Design, Synthesis and Anticancer Activity Studies of Selected Indole-Based Heterocycles

THESIS

Submitted in partial fulfilment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

by

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Under the Supervision of

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CERTIFICATE

This is to certify that the thesis entitled **"Design, Synthesis and Anticancer Activity Studies of Selected Indole-Based Heterocycles"** and submitted by **Mr. Tantak Mukund Pandurang** ID No **2010PHXF416P** for the award of Ph. D. Degree of the Institute embodies the original work done by him under our supervision.

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Dedicated to

My Family and Teachers

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ABSTRACT

Cancer is one of the prominent causes of death worldwide after heart disease. To identify potent and selective anticancer drugs with reduced side effects is a serious concern to medicinal chemists. The thesis deals with the design, synthesis and anticancer activity studies of some indole-based compounds. The thesis is divided into five chapters.

The **first chapter** briefly highlights chemical properties and synthesis of indole derivatives in addition to description about cancer and its treatments. Natural and synthetic indole-based anticancer agents have been categorized based on their structural features and rational approaches.

The **second chapter** is subdivided into two parts which deals with design, synthesis and anticancer activity studies of two different series of novel indolylthiazoles. **Part A** of the chapter reports one-pot microwave-assisted rapid and high yielding synthetic protocol for the construction of 2-aryl amino-4-(3′-indolyl)thiazoles from the reaction of *α*-tosyloxyketones with *N*-phenylthiourea in PEG-400 as a benign reaction medium within short time (5 min). The most potent aminothiazole found to display significant anticancer activity (IC_{50} = 1.86 μM; MCF-7) *via* apoptosis inducing pathway. **Part B** describes the synthesis and anticancer activity studies of sixteen 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides which led to a potent compound with an IC_{50} value of 3.41 μM against HeLa cell line. Further, preliminary mechanism of action studies indicated that thiazole carboxamide induces apoptosis in HeLa cells.

The **third chapter** illustrates rational design, synthesis and *in vitro* anticancer activity studies of two series of indolyloxadiazoles. **Part A** of the chapter includes a simple and convenient protocol for the preparation of 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles using IBD-mediated oxidative desulfurization of readily available acylthiosemicarbazides. The most potent analogue exhibited significant cytotoxicity with an IC_{50} value of < 0.001 μM against HeLa cancer cell line through apoptotic cell death. **Part B** of this chapter discloses a facile, efficient and high yielding synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles from the oxidative cyclization of acylhydrazones using molecular iodine. The most active compound exhibited cytotoxicity towards acute lymphoblastic leukemia SB cell line (IC_{50} = 0.8 μM) which induces caspase-dependent apoptotic cell death. Preliminary mechanism of action study of the most potent compound showed inhibition of tubulin polymerization.

The **fourth chapter** comprises the rational design, synthesis and cytotoxicity of bisindoles connected with linear chain linkers. **Part A** describes the microwave-assisted synthesis of *α*-cyano bis(indolyl)chalcones. Cytotoxicity study of bis(indolyl)chalcones revealed potent and selective anticancer agents against A549 lung cancer cell line (IC_{50} = 0.8 μM) which is likely through microtubule stabilization mechanism. In **part B** various bis(indolyl)ketohydrazide-hydrazones are reported as potent anticancer agents. The most active analogue was found broadly cytotoxic against MCF-7, MDA-MB-231, HCT-116 and JURKAT cancer cell lines with IC_{50} values of 0.8, 0.5, 0.15, and 0.22 μM, respectively. The most potent compounds found to be selectively cytotoxic (10-folds) against cancerous cells Preliminary mechanism of the action studies suggested that the bis(indolyl)ketohydrazide-hydrazones induced DNA fragmentation and caspase 3/7 activation. The most potent ketohydrazide-hydrazones displayed inhibition of tubulin polymerization (IC_{50} = 0.6 μM).

Finally, summary and conclusions of the thesis are reported in **chapter five**. Future directions in continuation of the results achieved in the thesis are also described in this chapter.

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation/Symbol	Description
α	Alpha
β	Beta
Δ	Delta
°C	Degree centigrade
Å	Angstrom
Ac	Acetyl
Ac_2O	Acetic anhydride
ACN	Acetonitrile
Ar	Aryl
ATP	Adenosine triphosphate
Bn	Benzyl
Bu	Butyl
t-BuOK	Potassium *tert*-butoxide
Calcd.	Calculated
^{13}C	Carbon-13
CA-4	Combretastatin A-4
Cat.	Catalyst
CAN	Ceric ammonium nitrate
$CDCl_3$	Deuterated chloroform
Chalcones	1,3-Diarylprop-2-en-1-ones
Conc	Concentration
CuAAC	Copper catalyzed Azide-Alkyne Cycloaddition

d	Doublet
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dd	Doublet of doublet
DCE	Dichloroethane
DCM	Dichloromethane
DMA	*N,N*-Dimethylacetamide
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
EGFR	Epidermal growth factor receptor
EI	Electron ionization
ESI	Electrospray ionization
EtOAc	Ethyl acetate
EtOH	Ethanol
Equiv	Equivalent
g	Gram
GPCR	G-protein coupled receptors
h	Hours
HCN	Hydrogen cyanide
HDNIB	[Hydroxy-(2,4-dinitrobenzenesulfonyloxy)iodo]benzene,
HRMS	High resolution mass spectra

5-HT	5-Hydroxytryptamine
HTIB	(Hydroxy(tosyloxy)iodo)benzene
IBX	2-Iodoxybenzoic acid
IC_{50}	Half maximal inhibitory concentration
IR	Infrared
HTS	High-throughput screening
Hz	Hertz
J	Coupling constant
Lit.	Literature
m-CPBA	*m*-Choroperbenzoic acid
MCR	Multi component reaction
Me	Methyl
MS	Mass spectrometry
mp	Melting point
m	Multiplet
MeOH	Methanol
mg	Milligram
MHz	Mega hertz
MIC	Minimum inhibitory concentration
min	Minutes
mL	Milliliter
mmol	Millimole
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
MW	Microwave
NCI	National Cancer Institute

NH_3	Ammonia
N_2	Nitrogen gas
NMP	*N*-Methyl pyrrolidine
NMR	Nuclear magnetic resonance
O_2	Oxygen gas
PPA	Polyphosphoric acid
PPAR	Peroxisome proliferator-activated receptor
PEG	Polyethylene glycol
PIDA	Phenyl iodonium diacetate
Ph	Phenyl
ppm	Parts per million
PS	Polymer supported
%	Percentage
psi	Per square inch
PTK	Protein tyrosine kinase
PTP1B	Protein tyrosine phosphatase 1B
p-TsOH	*p*-Toluenesulfonic acid
R	Hydrocarbon
rt	Room temperature
s	Singlet
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
nM	Nano molar
SAR	Structure-activity relationship
t	Triplet

t-Bu	Tertiary butyl
T3P	Propylphosphonic anyhydride
TCPTP	T-Cell protein tyrosine phosphatase
TBAI	Tetrabutylammonium iodide
TBHP	*tert*-Butyl hydroperoxide
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSCl	Trimethylsilyl chloride
TNF-α	Tumor necrosis factor α
δ	Parts per million
UV	Ultraviolet
w	Watt
μM	Micromolar

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Chapter 1

Introduction

1.1 Introduction

Heterocyclic compounds are of immense chemical and biological significance. In particular, azaheterocycles (nitrogen containing heterocycles) such as pyrrole, oxazoles, imidazoles, thiazoles, oxadiazoles, quinolines, pyrimidines and indoles are structural constituents of many natural as well as synthetic bioactive drug-like molecules.[1] Database of U.S. FDA approved drugs reveals that fifty nine percent of unique small-molecule drugs are having azaheterocycle scaffold.[2] Substituted azaheterocycles have been referred as "*privileged structures*" since they are capable of binding to many receptors with high affinity and hydrogen bonding capacity. Naturally occurring nitrogen-based heterocycles such as reserpine, vinca alkaloids, bisindoles, indoloquinolines, opioid analgesics, carbolines and cinchona alkaloids are established source of lead molecules for diverse therapeutic areas.[2] In recent past, several nitrogen containing novel chemical entities emerged as drug molecules, for example, Dasatinib (**1**) and Pazopanib (**2**) (anticancer);[3] Rilpivirine (**3**) and Atevirdine (**4**) (anti-HIV);[4] Chloroquine (**5**) and Pyrimethamine (**6**)[5] (antimalarial) showcase their potential in drug discovery research (Figure 1.1).

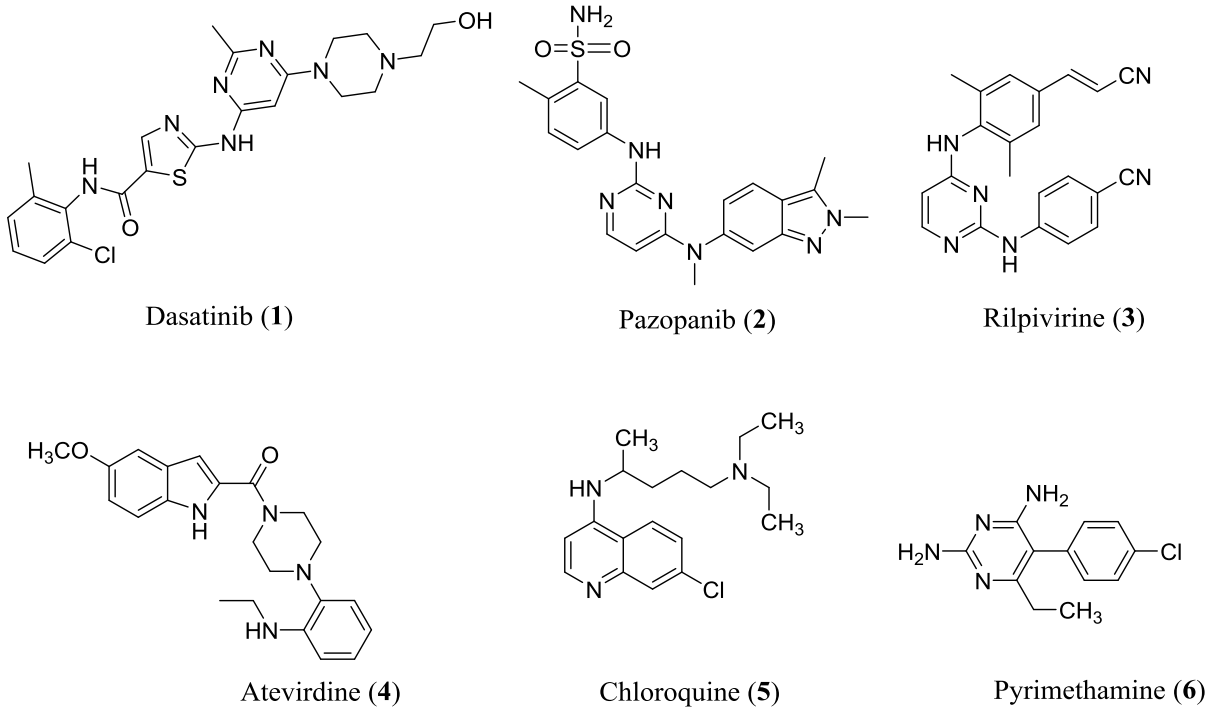


Figure 1.1 Representative examples of drug molecules **1-6** with azaheterocyclic units

Among the nitrogen containing heterocycles, indole is the parent core in a large number of bioactive naturally occurring compounds. Indole and its derivatives have received significant attention due to their wide range of biological activities including antimicrobial, anticancer, anti-HIV antileishmanial and anti-inflammatory.[6]

1.2 Indole

Etymologically, the word indole (**7**) is a trivial name for benzo[b]pyrrole originated from the combination of indigo and oleum. In 1866, Baeyer and Knop obtained two products, dihydroxyindole and oxindole from the reduction of indigo which they considered as derivative of C_8H_7N, later they proposed name "Indole".[7,8]

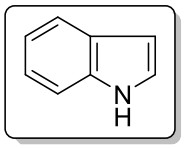


Figure 1.2 Indole (**7**)

Since, indole (**7**) is a structural component of a vast number of pharmaceuticals, fragrances, agrochemicals and pigments. Indole chemistry received a more attention during 1950-60s, when the alkaloid reserpine was introduced as a first drug for the treatment of diseases of central nervous system (CNS) and vincristine discovered as an antitumor agent. Plants including *Robinia pseudocacia*, the jasmines, some citrus plant and orange blossoms are the prime sources of indoles.

1.2.1 Chemical reactivity

Indole and simple alkyl indoles are colorless crystalline solids found to be stable in air and soluble in most of the organic solvents. Indole is planer conjugated system with 10 π-electrons and its IUPAC name is 1*H*-benzo[b]pyrrole. Like pyrrole, indole is a very weak base with pKa-3.63. Indole and its derivatives are reported to display diverse of physical and chemical properties in addition to their interesting chemical reactions as delineated below.

1.2.1.1 Reaction with acids

Indole being weak base reacts with dilute acids to form a β-protonated 3*H*-indolium cation **8**. While in concentrated acidic solution proton adds at both 1- and 2-positions to form cations **9** and **10** (Figure 1.3).[9]

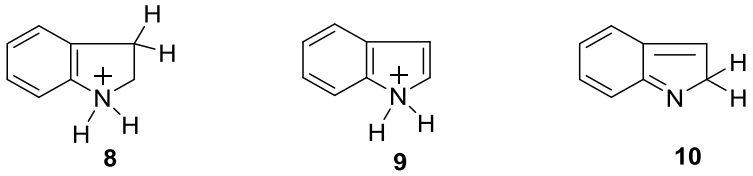
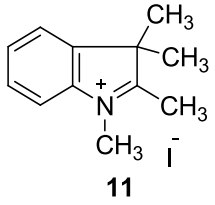


Figure 1.3 Reactivity of indole in weak and strong acids

1.2.1.2 Alkylation

Indole does not react with alkyl halides due to the involvement of nitrogen lone-pair in aromatic sextet. Indole itself react with methyl iodide in DMF at 100 °C to form 1,2,3,3-tetramethyl-3*H*-indolium iodide (**11**).



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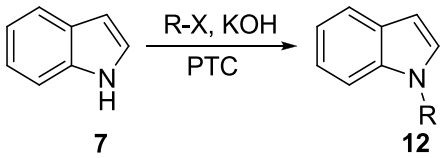


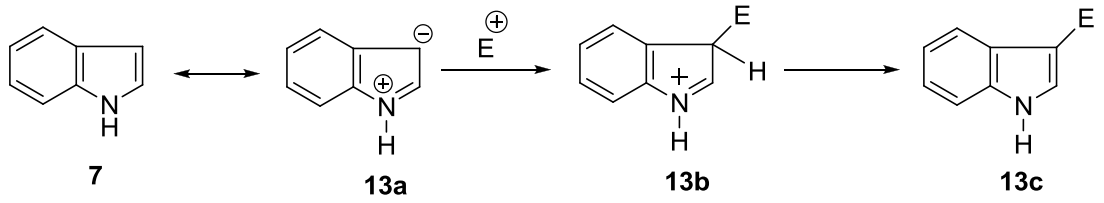
7 **12**

Scheme 1.1 Alkylation of indole (**7**)

Alkylation of indole *N*-H, requires bases such as sodium amide, potassium hydroxide (Scheme 1.1). In a biphasic system of sodium hydroxide and benzene, alkylation of indoles could be easily achieved in the presence of a phase transfer catalyst by employing an appropriate alkylating agent.[9]

1.2.1.3 Electrophilic substitution

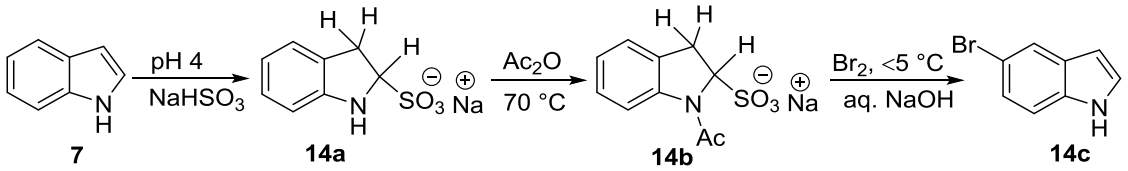
π-Excessive aromatic heterocycle indole (**7**) is highly reactive in classical electrophilic substitution reactions such as protonation, halogenation, alkylation and acylation. The C-3 position is the most reactive site on the unsubstituted ring, by virtue of its increased electron density and the greater stability of resulting intermediate **13a** when compared to **13b** (Scheme 1.2).[9]



Scheme 1.2 Electrophilic substitution of indole (**7**)

1.2.1.4 Reactions of *β*-protonated indoles

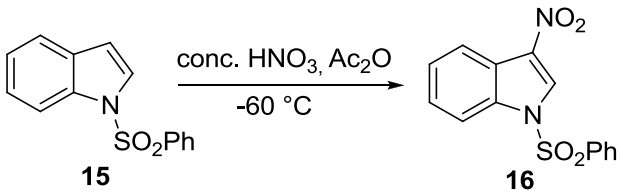
3*H*-Indolium cation (**14a**) are more electrophilic species compared to neutral indole. For example, the 3*H*-indolium cation itself react with bisulfite at pH 4, under condition that led to sodium salt of indoline-2-sulfonic acid (**14a**). The salt **14a** converts into **7** on dissolving in water, however, the salt **14a** can be *N*-acetylated **14b**, further which can be used for C-5 halogenation or nitration. Finally hydrolysis with loss of bisulfite led to 5-substituted indole (**14c**) as shown in Scheme 1.3.[10]



Scheme 1.3 Reaction of *β*-protonated indole

1.2.1.5 Nitration

Generally, nitration of indole requires a mixture of HNO_3 and H_2SO_4. For example, nitration of **15** using nitrating mixture (HNO_3 and H_2SO_4) led to intractable products, probably due to acid catalyzed polymerization of indole.

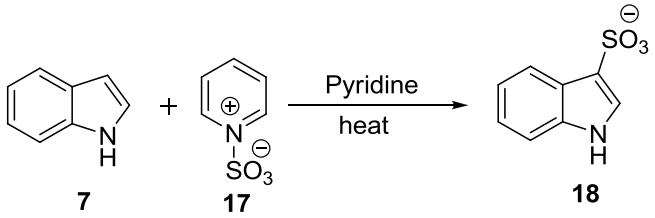


Scheme 1.4 Nitration of indole **15**

This polymerization can be avoided by performing the nitration reaction of *N*-alkylindole in concentrated nitric acid and acetic anhydride at low temperature (Scheme 1.4). However, this reaction fails with indole itself.[11]

1.2.1.6 Sulfonation

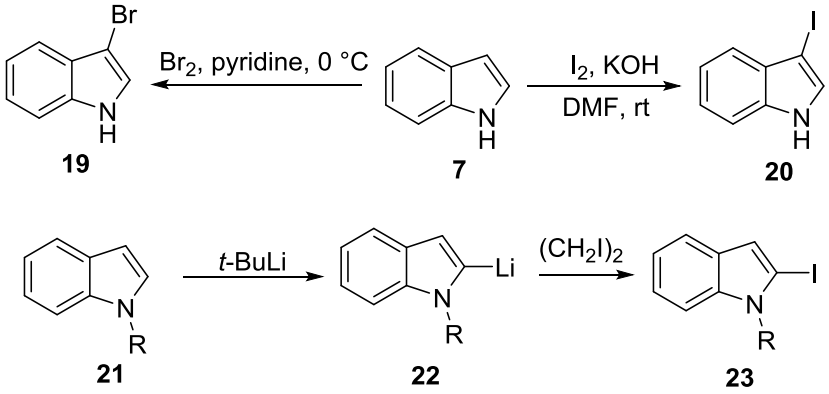
Sulfonation of indole (**7**) can be achieved by the pyridine-sulfur trioxide complex **17** in hot pyridine at C-3 position as depicted in Scheme 1.5.[12]



Scheme 1.5 Sulfonation of indole (**7**)

1.2.1.7 Halogenation

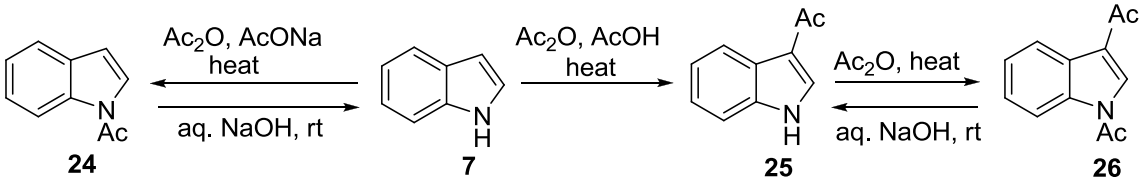
There are several reports pertaining to the *β*-halogenation of indole (**7**). Reaction of **7** with bromine or iodine in the presence of potassium hydroxide in dimethylformamide affords 3-bromo/iodo-indoles (**19-20**) in good yields.[13] On the other hand, 2-bromo-/iodoindoles **23** can be prepared *via* *α*-lithiation of *N*-protected indole **22** followed by treatment with appropriate halogenated reagent (Scheme 1.6).[14]



Scheme 1.6 Synthesis of halogenated indoles

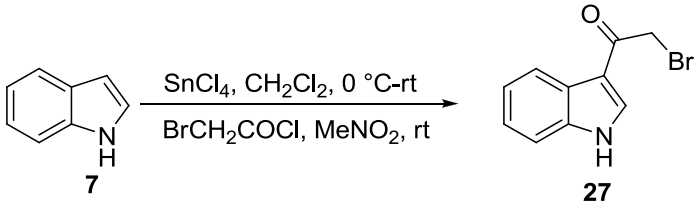
1.2.1.8 Acylation

Indole can react with appreciable rate with acetic anhydride alone above 140 °C to produce 1,3-diacetylindole (**26**) along with smaller amounts of *N*- and 3-acetylindoles (**24** and **25**) which upon alkaline hydrolysis led to 3-acetylindoles (**25**). In acetylation reaction, *β*-attack occurs first to produce 3-acetylindole (**25**) and then easy conversion of **25** to **26**. On the other hand, acetylation of indole in the presence of sodium acetate or 4-dimethylaminopyridine (DMAP) affords exclusively *N*-acetylindole probably through indolyl anion. *N*-Acylindoles are easily hydrolyzed in aqueous sodium hydroxide at room temperature (Scheme 1.7).[15]



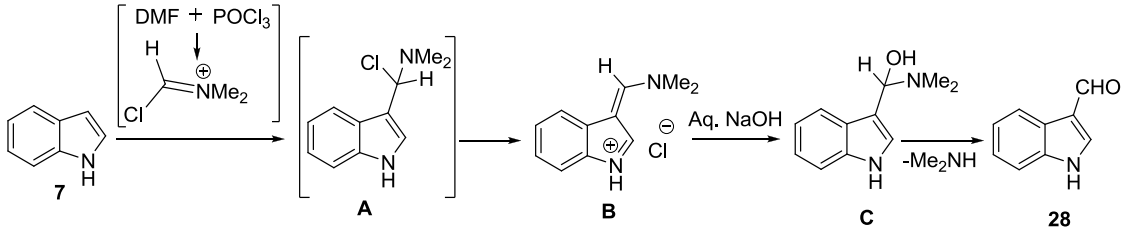
Scheme 1.7 Acylation of indole (**7**)

The Lewis acid catalyzed Friedel-Craft acylation must be carried out with care to avoid oligomerization of indole. Formation of α-bromoacetylindole (**27**) involves the reaction of **7** with tin(IV) chloride and followed by addition of acid chloride or anhydride (Scheme 1.8).[16]



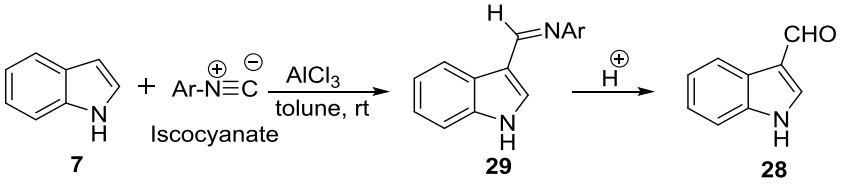
Scheme 1.8 Synthesis of α-bromoacetylindole (**27**)

The Vilsmeier-Haack reaction (Scheme 1.9) is a very efficient method for the synthesis of 3-formylindole (**28**) in good yield.[17]



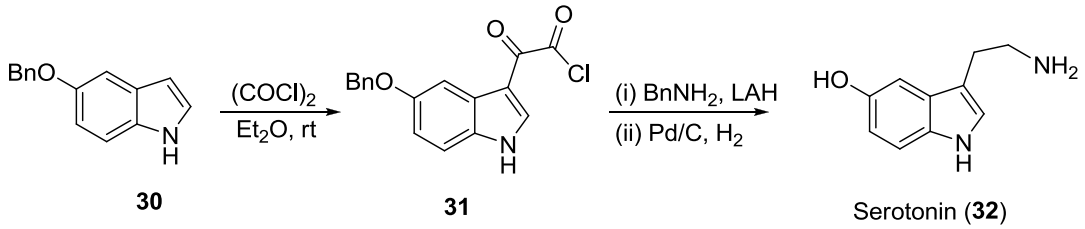
Scheme 1.9 Synthesis of 3-formylindole (**28**)

Alternatively, 3-formylindole (**28**) can be prepared (Scheme 1.10) from the reaction of isocyanates and indole (**7**) in the presence of aluminium chloride to produce imine **29** which upon hydrolysis led to 3-formylindole (**28**) in good yield.[18]



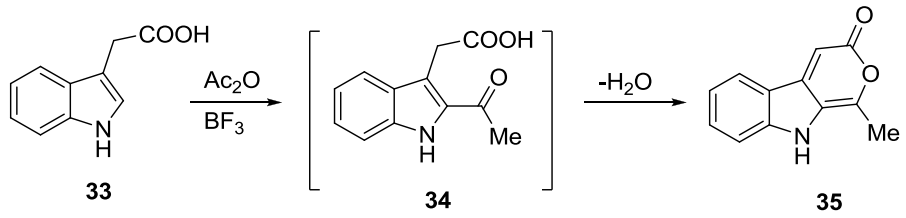
Scheme 1.10 Synthesis of 3-formylindole (**28**) using isocyanates

Another example of acylation reaction (Scheme 1.11) involves the reaction of 5-benzyloxy indole (**30**) with oxalyl chloride to obtain indole-3-glyoxylyl chloride (**31**) in very high yield. Intermediate **31** can be converted into various useful compounds like tryptamine or serotonin (**32**).[19]



Scheme 1.11 Synthesis of serotonin (**32**)

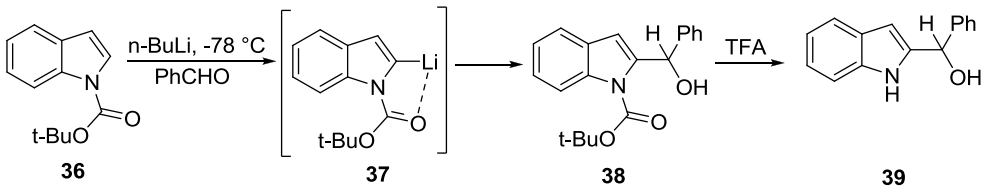
Reaction of indole acetic acid (**33**) with acetic anhydride in the presence of Lewis acid led to 2-acylated product **34**. Subsequent dehydration of **34** afforded enol-lactone **35** (Scheme 1.12).[20]



Scheme 1.12 Preparation of enol-lactone **35**

1.2.1.9 C-Metallation of indoles and their synthetic applications

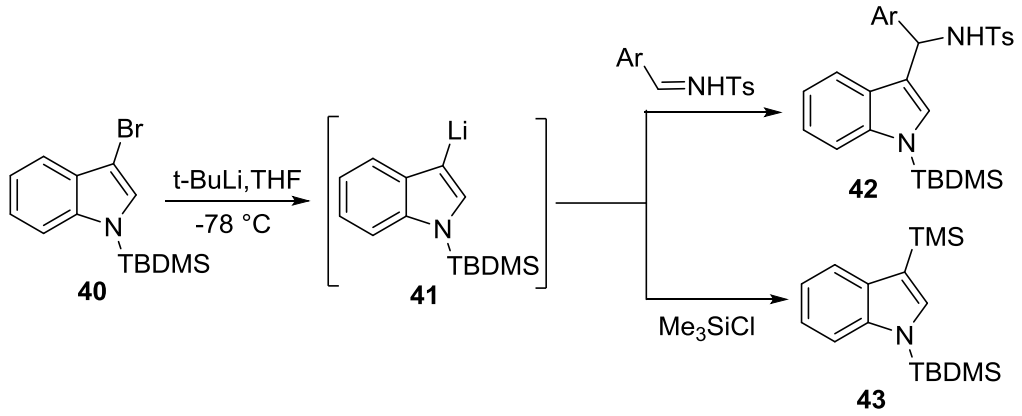
C-Metallation of indoles generally performed in the presence of *N*-substituents like methyl or removable groups like benzene sulfonyl, acetyl, *t*-butoxycarbonyl and methoxymethyl. Each of these removable groups assist metallation by intramolecular chelation as shown in Scheme 1.13. Further, C-metallated intermediate could be used for the synthesis of functionalized indoles.[21]



Scheme 1.13 C-Metallation of indole **36** and its functionalizations

1.2.1.10 Metal-halogen exchange reaction

3-Lithio-indole (**41**) can be prepared by halogen exchange reaction of 3-haloindole **40** with *t*-butyl lithium at -78 °C. Further, this lithiated indole **41** can be converted into functionalized indoles **42** and **43** by reacting with various electrophiles (Scheme 1.14).[22]



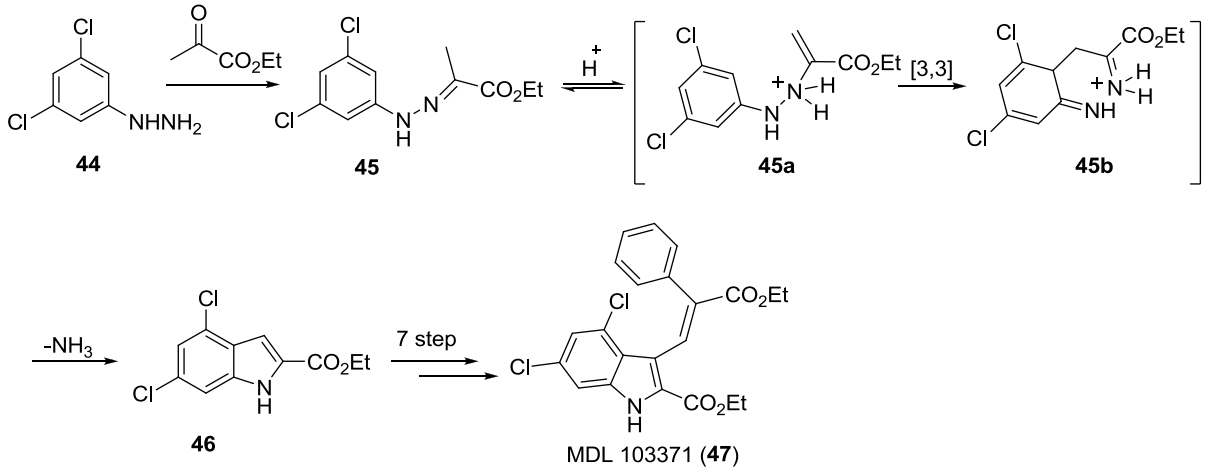
Scheme 1.14 Functionalizations of indole through metal-halogen exchange reaction

1.2.2 Synthesis of indoles

Indole nucleus is an important structural component that is embedded in a large number of biologically active natural and synthetic compounds. By considering the importance of indole unit, its synthesis has been the object of research for over 100 years and reports of new routes for the construction of indoles appears frequently.[23] Following well-established classical methods are now available for synthesis of indoles.

1.2.2.1 Fisher indole synthesis

Fisher indole synthesis was discovered by Hermann Emil Fischer and Jourdan in 1883 from the treatment of pyruvic acid 1-methylphenylhydrazone with alcoholic hydrogen chloride.[24] The Fischer reaction gives a simple and efficient method for the synthesis of indoles from enolizable *N*-arylhydrazones. In many cases, synthesis of indoles can be achieved by simply heating the ketone or aldehyde and arylhydrazine (**44**) in the presence of an acid catalyst without isolation of the hydrazone intermediate. Advantage of this reaction include the tolerance of various functional groups on the aromatic ring to form the new C-C and C-N bonds. Mechanism of Fischer indole synthesis involve a [3,3] sigmatropic rearrangement of an ene-hydrazine tautomer **45a** to bisiminobenzylketone **45b** (Scheme 1.15).

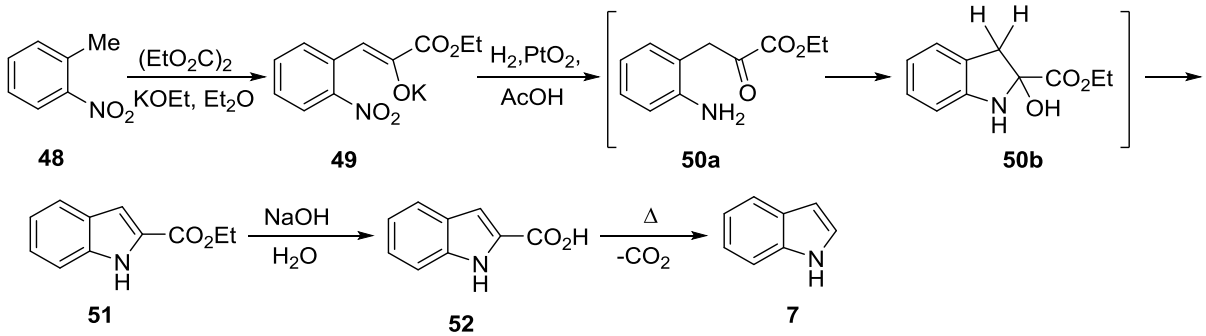


Scheme 1.15 Preparation of MDL 103371 (**47**) using Fisher indole synthesis

Later cyclization and aromatization with loss of ammonia gives the indole **46** which is a key intermediate in the synthesis of *N*-methyl-D-aspartate (NMDA)-type glycine receptor antagonist MDL 103371 (**47**); used for the potential treatment of stroke.[23] Industrial applications of Fischer synthesis have been demonstrated in the construction of an indole ring in the generic drugs namely, Almotriptan, Sumatriptan, Avitriptan, Indomethacin and Nosiheptide.[23]

1.2.2.2 Reissert synthesis

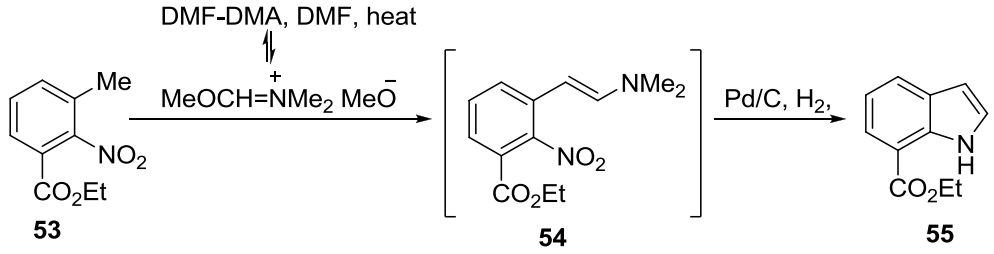
Reissert indole synthesis involves condensation of *o*-nitrotoluene (**48**) with diethyl oxalate to give ethyl *o*-nitrophenylpyruvate (**49**). Subsequent reductive cyclization of **49** with platinum catalyst led to indole-2-carboxylic acid ester **51** which upon hydrolysis produces indole-2-carboxylic acid (**52**). Finally, decarboxylation of **52** yielded indole **7** as depicted in Scheme 1.16.[25]



Scheme 1.16 Reissert indole synthesis

1.2.2.3 Leimgruber-Batcho synthesis

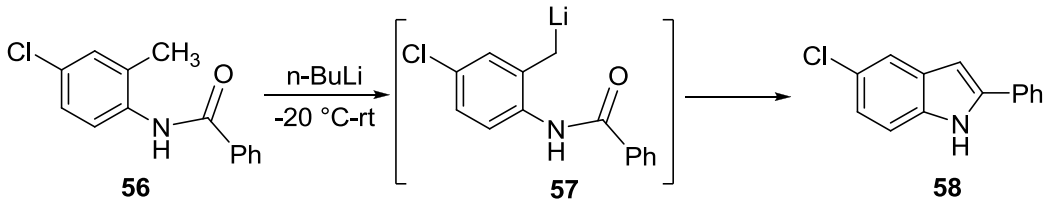
Leimgruber-Batcho synthesis is most commonly used method for the preparation of 2,3-unsubstituted indoles as shown in Scheme 1.17. Condensation of substituted *o*-nitrotolune (**53**) with hot *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) led to an enamine **54**. Subsequent, reduction of enamine **54** using Pd/C to produce indole **55**.[26]



Scheme 1.17 Leimgruber-Batcho synthesis of indole **55**

1.2.2.4 Madelung synthesis

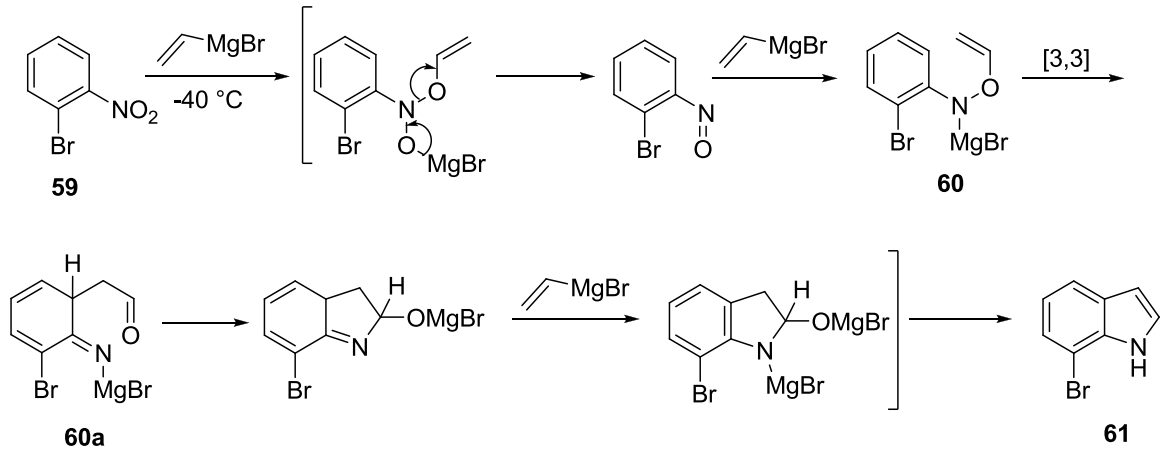
Madelung synthesis was reported in 1912 by Walter Madelung, when he observed that 2-phenylindole (**58**) was prepared from *N*-benzoyl-*o*-toluidine (**56**) by heating in the presence of sodium ethoxide. Madelung synthesis is important because it is one of few known reactions to produce indoles from a base-catalyzed thermal cyclization of *N*-acyl-*o*-toluidine **56**. Also, this reaction can be performed at low temperature using strong base, *n*-BuLi to afforded lithiated product **57**, which upon internal cyclization can furnish 2-phenylindole **58** (Scheme 1.18).[27]



Scheme 1.18 Madelung synthesis of indole

1.2.2.5 Bartoli indole synthesis

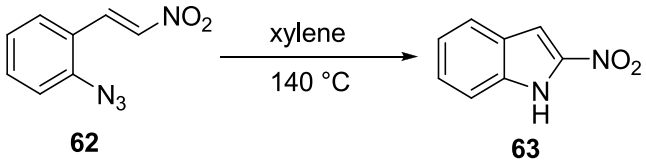
This protocol involves the reaction of *o*-substituted nitrobenzene (**59**) with three equivalents of vinylmagnesium bromide to produce 7-bromoindole (**61**). Formation of indole involves the [3,3]-sigmatropic rearrangement of **60**, and finally hetero ring closure of intermediate **60a** led to **61** as shown in Scheme 1.19.[28]



Scheme 1.19 Bartoli Synthesis of bromoindole (**61**)

1.2.2.6 Sundberg indole synthesis

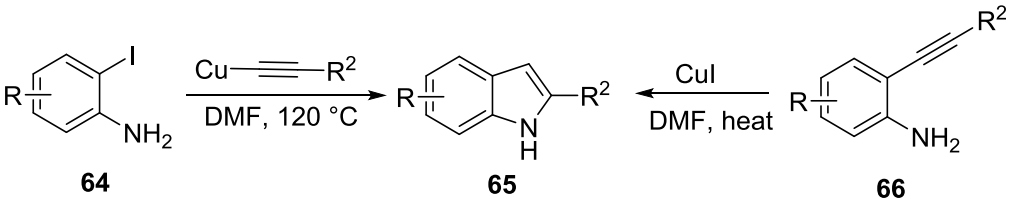
Sundberg indole synthesis involves thermolysis of 2-azidonitrostyrene (**62**) in xylene at 140 °C for 12 hours to produce 2-nitroindole (**63**) in good yield (Scheme 1.20).[29]



Scheme 1.20 Synthesis of 2-nitroindole (**63**)

1.2.2.7 Castro indole synthesis

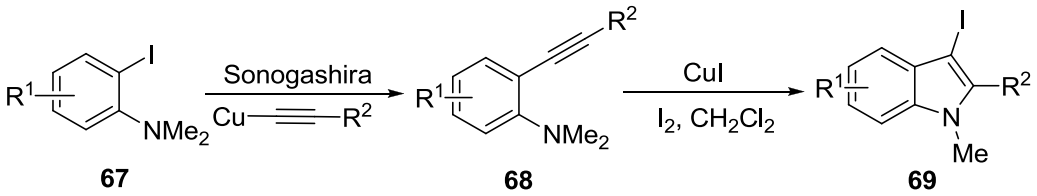
Castro indole synthesis formally involves the cyclization of either *o*-iodoaniline derivatives **64** with cuprous acetylides or 2-alkynylanilines **66** with copper(I) iodide as shown in Scheme 1.21.



Scheme 1.21 Copper-promoted Castro indole synthesis

Synthesis of indole **65** using cuprous acetylides are rare and it is difficult at gram scale, however, Cu-promoted cyclizations of 2-alkynylanilines **66** have received considerable attention as an attractive method for the construction of 2-alkylindoles **65**.[23] Yue and Larock have also reported the synthesis of *N*-methyl-3-iodoindoles (**69**) from iodine-mediated electrophilic cyclization of 2-alkynyldimethylanilines (**68**) which were prepared by the

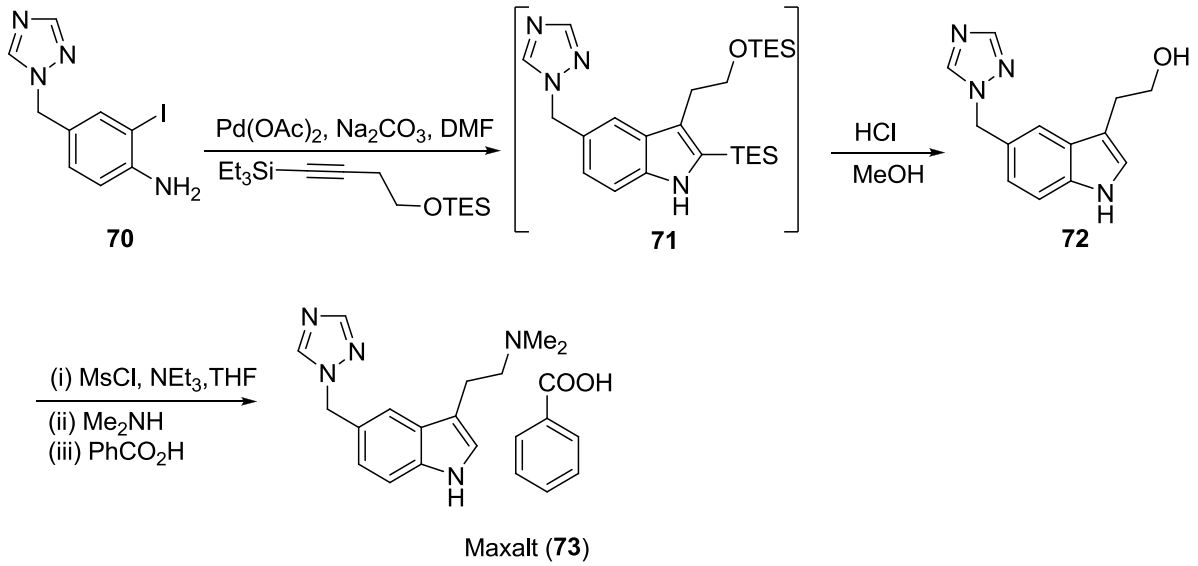
Sonogashira coupling reaction of 2-iodo-*N,N*-dimethylanilines (**67**) with various alkynes (Scheme 1.22).[23]



Scheme 1.22 Synthesis of 3-iodoindoles **69**

1.2.2.8 Larock Heteroannulation

Larock heteroannulation is one of the attractive methods for the synthesis of 2,3-disubstituted indoles. This reaction involves the Pd-catalyzed reaction of alkyne with *o*-iodoaniline (**70**). Chen *et al.* utilized this methodology for the synthesis of antimigraine drug, Maxalt **73**. Heteroannulation reaction of *o*-iodoaniline (**70**) and alkyne in the presence of palladium acetate give indole **71**, which on removal of TES group and reaction with dimethylamine produced desired drug Maxalt (**73**) as illustrated in Scheme 1.23.[30]



Scheme 1.23 Synthesis of Maxalt (**73**) by heteroannulation reaction

1.3 Cancer and its treatment

Cancer is a group of diseases characterized by the uncontrolled, rapid and pathological proliferation of abnormal cells, is one of the most formidable afflictions in the world. If the spread of uncontrolled cell is not controlled, it can result in death. In 2012, the worldwide burden of cancer rose to an estimated 14 million new cases per year, a figure expected to rise

to 22 million annually within the next two decades. Over the same period, cancer deaths are predicted to rise from an estimated 8.2 million annually to 13 million per year.[31] Globally, in 2012 the most common cancers diagnosed were those of the lung (1.8 million cases, 13.0% of the total), breast (1.7 million cases, 11.9%), liver (0.8 million, 9.1%), stomach (0.7 million, 8.8%).and large bowel (1.4 million cases, 9.7%). Generally, cancer caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (mutations that occur from metabolism, inherited mutations, immune conditions and hormones). Either these factors may be act together or in sequence to start or promote the growth of cancer. Cancer patients can be cure by treated with surgery, radiation, chemotherapy, hormone therapy, immune therapy, and targeted 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 cancer therapies are drugs or other substances that stop the growth and spread of cancer cells by interfering with specific molecules ("molecular targets") that are involved in the progression, growth, spread of cancer as well as the signals that cause cancer cells to die naturally.[32]

1.3.1 Chemotherapy

Chemotherapy is the use of drugs or chemical entities to kill cancer cells. It works by stop the cancer cells from growing and dividing to make more cells. Because cancer cells usually grow and divide faster than healthy cells, chemotherapy kills them faster than it healthy cells. Since chemotherapy drugs/agents are powerful, they cause damage to many growing cells, including some healthy cells. Due to this damage of cells causes the side effects of chemotherapy.[33]

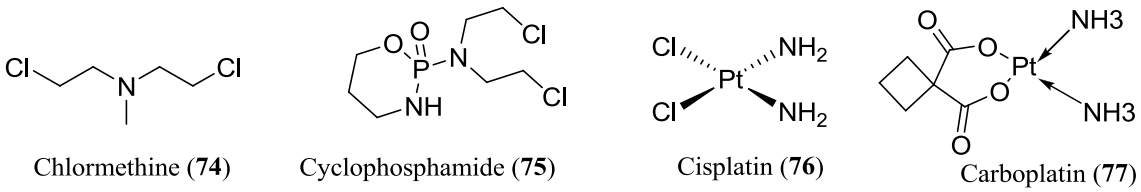
1.3.2 Types of chemotherapy drugs

Chemotherapy drugs can be divided into several groups based on various factors such as how they affect chemical substances within the cancer cell, what part of the cell cycle the drug affects, chemical structure and their relationship to another drug. Chemotherapy can be divided into following groups.

1.3.2.1 Alkylating agents

Alkylating agents were one of the earliest classes of drugs used in the treatment of cancer. The biggest weakness of most cancer cells is that they are very sensitive to DNA damage. Alkylating agents work by reacting with the proteins that bound together to form the very delicate double helix structure of a DNA molecule, adding an alkyl group to some or all of them. This structure of DNA prevents the proteins from linking up as they should and causing breakage of the DNA strands and ultimately the death of the cancer cells. These drugs will kill cells in all phases of the cell cycle.[34]

Chlormethine (**74**), also known as nitrogen mustard, is sold under the brand name Mustargen. This alkylating agent works by binding to DNA, crosslinking two strands and preventing cell multiplication. Chlormethine (**74**) binds to the *N*-7 nitrogen on the DNA base guanine. Cyclophosphamide (**75**) is nitrogen mustard from oxazaphosphorine group works like Chlormethine.



Chlormethine (**74**) Cyclophosphamide (**75**) Cisplatin (**76**) Carboplatin (**77**)

Figure 1.4 Representative alkylating agents as anticancer drugs **74-77**

Generally, Cyclophosphamide (**75**) is used to treat cancers including lymphomas, some forms of brain cancer, leukemia and some solid tumors. It is a chemotherapy drug that works by inducing the death of certain T cells. Cisplatin (**76**) and Carboplatin (**77**) are the member of a class of platinum-containing anticancer drugs (Figure 1.4). These platinum complexes react *in vivo*, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis. It is used to treat various types of cancers, including small cell lung cancer, ovarian cancer, lymphomas, bladder cancer, cervical cancer and germ cell tumors. Cisplatin is particularly effective against testicular cancer; the cure rate was improved from 10% to 85%.[35]

1.3.2.2 Antimetabolites

Antimetabolites generally interfere with a cell's RNA and DNA. These agents damage cells during the S-phase, when the cell's chromosomes are being copied. They are commonly used to treat leukemias, breast, ovary and the intestinal tract as well as other types of cancer.

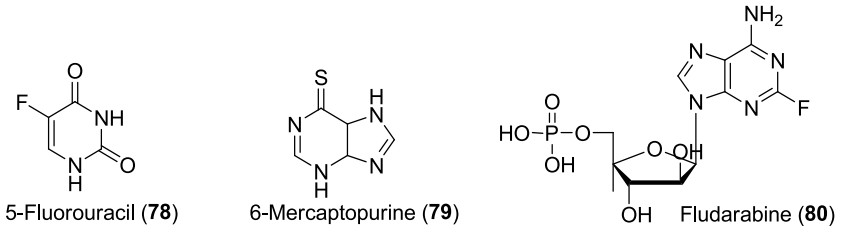
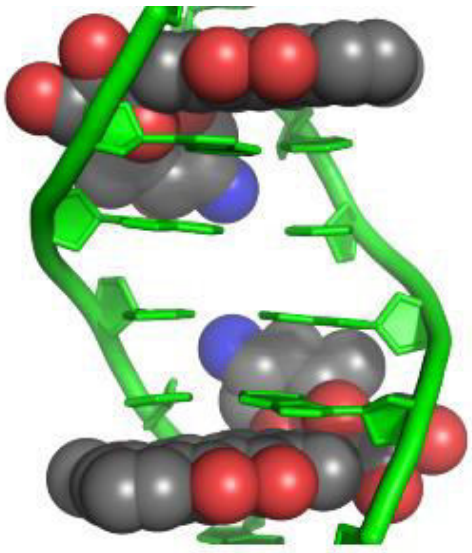


Figure 1.5 Examples of antimetabolites as anticancer agents **78-80**

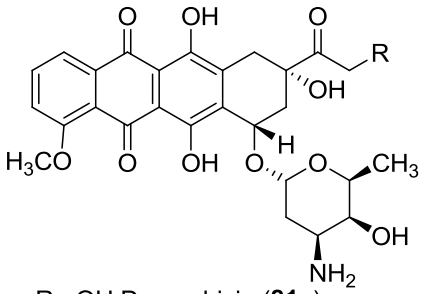
5-Fluorouracil (**78**) is the generic name for the drug Adrucil®. It is used for the treatment of various cancers such as colon, rectal, anal, breast, cervical and bladder. 6-Mercaptopurine (**79**) also sold under the trade name of Purinethol®. It is used in the treatment of acute lymphoblastic leukemia. Fludarabine (**80**) inhibits DNA synthesis by interfering with ribonucleotide reductase and DNA polymerase (Figure 1.5). It is active against both dividing and resting cells. Fludarabine used in the treatment of hematological malignancies (cancers of blood cells such as leukemias and lymphomas).[36]

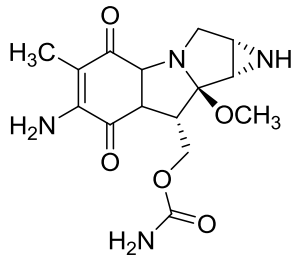
1.3.2.3 Anticancer antibiotics

These are natural products that are produced from Streptomyces bacteria. They interfere with nucleic acid synthesis/function and inhibit DNA/RNA synthesis. Anthracyclines (Doxorubicin, Daunorubicin), Bleomysin, Dactinomycin and Mitomycin are the classes of drugs that come under this category (Figure 1.6).[37]



Mechanism of action of doxorubicin[37]





Mitomycin C (**82**)

Figure 1.6 Anticancer antibiotics **81** and **82**

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) and pediatric tumors. Tetracycline rings (Doxorubicin **81a** and Daunorubicin **81b**) are DNA intercalating agents and inhibit the progression of topoisomerase II and block the synthesis of DNA and RNA (Figure 1.6). These drugs are used in acute leukemias, lymphoma and a number of solid tumors. Mitomycin C (**82**) is an aziridine containing natural product that is used in the treatment of upper gastro-intestinal (e.g. esophageal carcinoma), anal and breast cancers.[38]

1.3.2.4 Topoisomerase inhibitors

Topoisomerase is one of the important targets in anticancer drug discovery. Topoisomerase inhibitors are designed to interfere with the action of topoisomerase enzymes (topoisomerase I and II), which control the changes in DNA structure by catalyzing, breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. The typical way that both topoisomerase I and II inhibitors work is that the inhibitor binds to the topoisomerase molecule and it makes the enzyme nonfunctional by blocking the ability of the topoisomerase to bind the DNA back together after it has been cut. Therefore, cuts are made to either one or both strands of the DNA molecule which are never repaired, ultimately leading to death of the cell.

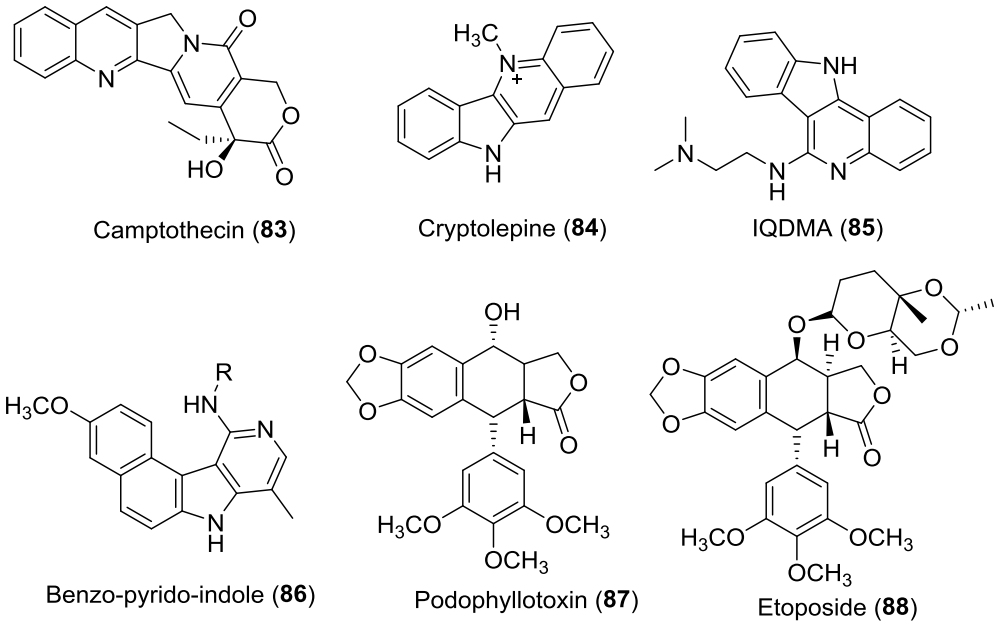


Figure 1.7 Structures of topoisomerase inhibitors **83-88**

Camptothecin and its derivatives are some of the well known inhibitors of topoisomerase I. Topoisomerase II inhibitors include doxorubicin, etoposides and mitoxantrone. Camptothecin (CPT) (**83**) was isolated in 1966 by M. E. Wall and M. C. Wani in systematic screening of natural products for anticancer drugs from the bark and stem of *Camptotheca acuminata* (Camptotheca, Happy tree). It has been demonstrated to be effective against a broad spectrum of tumors. Their molecular target has been firmly established to be human DNA topoisomerase I (topo I).[39] Cryptolepine (**84**) hydrochloride is an indoloquinoline alkaloid isolated from the roots of *Cryptolepis sanguinolenta*. It is intercalate into DNA and inhibit topoisomerase II as well as DNA synthesis.[40] Synthetic analogues of Cryptolepine such as IQDMA (**85**) and compound (**86**) exhibited potent anticancer activity *via* interaction of DNA.[41] Podophyllotoxin (PPT) (**87**) known as podofilox, is a non-alkaloid toxin lignan extracted from the roots and rhizomes of *Podophyllum* species endowed with potent anticancer activity. C-4-Modified Podophyllotoxin analogues named Etoposide (**88**), showed the improved activity against lung, testicular, lymphoma non lymphocytic leukemia and glioblastoma cancers (Figure 1.7).[42]

1.3.2.5 Antimitotic agents

Antimitotic agents have been the most successful pharmacological agents for the treatment of cancer. The term "antimitotic agent" has traditionally been synonymous with tubulin-targeting compounds. The vast majority of these molecules act by binding to the protein tubulin, an α, β-heterodimer that forms the core of the microtubule. Microtubules (MTs) are tubular polymers and are major cytoskeletal components in eukaryotic cells. The dynamic and mutual interconversion of tubulins and microtubules is responsible for the variety of cellular functions including maintaining the cellular structure, providing an intracellular rail-like transport platform for the motor proteins kinesin and dynein and enabling cell division through spindle formation during mitosis, which pulls apart the eukaryotic chromosomes. Due to important roles in cellular process, microtubules are attractive molecular targets in cancer chemotherapy. MTs disturbs not only mitosis but also arrest the cells during interphase. MTs are categorized accordingly to their binding sites (Figure 1.8) like laulimalide, taxane/epothilone, vinca alkaloid and colchicine sites.[43] The Vinca alkaloids have been used medicinally since the 1600s; however, their anti-proliferative activity was not explored until the late 1950. Vinca alkaloids, including vinblastine (**89a**), vincristine (**89b**), vinorelbine (**90a**) and vinflunine (**90b**) promote the inhibition of tubulin polymerization.

They generally bind with high affinity to one or a few tubulin molecules at the tip of microtubules but do not copolymerize into microtubules.

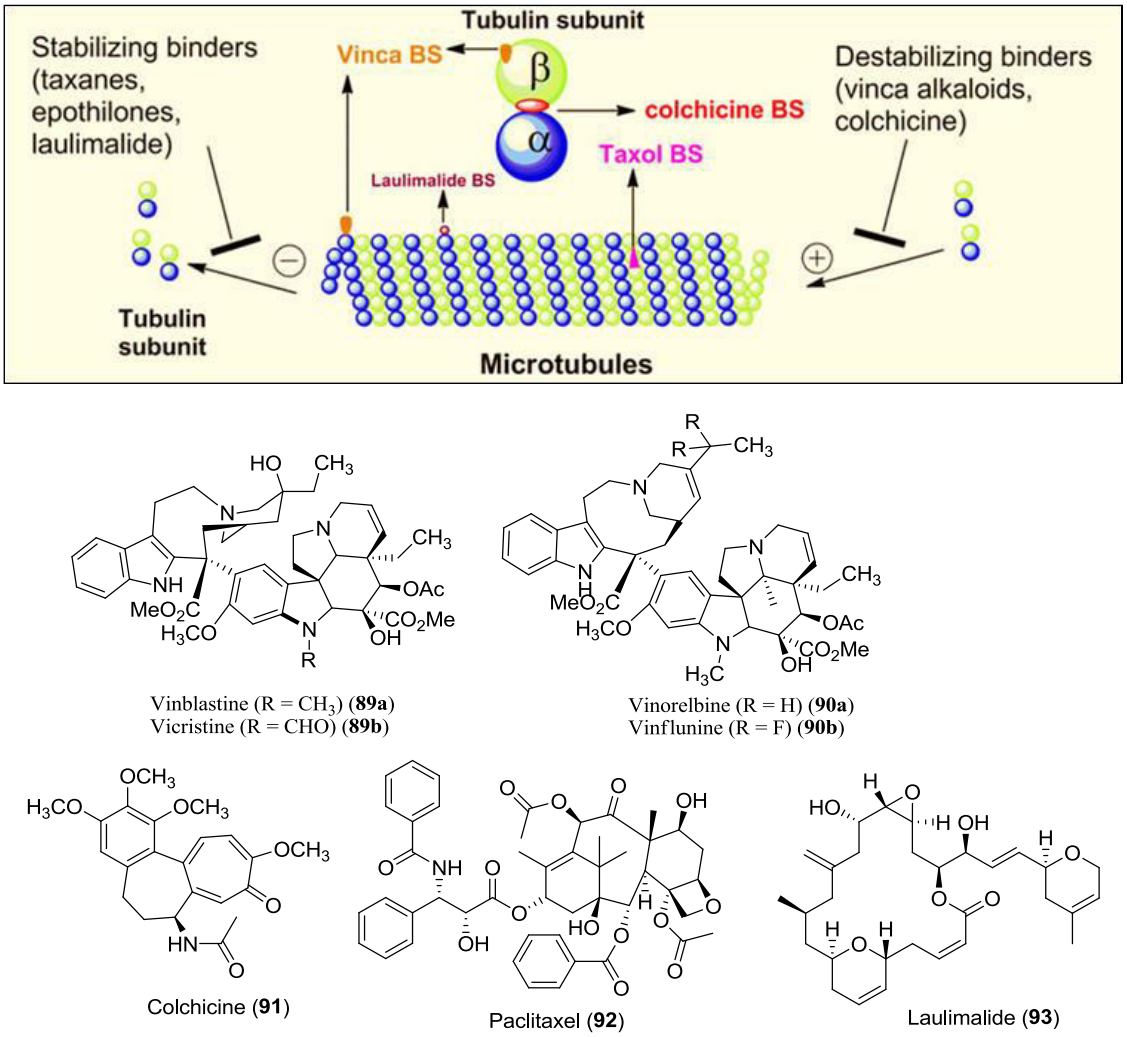


Figure 1.8 Binding sites of tubulin targeting agents[43]

Indeed, vinblastine prevents self-association of tubulin by interacting at the interface between two *α*, *β*–tubulin heterodimers. The second group of microtubule interfering agents is colchicines (**91**), which also induce microtubule depolymerization. Colchicine (**91**) binds with high affinity to tubulin than any other tubulin targeting agents that can become co-polymerized into microtubules. Colchicine binding to *β*-tubulin results in curved tubulin dimer and prevents it to adopt a straight structure, due to a steric clash between colchicine and *α*-tubulin, which inhibits microtubule assembly.[44] Both Paclitaxel (**92**) and Laulimalide (**93**) can promote the tubulin polymerizations, but they binds at different site on the microtubules. Taxanes, including paclitaxel and docetaxel, bind to the inner surface of the *β*-subunit at polymerized microtubules and are widely used in the treatment of various cancers

including, breast, lung, ovarian and bladder cancers. Paclitaxel (**92**) was the first identified microtubule-stabilizing agents and was FDA approved in 1992.[45]

1.4 Indole nucleus in anticancer research

Indole nucleus plays a vital role in anticancer drug discovery research. Many of the natural as well as synthetic indole analogues are found to be potent anticancer agents. In the late 1950s, vinca alkaloids were isolated from the periwinkle plant *Catharanthus roseus* and it was a milestone in the development of cancer chemotherapy. Many researchers were motivated from this achievement of vinblastine and vincristine (vinca alkaloids) and developed the indole-based new anticancer chemical entities. Indole containing anticancer molecules has shown their anticancer properties through diverse mechanism of actions. 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 can be categorized into three groups based on their structural features.

(a) Functionalized indoles

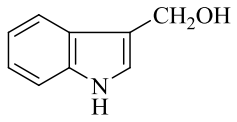
(b) Indolylazoles

(c) Bisindoles

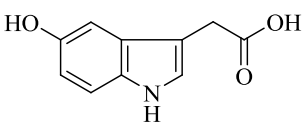
1.4.1 Functionalized indoles

Various substituted functional groups on indole ring and their anticancer potential are described under this category (Figure 1.9). Indole-3-carbinol (**94**) is a simple compound isolated from cruciferous vegetables (broccoli, cabbage, cauliflower, radish and sprouts). Compound **94** shows better *in vitro* efficacy against breast, cervical, prostate, colon and leukemia cancer.[46] Moreover, it is reported that indole-3-carbinol induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells.[47] It is also known that compound **94** stimulate detoxification enzymes in the gut and liver. One placebo controlled trial shows that indole-3-carbinol (**94**) is effective in treating precancerous cervical dysplasia. It is generally well effective when taken orally.[48] An indole derivative 5-hydroxyindoleacetic acid (**95**), is the crucial metabolite of serotonin in the human body. In 2011, Jeong *et al.* demonstrated that ultraviolet B (UVB)-activated 5-hydroxyindoleacetic acid (5-HIAA^{UVB}) induces apoptosis in prostate and bladder cancer cells through the stress signalling and

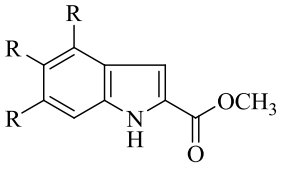
apoptotic pathway. Subsequently, this achievement provides a possible candidate for a novel photosensitizer in photodynamic therapy (PDT).[49]



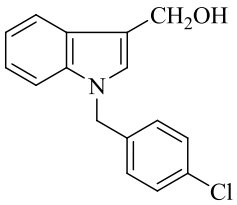
Indole-3-carbinol (**94**)



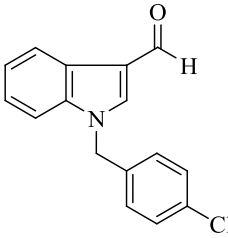
5-Hydoxyindole-3-acetic acid (**95**)



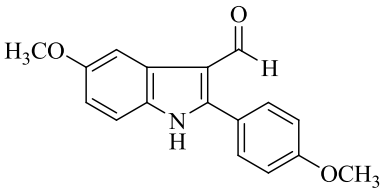
Indole-2-carboxylate (**96**)



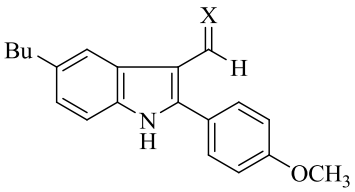
NSC-741909 (**97**)



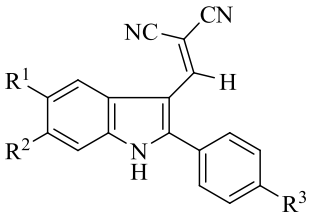
Oncrasin-1 (**98**)



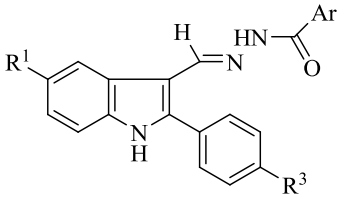
Indole-2-phenyl-3-carboxaldehyde (**99**)



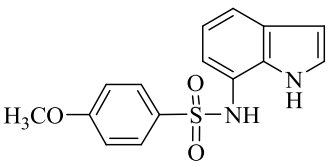
X = NCH_3 (**100a**)
X = NOH (**100b**)



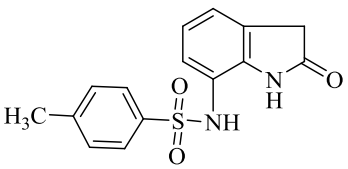
[(2-Phenylindol-3-yl)methylene] propanedinitriles (**101**)



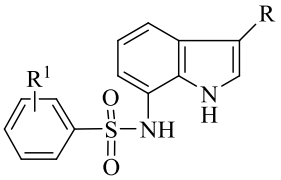
Aroyl hydrazones of 2-phenyl-indole-3-carbaldehydes (**102**)



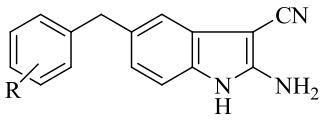
Indole-7-sulfonamide (**103**)



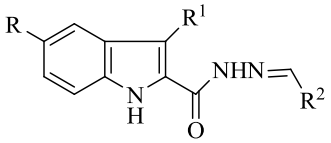
ER-67865 (**104**)



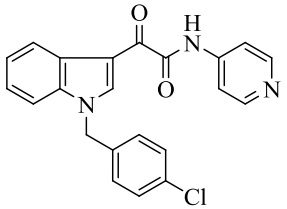
(E7070) R = Cl, R^1 = 4-SO_2NH_2 (**105a**)
(ER68487) R = Cl, R^1 = 4-CN (**105b**)



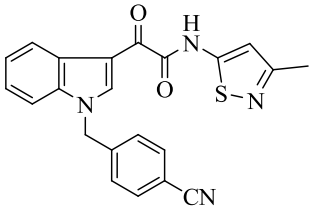
2-Amino-3-cyanoindole (**106**)



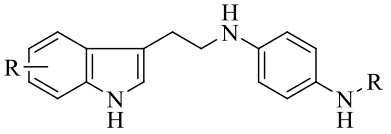
Indolylhydrazide-hydrazone (**107**)



Indibulin (**108**)



Indolyl glyoxylamide (**109**)



Tryptamine derivatives (**110**)

Figure 1.9 Functionalized indoles as anticancer agents **94-110**

Recently, Li. *et al.* prepared a new series of indole-2-carboxylate derivatives (**96**) and access for their *in vitro* antiproliferative activity against a panel of cancer cell lines (best compound IC_{50} = 1.76-24 μM). Further biological assay showed that this compound increased reactive oxygen species (ROS) generation dose-dependently and induced poly ADP ribose polymerase (PARP) cleavage in A549 cells, also this compound found to be arrested cell cycle in G2/M phase.[50] Indole-3-carbinol derivative NSC171909 (**97**) is a potent anticancer agent highly active against various NCI-60 cancer cell lines.[51] Compound **97** induces sustained activation of mitogen-activated protein kinases (MAPK), including JNK and p38 MAP kinases.[52] Oncrasin-1 (**98**) is a novel anticancer agent that was identified through synthetic lethality screening on isogenic human ovarian epithelial cells with or without oncogenic Ras expression.[53] Angerer and coworkers reported that methoxy-substituted 3-formyl-2-phenylindoles (**99**) found to be active against breast cancer cell lines (IC_{50} = 35 nM; MDA-MB-231), similarly this compound disrupted microtubule assembly with IC_{50} = 1.5 μM. By fluorescence microscopy study it was confirmed that compound **99** degrades the cytoskeleton similar to colchicine.[54] *In vivo* activity results of these compounds were not satisfactory most probably due to metabolically unstable nature of aldehyde functional group. In 2007, same group modify aldehydic functional group by converting in to the methyl imines **100a** and oximes **100b**. The methyl imine analogue **100a** was found to be prodrug by releasing aldehyde by hydrolysis. On the other hand, oxime **100b** was found to be stable but exhibited less activity compare to parent aldehyde **99**.[55] In the search of stable aldehyde derivative, same author prepared (2-phenylindol-3-yl)methylenepropanedinitriles (**101**) by Knoevenagel condensation of 2-aryl-3-formylindole with malononitrile. The resulting analogues endowed with similar SAR and inhibited the growth of breast (MDA-MB-231 and MCF-7) cancer cells with $IC_{50} < 100$ nM. These compounds failed to bind tubulin assembly but capable of cell cycle arrest in G2/M phase. The new analogues 2-phenylindole derivatives (**101**) also inhibited the growth of transplanted MXT mouse mammary tumors.[56] Further, 2-phenyl-3-formyl indoles were modified to aroyl hydrazones of 2-phenylindole-3-carbaldehydes (**102**) by condensation of 2-phenyl-3-formyl indole with various hydrazides inhibited the growth of breast (MDA-MB 231 and MCF-7) cancer cells (IC_{50} ~ 20–30 nM for the most potent derivatives). Similarly, it did not inhibit tubulin polymerization like aldehydes but were capable of blocking the cell cycle in G2/M phase.[57] Sulfonamide derivative (**103**) was found to be potent against tested cancer cell lines with IC_{50} values in 0.67-1.5 μM and arrested the cell growth at G2/M phase. Similarly, small molecules from sulfonamide-focused libraries including ER-67865 (**104**), ER-7070 (**105a**) and ER-68487

(**105b**) also demonstrated good anticancer spectrum.[58] The flow cytometric analysis revealed that the presence of two distinct classes of cell cycle inhibitors in this series; one **104** (ER-67865) arrested mitosis by preventing tubulin polymerization; and the second E7070 (**105a**) and ER-68487 (**105b**) caused a decrease in the S-phase fraction along with cell cycle perturbation in G1 and/or G2 *via* an unknown mechanism(s).[59] More recently, indole derivatives (**106**) (AstraZeneca) and indolyl-2-hydrazide-hydrazones (**107**) have been approved as angiogenesis (Abbott Lab) inhibitors that cause selective destruction of tumor vasculature.[60-61] Indolyl glyoxamide, Indibulin (D-24851) (**108**) the most promising antimitotic agent which showed good *in vitro* cytotoxicity towards human cancer cell lines including ovarian, glioblastoma, breast and pancreatic (IC_{50} = 0.036-0.285 μM), destabilizes tubulin polymerization in tumor cells and arrests tumor cell growth at the G2/M phase. Indibulin is also active in multidrug resistant tumor cell lines and its oral formulation is currently being evaluated in the clinical stage.[62-63] Based on anticancer profile of Indibulin (**108**), Li *et al.* synthesized a new series of *N*-heterocyclic indolyl glyoxylamides (**109**) and evaluated for *in vitro* and *in vivo* anticancer activities. This glyoxamides exhibited a broad spectrum of anticancer activity not only in murine leukemic cancer cells but also in human gastric, breast and uterus cancer cells. They also active against multidrug resistant sublines with a wide range of IC_{50} values and induced apoptosis and caused DNA fragmentation in human gastric cancer cells.[64] In 2013, Janssen Pharmaceuticals, USA, patented tryptamine derivatives (**110**) as potent anticancer agents. These tryptamine derivatives (**110**) exhibited admirable *in vitro* as well *in vivo* antitumor effects.[65]

1.4.2 Functionalized indoles as combretastatin-4 (CA-4) analogues

In 1982, Prof. George R. Pettit, the Director of the Cancer Research Institute based at Arizona State University in the USA, isolated Combretastatin (CA-4) (**111**) a small organic molecule from the bark of the African bush willow tree *Combretum caffrum.* The beautiful Bush Willow tree only grows on the banks of rivers in the Eastern Cape Province of South Africa.[66] CA-4 (**111**), is the simplest and most potent antimitotic agent that is known to bind at colchicine binding site of tubulin, with an IC_{50} of 0.53–1.2 μM against tubulin polymerisation. CA-4 displays potent antiproliferative activity against a variety of cancer cell lines including the MDR phenotype.[67] The unique features of CA-4 are its ability to selectively elicit vascular shutdown within solid tumours, while leaving normal vasculature intact.

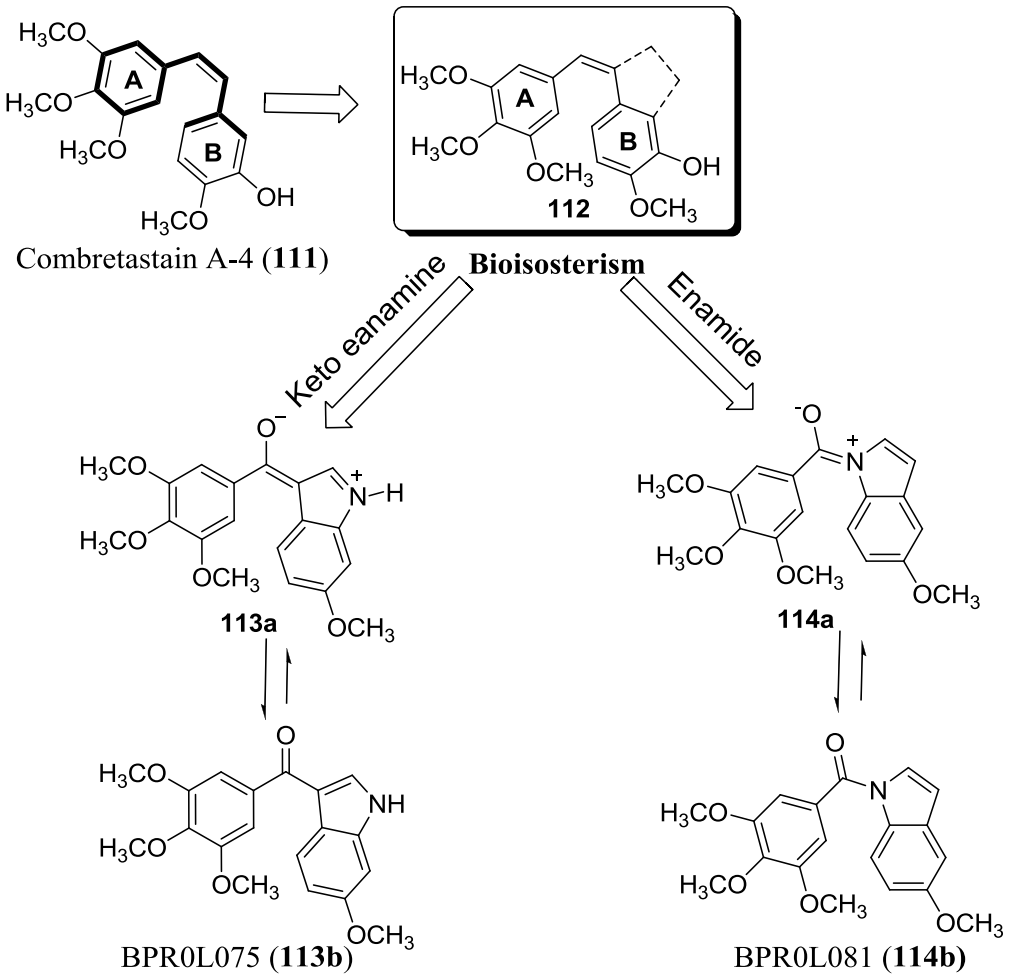


Figure 1.10 Design of 1-and 3-aroyl indoles **113-114**

However, the poor efficacy of CA-4 *in vivo* probably due to its poor water solubility and pharmacokinetic properties. The *cis*-stilbene and trimethoxyphenyl group that are present in CA-4 make it remarkably similar to colchicine.[68]

Bioisosterism is a strategy of medicinal chemistry for the rational design of new drugs, applied with a lead compound for molecular modification. 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 a lead compound. Indole motifs are frequently found in many recently discovered antimitotic agents, particularly those are colchicine-site binders. Inspired from the antitumor and antivascular activity profile of CA-4, Liou *et al.* designed and synthesized 1- and 3-aroylindoles as novel tubulin inhibitors. Due to importance of *cis* geometry for the activity in CA-4, hence this concept used for the design a series of CA-4 analogues by introducing five-membered heterocycles to the B ring of CA-4 analogue **112**. Olefinic moiety and B ring of CA-4 replaced with ketoenamine and

enamide group by incorporating 1-aroylindoles (**114**) and 3-aroylindoles (**113**), respectively (Figure 1.10). On the other hand, the ketoenamine and enamide groups were explored as bioisosteric replacement in order to study the effect on cytotoxicity and tubulin polymerization by novel CA-4 analogues.[69] Compound **113b** (BPR0L075) was found to be potent against the tested cancer cell lines (IC_{50} = 1-23 nM). It was observed that the cytotoxic activity of **113b** exhibited through the activation of the apoptotic cascade, and the arrest of cell cycle is accompanied by an increase of cyclin B1 levels and mobility shift of Cdc2 and Cdc25C. 3-Aroylindoles (**113b**) proved to be cytotoxic against several KB-derived cell lines, over expressing P-gp170/MDR and MRP, resistant to vincristine, paclitaxel, and colchicine. 1-Aroylindoles **114b**, with a methoxy group at C-5 position exhibited stronger cytotoxic activity towards the tested cancer cell lines (Figure 1.11).[69]

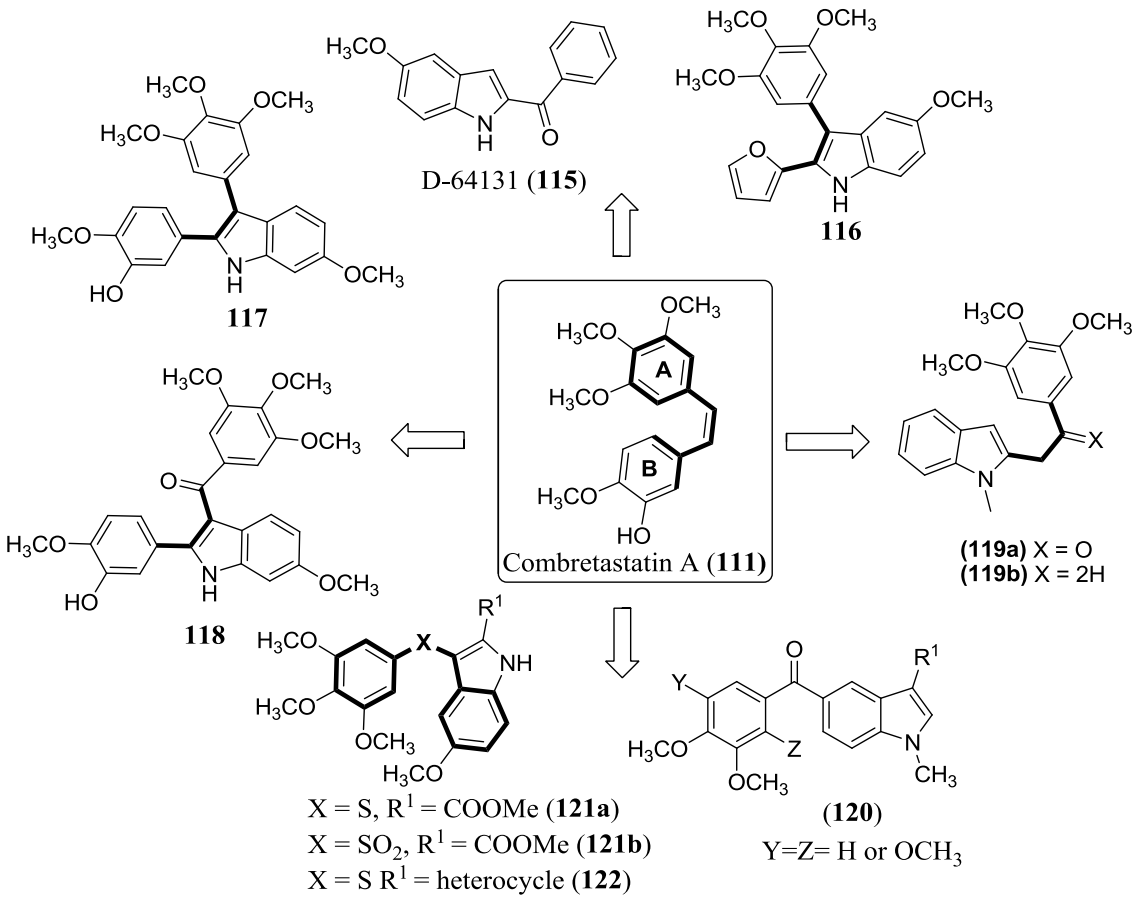


Figure 1.11 Functionalized indoles as combretastatin-4 (CA-4) analogues **115-122**

Mahboobi *et al.* synthesized various 2-aroylindoles and screened against divers cancer cell lines including, human cervical epitheloid carcinoma HeLa/ KB and the human ovarian adenocarcinoma SK-OV-3 cell line.[70] Among the synthesized analogues, 2-aroylindoles (D-

64131) **115** found to be potent antimitotic agents. *In vivo* study demonstrated that the compound **115** was highly active after oral administration at 100 and 200 mg/kg and these dose were well tolerated and showed no toxicity or body weight loss.[71] Medarde and co-workers synthesized some 2,3-diarylindole derivatives by mimicking the *cis* orientation of the aryls of combretastatin A-4. After screening the synthesized compounds against a panel of 60 tumoral cell lines at NCI, compound **116** displayed a remarkable cytostatic activity. The most potent activity was exerted against leukemia, non-small cell lung cancer and CNS cancer. The compound **116** exhibited significant activities for two CNS and two colon cancer cell lines.[72] Furthermore, modification of **116** led to **117**, exhibited tubulin inhibition activity with IC_{50} = 4.1 μM. Insertion of keto functionality in **117** resulted compound **118** with 1.3 times (IC_{50} = 1.6 μM) more active than CA-4 (IC_{50} = 2.1 μM) as a tubulin polymerization inhibitor.[73] In indole bearing hetero combretastatin analogues series, compound **119** showed significant anticancer activity against a panel of cancer cell lines (IC_{50} = 0.3-0.6 μM).[74] In 2007, Chang *et al.* prepared various aroylindoles including 2-, 3-, 4-, 5- and 6-aroylindoles and studied there antiproliferative and tubulin polymerizing inhibition activities. Interestingly, among these series of compounds, some of the analogues exhibited more potent inhibition of tubulin polymerization than the colchicine. Compound **120** was found to be the most potent compound in the series with IC_{50} values ranging from 10-15 nM in six different cancer cell lines including MDR-positive KB-vin 10. More importantly, it showed greater antitubulin activity (IC_{50}= 1.1 μM) than colchicine (IC_{50}= 2.9 μM) and comparable activity to CA-4 (IC_{50}= 1.3 μM).[75]

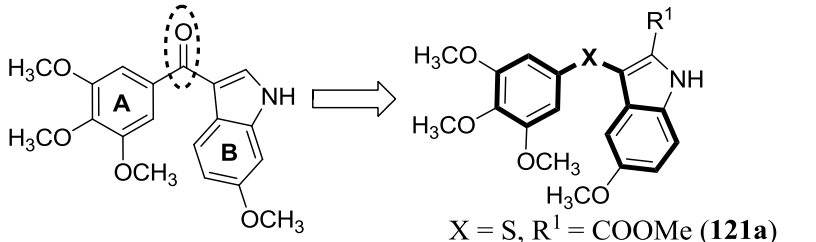


Figure 1.12 Rational design for the synthesis of arylthio/sulphonylindoles **121-122**

Arylthioindoles (**121a**, **122**) and arylsulphonylindoles (**121b**) were designed based on the structural modifications of 3-aroylindoles (**113b**), in which a keto functionality was replaced with sulphur or sulfide groups and C-2 position of the indole ring was substituted with an ester or various heterocyclic rings as shown in Figure 1.12. The detailed SAR of these scaffolds was studied by the introducing the methoxy group at different positions of indole

and phenyl rings. The study revealed that the methoxy group at C-5 position, trimethoxyphenyl thio group at C-3 position of the indole ring (compound **121**) and sulfur in sulfide state beneficial for the anticancer activity. The compound **121a** (IC_{50} = 2.0 μM) was 1.6 times more active than colchicine and about as active as CA-4 (**111**) as an inhibitor of tubulin polymerization. It has the potent growth inhibition of MCF-7 cells (IC_{50} = 13 nM) which is comparable to the activity of colchicine and CA-4 (IC_{50} = 17 nM).[76] Further, docking study of these arylthioindoles in colchicine binding site of tubulin clearly indicate that the trimethoxyphenyl ring is well situated in proximity to Cys241 and methoxy substituent of the indole is also very close to the corresponding group on ring C of colchicine, leading to a very similar general binding of the two inhibitors. Additionally, indole ring establishes the hydrogen bond between N-H and back bone of Thr179.[77] These observations are consistent with the highly efficient inhibition of [^{3}H]colchicine binding that occurs with compound **121**. Substituent at C-2 position of the arylthioindoles plays an important role in the potency of these analogues.

Recently, diverse arylthioindoles were reported by incorporating various cyclic substituents such as aryl/heteroaryl rings at C-2 position of the indole ring (Figure 1.13). Compounds with the thiophene and pyrrole substituents at C-2 of indole (**122a**) were found to be most active analogues. These analogues exhibited higher metabolic stability as compared to their parent ester derivative **121a** and were more effective than vinorelbine, vinblastine, and paclitaxel as growth inhibitors of the P-glycoprotein-over expressing cell line NCI/ADR-RES.[78]

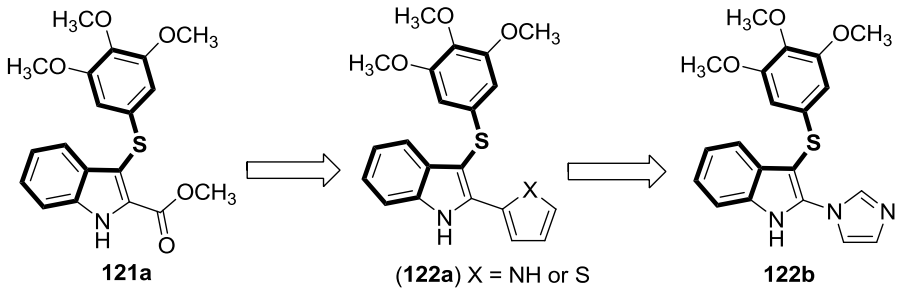


Figure 1.13 Structural modifications at C-2 position of arylthioindoles

In order to further improve the activity, thiophenes of **122a** have been replaced with several heterocycles to achieve most potent arylthioindoles. Compound with imidazole substituent **122b** endowed with improved anticancer activity (IC_{50} = 1.0 nM; MCF-7 cells) and found to be uniformly active in the whole panel of cancer cells and superior to colchicine and combretastatin A-4.[79]

1.4.3 Indolylazoles as anticancer agents

Indole connected to five/six-membered azaheterocycles are classified under this category. There are several indolylazoles with natural as well as synthetic origin exhibited interesting antitumor activity. Naturally occurring indolylazoles as shown in Figure 1.14. Indolylthiazole, Camalexine (**123**), isolated from phytoalexin of *Arabidopsis thaliana* which displayed significant cytotoxicity and induces apoptosis in T-leukemia Jurkat cells.[80] Pettit *et al.* isolated indolyloxazoles, Labradorins 1 (**124a)** and 2 (**124b**) from *Pseudomonas syringae* pv. *Coronafaciens*, were 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.[81] Meridianins A-E (**125**) are pyrimidine class of marine alkaloids that were isolated from the tunicate *Aplidium meridianum* and found to possess potent cytotoxic and kinase inhibition activities.[82,83] Recently, Reyes and co-workers isolated a family of indole alkaloids, Aplicyanins (**126**) from Antarctic tunicate *Aplidium cyaneum.* The Aplicyanins (**126**) with a bromoindole nucleus and 6-tetrahydropyrimidine at C-3 position were found to be cytotoxic against human tumor cell lines including MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma) and HT-29 (colorectal carcinoma) with inhibition of tubulin polymerization.[84] With (aminoimidazolinyl)bromoindole moiety, Discodermindole (**127**) was isolated from the marine sponge *Dirpcodermia polydiscus* and its *in vitro* anticancer activity study exhibited IC_{50} values ranges 1.8-12 μg/mL against P388 (murine leukemia), A-549 (human lung) and HT-29 (human against colon) cell lines.[85]

Inspired from above mentioned naturally occurring indolylazoles, various research groups prepared diverse indolylazoles by connecting azole ring through pyrrolic ring or benzene ring (**128**) and evaluated their anticancer activity (Figure 1.15). Recently, Kumar *et al.* identified indolyloxadiazoles (**129**) and indolylthiadiazoles (**130**) as analogues of Labradorins with potent cytotoxicity and selectivity against human cancer cell lines.[86] Thiadiazoles analogue **130** endowed with potent anticancer activity with best compound IC_{50} = 1.42 μM against the HeLa cell lines. Also, the analogue **130** exert their anticancer activity through inhibition of tubulin polymerization with an IC_{50} value of 17.5 μM.[87]

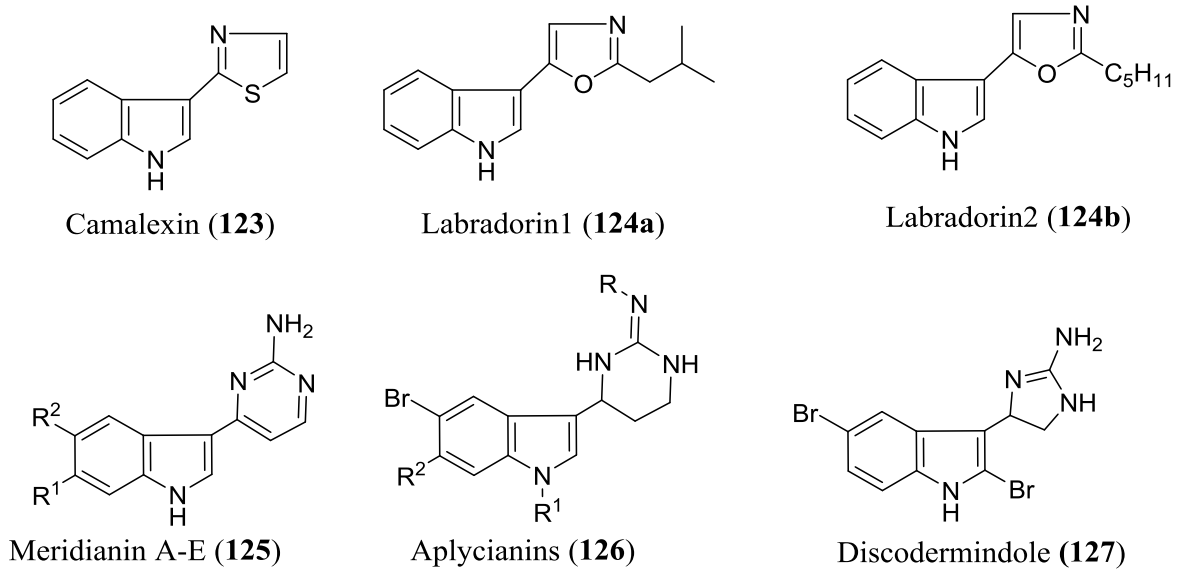


Figure 1.14 Naturally occurring cytotoxic indolylazoles **123-127**

Hu *et al.* reported indolylpyrimidine (**131**) as a potent antiproliferative agent with average IC_{50} values ranging from 16-62 nM against the tested cancer cell lines (Figure 1.15).

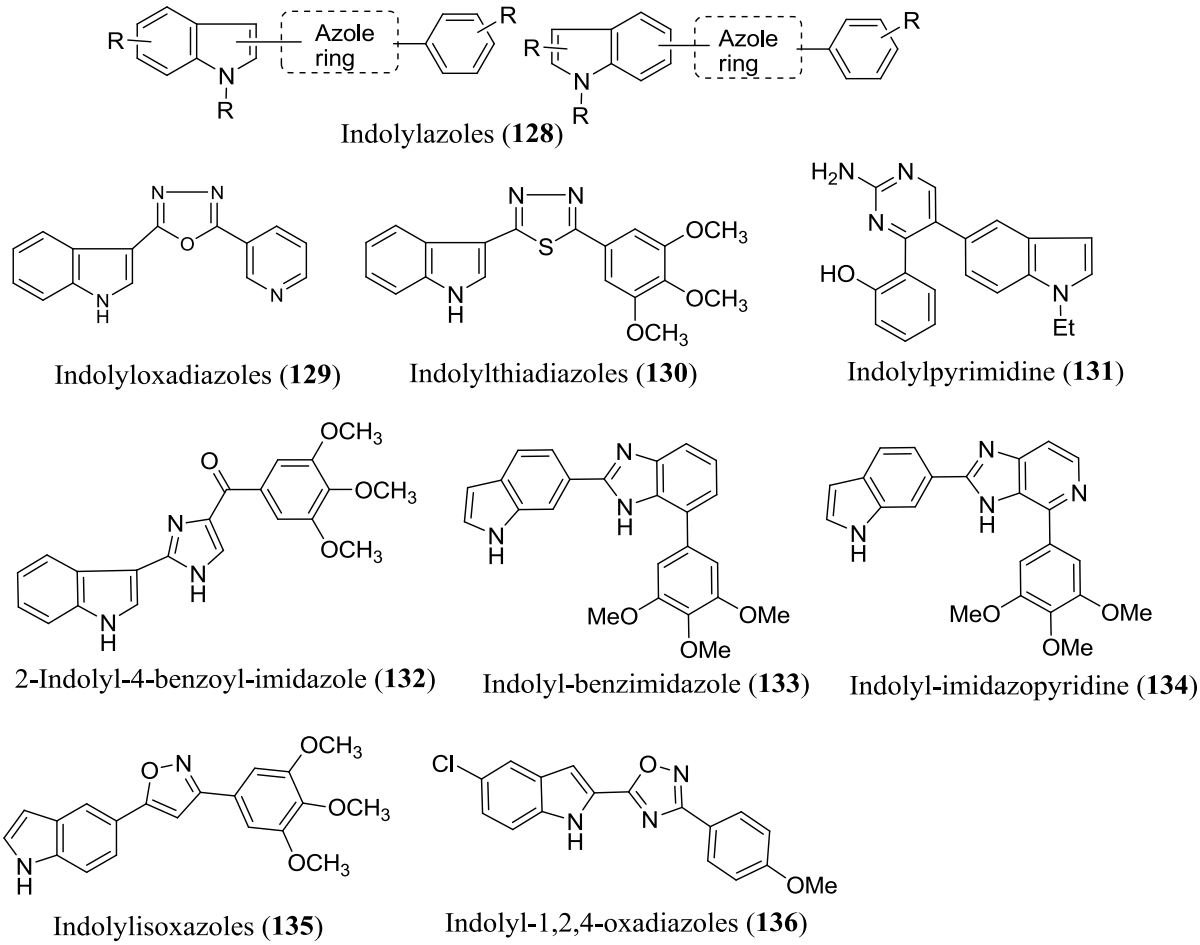


Figure 1.15 Representative synthetic indolylazoles as anticancer agents **129-136**

Compound **131** was observed to be an excellent inhibitor of tubulin polymerization (IC_{50} = 0.79 μM).[88] In 2012, Li *et al.* identified the indolyl-benzoylimidazole (**132**) as antiproliferative agents (average IC_{50}= 3.8 nM; melanoma and prostate cancers). Compound **132** can effectively overcome Pgp-mediated multidrug resistance and paclitaxel resistance cancer cell lines. Mechanism of action showed that the compound **132** exert their anticancer activity through inhibition of tubulin polymerizations.[89] Miller and co-workers synthesized a series of indolyl-imidazoles (**133**) and indolyl-imidazopyridines (**134**) as promising cytotoxic agents towards the melanoma and prostate cancer cell lines which exhibited their activity through modulation of tubulin polymerizations (IC_{50}= 5-57 nM), also were found to be effective against P-glycoprotein (P-gp) mediated multiple drug resistance (MDR) and taxol resistance cells.[90,91] Westwell *et al.* reported the indolylisoxazoles (**135**) as potent cytotoxic agents. Further analysis of indolylisoxazoles showed that these compounds induce apoptosis through expression of caspases-3 and -7.[92] Subsequently, same group synthesized indole-based 3,5-disubstituted 1,2,4-oxadiazoles (**136**) as potent pro-apoptotic inducing antitumour agents with an IC_{50} values in low micromolar range.[93]

1.4.4 Bisindoles as anticancer agents

Bisindoles are an important class of alkaloids, in which two indole nuclei are separated by a heterocyclic ring or linear spacer or any functional group as depicted in Figure 1.16.

Figure 1.16 Bisindole **137**

Bisindole alkaloids have drawn significant attention due to their diverse biological properties including antiviral, antimicrobial and anticancer.[94] Bisindole alkaloids generally isolated from marine source including sponges, coelenterates, tunicates, and bryozoans and exhibited anticancer activity (Figure 1.17). For examples, Topsentins A-C (**138**) which contain keto-imidazole moiety as a linker between two indole rings which isolated from marin sponge *Topsentia genitrix*, inhibit the proliferation of tumor cells (IC_{50} = 4–40 μM).[95] The imidazole bearing bisindole alkaloid, Nortopsentins A–C (**139**) were isolated from *Spongosorites ruetzleri* and found to display *in vitro* cytotoxicity against P388 cells (IC_{50} = 4.5–20.7 μM).[96] Rhopaladin B (**140**) in which two indole rings linked *via* an imidazolinone spacer, was

isolated from the Okinawan marine tunicate *Rhopalaea* sp. with potent inhibitory activity against CDK 4 (IC_{50} = 12.5 µg/mL) and c-erbβ-2 (IC_{50} = 7.4 µg/mL) kinases.[97]

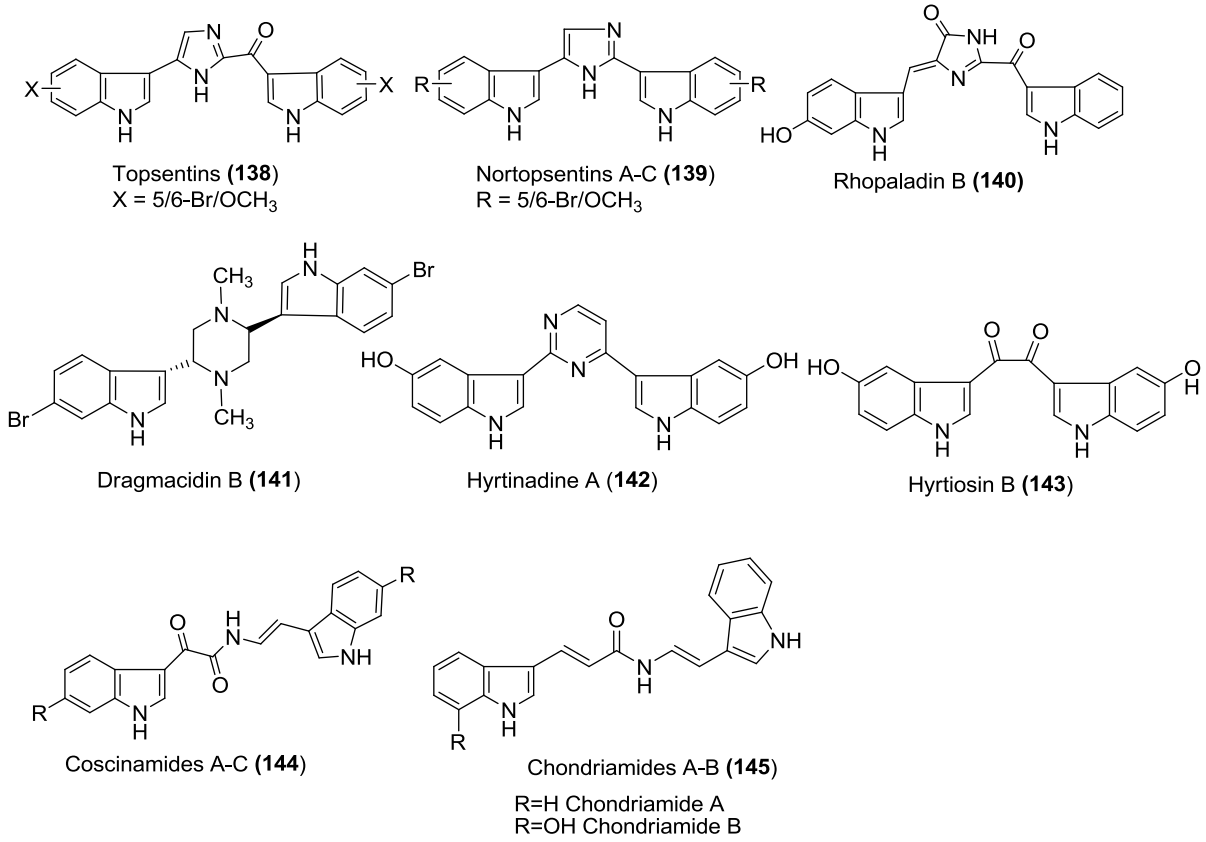


Figure 1.17 Naturally occurring bisindoles as cytotoxic agents **138-145**

In 1995, Capon and co-workers isolated Dragmacidin B (**141**) with a piperazine spacer from a deep-water marine sponge *Spongosorites* sp. and found to display good anticancer activity (IC_{50} = 15 µg/mL, P388; 1-10 µg/mL, A-549, HCT-8 and MDA-MB-231).[98] Hyrtinadine A (**142**) with a 2,5-disubstituted pyrimidine linker between two indole ring was isolated from the extracts of an Okinawan marine sponge *Hyrtios* sp. and showed *in vitro* cytotoxicity against murine leukemia L1210 (IC_{50}= 1 µg/mL) and human epidermoid carcinoma KB (IC_{50}= 3 µg/mL) cells.[99] Bisindole with 1,2-diketo linker, Hyrtiosin B (**143**) was isolated from marine sponge *Hyrtios erecta* at Ishigaki Island, Okinawa and found to possessed *in vitro* cytotoxicity against epidermoid carcinoma KB cell line.[100] In 2000, Bokesch and co-workers isolated Coscinamides A-C (**144**) with linear alpha-keto enamide spacers from an extract of marine sponge *Coscinoderma* sp. collected in Papua, New Guinea and were reported to exhibit partial cytoprotection against HIV[101] and antitumor activity against human prostate cancer cell line (IC_{50} = 7.6 µg/mL).[102] Two cytotoxic bisindole amides, Chondriamides A-B (**145**), were isolated from a red alga *Chondria* sp. which was found in abundance on the

rocky shores of the province of Buenos Aires, Argentina. Chondriamides A-B were found to be cytotoxic against KB and LOVO cell lines (IC_{50} values of 0.5 μg/mL and 10.0 μg/mL) as shown in Figure 1.17.[103]

Among these natural bisindoles, Nortopsentins (**139**) received much attention of medicinal chemist due to their structural simplicity and broad anticancer activities. In recent years, diverse synthetic analogues of Nortopsentins **139**, have been reported by replacing imidazole ring with variety of cyclic (five-or six-membered heterocyclic rings) as well as linear chain linkers (**146-158**) and evaluated their anticancer activities as shown in Figure 1.18.

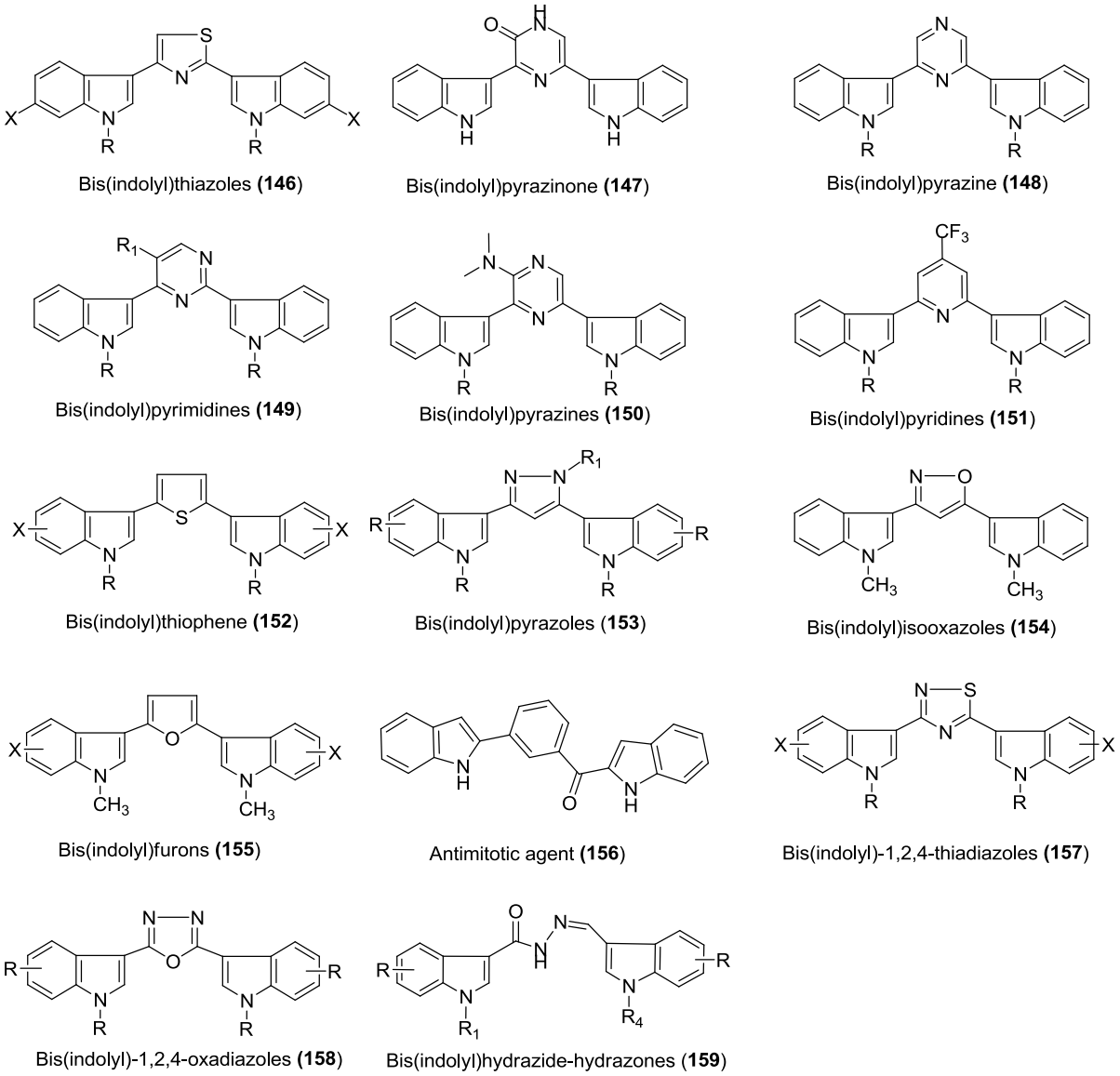


Figure 1.18 Synthetic bisindoles as anticancer agents **146-159**

In 1999, Jiang *et al.* prepared thiazole analogues (**146**) and exhibited potent cytotoxicity in low micromolar range against various cancers.[104] Subsequently, same group identified

bis(indolyl)pyrazinone (**147**) and bis(indolyl)pyrazine (**148**) as cytotoxic agents with strong inhibitory activity against a wide range of human tumor cell lines.[105] In 2001, same group replace thiazole scaffold in **146** by pyrimidines **149** and pyrazines **150** prepared library of compounds. Anticancer activity evaluation study showed that these analogues exhibited selective anticancer activity with the GI_{50} values of < 0.01 μM against IDROV1 cell lines.[106] Subsequently, bis(indolyl)pyridines (**151**) was prepared as moderate cytotoxic agents against murine leukemia cells (P388) and human lung cancer cells (A-549).[107] Diana and co-workers prepared a series of bis(indolyl)thiophenes (**152**) were evaluated for their anticancer activity against full panel of about 60 human tumor cell lines. Some of the bis(indolyl)thiophenes showed antiproliferative activity ranging GI_{50} values from 0.3-19 μM.[108] Subsequently the same group reported various bis(indolyl)pyrazoles **153** as strong cytotoxic agents against human tumor cell lines. The most active compound was effective against all the tested cell lines with a mean GI_{50} value of 3.23 μM.[109] After successful achievement of bisindoles **152** and **153**, same research group prepared bisindoles possessing isoxazoles (**154**) and furan (**155**) spacers with significant *in vitro* anticancer activity towards a panel of 29 cell lines.[110] In 2012, Dalton *et al.* patented bisindoles **156** with an aroyl linker as inhibitors of tubulin assembly with promising *in vitro* and *in vivo* activities against multidrug resistant prostate cancer cells.[111] Recently, Kumar and co-workers have designed and synthesized various bisindoles with cyclic as well as linear spacers including 1,2,4-thiadiazoles[112] (**157**), 1,3,4-oxadiazoles[113] (**159**) and hydrazide-hydrazones[114] (**158**). *In vitro* cytotoxicity study of these bisindoles against panel of cancer cells resulted in potent compounds with IC_{50} values in nanomolar to micromolar ranges.

1.5 Conclusions and present work

Over the years, the design of cancer chemotherapy has become increasingly sophisticated. Till date there is no cancer treatment that is hundred percent effective against disseminated cancer so there is a huge demand for the new chemical entities in the area of anticancer research for the development of effective anticancer drugs. Marketed drugs are suffering from various side effects including drug resistance, bioavailability and drug toxicity. Resistance to chemotherapeutics can be divided into two broad categories: intrinsic or acquired. Intrinsic resistance indicates that before receiving chemotherapy, resistance-mediating factors pre-exist in the bulk of tumour cells that make the therapy ineffective. Acquired drug resistance can develop during treatment of tumours that were initially sensitive

and can be caused by mutations arising during treatment, as well as through various other adaptive responses, such as increased expression of the therapeutic target and activation of alternative compensatory signalling pathways. 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 overcome these aforementioned problems and scientific understanding of the mechanisms of drug resistance may emerge new treatments which may develop to counteract resistance and decrease the toxicity.

The present study mainly focuses on the design and synthesis of novel indole-based chemical entities for the improved anticancer activity. Design of diverse series of indole-based compounds is based on natural as well as synthetic bioactive lead molecules. Synthesizing various heterocycles/functionalizations at C-3/C-2/N-H position of indole ring and screening of these synthesized compounds against wide range of cancer cell lines gave insight about their mechanism and required structural modifications. The preliminary mechanism of action studies of synthesized indole-based compounds encouraged us to evaluate the further structure-activity relationship and detailed mechanism of actions of the most potent compounds.

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Chapter 2

Synthesis and Anticancer Activity Studies of Novel Indolylthiazoles

Part A: ***Synthesis of 2-arylamino-4-(3′-indolyl)thiazoles as novel apoptosis inducing cytotoxic agents***

Part B: ***Synthesis of 2-(3′-indolyl)-N-arylthiazole-4-carboxamides as cytotoxic agents***

2.1 Indolylthiazoles

Indolylazoles (**1**), linking an indole ring with a five-membered heterocycle, have been found to occur from natural as well synthetic source. In the recent past, various indole-based compounds including pyrazole, isoxazoles, oxazole, imidazole, thiazole, triazole, oxadiazole and thiadiazole have been identified as potent anticancer agents.[1-3] Especially, indolylthiazoles and its congeners exhibited encouraging anticancer activities. For instance, Indolylthiazole, Camalexin (**2**), isolated from phytoalexin of *Arabidopsis thaliana* was found to show significant cytotoxicity effects and induces apoptosis in T-leukemia Jurkat cells.[4] In 1999, Jiang *et al.* prepared bis(indolyl)thiazole **3** endowed with potent cytotoxicity against a panel of cancer cell lines (GI_{50} = 2.9-14.4 μM).[5] Indolylthiazole **4** bearing pyridyl moiety was found to inhibit cancer cells growth significantly (leukemia HL-60, CNS cancer SF-295 and renal cancer RXF 393, GI_{50}= 1.9-2.5μM).[6] Diana and co-workers identified azindolylthiazole (**5**) with substantial cytotoxicity towards MiaPaCA-2 and STO cell lines with IC_{50} values of 4.3 and 0.41 μM, respectively.[7] Thiazole substituted indolequinone, BE 10988 (**6**) was isolated from the culture broth of a strain of actinomycetes which exhibited as a topoisomerase inhibitor (Figure 2.1.1).[8]

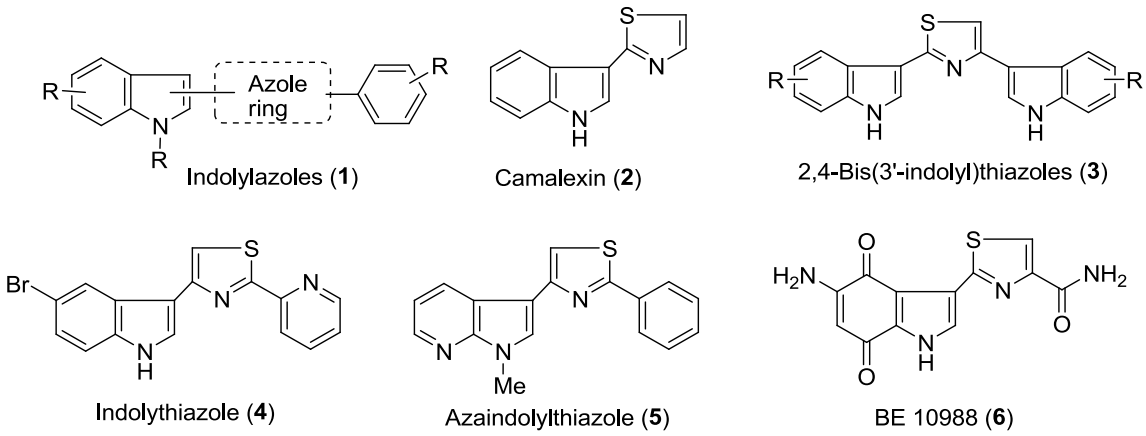


Figure 2.1.1 Representative examples of cytotoxic indolylthiazoles

In this chapter, synthesis and anticancer activity studies of two series of indolylthiazoles have been discussed. Part A includes the synthesis of 2-arylamino-4-(3′-indolyl)thiazoles as novel apoptosis inducing cytotoxic agents. Part B deals with the synthesis and biological evaluation of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides as anticancer agents.

2.2 Part A: Synthesis of 2-arylamino-4-(3′-indolyl)thiazoles as novel apoptosis inducing cytotoxic agents

2.2.1. Rational design

Indole containing compounds are frequently found in cancer drug discovery research in addition to their antiinflammatory, antimalarial, antibacterial, and antifungal properties.[9-11] Over the last few years, many indole-based anticancer agents have been identified from natural as well as synthetic sources endowed with interesting cytotoxic activities.[12,13] For example, vinblastine, vincristine, MKC-1[14] (**7**), LP-261[15] (**8**), and indibulin[16] (**9**) are some of the indole-based molecules with good tubulin inhibition activity in clinical trials. Recently, several indole-based small molecules like aroylindoles (**10**; IC_{50} = 1-23 nM),[17] arylthioindole (**11**; IC_{50} = 1.0 nM; MCF-7)[18] and 2-indolyl-4-benzoylimidazole[19] (**12**) (average IC_{50} = 3.8 nM) have been identified with potent anticancer properties (Figure 2.2.1).

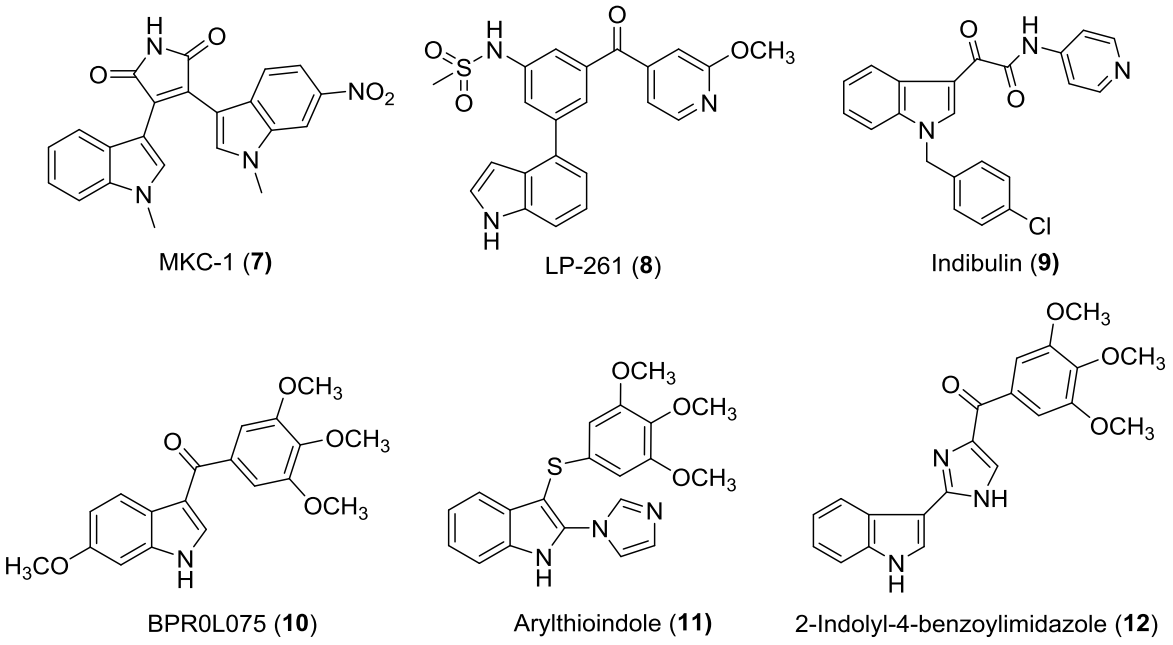


Figure 2.2.1 Chemical structures of indole-based anticancer agents

On the other hand, thiazole is an important pharmacophore embedded in many molecules possessing a wide range of biological activities like anticancer, antidiabetics, antibacterial, antiinflammatory, antimycobacterial, anti-HIV, antidepressant, antihypertension and anticonvulsant.[20-22] Several molecules containing thiazole scaffold exhibit potent anticancer activity against a panel of cancer cell lines. For example, thiazole amide INH (**13**) and its analogues, and SMART (**14**) (IC_{50} = 21-71 nM) showed excellent antiproliferative activity

against cancer cell lines.[23] Furthermore, aminothiazole is found to be common scaffold in many bioactive heterocycles of medicinal interests, especially in anticancer agents.

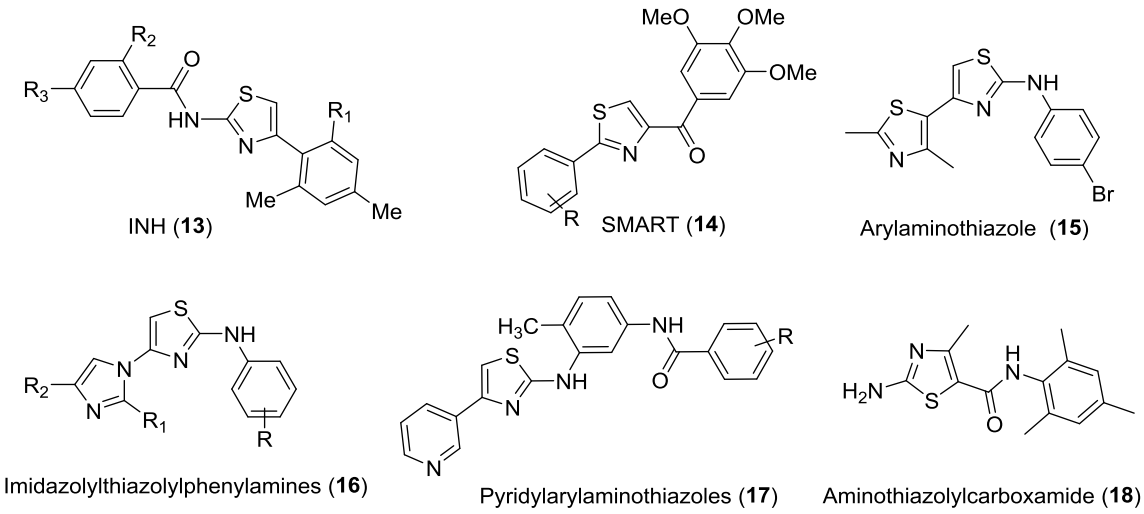


Figure 2.2.2 Anticancer lead molecules with thiazole scaffold

In 2006, Mahboobi *et al.* synthesized arylaminothiazoles (**15**; IC_{50} = 0.7 µM) and imidazolylthiazolylphenylamines (**16**) with interesting activity against genetically engineered human colon carcinoma cell line RKOp27 with IC_{50} values ranging between 0.25-49 µM.[24] Thiazolyl analogues of imatinib (**17**) exhibited potent anticancer activity by virtue of its kinase inhibition activity.[25] Similarly, Das and co-workers identified methylated aminothiazole (**18**) to be active against murine Lck (IC_{50} = 6.6 µM), human Lck (IC_{50} = 5 µM) and T-cell proliferation (IC_{50} > 10 µM) as a Lck inhibitor (Figure 2.2.2).[26] Gu *et al.* synthesized the thiazole analogue of Nortopsentin (**3**) endowed with a broad spectrum of anticancer activity (IC_{50} = 2.94-31.5 µM) against various cancer cell lines.[27]

The cytotoxicity of many chemotherapeutic drugs is accomplished through the induction of apoptosis.[28-31] Apoptosis is a programmed cell death that consists of a cascade of molecular events and occurs in response to variety of stimuli such as chemical reagents, radiation and viral infection.[32-34]

Recently, our group also prepared several indolylazoles with an indole motif as potent anticancer agents and could kill cancer cells through the induction of apoptosis.[1-3, 35-38] In continuation of our search for indole-based potent anticancer agents, we prepared a new library of 2-arylamino-4-(3′-indolyl)thiazoles (**25a-o**) by incorporating important scaffolds indole and aminothiazole in a single molecule (Figure 2.2.3), and studied their anticancer activity.

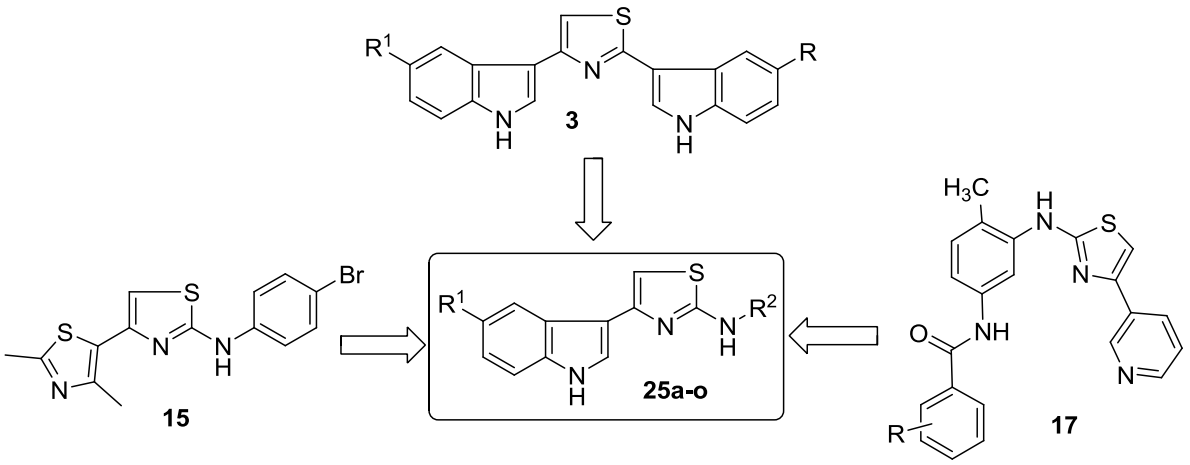
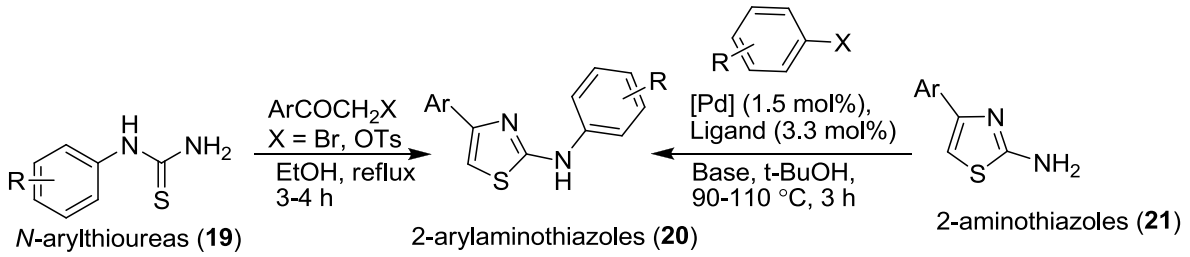


Figure 2.2.3 Rational design for 2-arylamino-4-(3'-indolyl)thiazoles **25a-o**

2.2.2 Results and discussion

2.2.2.1 Synthesis

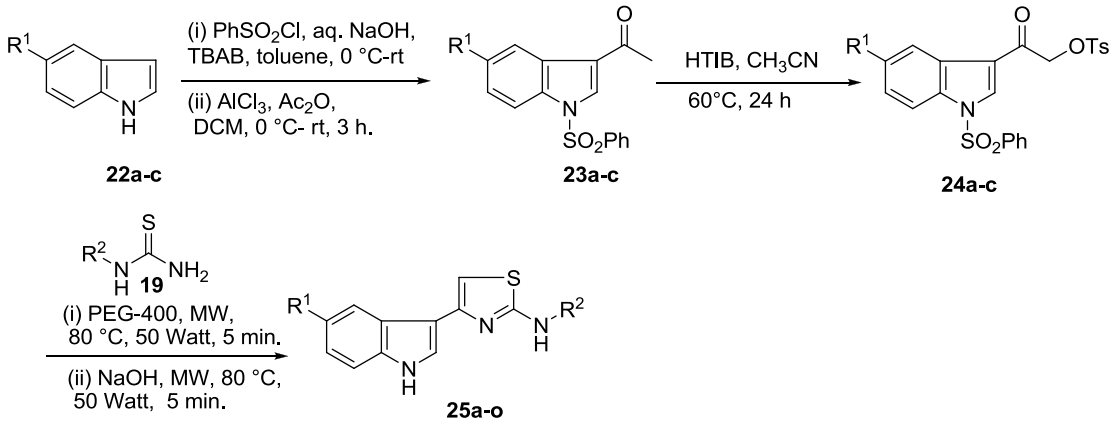
Typically, 2-arylaminothiazoles (**20**) are prepared by Hantzsch thiazole synthesis involving lachrymatory α-haloketones and thiourea (**19**) in polar protic solvents.[39] Alternatively, 2-aryl-aminothiazoles can be synthesized *via* palladium-catalyzed *N*-arylation of 2-aminothiazoles (**21**) (Scheme 2.2.1).[40]



Scheme 2.2.1 Reported methods for the synthesis of 2-arylaminothiazoles

Ugi four-component coupling (Ugi 4-CC) reaction of an arylaldehyde, a carboxylic acid, an isocyanate and ammonia was also reported to give intermediate diamides which upon treatment with Lawesson's reagent led to substituted aminothiazoles.[41] Generally, existing synthetic methods involve the use of lachrymatory α-haloketones, expensive palladium catalyst or multi-step protocol. In an efforts to develop efficient protocol, Koser´s group reported the reaction of enolisable ketones with [hydroxy(tosyloxy)iodobenzene] (HTIB)[42] to form α-tosyloxyketones, benign intermediates used as surrogate of lachrymatory α-haloketones. Under similar reaction conditions, we have successfully prepared α-tosyloxyketones **21a-c** from the reaction of 3-acetylindoles **24a-c** with HTIB in quantitative

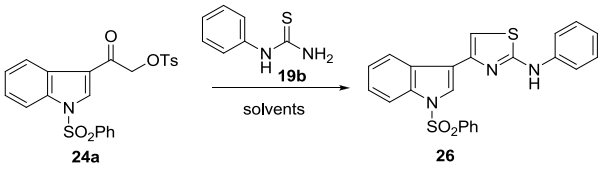
yields as shown in Scheme 2.2.2. On the other hand, substituted *N*-arylthioureas **18** were prepared from arylanilines, ammonium isothiocyanate and benzoyl chloride using known protocol.[43]



Scheme 2.2.2 Synthesis of 2-arylamino(indolyl)thiazoles (**25a-o**)

Reaction of *α*-tosyloxyketones **24a** with *N*-phenylthiourea (**19b**) was chosen as a model reaction to prepare 2-arylaminothiazoles **25b**. Initially, the reaction of *α*-tosyloxyketones **24a** with *N*-phenylthiourea (**19b**) in ethanol at 80 °C led to intermediate thiazole **26** in 85% yield. Further, treatment of **26** with solid sodium hydroxide in refluxing ethanol for 12 h. afforded 2-arylaminothiazole **25b** in moderate yield (50%). Over the years, there has been growing recognition that PEG-400 has become an attractive and alternate solvent of choice for many useful organic transformations due to its benign characteristic properties such as non-toxicity, non-hazardousness, air and moisture stability and low volatility.[44,45] Therefore, we used PEG-400 as a benign reaction medium to prepare 2-arylaminothiazole **25b** (Table 2.2.1). Interestingly, when the reaction of **24a** with *N*-phenylthiourea (**19b**) was performed in focused microwave (50 watt power) in PEG-400 at 80 °C, intermediate thiazole **26** was formed within 5 min in excellent yield (95%, Table 2.2.1; entry 8). Subsequently, the benzenesulfonyl group of **26** was successfully removed under microwave irradiation and furnished **25b** in 70% yield. Reaction of **24a** with *N*-phenylthiourea (**19b**) was also performed in other polar protic (ethanol, methanol, water, 2-propanol) and polar aprotic (DMF, toluene) solvents under conventional as well as microwave conditions.

Table 2.2.1. Optimization of the reaction conditions to prepare 2-arylamino(indolyl)thiazole



Entry	Reaction solvent	Temperature (°C)	Conventional Heating		Focused Microwave	
			Time (min)	Yield (%)	Time (min)	Yield (%)
1.	Toluene	100	120	20	10	30
2.	Dimethylformamide	100	120	40	10	50
3.	Ethanol	80	120	78	05	85
4.	Methanol	70	120	75	05	75
5.	Water	100	180	50	12	62
6.	Solvent free	100	180	30	15	30
7.	Ethylene glycol	100	120	79	05	84
8.	PEG-400	80	120	85	**05**	**95**
9.	PEG-400	40	120	trace	20	40
10.	2-Propanol	80	120	72	05	80

However, reaction yields were inferior to PEG-400 (Table 2.2.1; entry 8). Reaction of **24a** with *N*-phenylthiourea below 80 °C resulted in lower product yields, prolonged reaction times and recovery of unchanged starting materials (Table 2.2.1; entry 9). For the formation of intermediate thiazole **26** in good yield, 80 °C temperature was found to be optimum. To make the procedure more efficient and economical, next we accomplished both the steps in one-pot (formation of thiazole **26** and removal of benzenesulfonyl group). After the reaction of **24a** with *N*-phenylthiourea (**19b**), *in situ* generated thiazole **26**, was treated with solid sodium hydroxide and exposed to microwave irradiation (5 min) to afford **25b** in 85% yield. These optimized reaction conditions were generalized by reacting various α-tosyloxyketones **24** with *N*-arylthioureas **19** to obtain diverse 2-arylaminothiazoles (**25a-o**) in good yields. Synthesized 2-arylamino-4-(3'-indolyl)thiazoles (**25a-o**) were well characterised by their IR, NMR (^{1}H and ^{13}C) and mass spectral data (Figures 2.2.4-2.2.6). ^{1}H NMR spectra of **25a-o** displayed a characteristic singlet between δ values 6.5 to 7.0, corresponding to thiazole C5-H. Similarly, in ^{13}C NMR spectra, thiazolyl C5-carbon was observed between δ values 110 to 115 ppm.

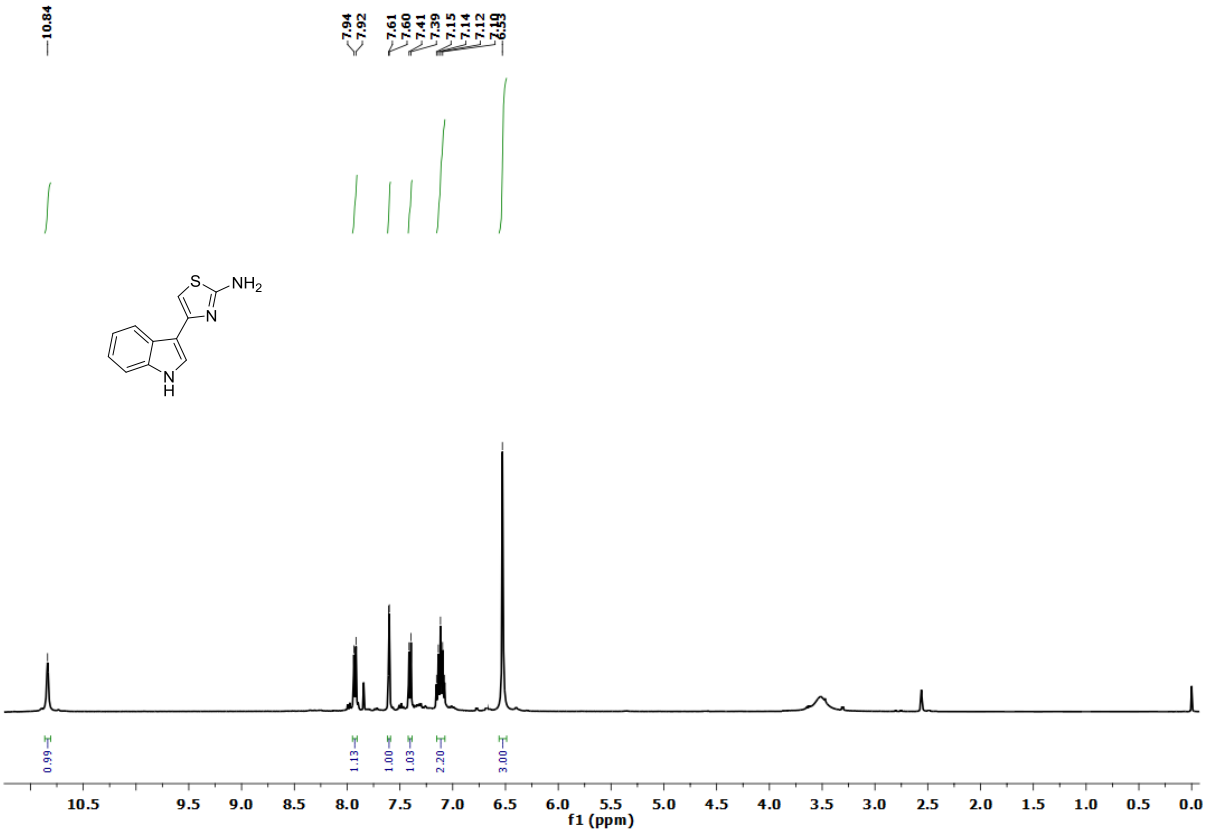


Figure 2.2.4 ^{1}H NMR spectrum of **25a**

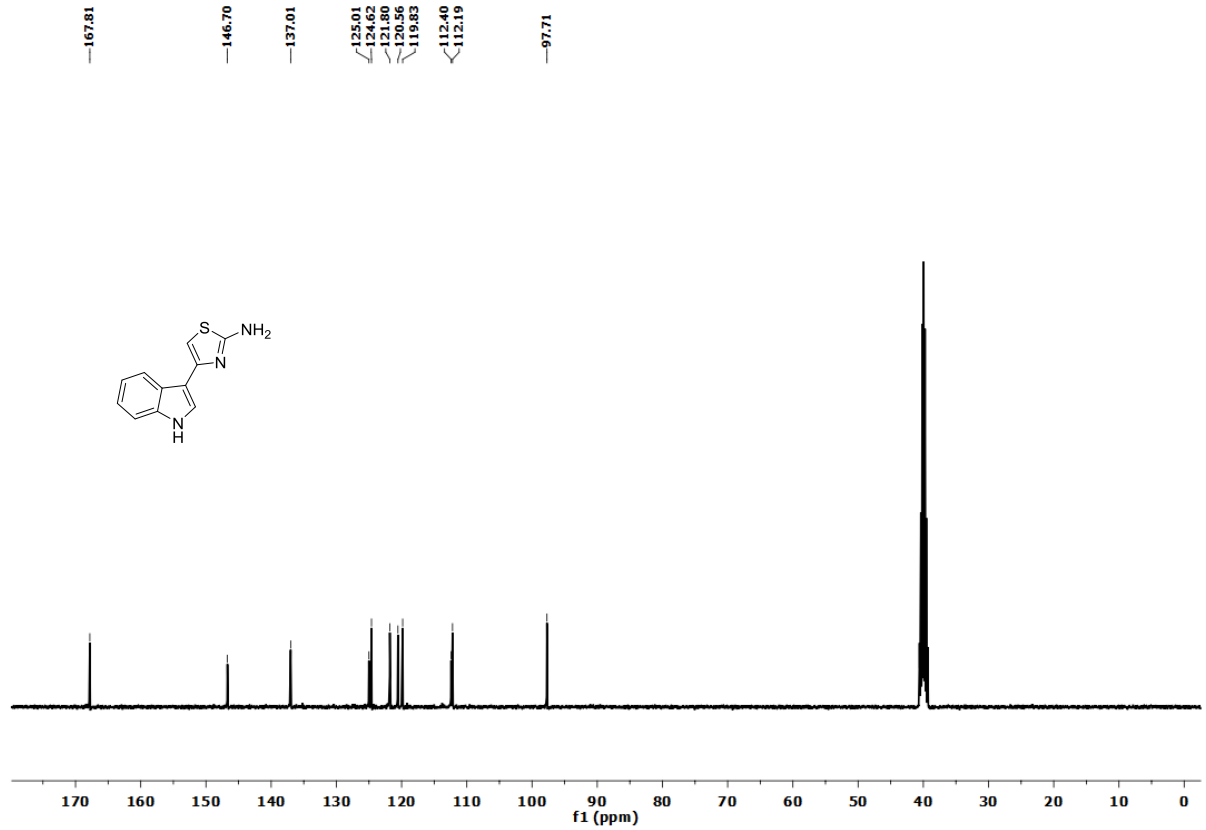


Figure 2.2.5 ^{13}C NMR spectrum of **25a**

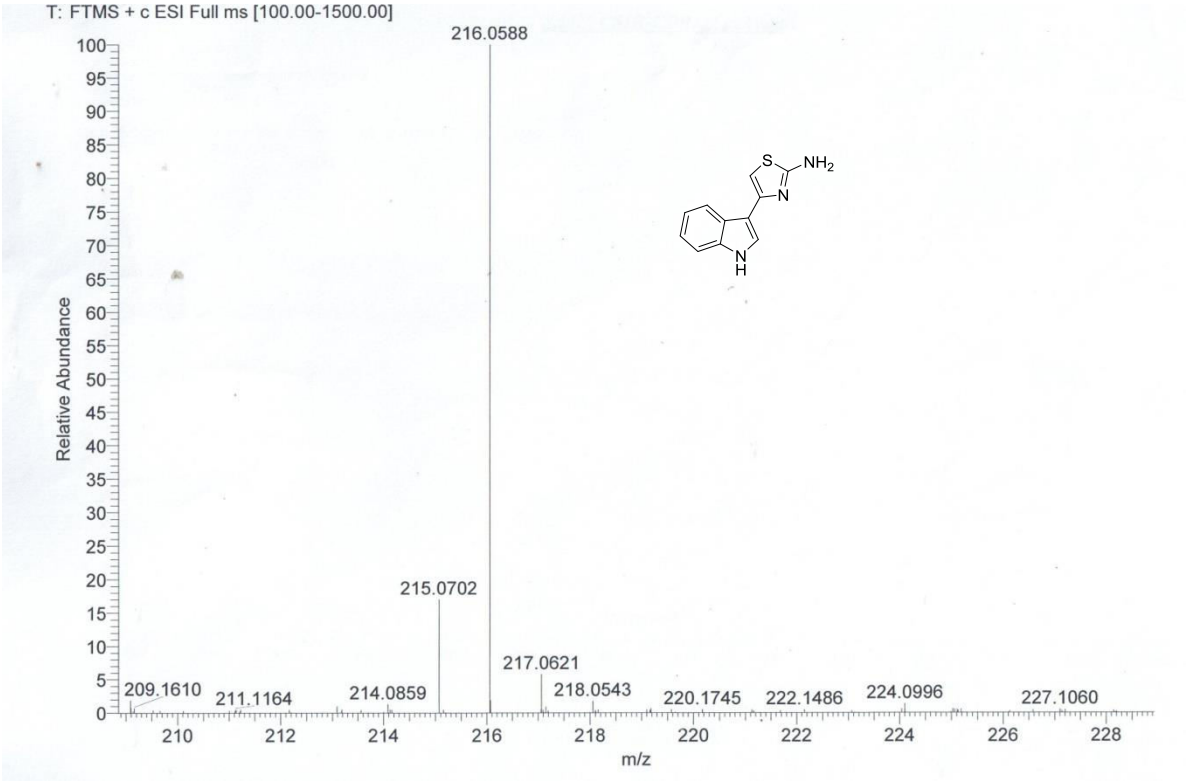


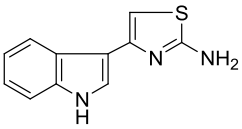
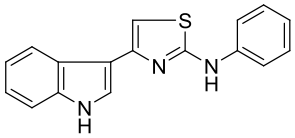
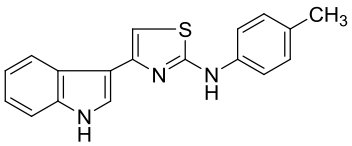
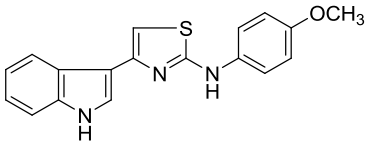
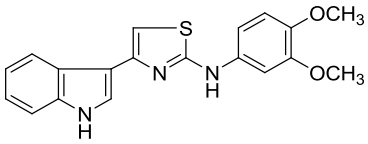
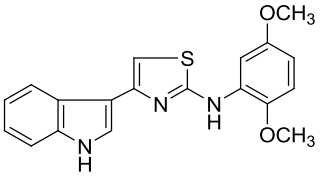
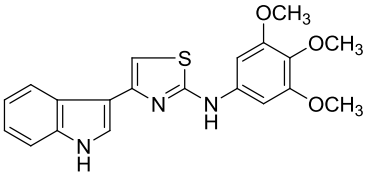
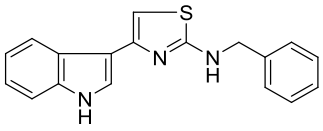
Figure 2.2.6 HRMS spectrum of **25a**

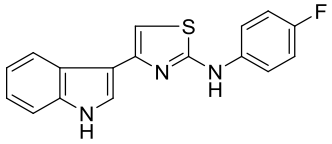
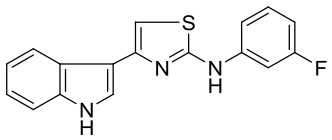
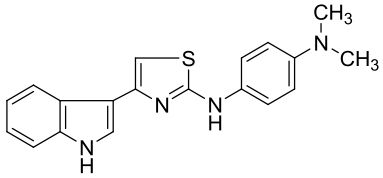
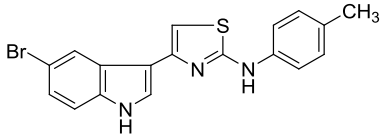
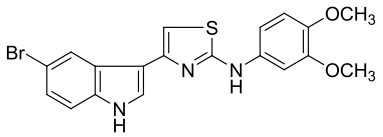
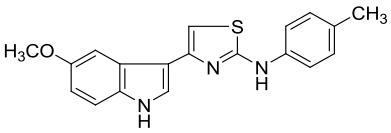
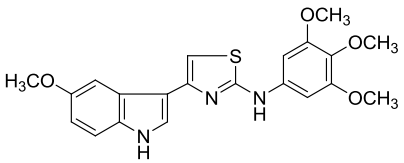
2.2.2.2 Anticancer activity

Synthesized 2-arylamino-4-(3'-indolyl)thiazoles (**25a-o**) were assessed for their *in vitro* cytotoxicity against selected human cancer cell lines of lung (A549), cervix (HeLa), liver (HepG2) and breast (MCF-7 and MDA-MB-231) by MTT assay with doxorubicin, an effective anticancer drug and as a positive control. Structure–activity relationship (SAR) study was carried out by varying substituents on the indole (R^1) and *N*-arylamino (R^2) moieties. The ability of these aminothiazoles to inhibit the growth of cancer cell lines is summarized in Table 2.2.2.

Compound **25a** without any substituent both on indole as well as on arylamino moieties was found to be moderately active against a panel of cancer cell lines (IC_{50} 19.75-40.2 µM). Replacement of an amine hydrogen with a phenyl ring led to compound **25b** with good cytotoxicity (IC_{50} 1.86-12 µM). Compound **25b** was found to be 21 and 4 fold more potent against the breast (MCF-7) cancer line with an IC_{50} value of 1.86 µM when compared with **25a** and doxorubicin, respectively. Analogue **25c** with *p*-tolyl substituent displayed reduced activity when compared to their phenyl analogue **25b** and doxorubicin but almost similar to **25a.**

Table 2.2.2 IC_{50} values[a] (in μM) of 2-arylamino-4-(3'-indolyl)thiazoles (**25a-o**) against selected human cancer cell lines

2-arylamino-4-(3'-indolyl)thiazoles		A549	HeLa	HepG2	MDA-MB-231	MCF-7
	**(25a)**	39 ± 3.17	19.75 ± 1.42	35.2 ± 2.45	40.2 ± 2.12	39.5 ± 2.67
	**(25b)**	12 ± 1.82	**5.25 ± 1.35**	**9.75 ± 1.32**	**4.75 ± 0.86**	**1.86 ± 0.14**
	**(25c)**	47.5 ±3.25	21 ± 1.46	42.5 ± 3.63	25.3 ± 1.55	34 .6 ± 2.65
	**(25d)**	24 ± 2.86	20.6 ± 1.42	27.6 ± 1.8	**9.75 ± 0.92**	16.5 ± 2.23
	**(25e)**	52.1 ± 4.5	35 ± 2.5	>100	51.5 ± 1.78	49.7 ± 3.15
	**(25f)**	47.5 ±3.25	21 ± 1.46	42.5 ± 3.63	20 ± 2.25	15 ± 1.34
	**(25g)**	27 ± 2.14	**10.2 ± 1.38**	39.8 ± 2.57	15.5 ± 1.29	**7.25 ± 1.12**
	**(25h)**	17 ± 1.97	12.5 ± 1.76	20.3 ± 2.9	16.25 ± 1.92	27.3 ± 2.16

	**(25i)**	25.2 ±2.17	**10.5 ± 0.45**	43.5 ± 3.61	**8.5 ± 0.1.25**	**9.1 ± 1.89**
	**(25j)**	22.6 ±1.71	**8.0 ± 0.22**	24.5 ± 1.49	21.5 ± 1.63	12.75 ±1.55
	**(25k)**	27 ± 2.14	**10.2 ± 1.38**	39.8 ± 2.57	17.5 ± 1.59	12.5 ± 1.45
	**(25l)**	33 ± 3.52	14 ± 1.29	20 ± 1.26	29.7 ± 2.02	**6.25 ± 1.47**
	**(25m)**	34.6 ± 2.2	26.25 ± 1.49	35 ± 2.58	32 ± 3.05	65 ± 4.78
	**(25n)**	17 ± 1.97	12.5 ± 1.76	20.3 ± 2.9	27.3 ± 1.52	46.8 ± 4.12
	**(25o)**	25.2 ±2.17	**10.5 ± 0.45**	43.5 ± 3.61	18.75 ± 1.32	45 ± 3.97
Doxorubicin		15.1 ±1.75	11.3 ± 1.02	7.8 ± 1.24	9.2 ± 1.11	7.75 ± 1.15

[a] 50% Growth inhibitory concentration after treatment with the ligand for 48 h. and the values are represented as mean ± S.D. of three individual experiments.

Compound **25d** was obtained by introducing a 4-methoxy group in **25c** and found to be selectively cytotoxic to breast (MDA-MB-231) cancer cell line with an IC_{50} value of 9.75 µM, which was similar to that of doxorubicin. Compounds with dimethoxyphenyl (**25e** and **25f**) and benzyl (**25h**) moieties showed moderate cytotoxicity ranging from 12.5 to 52.1 µM but were less active in comparison to doxorubicin. A 3,4,5-trimethoxyphenyl is a crucial

fragment, which is frequently found in many anticancer entities.[46] Therefore, we synthesized 3,4,5-trimethoxyphenyl bearing analogue **25g** with improved anticancer activity against selected cancer cell lines: HeLa (IC_{50} = 10.2 µM) and MCF-7 (IC_{50} = 7.25 µM) where it was more potent than doxorubicin. Fluorophenyl substituted analogues **25i** and **25j** showed overall improvement in activity against MDA-MB-231 (**25i**, IC_{50} = 8.5 µM) and HeLa (**25j**, IC_{50} = 8.0 µM) with cytotoxicity better than doxorubicin. Introduction of an electron-donating *N,N′*-dimethylphenyl group led to compound **25k** with similar activity. The presence of a bromo substituent in the indole ring was found to be beneficial for the activity; compounds **25l** and **25m** exhibited enhanced cytotoxicity against MCF-7 cells compared to doxorubicin. Incorporation of a methoxy group in the indolyl moiety does not significantly improve the activity (**25n** *vs* **25c**; **25g** *vs* **25o**). The investigation of anticancer screening data revealed that phenyl, *p*-tolyl, trimethoxyphenyl and fluorophenyl as C2-arylamino moieties are good for the activity of arylaminothiazoles **25a-o**.

Based on the cytotoxicity profile of newly synthesized 2-arylamino-4-(3'-indolyl)thiazoles against different human cancer cell lines, **25b** was recognized as the most effective compound in inhibiting the proliferation of MCF-7 breast cancer cells. Thus, **25b** was selected as the ligand of interest for further mechanistic study on MCF-7 cells.

2.2.2.3 Dose dependent anticancer activity of 25b against MCF-7 cells

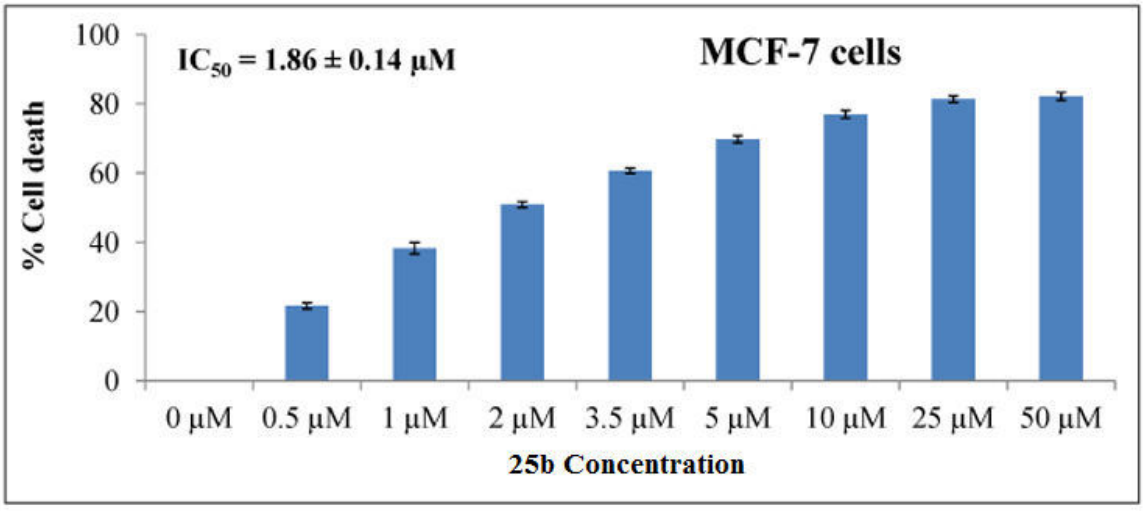


Figure 2.2.7 Dose dependent effect of compound **25b** on the cell viability and morphology of MCF-7 breast cancer cells. The cells were treated with varying concentration of **25b**, as indicated for 48 h.: The cell viability was measured by MTT assay. The result represents the mean ± S.D. of three experiments.

The effect of **25b** on the proliferation of MCF-7 cells was determined by MTT assay. We found that **25b** inhibited the growth of MCF-7 in dose dependent manner, upon 48 h. treatment and maximum proliferation inhibition of ~ 85% was attained at a concentration of 50 µM (Figure 2.2.7). As summarised in Table 2.2.2, the half-maximal inhibitory concentration (IC_{50}) value of 1.86 ± 0.14 µM prompted us to elucidate the anticancer mechanism of **25b** against human breast cancer MCF-7 cells.

2.2.2.4 Compound 25b induces apoptosis in MCF-7 breast cancer cells

Compound **25b** induced concentration dependent apoptosis in MCF-7 cells were detected using Annexin-V-FITC and propidium iodide (PI) double staining assay by flow cytometry. We observed a significant increase in early apoptotic cells when treated with increasing doses of the compound for 48 h.

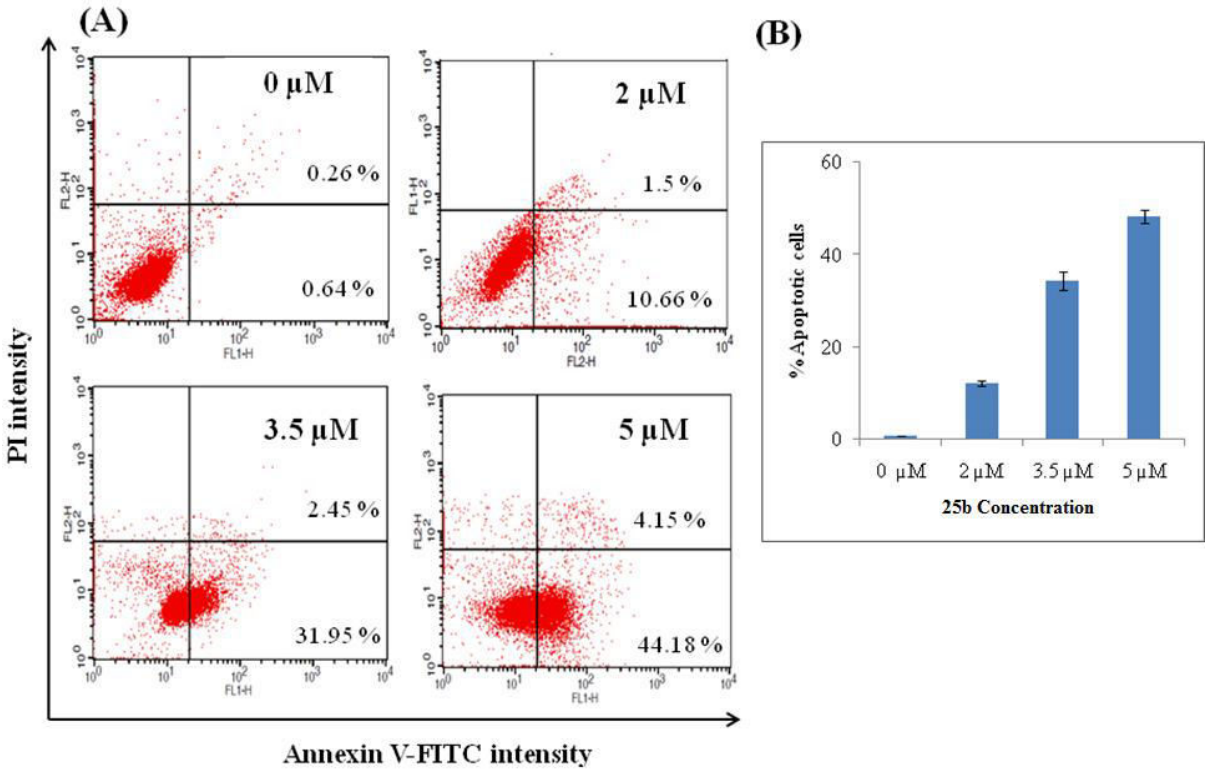


Figure 2.2.8 Annexin V- PI assay of **25b** induced apoptosis in MCF-7 cells. The effect of the compound **25b** on the apoptosis induction in MCF-7 cells was monitored by treating the cells with increasing concentration (0-5 µM) of **25b** for 48 h. and staining them with Annexin-V-FITC and PI, as described in the experimental section. (A) Representation of the apoptotic population of MCF-7 cells induced in response to the ligand. (B) Quantification of the apoptotic cells obtained from the above experiments performed thrice. Data represents mean ± S.D.

In presence of 2 μM, 3.5 μM and 5 μM of **25b**, percent of cells in early and late apoptotic phases were, 10.66% and 1.5%, 31.96% and 2.45%, and 44.18% and 4.15%, respectively (Figure 2.2.8A and 2.2.8B). These results indicated that **25b** causes cell death by activating apoptosis in MCF-7 cells.

In this study, we first developed a microwave-assisted high yielding and rapid synthetic protocol to prepare a new series of 2-arylamino-4-(3′-indolyl)thiazoles **25a-o** from the reaction of *α*-tosyloxyketones and *N*-arylthioureas in PEG-400. This synthesized series of 2-arylamino-4-(3′-indolyl)thiazole derivatives **25a-o** screened against a panel of human cancer cell lines and found that **25b** was the most potent compound among all the ligands and its anticancer potency was most apparent against MCF-7 human breast adenocarcinoma cells. Hence, we selected the compound **25b** as the test ligand for further investigation of the anti-cancer mechanism on MCF-7 cells. Evaluation of the antiproliferative activity by MTT assay showed that **25b** inhibited the growth of human breast cancer MCF-7 cells in concentration dependent manner with IC_{50} value of 1.86 ± 0.14 μM (Figure 2.2.7A). It is well-known that apoptosis is the prevalent mode of cancer cell death by most chemotherapeutic agents and thus, has remained preferred approach for anticancer therapy.[28,47] Externalization of the phosphatidylserine from inner to outer leaflet of the cell membrane, which is characteristic feature of apoptosis, was exploited to monitor the apoptosis induction by Annexin-V-FITC/PI assay. In comparison to control cells, incubation with increasing ligand concentration caused significant increase in apoptotic population of MCF-7 cells (Figure 2.2.8).

2.2.3 Conclusions

In summary, we have developed a facile and microwave-assisted rapid synthetic protocol for the construction of a new series of 2-arylamino-4-(3′-indolyl)thiazoles. This eco-friendly protocol offers several advantages such as easy workup and isolation procedures, shorter reaction times and use of benign reaction solvent and readily available starting materials. The synthesized 2-arylamino-4-(3′-indolyl)thiazoles were analyzed for cytotoxicity in A549 (lung), HeLa (cervix), HepG2 (liver), MCF-7 and MDA-MB-231 (breast) cancer cell lines. Compound **25b** was found to be the most effective with IC_{50} values between 1.86 and 12 μM. Moreover, **25b** induces apoptosis in MCF-7 breast cancer cells.

2.2.4 Experimental

Chemistry

Solvents and reagents were procured commercially and used as such without any further purification. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 precoated aluminium sheets. Melting points were determined with electrothermal capillary melting point apparatus (*E-Z*-melting) and are uncorrected. Infrared spectra were recorded on Shimadzu IR Prestige-21 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra (400 & 100 MHz, respectively) were recorded on Bruker Avance II 400 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in parts per million (δ) and coupling constants (J) in Hz. Mass spectra were obtained on a 'Hewlett–Packard' HP GS/MS 5890/ 5972.

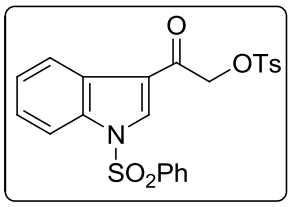
General procedure for synthesis of 1-(phenylsulfonyl)-1*H*-indole:[6] To a solution of indole **22** (10 mmol) in toluene (30 mL) was added 50% NaOH (35 mL), tetrabutylammonium bromide (TBAB) (0.322 g, 1 mmol) and benzensulfonyl chloride (21.12 g, 12 mmol) in toluene (20 mL) with continuous stirring at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After completion of reaction, contents were diluted with ethyl acetate (200 mL) and washed with water (2 × 20 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated under *vaccuo* to obtain off-white solid in 85-88% yields.

General procedure for synthesis of 3-acetyl-1-benzenesulfonylindoles (23a-c):[6] To a cooled (0 °C) solution of $AlCl_3$ (7.64 g, 58.35 mmol) in dichloromethane (60 mL) was added acetic anhydride (3.30 mL, 35.01 mmol). A solution of *N*-benzenesulfonylindole (11.67 mmol) in dichloromethane (15 mL) was added to the above solution and stirred for 2 h. After completion of the reaction as indicated by TLC, product was extracted by using dichlomethane (2 × 300 mL) and washed with brine solution (2 × 100 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated under *vaccuo* to obtain an off-white solid which upon recrystallization with methanol afforded pure **23a-c** in 85-90% yields.

General procedure for the synthesis of 3-(*α*-tosyloxyacetyl) indoles (24a-c):[48] To a solution of 3-acetyl-1-benzenesulfonylindoles **23a-c** (10.0 mmol) in acetonitrile (50 mL) was added [hydroxy(tosyloxy)iodo]benzene (4.70 g, 12.0 mmol) in portions. The resulting

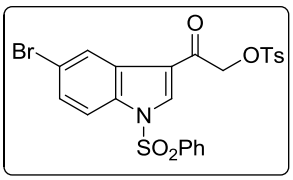
reaction mixture was stirred at 60 °C for 18 h. After completion of the reaction, solvent was distilled off to obtain solid residue which was crystallized from methanol to afford pure 3-(*α*-tosyloxyacetyl)-1-benzenesulfonylindoles **24a-c** in 70–84% yields.

3-(*α*-Tosyloxyacetyl)-1-benzenesulfonylindole (24a)[48]



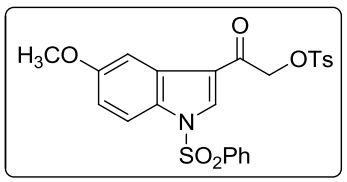
Yield 84%; White solid; mp 137-139 °C; IR (KBr, *v,* cm^{-1}): 3147, 2983, 1693, 1597, 1174, 1143, 1087, 873; ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.36 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 8.00–7.94 (m, 3H), 7.83 (d, *J* = 8.40 Hz, 2H), 7.63–7.61 (m, 1H), 7.55–7.51 (m, 2H), 7.40–7.30 (m, 4H), 5.02 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 186.9, 145.6, 137.4, 135.0, 134.5, 132.8, 132.4, 130.1, 129.8, 128.2, 127.5, 127.3, 126.3, 125.5, 123.0, 117.7, 113.1, 70.8, 21.7; ESI (FAB) *m/z* calcd for $C_{23}H_{20}NO_6S_2$: 470.1 $(M + H)^+$, found 470.0.

3-(*α*-Tosyloxyacetyl)-1-benzenesulfonyl-5-bromoindole (24b)[48]



Yield 75%; Off white solid; mp 148-149 °C; IR (KBr, *v,* cm^{-1}): 3150, 2978, 1685, 1583, 1170, 1135, 1090, 865; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 8.91 (s, 1H), 8.21 (s, 1H), 8.06 (d, *J* = 7.4 Hz, 2H), 7.85-7.83 (m, 3H), 7.68 (t, *J* = 7.1 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 2H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 2H), 5.39 (s, 2H), 2.38 (s, 3H);^{13}C NMR (100 MHz, DMSO-d_6): *δ* 187.0, 145.3, 136.7, 135.5, 133.1, 132.9, 130.2, 129.0, 128.9, 128.4, 128.1, 127.6, 125.9, 125.0, 118.4, 116.4, 115.0, 70.9, 21.5; ESI (FAB) *m/z* calcd for $C_{23}H_{19}BrNO_6S_2$: 547.98 $[M + H]^+$, found 547.98.

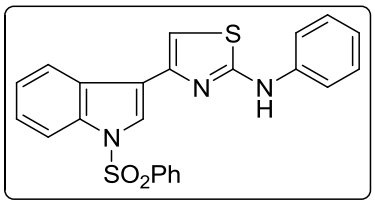
3-(*α*-Tosyloxyacetyl)-1-benzenesulfonyl-5-methoxyindole (24c)[48]



Yield 77%; White solid; mp 128-129 °C; IR (KBr, *v,* cm^{-1}): 3161, 2972, 1687, 1579, 1269, 1173, 1141, 1093, 868; ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 8.36 (s, 1H), 7.72 (dd, *J* = 7.5, 4.8 Hz, 3H), 7.80 (dd, *J* = 7.4, 1.3 Hz, 2H), 7.67 (d, *J* = 1.4 Hz, 1H), 7.64–7.57 (m, 1H), 7.54–7.51 (m, 2H), 7.45 (s, 2H), 6.92 (dd, *J* = 7.5, 1.4 Hz, 1H), 4.88 (s, 2H), 3.78 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): *δ* 189.0, 156.4, 146.4, 141.7, 139.5, 138.0, 133.2, 132.5, 129.6, 129.4, 129.1, 128.9, 128.2, 122.3, 116.4, 115.7, 109.1, 68.1, 56.0, 21.1; ESI (FAB) *m/z* calcd for $C_{24}H_{22}NO_7S_2$: 500.08 $[M + H]^+$, found 500.09.

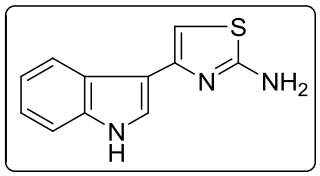
General procedure for the synthesis of 4-(1*H*-indol-3-yl)-*N*-phenylthiazol-2-amines (25a-o): PEG-400 (0.2 mL) was added to a mixture of α-tosyloxyketone **24** (0.01 mmol) and *N*-arylthiourea **19** (0.01 mmol) in sealed vial (10 mL) and mixed thoroughly. The resulting reaction mixture was irradiated in a CEM focused microwave oven with P = 50 w/100 psi at 80 °C for 5 min. After completion of the reaction as indicated by TLC, *in situ* solid NaOH (0.083 g, 0.20 mmol) was added and again irradiated in a CEM focused microwave oven for 5 min using same reaction condition. Upon completion of reaction as confirmed by TLC, water was added (7 mL) and thus obtained suspension was filtered, washed with cold water (10 mL), dried and recrystallized from ethanol to afford **25a-o** in 70–85% yields.

***N*-Phenyl-4-(1-(phenylsulfonyl)-1*H*-indol-3-yl)thiazol-2-amine (26)**



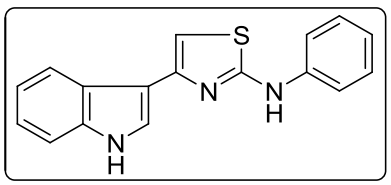
Yield 95%; White solid; mp 201-203 °C; IR (KBr, *v,* cm^{-1}): 3404, 3050, 1628, 1582, 1512, 1180, 679; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.2 (d, *J* = 7.6 Hz, 2H), 8.04–8.00 (m, 2H), 7.7 (d, *J* = 8.0 Hz, 2H), 7.61–7.59 (m, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.43–7.36 (m, 4H), 7.10 (d, *J* = 8.0 Hz, 2H), 6.98 (t, *J* = 7.2 Hz, 1H); ESI (FAB) *m/z* calcd for $C_{23}H_{18}N_3O_2S_2$: 432.08 $(M + H)^+$, found 432.10.

4-(1*H*-Indol-3-yl)thiazol-2-amine (25a)



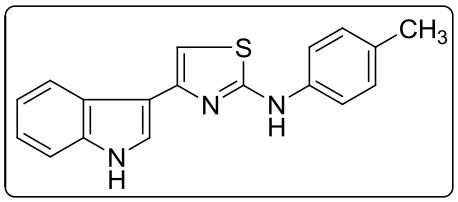
Yield 85%; Reddish brown solid; mp 152-153 °C; IR (KBr, *v,* cm^{-1}): 3425, 3402, 3117, 1643, 1528, 1420, 1366, 1234, 1011, 818, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.84 (s, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 2.5 Hz, 1H), 7.40 (d, *J* = 7.4 Hz, 1H), 7.15–7.08 (m, 2H), 6.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8, 146.7, 137.0, 125.0, 124.6, 121.8, 120.6, 119.8, 112.4, 112.2, 97.7; ESI (FAB) *m/z* calcd for $C_{11}H_{10}N_3S$: 216.0 $(M + H)^+$, found 216.1.

4-(1*H*-Indol-3-yl)-*N*-phenylthiazol-2-amine(25b)



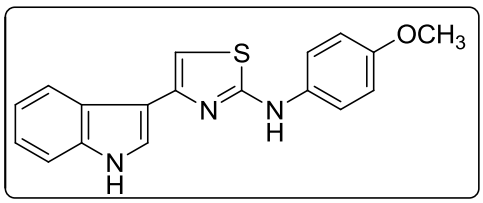
Yield 85%; Orange solid; mp 168-169 °C; IR (KBr, *v,* cm^{-1}): 3387, 3117, 3055, 1597, 1535, 1420, 1311, 1242, 1095, 741; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (s, 1H), 8.00 (d, *J* = 9.0 Hz, 1H), 7.77 (d, *J* = 2.6 Hz, 1H), 7.46–7.35 (m, 5H), 7.24–7.26 (m, 3H), 7.12–7.08 (m, 1H), 6.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.8, 147.0, 141.9, 137.1, 129.4, 125.1, 125.0, 121.9, 121.4, 120.4, 120.0, 117.2, 112.3, 112.1, 98.8; ESI (FAB) *m/z* calcd for $C_{17}H_{14}N_3S$: 292.10 $(M + H)^+$, found 292.16.

4-(1*H*-Indol-3-yl)-*N*-*p*-tolylthiazol-2-amine (25c)



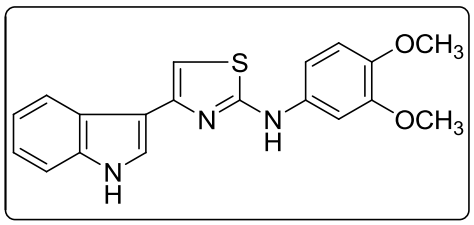
Yield 75%; Reddish brown solid; mp 189–190 °C; IR (KBr, *v,* cm^{-1}): 3433, 3394, 3117, 1597, 1535, 1420, 1242, 879, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (s, 1H), 9.98 (s, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.80 (s, 1H), 7.67 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 7.8 Hz, 1H), 7.16–7.12 (m, 2H), 6.98–6.90 (m, 3H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.6, 146.6, 139.0, 136.6, 129.6, 129.1, 124.5, 124.2, 121.2, 119.8, 119.4, 116.9, 111.7, 99.5, 97.5, 20.4; ESI (FAB) *m/z* calcd for $C_{18}H_{16}N_3S$: 306.1 $(M + H)^+$, found 306.1.

4-(1*H*-Indol-3-yl)-*N*-(4-methoxyphenyl)thiazol-2-amine (25d)



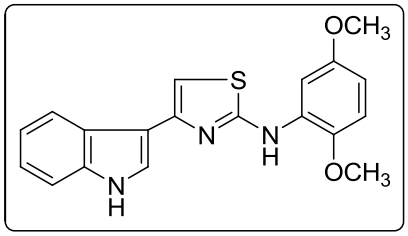
Yield 72%; Light brown solid; mp 189-190 °C; IR (KBr, *v,* cm^{-1}): 3271, 3055, 2932, 1612, 1512, 1242, 1026, 825, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 11.08 (s, 1H), 7.93–7.91 (m, 1H), 7.74–7.70 (m, 1H), 7.44–7.41 (m, 1H), 7.29–7.26 (m, 2H), 7.18–7.12 (m, 2H), 6.94–6.90 (m, 2H), 6.64–6.62 (m, 1H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.8, 158.7, 137.0, 136.1, 130.0, 125.8, 124.1, 123.9, 122.8, 120.9, 118.9, 115.3, 112.6, 104.6, 95.0, 55.6; ESI (FAB) *m/z* calcd for $C_{18}H_{16}N_3OS$: 322.1 $(M + H)^+$, found 322.0.

***N*-(3,4-Dimethoxyphenyl)-4-(1*H*-indol-3-yl)thiazol-2-amine (25e)**



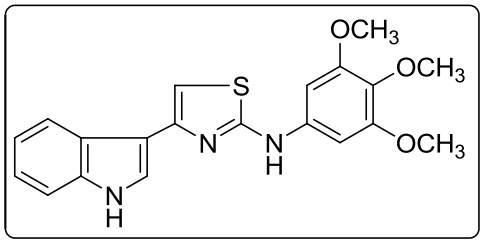
Yield 70%; Light brown solid; mp 148-149 °C; IR (KBr, *v,* cm^{-1}): 3334, 3286, 2939, 1597, 1512, 1234, 1134, 1026, 849, 764; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 10.91 (s, 1H), 8.31 (d, J = 7.4 Hz, 1H), 8.07–7.98 (m, 1H), 7.55–7.46 (m, 2H), 7.18 (d, J = 7.5 Hz, 1H), 7.14–7.07 (m, 3H), 6.98 (s, 1H), 3.81 (s, 3H), 3.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.2, 149.7, 149.4, 144.8, 136.3, 134.7, 127.2, 122.4, 122.2, 120.3, 120.2, 113.1, 112.9, 112.4, 110.3, 103.9, 98.3, 56.3, 55.9; ESI (FAB) *m/z* calcd for $C_{19}H_{18}N_3O_2S$: 352.1 $(M + H)^+$, found 352.0.

***N*-(2,5-Dimethoxyphenyl)-4-(1*H*-indol-3-yl)thiazol-2-amine (25f)**



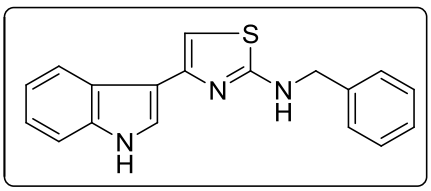
Yield 58%; Light brown solid; mp 141-143 °C; IR (KBr, *v,* cm^{-1}): 3217, 3132, 2939, 1605, 1535, 1219, 1119, 1041; ^1H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 9.65 (s, 1H), 8.29 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.17–7.07 (m, 2H), 6.99–6.94 (m, 2H), 6.54 (d, J = 8.0 Hz, 1H), 3.82 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 154.3, 145.9, 137.6, 127.2, 126.7, 126.6, 124.6, 123.4, 121.5, 119.6, 113.6, 113.3, 112.1, 107.9, 105.3, 96.4, 57.1, 56.5; ESI (FAB) *m/z* calcd for $C_{19}H_{18}N_3O_2S$: 352.1 $(M + H)^+$, found 352.1.

4-(1*H*-Indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine (25g)



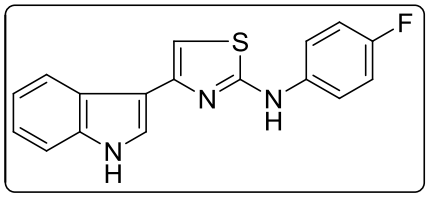
Yield 75%; Light brown solid; mp 226-228 °C; IR (KBr, *v,* cm^{-1}): 3379, 3340, 3117, 1612, 1535, 1458, 1420, 1234, 1126, 1011, 825, 748; ^1H NMR (400 MHz, DMSO-d_6): δ 11.27 (s, 1H), 10.12 (s, 1H), 8.14 (d, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.43–7.41 (m, 1H), 7.16–7.09 (m, 3H), 7.06 (d, J = 8.0 Hz, 1H), 6.97 (s, 1H), 3.82 (s, 6H), 3.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.5, 152.9, 146.6, 137.6, 136.5, 131.6, 124.6, 123.8, 121.3, 120.0, 119.2, 111.7, 99.5, 97.7, 94.5, 60.1, 55.5; ESI (FAB) *m/z* calcd for $C_{20}H_{20}N_3O_3S$: 382.1 $(M + H)^+$, found 382.0.

***N*-Benzyl-4-(1*H*-indol-3-yl)thiazol-2-amine (25h)**



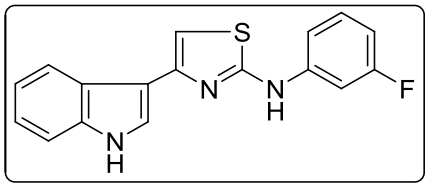
Yield 79%; Light brown solid; mp 130-131 °C; IR (KBr, *v,* cm^{-1}): 3394, 3217, 1620, 1536, 1427, 1342, 1234, 1103, 972, 741; ^1H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 8.01 (dd, J = 6.7, 2.4 Hz, 1H), 7.66 (d, J = 2.6 Hz, 1H), 7.44–7.29 (m, 6H), 7.26–7.21 (m, 2H), 6.68 (s, 1H), 5.89 (s, 1H), 4.52 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 146.7, 137.8, 136.6, 128.7, 127.7, 127.8, 125.1, 123.7, 122.3, 120.4, 120.2, 113.1, 111.4, 98.7, 49.9; ESI (FAB) *m/z* calcd for $C_{18}H_{16}N_3S$: 306.1 $(M + H)^+$, found 306.1.

***N*-(4-Fluorophenyl)-4-(1*H*-indol-3-yl)thiazol-2-amine (25i)**



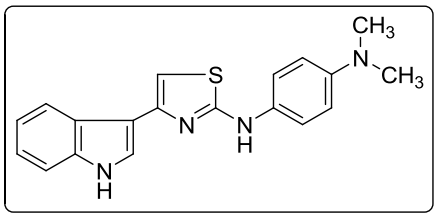
Yield 73%; Light brown solid; mp 169-171 °C; IR (KBr, *v,* cm^{-1}): 3394, 3302, 1620, 1512, 1219, 1011, 833, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 10.51 (s, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.89 (s, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.40–7.36 (m, 2H), 7.17–7.13 (m, 2H), 7.11 (s, 1H), 6.78–6.74 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2, 167.9 (d, $J_{C\text{-}F}$ = 248.4 Hz), 141.8, 136.3 (d, $J_{C\text{-}F}$ = 7.3 Hz), 130.7 (d, $J_{C\text{-}F}$ = 7.3 Hz), 128.7, 127.6, 125.8, 123.7, 120.7, 118.1, 118.0 (d, $J_{C\text{-}F}$ = 22.1 Hz), 112.6 (d, $J_{C\text{-}F}$ = 22.1 Hz), 109.4, 100.6; ESI (FAB) *m/z* calcd for $C_{17}H_{13}FN_3S$: 310.1 $(M + H)^+$, found 310.2.

***N*-(3-Fluorophenyl)-4-(1*H*-indol-3-yl)thiazol-2-amine (25j)**



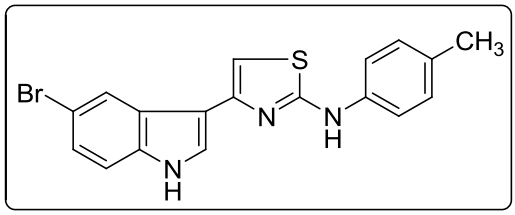
Yield 74%; Orange solid; mp 143-144 °C; IR (KBr, *v,* cm^{-1}): 3394, 3305, 2924, 1612, 1520, 1458, 1366, 1234, 1111, 856, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (s, 1H), 10.49 (s, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.80 (s, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.39–7.33 (m, 2H), 7.17–7.09 (m, 2H), 7.04 (s, 1H), 6.78–6.74 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.3, 161.8 (d, $J_{C\text{-}F}$ = 243.5c Hz), 147.9, 135.0, 133.2 (d, $J_{C\text{-}F}$ = 7.7 Hz), 126.0, 122.5, 121.8, 121.4 (d, $J_{C\text{-}F}$ = 21.3 Hz), 121.1, 120.7, 119.5, 119.2, 118.9, 113.6 (d, $J_{C\text{-}F}$ = 21.3 Hz), 110.6, 108.4; ESI (FAB) *m/z* calcd for $C_{17}H_{13}FN_3S$: 310.1 $(M + H)^+$, found 310.1.

***N*-1-(4-(1*H*-Indol-3-yl)thiazol-2-yl)-*N4,N4*-dimethylbenzene-1,4-diamine (25k)**



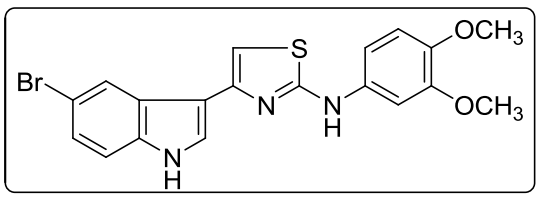
Yield 70%; Brown solid; mp 145-147 °C; IR (KBr, *v,* cm^{-1}): 3387, 3225, 2924, 1612, 1520, 1427, 1227, 1126, 941, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.06 (s, 1H), 9.59 (s, 1H), 8.01 (d, J = 7.0 Hz, 1H), 7.71 (d, J = 2.5 Hz, 1H), 7.55 (d, J = 9.0 Hz, 2H), 7.43 (d, J = 7.1 Hz, 1H), 7.16–7.09 (m, 2H), 6.75 (d, J = 9.0 Hz, 2H), 6.67 (s, 1H), 2.90 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 147.0, 146.3, 137.0, 132.5, 124.9, 124.6, 121.6, 119.8, 119.5, 113.7, 112.2, 112.0, 97.2, 41.3; ESI (FAB) *m/z* calcd for $C_{19}H_{19}N_4S$: 335.1 $(M + H)^+$, found 335.0.

4-(5-Bromo-1*H*-indol-3-yl)-*N*-*p*-tolylthiazol-2-amine (25l)



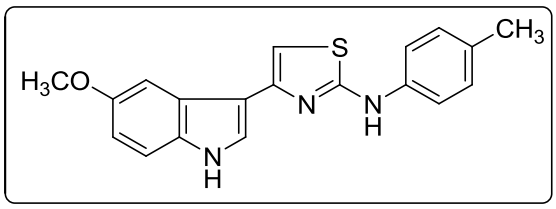
Yield 73%; Brown solid; mp 149-150 °C; IR (KBr, *v,* cm^{-1}): 3376, 3356, 1589, 1520, 1450, 1396, 1227, 1049, 879, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H), 10.13 (s, 1H), 8.35 (s, 1H), 7.88 (s, 1H), 7.62 (s, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 7.2 Hz, 2H), 7.01 (s, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 146.3, 139.4, 135.6, 130.4, 130.3, 129.8, 126.9, 126.1, 124.3, 123.1, 117.5, 112.7, 111.7, 98.9, 20.9; ESI (FAB) *m/z* calcd for $C_{18}H_{15}BrN_3S$: 384.0 $(M + H)^+$, found 383.9.

4-(5-Bromo-1*H*-indol-3-yl)-*N*-(3,4-dimethoxyphenyl)thiazol-2-amine (25m)



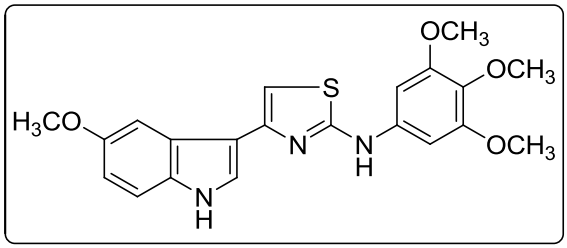
Yield 70%; Brown solid; mp 185-186 °C; IR (KBr, *v,* cm^{-1}): 3340, 3302, 2932, 1605, 1512, 1450, 1234, 1134, 1018, 849, 764; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.35–8.33 (m, 1H), 8.31–8.25 (m, 1H), 8.17–8.12 (m, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.43 (t, *J* = 8.6 Hz, 1H), 7.34–7.28 (m, 2H), 7.13 (d, *J* = 7.9 Hz, 1H), 3.5 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 149.7, 148.54, 145.45, 136.58, 131.79, 127.53, 124.01, 122.54, 121.1, 121.0, 120.3, 112.7, 112.3, 111.9, 110.0, 105.9, 55.7, 55.5; ESI (FAB) *m/z* calcd for $C_{19}H_{17}BrN_3O_2S$: 430.0 $(M + H)^+$, found 430.0.

4-(5-Methoxy-1*H*-indol-3-yl)-*N*-*p*-tolylthiazol-2-amine (25n)



Yield 79%; Light brown solid; mp 257-259 °C; IR (KBr, *v,* cm^{-1}): 3294, 3094, 3009, 1636, 1589, 1435, 1366, 1273, 1119, 810; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 1H), 10.71 (s, 1H), 7.85 (d, *J* = 1.8 Hz, 1H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 1.8 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 2H), 7.01 (s, 1H), 6.83 (dd, *J* = 8.8, 2.3 Hz, 1H), 3.84 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 154.5, 149.5, 138.9, 132.1, 131.3, 130.1, 129.8, 119.0, 118.0, 113.1, 112.6, 112.5, 109.6, 98.1, 55.9, 20.9; ESI (FAB) *m/z* calcd for $C_{19}H_{18}N_3OS$: 336.12 $(M + H)^+$, found 336.10.

4-(5-Methoxy-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazol-2-amine (25o)



Yield 71%; Light brown solid; mp 130-132 °C; IR (KBr, *v,* cm^{-1}): 3340, 3117, 2939, 1605, 1512, 1458, 1342, 1273, 1126, 926, 795; ^1H NMR (400 MHz, DMSO-d_6): δ 11.13 (s, 1H), 10.11 (s, 1H), 7.73 (d, J = 2.6 Hz, 1H), 7.53 (d, J = 2.3 Hz, 1H), 7.37–7.30 (m, 1H), 7.15 (s, 2H), 6.96 (s, 1H), 6.82 (dd, J = 8.8, 2.4 Hz, 1H), 3.81 (s, 6H), 3.80 (s, 3H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.9, 154.3, 153.5, 147.0, 138.2, 132.3, 125.5, 125.4, 112.9, 112.7, 112.0, 111.7, 103.1, 98.5, 95.3, 60.6, 56.1, 56.0; ESI (FAB) *m/z* calcd for $C_{21}H_{22}N_3O_4S$: 412.13 $(M + H)^+$, found 412.10.

Materials and methods

Materials

Nutrient mixture DMEM (supplemented with L-glutamine), Penicillin- streptomycin and Fetal Bouvine Serum (FBS) were purchased from GIBCO, Invitrogen, USA. 0.25% trypsin-EDTA was purchased from Himedia (Mumbai, India). The primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The HRP-tagged secondary antibodies and the Bradford protein estimation kit were purchased from Genei, India. All other chemicals and reagents were obtained from Sisco Research Laboratories, India.

Cell culture and treatment

Non-small cell lung carcinoma (A549), cervical carcinoma (HeLa), hepatocellular carcinoma (HepG2) and breast carcinoma (MDA-MB-231 and MCF-7) cells were cultured in DMEM medium containing 3.7 g/L $NaHCO_3$, 1mM L-glutamine, 10% FBS, 100 IU penicillin and 100mg/ml streptomycin and incubated in the humified atmosphere at 37 °C in presence of 5% CO_2. When the cells reached about 80% confluence, they were trypsined with 0.25% trypsin- EDTA and subcultured at required density and treated with the ligands (**25a-o**) for 48h. To measure the protective effect of NAC, the ROS scavenger, against ligand (**25b)** induced cytotoxicity on MCF-7 cells, the cells were pre-incubated with 10 mM NAC for 1 h. followed by media replacement before adding the test ligand. The cells were maintained under observation and the MCF-7 cells were photographed by Olympus model CKX41 inverted microscope.

Cell viability assay

The cell viability was measured by MTT assay. The mammalian cells (A549, HeLa, HepG2, MDA-MB-231 and MCF-7) were seeded in 96 well plate at a density of ~2 X 10^3 cells per well were allowed to attach overnight and then, treated with the ligands (**25a-o)** and doxorubicin at varying concentration (0-100 µM) for 48 h. Then, the cells were incubated with MTT (5 mg/mL) solution at 37 °C for 4 h. and the formazan crystals formed were dissolved by adding 150 µL DMSO to each well under shaking condition in dark for 2 h. The absorbance was measured at 570 nm wavelength using an ELISA reader (MultiskanEX, Lab systems, Helsinki, Finland) and the Data were calculated as the percentage of inhibition by the following formula:

$$\% \text{ inhibitioin} = (1-A_t/A_s) \text{ X } 100\% \qquad [1]$$

Where A_t and A_s denoted the absorbance of the test sample and solvent control, respectively.[49]

Assessment of apoptosis

Cultured mammalian breast cancer cells (MCF-7) were exposed to varying concentration of **25b** (0-5 µM), in absence or presence of NAC pre-treatment, for 48 h. and the live cells were stained with Annexin-V-FITC in Ca^+- enriched binding buffer (15 min, room temperature) and counterstained with Propidium Iodide (PI). The results were obtained by FACS Calibur flow cytometer (from Becton- Dickson) using FL1 and FL2 channels for detecting the Annexin V FITC and PI, respectively, and analysed using Cell Quest software.[30]

2.3 Part B: Synthesis of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides as cytotoxic agents

2.3.1 Rational design

Indole derivatives are recognized as a class of important heterocycles for their useful pharmacological properties.[50] Existing studies have proved their wide range of biological activities such as antimicrobial, antiviral and antitumor agents.[51-53] Indole ring system is found in a number of clinically useful therapeutic agents, such as, indomethacin, indoramin, and indorenate.[54] Moreover, several indole containing molecules have been documented for their interesting anticancer activities. For example, indolylthiazole (Camalexin) **2** is the characteristic phytoalexin of *Arabidopsis thaliana*, which is induced by a variety of plant pathogens as well as human tumor cell lines.[4] Recently, an indole-based small molecule, indibulin (**9**) was found to display significant activity against a panel of cancer cell lines (IC_{50} = 0.036-0.28 μM) *via* destabilization of tubulin dynamics.[16,55] Very recently, antimitotic agents, 3-aroylindole (**10**) and arylthioindole (**11**), have been reported to possess interesting antitumor activity.[56] In 2012, Li *et al.* explored 2-indolyl-4-benzoyl-imidazole (**12**) as tubulin targeting anticancer agent with an average IC_{50} value of 3.8 nM against the tested cancer cell lines.[57] Isolated from marine sponges, the bisindole alkaloids, Topsentins[58] (**27**) and Nortopsentins[59] (structure not shown) have received increasing attention due to their interesting anticancer activities (Figure 2.3.1).

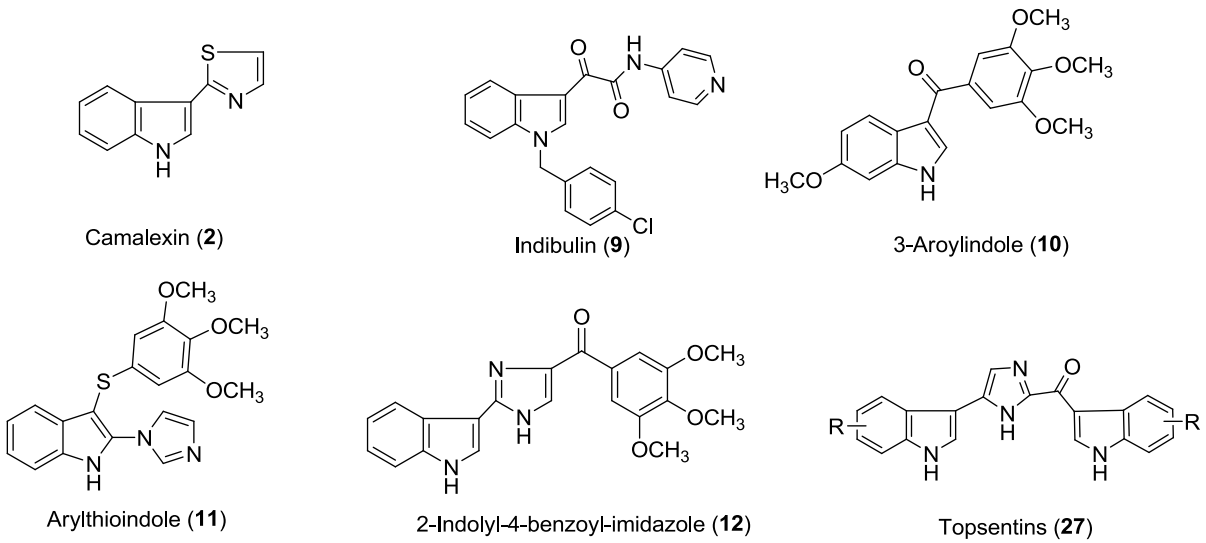


Figure 2.3.1 Representative indole based anticancer agents

Over the past few years, various substituted thiazole analogs have been reported to demonstrate diverse biological activities including antimicrobial, antiinflammatory,

antimalarial, antitubercular, antiviral and anticancer activities.[22, 60-62] Among the thiazoles, substituted thiazole carboxamides exhibited encouraging anticancer activities.[63] For examples; Hofle and co-workers isolated peptides, Tubulysins A and D (**28a** and **28b**) from the myxobacteria *Archangium gephyra* and *Angiococcus disciformis,* respectively.[64,65] Tubulysin A **28a** is a highly cytotoxic peptide with antimitotic activity that induces microtubule polymerisation and triggers the apoptotic process.[66] Tubulysin D **28b** showed potent anticancer activity against the multidrug-resistant cervix carcinoma cell line (P-glycoprotein-expressing human KBV1; IC_{50} = 0.31 nM).[67] In 2012, Huang and coworker identified 2-aryl-*N*-(2-(piperazin-1-yl)phenyl)-thiazole-4-carboxamide (ALIS hit **29a**), as a potent CHK1 inhibitor.[68] Various 2-phenylthiazole-4-carboxamides **29b** synthesized by Foroumadi *et al.* were also found to be potent anticancer agents with interesting cytotoxic activity (average IC_{50} = 2-10 μM).[69] Similarly, Dasatinib (**30**), with thiazole carboxamide scaffold was reported to possess activity against cultured human prostate and breast cancer cells (Figure 2.3.2).[70,71] Recently, tiazofurin mimics 2-Substituted thiazole-4-carboxamide (**31**) derivatives prepared by Popsavin *et al.* as an apoptosis inducing anticancer agent (Figure 2.3.3).[72]

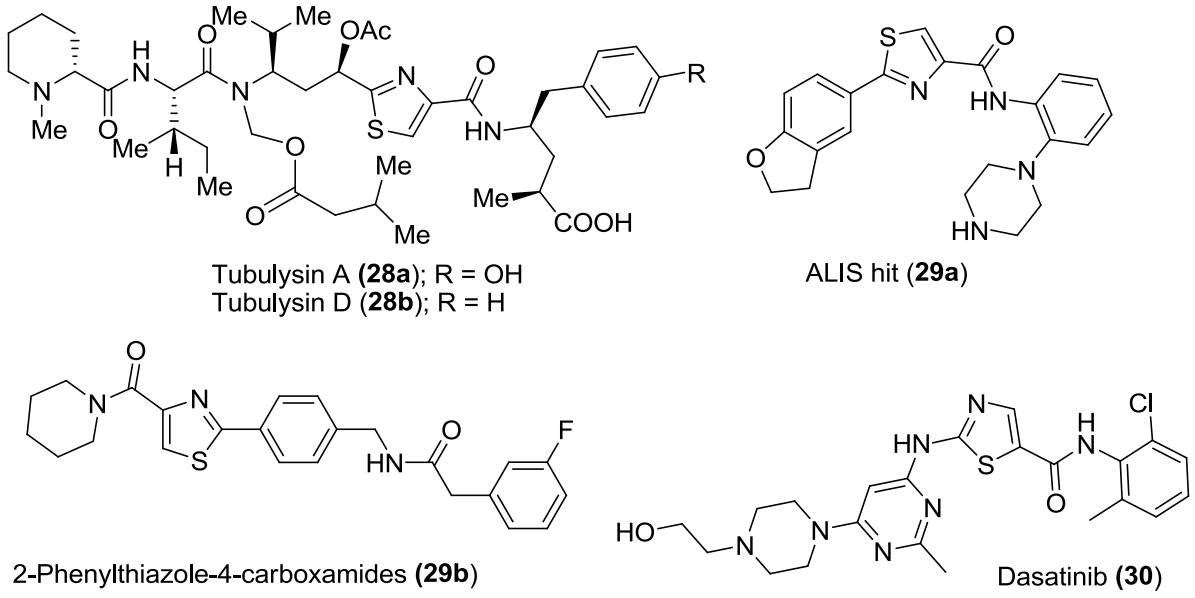


Figure 2.3.2 Anticancer agents with thiazole carboxamide scaffold

Cancer is one of the most formidable afflictions in the world. Therefore, new cancer chemotherapeutic agents are urgently needed to combat this challenging and most difficult disease.[73-75] Apoptosis (programmed cell death) is a set of ordered events that enables the selective removal of cells from tissue and is essential for homeostasis and proper functioning

of multi-cellular organisms. Apoptosis is one of the major pathways that leads to the process of cell death for many chemotherapeutical agents, hence, it remained an attractive approach in anticancer drug discovery research.[76] In continuation of our efforts to identify potent anticancer agents, recently, we have identified indole-based heterocycles which exerts their anticancer effects through the induction of apoptosis.[3,37,38] In the light of above mentioned observations regarding potential of indole-based anticancer agents, thiazole carboxamides and our previous results, we designed a new library of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**) by incorporating crucial structural features of both the bioactive scaffolds (Figure 2.3.3).

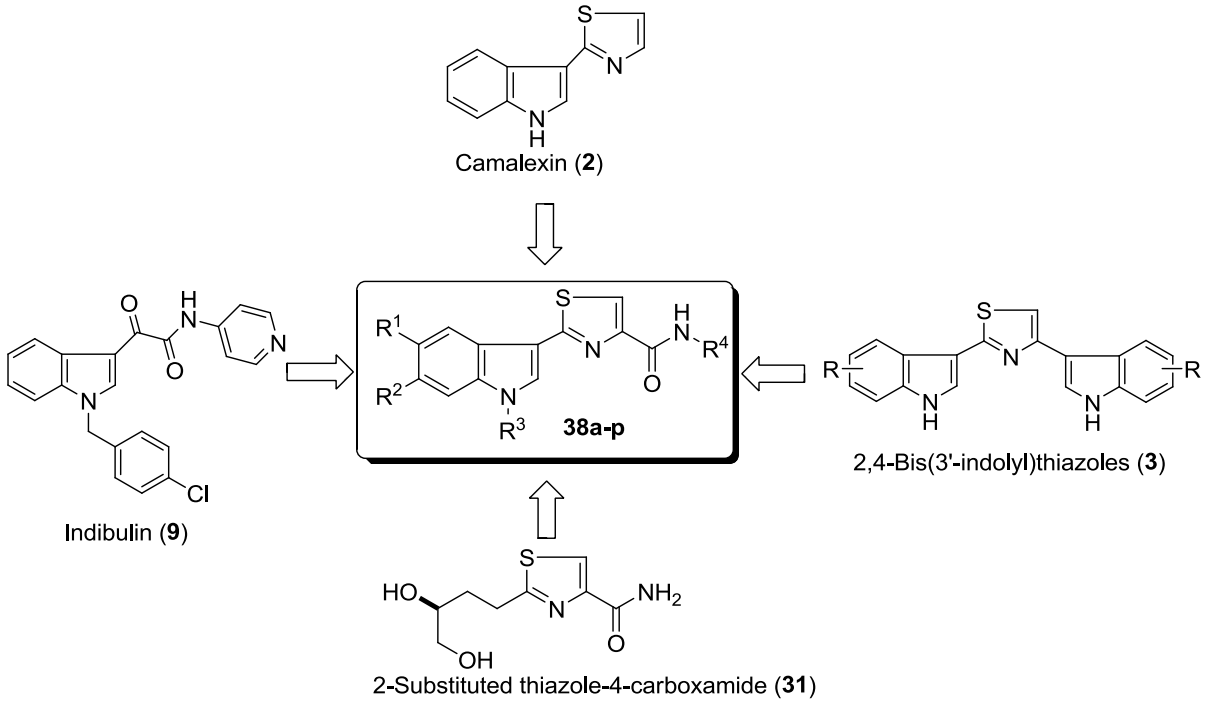
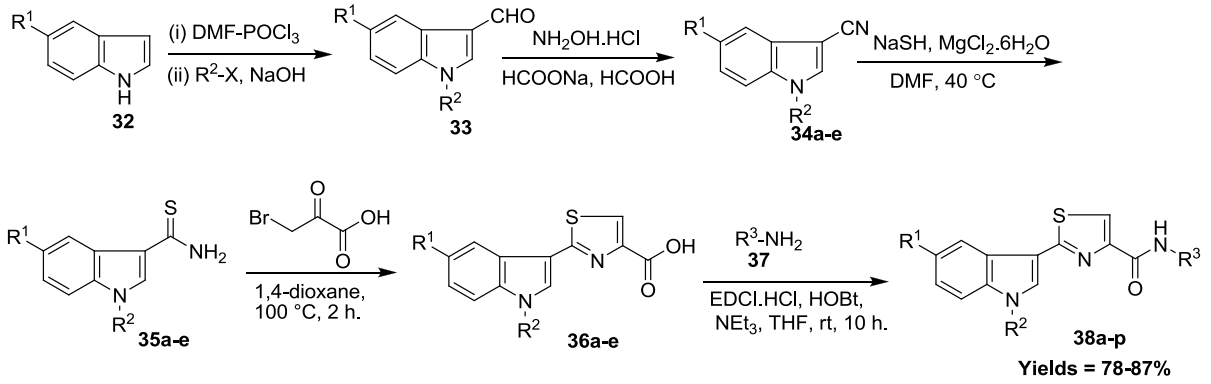


Figure 2.3.3 Rational approach to 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**)

2.3.2 Results and discussion

2.3.2.1 Synthesis

Synthesis of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**) were achieved as outlined in the Scheme 2.3.1. Initially, we have synthesized indole-3-carboxaldehydes and their *N*-alkylated derivatives **33** from corresponding indoles **32**.



Scheme 2.3.1 Synthesis of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides **38a-p**

The indole-3-carbonitriles **34** were prepared in good yields by reacting indole-3-carboxaldehydes **33** with hydroxylamine hydrochloride and formic acid. The reaction of indole-3-carbonitriles **34** with sodium hydrosulfide and magnesium chloride in presence of dimethylformamide led to the formation of indole-3-thiocarboxamides **35a-e** as illustrated in Scheme 2.3.1.[77,78] Reaction of thioamide **35** with bromopyruvic acid under refluxing conditions led to thiazole carboxylic acids **36** in good yields. Diverse carboxylic acids **36a-e** were coupled with various arylamines **37** in the presence of coupling reagent, EDCI.HCl and HOBt to prepare arylthiazole-4-carboxamides **38a-p** in 78-87% yields.

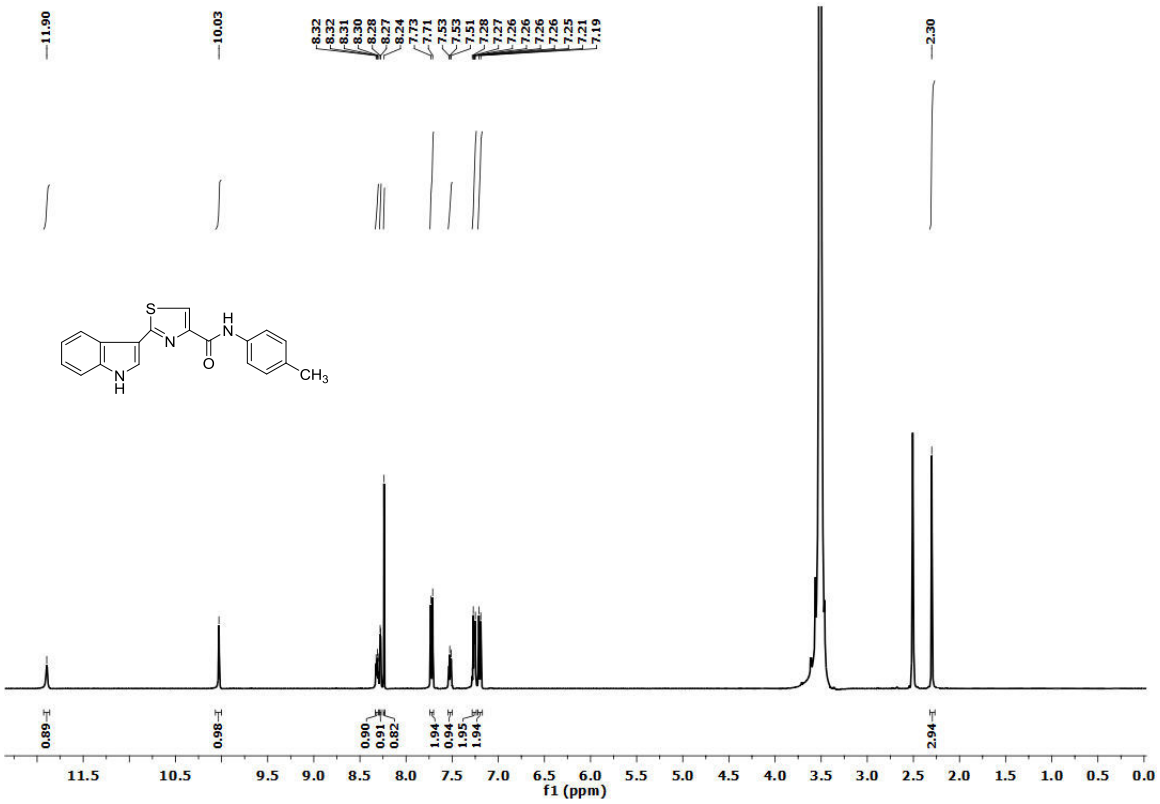


Figure 2.3.4 ^{1}H NMR spectrum of **38b**

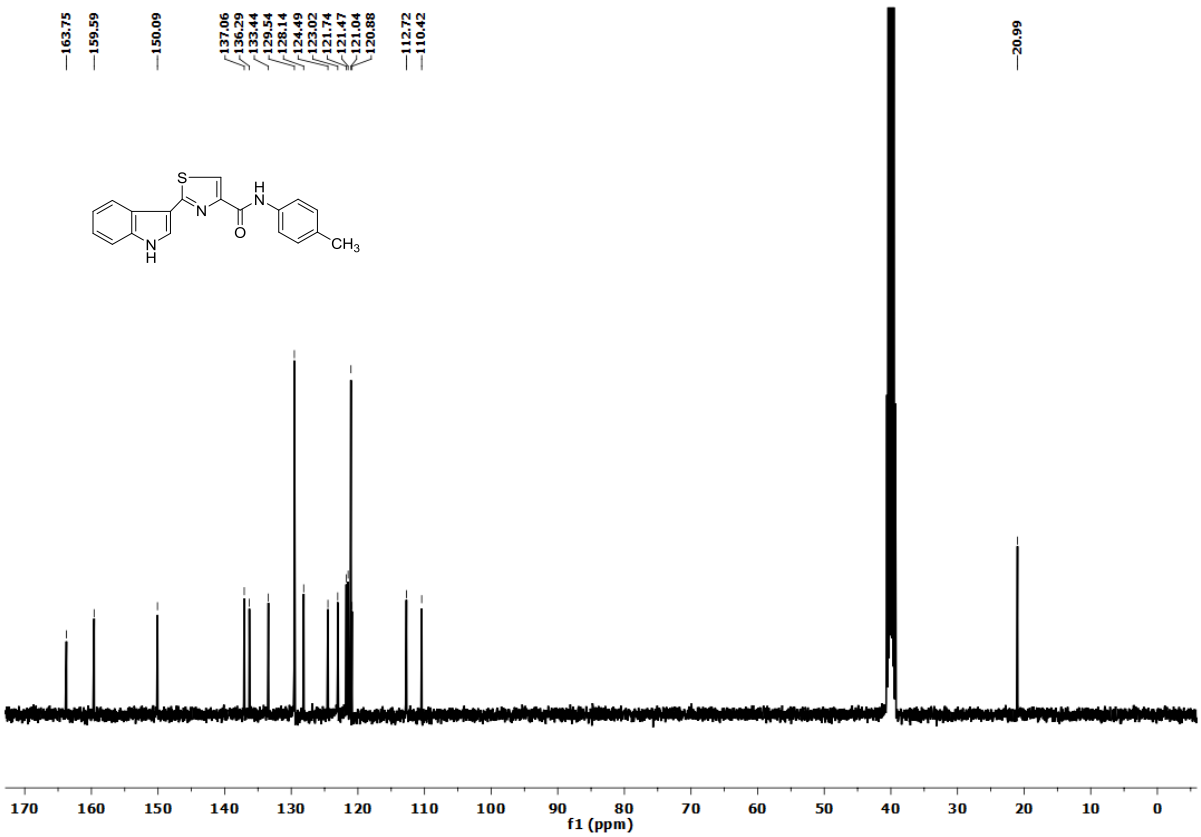


Figure 2.3.5 ^{13}C NMR spectrum of **38b**

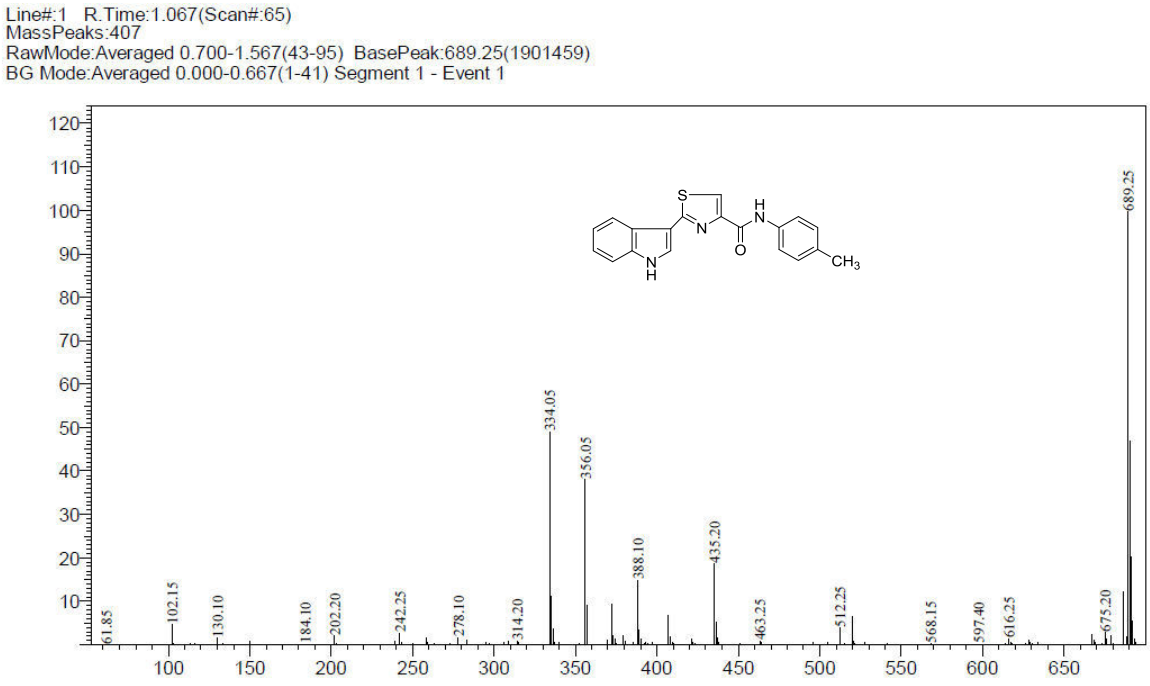


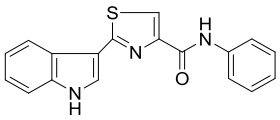
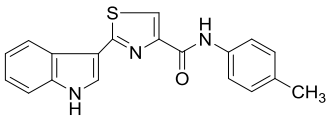
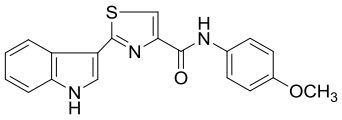
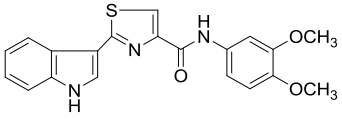
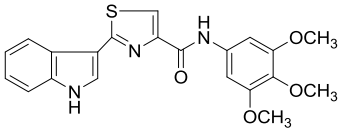
Figure 2.3.6 Mass spectrum of **38b**

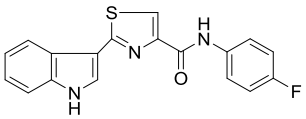
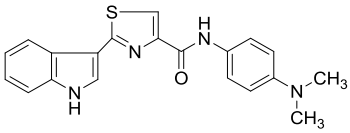
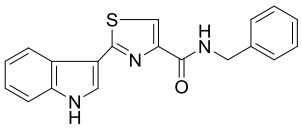
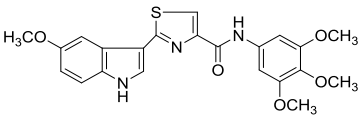
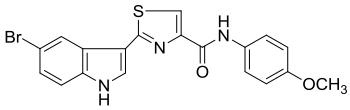
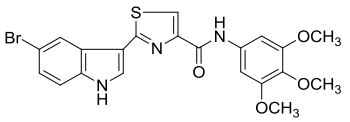
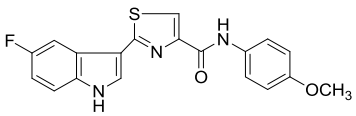
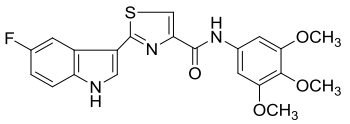
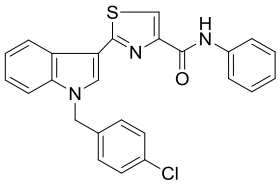
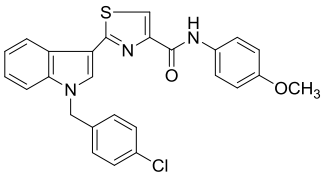
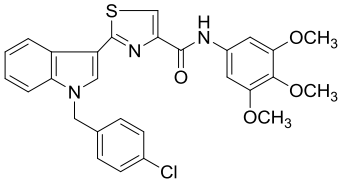
Structures of thiazole-carboxamides (**38a-p**) were elucidated through their IR, NMR (^{1}H & ^{13}C) and mass spectral analysis (Figures 2.3.4-2.3.6). In IR spectra, a characteristic sharp peak at ~1660 cm^{-1} was observed due to C=O stretching of an amide functional group. Carbon of an amide moiety (CONH) resonated at ~163 ppm in the ^{13}C NMR spectra of compounds **38a-p**.

2.3.2.2 Anticancer activity

To conduct a structure-activity relationship (SAR) study, we synthesized various thiazole-carboxamides (**38a-p**) with varying substituents either on indole, indole *N*-H or arylamido part. *In vitro* anticancer activities of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**) were determined against human embryonic kidney 293 cells (HEK 293T), human prostate (PC3 and LNCaP), cervical (HeLa), castration-resistant prostate (C4-2) and breast (MDA-MB-231) cancer cell lines using MTT assay. Doxorubicin was used as a reference drug. We initially analyzed potential cytotoxicity of these compounds in aforementioned cancer cell lines in the presence of FBS (Table 2.3.1). The cells growing in 10% FBS were treated with varying concentrations of (3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**) for 48 h. and cytotoxicity evaluated using MTT assay.

Table 2.3.1 *In vitro* cytotoxicity (with FBS) of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a-p**) towards selected cancer cell lines (IC_{50} in μM)

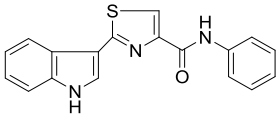
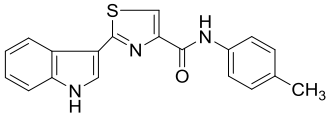
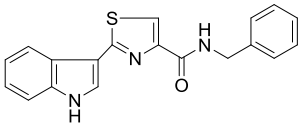
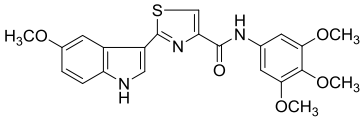
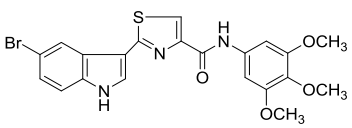
Compounds		HEK 293T	C4-2	HeLa	PC3	MDA-MB-231	LNCaP
	**(38a)**	>100	>100	>100	>100	>100	>100
	**(38b)**	>100	>100	>100	>100	>100	>100
	**(38c)**	ND	ND	ND	ND	ND	ND
	**(38d)**	ND	ND	ND	ND	ND	ND
	**(38e)**	ND	ND	ND	ND	ND	ND

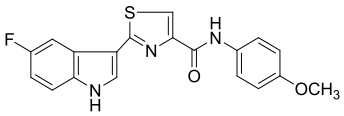
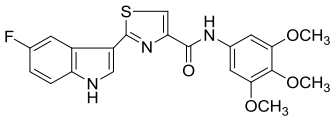
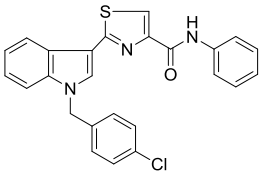
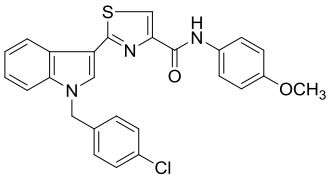
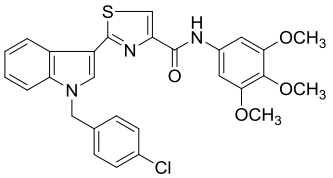
	**(38f)**	ND	ND	ND	ND	ND	ND
	**(38g)**	ND	ND	ND	ND	ND	ND
	**(38h)**	74.50**±**1.19	>100	41.38±2.03	>100	>100	>100
	**(38i)**	**8.74±1.26**	>100	**9.98±0.01**	>100	>100	84.80±1.21
	**(38j)**	ND	ND	ND	ND	ND	ND
	**(38k)**	32.82±0.75	>100	93.03±3.96	>100	>100	>100
	**(38l)**	>100	>100	33.48±0.98	>100	>100	75.42±1.88
	**(38m)**	66.02±3.2	>100	48.85±1.35	>100	>100	88.51±3.25
	**(38n)**	>100	>100	32.07±0.87	>100	>100	68.94±2.49
	**(38o)**	>100	>100	90.85±3.73	>100	>100	>100
	**(38p)**	>100	>100	>100	>100	>100	>100
Doxorubicin		0.75±0.03	0.57±0.08	0.43±0.10	9.8±0.40	6.29±0.24	7.40±1.10

[a]IC_{50} values are the mean of three different experiments performed in duplicate; ND: Not determined

The cytotoxicity results of compounds **38a-p** are expressed as IC_{50} values in micromolar (μM) as shown in Table 2.3.1. Surprisingly, most of the compounds showed minimal cytotoxic effect following 48 h. exposure of the micromolar concentrations of the compounds. A few exceptions, including compound **38i** (Table 2.3.1) was highly potent and selective for HEK293T and HeLa cells. Compound **38k**, which contains a bromo group instead of methoxy at indole C-5 position compared to **38i**, selectively inhibited HEK293T cells, but had negligible effect on HeLa cells. These results suggested that either most of these (3′-indolyl)-*N*-arylthiazole-4-carboxamides are ineffective as anticancer agents or they are not readily available to cells. The latter concern was also prompted by the fact that compounds **38c-g** and **38j** in this series displayed poor water solubility and were precipitated out of solution when added to cells in an overall DMSO concentration of 0.05-0.1% (Table 2.3.1). Similar to fatty acids, several water-insoluble compounds are known to avidly bind serum proteins, which drastically reduces the effective concentration of free molecules to enter the cells.[79] Further, serum in growth media can also interfere with MTT reagent leading to overestimation of cell growth and an underestimation of potential cytotoxicity of compounds.[80,81] As a result, these compounds are rendered ineffective in the presence of serum, although when serum is removed, they promote significant cell death.

Table 2.3.2 *In vitro* cytotoxicity (without FBS) of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides toward selected cancer cell lines (IC_{50} in μM)

Compounds		HEK 293T	C4-2	HeLa	PC3	MDA-MB-231	LNCaP
	**(38a)**	1.07±2.64	>100	>100	>100	79.37±3.98	>100
	**(38b)**	>100	>100	>100	>100	>100	>100
	**(38h)**	>100	>100	**9.51±0.22**	>100	>100	>100
	**(38i)**	**8.60±0.91**	>100	29.64±1.44	94.34±2.04	>100	>100
	**(38k)**	>100	>100	55.79±4.73	>100	>100	>100

	**(38l)**	**12.10±0.61**	>100	**3.41±0.07**	>100	49.41±5.91	66.41±4.94
	**(38m)**	>100	>100	>100	89.00±0.81	>100	>100
	**(38n)**	>100	>100	24.27±2.93	76.40±3.22	50.51±4.92	64.07±4.16
	**(38o)**	>100	>100	>100	>100	84.90±2.38	>100
	**(38p)**	58.69±1.90	>100	91.39±3.31	>100	>100	97.70±1.76
Doxorubicin		0.84±0.05	1.02±0.06	0.45±0.06	7.65±0.11	6.03±0.22	6.46±0.09

[a]IC_{50} values are the mean of three different experiments performed in duplicate

Therefore, we examined the potential cytotoxicity of these compounds in cells in the absence of serum. The cells were freshly plated and grown in serum containing media. After 12 h., the media was replaced with serum free media and varying concentrations of the compounds were added. After 48 h., cell viability was analyzed using MTT assay. As expected, we observed ~10-20% cell growth with no cell death in DMSO-treated cells after 48 h. of treatment, thereby ruling out any artifact that can potentially interfere with cytotoxicity assays. Importantly, in the absence of serum, a number of compounds showed significant and selective cytotoxicity, suggesting that this set of compounds indeed bind serum proteins, which interfere with their ability to penetrate the cells (Table 2.3.2).

From the cytotoxicity results (Table 2.3.2) it was found that compounds **38a** and **38b** with unsubstituted indole and phenyl and *p*-tolyl groups on arylamido part were inactive. Replacement of a phenyl moiety in **38a** with a benzyl substituent led to compound **38h** with selective cytotoxicity against HeLa cells (IC_{50} = 9.51 μM). Analogue **38i** having 5-methoxyindole and trimethoxyphenyl substituents was found to exhibit selective cytotoxic against the HEK293T cells (IC_{50} = 8.60 μM). Replacement of a 5-methoxyindole with 5-bromoindole (**38i** *vs* **38k**) was unfavourable for the activity. Interestingly, analogue **38l** with

5-fluoroindole and methoxyphenyl moieties was found to be the most potent compound of the series and selectively cytotoxic towards HEK293T and HeLa cells with IC_{50} values of 12.10 and 3.41 μM, respectively. Introduction of additional methoxy groups in compound **38l**, resulted in an inactive analogue **38m**. Protection of an indole ring nitrogen with *p*-chlorobenzyl moiety led to compounds **38n-p** endowed with moderate activity against the tested cancer cell lines. As noted before, when the MTT assay was performed in the presence of FBS, only compound **38i** was found to exhibit selective cytotoxicity against HEK293T (IC_{50} = 8.74 μM) and HeLa (IC_{50} = 9.98 μM) cells. Compounds **38h**, **38k**, **38l** and **38n** showed moderate activity (IC_{50} = 32-41 μM) against the tested cell lines.

2.3.2.3 Apoptosis study

We next examined whether these compounds induce cytotoxicity by inducing apoptosis in cancer cell lines. As compound **38i** exhibited high potency in HeLa cells (IC_{50} = 9.98 μM), these cells were treated with **38i** (10 μM) for 24 h., fixed and stained with propidium iodide, and their nuclear morphology was analyzed using fluorescence microscopy. DMSO-treated cells were used as negative control and doxorubicin (10 μM), which exerts cytotoxicity by promoting apoptosis, was used as a positive control.

Figure 2.3.7 Propidium iodide staining of HeLa cell treated with compound **38i** for 48 h. DMSO was used as a control. Scale: 10 mm.

As shown in Figure 2.3.7, nuclei in DMSO-treated HeLa cells retained their normal size and shape while HeLa cells treated with **38i** or doxorubicin showed large percentage of apoptotic nuclei, thereby confirming that **38i** indeed induces apoptosis in HeLa cells.

2.3.3 Conclusions

In summary, we synthesized a diverse series of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a–p**) from the initial reaction of thioamides and bromopyruvic acid to afford thiazole carboxylic acids **36**, which were coupled with appropriate arylamines. *In vitro* cytotoxicity study of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a–p**) were resulted in **38i** and **38l** as the most potent compounds of the series. Our preliminary mechanism of action studies further indicated that thiazole carboxamide **38i** induces apoptosis in HeLa cells. Overall, these results suggest that appropriate substituents in indole and arylamide moieties of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides (**38a–p**) are crucial for their targeted selectivity and potency for their anticancer activities. Further, it may be necessary to perform MTT or XTT assays for testing potential cytotoxicity of test compounds both in the absence and presence of FBS to rule out any interference from serum binding proteins which may drastically impact cellular availability. As exemplified by our SAR studies, this series of compounds are very versatile and can be exploited to develop highly specific and potent anticancer agents.

2.3.4 Experimental section

Chemistry

All commercially available reagents and solvents were purchased from Merck and Aldrich and used as such without further purification.The progress of reaction was monitored by thin layer chromatography, which was performed on Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm). Solvents were evaporated using Büchi rotary evaporator. Melting points were determined with electrothermal capillary melting point apparatus (E-Z-melting) and are uncorrected. IR spectra were recorded on Shimadzu FT-IR spectrophotometer. NMR (^{1}H & ^{13}C) spectra were recorded on a Bruker Advance II (300/400 MHz & 75/100 MHz) spectrometer. The coupling constant (*J*) values are mentioned in Hz. Mass spectra were obtained on a ‘Hewlett-Packard' HP GS/MS 5890/5972.

General procedure for the synthesis of indole-3-carboxaldehyde:[82] A round-bottomed flask containing freshly distilled dimethylformamide (DMF) (370 mmol) was cooled in an ice-salt bath for about 0.5 h and 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 (**32**, 85.47 mmol) in DMF (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

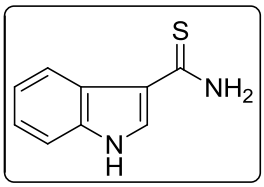
crushed ice was added to the paste with stirring which becomes a clear cherry-red aqueous solution. A solution of sodium hydroxide (94 mmol) in 100 mL of water was added dropwise with stirring to the cherry-red solution. The resulting suspension was heated rapidly to 90 °C and allowed to cool to room 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 pure indole-3-carboxaldehydes in 85-90% yields.

***N*-Alkylindole-3-carboxaldehydes:** To a stirred mixture of indole-3-carboxaldehyde (34 mmol) in 50% aqueous NaOH (40 mL), water (60 mL) and tetrabutylammonium bromide (0.1 g, 3.4 mmol) was added 4-chlorobenzylchloride (34 mmol) in toluene (30 mL). After the completion of reaction, organic phase was washed twice with aqueous $NaHCO_3$ (50 mL), water (100 mL) and saturated brine (100 mL) solution, and then dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and residue so obtained was washed with anhydrous ether to give crude alkylated product which upon recrystallization with ethylacetate and hexane led to pure alkylated 3-carboxaldehydes in 85-90% yields.[83, 84]

Synthesis of indole-3-carbonitriles (34): To a stirred solution of indole-3-carboxaldehyde (**33**, 0.02 mol) in formic acid (25mL) 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 extract was washed with saturated sodium bicarbonate solution (40 mL) and brine solution (40 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) to afforded pure indole-3-carbonitriles **34** in 65-70% yields.

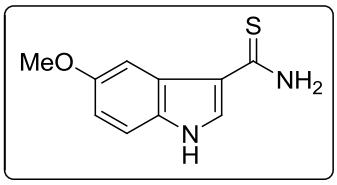
Indole-3-thiocarboxamides (**35a-e**): A mixture of indole-3-carbonitrile (**34**, 0.02 mol), sodium hydrosulfide (0.04 mol) and magnesium chloride hexahydrate (0.02 mol) was taken in a round bottomed flask and stirred for 10 h. at 40 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, contents were poured into cold water (50 mL) and the solid obtained was filtered. The residue was re-suspended in 100 mL HCl solution (3N) and stirred for 10 min and then filtered-off the solid to obtain indole-3-thiocarboxamides **35a-e** in 65-75% yields.

1*H*-Indole-3-carbothioamide (35a)



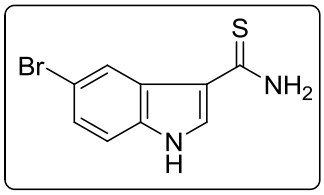
Yield 75%; Off white solid; mp 149-151 °C (Lit[85] 148-152 °C); IR (KBr, *v,* cm^{-1}): 3386, 3276, 3180, 1623, 1529, 1442,1365, 1155, 765.

5-Methoxy-1*H*-indole-3-carbothioamide (35b)



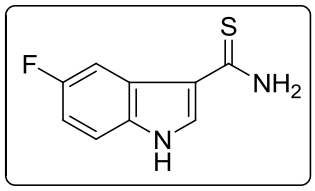
Yield 65%; Off white solid; mp 225-227 °C (Lit[85] 226-228 °C); IR (KBr, *v,* cm^{-1}): 3200, 2837, 1630, 1510, 1435, 1234,790.

5-Bromo-1*H*-indole-3-carbothioamide (35c)



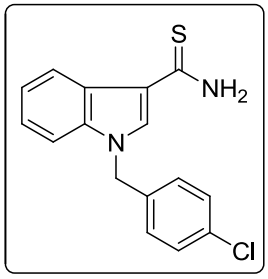
Yield 67%; Off white solid; mp 241-242 °C (Lit[85] 240-242 °C); IR (KBr, *v,* cm^{-1}): 3381, 3190, 1623, 1525, 1435, 1295,1191, 794.

5-Fluoro-1*H*-indole-3-carbothioamide (35d)



Yield 70%; Off white solid; mp 230-232 °C; IR (KBr, *v,* cm^{-1}): 3384, 3193, 1620, 1522, 1437, 1290,1194, 793.

1-(4-Chlorobenzyl)-1*H*-indole-3-carbothioamide (35e)



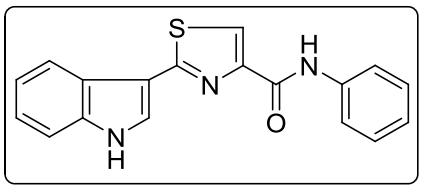
Yield 70%; Off white solid; mp 137-139 °C; IR (KBr, *v,* cm^{-1}): 2945, 1645, 1565, 1475, 1442, 1223, 786.

General procedure for the synthesis of indolyl thiazole carboxylic acid (36): To a solution of thioamide **35** (1.7 mmol) in 1,4-dioxane (3 mL) was added bromopyruvic acid (1.7 mmol) portion wise and resulting reaction mixture was heated at 100 °C for 2 h. After completion of reaction as indicated by TLC, solvent was concentrated under reduced pressure to final volume of 1 mL; water was added (10 mL) and contents were cooled to 0 °C and filtered. The

filtered cake was washed with water (10 mL) and dried to give pure thiazole carboxylic acids **36** in 80-85% yields.

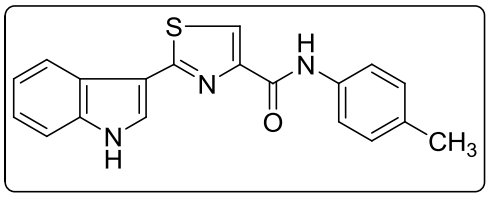
General procedure for the synthesis of thiazole carboxamides (38a-p): To a mixture of thiazole carboxylic acid **36** (0.8 mmol) in dry THF (3 mL) was added EDCI.HCl (0.9 mmol), HOBT (0.9 mmol) and triethylamine (1.6 mmol) and the contents were stirred at 25 °C for 30 min. Arylamine **37** (0.8 mmol) was added and the resulting mixture was stirred for 10 h. at 25 °C. Upon completion of reaction as indicated by TLC, solvent was evaporated *in vaccuo*, water was added (15 mL) and extracted with ethyl acetate (2 × 20 mL). Combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, the residue so obtained was purified through column chromatography to afford pure thiazole carboxamides **38a-p** in 78-87% yields.

2-(1*H*-Indol-3-yl)-*N*-phenylthiazole-4-carboxamide (38a)



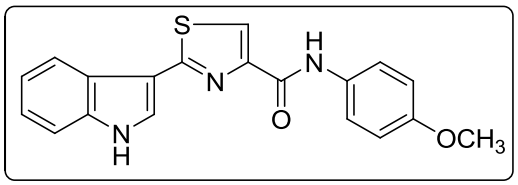
Yield 80%; Off white solid; mp 206-208 °C; IR (KBr, *v,* cm^{-1}): 3348, 3263, 1666, 1597, 1545, 1435, 1126, 741, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.91 (s, 1H), 10.11 (s, 1H), 8.35–8.26 (m, 3H), 7.87 (d, J = 7.8 Hz, 2H), 7.56–7.54 (m, 1H), 7.41–7.39 (m, 2H), 7.28–7.25 (m, 2H), 7.15 (t, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 159.3, 149.5, 138.3, 136.6, 128.6, 127.6, 124.0, 124.0, 122.5, 121.4, 121.0, 120.6, 120.4, 112.2, 109.9; ESI (FAB) *m/z* calcd for $C_{18}H_{13}N_3NaOS$: 342.07 $[M + Na]^+$, found 342.05.

2-(1*H*-Indol-3-yl)-*N*-*p*-tolylthiazole-4-carboxamide (38b)



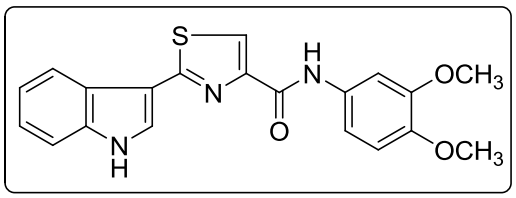
Yield 82%; Pale yellow solid; mp 221-223 °C; IR (KBr, *v,* cm^{-1}): 3340, 3256, 1666, 1548, 1242, 1126, 810, 741, 671; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1H), 10.03 (s, 1H), 8.32–8.30 (m, 1H), 8.28 (d, J = 2.4 Hz, 1H), 8.24 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 7.54–7.50 (m, 1H), 7.28–7.24 (m, 2H), 7.20 (d, J = 8.3 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.7, 159.6, 150.1, 137.1, 136.3, 133.4, 129.5, 128.1, 124.5, 123.0, 121.7, 121.5, 121.0, 120.9, 112.7, 110.4, 21.0; ESI (FAB) *m/z* calcd for $C_{19}H_{16}N_3OS$: 334.10 $[M + H]^+$, found 334.05.

2-(1*H*-Indol-3-yl)-*N*-(4-methoxyphenyl)thiazole-4-carboxamide (38c)



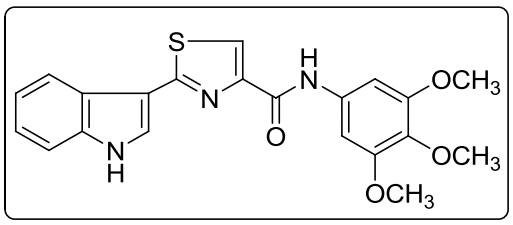
Yield 80%; Off white solid; mp 200-202 °C; IR (KBr, *v,* cm^{-1}): 3364, 3232, 1666, 1512, 1242, 1111, 748, 617; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.94 (s, 1H), 10.02 (s, 1H), 8.36–8.29 (m, 1H), 8.28 (d, J = 2.6 Hz, 1H), 8.23 (s, 1H), 7.77 (s, 2H), 7.55–7.51 (m, 1H), 7.29–7.22 (m, 2H), 6.97 (d, J = 9.0 Hz, 2H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.3, 159.0, 155.8, 149.7, 136.6, 131.3, 127.6, 124.0, 122.5, 122.3, 121.0, 121.0, 120.4, 113.8, 112.2, 109.9, 55.2; ESI (FAB) *m/z* calcd for $C_{19}H_{14}N_3O_2S$: 348.09 $[M - H]^+$, found 348.15.

***N*-(3,4-Dimethoxyphenyl)-2-(1*H*-indol-3-yl)thiazole-4-carboxamide (38d)**



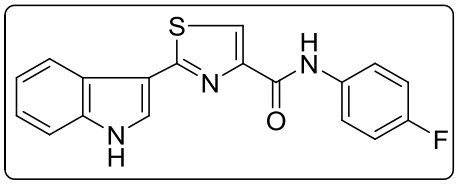
Yield 79%; Off white solid; mp 176-178 °C; IR (KBr, *v,* cm^{-1}): 3340, 3132, 1643, 1520, 1458, 1219, 1018, 741, 633; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.90 (s, 1H), 9.99 (s, 1H), 8.29 (s, 2H), 8.23 (s, 1H), 7.56–7.53 (m, 2H), 7.43 (d, J = 7.1 Hz, 1H), 7.28–7.25 (m, 2H), 6.97 (d, J = 8.7 Hz, 1H), 3.80 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.3, 159.0, 149.7, 148.5, 145.4, 136.6, 131.8, 127.5, 124.0, 122.5, 121.1, 121.0, 120.3, 112.7, 112.3, 111.9, 111.0, 105.9, 69.7, 55.7, 55.5; ESI (FAB) *m/z* calcd for $C_{20}H_{16}N_3O_3S$: 378.10 $[M - H]^+$, found 378.20.

2-(1*H*-Indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (38e)



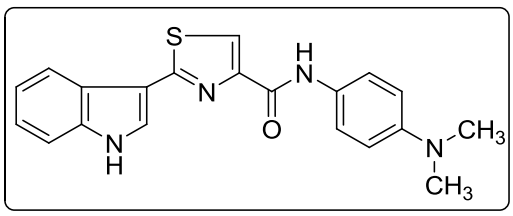
Yield 85%; Off white solid; mp 193-195 °C; IR (KBr, *v,* cm^{-1}): 3325, 3117, 1666, 1612, 1551, 1512, 1227, 1126, 956, 748, 633; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.91 (s, 1H), 10.02 (s, 1H), 8.31–8.23 (m, 3H), 7.55–7.52 (m, 1H), 7.33 (s, 2H), 7.28–7.25 (m, 2H), 3.81 (s, 6H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.3, 159.2, 152.7, 149.5, 136.6, 134.4, 134.0, 127.5, 124.0, 122.5, 121.4, 121.0, 120.2, 112.3, 109.9, 98.5, 60.1, 55.8; ESI (FAB) *m/z* calcd for $C_{21}H_{20}N_3O_4S$: 410.12 $[M + H]^+$, found 410.10.

***N*-(4-Fluorophenyl)-2-(1*H*-indol-3-yl)thiazole-4-carboxamide (38f)**



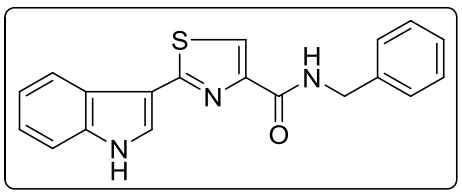
Yield 78%; Off white solid; mp 225-227 °C; IR (KBr, *v,* cm^{-1}): 3263, 3117, 1666, 1551, 1512, 1211, 1126, 741, 640; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.89 (s, 1H), 10.18 (s, 1H), 8.37–8.31 (m, 1H), 8.27 (s, 2H), 7.89–7.87 (m, 2H), 7.55–7.50 (m, 1H), 7.28–7.20 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.7 (d, $J_{C\text{-}F}$ = 244.3 Hz), 159.3, 156.9, 149.4, 136.6, 134.7 (d, $J_{C\text{-}F}$ = 2.5 Hz), 127.6, 126.3, 124.0, 122.6 (d, $J_{C\text{-}F}$ = 8.1 Hz), 121.5, 120.9, 120.4, 115.2 (d, $J_{C\text{-}F}$ = 22.2 Hz), 112.2, 109.9; ESI (FAB) *m/z* calcd for $C_{18}H_{13}FN_3OS$: 338.08 $[M + H]^+$, found 338.05.

***N*-(4-(Dimethylamino)phenyl)-2-(1*H*-indol-3-yl)thiazole-4-carboxamide (38g)**



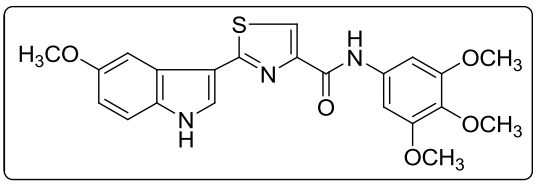
Yield 80%; Brown solid; mp 219-221 °C; IR (KBr, *v,* cm^{-1}): 3379, 3209, 1659, 1528, 1466, 748; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.87 (s, 1H), 9.84 (s, 1H), 8.34–8.27 (m, 2H), 8.19 (s, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.56–7.49 (m, 1H), 7.30–7.22 (m, 2H), 6.76 (d, *J* = 8.7 Hz, 2H), 3.39 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.2, 158.7, 149.9, 147.5, 136.6, 127.9, 127.5, 124.0, 122.5, 122.0, 120.9, 120.5, 120.4, 112.4, 112.2, 110.0, 40.4; ESI (FAB) *m/z* calcd for $C_{20}H_{19}N_4OS$: 363.13 $[M + H]^+$, found 363.10.

***N*-Benzyl-2-(1*H*-indol-3-yl)thiazole-4-carboxamide (38h)**



Yield 78%; Brown solid; mp 203-205 °C; IR (KBr, *v,* cm^{-1}): 3402, 3225, 1651, 1543, 1234, 941, 879, 733; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (d, *J* = 7.4 Hz, 2H), 7.56 (t, *J* = 7.2 Hz, 2H), 7.38–7.17 (m, 5H), 6.98–6.87 (m, 3H), 6.32 (s, 1H), 4.12 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.5, 164.6, 163.9, 152.3, 131.5, 129.6, 124.0, 123.4, 122.8, 118.7, 115.7, 113.5, 113.2, 112.1, 111.9, 103.4, 36.0; ESI (FAB) *m/z* calcd for $C_{19}H_{16}N_3OS$: 334.10 $[M + H]^+$, found 334.05.

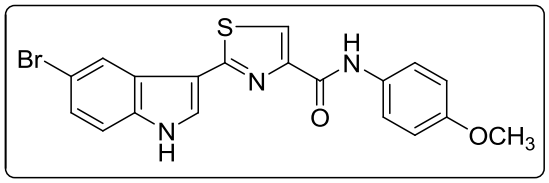
2-(5-Methoxy-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (38i)



Yield 78%; Off white solid; mp 217-219 °C; IR (KBr, *v,* cm^{-1}): 3333, 3066, 1674, 1543, 1420, 1203, 802, 687; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.77 (s, 1H), 10.05 (s, 1H), 8.22 (s, 2H), 7.84 (d, *J* = 2.2 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.32 (s, 2H), 6.89 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.92

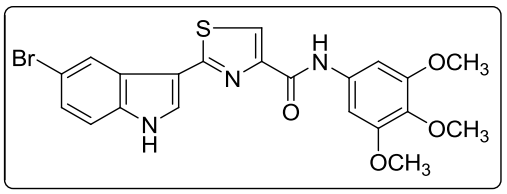
(s, 3H), 3.80 (s, 6H), 3.66 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.5, 159.0, 154.8, 152.7, 149.4, 134.6, 133.9, 131.5, 127.9, 124.6, 120.9, 113.0, 112.8, 109.7, 102.0, 97.9, 60.1, 55.7, 55.1; ESI (FAB) *m/z* calcd for $C_{22}H_{20}N_3O_5S$: 438.12 $[M - H]^+$, found 438.20.

2-(5-Bromo-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)thiazole-4-carboxamide (38j)



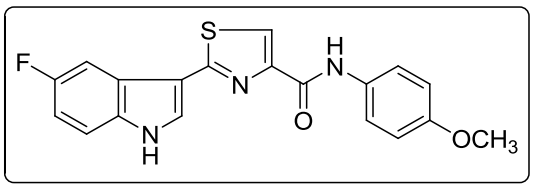
Yield 79%; Off white solid; mp 233-235 °C; IR (KBr, *v,* cm^{-1}): 3371, 3256, 1659, 1520, 1242, 810, 609; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.05 (s, 1H), 10.11 (s, 1H), 8.52 (d, *J* = 1.6 Hz, 1H), 8.31 (d, *J* = 2.8 Hz, 1H), 8.24 (s, 1H), 7.75 (d, *J* = 8.9 Hz, 2H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.38 (dd, *J* = 8.6, 1.8 Hz, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6 159.0, 155.8, 149.9, 135.3, 131.5, 129.0, 125.7, 125.2, 122.8, 122.2, 121.3, 114.2, 113.8, 113.7, 109.6, 55.2; ESI (FAB) *m/z* calcd for $C_{19}H_{15}BrN_3O_2S$: 430.00 $[M + H + 2]^+$, found 430.00.

2-(5-Bromo-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (38k)



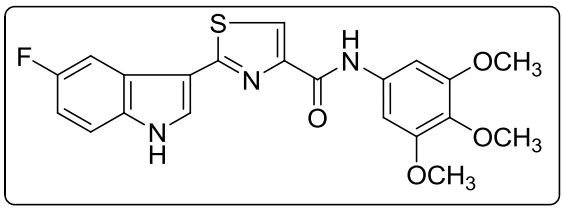
Yield 81%; Off white solid; mp 238-240 °C; IR (KBr, *v,* cm^{-1}): 3348, 3217, 1674, 1543, 1512, 1126, 625; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.06 (s, 1H), 10.10 (s, 1H), 8.49 (d, *J* = 1.7 Hz, 1H), 8.32 (d, *J* = 2.8 Hz, 1H), 8.25 (s, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.38 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.29 (s, 2H), 3.81 (s, 6H), 3.67 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6, 159.1, 152.7, 149.7, 135.3, 134.5, 134.0, 129.0, 125.7, 125.2, 122.6, 121.6, 114.3, 113.7, 109.53, 98.3, 60.1, 55.8; ESI (FAB) *m/z* calcd for $C_{21}H_{19}BrN_3O_4S$: 490.03 $[M + H + 2]^+$, found 490.05.

2-(5-Fluoro-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)thiazole-4-carboxamide (38l)



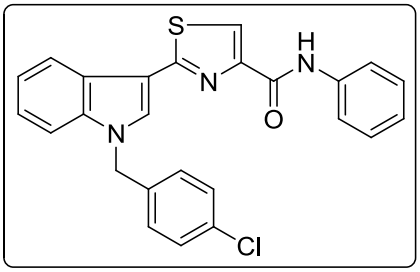
Yield 80%; Off white solid; mp 239-241 °C; IR (KBr, *v,* cm^{-1}): 3364, 3232, 1666, 1512, 1242, 1180, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.99 (s, 1H), 10.10 (s, 1H), 8.33 (d, *J* = 2.6 Hz, 1H), 8.23–8.21 (m, 2H), 7.75 (d, *J* = 8.9 Hz, 2H), 7.55–7.50 (m, 1H), 7.14–7.09 (m, 1H), 6.98 (d, *J* = 8.9 Hz, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.6 (d, $J_{C\text{-}F}$ = 233.6 Hz), 159.8, 157.4, 156.3, 150.4, 133.7, 131.8, 129.9, 124.8 (d, $J_{C\text{-}F}$ = 8.7 Hz), 123.1, 121.7, 114.2, 113.8 (d, $J_{C\text{-}F}$ = 9.0 Hz), 111.3 (d, *J* = 22.4 Hz), 110.7 (d, *J* = 3.3 Hz), 106.4 (d, *J* = 22.4 Hz), 55.6; ESI (FAB) *m/z* calcd for $C_{19}H_{15}FN_3O_2S$: 368.09 $[M + H]^+$, found 368.05.

2-(5-Fluoro-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (38m)



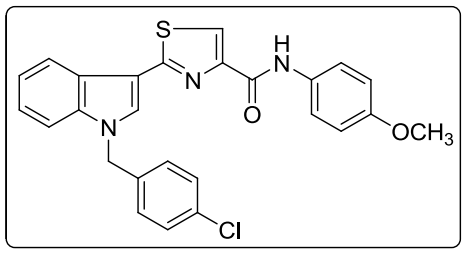
Yield 78%; Off white solid; mp 208-210 °C; IR (KBr, *v,* cm^{-1}): 3256, 3101, 1666, 1551, 1180, 1126, 995, 795, 633; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.00 (s, 1H), 10.10 (s, 1H), 8.35 (d, J = 2.9 Hz, 1H), 8.26 (s, 1H), 8.14 (dd, J = 8.5, 2.7 Hz, 1H), 7.54–7.51 (m, 1H), 7.31 (s, 2H), 7.14–7.09 (m, 1H), 3.81 (s, 6H), 3.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 158.5 (d, $J_{C\text{-}F}$ = 240.0 Hz), 157.4, 153.1, 150.2, 134.9, 134.4, 133.7, 129.9, 124.8 (d, $J_{C\text{-}F}$ = 8.4 Hz), 122.1, 113.9 (d, $J_{C\text{-}F}$ = 8.4 Hz), 111.3 (d, $J_{C\text{-}F}$ = 22.1 Hz), 110.7 (d, $J_{C\text{-}F}$ = 2.5 Hz), 106.1 (d, $J_{C\text{-}F}$ = 22.2 Hz), 99.2, 60.6, 56.3; ESI (FAB) *m/z* calcd for $C_{21}H_{19}FN_3O_4S$: 428.11 $[M + H]^+$, found 428.05.

2-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-*N*-phenylthiazole-4-carboxamide (38n)



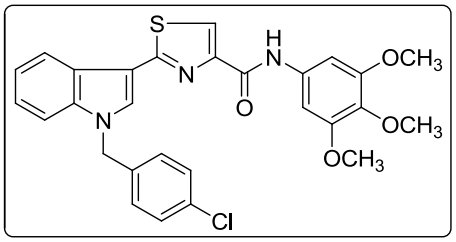
Yield 87%; White solid; mp 183-185 °C; IR (KBr, *v,* cm^{-1}): 3356, 1666, 1543, 1443, 1173, 741; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.13 (s, 1H), 8.50 (s, 1H), 8.41-8.38 (m, 1H), 8.32 (s, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.61–7.59 (m, 1H), 7.42–7.38 (m, 4H), 7.34–7.27 (m, 4H), 7.15 (t, J = 7.4 Hz, 1H), 5.56 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.2, 159.7, 150.2, 138.8, 136.9, 136.8, 132.7, 131.3, 129.5, 129.1, 125.1, 124.4, 123.4, 122.3, 122.0, 121.4, 121.1, 111.5, 110.2, 49.2; ESI (FAB) *m/z* calcd for $C_{25}H_{19}ClN_3OS$: 444.09 $[M + H]^+$, found 444.10.

2-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)thiazole-4-carboxamide (38o)



Yield 82%; Off white solid; mp 162-164 °C; IR (KBr, *v,* cm^{-1}): 3304, 1659, 1512, 1350, 1173, 795, 748, 617; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.99 (s, 1H), 8.46 (s, 1H), 8.39–8.36 (m, 1H), 8.25 (s, 1H), 7.75–7.71 (m, 2H), 7.60–7.57 (m, 1H), 7.43–7.39 (m, 2H), 7.33 (s, 1H), 7.31–7.26 (m, 3H), 6.99–6.94 (m, 2H), 5.55 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6, 159.0, 155.8, 149.8, 136.4, 136.3, 132.2, 131.3, 130.7, 129.0, 128.6, 124.7, 122.9, 122.3, 121.4, 121.4, 120.9, 113.8, 110.8, 109.8, 55.2, 49.1; ESI (FAB) *m/z* calcd for $C_{26}H_{21}ClN_3O_2S$: 474.10 $[M + H]^+$, found 474.15.

2-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)thiazole-4-carboxamide (38p)



Yield 82%; Off white solid; mp 207-209 °C; IR (KBr, *v,* cm^{-1}): 3325, 1674, 1597, 1555, 1450, 1219, 935, 741, 656; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.02 (s, 1H), 8.46 (s, 1H), 8.36–8.30 (m, 1H), 8.28 (s, 1H), 7.61–7.56 (m, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.32–7.27 (m, 6H), 5.56 (s, 2H), 3.80 (s, 6H), 3.66 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.6, 159.1, 152.6, 149.7, 136.4, 136.3, 134.4, 134.0, 132.2, 130.6, 129.0, 128.6, 124.6, 122.9, 121.8, 121.5, 120.7, 111.1, 109.7, 98.6, 60.1, 55.8, 48.7; ESI (FAB) *m/z* calcd for $C_{28}H_{25}ClN_3O_4S$: 534.13 $[M + H]^+$, found 534.20.

2.3.3.2 *In vitro* anticancer screening

MTT assay

Prostate cancer cell lines (LNCaP, PC3 and C4-2) were cultured in RPMI-1640 media and cervical (HeLa), breast (MDA-MB-231) and human embryonic kidney cells (HEK293T) were cultured in DMEM media supplemented with 10% heat inactivated fetal bovine serum and 1% penicillin/streptomycin. They were seeded in 96-well plates at a density of 2.5×10^3 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 0.5 mg/mL and incubated for 30 min. The cells were washed twice with PBS and lysed in 100 µL DMSO, and the absorbance was measured at 590 nm using Tecan Spectrafluor Plus.

2.3.3.3 Nuclear staining using propidium iodide

HeLa cells plated on coverslips were treated either with 0.01% DMSO (control), or 10 µM doxorubicin or **38i** for 24 h. After the treatment, cells were fixed with 4% cold formaldehyde for 10 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 RNase A in PBS for 1 h., rinsed, and stained with 2.5 µg/mL propidium iodide in PBS for 20 min. Before mounting with Mowiol, coverslips were washed twice with PBS and once with H_2O.

2.3.5 References

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Chapter 3

Synthesis and Biological Evaluation of Some Novel Indolyloxadiazoles as Potent Anticancer Agents

Part A: ***2-Arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles as novel cytotoxic agents***

Part B: ***Design and facile synthesis of indolyl-α-keto-1,3,4-oxadiazoles as tubulin interacting anticancer agents***

3.1 Indolyloxadiazoles

Natural and synthetic bioactive heterocycles containing indole linked five-membered azole are extensively studied in literature for their wide spectrum of biological activities including antimicrobial, antiinflammatory, antioxidant, antiepileptic, antiviral and anticancer.[1,2] In the recent past, several indole-based molecules exhibited potent anticancer activities leading to their further exploration in cancer drug discovery research to identify potent and safer drug candidate (Figure 3.1.1). For example, Pettit *et al.* isolated natural products Labradorins (**1**) having indolyloxazole system from the *Pseudomonas syringae* pv., as potent *in vitro* growth inhibitors of human cancer cells (GI_{50} =25.8-40.8 μM).[3] In 2009, Kumar *et al.* prepared indolyl-1,3,4-oxadiazoles (**2**) as synthetic analogue of Labradorins and found to exhibit improved anticancer activity (IC_{50} = 1 μM; MCF-7).[4] Subsequently, in analogy with bisindole alkaloids, Kumar *et al.* prepared a series of bis(indolyl)-1,3,4-oxadiazoles (**3**) which exerted anticancer activity *via* the induction of apoptosis (IC_{50} = 20 nM; HeLa).[5] In 2011, Gavagnin *et al.* isolated two cytotoxic (IC_{50} = 0.4-1.5 μM; HeLa) indole alkaloids, Phidianidine A and Phidianidine B (**4**) from the marine ophisthobranch mollusk *Phidiana military*. Although Phidianidine A and B are not toxic to normal HEK293 human epithelial kidney cells.[6] Westwell and co-workers identified 3,5-disubstituted oxadiazoles **5** (IC_{50} = 7.7-28.3 μM; COLO 320) as potential pro-apoptotic antitumour agents.[7]

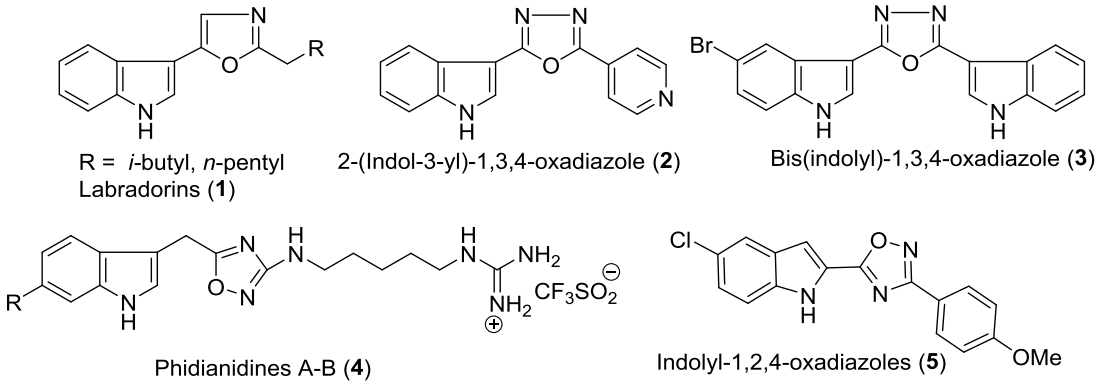


Figure 3.1.1 Representative chemical structures of indole-based cytotoxic agents

In view of potent cytotoxic activities of indolyloxadiazoles and continuation of our efforts to identify safer and novel indole-based bioactive heterocycles, in this chapter (parts A and B) we report synthesis and *in vitro* anticancer activities of novel indolyl-1,3,4-oxadiazoles. Efficient synthesis and anticancer activity studies of 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles and indolyl-*α*-keto-1,3,4-oxadiazoles have been described in part A and part B, respectevely.

3.2 Part A: 2-Arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles as novel cytotoxic agents

3.2.1 Rational design

Indole nucleus is a privileged scaffold that is highly prevalent in natural and synthetic compounds of medicinal interest.[8] It is found in many clinical therapeutic agents (e.g., indomethacin, indorenate and indoramin) and endogenous biologically active substances (e.g., serotonin, melatonin, tryptophan and brassinin). Molecules containing an indole nucleus, are known to display a diverse range of biological activities such as anticancer, antidiabetic, antirheumatoidal, antioxidant and antiviral properties[9] in addition to their crucial role in the immune system.[10,11]

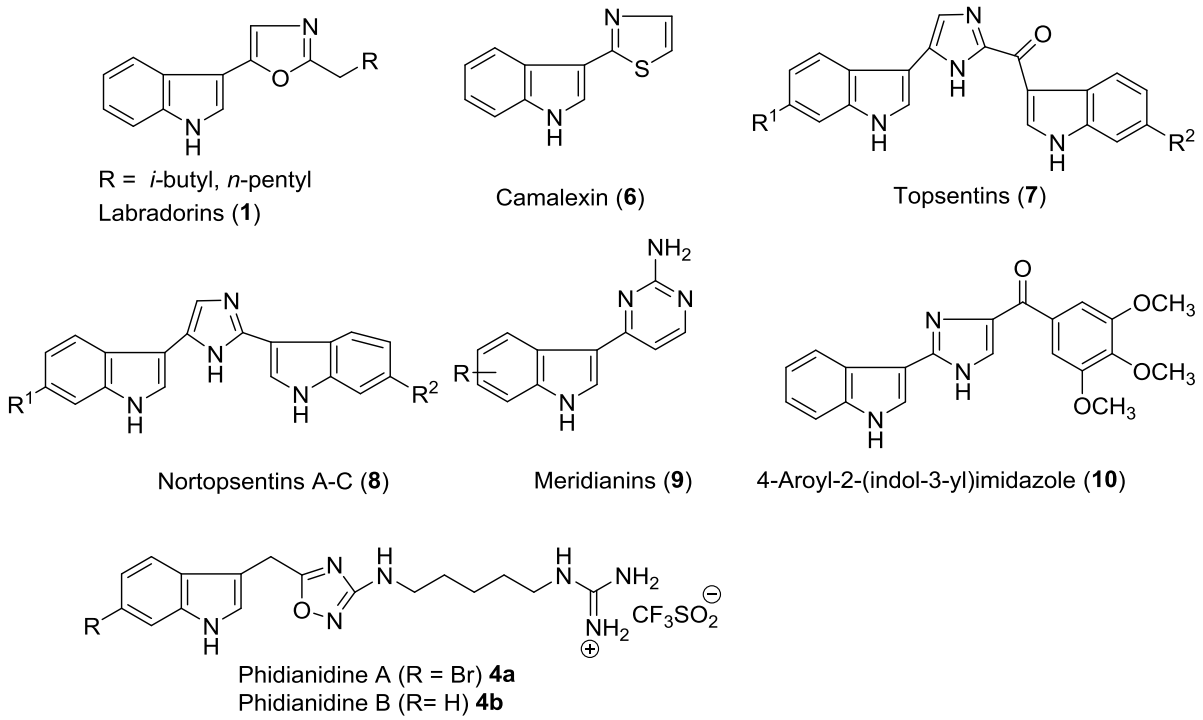


Figure 3.2.1 Indole-based some natural and synthetic anticancer agents

In recent years, several indole-containing heterocyclic compounds have been isolated or synthesized with interesting activities. For example, Labradorins (**1**), indolyloxazole-derived natural products, have been reported to exhibit potent *in vitro* growth inhibitors of human cancer cells with GI_{50} values of 40.8 μM (non-small-cell lung carcinoma) and 25.8 μM (pancreatic adenocarcinoma).[3] Camalexin (**6**) is a characteristic phytoalexin of *Arabidopsis thaliana* that is induced by a variety of plant pathogens.[12] Topsentins (**7**), a class of bisindole alkaloid isolated from the marine sponge *Topsentina genitrix* as growth inhibitors of

leukemia cells *in vitro*.[13] Nortopsentins A–C (**8**) exhibit *in vitro* cytotoxicity against murine leukemia (P388) cells with IC_{50} values of 4.5–20.7l μM.[14] Meridianins (**9**) and their analogues have been known to display good anticancer activities against human breast adenocarcinoma (MCF-7) cells (IC_{50}=1.10 μM).[15] A recent report from Li *et al.* described a series of 2-aryl-4-benzoylimidazoles (ABI-III) **10** where replacement of the aryl ring with an indole nucleus enhanced the antiproliferative activity. Indole derivative **10** showed remarkable activity against melanoma and prostate cancers through inhibition of tubulin polymerization, with typical IC_{50} values around 3.8 nM.[16] In 2011, Gavagnin *et al.* reported the isolation of Phidianidines[17] A and B (**4**) from marine opisthobranch mollusk *Phidiana militaris*; these natural products were found to display high cytotoxicity against both tumor and non tumor cell lines (Figure 3.2.1).

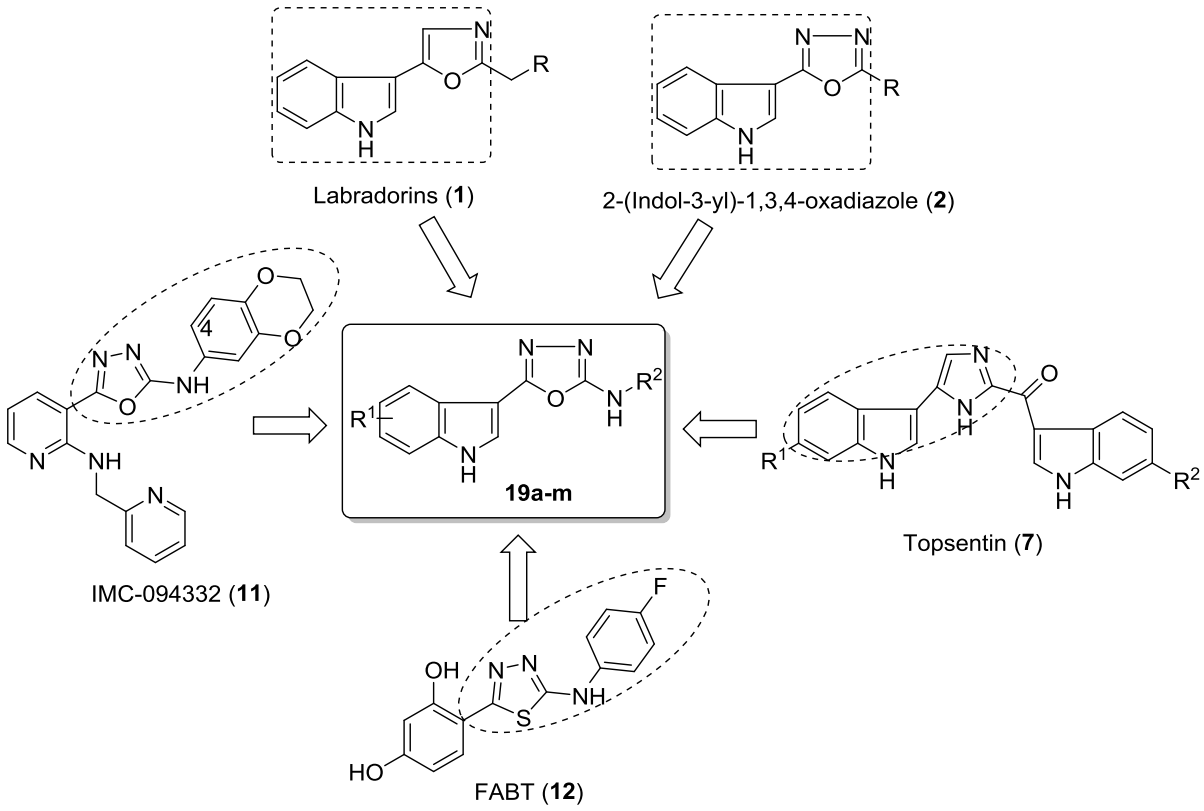


Figure 3.2.2 Design strategy of 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles

The five-membered oxadiazole ring system is an important structural motif, found in a wide range of biologically potent compounds and known to have interesting applications in medicinal chemistry as bioisosteres for ester and amide functionalities.[18-23] In particular, 2-arylamino-1,3,4-oxadiazoles are important chemical entities due to their interesting biological properties[24,25] and various applications in materials science as photosensitizers and organic light-emitting diodes.[26] Ouyang *et al.* identified arylamino-1,3,4-oxadiazoles (IMC-094332)

11 as a potent tubulin inhibitor.[27] Indolyl-1,3,4-oxadiazoles (**2**) reported as analogues of naturally occurring Labradorins (**1**) and found to possess *in vitro* cytotoxicity in the micromolar ranges.[28] Furthermore, 2-arylamino-5-(2,4-dihydroxy-phenyl)-1,3,4-thiadiazoles (e.g., FABT (**12**) have also emerged as potent cytotoxic agents (Figure 3.2.2).[29] Several bis(indolyl)thiophenes,[30] pyrazoles,[31] furans,[32] isoxazoles,[33] pyrroles,[34] indolyl-phenyl and azaindolylphenylthiazoles[35] have been reported as anticancer agents. Subsequently, indolyloxadiazoles and indolylthiadiazoles have also been identified as potent anticancer agents.[5,36-38]

On the basis of the above-mentioned observations regarding the potential of oxadiazoles and indoles and our own observations regarding the importance of the indole motif in conferring anticancer activity, 2-arylamino-1,3,4-oxadiazoles (**19a–m**) were designed. Newly designed 2-arylamino-1,3,4-oxadiazoles consist of key scaffolds indolyloxadiazole and arylamino-oxadiazole (Figure 3.2.2).

3.2.2 Results and discussion

3.2.2.1 Synthesis

General methods for the synthesis of 2-arylamino-1,3,4-oxadiazoles involves cyclodehydration of semicarbazides and cyclodesulfurization of thiosemicarbazides.[39] Cyclodehydration of semicarbazides to form 1,3,4-oxadiazoles employs concentrated sulfuric acid, *p*-toluenesulfonyl chloride, phosphorousoxychloride, thionylchloride, bromine, and methyl *N*-(triethylammonium-sulfonyl)carbamate (Burgess reagent).[40-42] Cyclodesulfurization of acylthiosemicarbazides to 1,3,4-oxadiazoles involves the use of mercury and lead salts, molecular iodine in basic medium, tosyl chloride in combination with pyridine, 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent), 2-iodoxybenzoic acid (IBX), hydantoin-potassium iodide, and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-tetrafluoroborate (TBTU) in the presence of *N*,*N*-diisopropylethylamine (DIEA).[23, 39, 43-45] Another approach involves the reaction of carboxylic acids with thiosemicarbazides to generate acylthiosemicarbazides, which were cyclized to the corresponding 1,3,4-oxadiazoles by employing coupling reagents namely, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI.·HCl), *N*,*N'*-dicyclohexylcarbodiimide (DCC), and PS-carbodiimide (Figure 3.2.3).[39,45]

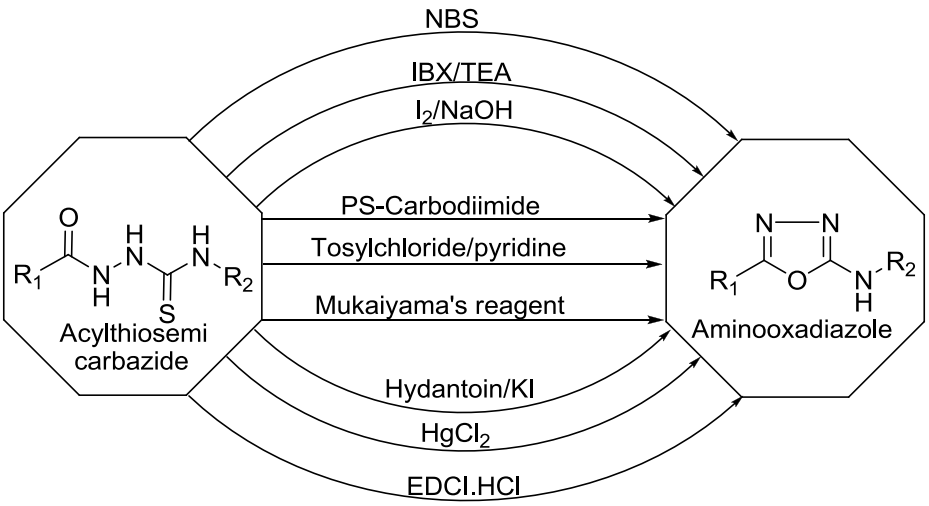
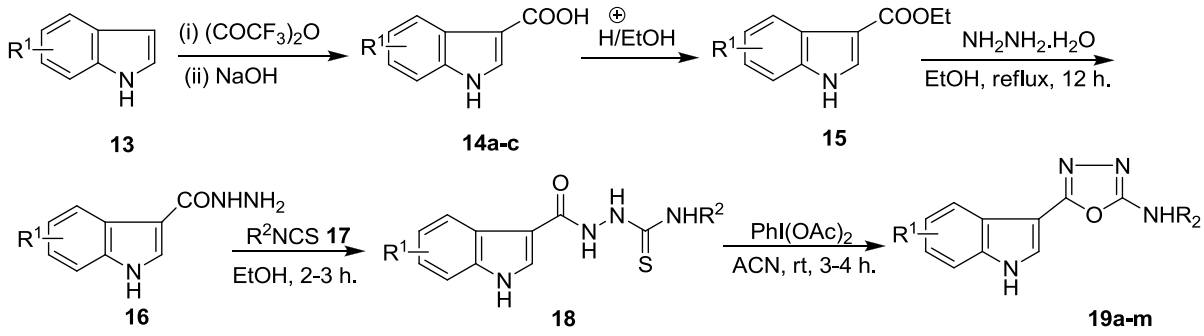


Figure 3.2.3 Conversion of acylthiosemicarbazides into 1,3,4-oxadiazoles

Alternatively, 2-arylamino-1,3,4-oxadiazoles were synthesized by amination of oxadiazol-2-ones using phosphorous oxychloride and an appropriate amine. In 2008, wan *et al.* synthesized 2-arylamino-1,3,4-oxadiazoles from activation of oxadiazol-2-ones through S_NAr substitution using phosphonium reagents (e.g., BOP).[46] Recently, Telvekar *et al.* reported the preparation of 2-arylamino-1,3,4-oxadiazoles by using activated iodine(III) species, generated *in situ* from iodobenzene and oxone in the presence of triethylamine.[44] In view of the advantages associated with hypervalent iodine(III) reagents, the later protocol was extended to prepare 2-arylamino-1,3,4-oxadiazoles (**19a–m**). Synthesis of the designed 2-arylamino-1,3,4-oxadiazoles (**19a–m**) was carried out from readily available indolylhydrazides **16** *via* iodobenzene diacetate (IBD)-mediated desulfurization of the intermediate acylthiosemicarbazides **18** (Scheme 3.2.1).



Scheme 3.2.1 Synthesis of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles (**19a-m**)

Readily accessible indolylhydrazides **16** were prepared from the corresponding indoles **13**. Reaction of **13** with trifluoroacetic anhydride and followed by hydrolysis using sodium hydroxide produced acid **14**. Treatment of **14** with H_2SO_4 in presence of ethanol led to the

corresponding indole-3-carboxylates **15**. Further reaction of **15** with hydrazine hydrate afforded indolylhydrazides **16** in good yields. Reaction of indolylhydrazides **16** with aryl isothiocyanates **17** in ethanol gave acylthiosemicarbazides **18** in almost quantitative yields. Subsequent cyclodesulfurization of acylthiosemicarbazides **18** with IBD in acetonitrile afforded **19a–m** in good yields (72–85%). Attempts using other hypervalent iodine reagents such as Dess–Martin periodinane (DMP), *o*-iodoxybenzoic acid (IBX) and [bis(trifluoroacetoxy)iodo]benzene required extended reaction times (8–10 h.) and produced **19** in poor yields (40–50%). The cyclodesulfurization reaction remained incomplete in dichloromethane or chloroform, probably due to poor solubility of acylthiosemicarbazides **18**. Newly synthesized 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles (**19a-m**) were well characterized using IR, NMR (^{1}H & ^{13}C) and mass spectral data. Purity of all the compounds **19a-m** were more than 97% as checked by HPLC analysis. Copies of NMR (^{1}H & ^{13}C) spectra and HPLC traces for a representative compound **19a** are given in Figures 3.2.4-3.1.6.

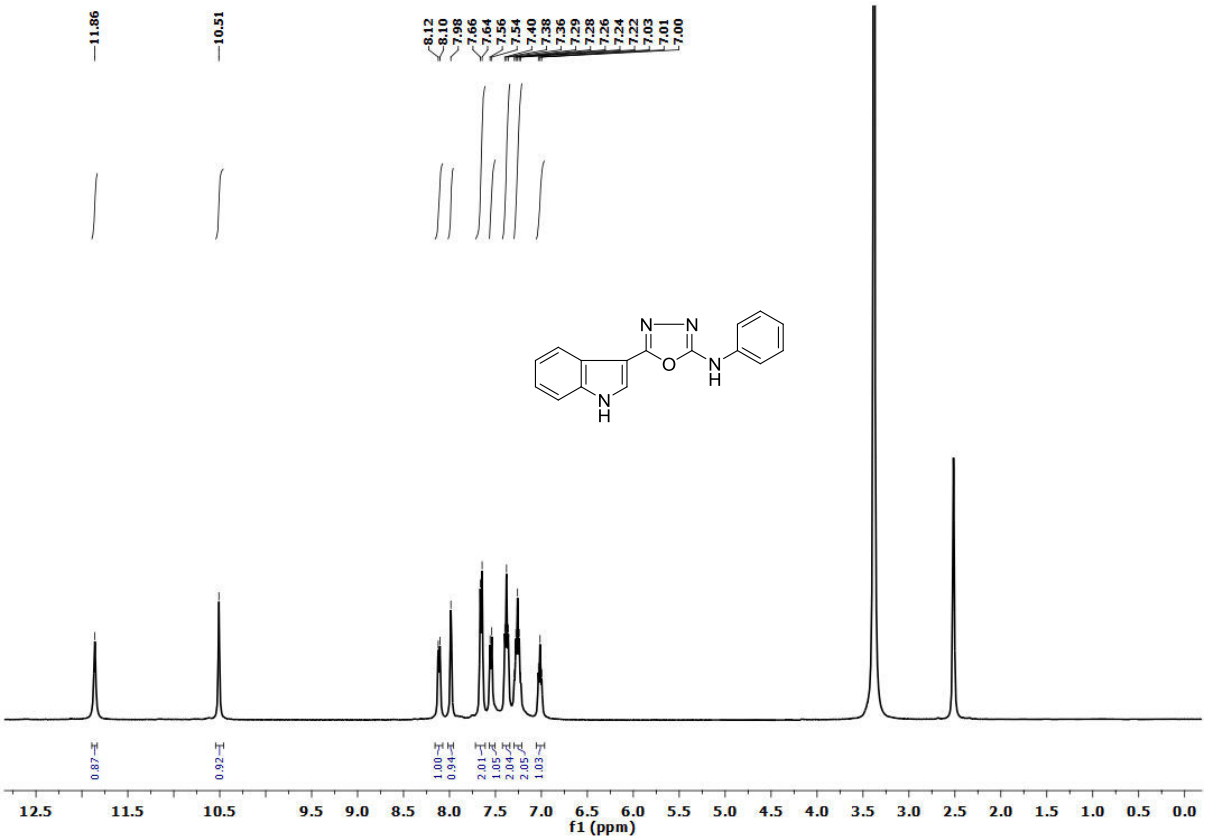


Figure 3.2.4 ^{1}H NMR spectrum of **19a**

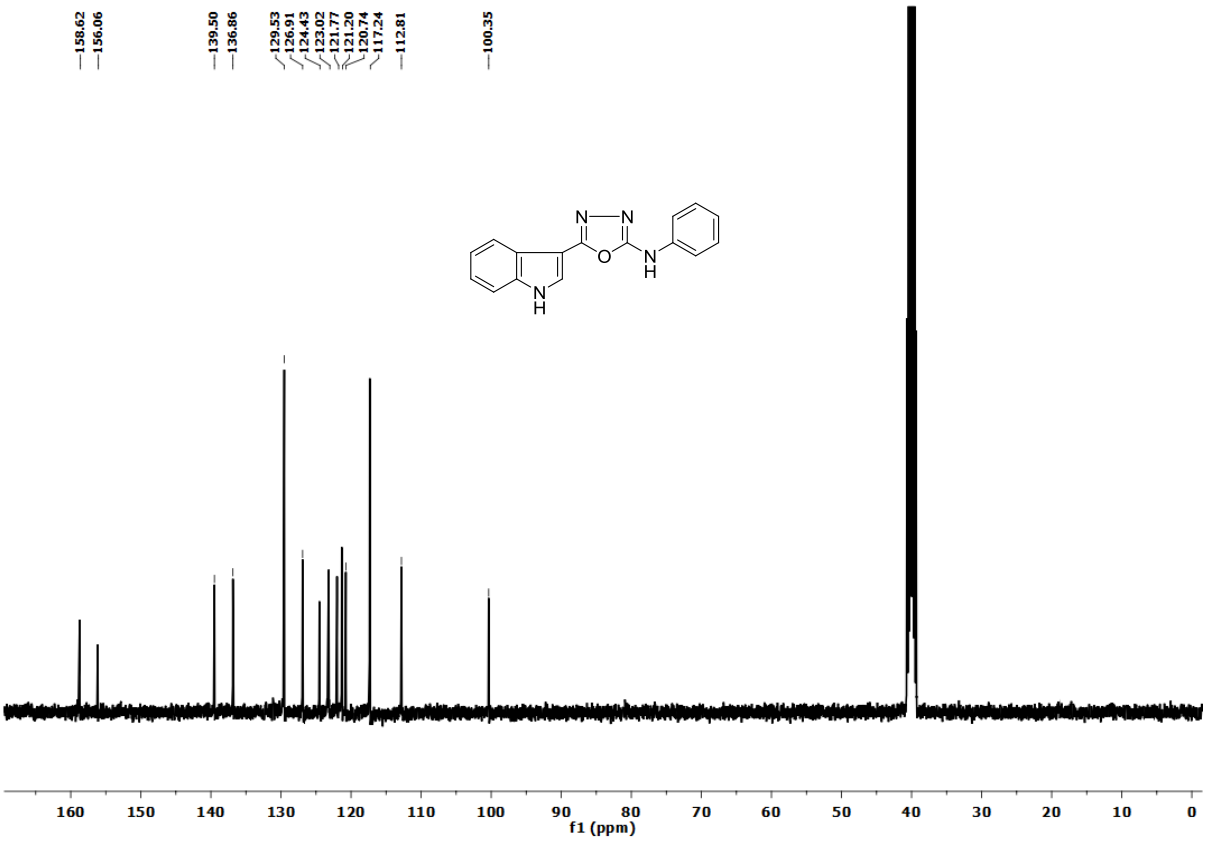
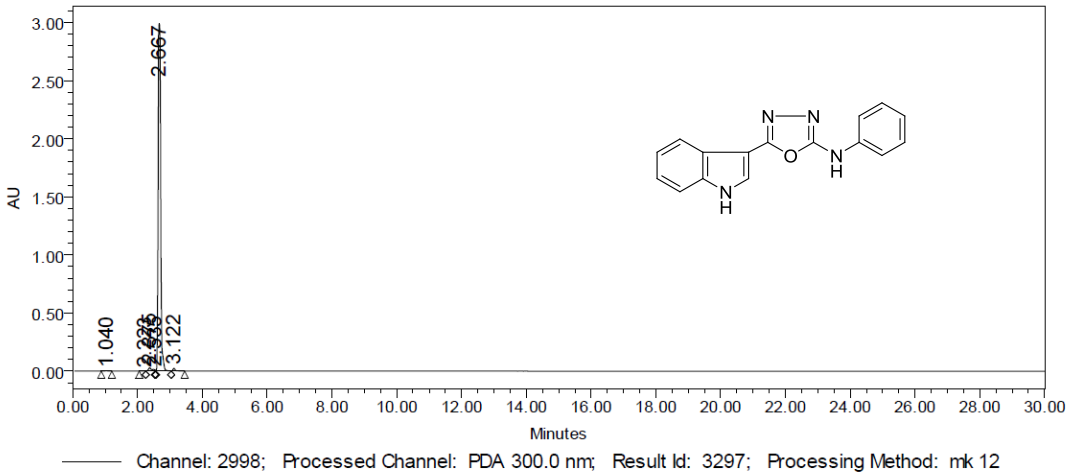


Figure 3.2.5 ^{13}C NMR spectrum of **19a**

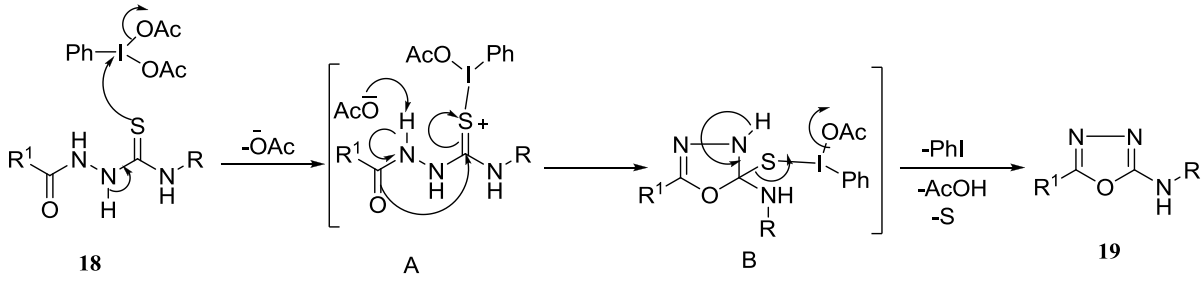


Processed Channel Descr.: PDA 300.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 300.0 nm	1.040	18532	0.11	2652
2	PDA 300.0 nm	2.223	12625	0.07	2806
3	PDA 300.0 nm	2.375	226374	1.33	27258
4	PDA 300.0 nm	2.535	14409	0.08	10034
5	PDA 300.0 nm	2.667	16627668	97.71	2991084
6	PDA 300.0 nm	3.122	118340	0.70	24197

Figure 3.2.6 HPLC traces of **19a**

3.2.2.2 Plausible reaction mechanism



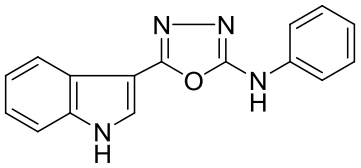
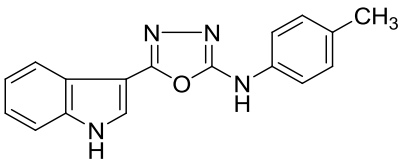
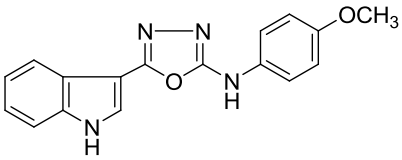
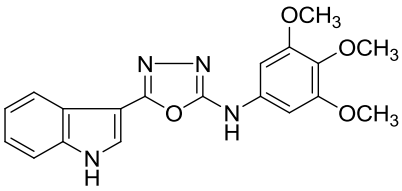
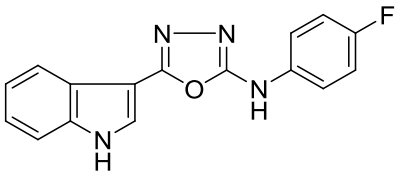
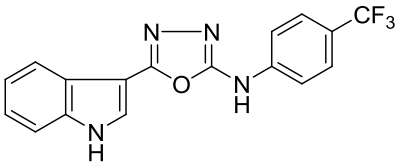
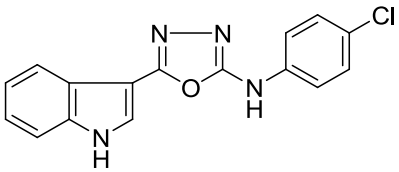
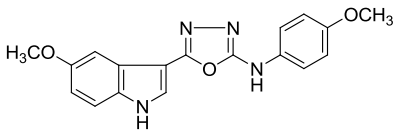
Scheme 3.2.2 Plausible reaction mechanism for the formation of **19a-m**

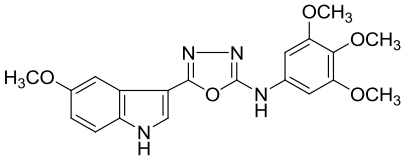
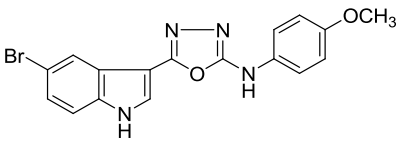
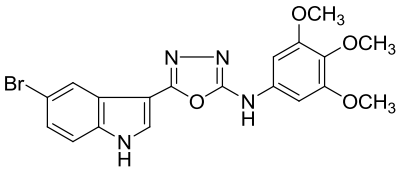
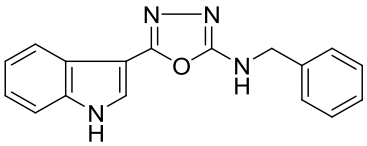
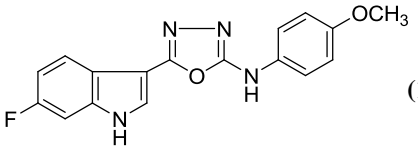
Initial nucleophilic attack of thiosemicarbazide **18** through sulfur on iodobenzenediacetate forms an adduct **A** which upon internal cyclization forms oxadiazoline **B**. Finally, adduct **B** by the loss of sulfur, iodobenzene and acetic acid may led to 2-arylaminooxadiazoles **19** (Scheme 3.2.2).

3.2.2.3 Anticancer activity

2-Arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles (**19a–m**) were assessed for their *in vitro* cytotoxicity against human embryonic kidney cells (HEK293), and a panel of cancer cell lines: cervical (HeLa), prostate (LNCaP and PC3) and breast (MCF-7 and MDA-MB-231). Doxorubicin was evaluated in parallel as a positive control, and IC_{50} values were determined from the results of MTT assays (Table 3.2.1). The activity results indicate that substituents at the C-2 and C-5 positions of 1,3,4-oxadiazole ring have significant impact on the potency of derivatives **19a–m**. Most of the compounds exhibited significant anticancer activities in tested cell lines with IC_{50} values in nanomolar to micromolar ranges. Structure–activity relationships (SAR) studies were probed through altering substituents in the indole and *N*-arylamino moieties. Unsubstituted derivative **19a** was found to be active against the tested cancer cell lines. In general, electron-donating groups on the aryl ring of the *N*-arylamino moiety were found to be beneficial for the activity. Analogue **19b** with *p*-tolyl substituent displayed enhanced activity against all tested cell lines, but was particularly active in HeLa cells with an IC_{50} value of **<** 0.001 µM. Introduction of *p*-methoxyphenyl (**19c**) or trimethoxyphenyl (**19d**) groups in the anilino part led to compounds with substantial activity, particularly against prostate cancer cell line (PC-3 and LNCaP). The trimethoxyphenyl derivative (**19d**) was also found to exhibit potent cytotoxicity in HEK293 cells, with an IC_{50} value of 0.1 µM.

Table 3.2.1 *In-vitro* cytotoxicity data of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles (**19a-m**) IC_{50} (μM)[a]

Compounds		HEK293	HeLa	LNCaP	MCF-7	PC3	MDA-MB-231
	**(19a)**	1.5± 0.01	2.2 ± 0.04	3.0 ± 0.01	1.6 ±0.01	4.7±0.40	**0.8**±0.01
	**(19b)**	2.4±0.04	**< 0.001**	2.8±0.01	0.2±0.01	**0.8**±0.07	**0.2**±0.01
	**(19c)**	1.0±0.01	3.1±0.1	**0.5**±0.01	1.0±0.01	**0.1**±0.01	7.6±0.19
	**(19d)**	**0.1**±0.01	**0.5**±0.01	**0.9**±0.01	**0.5**±0.03	**0.2**±0.01	**0.9**±0.06
	**(19e)**	7.2±0.14	1.0±0.04	11.2±0.13	**0.4**±0.03	9.7±0.38	4.3±0.31
	**(19f)**	10.6±0.1	**0.2**±0.01	**0.6**±0.01	**0.2**±0.01	2.7±0.01	5.8±0.1
	**(19g)**	4.7±0.14	**0.3**±0.03	1.0±0.06	**0.1**±0.01	**0.2**±0.02	1.5±0.06
	**(19h)**	**0.1**±0.01	10.3±0.30	4.6±0.03	**0.1**±0.004	**0.7**±0.01	1.9±0.04

 **(19i)**	**0.3**±0.01	**< 0.001**	**0.7**±0.03	6.4±0.4	2.0±0.1	55.0±3.5
 **(19j)**	**0.6**±0.006	**0.1**±0.002	**0.2**±0.01	1.1±0.01	**0.2**±0.01	**0.9**±0.03
 **(19k)**	1.1±0.01	1.7±0.03	**0.6**±0.01	1.4±0.01	**0.2**±0.01	**0.3**±0.03
 **(19l)**	1.1±0.02	**0.8**±0.03	5.7±0.47	**< 0.001**	**0.1**±0.01	64.4±0.51
 **(19m)**	**0.2**±0.01	1.5±0.12	3.7±0.07	**0.1**±0.003	**0.1**±0.01	**0.8**±0.01
Doxorubicin	**0.4**±0.02	**0.9**±0.04	**0.3**±0.01	1.4±0.06	**0.7**±0.02	1.5±0.11
Labradorin 1	-	-	-	25	**-**	-

[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. SEM was < 10% for each value. [b] Bold values shows IC_{50} of less than 0.1 μM.

Compounds **19e** and **19g** with electronegative substituents (F and Cl) on the phenyl group of the C-2 *N*-arylamino motif exhibited good cytotoxicity across all cell lines tested, however, the cytotoxicity observed against MCF-7 (**19e**, IC_{50} = 0.4 μM; **19g**, IC_{50} = 0.1 μM) and HeLa (**19e**, IC_{50} = 1.0 μM; **19g**, IC_{50} = 0.3 μM) cancer cell lines was particularly potent. Furthermore, *p*-chlorophenyl analogue **19g** showed improved activity against a pancreatic cancer cell line (PC-3, IC_{50} = 0.2 μM), whereas *p*-fluorophenyl analogue **19e** was found to be selectively cytotoxic against HeLa and MCF-7 cells. Replacement of the methyl moiety in compound **19b** by a trifluoromethyl (**19f**) imparted activity against LNCaP cells, however, activity was decreased against the other cell lines tested. Introduction of a methoxy group at C_5 position of indole ring in compound **19c** led to analogue **19h** with ten-fold improvement in

activity against HEK293 and MCF-7 cells, and four-fold improvement against MDA-MB-231 cells. The same modification to trimethoxyphenyl analogue **19d** to give compound **19i** resulted in significantly improved cytotoxicity against HeLa cells (IC_{50} < 0.001 μM). The presence of a bromo substituent at the indole 5 position was found to be beneficial for activity. 5-Bromoindoles **19j** and **19k** displayed improved activity against the MDA-MB-231 cell line over the unsubstituted indole analogues **19c** and **19d**, however, derivative **19j** with a single methoxy group in the para position of *N*-arylamino moiety also exhibited a 30-fold improvement in cytotoxicity (HeLa cells). Compound **19l** with indole and *N*-benzyl moieties exhibited cytotoxicity in nanomolar range against MCF-7 cells and good cytotoxicity against all other tested cell lines except MDA-MB-231. Incorporation of a fluoro substituent at the 6 position of indole ring was found to be beneficial for activity. 6-Fluoroindole containing analogue **19m** showed improved cytotoxicity when compared to analogue **19c** against HEK293 (IC_{50} = 0.2 μM) and breast cancer cells (MCF-7, IC_{50} = 0.1 μM; MDA-MB-231, IC_{50} = 0.8 μM).

Structure-activity relationship studies of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles revealed that indole with C5/6-methoxy or halogen and arylamino part with benzyl, *p*-tolyl or trimethoxyphenyl moieties are beneficial for the anticancer activity (Figure 3.2.7).

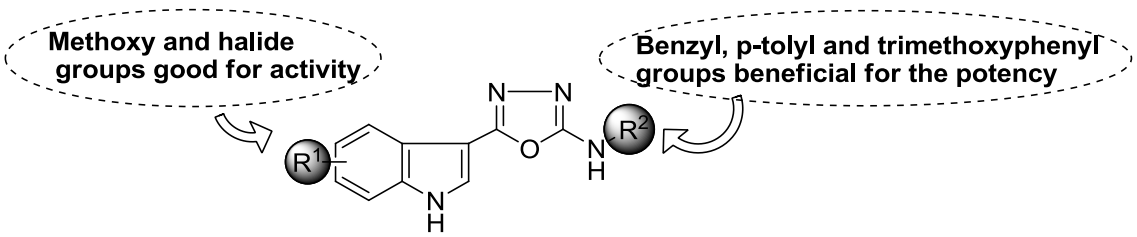


Figure 3.2.7 Structure-activity relationship of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles

3.2.2.4 Apoptosis study

Finally, we investigated the mechanism of cell death in MDA-MB-231 cells using compounds (10 μM) **19a** and **19e**, selected for their potency against the cell line (IC_{50} = 0.8 and 4.3 μM). After incubation for 48 h., MDA-MB-231 cells were fixed and stained with propidium iodide, and their nuclear morphology was analyzed using fluorescence microscopy. While DMSO-treated control cells revealed normal healthy nuclei, inhibitor-treated cells showed apoptotic nuclei (Figure 3.2.8), suggesting that apoptosis is the major mechanism by which these 2-arylamino-1,3,4-oxadiazoles promote cell death in MDA-MB-231 cancer cells.

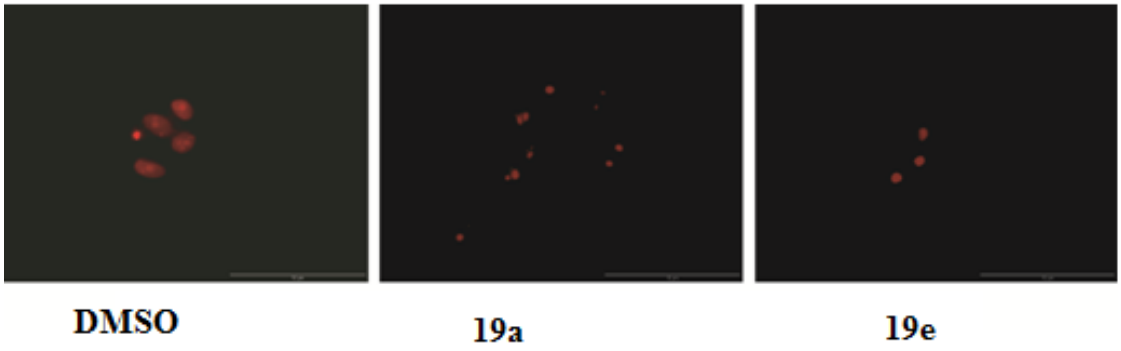


Figure 3.2.8 Propidium iodide staining of MDA-MB-231 cells treated with compounds **19a** and **19e** for 48 h. DMSO was used as a control. Scale: 10 mm

3.2.3 Conclusions

In summary, a convenient and high yielding synthesis of 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles by employing IBD-mediated oxidative desulfurization of readily available acylthiosemicarbazides **18** has been developed. Results from anticancer screening showed that compounds **19a–m** possess significant antiproliferative activity against a panel of cell lines. The most potent compounds (**19b**, **19i** and **19l**), with *N*-(tolyl), *N*-(trimethoxyphenyl) and *N*-(benzyl) motifs at the C-2 position of the 1,3,4-oxadiazole core, exhibited IC_{50} values in the nanomolar range against most of the cell lines tested. Interestingly, compounds **19i** and **19l** although exceedingly potent against most cell lines tested, were not as cytotoxic in MDA-MB-231 cells. Compounds with electron-withdrawing substituents in the C-2 *N*-aryl ring (**19e–g**) exhibited a greater range of IC_{50} values across the tested cell lines, indicating potential selective cytotoxicity. Also, the introduction of electron-donating (MeO) and electron-withdrawing (Br and F) substituents in the indole ring resulted in derivatives with enhanced cytotoxicity. Finally, preliminary mechanism of action studies indicated that these compounds induce apoptosis in MDA-MB-231 cancer cells.

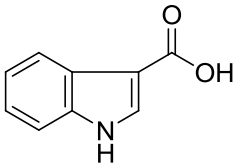
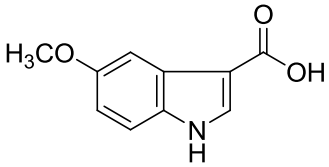
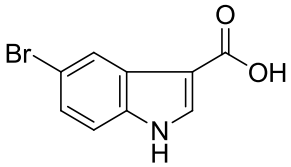
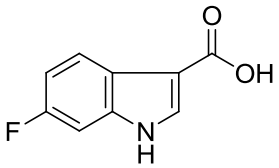
3.2.4 Experimental Section

Chemistry

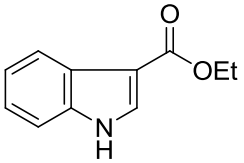
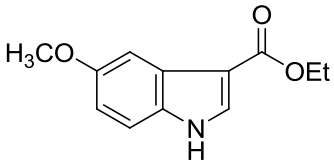
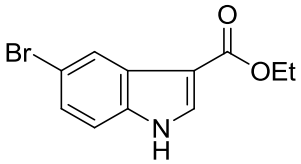
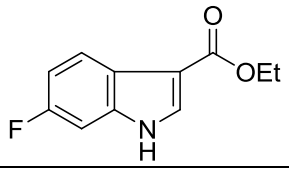
All the laboratory grade reagents were obtained commercially either from Aldrich or Spectrochem. The reactions were monitored by thin layer chromatography, which was performed on commercially available Merck precoated plates (silicagel 60 F_{254}, 0.25 mm). Organic solvents were evaporated using rotary evaporator. Melting points were determined

on a *E-Z* melting apparatus. NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker advance II (400 and 100 MHz, respectively) spectrophotometer using DMSO-d_6 as a solvent. Mass spectra were obtained on Bruker ProFLEX III MALDI-TOF (using DHB as the matrix) and ABI Sciex 5800 TOF (ESI-MS) mass spectrometers. Analyses were performed on a Waters 515 HPLC system equipped with a reversed phase Sunfire C-18 column (5 µm, 4.6 mm × 250 mm) and PDA detector, flow rate 1 mL/min and a gradient of solvent A (methanol) and solvent B (acetonitrile).

Synthesis of indole-3-carboxylic acids (**14a-c**):[5] To a stirred solution of indole **13** (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 the aqueous solution was acidified with hydrochloric acid. The precipitate was isolated by filtration and dried to obtain pure indole-3-carboxylic acids **14** in 71-82% yields.

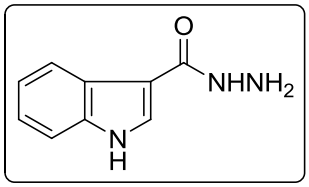
Indole-3-carboxylic acid		**mp (°C)**	**Yield (%)**
	**14a**	232-234 (Lit.[5] 229-234)	82
	**14b**	174-175 (Lit.[47] 172-175)	71
	**14c**	236-238 (Lit.[48] 238-240)	75
	**14d**	240-242 (Lit.[49] 242-244)	78

General procedure for the sythesis of ethyl ester of indole-3-carboxylic acid (15):[5] To a solution of indole-3-carboxylic acid **14** (1 mmol) in ethanol (20 mL) was added a catalytic amount of concentrated sulfuric acid (0.2 mL) and allowed to reflux for 20 h. After completion of the reaction, ethanol was removed *in vacuo* and the residue was extracted with ethyl acetate (2 × 15 mL) and washed with saturated sodium bicarbonate solution (25 mL). Organic layer was dried over anhydrous sodium sulphate and evaporated to furnish the corresponding esters **15** in good yields (75-85%).

Ethyl-1*H*-indole-3-carboxylate		mp (°C)	Yield (%)
	**15a**	120-123 (Lit.[5] 120-124)	85
	**15b**	145-146	75
	**15c**	135-137	75
	**15d**	142-144	78

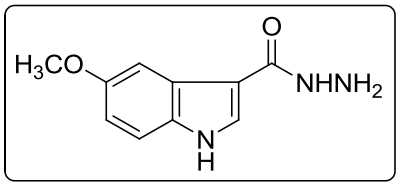
General procedure for the preparation of indole-3-carbohydrazides (16a-d): A solution of an appropriate ester **15** (1 mmol) and hydrazine hydrate (2 mmol) in ethanol (15 mL) was refluxed for 4 h. Cooled the reaction mixture, the solid so obtained was filtered and recrystallized from ethanol to obtain pure hydrazides **16a-d** in 85-90% yields.

Indole-3-carbohydrazide (16a)



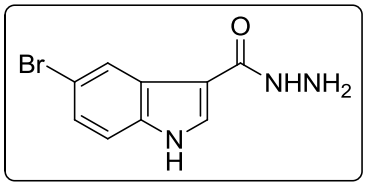
Yield 85%; White solid; mp 232-234 °C; IR (KBr, *v,* cm^{-1}): 3256, 3109, 1660, 1607, 1583, 1523, 1433, 1240, 736; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 9.14 (s, 1H), 8.17 (d, J = 8.10 Hz, 1H), 7.95 (d, J = 2.80 Hz, 1H), 7.41 (d, J = 8.10 Hz, 1H), 7.20–6.99 (m, 2H), 3.95 (s, 2H); MS (ESI) *m/z* calcd for $C_9H_9N_3O$: 175.1 $[M]^+$, found: 175.2.

5-Methoxyindole-3-carbohydrazide (16b)



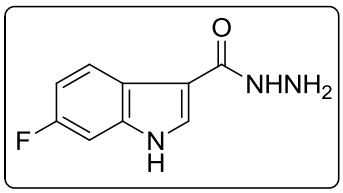
Yield 90%; White solid; mp 178-179 °C; IR (KBr, *v,* cm^{-1}): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.68 (s, 1H), 9.64 (s, 1H), 7.61 (s, 1H), 7.49 (s, 1H), 7.41–7.39 (m, 2H), 4.18 (s, 2H), 3.86 (s, 3H); MS (ESI) *m/z* calcd for $C_{10}H_{12}N_3O_2$: 206.1 $[M + H]^+$, found: 206.2.

5-Bromo-indole-3-carbohydrazide (16c)



Yield 90%; White solid; mp 255-257 °C; IR (KBr, *v,* cm^{-1}): 3340, 3290, 3050, 2920, 1646, 1605, 1545, 778, 724; ^{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), 7.47–7.45 (m, 2H), 4.23 (s, 2H); MS (ESI): *m/z* calcd. for $C_9H_8BrN_3O$: 253.0 $[M]^+$, found: 253.0.

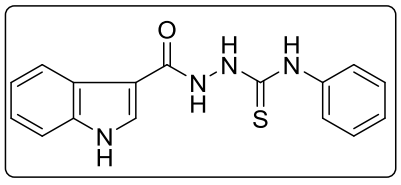
6-Fluoroindole-3-carbohydrazide (16d)[5]



Yield 85%; Off-white solid; mp 204–205 °C; IR (KBr *v,* cm^{-1}): 3310, 3163, 3070, 2931, 1620, 1542, 1442, 1218, 1141, 778, 724; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 9.61 (s, 1H), 8.29–8.24 (m, 1H), 7.25–7.19 (m, 1H), 7.18–7.15 (m, 1H), 7.10–7.04 (m, 1H), 4.20 (s, 2H).

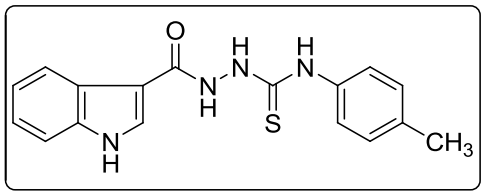
Synthesis of 1-(indole-3-carbonyl)-4-arylthiosemicarbazides (18a-m): Indole-3-carbohydrazide **16** (2.8 mmol) and aryl isothiocyanate **17** (2.8 mmol) were taken into a round bottom flask containing 10 mL of ethanol and stirred the reaction mixture at 60 °C for 2-3 h. The solid so formed was filtered and dried well to obtain thiosemicarbazides **18a-m** in good yields (85-95%). The products were sufficiently pure and used as such for the next step.

2-(1*H*-Indole-3-carbonyl)-*N*-phenylhydrazinecarbothioamide (18a)



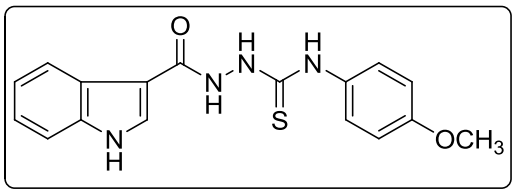
Yield 95%; White solid; mp 205-206 °C; IR (KBr, *v,* cm^{-1}): 3253, 3197, 1645, 1597, 1498, 1355, 1242, 740, 684; MS (ESI) *m/z* calcd. for $C_{16}H_{14}N_4OS$: 310.1, found: 311.1 $[M + H]^+$.

2-(1*H*-Indole-3-carbonyl)-*N*-*p*-tolylhydrazinecarbothioamide (18b)



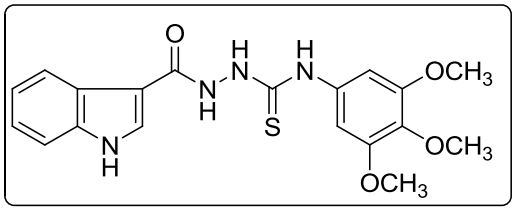
Yield 90%; White solid; mp 202-203 °C; IR (KBr, *v,* cm^{-1}): 3394, 3142, 1666, 1585, 1495, 1354, 1242, 736, 688; MS (ESI) *m/z* calcd. for $C_{17}H_{16}N_4OS$: 324.1, found: 325.0 $[M + H]^+$.

2-(1*H*-Indole-3-carbonyl)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide (18c)



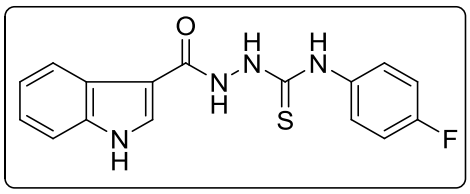
Yield 85%; White solid; mp 210-211 °C; IR (KBr, *v,* cm^{-1}): 3394, 3142, 1666, 1585, 1495, 1354, 1242, 840, 736, 688.

2-(1*H*-Indole-3-carbonyl)-*N*-(3,4,5-trimethoxyphenyl)hydrazinecarbothioamide (18d)



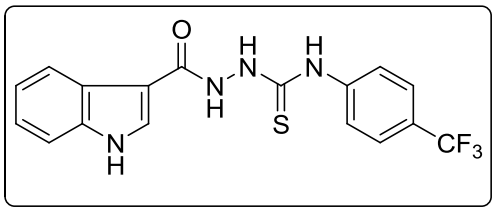
Yield 90%; White solid; mp 204-206 °C; IR (KBr, *v,* cm^{-1}): 3296, 3213, 1655,1600, 1483, 1359, 1272, 1176, 1130, 864, 748, 663; MS (ESI) *m/z* calcd. for $C_{19}H_{20}N_4O_4S$: 400.1, found: 401.0 $[M + H]^+$.

***N*-(4-Fluorophenyl)-2-(1*H*-indole-3-carbonyl)hydrazinecarbothioamide (18e)**



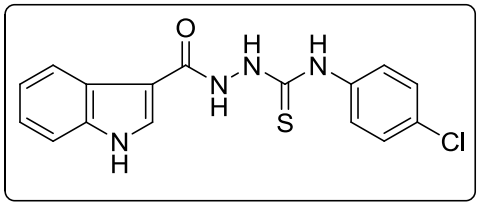
Yield 85%; White solid; mp 205-207 °C; IR (KBr, *v,* cm^{-1}): 3272, 3232, 1647, 1598, 1495, 1356, 1245, 829, 741, 686.

2-(1*H*-Indole-3-carbonyl)-*N*-(4-(trifluoromethyl)phenyl)hydrazinecarbothioamide (18f)



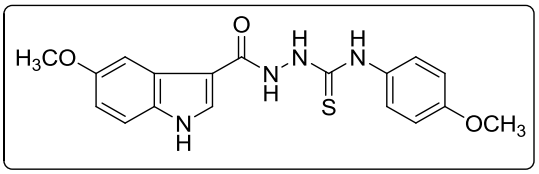
Yield 80%; White solid; mp 213-214 °C; IR (KBr, *v,* cm^{-1}): 3257, 3199, 1645, 1590, 1487, 1336, 1242, 836, 742, 682.

***N*-(4-Chlorophenyl)-2-(1*H*-indole-3-carbonyl)hydrazinecarbothioamide (18g)**



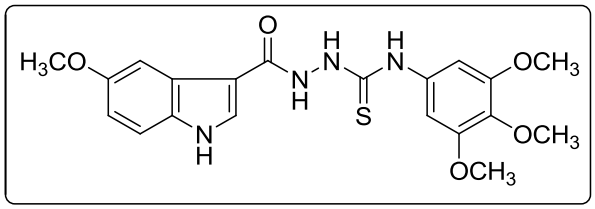
Yield 85%; White solid; mp 205-207 °C; IR (KBr, *v,* cm^{-1}): 3275, 3230, 1645, 1597, 1494, 1352, 1242, 827, 744, 690.

2-(5-Methoxy-1*H*-indole-3-carbonyl)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide (18h)



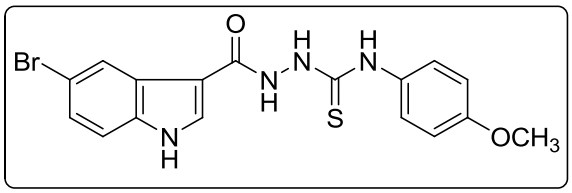
Yield 80%; White solid; mp 183-184 °C; IR (KBr, *v,* cm^{-1}): 3269, 3176, 1629, 1586, 1485, 1247, 1176, 865, 794, 654.

2-(5-Methoxy-1*H*-indole-3-carbonyl)-*N*-(3,4,5-trimethoxyphenyl)hydrazine-carbothioamide (18i)



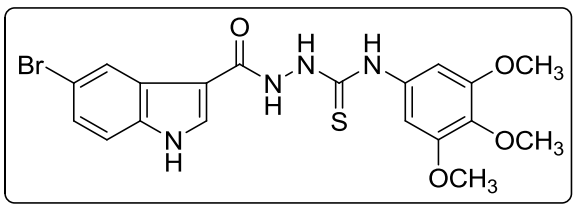
Yield 84%; White solid; mp 191-192 °C; IR (KBr, *v,* cm^{-1}): 3365, 3245, 1665, 1584, 1459, 1237, 1165, 835, 785, 680.

2-(5-Bromo-1*H*-indole-3-carbonyl)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide (18j)



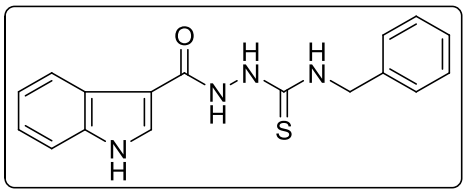
Yield 86%; White solid; mp 230-232°C; IR (KBr, *v,* cm^{-1}): 3292, 3210, 1651, 1609, 1479, 1361, 1270, 1171, 1138, 865, 742, 664.

2-(5-Bromo-1*H*-indole-3-carbonyl)-*N*-(3,4,5-trimethoxyphenyl)hydrazine-carbothioamide (18k)



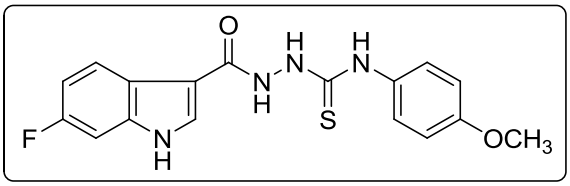
Yield 90%; White solid; mp 216-218 °C; IR (KBr, *v,* cm^{-1}): 3292, 3210, 1651, 1609, 1479, 1361, 1270, 1171, 1138, 865, 742, 664.

2-(1*H*-Indole-3-carbonyl)-*N*-(4-methoxybenzyl)hydrazinecarbothioamide (18l)



Yield 86%; White solid; mp 230-232 °C; IR (KBr, *v,* cm^{-1}): 3313, 3253, 1665, 1603, 1498, 1379, 1244, 1168, 738, 698.

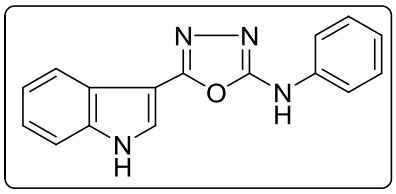
2-(6-Fluoro-1*H*-indole-3-carbonyl)-*N*-(4-methoxyphenyl)hydrazinecarbothioamide (18m)



Yield 95%; White solid; mp 215-217 °C; IR (KBr, *v,* cm^{-1}): 3319, 3257, 1667, 1601, 1491, 1374, 1240, 1165, 731, 696.

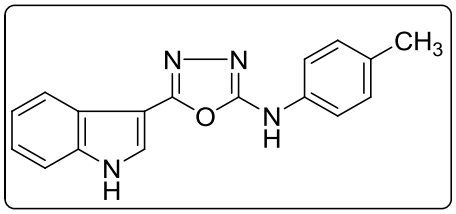
Synthesis of 2-arylamino-5-(indolyl)-1,3,4-oxadiazoles (19a-m): To a cooled (10 °C) solution of thiosemicarbazides **18** (0.85 mmol) in dry acetonitrile (5 mL) was added iodobenzene diacetate (1 mmol) portion wise. The reaction mixture was allowed to stir at room temperature for 3-4 h. After completion of the reaction, solvent was removed at reduced pressure and the residue so obtained was triturated with hexane to afford **19a-m**. Crude products were recrystalised from ethanol to obtain pure **19a-m** in 72-85% yields.

5-(1*H*-Indol-3-yl)-*N*-phenyl-1,3,4-oxadiazol-2-amine (19a)



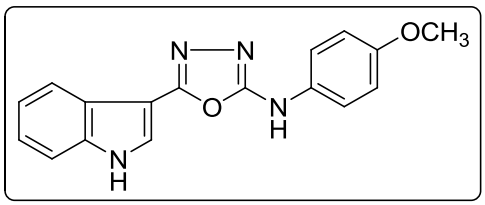
Yield 75%; Light brown powder; mp 212-214 °C; IR (KBr, *v,* cm^{-1}): 3193, 1620, 1550, 1450, 1178, 750, 694; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.73 (s, 1H), 9.87 (s, 1H), 8.07 (d, J = 7.3 Hz, 1H), 7.86 (s, 1H), 7.98 (d, J = 2.8 Hz, 1H), 7.53–7.50 (m, 3H), 7.22–7.20 (m, 2H), 7.13 (d, J = 8.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.6, 156.0, 139.5, 136.9, 129.5, 126.9, 124.4, 123.0, 121.8, 121.2, 120.7, 117.3, 112.8, 100.3; MS (ESI) *m/z* calcd. for $C_{16}H_{13}N_4O$: 277.1 $[M + H]^+$, found: 277.1; HPLC: t_R = 2.667 min (97.71% purity).

5-(1*H*-Indol-3-yl)-*N*-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (19b)



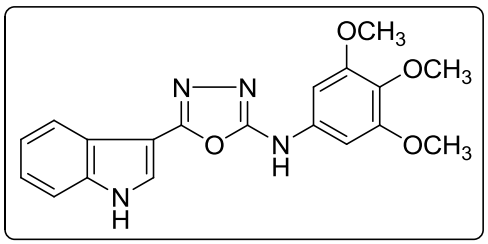
Yield 78%; Redish brown powder; mp 240-242 °C; IR (KBr, *v,* cm^{-1}): 3178, 1612, 1581, 1504, 1420, 1160, 954, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.71 (s, 1H), 10.22 (s, 1H), 8.12 (d, J = 7.3 Hz, 1H), 7.87 (d, J = 2.8 Hz, 1H), 7.52–7.54 (m, 3H), 7.18–7.25 (m, 2H), 7.14 (d, J = 8.3 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.2, 155.5, 136.3, 136.2, 130.2, 129.1, 125.5, 123.9, 122.4, 120.5, 120.3, 116.8, 111.9, 100.2, 20.3; MS (MALDI) *m/z* calcd. for $C_{17}H_{15}N_4O$: 291.1246 $[M + H]^+$, found: 291.1237; HPLC: t_R = 3.153 min (98.04% purity).

5-(1*H*-Indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (19c)



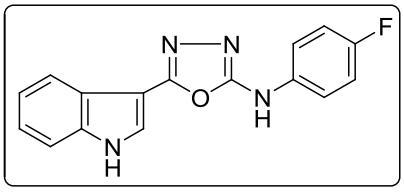
Yield 75%; Light brown powder; mp 215-217 °C; IR (KBr, *v,* cm^{-1}): 3340, 3139, 1635, 1570, 1512, 1458, 1226, 1141, 1030, 833, 794; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.83 (s, 1H), 10.27 (s, 1H), 8.09 (d, *J* = 7.0 Hz, 1H), 7.93 (s, 1H), 7.56–7.51 (m, 3H), 7.27–7.21 (m, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 3.73 (s, 3H); MS (ESI) *m/z* calcd. for $C_{17}H_{15}N_4O_2$: 307.1 $[M + H]^+$, found: 307.1; HPLC: t_R = 3.479 min (98.25% purity).

5-(1*H*-Indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (19d)



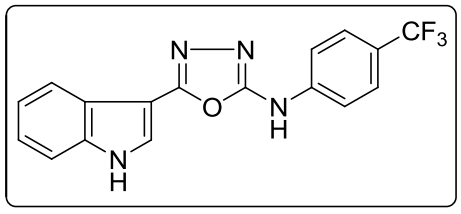
Yield 80%; Light brown powder; mp 263-265 °C; IR (KBr, *v,* cm^{-1}): 3494, 3209, 1650, 1560, 1504, 1450, 1234, 1126, 955, 817, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 1H), 10.23 (s, 1H), 8.14 (d, *J* = 7.5 Hz, 1H), 7.85 (s, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.26–7.18 (m, 2H), 7.03 (s, 2H), 3.85 (s, 6H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.2, 155.5, 152.9, 136.3, 135.1, 131.9, 125.7, 123.9, 122.4, 120.6, 120.3, 112.0, 99.9, 94.6, 60.1, 55.5; MS (MALDI) *m/z* calcd. for $C_{19}H_{19}N_4O_4$: 367.1406 $[M + H]^+$, found: 367.0526; HPLC: t_R = 2.854 min (98.19% purity).

***N*-(4-Fluorophenyl)-5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (19e)**



Yield Light 81%; Brown powder; mp 215-217 °C; IR (KBr, *v,* cm^{-1}): 3409, 3294, 1643, 1581, 1535, 1488, 1350, 1126, 940, 740; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.58 (s, 1H), 10.24 (s, 1H), 8.15 (d, *J* = 7.0 Hz, 1H), 7.81 (d, *J* = 3.4 Hz, 1H), 7.68–7.64 (m, 2H), 7.51–7.49 (m, 1H), 7.26–7.18 (m, 2H), 7.09–7.02 (m, 2H); MS (MALDI) *m/z* calcd. for $C_{16}H_{12}FN_4O$: 295.0995 $[M + H]^+$, found: 295.1038; HPLC: t_R = 2.966 min (98.97% purity).

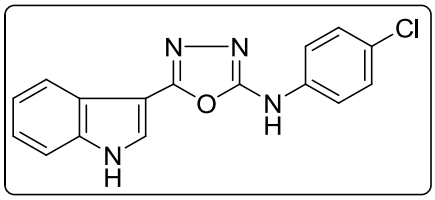
5-(1*H*-Indol-3-yl)-*N*-[4-(trifluoromethyl)phenyl]-1,3,4-oxadia-zol-2-amine (19f)



Yield 85%; Off-white powder; mp 206-208 °C; IR (KBr, *v,* cm^{-1}): 3425, 3271, 1643, 1572, 1427, 1326, 1249, 1118, 804, 725; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.57 (s, 1H), 10.65 (s, 1H), 8.17 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 7.7 Hz, 2H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.51 (d, *J* = 7.4

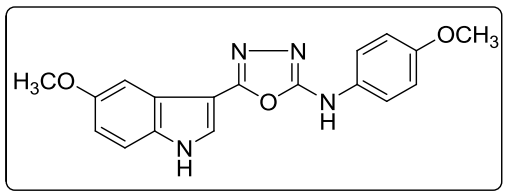
Hz, 1H), 7.28–7.19 (m, 2H); MS (MALDI) *m/z* calcd. for $C_{17}H_{12}F_3N_4O$: 345.0963 $[M + H]^+$, found: 345.0812; HPLC: t_R = 3.127 min (98.94% purity).

_N_-(4-Chlorophenyl)-5-(1_H_-indol-3-yl)-1,3,4-oxadiazol-2-amine (19g)



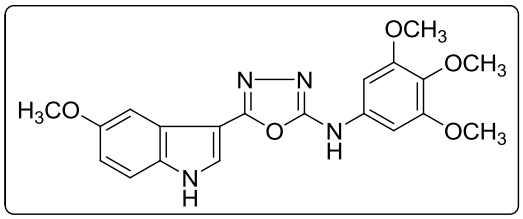
Yield 77%; Off-white powder; mp 237–239 °C; IR (KBr, *v,* cm^{-1}): 3409, 3294, 1643, 1581, 1535, 1488, 1215, 1126, 740, 680; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.85 (s, 1H), 10.67 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.96 (s, 1H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 7.4 Hz, 2H), 7.27–7.24 (m, 2H); MS (ESI) *m/z* calcd. for $C_{16}H_{12}ClN_4O$: 311.1 $[M + H]^+$, found: 311.2; HPLC: t_R = 3.198 min (99.17% purity).

5-(5-Methoxy-1_H_-indol-3-yl)-_N_-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (19h)



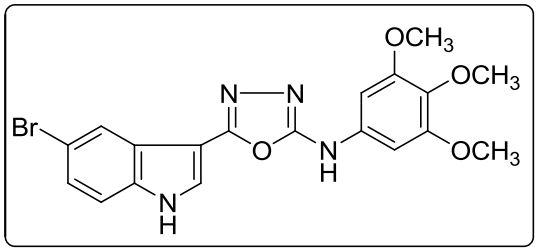
Yield 77%; Off-white powder; mp 210-212 °C; IR (KBr, *v,* cm^{-1}): 3317, 3178, 1650, 1581, 1512, 1419, 1234, 1180, 970, 720; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.51 (s, 1H), 10.05 (s, 1H), 8.10 (s, 1H), 7.77 (d, *J* = 2.6 Hz, 1H), 7.61 (s, 1H), 7.56 (d, *J* =8.8 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 6.90–6.78 (m, 3H), 3.86 (s, 3H), 3.77 (s, 3H); MS (MALDI) *m/z* calcd. for $C_{18}H_{17}N_4O_3$: 337.1301 $[M + H]^+$, found: 337.1664; HPLC: t_R = 3.097 min (98.68% purity).

5-(5-Methoxy-1_H_-indol-3-yl)-_N_-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (19i)



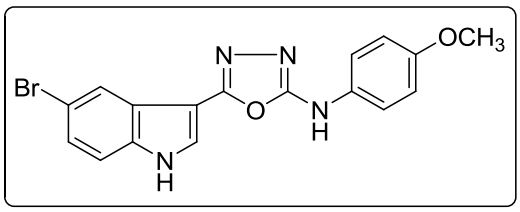
Yield 72%; Light brown powder; mp 250-252 °C; IR (KBr, *v,* cm^{-1}): 3217, 3186, 1658, 1604, 1350, 995, 833, 702; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (s, 1H), 10.06 (s, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.59 (s, 1H), 6.99 (s, 2H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.77 (d, *J* = 8.6 Hz, 1H), 3.81 (s, 6H), 3.78 (s, 3H), 3.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.3, 156.3, 153.3, 152.9, 137.2, 137.1, 132.0, 125.1, 121.3, 118.4, 110.5, 106.9, 95.0, 94.6, 60.1, 55.6, 55.0; MS (MALDI) *m/z* calcd. for $C_{20}H_{21}N_4O_5$: 397.1512 $[M + H]^+$, found: 397.1409; HPLC: t_R = 2.883 min (97.18% purity).

5-(5-Bromo-1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (19j)



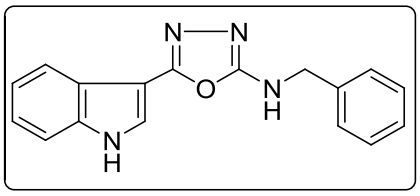
Yield 75%; Brown powder; mp 263-265 °C; IR (KBr, *v,* cm^{-1}): 3260, 3240, 1643, 1550, 1512, 1427, 1242, 1141, 686; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.65 (s, 1H), 10.32 (s, 1H), 8.36–8.32 (m, 2H), 8.18 (s, 1H), 7.61 (s, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.45 (dd, J = 8.6, 1.9 Hz, 1H), 4.08 (s, 3H), 3.99 (s, 3H), 3.94 (s, 3H); MS (MALDI) *m/z* calcd. for $C_{19}H_{18}BrN_4O_4$: 447.0491 $[M + H]^+$, found: 446.9796; HPLC: t_R = 3.205 min (98.67% purity).

5-(5-Bromo-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (19k)



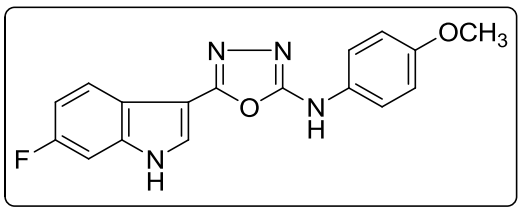
Yield 78%; Light brown powder; mp 199-201 °C; IR (KBr, *v,* cm^{-1}): 3240, 3109, 1674, 1473, 1419, 1311, 1226, 1134, 933, 765; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 1H), 9.98 (s, 1H), 9.47 (d, J = 7.7 Hz, 1H), 8.36–8.28 (m, 1H), 8.17 (d, J = 8.7 Hz, 1H), 7.45–7.25 (m, 3H), 6.86 (d, J = 8.7 Hz, 2H), 3.77 (s, 3H); MS (MALDI) *m/z* calcd. for $C_{17}H_{14}BrN_4O_2$: 387.0280 $[M + H]^+$, found: 387.0356; HPLC: t_R = 2.963 min (97.28% purity).

***N*-Benzyl-5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-amine (19l)**



Yield 76%; Off-white powder; mp 268-270 °C; IR (KBr, *v,* cm^{-1}): 3217, 3186, 1658, 1604, 1350, 995, 833, 702; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.73 (s, 1H), 9.87 (s, 1H), 8.07 (d, J = 7.3 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.86 (s, 1H), 7.48–7.47 (m, 1H), 7.41–7.33 (m, 3H), 7.30–7.23 (m, 3H), 4.44 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.5, 155.8, 139.2, 136.6, 128.8, 127.8, 127.5, 126.4, 124.5, 122.9, 121.0, 120.7, 112.8, 100.8, 46.7; MS (ESI) *m/z* calcd. for $C_{17}H_{15}N_4O$: 291.1 $[M + H]^+$, found: 291.3; HPLC: t_R = 2.993 min (98.28% purity).

5-(6-Fluoro-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (19m)



Yield 80%; Off-white powder; mp 259-261 °C; IR (KBr, *v,* cm^{-1}): 3201, 3155, 1620, 1551, 1419, 1218, 756, 700; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.74 (s, 1H), 10.13 (s, 1H), 8.07–8.09 (m, 1H), 7.84 (s, 1H), 7.54 (d, J = 8.2 Hz, 2H), 7.23 (dd, J = 8.2, 2.0 Hz, 1H), 7.02–6.97 (m, 1H), 6.88

(d, J = 8.2 Hz, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.5, 158.0 (d, $J_{C\text{-}F}$ = 240.0 Hz), 154.1, 136.5 (d, $J_{C\text{-}F}$ = 22.1 Hz), 132.2, 126.3, 121.4 (d, $J_{C\text{-}F}$ = 8.1 Hz), 120.7, 118.3, 114.0, 109.2 (d, $J_{C\text{-}F}$ = 22.2 Hz), 100.3, 99.5, 98.3 (d, $J_{C\text{-}F}$ = 8.2 Hz), 55.0; MS (MALDI) *m/z* calcd. for $C_{17}H_{14}FN_4O_2$: 325.1101 $[M + H]^+$, found: 325.1384; HPLC: t_R = 2.912 min (97.72% purity).

MTT Assay

Six human cancer cell lines: prostate (LNCaP and PC3), cervical (HeLa), breast (MCF-7 and MDA-MB-231) and human embryonic kidney cells (HEK293) 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. Final DMSO concentration was 0.05% in each sample including control. After 48 h., MTT (3-(4,5-dimethyl-diazol-2-yl)-2,5-diphenyltetrazolium bromide) 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.

Nuclear staining using propidium iodide

MDA-MB-231 cells plated on coverslips were treated either with 0.01% DMSO (control), or 10 μM **19a** or **19e** for 48 h. After the treatment, cells were fixed with cold methanol for 5 min, followed by rehydration in PBS and and permeabilization using 0.1% Triton X-100 in PBS plus 2% BSA. Cells were treated with 0.1 μg/mL RNase A in PBS for 1 h., 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.

3.3 Part B: Design and facile synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles as tubulin interacting anticancer agents

3.3.1 Rational design

2, 5-Disubstituted 1,3,4-oxadiazole is an active pharmacophore in many of the anticancer lead molecules. For example; in 2009, Kumar group identified indolyl-1,3,4-oxazdiazoles (**2**) as potent cytotoxic agents (IC_{50} = ~1 μM).[28] Subsequently, bis(indolyl)-1,3,4-oxadiazoles were synthesized and reported as potent cytotoxic agents (IC_{50} = 20 nM; HeLa). Preliminary mechanism of action studies in MDA-MB-231 breast cancer cells suggested that bis(indolyl)-1,3,4-oxadiazoles **3** induces apoptosis to promote cell death.[5] In 2012, Li *et al.* reported a series of 2-indolyl-4-benzoylimidazoles (**10**) as antiproliferative agents. Compound **10** exhibited anticancer activity through the inhibition of tubulin polymerization with an IC_{50} value of 3.8 nm.[16]

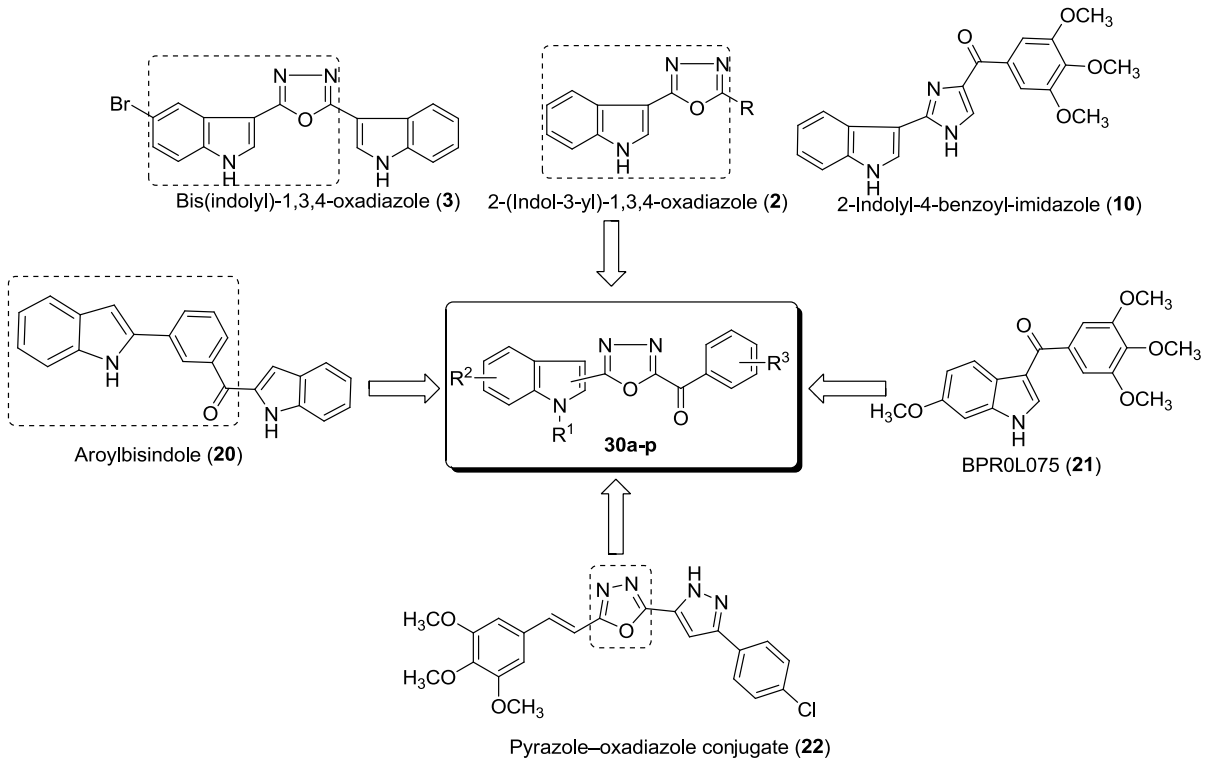


Figure 3.3.1 Rational design for the synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles

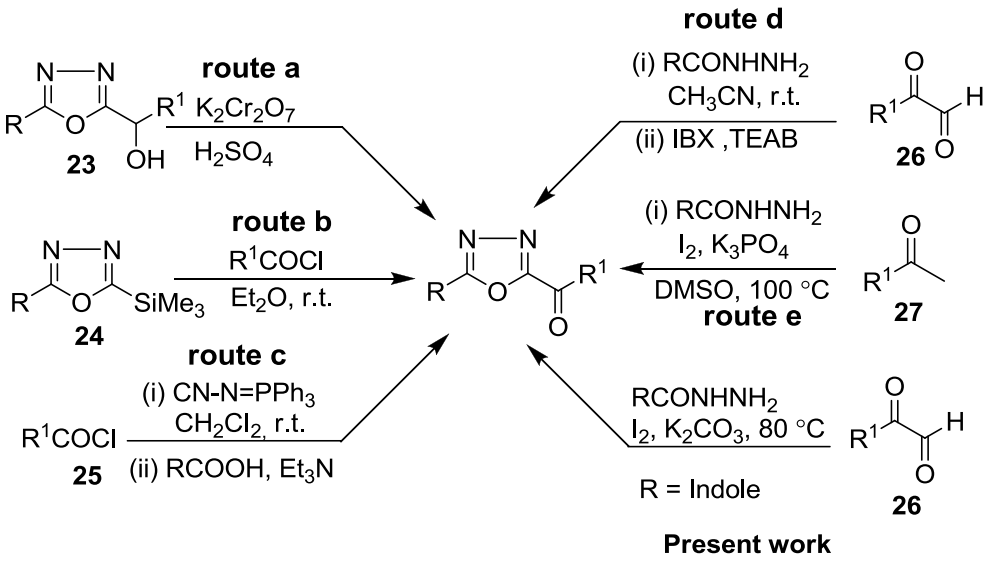
Dalton and co-workers patented a series of aroylbisindole **20** as inhibitors of tubulin assembly with promising *in vitro* and *in vivo* activities against multidrug resistant prostate cancer cells.[50] 3-Aroylindole (BPR0L075) **21** was found to be potent against the tested cancer cell lines (IC_{50} = 1-23 nM). It was observed that BPR0L075 exhibited the anticancer activity

through the activation of apoptotic cascade.[51] Recently, Kamal group prepared a number of pyrazole–oxadiazole conjugates and evaluated their ability on a panel of human cancer cell lines to function as antiproliferative agents. The conjugates are comprised of pyrazole and oxadiazole scaffolds closely attached to each other without any spacer. The most potent compound **22** of this series (IC_{50} ranging 1.5-11.2 μM) inhibited tubulin polymerization with an IC_{50} value of 1.3 μM. Also, the compound **22** arrested cell cycles at G2/M phase and disturbed microtubule network. Further docking study of the conjugate **22** showed its binding at the colchicine site of tubulin.[52] In view of encouraging anticancer potential of indole-based compounds and oxadiazole scaffold, in this chapter we designed indolyl-*α*-keto-1,3,4-oxadiazoles by incorporating indole and oxadiazole motifs in a single molecule as depicted in Figure 3.3.1.

3.3.2 Results and discussion

3.3.2.1 Synthesis

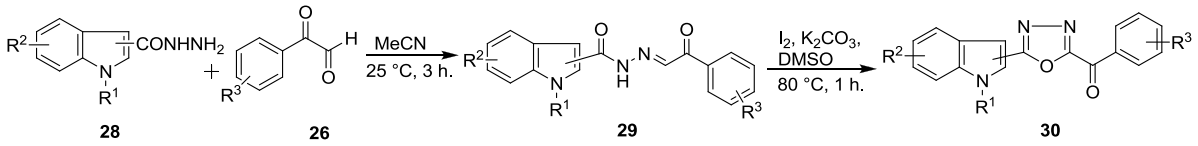
General methods for the synthesis of *α*-keto-1,3,4-oxadiazoles are described in Scheme 3.3.1. An earlier strategy involved the oxidation of 2-(1-hydroxy-1-phenylmethyl)-1,3,4-oxadiazoles **23** with a mixture of $K_2Cr_2O_7$ and H_2SO_4 (route a).[53] Pervak and co-workers reported *α*-keto-1,3,4-oxadiazoles in moderate to good yields from the acylation of 2-aryl-5-trimethylsilyl-1,3,4-oxadiazoles **24** with an appropriate acid chloride (route b).[54]



Scheme 3.3.1 Reported protocols for the preparation of *α*-keto-1,3,4-oxadiazoles

In 2011, Cui *et al.* synthesized various *α*-keto-1,3,4-oxadiazole derivatives in moderate yields (36–69%) by employing acyl chlorides **25** and (*N*-isocyanimine)triphenylphosphorane *via* an *α*-keto-imidoyl chloride intermediate, which was trapped by carboxylic acids (route c).[55] Further, Kudelko *et al.* reported an efficient approach to symmetrically substituted bis(1,3,4-oxadiazol-2-yl-phenylmethyl) sulfides by acetic acid catalyzed reactions of 1,1′-diphenylthiodiacetic acid dihydrazides with triethyl orthoformate but, unexpectedly, the *α*-keto-1,3,4-oxadiazole was also formed as a minor by-product.[56] Recently, Kumar *et al.* reported IBX-mediated oxidative cyclizations of hydrazide-hydrazones to prepare *α*-keto-1,3,4-oxadiazoles (route d).[57] Very recently, Wu *et al.* described the synthesis of *α*-keto-1,3,4-oxadiazoles from the reaction of arylmethylketones **27** and arylhydrazides in the presence of molecular iodine and K_3PO_4 (route e).[58] The reported general methods require either multi-step synthetic protocol or expensive reagents or high temperature. To overcome these drawbacks and in our efforts to develop a convenient protocol for the preparation of novel indolyl-*α*-keto-1,3,4-oxadiazoles, we utilized molecular iodine as an oxidant for the cyclodehydration of acylhydrazone **29** (Scheme 3.3.2).

Complete synthetic route to prepare various indolyl-*α*-keto-1,3,4-oxadiazoles is presented in Scheme 3.3.2. Initial reaction of indole hydrazides **28** with arylglyoxals **26** in acetonitrile at room temperature afforded the key intermediate hydrazide-hydrazones **29** in 90-95% yields.

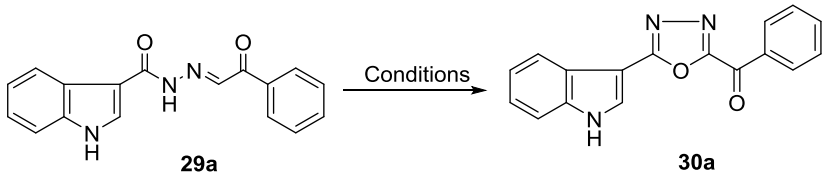


28 **26** **29** **30**

Scheme 3.3.2 Synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles **30**

For the optimization of reaction conditions, we chose oxidative cyclization of acylhydrazone **29a** as a model reaction. In literature, hypervalent iodine reagents including iodobenzene diacetate (IBD), 2-iodoxybenzoic acid (IBX) Dess–Martin periodinane (DMP) and (bis(trifluoroacetoxy)iodo)benzene are well known for oxidative cyclization of acylhydrazones.[43,59] Inspired from literature reports, we utilized IBD for the cyclization of **29a** in DCM, however, it produced only trace amount of desired keto-oxadiazole along with arylglyoxal **26** and unreacted starting material (entry 1; Table 3.3.1). Also, use of IBD in acetonitrile or DMF as a solvent failed to give desired product (entries 2-5; Table 3.3.1). Next, we explored various hypervalent iodine reagents including IBX, DMP and (bis(trifluoroacetoxy)iodo)benzene with or without additives and base but all the attempts failed to deliver **30a** in good yield (entries 6-10, Table 3.3.1).

Table 3.3.1 Screening of reaction conditions to prepare indolyl-α-keto-1,3,4-oxadiazole



29a **30a**

Entry	Reagents	Additive	Base	Solvent	Temp (°C)	Yield (%)
1.	Iodobenzene diacetate	-	-	DCM	25	trace
2.	Iodobenzene diacetate	-	-	CH_3CN	25	trace
3.	Iodobenzene diacetate	-	-	CH_3CN	0	no reaction
4.	Iodobenzene diacetate	-	DIPEA	CH_3CN	25	trace
5.	Iodobenzene diacetate	-	-	DMF	25	trace
6.	2-Iodoxybenzoic acid		-	CH_3CN	25	trace
7.	2-Iodoxybenzoic acid	TEAB	-	CH_3CN	25	trace
8.	Dess–Martin periodinane		-	CH_3CN	25	trace
9.	Dess–Martin periodinane	TEAB	-	CH_3CN	25	trace
10.	(Bis(trifluoroacetoxy)iodo)benzene	-	-	CH_3CN	25	trace
11.	I_2	-	K_2CO_3	DMSO	50	40
12.	**I_2**	**-**	**K_2CO_3**	**DMSO**	**80**	**85**
13.	I_2		Na_2CO_3	DMSO	80	45
14.	I_2		Cs_2CO_3	DMSO	80	50
15.	I_2	-	-	DMSO	80	no reaction

Next, we performed the oxidative cyclization of **29a** using molecular iodine and K_2CO_3 as a base in DMSO at 50 °C for 7 h., delightfully the desired product **30a** was obtained in 40% yield (entry 11). Notably, by changing the temperature from 50 °C to 80 °C led to **30a** with 85% yield within 1 h. (entry 12, Table 3.3.1). Use of Na_2CO_3 or Cs_2CO_3 as a base led to **30a** in moderate yields (entries 13-14). No product was formed in absence of base (entry 15, Table 3.3.1). Utilizing the identified optimized reaction conditions (1.2 equivalent iodine, DMSO, 80 °C, 1 h.), we prepared fifteen diverse indolyloxadiazoles **30a-p** in excellent yields (80-87%).

All the synthesized indolyl-α-keto-1,3,4-oxadiazole were characterized using IR, NMR (^{1}H & ^{13}C) and HRMS spectral data. In ^{13}C NMR spectrum of **30a**, characteristic signals for ketonic (C=O), C-2 and C-5 (oxadiazole moiety) carbon were observed at δ values 178, 163 and 159 ppm, respectively. The HRMS spectrum of **30a** displayed a molecular ion peak at 290.0924 which is in agreement with the calculated mass 290.0930 $[M + H]^+$. Copies of NMR (^{1}H and ^{13}C) and HRMS spectra for a representative compound **30a** are given in Figures 3.3.2-3.3.4.

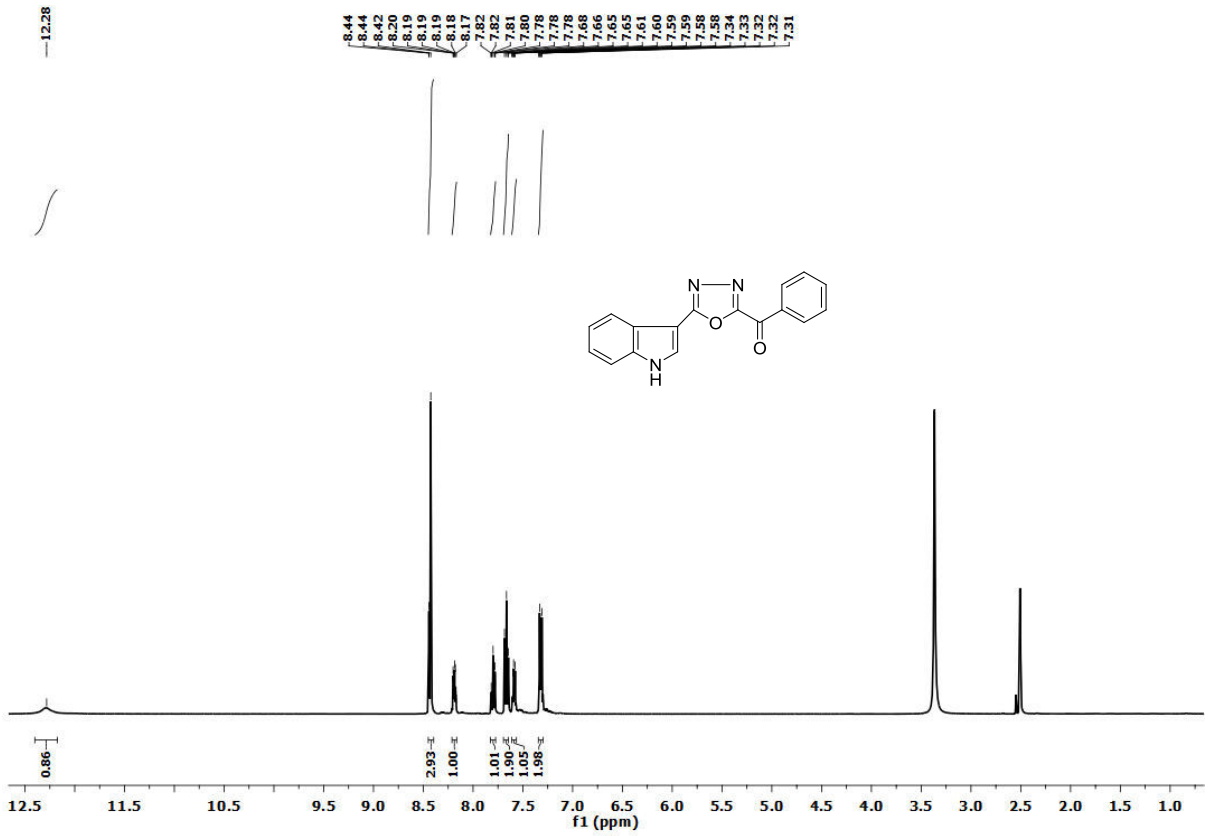


Figure 3.3.2 ^{1}H NMR spectrum of **30a**

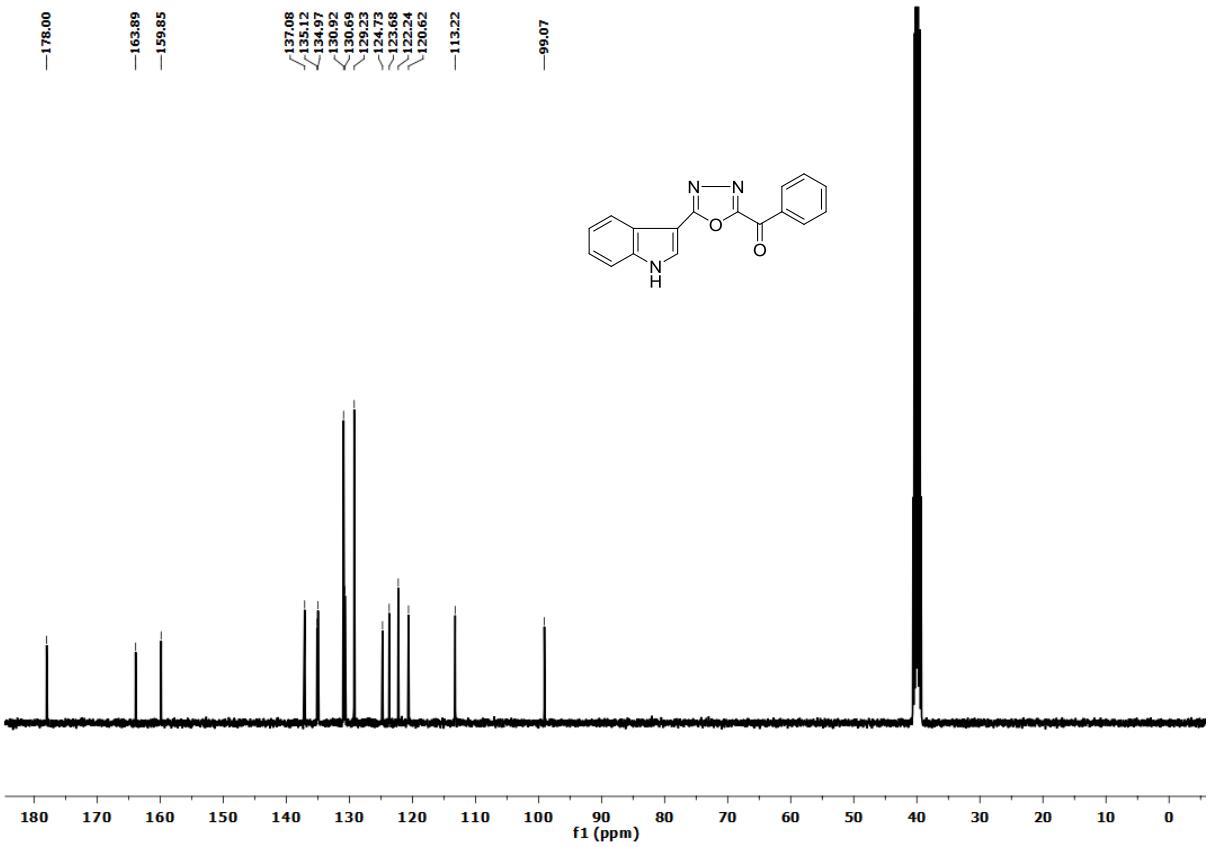


Figure 3.3.3 ^{13}C NMR spectrum of **30a**

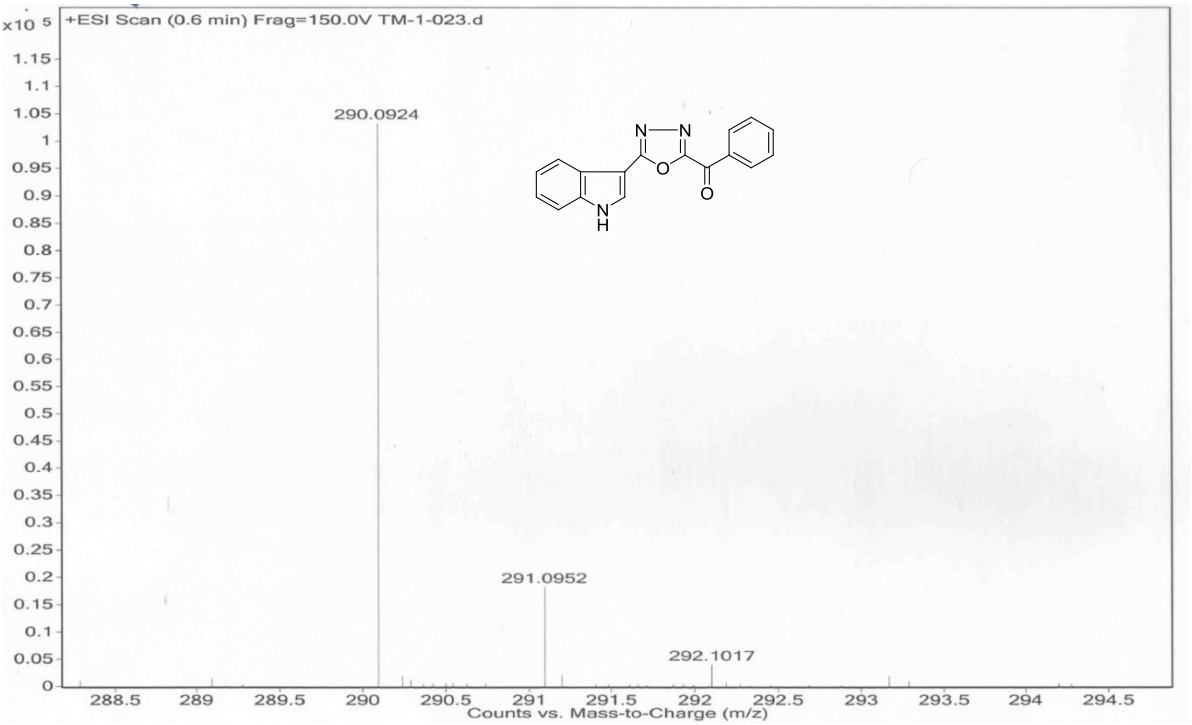
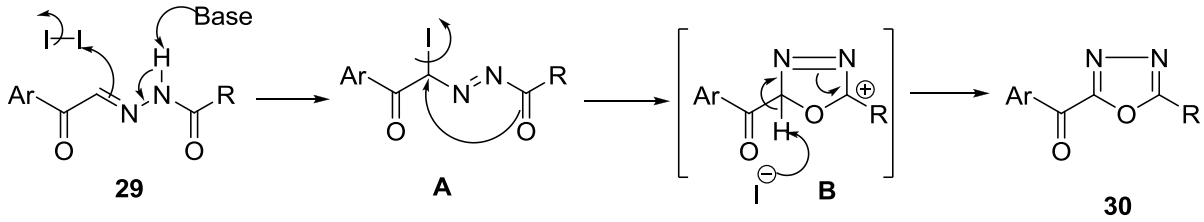


Figure 3.3.4 HRMS spectrum of **30a**

3.3.2 Reaction mechanism

A proposed reaction mechanism for the formation of *α*-keto-oxadiazoles **30** is depicted in Scheme 3.3.3. Initially base promoted oxidative iodination of hydrazide-hydrazone **29** likely to generate iodinated intermediate **A**, which upon internal cyclization and aromatization through the intermediacy of **B** may produce *α*-keto-oxadiazole **30**.



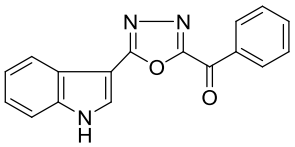
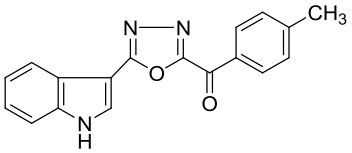
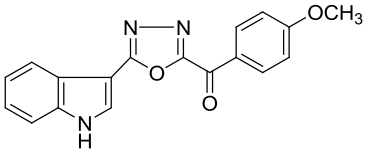
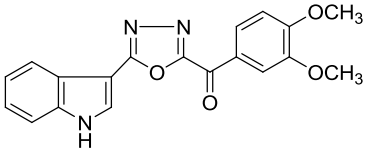
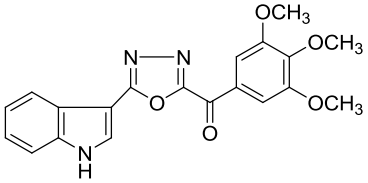
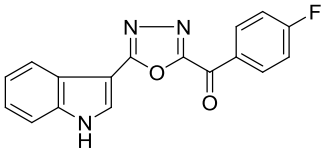
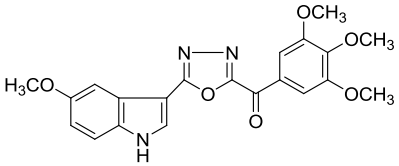
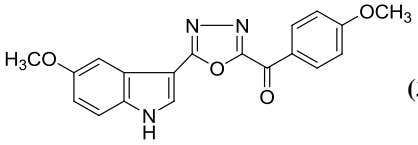
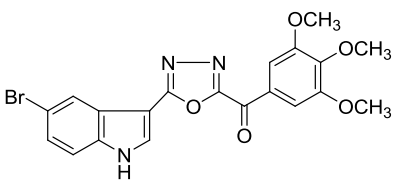
Scheme 3.3.3 Mechanism for the formation of indolyl-*α*-keto-1,3,4-oxadiazole **30**

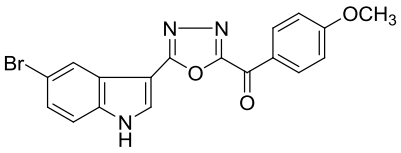
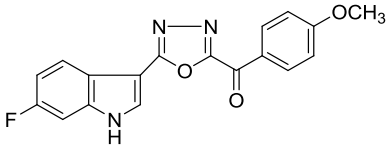
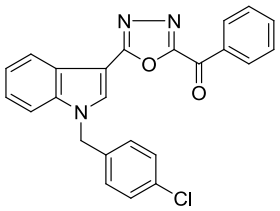
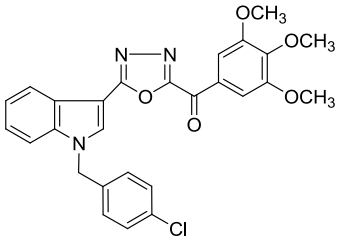
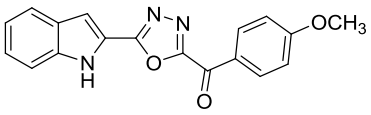
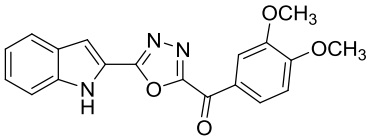
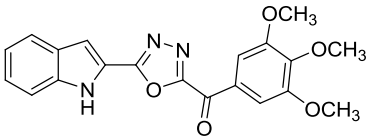
3.3.3 Anticancer activity

All the synthesized indolyl-*α*-keto-1,3,4-oxadiazoles (**30a-p**) were screened *in vitro* for their cytotoxicity against a panel of cancer cell lines including human lymphoblast lung (U937), leukemia (Jurkat & SB) and human breast (BT474) cancer cell lines. Doxorubicin and Indibulin were used as reference drugs. Initially, we screened all the indolyl-*α*-keto-1,3,4-

oxadiazoles (**30a-p**) at 10 μM concentration against a selected panel of cancer cells and the results are given in Table 3.3.2.

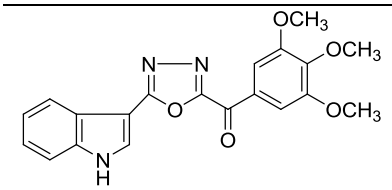
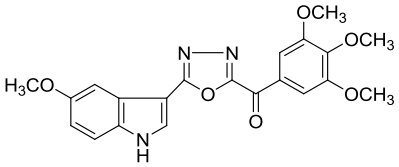
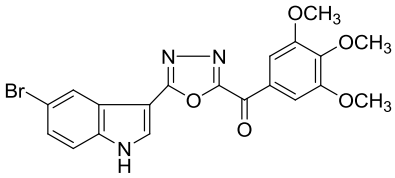
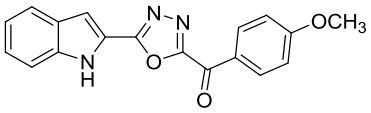
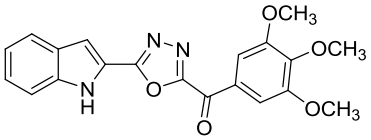
Table 3.3.2 *In vitro* screening of indolyl-α-keto-1,3,4-oxadiazoles
percentage cell survival (@ 10 μM)

Compounds		U 937	Jurkat	BT474	SB
Cells		100.0 ± 8.7	104.3 ± 10.2	100.0 ± 6.3	100.0 ± 6.0
	**(30a)**	98.7 ± 3.4	82.1 ± 8.9	59.3 ± 1.0	87.6 ± 16.2
	**(30b)**	101.7 ± 21.2	78.9 ± 4.2	67.9 ± 6.6	66.8 ± 18.7
	**(30c)**	107.8 ± 16.8	99.6 ± 4.7	**88.4 ± 1.4**	96.2 ± 2.3
	**(30d)**	90.3 ± 20.4	92.5 ± 17.1	70.2 ± 2.5	93.5 ± 5.4
	**(30e)**	**27.5 ± 2.1**	**35.2 ± 1.9**	**41.1 ± 3.6**	**11.6 ± 0.8**
	**(30f)**	95.3 ± 7.8	90.9 ± 2.5	62.3 ± 0.8	76.8 ± 2.8
	**(30g)**	85.5 ± 4.6	88.9 ± 13.5	**54.0 ± 1.2**	74.3 ± 14.5
	**(30h)**	98.7 ± 19.8	99.9 ± 9.3	70.3 ± 2.8	70.7 ± 0.6
	**(30i)**	92.1 ± 8.7	77.4 ± 8.0	**56.8 ± 5.3**	70.9 ± 2.6

	**(30j)**	105.1 ± 5.9	88.8 ± 14.4	65.8 ± 1.5	94.8 ± 0.1
	**(30k)**	108.5 ± 5.9	86.4 ± 4.1	72.6 ± 0.3	101.0 ± 7.6
	**(30l)**	85.0 ± 5.8	82.0 ± 0.4	68.9 ± 0.3	87.9 ± 12.8
	**(30m)**	86.0 ± 4.6	80.6 ± 5.9	68.5 ± 0.7	96.2 ± 0.1
	**(30n)**	84.1 ± 6.2	75.1 ± 4.8	**56.1 ± 5.4**	79.9 ± 4.2
	**(30o)**	75.2 ± 3.2	67.4 ± 3.5	67.4 ± 2.3	76.1 ± 19.1
	**(30p)**	91.2 ± 5.3	**44.9 ± 1.3**	111.2 ± 7.1	**35.7 ± 2.8**
Indibulin		27.1 ± 0.4	31.6 ± 1.1	40.9 ± 2.4	32.9 ± 5.8
Doxorubicin		22.9 ± 0.8	30.1 ± 0.9	31.8 ± 2.2	15.1 ± 0.4

In vitro screening of indolyl-*α*-keto-1,3,4-oxadiazoles **30a-p** at 10 μM concentration led to analogues **30e**, **30g**, **30i**, **30n** and **30p** with ~ 50% cell survival activity against the tested cancer cell lines. Therefore, IC_{50} values of only these potent compounds were determined (Table 3.3.3). The 3,4,5-trimethoxyphenyl fragment present in various anticancer agents is known to play a crucial role in tubulin interaction.[60] With the observed beneficial effects of 3,4,5-trimethoxyphenyl group and in our efforts to identify compound with better activity, five analogues of indolyl-*α*-keto-1,3,4-oxadiazoles (**30e. 30g, 30i, 30m** and **30p**) having similar structural moiety, were prepared.

Table 3.3.3 *In vitro* cytotoxic activity of the selected indolyl-*α*-keto-1,3,4-oxadiazoles

Compound		Cancer cell lines (IC_{50}, μM)			
		U 937	**Jurkat**	**BT474**	**SB**
	**(30e)**	3.1 ± 1.6	1.4 ± 0.21	1.3 ± 0.38	0.8 ± .05
	**(30g)**	>30	>30	13.8 ± 2.4	>30
	**(30i)**	>30	>30	16.7 ± 3.1	>30
	**(30n)**	>30	20.2 ± 2.2	15.8 ± 2.5	>30
	**(30p)**	>30	10.2 ± 1.1	>30	8.8 ± 2.5

The analogue **30e** with unsubstituted indole and trimethoxyphenyl group was found to be broadly cytotoxic towards tested cancer cells (IC_{50} = 0.8-3.1 μM). Moreover, compound **30e** was selectively cytotoxic to SB cell line with an IC_{50} value of 0.8 μM. Introduction of a methoxy or bromo group at C-5 position of indole in compound **30i** led to compounds **30g** and **30i**, with reduced cytotoxicity (10-12-fold) against BT474 cells. In 2012, Dalton *et al.* prepared bisindoles with aroyl spacer linking through C-2 of indole ring and found to exhibit potent anticancer activity through inhibition of tubulin polymerization.[50] By using the same analogy, we prepared three analogues indolyl-*α*-keto-1,3,4-oxadiazoles linking through C-2 position of indole. The 4-methoxyphenyl derivative **30n**, showed moderate activity against Jurkat and BT474 cell lines with IC_{50} values of 20.2 and 15.8 μM, respectively. Replacement of 4-methoxyphenyl group in compound **30n** with 3,4,5-trimethoxyphenyl unit resulted in compound **30p** with improved cytotoxicity against Jurkat (IC_{50} = 10.2 μM) and SB (IC_{50} = 8.8 μM) cell lines (compound **30n** *vs* **30p**).

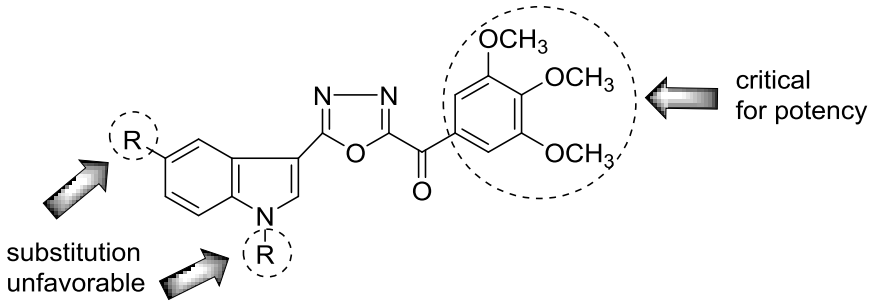


Figure 3.3.5 Structure-activity relationship of indolyl-α-keto-1,3,4-oxadiazoles

Structure-activity relationship (SAR) studies of indolyl-α-keto-1,3,4-oxadiazoles (**30a-p**) revealed that substituents such as methoxy, bromo and fluoro on indole ring, are unfavourable for the activity. As observed in literature, the 3,4,5-trimethoxyphenyl group was found to be critical for the potency of indolyloxadiazoles **30a-p** (Figure 3.3.5).

3.3.3.1 Caspase-3/7 activation

To characterize the mode of cell death induced by most cytotoxic analogue **30e**, caspase-3/7 activation assay was performed. The caspases are a unique family of cysteine proteases which play a crucial role in the induction of apoptosis and amongst them caspases 3 and 7 happens to be one of the effector caspases.[61] This prompted us to treat Jurkat cells with compound **30e** to examine the activation of caspase-3/7 while using Camptothecin as a standard drug. The results indicated that compound **30e** and Camptothecin enhanced the fluorescence by 9-fold and 8-fold, respectively which is indicative of caspases 3/7 activation (Figure 3.3.6). This enhanced fluorescence suggests that these compounds could kill the cancer cells *via* apoptosis induction.

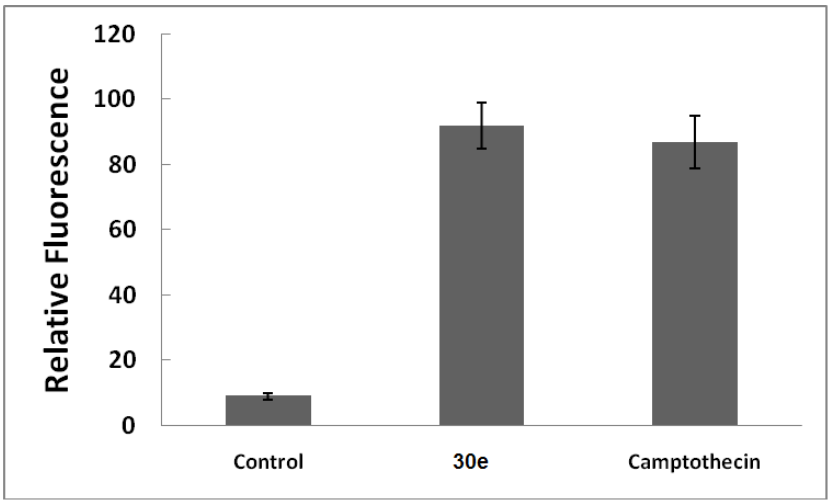


Figure 3.3.6 Caspase activation-induced by **30e** in Jurkat cells

3.3.3.2 Microtubule instability

To investigate whether the antiproliferative activity of indolyl-α-keto-1,3,4-oxadiazoles are due to an interaction with tubulin, the most cytotoxic analogue **30e** was evaluated for its tubulin activity. Compound **30e** caused a decrease in tubulin polymerization in a dose dependent manner as shown in Figure 3.3.7. Compound **30e** inhibited microtubule formation upto 50% at 10.66 µM. These results indicated that the most potent compound **30e** inhibits microtubule formation, a critical part of cell division.

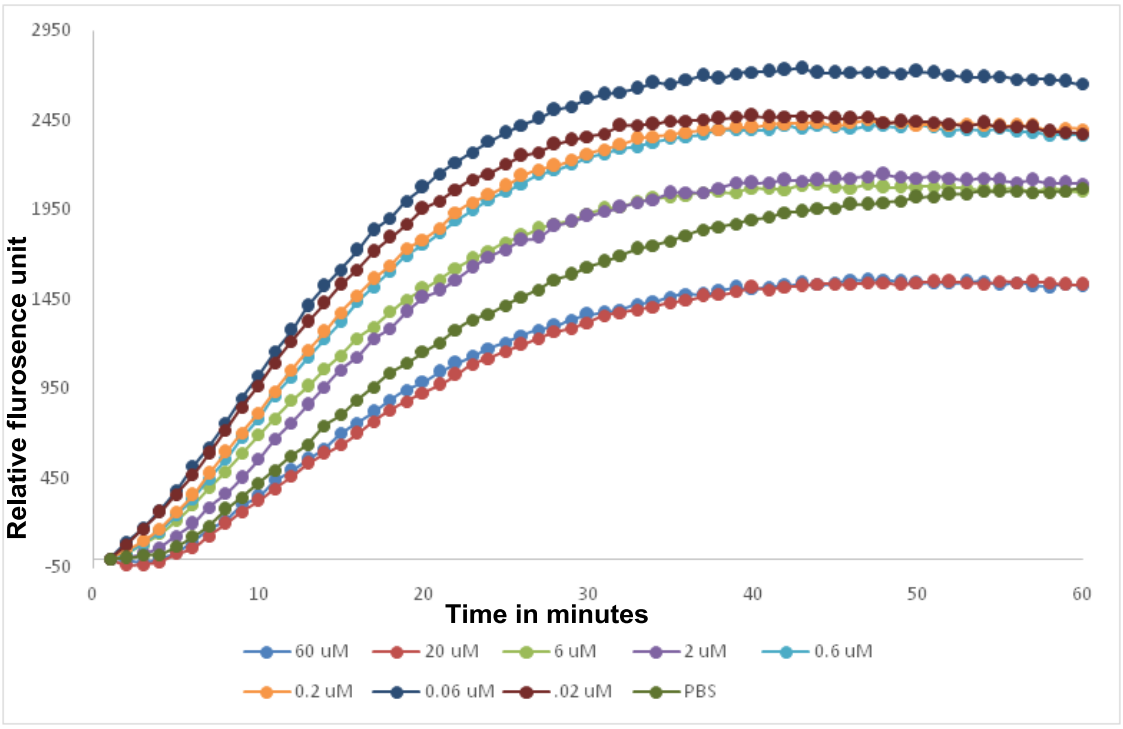


Figure 3.3.7 Effect of **30e** on *in vitro* tubulin polymerization

3.3.3.3 Molecular docking studies

Tubulin polymerization assay suggests that the indolyl-α-keto-1,3,4-oxadiazoles exhibited their anticancer activity effect through the inhibition of tubulin polymerization. To gain better understanding on the potency of **30e** and guide further SAR studies, we proceeded to examine the interaction of compound **30e** with tubulin crystal structure (PDB code: 1SA0) using Schrodinger 2011 molecular modeling suite (Schrodinger, Inc., New York, NY). Docking studies were performed on reported high-resolution crystal structure of the tubulin-DAMA-colchicine (CN-2) complex (PDB ID: 1SA0).[62] Compound **30e** demonstrated good binding in this model with a glide score of -7.37. The close view of potential binding pos of

30e with tubulin is illustrated in Figure 3.3.8. In the binding model, compound **30e** is bound to tubulin protein *via* two hydrogen bonds. The indole N-H atom contributes to the hydrogen bonding interaction with VAL315B (2.14 Å). The methoxy group on aroyl part contributes to the hydrogen bonding interactions (2.51 Å) with the CYS241.

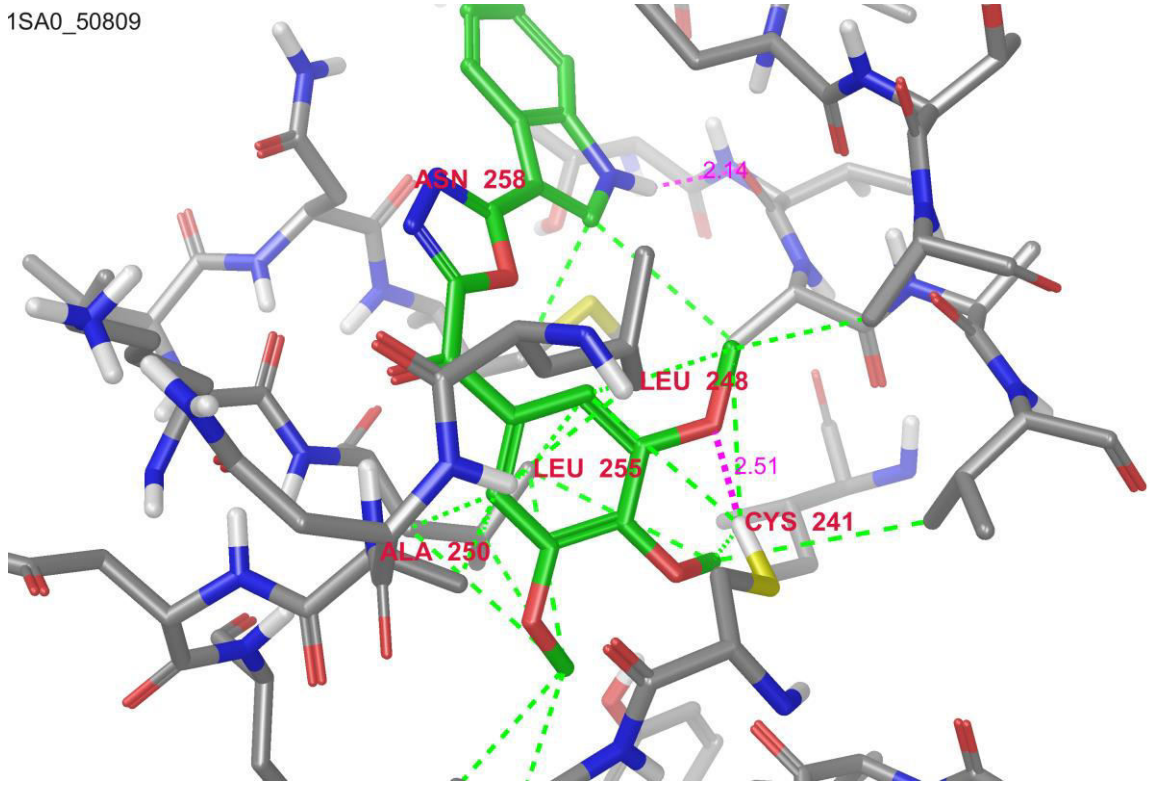


Figure 3.3.8 Binding modes of **30e** in the colchicine binding site of tubulin: Showing hydrogen bonding (pink dotted lines) and hydrophobic interactions (green dotted lines)

3.3.4 Conclusions

In conclusion, developed a facile, efficient and high yielding synthetic route for the construction of indolyl-*α*-keto-1,3,4-oxadiazoles by employing molecular iodine-mediated oxidative cyclization of acylhydrazones. *In vitro* anticancer activity screening study indicated that some of the indolyl-*α*-keto-1,3,4-oxadiazoles exhibited significant antiproliferative activity against a panel of cell lines. Compound **30e** with 3,4,5-trimethoxyphenyl motif endowed with most potent activity against U 937, Jurkat, BT474 and SB cancer cells with IC_{50} values of 3.1, 1.4, 1.3 and 0.8 µM, respectively. Mechanism of the action studies suggested that the indolyl-*α*-keto-1,3,4-oxadiazoles induced apoptosis through caspase 3/7 activation, and exerted anticancer activity through inhibition of tubulin polymerization (IC_{50} = 10.66 µM). Moreover, molecular docking studies suggested a potential binding mode for

compound **30e** in the colchicine binding site of tubulin. These results strongly suggest that novel indolyl-*α*-keto-1,3,4-oxadiazoles can be further explored to develop promising anticancer agents for the more efficacious treatment of cancers.

3.3.5 Experimental

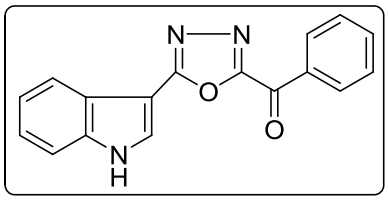
Chemistry

All the laboratory grade reagents and solvents were obtained commercially either from Spectrochem or Aldrich and used as such without any purification. Organic solvents were distilled off using Buchi rotary evaporator. Melting points (mps) were determined on *E-Z* melting apparatus. Reactions were monitored by thin layer chromatography, performed on commercially available Merck pre-coated plates (silica gel. 60 F_{254}, 0.25 mm). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker advance II spectrometer using DMSO-d_6 and $CDCl_3$ as solvents and the chemical shifts are expressed in δ units (ppm) from tetramethylsilane. The proton multiplicities were described as: s = singlet, d = doublet, t= triplet, dd = doublet of doublet and m = multiplet. HRMS spectra were obtained on a Bruker micro TOF-Q II 10348 (ESI) spectrometer. The IR spectra were recorded on ABB Bomen MB 3000 FTIR machine using KBr and are reported in wave numbers (cm^{-1}).

General procedure for the synthesis of hydrazones (29): To a suspension of indolylhydrazides **28** (1 mmol) in dry acetonitrile (5 mL) was added arylglyoxals **26** (1 mmol) at room temperature. The resulting mixture was stirred at 25 °C for 3 h. After completion of reaction as indicated by TLC, solvent was removed under reduced pressure. The residue so obtained was recrystallized from ethanol to obtain pure hydrazones **29** in 90-95% yields.

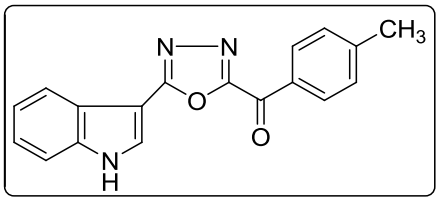
General procedure for the synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles (30a-p): To a solution of hydrazone **29** (0.68 mmol) in DMSO (2 mL) was added molecular iodine (0.82 mmol) in portions and followed by K_2CO_3 (2 mmol). Reaction contents were heated at 80 °C for 1 h. Upon completion of the reaction as confirmed by TLC, reaction mixture was allow to cool at room temperature and the contents were poured over crushed ice (20 g). Obtained solid was filtered, washed with cold water (20 mL) and dried to give crude product which was purified through column chromatography using ethylacetate:hexane as eluent to afford pure indolyl-*α*-keto-1,3,4-oxadiazoles (**30a-p**) in 80-87% yields.

(5-(1*H*-Indol-3-yl)-1,3,4-oxadiazol-2-yl)(phenyl)methanone (30a)



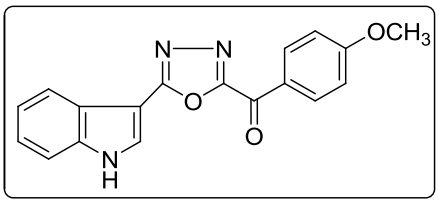
Yield 85%; Light yellow powder; mp 256-257 °C; IR (KBr, *v,* cm^{-1}): 3356, 1651, 1589, 1512, 1481, 1420, 1335, 1250, 1041, 910, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (s, 1H), 8.45–8.40 (m, 3H), 8.20–8.17 (m, 1H), 7.82–7.77 (m, 1H), 7.68–7.65 (m, 2H), 7.61–7.56 (m, 1H), 7.34–7.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.0, 163.9, 159.8, 137.1, 135.1, 135.0, 130.9, 130.7, 129.2, 124.7, 123.7, 122.2, 120.6, 113.2, 99.1; HRMS (ESI$^+$) *m/z* calcd. for $C_{17}H_{12}N_3O_2$: 290.0930 $[M + H]^+$, found: 290.0924.

(5-(1*H*-Indol-3-yl)-1,3,4-oxadiazol-2-yl)(*p*-tolyl)methanone (30b)



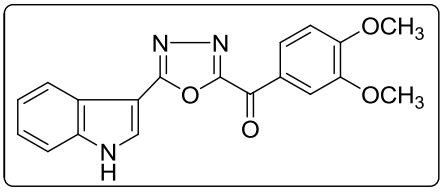
Yield 80%; Yellow powder; mp 251-253 °C; IR (KBr, *v,* cm^{-1}): 3364, 1651, 1589, 1512, 1427, 1335, 1250, 1041, 910, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H), 8.43–8.37 (m, 3H), 8.17–8.13 (m, 1H), 7.80–7.74 (m, 1H), 7.66–7.62 (m, 1H), 7.57–7.54 (m, 1H), 7.30–7.28 (m, 2H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.0, 163.9, 159.8, 145.8, 137.1, 135.1, 134.9, 131.0, 130.9, 130.7, 129.8, 129.2, 124.7, 123.7, 122.2, 120.6, 113.2, 99.1, 21.8; HRMS (ESI$^+$) *m/z* calcd. for $C_{18}H_{14}N_3O_2$: 304.1086 $[M + H]^+$, found: 304.1081.

(5-(1*H*-Indol-3-yl)-1,3,4-oxadiazol-2-yl)(4-methoxyphenyl)methanone (30c)



Yield 81%; Yellow powder; mp 263-264 °C; IR (KBr, *v,* cm^{-1}): 3375, 1643, 1597, 1504, 1450, 1327, 1257, 1157, 1026, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (s, 1H), 8.52 (d, J = 9.0 Hz, 2H), 8.25–8.19 (m, 2H), 7.57–7.55 (m, 1H), 7.29–7.27 (m, 2H), 7.10 (d, J = 9.0 Hz, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.2, 164.3, 163.3, 159.1, 136.5, 132.8, 129.3, 127.2, 124.2, 122.9, 121.4, 120.2, 113.8, 112.4, 98.7, 55.4; HRMS (ESI$^+$) *m/z* calcd. for $C_{18}H_{14}N_3O_3$: 320.1035 $[M + H]^+$, found: 320.1025.

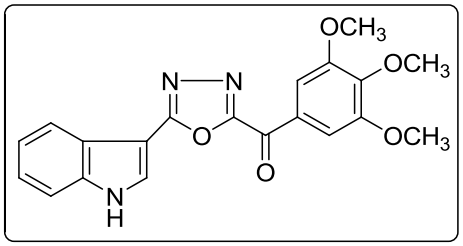
(5-(1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(3,4-dimethoxyphenyl)methanone (30d)



Yield 83%; Yellow powder; mp 226-227 °C; IR (KBr, *v,* cm^{-1}): 330, 1650, 1595, 1502, 1450, 1326, 1256, 1158, 1022, 687; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (s, 1H), 8.40 (d, J = 3.0 Hz, 1H), 8.29 (dd, J = 8.5, 2.0 Hz,

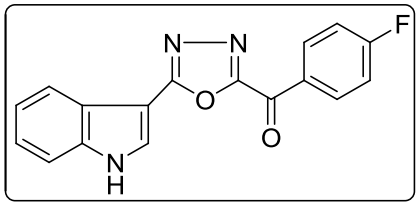
1H), 8.20–8.16 (m, 1H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.59–7.56 (m, 1H), 7.33–7.29 (m, 2H), 7.23 (d, *J* = 8.7 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): *δ* 176.0, 163.6, 159.8, 155.0, 149.1, 137.0, 130.5, 127.6, 126.7, 124.7, 123.6, 122.2, 120.6, 113.2, 112.4, 111.6, 99.1, 56.4, 56.0; HRMS (ESI$^+$) *m/z* calcd. for $C_{19}H_{16}N_3O_4$: 350.1141 $[M + H]^+$, found: 350.1135.

(5-(1*H*-Indol-3-yl)-1,3,4-oxadiazol-2-yl)(3,4,5-trimethoxyphenyl)methanone (30e)



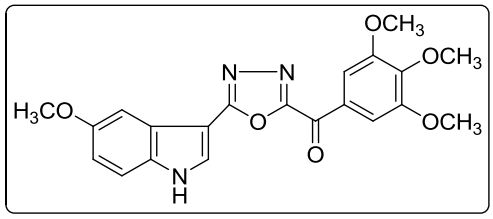
Yield 82%; Yellow powder; mp 223-224 °C; IR (KBr, *v,* cm^{-1}): 3441, 1659, 1582, 1504, 1458, 1342, 1242, 1134, 995, 756, 656; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* 12.54 (s, 1H), 8.41–8.39 (m, 1H), 8.19–8.21 (m, 1H), 7.82–7.80 (m, 2H), 7.59 (s, 1H), 7.31 (s, 2H), 3.90 (s, 6H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): *δ* 176.5, 163.8, 159.8, 153.1, 143.8, 137.1, 130.7, 129.9, 124.7, 123.6, 122.2, 120.6, 113.3, 108.6, 99.0, 60.8, 56.6; HRMS (ESI$^+$) *m/z* calcd. for $C_{20}H_{18}N_3O_5$: 380.1246 $[M + H]^+$, found: 380.1242.

(5-(1*H*-Indol-3-yl)-1,3,4-oxadiazol-2-yl)(4-fluorophenyl)methanone (30f)



Yield 81%; Yellow powder; mp 249-250 °C; IR (KBr, *v,* cm^{-1}): 3380, 1648, 1595, 1540, 1327, 1260, 1154, 1027, 668; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* 12.29 (s, 1H), 8.54–8.51 (m, 2H), 8.41 (d, *J* = 2.4 Hz, 1H), 8.18–8.16 (m, 1H), 7.59–7.56 (m, 1H), 7.51–7.49 (m, 2H), 7.32–7.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): *δ* 176.4, 167.5, 165.0, 163.9, 159.7, 137.0, 134.1, 134.0, 131.8, 130.7, 124.7, 123.7, 122.2, 120.6, 116.5, 116.3, 113.2, 99.0; HRMS (ESI$^+$) *m/z* calcd. for $C_{17}H_{11}FN_3O_2$: 308.0835 $[M + H]^+$, found: 308.0828.

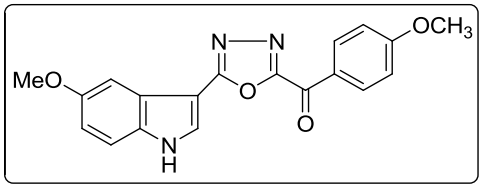
(5-(5-Methoxy-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(3,4,5-trimethoxyphenyl)methanone (30g)



Yield 87%; Yellow powder; mp 246-247 °C; IR (KBr, *v,* cm^{-1}): 3340, 2947, 1658, 1582, 1504, 1458, 1327, 1242, 1126, 771, 658; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): *δ* 12.14 (s, 1H), 8.31 (s, 1H), 7.84 (s, 2H), 7.65 (s, 1H), 7.46 (d, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 3.90 (s, 6H), 3.85 (s, 3H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$): *δ* 176.3, 163.8, 159.6, 155.8, 153.1, 143.7, 131.9, 130.6, 129.9, 125.5, 114.0, 113.8, 108.6,

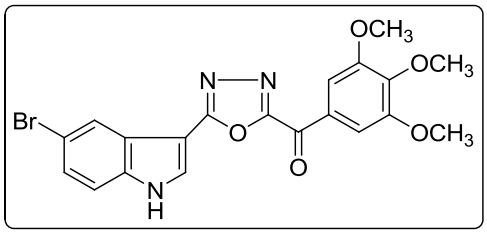
102.1, 98.9, 60.8, 56.6, 55.8; HRMS (ESI$^+$) calcd for $C_{21}H_{20}N_3O_6$:410.1352 [M + H]$^+$, found: 410.1347.

(5-(5-Methoxy-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(4-methoxyphenyl) methanone (30h)



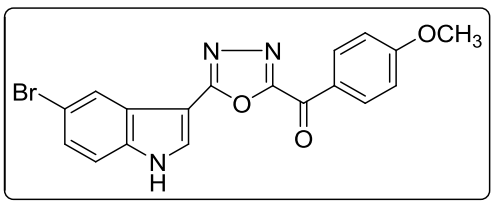
Yield 84%; Yellow powder; mp 2556-256 °C; IR (KBr, *v,* cm^{-1}): 3240, 2924, 1651, 1597, 1512, 1481, 1265, 1165, 1034, 918, 633; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.13 (s, 1H), 8.48 (d, J = 8.9 Hz, 2H), 8.32 (s, 1H), 7.66 (d, J = 2.3 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.20 (d, J = 9.0 Hz, 2H), 6.95 (dd, J = 8.8, 2.4 Hz, 1H), 3.92 (s, 3H), 3.86 (S, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.2, 164.9, 163.7, 159.7, 155.8, 133.5, 131.7, 130.5, 127.8, 125.5, 114.7, 114.0, 113.7, 102.3, 98.9, 56.3, 55.8; HRMS (ESI$^+$) *m/z* calcd. for $C_{19}H_{16}N_3O_4$: 350.1141 [M + H]$^+$, found: 350.1131.

(5-(5-Bromo-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(3,4,5-trimethoxyphenyl)methanone (30i)



Yield 83%: Yellow powder; mp 262-263 °C; IR (KBr, *v,* cm^{-1}): 3171, 2939, 1651, 1589, 1504, 1458, 1335, 1234, 1120, 1003, 818, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.37 (s, 1H), 8.05 (s, 1H), 7.88 (s, 2H), 7.36 (d, J = 8.6 Hz, 1H), 7.29 (d, J = 8.7 Hz, 1H), 3.90 (s, 6H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.2, 164.9, 163.6, 159.7, 155.7, 133.5, 131.9, 130.6, 127.8, 125.5, 114.7, 114.0, 113.7, 102.2, 98.9, 56.2, 55.8; HRMS (ESI$^+$) *m/z* calcd. for $C_{20}H_{17}BrN_3O_5$: 458.0352 [M + H]$^+$, found: 458.0492 [M + H]$^+$ and 460.0466 [M +2 + H]$^+$.

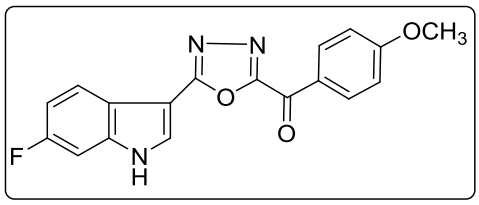
(5-(5-Bromo-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(4-methoxyphenyl)methanone (30j)



Yield 80%; Yellow powder; mp 299-301 °C; IR (KBr, *v,* cm^{-1}): 3320, 2932, 1654, 1589, 1507, 1460, 1336, 1230, 1120, 1005, 818, 668; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.43 (s, 1H), 8.49–8.43 (m, 3H), 8.29 (d, J = 1.8 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.44 (dd, J = 8.7, 1.9 Hz, 1H), 7.19 (d, J = 9.0 Hz, 2H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.1, 165.0, 163.1, 159.9, 135.8, 133.5, 131.7, 127.7, 126.5, 126.3, 122.8, 115.3, 114.8, 114.7, 98.8, 56.3; HRMS

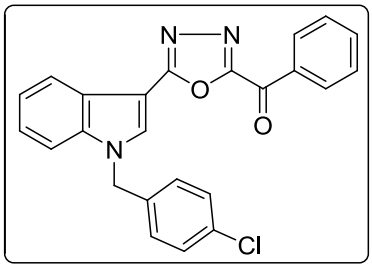
(ESI$^+$) *m/z* calcd. for $C_{18}H_{13}BrN_3O_3$: 398.0140 [M + H]$^+$, found: 398.0155 [M + H]$^+$ and 400.0130 [M +2 + H]$^+$.

(5-(6-Fluoro-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(4-methoxyphenyl)methanone (30k)



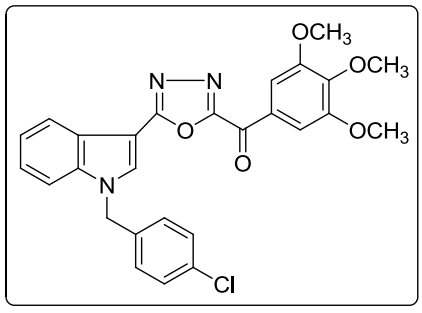
Yield 82%; Light brown powder; mp 254-255 °C; IR (KBr, *v,* cm^{-1}): 3320, 2932, 1654, 1589, 1507, 1460, 1336, 1230, 1120, 1005, 818, 668; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.29 (s, 1H), 8.46 (d, J = 9.0 Hz, 2H), 8.39 (s, 1H), 8.17–8.13 (m, 1H), 7.37 (dd, J = 9.0, 2.3 Hz, 1H), 7.22–7.16 (m, 3H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.1, 165.0, 162.3 (d, $J_{C\text{-}F}$ = 243.7 Hz), 159.9, 158.8, 137.1 (d, $J_{C\text{-}F}$ = 21.7 Hz), 133.5, 131.2, 131.1, 127.7, 121.8 (d, $J_{C\text{-}F}$ = 9.2 Hz), 121.5, 114.7, 110.8 (d, $J_{C\text{-}F}$ = 21.6 Hz), 99.3 (d, $J_{C\text{-}F}$ = 9.2 Hz), 56.3; HRMS (ESI$^+$) *m/z* calcd. for $C_{18}H_{13}FN_3O_3$: 338.0941 [M + H]$^+$, found: 338.0934.

(5-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(phenyl)methanone (30l)



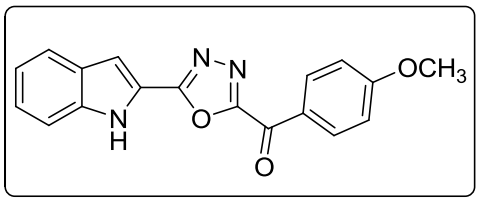
Yield 80%; Light yellow powder; mp 173-175 °C; IR (KBr, *v,* cm^{-1}): 2932, 1674, 1587, 1498, 1468, 1405, 1188, 1084, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.43 (d, J = 7.9 Hz, 2H), 8.23–8.17 (m, 1H), 7.80 (t, J = 7.0 Hz, 1H), 7.67–7.64 (m, 3H), 7.43–7.39 (m, 3H), 7.37–7.31 (m, 3H), 5.61 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.0, 163.4, 159.9, 136.8, 136.4, 135.1, 135.0, 133.6, 132.9, 130.9, 129.7, 129.2, 129.2, 125.3, 124.0, 122.7, 121.0, 112.1, 98.9, 49.6; HRMS (ESI$^+$) *m/z* calcd. for $C_{24}H_{17}ClN_3O_2$: 414.1009 [M + H]$^+$, found: 414.0999.

(5-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-1,3,4-oxadiazol-2-yl)(3,4,5-trimethoxyphenyl)-methanone (30m)



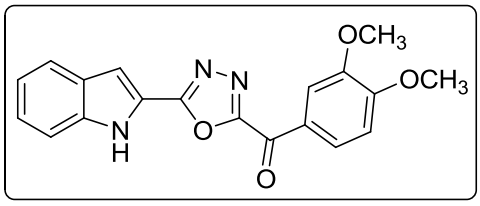
Yield 84%; Light brown powder; mp 159-161 °C; IR (KBr, *v,* cm^{-1}): 2930, 1658, 1589, 1497, 1466, 1404, 1188, 1088, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (d, J = 7.4 Hz, 1H), 8.03–8.01 (m, 3H), 7.38–7.29 (m, 5H), 7.13 (d, J = 8.3 Hz, 2H), 5.39 (s, 2H), 3.99 (s, 3H), 3.99 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 163.5, 159.7, 153.0, 144.1, 136.8, 134.3, 134.0, 131.5, 129.4, 129.3, 128.5, 125.4, 124.0, 122.7, 121.8, 110.5, 108.4, 100.1, 61.1, 56.4, 50.3; 748; HRMS (ESI$^+$) *m/z* calcd. for $C_{27}H_{23}ClN_3O_5$: 504.1326 [M + H]$^+$, found: 504.1320.

(5-(1*H*-Indol-2-yl)-1,3,4-oxadiazol-2-yl)(4-methoxyphenyl)methanone (30n)



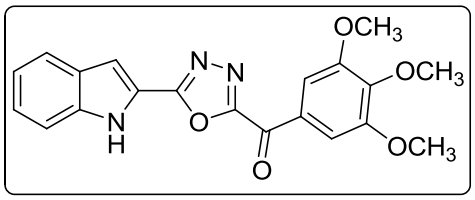
Yield 80%; Yellow powder; mp 226-227 °C; IR (KBr, *v,* cm^{-1}): 3300, 2988, 1658, 1610, 1582, 1504, 1420, 1242, 1134, 1003, 733, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (s, 1H), 8.45 (dd, *J* = 9.0, 2.0 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.53 (dd, *J* = 8.3, 0.8 Hz, 1H), 7.41 (d, *J* = 1.1 Hz, 1H), 7.33–7.29 (m, 1H), 7.21 (dd, *J* = 9.0, 2.0 Hz, 2H), 7.16–7.12 (m, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.0, 165.0, 160.8, 160.7, 138.7, 133.6, 127.7, 127.6, 125.3, 122.2, 121.1, 120.7, 114.8, 113.0, 107.2, 56.3; HRMS (ESI^+) *m/z* calcd. for $C_{18}H_{14}N_3O_3$: 320.1035 $[M + H]^+$, found: 320.1030.

(5-(1*H*-Indol-2-yl)-1,3,4-oxadiazol-2-yl)(3,4-dimethoxyphenyl)methanone (30o)



Yield 80%; Yellow powder; mp 230-232 °C; IR (KBr, *v,* cm^{-1}): 3320, 2987, 1657, 1598, 1510, 1420, 1244, 1134, 1005, 733, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (s, 1H), 8.31 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.85 (d, *J* = 2.0 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.41 (s, 1H), 7.33–7.29 (m, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 7.16–7.11 (m, 1H), 3.92 (s, 3H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.9, 160.8, 155.2, 149.2, 138.7, 127.7, 127.4, 127.0, 125.3, 122.2, 121.1, 120.7, 113.0, 112.3, 111.6, 107.2, 56.5, 56.1; HRMS (ESI^+) *m/z* calcd. for $C_{19}H_{16}N_3O_4$: 350.1141 $[M + H]^+$, found: 350.1131.

(5-(1*H*-Indol-2-yl)-1,3,4-oxadiazol-2-yl)(3,4,5-trimethoxyphenyl)methanone (30p)



Yield 80%; Yellow powder; mp 201-202 °C; IR (KBr, *v,* cm^{-1}): 3240, 1659, 1612, 1582, 1504, 1458, 1350, 1242, 1134, 1003, 818, 733, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.50 (s, 1H), 7.80 (s, 2H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.42 (s, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.14 (t, *J* = 7.4 Hz, 1H), 3.90 (s, 6H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.5, 160.8, 153.2, 153.1, 143.9, 138.7, 129.8, 127.7, 125.3, 122.3, 121.1, 120.6, 113.0, 108.7, 107.3, 60.8, 56.6; HRMS (ESI^+) *m/z* calcd. for $C_{20}H_{18}N_3O_5$: 380.1246 $[M + H]^+$, found: 380.1242.

Cell culture

Human lymphoblast lung (U937), leukemia (Jurkat and SB) and human breast (BT474) cancer cell lines were cultured in DMEM supplemented with 10% fetal serum albumin and

50 µg/mL of penicillin and streptomycin. Jurkat (leukemia cancer) cells were cultured in RPMI supplemented with 10% fetal serum albumin and 50 µg/mL of penicillin and streptomycin. All cell lines were maintained in an incubator containing 5% CO_2 at 37 °C.

Cell viability assay

Cells were seeded in a 96-well plate at a density of 100,000/mL and grown overnight. Cells were treated with various compounds at a final concentration of 10 µM and incubated for 48 h. Cell viability assay was performed using a MTT cell proliferation kit from ATCC (#30-1010K). In summary, 10 µL MTT reagent was added to each well, and cells were placed back in incubator for 4 h. 100 µL of detergent (from kit) was added and absorbance data was collected at 570 nm using Biotek synergy 2 spectrophotometer. Data was calculated as percentage of cell survival using the following formula;

$$\% \text{ cell survival} = (100/At*As)$$

Where At and As are the absorbances of wells treated with test compounds and solvent control, respectively.

Caspase assay

100,000 cells were plated in a 24 well plate and treated with 1 µM **30e**. 24 h. Later, 100 µL sample was taken and analyzed as per kit (Promega G7790). Fluorescence for the samples was measured at 0 min and 180 min. Camptothecin was used as a positive control for inducing apoptotic cell death.

***In vitro* tubulin polymerization**

Tubulin (1.2 mg/mL) was mixed with varying concentrations of **30e** (0-0.2µM) for 5 min. at room temperature in a 96 well plate and reaction was initiated by adding polymerization buffer (as per a kit from Cytoskeleton; BK011P) and the reaction was monitored for change in fluorescence (Ex: 360 nm and Em: 420 nm) using a Biotek fluorimeter.

3.3.6 References

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Chapter 4

Synthesis and Anticancer Activity Studies of Novel Bisindoles

Part A: ***A novel α-cyano bis(indolyl)chalcones as anticancer agents***

Part B: ***Bis(indolyl)ketohydrazide-hydrazones as novel tubulin modulating anticancer agents***

4.1 Bisindoles

Bisindole alkaloids, isolated from marine sources, continues to inspire the development of novel anticancer agents. In most of the bisindole alkaloids possess two indole units connecting through a linear or heterocyclic ring spacer (**1**) as shown in Figure 4.1.1. Isolated from marin sponges *Topsentia genitrix* and *Spongosorites*, bisindole alkaloids, Topsentin (**2**) and Nortopsentin (**3**) with an imidazole linker, have been reported to display potent cytotoxicity towards diverse cancers.[1, 2] Another emerging class of marine alkaloid, Dragmacidin B (**4**) with a piperazine linker, was isolated from the deep water marine sponge *Hexadella* sp. and found to display good anticancer activity (IC_{50} = 15 µg/mL, P388; 1-10 µg/mL, A-549, HCT-8 and MDA-MB-231).[3]

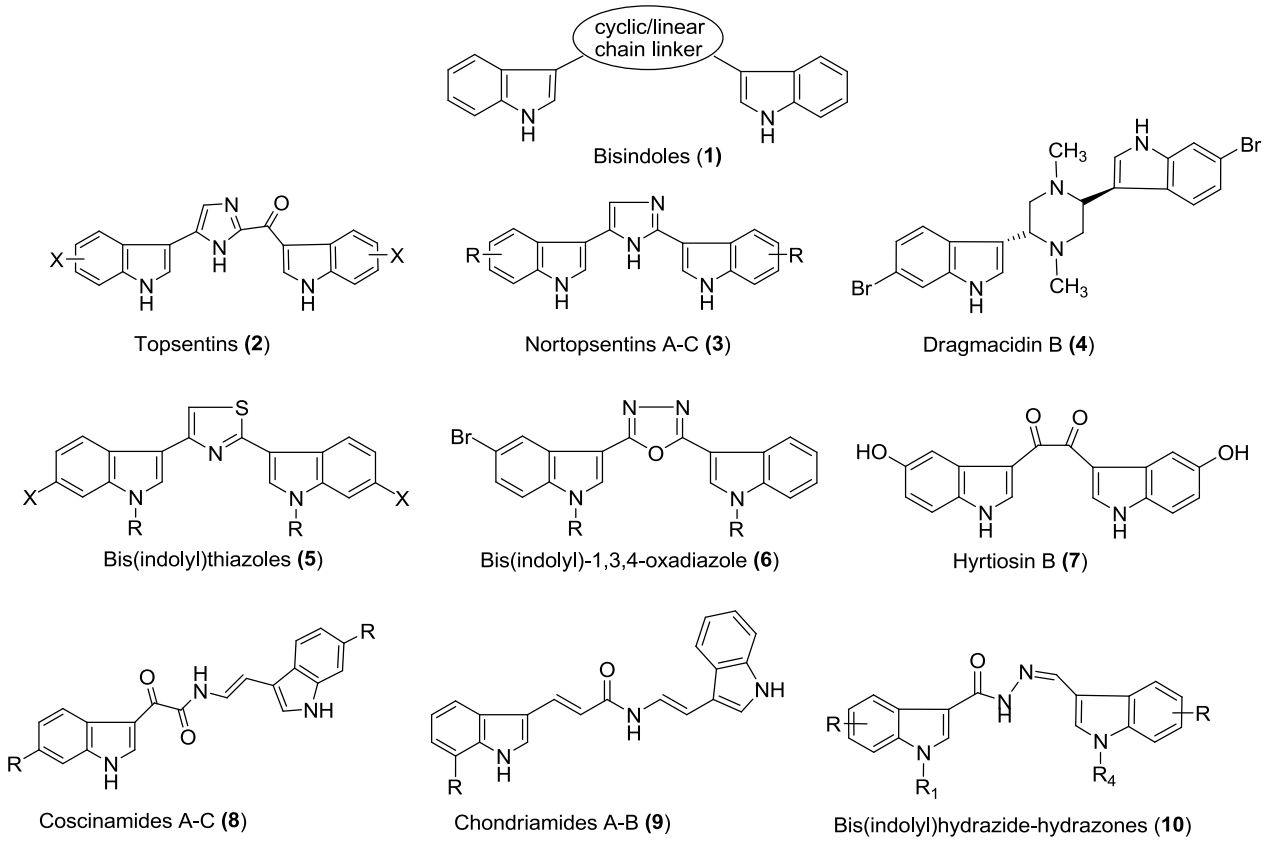


Figure 4.1.1 Structures of some cytotoxic bisindoles

Despite the interesting anticancer activity exerted by bisindole alkaloids, in the recent past, researchers have identified several synthetic analogues of bisindole alkaloids with improved anticancer properties.[4,5] Subsequently, many reports describing the synthesis and antiproliferative activities of Nortopsentin analogues by replacing the imidazole spacer with

five-membered heterocycles such as thiazole, pyrazole, furan, isoxazoles, 1,2,4-thiadiazoles, oxadiazoles have been appeared in the literature. Jiang and Gu prepared 2,4-bis(3'-indolyl)thiazole (**5**) endowed with selective cytotoxicity against certain leukemia cell lines with GI_{50} values in low micromolar ranges.[6] Very recently, Kumar *et al.* identified bis(indolyl)-1,3,4-oxadiazoles (**6**) as apoptosis inducing cytotoxic agents (IC_{50} = 20 nM; HeLa).[7] Many bisindole alkaloids with linear spacers have been reported to exhibit interesting anticancer activities. For examples; bisindole with 1,2-diketo linker, Hyrtiosin B (**7**) isolated from marine sponge *Hyrtios erecta* was found to possess *in vitro* cytotoxicity against epidermoid carcinoma KB cell line.[8] Bokesch and co-workers isolated Coscinamides A-C (**8**) with a linear alpha-keto enamide spacer from the extract of marine sponge *Coscinoderma* sp., were reported to exhibit antitumor activity against human prostate cancer cell line (IC_{50} = 7.6 µg/mL).[9] Isolated from a red alga *Chondria* sp. two cytotoxic bisindole amides, Chondriamides A-B (**9**), were found to be cytotoxic against KB and LOVO cell lines (IC_{50} = 0.5-10 µg/mL).[10] Inspired from the interesting anticancer activities of naturally occurring bisindoles with linear chain spacers, in 2012, Kumar *et al.* reported bis(indolyl)hydrazide–hydrazones as potent cytotoxic agents (IC_{50} = 1 µM; MDA-MB-231)[11] as illustrated in Figure 4.1.1.

The present chapter divided into part A and part B, reports synthesis and anticancer activity studies of two series of bisindoles. Part A deals with the synthesis and anticancer activity study of α-cyano bis(indolyl)chalcones and part B includes design and synthesis of bis(indolyl)ketohydrazide-hydrazones as tubulin interacting agents.

4.2 Part A: A novel *α*-cyano bis(indolyl)chalcones as anticancer agents

4.2.1 Rational Design

Literature reports show that the indole scaffold frequently encounters in anticancer drug discovery research as mentioned in 4.1. On the other hand, the *α*, *β*-unsaturated ketones also known as chalcones (**11**), are reported to play a vital role in the identification of bioactive molecules (Figure 4.2.1).[12,13]

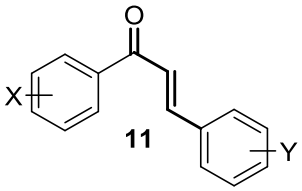


Figure 4.2.1 General structure of chalcones (**11**)

Natural and synthetic chalcones have been reported to show diverse biological activities such as antiinflammatory,[14] antimalarial,[15] antileishmanicidal, antiviral, antifungal, antibacterial and anticancer.[16-20] Particularly, synthetic and natural chalcones had a significant impact in anticancer drug discovery research.[21] Anticancer activities of some chalcones are reported owing to their bindings to tubulin assembly which effects tubulin-microtubule dynamic equilibrium.[22-24]

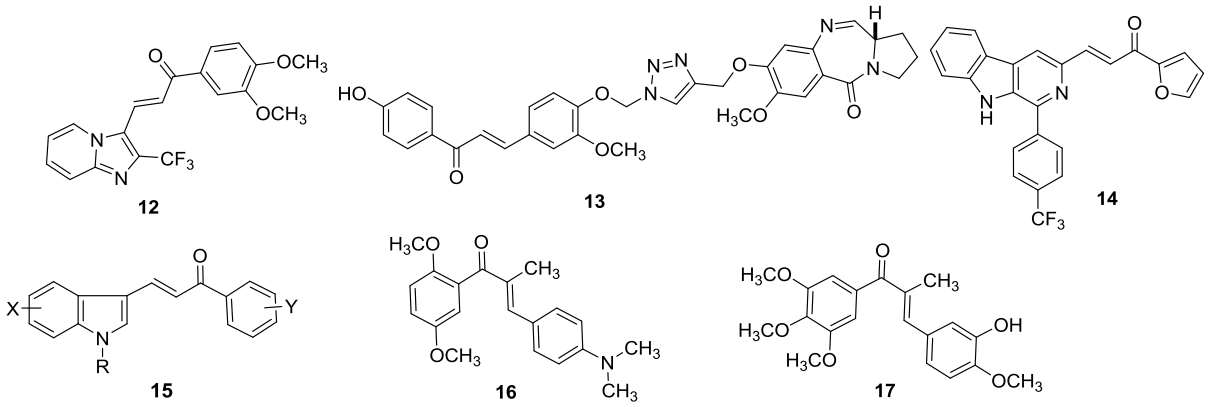


Figure 4.2.2 Representative examples of chalcones as potent anticancer agents (**12-17**)

Over a period of time, several new chalcones have been reported with structural modifications around the basic enone template as shown in Figure 4.2.2. Recently, Kamal′s research group explored diverse heterocyclyl chalcones as potential anticancer agents.[25] For example, imidazopyridine/pyrimidine and pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) conjugated chalcones **12** and **13** induced G1 phase cell cycle arrest and exhibited inhibitory

effect on NF-kβ, Bcl-XL proteins.[25,26] Subsequently, same group in 2014 explored chalcone-linked *β*-carboline hybrids **14** as antitopoisomerase-I, DNA-interactive, and apoptosis-inducing anticancer agents.[27] Recently, Kumar group reported a series of indolyl chalcones **15** as potential cytotoxic agents.[28] Edwards *et al.* first reported that *α*-substituted chalcones **16** are more potent than their unsubstituted counter parts.[29] Later, Duke *et al.* supported the same concept by synthesizing the *α*-methylated chalcone (SD400) **17**, the most active compound reported to inhibit tubulin assembly (IC_{50} = 1.8 μM).[19,30-32] Further, same group studied the improved cytotoxic effects of *α*-substituents such as cyano, fluoro, phenyl and ester groups on *α, β*-unsaturated enone system.[33] Ikeda *et al.* disclosed a series of *α*-methylated chalcones with 10-folds enhanced activity when compared to their parent derivatives.[34] It is believed that *α*-substitution imparts *s*-trans geometry to the molecule which may be responsible for the enhanced cytotoxic effects over unsubstituted analogues.[13,35]

Bisindole alkaloids drawn significant interest due to their diverse biological properties including antiviral, antimicrobial and anticancer.[36] These alkaloids are majorly discovered from marine invertebrates such as sponges, bryozoans coelenterates and tunicates.[37,38]

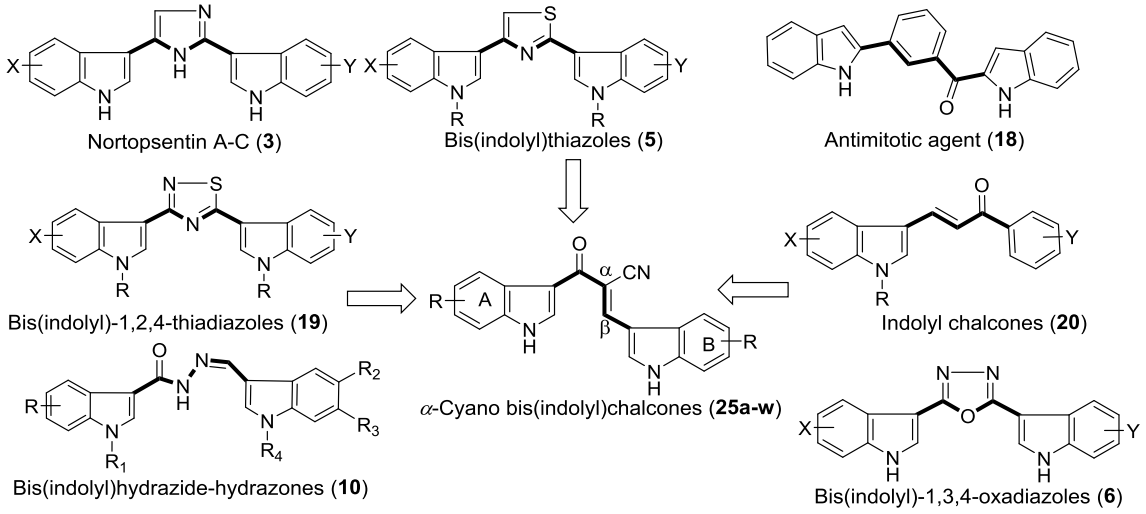


Figure 4.2.3 Rational for the synthesis of *α*-cyano bis(indolyl)chalcones

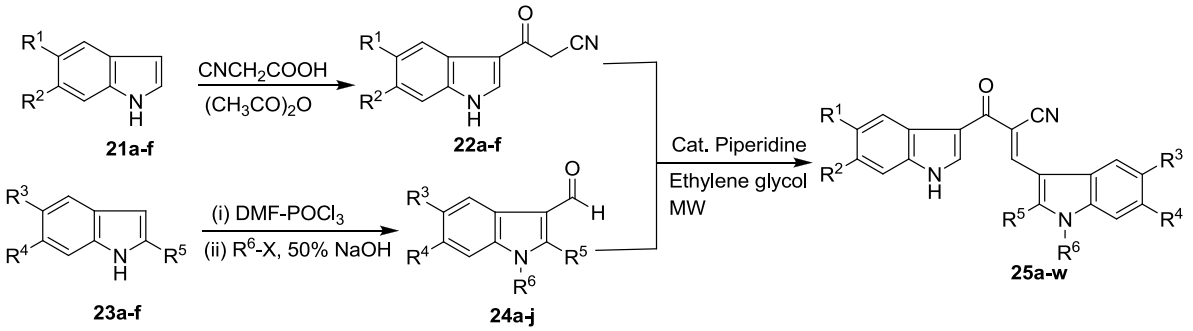
For example, Nortopsentins A-C (**3**) were isolated from a marine sponge *Spongosorites ruetzleri,* and exhibited *in vitro* cytotoxicity (IC_{50} = 4.5-20.7 μM, P388 cells). *N*-Methylated counterparts of Nortopsentin reported to display improved P388 activity (IC_{50} = 0.8-2.1 μM) as shown in Figure. 4.2.3.[39] With the interesting cytotoxic properties, Nortopsentins and Topsentins are considered as lead compounds for the development of novel synthetic bisindoles. Several synthetic analogues of bisindole alkaloids with variety of cyclic spacers such as thiazole **5**,[40] thiophene,[41] pyrazole,[42] isoxazole,[43] pyridine[44] and pyrimidine[45] have

been reported for their cytotoxic properties. Recently, Sunjoo *et al.* showed that Topsentin derivative **18** displays potent *in vitro* and *in vivo* anticancer activity by destabilizing the microtubule assembly.[46] Bisindoles containing long chain linkers such as enamides (Coscinamide A-C)[47] and glyoxylamide (Indibulin) exhibited significant HIV-inhibitory and anticancer activities.[48] Our ongoing efforts to identify indole-based heterocycles as potent anticancer agents include syntheses of various indolylazoles, for example,5-(3′-indolyl)-1,3,4-oxadiazoles, 4-(3′-indolyl)oxazoles, 5-(3′-indolyl)-1,3,4-thiadiazoles and indolyl-1,2,4-triazoles.[49-52] Recently, we synthesized a novel series of bis(indolyl)-1,2,4-thiadiazoles **19**, bis(indolyl)hydrazide-hydrazones **10**, and bis(indolyl)-1,3,4-oxadiazoles **6** and evaluated their anticancer activities (Figure 4.2.3).[7,53,54] In this study, it was realized that the central spacers in bisindoles play a crucial role in altering their cytotoxicity. In order to improve the anticancer efficacy of bisindoles, herein we designed and synthesized a series of novel *α*-cyano bis(indolyl)chalcones **18** having *α*-cyano 2-propen-1-one as a novel spacer. A recent report on the synthesis of *α*-cyano substituted compounds as potent anticancer agents also encouraged us to prepare these bisindole compounds.[55]

4.2.2 Results and discussion

4.2.2.1 Synthesis

The common methods for the preparation of chalcones utilizes the Claisen–Schmidt condensation, wherein aldehydes and ketones react in the presence of base/acid catalysts using polar protic solvents.[28,56-58] The *α*-substituted chalcones are usually prepared by the Knoevenagel like condensation of substituted propiophenones and appropriate benzaldehydes. Knoevenagel like condensation has been documented in bases such as pyridine under reflux and followed by water removal,[59,60] pyrrolidine/acetic acid,[61] Ti(OPr-i)$_4$/pyridine,[62] potassium exchanged zirconium hydrogen phosphate Zr(O_3POK)$_2$,[63] or by a Lewis acid ($CeCl_3$)[64] or in ionic liquids,[65] or in solvents such as DMF and DMSO[66] or using surfactants.[67] Knoevenagel condensations are also reported in water or under microwave irradiation.[68,69] For the construction of *α*-cyano chalcones, limited protocols are available with lower product conversions and extended reaction times.[33,70,71] In this report, we have optimized the reaction conditions involving a simple experimental setup for the successful preparation of various *α*-cyano bis(indolyl)chalcones (**25**) in good yields. Synthesis of *α*-cyano bis(indolyl)chalcones (**25**) was achieved as described in Scheme 4.2.1.

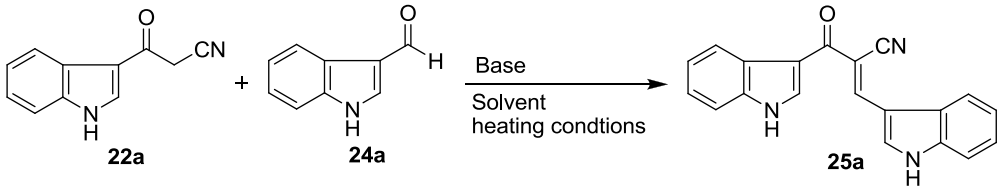


Scheme 4.2.1 Synthesis of α-cyano bis(indolyl)chalcones **25a-w**

The 3-cyanoacetyl indoles (**22a-f**) were synthesized in good yields from the reaction of indoles **21a-f** with cyanoacetic acid in the presence of acetic anhydride. This rapid protocol allowed us to isolate various α-cyano bis(indolyl)chalcones (**25**) by simple filtration.[72] On the other hand, the reaction of indoles **23a-f** with phosphorus oxychloride in presence of dimethylformamide (DMF) followed by alkylation resulted in the formation of *N*-substituted indole-3-carboxaldehydes **24a-j** in good yields.[53,73] In our efforts, α-cyano bis(indolyl)chalcones (**25a-w**) were prepared by Knoevenagel condensation of compounds **22** and **24** under microwave irradiation in the presence of ethylene glycol using piperidine as a base catalyst. The condensation step was optimized using different polar protic and aprotic solvents under conventional and microwave (MW) irradiation techniques. Initially, a model reaction was carried out conventionally by heating 3-cyano-acetyl indole (**22a**) and indole-3-carboxaldehyde (**24a**) in various solvents using piperidine as a base catalyst (Table 4.2.1). The conventional synthesis involves the longer reaction times and lower product yields. It is well documented that advantages of MW-assisted organic transformations are shorter reaction time and it proceed with high yields.[74-77] Hence to improve the product yield as well as rate of reaction we were further optimized the reaction conditions under microwave-irradiation technique at different parameters.

As reported in the literature that efficient coupling in MW with polar molecules/polar intermediates likely to enhance the reaction rate or formation of target compounds. We observed that 100 watt MW power, 80 °C set temperature and 100 psi pressure were the optimum for the reaction to give desired chalcones **25**. Most of the screened solvents afforded comparable yields of the product **25a** under focused microwave irradiation.

Table 4.2.1 Optimization of **25a** in various solvents using conventional and MW method



Entry	Base[a]	Solvent	Conventional Heating[b]		Focused MW[c]	
			Time (min)	Yield (%)	Time (min)	Yield (%)
1.	Piperidine	Toluene	600	0	10	86
2.	Piperidine	DMF	600	10	12	75
3.	Piperidine	Ethanol	600	45	04	84
4.	Piperidine	Methanol	900	30	04	80
5.	Piperidine	Water	900[b]	0	06	79
6.	-	-	120[c]	0	10	0
7.	-	Ethylene glycol	360	35	15	trace
8.	Piperidine	Ethylene glycol	600	50	**05**	**96**
9.	Piperidine	-	300	10	07	81
10.	Piperidine	PEG-400	600	45	10	74

[a] 0.2 mL of piperidine was used for 1 mmol of the reactants.
[b] Heated at reflux temperatures.
[c] at 100 °C.

The progress of the reaction was also impressive in water as well as under solvent-free conditions (entries 5 and 9, Table 4.2.1) but in terms of yield, ethylene glycol was proved to be superior among the screened solvents (entry 9, Table 4.2.1). The low solubility of the reactants may be the primary reason for the lowering of the yields in most of the screened solvents. The efforts were made to find the role of piperidine in reaction progress. After 15 min of the MW-irradiation, the product was formed in trace amount under base-free conditions (entry 7, Table 4.2.1). Optimized reaction conditions were generalized by reacting several indole-3-carboxaldehydes and 3-cyanoacetyl indoles to produce diverse series of α-cyano bis(indolyl)chalcones (**25a-w**) in 72-92% yields. The structures of compounds **25a-w** were elucidated through their IR, NMR (^{1}H & ^{13}C) and Mass spectral analysis. In IR spectra, a characteristic sharp peak at ~ 2200 cm^{-1} was observed due to the presence of a nitrile functional group. The ^{1}H NMR spectra of **25a-w** displayed a characteristic singlet between δ values 8.5 to 8.7 ppm corresponding to an alkenyl proton (=CH). The carbonyl and nitrile carbons were observed at about δ 180 and 120 ppm in the ^{13}C NMR spectra of **25a-w**. Mass spectral data is well in agreement with their respective structures (Figures 4.2.4-4.2.6).

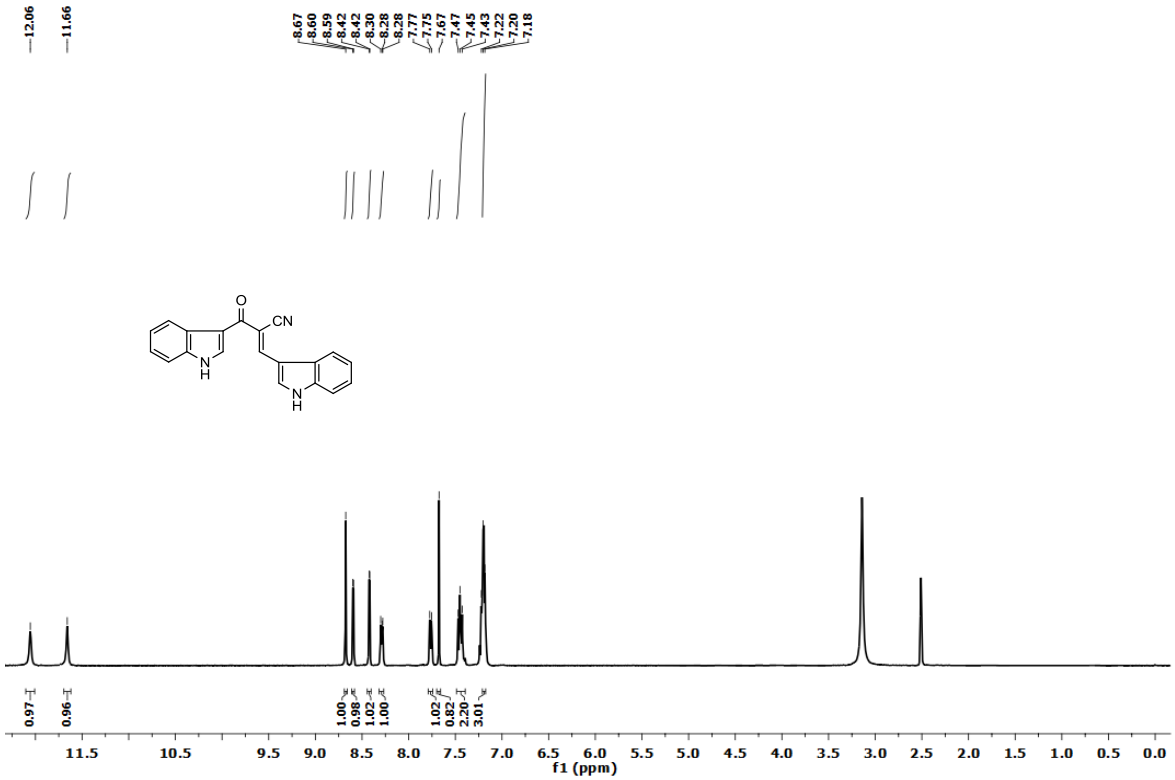


Figure 4.2.4 ^{1}H NMR spectrum of **25a**

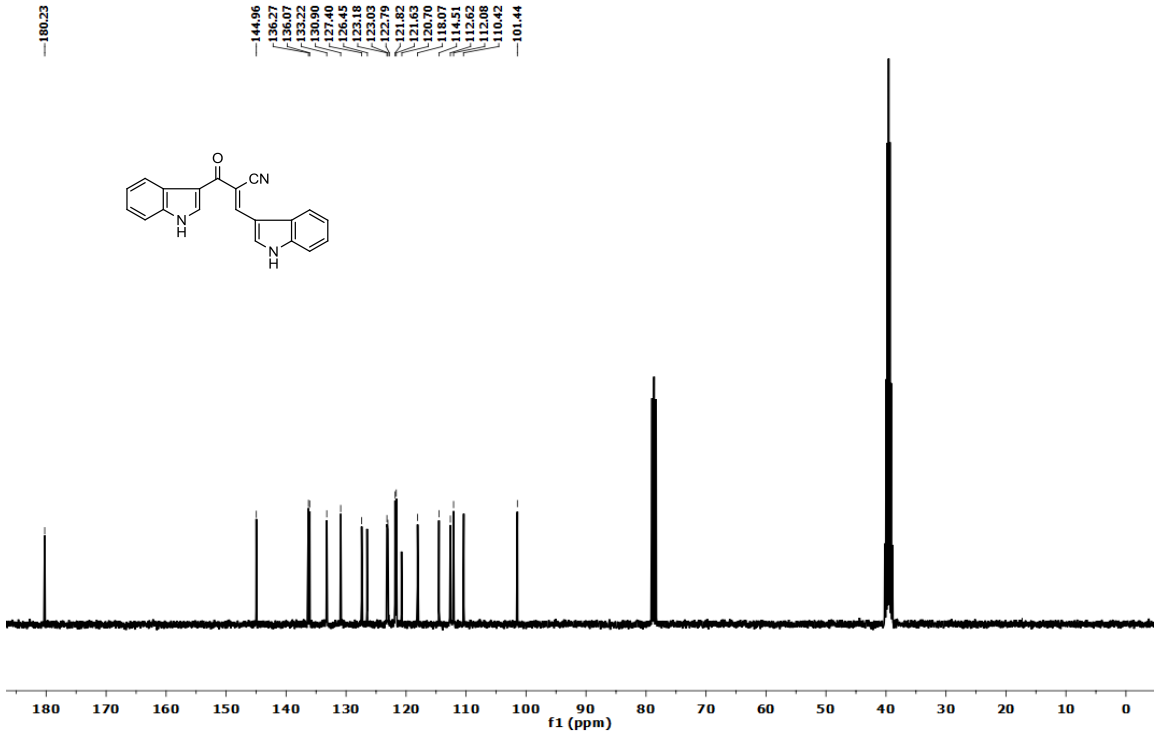


Figure 4.2.5 ^{13}C NMR spectrum of **25a**

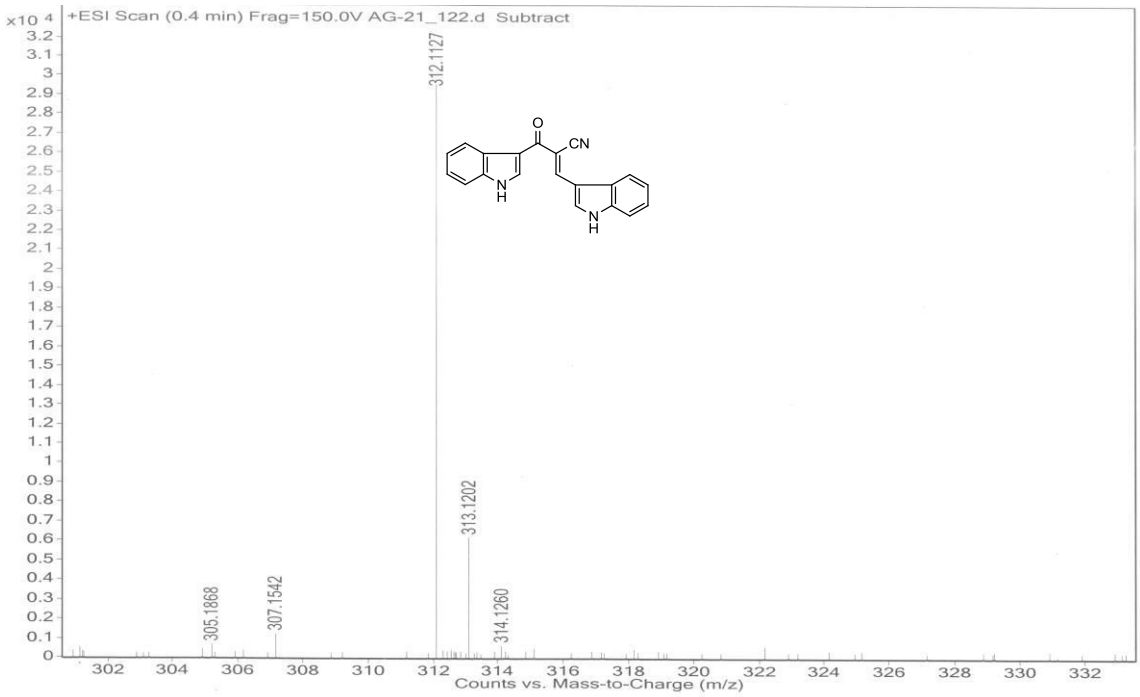
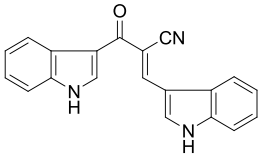
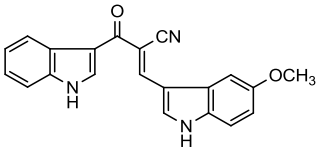
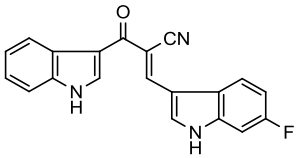
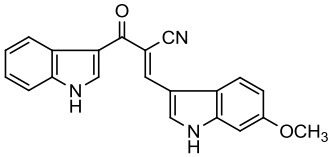
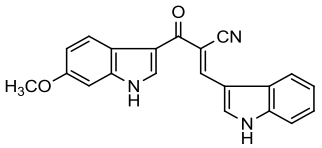
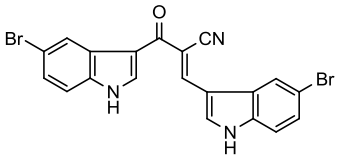
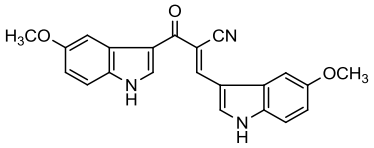
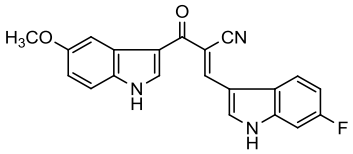
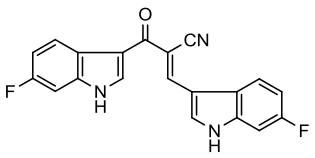


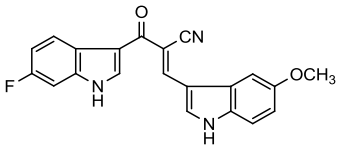
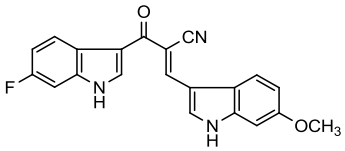
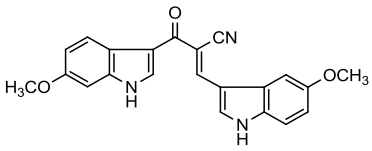
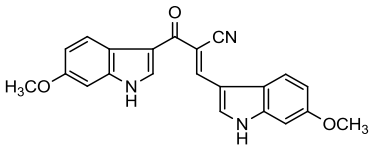
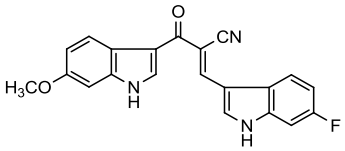
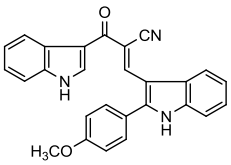
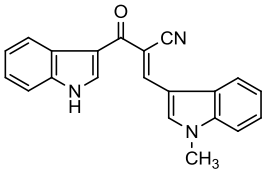
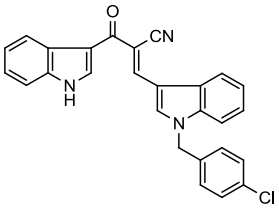
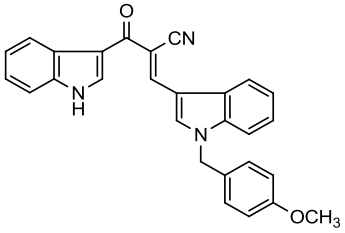
Figure 4.2.6 HRMS spectrum of **25a**

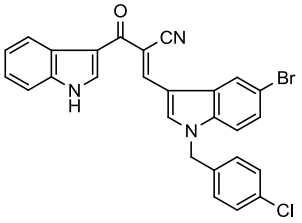
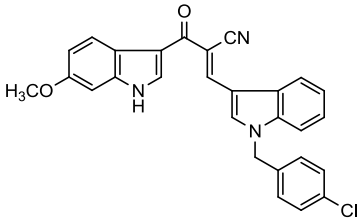
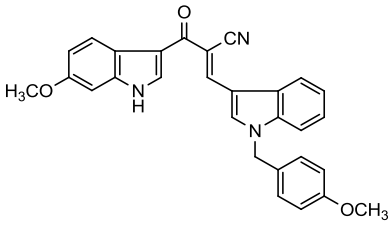
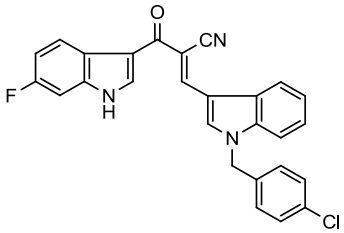
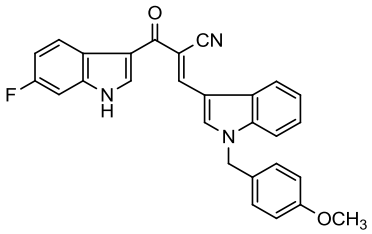
4.2.2.2 Anticancer activity

The cytotoxicity of the synthesized α-cyano bis(indolyl)chalcones **25a-w** were evaluated against three human cancer cell lines; lung (A549), prostate (PC3) and pancreas (PaCa2) cell lines by using WST-8 assay. The anticancer activity was expressed in terms of IC_{50} values for inhibition of tumor cell growth in micromolar concentrations (Table 4.2.2 and Figure 4.2.7). A study of structure-activity relationship (SAR) was carried out by synthesizing diverse α-cyano bis(indolyl)chalcones having substituents at various positions of the both indole rings. Compound **25a** having no substituent showed moderate activity against A549 and PaCa2 cancer cell lines. Among the disubstituted α-cyano bis(indolyl)chalcones, compounds **25f-h** processing C-5 Br/OCH_3 groups on 3-cyanoacetyl indole did not show any impact on anticancer activity, except compound **25g** with moderate selectivity towards A549 and PaCa2 cells. On the other hand, F or OCH_3 substituent at C-6 position of 3-cyanoacetyl indole (compounds **25i-n**) imparted activity as well as selectivity. Compounds **25i** and **25j** with C-6 fluoro on 3-cyanoacetyl indole showed selectivity towards A549 lung cancer cell line (IC_{50} = 36.1 and 18.0 µM). Methoxy substituent on C-5/C-6 position of indole carboxaldehyde and C-6 position of 3-cyano-acetyl indole led to compounds **25l** and **25m** with improved activity against lung cancer cells (IC_{50} = 3.7 and 1.7 µM, respectively).

Table 4.2.2 IC_{50} (μM) of *α*-cyano bis(indolyl)chalcones (**25a-w**) for growth inhibition of selected human cancer cell lines

***α*-Cyano bis(indolyl)chalcones**		**A549**		**PC3**		**PaCa2**	
		24 h	48 h	24 h	48 h	24 h	48 h
	**(25a)**	16.1	22.0	>50	>50	17.9	36.8
	**(25b)**	**6.6**	13.7	33.2	30.5	21.2	25.1
	**(25c)**	17.0	22.7	>50	>50	23.5	31.6
	**(25d)**	**9.6**	**5.8**	33.3	12.5	27.5	>50
	**(25e)**	**6.4**	**7.5**	>50	12.0	13.5	>50
	**(25f)**	>50	>50	>50	>50	>50	>50
	**(25g)**	>50	24.5	>50	>50	28.5	>50
	**(25h)**	>50	>50	>50	>50	>50	>50
	**(25i)**	>50	36.1	>50	>50	>50	>50

	**(25j)**	18.0	26.3	>50	>50	>50	>50
	**(25k)**	10.3	**5.5**	>50	21.4	>50	>50
	**(25l)**	**3.7**	**5.5**	31.1	37.1	>50	>50
	**(25m)**	**1.9**	**1.7**	>50	27.0	>50	>50
	**(25n)**	**2.4**	**0.8**	36.0	22.5	>50	>50
	**(25o)**	**4.9**	**3.0**	17.2	**8.1**	24.0	>50
	**(25p)**	27.9	>50	>50	>50	31.6	>50
	**(25q)**	>50	>50	>50	>50	>50	>50
	**(25r)**	>50	>50	>50	>50	>50	>50

	**(25s)**	14.0	24.9	47.1	36.5	37.7	>50
	**(25t)**	>50	>50	>50	>50	>50	40.4
	**(25u)**	37.4	28.9	>50	>50	>50	>50
	**(25v)**	>50	>50	>50	>50	>50	16.6
	**(25w)**	>50	36.3	>50	>50	>50	29.4
Mitomycin C		0.45	–	–	–	–	–

Fluoro and methoxy substituents at C-6 position of both the indole rings (compound **25k** *vs* **25n**) exhibited good anticancer activity and particularly, **25n** was found to be the best compound of series with an IC_{50} value of 0.8 μM against lung cancer cells. Compound **25o** having *p*-methoxyphenyl substituent at C-2 of the indole ring showed overall improvement in activity against tested cancer cell lines with best activity against A549 cells (IC_{50} = 3.0 μM). *N*-Alkylation (methyl, *p*-chlorobenzyl and *p*-methoxybenzyl) of **25a** did not improve the anticancer activity (compounds **25p-r**). Compound **25s** with C-5 bromo and *N*-1 *p*-chlorobenzyl substituents showed moderate activity against tested cancer cell lines. Introducing *N*-chloro/methoxybenzyl and C-6 methoxy/fluoro groups led to compounds

25t-w with moderate selectivity against PaCa2 cancer cells. *α*-Cyano bis(indolyl)chalcones **25v** and **25w** with C-6 fluoro substituent showed selective cytotoxicity against PaCa2 cancer cell lines (IC_{50} = 16.6 and 29.4 µM, respectively). The mono substituted bis(indolyl)-chalcones (**25b-e**) were obtained by introducing methoxy or fluoro group at C-5/C-6 position of indole rings. Compounds (**25b**, **25d** and **25e**) having methoxy substituents showed improved anticancer activity against A549 cancer cell line (5-7 µM).

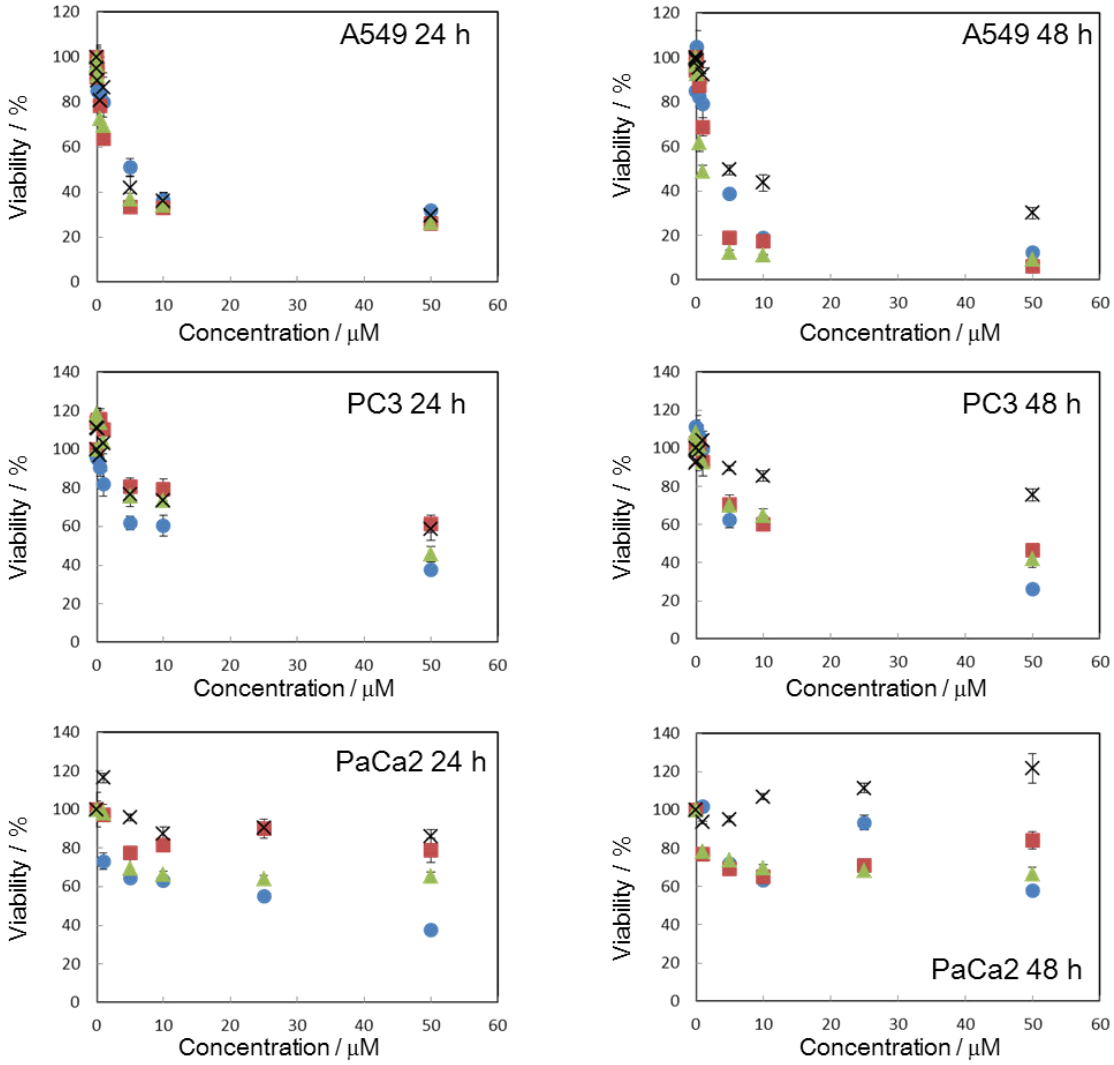


Figure 4.2.7 Effects of chalcones (●, **25o**; ■, **25m**; ▲, **25n**; ×, **25l**) on the growth of (a) A549 and (b) PC3 cells after 24 h. incubation. The results of studies are expressed as mean values ± SD from at least four separate experiments

Most of the synthesized *α*-cyano bis(indolyl)chalcones showed anticancer activity less than 10 µM. Elaborate study on the effect of substituent pattern on anticancer activity of *α*-cyano-bis(indolyl)chalcones emphasizes the importance of C-6 methoxy group. Overall, the anticancer activity of novel *α*-cyano bis(indolyl)chalcones **25a-w** was encouraging and found

to be comparable with reported bis(indolyl)hydrazide-hydrazones and bis(indolyl)-heterocycles.[28,6-8,10]

4.2.2.3 Tubulin binding study

To investigate whether the cytotoxic activity of *α*-cyano bis(indolyl)chalcones were related to the binding to tubulin, the selected *α*-cyano bis(indolyl)chalcones (compounds **25e**, **25m**, **25n** and **25u**) were evaluated for their preliminary mechanistic studies by investigating their tubulin binding properties.[11,19] The experiments were conducted using Porcine brain tubulin and compounds were compared with paclitaxel at 5 μM concentration. Tubulin polymerization was measured by absorbance at 340 nm over time as indicated in the Figure 4.2.8. The results showed that compounds **25e**, **25m** and **25u** enhanced the polymerization rate to some extent, which suggesting that these compounds could work as microtubule stabilizing agents.

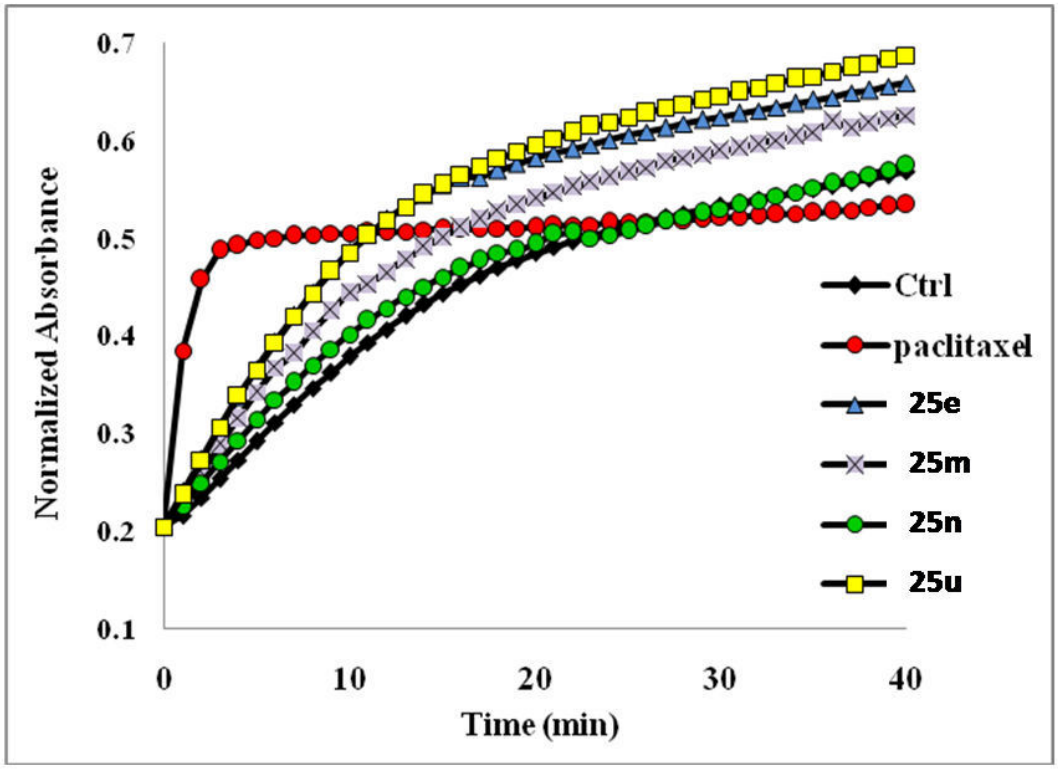


Figure 4.2.8 Tubulin polymerization kinetics of compounds in the presence or absence of (●) paclitaxel, (▲) compound **25e**, (×) **25m** (○), **25n** or (□) **25u** (5 μM each)

4.2.3 Conclusions

In summary, we have reported a facile microwave-assisted synthesis of *α*-cyano bis(indolyl)chalcones (**25a-w**) and their *in vitro* anticancer activities. Some of the compounds

showed significant anticancer activity and the most active compound **25n** with C-6 methoxy and fluoro substituents on indole rings exhibited selective cytotoxicity against A549 lung cancer cells (IC_{50} = 0.8 μM). Compounds **25b**, **25d**, **25e**, **25k-m** and **25o** having mono or di-methoxy groups at various positions of indole ring were found to be potent against multiple cancer cell lines. Preliminary mechanistic studies revealed that some of these compounds enhance tubulin polymerization thereby imparting anticancer activity to the compounds.

4.2.4 Experimental section

General Remarks

All the reagents were obtained commercially and used as received, unless otherwise stated. Solvents used for all reaction were dried prior to use by standard procedure. All the reactions were performed in a CEM Focused microwave. The reactions were 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). The solvents were evaporated using Buchi rotary evaporator. Melting points were determined with electrothermal capillary melting point apparatus (*E-Z*-melting). IR spectra were recorded on Shimadzu FT-IR spectrophotometer. NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker Avance II (400 and 100 MHz, respectively) spectrometer and TMS used as reference and $CDCl_3$ or DMSO-d_6 used as solvents for recoding the NMR spectra. The coupling constant (*J*) values are in Hz. Mass spectra were obtained on a 'Hewlett–Packard' HP GS/MS 5890/ 5972.

General Procedure for the synthesis of 3-cyanoacetyl indoles (22a-f):[72] Indole (10 mmol) **21** was added to a stirred solution of cyanoacetic acid (10 mmol) in acetic anhydride (10 mL) at 50 °C. The solution was heated at 85 °C for 5-10 min. During that period 3-cyanoacetylindole started to crystallize. After completion of the reaction as monitored by TLC, the mixture was allowed to cool and the solid was collected, washed with methanol and dried to give pure 3-cyanoacetyl indoles (**22a-f**) in 85-92% yields.

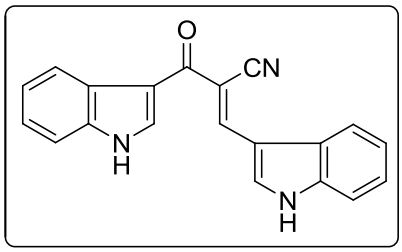
General procedure for the synthesis of indole-3-carboxaldehyde (24a-j):[73] A round-bottomed flask containing freshly distilled dimethylformamide (DMF) (370 mmol) was cooled in an ice-salt bath for about 0.5 h. and 90 mmol of freshly distilled phosphorus oxychloride ($POCl_3$) was added with stirring to the DMF over a period of 0.5 h. A solution of indole (**23**, 85.47 mmol) in DMF (130 mmol) was added to the yellow solution over a period

of 1 h. The solution was stirred at 35 °C till it became 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. To this cherry-red solution, a solution of sodium hydroxide (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 at room temperature, after which it was placed in refrigerator for overnight. The product was filtered, washed with water (2 × 100 mL) and air-dried to afforded the pure indole-3-carboxaldehyde **24a-j** in 85-90% yields.

Alkylation of indole-3-carboxaldehydes:[78,79] To a stirred mixture of indole-3-carboxaldehyde (34 mmol) in 50% aqueous NaOH (40 mL), water (60 mL) and tetrabutylammonium bromide (0.1 g, 3.4 mmol) was added methyl iodide/4-chlorobenzylchloride/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 alkylated product which upon recrystallization with ethylacetate and hexane led to pure alkylated 3-carboxaldehydes.

General procedure for the preparation of *α*-cyano bis(indolyl)chalcones (25a-w): 3-Cyanoacetylindole (**22**, 1 mmol) and indole-3-carboxaldehyde (**24**, 1 mmol) were taken in a sealed vial (10 mL) containing ethylene glycol (2 mL) and piperidine (0.2 mL). The contents were thoroughly mixed and irradiated in a CEM focused microwave oven with P = 100 w/ 100 psi at 80 °C for 5 min. After completion of the reaction as indicated by thin layer chromatography, contents were poured into ice cold water (50 mL) and neutralized with dilute acetic acid. The solid thus obtained was filtered, dried and recrystallized from ethanol to get pure chalcones **25a-w** in 72-92% yields.

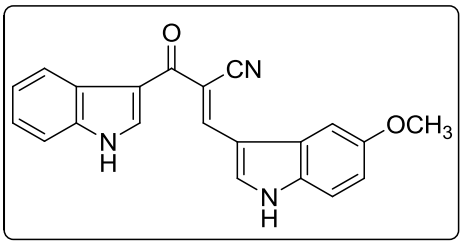
2-Cyano-1,3-bis(3′-indolyl)-prop-2-en-1-one (25a)



Yield 92%; Pale yellow solid; mp 270–271 °C; IR (KBr, *ν*, cm^{-1}): 3178, 2205, 1625, 1497, 1420, 1232, 792, 648; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 1H), 11.66 (s, 1H), 8.67 (s, 1H), 8.59 (d, J = 3.1 Hz, 1H), 8.42 (d, J = 3.1 Hz, 1H), 8.32–8.26 (m, 1H), 7.76 (d, J = 8.0 Hz), 7.67 (s, 1H), 7.50–7.39 (m, 2H), 7.22–7.18 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.2, 144.9, 136.3, 136.1, 133.2, 130.9, 127.4, 126.4, 123.2, 123.0, 122.8, 121.8, 121.6, 120.7, 118.1,

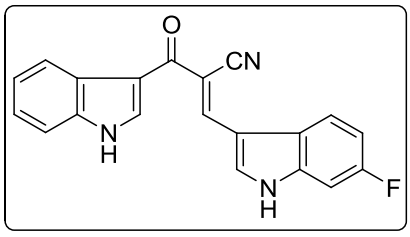
114.5, 112.6, 112.1, 110.4, 101.4; ESI (FAB$^+$) *m/z* calcd.for $C_{20}H_{14}N_3O$: 312.1137 $[M + H]^+$, found: 312.1127.

2-Cyano-1-(3′-indolyl)-3-[3′-(5′-methoxyindolyl)]-prop-2-en-1-one (25b)



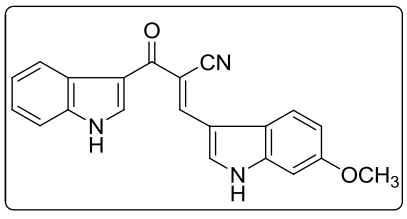
Yield 85%; Yellow solid; mp 238–239 °C; IR (KBr, *v,* cm^{-1}): 3225, 2191, 1605, 1496, 1219, 741, 633; ^1H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 11.81 (s, 1H), 8.71 (s, 1H), 8.59 (d, *J* = 3.0 Hz, 1H), 8.50 (d, *J* = 3.1 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.29–7.24 (m, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.2, 155.5, 145.0, 136.1, 133.14, 131.2, 130.9, 128.4, 126.5, 123.0, 121.8, 121.7, 121.0, 114.6, 113.4, 113.3, 112.1, 110.5, 100.5, 100.1, 55.3; ESI (FAB$^+$) *m/z* calcd.for $C_{21}H_{16}N_3O_2$: 342.1 $[M + H]^+$, found: 342.1.

2-Cyano-1-(3′-indolyl)-3-[3′-(6′-fluoroindolyl)]-prop-2-en-1-one (25c)



Yield 85%; Pale yellow solid; mp 246–248 °C; IR (KBr, *v,* cm^{-1}): 3194, 2206, 1605, 1512, 1298, 738, 680; ^1H NMR (400 MHz, DMSO-d_6): δ 12.25 (s, 1H), 11.87 (s, 1H), 8.64 (s, 1H), 8.61 (s, 1H), 8.47 (d, *J* = 3.2 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 2H), 7.91 (s, 1H), 7.81 (dd, *J* = 8.6, 5.1 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.29–7.22 (m, 1H), 7.05–7.00 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.2, 160.8, 158.5, 144.7, 136.3, 136.0, 133.4, 131.3, 126.5, 123.9, 123.1, 121.9, 121.7, 120.5, 119.3, 114.4, 112.1, 110.4, 110.1, 109.9, 102.3, 99.0, 98.7; ESI (FAB$^+$) *m/z* calcd. for $C_{20}H_{13}FN_3O$: 330.1 $[M + H]^+$, found: 330.0.

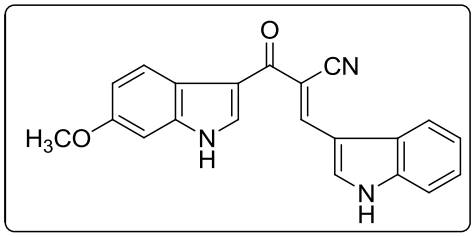
2-Cyano-1-(3′-indolyl)-3-[3′-(6′-methoxyindolyl)]-prop-2-en-1-one (25d)



Yield 82%; Yellow solid; mp 263–264 °C; IR (KBr, *v,* cm^{-1}): 3194, 2206, 1605, 1512, 1298, 738, 680; ^1H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 11.77 (s, 1H), 8.66 (s, 1H), 8.53 (s, 1H), 8.47 (d, *J* = 3.2 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.28–7.22 (m, 2H), 7.00 (d, *J* = 1.9 Hz, 1H), 6.90 (d, *J* = 6.6 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.2, 156.8, 145.1, 136.9, 136.2, 133.1, 130.1, 126.4, 123.0, 121.8, 121.7, 121.1, 120.8, 118.8,

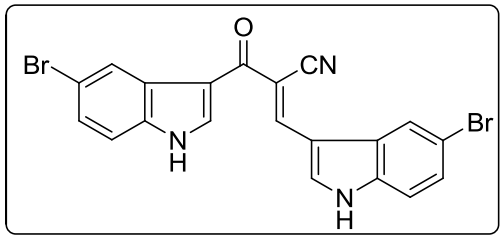
114.5, 112.0, 111.6, 110.5, 101.2, 95.5, 55.1; ESI (FAB$^+$) *m/z* calcd. for $C_{21}H_{16}N_3O_2$: 341.1 $[M + H]^+$, found: 341.1.

2-Cyano-1-[3′-(6′-methoxyindolyl)]-3-(3′-indoly)-prop-2-en-1-one (25e)



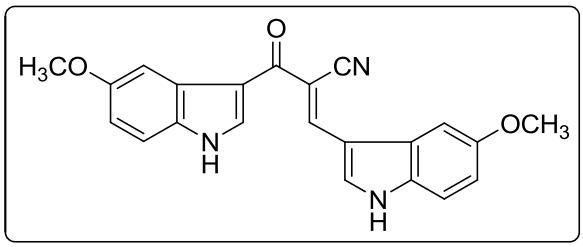
Yield 85%; Yellow solid; mp 243–244 °C;IR (KBr, *v,* cm^{-1}): 3286, 2206, 1605, 1497, 1219, 864, 741; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.18 (s, 1H), 11.60 (s, 1H), 8.71 (s, 1H), 8.64 (d, *J* = 2.9 Hz, 1H), 8.38 (d, *J* = 2.8 Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 7.2 Hz, 1H), 7.28–7.26 (m, 2H), 6.98 (d, *J* = 7.8 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 3.86 (s, 3H); ESI (FAB$^+$) *m/z* calcd. for $C_{21}H_{16}N_3O_2$: 342.1 $[M]^+$, found: 342.1.

2-Cyano-1,3-bis[3′-(5′-bromoindolyl)]-prop-2-en-1-one (25f)



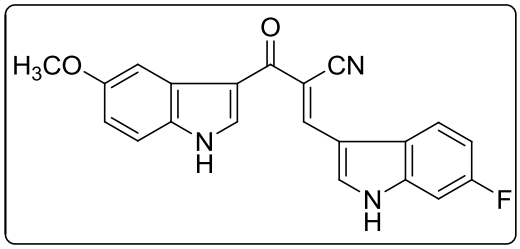
Yield 78%; Yellow solid; mp 240–242 °C; IR (KBr, *v,* cm^{-1}): 3194, 2204, 1600, 1548, 1234, 740, 668; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.31 (s, 2H), 8.63–8.61 (m, 2H), 8.49 (s, 1H), 8.40 (d, *J* = 1.9 Hz, 1H), 8.13 (d, *J* = 1.7 Hz, 1H), 7.50–7.48 (m, 2H), 7.37–7.35 (m, 2H); ESI (FAB$^+$) *m/z* calcd. for $C_{20}H_{11}Br_2N_3O$: 466.9 $[M]^+$, found: 466.9, 468.9 $[M + 2]^+$.

2-Cyano-1,3-bis[3′-(5′-methoxyindolyl)]-prop-2-en-1-one (25g)



Yield 85%; Yellow solid; mp 277–278 °C; IR (KBr, *v,* cm^{-1}): 3194, 2206, 1605, 1497, 1241, 746, 669; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.07 (s, 1H), 11.68 (s, 1H), 8.74 (s, 1H), 8.60 (d, *J* = 3.3 Hz, 1H), 8.50 (d, *J* = 3.3 Hz, 1H), 7.91 (d, *J* = 2.5 Hz, 1H), 7.41–7.39 (m, 2H), 7.28 (d, *J* = 2.3 Hz, 1H), 6.91–6.88 (m, 2H), 3.88 (s, 6H); ESI (FAB$^+$) *m/z* calcd. for $C_{22}H_{17}N_3O_3$: 371.1 $[M]^+$, found: 371.1.

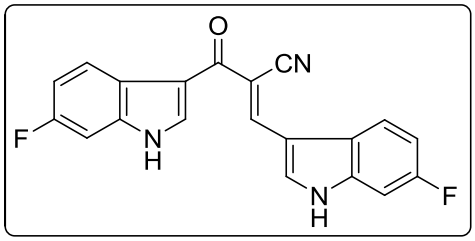
2-Cyano-1-[3′-(5′-methoxyindolyl)]-3-[3′-(6′-fluoroindoly)]-prop-2-en-1-one (25h)



Yield 85%; Yellow solid; mp 241–242 °C; IR (KBr, *v,* cm^{-1}): 3214, 2206, 1605, 1493, 1234, 741, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.26 (s, 1H), 11.78 (s, 1H), 8.66 (s, 1H), 8.65 (d, *J* = 2.9

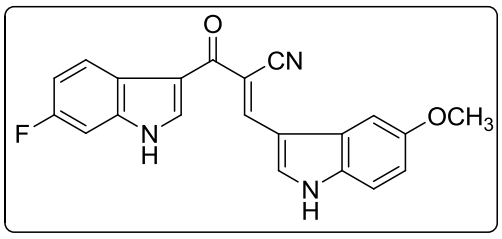
Hz, 1H), 8.45 (d, *J* = 3.3 Hz, 1H), 7.86 (d, *J* = 2.5 Hz, 1H), 7.80 (dd, *J* = 8.7, 5.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.08–6.99 (m, 1H), 6.89 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.87 (s, 3H); ESI (FAB^+) *m/z* calcd. for $C_{21}H_{14}FN_3O_2$: 360.1 $[M + H]^+$, found: 360.1.

2-Cyano-1,3-bis[3′-(6′-fluoroindolyl)]-prop-2-en-1-one (25i)



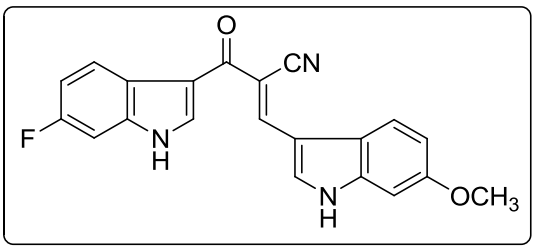
Yield 80%; Yellow solid; mp 289–290 °C; IR (KBr, *v,* cm^{-1}): 3230, 2206, 1605, 1532, 1219, 736, 663; ^1H NMR (400 MHz, DMSO-d_6): δ 12.25 (s, 1H), 11.94 (s, 1H), 8.66 (s, 1H), 8.62 (d, *J* = 2.8 Hz, 1H), 8.53 (d, *J* = 3.3 Hz, 1H), 8.00 (dd, *J* = 9.9, 2.6 Hz, 1H), 7.89 (s, 1H), 7.82 (dd, *J* = 8.8, 5.1 Hz, 1H), 7.47 (dd, *J* = 8.8, 4.5 Hz, 1H), 7.25 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.06–7.02 (m, 3H); ESI (FAB^+) *m/z* calcd. for $C_{20}H_{11}F_2N_3O$: 348.0 $[M + H]^+$, found: 348.0.

2-Cyano-1-[3′-(6′-fluoroindolyl)]-3-[3′-(5′-methoxyindoly)]-prop-2-en-1-one (25j)



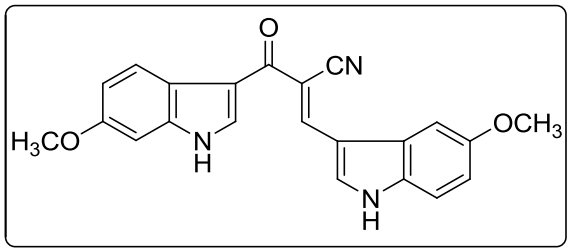
Yield 85%; Pale yellow solid; mp 276–277 °C; IR (KBr, *v,* cm^{-1}): 3256, 2206, 1598,1466, 1203 710, 617; ^1H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H), 11.93 (s, 1H), 8.65 (s, 1H), 8.58–8.56 (m, 2H), 8.00 (dd, *J* = 8.0, 2.6 Hz, 1H), 7.89 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.47 (dd, *J* = 8.8, 4.5 Hz, 1H), 7.05–6.99 (m, 2H), 6.90 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.86 (s, 3H); ESI (FAB^+) *m/z* calcd. for $C_{21}H_{14}FN_3O_2$: $[M + H]^+$ 360.1, found: 360.0.

2-Cyano-1-[3′-(6′-fluoroindolyl)]-3-[3′-(5′-methoxyindoly)]-prop-2-en-1-one (25k)



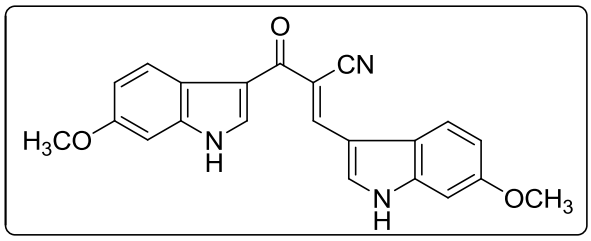
Yield 90%; Pale yellow solid; mp 257–258 °C; IR (KBr, *v,* cm^{-1}): 3148, 2191, 1605,1481, 1219, 802, 673; ^1H NMR (400 MHz, DMSO-d_6): δ 12.12 (s, 1H), 11.64 (s, 1H), 8.68 (s, 1H), 8.57 (d, *J* = 3.1 Hz, 1H), 8.39 (d, *J* = 3.1 Hz, 1H), 8.19 (d, *J* = 8.7 Hz, 1H), 7.91 (d, *J* = 1.9 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 1.7 Hz, 1H), 6.98 (d, *J* = 1.9 Hz, 1H), 6.90–6.86 (m, 2H), 3.85 (s, 3H); ESI (FAB^+) *m/z* calcd. for $C_{21}H_{14}FN_3O_2$ $[M]^+$ 359.1, found: 359.1.

2-Cyano-1-[3′-(6′-methoxyindolyl)]-3-[3′-(5′-methoxyindoly)]-prop-2-en-1-one (25l)



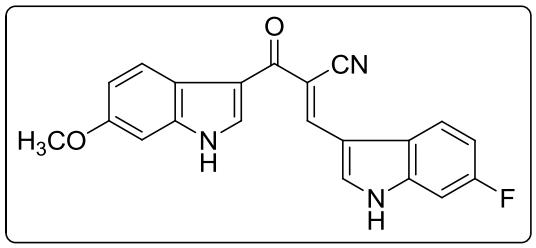
Yield 85%; Pale yellow solid; mp 269–270 °C; IR (KBr, *v,* cm^{-1}): 3148, 2191, 1605,1481, 1219, 802, 710; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11 (s, 1H), 11.88 (s, 1H), 8.72 (s, 1H), 8.57 (d, *J* = 3.1 Hz, 2H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.82 (s, 1H), 7.47 (d, *J* = 8.0 Hz), 7.41 (d, *J* = 8.8 Hz, 1H), 7.02 (t, *J* = 8.3 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H); ESI (FAB$^+$) *m/z* calcd. for $C_{22}H_{18}N_3O_3$: 372.1 $[M + H]^+$, found: 372.1.

2-Cyano-1,3-bis[3′-(6′-methoxyindolyl)]-prop-2-en-1-one (25m)



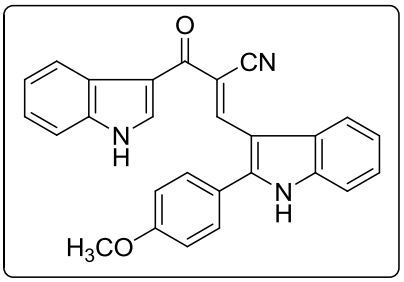
Yield 84%; Yellow solid; mp 258–260 °C; IR (KBr, *v,* cm^{-1}): 3137, 2206, 1604, 1512, 1281, 741, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.94 (s, 1H), 11.56 (s, 1H), 8.65 (s, 1H), 8.53 (d, *J* = 3.0 Hz, 1H), 8.38 (d, *J* = 3.1 Hz, 1H), 8.20 (d, *J* = 8.8 Hz, 1H), 7.78 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 1H), 6.99 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.91–6.82 (m, 2H), 3.86 (s, 6H); ESI (FAB$^+$) *m/z* calcd. for $C_{21}H_{18}N_3O_2$: 372.1 $[M + H]^+$, found: 372.0.

2-Cyano-1-[3′-(6′-methoxyindolyl)]-3-[3′-(6′-fluoroindoly)]-prop-2-en-1-one (25n)



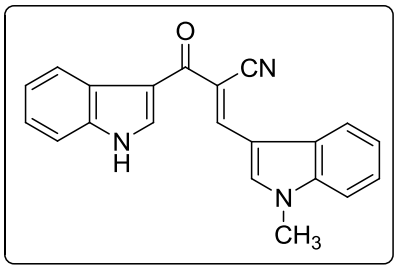
Yield 75%; Pale yellow solid; mp 292–294 °C; IR (KBr, *v,* cm^{-1}): 3209, 2206, 1605, 1558, 1219, 741, 662; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H), 11.64 (s, 1H), 8.64 (s, 1H), 8.61 (d, *J* = 2.8 Hz, 1H), 8.37 (d, *J* = 3.3 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 7.87 (s, 1H), 7.80 (dd, *J* = 8.7, 5.1 Hz, 1H), 7.24 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.05–7.00 (m, 1H), 6.98 (d, *J* = 2.2 Hz, 1H), 6.88 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.80, 160.94, 158.33, 156.65, 144.55, 137.12, 136.14, 135.95, 132.40, 131.23, 123.89, 122.33, 120.52, 120.41, 119.33, 114.59, 111.66, 110.35, 110.08, 109.84, 102.14, 98.93, 98.67, 95.08, 54.97; ESI (FAB$^+$) *m/z* calcd. for $C_{21}H_{15}FN_3O$: 360.1 $[M + H]^+$, found: 360.0.

2-Cyano-1-(3′-indolyl)]-3-[3′-2′-(4′′-methoxyphenyl)-indoly))]-prop-2-en-1-one (25o)



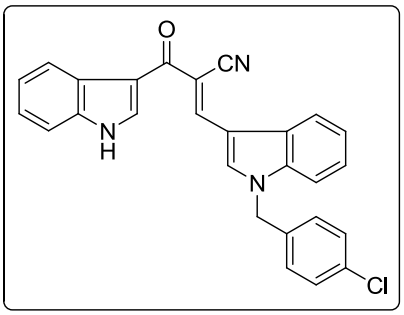
Yield 72%; Yellow solid; mp 192–193 °C; IR (KBr, *v,* cm^{-1}): 3225, 2191, 1605, 1549, 1497, 1250, 833, 741; ^1H NMR (400 MHz, DMSO-d_6): δ 11.78 (s, 1H), 11.25 (s, 1H), 8.66 (s, 1H), 8.47 (d, *J* = 3.2 Hz, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 8.23–8.19 (m, 2H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.7 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 1H), 7.58–7.38 (m, 3H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.1 Hz, 1H), 3.88 (s, 3H); ESI (FAB^+) *m/z* calcd. for $C_{27}H_{20}N_3O_2$: 418.0 $[M + H]^+$, found: 418.0.

2-Cyano-1-(3′-indolyl)-3-[3′-(1′-methylindolyl)]-prop-2-en-1-one (25p)



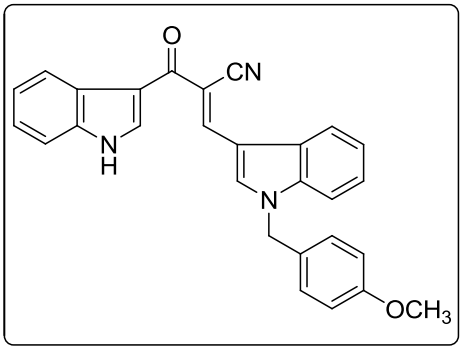
Yield 82%; Yellow solid; mp 218–220 °C; IR (KBr, *v,* cm^{-1}): 3194, 2206, 1632, 1574, 1497, 1429, 1219, 849, 725; ^1H NMR (400 MHz, DMSO-d_6): δ 11.68 (s, 1H), 8.64 (s, 1H), 8.51 (s, 1H), 8.42 (d, *J* = 3.2 Hz, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 7.5 Hz, 1H), 7.69 (s, 1H), 7.45–7.39 (m, 2H), 7.33–7.25 (m, 2H), 3.94 (s, 3H); ESI (FAB^-) *m/z* calcd. for $C_{21}H_{16}N_3O$ 325.1 $[M + H]^+$, found: 325.1.

2-Cyano-1-(3′-indolyl)-3-[3′-(1′-(4′′-chlorobenzylindolyl))]-prop-2-en-1-one (25q)



Yield 85%; Yellow solid; mp 219–221 °C; IR (KBr, *v,* cm^{-1}): 3178, 2176, 1628, 1605, 1450, 1234, 741, 646; ^1H NMR (400 MHz, DMSO-d_6): δ 11.35 (s, 1H), 8.56 (s, 1H), 8.40 (d, *J* = 3.2 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 2H), 8.01–8.03 (m, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.66 (s, 1H), 7.40–7.28 (m, 2H), 7.33–7.25 (m, 2H), 7.15–6.98 (m, 2H), 5.36 (s, 2H); ESI (FAB^+) *m/z* calcd. for $C_{27}H_{18}ClN_3O$ $[M]^+$ 436.1, found: 436.1.

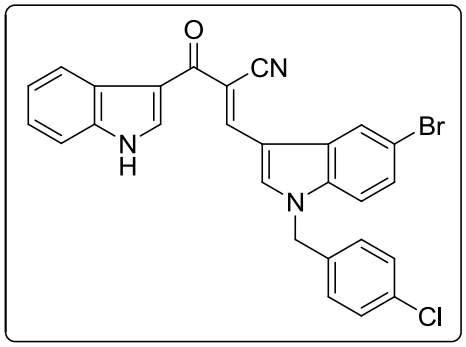
2-Cyano-1-(3′-indolyl)-3-[3′-(1′-(4′′-methoxybenzylindolyl))]-prop-2-en-1-one (25r)



Yield 82%; Yellow solid; mp 206–208 °C; IR (KBr, *v,* cm^{-1}): 3271, 2191, 1605, 1497, 1298, 741, 679; ^1H NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H), 8.72 (s, 1H), 8.66 (s, 1H), 8.57 (d, *J* = 3.2 Hz, 1H), 8.51–8.48 (m, 1H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.47–7.44 (m, 1H), 7.37–7.30 (m, 5H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8

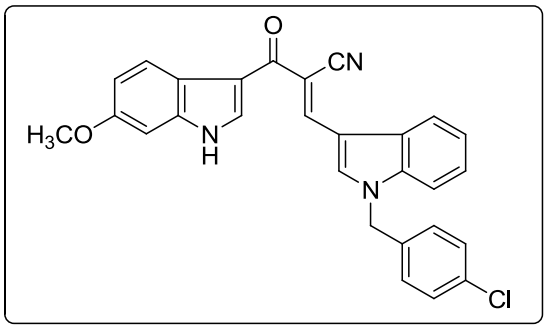
Hz, 2H), 5.38 (s, 2H), 3.78 (s, 3H); ESI (FAB$^+$) *m/z* calcd. for $C_{28}H_{22}N_3O_2$ $[M + H]^+$ 432.1, found: 432.1.

2-Cyano-1-(3′-indolyl)-3-[3′-(1′-(4′′-chlorobenzyl)-5′-bromoindolyl))]-prop-2-en-1-one (25s)



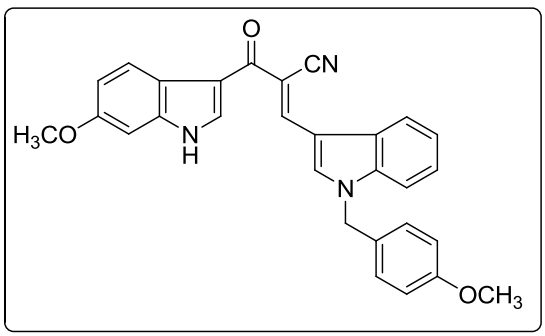
Yield 80%; Yellow solid; mp 180–181 °C; IR (KBr, *v,* cm^{-1}): 3190, 2197, 1635, 1496, 1256, 741, 694; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 8.62 (d, J = 7.5 Hz, 2H), 8.45 (s, 1H), 8.32–8.28 (s, 2H), 8.06 (s, 1H), 8.01 (d, J = 1.6 Hz, 1H), 7.97 (s, 1H), 7.53–7.46 (m, 2H), 7.36–7.32 (m, 2H), 7.25–7.23 (m, 2H), 5.34 (s, 2H); ESI (FAB$^+$) *m/z* calcd. for $C_{27}H_{17}BrClN_3O$ $[M]^+$ 513.0, found: 513.0.

2-Cyano-1-[3′-(6′-methoxyindolyl)]-3-[3′-(1′-(4′′-chlorobenzylindolyl))]-prop-2-en-1-one (25t)



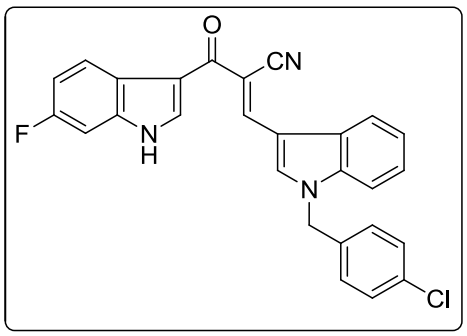
Yield 85%; Yellow solid; mp 215–217 °C; IR (KBr, *v,* cm^{-1}): 3163, 2191, 1605,1519, 1281, 741, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.64 (s, 1H), 8.68 (s, 1H), 8.38 (d, J = 3.2 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 7.89–7.85 (m, 3H), 7.45–7.42 (m, 1H), 7.35–7.29 (m, 3H), 7.23 (d, J = 8.0 Hz, 2H), 6.98 (d, J = 2.0 Hz, 1H), 6.91–6.86 (m, 1H), 5.57 (s, 2H), 3.86 (s, 3H); ESI (FAB$^+$) *m/z* calcd. for $C_{28}H_{20}ClN_3O_2$ $[M + H]^+$ 465.1, found: 465.1.

2-Cyano-1-[3′-(6′-methoxyindolyl)]-3-[3′-(1′-(4′′-methoxybenzylindolyl))]-prop-2-en-1-one (25u)



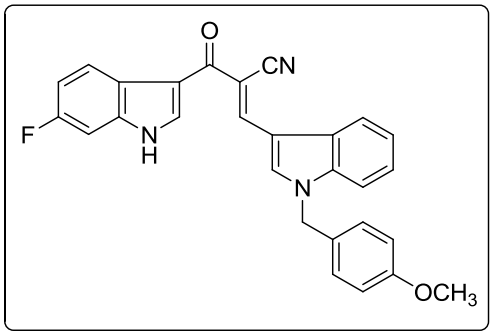
Yield 85%; Yellow solid; mp 217–219 °C; IR (KBr, *v,* cm^{-1}): 3163, 2191, 1605,1512, 1234, 741, 663; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.74 (s, 1H), 8.67 (s, 1H), 8.64 (s, 1H), 8.36 (d, J = 3.2 Hz, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.01 (s, 1H), 7.89–7.85 (m, 1H), 7.54–7.52 (m, 1H), 7.30–7.24 (m, 3H), 6.99 (d, J = 2.2 Hz, 1H), 6.89–6.85 (m, 3H), 5.56 (s, 2H), 3.84 (s, 3H), 3.76 (s, 3H); ESI (FAB$^+$) *m/z* calcd. for $C_{29}H_{24}N_3O_3$ $[M + H]^+$ 462.2, found: 462.2.

2-Cyano-1-[3′-(6′-fluoroindolyl)]-3-[3′-(1′-(4′′-chlorobenzylindolyl))]-prop-2-en-1-one (25v)



Yield 85%; Yellow solid; mp 224-226 °C; IR (KBr, *v,* cm^{-1}): 3213, 2191, 1606,1514, 1276, 740, 665; ^1H NMR (400 MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.70 (s, 1H), 8.68 (s, 1H), 8.54 (d, J = 3.2 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.90–7.86 (m, 2H), 7.49–7.43 (m, 2H), 7.34–7.22 (m, 3H), 7.23 (d, J = 8.0 Hz, 2H), 7.02 (t, J = 9.0 Hz, 1H), 5.56 (s, 2H); ESI (FAB^+) *m/z* calcd. for $C_{27}H_{18}ClFN_3O$ $[M + H]^+$ 454.1, found: 454.1.

2-Cyano-1-[3′-(6′-fluoroindolyl)]-3-[3′-(1′-(4′′-methoxybenzylindolyl))]-prop-2-en-1-one (25w)



Yield 82%; Pale yellow solid; mp 233-235 °C; IR (KBr, *v,* cm^{-1}): 3256, 2191, 1605,1497, 1281, 741, 663; ^1H NMR (400 MHz, DMSO-d_6): δ 12.03 (s, 1H), 8.68 (s, 1H), 8.60 (s, 1H), 8.53 (d, J = 3.2 Hz, 1H), 7.98 (dd, J = 9.9, 2.5 Hz, 1H), 7.91–7.84 (m, 1H), 7.56–7.51 (m, 1H), 7.49 (dd, J = 8.8, 4.5 Hz, 1H), 7.31–7.27 (m, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.03 (t, J = 8.4 Hz, 1H), 6.88 (d, J = 8.6 Hz, 2H), 5.55 (s, 2H), 3.73 (s, 3H); ESI (FAB^+) *m/z* calcd. for $C_{28}H_{21}FN_3O_2$ $[M + H]^+$ 450.2, found: 450.1.

Cytotoxicity 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) with100 U/mL penicillin and 100 μg streptomycin. PaCa2, a human pancreatic carcinoma cell line, was obtained from Health Science Research Resources Bank (HSRRB) (Osaka, Japan). PaCa2 were cultured in minimum essential medium with Earle's salts, L-Gln and non-essential aminoacids (NacaliTesque Inc., Kyoto, Japan) containing 10% FBS with 100 U/mL penicillin and 100 μg streptomycin. PC3, an androgen-independent human prostatic adenocarcinoma cell line, was obtained from HSRRB. PC3 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%). A-549 and PaCa-2 cells were plated 5000 cells per well, and PC3 cells were plated 1 x 10^4 cells well in 96-well plates, the day before chalcones treatment. All the compounds were dissolved in DMSO at room temperature. Aliquots of these stock solutions at 100 mM were stored at 20 °C. The cell viability was measured by the cell Counting Kit-8 (Dojin, Kumamoto, Japan) using a spectrophotometer (xMark; 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. Mitomycin-C (MMC) was used as a positive control.

Tubulin polymerization assay

The tubulin polymerization assay was done according to the manufacturer's instructions (Tubulin Polymerization HTS Assay Kit (BK004P), Cytoskeleton, Inc.). Briefly, α-cyano-bis(indolyl)chalcones or paclitaxel (final concentration of 5 μM) were added to tubulin heterodimer (final concentration of 4 mg/mL) in cold assay buffer containing 10% glycerol, 80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, and 1 mM GTP. Solutions were immediately placed in a spectrophotometer at 37 °C and absorbance at 340 nm was measured once every 1 min for 40 min.

4.3 Part B: Bis(indolyl)ketohydrazide-hydrazones as novel tubulin modulating anticancer agents

4.3.1 Rational design

Hydrazide-hydrazone (-CO-NH-N=CH-) is an important moiety known for its significant roles in many antitumor agents (Figure 4.3.1). For examples; in 2004, Cai and co-workers identified a series of indole-2-carboxylic acid benzylidene-hydrazides as a new class of potent apoptosis inducers through a novel cell-based caspase HTS assay. The most potent analogue **26** (EC_{50} = 1.4-2.2 μM) of this series, shows there activity through arresting cells (T47D cells) in G2/M phase and induce apoptosis.[78]

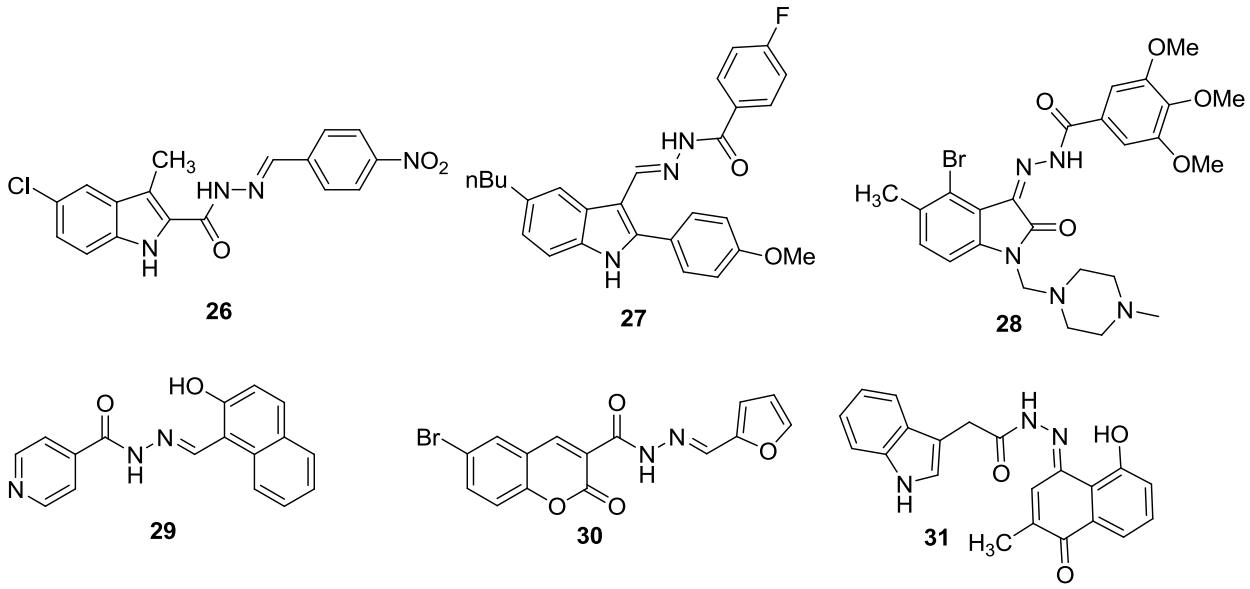


Figure 4.3.1 Some examples of hydrazide-hydrazone as anticancer agents (**26-31**)

Angerer group reported aroyl hydrazones of 2-phenylindole-3-carbaldehydes (**27**) as potent anticancer agents towards the breast (MDA-MB 231 and MCF-7) cancer cell lines (IC_{50}= 20–30 nM).[79] In 2009, Cai *et al.* discovered the substituted *N′*-(2-oxoindolin-3-ylidene)benzohydrazides as new apoptosis inducers using a cell- and caspase-based HTS assay which also found to function as inhibitors of tubulin polymerization. Among them, a methyl piperazine moiety bearing analogues **28** exhibited strong cytotoxicity against human colorectal carcinoma cells HCT116, hepatocellular carcinoma cancer SNU398 cells and human colon cancer RKO cells (EC_{50} values of 0.17, 0.088 and 0.14 μM, respectively).[80] In 2014, Kumar *et al.* synthesized a series of isonicotinoyl hydrazone derivatives **29** which exhibited potent cytotoxicity with IC_{50} values of 0.29 μM against HCT 116 cell line.[81] Same

year, Nasr group designed and synthesized coumarins-hydrazide hydrazone and screened them against human drug-resistant pancreatic carcinoma (Panc-1) cells and drug-sensitive (hepatic carcinoma; Hep-G2 and leukemia; CCRF) cell lines *in vitro*. Of these synthesized compounds, the brominated coumarin hydrazide-hydrazone derivative **30** exhibited potent activity than doxorubicin (DOX) against resistant Panc-1 cells (IC_{50} = 2.02 μM).[82] Padhye *et al.* identified novel plumbagin hydrazones (**31**) which showed promising cytotoxicity against breast cancer cell lines (IC_{50} = 1.5 μM; MCF-7 and MDA-MB-231).[83]

Microtubules, composed of *α*- and *β*-tubulin heterodimers, play an important role in various cellular functions including mitosis, shape maintenance and intracellular transport.[84-86] Due to the involvement of microtubules, especially in mitosis, the discovery of natural and synthetic compounds that could inhibit tubulin polymerization or interrupt microtubule depolymerization is one of the useful targets in anticancer drug discovery. Tubulin targeting substances are broadly categorized into microtubule stabilizing (taxanes, epothilones and discodermolide) and destabilizing (colchicine, vinca alkaloids and cryptophycins) agents.[87-89] Generally, anticancer agents that inhibit microtubule assembly are known as antimitotic agents.[90]

On the other hand, indole skeleton is frequently found in many natural as well as synthetic chemical entities of immense biological significance.[20,91-94] In the recent past, several indole containing small molecules have been identified as potent tubulin inhibitors **32-37**, for example, 2-aryl-3-formylindoles (**32**), 3-aroylindoles **33** and **34**, *N*-aroylindoles **35**, 2-heteroarylthioindoles **36**, and 2-indolyl-4-benzoylimidazoles (**37**) (Figure 4.3.2).[95-98]

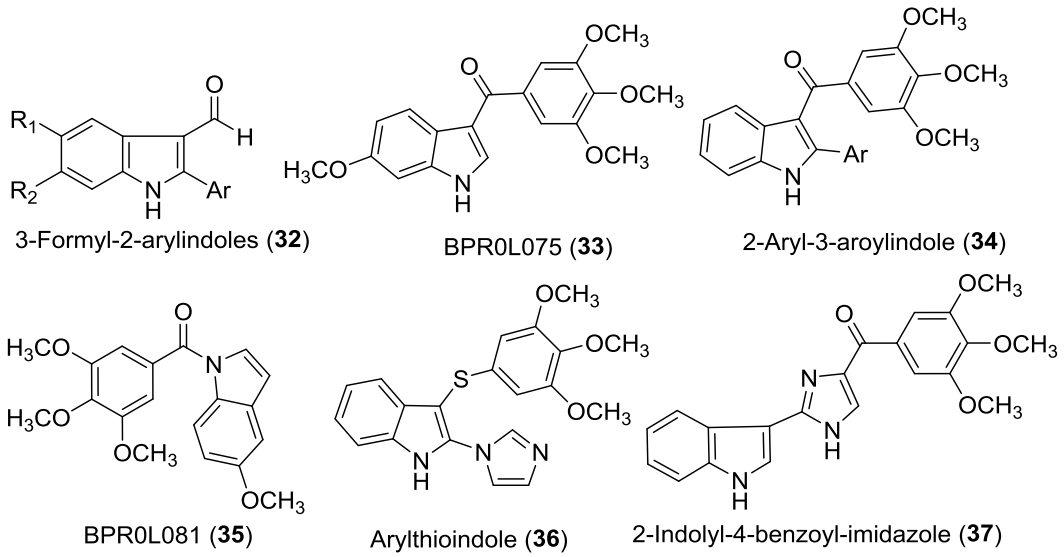


Figure 4.3.2 Indole-based small molecules as tubulin inhibitors (**32-37**)

The anticancer activity of these indole-based tubulin inhibitors is due to the blocking of tubulin polymerization.[95] Particularly, bisindole class of compounds isolated from marine sources including sponges, tunicates, and bryozoans are reported to possess a broad spectrum of interesting biological activities.[99] In bisindole compounds, two indole moieties are linked either through a five/six-membered heterocyclic ring or a linear spacer. For example, Topsentins (**2**) having an 2-acylimidazole unit, were isolated from the sponge *Topsentia genitrix* and found to inhibit the proliferation of tumor cells (IC_{50} = 4–40 μM).[100] Nortopsentins A–C (**3**) involve an imidazole moiety, were isolated from the marine sponge *Spongosorites ruetzleri* and found to exhibit cytotoxicity against P388 cells (IC_{50} = 4.5–20.7 μM).[6]

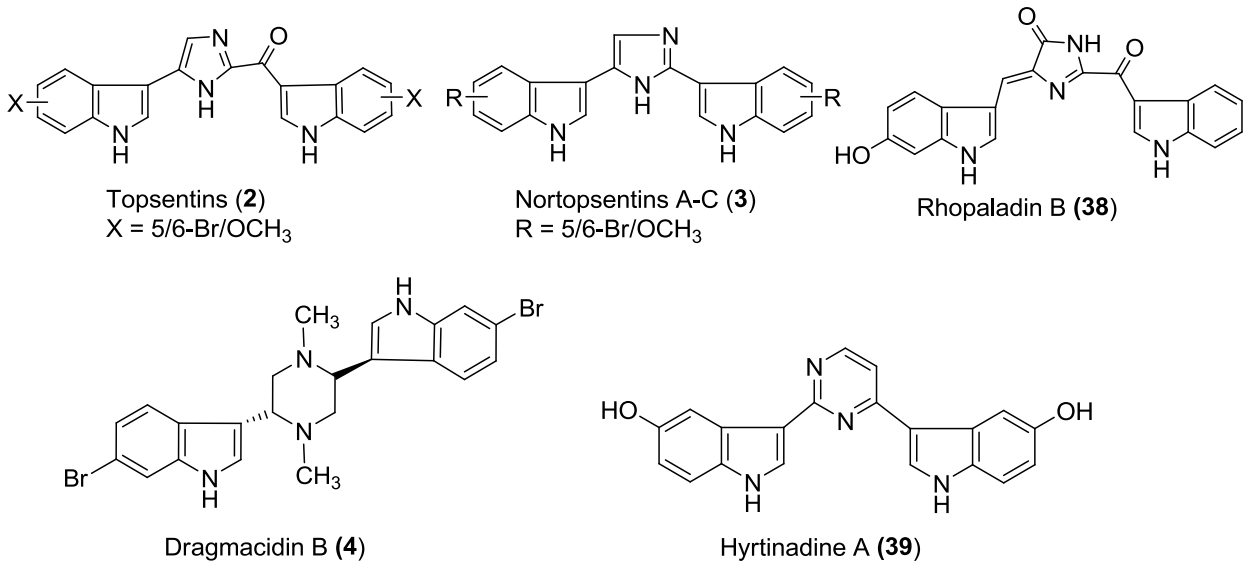


Figure 4.3.3 Structures of selected bisindole alkaloids

Rhopaladin B (**38**) linking two indole rings *via* an imidazolinone spacer, was described to show inhibitory activity against CDK 4 (IC_{50} = 12.5 μg/mL) and c-erbβ-2 (IC_{50} = 7.4 μg/mL) kinases.[101,102] Dragmacidin B (**4**) with a piperazine spacer, was found to display good anticancer activity (IC_{50} = 15 μg/mL, P388; 1-10 μg/mL, A-549, HCT-8 and MDA-MB).[103] Hyrtinadine A (**39**) with a pyrimidine moiety was isolated from the extracts of an Okinawan marine sponge *Hyrtios sp*. and showed *in vitro* cytotoxicity against murine leukemia L1210 (IC_{50}= 1 μg/mL) and human epidermoid carcinoma KB (IC_{50}= 3 μg/mL) cells (Figure 4.3.3).[104] Jiang *et al.* have synthesized various bisindoles with thiazole, pyrazinone, pyrazine and pyridine linkers as potential anticancer agents.[105-106] Similarly, Diana and co-worker identified diverse bis(indolyl) heterocycles as potential antitumor agents by linking two units

through thiophene, pyrazole, furan, isoxazole and pyrrole moieties.[107-110] Bisindole containing linear chain spacers such as 1,2-diketo, glyoxylamide, enamide, hydrazide-hydrazones, have also been reported to display interesting biological activities.[111-112]

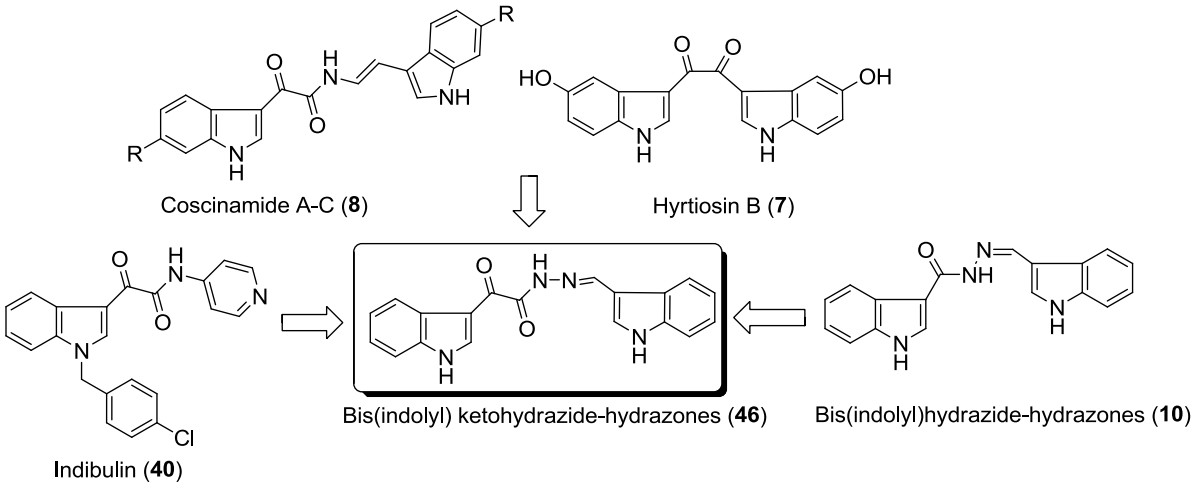


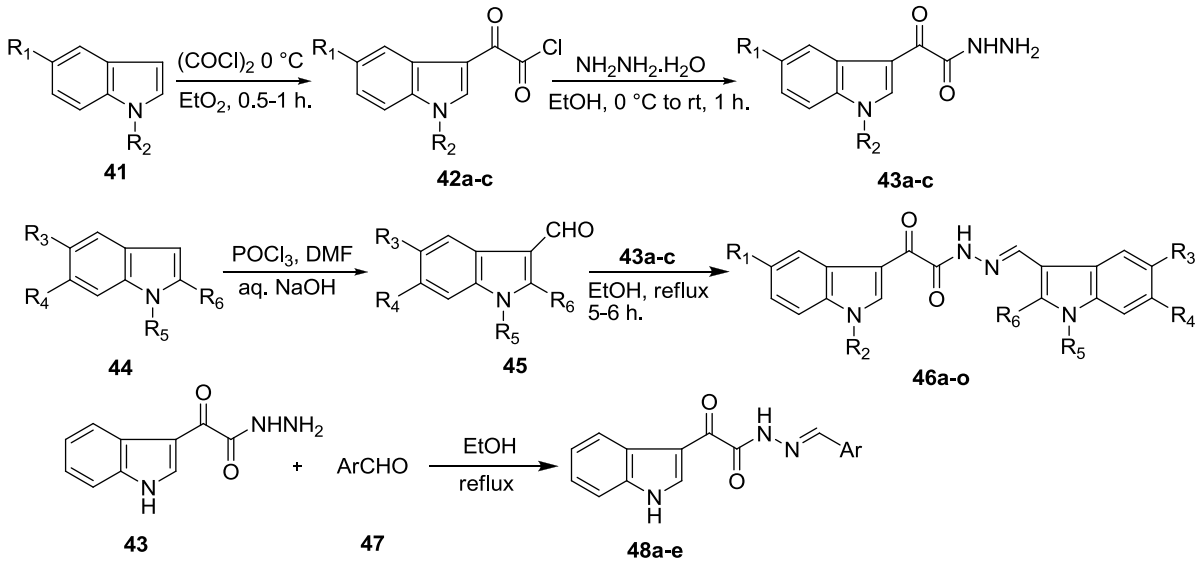
Figure 4.3.4 Rational design for ketohydrazide-hydrazones

In 2000, Bokesch *et al.* isolated Coscinamides A-C (**8**) with linear enamide spacers from an extract of marine sponge *Coscinoderma sp* and were reported to exhibit partial cytoprotection against HIV[113] and antitumor activity against human prostate cancer cell line (IC_{50} = 7.6 µg/mL).[114] Another indolic enamide, Igzamide was isolated from the extract of sponge *Plocamissa igzo* and exhibited modest cytotoxic activity against L1210 murine leukemia cell line.[115] Hyrtiosin B (**7**) bearing a 1,2-diketo moiety as a spacer, was isolated from marine sponge *Hyrtios erecta* and possessed *in vitro* maintain uniformity cytotoxicity against epidermoid carcinoma KB cell line.[8] Didemnidine B, an indole-3-glyoxylamide was isolated from the New Zealand ascidian *Didemnum sp* with moderate cytotoxicity towards non malignant L6 cell line.[116] Coscinamides and related glyoxylamide analogues have attracted significant attention of medicinal chemists due to their unique chemical structures and a wide range of biological properties.[112,117] Indibulin (D-24851) **40** with a glyoxylamide moiety showed good *in vitro* cytotoxicity towards human cancer cell lines including ovarian, glioblastoma, breast and pancreatic (IC_{50} = 0.036-0.285 µM).[10,118] Further, biological potential of these indole-based ketoamides are largely unexplored in new drug discovery. Recently, we have identified several synthetic indolylheterocycles and analogues of bisindole alkaloids **6**, **10** and **19** as novel and potent anticancer agents.[7,11,5] In continuation of our efforts, herein we report novel bis(indolyl)ketohydrazide-hydrazones by incorporating a ketohydrazide-hydrazone scaffold between the two indole rings while maintaining the crucial features of Coscinamide, Indibulin and bis(indolyl)hydrazide-hydrazones (Figure 4.3.4).

4.3.2 Results and discussion

4.3.2.1 Synthesis

Coscinamide analogues **46a-o** and **48a-e** were synthesized in high yields by reacting indolyl glyoxalylhydrazides **43** with appropriate aldehydes **45**. Indolyl glyoxalylhydrazides **43** were synthesized by the initial reaction of indole **41** with oxalyl chloride to produce indolyloxalyl chlorides **42a-c**. Subsequent reaction of **42a-c** with hydrazine hydrate led to keto hydrazides **43a-c** in good yields.



Scheme 4.3.1 Synthesis of ketohydrazide-hydrazones (**46a-o** and **48a-e**)

On the other hand, indole-3-carboxaldehydes **45** were achieved from the reaction of indoles **44** with phosphorus oxychloride in presence of dimethylformamide (DMF) followed by alkylation.[53, 73] In an effort to identify potent compounds, one of the indole rings in hydrazide-hydrazones **46a-o** was protected with *p*-chlorobenzyl moiety; such structural modification was found to be good in case of Indibulin.[119] Similarly, coscinamide analogues **48a-e** having diversely substituted aryl ring were prepared from the reaction of indolyl glyoxalyl-hydrazides **43** and arylaldehydes **47** in ethanol under refluxing conditions (Scheme 4.3.1).

The structures of bis(indolyl)ketohydrazide-hydrazones **46a-o** and **48a-e** were confirmed by detailed IR, NMR (^{1}H & ^{13}C) and HRMS spectral analysis. Formation of ketohydrazide-hydrazone **46b** was indicative by the presence of bands at 3440 (*N*-H), 3193 (*N*-H) and 1658 (C=N) cm^{-1} in its IR spectrum. ^{1}H NMR spectrum of **46b** (Figure 4.3.5) exhibited two sets of

nuclei for imine protons (*N*=CH) which is believed to be due to its existence as conformational isomers (rotation around *N*-C(O)) or geometrical isomers (*E/Z*, *N*=CH).[120] ^{13}C NMR spectrum of **46b** also showed two sets of signals for most of its carbon nuclei (Figure 4.3.6). The HRMS spectroscopic analysis of **46b** displayed a molecular ion $[M + Na]^+$ at 431.0114 in agreement with the expected mass for $C_{19}H_{13}BrN_4NaO_2$ $[M + Na]^+$, 431.0120 (Figure 4.3.7). Purity of all the newly synthesized keto-hydrazide hydrazones was > 98% as analyzed by using HPLC (Figure 4.3.8).

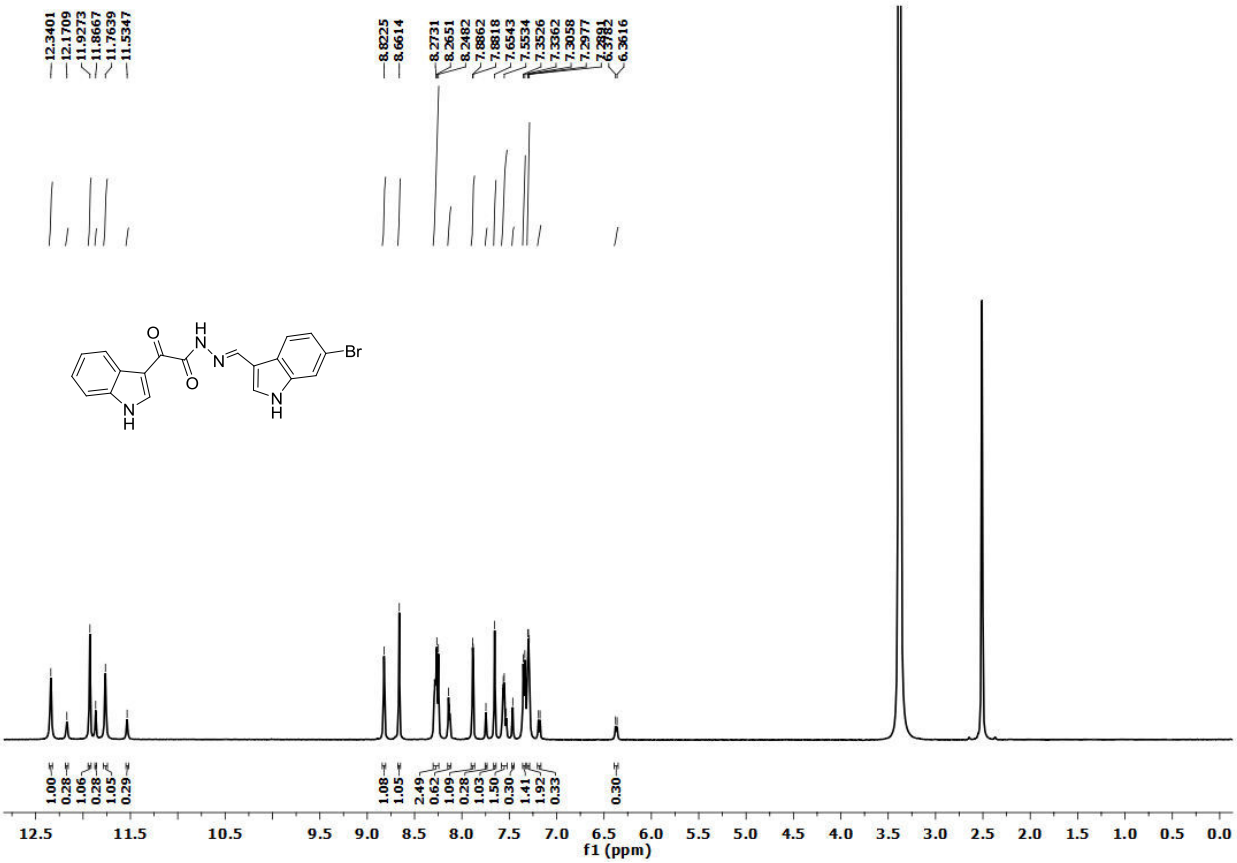


Figure 4.3.5 ^{1}H NMR spectrum of compound **46b**

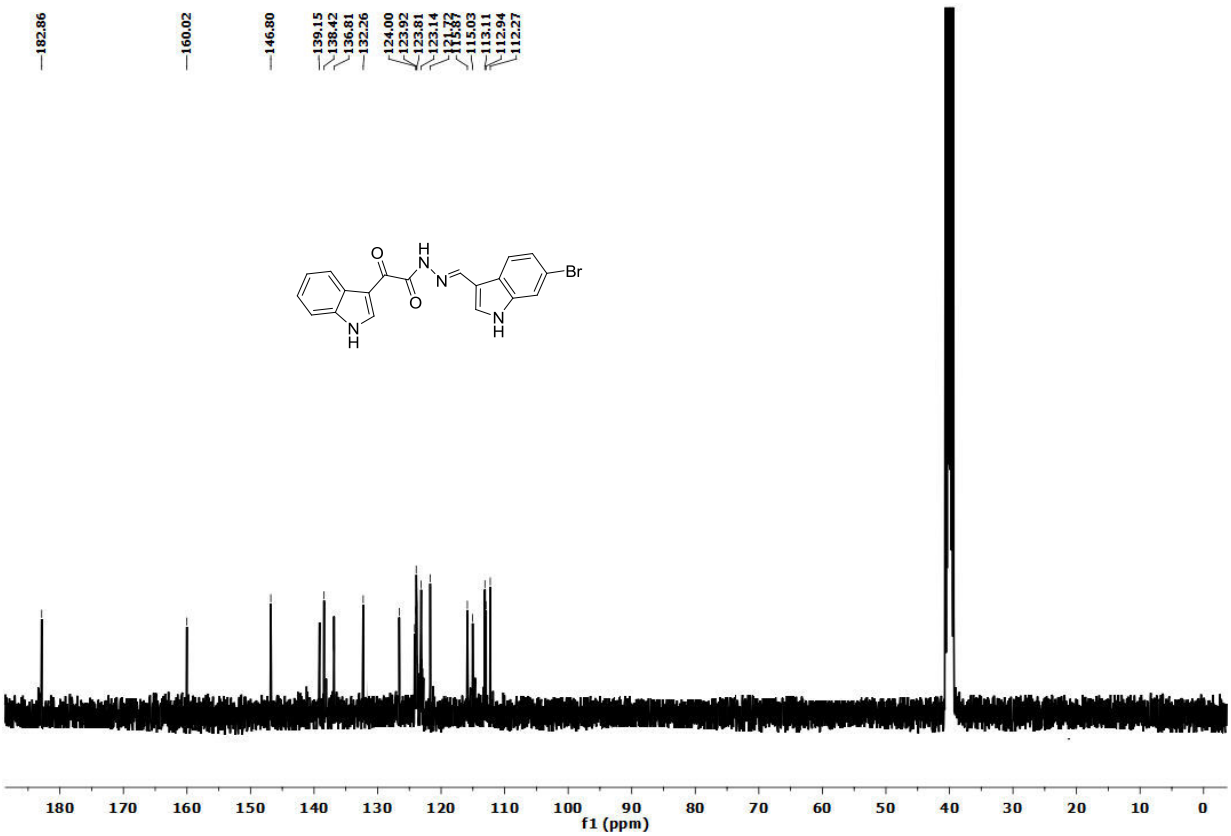


Figure 4.3.6 ^{13}C NMR spectrum of compound **46b**

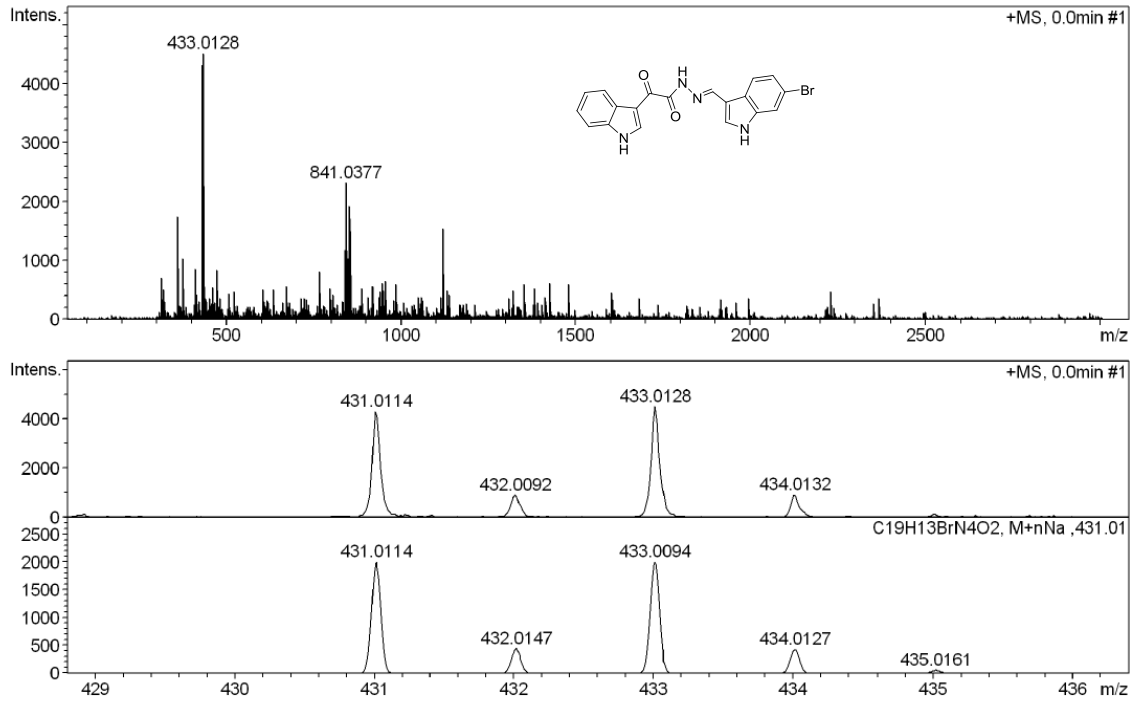
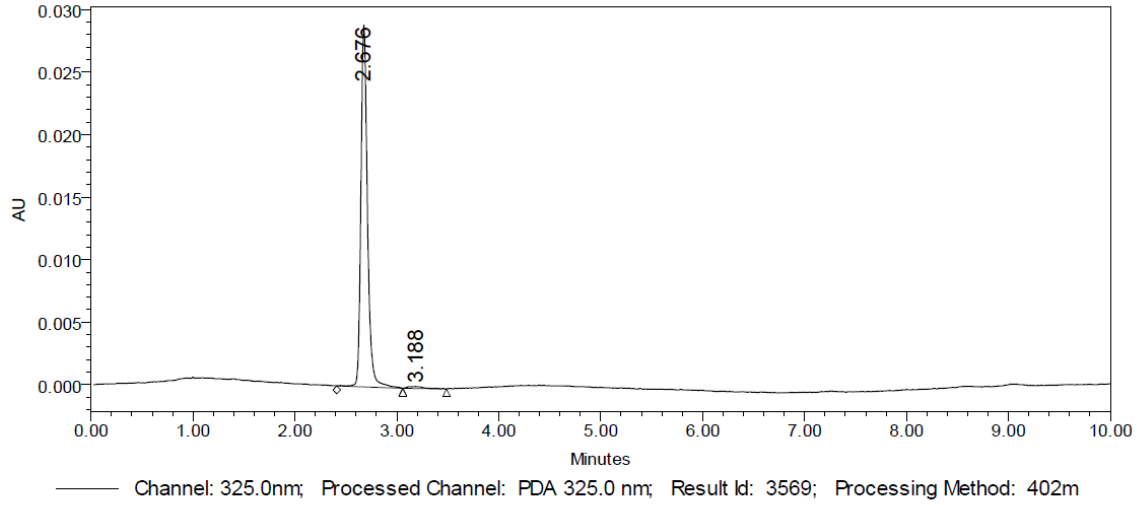


Figure 4.3.7 HRMS spectrum of compound **46b**



Processed Channel Descr.: PDA 325.0 nm

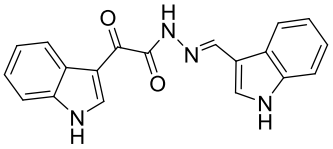
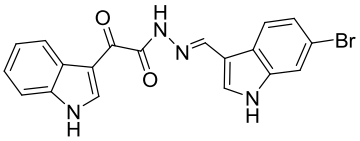
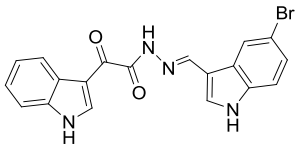
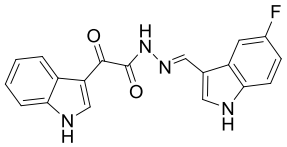
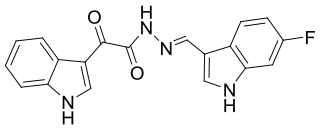
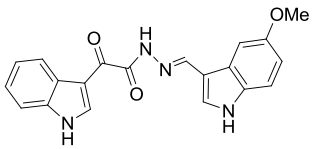
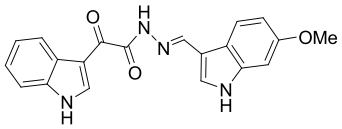
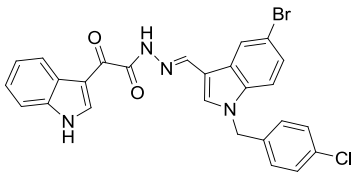
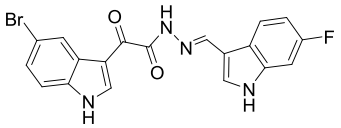
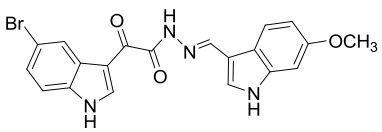
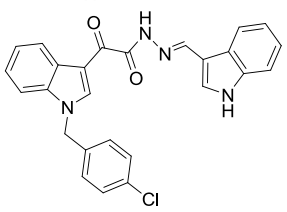
	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 325.0 nm	2.676	129835	98.65	28935
2	PDA 325.0 nm	3.188	1778	1.35	153

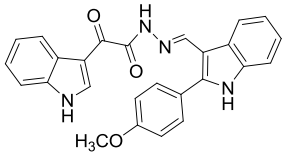
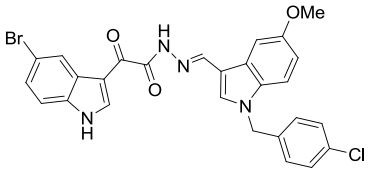
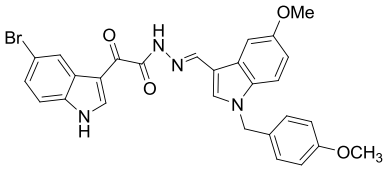
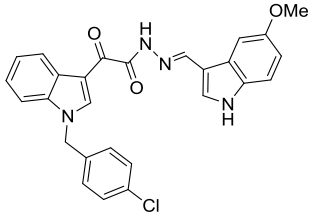
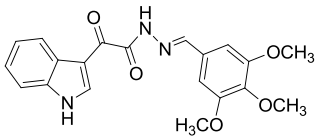
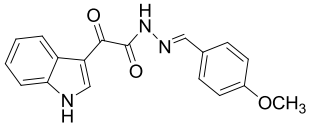
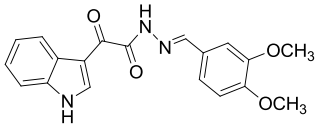
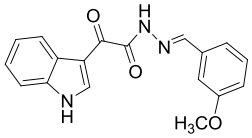
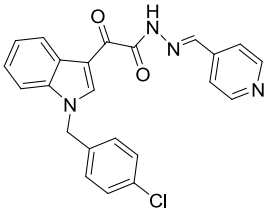
Figure 4.3.8 HPLC traces of compound **46b**

4.3.2.2 Anticancer activity

Synthesized ketohydrazide-hydrazones **46a-o** and **48a-e** were assessed *in vitro* for their cytotoxicity against a panel of cancer cell lines; human breast cell lines (BT474, MCF-7 and MDA-MB-231), human colon carcinoma cell lines (HCT-116), leukemia cell line (Jurkat). Firstly, we screened all the ketohydrazide-hydrazones at 10 µM concentration against a panel of five different cancer cell lines (Table 4.3.1). Compounds **46a**, **46c**, **46e-g**, **46k** and **46o** showed cytotoxic effects less than 50% cell survival against the tested cancer cell lines. Replacement of one indole ring in **46** with an aryl or pyridyl group resulted in compounds **48** with percentage cell survival more than 90% except for Jurkat cancer cell line. These results suggested that both the indole rings are necessary for the activity of ketohydrazide-hydrazones. Compounds **46a**, **46c**, **46e-g**, **46k** and **46o** with less than 50% cell survival were further investigated for their cytotoxicity (Table 4.3.2).

Table 4.3.1 *In vitro* screening of bis(indolyl)ketohydrazide-hydrazones and indolyl ketohydrazide-hydrazones

Bis(indolyl)ketohydrazide-hydrazones		Percentage cell survival (@ 10 µM)				
		BT-474	**MCF-7**	**MDA-MB-231**	**HCT-116**	**Jurkat**
	**(46a)**	83 ± 8.5	99.2 ± 4.7	98.6 ± 0.35	88.4 ± 2.7	33.6 ± 7.7
	**(46b)**	86.8 ± 0.6	92.7 ± 8.1	95.1 ± 5.4	94.5 ± 5.5	75.4 + 8.3
	**(46c)**	82.4 ± 7.7	35.0 ± 5.0	34 ± 5.2	26.3 ± 5.1	25.5 ± 3.2
	**(46d)**	112 ± 5.5	105 ± 9.0	102 ± 8.7	85.1 ± 5.9	56.7 ± 4.7
	**(46e)**	88.2 ± 0.5	40.2 ± 2.1	105.5 ± 8.2	103.9 ± 4.4	69.9 ± 3.9
	**(46f)**	20.3 ± 2.1	17.2 ± 3.1	21.6 ± 3.5	17.6 ± 2.5	19.3 ± 1.5
	**(46g)**	95.6 ± 2.9	34.6 ± 5.1	103.4 ± 9.6	38.8 ± 4.3	52.1 ± 8.5
	**(46h)**	94.7 ± 5.2	107.2 ± 5.1	88.8 ± 2.3	72.6 ± 2.5	93.2 ± 1.3
	**(46i)**	94.8 ± 1.6	97.5 ± 5.3	111.1 ± 4.7	84.2 ± 5.4	95.8 ± 2.9
	**(46j)**	92.0 ± 3.1	91.4 ± 9.9	111.5 ± 5.5	71.4 ± 6.1	74.1 ± 2.2
	**(46k)**	22.5 ± 2.5	15.0 ± 4.6	17.1 ± 4.4	13.3 ± 1.5	20.6 ± 2.8

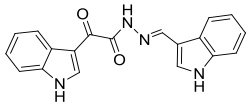
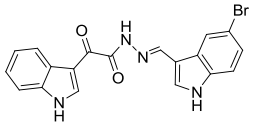
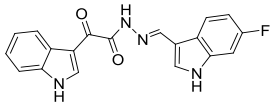
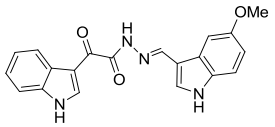
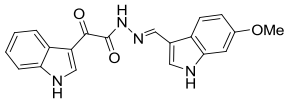
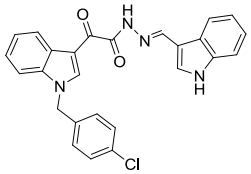
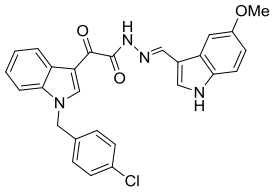
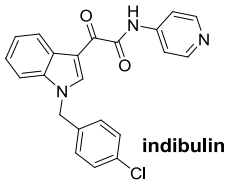
	**(46l)**	94.2 ± 7.1	107.1 ± 2.5	98.8 ± 10.1	71.8 ± 8.1	81.6 ± 1.4
	**(46m)**	99.1 ±	96.1 ±	103.2 ±	86.1 ± 7.2	46.6 ± 3.2
	**(46n)**	91.1 ± 9.4	112.2 ± 9.9	102.2 ± 4.0	65.7 ± 7.4	81.9 ± 1.3
	**(46o)**	21.2 ± 2.3	33.7 ± 1.8	29.4 ± 2.2	38.2 ± 1.2	36.1 ± 1.6
	**(48a)**	101.1 ± 4.1	110.2 ± 1.5	102.8 ± 6.1	99.1 ± 2.9	69.6 ± 3.5
	**(48b)**	97.5 ± 1.7	106.2 ± 5.4	102.4 ± 3.2	101.3 ± 6.3	61.9 ± 1.5
	**(48c)**	103.4 ± 7.7	99.8 ± 4.9	94.6 ± 9.7	92.8 ± 2.3	78.9 ± 1.1
	**(48d)**	95.1 ± 4.6	96.8 ± 2.2	97.2 ± 2.5	72.4 ± 5.5	59.7 ± 6.4
	**(48e)**	78.7 ± 6.5	82.2 ± 5.8	71.2 ± 4.3	69.1 ± 5.5	66.7 ± 5.7
doxorubicin		**17.6 ± 1.1**	**27 ± 1.4**	**22.5 ± 3.5**	**25 ± 4.2**	**21 ± 1.5**

The activity data represents mean values ± SD of experiments conducted in triplicates at three independent times. N.D = not determined.

Without any substituent on indole ring, compound **46a** was moderately active against Jurkat cancer cell line with an IC_{50} value of 3.0 µM. Further modification in compound **46a** by the introduction of a 5-bromo substituent in the indole ring led to **46c** with moderately active

against the tested cancer cells. Compound **46e** having 6-fluoroindole moiety, exhibited moderate cytotoxicity against MCF-7 cells with an IC_{50} value of 2.3 µM. Interestingly, replacement of bromine in **46c** with methoxy group resulted analogue **46f** which showed enhanced cytotoxicity against tested cancer cell lines with 16 and 10-fold selective cytotoxicity towards HCT-116 (IC_{50} = 0.4 µM) and Jurkat (IC_{50} = 0.5 µM) cell lines (**46c** *vs* **46f**).

Table 4.3.2 *In vitro* cytotoxic activity of the selected bis(indolyl)ketohydrazide-hydrazones

Bis(indolyl)ketohydrazide-hydrazones		Cancer cell lines (IC_{50}, µM)				
		BT-474	**MCF-7**	**MDA-MB-231**	**HCT-116**	**Jurkat**
	**(46a)**	N.D.	N.D.	N.D.	N.D.	3.0
	**(46c)**	N.D.	3.3 ± 1.2	5.6 ± 0.9	6.6 ± 1.1	5.0 ± 0.9
	**(46e)**	N.D.	2.3	N.D.	N.D.	N.D.
	**(46f)**	1.1 ± 0.3	1.8 ± 0.4	1.7 ± 0.3	**0.4 ±0.1**	**0.5 ± 0.2**
	**(46g)**	N.D.	6.1 ± 1.2	N.D.	2.5 ± 0.4	10.0 ± 1.2
	**(46k)**	2.1 ± 0.3	**0.8 ± 0.11**	**0.5 ± 0.1**	**0.15 ± 0.03**	**0.22 ± 0.04**
	**(46o)**	2.7 ± 0.5	3.8 ± 0.7	2.9 ± 0.35	4.2 ± 0.9	3.6 ± 0.7
 **indibulin**		-	1.1[111]	**0.074**[119]	-	-
doxorubicin		0.3 ± 0.12	0.39 ± 0.16	0.55 ± 0.22	0.5 ± 0.11	0.26 ± 0.12

Replacement of fluorine with a methoxy group (compound **46g**) was found to be unfavourable for the activity as compound **46g** showed ~ 2.5-fold decrease in activity against MCF-7 cells (IC_{50} = 6.1 μM). Protection of indole nitrogen with *p*-chlorobenzyl moiety led to the compound **46k** with significantly enhanced cytotoxicity against the tested cell lines (IC_{50} = 0.15-2.1 μM). Compound **46k** was found to be comparable or superior than lead anticancer agent, indibulin and standard drug doxorubicin. Furthermore, to improve the activity of ketohydrazide-hydrazones, we combined these two key substituents of potent compounds **46f** and **46k** in a single molecule and prepared derivative **46o.** However, this structural medication did not improve the cytotoxicity (IC_{50} = 2.7-4.2 μM) of **46**. SAR studies revealed that C-5 methoxy, bromo and *N*-(4-chlorobenzyl) groups in indole ring are beneficial for the activity. Both the indole rings are necessary for the potency of coscinamide analogues (Figure 4.3.9). Compound **46f** with 5-methoxyindole is more potent than the corresponding 6-methoxy analogue **46g**. The most potent compound **46k** was found to be 10-fold selectively cytotoxic towards tumor cells when compared to non-tumor cells. Also, it was observed that the identified bis(indolyl)ketohydrazide-hydrazones **46a-o** are superior than the reported bisindoles having a five/six membered heterocyclic system or a linear spacer as a linker between two indole rings.[18-20,28,29]

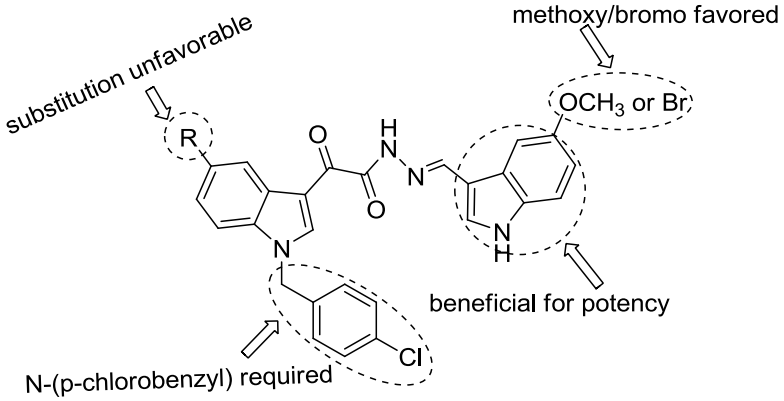


Figure 4.3.9 Structure-activity relationship for bis(indolyl)ketohydrazide-hydrazones

4.3.2.3 Apoptosis induction

Apoptosis is a programmed cell death which plays a key role in the maintenance of tissue homeostasis and cell survival. Thus, inducing apoptosis in cancer cells has emerged as an attractive strategy in cancer therapy.[121] In an attempt to find out the apoptosis induction, Jurkat cells were treated with **46a**, **46c**, **46f**, **46g** and **46k** for 24 h. Apoptosis induction was observed by DNA fragmentation analysis and caspases 3/7 activation. As shown in Figure 4.3.10, all the tested compounds induced DNA fragmentation indicative of apoptosis.

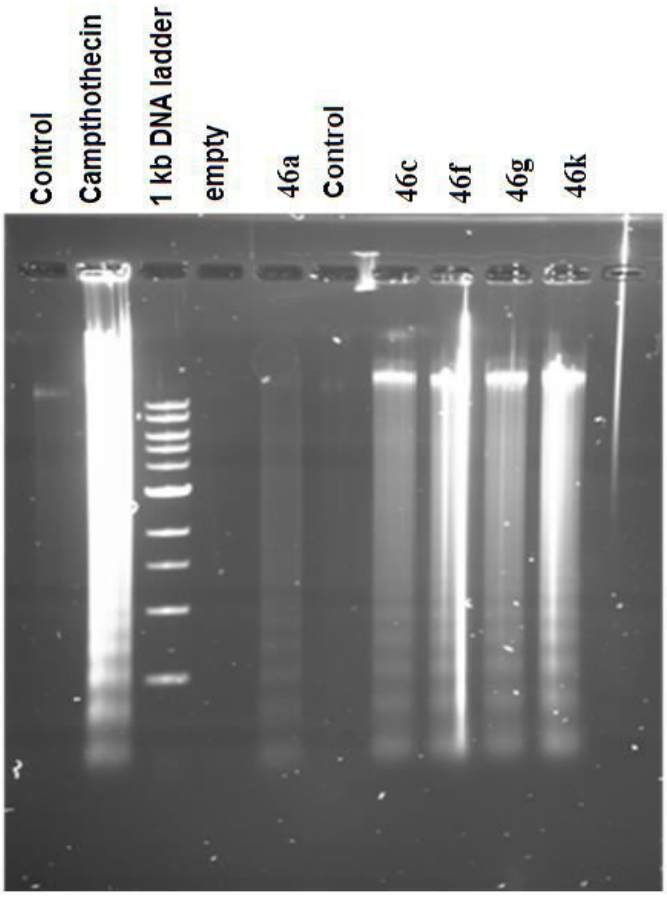


Figure 4.3.10 DNA Fragmentation-induced by **46a**, **46c**, **46f**, **46g** and **46k** in Jurkat cells

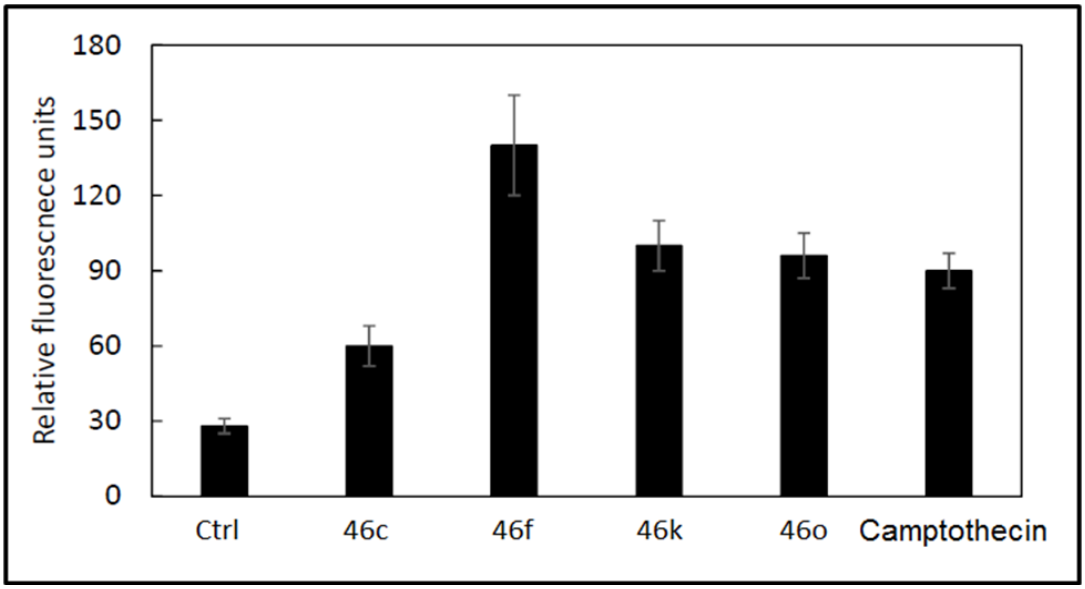


Figure 4.3.11 Caspase activation-induced by **46c**, **46f**, **46k** and **46o** in Jurkat cells

Caspase-3, one of the main executioner caspases, is activated in the apoptotic cells in both extrinsic and intrinsic pathways. The activation of caspase-3 is useful in the discovery of many potential anticancer agents. In order to find out whether the induction of apoptosis by

compounds **46c**, **46f**, **46k** and **46o** are dependent on caspase-3 activity or not, we measured the activation of caspase-3 in Jurkat cells. Compounds **46c**, **46f**, **46k** and **46o** showed 2-5-folds enhancement in caspase level compared to the control, which is indicative of caspases 3/7 activation (Figure 4.3.11). This enhanced fluorescence suggests that these compounds could kill the cancer cells *via* induction of apoptosis.

4.3.2.4 Microtubule instability:

To investigate whether the antiproliferative activities of potent compounds **46k** and **46o** are related to the interaction with tubulin or not, both the compounds were evaluated for tubulin inhibition activity in a cell-free *in vitro* assay. Addition of **46k** and **46o** caused a decrease in tubulin polymerization in a dose dependent manner as shown in Figure 4.3.12. Colchicine, compounds **46k** and **46o** inhibited microtubule formation upto 50% at 0.3 µM, 0.6 µM and 3.2 µM, respectively. These results indicated that analogues **46k** and **46o** inhibit microtubule formation, a critical part of cell division.

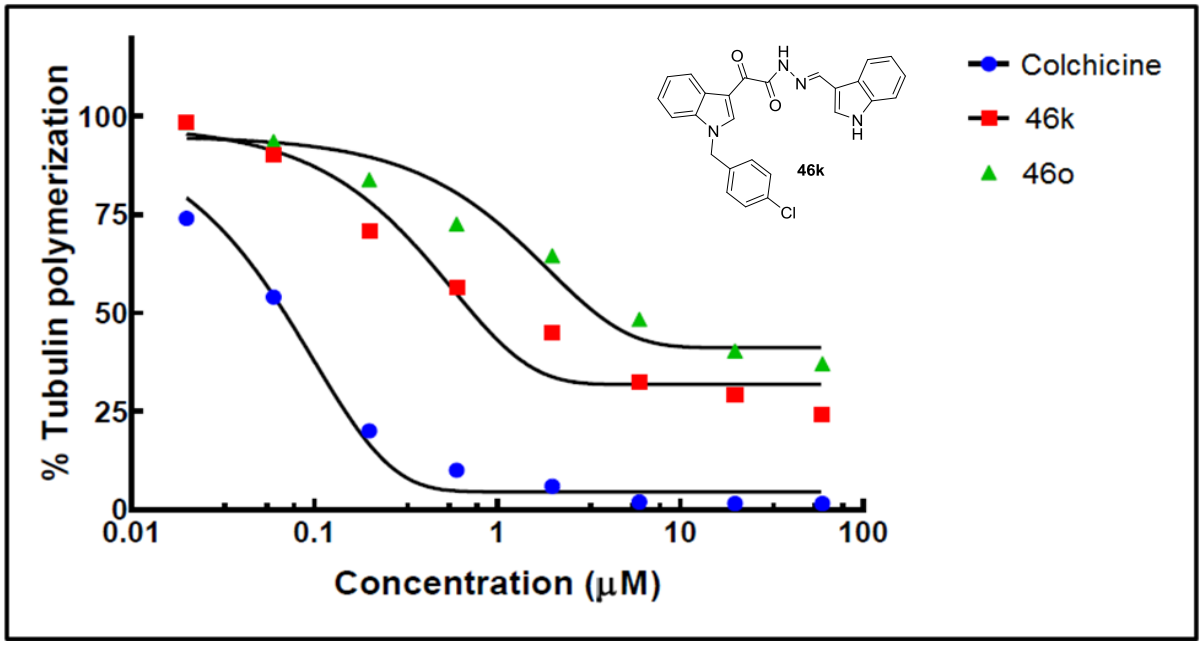


Figure 4.3.12 Dose-dependent inhibition of tubulin polymerization by **46k** and **46o**

4.3.2.5 Cell cycle progression analysis

Antimitotic drugs induced cell cycle arrest at G2/M phase in various cancer cell lines and led to an increment of the relative peak in the DNA histogram. To gain further insight into the mode of action, the effect of compound **46k** on the cell cycle was measured by flow cytometry against MCF-7 human cancer cells. To test this, effect of **46k** on cell cycle

progression of MCF-7 cells was analyzed using flow cytometry (Figure 4.3.13). Nearly 45% ± 5.6 of the cells were arrested at G2/M phase of the cell cycle upon treatment with **46k** (3.0 µM) for 24 h., whereas, only 15 ± 1.5% untreated cells were arrested in G2/M phase. Cells treated with colchicine (3.0 µM) were used as a positive control in the experiment.

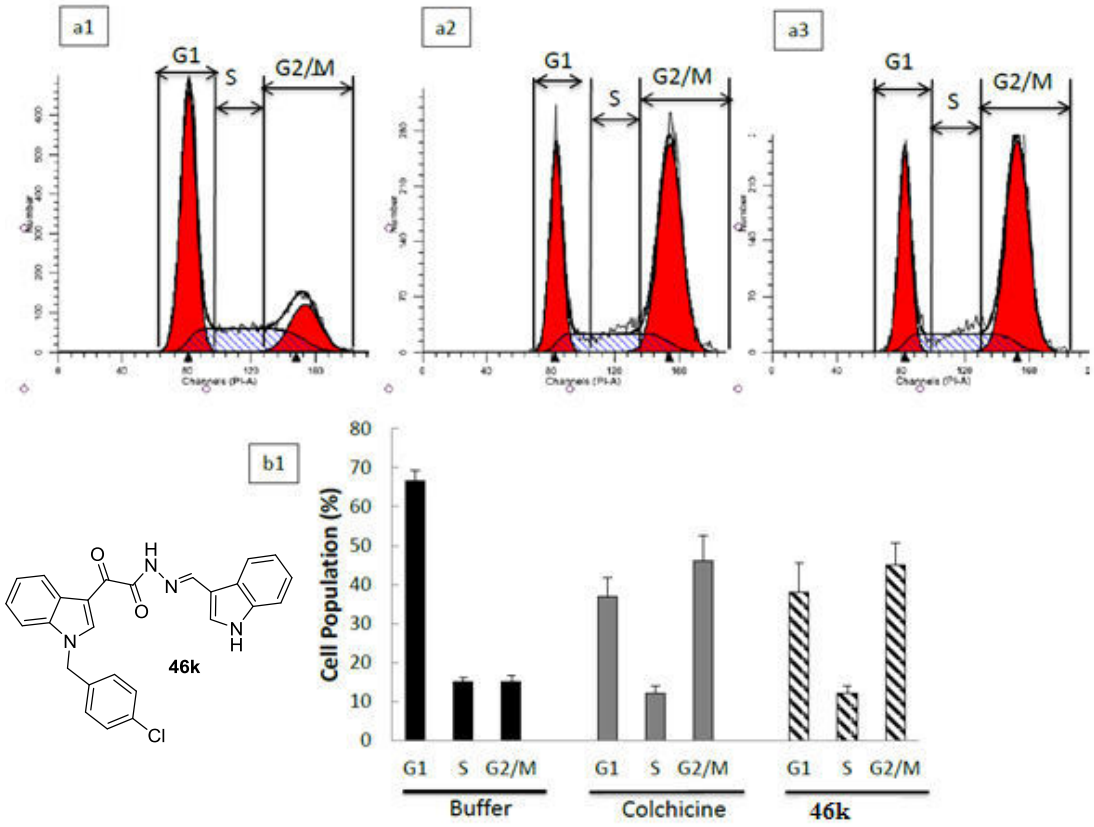


Figure 4.3.13 Cell cycle progression analysis in **46k** treated MCF-7cells: (**a1**) cells treated with vehicle, (**a2**) cells treated with colchicine (3.0 µM) and **(a3)** cells treated with **46k** (3.0 µM) for 24 h. After incubation, cells were processed with RNase A and stained with PI for detection through flow cytometer. Data are representative of two experiments. **(b1)** A graph representing % of cells in G1, S and G2/M phase

4.3.2.6 Toxicity study

The lactate dehydrogenase (LDH) assay is a commonly-used tool for assessing toxicity *in vitro*.[122] The toxicity of potent compounds **46k** and **46o** was evaluated using LDH assay as illustrated in Figure 4.3.14. The LDH activity result shows that the tested compounds **46k** and **46o** are very less (20-25%) toxic as compared to LDH (+ve control; 140%) and there toxicity is similar to untreated cells.

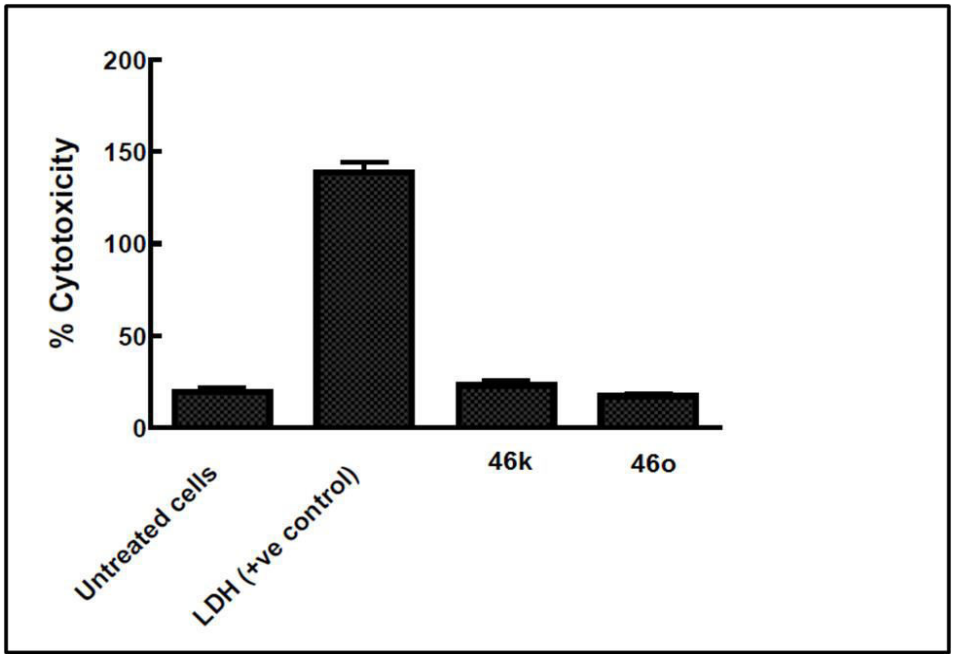


Figure 4.3.14 Toxicity induced by **46k** and **46o** in terms of LDH release

4.3.2.7 Molecular modeling and docking studies on 46k

Tubulin polymerization assay suggests that bis(indolyl)ketohydrazide-hydrazones **46** exhibits cytotoxicity through the inhibition of tubulin polymerization. To better understand how ketohydrazide-hydrazones interact with tubulin, we investigated *in silico* binding modes of the most potent compound **46k** of the series at colchicine binding site in tubulin dimer using Schrodinger 2011 molecular modeling suite (Schrodinger, Inc., New York, NY). Docking studies were performed on reported high-resolution crystal structure of tubulin-DAMA-colchicine (CN-2) complex (PDB ID: 1SA0)[123] using Glide module of Schrodinger. Compound **46k** demonstrated good binding in this model with a glide score of -6.54. The close view of potential binding pos is illustrated in Figure 4.3.15A. Interactions of **46k** (green tube model) are strongly stabilized by two hydrogen bonds: first one between C=O (linked to indole) and CYS241; the second one between *N*-H of glyoxalic amide and LYS352. Similar hydrogen bonding interactions were also observed between the oxygen of one methoxy group in colchicine and -SH of *β*-CYS241. Apart from hydrogen bonding interactions, compound **46k** also strongly stabilized by hydrophobic contacts with ALA354, LEU248, ALA316, MET259 and LEU255 (Figure 4.3.15B).

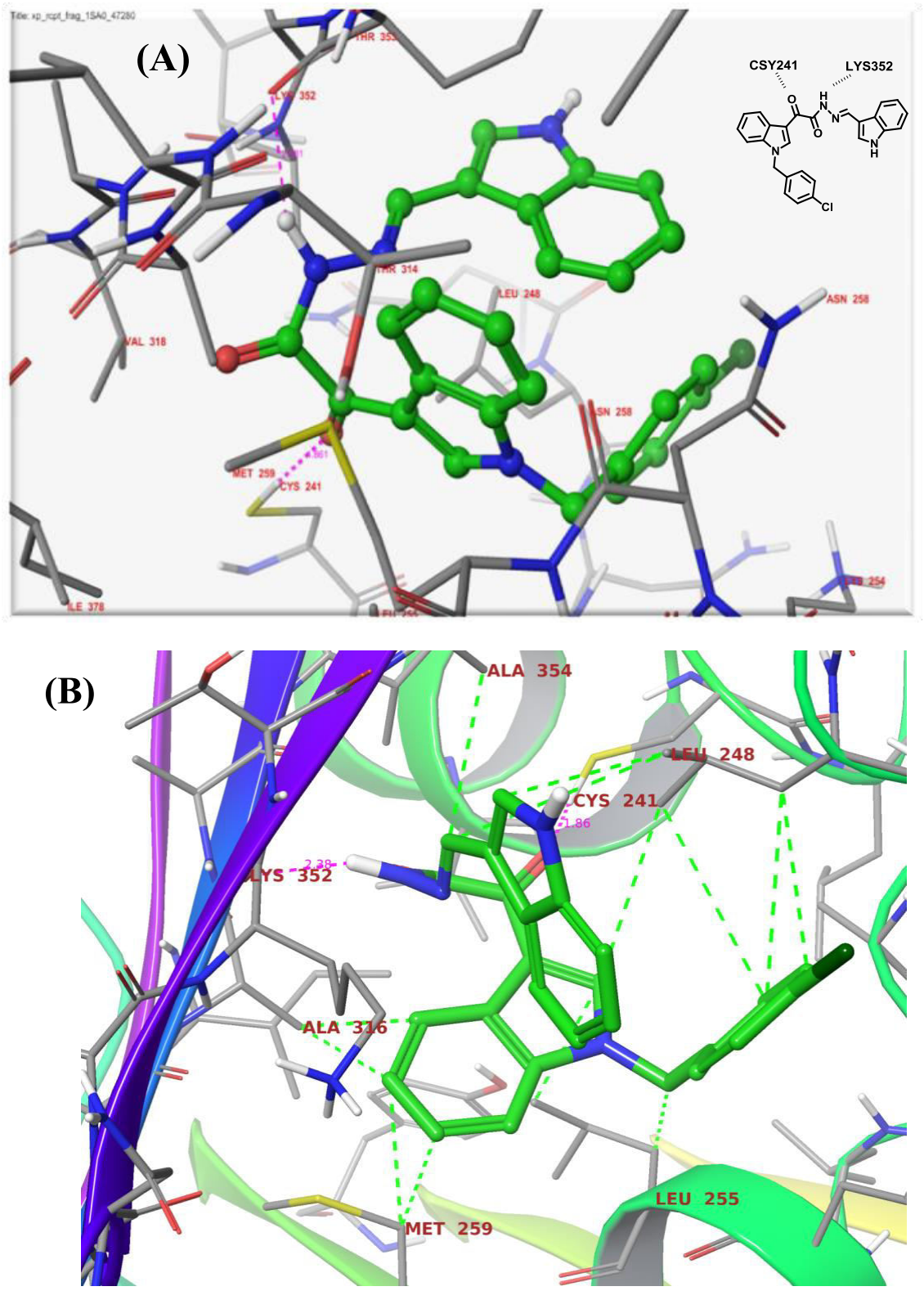


Figure 4.3.15 (**A**) Binding modes of **46k** in the colchicine binding site of tubulin showed hydrogen bonding interactions (pink dotted lines) and (**B**) hydrophobic interactions (green dotted lines)

4.3.3 Conclusions

A new series of bis(indolyl)ketohydrazide-hydrazones was synthesized from the reaction of indolyl glyoxalylhydrazides **43** with appropriate aldehydes and evaluated their *in vitro* anticancer activity. Compound **46k** exhibited the most potent anticancer activity against MCF-7, MDA-MB-231, HCT-116 and Jurkat cancer cell lines with IC_{50} values of 0.8, 0.5, 0.15, and 0.22 μM, respectively. The most potent compound **46k** was selectively cytotoxic to cancerous cells as compared to non-cancerous cells by approximate 10-folds. SAR studies revealed that both side indole rings are critical for the excellent *in vitro* anticancer activity of ketohydrazide-hydrazones. Mechanism of the action studies suggested that the bis(indolyl)ketohydrazide-hydrazones induced DNA fragmentation and caspase 3/7 activation, and exerted anticancer activity through the inhibition of tubulin polymerization (IC_{50} = 0.6 μM). Moreover, molecular docking studies corroborated a potential binding mode for compound **46K** in the colchicine binding site of tubulin.

4.3.4 Experimental section

General remarks

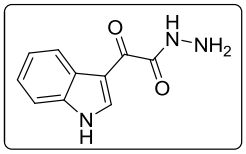
The laboratory grade reagents were obtained commercially either from Spectrochem or Aldrich and used without further purification otherwise noted. Organic solvents were distilled off using Buchi rotary evaporator. The reactions were monitored by thin layer chromatography (TLC), which was performed on commercially available Merck pre-coated plates (silica gel 60 F_{254}, 0.25 mm). Melting points (mps) were determined on *E-Z* melting apparatus. NMR (^{1}H &^{13}C) spectra were recorded on a Bruker advance II (400/500 & 100/125 MHz, respectively) spectrometer using DMSO-d_6 as a solvent and the chemical shifts are expressed in δ units (ppm) from tetramethylsilane. HRMS spectra were obtained on a Bruker microTOF-Q II 10348 (ESI) spectrometer. Purity of all the synthesized compounds was > 98% as determined by WATERS 515 HPLC system with a Sunfire C-18 column (5 μm, 4.6 × 250 mm) and PDA detector using a flow rate of 1 mL/min. and a gradient of acetonitrile.

General procedure for synthesis of indolyl oxoacetyl chlorides (**42**):[111,124] To a solution of indole **41** (8.5 mmol) in diethyl ether (10 mL) was added oxalyl chloride (1.3 g, 10.25 mmol) while maintaining the temperature at 0 °C. The resulting yellowish mixture was allowed to

stir at same temperature for 30 min. After completion of the reaction, suspension was filtered, washed with cold diethyl ether to afford **42** as yellow solids in 81-90% yields.

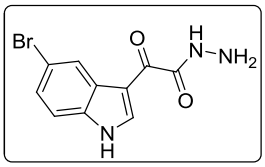
General procedure for synthesis of indolyl oxoacetohydrazides (**43**): To a solution of glyoxalyl chloride **42** (2.40 mmol) in dry methanol (5 mL) was added hydrazine hydrate (0.36 g, 7.20 mmol) at 0 °C and the contents were allowed to stir at same temperature for 30 min. Upon completion of the reaction, suspension obtained was filtered and washed with methanol (10 mL) to afford a solid product which was recrystallized from methanol to obtain pure glyoxalyl-hydrazides **43** in 80-85% yields as light yellow solids.

2-(1*H*-Indol-3-yl)-2-oxoacetohydrazide (**43a**)



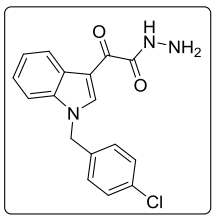
Yield 85%; Light yellow solid; mp 220–222 °C; IR (KBr, *v,* cm^{-1}): 3256, 3109, 1680, 1660, 1607, 1583, 1523, 1433, 1240, 736; (ESI-MS) $[M + H]^+$ *m/z* 204.2.

2-(5-Bromo-1*H*-indol-3-yl)-2-oxoacetohydrazide (**43b**)



Yield 83%; Light yellow solid; mp 249–251 °C; IR (KBr, *v,* cm^{-1}): 3340, 3290, 3050, 2920, 1686, 1646, 1605, 1545, 724. (ESI-MS) $[M + H]^+$ *m/z* 282.2 and 284.1 $[M + H + 2]^+$.

2-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-2-oxoaceto-hydrazide (**43c**)



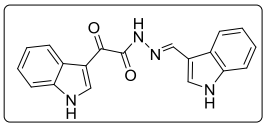
Yield 80%; Light yellow solid; mp 155–157 °C; IR (KBr, *v,* cm^{-1}): 3310, 3163, 2931, 1682, 1620, 1542, 1442, 1218, 1141, 778, 724; (ESI-MS) $[M + H]^+$ *m/z* 328.0.

General procedure for the synthesis of indole-3-carboxaldehydes (**45**):[7, 11] A round-bottomed flask containing (28 mL, 370 mmol) freshly distilled dimethylformamide (DMF) was cooled to 0 °C for about 30 min and freshly distilled phosphorus oxychloride (8.41 mL, 90 mmol) was subsequently added with stirring to the DMF over a period of 30 min. A solution of indole **44** (85.47 mmol) in DMF (10 mL, 130 mmol) was added to the yellow solution over a period of 1 h. The solution was stirred at room temperature till it become a yellow paste. At the end of the reaction, 30 g of crushed ice was added to the paste with stirring to make a clear cherry-red aqueous solution. Sodium hydroxide solution (1N, 100 mL) was added dropwise with stirring to this cherry-red solution. The resulting suspension was heated rapidly to 90 °C and allowed to cool at room temperature, after which it was

refrigerated for overnight. The product was filtered, washed with water (2 × 100 mL) and air-dried to afford pure indole-3-carboxaldehydes **45** in 80-90% yields.

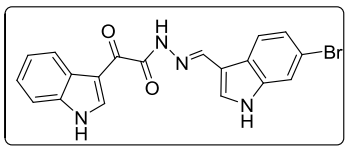
General procedure for the synthesis of bis(indolyl)ketohydrazide-hydrazones (**46a-o**) **and indolyl ketohydrazide-hydrazones** (**48a-e**): A mixture of glyoxalylhydrazide **43** (1.47 mmol) and aldehyde **45** or **47** (1.47 mmol) in ethanol (5 mL) was refluxed for 6 h. After completion of the reaction, ethanol was distilled off and the residue was taken into water (10 mL) and allowed to stir at room temperature for 30 min. The obtained suspension was filtered, washed with water and dried to obtain yellow solid ketohydrazide-hydrazones **46a-n** and **48a-d** in 88-96% yields.

***N'*-((1*H*-Indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide** (**46a**)



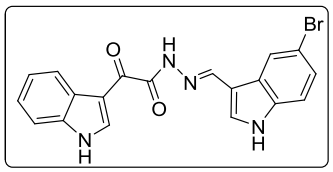
Yield 90%; Yellow solid; mp 248–250 °C; IR (KBr, *v,* cm^{-1}): 3440, 3193, 1658, 1603, 1496, 1434, 1234, 1157, 1126, 740; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.34 (s, 1H), 11.87 (s, 1H), 11.66 (s, 1H), 8.83 (s, 1H), 8.68 (s, 1H), 8.29–8.33 (m, 2H), 7.85 (s, 1H), 7.56–7.58 (m, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.29–7.31 (m, 2H), 7.25–7.37 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.2, 183.0, 169.2, 159.9, 147.4, 141.9, 138.9, 137.5, 137.3, 137.2, 136.8, 136.3, 131.4, 130.6, 126.6, 125.6, 124.8, 124.3, 124.0, 123.7, 123.2, 123.1, 122.8, 122.7, 122.5, 122.2, 121.7, 121.3, 121.0, 120.3, 113.8, 113.1, 113.0, 112.4, 112.1, 111.8, 111.7; Anal. RP-HPLC t_R = 2.699 min, purity 99.73%; (ESI-MS) $[M + H]^+$ *m/z* 331.20; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{14}N_4O$ $[M + H]^+$: 331.1195, found: 331.1191.

***N'*-((6-Bromo-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide** (**46b**)



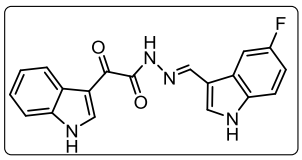
Yield 88%; Yellow solid; mp 279–281 °C; IR (KBr, *v,* cm^{-1}): 3302, 1689, 1604, 1488, 1427, 1242, 1126, 810, 740; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.34 (s, 1H), 11.93 (s, 1H), 11.76 (s, 1H), 8.82 (s, 1H), 8.66 (s, 1H), 8.24–8.29 (m, 2H), 7.88 (d, J = 2.2 Hz, 1H), 7.65 (s, 1H), 7.55–7.57 (m, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.29–7.31 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.9, 160.0, 146.8, 139.1, 138.4, 136.8, 132.3, 126.6, 124.2, 124.0, 123.9, 123.8, 123.8, 123.6, 123.1, 123.0, 121.7, 115.9, 115.0, 113.1, 112.9, 112.3; Anal. RP-HPLC t_R = 2.676 min, purity 98.65%; (ESI-MS) $[M + H]^+$ *m/z* 409.35; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{13}BrNaN_4O_2$ $[M + Na]^+$: 431.0120, found: 431.0114 $[M + Na]^+$ and 433.0128 $[M + 2 + Na]^+$.

***N'*-((5-Bromo-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46c)**



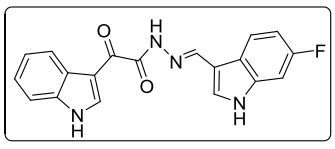
Yield 89%; Yellow solid; mp 239–240 °C; IR (KBr, *v,* cm^{-1}): 3440, 3255, 1658, 1597, 1496, 1427, 1226, 1118, 794, 740; ^1H NMR (400 MHz, DMSO-d_6): δ 12.12 (s, 1H), 11.72 (s, 1H), 11.60 (s, 1H), 8.87 (d, *J* = 3.3 Hz, 1H), 8.65 (s, 1H), 8.45 (d, *J* = 1.8 Hz, 1H), 8.27–8.30 (m, 1H), 7.65 (d, *J* = 2.8 Hz, 1H), 7.46–7.49 (m, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.24–7.26 (m, 1H), 7.20–7.23 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.8, 160.0, 146.9, 141.7, 139.2, 137.4, 136.8, 136.3, 132.8, 131.9, 126.6, 126.5, 125.7, 125.5, 124.7, 124.4, 124.0, 123.7, 123.1, 122.7, 121.7, 114.4, 113.7, 113.1, 112.9, 111.7, 111.2; Anal. RP-HPLC t_R = 2.721 min, purity 98.96%; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{13}BrNaN_4O_2$ $[M + Na]^+$: 431.0120, found: 431.0115 $[M + Na]^+$ and 433.0109 $[M + 2 + Na]^+$.

***N'*-((5-Fluoro-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46d)**



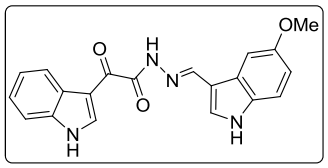
Yield 90%; Yellow solid; mp 267–269 °C; IR (KBr, *v,* cm^{-1}): 3448, 3217, 1658, 1596, 1488, 1442, 1242, 1157, 1126, 794, 740; ^1H NMR (500 MHz, DMSO-d_6): δ 8.84 (s, 1H), 8.67 (s, 1H), 8.27 (d, *J* = 3.8 Hz, 1H), 8.05–8.07 (m, 1H), 7.93 (s, 1H), 7.54–7.58 (m, 1H), 7.48 (s, 1H), 7.28–7.28 (m, 3H), 7.07–7.31 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.9, 181.9, 160.4, 159.4, 157.1, 146.6, 141.7, 141.2, 134.2, 133.8, 133.0, 127.4, 123.5, 123.4, 122.6, 122.5, 121.6, 113.8, 113.1, 113.0, 112.3, 112.2, 111.4, 111.1, 107.4, 107.1, 79.6; Anal. RP-HPLC t_R = 2.710 min, purity 98.50%; (ESI-MS) $[M + H]^+$ *m/z* 349.12; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{13}FNaN_4O_2$ $[M + Na]^+$: 371.0920, found: 371.0925.

***N'*-((6-Fluoro-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46e)**



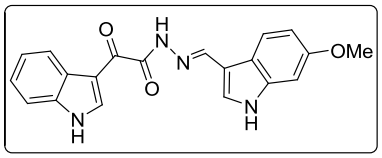
Yield 91%; Yellow solid; mp 240–242 °C; IR (KBr, *v,* cm^{-1}): 3440, 3224, 1666, 1596, 1496, 1442, 1234, 1126, 810, 740; ^1H NMR (500 MHz, DMSO-d_6): δ 8.80 (s, 1H), 8.65 (s, 1H), 8.31–8.33 (m, 1H), 8.26–8.27 (m, 1H), 7.85 (s, 1H), 7.53–7.56 (m, 1H), 7.31–7.36 (m, 1H), 7.25–7.26 (m, 3H), 7.05–7.08 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.1, 186.7, 182.5, 169.3, 161.1, 160.2, 158.7, 146.8, 141.3, 139.9, 137.7, 137.5, 136.6, 132.1, 126.9, 125.6, 123.8, 123.7, 123.6, 122.9, 122.7, 121.7, 121.2, 113.8, 113.4, 113.2, 113.0, 112.2, 111.8, 109.5, 109.2, 108.2; Anal. RP-HPLC t_R = 2.707 min, purity 99.31%; (ESI-MS) $[M + H]^+$ *m/z* 349.13; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{14}FN_4O_2$ $[M + H]^+$: 349.1101, found: 349.1091.

***N'*-((5-Methoxy-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46f)**



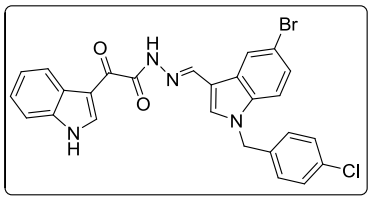
Yield 89%; Yellow solid; mp 236–238 °C; IR (KBr, *v,* cm^{-1}): 3286, 3085, 1681, 1604, 1488, 1434, 1242, 1157, 1126, 802, 740; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.62 (s, 1H), 8.84 (s, 1H), 8.67 (s, 1H), 8.28–8.30 (m, 1H), 8.33–8.37 (m, 1H), 7.87 (d, *J* = 2.3 Hz, 1H), 7.79 (s, 1H), 7.55–7.57 (m, 1H), 7.36 (d, *J* = 8.7 Hz, 1H), 7.27–7.29 (m, 3H), 6.88 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.9, 182.8, 169.0, 159.9, 155.0, 154.6, 147.5, 142.9, 139.6, 137.5, 137.3, 137.0, 132.6, 132.1, 131.8, 131.2, 126.8, 125.6, 125.5, 124.7, 123.8, 123.7, 123.0, 122.6, 121.7, 121.3, 113.6, 113.3, 113.1, 113.0, 112.9, 112.7, 111.9, 111.4, 104.9, 102.6, 55.9, 54.6; Anal. RP-HPLC t_R = 2.702 min, purity 98.31%; (ESI-MS) $[M + H]^+$ *m/z* 361.18; HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{17}N_4O_3$ $[M + H]^+$: 361.1301, found: 361.1295.

***N'*-((6-Methoxy-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46g)**



Yield 90%; Yellow solid; mp 238–240 °C; IR (KBr, *v,* cm^{-1}): 3394, 3271, 1674, 1612, 1496, 1442, 1357, 1242, 1157, 1126, 810, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.05 (s, 1H), 11.58 (s, 1H), 11.15 (s, 1H), 8.94 (d, *J* = 2.3 Hz, 1H), 8.66 (s, 1H), 8.35–8.37 (m, 1H), 8.23 (d, *J* = 8.7 Hz, 1H), 7.49–7.52 (m, 2H), 7.25–7.29 (m, 2H), 6.92 (d, *J* = 2.2 Hz, 1H), 6.79 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.3, 182.8, 169.2, 160.0, 156.9, 156.5, 147.2, 139.5, 138.4, 137.2, 130.4, 126.8, 123.9, 123.1, 123.0, 121.7, 118.9, 118.4, 113.8, 113.3, 113.0, 112.2, 111.8, 110.9, 110.3, 95.4, 55.7; Anal. RP-HPLC t_R = 2.683 min, purity 98.94%; HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{16}NaN_4O_3$ $[M + Na]^+$: 383.1120, found: 383.1115.

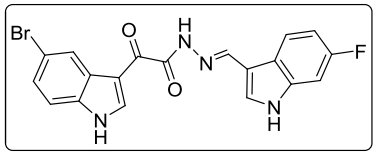
***N'*-((1-(4-Chlorobenzyl)-5-bromo-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (46h)**



Yield 95%; Yellow solid; mp 233–235 °C; IR (KBr, *v,* cm^{-1}): 3178, 1674, 1612, 1496, 1442, 1388, 1242, 1157, 1126, 802, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H), 11.85 (s, 1H), 8.87 (d, *J* = 3.1 Hz, 1H), 8.66 (s, 1H), 8.49 (d, *J* = 1.4 Hz, 1H), 8.28–8.30 (m, 1H), 7.87 (s, 1H), 7.48–7.50 (m, 1H), 7.36 (d, *J* = 8.7 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 3H), 7.20–7.24 (m, 4H), 5.41 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.7, 159.9, 146.3, 139.3, 136.8, 136.7, 136.1, 135.6, 132.8, 129.6, 129.3, 129.2, 129.1, 127.0, 126.6, 126.0, 125.0, 124.0, 123.2, 121.7, 114.4, 114.1, 113.6, 113.4, 113.1, 113.0,

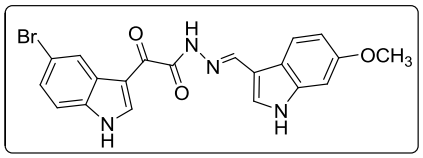
112.9, 111.5, 111.0, 49.3; Anal. RP-HPLC t_R = 3.173 min, purity 98.74%; HRMS (ESI^+): *m/z* calcd. for $C_{26}H_{18}BrClNaN_4O_2$ $[M + Na]^+$: 555.0199, found: 555.0184 $[M + Na]^+$ and 557.0174 $[M + 2 + Na]^+$.

_N'_-((6-Fluoro-1*H*-indol-3-yl)methylene)-2-(5-bromo-1*H*-indol-3-yl)-2-oxoaceto-hydrazide (46i)



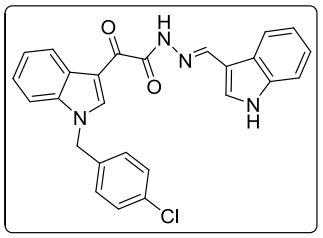
Yield 96%; Yellow solid; mp 290–292 °C; IR (KBr, *v,* cm^{-1}): 3348, 3301, 1689, 1604, 1489, 1434, 1226, 1134, 1126, 825, 601; ^1H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H), 11.57 (s, 1H), 11.35 (s, 1H), 8.97 (d, *J* = 3.4 Hz, 1H), 8.69 (s, 1H), 8.51 (d, *J* = 1.8 Hz, 1H), 8.33–8.37 (m, 1H), 7.60 (d, *J* = 2.7 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.35 (dd, *J* = 8.6, 1.9 Hz 1H), 7.14 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.90–6.95 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.4, 182.8, 168.8, 161.1, 159.6, 158.7, 147.2, 141.6, 140.1, 137.6, 137.5, 137.4, 136.1, 135.6, 132.2, 131.4, 128.4, 127.3, 126.6, 126.4, 123.9, 123.7, 123.6, 123.4, 121.5, 121.1, 115.9, 115.6, 115.2, 113.3, 112.4, 112.2, 111.8, 109.5, 109.3, 98.7, 98.5, 98.2; Anal. RP-HPLC t_R = 2.903 min, purity 98.70%; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{12}BrFNaN_4O_2$ $[M + Na]^+$: 449.0025, found: 449.0022 $[M + Na]^+$ and 451.0009 $[M + 2 + Na]^+$.

_N'_-((6-Methoxy-1*H*-indol-3-yl)methylene)-2-(5-bromo-1*H*-indol-3-yl)-2-oxoaceto-hydrazide (46j)



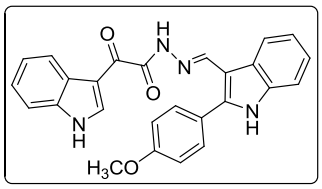
Yield 90%; Yellow solid; mp > 300 °C; IR (KBr, *v,* cm^{-1}): 3417, 3278, 1666, 1604, 1504, 1434, 1357, 1234, 1141, 817, 609; ^1H NMR (500 MHz, DMSO-d_6): δ 12.49 (s, 1H),11.94 (s, 1H), 11.72 (s, 1H), 8.88 (s, 1H), 8.67 (s, 1H), 8.41 (s, 1H), 8.30–8.33 (m, 1H), 7.87 (s, 1H), 7.5–7.56 (m, 1H), 7.43–7.48 (m, 1H), 7.26–7.24 (m, 1H), 7.04–7.10 (m, 1H), 3.40 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.5, 182.9, 168.8, 159.5, 156.94, 156.5, 147.5, 141.8, 140.1, 138.4, 138.1, 137.3, 136.1, 135.6, 130.6, 129.6, 128.4, 127.3, 126.6, 126.4, 123.8, 123.4, 123.1, 122.7, 118.9, 118.4, 115.9, 115.5, 115.2, 113.4, 112.5, 112.2, 111.7, 111.0, 110.0, 95.4, 95.1, 55.7; Anal. RP-HPLC t_R = 2.870 min, purity 98.13%; HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{15}BrNaN_4O_3$ $[M + Na]^+$: 461.0225, found: 461.0228 $[M + Na]^+$ and 463.0210 $[M + 2 + Na]^+$.

***N'*-((1*H*-Indol-3-yl)methylene)-2-(1-(4-chlorobenzyl)-1*H*-indol-3-yl)-2-oxoaceto-hydrazide (46k)**



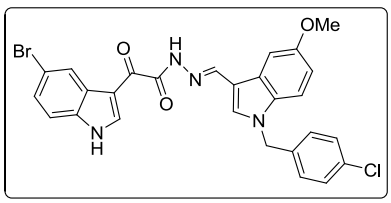
Yield 89%; Yellow solid; mp 225–227 °C; IR (KBr, *v,* cm^{-1}): 3186, 3055, 1689, 1627, 1504, 1442, 1365, 1234, 1172, 810, 740; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 11.30 (s, 1H), 9.02 (s, 1H), 8.66 (s, 1H), 8.29–8.35 (m, 2H), 7.56 (d, *J* = 2.7 Hz, 1H), 7.36–7.38 (m, 2H), 7.19–7.27 (m, 6H), 7.08–7.14 (m, 2H), 5.43 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.0, 182.8, 159.9, 159.9, 155.5, 147.4, 141.9, 137.7, 137.5, 137.3, 137.1, 136.7, 136.2, 136.2, 133.0, 132.5, 132.3, 131.4, 129.8, 129.2, 129.1, 128.8, 127.3, 125.2, 124.8, 124.3, 124.2, 124.0, 123.6, 123.2, 123.2, 123.1, 122.8, 122.6, 122.2, 122.1, 121.0, 121.0, 112.6, 112.4, 112.4, 112.4, 112.1, 112.0, 111.9, 111.7, 49.6, 49.3; Anal. RP-HPLC t_R = 2.727 min, purity 98.04%; (ESI-MS) $[M + H]^+$ *m/z* 455.6; HRMS (ESI^+): *m/z* calcd. for $C_{26}H_{20}ClN_4O_2$ $[M + H]^+$: 455.1275, found: 455.1269.

***N'*-((2-(4-Methoxyphenyl)-1*H*-indol-3-yl)methylene)-2-(1*H*-indol-3-yl)-2-oxoaceto-hydrazide (46l)**



Yield 91%; Yellow solid; mp >300 °C; IR (KBr, *v,* cm^{-1}): 3394, 3271, 1674, 1612, 1496, 1442, 1357, 1242, 1157, 1126, 810, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H), 11.74 (s, 1H), 11.54 (s, 1H), 8.86 (d, *J* = 3.2 Hz, 1H), 8.82 (s, 1H), 8.43 (d, *J* = 7.1 Hz, 1H), 8.25–8.28 (m, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.43–7.46 (m, 1H), 7.36 (d, *J* = 7.3 Hz, 1H), 7.17–7.21 (m, 2H), 7.07–7.17 (m, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.4, 182.9, 169.0, 160.3, 159.7, 147.8, 142.8, 142.1, 141.8, 139.1, 137.3, 136.9, 136.8, 136.5, 131.0, 130.8, 126.6, 126.2, 125.7, 125.6, 124.0, 123.8, 123.3, 123.1, 122.9, 121.8, 121.2, 114.9, 113.9, 113.1, 113.0, 111.8, 111.5, 107.9, 107.4, 55.8; Anal. RP-HPLC t_R = 2.780 min, purity 99.42%; HRMS (ESI^+): *m/z* calcd. for $C_{26}H_{20}N_4O_3$ $[M + H]^+$: 437.1614, found: 437.1613.

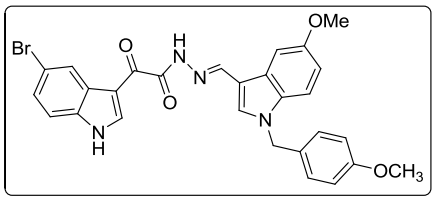
***N'*-((1-(4-Chlorobenzyl)-5-methoxy-1*H*-indol-3-yl)methylene)-2-(5-bromo-1*H*-indol-3-yl) -2-oxoacetohydrazide (46m)**



Yield 91%; Yellow solid; mp 223–225 °C; IR (KBr, *v,* cm^{-1}): 3340, 1689, 1620, 1489, 1411, 1350, 1226, 1172, 833, 702; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.44 (s, 1H), 11.89 (S, 1H), 8.91 (s, 1H), 8.70 (s, 1H), 8.42 (s, 1H), 8.23 (d, *J* = 7.5 Hz, 1H), 8.0 (s, 1H), 7.88–7.89 (m, 1H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.39–7.45 (m, 3H), 7.35 (d,

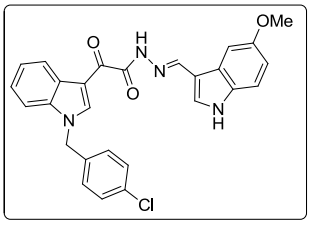
J = 8.3 Hz, 1H), 7.26–7.30 (m, 2H), 7.17 (d, *J* = 8.2 Hz, 1H), 6.9 (dd, *J* = 8.8, 2.0 Hz, 1H) 5.45 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.7, 182.7, 168.6, 159.4, 155.4, 155.0, 147.1, 142.6, 140.2, 137.9, 137.0, 136.1, 135.7, 134.7, 134.4, 132.7, 132.6, 132.4, 132.1, 129.5, 129.3, 129.1, 129.0, 128.5, 127.2, 126.6, 126.4, 126.2, 125.4, 123.8, 123.5, 115.9, 115.5, 115.2, 115.1, 113.6, 113.0, 112.9, 112.4, 111.9, 111.5, 111.0, 105.3, 103.2, 55.9, 54.6, 49.3, 49.2; Anal. RP-HPLC t_R = 3.328 min, purity 98.67%; HRMS (ESI$^+$): calcd. for $C_{27}H_{21}BrClN_4O_3$ $[M + H]^+$: 563.0486; found: 563.0430 and 565.0418 $[M + H + 2]^+$.

_N'_-((1-(4-Methoxybenzyl)-5-methoxy-1_H_-indol-3-yl)methylene)-2-(5-bromo-1_H_-indol-3-yl)-2-oxoacetohydrazide (46n)



Yield 91%; Yellow solid; mp 156–158 °C; IR (KBr, *v,* cm^{-1}): 3340, 3301, 1689, 1612, 1488, 1419, 1234, 1172, 1141, 877, 748; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.80 (d, *J* = 8.1 Hz, 1H), 8.66 (s, 1H), 8.34 (s, 1H), 8.24 (s, 1H), 7.97 (s, 1H), 7.87 (s, 1H), 7.47–7.48 (m, 1H), 7.39–7.42 (m, 3H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.24–7.29 (m, 3H), 7.16 (d, *J* = 8.4 Hz, 1H), 6.89 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.44 (s, 2H), 3.81 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.4, 159.6, 159.2, 159.1, 155.3, 154.9, 147.1, 142.6, 141.1, 136.6, 134.5, 134.3, 132.4, 129.8, 129.3, 129.0, 128.8, 127.3, 126.3, 126.2, 125.4, 123.8, 123.4, 115.7, 115.5, 115.3, 114.5, 114.4, 113.5, 113.0, 112.7, 112.4, 112.0, 111.2, 111.0, 110.7, 105.2, 103.2, 56.0, 55.5, 54.6; Anal. RP-HPLC t_R = 3.189 min, purity 98.97%; (ESI-MS) $[M + H]^+$ *m/z* 559.2; HRMS (ESI$^+$): *m/z* calcd. for $C_{28}H_{24}BrN_4O_4$ $[M + H]^+$: 559.0981, found: 559.0973 and 561.970 $[M + 2 + Na]^+$.

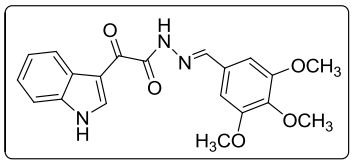
2-(1-(4-Chlorobenzyl)-1H-indol-3-yl)-_N'_-((5-methoxy-1_H_-indol-3-yl)methylene)-2-oxoacetohydrazide (46o)



Yield 86%; Yellow solid; mp 217–219 °C; IR (KBr, *v,* cm^{-1}): 3184, 3057, 1688, 1627, 1502, 1446, 1367, 1236, 1175, 811, 740; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.61 (s, 1H), 11.32 (s, 1H), 9.08 (s, 1H), 8.67 (s, 1H), 8.29–8.34 (m, 2H), 7.56 (d, *J* = 2.7 Hz, 1H), 7.36–7.38 (m, 2H), 7.19–7.26 (m, 5H), 7.09–7.13 (m, 2H), 5.43 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.6, 182.5, 168.5, 159.6, 155.3, 155.0, 147.3, 142.8, 140.0, 137.8, 137.1, 136.0, 135.4, 134.7, 134.3, 132.7, 132.6, 132.4, 132.0, 129.5, 129.2, 129.1, 129.0, 128.5, 127.0, 126.5, 126.4, 126.2, 125.3, 123.7, 123.5, 116.0, 115.5, 115.2, 115.1,

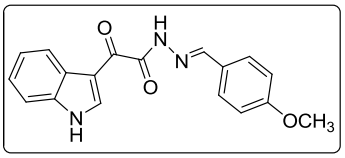
113.5, 113.0, 112.8, 112.3, 111.9, 111.4, 111.0, 105.3, 103.2, 56.0, 54.9, 49.8, 49.2; Anal. RP-HPLC t_R = 2.827 min, purity 98.15%; HRMS (ESI^+): *m/z* calcd. for $C_{27}H_{22}ClN_4O_3$ $[M + H]^+$: 485.1380, found: 485.1375.

***N'*-(3,4,5-Trimethoxybenzylidene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (48a)**



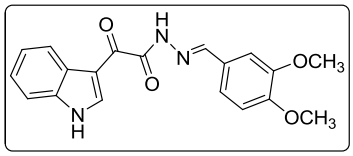
Yield 96%; Yellow solid; mp 155–157 °C; IR (KBr, *v,* cm^{-1}): 3232, 2985, 1681, 1610, 1496, 1434, 1234, 1126, 995, 810, 740; ^1H NMR (400 MHz, DMSO-d_6): δ 8.79 (s, 1H), 8.46 (s, 1H), 8.30–8.32 (m, 1H), 8.12 (s, 1H), 7.52–7.54 (m, 1H), 7.25–7.27 (m, 2H), 7.02 (s, 2H), 3.88 (s, 6H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.2, 160.9, 153.7, 153.3, 150.1, 140.0, 139.9, 137.8, 130.1, 126.8, 123.8, 123.0, 121.6, 113.5, 112.8, 104.9, 104.2, 60.6, 56.4, 55.8; Anal. RP-HPLC t_R = 2.855 min, purity 98.62%; HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{19}N_3O_5$ $[M + H]^+$: 382.1403; found: 382.1395.

***N'*-(4-Methoxybenzylidene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (48b)**



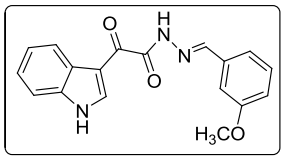
Yield 91%; Yellow solid; mp 247–249 °C; IR (KBr, *v,* cm^{-1}): 3224, 3062, 1674, 1604, 1496, 1434, 1249, 1164, 1118, 810, 740; ^1H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H), 11.99 (s, 1H), 8.80 (d, *J* = 3.3 Hz, 1H), 8.50 (s, 1H), 8.31–8.33 (m, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.51–7.53 (m, 1H), 7.25–7.27 (m, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.5, 161.6, 160.4, 150.3, 145.4, 139.1, 136.7, 129.3, 128.8, 127.1, 126.5, 124.1, 123.2, 121.7, 114.9, 113.1, 112.8, 55.8; Anal. RP-HPLC t_R = 2.866 min, purity 97.77%; HRMS (ESI^+): *m/z* calcd. for $C_{18}H_{15}NaN_3O3$ $[M + Na]^+$: 344.1011, found: 344.1020.

***N'*-(3,4-Dimethoxybenzylidene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (48c)**



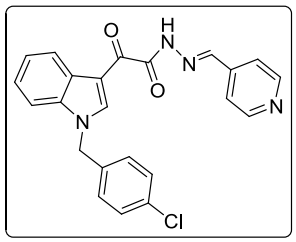
Yield 95%; Yellow solid; mp 198–200 °C; IR (KBr, *v,* cm^{-1}): 3309, 1666, 1627, 1504, 1435, 1265, 1134, 810, 740; ^1H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H), 12.01 (s, 1H), 8.81 (d, *J* = 3.3 Hz, 1H), 8.48 (s, 1H), 8.33–8.33 (m, 1H), 7.50–7.53 (m, 1H), 7.41 (d, *J* = 1.8 Hz, 1H), 7.24–7.28 (m, 2H), 7.20 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.6, 160.4, 151.5, 150.5, 149.5, 139.1, 136.9, 127.2, 126.5, 124.1, 123.2, 122.7, 121.7, 113.2, 112.8, 111.9, 108.7, 56.1, 55.9; Anal. RP-HPLC t_R = 2.838 min, purity 98.31%; HRMS (ESI^+): *m/z* calcd. for $C_{19}H_{17}NaN_3O_4$ $[M + Na]^+$: 374.1117; found: 374.1090.

***N'*-(3-Methoxybenzylidene)-2-(1*H*-indol-3-yl)-2-oxoacetohydrazide (48d)**



Yield 92%; Yellow solid; mp 227–229 °C; IR (KBr, *v,* cm^{-1}): 3386, 3247, 1689, 1627, 1504, 1427, 1288, 1242, 1149, 1118, 810, 748, 601; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.06 (s, 2H), 8.83 (d, *J* = 3.3 Hz, 1H), 8.54 (s, 1H), 8.33–8.35 (m, 1H), 7.50–7.53 (m, 1H), 7.25–7.35 (m, 5H), 6.95–6.98 (m, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.4, 160.6, 160.0, 150.3, 139.2, 136.9, 136.0, 130.5, 126.5, 124.1, 123.2, 121.7, 120.8, 117.2, 113.2, 112.8, 111.7, 55.7; Anal. RP-HPLC t_R = 2.922 min, purity 98.84%; HRMS (ESI^+): *m/z* calcd. for $C_{18}H_{15}NaN_3O_3$ $[M + Na]^+$: 344.1011, found: 344.1004.

2-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-2-oxo-*N*'-(pyridin-4-ylmethylene)acetohydrazide (48e)



Yield 90%; Yellow solid; mp 220–221 °C; IR (KBr, *v,* cm^{-1}): 3226, 3061, 1672, 1609, 1500, 1437, 1248, 1163, 1119, 810, 740; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.74 (s, 1H), 8.70 (d, *J* = 7.4 Hz, 2H), 8.22 (s, 1H), 8.11–8.03 (m, 2H), 7.74 (d, *J* = 7.2 Hz, 2H), 7.70 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.39–7.35 (m, 2H), 7.23 (d, *J* = 7.5 Hz, 2H), 7.14 (d, *J* = 7.5 Hz, 2H), 5.22 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): 187.2, 168.1, 151.0, 149.3, 140.8, 140.2, 137.8, 136.0, 132.9, 130.2, 129.3, 127.5, 124.3, 123.6, 123.2, 122.5, 118.3, 111.6, 52.9; Anal. RP-HPLC t_R = 2.922 min, purity 98.77%; HRMS (ESI^+): *m/z* calcd. for $C_{23}H_{18}ClN_4O_2$ $[M + H]^+$: 417.1118, found: 417.1112.

Cell culture

Colon cancer cell line (HCT-116), and breast cancer cell lines (BT-474, MCF-7 and MDA-MB-231) were cultured in DMEM supplemented with 10% fetal serum albumin and 50µg/mL of penicillin and streptomycin. Jurkat (leukemia cancer) cells were cultured in RPMI supplemented with 10% fetal serum albumin and 50 µg/mL of penicillin and streptomycin. All cell lines were maintained in an incubator containing 5% CO_2 at 37 °C.

Cell viability assay

Cells were seeded in a 96-well plate at a density of 100,000/mL and grown overnight. Cells were treated with various compounds at a final concentration of 10 µM and incubated for 48 h. Cell viability assay was performed using a MTT cell proliferation kit from ATCC (#30-1010K). In summary, 10 µL MTT reagent was added to each well, and cells were placed back

in incubator for 4 h. 100 µL of detergent (from kit) was added and absorbance data was collected at 570 nm using Biotek synergy 2 spectrophotometer. Data was calculated as percentage of cell survival using the following formula;

% cell survival = (100/At*As)

Where At and As are the absorbances of wells treated with test compounds and solvent control respectively.

DNA fragmentation

100,000 cells were plated in a 24 well plate and treated with 1 µM of **46a**, **46c**, **46f**, **46g** and **46k**. 24 h. later, genomic DNA was isolated using a kit from Abcam (Ab66090) and analyzed *via* agarose gel and UV-visualization. Camptothecin was used as a positive control for inducing apoptotic cell death.

Caspase assay

100,000 cells were plated in a 24 well plate and treated with 1 µM **46c**, **46f**, **46k** and **46o**. Later, 100 µL sample was taken and analyzed as per kit (Promega G7790). Fluorescence for the samples was measured at 0 min and 180 min. Camptothecin was used as a positive control for inducing apoptotic cell death.

***In vitro* tubulin polymerization**

Tubulin (1.2 mg/mL) was mixed with varying concentrations of **46k** and **46o** (0-0.2µM) for 5 min. at room temperature in a 96 well plate and reaction was initiated by adding polymerization buffer (as per a kit from Cytoskeleton; BK011P) and the reaction was monitored for change in fluorescence (Ex: 360 nm and Em: 420 nm) using a Biotek fluorimeter. Colchicine was used as a positive control inhibitor of tubulin polymerization.

LDH assay

50,000 Cells per 100 microliter were plated per well in a 96-well format and were treated with 20 micro molar test compounds. After 40 h., samples were processed and analyzed for LDH activity using LDH cytotoxicity kit from Cayman chemicals (cat no 601170).

4.3.5 References

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Chapter 5

Conclusions

5.1 General conclusions

Azaheterocycles are one of the most valuable sources of novel compounds with a wide range of biological activities, mainly because of the distinctive ability of the resulting compounds to mimic the structures of peptides and to bind reversibly to receptors. Among the azaheterocycles, indole has become leading pharmacophore which continues to receive increasing attention from medicinal chemists due to unique biological properties of indole derived compounds, especially, anticancer activity. Chemotherapy is very important and popular treatment among the available cancer therapies. In recent past, various chemotherapeutic agents such as paclitaxel, vincristine, and doxorubicin have lost their efficacy due to the development of drug resistance from prolonged exposure. Therefore, the development of novel and effective anticancer agents with an improved pharmacological profile remains a major challenge.

The thesis deals with the design, synthesis and *in vitro* cytotoxicity studies of indolylthiazoles, indolyloxadiazoles and bisindoles. While synthesizing the indole-based compounds we developed mild, efficient, metal-free and high yielding synthetic protocols for the construction of 2-arylamino-4-(3′-indolyl)thiazoles, 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles and indolyl-α-keto-1,3,4-oxadiazoles. In the search of potent indole-based analogues we explored the linking positions (C-2/C-3) in indole. In the present thesis, we identified 2-aryl amino-4-(3′-indolyl)thiazoles, 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles and 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides as potent apoptosis inducing cytotoxic agents in addition to indolyl-α-keto-1,3,4-oxadiazoles, α-cyano bis(indolyl)chalcones and bis(indolyl)ketohydrazide-hydrazones as tubulin interacting anticancer agents. Moreover, *in silico* binding modes for some of the potent analogues of indolyl-α-keto-1,3,4-oxadiazoles and bis(indolyl)ketohydrazide-hydrazones have also been investigated using molecular modeling.

5.2 Specific conclusions

The **first chapter** deals the physical and chemical properties of indole and a brief overview of anticancer research and treatments of various types of cancers with special emphasizes on chemotherapy and classification of anticancer drugs present in market. This chapter described the utilities of natural and synthetic indole-based molecules in anticancer drug discovery. Further, this chapter provides the information for the rational design of novel indole

containing chemical entities as potent anticancer agents. Also, it describes the current problems associated with the existing anticancer drugs and the scope for developing novel indole-based compounds by structural modifications of existing indole-based lead anticancer drug candidates with improved anticancer properties.

The **second chapter** highlights the design, synthesis and anticancer activities of two different novel indolyl thiazoles series. **Part A** of this chapter reports, synthesis of 2-arylamino-4-(3′-indolyl)thiazoles (**22a-o**) involving microwave-assisted rapid reaction of *α*-tosyloxyketones with *N*-phenylthiourea in PEG-400 as a benign reaction medium. Among the synthesized aminothiazoles, 2-phenylamino-4-(3′-indolyl)thiazole (**22b**) exhibited potent cytotoxicity (1.86 μM; MCF-7) through induction of apoptosis in MCF-7 cells. In **part B**, diverse series of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides **38a-p** were synthesized from the initial reaction of thioamides and bromopyruvic acid to afford thiazole carboxylic acids **36**, which were coupled with appropriate arylamines. *In vitro* cytotoxicity study of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides were resulted in **38i** and **38l** as the most potent compounds. Further, preliminary mechanism of action studies indicated that thiazole carboxamide **38i** induces apoptosis in HeLa cells.

The **third chapter** of thesis deals with the anticancer activity potential of two series of interesting novel indolyloxadiazoles. The chapter is divided in two parts. In **part-A**, various 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles (**19a-m**) were synthesized from IBD-mediated oxidative desulfurization of readily available acylthiosemicarbazides. Of the synthesized 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles **19b**, **19i** (IC_{50} = < 0.001; HeLa), and **19l** (IC_{50} = < 0.001; MCF-7) exhibited strongest cytotoxicity. Preliminary mechanism of action studies indicated that these aminooxadiazoles induce apoptosis in MDA-MB-231 cancer cells. In part B, design and synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles (**30a-p**) have been described. Synthesis of indolyl-*α*-keto-1,3,4-oxadiazoles was achieved from the oxidative cyclization of acylhydrazones using molecular iodine in good yields. Most of the compounds exhibited prominent cytotoxicity towards tested cancer cell line. Indolyl-*α*-keto-1,3,4-oxadiazole (**30e**) showed the highest cytotoxicity against acute lymphoblastic leukemia SB cell line (IC_{50} = 0.8 μM). The preliminary anticancer mechanistic studies showed that indolyl-*α*-keto-1,3,4-oxadiazoles induces caspase-dependent apoptotic cell death and exerted their anticancer activity through inhibition of tubulin polymerization.

Chapter four illustrates the synthesis and anticancer activity studies of novel bisindoles which is further divided into two parts. **Part A** describes the microwave-assisted synthesis of

α-cyano bis(indolyl)chalcones (**25a-w**) and their *in vitro* anticancer activity against three human cancer cell lines. Among the synthesized chalcones, compound **25n** was found to be the most potent and selective against A549 lung cancer cell line (IC_{50} = 0.8 μM). In a preliminary mechanism of action studies, some *α*-cyano bis(indolyl)chalcones were found to enhance tubulin polymerization suggesting these compounds could act as microtubule stabilizing agents. **Part B** illustrates the synthesis and anticancer activity study of a series of bis(indolyl)ketohydrazide-hydrazones, which were achieved in excellent yields from the reaction of indolyl glyoxalylhydrazide with appropriate aldehydes. The *in vitro* cytotoxicity evaluation of synthesized bis(indolyl)ketohydrazide-hydrazones resulted analogue **46k** with most potent anticancer activity against MCF-7, MDA-MB-231, HCT-116 and JURKAT cancer cell lines with IC_{50} values of 0.8, 0.5, 0.15, and 0.22 μM, respectively. Also, the analogue **46k** was selectively cytotoxic (10-folds) to cancerous cells. Mechanism of the action studies suggested that the bis(indolyl)ketohydrazide-hydrazones induced DNA fragmentation and caspase 3/7 activation, and exerted their anticancer activity through inhibition of tubulin polymerization (IC_{50} = 0.6 μM). Moreover, molecular docking studies demonstrated a potential binding mode for compound **46K** in the colchicine binding site of tubulin.

5.3 Future scope of the research work

In the recent past significant improvement has been made in the area of anticancer drug discovery. Various efficient cancer therapies such as surgery, chemotherapy, radiation therapy and advanced immunotherapy have been used for the treatment of a wide range of cancers. Also, the knowledge of cancer biology has exploded during the past decades and this may help in understanding the mechanistic path ways of cancer cell growth. It was evidenced that several natural occurring anticancer entities such as taxol, vinca alkaloids and combretastatin A-4 made significant impact in the discovery and development of potent antitumor agents. Although, in addition to natural products, a large number of synthetic lead clinical candidates has been designed with improved efficacy, however, so far none of them find a solution for complete cure. Tumor specificity, solubility, drug resistance and toxicity associated side effects are the major challenges for the medicinal chemists to design novel drugs with improved safety profile. The mode of action of various leading anticancer entities encourages the medicinal chemist to develop potent and selective anticancer drugs.

The scope of this thesis is to achieve diverse structural class of indoles as novel anticancer agents. The anticancer activity potential of identified (2-arylamino-4-(3′-indolyl)thiazoles, (2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles, indolyl-α-keto-1,3,4-oxadiazoles, α-cyano bis(indolyl)chalcones and bis(indolyl)ketohydrazide-hydrazones further can be enhanced with extensive SAR. Based on *in vitro* cytotoxicity results, further structural modification of most potent compounds identified in different series is likely to produce drug-like compounds. Subsequently, molecular target and *in vivo* screening studies of the most potent compounds may also be investigated.

The relatively benign synthetic protocols developed for the preparation of 2-arylamino-4-(3′-indolyl)thiazoles, 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles, 2-aroyl-5-(2′/3′-indolyl)-1,3,4-oxadiazoles and α-cyano bis(indolyl)chalcones have greater potential to produce a library of bioactive important scaffolds.

Appendices

List of publications *[A-1]*

1. **Mukund P. Tantak**, Dipanwita Das Mukherjee, Anil Kumar, Gopal Chakrabarti, Dalip Kumar, A facile and microwave-assisted rapid synthesis of 2-arylamino-4-(3′-indolyl)-thiazoles as apoptosis inducing cytotoxic agents, ***Anti-Cancer Agents in Medicinal Chemistry,*** **2016**, 0000.
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11. Surela Bhattacharya, N. Maruthi Kumar, Arnab Ganguli, **Mukund P. Tantak**, Dalip Kumar, Gopal Chakrabarti, NMK-TD-100, a novel microtubule modulating agent, blocks mitosis and induces apoptosis in HeLa cells by binding to tubulin, ***PLOS ONE,*** **2013,** *10*, e76286.
12. **Mukund P. Tantak**, Anil Kumar, Brett Noel, Kavita Shah, Dalip Kumar, Synthesis and biological evaluation of 2-arylamino-5-(3'-indolyl)-1,3,4-oxadiazoles as potent cytotoxic agents, ***ChemMedChem***, **2013**, *8*, 1468.
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❖ **Communicated/Under Revision**

14. Surela Bhattacharya, N. Maruthi Kumar, Arnab Ganguli, **Mukund P. Tantak**, Dalip Kumar, Gopal Chakrabarti, Attenuation of ROS mediated autophagy augments the cytotoxicity of the microtubule targeting agent NMK-TD-100 in A549 cells, ***Apoptosis*** **(Communicated).**

List of papers presented in conferences *[A-2]*

❖ **Oral presentations:**

1. **Mukund P. Tantak**, Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and facile synthesis of indolyloxadiazoles as tubulin interacting anticancer agents" at International Conference on Frontiers at The Chemistry-Allied Sciences Interface (FCASI-2016), Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan, India. 25-26 April, 2016. (*Best oral presentation award*)
2. **Mukund P. Tantak,** Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and synthesis of bis(indolyl)ketohydrazide-hydrazones as potent and selective novel tubulin inhibitors." at National Conference on Frontiers at The Chemistry-Allied Sciences Interface (FCASI-2015), Department of Chemistry, University of Rajasthan, Jaipur, India, 13-14, March, 2015.
3. **Mukund P. Tantak,** Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and synthesis of bis(indolyl)ketohydrazide-hydrazones as potent antimitotic agents" at International Conference on Current Challenges in Drug Discovery Research (CCDDR 2015), Department of Chemistry, Malaviya National Institute of Technology, Jaipur, India, 23- 25, November, 2015.

❖ **Poster presentations:**

1. **Mukund P. Tantak,** Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and synthesis of bis(indolyl)ketohydrazide-hydrazones as potent antimitotic agents" at National Conference on organic synthesis and catalysis (NCOSC-2016) organized by Department of Chemistry, Guru Jambheshwar University of Science and Technology, Hisar, Haryana , India, February 17-18, 2016. (*Best poster presentation award*)
2. **Mukund P. Tantak**, Anil Kumar, Rachna Sadana and Dalip Kumar, "Design and synthesis of bis(indolyl) ketohydrazide-hydrazones as potent antimitotic agents" at International Conference on Nascent Developments in Chemical Sciences: Opportunities for Academia-Industry Collaboration, Department of Chemistry BITS Pilani, Pilani Campus, India October 16-18, 2015.
3. P. O. Venkataramana Reddy, **Mukund P. Tantak**, Rajnish Prakash Singh, Okram Mukherjee Singh, Rachana Sadana, Dalip Kumar "Synthesis and biological evaluation of novel carbazolyl glyoxamides as anticancer agents" at International

4. Conference on Nascent Developments in Chemical Sciences: Opportunities for Academia-Industry Collaboration, Department of Chemistry BITS Pilani, Pilani Campus, India October 16-18, 2015.
5. Manish Kumar Mehra, **Mukund P. Tantak**, Indresh Kumar, Dalip Kumar, "Microwave-assisted metal and ligand-free O-arylation of quinolones using diaryliodonium salts" at International Conference on Nascent Developments in Chemical Sciences: Opportunities for Academia-Industry Collaboration, Department of Chemistry BITS Pilani, Pilani Campus, India October 16-18, 2015.
6. **Mukund P. Tantak**, Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and synthesis of bis(indolyl)ketohydrazide-hydrazones as potent and selective novel tubulin inhibitors" at 21st ISCBC-2015, Department of chemistry, CDRI, Lucknow, India February 25-28, 2015.
7. Manish Mehra, **Mukund P. Tantak**, Indresh Kumar, Dalip Kumar, "An expeditious synthesis and anticancer activity of 2-arylamino-5-(indolyl)-1,3,4-oxadiazoles" at 21st ISCBC-2015, Department of chemistry, CDRI, Lucknow, India February 25-28, 2015.
8. P. O. Venkataramana Reddy, **Mukund P. Tantak**, Dalip Kumar "Synthesis and in-vitro anticancer activity of α-cyano bis(indolyl)chalcones" at 21st ISCBC- 2015, Department of Chemistry, CDRI, Lucknow, India February 25-28, 2015.
9. Vishakha Gupta, **Mukund P. Tantak**, Dalip Kumar "Design, synthesis and biological evaluation of 2-(3′-indolyl)-*N*-arylthiazole-4-carboxamides as cytotoxic agents" at National Conference on Frontiers at The Chemistry-Allied Sciences Interface (FCASI-2015), Department of Chemistry, University of Rajasthan, Jaipur, India, March 13-14, 2015.
10. Manish Kumar Mehra, **Mukund P. Tantak**, Indresh Kumar, Dalip Kumar, "An expeditious synthesis and anticancer activity of 2-arylamino-5-(indolyl)-1,3,4-oxadiazoles" at National Conference on Frontiers at The Chemistry-Allied Sciences Interface (FCASI-2015), Department of Chemistry, University of Rajasthan, Jaipur, India March 13-14, 2015.
11. **Mukund P. Tantak,** Anil Kumar, Rachna Sadana, Dalip Kumar, "Design and synthesis of bis(indolyl)ketohydrazide-hydrazones as potent and selective novel

12. tubulin inhibitors." National Conference on Frontiers at The Chemistry-Allied Sciences Interface (FCASI-2015) Department of Chemistry, University of Rajasthan, Jaipur, India March 13-14, 2015.
13. **Mukund P. Tantak,** N. Maruthi Kumar, Anil Kumar, Dalip Kumar, "Hydrazones of indole-2(3)-carboxylic acid hydrazides: Synthesis and preliminary evaluation as anticancer agents." 20th ISCB International Conference (ISCBC-2014) on Chemistry and Medicinal Plants in Translational Medicine for Healthcare at Department of Chemistry, University of Delhi, Delhi, India March 1-4, 2014.
14. P. O. Venkataramana Reddy, **Mukund P. Tantak**, Dalip Kumar, "Design and synthesis of 2-arylamino-5-(indolyl)-1,3,4-oxadiazoles as anticancer agents" at National Conference on Recent Developments in Chemical Sciences, Department of Chemistry, Guru Jambheswar University, Hisar, Haryana, India, February 25-26, 2014.
15. **Mukund P. Tantak,** N. Maruthi Kumar, Anil Kumar, Dalip Kumar, "Synthesis and biological evaluation of α-cyano bis(indolyl)chalcones as novel anticancer agents." International Symposium on Recent Advances in Medicinal Chemistry *at* National Institute of Pharmaceutical Education & Research (NIPER) S.A.S.Nagar-160062, Punjab, India September 8-10, 2014.
16. **Mukund P. Tantak,** N. Maruthi Kumar, Anil Kumar, Dalip Kumar, "Synthesis and biological evaluation of α-cyano bis(indolyl)chalcones as novel anticancer agents." at National Conference on Emerging Areas in Chemical Education & Research and National Convention of Chemistry Teachers" NCCT 2014, The IIS University, Jaipur, India, October 16-18, 2014. (*Best poster presentation award*)
17. **Mukund P. Tantak,** Anil Kumar, Dalip Kumar, "A facile and efficient synthesis of 2-arylamino-5-(3$^{'}$-indolyl)-1,3,4-oxadiazoles as cytotoxic agents" 19th ISCBC-2013, at Department of Chemistry, Mohanlal Sukhadia University, Udaipur, Rajasthan, India March 2-5, 2013.

BRIEF BIOGRAPHY OF THE CANDIDATE [A-3]

Tantak Mukund Pandurang born in Shrirampur, Maharashtra, India. He pursued his Bachelor (Chemistry) and Master degree (Organic Chemistry) from R.B.N.B College, Shrirampur, University of Pune, India during 2007 and 2009, respectively. In Novemberv2009, he joined Jubilant Chemsys Ltd, Noida as a Trainee Research Associate and continued till December 2010. During this period, he involved in a variety of projects dealing with the synthesis of diverse bioactive organic molecules. In this period, he was promoted to Research Associate. In December 2010, he joined Department of Chemistry, BITS Pilani as project fellow in Ranbaxy Sponsored Research Project under the supervision of Prof. Dalip Kumar and Prof. Anil Kumar. Subsequently, in 2011 January, he registered for Ph.D program under the guidance of Prof. Dalip Kumar and Prof. Anil Kumar with the financial assistance from the Ranbaxy Sponsored Research Project. During the Ph.D tenure, he has presented (oral/poster) his research work in various national and international conferences and received one time best oral presentation and two times best poster presentation awards. He has published research articles in well renowned international journals.

BRIEF BIOGRAPHY OF THE SUPERVISOR [A-4]

Dr. Dalip Kumar is a Professor of Chemistry at 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. Rajender S. Varma at Sam Houston State University, TX, USA. He was also associated with Prof. Sean M. Kerwin as a post-doctoral fellow (1999-2000), College of Pharmacy, University of Texas at Austin, TX, USA. He joined BITS Pilani, Pilani campus, as a lecturer during 2000-2002. Later, in December 2002, he moved to University of Maryland, College Park, MD, USA as a Research Associate. In 2004, Prof. Kumar rejoined BITS Pilani, Pilani campus, as an Assistant Professor, at Department of Chemistry and since then he is continuing there. He was promoted to professor in year 2012. He has been involved in research for the last 20 years and in teaching for 13 years. As a result of his research accomplishment, he has around 120 international publications in peer reviewed journals. Prof. Kumar has guided six Ph.D students and currently he is supervising six Ph.D. students. He has one US patent, one Indian patent and handled several projects from DST, DRDO, UGC, CSIR and DBT. Currently, he has one DST-JSPS Indo-Japan project and a collaborative industrial project from Ranbaxy Research Laboratory Ltd.

Prof. Kumar is recipient of Honorary Diploma for Scientific Achievements and International Scientific Collaboration by Russian International Charitable Foundation “Scientific Partnership”, Moscow, Russia (March 2013). He received the Prof. R. D. Desai 80th Birthday Commemoration Medal and Prize from Indian Chemical Society for year 2015. He is an Associate Editor of Chemistry & Biology Interface Journal published by Indian Society of Chemists and Biologists, Lucknow. Prof. Kumar is life members of Indian Chemical Society, Indian Society of Chemists and Biologists, and Indian Council of Chemists. His current research pursuit is focused on synthesis of indole and porphyrin derived potential anticancer agents by employing transition-metals and organoiodine reagents.

BRIEF BIOGRAPHY OF THE CO-SUPERVISOR [A-5]

Dr. Anil Kumar is Associate Professor of Chemistry at the Birla Institute of Technology and Science, Pilani. He obtained his Ph.D degree from Department of Chemistry, University of Delhi, Delhi, India under the supervision of Prof. S. M. S. Chauhan in 2004. Dr. Anil Kumar worked on development of heterogeneous catalyst for organic synthesis with emphasis on green chemistry for his doctoral studies. During May 2004 to April 2006, he worked in Prof. Keykavous Parang group as postdoctoral fellow at Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, USA. He joined Department of Chemistry, Birla Institute of Technology and Science, Pilani, India as Assistant Professor in 2006 and was promoted to Associate Professor in February 2013. He was appointed as Head of Department of Chemistry in September 2014. He has visited University of Rhode Island, Kingston, USA as visiting scientist and Acadia University, Wolfville, Canada as Harrison McCain visiting professor.

Dr. Kumar is recipient of Harrison McCain Foundation award from Acadia University, Canada for 2012, ISCB Young Scientist award in Chemical Sciences from Indian Society of Chemists and Biologists, Lucknow for 2013 and Dr. Aravind Kumar memorial award from Indian Council of Chemist for 2014. He has 16 year of research experience and 10 year of teaching experience. He has published over 125 research papers in international journals of repute and contributed two book chapters and one US patent. He has participated in several national and international symposia/conferences and delivered more than 25 invited lectures. He has guided four Ph.D students and currently supervising 9 Ph.D students. He is editor for Canadian Chemical Transactions and member of editorial advisory board for The Open Catalysis Journal. He has completed three research projects as Principle Investigator and one as Co-PI sponsored by DST, CSIR and UGC. Currently, he has one research project from CSIR and one industry project from Ranbaxy in collaboration with Prof. Dalip Kumar. He is member of American Chemical Society and life member of Chemical Research Society of India, Bangalore; Indian Society of Chemists and Biologists, Lucknow and Indian Council of Chemists, Agra. His research interest lies in transition metal catalyzed C−H activation, green chemistry, ionic liquids and medicinal chemistry.