SYNOPSIS

SYNOPSIS

The work carried in the research tenure has been compiled in the form of a thesis entitled "**Synthesis and biological evaluation of new pyrrolobenzodiazepines, tetrazoles and quinazolinone derivatives as potential anticancer agents**". The main aim of this work is the design and synthesis of diaryl ether linked pyrrolobenzodiazepine conjugates, to explore their DNA binding ability as well as anticancer activity and synthesis of tetrazolo linked isoxazoline/cinnamide hybrids, to investigate their anticancer activity and inhibition of tubulin polymerization. Further, chalcone linked quinazolinone hybrids has been prepared and evaluated for their anticancer activity. The thesis has been divided into four chapters.

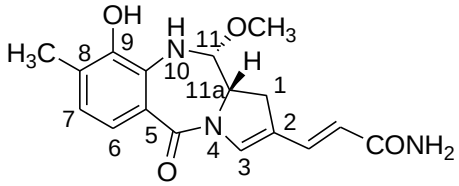
- **CHAPTER I:** This chapter gives the general introduction about cancer chemotherapy, covalent interactions of drug-DNA, particularly of pyrrolo[2,1-*c*]-[1,4]benzodiazepine (PBD) antitumor antibiotics, combretastatins, quinazolinones and the objectives of the present work.
- **CHAPTER II:** This chapter describes the synthesis of diaryl ether-PBD conjugates linked through five carbon spacer or a piperazine and evaluation of their anticancer activity against a panel of eleven human cancer cell lines, and also discussed the DNA-binding ability.
- **CHAPTER III:** This chapter deals with the synthesis and biological evaluation of new tetrazole linked isoxazoline/cinnamide hybrids as potential anticancer agents. Further, the effect of these compounds on cell cycle and inhibition of tubulin polymerization has been studied.
- **CHAPTER IV:** This chapter illustrates the design, synthesis and biological evaluation of new chalcone linked quinazolinone hybrids as potential anticancer agents.

CHAPTER-I

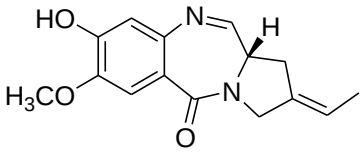
This chapter describes the general introduction about pyrrolobenzodiazepines, combretastatins, quinazolinones and the objectives of the present work.

PYRROLO[2,1-*c*][1,4]BENZODIAZEPINES

Cancer is a group of diseases characterized by uncontrolled growth or spread of abnormal cells. Since it involves the conversion of any normal cells to a cancerous cell exhibiting tandem replication and cell divisions at much faster rate in comparison to the normal cells and thus provides a potential target area for the development of chemotherapeutic agents. It is now clear that chemotherapy's most effective role in solid tumors is as an adjuvant to initial therapy by surgical or radiotherapeutic procedures. Chemotherapy becomes critical to effective treatment because only systemic therapy can attack micrometastases. These agents can be categorized into functional sub groups, alkylating agents, antimetabolites, antibiotics, and antimitotics. The pyrrolo[2,1-*c*][1,4]-benzodiazepines (PBDs) belonging to the class of DNA-interactive antitumor antibiotics have the potential as regulators of gene expression with possible therapeutic application in the treatment of genetic disorders including cancer. The first PBD antitumor antibiotic anthramycin (Figure 1) has been described by Leimgruber coworkers about 40 years back and since then a number of compounds have been developed based on PBD ring system leading to DNA binding ligands.



Anthramycin



Tomaymycin

Figure 1. Chemical structures of anthramycin and tomaymycin

Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of potent naturally occurring low molecular weight antitumor antibiotics originally isolated from various

Streptomyces species. Their common interaction with DNA has been extensively investigated and it is considered unique since they bind within the minor groove of DNA forming a covalent aminal bond between the C11-position of the central B-ring and the N2 amino group of guanine base as depicted in Figure 2. A number of naturally occurring and synthetic compounds based on PBD ring system, such as anthramycin, tomaymycin, DC-81 and its dimers (presently, one of the dimer SJG-136 is under clinical evaluation), have shown varying degrees of DNA binding affinity and anticancer activity.

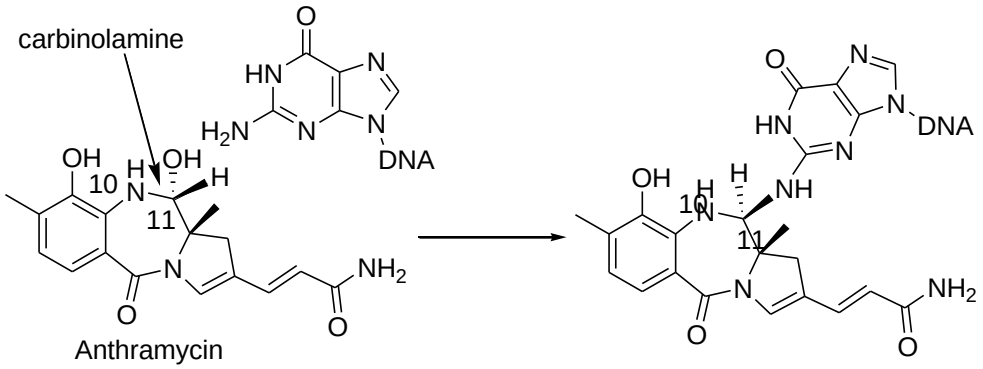


Figure 2. Formation covalent bond between PBD and DNA

Combretastatin A-4 (CA-4, **1**, Figure 3), a natural product isolated by Pettit and co-workers from the bark of South African tree *Combretum caffrum*, exhibits cytotoxicity against a broad range of human cancer cell lines as well as multidrug resistant cell lines by inhibiting tubulin polymerization and binding to the colchicine binding site. A water soluble disodium phosphate prodrug (CA-4P) of CA-4 and AVE8062, are currently under clinical trials for the treatment of cancer.

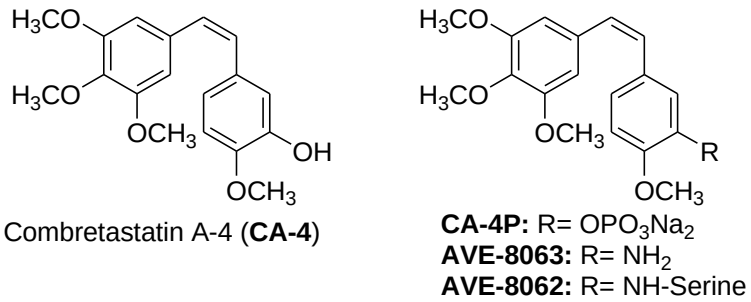
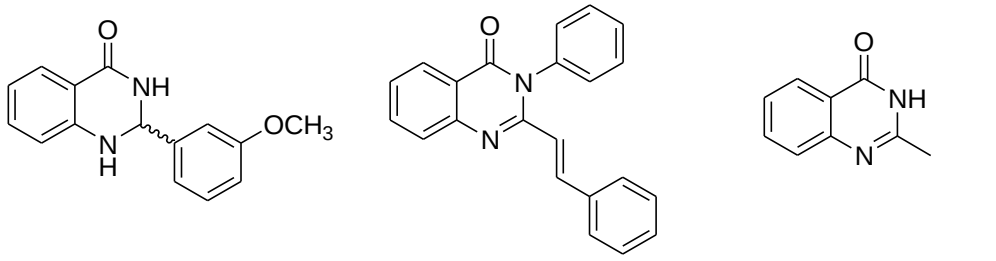


Figure 3. Chemical structures of Combretastatin A-4 derivatives

In recent years there has been an increasing interest in the chemistry of quinazolinones because of their wide range of biological significance. Among the various classes of quinazolinones, 2,3-dihydro/2-styryl quinazolinone and 2-methyl quinazolinone derivatives (Figure 4) exhibited anticancer activity by inhibitory effects on tubulin polymerization or inhibition the DNA repair enzyme poly(ADP-ribose)polymerase (PARP).



2,3-dihydroquinazolinone 2-styrylquinazolinone 2-methylquinazolinone

Figure 4. Chemical structures of various quinazolinones

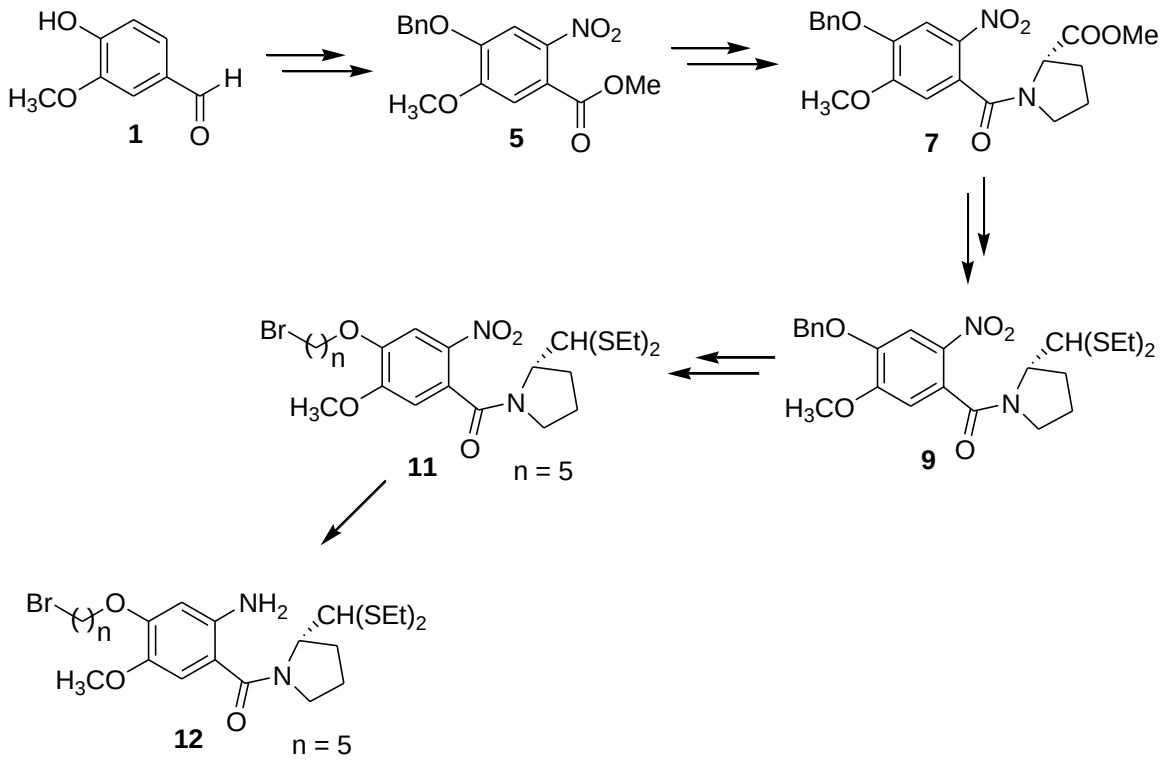
CHAPTER-II

Chemotherapy is often the treatment of choice for many types of cancer as a result the search for newer chemotherapeutic agents still plays a major role in the fight against cancer. In recent years there has been increasing interest in the design of conjugate molecules that could act in a specific manner on more than one target. The development of such conjugates lowers the risk of drug-drug interaction in comparison to cocktails but could also enhance the efficacy as well as improve the safety aspects in relation to the drugs that interact on a single target. Several conjugate compounds, in which a known antitumor compound or some simple active moiety tethered to PBD, have been designed, synthesized and evaluated for their biological activity. Recently, Wang and co-workers have synthesized indole-PBD conjugates as potential antitumor agents and a correlation between antitumor activity and apoptosis has been well explained. More recently, we have also reported some of the PBD conjugates that demonstrate potent apoptotic activity through mitochondrial-mediated pathway.

In continuation of these efforts, substituted diaryl ether derivatives were linked to the C8-position of the PBD scaffold (DC-81) through the stable alkane spacer and also by incorporating a piperazine moiety in spacer linker. This led to a new library of PBD conjugates that were evaluated for their anticancer potential. Therefore this chapter describes the synthesis, DNA-binding ability and anticancer activity of some new PBD conjugates.

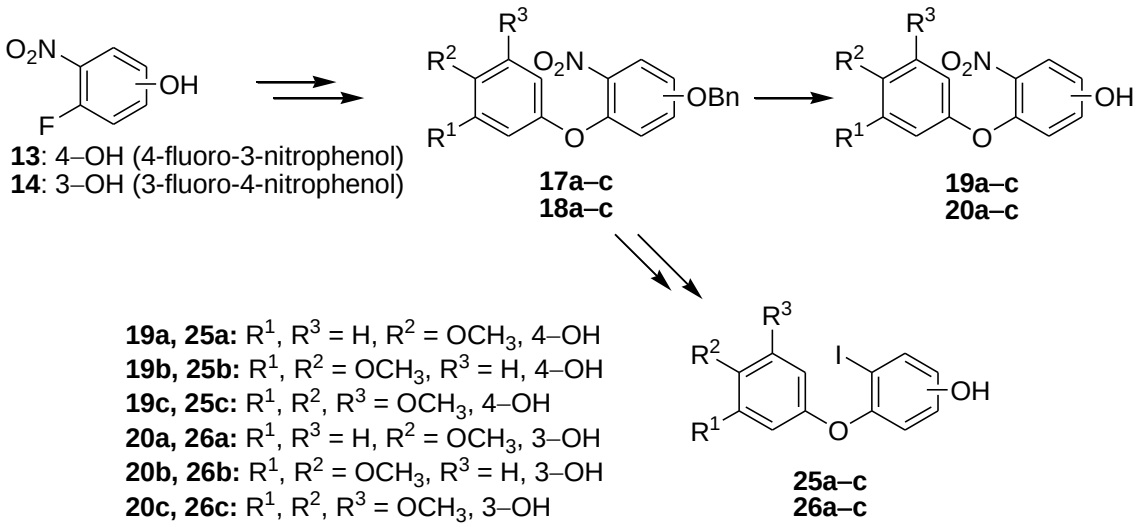
SYNTHESIS OF DIARYL ETHER LINKED PYRROLOBENZODIAZEPINE CONJUGATES

Synthesis of the PBD subunit precursors (**11**, **12**) is carried out by employing the commercially available vanillin (**1**) as the starting material. Oxidation of vanillin in the presence of sulphamic acid and sodium chlorite to form the corresponding carboxylic acid (**2**) and followed by acid-catalyzed esterification with methanol provided methyl benzoate (**3**) in quantitative yield. This is followed by benzylation, nitration and basic hydrolysis to provide 4-benzyloxy-5-methoxy-2-nitrobenzoic acid (**6**). Later this has been coupled to L-proline methyl ester in the presence of triethylamine to afford the compound **7**, which upon reduction with DIBAL-*H* produces the corresponding aldehyde **8**. This product upon protection with EtSH/TMSCl gives compound **9**, which on debenzylation with $BF_3.OEt_2$/EtSH provides the hydroxyl intermediate compound **10**, which upon etherification by dibromoalkane provide **11**, which upon reduction with $SnCl_2 \cdot 2H_2O$ provides **12** (Scheme 1).



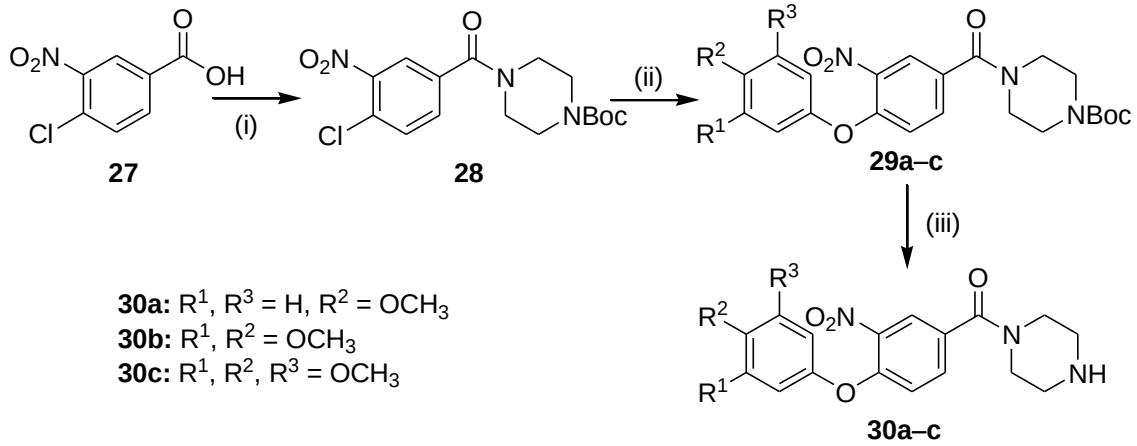
Scheme 1: *Reagents and conditions*: (i) NH_2SO_3H, $NaClO_2$, H_2O, rt, 2 h; (ii) H_2SO_4, MeOH, reflux, 4 h; (iii) benzylbromide, K_2CO_3, acetone, reflux, 24 h; (iv) $SnCl_4$, fuming HNO_3, CH_2Cl_2, 5 min, -25 ^oC; (v) 2N LiOH, MeOH, H_2O, THF (1:1:3), rt, 12 h; (vi) $SOCl_2$, C_6H_6, *L*-proline methylester hydrochloride, THF-H_2O, 1-2 h, rt; (vii) DIBAL-*H*, CH_2Cl_2, 1-1.3 h, -78 ^oC; (viii) EtSH, TMSCl, CH_2Cl_2, 8-12 h, rt; (ix) $BF_3.OEt_2$, EtSH, $CHCl_3$, rt, 8 h; (x) dibromoalkane, K_2CO_3, acetone, reflux, 24 h; (xi) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 4 h.

The synthesis of substituted diaryl ether precursors (**19a–c, 20a–c, 25a–c** and **26a–c**) is outlined in Scheme 2. Compounds 3-fluoro-4-nitrophenol (**13**) and 4-fluoro-3-nitrophenol (**14**) upon benzylation to obtain **15** and **16** which upon substitution with substituted phenols in dry DMF gave the compounds **17a–c** and **18a–c**, which on debenzylation gives **19a–c** and **20a–c** respectively. Compounds **17a–c** and **18a–c** on reduction with $SnCl_2 \cdot 2H_2O$ gives amino compound of **21a–c** and **22a–c**, which on treating with HI and DMSO gives the iodo compound of **23a–c** and **24a–c**. Compound **23a–c** and **24a–c** on debezylation gives the compounds of **25a–c** and **26a–c** respectively.



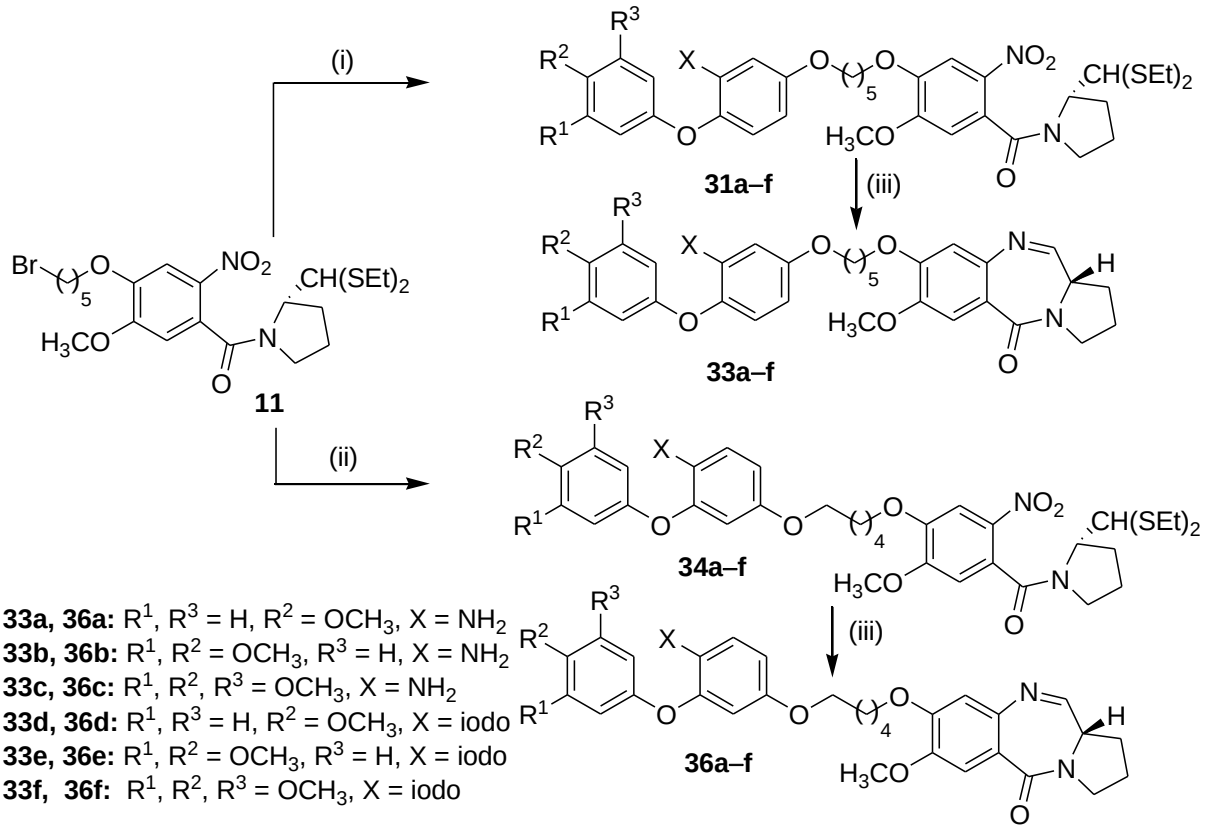
Scheme 2: *Reagents and conditions*: (i) BnBr, dry acetone, reflux, 10-12 h; (ii) substituted phenol, Cs_2CO_3, dry DMF, 70 °C, 10-12 h; (iii) $TiCl_4$, dry DCM, 0 °C, 30 min.; (iv) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 3-4 h; (v) HI, DMSO, CuI, rt, 30 min.

The synthesis of piperazine containing diaryl ether precursors (**30a–c**) is outlined in Scheme 3. Compound **28** is obtained by coupling of commercially available 4-chloro-3-nitrobenzoic acid (**27**) with *tert*-butyl piperazine-1-carboxylate. Compound **28** upon substitution with substituted phenols in dry DMF gives the compound **29a–c**, which on deprotection gives compound **30a–c** (Scheme 3).

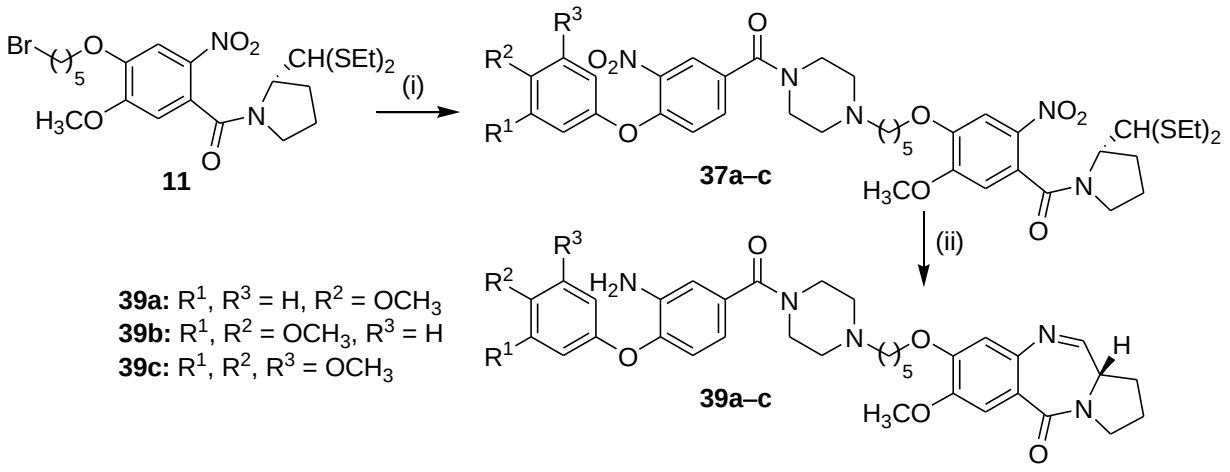


Scheme 3: *Reagents and conditions*: (i) (a) $SOCl_2$, C_6H_6, DMF (cat.); (b) *tert*-butyl piperazine-1-carboxylate, Et_3N, dry THF, 0 °C, 1-2 h; (ii) substituted phenol, Cs_2CO_3, dry DMF, 70 °C, 10-12 h; (iii) CF_3COOH, DCM, 0 °C, 10-12 h.

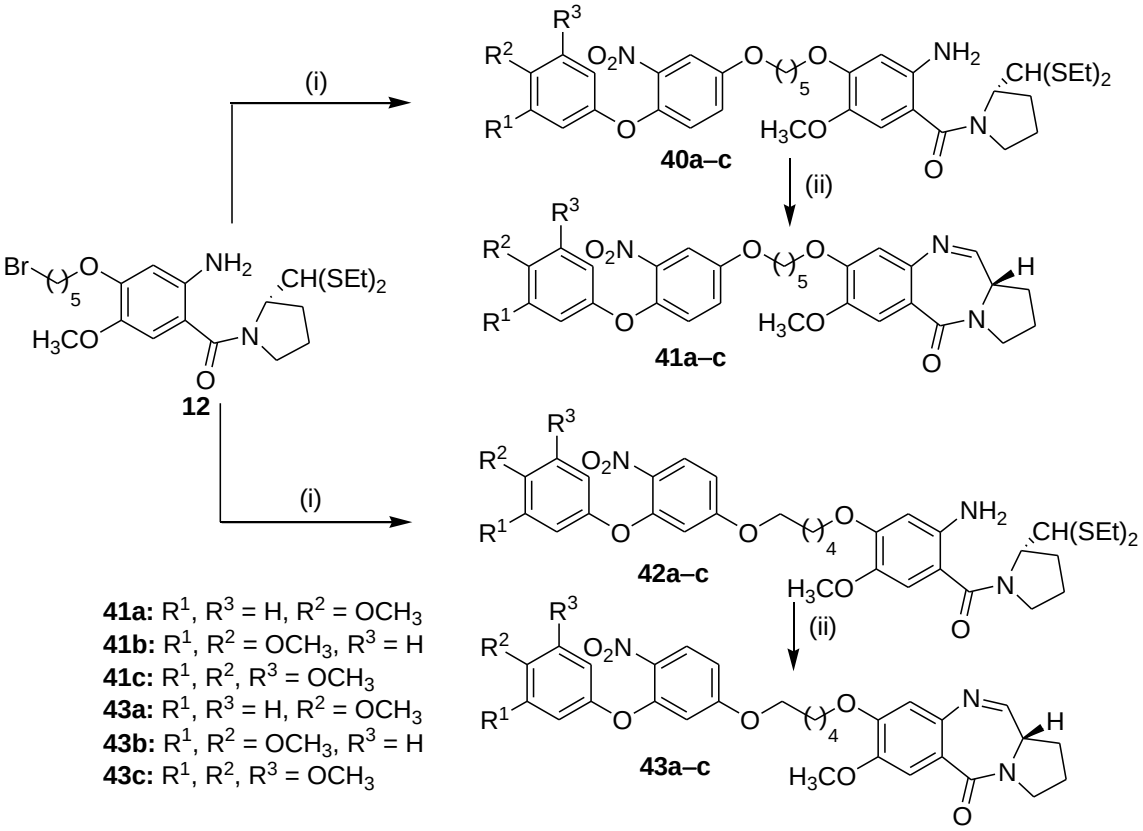
Synthesis of C8-linked diaryl ether-PBD conjugates (**33a–f, 36a–f, 39a–c, 41a–c, 43a–c** and **45a–c**) has been carried out from the (2*S*)-*N*-{4-[5-bromopentyloxy]-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal (**11**) and (2*S*)-*N*-{4-[5-bromopentyloxy]-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehydediethyl thioacetal (**12**), these upon etherification with the phenolic diethyl ether precursors (**17a–c, 18a–c, 25a–c, 26a–c** and **30a–c**) using K_2CO_3 in acetone provided the corresponding nitro thioacetals (**31a–f**, **34a–f** and **37a–c**) and amino thioacetals (**40a–c, 42a–c** and **44a–c**). These nitro thioacetals **31a–f**, **34a–f** and **37a–c** are reduced to the amino thioacetals **32a–f**, **35a–f** and **38a–c** by employing $SnCl_2 \cdot 2H_2O$ in refluxing methanol and then cyclized by treatment with $HgCl_2$ and $CaCO_3$ in MeCN-H_2O to yield the target products **33a–f, 36a–f, 39a–c, 41a–c, 43a–c** and **45a–c** (Schemes 4–7).



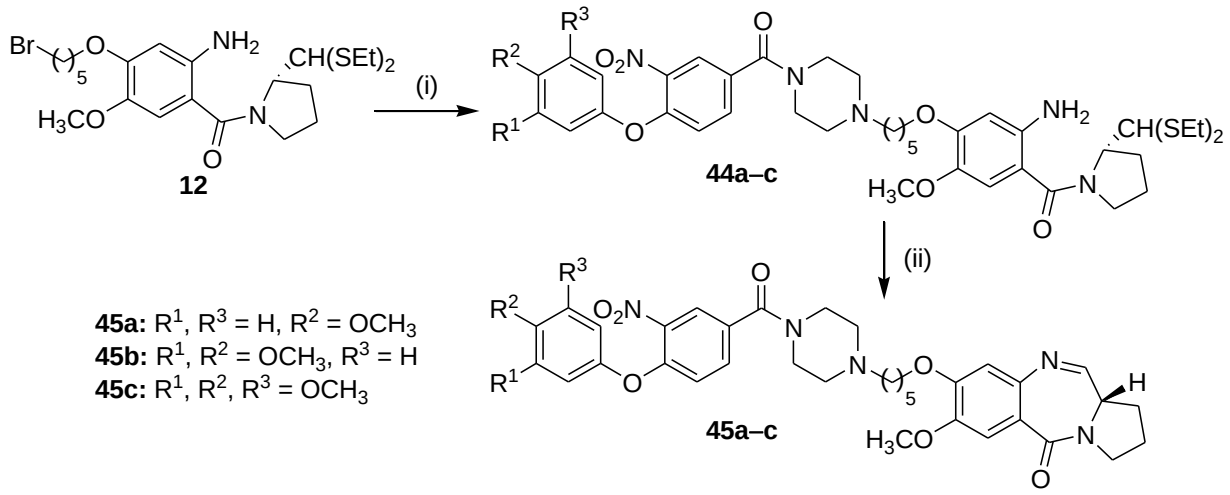
Scheme 4: *Reagents & conditions:* (i) **17a–c** and **25a–c**, K_2CO_3, dry acetone, reflux, 24 h; (ii) **18a–c** and **26a–c**, K_2CO_3, dry acetone, reflux, 24 h; (iii) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 3-4 h; (iv) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O, (4:1), rt, 12 h.



Scheme 5: *Reagents & conditions:* (i) **30a–c**, K_2CO_3, dry acetone, reflux, 24 h; (ii) $SnCl_2.2H_2O$, MeOH, reflux, 3-4 h; (iii) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O, (4:1), rt, 12 h.



Scheme 6: *Reagents & conditions:* (i) **17a–c** and **18–c**, K_2CO_3, dry DMF, rt, 12 h; (ii) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O, (4:1), rt, 12 h.



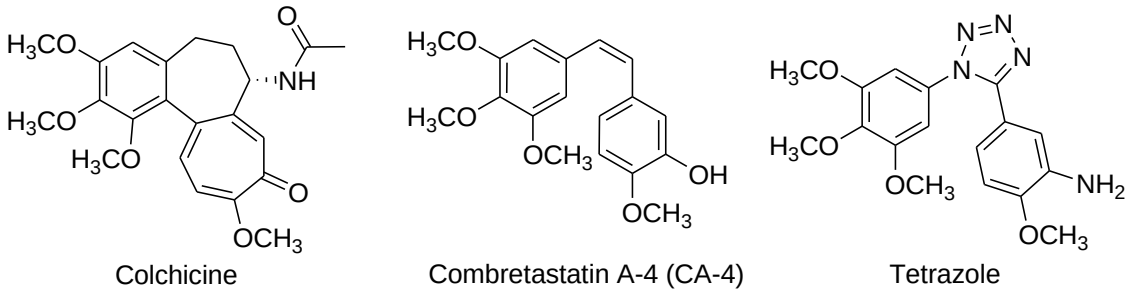
Scheme 7: *Reagents & conditions:* (i) **30a–c**, K_2CO_3, dry DMF, rt, 12 h; (ii) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O, (4:1), rt, 12 h.

Thermal denaturation studies showed that these conjugates exhibit higher DNA binding affinity than the naturally occurring DC-81. All these PBD conjugates **25a–x** and **26a–h** showed significant anticancer activity, with GI_{50} values ranging from 0.01 to 3.41 μM, whereas the positive controls adriamycin and DC-81 demonstrated the GI_{50} in the range of 0.10 to 7.25 μM and 0.10 to 2.37 μM, respectively (Patent filed 0390/DEL/11 (India), PCT/IB2011/000670, manuscript accepted in *Anri-cancer Agents Med. Chem.*).

CHAPTER-III

This chapter describes the design, synthesis, and biological evaluation of tetrazole linked isoxazoline/cinnamides as anticancer agents and tubulin polymerarization inhibitors. Tubulin is a heterodimeric protein consisting of α and β subunits. During cellular division upon binding of GTP, tubulin polymerizes into microtubules. This formation of microtubule is essential for chromosome separation and formation of two daughter cells. Hence, microtubules are the important targets for the development of new potential anticancer therapeutics. When ligands that interact with tubulin are present, a reduction in cellular division is observed and shows

anticancer activity. Tubulin having colchicine binding site and if any ligand binds to this site prevents the tubulin polymerization. Colchicine and combretastatin-A-4 (CA-4) are the good examples as tubulin binding agents. Combretastatin A-4 is a naturally occurring stilbene and isolated from the African willow tree (*Combretum caffrum*). CA-4 shows interesting anticancer potential due to its inhibition of tubulin polymerization property. It strongly binds to the colchicine site of tubulin thus preventing tubulin polymerization and causes antimitotic effect. The *cis* configuration at olefinic bidge is essential for biological activity, unfortunately this *cis* olefin is converted into thermodynamically stable *trans* form during storage and metabolism, which eventually leads to loss of cytotoxicity as well as tubulin polymerization inhibition. To overcome this problem, many heterocyclic five membered rings (thiazole, oxazole, imidazole, triazole, tetrazole etc.) have incorporated to retain *cis* configuration at the olefinic bridge.



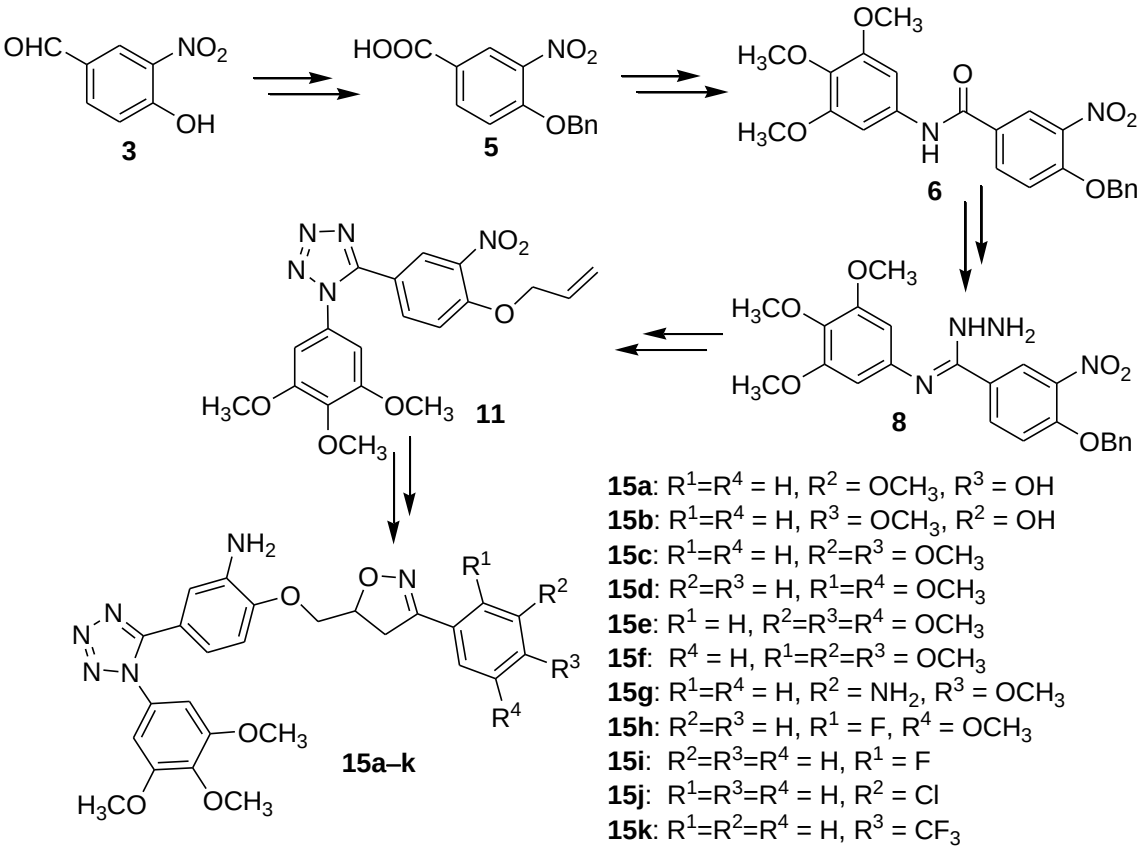
Colchicine | Combretastatin A-4 (CA-4) | Tetrazole

Figure 1. Potential inhibitors of tubulin polymerization

SYNTHESIS OF TETRAZOLE LINLED ISOXAZOLINES

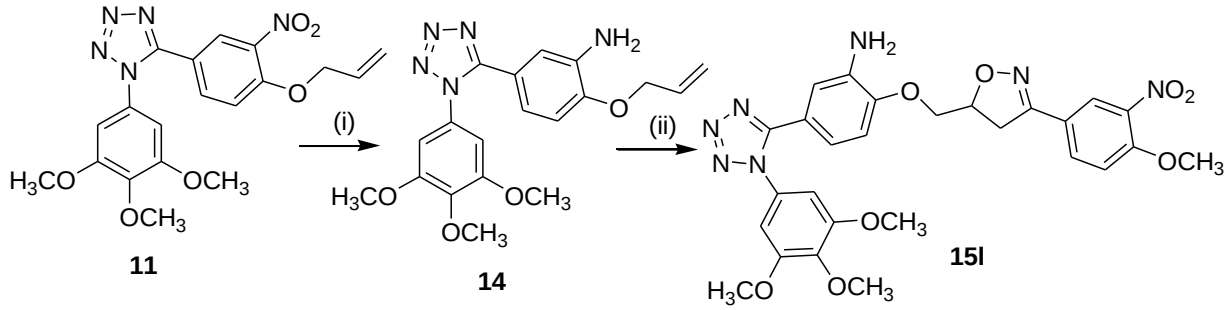
The synthesis of tetrazole linked isoxazoline derivatives (**15a–l**) was carried out by using the commercially available aldehydes (**1a–k, 3**) as the starting material. Reaction of these aldehydes (**1a–k**) with hydroxylamine in a MeOH/H_2O (3:1) solution produced the corresponding oximes (**2a–k)** in high yields. 4-Hydroxy-3-nitrobenzaldehyde (**3)** was benzylated with benzyl bromide to afford the compound **4**, which is oxidized to the acid (**5**) by using oxone. Later this acid (**5**) was coupled with 3,4,5-trimethoxyaniline to afford the amide compound (**6**). Further, amide (**6**) was

converted into thioamide (**7**) by using Lawesson's reagent followed by conversion to its amidrazide (**8**) by using hydrazine hydrate, this was cyclized in the presence of $NaNO_2$ and acetic acid to afford the tetrazole (**9**) in quantitative yield. Later this tetrazole (**9**) was debenzylated with $TiCl_4$ to provide the nitro phenolic compound (**10**), which upon etherification with allyl bromide using K_2CO_3 and DMF to provide the nitro allyloxy compound **11**. This was then coupled to different oximes (**2a–k**) to provide the corresponding tetrazole linked isoxazolines (**12a–k**). The nitro group of tetrazole (**11** and **12a–k**) was reduced to the amine with Zn and acetic acid to afford the amino allyloxy compound (**14**) and the desired tetrazole-isoxazoline hydrids (**15a–k**) as shown in Scheme 1. This amino allyloxy compound (**14**) was coupled to the oxime (**2g**) to provide the desired tetrazole linked isoxazoline (**15l**) as shown in Scheme 2.



Scheme 1. *Reagents & conditions:* (i) $NH_2OH \cdot HCl$, $NaHCO_3$, methanol, water, 0 °C to rt, 3h; (ii) BnBr, K_2CO_3, DMF, rt, 12h; (iii) Oxone, DMF, rt, 10h; (iv) $SOCl_2$, DMF, C_6H_6, 3,4,5-

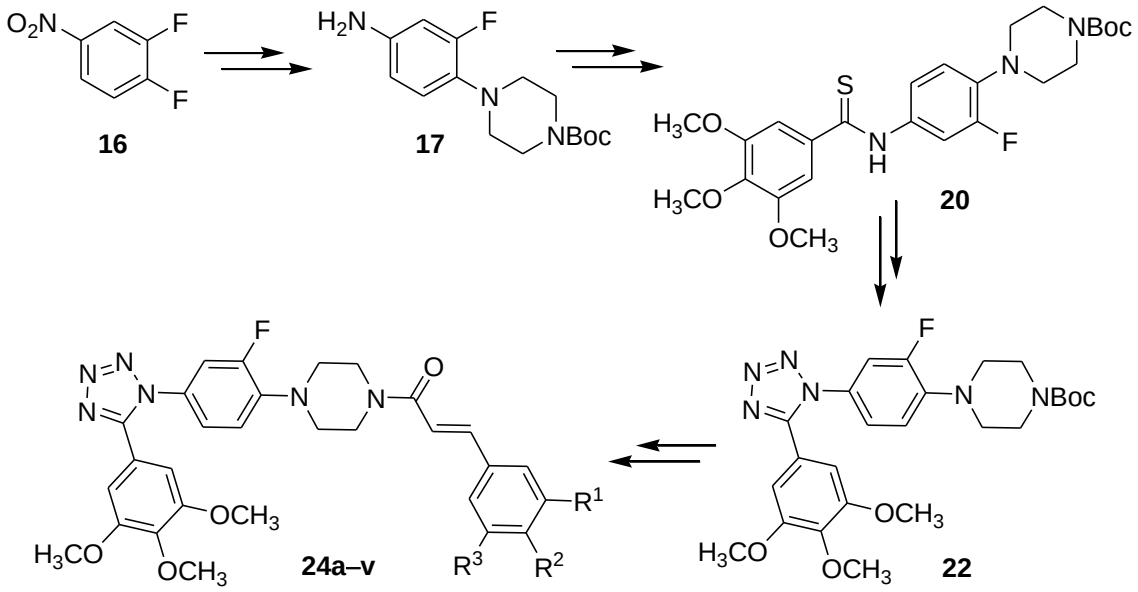
trimethoxyaniline, TEA, dry THF, 0 °C to rt, 2h; (v) Lawesson's reagent, dry toluene, reflux, 4h; (vi) $NH_2NH_2 \cdot H_2O$, DCM, EtOH, rt, 6h; (vii) $NaNO_2$, AcOH, rt, 4h; (viii) $TiCl_4$, DCM, 0 °C, 30 min; (ix) Allyl bromide, K_2CO_3, DMF, 0 °C to rt, 10h; (x) **2a-k**, NaOCl, DCM, TEA, 0 °C to rt, 12h; (xi) TBAF, dry THF, rt, 30 min; (xii) Zn dust, AcOH, rt, 3h.



Scheme 2. *Reagents & conditions:* (i) Zn dust, AcOH, rt, 3h; (ii) **2g**, NaOCl, DCM, TEA, 0 °C to rt, 12h.

SYNTHESIS OF TETRAZOLE LINKED CINNAMIDES

The synthesis of tetrazole linked cinnamide derivatives (**24a–v**) was carried out by using the commercially available 1,2-difluoro-4-nitrobenzene (**16**) as shown in Scheme 3. Compound **16** on nucleophilic substitution with *tert*-butyl piperazine-1-carboxylate by using K_2CO_3 forms *N*-Boc protected nitro compound (**17**) in high yields. This on reduction with Zn/CH_3COOH forms the amino compound (**18**), which on coupled with 3,4,5-trimethoxybenzoyl chloride to afford the amide compound (**19**) in good yields. Later, amide was converted into thioamide (**20**) by using Lawesson's reagent followed by conversion to its amidrazide (**21**) by using hydrazine hydrate. This amidrazide was cyclized by using $NaNO_2$ in acetic acid to afford the *N*-Boc protected tetrazole (**22**) in quantitative yields, this on deprotection with tirfluoro acetic acid to afford secondary amino compound (**23**). Further this on coupled with different cinnamic acids to provide the desired tetrazole linked cinnamide hybrids (**24a–v**) in good yields.



Scheme 3. *Reagents & conditions:* (i) *tert*-Butyl piperazine-1-carboxylate, K_2CO_3, DMSO, 70 °C, 10h; (ii) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 4h; (iii) 3,4,5-Trimethoxybenzoyl chloride, TEA, dry THF, 0 °C, 2h; (iv) Lawesson's reagent, dry toluene, reflux, 4h; (v) $NH_2NH_2 \cdot H_2O$, DCM, EtOH, rt, 6h; (vi) $NaNO_2$, AcOH, rt, 4h; (vii) TFA, DCM, 0 °C to rt, 12h; (viii) Substituted cinnamic acids, EDCI, HOBt, DCM, rt, 6h.

BIOLOGICAL ACTIVITY OF TETRAZOLE LINKED ISOXAZOLINE/CINNAMIDE HYBRIDS

A new series of tetrazole linked isoxazoline/cinnamide hybrids were synthesized and evaluated for their anticancer and inhibition of tubulin polymerization activities. These compounds exhibited significant anticancer activity with IC_{50} values ranging from 0.86 to 26.80 μM. The FACS analysis has shown more population in G2/M phase, which is suggestive of G2/M cell cycle arrest. Further these compounds (**15h**, **15i**) induce apoptotic cell death by inhibition of tubulin polymerization leading to cell cycle arrest at G2/M phase of the cell cycle followed by caspase-3 activity (Accepted in *Med.Chem.Comm.*).

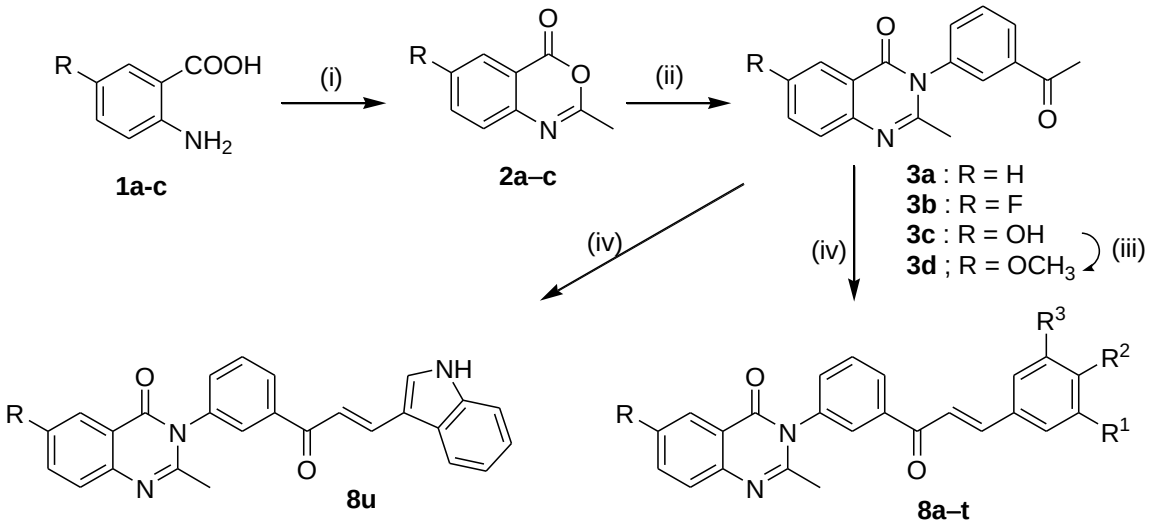
CHAPTER IV

In continuation to our efforts on the design of new anticancer agents, a series of quinazoline linked chalcones and 2-vinylfuryl quinazolinone derivatives have been

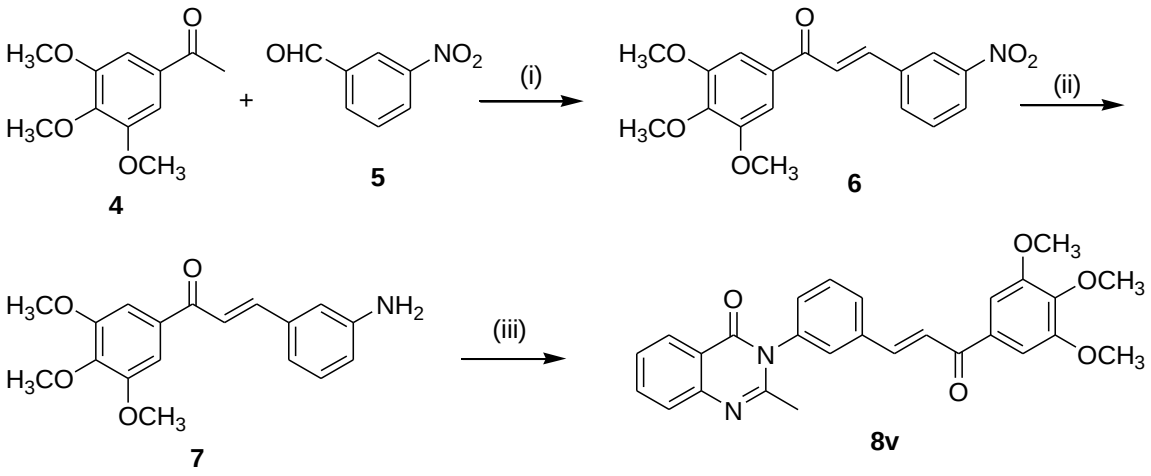
synthesized and evaluated for their anticancer activity against 4 human cancer cell lines.

SYNTHESIS OF QUINAZOLINONE LINKED CHALCONES

The quinazolinone linked chalcone derivatives (**8a–v**) are synthesized from the commertially available substituted antranilic acids (**1a–c**) as outlined in Schemes 1. The first synthetic step involved the condensation of anthranilic acids (**1a–c**) with acetic anhydride to afford the corresponding benzoxazinones (**2a–c**) respectively in quantitative yields. Later these benzoxazinones (**2a–c**) was coupled to 3-amino acetophenone to provide the quinazolinone compounds (**3a–c**). The 6-hydroxy quinazolinone (**3c**) on methylation gives the 6-methoxy quinazolinone (**3d**). These quinazolinones (**3a–d**) undergoes condensation reaction with substituted benzaldehydes in presence of $Ba(OH)_2$ to afford the desired quinazolinone linked chalcones (**8a–t**) in good yields. The quinazolinone linked indolylchalcone (**8u**) was obtained by reacting quinazolinone (**3a**) with indole-3-aldehyde in presence of base. The other types of quinazolinone linked chalcone (**8v**) was obtained by the condensation of 3,4,5-trimethoxy acetophenone (**4**) with 3-nitro benzaldehyde (**5**) in presence of base to give the nitro chalcone (**6**), this on reduction with Zn/AcOH provided the amino compound (**7**). Further, this amino compound undergoes insertion reaction with benzoxazinone (**2a**) to afford the quinazolinone linked chalcone (**8v**) (Scheme 2).



Scheme 1. *Reagents & conditions:* (i) $(CH_3CO)_2O$, 150 °C, 30 min.; (ii) 3-aminoacetophenone, AcOH, reflux, 4 h; (iii) NaOH, EtOH, rt, 12 h.



Scheme 2. *Reagents & conditions:* (i) NaOH, EtOH, rt, 4 h; (ii) $SnCl_2.2H_2O$, MeOH, reflux, 4 h; (iii) AcOH, reflux, 4 h.

A new class of chalcone linked quinazolinone conjugates have been synthesized and evaluated for their *in vitro* anticancer activities against four human cancer cell lines. All the compounds showed significant anticancer potency against the tested cancer cell lines with IC_{50} values in the range of 0.017 to 24.82 μM, however some of the compounds (**8p**, **8v**) exhibited significant anticancer potency with IC_{50} values in the range of 0.017 to 4.60 μM (Manuscript under preparation).