (C=C), 1568, 1528, 1462, 865, 748; HRMS (FAB$^+$): *m/z* calcd. for $C_{18}H_{15}NO_2$: 277.1103, found: 278.1180 $(M+H)^+$.

1-(3,4,5-Trimethoxyphenyl)-3-(1-methyl-indol-3-yl)prop-2-en-1-one (3m)

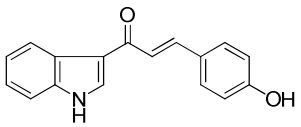


Yield 80%; Yellow solid; mp 152-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H, NCH_3), 3.90 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3), 7.22-7.39 (m, 5H, Ar-H), 7.39 (s, 1H, Ar-H), 7.47 (d, 1H, *J* = 14.88 Hz, H_α), 7.97 (d, 1H, *J* =8.04 Hz, Ar-H), 8.07 (d, 1H, *J* = 15.36 Hz, H_β); IR (KBr, *v* cm^{-1}): 3081 (C-H), 1641 (C=O), 1589 (C=C), 1558, 1527, 1471, 1252 (C-O), 812, 727; HRMS (FAB$^+$): *m/z* calcd. for $C_{21}H_{21}NO_4$: 351.1504, found: 352.1542 $(M+H)^+$.

General procedure for the synthesis of indolyl chalcones (4a-j)

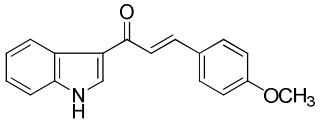
To a solution of acetylindole (**9** or **10**, 1 mmol) and appropriate aldehyde (**11**, 1 mmol) in ethanol (20 mL) was added 10% sodium hydroxide (2 mL) and refluxed the reaction mixture for 15 h. The contents of reaction mixture were poured into ice-cold water and neutralized with dilute hydrochloric acid. The solid so obtained was filtered, dried and recrystallized from ethanol to afford pure **4a–j**.

3-(4-Hydroxyphenyl)-1-(1H-indol-3-yl)prop-2-en-1-one (4a)



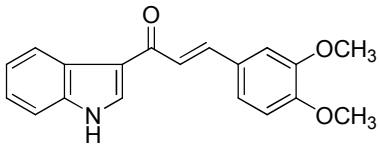
Yield 60%; Dark blue solid; mp 213-215 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.60 (s, 1H, OH), 6.90-7.26 (m, 4H, Ar-H), 7.27 (d, 1H, *J* = 15.52 Hz, H_α), 7.30-7.38 (m, 3H), 7.75 (d, 1H, *J* = 15.5 Hz, H_β), 8.37 (d, 2H, *J* = 8.08 Hz), 11.02 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3539-3397 (OH), 3180 (NH), 1612 (C=O), 1587 (C=C), 1518, 1485, 966, 794. 754; HRMS (FAB$^+$): *m/z* calcd. for $C_{17}H_{13}NO_2$: 263.0946, found: 263.0870 $(M)^+$.

1-(Indol-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (4b)



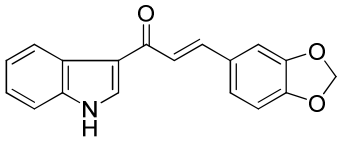
Yield 65%, Yellow solid; mp 200-202 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16 (s, 3H, OCH_3), 6.94 (d, 2H, *J* = 8.76 Hz, Ar-H), 7.24-7.29 (m, 2H, Ar-H), 7.35 (d, 1H, *J* = 15.2 Hz, H_α), 7.46-7.48 (m, 1H, ArH), 7.61 (d, 2H, *J* = 8.76 Hz, Ar-H), 7.75 (d, 1H, *J* = 15.5 Hz, H_β), 8.08 (d, 1H, *J* = 3.12 Hz, Ar-H), 8.45-8.48 (m, 1H, Ar-H), 11.24 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3120 (NH), 1639 (C=O), 1566, 1543, 1268, 819, HRMS (FAB: *m/z* calcd. for $C_{18}H_{15}NO_2$: 277.1103, found: 278.1179 $(M+H)^+$.

1-(Indol-3-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (4c)



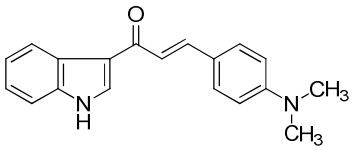
Yield 75%; Pale yellow solid; mp 202-205; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.91 (d, 1H, *J* = 8.32, Ar-H), 7,16 (d, 1H, *J* = 1.72 Hz), 7.24 (d, 1H, *J* = 16.04 Hz , H_α), 7.30-7.36 (m, 3H), 7.43-7.46 (m, 1H), 7.79 (d, 1H, *J* = 15.5, H_β), 8.04 (s, 1H), 8.51-8.53 (m, 1H). 8.74 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3140 (NH), 1641 (C=O), 1579 (C=C), 1556, 1514, 1440, 1269 (C-O), 794, 748; HRMS (FAB^+): *m/z* calcd. for $C_{19}H_{17}NO_3$: 307.1208, found: 308.1291 $(M+H)^+$.

3-(3,4-Methylenedioxyphenyl)-1-(indol-3-yl)prop-2-en-1-one (4d)



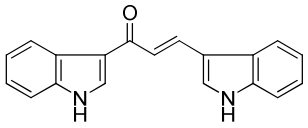
Yield 72%; Off-white solid; mp 214-218 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (s, 2H, CH_2), 6.78 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.05 (d, 1H, *J* = 1.56 Hz, Ar-H), 7.09 (d, 1H, *J* = 18.56 Hz, H_α), 7.24-7.38 (m, 4H, Ar-H), 7.69 (d, 1H, *J* = 15.48 Hz, H_β), 7.93 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.43-8.46 (m, 1H, Ar-H), 10.51 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3140 (NH), 1637 (C=O), 1602, 1566, 1517, 1489, 1246, 754; HRMS (FAB^+): *m/z* calcd. for $C_{18}H_{13}NO_3$: 291.0950, found: 292.0981 $(M+H)^+$.

3-(4-(Dimethylamino)phenyl)-1-(indol-3-yl)prop-2-en-1-one (4e)



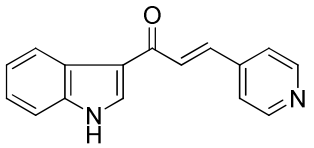
Yield 80%, Light brown solid; mp 191-192 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.97 (s, 6H, NCH_3), 6.89 (d, 1H, *J* = 8.28 Hz, Ar-H), 7.15 (d, 1H, *J* = 1.88 Hz, Ar-H), 7.23 (d, 1H, *J* = 14.04 Hz, H_α), 7.32-7.37 (m, 5H, Ar-H), 7.77 (d, 1H, *J* = 15.52 Hz, H_β), 7.89 (s, 1H, Ar-H), 8.50-8.52 (m, 1H, Ar-H), 10.10 (s, 1H, NH); IR (KBr, *v* cm^{-1}): 3180 (NH), 1612 (C=O), 1573, 1521, 1494, 1435, 798, 754; HRMS (FAB^+): *m/z* calcd. for $C_{19}H_{18}N_2O$: 290.1497, found: 291.1506 $(M+H)^+$.

1,3-Di(indol-3-yl)prop-2-en-1-one (4f)



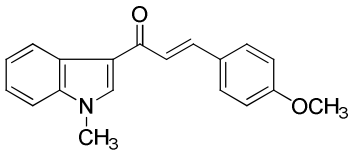
Yield 55%; Pale yellow, mp 180-183 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.13-7.26 (m, 5H, Ar-H), 7.23 (d, 1H, J = 14.04 Hz, H_α), 7.26 (d, 1H, J = 7.2 Hz), 7.47 (d, 1H, J = 15.98 Hz, H_β), 8.15 (d, 1H, J = 8.00 Hz, Ar-H), 8.28 (d, 2H, J = 8.00 Hz, Ar-H), 9.92 (s, 1H, Ar-H), 11.80 (s, 1H, NH), 11.89 (s, 1H, NH); IR (KBr, v cm^{-1}): 3197 (NH), 1641 (C=O), 1612, 1577 1529, 1444, 788, 754; HRMS (FAB$^+$): *m/z* calcd. for $C_{19}H_{14}N_2O$: 286.1187, found: 287.2105 $(M+H)^+$.

1-(Indol-3-yl)-3-(pyridin-4-yl)prop-2-en-1-one (4g)



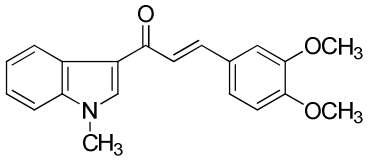
Yield 55%; Dark brown solid; mp 210-213 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.32 (m, 2H, Ar-H), 7.47-7.52 (m, 3H, Ar-H), 7.60 (d, 1H, J = 15.2 Hz, H_α), 7.68 (d, 1H, J = 15.6 Hz, H_β), 8.11 (d, 1H, J = 3.16 Hz), 8.45-8.48 (m, 1H, Ar-H), 8.66 (d, 2H, J = 5.96 Hz, Ar-H), 11.37 (s, 1H, NH); IR (KBr, v cm^{-1}): 3196 (NH), 1649 (C=O), 1589, 1570, 1517, 1448, 979, 794, 748; HRMS (FAB$^+$): *m/z* calcd. for $C_{16}H_{12}N_2O$: 248.0950, found: 249.1031 $(M+H)^+$.

3-(4-Methoxyphenyl)-1-(1-methyl-indol-3-yl)prop-2-en-1-one (4h)



Yield 60%; Yellow solid; mp 126-130 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H, NCH_3), 3.86 (s, 3H, OCH_3), 6.91 (d, 2H, J = 8.76 Hz, Ar-H), 7.26 (d, 1H, J = 15.56 Hz, H_α), 7.30-7.35 (m, 3H, Ar-H), 7.58 (d, 2H, J = 8.8 Hz, Ar-H), 7.80 (d, 1H, J = 15.52 Hz, H_β), 7.86 (s, 1H, Ar-H), 8.48-8.52 (m, 1H, Ar-H); IR (KBr, v cm^{-1}): 3040 (C-H), 1644 (C=O), 1569, 1531, 1511, 1454, 1259, 813, 769; HRMS (FAB$^+$): *m/z* calcd. for $C_{19}H_{17}NO_2$: 291.1259, found: 292.1335 $(M+H)^+$.

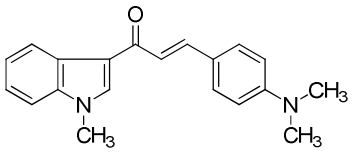
3-(3,4-Dimethoxyphenyl)-1-(1-methyl-indol-3-yl)prop-2-en-1-one (4i)



Yield 85%; Pale yellow solid; mp 158-159 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H, NCH_3), 3.92 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.88 (d, 1H, J = 8.32 Hz, Ar-H), 7.14 (d, 1H, J=1.96 Hz, Ar-H), 7.22 (d, 1H, J=14.68 Hz, H_α), 7.32-7.35 (m, 4H, Ar-H), 7.87 (d, 1H, J = 15.96, H_β), 7.89 (s, 1H, Ar-H), 8.48-8.50 (m, 1H, Ar-H); IR (KBr,

v cm^{-1}): 1641 (C=O), 1604 C=C), 1581, 1512, 1467, 1263 (C-O), 794; HRMS (FAB^+): *m/z* calcd. for $C_{20}H_{19}NO_3$: 321.1365, found: 322.1436 $(M+H)^+$.

3-(4-(Dimethylamino)phenyl)-1-(1-methyl-indol-3-yl)prop-2-en-1-one (4j)



Yield 75%; Light brown solid; mp 153-156 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 3.07 (s, 6H, 2 × NCH_3), 3.87 (s, 3H, OCH_3), 6.74 (d, 2H, *J* = 6.64 Hz, Ar-H), 7.28 (d, 1H, *J* = 15.56 Hz, H_α), 7.32-7.37 (m, 3H, Ar-H), 7.55 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.70 (s, 1H, Ar-H), 7.78 (d, 1H, *J* = 15.44, H_β), 8.51-8.53 (m, 1H, Ar-H); IR (KBr, *v* cm^{-1}): 1641 (C=O), 1604 (C=C), 1581, 1512, 1467, 1263 (C-O), 794, 732; HRMS (FAB^+): *m/z* calcd. for $C_{20}H_{20}N_2O$: 304.1575, found: 305.1656 $(M+H)^+$.

4.5.2 Anticancer assay

A549, a human epithelial cell line derived from a lung carcinoma was obtained from American Type Culture Collection. A549 were maintained in Dulbecco's modified Eagle medium with high glucose (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) with 100 µg/mL penicillin and 100 µg streptomycin. PaCa-2, a human pancreatic carcinoma cell line was obtained from Health Science Research Resources Bank (HSRRB) (Osaka, Japan) and were cultured in minimum essential medium with Earle's salts, L-Gln and non-essential amino acids (Nacali Tesque Inc, Kyoto, Japan) containing 10% FBS with 100U/mL penicillin and 100 µg streptomycin. PC-3, an androgen-independent human prostatic adenocarcinoma cell line was obtained from HSRRB. PC-3 were maintained in RPMI-1640 (Wako Pure Chem. Ind. Ltd.,Osaka, Japan) containing 10% FBS with 100 µg/mL penicillin and 100 µg streptomycin. Cell lines were kept at 37°C in a humidified atmosphere consisting of air (CO_2 5%). A549 and PaCa-2 cells were plated 5000 cells well, and PC-3 cells were plated 1×10^4 cells well in 96-well plates, the day before chalcones (**3** and **4**) treatment. All the compounds were dissolved in DMSO at room temperature. Aliquots of these stock solutions at 100 mM were stored at -20^{o}C. The cell viability was measured by the cell counting Kit-8 (Dojin, Kumamoto, Japan) using a spectrophotometer (xMax; Bio-Rad, Hercules, CA, USA) at 450 nm after 24 h and 48 h of chalcones treatment. Final concentrations of the vehicle were 1% DMSO in culture medium. The cell viability of A549, PaCa-2 and PC-3 human cancer cells was inhibited by the chalcone analogues **3** and **4** for 24-48 h in a dose-dependent manner.

4.6 References

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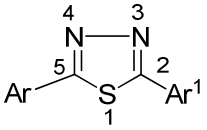
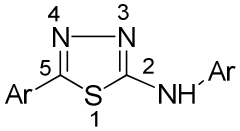
Chapter 5

5.1 Introduction-1,3,4-thiadiazoles in drug discovery

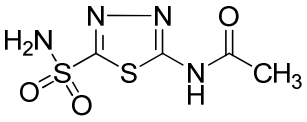
1,3,4-Thiadiazole is a five-membered heterocyclic system with a wide range of biological activities. The two major classes of biologically important 1,3,4-thiadiazoles are 2,5-diaryl-1,3,4-thiadiazoles (**1a**) and 2-arylamino-5-aryl-1,3,4-thiadiazoles (**1b**). Past several years of medicinal chemistry research has produced several drugs with thiadiazole scaffold and also it has become a target for anticancer drug research. For example, acetazolamide (**1c**) and methazolamide (**1d**) are carbonic anhydrase inhibitors and used for the treatment of glaucoma,[1] epileptic seizures, altitude sickness and cystinuria .[2]



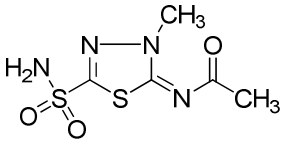
2,5-Diaryl-1,3,4-thiadiazoles (**1a**)



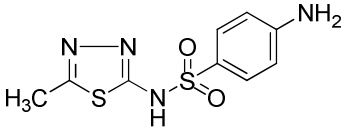
2-Arylamino-5-aryl-1,3,4-thiadiazole (**1b**)



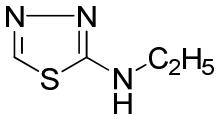
Acetazolamide **(1c)**



Methazolamide **(1d)**

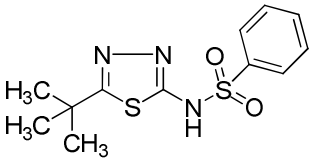


Sulfamethizole **(1e)**

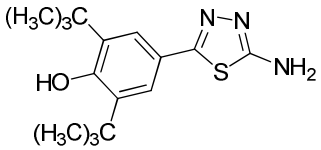


ETDA (**1f**)

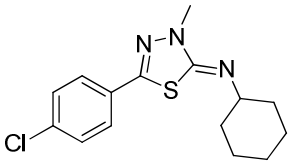
Furidiazine (**1g**)



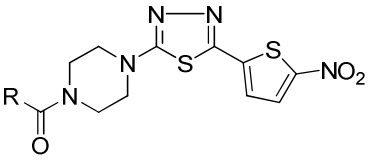
Glybuzole (**1h**)



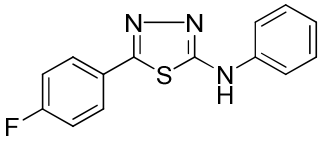
COX-2 inhibitor (**1i**)



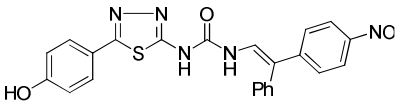
PDE7 inhibitors **(1j)**



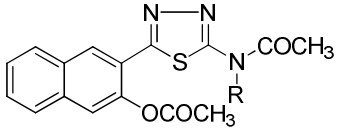
Antileishmanial agent **(1k)**



Antituberculosis agent **(1l)**



Antiepileptic agent **(1m)**



Antimicrobial agent **(1n)**

Figure 5.1 1,3,4-Thiadiazole scaffold in drug discovery (**1a-n**)

Sulfamethizole (**1e**) is an antibiotic used for the treatment of urinary tract infections caused by several gram-positive and gram-negative bacteria. A competitive inhibitor of bacterial enzyme dihydropteroate synthetase, the Sulfamethizole (**1e**) has significant activity against *E. coli* strains (MIC=512 μg/mL).[3] The 2-ethyl-1,3,4-thiadiazole **1f** (ETDA) increases the excretion of uric acid in urine and reduces the concentration of uric acid in blood plasma, hence **1f** is suggested to the patients suffering from hyperuricemia.[4] The drug Furidiazine (**1g**) is an effective antimicrobial agent. The drug Glybuzole (**1h**) increases the insulin sensitivity primarily in muscles and adipocytes, is an oral antidiabetic and suggested for the patients suffering from type 2 diabetics. The significance of the 1,3,4-thiadiazoles is spread over a broad spectrum of biological activities and as intermediates in several organic preparations.[5-8] Aromatic character of thiadiazole ring confers its *in-vivo* stability and reduced toxicity in several biological assays.[9] Some of the thiadiazoles **1i** are reported as potent inhibitors of 5-lipoxygenase (IC_{50} = 2.8 μM) and cyclooxygenase (IC_{50} = 0.8 μM) and orally active in rat models of inflammation and nonulcerogenic.[10] The novel thiadiazole **1j** acts as a potent and selective PDE7 inhibitor (IC_{50} = 1.5 μM),[11] *N*-(5-benzylsulfonyl-1,3,4-thiadiazol-2-yl) derivative **1k** of piperazinyl quinolone exhibits high antibacterial activity against gram-positive bacteria *S. aureus* and *S. epidermis* (MIC = 0.03-4 μg/mL)[12]. The 5-(4-fluorophenyl)-2-phenylamino-1,3,4-thiadiazole (**1l**) showed highest antituberculosis activity against *Mycobacterium tuberculosis* H37Rv with 69% of inhibitory activity.[13] Rajak and coworkers synthesis of 1,3,4-thiadiazoles and studied for their antiepileptic activity. The compound **1m** was found to possess significant anticonvulsant activity at 100 mg/kg in MES and scPTZ models employed for anticonvulsant evaluation.[14] Naphthyl derivatives of 1,3,4-thiadazoles (**1n**) exhibited potent antimicrobial activity against *Pseudomanas aeruginosa* which is equal to Penicillin.[12] Thiadiazoles are also known for their complexing capacity towards dyes and metal ions.[15-17]

5.1.1 1,3,4-Thiadiazoles in anticancer research

"Cancer" has emerged as a major life threatening disease in the world for past several decades and to address this issue many heterocycles emerged out as anticancer agents. Among them, 1,3,4-thiadiazoles found to be potential anticancer candidates. For example, 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) (**1o**) is a

most promising thiadiazole containing anticancer agent and inhibits proliferation in tumor cells by decreasing cell division and inhibiting metastasis (figure 5.2).[18-20]

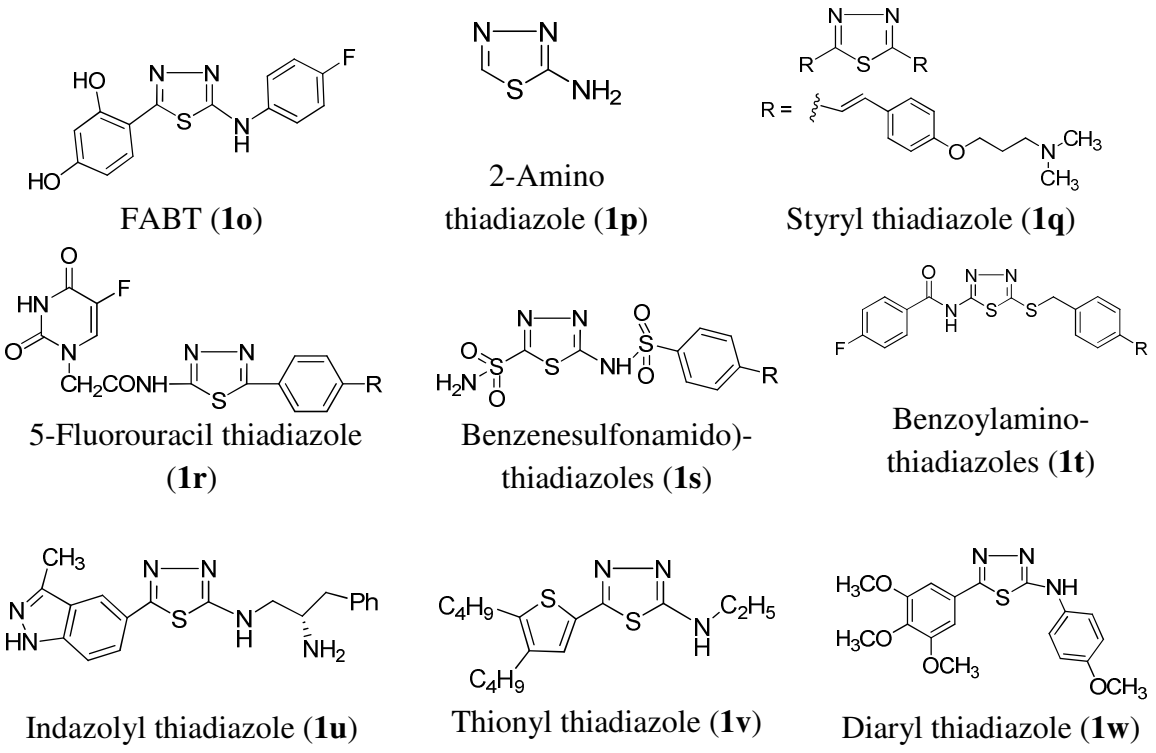


Figure 5.2 2-Amino-1,3,4-thiadiazoles (**1o-w**) as anticancer agents

2-Amino-1,3,4-thiadiazole (**1p**) and its derivatives are well known as compounds with a wide range of anticancer activities.[21-22] The mechanism of action of 1,3,4-thiadiazole ring is connected with the apoptosis and angiogenesis, which are crucial steps in the tumorigenesis. The (*E,E*)-2,5-bis[4-(3-dimethylaminopropoxy)styryl]-1,3,4-thiadiazole (**1q**) induces apoptosis in human non-small lung cancer A549 cells. The mechanistic studies indicate that **1q** induces the early-phase apoptosis in A549 cells *via* the down-regulation of Bcl-xL expression and the late-phase apoptosis through up-regulation of Bax expression as well as inhibition of Akt/PKB activation.[23] Zheng *et al.* studied the 5-fluorouracil derivatives of 1,3,4-thiadiazole for their antitumor activity against human lung cancer cell lines. The 1,3,4-thiadiazole derivative **1r** showed more potency than the 5-fluorouracil (IC_{50} = 12.07 µg/mL).[24] The thiadiazole-2-sulphonamide derivative (**1s**) was effective as a tumor cell growth inhibitor (leukaemia, non-small lung cancer, melanoma, ovarian, renal, prostate and breast cancers).[25] Targeting specific molecular functions is one of the innovative trends in the recent anticancer research, in this regard a series of benzoylamino derivatives of 1,3,4-thiadiazole (**1t**) have been discovered as Abl tyrosine kinase inhibitor (THI).[26] Indazole containing 1,3,4-thiadiazole (**1u**) found to be

an inhibitor of AKT (protein kinase B) and used as potential cancer therapeutics (IC_{50} = 76 nM).[5] Mavrova *et al.* reported thiophene containing 1,3,4-thiadiazole (**1v**) for their cytotoxicity against lymphocytes (IC_{50} = 5.2 μM).[27] Very recently, we have reported a series of 2-arylamino-5-aryl-1,3,4-thiadiazoles **6** as potent cytotoxic agents. The 5-(3,4,5-trimethoxyphenyl)-2-(4-methoxyphenylamino)-1,3,4-thiadiazole (**1w**) was identified to display highest tumor growth suppressing against PaCa2 cancer cell line (IC_{50} = 4.3 μM).[28]

5.2 Rational design

2-Arylamino-1,3,4-thiadiazoles have been explored for variety of biological activities. The 1,3,4-thiadiazole moiety is an active pharmacophore in the drug molecules such as Acetazolamide (**1c**), Methazolamide (**1d**) and Sulfamethizole (**1e**). The anticancer activity of 1,3,4-thiadiazoles is remarkable and it has become an attractive scaffold for the development of new anticancer entities. The simple 2-aminothiadiazole (**1p**) (ATDA) and related compounds including 2-ethylamino-1,3,4-thiadiazole (**1f**) (ETDA) and 2,20-(methylene diamino)bis-1,3,4-thiadiazole (**1q**) are promising anticancer candidates. The compound **1p** was used in phase II clinical trials in patients with different tumors (renal, colon, ovarian, and others). A thorough examination of the reported anticancer molecules like FABT (**1o**) shows the importance of substituents at C-2 and C-5 positions of 1,3,4-thiadiazole.

The remarkable anticancer activities of various indolylazoles such as indolyloxazoles (**7**), labradorins **1** & **2**,[29] camalexin (**8**),[30] indolyl-1,3,4-thiadiazoles (**5**), indolyl-1,3,4-oxadiazoles (**9**) and indole-based Combretastatin analogues, BPR0L075 (**10**) encouraged us to design and synthesize the present series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11**). In addition, indole nucleus is one of the "*privileged structures*" and being part of many anticancer drug candidates, incorporation of indole ring may increase the anticancer activity of 1,3,4-thiadiazoles.[31-33] Several antimitotic agents such as Combretastatin A-4 (**2**), Colchicine (**3**), Podophyllotoxin (**4**) and indole-based combretastatin analogues,[34] containing the 3,4,5-trimethoxyphenyl group as an active pharmacophore which is known to bind the colchicine site of the tubulin.[35-37] Our recent study on trimethoxyphenyl substituted 1,3,4-thiadiazoles (**6**) led to identify potent cytotoxic agents with IC_{50} values less than 5 μM against various human cancer cell lines.[28]

The incorporation of crucial structural features of two classes of anticancer agents on both the sides of 1,3,4-thiadiazole ring led to the present series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11**) (figure 5.3).

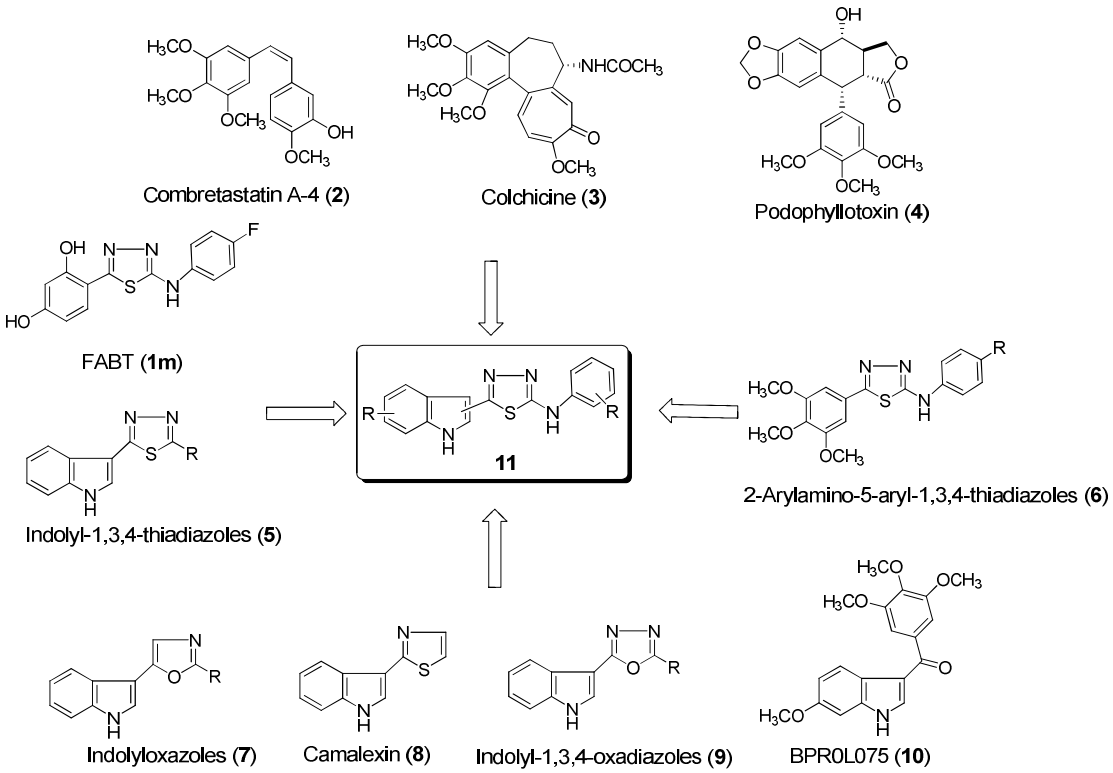


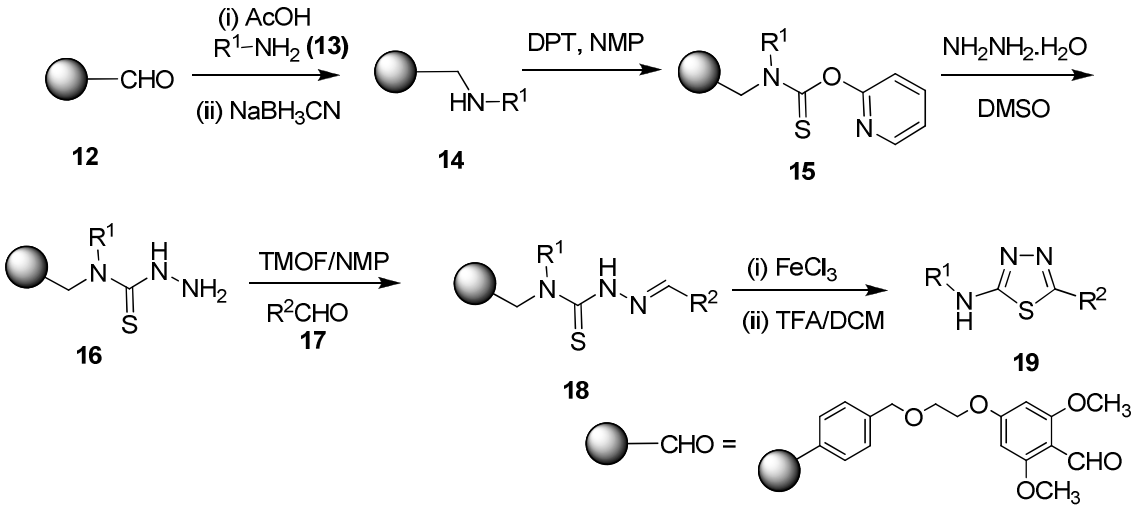
Figure 5.3 Rational for the synthesis of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11**)

5.3 Synthetic routes for 2,5-disubstituted-1,3,4-thiadiazoles

Synthesis of bio-active heterocycles such as 2,5-disubstitueted-1,3,4-thiadiazoles has been of always prime importance for the organic and medicinal chemists. Generally, preparation of 1,3,4-thiadiazoles involve use of diacylhydrazide as a precursors under different reaction conditions. Symmetrical 2,5-disubstituted-1,3,4-thiadiazoles are prepared by condensation of aryl aldehydes, hydrazine, and sulfur in ethanol under microwave irradiation.[38] The general routes for their preparation involve synthesis of acyl thiohydrazides and cyclization under acidic conditions, thionation of acyl hydrazides followed by cyclization under acidic conditions or cyclization of thiosemicarbazones. Some of the widely used and important protocols reported for the synthesis of 1,3,4-thiadiazoles are given below.

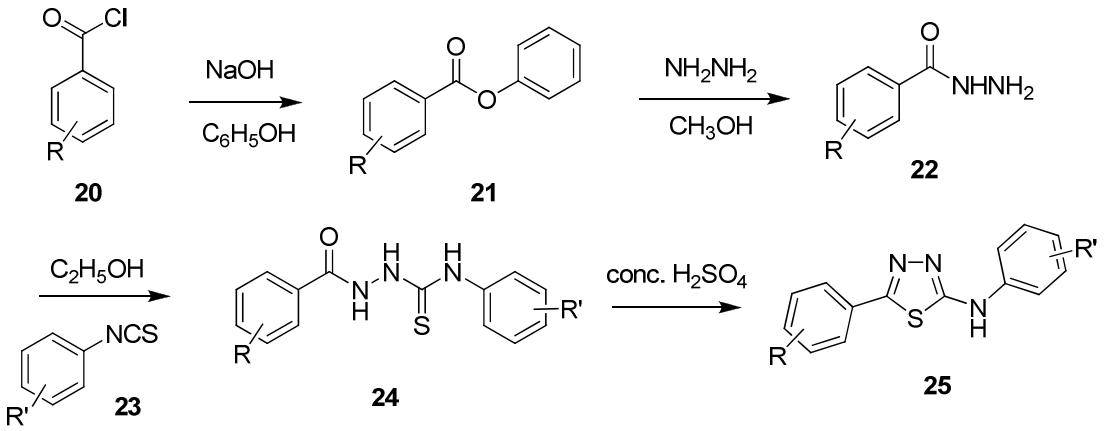
A robust protocol for the solid phase synthesis of 5-alkyl/aryl-2-alkylamino-1,3,4-thiadiazoles was described from resin bound thiosemicarbazide.[39] The protocol has been

verified by the preparation of a library with diverse substitutions that gave 1,3,4-thiadiazoles in good to excellent yields (scheme 5.1). The 2-(3,5-dimethoxy-4-formylphenoxy)ethoxymethyl polystyrene (**12**) was treated with a range of primary amines (**13**) under standard reductive amination conditions to yield the respective resin bound benzyl amine derivatives **14**. The transformation of a resin bound primary amine to an isothiocyanate (**15**) was achieved upon treatment with di-(2-pyridyl)-thionocarbonate (DPT). The isothiocyanate (**15**) was subsequently reacted with hydrazine to give a thiosemicarbazide (**16**). Treatment of **16** with aldehydes **17** in a mixture of trimethyl orthoformate (TMOF) and *N*-methyl pyrrolidine (NMP) yielded the thiosemicarbazone (**18**). Cyclization of **18** was achieved by treating the resin with a solution of iron(III) chloride. Finally, cleavage of resin with trifluoroacetic acid yielded 2-amino-5-substituted-1,3,4-thiadiazoles (**19**).



Scheme 5.1 Solid phase synthesis of 2,5-disubstituted-1,3,4-thiadiazoles (**19**)

Oruc *et al.* prepared 1,3,4-thiadiazoles in four steps utilizing acyl halides and aryl isothiocyanates (scheme 5.2).[13] The acyl halides (**20**) were reacted with phenol under basic conditions to generate esters (**21**) which were then reacted with hydrazine to yield hydrazides (**22**). Reaction of isothiocyanates **23** with **22** afforded acyl thiosemicarbazides (**24**) which were further cyclized to thiadiazoles (**25**) in presence of concentrated sulphuric acid.

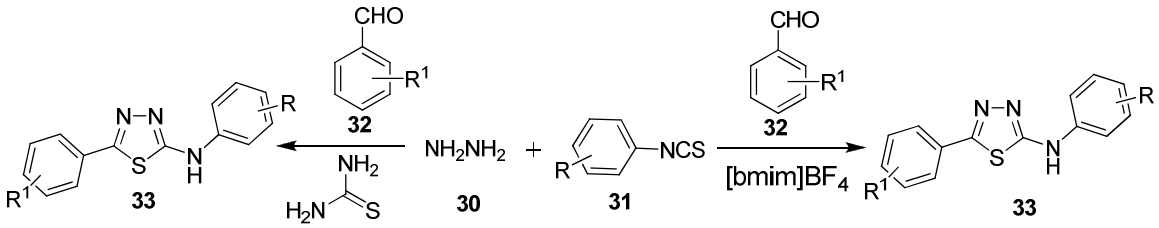


Scheme 5.2 Synthesis of 2-arylamino-1,3,4-thiadiazoles (**25**)

The 1,3,4-thiadiazoles (**29**) were also achieved in good yields from the reaction of 1,3,4-oxadiazoles (**28**) with thiourea.[40] The 1,3,4-oxadiazoles (**28**) were prepared from acid hydrazides (**26**) on treatment with different carboxylic acids (**27**) in the presence of phosphorus oxychloride (scheme 5.3).

Scheme 5.3 Synthesis of 1,3,4-thiadiazoles **(29**) from 1,3,4-oxadiazoles (**28**)

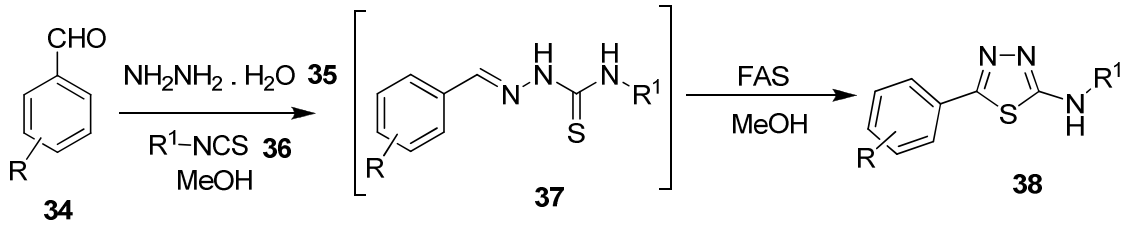
Rostamizadeh *et al.* utilizes ionic liquid/thiourea mediated oxidation of *in situ* generated thiosemicarbazones for one-pot the synthesis of 1,3,4-thiadiazoles (**33**).[41-42] The one-pot reaction of hydrazine hydrate (**30**) with variety of aryl isothiocyanates (**31**) followed by the addition of substituted aldehydes (**32**) in presence of ionic liquid or thiourea generates 1,3,4-thiadiazoles (**33**) in good yields (scheme 5.4).



Scheme 5.4 Ionic liquid/ thiourea mediated one-pot synthesis of 1,3,4-thiadiazoles (**33**)

Very recently, Kumar *et al.* reported one-pot synthesis of 1,3,4-thiadiazoles (**38**), which involves refluxing of aryl aldehydes (**34**), hydrazine hydrate (**35**) and aryl isothiocyanates (**36**) followed by oxidative cyclization of intermediate (**37**) with ferric ammonium sulfate

(FAS). The synthesized 2,5-diarylamino-1,3,4-thiadiazoles (**38**) were evaluated for their anticancer activity (scheme 5.5).[28]

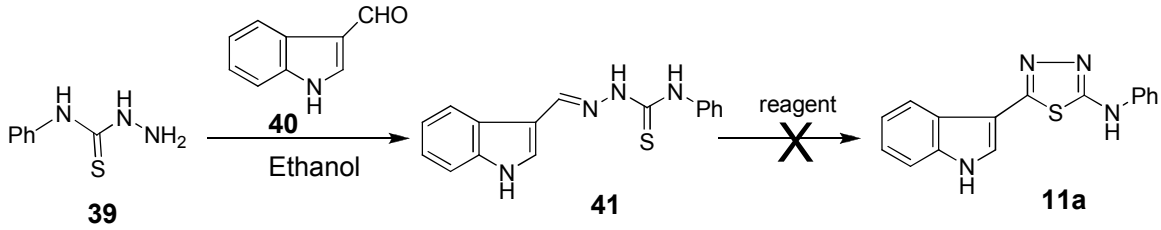


Scheme 5.5 FAS mediated one-pot synthesis of 1,3,4-thiadiazoles (**38**)

5.4 Results and discussion

5.4.1 Chemistry

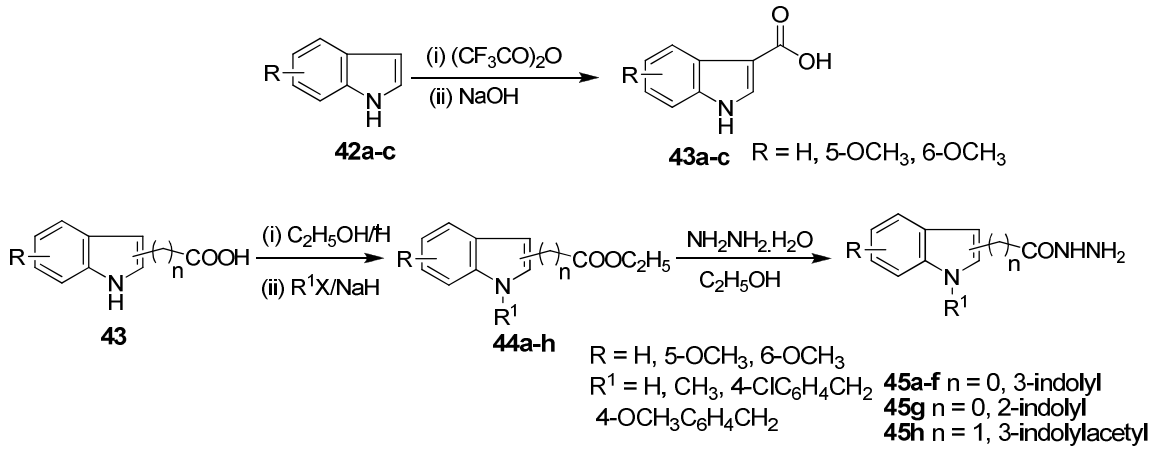
Our initial attempt to synthesize 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11**) involve the preparation of thiosemicarbazone **41** from the reaction of equimolar quantities of phenylthiosemicarbazide (**39**) and indole-3-carboxaldehyde (**40**) in ethanol under refluxing conditions. It was isolated by simple filtration and subjected to oxidative cyclization. Taking a cue from literature reports, the cyclization of (**41**) was attempted in refluxing ethanol using different reagents such as ferric chloride, zinc chloride, ferric ammonium sulfate (FAS), Chloramine-T, iodobenzene diacetate (IBD) and [hydroxy(tosyloxy)iodo] benzene (HTIB) (scheme 5.6). Unfortunately, none of the these reagents led to the desired 1,3,4-thiadaizole (**11a**).



Scheme 5.6 Attempted synthesis of indolyl-1,3,4-thiadiazole (**11a**)

On an attempt towards oxidative cyclization of **41** using reagents such as FAS and chloramine T produced indole-3-carboxaldehyde. In other cases either the reaction failed to initiate or led to complex mixtures without forming the desired indolyl-1,3,4-thiadiazole **11a**. After these unsuccessful attempts, we explored an alternative synthetic route to achieve the desired 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles. Modified route involves the reaction of indolyl hydrazides (**45**) with aryl isothiocyanates (**46**) to give an intermediate **47** which upon cyclization in acidic conditions afforded the desired

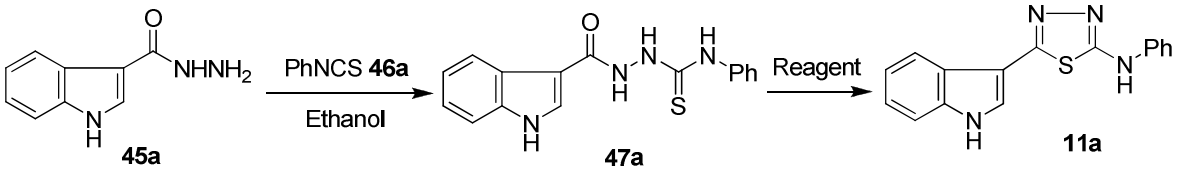
1,3,4-thiadiazoles (**11**). The synthesis was initiated with the preparation indolyl-2(3)-carbohydrazides (**45**) from corresponding indole 2(3)-carboxylic acids (**43**) which intern were prepared from the reaction of corresponding indoles (**42a-c**) with trifluoroacetic anhydride and followed by hydrolysis with sodium hydroxide (scheme 5.7).



Scheme 5.7 Synthesis of indolyl-2(3)-carbohydrazides (**45a-h**)

The indole-2-carboxylic acid and indole-3-acetic acids were procured commercially. Esterification and *N*-alkylation of indole-2(3)-carboxylic acids (**43**) afforded the ethyl-indole-2(3)-carboxylates (**44**) which upon reaction with hydrazine hydrate resulted in the formation of indole-2(3)-carbohydrazides (**45a-h**) in good yields (scheme 5.7).

The preparation of 1,3,4-thiadiazole (**11**) was instigated with a trial reaction of indole-3-carbohydrazide (**45a**) and phenyl isothiocyanate (**46a**) in ethanol (scheme 5.8). The reaction mixture was refluxed for 2 h in ethanol without additives to afford indolyl thiosemicarbazide (**47a**) in 95% yield. Upon confirming the intermediate **47a** by its IR, it was further cyclized under different acidic conditions to afford the desired 2-phenylamino-5-indoly-1,3,4-thiadiazole (**11a**) as shown in the scheme 5.8.



Scheme 5.8 Synthesis of 2-phenylamino-5-(indolyl)-1,3,4-thiadiazole (**11a**)

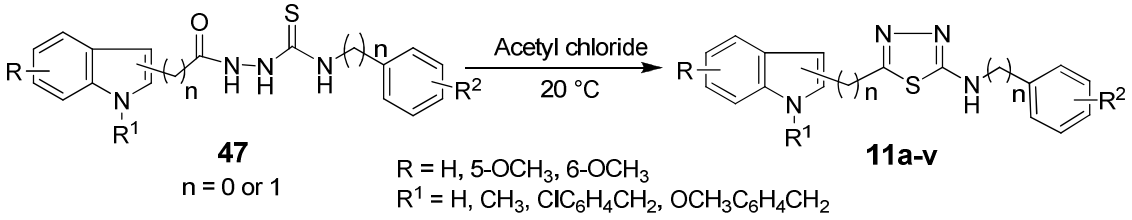
The cyclization of **47a** was attempted using concentrated sulfuric acid, polyphosphoric acid, phosphorus oxychloride and ortho phosphoric acid which resulted in poor yield of **11a** and generated complex mixtures along with product (table 5.1). Finally, our efforts to

cyclize **47a** using neat acetyl chloride at 20 °C were successful in preparing **11a** with good yield (scheme 5.8).

Table 5.1 Cyclization of **47a** to **11a** in various acidic reagents

Acidic reagent	Temp (°C)	Time (h)	Isolated yield of **11a** (%)
Conc.H_2SO_4	30	2.0	20
PPA	30	3.0	10
$POCl_3$	30	2.0	0
H_3PO_4	30	1.0	5
CH_3COCl	20	0.5	65

The optimized protocol was extended to other aryl isothiocyanates (**45**) and indolyl hydrazides (**47**) to synthesize a series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**). Variety of aryl isothiocyanates (**46**) with electron-withdrawing/electron-donating substituents and diverse indolyl-2(3)-carbohydrazides (**45**) were reacted smoothly to generate intermediates **47**, which was treated with acetyl chloride to afford **11** in good yields (scheme 5.9).



Scheme 5.9 Preparation of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**)

Our protocol to prepare 1,3,4-thiadiazoles is fairly general and involves simple experimental procedures. All the products were isolated in good yields by simple recrystallization. Synthesized 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**) were well characterized by their IR, NMR and Mass spectral data.

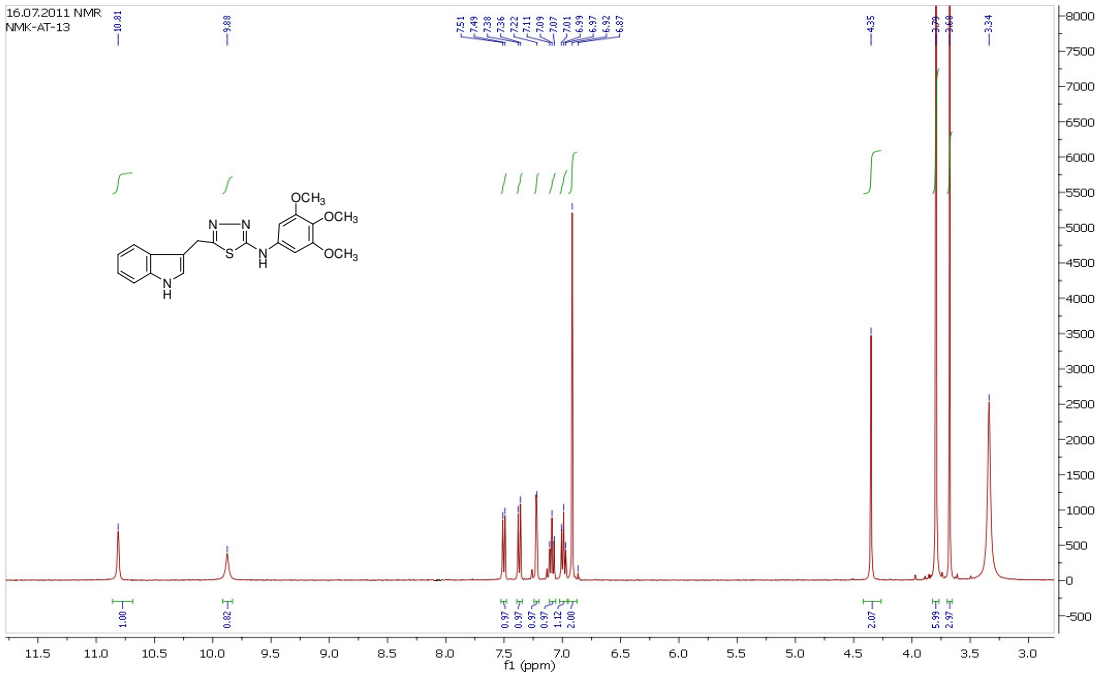


Figure 5.4 ^{1}H NMR spectrum of indolyl-1,3,4-thiadiazole **11v**

The ^{1}H and ^{13}C NMR spectra of the indolyl-1,3,4-thiadiazole (**11v**) are shown in the figures 5.4 and 5.5. ^{1}H NMR spectrum of the compound **11v** showed the characteristic singlets at δ 10.81 and 9.88 for two NH protons and 4.35 for methylene proton. ^{13}C NMR **11v** displayed two characteristic signals at δ values 160.46 and 164.03 corresponds to C-2 and C-5 carbons of 1,3,4-thiadaizoles. The IR spectrum of **11v** showed sharp bands at 3383 and 3221 cm^{-1} (N-H stretching) (figure 5.6).

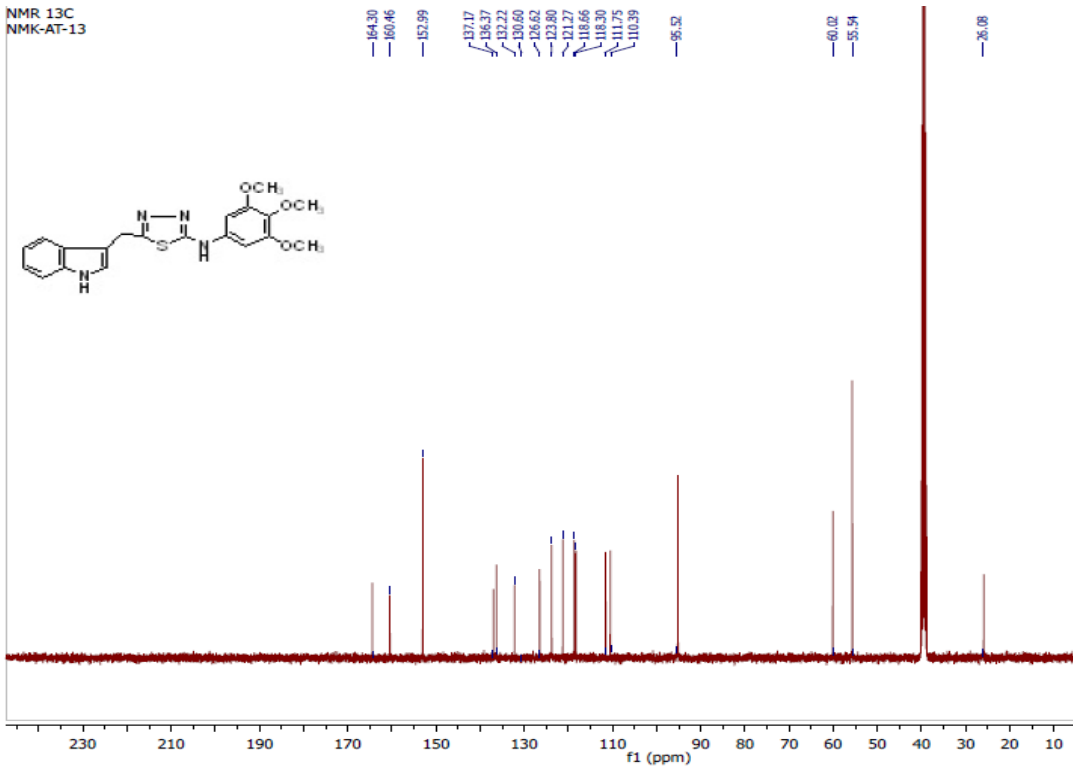


Figure 5.5 ^{13}C NMR spectrum of indolyl-1,3,4-thiadiazole **11v**

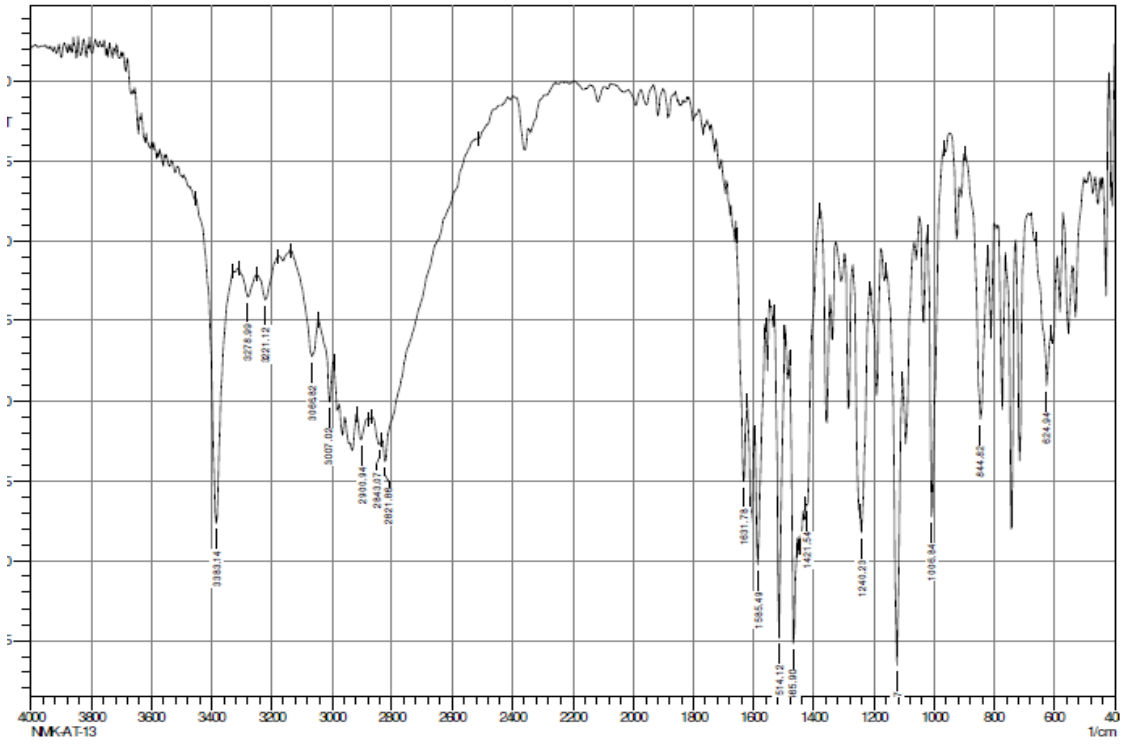


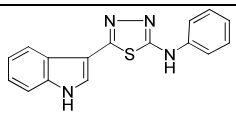
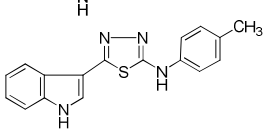
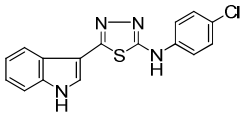
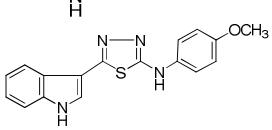
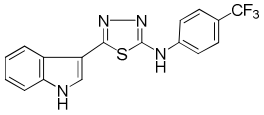
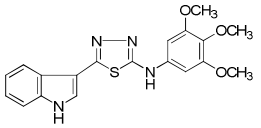
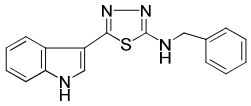
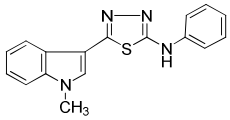
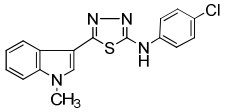
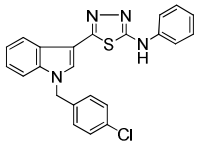
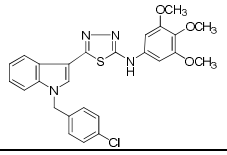
Figure 5.6 IR spectrum of indolyl-1,3,4-thiadiazole **11v**

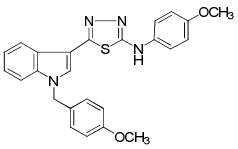
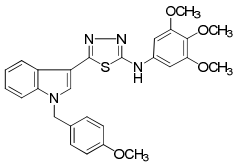
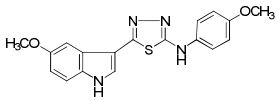
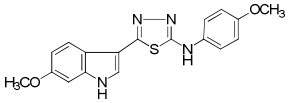
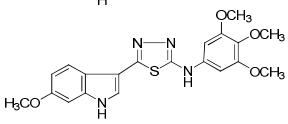
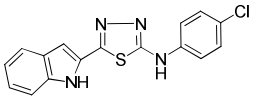
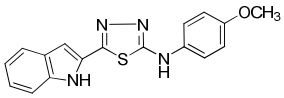
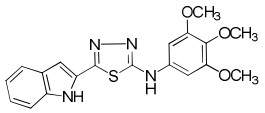
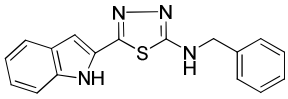
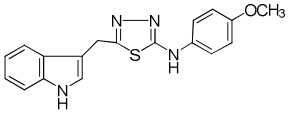
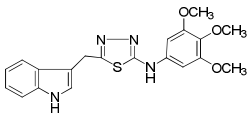
5.4.2 Anticancer activity

All the synthesized 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**) were screened against prostate (DU145 and LnCaP), breast (MCF-7 and MDA-MB-231), cervical (HeLa) and ovarian (Ovcar-3) human cancer cell lines. The activity results as mentioned in the table 5.2 show that the compounds (**11a-v**) are most sensitive against all the cancer cell lines. Most of the compounds are highly selective towards breast cancer cell line (MBA-MB-231) and the IC_{50} values are less than 1 μM and have almost similar activity against ovarian (Ovcar-3) cancer cell line (IC_{50} ~ 3-4 μM). Activity results proved that substituents at C-2 and C-5 positions of the 1,3,4-thiadiazole ring plays a crucial role in imparting the anticancer activity. A remarkable improvement in anticancer activity was observed by the introduction of indole ring at C-5 position, instead of aryl ring as reported in our previous series of 2-arylamino-5-aryl-1,3,4-thiadiazoles. For example the compound (**11a**) showed 100-folds improved cytotoxicity (IC_{50} = 0.9 μM), when compared with previously reported 2-phenylamino-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadizole against breast cancer cell line MDA-MB-231 (IC_{50} = 122.4 μM).[28] The compound **11a** has sixty folds selective cytotoxicity against breast cancer cell line MDA-MB-231 (IC_{50} = 0.9 μM) over MCF7 (IC_{50} = 55.58 μM) and has moderate cytotoxic effect against Ovcar-3, LnCap and HeLa cancer cell lines (< IC_{50} = 10 μM). The para-substitutions (CH_3, Cl, OCH_3 and CF_3) on C-2 arylamino moiety led to compounds (**11b-e**) without any significant improvement in cytotoxicity, except the compounds **11c** and **11e** having electron with-drawing groups (Cl and CF_3) exhibited slight increase in cytotoxicity against LnCap (IC_{50} = 3.14 and 5.24 μM) and MDA-MB-231 (IC_{50} = 0.55 and 0.51μM) when compared to compound **11a**. Our earlier studies on indolyl-1,2,4-triazoles and indolyl chalcones delivered potent compounds (IC_{50} = 0.8 μM and 0.03 μM) with C-2, trimethoxyphenyl moiety. On similar grounds the compound **11f** was synthesized which resulted in improvement of cytotoxicity against all the tested cancer cell lines with remarkable activity against LnCap (IC_{50} = 0.15 μM) and MCF-7 (IC_{50} = 0.91 μM), MDA-MB-231 (IC_{50} = 0.44 μM) and Ovcar (IC_{50} = 1.18 μM) cancer cell lines. Benzylamino group at C-2 position (compound **11g**) has no impact on cytotoxicity. *N*-Alkylation of indole (*N*-methyl, *N*-(*p*-chlorobenzyl) and *N*-(*p*-methoxybenzyl) compounds **11h-m**) was found to be beneficial for the anticancer activity when compared with their parent compounds (**11a,c,d** and **f**). In particular, *N*-methylindole analogues **11h** and **11m** exhibited improved cytotoxicity with IC_{50} values

0.47 and 0.44 μM, respectively against MDA-MB-231 cancer cell line. Introduction of methoxy substituent at C-5/C-6 of indole ring of **11d** led to the compounds **11n** and **11o** with improved cytotoxicity and selectivity with IC_{50} values 7.58 and 2.16 μM, respectively against LnCap cancer cell line.

Table 5.2 *In-vitro* anticancer activity of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11**)

1,3,4-Thiadiazoles (**11**)		IC_{50} (μM)					
		MCF-7	LnCap	MDA-MB-231	DU145	HeLa	Ovcar3
	(**11a**)	55.58	7.57	**0.91**	19.70	8.41	3.49
	(**11b**)	21.10	7.37	**0.82**	31.97	27.14	3.25
	(**11c**)	10.19	3.14	**0.55**	22.88	10.08	4.05
	(**11d**)	8.79	10.65	**0.94**	22.17	19.44	3.58
	(**11e**)	16.50	5.24	**0.51**	20.95	21.58	3.70
	(**11f**)	**0.91**	**0.15**	**0.44**	22.35	3.64	1.18
	(**11g**)	34.56	10.51	**0.62**	63.92	>100	3.81
	(**11h**)	11.77	6.63	**0.47**	21.63	47.88	3.85
	(**11i**)	12.81	8.31	**0.44**	19.44	10.60	3.96
	(**11j**)	9.13	18.32	**0.99**	18.04	30.80	3.55
	(**11k**)	10.59	7.53	**0.81**	24.37	40.00	3.69

	(**11l**)	8.88	11.45	**0.90**	21.16	72.87	3.68
	(**11m**)	23.15	12.38	**0.90**	90.33	50.73	3.54
	(**11n**)	21.68	7.58	**0.58**	16.11	24.36	3.43
	(**11o**)	23.38	2.16	**0.93**	19.81	63.16	3.56
	(**11p**)	13.37	3.69	**0.64**	18.95	32.34	3.46
	(**11q**)	7.55	11.38	**0.64**	16.42	21.79	3.30
	(**11r**)	8.04	2.93	**0.23**	28.83	14.39	3.10
	(**11s**)	6.48	8.67	**0.25**	42.02	9.74	2.00
	(**11t**)	9.92	5.92	**0.46**	53.75	86.57	3.75
	(**11u**)	12.72	2.30	5.94	27.74	>100	3.82
	(**11v**)	>100	5.91	1.34	24.46	>100	3.29
Doxorubicin		8.62	2.76	19.01	10.86	20.93	10.53

Introduction of methoxy group in compound **11f** led to **11p** without any improvement in cytotoxicity. Shifting the connectivity of 1,3,4-thadiazole with indole from C-3 to C-2 position led to the compounds **11q-t** with improved cytotoxicity. The compound **11r** found to be more cytotoxic against LnCap (IC_{50} = 2.93 μM) and MDA-MB-231 (IC_{50} = 0.23 μM) cell lines than the compound **11d**. The compounds **11s** and **11t** with 3,4,5-trimethoxyphenylamino and benzylamino substituents improved selectivity with

IC_{50} values 0.25 and 0.46 μM, respectively against MDA-MB-231 when compared with their regioisomers **11f** and **11g**. Further, introduction of a methylene unit between indole and 1,3,4-thiadiazole ring (compounds **11u** and **11v**) showed no appreciable improvement in the activity and resulted in poor cytotoxicity against HeLa cancer cell line (IC_{50}>100 μM). The compound **11v** with 3,4,5-trimethoxyphenylamino substituent induced the selective cytotoxicity against MDA-MB-231 (IC_{50} = 1.34 μM) cancer cell line. Doxorubicin was used as a control, which showed higher cytotoxicity towards LnCap, DU145, and MCF-7 cancer cell lines. The synthesized compounds were found to be more cytotoxic than the control Doxorubicin.

5.5 Conclusions

In summary, we have developed a novel synthetic protocol to generate a diverse library of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**) from readily available starting materials. Most of the synthesized 2-arylamino-indolyl-1,3,4-thiadiazoles (**11a-v**) exhibited good anticancer activity (IC_{50} < 1 μM) and selective towards breast cancer cell line (MDA-MB-231). Among the synthesized thiadiazoles (**11a-v**), the compound **11f** with 3,4,5-trimethoxyphenylamino moiety showed highest cytotoxicity against prostate cancer cell line (LnCap IC_{50} = 0.15 μM). The anticancer activity results of **11a-v** disclosed the significance of indole substituent at C-5 position of 1,3,4-thiadiazole ring when compared with anticancer activity data of previously reported C-5 aryl substituetnets.[28] The studies to further improve anticancer activity and identify the cellular targets of 2-arylamino-5-(indolyl)-1,3,4-thiadaizoles **11a-v** are in progress.

5.6 Experimental procedures

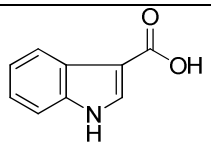
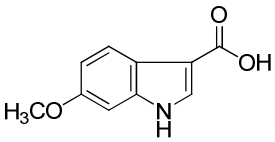
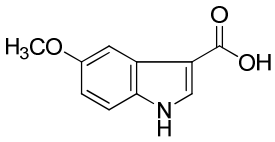
5.6.1 Synthesis of 2-arylamino-5-indolyl-1,3,4-thiadiazoles (11a-v)

Synthesis of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (**11a-v**) was achieved in five-steps (scheme 5.7 & 5.9) as given below.

Step 1. Synthesis of indole-3-carboxylic acid (43a-c)

To a stirred solution of indole or 5/6-methoxyindole (**42**, 38 mmol) in dimethylformamide (50 mL), trifluoroacetic anhydride (44 mmol) was added dropwise at 0 °C. After stirring the reaction mixture for 3 h, the contents were poured into water (200 mL) and the product was isolated by filtration. The solid residue was washed with water (3 × 50 mL) and the crude product was suspended in 20% aqueous NaOH (200 mL) and heated at

reflux for 6 h. Cooled the reaction contents, washed with dichloromethane (2 × 100 mL) and aqueous solution was acidified with hydrochloric acid. The precipitate was isolated by filtration and dried to get pure indole-3-carboxylic acid **43**.

Indole-3-carboxylic acid (**43**)		mp (°C)	Yield (%)
	(**43a**)	230-235 (Lit.[43] 229-234)	80
	(**43b**)	185-187	75
	(**43c**)	182-184	70

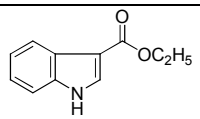
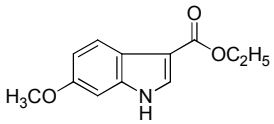
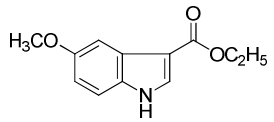
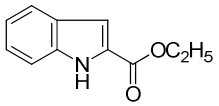
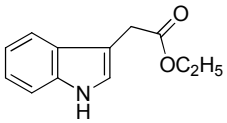
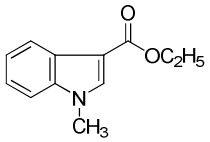
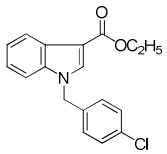
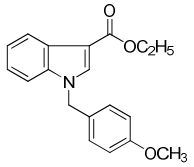
Step 2. General synthesis of ethyl ester of indole-2(3)-carboxylate (44a-e)

To a solution of indole-2(3)-carboxylic acid (**43**, 10 mmol) in 50 mL of ethanol was added 0.5 mL of concentrated sulphuric acid and refluxed for 20 h. After completion of the reaction, ethanol was evaporated and the residue so obtained was taken into water and extracted with ethyl acetate (50 mL). Organic layer was washed with brine solution (25 mL), water (25 mL) and dried over anhydrous sodium sulphate. Excess of solvent was removed to afford pure ethyl ester of indole-2(3)-carboxylate in good yields.

General procedure for alkylation of ethyl esters of indole-3-carboxylic acids

To a solution of indole-3-carboxylate (**43a**, 10 mmol) in tetrahydrofuran (20 mL) was added slowly sodium hydride 60% dispersed in mineral oil (15 mmol). Stirred the reaction mixture for 15 min at 10 °C and methyl iodide/4-chlorobenzyl chloride/ 4-methoxybenzyl chloride (11 mmol) was added and continued stirring at room temperature for 5 h. After completion of the reaction, the contents were poured into ice-cold water and the product was extracted with ethyl acetate (50 mL). Organic phase was dried over anhydrous sodium sulfate and concentrated to give pure *N*-alkylated indole esters (**44f-h**) in good yields.

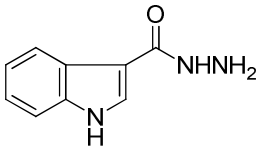
Physical data for the ethyl indole-2(3)-carboxylates (**44**)

Indole-2(3)-carboxylates (**44**)		mp (°C)	Yield (%)
	(**44a**)	120-123 (Lit.[44]120-124)	82
	(**44b**)	135-135 (Lit.[44]130-134)	85
	(**44c**)	145-146	85
	(**44d**)	195-196 (Lit. [45]194-196)	90
	(**44e**)	43-45 (Lit.[46] 43-45)	90
	(**44f**)	80- 83 (Lit.[47] 81-84)	80
	(**44g**)	Oily gum	75
	(**44h**)	120-125	70

Step 3. General synthesis of indole-2(3)-carbohydrazide (45a-h)

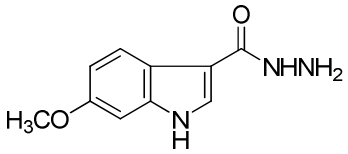
To a stirred solution of esters (**44**, 10 mmol) in ethanol (25 mL) was added hydrazine hydrate (2 mL) and heated to reflux for 24 h. After completion of the reaction, the contents were cooled and solid so obtained was filtered off to get pure indole-2(3)-carbohydrazides (**45a-h**).

Indole-3-carbohydrazide(45a)



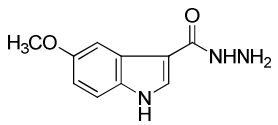
Yield 85%; White solid; mp 232-234 °C (lit.[48]); ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 9.14 (s, 1H), 8.17 (d, J = 7.10 Hz, 1H), 7.95 (d, J = 2.80 Hz, 1H), 7.41 (d, J = 8.10 Hz, 1H), 7.20-6.99 (m, 2H), 3.95 (s, 2H); IR (KBr, v cm^{-1}): 3256, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736; MS (ESI): *m/z* calcd. for $C_9H_9N_3O$: 175.1 found: 175.2 $(M)^+$.

6-Methoxy-indole-3-carbohydrazide (45b)



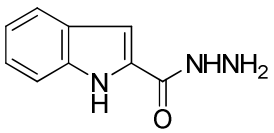
Yield 60%; Off-white, solid mp 213-214 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (s, 1H), 9.58 (s, 1H), 7.64 (s, 1H), 7.53 (s, 1H), 7.38 (d, J = 8.00 Hz, 2H), 4.18 (s, 2H), 3.83 (s, 3H). IR (KBr, v cm^{-1}): 3345, 3265, 2935, 1653, 1610, 1543, 736, 720; MS (ESI) *m/z* calcd. for $C_{10}H_{11}N_3O_2$: 205.1, found: 205.1 $(M)^+$.

5-Methoxy-indole-3-carbohydrazide (45c)



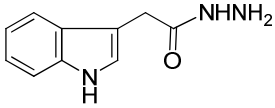
Yield 60%, Off-white solid; mp 178-179 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 11.68 (s, 1H), 9.64 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.40 (d, J = 8.00 Hz, 2H), 4.18 (s, 2H), 3.86 (s, 3H); IR (KBr, v cm^{-1}): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724; MS (ESI): *m/z* calcd. for $C_{10}H_{11}N_3O_2$: 205.2, found: 206.2 $(M+H)^+$.

Indole-2-carbohydrazide (45d)



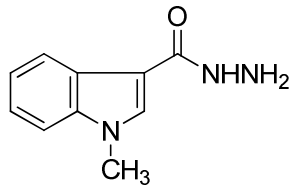
Yield 90%; White solid; mp 247-248 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.70 (s, 1H), 9.85 (s, 1H), 7.80-7.10 (m, 4H), 8.17 (s, 1H), 4.2 (s, 2H). IR (KBr, v cm^{-1}): 3356 3217, 3080, 2900, 1640, 1615, 1550, 780; MS (ESI): *m/z* calcd. for $C_9H_9N_3O$: 175.2, found: 176.2 $(M+H)^+$.

Indole-3-acetohydrazide (45e)



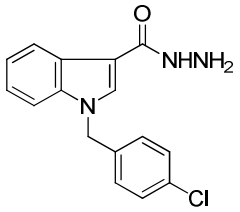
Yield 85%; Off-white solid; mp 145-146 °C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 11.0 (s, 1H), 9.65 (s, 1H), 7.59 (dd, J =7.8Hz, J =1.6 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.09 (d, J = 7.8 Hz, 1H), 6.98 (d, J = 7.8 Hz, 2H), 4.45 (s, 2H), 3.40 (s, 2H); IR (KBr, v cm^{-1}): 3317, 1649, 1623, 1235, 1063, 812; MS (ESI): *m/z* calcd. for $C_{10}H_{11}N_3O$: 189.1, found: 190.1 $(M+H)^+$.

1-Methyl-indole-3-carbohydrazide (45f)



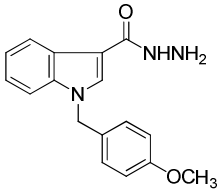
Yield 80%; White solid; mp 149-150 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (s, 1H), 8.14 (d, J = 7.80 Hz, 1H), 7.92 (d, J = 7.80 Hz, 1H), 7.37 (d, J = 8.10 Hz, 1H), 7.20-6.99 (m, 2H), 4.03 (s, 2H), 3.65 (s, 3H); IR (KBr, v cm^{-1}): 3212, 3098,1658, 1604, 1582, 1520, 1465, 1235, 740; MS (ESI): *m/z* calcd. for $C_{10}H_{11}N_3O$: 189.09, found: 189.1 $(M)^+$.

1-(4-Chlorobenzyl)-indole-3-carbohydrazide (45g)



Yield 75 %; White solid; mp 173-174 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.19 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.37 (d, J = 6.2 Hz, 1H), 7.15 (d, J = 7.0 Hz, 4H), 6.83 (d, J = 7.45 Hz, 2H), 5.28 (s, 2H), 4.43 (s, 2H); IR (KBr, v cm^{-1}): 3117, 3098, 1648, 1601, 1576, 1518, 1456, 1228, 764; MS (ESI): *m/z* calcd. for $C_{16}H_{14}ClN_3O$: 299.0, found: 300.0 $(M+H)^+$and 301.0 $(M+2)^+$.

1-(4-Methoxybenzyl)-indole-3-carbohydrazide (45h)

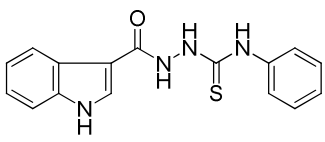


Yield 75 %; White solid; mp 193-194 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, J = 7.3 Hz, 1H), 7.83 (s, 1H), 7.33-7.23 (m, 6H), 7.06-7.09 (m, 2H), 5.30 (s, 2H), 4.45 (s, 2H), 3.91 (s, 3H); IR (KBr, v cm^{-1}): 3112, 3098, 1650, 1602, 1580, 1521, 1448, 1223, 750; MS (ESI): *m/z* calcd. for $C_{17}H_{17}N_3O_2$: 295.1, found: 296.1 $(M+H)^+$.

Step 4. 1-(Indole-3-carbonyl)-4-arylthiosemicarbazides (47a-v)

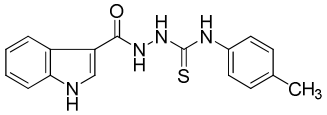
Indole-2(3)-carbohydrazide (**45**, 10 mmol) and aryl isothiocyanate (**46**, 10 mmol) was taken in round bottom flask containing 10 mL of ethanol and stirred the mixture at 60 °C for 2-3 h. Solid product so obtained was filtered and dried well to obtain desired thiosemicarbazide **47a-v** in good yields (80-95 %).

1-(Indole-3-carbonyl)-4-phenylthiosemicarbazide (47a)



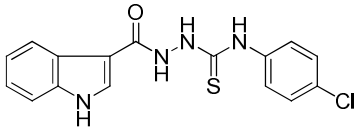
Yield 95%; mp 205 °C; IR (KBr, v cm^{-1}): 3253, 3197, 1645, 1597, 1498, 1355, 1242, 740, 684.MS (ESI): *m/z* calcd. for $C_{16}H_{14}N_4OS$: 310.1, found: 311.1 $(M+H)^+$.

1-(Indole-3-carbonyl)-4-toluyllthiosemicarbazide (47b)



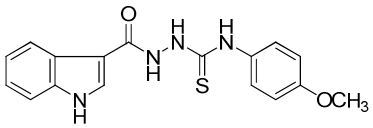
Yield 90 %; mp 202 °C; IR (KBr, *v* cm^{-1}): 3394, 3142, 1666, 1585, 1495, 1354, 1242, 736, 688; MS (ESI): *m/z* calcd. for $C_{17}H_{16}N_4OS$: 324.1, found: 325.0 $(M+H)^+$.

1-(Indole-3-carbonyl)-4-(*p*-chlorophenyl)thiosemicarbazide (47c)



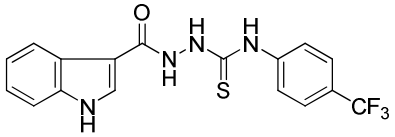
Yield 85%; mp 205-207 °C; IR (KBr, *v* cm^{-1}): 3275, 3230, 1645, 1597, 1494, 1352, 1242, 827, 744, 690.

1-(Indole-3-carbonyl)-4-(*p*-methoxyphenyl)thiosemicarbazide (47d)



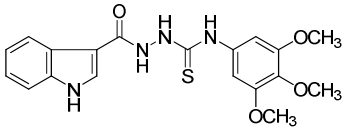
Yield 85%; mp 210 °C; IR (KBr, *v* cm^{-1}): 3394, 3142, 1666, 1585, 1495, 1354, 1242, 840, 736, 688.

1-(Indole-3-carbonyl)-4-(4′-trifluoromethylphenyl)thiosemicarbazide (47e)



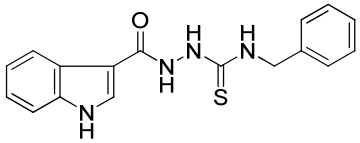
Yield 80%; mp 213 °C; IR (KBr, *v* cm^{-1}): 3257, 3199, 1645, 1590, 1487, 1336, 1242, 836, 742, 682.

1-(Indole-3-carbonyl)-4-(3′,4′,5′-trimethoxyphenyl)thiosemicarbazide (47f)



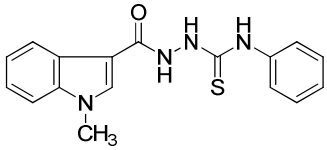
Yield 90%; mp 204 °C; IR (KBr, *v* cm^{-1}): 3296, 3213, 1655, 1600, 1483, 1359, 1272, 1176, 1130, 864, 748, 663; MS (ESI): *m/z* calcd. for $C_{19}H_{20}N_4O_4S$: 400.1, found: 401.0 $(M+H)^+$.

1-(Indole-3-carbonyl)-4-(benzyl)thiosemicarbazide (47g)



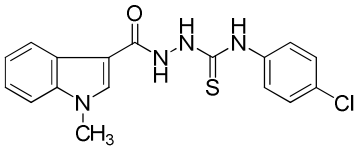
Yield 84%; mp 230 °C; IR (KBr, *v* cm^{-1}): 3313, 3253, 1665, 1603, 1498, 1379, 1244, 1168, 738, 698.

1-(1′-Methyl-indole-3-carbonyl)-4-phenylthiosemicarbazide (47h)



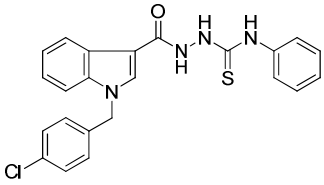
Yield 90%; mp 191-192 °C; IR (KBr, *v* cm^{-1}): 3257, 2908, 1648, 1596, 1495, 1345, 1240, 1148, 740, 688.

1-(1′-Methyl-indole-3-carbonyl)-4-(*p*-chlorophenyl)thiosemicarbazide (47i)



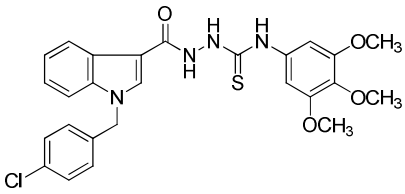
Yield 90%; mp 181-182 °C; IR (KBr, *v* cm^{-1}): 3234, 2943, 1654, 1589, 1487, 1373, 1236, 1155, 860, 748, 654.

1-(1′-(*p*-Chlorophenyl)-indole-3-carbonyl)-4-phenylthiosemicarbazide (47j)



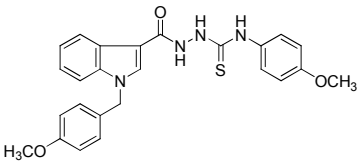
Yield 90%; mp 175 °C; IR (KBr, cm^{-1}): 3226, 2927, 1664, 1565, 1484, 1354, 1242, 1112, 858, 740, 684.

1-(1′-(*p*-Chlorophenyl)-indole-3-carbonyl)-4-(3′,4′,5′-trimethoxyphenyl)thiosemicar bazide (47k)



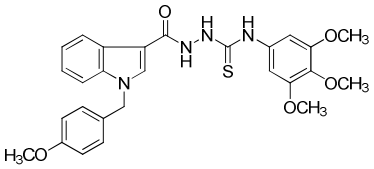
Yield 80%; mp 184 °C; IR (KBr, *v* cm^{-1}): 3245, 2930, 1654, 1545, 1492, 1265, 1185, 834, 748, 680.

1-(1′-(*p*-Methoxyphenyl)-indole-3-carbonyl)-4-(4′-methoxyphenyl)thiosemicarbazide (47l)



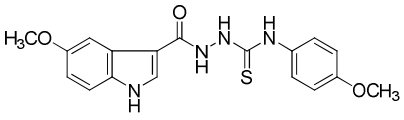
Yield 84%; mp 188-189 °C; IR (KBr, *v* cm^{-1}): 3215, 2966, 1668, 1560, 1465, 1294, 1186, 831, 752, 645; MS (ESI): *m/z* calcd. for $C_{25}H_{24}N_4O_3S$: 460.1, found: 461.0 $(M+H)^+$.

1-(1′-(*p*-Methoxyphenyl)-indole-3-carbonyl)-4-(3′,4′,5′-trimethoxyphenyl)thiosemicabazide (47m)



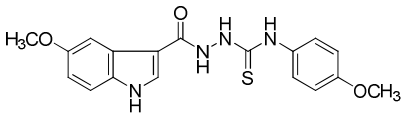
Yield 84%; mp 181-182 °C; IR (KBr, *v* cm^{-1}): 3176, 2946, 1668, 1597, 1434, 1296, 1180, 844, 746, 685.

1-(5′-Methoxy-indole-3-carbonyl)-4-(4′-methoxyphenyl)thiosemicarbazide (47n)



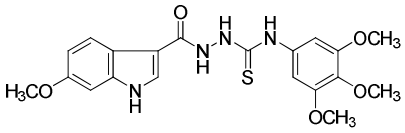
Yield 80%; mp 183-184 °C; IR (KBr, *v* cm^{-1}): 3269, 3176, 1629, 1586, 1485, 1247, 1176, 865, 794, 654.

1-(6′-Methoxy-indole-3-carbonyl)-4-(4′-methoxyphenyl)thiosemicarbazide (47o)



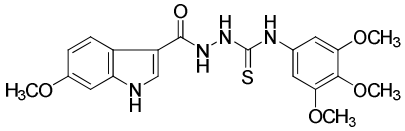
Yield 90%; mp 198-200 °C; IR (KBr, ν cm^{-1}): 3388, 3255, 1645, 1594, 1454, 1247, 1180, 845, 795, 690.

1-(6′-Methoxy-indole-3-carbonyl)-4-(3′,4′,5′-trimethoxyphenyl)thiosemicarbazide (47p)



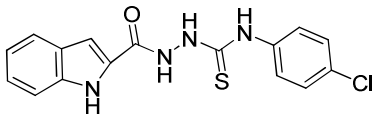
Yield 84%; mp 191-192 °C; IR (KBr, ν cm^{-1}): 3365, 3245, 1665, 1584, 1459, 1237, 1165, 835, 785, 680.

1-(6′-Methoxy-indole-3-carbonyl)-4-(3′,4′,5′-trimethoxyphenyl)thiosemicarbazide (47p)



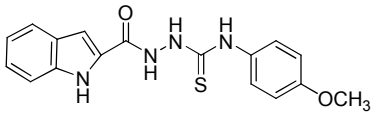
Yield 84%; mp 191-192 °C; IR (KBr, ν cm^{-1}): 3365, 3245, 1665, 1584, 1459, 1237, 1165, 835, 785, 680.

1-(Indole-2-carbonyl)-4-(*p*-chlorophenyl)thiosemicarbazide (47q)



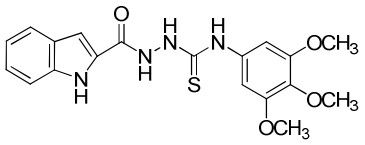
Yield 90%; mp 188 °C; IR (KBr, ν cm^{-1}): 3343, 3225, 1656, 1595, 1492, 1247, 1180, 840, 745, 690.

1-(Indole-2-carbonyl)-4-(*p*-methoxyphenyl)thiosemicarbazide (47r)



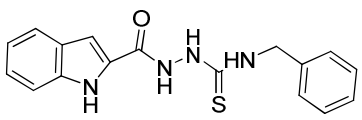
Yield 84%; mp 207-208 °C; IR (KBr, ν cm^{-1}): 3358, 3302, 1676, 1545, 1489, 1314, 1226, 1178, 836, 750, 677.

1-(Indole-2-carbonyl)-4-(3′,4′,5′-chlorophenyl)thiosemicarbazide (47s)



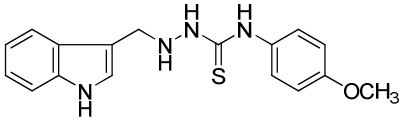
Yield 80%; mp 217-218 °C; IR (KBr, ν cm^{-1}): 3360, 3317, 1680, 1600, 1495, 1371, 1188, 826, 746, 696.

1-(Indole-2-carbonyl)-4-(benzyl)thiosemicarbazide (47t)



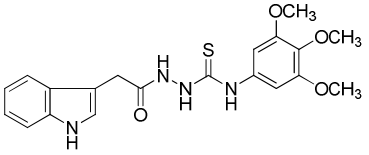
Yield 90%; mp 226 °C; IR (KBr, ν cm^{-1}): 3360, 3317, 1680, 1600, 1495, 1371, 1188, 826, 746, 696.

1-(Indole-3-acetyl)-4-(*p*-methoxyphenyl)thiosemicarbazide (47u)



Yield 95%; mp 186-187 °C; IR (KBr, *v* cm^{-1}): 3396, 3296, 1685, 1565, 1487, 1365, 1298, 833, 794, 698.

1-(Indole-3-acetyl)-4-(3,4′,5′-trimethoxyphenyl)thiosemicarbazide (47v)

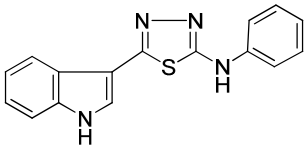


Yield 90%; mp 166-167 °C; IR (KBr, *v* cm^{-1}): 3298, 3251, 1693, 1598, 1488, 1393, 1232, 734, 671.

Step 5. General procedure for the synthesis of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles (11)

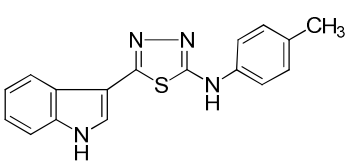
The semicarbazide (**47**, 10 mmol) was charged into 10 mL round bottom flask containing freshly distilled acetyl chloride (4 mL). The reaction mixture was stirred at 20 °C temperature till the completion of the reaction as monitored by thin layer chromatography. The reaction contents were poured over crushed ice and neutralized with aqueous ammonia solution. The solid so obtained was filtered, dried and recrystallized from ethanol to obtain the arylamino-1,3,4-thiadiazoles (**11**) in good yields.

2-Phenylamino-5-(3′-indolyl)-1,3,4-thiadiazole (11a)



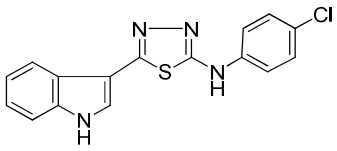
Yield 65%; White solid; mp 210-212 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 10.16 (s, 1H), 8.18 (d, *J* = 7.1 Hz, 1H), 7.94 (s, 1H), 7.65-7.59 (m, 3H), 7.30-7.24 (m, 3H), 7.20-7.11 (m, 2H); IR (KBr, *v* cm^{-1}): 3377, 3250, 1612, 1570, 1431, 1124, 746, 675; MS (ESI): *m/z* calcd. for $C_{16}H_{12}N_4S$: 292.1, found: 292.2 $(M)^+$.

2-Tolylamino-5-(3′-indolyl)-1,3,4-thiadiazole (11b)



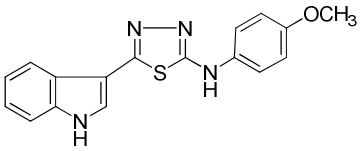
Yield 70%; Off-white solid; mp 205 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 10.07 (s, 1H), 8.20 (s, 1H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.39-7.26 (m, 2H), 7.21-7.07 (m, 3H), 2.30 (s, 3H); IR (KBr, *v* cm^{-1}): 3236, 3122, 1610, 1534, 1498, 1247, 747, 685; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4S$: 306.0, found: 307.0 $(M+H)^+$.

2-(4-Chorophenyl)amino-5-(3′-indolyl)-1,3,4-thiadiazole (11c)



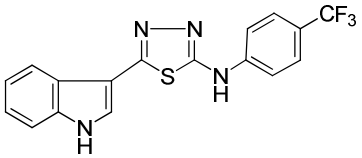
Yield 58%; White solid; mp 232-234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59 (s, 1H), 10.42 (s, 1H), 8.17 (d, J = 7.4 Hz, 1H), 8.09 (d, J = 7.2 Hz, 1H), 8.05 (s, 1H), 7.71 (d, J = 8.9 Hz, 2H), 7.46 (d, J = 7.5 Hz, 2H), 7.22-7.10 (m, 2H); IR (KBr, v cm^{-1}): 3329, 3253, 1622, 1546, 1496, 1116, 745, 669; MS (ESI): *m/z* calcd. for $C_{16}H_{11}ClN_4S$: 326.0, found: 327.0 $(M+H)^+$ and 328.0 (M+2).

2-(4-Methoxyphenyl)amino-5-(3′-indolyl)-1,3,4-thiadiazole (11d)



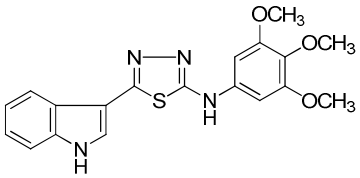
Yield 65%; White solid; mp 200-201 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 10.05 (s, 1H), 8.16 (t, J = 8.6 Hz, 1H), 7.79 (dd, J = 8.0, 2.7 Hz, 1H), 7.57 (d, J = 8.9 Hz, 2H), 7.52-7.45 (m, 1H), 7.25-7.16 (m, 2H), 6.90-6.87 (m, 2H), 3.84 (s, 3H). IR (KBr, v cm^{-1}): 3319, 3182, 1616, 1583, 1450, 1247, 1180, 819, 750; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4OS$: 322.1, found: 323.2 $(M+H)^+$.

2-(4-Trifluoromethylphenyl)amino-5-(3′-indolyl)-1,3,4-thiadiazole (11e)



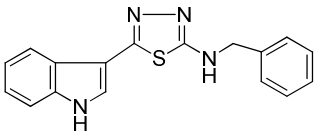
Yield 72%; White solid; mp 239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (s, 1H), 10.65 (s, 1H), 8.21 (d, J = 7.3 Hz, 1H), 8.10 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 7.5 Hz, 1H), 7.23-7.18 (m, 2H); IR (KBr, v cm^{-1}): 3396, 3194, 1614, 1547, 1419, 1247, 1196, 840, 748, 671; MS (ESI): *m/z* calcd. for $C_{17}H_{11}F_3N_4S$: 360.0, found: 361.0 $(M+H)^+$.

2-(3′,4′,5′-Trimethoxylphenyl)amino-5-(3′-indolyl)-1,3,4-thiadiazole (11f)



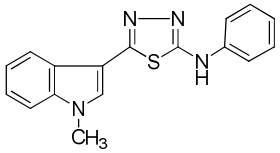
Yield 75%; White solid; mp 223-224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.55 (s, 1H), 10.16 (s, 1H), 8.18 (d, 7.5 Hz, 1H), 8.01 (s, 1H), 7.75 (d, J = 2.8 Hz, 1H), 7.53-7.41 (m, 1H), 7.23-7.15 (m, 2H), 7.04 (d, J = 2.2 Hz, 1H), 3.86 (s, 6H), 3.72 (s, 3H); IR (KBr, cm^{-1}): 3313, 32113, 1608, 1550, 1433, 1238, 1146, 827, 746, 621; MS (ESI): *m/z* calcd. for $C_{19}H_{18}N_4O_3S$: 382.0, found: 383.0 $(M+H)^+$.

2-(Benzyl)amino-5-(3′-indolyl)-1,3,4-thiadiazole (11g)



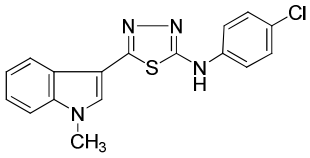
Yield 62%; Off-white solid; mp 150-151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59 (s, 1H), 10.01 (s, 1H), 8.10 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 2.5 Hz, 1H), 7.48-7.42 (m, 3H), 7.36-7.28 (m, 3H), 7.19-7.14 (m, 2H) 4.64 (s, 2H). IR (KBr, *v* cm^{-1}): 3221, 3140, 1645, 1583, 1429, 1244, 1135, 745, 696; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4S$ 307.0 $(M+H)^+$, found: 307.0

2-(Phenyl)amino-5-(1′-methyl-3′-indolyl)-1,3,4-thiadiazole (11h)



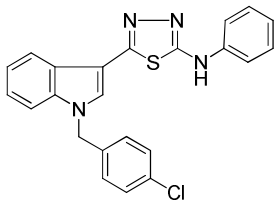
Yield 75%; White solid; mp 217-218 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H), 8.18 - 8.24 (m, 3H), 7.92 (s, 1H), 7.75-7.57 (m, 2H), 7.40 (d, *J* = 8.0. Hz, 1H), 7.34-7.28 (m, 3H), 3.88 (s, 3H); IR (KBr, *v* cm^{-1}): 3194, 1624, 1546, 1456, 1246, 747, 653; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4S$: 306.0, found: 307.0 $(M+H)^+$.

2-(4′Chlorophenyl)amino-5-(1′-methyl-3′-indolyl)-1,3,4-thiadiazole (11i)



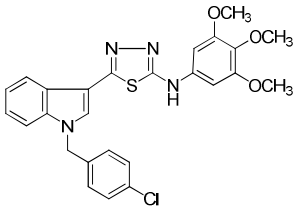
Yield 72%; White solid; mp 188-189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.23 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.90 (s, 1H), 7.75 - 7.57 (m, 3H), 7.44 (d, *J*= 8.2 Hz, 1H), 7.37 – 7.20 (m, 3H), 3.89 (s, 3H); IR (KBr, *v* cm^{-1}): 3255, 1616, 1514, 1428, 1219, 815, 736, 667; MS (ESI): *m/z* calcd. for $C_{17}H_{13}ClN_4S$: 340.0, found: 341.0 and 342.0 $(M+2)^+$.

2-(Phenyl)amino-5-(1′-(*p*-chlorobenzyl)-3′-indolyl)-1,3,4-thiadiazole (11j)



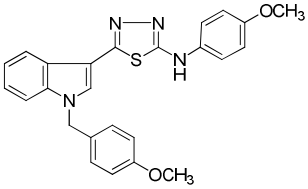
Yield 70%; White solid; mp 159-160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.98 (s, 1H), 8.24-8.21 (m, 2H), 7.97 (s, 1H), 7.54-7.39 (m, 3H), 7.46-7.10 (m, 8H), 5.42 (s, 2H); IR (KBr, *v* cm^{-1}): 3192, 2943, 1608, 1527, 1431, 1246, 834 738; MS (ESI): *m/z* calcd. for $C_{23}H_{17}ClN_4S$: 416.0, found: 417.0 $(M+H)^+$ and 418. $(M+2)^+$.

2-(3′,4′,5′-Trimethoxyphenyl)amino-5-(1′-(*p*-chlorobenzyl)-3′-indolyl)-1,3,4-thiadiazole(11k)



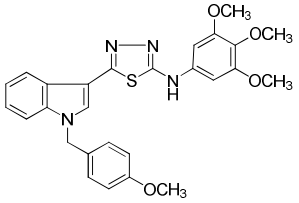
Yield 65%; White solid; mp 238-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.57 (s, 1H), 7.31-7.29 (m, 5H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.72 (s, 2H), 5.33 (s, 2H), 3.89 (s, 6H), 3.85 (s, 3H); IR (KBr, *v* cm^{-1}): 3124, 2937, 1597, 1537, 1429, 1234, 1182, 819, 769, 738; MS (ESI): *m/z* calcd. for $C_{26}H_{23}ClN_4O_3S$: 506.3, found: 507.3 $(M+H)^+$ and 508.1 $(M+2)^+$

2-(4′-Methoxyphenyl)amino-5-(1′-(*p*-methoxybenzyl)-3′-indolyl)-1,3,4-thiadiazole (11l)



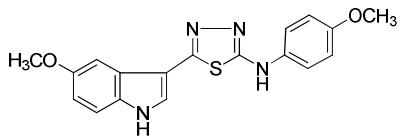
Yield 55%; White solid; mp 174-175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 7.54 (s, 1H), 7.35 (d, *J* = 8.8 Hz, 3H), 7.29-7.26 (m, 2H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.26 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H); IR (KBr, *v* cm^{-1}): 3215, 2856, 1616, 1587, 1497, 1246, 809, 746, 685; MS (ESI): *m/z* calcd. for $C_{25}H_{22}N_4O_2S$: 442.2, found: 443.3 $(M+H)^+$.

2-(3′,4′,5′-Trimethoxyphenyl)amino-5-(1′-(*p*-methoxybenzyl)-3′-indolyl)-1,3,4-thiadiazole (11m)



Yield 65%; Pale yellow solid; mp 189-190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.99 (s, 1H), 8.23 (d, *J* = 7.4 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.29-7.26 (m, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 6.92 -6.82 (m, 2H), 6.77 (s, 2H), 5.28 (s, 2H), 3.90 (s, 6H), 3.86 (s, 3H), 3.78 (s, 3H); IR (KBr, *v* cm^{-1}): 3263, 2937, 1608, 1583, 1249, 831, 744, 707; MS (ESI): *m/z* calcd. for $C_{27}H_{26}N_4O_4S$: 502.2, found: 503.6 $(M+H)^+$.

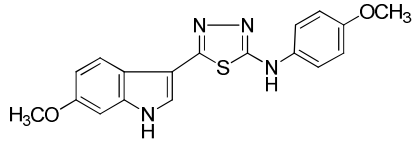
2-(4′-Methoxyphenyl)amino-5-(5′-methoxy-3′-indolyl)-1,3,4-thiadiazole (11n)



Yield 50%; White solid; mp 196-197 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 9.84 (s, 1H), 7.89 (s, 1H), 7.74 (d, *J* = 2.1 Hz,1H), 7.60 (dd, *J* = 9.3, 5.8 Hz, 2H), 7.36-7.30 (m, 2H), 6.85 (d, *J* = 8.0 Hz, 2H), 3.86 (s, 3H), 3.79 (s, 3H); IR (KBr, *v* cm^{-1}): 3415, 3219, 1619, 1581, 1485, 1257, 1185, 825, 794, 685; MS

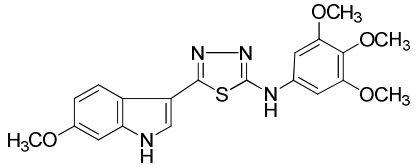
(ESI): *m/z* calcd. for $C_{18}H_{16}N_4O_2S$: 352.0, found: 353.0 $(M+H)^+$.

2-(4′-Methoxyphenyl)amino-5-(6′-methoxy-3′-indolyl)-1,3,4-thiadiazole (11o)



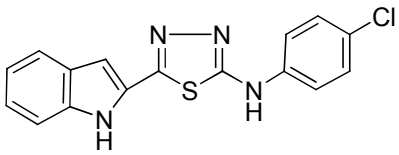
Yield 55%; Off-white solid; mp 182-183 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.19 (s, 1H), 9.86 (s, 1H), 8.08 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.0 Hz, 2H), 3.84 (s, 3H), 3.78 (s, 3H); IR (KBr, *v* cm^{-1}): 3240, 3185, 1614, 1579, 1454, 1246, 1165, 817, 746, 673; MS (ESI): *m/z* calcd. for $C_{18}H_{16}N_4O_2S$: 352.0, found: 353.0 $(M+H)^+$.

2-(3′,4′,5′-Trimethoxyphenyl)amino-5-(6′-methoxy-3′-indolyl)-1,3,4-thiadiazole (11p)



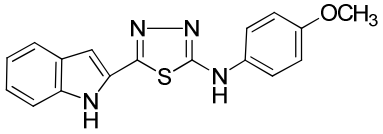
Yield 60%; Light brown solid; mp 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 10.04 (s, 1H), 8.06 (d, *J* = 8.7 Hz, 1H), 7.99 (s, 1H), 7.61 (d, *J* = 2.7 Hz, 1H), 7.02 (s, 2H), 6.81 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.86 (s, 6H), 3.84 (s, 3H), 3.72 (s, 3H). IR (KBr, *v* cm^{-1}): 3329, 3215, 1614, 1577, 1455, 1240, 833, 773, 709; MS (ESI): *m/z* calcd. for $C_{20}H_{20}N_4O_4S$: 412.0, found: 413.0 $(M+H)^+$.

2-(4′-Chlorophenyl)amino-5-(2′-indolyl)-1,3,4-thiadiazole (11q)



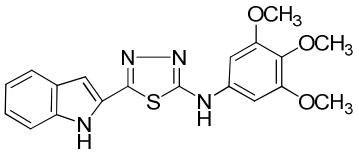
Yield 65%; White sold; mp 238-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.57 (s, 1H), 10.39 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.48 – 7.39 (m, 3H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.75 (s, 1H); IR (KBr, *v* cm^{-1}): 3342, 3199, 1624, 1535, 1458, 1230,798, 734, 648; MS (ESI): *m/z* calcd. for $C_{16}H_{11}ClN_4S$: 326.0, found: 327.0 $(M+H)^+$and 328.0 $(M+2)^+$.

2-(4′-Methoxyphenyl)amino-5-(2′-indolyl)-1,3,4-thiadiazole (11r)



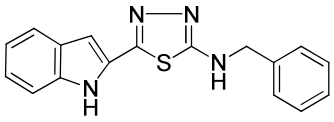
Yield 74%; pale yellow solid; mp 206 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 10.10 (s, 1H), 7.56-7.48 (m, 3H), 7.20-7.16 (m, 2H), 7.09-7.05 (m, 1H), 6.89 (d, *J* = 7.2 Hz, 2H), 6.77 (s, 1H), 3.79 (s, 3H); IR (KBr, *v* cm^{-1}): 3388, 3317, 1606, 1539, 1413, 1234, 833, 795, 676; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4OS$: 322.3, found: 323.4 $(M+H)^+$.

2-(3′,4′,5′-Trimethoxyphenyl)amino-5-(2′-indolyl)-1,3,4-thiadiazole (11s)



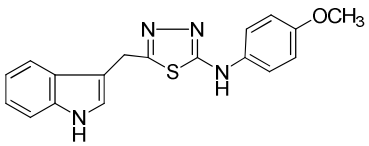
Yield 78%; White solid; mp 232-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.63 (s, 1H), 10.25 (s, 1H), 7.56 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.9 Hz, 2H), 7.18 (d, J = 2.4 Hz, 2H), 6.81 (s, 1H), 3.87 (s, 6H), 3.75 (s, 3H); IR (KBr, ν cm^{-1}): 3369, 3215, 1604, 1587, 1462, 1238, 1186, 833, 750, 713; MS (ESI): *m/z* calcd. for $C_{19}H_{18}N_4O_3S$: 383.1, , found: 383.4 $(M+H)^+$.

2-(Benzyl)amino-5-(2′-indolyl)-1,3,4-thiadiazole (11t)



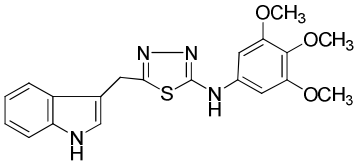
Yield 60%; White solid; mp 175-176 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 8.17 (s, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.45-7.40 (m, 3H), 7.36 (t, J = 7.2 Hz, 2H), 7.27 (d, J = 7.2 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.69 (d, J = 1.6 Hz, 1H), 4.60 (d, J = 7.2 Hz, 2H); IR (KBr, ν cm^{-1}): 3364, 3271, 1610, 1543, 1358, 1246, 745, 685; MS (ESI): *m/z* calcd. for $C_{17}H_{14}N_4S$: 306.1, found: 307.3 $(M+H)^+$.

2-(4′-Methoxyphenyl)amino-5-(3′-indolyl-methyl)-1,3,4-thiadiazole (11u)



Yield 65%; White solid; mp193-194 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 9.72 (s, 1H), 7.50 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.09 (t, J = 8.0 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 4.28 (s, 2H), 3.74 (s, 3H); IR (KBr, cm^{-1}): 3344, 3199, 1624, 1566, 1436, 1230, 1126, 829, 734, 648; MS (ESI): *m/z* calcd. for $C_{18}H_{16}N_4OS$: 336.1, found: 337.0 $(M+H)^+$.

2-(3′,4′,5′-Trimethoxyphenyl)amino-5-(3′-indolyl-methyl)-1,3,4-thiadiazole (11v)



Yield 65%; Off white solid; mp 235 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.81 (s, 1H), 9.88 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 2.4 Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 6.99 (t, J = 7.8 Hz, 1H), 6.92 (s, 2H), 4.35 (s, 2H), 3.79 (s, 6H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 164.30, 160.46, 152.99, 137.17, 136.37,

132.22, 130.60, 126.62, 123.80, 121.27, 118.66, 118.30, 111.75, 110.39, 95.52, 60.02, 55.54, 26.08; IR (KBr, ν cm^{-1}): 3383, 3221, 1616, 1585, 1421, 1240, 1165, 844, 746, 624; MS (ESI): *m/z* calcd. for $C_{20}H_{20}N_4O_3S$: 396.2, found: 397.3 $(M+H)^+$.

5.7 MTT assay

Six human cancer cell lines (LnCaP, DU145, MCF-7, MDA-MB-231, HeLa and Ovcar-3) were cultured in RPMI-1640 media supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/ streptomycin. They were seeded in 96-well plates at a density of 4×10^3 cells per well for 12 h. Cells were incubated with various concentrations of the compounds ranging from 10 nM–1 mM. After 48 h, MTT (3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was added to the final concentration of 0.2 mg/ml and incubated for 30 min. The cells were washed twice with PBS and lyses in 100 µL dimethylsulfoxide, and the absorbance was measured at 570 nm using Tecan Spectrafluor Plus.

5.8 References

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Chapter 6

6.1 Introduction

Cryptosanguinolentine is one of the naturally occurring indoloquinoline alkaloids with a broad range of biological applications. Indoloquinolines are unique natural alkaloids, characterized by two privileged units' indole and quinoline fused rings, thus gained special attention for their medical usage for curing various diseases. These tetracyclic alkaloids were isolated mainly from "*Cryptolepis Sanguinolenta",* a shrub indigenous to tropical West Africa,[1-3] and it had long been employed in the dyeing of textiles and leather. Use of plants for medicinal purposes is an important part of the culture and the tradition in Africa. Majority of the population depends directly on the traditional medicine for the primarily health care. The first medical use of these isomeric indoloquinolines was found as a folk medicine in treating malarial fevers. Out of thirteen characterized alkaloids from the plant *Cryptolepis Sanguinolenta*, the major three indoloquinoline alkaloids are, Cryptolepine (5-methyl-5H-indolo[3,2-*b*]quinoline) (**1**), Neocryptolepine (5-methyl-5H-indolo[2,3-*b*]quinoline) (**2**), Isocryptolepine (5-methyl-5H-indolo[3,2-*c*]quinoline) (cryptosanguinolentine) (**3**) and their synthetic analogue isoneocryptolepine (**4**) as shown in figure 6.1. Chemically these compounds (**1-4**) are isomeric indoloquinolines. Indoloquinolines (**1**) and (**2**) are linearly linked, whereas compounds (**3**) and (**4**) possess angular linkage of the indole and quinoline rings.

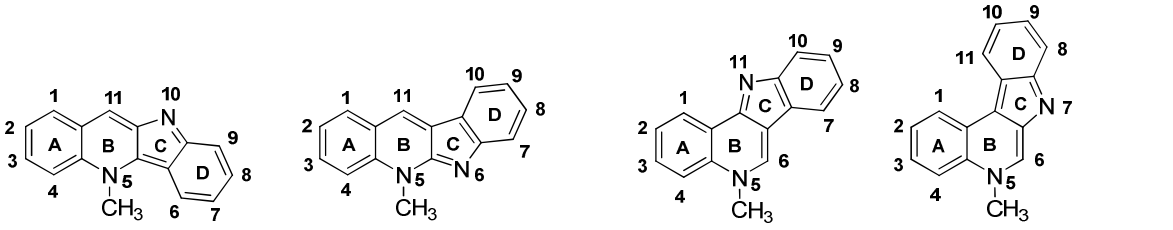


Figure 6.1 Indoloquinoline alkaloids (**1-4**)

6.2 Indoloquinolines in drug discovery

Plant alkaloids and their analogues contributed greatly for the discovery and development of new therapeutic agents and particularly, indoloquinoline alkaloids represent a new class of drug leads. The natural indoloquinolines, cryptolepine (**1**), neocryptolepine (**2**),

cryptosanguinolentine (**3**) and synthetic analogues isoneocryptolepine (**4**) compounds are of particular interest due to their broad spectrum of biological activities. These four indoloquinolines have been widely used as reference for the design and development of many new drug candidates.

Biological activities of indoloquinolines:

(i) Antibacterial activity: Cryptolepine (**1**), the most extensively studied indoloquinoline for medicinal applications and is known to exhibit bacteriostatic and bactericidal actions occurring in the initial stages of drug bacterial interactions. Cryptolepine (**1**) has highest antimicrobial activity (MIC = 12.5 µg/mL) against 106 strains of *Campylobacter* which is higher than that of marketed drug co-trimoxazole and equal to ampicillin.[4] The direct inhibition of DNA or peptidoglycan synthesis may be responsible for the antibacterial activity of **1**.[5] Neocryptolepine (**2**) was found to be only bacteriostatic rather than bactericidal which inhibits the growth of Gram-positive bacteria (MIC = 58.5 µg/mL) but in less extent than that of Gram-negative bacteria.[6]

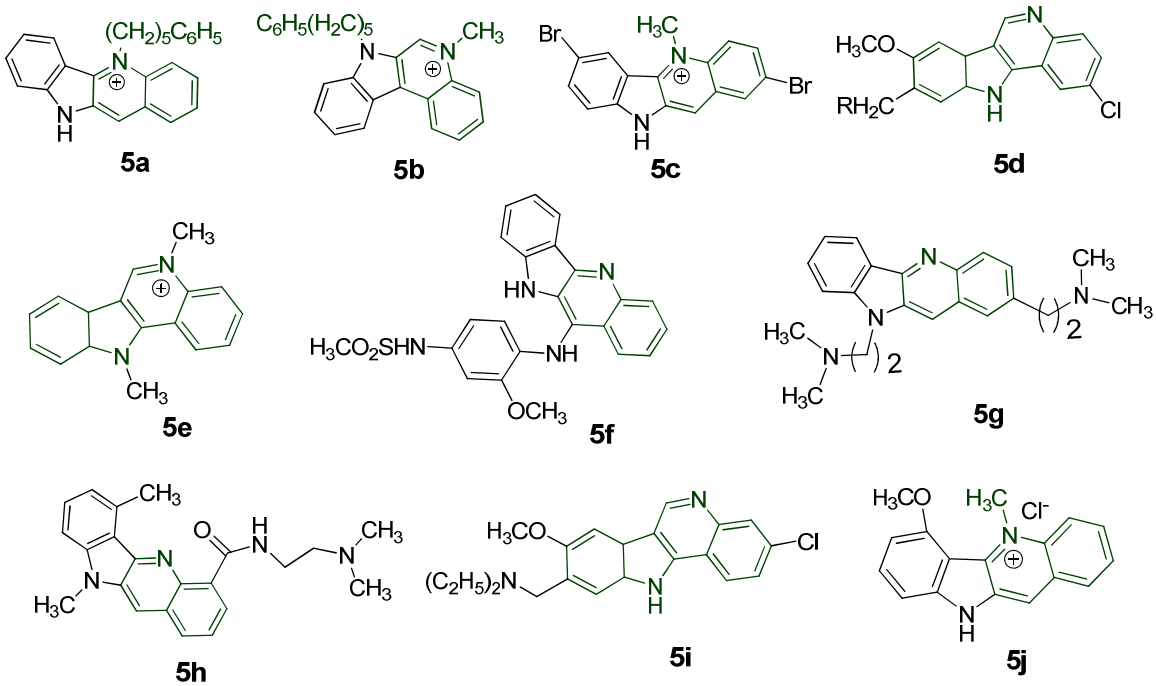


Figure 6.2 Indoloquinolines (**5a-j**) for drug discovery

(ii) Antifungal activity: Cryptolepine **1** showed *in-vitro* antifungal activity against *Candidaalbicans* (MIC = 320 µg/mL) and *Saccharomyces cerevisiae* (MIC = 40 µg/mL) by interacting with the cytoplasmatic membrane of cell which leads to cell death.[7]

The *N*-alkylated analogues **5a** of Cryptolepine showed antifungal activity against *Cryptococcus neoformans* and *Candida albicans* (MIC < 1.9 µg/mL) which demonstrated the essentiality of *N*-5 alkylation of **1** for the antifungal activity.[8] The isoneocryptolepine (**4**) displayed weak antifungal activity while its analogues (**5b**) was found to be a potent antifungal agent (MIC = 0.6-2.5 µg/mL).[9] Neocryptolepine (**2**) has shown weak antifungal activity at higher MIC values (>100 µg/mL).[10]

(iii) Antiprotozoal activity: Malaria is a global health problem with 109 endemic countries and killing over one million people every year. Cryptolepine (**1**) is the most studied indoloquinoline against malaria parasite. Cryptolepine (**1**) and its hydrochloride salt, and neocryptolepine (**2**) showed strong antiplasmodial activity against *Plasmodium falciparum* chloroquine-resistant strains (IC_{50} = 134 nM). Cryptolepine hydrochloride salt exhibited significant chemo suppressive effect against *Plasmodium berghei yoelii* and *Plasmodium berghei* infected mice.[11] Isoneocryptolepine (**4**) and *N*-methyl-isocryptolepinium iodide showed a high antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain K1 (IC_{50} = 0.23 and 0.017 µM).[12] Wright *et al.* reported dibromoindoloquinoline (**5c**) which is about 10 times more potent against *Plasmodium falciparum* K1 strain (IC_{50} = 49 nM) than **1** (IC_{50} = 440 nM).[13] Cryptosanguinolentine (**3**) and its analogues (**5d** and **5e**) have IC_{50} values ranging from 0.024 to 13.7 nM against *P. falciparum*.[14]

(iv) Anticancer activity: The indoloquinolines (**1-3**) are known for their remarkable anticancer activity.[15-16] Initially Kirby *et al.* reported that cryptolepine (**1**) intercalates to DNA suggested that a stabilized intercalation complex is made through π-π charge transfer complexes between nitrogen′s of purine-pyrimidine bases and cryptolepine.[17] Cryptolepine (**1**) and neocryptolepine (**2**) provoke a massive accumulation of P388 murine leukemia cells in the G2/M phase.[18] Cryptolepine (**1**) has been shown to intercalate into DNA preferentially at GC rich sequences and non-alternating CC sites and inhibit topoisomerase II as well as DNA synthesis.[19] The crystal structure of this antimalarial drug-DNA complex provides evidence for the first non-alternating intercalation and, as such, provides a basis for the design of new anticancer drugs, whereas analogues of (**1**) were found to follow different mechanism to exhibit the cytotoxicity.[20] Cryptolepine binds 10-fold more tightly to DNA than other alkaloids and

proves to be much more cytotoxic toward B16 melanoma cells.[21] Over past 20 years, several synthetic analogues of cryptolepine (**1**) have been emerged as cytotoxic agents.

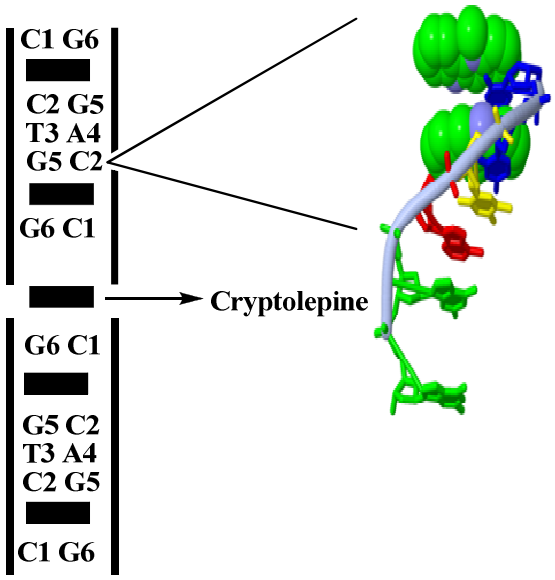


Figure 6.3 Cryptolepine-DNA complex[19]

Yamato *et al.* reported compound **5f** which intercalated DNA and induced topoisomerase II dependent DNA cleavage at low dose.[22] The other analogues (**5g**) was reported as a telomerase inhibitor (IC_{50} = 16 μM)[23] and compound (**5h**) showed antitumor activity against Lewis Lung carcinoma cell lines (IC_{50} = 18 nM).[24] Miert and co-workers investigated and proved the DNA binding properties of cryptosanguinolentine (**3**) and isoneocryptolepine (**4**).[12] Ibrahim and co-workers synthesized the cryptosanguinolentine (**5i**) that formed complex with DNA (K_a ranging from 1.5 to 4.9 × 10^5 M^{-1}).[25]

(v) Antihyperglycemic activity: Cryptolepine (**1**) is the only natural indoloquinoline with known antihyperglycemic properties.[26] *In-vitro e*valuation of cryptolepine and its salt for the stimulation of glucose transport in 3T3-L1 adipocytes showed a dose-dependent capacity to stimulate the glucose transport at 3-10 μM. Bierer and co-workers synthesized a series of indoloquinolines (**5**) and evaluated for their *in-vitro* and *in-vivo* antihyperglycemic activity. The compound **5j** has displayed true antihyperglycemic activity.[26]

(vi) Antiinflammatory activity: Bamgbose and co-workers investigated the antiinflammatory activity of cryptolepine (**1**) and found to be less potent (ID_{70} = 20 $mg.kg^{-1}$) than aspirin (ID_{70} = 8 mg/kg) and indomethacin (ID_{70} = 2 mg/kg).[27] Among all

the indoloquinoline alkaloids isolated from *C. sanguinolenta* only 11-hydroxycryptolepine is an active inhibitor of xanthine oxidase (IC_{50} = 32.3 μM) and scavenger of superoxide anions (IC_{50} = 28 μM).[28]

(vii) Hypotensive and vasodilation activities: Cryptolepine (**1**) exhibits antihypotensive properties. Cryptolepine (**1**) at 0.3 μM concentration exhibits sympathomimetic effect by blocking the α_2-adrenoceptors, which suggests its preferential prejunctional α-adrenoceptor antagonist properties.[29] Oyekan *et al.* reported renal vasodilation and ADP-induced aggregation properties of *N*-alkylated cryptolepine (**1**).[30]

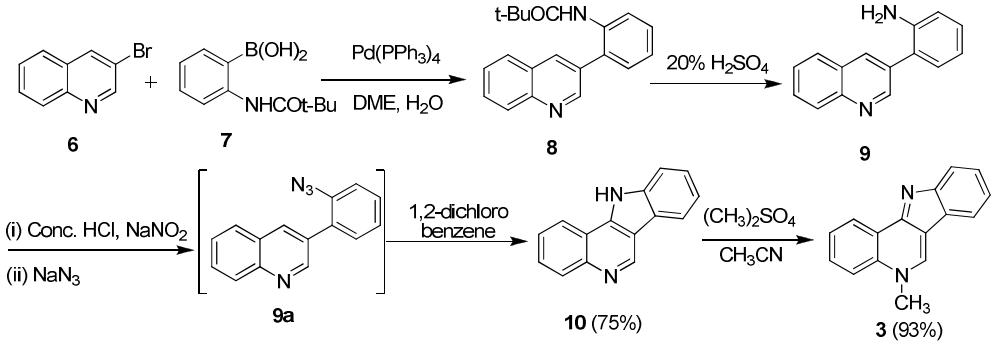
Due to the structural diversity and wide spread biological applications of indoloquinoline alkaloids and their analogues; they are used as lead compounds for the design of novel anticancer drugs. To prepare novel synthetic analogues of bio-active indoloquinoline scaffold it is also imperative to discover new eco-friendly synthetic protocol which utilizes readily available starting materials. We have developed a novel and facile synthetic route for the preparation of naturally occurring anticancer agent, cryptosanguinolentine **3** and its analogues.

6.3 Reported methods for the preparation of cryptosanguinolentine (3)

Several synthetic protocols were reported to achieve the medicinally important indoloquinolines **1-4**. The cryptosanguinolentine (**3**) (indolo[3,2-*c*]-quinoline) is a class of indoloquinoline derivatives having an angularly fused indole and quinoline rings together. In the following section, major classes of synthetic approaches used for the preparation of **3** are being discussed.

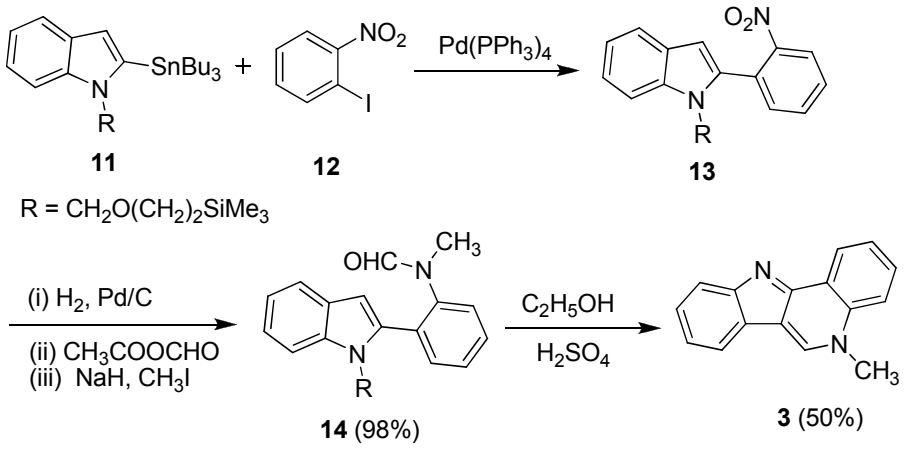
(i) Palladium-catalyzed synthesis of cryptosanguinolentine (3)

Timari *et al.* reported the synthesis of cryptosanguinolentine (**3**) by using Suzuki-coupling reaction. The reaction of 3-bromoquinoline (**6**) with *N*-pivaloylaminophenyl boronic acid **7** in presence of Pd(0) catalyst afforded the desired biaryl compound **8** which, upon hydrolysis with sulfuric acid gave amine (**9**). The amine (**9**) was converted to azide (**9a**) which upon heating led to the indolo[3,2-*c*]quinoline (**10**). Regioselective methylation of **10** using dimethyl sulfate produced cryptosanguinolentine (**3**) in good yield (scheme 6.1).[31]



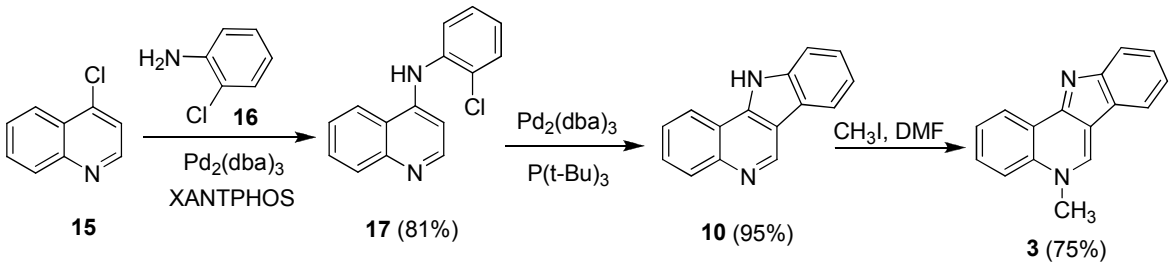
Scheme 6.1 Pd-catalyzed synthesis of cryptosanguinolentine (**3**)

Murray *et al.* achieved the synthesis of cryptosanguinolentine (**3**) as depicted in scheme 6.2. Pd(0)-catalyzed Stille coupling reaction of 2-tributylstannyl-*N*-protected indole (**11**) with 2-iodonitrobenzene (**12**) gave 2-(*o*-nitrophenyl)indole (**13**) which upon reduction, *N*-formylation and *N*-methylation afforded the desired formamide (**14**). Finally ring closure of **14** under refluxing conditions in presence of sulfuric acid produced cryptosanguinolentine (**3**) (scheme 6.2).[32]



Scheme 6.2 Pd-catalyzed synthesis of cryptosanguinolentine (**3**)

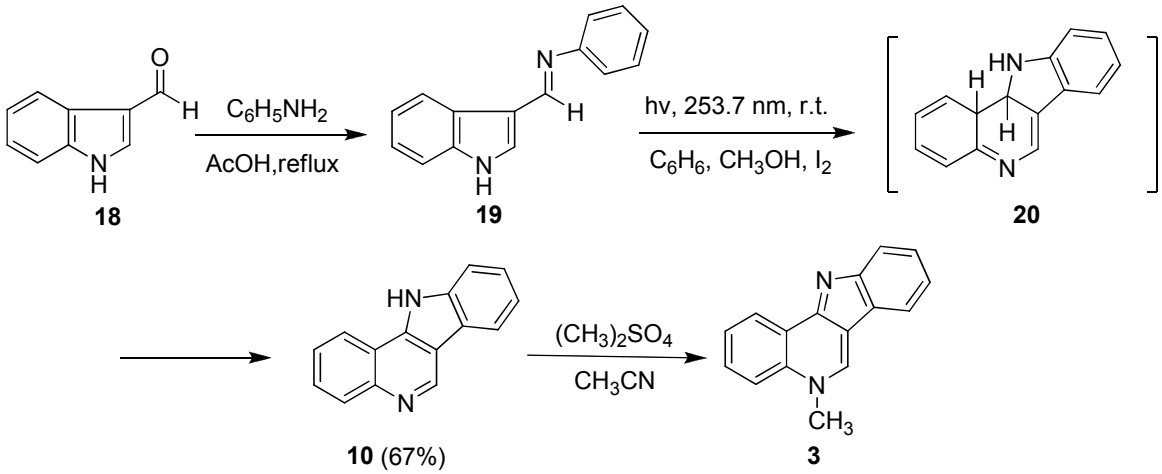
Joncker *et al.* reported the synthesis of cryptosanguinolentine (**3**) *via* selective Buchwald-Hartwig reaction. The protocol includes the reaction of 2-chloroaniline (**16**) with 4-chloroquinoline (**15**) followed by an intramolecular arylation of compound (**17**) to afford indoloquinoline (**10**). Finally, the compound (**10**) was methylated to give cryptosanguinolentine (**3**) in 75% yield (scheme 6.3).[33]



Scheme 6.3 Pd-catalyzed synthesis of cryptosanguinolentine (**3**)

(ii) Synthesis of cryptosanguinolentine (3) *via* photochemical reactions

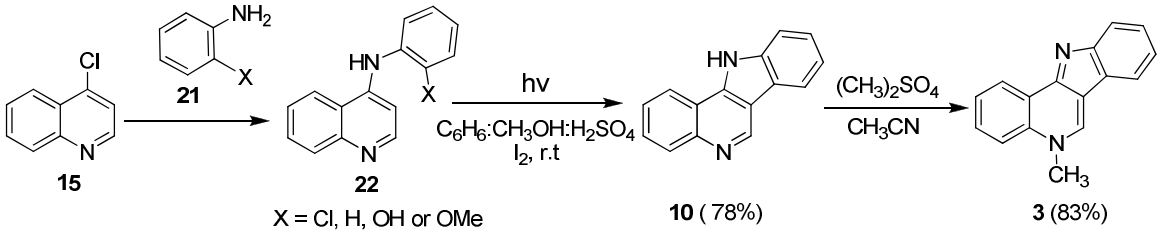
Photochemical reactions are valuable in organic chemistry as they proceed differently than thermal reactions and have the advantage of forming thermodynamically disfavoured and inaccessible products with prevention of by products. Mohan *et al.* described the first photochemical synthesis of cryptosanguinolentine (**3**) as given in scheme 6.4.[34]



Scheme 6.4 Photochemical route to cryptosanguinolentine (**3**)

Schiff's base (**19**) obtained by heating indole-3-carboxaldehyde (**18**) with aniline in acetic acid, when irradiated at wave length 253.7 nm underwent cyclization to give 11*H*-indolo[3,2-c]quinoline (**20**) *via* initial photo-isomerization of the Schiff's base (**19**) from *E*- to *Z*-isomer followed by conrotatory ring closure and subsequent oxidation by iodine led to indoloquinoline (**10**) which upon methylation provides **3** (scheme 6.4).

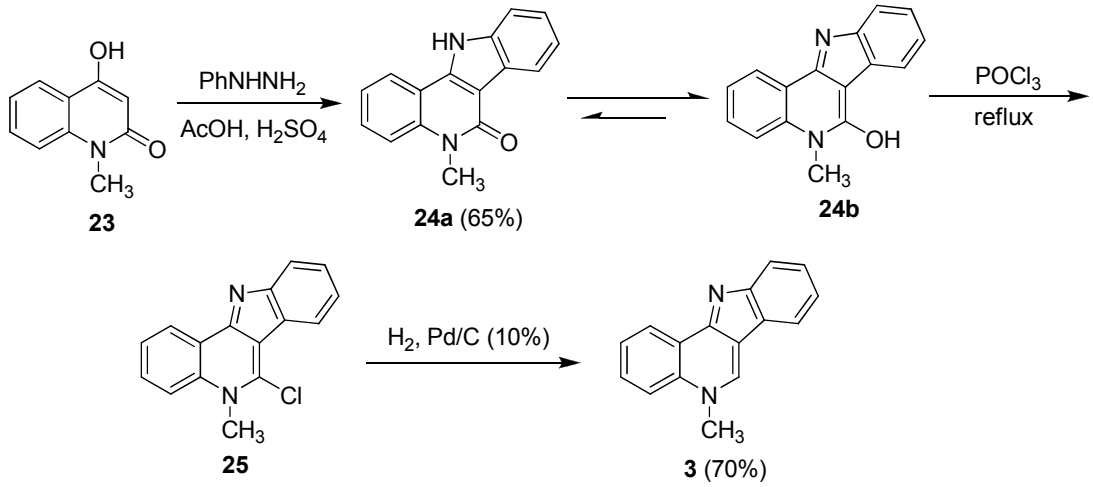
Mohan *et al.* have also reported synthesis of **3** *via* heteroatom directed photoannulation technique (scheme 6.5). Photocyclization of anilinoquinolines (**22**) followed by treatment with iodine generated the indoloquinoline (**10**). Methylation of **10** by dimethyl sulfate afforded the desired cryptosanguinolentine (**3**) in 83% yeild.[21]



Scheme 6.5 Photoannulation method to synthesize cryptosanguinolentine (**3**)

(iii) Fischer indole approach for the synthesis of cryptosanguinolentine (3)

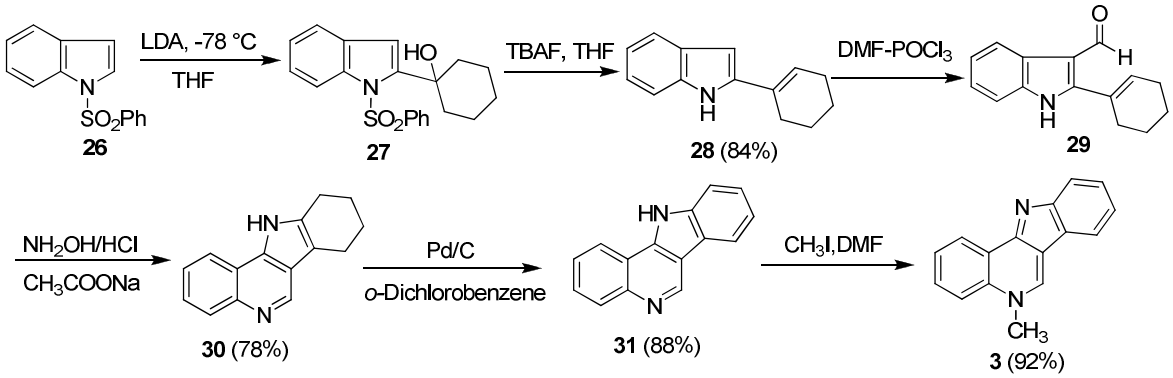
Dhanabal *et al.* reported the synthesis of cryptosanguinolentine (**3**) by using a Fischer indole cyclization reaction (scheme 6.6).[35] Fischer indole reaction of **23** with phenylhydrazine produced indoloquinolone (**24**) in which upon treatment with phosphorus oxychloride afforded the corresponding chloride (**25**). Finally catalytic hydrogenation of **26** afforded cryptosanguinolentine (**3**).



Scheme 6.6 Fisher indole synthesis of cryptosanguinolentine (**3**)

(iv) Intramolecular thermal electrocyclization method

More recently, the synthesis of **3** was reported by Dattatray *et al. via* intramolecular thermal electrocyclization strategy.[36] Initial preparation of alcohol (**27**) was achieved by the reaction of **26** with cyclohexanone in presence of LDA and further deprotection and dehydration afforded the compound **28** in 84 % yield. Formylation of **28** produced **29** which upon reaction with hydroxylamine hydrochloride furnished the compound **30** *via* intramolecular thermal electrocyclization. Further dehydration of **30** by using Pd/C resulted in indoloquinoline **31**. Finally, *N*-methylation of **31** produced cryptosanguinolentine (**3**) in 92% yield (scheme 6.7).

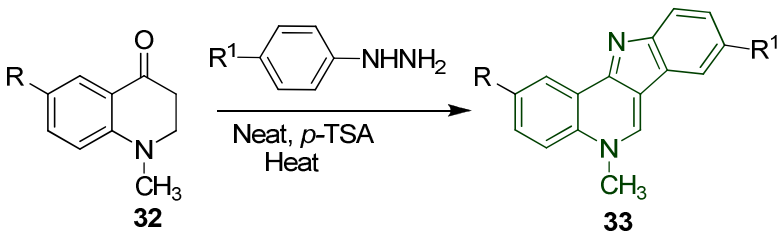


Scheme 6.7 Intramolecular thermal electrocyclization of **29** to **30**

6.4 Results and discussion

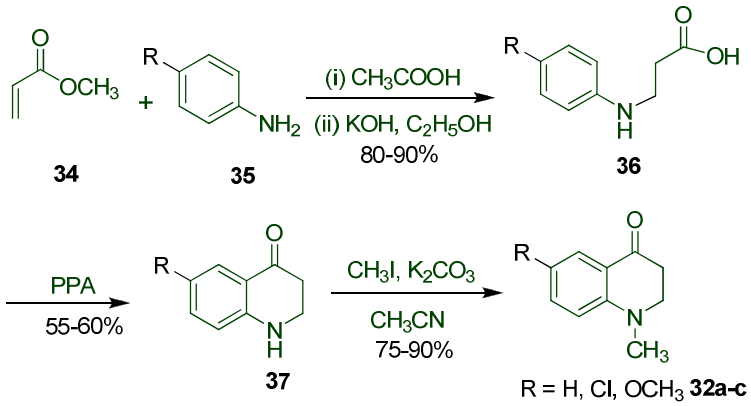
6.4.1 Chemistry

Our facile and rapid protocol to prepare cryptosanguinolentines (**33**) utilizes readily available 1,2,3,4-tetrahydroquinolones and arylhydrazines under solvent-free conditions. There is an increasing interest in developing eco-friendly organic reactions due to the growing concern for the influence of organic solvents on the environment. In this context, the replacement of toxic and volatile conventional organic solvents as reaction media with environmentally acceptable alternatives such as water,[37-38] fluorous media,[39] ionic liquids, PEG[40-42] etc. is highly desirable. Particularly, reactions under solvent-free conditions are of tremendous importance in modern organic synthesis as it avoids the use of toxic solvents and the formation of undesired products and generally afford the desired products in greater yield. In view of the environmental constraints and interesting biological activities displayed by cryptosanguinolentine, it is desirable to develop a benign and facile protocol. Inspired by recently reported easy and short synthesis of 1,2,3,4-tetrahydroquinolones (**32**), we envisioned this to be a starting material for the Fischer indole reaction with arylhydrazines to obtain cryptosanguinolentine (cryptosanguinolentine) and its analogues (scheme 6.8).



Scheme 6.8 Synthesis of cryptosanguinolentines (**33**)

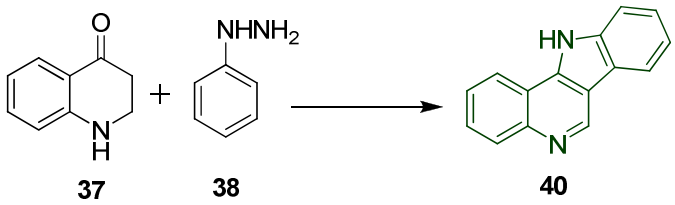
Initially we synthesised the 1,2,3,4-tetrahydroquinolines[43] starting from methylacrylate (**34**) and anilines (**35**) as mentioned in the scheme 6.9. The reaction of methylacrylate (**34**) and anilines (**35**) in acetic acid and followed by hydrolysis of *in situ* generated ester with potassium hydroxide produced *N*-arylpropionic acids (**36**) in good yields. Cyclization of **36** with PPA afforded 4-keto-1,2,3,4-tetrahydroquinolines (**37**) in good yields. Finally, reaction of **37** with methyl iodide in presence of potassium carbonate afforded the desired **32a-c** (scheme 6.9).



Scheme 6.9 Synthesis of *N*-methyl-1,2,3,4-tetrahydroquinolines (**32**)

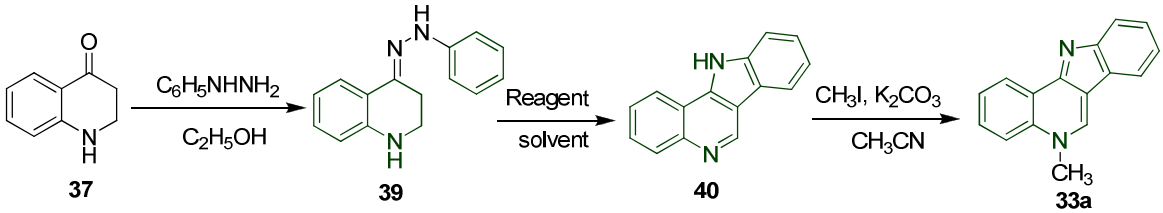
Initially the treatment of **37** with phenylhydrazine (**38**) was carried out in presence of acid catalysts under polar protic solvents led to the formation of **40** in poor yield. The reaction conditions were optimized by performing reaction in presence of various acids and at different temperatures. The results of this study are given in table 6.1. We found that reaction was very slow in ethanol and proceeded well in presence of *p*-TSA at 100 °C temperature without any solvent. In order to confirm the step-wise formation of **40**, the hydrazone **39** was isolated and subjected to similar conditions as shown in the table 6.1. Formation of **40** was noticed in good yield in presence of *p*-TSA under solvent-free condition.

Table 6.1 Fischer indole cyclization of (**37**) to (**40**)



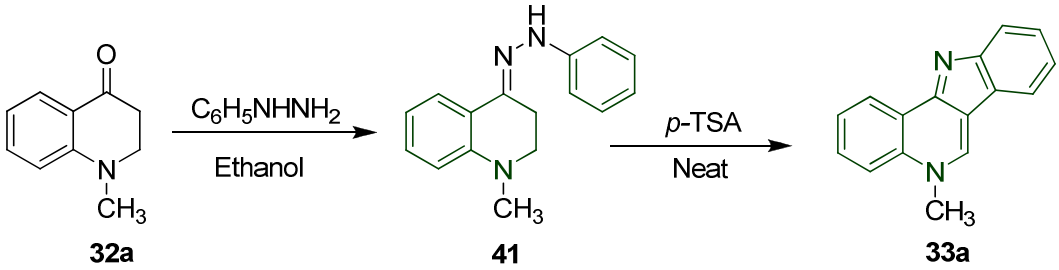
Reagent	Solvent	Temp (°C)	time (h)	Yield (%)
H_2SO_4	Ethanol	80	10	10
AcOH	Ethanol	80	10	10
AcOH	-	100	10	-
TFAA	Ethanol	80	10	-
HCl	Ethanol	80	10	-
PPA	-	100	1	15
p-TSA	Ethanol	80	10	25
p-TSA	-	100	0.5	50

The formation of product **40** was confirmed by comparing its melting point (245 °C) with the reported melting point (Lit.[34] >240 °C). Also its spectral data (NMR & Mass) were found in agreement with reported in literature. Finally, methylation of the indoloquinoline **40** led to the cryptosanguinolentine **33a** (scheme 6.10).



Scheme 6.10 Three-step synthesis of cryptosanguinolentine (**33a**)

Alternatively, we also prepared **33a** in 60 % yield from the initial reaction of *N*-methyl-1,2,3,4-tetrahydroquinolone (**32a**) with phenylhydrazine (**38**) generated hydrazone (**41**) was cyclized using *p*-TSA under solvent-free condition (scheme 6.11).



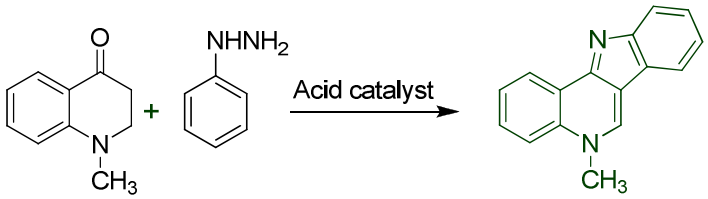
32a 41 33a

Scheme 6.11 Synthesis of cryptosanguinolentine (**33a**) *via* hydrazone (**41**)

The synthesis of cryptosanguinolentine (**33a**) was further reduced to single step, by reacting *N*-methyl-1,2,3,4-tetrahydroquinolone (**32a**) and phenylhydrazine (**38**) under solvent-free condition using *p*-TSA a catalyst.

In our efforts to prepare cryptosanguinolentine from the reaction of 1-methyl-1,2,3,4-tetrahydroquinolin-4-one (**32a**) with phenylhydrazine (**38**) under solvent-free conditionsat lower temperatures (<100 °C) the reaction was very sluggish and resulted in poor yield of **33a**. Among the acids, *p*-toluenesulfonic acid (*p*-TSA) was found superior in terms of reaction time and product yield (table 6.2).

Table 6.2 Preparation of **33a** in the presence of various acid catalysts



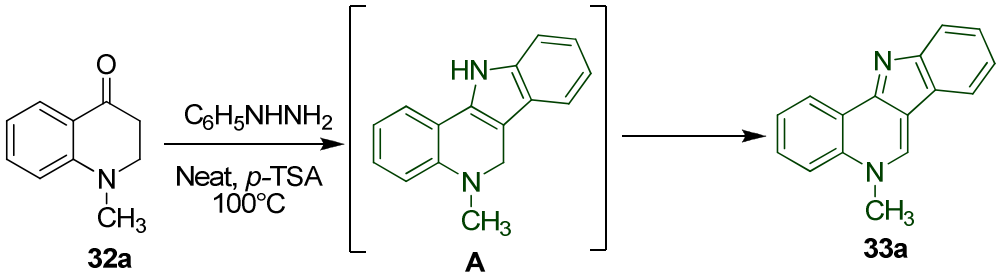
32a 38 33a

Acid catalyst[a]	Reaction time (min)	Yield (%)
$ZnCl_2$	120	40
$SnCl_4$	90	35
TFA	240	40
Phosphotungstic acid	150	28
p-TSA	20	83
$FeCl_3$	120	30

[a] 1.1 Equivalent used.

The neat reaction of 1-methyl-1,2,3,4-tetrahydroquinolin-4-one (**32a**) with phenylhydrazine (**38**) at 100 °C in the presence of *p*-TSA afforded cryptosanguinolentine **33a** in 83% yield. After completion of reaction, cryptosanguinolentine (**33a**) was isolated simply by neutralization of the reaction mixture.

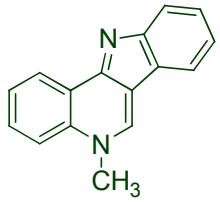
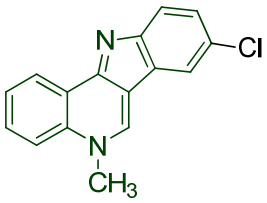
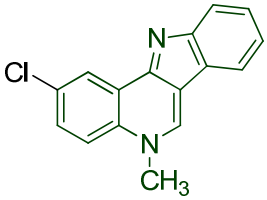
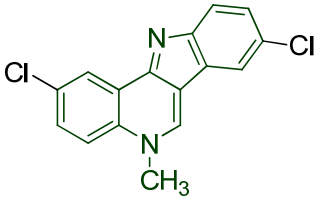
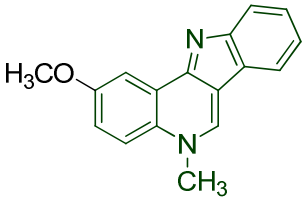
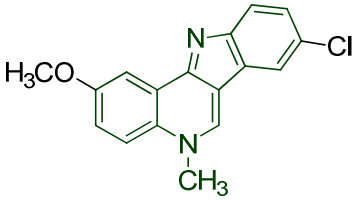
Mechanistically, Fischer indole cyclization of 1-methyl-1,2,3,4-tetrahydroquinolin-4-one (**32a**) with phenylhydrazine, initially forms intermediate **A**, which upon dehydrogenation produces cryptosanguinolentine **33a** (scheme 6.12). Dehydrogenation of intermediate **A** generates more stable **33a** in which both the nitrogen's are in conjugation. Similar dehydrogenations are also reported in the literature under the influence of heat, light or oxygen.[34, 44]



Scheme 6.12 Proposed reaction pathway for the formation of (**33**)

With the optimized protocol in hand, several cryptosanguinolentine analogues were synthesized by using different 1-methyl-1,2,3,4-tetrahydroquinolones (**32**) and arylhydrazines with electron-donating and electron-withdrawing groups. The reaction with differently substituted 1-methyl-1,2,3,4-tetrahydroquinolones (**32**) and arylhydrazines proceeded smoothly to afford analogues **33a-f** in good yields as shown in the table 6.3. The scope of this useful synthetic transformation was further broadened by the successful reaction of 1-methyl-1,2,3,4-tetrahydroquinolin-4-one (**32a**) and phenylhydrazine at larger scale (1 mmol) to obtain cryptosanguinolentine (**33a**) in 80% yield.

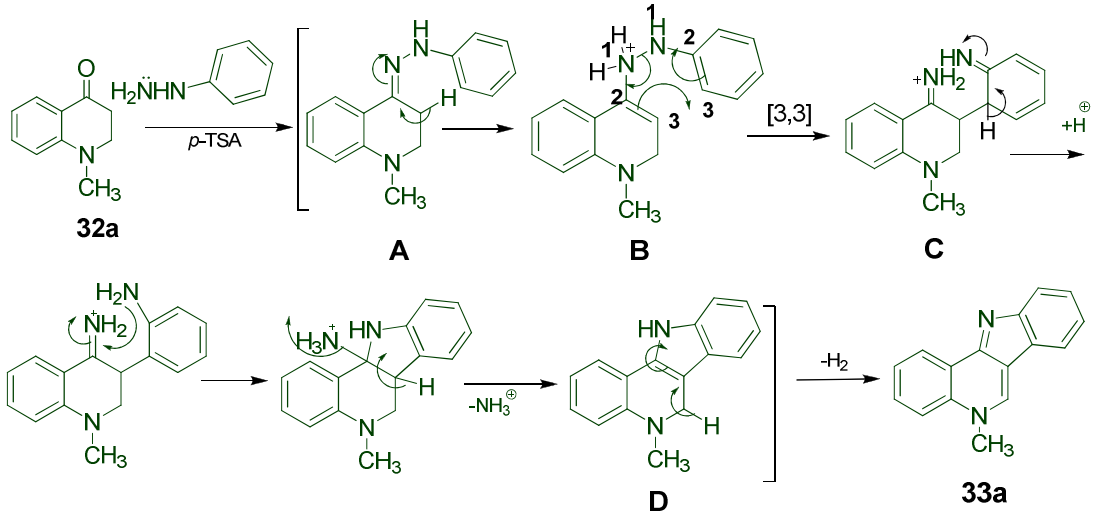
Table 6.3 Solvent-free synthesis of cryptosanguinolentine and its analogues **33a-f**

Cryptosanguinolentines **33**		Time (min)	Yield (%)[a]
	(**33a**)	20	83
	(**33b**)	25	77
	(**33c**)	25	74
	(**33d**)	30	84
	(**33e**)	25	82
	(**33f**)	30	78

[a]Yields of pure products

6.4.2 Proposed reaction mechanism

A plausible mechanism would be the initial formation of the hydrazone **A** from the reaction of quinolinones (**32**) and arylhydrazine (**38**), further hydrazone under goes fisher indole cyclization. The hydrazone **A** tautomerizes to give ene-hydrazine tautomer **B**, which undergoes [3,3]-sigmatropic rearrangement to bis iminobenzylketone **C**. Cyclization and aromatization of **C** with loss of ammonia led to indoloquinoline **D**, Which then rearranges to **33a** (scheme 6.13)



Scheme 6.13 Plausible mechanism for the formation of cryptosanguinolentine (**33a**)

All the synthesized compounds were well characterized by IR, NMR and mass spectral data. ^{1}H NMR spectrum of compound (**33b**) is given in the figure 6.4. Mass spectrum of compound **33b** showed the molecular ion peak *m/z* = 267.1 $(M+H)^{+}$ which is in agreement with the calculated value (figure 6.5).

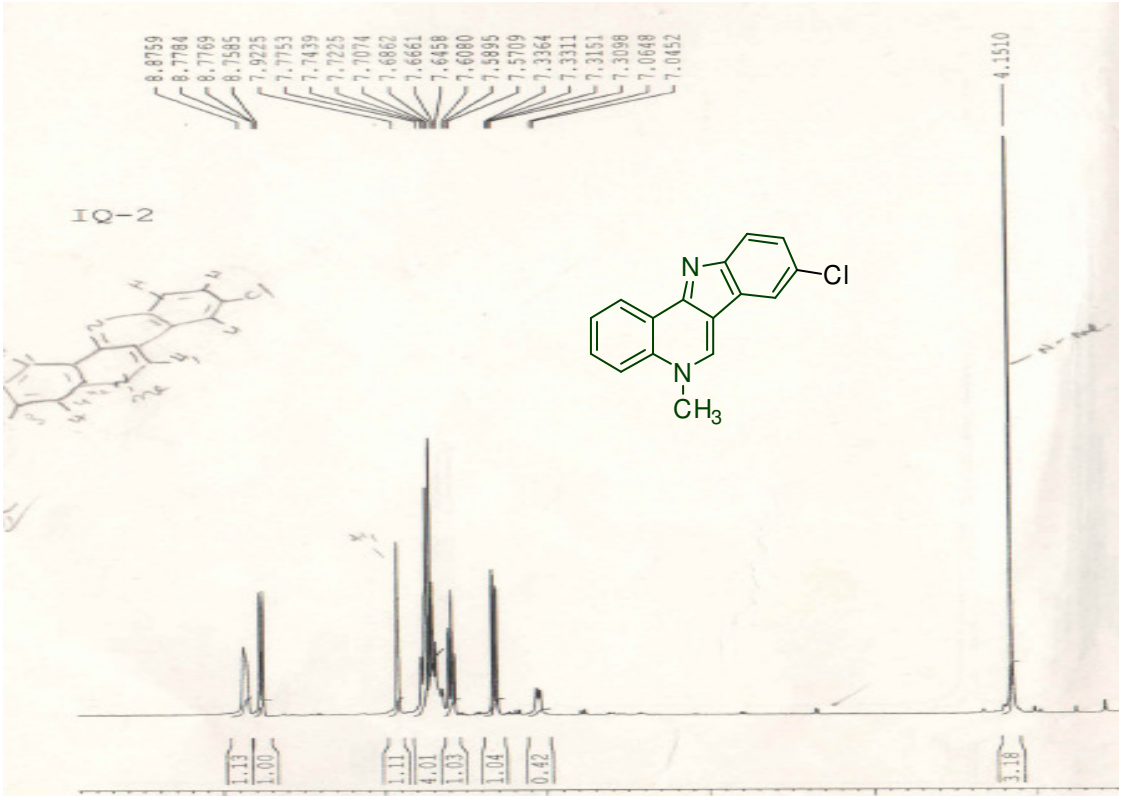


Figure 6.4 ^{1}H NMR spectrum of compound **33b**

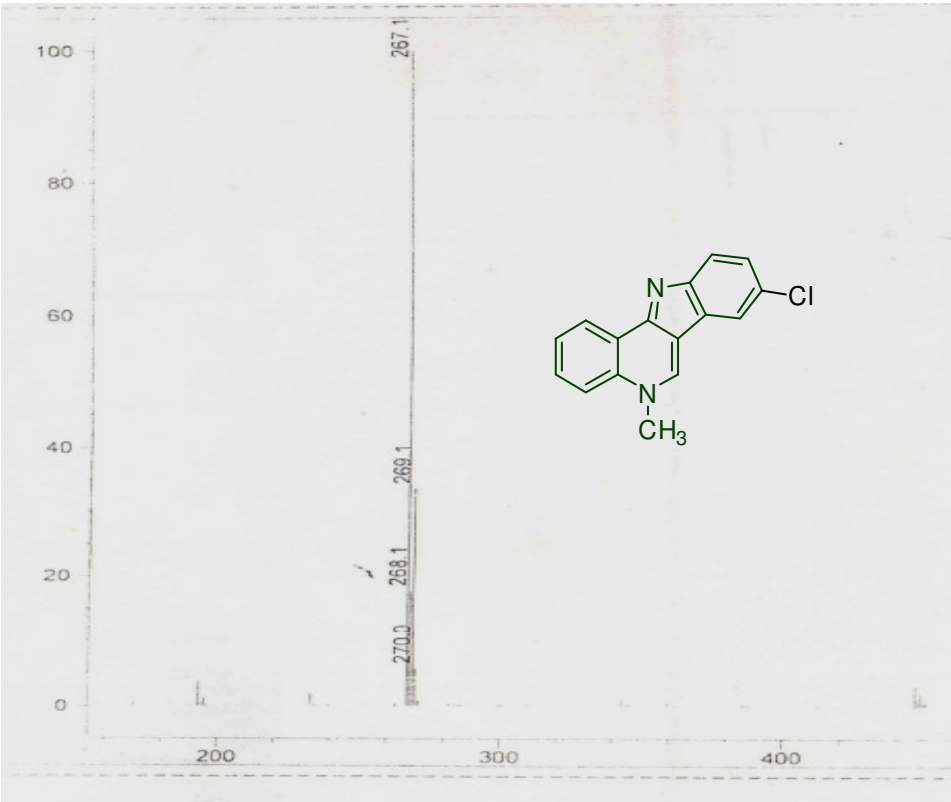


Figure 6.5 Mass spectrum of compound **33b**

6.5 Conclusions

In conclusion, we have developed a novel, short, and high yielding solvent-free synthesis of biologically important cryptosanguinolentine and its analogues from easily accessible starting materials 1-methyl-tetrahydroquinolines and arylhydrazines. Utilizing substituted anilines and various tetrahydroquinolines, a diverse library of biologically important indoloquinolines can be prepared. The protocol utilizes *p*-toluenesulfonic acid which can be reused and recycled. Overall, the protocol provides an alternative convenient route to prepare indoloquinolines.

6.6 Experimental Procedures

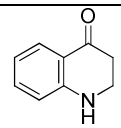
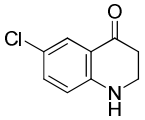
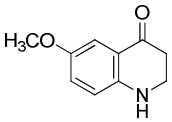
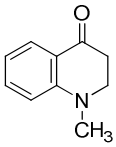
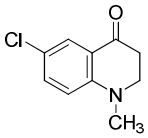
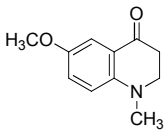
Melting points were determined in open capillary tubes on a MPA120-Automated Melting Point System and are uncorrected. IR spectra were recorded with a JASCO IR-Report-100. ^{1}H and ^{13}C spectra were recorded on a Bruker Advance II 400 NMR spectrometer at 400 and 100 MHz, respectively. DMSO-d_6 was used as a solvent and tetramethylsilane as internal standard. Mass spectra were taken on Agilent Mass spectrometer using FAB mode. All the reagents and solvents were commercially purchased and further purified according to the standard procedures.

General procedure for the synthesis of 4-keto-1,2,3,4-tetrahydroquinolones (37)

A mixture of methylacrylate (**34**, 10 mmol), aniline (**35**, 10 mmol) and acetic acid (2 mL) was refluxed for 24 h. After completion of the reaction, the crude product so obtained was extracted with ethyl acetate and the organic layer was washed with 100 mL water. After removal of excess solvent at reduced pressure, obtained product was taken for the next step. The ester was dissolved in solution containing KOH (20 mmol) in 100 mL of water and refluxed for 16 h. After complertion of the reacation, contents were cooled and acidified by using 2N HCl to produced acid **36**. The anilino-propionic acid (**36**, 5g 30 mmol) was taken in polyphosphoric acid (100 g) and heated at 120 °C for 40 min. Reaction mixture was poured into 300 mL of ice-cold water with constant stirring. After few hours, a yellow coloured oily product was extracted with ethyl acetate and the residue so obtained was purified by silica-gel column chromatography using ethyl acetate: hexane (3:7) to obtain pure 4-keto-1,2,3,4-tetrahydroquinoline (**37**).

***N*-Methyl 4-keto-1,2,3,4-tetrahydroquinolines (32a-c):** To a stirred solution of 4-keto-1,2,3,4-tetrahydroquinoline (**37**, 1 mmol) and potassium carbonate (1.1 mmol) in acetonitrile (10 mL), was added methyl iodide (6 mmol). The reaction mixture was heated at 80 °C for 4h. The crude product was poured into water (20 mL) and extracted with ethyl acetate (2 × 20 mL).The organic phase was separated and dried over anhydrous sodium sulphate and removed excess of ethyl acetate at reduced pressure to afford **32** in good yields.[34]

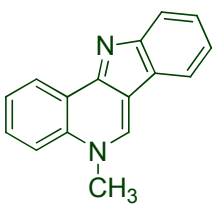
Physical data for the 4-keto-1,2,3,4-tetrahydroquinolines **32** and **37**

Tetrahydro-quinolines **32** and **37**		mp (°C)	Yield (%)
	**(37a)**	44 (Lit.[45] 43-44)	55
	**(37b)**	124-125 (Lit.[43] 125)	60
	**(37c)**	113-115 (Lit.[43-45] 112)	50
	**(32a)**	74-75	85
	**(32b)**	87-90	90
	**(32c)**	90-93	75

6.6.1 General procedure for the synthesis of cryptosanguinolentines (33a-f)

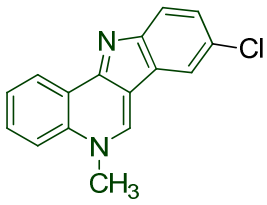
A mixture of *N*-methyl-keto-1,2,3,4-tetrahydroquinolone (**32**, 1.0 mmol), arylhydrazine (**38**, 1.0 mmol) and *p*-toluenesulfonic acid (1.1 mmol) was heated at 100 °C for 20-30 min (table 6.2). After completion of reaction, the mixture was allowed to cool at room temperature and the solid residue was taken into dilute solution of sodium bicarbonate. Filtered the solid product, washed with water (10 mL) and recrystallized from ethanol to afford pure product **33**.

5-Methylindolo[3,2-*c*]quinoline (33a)



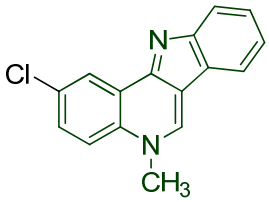
Off-white solid, mp 132-135 °C (Lit.[34] mp.133-135 °C). IR KBr ν cm^{-1}1633, 1617, 1460, 1354, 1228, 762, 715; MS (FAB): *m/z* calcd. for $C_{16}H_{12}N_2$: 232.1, found: 233.10 $(M+H)^+$.

8-Chloro-5-methylindolo[3,2-*c*]quinoline (33b)



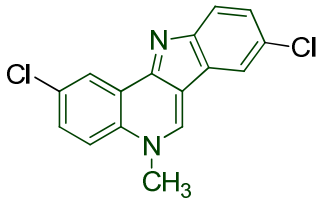
Off-white solid, mp 170-172 °C. IR (KBr, ν cm^{-1}): 1633, 1617, 1460, 1354, 1228, 762, 715; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.15 (3H, s, CH_3), 7.32 (1H, dd, *J* = 2.12, 8.52 Hz), 7.57-7.60 (1H, m), 7.64-7.77 (3H, m), 7.92 (1H, s), 8.76 (1H, d, *J* = 7.96 Hz), 8.88 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 42.13, 116.33, 118.69, 118.91, 120.76, 123.92, 124.71, 125.04, 125.36, 126.20, 127.83, 129.19, 135.12, 137.52, 152.17, 153.23; MS (FAB): *m/z* calcd. for $C_{16}H_{11}ClN_2$: 266.06, found 267.1 $(M+H)^+$, 269.1 $(M+2)^+$.

2-Chloro-5-methylindolo[3,2-c]quinoline (33c)



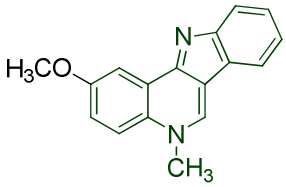
Brown solid, mp 190-192 °C. IR (KBr, ν cm^{-1}): 1640, 1434, 1218, 768, 695 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.94 (3H, s), 7.24-7.28 (1H, m), 7.43-7.52 (3H, m), 7.81-8.00 (2H, m), 8.12 (1H, s), 8.66 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): δ 42.31, 116.46, 117.33, 118.11, 118.82, 120.26, 121.01, 123.02, 124.51, 125.90, 128.80, 130.40, 132.99, 135.43, 151.40, 154.20; MS (FAB): *m/z* calcd. for $C_{16}H_{11}ClN_2$: 266.06, found 267.2 $(M+H)^+$, 269.1 $(M+2)^+$.

2,8-Dichloro-5-methylindolo[3,2-*c*]quinoline (33d)



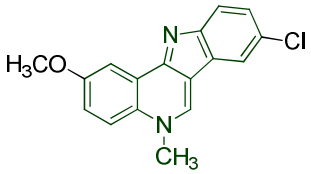
Off-white solid, mp 240-242 °C, (KBr, ν cm^{-1}): 1647, 1610, 1434, 1219, 1169, 762, 680 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.22 (3H, s), 7.36 (1H, dd, *J* = 2.14, 8.54 Hz), 7.67-7.74 (2H, m), 7.87 (1H, d, *J* = 9.16 Hz), 8.02 (1H, d, *J* = 2.08 Hz), 8.74 (1H, d, *J* = 2.4 Hz), 9.10 (1H, s); ^{13}C NMR (100 MHz,DMSO-d_6, δ): δ 42.31, 116.33, 118.58, 118.98, 121.75, 122.95, 124.71, 125.65, 126.16, 127.83, 129.26, 133.74, 135.60, 138.30, 151.90, 157.87; MS (FAB): *m/z* calcd. for $C_{16}H_{10}Cl_2N_2$: 300.0, found 301.0 $(M+H)^+$.

2-Methoxy-5-methylindolo[3,2-*c*]quinoline (33e)



Brown solid, mp 215-217 °C, (KBr, ν cm^{-1}): 1642, 1594, 1428, 1220, 1125, 764, 678 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.17 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 6.77 (d, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 8.9 Hz, 1H), 7.15-7.19 (m, 3H), 7.25 (d, *J* = 1.4 Hz, 1H), 7.36 (d, *J* = 8.9 Hz, 1H), 9.89 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.31, 56.06, 112.33, 113.58, 117.98, 118.73, 120.88, 121.12, 122.64, 124.68, 124.99, 131.11, 134.74, 136.60, 150.90, 158.47; MS (FAB): *m/z* calcd. for $C_{17}H_{14}N_2O$: 262.1, found 263.1$(M+H)^+$.

8-Chloro-2-methoxy-5-methylindolo[3,2-c]quinoline (33f)



Off-white solid, mp 222-224 °C. (KBr, ν cm^{-1}): 1648, 1596, 1428, 1235, 1158, 790, 680 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.45 (3H, s, CH_3), 3.83 (s, 3H, CH_3), 6.94 (d, *J* = 8.0 Hz, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 1.4 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 1.4 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 9.89 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 46.31, 55.8, 112.45, 112.95, 116.45 118.30, 120.34, 121.45, 122.14, 125.32, 126.62, 133.35, 135.44, 137.45, 150.90, 158.47; MS (FAB): *m/z* calcd. for $C_{17}H_{13}ClN_2O$: 296.0, found 296.0 $(M)^+$.

6.7 References

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Appendices

List of Publications *[A-1]*

1. Dalip Kumar, **Maruthi Kumar. N** and V. S. Rao "A Facile and Expeditious Synthesis of Cryptosanguinolentines" ***Chemistry Letters*** **2009**, 38, 156-157.

2. Dalip Kumar, **N. Maruthi Kumar**, Swapna Sundaree, Emmanuel O. Johnson and Kavita Shah "An expeditious synthesis and anticancer activity of novel 4-(3′-indolyl)oxazoles" ***European Journal of Medicinal Chemistry*** **2010**, 45, 1244-1249.

3. Dalip Kumar, **N. Maruthi Kumar**, Kanako Akamatsu, Eriko Kusaka,Hiroshi Harada, Takeo Ito "Synthesis and biological evaluation of indolyl chalcones as antitumor agents" ***Bioorganic & Medicinal Chemistry Letters*** **2010**, 20, 3916–3919.

4. Dalip Kumar, **N. Maruthi Kumar**, Kuei-Hua Chang, Kavita Shah "Synthesis and anticancer activity of 5-(3′-indolyl)-1,3,4-thiadiazoles" ***European Journal of Medicinal Chemistry*** **2010***,* 45, 4664-4668

5. Prasun Ghosh, S. Syed Jaffer, Tarasankar Das, Arnab Maity, **N. Maruthi Kumar**, Dalip Kumar, and Pradipta. "Solvatochromic study of three indoloquinoline derivatives: Effect of chloro group/s on the photophysics of the compounds." ***Journal of Luminescence*** **2011**, 131*,* 147–154.

6. Dalip Kumar, **N. Maruthi Kumar**, Kuei-Hua Chang, Kavita Shah. "Synthesis and anticancer activity of novel 5-(3′-indolyl)-1,2,4-triazoles" ***Chemical Biology and Drug Design*** **2011***,* 77, 182–188.

7. Dalip Kumar, **N. Maruthi Kumar,** Gautam Patel, Sudeep Gupta and Rajender S. Varma "A facile and eco-friendly synthesis of diarylthiazoles and diarylimidazoles in water." ***Tetrahedron Letters*** **2011***,* 52, 1983–1986.

8. Dalip Kumar, **N. Maruthi Kumar**, Kuei-Hua Chang, Ritika Gupta, Kavita Shah. "Synthesis and *in-vitro* anticancer activity of bis(indolyl)-1,2,4-thiadiazoles" ***Bioorganic & Medicinal Chemistry Letters*** **2011**, 21,5897–5900

9. Dalip Kumar, **N. Maruthi Kumar**, Soumitra Ghosh, Kavita Shah. "Novel bis(indolyl)hydrazide-hydrazones as potent cytotoxic agents". ***Bioorganic & Medicinal Chemistry Letters*** **2012**, 22, 212-215.

10. Dalip Kumar, **N. Maruthi Kumar**, Kavita Shah. "A series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles as anticancer agents" ***European Journal of Medicinal Chemistry*** *(**2012-** Communicated*)

11. Dalip Kumar, **N. Maruthi Kumar**, Kavita Shah. "Indole-2(3)-carboxylic acid hydrazides as novel anticancer agents." ***European Journal of Medicinal Chemistry*** *(**2012-** Communicated*)

List of papers presented in conferences *[A-2]*

1. **N. Maruthi Kumar** "Synthesis and anticancer activity of indolyl-1,2,4-triazoles" at **Symposium on Advances in Chemistry,** BITS-Pilani, **March 26, 2011**. **(Oral presentation)**

2. **N. Maruthi Kumar**, Sudeep Gupta and Dalip Kumar "A facile and eco-friendly Synthesis of diarylthiazoles and diarylimidazoles in water" at **Symposium on Advances in Chemistry,** BITS-Pilani, **March 26, 2011**. **(Poster)**

3. **N. Maruthi Kumar** and Dalip Kumar "Synthesis and anticancer activity of novel 5-(3-indolyl)-1,3,4-thiadiazoles" at **Symposium on Advances in Chemistry,** BITS-Pilani, **March 26, 2011**. **(Poster)**

4. **N. Maruthi Kumar** and Dalip Kumar "Synthesis of Novel Indolyl-1,2,4-triazoles as Potent Anticancer Agents" at **15th ISCBC International Conference on the Interface of Chemistry-Biology n Biomedical Research,** organized at Saurashtra University, Rajkot, during **February 4-7, 2011 (Poster)**

5. **N. Maruthi Kumar** and Dalip Kumar "A Facile Synthesis of Naturally Occurring 2,5-Diaryloxazoles" at the **National Conference on Green and Sustainable Chemistry (NCGSC-2010)** organized at Birla Institute of Technology and Science, Pilani during **February 19-21, 2010 (Poster)**

6. **N. Maruthi Kumar** and Dalip Kumar "Synthesis and Anticancer Activity of Novel 4-(3′-Indolyl)oxazoles" at the **14th ISCBC International Conference on the Interface of Chemistry-Biology in Biomedical Research** organized at Central Drug Research Institute (CDRI), Lucknow, during **January 15-18, 2010 (Poster)**

7. **N. Maruthi Kumar** and Dalip Kumar "A Facile and Expeditious Synthesis of Cryptosanguinolentines" at the **National Symposium on Green Chemistry: Applications in Science & Engineering (NSGC-2009)** organized by School of Chemistry & Biochemistry, Thapar University at Thapar University, Patiala, Punjab during **February 5-6, 2009 (Poster)**

8. **N. Maruthi Kumar** and Dalip Kumar "A Facile and short Synthesis of Cryptosanguinolentines" at the **13th ISCBC International Conference on Interplay of Chemical and Biological Sciences: Impact on Health and Environment** organized by Department of Chemistry, University of Delhi, Delhi during **26th February to 1st March 2009 (Poster)**

9. **N. Maruthi Kumar** and Dalip Kumar "Rapid and Short Synthesis of Indoloquinoline Alkaloid Cryptosanguinolent -ines" at the **12th ISCBC (International Conference on the Interface of Chemistry-Biology in Biomedical Research)** organized at Birla Institute of Technology and Science, Pilani during **February 22-24, 2008 (Poster)**

BRIEF BIOGRAPHY OF THE CANDIDATE *[A-3]*

Maruthi Kumar. Narayanam completed his M.Sc in Organic Chemistry from Kakatiya University, Warangal, in 2005 and secured University rank during the academic years 2003-05. He worked as a Research Executive in Medicinal Chemistry Department, Orchid Research Laboratories Limited, Chennai (T.N) from July 2005 to May 2007. He worked in the area of PDE4 inhibitors. He is having one US patent to his credit from this assignment. In 2007 he joined in BITS-Pilani, Pilani Campus, for his doctoral studies under the supervision of Dr. Dalip Kumar. During his doctorial tenure, he worked as a Project Fellow in UGC sponsored project (2007-2010). In the year 2010, he has received Senior Research Fellowship (SRF) from Council of Scientific and Industrial Research (CSIR) for his research work. He has published research articles in well renowned international journals and presented papers in conferences/symposium.

BRIEF BIOGRAPHY OF THE SUPERVISOR *[A-4]*

Dr. Dalip Kumar is an Associate Professor & Head, Department of Chemistry, Birla Institute of Technology and Science, Pilani. He received his Ph.D. degree from Kurukshetra University, Kurukshetra, Haryana in 1997. For his doctoral degree, he worked with Prof. Shiv P. Singh in the research area of heterocyclic chemistry. After his doctorate, he worked as a post-doctoral fellow (1997-1999) with Prof. Rajendra S. Verma at Sam Houston State University, TX, USA. He was also associated with Prof. Sean M. Kerwin as a postdoctoral fellow (1999-2000), College of Pharmacy, University of Texas at Austin, TX, USA. He joined BITS, Pilani as a lecturer in 2000-2002. Later, in 2002-2004 he moved to University of Maryland, College Park, MD, USA as a research Associate. He rejoined BITS, Pilani as an Assistant Professor, Chemistry Group in 2004 and he was promoted to associate professor in year 2010. At present, he is also Head, Department of Chemistry, BITS, Pilani. He has been involved in research for the last 17 years and in teaching for 8 years. As a result of his research accomplishment, he has around 70 publications in peer reviewed international journals. Under the supervision of Prof. Kumar, three students have completed their Ph D. degree and six more students are continuing with him. Additionally, to his credit he has US and Indian patents and handled several major research projects funded by DST, DRDO, UGC and presently handling two academic projects from CSIR, UGC and an industrial project from Ranbaxy Laboratories Ltd.

Chapter 7

7.1 General conclusions

Indole scaffolds have been widely explored since last several decades due to their therapeutic potential in several areas of drug discovery, dye and agro products. Indoles continue to impact both of pharmaceutical and textile markets today. In many of the drug discovery laboratories and academic research institutes indole has become one of the active pharmacophores to design new chemical entities. The indole nucleus plays an enormous role in the anticancer research. Cancer poses serious threat on human health and significantly contributes to mortality rate. Among the existing cancer therapies, chemotherapy has proven to be one of the most significant treatments in cancer management. Natural product derived Paclitaxel and Docetaxel are extensively used in the treatment of large types of cancers, however, they suffer from hematopoietic and neurologic toxicities. Several other classes of natural and synthetic anticancer agents are under clinical investigations, nevertheless dose limiting side effects, toxicity to normal tissues of the body and drug resistance are the major challenges in development of novel small molecule chemotherapeutics. The first foremost plant based anticancer agents to advance into clinical use; the so-called vinca alkaloids vinblastine and vincristine contain indole as active pharmacophore. Vinca alkaloids success in chemotherapy motivated to synthesize analogues of indole-based natural products (semi-synthetic and synthetic) and some of them has emerged as clinical candidates.

Anticancer agents that interfere with microtubule function are in widespread use and have a broad spectrum of activity against both hematological malignancies and solid tumors. The mechanisms of actions of these agents have been better defined during the past decade, indicating that there are distinct binding sites for these agents and they interfere with microtubule dynamics. Indole nucleus has ability to interfere and alter the microtubule dynamics. Since two decades several structurally modified indoles were reported as tubulin polymerization inhibitors. The clinical candidates' indibulin and indole-3-carbinol are newly emerging chemical entities for chemotherapy. With the help of innovative chemistry and structure based rational drug design, the challenges posed by existing marketed drugs can be tackled to develop novel indole-based anticancer agents.

7.2 Specific Conclusions

The first chapter introduces the physical and chemical properties of indole nucleus and its significance in various areas of drug discovery. This chapter introduces the anticancer research and treatments of various types of cancers, specially emphasizes on chemotherapy and classification of anticancer drugs present in market. This chapter elaborates the application of natural and synthetic indoles in anticancer research and also explains rational design and development of novel indole-based chemical entities as tubulin polymerization inhibitors. Chapter 1 also describes the problems associated with the existing anticancer drugs and the scope for developing novel indole-based heterocycles and need for the structural modifications to existing indole-based lead anticancer drug candidates.

The second chapter deals with the synthesis and biological activities of indolylazoles; including 4-(3′-indolyl)oxazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles and 5-(3′-indolyl)-1,2,4-triazoles against various human cancer cell lines. The key-step in the first series (part-A) 4-(3′-indolyl)oxazoles involves microwave-assisted solvent-free reaction of 3-tosyloxyacetyl-1′-benzenesulfonylindole with variety of amides. The novel 4-(3′-indolyl)oxazole (**56**) exhibited good cytotoxicity against breast cancer cell line (IC_{50} = 14.1 μM, MCF-7). The second series (part-B) involves convenient synthesis of 5-(3′-indolyl)-1,3,4-thiadiazoles and their *in-vitro* anticancer activity. The most active indolyl-1,3,4-thiadiazole (**65m**) suppressed PaCa2 cancer cell growth with an IC_{50} value of 1.5 μM. In the third series (part-c), 5-(3′-indolyl)-1,2,4-triazoles were achieved in a single-step reaction of 3-cyanoindoles with aryl/heteroaryl hydrazides. Among the synthesized 5-(3′-indolyl)-1,2,4-triazoles, the compound (**82i**) with 3,4,5,-trimethoxyphenyl moiety showed highest cytotoxicity (IC_{50} = 0.8 μM, PaCa2). Preliminary anticancer mechanistic studies revealed that indolylazoles probably inhibit the cancer cell growth by disrupting microtubule polymerization process.

In the part-A of third chapter, we have successfully prepared Nortopsentin analogues bis(indolyl)-1,2,4-thiadiazoles. The key synthetic step involves iodobenzene diacetate-mediated oxidative dimerization of indole-3-thiocarboxamide to prepare desired bis(indolyl)-1,2,4-thiadiazoles in good yields. The most active bis(indolyl)-1,2,4-thiadiazole (**45h**) showed an IC_{50} value of 14.6 μM against LnCap cancer cell line. In the part-B of this chapter, synthesis and anticancer activity of novel bis(indolyl)hydrazide-hydrazones have been described. The bis(indolyl)hydrazide-hydrazones were achieved

from the reaction of indole-3-carbohydrazides with different indole-3-carboxaldehydes. Most of the Bis(indolyl)hydrazide-hydrazones exhibited prominent selective cytotoxicity towards breast cancer cell line (IC_{50} = 5-0 µM .7- µM, MDA-MB-231). The preliminary anticancer mechanistic studies showed that bis(indolyl)hydrazide-hydrazones induces apoptotic cancer cell death.

The fourth chapter illustrates the synthesis and anticancer activity of a series of indolyl chalcones. The protocol for the synthesis of chalcones involves Claisen–Schmidt condensation of indole-3-aldehydes/3-acetylindoles with appropriate arylketones/ arylaldehydes. The cytotoxicity studies of synthesized indolyl chalcones resulted in potent and selective anticancer agent **3g** with highest activity against PaCa2 cell line (IC_{50} = 0.03 µM).

The synthesis and anticancer activity of 2-arylaminoindolyl-1,3,4-thiadiazoles have been described in the fifth chapter. The protocol for the synthesis of 2-arylaminoindolyl-1,3,4-thiadiazoles involves the cyclization of *N*-arylindolylthiosemicarbazides in presence of acetyl chloride. All the synthesized compounds exhibited excellent activity with selective cytotoxicity towards MDA-MB-231 breast cancer cell line. The most active compound **11f** with 3,4,5-trimethoxyphenylamino and indole moieties exhibited highest cytotoxicity against prostate cancer cell line (IC_{50} = 0.15 µM, LnCap).

A simple and convenient synthesis of indoloquinoline alkaloids, cryptosanguinolentine and its analogues has been reported in the sixth chapter. The facile synthesis of cryptosanguinolentines involves the neat reaction of easily accessible 1-methyl-1,2,3,4-tetrahydroquinolin-4-ones and aryl hydrazines in the presence of *p*-toluenesulfonic acid at 100 °C. This novel synthetic protocol is appreciable in terms of the high exploratory power, short reaction time and ease of availability of starting materials.

7.3 Future scope of the work

Remarkable progress has been made in the past several years in the area of anticancer drug discovery. Efficient cancer therapies such as surgery, radiation therapy, chemotherapy and advanced immunotherapy are used for the treatment of several types of cancers. For many solid tumors, as in colon cancer, improved methods for early diagnosis and combination therapies have had an important impact on survival. However, once the tumor has metastasized, treatment becomes more complicated. Even in such cases, current treatment strategies can relegate cancer to more of a chronic disease. Knowledge

of cancer biology has exploded during the past decades and this may help us in understanding the mechanistic pathways of cancer cell growth. It was evidenced that numerous natural plant-based alkaloids such as taxol, vinca alkaloids and combretastatin A-4 made an impact in the discovery and development of potent anticancer agents. Although enormous efforts have brought out large number of lead clinical candidates designed for improved tolerability and efficacy, so far none of them fulfilled expectations of drug regulatory agencies and find a solution for complete cure. Tumor specificity and toxicity associated side effects are the major challenges for the medicinal chemists to design novel drugs with better safety profile. Up-to-date knowledge of various targets and mechanisms of diverse cancer cells will fortify the medicinal chemists to take-up the fascinating challenge to develop potent and selective anticancer drugs.

The scope of this thesis is to synthesize and evaluate diverse structural class of indoles as novel anticancer agents. The *in-vitro* activity results of synthesized 4-(3′-indolyl)-oxazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles, 5-(3′-indolyl)-1,2,4-triazoles and 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles can be further optimized with extensive structure-activity relationship studies and evaluated their cell cycle specificity, effect on tubulin network and possible molecular targets can be identified for observed cytotoxic effects. may be taken up further for extensive *in-vivo* study in animal models.

With the encouraging *in-vitro* anticancer activity results of bis(indoles) and indolyl chalcones, there is a fair scope to identify potent lead molecules. The bis(indole) series can be undertaken for further mechanistic studies to identify exact molecular target and breast cancer cell specificity. Indolyl chalcones due to their similitude to antimitotic agent, combretastatin A4 envisage their potential *in-vivo* activity to discover a potent and selective anticancer agent.

The synthetic protocol developed for Cryptosanguinolentine can be extended by using substituted tetrahydroquinolones and arylhydrazines to prepare a diverse library of indoloquinolines for further anticancer and antimalarial studies.