Synopsis

The work carried out in the research tenure has been compiled in the form of a thesis entitled "**Design and Development of Indole and Biphenyl Linked Heterocycles as Potent Anticancer Agents**". The main aim of this work is design and synthesis of biologically active trimethoxyaroyl indole-benzimidazole conjugates, arylindole-glyoxamides, arylamino linked biphenyl propenones and aryl (biphenylamino) Propenone-quinazolinone conjugates and evaluated for their anticancer activity and apoptosis inducing ability. The thesis has been divided into four chapters.

- **CHAPTER I:** This chapter gives the general introduction about cancer chemotherapy, DNA interchlators, tubulin polymerization inhibitors and objectives of the present work.
- **CHAPTER II:** This chapter describes the desige and synthesis of trimethoxyaroyl indole-benzimidazole conjugates and evaluated their cytotoxicity against a panel of four human cancer cell lines. Further, the apoptosis inducing ability of these derivatives was confirmed by Hoechst staining, measurement of mitochondrial membrane potential ROS generation and Annexin V-FITC assays.
- **CHAPTER III:** This chapter deals with the design and synthesis of trimethoxyaroyl indole-glyoxamide conjugates and evaluated their cytotoxicity against a panel of four human cancer cell lines.
- **CHAPTER IV (SECTION-A):** This chapter illustrates the synthesis and biological evaluation of arylamino linked biphenylpropenones conjugates as tubulin polymerization inhibitors. Further these compounds have been evaluated for their ability to induce apoptosis**.**
- **CHAPTER IV (SECTION-B):** This chapter deals with the design and synthesis of aryl (biphenylamino) Propenone-quinazolinone conjugates and evaluated their cytotoxicity against a panel of four human cancer cell lines

Chapter-I

Introduction

Cancer, characterized by uncontrolled growth or spread of abnormal cells, posses a significant challenge with high rates of diagnosis and mortality. Chemotherapy plays a very important role in treatment of solid tumors and is used as an adjuvant to surgical or radio therapeutic procedures. Chemotherapy becomes critical to effective treatment because only systemic therapy can attack micro metastases. The chemotherapeutic agents target the fast dividing abnormal cells and can be categorized into functional sub groups: alkylating agents, anti metabolites, antibiotics and antimitotics such as microtubule targeting agents.

Microtubules are protein biopolymers formed through polymerization of of α- and β-tubulin. Tubulin polymerization is reversible and the dynamic assembly and disassembly of microtubules are involved in a number of cell functions including cell division, migration and shape change. Many natural and synthetic compounds are reported to target the tubulin-microtubule system. Anti mitotic agents derived from natural or synthetic products, generally exert their effect as microtubule stabilizers or polymerizing agents like taxol, paclitaxel and docetaxal which block the microtubule disassembly. They bind at the α-tubulin site in the microtubules and are used in the treatment of carcinomas like lung, breast, ovarian and bladder. In contrast, microtubule destabilizers such as colchicine, vinca alkaloids, combretastatin A-4 and E7010 bind at the β-tubulin site in microtubules and cause the depolymerization of microtubules. Many of such agents manifest different limitations in their clinical utility, therefore development of new microtubule targeting agents is of significance.

Combretastatin A-4 (**4a**), is another excellent tubulin polymerization inhibitor that binds to colchicine binding site of the tubulin and demonstrates cytotoxicity against a broad spectrum of human cancer cell lines including MDR cancer cells. However, the *in vivo* efficiency of CA-4 is limited due to poor pharmacokinetics resulting from its high lipophilicity and low water solubility. The structural modifications on CA-4 has led to the development of a number of new CA-4 derivatives that exhibit potent tubulin polymerization inhibitors such as combretastatin A-4 phosphate (**4b**-CA4P, **Figure 1A**) and CA-1 disodium phosphate (CA1P-Oxi 4503) as prodrugs. Similarly, amino substituted combretastatin

derivative (**4c**-AVE-8063) and serine prodrug of combretastatin amine (**4d**-Ombrabulin) have been developed. These prodrugs of CA-4 are in advanced stages of clinical studies.

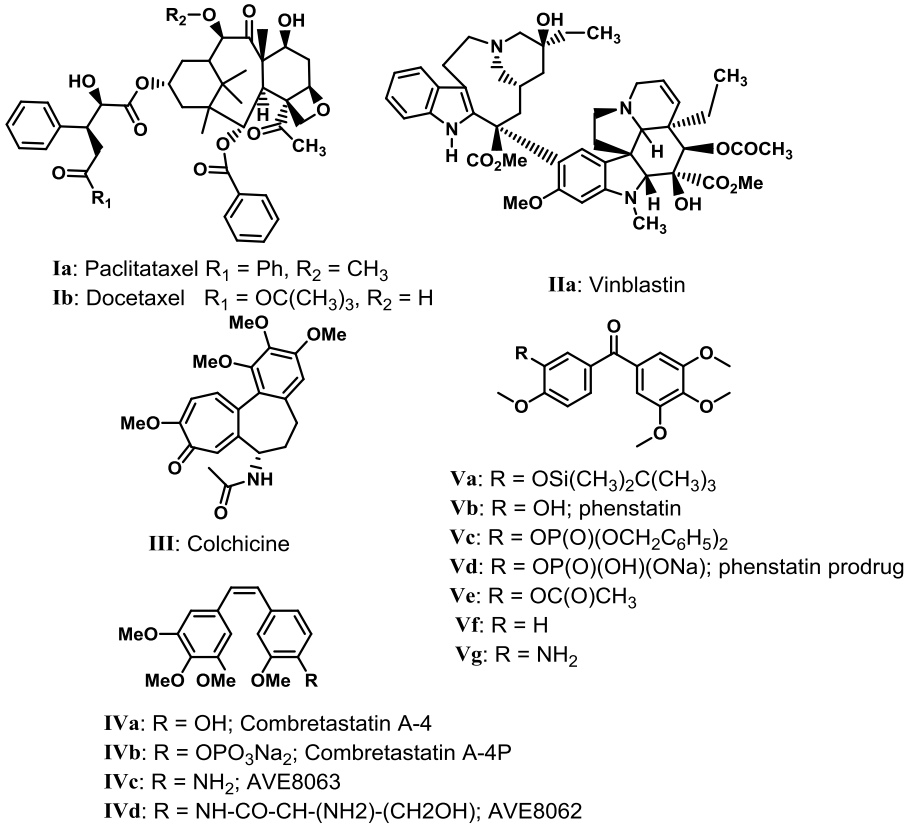


Figure 1. Tubulin depolimerization inhibitos.

Chapter-II

Design and synthesis of trimethoxyaryl indol-benzimidazole conjugates as tubulin polymerization inhibitors.

The main drawback associated with combretastatin A-4 are bioavailability and isomerization of the biologically active *Z*-configured double bond into the inactive *E*-configuration during long storage and administration. To overcome this, the ethynyl bridge of the stilbene moiety was replaced by a biologically stable keto group (phenstatin) and bioisosteric replacement of the (Z)-1,2-ethylene group with a 1,1-ethylene bridge of stilbene

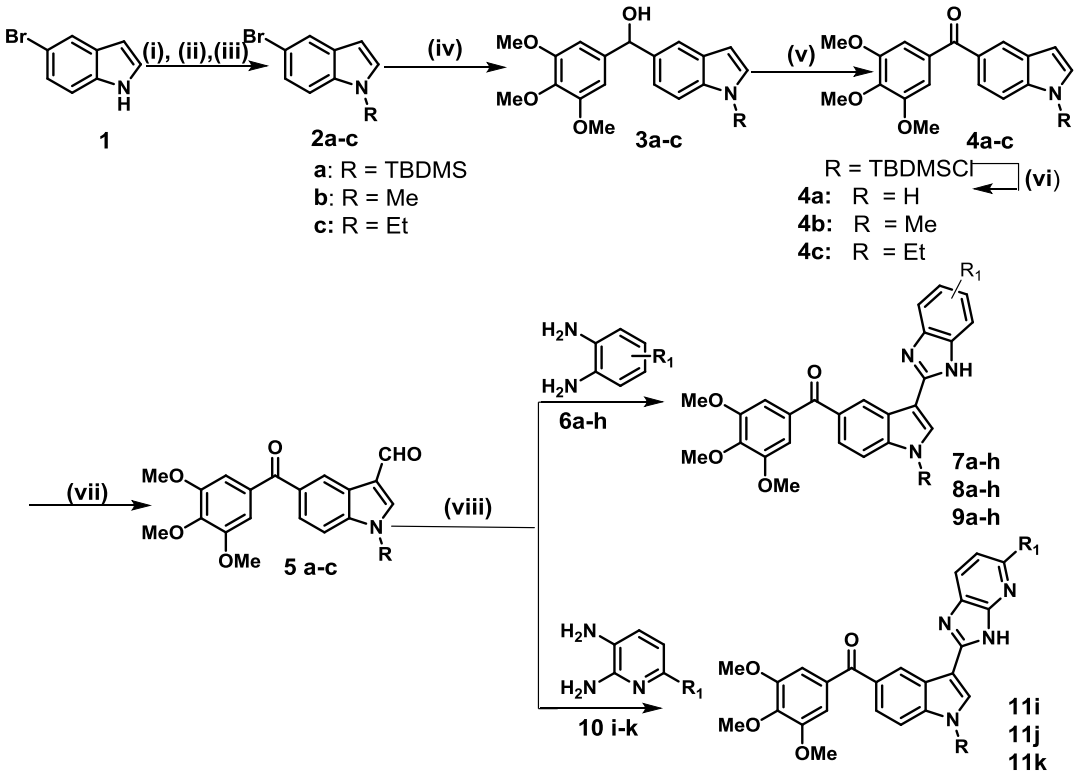
moiety (*iso*combretastatin) that resulted in the retention of the biological activity with improved physical properties. Phenstatin derivatives and *iso*combretastatin A-4 (*iso*CA-4) derivatives exhibited substantial anti-cancer activity and its derivatives were easier to synthesize than the combretastatins (which require control of geometric selectivity) and have greater pharmacological potential due to improved metabolic stability.

On the other hand, benzimidazoles are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Extensive biochemical and pharmacological studies have confirmed that benzimidazole molecules are effective against various strains of microorganisms. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B12. This ring system is present in numerous antiprotozoal, antihelmintics, anti-HIV4, anticonvulsant, antiinflammatory, antihepatic and antineoplastic, antiulcer activities.

Considering, the potent bioactivity of the molecules, phenstatin and *iso*combretastatin A-4 some new benzimidazole molecules have been synthesized that incorporate phenstatin and indole skeleton evaluated their cytotoxic activity. The promising activity observed prompted us to investigate the role of these new compounds in the proliferation and apoptosis of the human prostate cancer cell line.

The trimethoxy aroyl indole-benzimidazole conjugates **7-9(a–h), 11(i-k)** were synthesized in six straight forward reactions. Initially, the commercially available 5-bromoindole (**1**) was protected with tert-butylchlorodimethylsilane (TBDMSCl), alkylated with iodomethane (MeI) and bromoethane (EtBr) in the presence of sodium hydride and DMF to produce corresponding 1- substituted indoles (**2a-c)** respectively. The products (**2a-c)** were further treated with *n*-butyllithium (*n*-BuLi) and 3,4,5-trimethoxybenzaldehyde at -78 ^{o}C to furnish the corresponding alcohols (**3a-c)** with moderate yields. The oxidized products **5a-c** were isolated with good yields from the reaction of **4a-c** with 2-iodoxybenzoic acid (IBX) in DMSO and after oxidation, the deprotection of TBDMS group with tetra-*n*-butylammonium fluoride (TBAF) provided **4a**. Next, Vilsmeier reaction of **6a-c** with phosphoryl chloride ($POCl_3$) and DMF afforded **(7a-c)** in good yields. Finally, the derivatives **7-9(a–h)** and **11(i-k)** were generated by condensation of various aldehydes **7a-c**

with substituted benzene-1,2-diamine (OPDs) in the presence of sodium metabisulfite ($Na_2S_2O_5$) as shown in **Scheme 1**.



7a: R = H, R_1 = H	**8a**: R = Me, R_1 = H	**9a**: R = Et, R_1 = H
7b: R = H, R_1 = 4CH_3	**8b**: R = Me, R_1 = 4CH_3	**9b**: R = Et, R_1 = 4CH_3
7c: R = H, R_1 = 4F	**5c**: R = Me, R_1 = 4F	**9c**: R = Et, R_1 = 4F
7d: R = H, R_1 = 4Cl	**8d**: R = Me, R_1 = 4Cl	**9d**: R = Et, R_1 = 4Cl
7e: R = H, R_1 = 4 CF_3	**8e**: R = Me, R_1 = 4 CF_3	**9e**: R = Et, R_1 = 4 CF_3
7f: R = H, R_1 = 4,5-diCH_3	**8f**: R = Me, R_1 = 4,5-diCH_3	**9f**: R = Et, R_1 = 4,5-diCH_3
7g: R = H, R_1 = 4,5-diCl	**8g**: R = Me, R_1 = 4,5-diCl	**9g**: R = Et, R_1 = 4,5-diCl
7h: R = H ,R_1 = 4F,5Cl	**8h**: R = Me ,R_1 = 4F,5Cl	**9h**: R = Et ,R_1 = 4F,5Cl
11i: R = H, R_1 = OMe	**11j**: R = Me, R_1 = OMe	**11k**: R = Et, R_1 = OMe

Scheme 1: *Reagents and conditions*: (i) TBDMSCl, NaH, THF, 0 °C-rt, 3 h, 93%; (ii) MeI, NaOH,DMSO, 0 °C-rt, 3 h, 95%; (iii) EtBr, NaH, THF,0 °C-rt, 3 h, 95% (iv) 3,4,5-trimethoxy benzaldehyde, n-BuLi, THF, -78 °C, ,4 h, 73-75%, (v) IBX, DMSO, 0 °C–rt, 3 h, 95%; (vi) TBAF, THF, 0 °C-rt, 4 h, 90% (vii) $POCl_3$, DMF, $CHCl_3$, reflux, 12 h, 78-82%; (viii) $Na_2S_2O_5$, EtOH:H_2O, 80 °C, 2 h, 68-85%.

The conjugates **7-9(a–h) 11(i-k)** were evaluated for their cytotoxic activity on a panel of different human cancer cell lines such as A549 (lung), HeLa (cervical), MCF-7 (breast) and DU-145 (prostate) by employing MTT assay. IC_{50} values are expressed in

μM and compared with nocodazole as control. Some of the derivatives show significant activity against most of the cell lines tested with IC_{50} values ranging from 0.54 to 31.86 μM. Among them derivatives **7c-e**, **7i**, **8b**, **8c**, **8f-h**, **9a**, **9b**, **9d-f** and **9h** showed considerable activity with IC_{50} values of <5 μM and majority of derivatives show good cytotoxicity against prostate cancer cell line (DU-145). However, derivatives **8g** and **9f** were found to be very active against DU-145 cell line with IC_{50} values of 0.68 μM and 0.54 μM respectively. Therefore, the DU-145 cell line was chosen as a model cell line for subsequent experiments.

Chapter-III

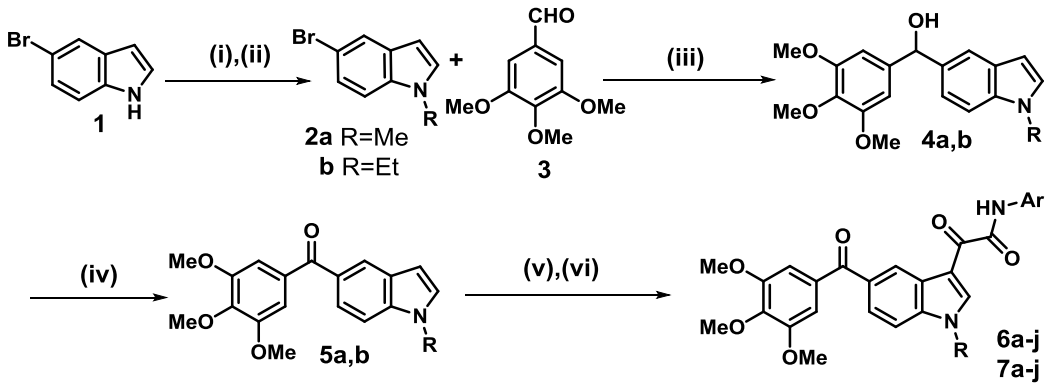
Synthesis and biological evaluation of tirmethoxyaryl indole glyoxamide conjugates as potential anticancer agents.

Indoles are the common components of fragrances and precursors of many pharmaceuticals. The amino acid tryptophan is an indole derivative and a precursor of the neurotransmitter serotonin and many biological macromolecules in our body. Although indole derivatives were used as monomers to synthesis phthalocyanines which can then form complexes with different metal ions, and these complexes exhibit some antiprion activities, the inhibitory effects of some indole derivatives on the amyloid fibrillation of the hen egg white lysozyme (HEWL) were the first example of indole derivatives as small molecules studied in the context of amyloidogenesis. Among the indole derivatives tested, indole-3-acetic acid, indole-3-carbinol and tryptophol had the most inhibitory effects on the amyloid formation. Disaggregation of previously formed HEWL amyloid fibrils was also enhanced by indole-3-acetic acid.

On this representation we designed and synthesized aryl indole based glyoxamides and evaluated their cytotoxic activity against four human cancer cell lines.

The trimethoxy aroyl indole-glyoxamide conjugates **6(a–j)**,**7(a–j)** were synthesized in four straight forward reactions. Initially, the commercially available 5-bromoindole (**1**) was alkylated with iodomethane (MeI) and bromoethane (EtBr) in the presence of sodium hydride and DMF to produce corresponding 1- substituted indoles (**2a, 2b)** respectively. The products **(2a, 2b)** were further treated with n-butyllithium (*n*-BuLi) and 3,4,5-trimethoxybenzaldehyde

at -78 ^{o}C to furnish the corresponding alcohols (**4a,4b)** with moderate yields. The oxidized products (**5a, 5b)** were isolated with good yields from the reaction of **(4a,4b)** with 2-iodoxybenzoic acid (IBX) in DMSO and after oxidation, products (**5a, 5b)** were treated with oxalyle chloride in dry THF for 3 h a yellow scurvy was formed. To this, add *N,N*-diisopropyl ethyl amine and different substituted anilines and allow it for 3h on heating 60 ^{o}C as shown in **Scheme 2**.



6a: R= Me, Ar = 3-fluoroaniline
6b: R= Me, Ar = 3-(trifluoromethyl)aniline
6c: R= Me, Ar = 4-fluoroaniline
6d: R= Me, Ar = 4-methoxyaniline
6e: R= Me, Ar = 3,4-difluoroaniline
6f: R= Me, Ar = 3,4-dimeyhoxyaniline
6g: R= Me, Ar = 3,5-dimethoxyaniline
6h: R= Me, Ar = 2-fluoro-3-(trifluoromethyl)aniline
6i: R= Me, Ar = 3,4,5-trimethoxyaniline
6j: R= Me, Ar = 3,4,5-trifluoroaniline

7a: R= Et, Ar = 3-fluoroaniline
7b: R= Et, Ar = 3-(trifluoromethyl)aniline
7c: R= Et, Ar = 4-fluoroaniline
7d: R= Et, Ar = 4-methoxyaniline
7e: R= Et, Ar = 3,4-difluoroaniline
7f: R= Et, Ar = 3,4-dimeyhoxyaniline
7g: R= Et, Ar = 3,5-dimethoxyaniline
7h: R= Et, Ar = 2-fluoro-3-(trifluoromethyl)aniline
7i: R= Et, Ar = 3,4,5-trimethoxyaniline
7j: R= Et, Ar = 3,4,5-trifluoroaniline

Scheme 2. *Reagents and conditions*: (i) MeI, NaOH, DMSO, 0 °C-rt, 3 h, 95%; (ii) EtBr, NaH, THF,0 °C-rt, 3 h, 95% (iii) n-BuLi, THF, -78 °C, ,4 h, 73-75%, (iv) IBX, DMSO, 0 °C–rt, 3 h, 95%; (v) Oxalyl chloride, THF, rt, 3 h (vi) *N,N*-diisopropyl amine, substututed anilins, 60 °C 3h.

The conjugates **6(a–j)7(a-j)** were evaluated for their cytotoxic activity on a panel of deferent human cancer cell lines such as DU-145 (prostate), A549 (lung), HCT-116 (colon), HepG2 (liver) and by employing MTT assay. IC_{50} values expressed in µM and compared with nocodazole as control. Some of the derivatives show significant activity against most the cell lines tested with IC_{50} values ranging from 1.24 to 138.03 µM.

Chapter-IV (Section-A)

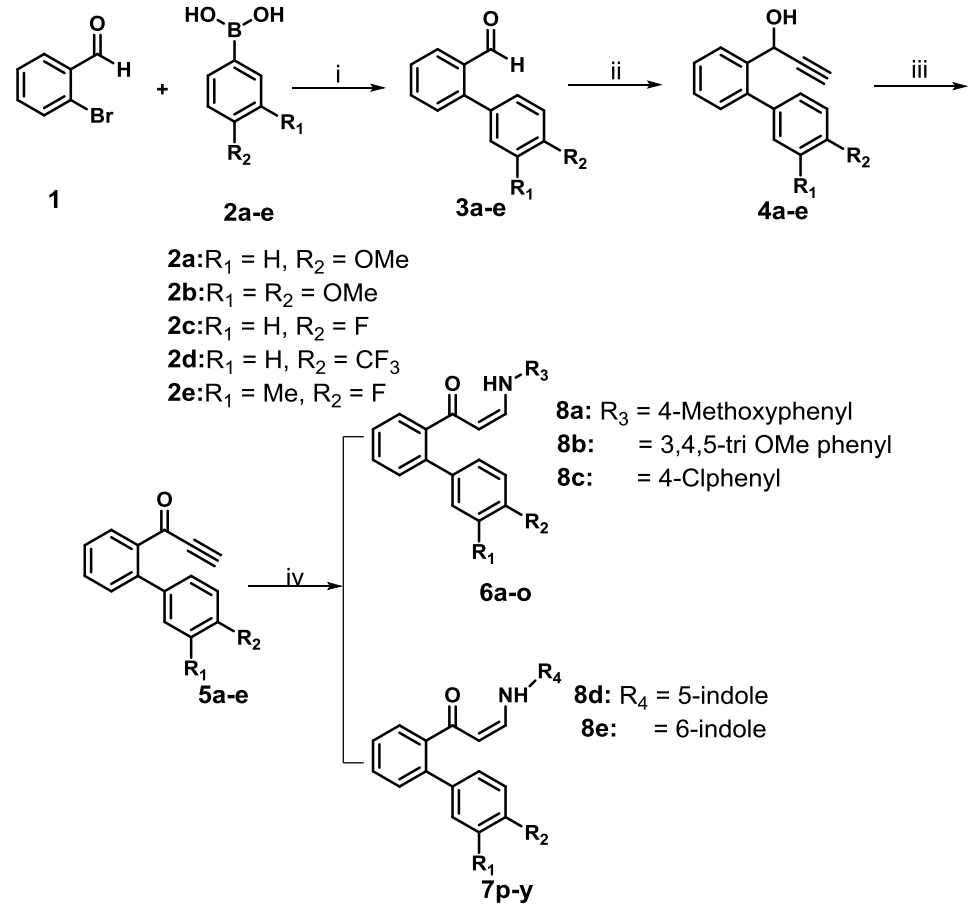
Design, synthesis and biological evaluation of arylamino linked biphenylpropenone conjugates as tubulin polymerization inhibitors.

Because of the biaryl configuration, Colchicine and allocolchicinoid derivatives are effective binding agents with tubulin. This biaryl configuration is also present in a wide range of cytotoxic natural products such as steganacin, steganone, eupomatilone, buflavine, dibenzocyclooctadiene lignans and many synthetic derivatives. B. Sridhar and coworkers designed, synthesized a new class of nitrovinyl biphenyl compounds **.**On the basis of structure of colchicines and allocolchicines by the modification of B ring, which exhibit to obstruct tubulin polymerization and cause mitotic arrest. Compared with colchicine in MCF-7 and HeLa cells, majorty of these compounds were found to possess potent anticancer properties, with IC_{50} values in the range of 0.05–7 μm. These compounds inhibited tubulin assembly by more than 60%, and by the studying of flow cytometry analysis, it indicates the growth arrest of cells in the G2/M phase of the cell cycle in a concentration-dependent manner. Treatment of cells with resulted in up regulation of cyclin B1 and aurora kinase B mRNA levels, corresponding to growth arrest in the G2/M phase of the cell cycle as the mode of action.

Reddy and co-workers synthesized a series of (*Z*)-1-aryl-3-arylamino- 2-propen-1-ones and were evaluated for their anti-proliferative activity against two different human tumor cell lines derived from human prostate (DU145) and leukemic (K562) cancers. These compounds displayed good anticancer activity with IC_{50} ranging from 0.06 to100 μM. The most active compound (*Z*)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4 methoxyphenylamino)prop-2-en-1-one exhibited promising activity with IC_{50} 0.06, 0.1 μM against DU145 and K562 cell lines respectively. Cytotoxicity data demonstrated that presence of a halo group at the ortho position moderately improved the cytotoxic activity of the molecules compared to the corresponding ortho-unsubstituted compounds. Further studies demonstrated that cells are arrested G2/M phase of the cell cycle and these compounds caused microtubule stabilization like paclitaxel and induced apoptosis *via*

activation of the caspase family. Therefore (*Z*)-1-aryl-3-arylamino-2-propen-1-one represents a new class of microtubule-stabilizing agents

On the basis of above literature we designed, synthesized and evaluated a series of arylamino linked biphenylpropenone conjugates as shown in **scheme 3.**



Scheme 3. *Reagents and conditions*: (i) $Pd(OAc)_2$, K_2CO_3, DMF:H_2O (1:2), 3 h, rt, 85%, (ii) Ethynyl magnesium bromide, THF, 0 °C-rt, 3 h, 65%, (iii) IBX, DMSO, 0 °C, 2 h, 75% (iv) Substituted anilines (**10a-e**), EtOH, 4 h, rt, 80-85%.

A series of arylamino-biphenylpropenones conjugates **6(a-o)** and **7(p-y)** were synthesized and developed a potent conjugates **6j** and **7x** which displayed a strong cytotoxic activity with IC_{50} values of 0.9 and 0.7 μM against the human A549 cancer cell lines. Flow cytometry analysis indicates that these compounds induce cell cycle arrest at G2/M phase in A549 cells. Tubulin polymerization assay showed that they are potent inhibitors of tubulin

polymerization and immunocytochemistry revealed loss of intact microtubule structure in cells treated by these conjugates **6j** and **7x**. In addition, they induce apoptosis in A549 cells by studying their effect on Hoechst staining and tunnel assay. Overall, these results demonstrate that the arylamino-biphenylpropenones conjugate have the potential to be developed as leads and their further amenable structural modifications may produce promising anticancer agents for A549 cancer cells.

Chapter-IV (Section-B)

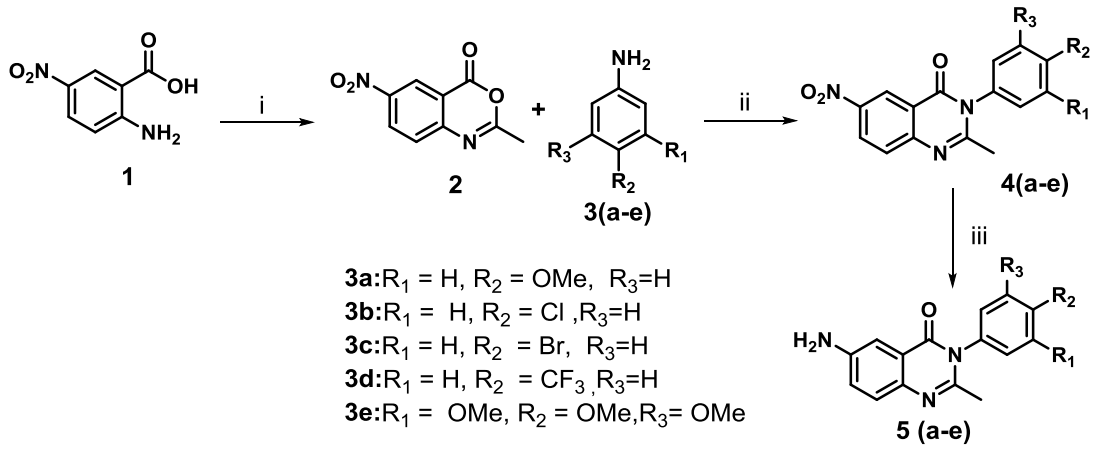
Design and synthesis of aryl biphenylamino propenone quinazolinone conjugates and evaluated their cytotoxicity.

In continuation to these efforts on the design of new anticancer agents, a series of quinazolinone amino linked biphenyl propenone derivatives have been synthesized and evaluated for their cytotoxic activity against four human cancer cell lines.

Synthesis of quinazolinone amino linked biphenyl propenones

1. Synthesis of 6-amino-2-methyl-3-phenylquinazolin-4(3*H*)-ones

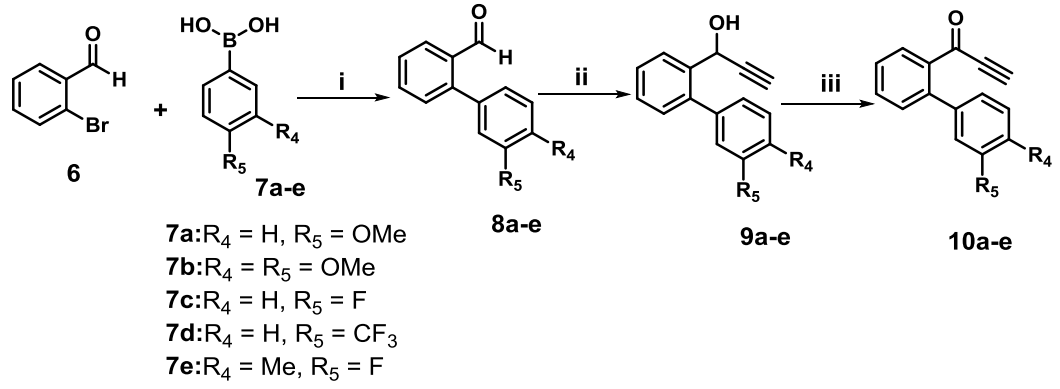
Aminoquinazolinones **(5a-e)** were prepared as shown in **scheme.4**. Anthranilic acid (**1**) on reaction with acetic anhydride at 150 ^{o}C afford the cyclized product 2-methyl-6-nitro-4*H*-benzo[d][1,3]oxazin-4-one (**2**). This upon reaction with different substituted aryl anilines (**3a–e**) in acetic acid under reflux conditions provide the corresponding 2-methyl-6-nitro-3-phenylquinazolin-4(3*H*)-ones (**4a–e**). Which on further reduced with palladium-carbon formed the 6-amino-2-methyl-3-phenylquinazolin-4(3*H*)-ones **(5a-e)**.



Schem 4. *Reagents and conditions*: (i) AC_2O, 3h, 150 0 °C, 85%, (ii) AcOH, 90 °C 3 h, 65%, (iii) EtOAc/Pd-C, rt, 3h 90%.

Synthesis of 1-([1,1'-biphenyl]-2-yl)prop-2-yn-1-ones

Biphenyl propenones were prepared as shown in **scheme 5,** a mixture of 2-bromo benzaldehyde **(1)** corresponding boronic acids **(2a-e)** palladium(II) diacetate, sodium carbonate (Na_2CO_3) and *N*,*N*-dimethyl farmamide(DMF):water (2:1) was stirred for 3 hours at room temperature to produce [1,1'-biphenyl]-2-carbaldehydes **(3a-e).** After that, these aldehydes (3a-e) were treated with ethynyl magnesium bromide in dry tetrahydro furon (THF) at 0 °C to room temperature for 3 hours to produce 1-([1,1'-biphenyl]-2-yl)prop-2-yn-1-ol **4a-e**. Oxidation of 4a-e with 2-iodoxybenzoic acid (IBX) in the presence of dimethyl sulfoxide (DMSO) to give 1-([1,1'-biphenyl]-2-yl)prop-2-yn-1-one **(5a-e)**.



Schem 5. *Reagents and conditions*: (i) $Pd(OAc)_2$, K_2CO_3, DMF:H_2O (1:2), 3 h, rt, 85%, (ii) Ethynyl magnesium bromide, THF, 0 °C-rt, 3 h, 65%, (iii) IBX, DMSO, 0 °C, 2 h, 75%

Condensation of **(10a-e)** with aminoquinazolinones (**5(a-e)** from **Scheme 4)** in ethanol at room temperature resulted in the formation of (*Z*)-6-((3-([1,1'-biphenyl]-2-yl)-3-oxoprop-1-en-1-yl)amino)-2-methyl-3-phenylquinazolin-4(3H)-ones in good yields. as shown in following reaction.