Search for New and Novel Chemotherapeutics for the Treatment of Human Malignancies

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Abstract: One of the hallmarks of cancer is the uncontrolled cell proliferation which causes more deaths among the human diseases throughout the globe. One in eight deaths worldwide are due to cancer, it is the second and third leading cause of death in economically developed and developing countries, respectively. As it is caused by both external and internal factors, a balanced approach to cancer control includes prevention, early detection, and effective treatment. In the treatment of cancer, chemotherapy is one of the practical methods and is widely used employing drugs that can destroy cancer cells by impeding their growth and reproduction. Despite the great strides made in the treatment of cancer over the past 50 years, it continues to be a major health concern and therefore, extensive efforts have been devoted to search for new scaffolds to develop chemotherapeutics. In this perspective, over the past two decades from this laboratory extensive efforts have been made in the development of new chemotherapeutic agents for the treatment of cancer. In this review, glimpses on types of current chemotherapeutic agents based on their action of inhibition and the new molecules that are being developed based on the scaffolds such as pyrrolo[2,1-*c*][1,4]benzodiazepines, podophyllotoxins, benzothiadiazine 1,1-dioxides, naphthalimides and monastrol across the world as well as in this laboratory have been articulated.

Keywords: Cancer, chemotherapeutics, pyrrolo[2,1-*c*][1,4]benzodiazepines, podophyllotoxins, benzothiadiazine1,1-dioxides, naphthalimides, monastrol.

BACKGROUND

Cancer is one of the most deadly diseases that remain as a challenge to both physicians as well as scientists. Every year more than 20% of the population is affected by cancer and the rate of its induction throughout the world is increasing annually. By 2020, it is estimated that there will be 27 million new cancer cases and 17.5 million cancer deaths making it an area for major focus for researchers [1]. Cancer is not a single disease but a broad group characterized by uncontrolled proliferate growth and spread of aberrant cells from their site of origin. At the simplest level, cancer cells may be regarded as having lost touch with their environment and they are no longer responsive to the controlling signals and interactions that occur continuously in normal healthy tissues. A balanced approach to cancer control includes prevention, early detection, and effective treatment. In the treatment of cancer, chemotherapy is widely used method to destroy cancer cells by impeding their growth and reproduction by different agents [2]. The chemotherapeutic agents include drugs interfering with DNA synthesis, inhibiting the function of mitotic spindle and drugs with complex action in various cellular processes within the cancer cells [3]. Despite the improvements in prevention and immense advances in the field of basic and clinical research, cancer remains with a strong need to develop more potent selective chemotherapeutics. Therefore, the discovery of potent, selective and less toxic anticancer agents is still a major challenge.

CAUSES FOR CANCER

Almost all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals or infectious agents [4]. Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication or are inherited, and present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the hosts genome [5]. Genetic abnormalities found in cancer typically affect two classes of genes. Cancer promoting oncogenes, activated in cancer cells that give new properties such as hyperactive growth and division. Tumor suppressor genes, inactivated in cancer cells that results in the loss of normal functions such as accurate DNA replication, control over the cell cycle, orientation and adhesion with in tissues and interaction with protective cells of the immune system.

TYPES OF CHEMOTHERAPEUTICS

The current chemotherapeutic drugs are divided into several categories based on their effect on specific chemical substances within the cancer cells, the cellular activities or processes the drug interferes with, and the specific phases of the cell cycle the drug effects. These includes DNA interactive agents, DNA topoisomerase I and II inhibitors, antimitotic agents, tubulin polymerization inhibitors, carbonic anhydrase (CA) inhibitors, CDK inhibitors, antimetabolites, and miscellaneous agents and these are briefly discussed below.

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DNA Interactive Agents

With the well-established characteristics and its role in the control of cellular functions as genetic material, DNA has been considered as a favored target for cancer chemotherapeutic agents [6, 7]. The double helical structure of deoxyribonucleic acid (DNA) represents the richest source of information within a living organism and its sequence codes for protein/enzyme synthesis *via* the process of translation [8]. The major groups of clinically important DNA reactive agents are covalent and non-covalent binders. The covalent binders include alkylators such as *cis*-platin and reversible or irreversible major and minor groove binders. Distamycin and anthramycin are examples for selective minor groove binders [9]. Intercalators are the molecules that insert perpendicularly into the DNA without forming covalent bonds such as anthracycline, actinomycin-D. The only recognized forces that maintain the stability of the DNA-intercalators complex, even more than DNA alone, are van der Waals, hydrogen bonding, hydrophobic, and/or charge transfer forces [10-13]. Amongst this class, minor groove covalent binders have gained significant attention in the development of new anticancer agents.

DNA Topoisomerase I and II Inhibitors

Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibitions of type I or II topoisomerases interferes with both transcription and replication of DNA by upsetting proper DNA super- coiling. Some type I topoisomerase inhibitors include camptothecins such as irinotecan and topotecan. Type II inhibitors include amsacrine, teniposdie, etoposide and its phosphate which are the semisynthetic derivatives of naturally occurring epipodophyllotoxins [14-16].

Antimitotic Agents or Tubulin Binders

Antimitotic agents interact with proteins rather than DNA, which arrests mitosis in metaphase and these include vinca alkaloids, colchicines [17-18] and taxol [19]. They exert their activity through the interaction with tubulin, a protein essential for the formation of microtubules in mitotic spindles, which is essential for cell division [20]. Promising anticancer drugs block cell division by stabilizing and destabilizing microtubule activity. As these compounds possess neurotoxicity and myelosuppression [21], their use is limited in cancer therapy. The colchicine derivative, democochicine shows very good antitumor activity and vindesine, a new semisynthetic analogue of vincristine alkaloid is being widely used in clinic [22-24].

Carbonic Anhydrase (CA) Inhibitors

The carbonic anhydrase (CA) family of Zn(II) metalloenzymes catalyzes the reversible hydration of CO_2 to HCO_3^-. These are involved in various physiological processes associated with pH control, respiration, transport of CO_2/HCO_3^- between metabolizing tissues and the lungs, fluid secretion, biosynthetic reactions such as the lipogenesis, gluconeogenesis and ureagenesis. More recently, CA inhibition has been implicated as playing an important role in cancer progression [25-27]. Generally, an aromatic or heteroaromatic sulfonamide moiety ($ArSO_2NH_2$) is the primary recognition element necessary for small molecules to bind the active site of CA. Some of the clinical agents from this class of CA inhibitors include acetazolamide, methazolamide, and indisulam, which is in phase II clinical trials as an anticancer agent to treat solid tumors [28].

CDK Inhibitors

Cyclin-dependent kinases (CDK) belong to a group of protein kinases, involved in the regulation of the cell cycle. CDKs are also involved in the regulation of transcription and *m*RNA processing. Serine and threonine kinases are those in which CDKs phosphorylate proteins on serine and threonine amino acid residues. CDK is activated by association with cyclin, forming a cyclin-dependent kinase complex. CDKs are considered a potential drug target for anti-cancer medication and CDK inhibitor such as seliciclib is currently under clinical trials [29-32].

Antimetabolites

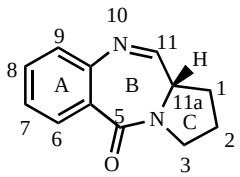
Antimetabolites (azathioprine, mercaptopurine) masquerade as purines or pyrimidines, which are the building blocks of DNA. They prevent the purines and pyrimidines from incorporating into DNA during the "*S*" phase of cell cycle, thus stopping the normal development and division. Because of their efficiency, these drugs are the most widely used cytostatics [33].

NEW CHEMOTHERAPEUTICS UNDER DEVELOPMENT

Cancer is a group of diseases characterized by uncontrolled growth or spread of abnormal cells. It involves the conversion of any normal cells to a cancerous cell showing tandem replication and cell divisions at much faster rate in comparison to the normal cells. Chemotherapy plays most effective role in solid tumors as an adjuvant to initial therapy by surgical or radiotherapeutic procedures. As mentioned earlier, the chemotherapeutic agents can be categorized into functional sub groups: DNA interactive agents, DNA topoisomerase I and II inhibitors, carbonic anhydrase (CA) inhibitors, CDK inhibitors, tubulin polymerization inhibitors, antimitotic agents, anti-metabolites, etc. In this review an effort has been made to outline the development of new chemotherapeutic agents based on pyrrolo[2,1-*c*][1,4]benzodiazepines, podophyllotoxins, benzothiadiazine1,1-dioxides, naphthalimides and monastrol type scaffolds.

PYRROLO[2,1-C][1,4]BENZODIAZEPINES AS DNA BINDING ANTITUMOR ANTIBIOTICS

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) belonging to the class of DNA-interactive antitumor antibiotics produced by various *Streptomyces* species, are one of the promising type of lead compounds. They differ in the number, type and position of substituent in both their aromatic A-ring and pyrrolidine C-rings and in the degree of saturation of the C-ring which can be either fully saturated or unsaturated at either the C2-C3 (endocyclic) or C2 (exocyclic) positions. To date, about thirteen PBD based antibiotics have been isolated, which includes anthramycin [34, 35], mazethramycin [36], porothramycin [37], prothracarcin [38, 39], sibanomycin [40], tomaymycin [41, 42], sibiromycin [43], chicamycin A [44], neothramycin A, B [45] and DC-81



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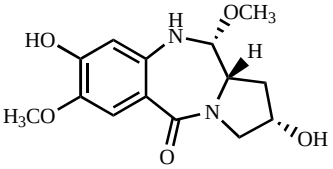
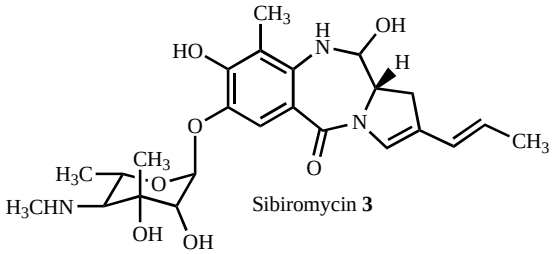
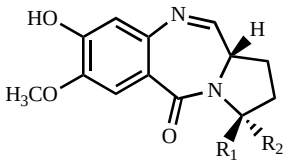


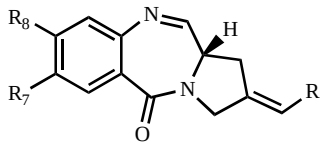
Chicamycin A **2**



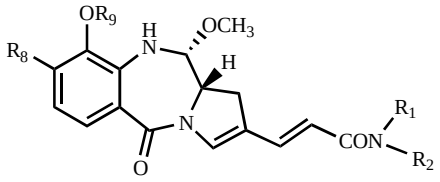
Sibiromycin **3**



Neothramycin A **4** (R_1 = H; R_2 = OH)
Neothramycin B **5** (R_1 = OH, R_2 = H)
DC-81 **6** (R_1 = R_2 = H)



Tomaymycin **7** (R_7 = OCH_3, R_8 = OH, R = CH_3)
Prothracarcin **8** (R_7 = R_8 = H, R = CH_3)
Sibanomicin **9** (R_8 = H, R_7 = sibirosamine pyranoside as in , R = Et)



Anthramycin **10** (R_8 = CH_3, R_9 = R_1 = R_2 = H)
Mazethramycin **11** (R_8 = R_1 = CH_3, R_9 = R_2 = H)
Porothramycin B **12** (R_8 = H, R_9 = R_1 = R_2 = CH_3)

Fig. (1). Naturally occurring PBDs.

[46-48] (Fig. **1**). The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) interactions with DNA are unique because they bind within its minor groove forming a covalent aminal bond between the C11-position of the central B-ring and the N2 amino group of a guanine base [49]. The cytotoxic and antitumor activity of PBDs is attributed to their ability to form covalent DNA adducts. Molecular modeling, solution NMR, fluorimetry and DNA foot printing experiments have shown that these molecules have a preferred selectivity for Pu-G-Pu sequences [50, 51], and are oriented with their A-rings pointed either towards the 3' or 5' end of the covalently bonded DNA strand (as in case of anthramycin and tomaymycin). The PBDs have been shown to interfere with the action of endonuclease enzymes on DNA and to block the transcription by inhibiting DNA polymerase in a sequence specific manner, which is relevant for the biological activity [52, 53].

The known PBD natural products have a (*S*) configuration at the C11a-position, which provides them with a right handed twist when viewed from the C-ring towards the A-ring. This has given the appropriate three-dimensional shape for isohelicity with the minor groove of DNA, leading to a snug fit at the binding site. The racemization at C11a can significantly reduce both DNA binding affinity and *in vitro* cytotoxicity. The synthetic PBD with the (*R*) configuration at C11a have shown to be devoid of both DNA binding affinity and *in vitro* cytotoxicity [54]. The N10-C11 imine moiety may exist in the hydrated form depending upon precise structure of the compound and the method of isolation or synthetic workup. Imines and methyl ether forms are interconvertable by dissolution of imine in methanol or by several cycles of refluxing the methyl ether in chloroform followed by evaporation of the solvent under vacuum (Fig. **2**).

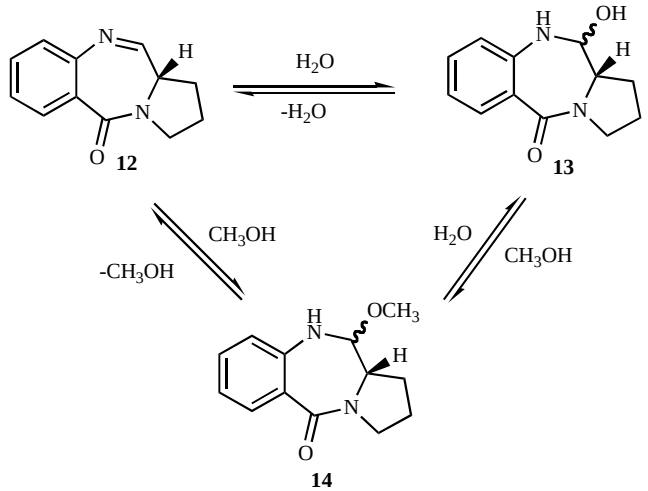


Fig. (2). Carbinolamine-methylether-imine interconversions of PBDs.

PBD-DNA Interactions

The mechanism of action of the PBDs is associated with their ability to form an adduct in the minor groove and thus

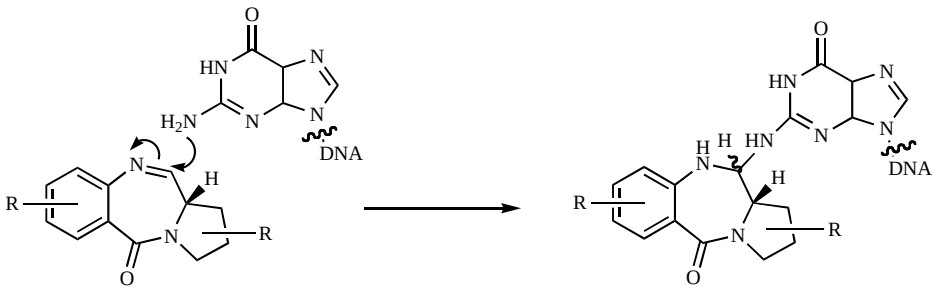


Fig. (3). Formation of PBD-DNA covalent adduct.

interfering with DNA processing. After insertion into the minor groove, an aminal bond is formed through nucleophilic attack of the N2 of a guanine base at the electrophilic C11 position of PBD. The X-Ray diffraction studies on crystals of anthramycin methylethers have shown that the molecule is twisted 0-50^{o} from one end to the other along the axis and this might fit into one of the grooves of DNA. In the CPK models, the drug fits snugly within the narrow groove without distortion of the DNA helix. The structure of the anthramycin DNA adduct was initially studied independently by Hurley and Kohn using indirect techniques, but more recently fluorescence spectroscopy, high field NMR and molecular modeling have been employed [55-61] (Fig. **3**).

Synthetic Approaches of Pyrrolo[2,1-*c*][1,4] Benzodiazepines

Biosynthesis of the naturally occurring PBDs has been extensively elucidated and the first total synthesis of a carbinolamine containing PBD of anthramycin has been reported in 1968 [62]. The various synthetic approaches to the PBDs scaffold have been reviewed in 1994, 1998, and 2002 [63-65]. These includes hydride reduction of seven-membered cyclic dilactams [66, 67], reductive cyclization of acyclic nitroaldehydes [68], iminothioether approach [69, 70], cyclization of aminothioacetals [71, 72], deprotective cyclization of the diethylthioacetals *via* N10 protected precursors [73], oxidation of cyclic secondary amines [74-76], reductive cyclizations [77] and solid phase approaches [78, 79].

Kaneko Approach (Iminothioether Reduction)

Kaneko and co-workers [80] have developed a mild method for the reduction of PBD dilactams to the carbinolamine using aluminium amalgam (Scheme **1**).

This methodology has been employed for the preparation of bicyclic and tricyclic analogues of anthramycin, as well as in the total synthesis of some naturally occurring PBDs like chicamycin. By using this approach Baraldi and co-workers have synthesized some heterocyclic PBD analogues in which the A ring of PBD skeleton is replaced with a 1,3 or 1,5-disubstituted pyrazole nucleus [81, 82].

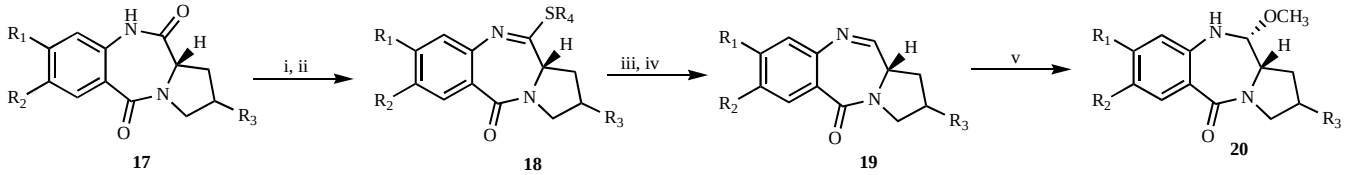
Thurston's Approach (Cyclization by Deprotection of Diethylthioacetal)

Thurston and co-workers have developed an efficient method for the synthesis of various PBDs containing carbinolamine moiety by employing mercuric chloride in the key cyclization step. In this procedure, the products are generally isolated in the imine form and this approach has been utilized for the synthesis of a variety of naturally occurring and synthetic PBDs such as DC-81, C8-linked DC-81 dimers, A-ring modified analogues of PBD, PBD-EDTA conjugates, lexitropsin conjugates of PBD, C2 linked PBD dimers, imine-amide PBD dimers and napthalimide conjugates of PBD (Scheme **2**) [83, 89].

Fukuyama's Approach

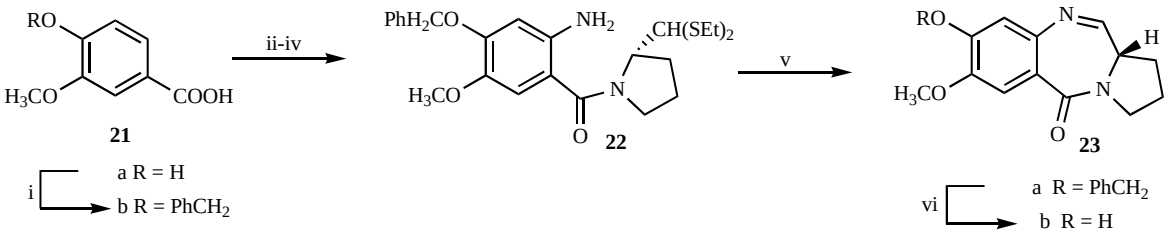
It has been developed to incorporate certain labile functionalities such as C8-epoxide moiety in the PBD system as the conventional approaches failed to give the desired results. In this 9-fluorenyl methyloxy carbonyl (Fmoc) group has been used to protect the amine group and which can be easily removed by cleavage by $Bu_4N^+F^-$ (TBAF) to provide C8 epoxide PBD system (Scheme **3**).

By using this approach, hybrid molecules containing PBD-oligo-pyrrole carriers, AT-groove binding hybrids and C7 aryl substituted PBDs have been synthesized as minor

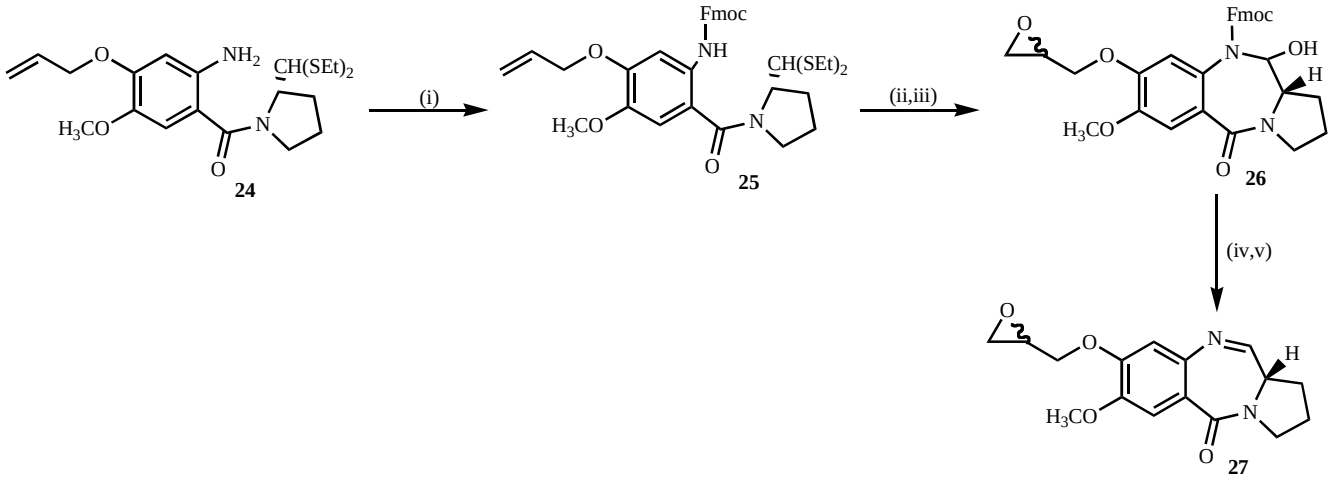


R_1 = H, OH, OBn, OCH_3, OAc; R_2 = H, OCH_3
R_3 = H, = CH-CH_3 (E), OH (a), OAc (b), = CH-COOEt (E); R_4 = CH_3

Scheme 1. *Reagents and conditions* : (i) P_2S_5, C_6H_6, 80 ^{o}C or P_2S_5, $NaHCO_3$, CH_3CN, D, 15 min, or (p-$CH_3OC_6H_4PS_2)_2$, C_6H_6, 80 ^{o}C; (ii) Et_3OBF_4, CH_2Cl_2, $KHCO_3$ or CH_3I, K_2CO_3, THF or DMF; (iii) Al-Hg, aq.THF or KH_2PO_4, 0-5 ^{o}C,14 h; (iv) 0.1 N methanolic $HgCl_2$, 0 ^{o}C or SiO_2 chromatography, 5 ^{o}C; (v) CH_3OH.



Scheme 2. *Reagents and conditions* : (i) $PhCH_2Cl$, THF, NaOH, H_2O, reflux, 48 h; (ii) $SnCl_4$, HNO_3, CH_2Cl_2, -25 ^oC,5 min; (iii) $(COCl)_2$, THF, DMF, 3 h then, pyrrolidine-2-carboxaldehyde diethyl thioacetal, Et_3N, H_2O, 0 ^oC, 1.5 h; (iv) $SnCl_2.2H_2O$, MeOH, reflux, 45 min; (v) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O, 12 h; (vi) 10% Pd-C, EtOH, cyclohexadiene, 3 h.



Scheme 3. *Reagents and conditions*: (i) CH_3OH, $SnCl_2.2H_2O$, reflux; (ii) Dioxane, Na_2CO_3 (aq), FmocCl, 0 ^oC; (iii) CH_3CN-H_2O, $HgCl_2$, $CaCO_3$; (iv) CH_2Cl_2, MCPBA; (v) DMF, TBAF.

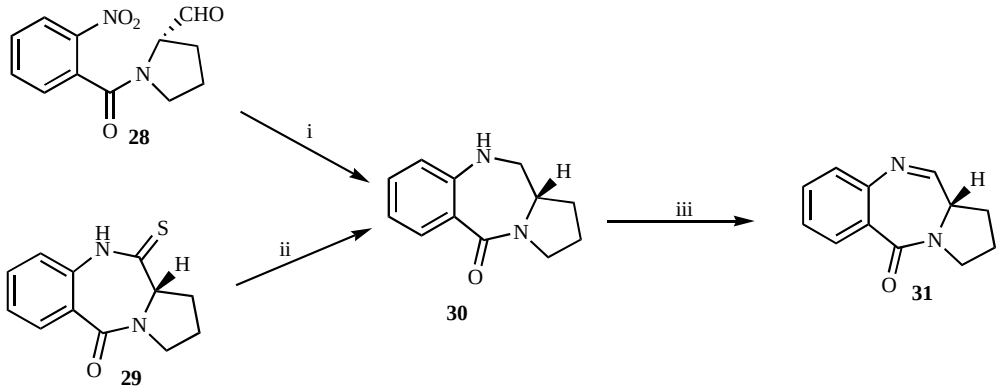
groove binders [90, 91]. Further, this strategy has also been employed in the synthesis of C2/C2'-*exo*-and C2-C3/C2'-C3'-*endo* unsaturated PBD dimers with remarkable DNA binding affinity [92, 93].

Kamal's Approach (Oxidation of Cyclic Secondary Amine)

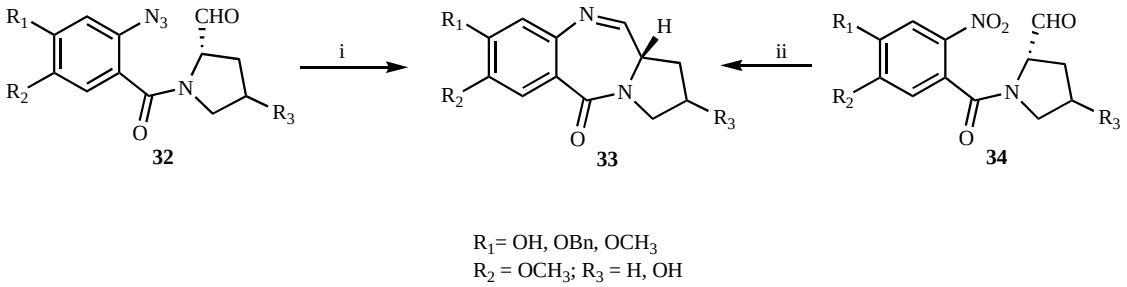
The PBDs with either a secondary amine or amide functionality at N10-C11 can be readily synthesized, but the introduction of imine or carbinolamine at this position is difficult due to high reactivity of these functional groups. In this elegant approach, the imine or carbinolamine moiety has been introduced by the oxidation of PBD secondary amines with DMSO/$(COCl)_2$ or TPAP (tetra-*n*-propyl ammonium perruthenate) in good yields as illustrated in Scheme **4** [94, 95].

Azido/Nitro Reductive Cyclization

The synthesis of N10-C11 imine containing PBDs *via* reductive cyclization has been reported by Miyamoto and coworkers [96]. Further, in an endeavor to explore new practical methods for the synthesis of PBDs particularly by the azido reductive process extensive investigations has been carried out in this laboratory. Recently, a facile intramolecular azido/amido reductive cyclization approach for the syn-



Scheme 4. *Reagents and conditions* : (i) Pd/C; (ii) Raney Ni; (iii) swern/TPAP.



Scheme 5. *Reagents used for Azide reduction:* (i) HMDST; Bakers' yeast; N,N-Dimethyl hydrazine/$FeCl_3$; TMSI; SmI_2; HI; $FeSO_4.7\ H_2O$. *Reagents used for Nitro reduction:* (ii) Fe/AcOH; N,N-Dimethyl hydrazine/$FeCl_3$

thesis of PBD and their dimers [97-99] has also been reported (Scheme **5**).

Hydride Reduction Approach

This approach involves the synthesis of imine form of PBD analogues through hydride reduction of N-10 MOM and SEM protected dilactams by employing hydride transfer reagents such as $LiBH_4$, $NaBH_4$ [100, 101].

Solid Phase Approach

Thurston and co-workers [102] have developed a solid phase approach for the synthesis of PBD imines on *p*-nitrophenylcarbonate Wang resin using a variety of oxidation and cyclization procedures. In this laboratory, new solid phase approach [103, 104] has been employed for the synthesis of PBD dilactams and PBD imines by using Wang resin (Scheme **6**).

Structure Activity Relationship

The naturally occurring PBDs namely anthramycin, tomaymycin, sibiromycin, neothramycin and DC-81 have different type of substitutions. The electron-donating substituents are required in the aromatic A ring for biological activity. Bulky substituent like a sugar moiety at C7 position enhances the DNA binding affinity and cytotoxicity. It is interesting to note that C ring modified PBDs appear to provide both greater differential thermal stabilization of DNA duplex and significantly enhance kinetic reactivity during covalent adduct formation. Similarly, the C2-substituted naturally occurring PBDs exhibit more cytotoxicity compared to their unsubstituted counter parts. Based on these considerations a structure activity relationship has been derived by Thurston and co-workers.

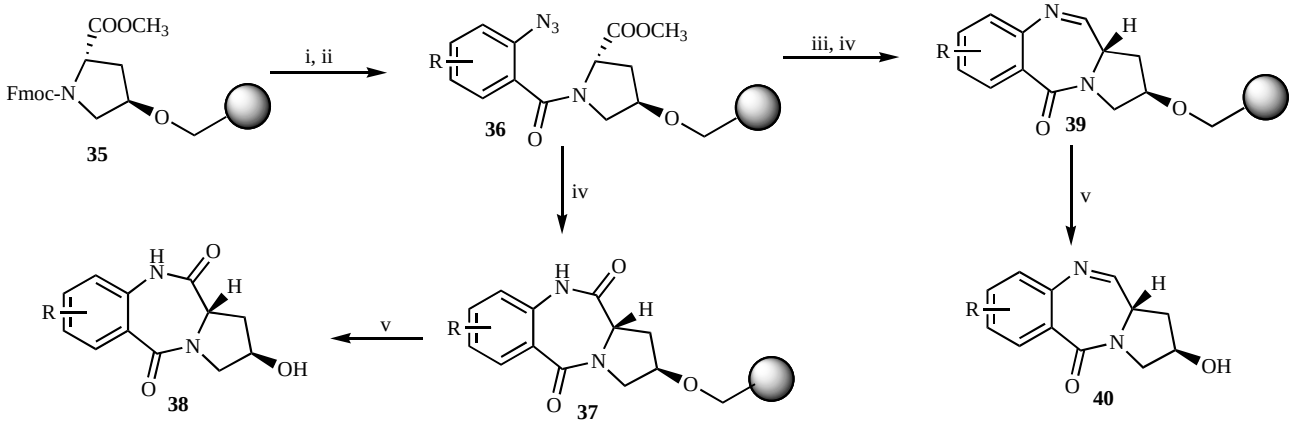
Synthesis of Ring-Modified PBDs

A-Ring Modifications

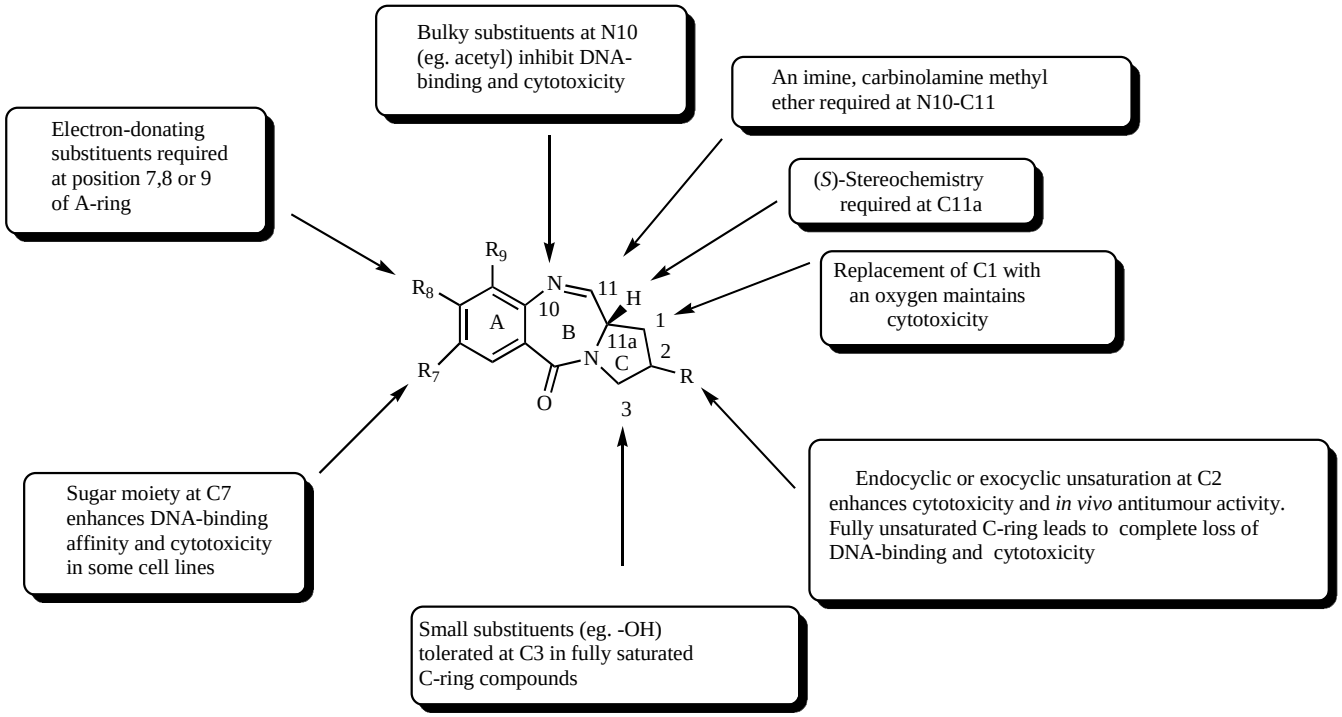
Baraldi and co-workers [105] have investigated heterocyclic analogues of pyrrolo-[2,1-*c*][1,4]benzodiazepine (PBD) by replacing A ring with pyrazolo[4,3-*e*]pyrrolo[1,2-*c*][1,4]diazepinone ring system. Some of these pyrazole PBD analogues have displayed an interesting cytotoxicity profile. Similarly, Thurston and co-workers [106] have synthesized some pyridine, pyrazine and pyrimidine A-ring analogues of PBDs (**41-43**) and evaluated for their DNA binding affinity (Fig. **4**). It has been observed that the aromatic A-ring has a modest influence on thermal denaturation of DNA. Further, they have also synthesized some tetracyclic PBD analogues by modification of A-ring, however the DNA binding affinity and cytotoxicity has been reduced compared to DC-81.

B-Ring Modifications

Very few attempts have been made on B-ring modifications. Robba and co-workers [107] have synthesized a series of PBDs having N10-C11 amidines functionality (**44-46**) and evaluated the *in vitro* DNA binding through thermal denaturation studies. It has been observed that some of these



Scheme 6. *Reagents and conditions*: (i) 20% piperidine/DMF; (ii) Subistituted 2-azido benzoic acid DCC, DMAP, CH_2Cl_2, 0 ^oC; (iii) DI-BAL-H, CH_2Cl_2, -78 ^oC; (iv) PPh_3, toluene; (v) TFA/CH_2Cl_2 (1:3).



Structure activity relationship of PBD ring system.

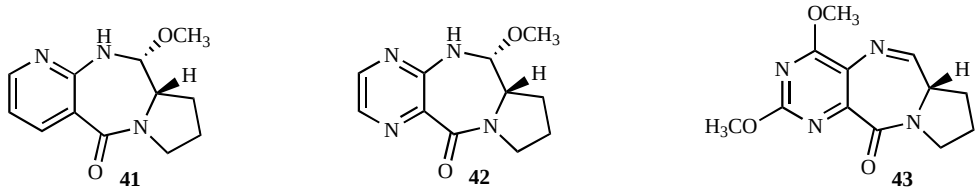


Fig. (4).

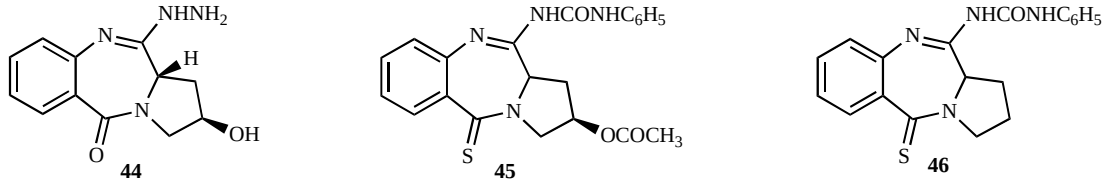


Fig. (5).

compounds show a significant increase in melting for calf thymus DNA comparable to the natural product DC-81 (Fig. **5**).

C-Ring Modifications

A number of naturally occurring PBDs namely anthramycin, tomaymycin, sibiromycin and neothramycin have different type of substitutions on the C ring. The modifications on C-ring provide both greater thermal stabilization of DNA duplex and significantly enhanced reactivity during the covalent adduct formation. Thurston and co-workers have synthesized a series of C2-*exo* unsaturated, C2/C3-*endo* unsaturated, C2-aryl 1,2/2,3-endo unsaturated and C2-C3 unsaturated PBDs (**47-51**). These PBDs with C2 modifications have been evaluated for both DNA-binding reactivity and *in vitro* cytotoxic potency. Some of them have shown significant activity comparable to anthramycin potency [108-110]. Recently, a series of C2-fluorinated PBDs **52** have been synthesized and screened for *in vitro* cytotoxicity against a number of cancer cell lines [111]. These PBDs have shown 550 fold increases in activity against the CH1 cell line when compared to the unsubstituted PBDs along with good DNA binding affinity. In this laboratory C2-fluorinated monomers of PBD and DC-81 dimers **53** have been synthesized and biologically evaluated. These new fluorinated compounds possess more potent *in vitro* anticancer activity in a number

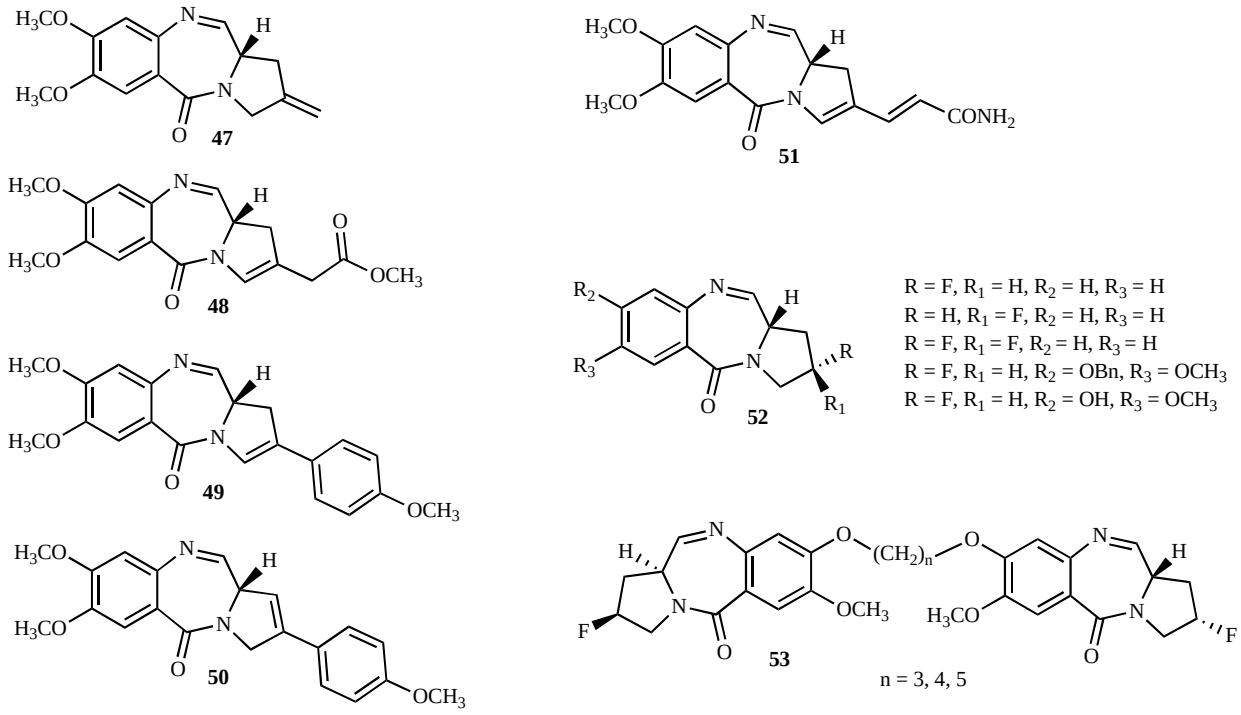


Fig. (6).

of human cancer cell lines. Moreover, they have also exhibited good DNA binding ability compared to A-ring unsubstituted C2-fluorine compounds (Fig. **6**). Recently, from this laboratory 1,2,3-triazolo linked PBD at C2 position have been reported with promising antitumor activity and DNA binding affinity [112-114].

Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimers

C7/C2--Linked Dimers

Suggs and coworkers [115, 116] have reported the first PBD dimer comprising of two PBD units joined through A-C7/A-C7' positions by alkanediyldioxy linker **54** (some including nitrogen heteroatoms) or alkanediyldisulfide linkers. The C7-linked dimers have been considered as unique among the DNA-cross linkers in their specificity for dG-containing duplex DNA. Lown and co-workers [117] have designed and synthesized PBD dimers that are linked at C2 position of the PBD subunits through alkylamido spacer **55**. A series of these dimers have been evaluated for their cytotoxicity against 60 human tumor cell line screen (Fig. **7**). It has been observed that these compounds exhibited moderate to promising cytotoxic potency against different cancer cells.

C8-Linked Dimers

In this laboratory after the discovey of DC-81 dimers by Thurtson and coworkers, extensive work on design and synthesis of C8-linked dimers has been carried out and their DNA binding, as well as cytotoxic potency has been evaluated [118]. This includes C8-linked dimers **56**, C2 exocyclic dimers **57**, C2-fluoro dimers **58** and C8-linked imine-amide mixed dimers **59** with varying alkyl chain spacers. These dimers have displayed potent antiproliferative activity in different cell lines [119]. Lown and coworkers have reported a novel *bis*-pyrrolo[2,1][1,4]benzodiazepine-pyrrole and imidazole polyamide conjugates **60** with potent antiproliferative activity in many human cancer cell lines [120, 121]. Recently, Thurston coworkers have reported an asymmetric tripyrrole-linked sequence selective PBD dimer **61** that binds with high affinity to an interstrand cross linking site spanning 11 DNA base pairs (Fig. **8**) [122].

C8-Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Conjugates

In the search for compounds with better antitumor selectivity and DNA sequence specificity many C8-linked hybrids of pyrrolo[2,1-*c*][1,4]benzodiazepines have been designed. Baraldi and co-workers [74] have designed and synthesized distamycin-PBD and netropsin-PBD conjugates **62** as novel sequence selective C8-linked PBD hybrids and investigated for the sequence selectivity and stability of DNA drug complexes. Hurley and co-workers [123] have synthesized novel DNA-DNA interstrand adenine-guanine cross-linking UTA-6026 compound **63**. Preliminary *in vitro* tests showed that

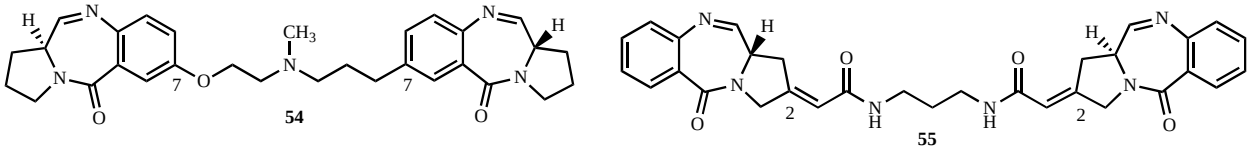


Fig. (7).

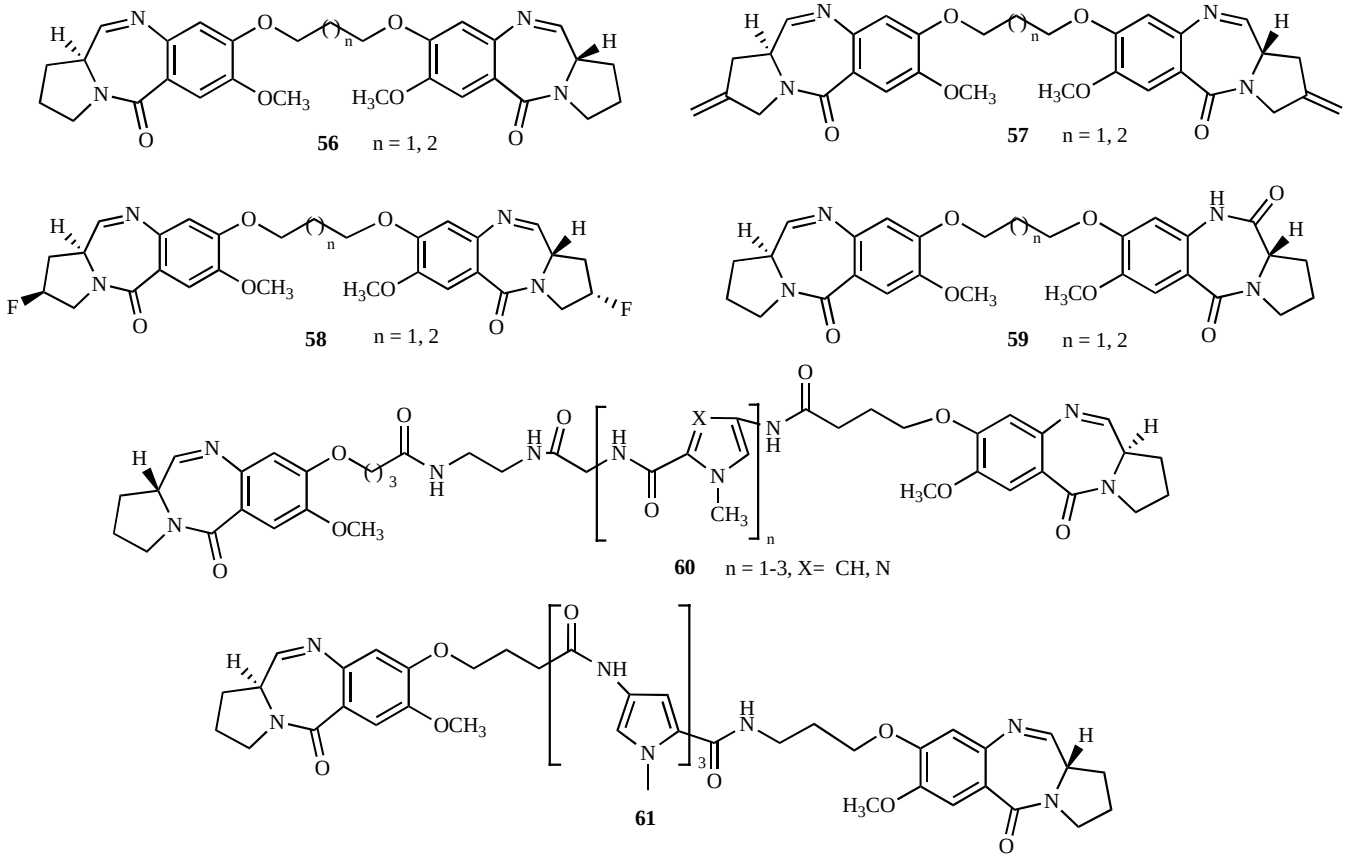

56 n = 1, 2
57 n = 1, 2
58 n = 1, 2
59 n = 1, 2
60 n = 1-3, X= CH, N
61

Fig. (8).

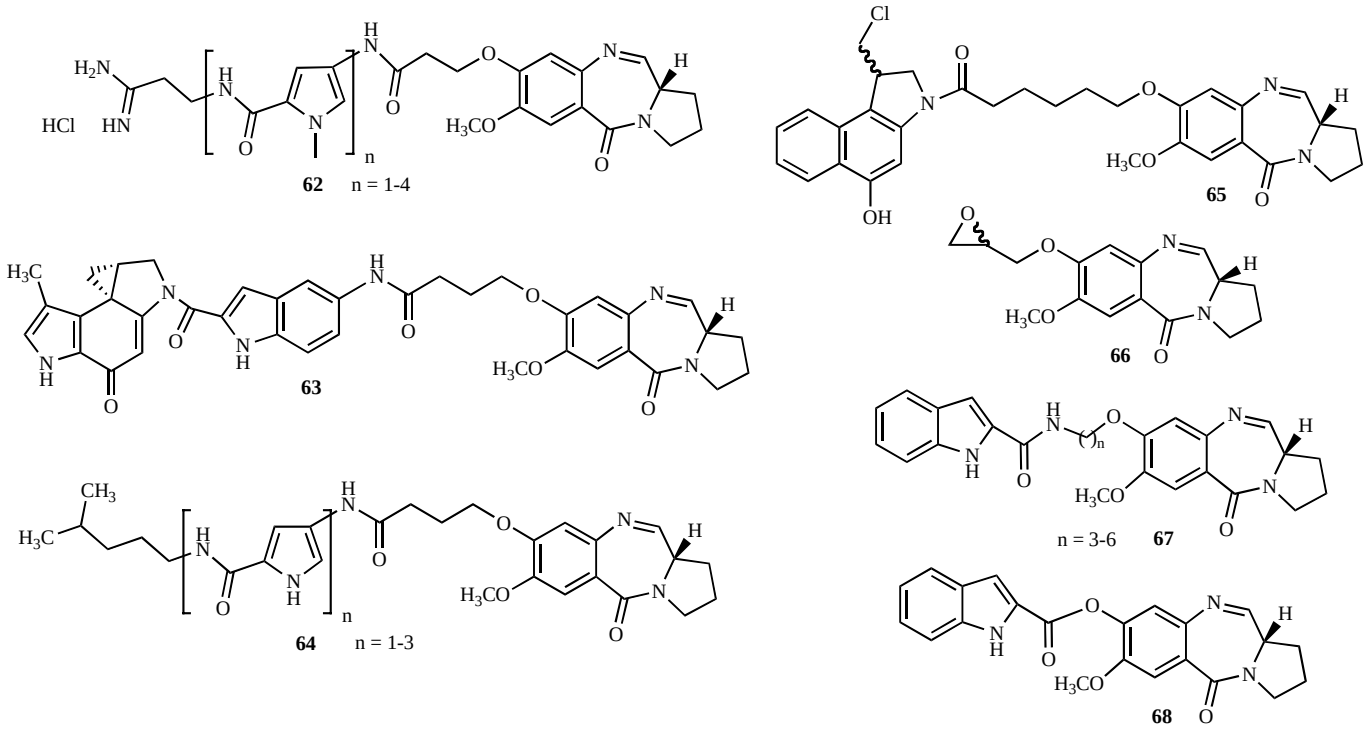

62 n = 1-4
63
64 n = 1-3
65
66
n = 3-6 67
68

Fig. (9).

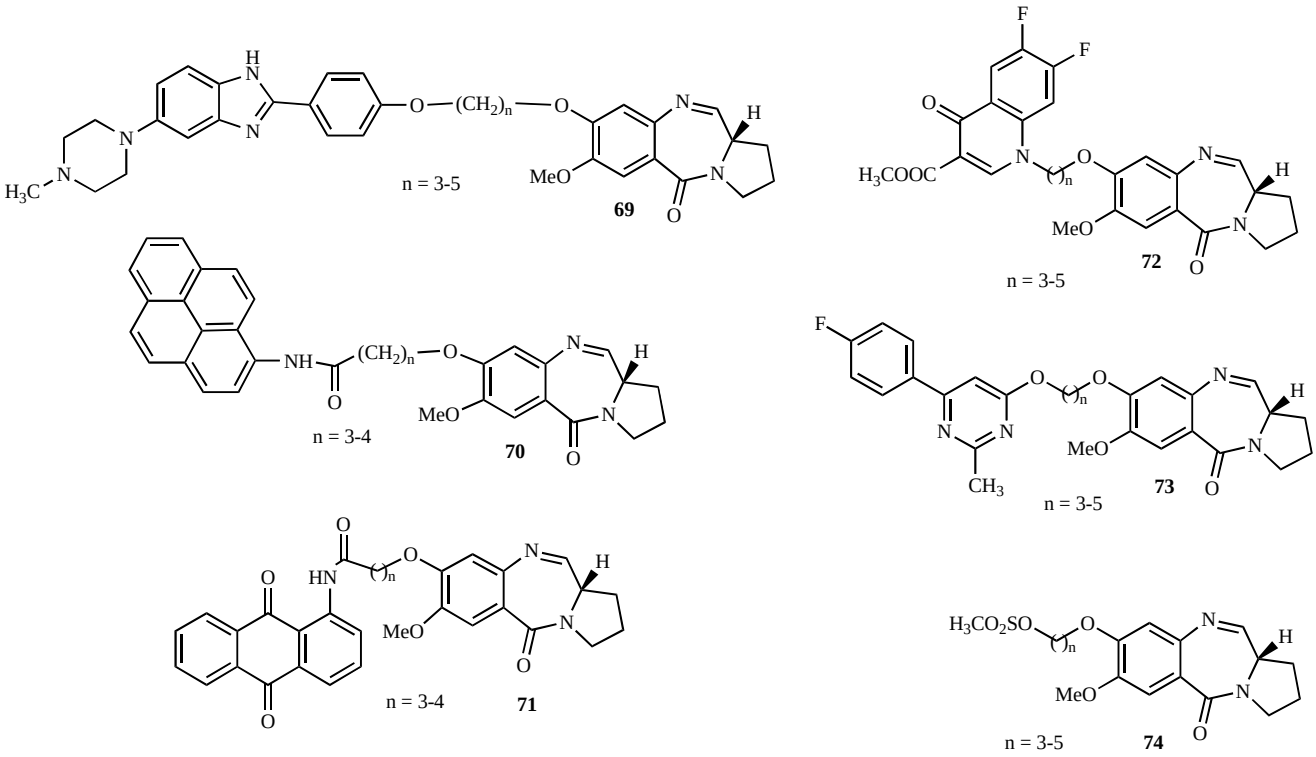


Fig. (10).

UTA-6026 has remarkably potent cytotoxicity to several tumor cell lines (IC_{50} = 0.28 nM in MCF-7 breast cancer cell line, IC_{50} = 0.047 nM in colon tumor cell line SW-480 and IC_{50} = 5.1 nM in human lung tumor cell line A549). Lown and co-workers [124] have also reported the synthesis of a series of PBD-lexitropsin conjugates **64** linked through the C8 position with a suitable linker. These compounds have been synthesized in view of the effect with sequence selective binding in DNA duplex. Similarly, unsymmetrical DNA cross-linkers **65**, C8-epoxide linked pyrrolo[2,1-*c*][1,4]benzodiazepine **66**, PBD-indole conjugates (**67**, **68**) have been synthesized and evaluated to possess higher cytotoxicity against selected human cancer cell lines. Moreover, novel water insoluble and soluble PBD-gylcosylated pyrrole and imidazole polyamide conjugates have been reported (Fig. **9**) [125-127].

In the course of a program to develop new antitumor drugs from this laboratory novel C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates have been designed, synthesized and evaluated as potential DNA binding agents. A series of PBD conjugates having different DNA interacting ligands such as benzimidazole **69** [128], polyaromatic hydrocarbons (pyrene amine and chrysene amine [129, 130] **70**, anthraquinones **71** [131], naphthalene [132] and acridones [133] by using varying linker length have been synthesized to enhance the DNA binding affinity and antitumor activity.

All these designed molecules have shown good DNA binding affinity with better anticancer activity. In addition to above derivatives, quinolone **72** [134], pyrimidine hybrids **73** [135], C2/C8 dimers [136], azepine conjugates and methanesulfonate derivatives **74** [137, 138] of pyrrolo[2,1-*c*][1,4]benzodiazepines has also been reported from this group (Fig. **10**). More recently, from this laboratory yet another series of a novel quinazolinone linked PBD conjugates have been synthesized and evaluated as potent antitumor agents with effective DNA affinity. Similarly naphthalimide, triazolo[1,2,4]benzothiadiazine linked PBD conjugates have also been reported with remarkable cytotoxicity and good binding affinity [139-144].

Owing to the increasing interest in the design and synthesis of DNA interstrand cross linking conjugates, extensive research endeavors has been carried out in this laboratory to improve the DNA binding affinities and cytotoxicity of novel PBD conjugates. Amongst them the C8-linked PBD hybrids have shown excellent DNA binding affinity as well as *in vitro* cytotoxicity, and the most potent compounds have been patented for potential commercial exploitation. Herein, the designed and synthesized coumarin-piperazine linked PBDs with varying alkane spacers is discussed. These conjugates have been tested against sixty human cancer cell lines derived from nine cancer types as per NCI protocol. Amongst them one analogue exhibited significant activity against forty one cell lines in nine cell panels, with GI_{50} value of < 20 nM. The DNA binding affinity for this novel coumarin linked PBD conjugate has been examined by thermal denaturation studies using calf thymus (CT) DNA. One of the coumarin-PBD conjugate elevates the helix melting temperature of CT-DNA by a margin of 7.9 ^{o}C after incubation for 18h at 37 ^{o}C [145]. Similarly several series of C8-linked isoxazoline [146], chalcone [147], benzimidazole [148], phenanthrylphenol [149], benzophenone [150], benzothiazole and benzoxazole PBD conjugates [151] have been designed, synthesized and evaluated for their antiprolifera-

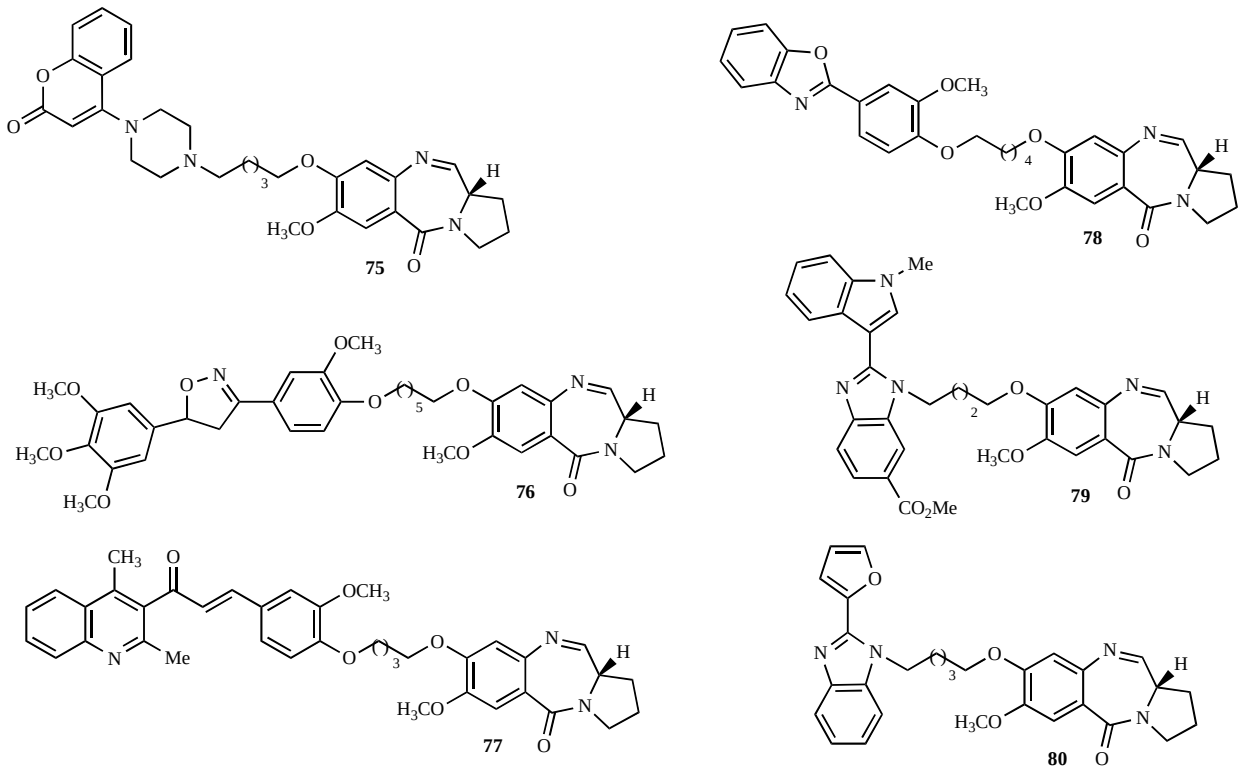


Fig. (11).

Table 1. $Log_{10}GI_{50}$ *In Vitro* Cytotoxicity Values of PBD Conjugates

Moiety linked to PBD	Cancer cell lines								
	Leukamia	NSCl	Colon	CNS	Melanoma	Ovarian	Renal	Prostate	Breast
Isoxazoline (**75**)	-6.76	-6.59	-6.41	-6.51	-6.68	-6.44	-6.58	-6.49	-6.74
Quinolinechalcone (**76**)	-6.94	-6.87	-6.41	-6.16	-6.59	-5.28	-6.17	-5.85	-6.52
Benzoxazole (**77**)	-7.51	-7.02	-7.08	-7.00	-7.08	-6.79	-6.96	-7.47	-7.25
Benzimidazole-indole (**78**)	-6.88	-6.08	-6.04	-6.00	-6.27	-6.04	-6.10	-6.62	-6.14
Benzimidazolefuryl (**79**)	< -8.00	< -7.92	< -7.92	< -8.00	< -7.92	< -8.00	< -7.92	< -7.64	< -7.79
Coumarin (**80**)	-7.68	-7.51	-7.68	-7.50	-7.28	-7.59	-7.66	-7.57	-7.55

Table 2. Thermal Denaturation Data for PBD Conjugates with Calf Thymus CT-DNA

Moiety linked to PBD	[PBD]:[DNA] molar ratio	(ΔT_m °C) after incubation at 37°c for	
		0 h	18h
Isoxazoline (**75**)	1:5	4.1	6.2
Quinolinechalcone (**76**)	1:5	4.3	4.9
Benzoxazole (**77**)	1:5	6.2	6.3
Benzimidazole-indole (**78**)	1:5	1.5	2.0
Benzimidazolefuryl (**79**)	1:5	5.1	7.0
Coumarin (**80**)	1:5	7.3	7.9
DC-81	1:5	0.3	0.7

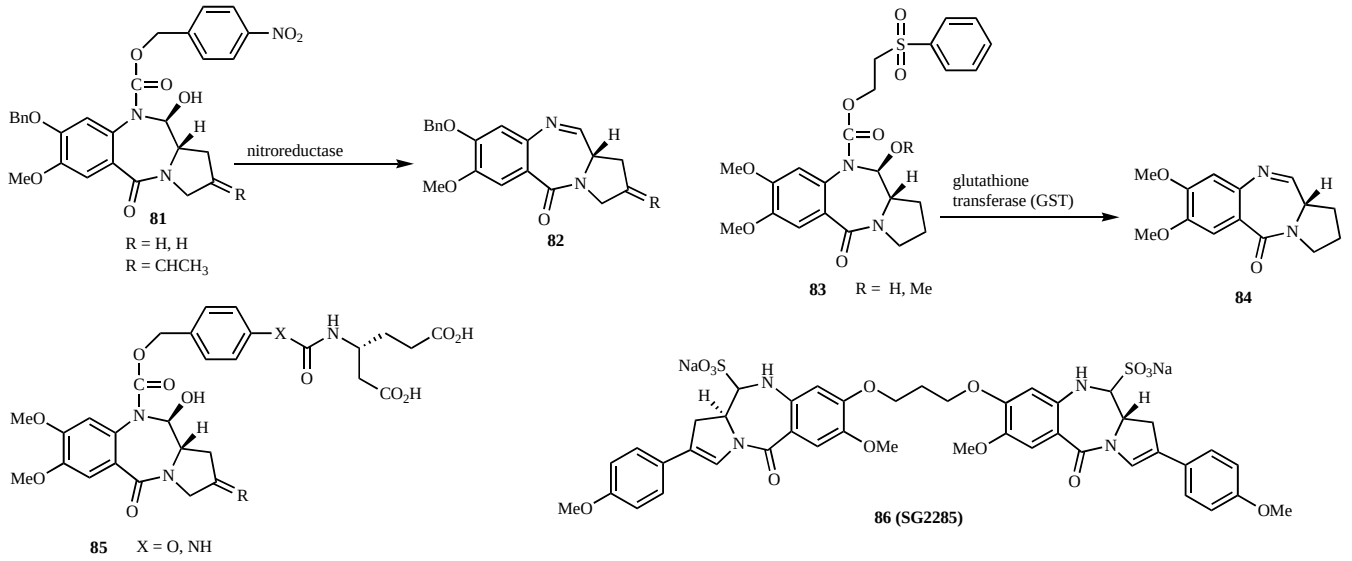


Fig. (12).

tive activity at NCI. All these PBD conjugates have shown excellent cytotoxic activity against tested cell lines with good binding affinity (Fig. **11**). The cytotoxic and DNA binding affinity values of the most potent molecules (75-80) have been summarized in Tables **1** and **2** and some compounds are undergoing preclinical studies.

Prodrug Monotherapy

A major limitation of cancer chemotherapy results from the lack of tumor specificity shown by most anticancer drugs leading to severe side-effects due to the destruction of healthy tissues [152]. One approach to overcome these drawbacks is the development of relatively non-toxic anticancer agents, in a prodrug form, specifically activated in and around the tumor tissue [153]. An ideal prodrug needs to be stable at *in vivo*, far less toxic than its parent form, and activated specifically in or within the microenvironment of the tumor cells or tumor site [154]. The prodrug monotherapy (PMT) [155-160] strategies, antibody directed enzyme prodrug therapy (ADEPT) [161] and gene directed enzyme prodrug therapy (GDEPT) [162] have been developed to target tumor cells selectively. PMT is a much safer and convenient method to activate the prodrugs keeping in view the immune response of the biological system towards ADEPT strategy and the intricacy to transfect the genes specifically to the tumor cells by GDEPT.

In view of the high therapeutic efficacy of PBDs and to overcome the limitations such as, lack of tumor selectivity, high reactivity of the pharmacophore and low water solubility, the prodrugs have been developed. Thurston and coworkers have synthesized some PBD prodrugs (**81-85**) based on ADEPT and GDEPT strategies, that can be activated by nitroreductase, glutathione transferase (GST) and carboxypeptidase G2 enzymes [162-165]. Recently, they have also reported another C2/C2′-aryl-substituted PBD dimer prodrug **86** by introducing sodium bisulfite groups to the C11/C11′-positions of the parent *bis*-imine with improved solubility (Fig. **12**). This newly synthesized prodrug is highly water soluble, stable in aqueous conditions and the rate of DNA cross-link formation is much slower than parent bisimine. Further, it has also shown significant antitumor *in vivo* activity across a wide range of human tumor xenograft models.

In this laboratory, PBD prodrugs containing a G-galactoside moiety have been synthesized for application in selective chemotherapy of cancer. Prodrugs having the general structure pyrrolobenzodiazepine-spacer-G-galactoside have been designed, synthesized and evaluated for activation by the enzyme G-galactosidase [166]. In the prodrugs **87** and **88** the PBDs are connected to the G-D-galactoside moiety through a self-immolative spacer. The results interpret that the prodrugs possess a potential to be efficiently used for the targeted delivery of PBD anticancer agents to solid tumors by ADEPT (Table **3**). Finally, the new PBD glycoside prodrugs possess all the essential requirements for their potential application in targeted therapy of cancer by enzyme directed therapies. The *in vivo* targeting ability of the prodrugs is being initiated. Further, another PBD-β-glucuronide prodrugs **89** have been designed synthesized for potential application in selective chemotherapy of cancer and evaluated to possess highly reduced toxicity by aforesaid strategies (Fig. **13**) [167].

PODOPHYLLOTOXIN AND ITS DERIVATIVES

Natural products have historically provided new drugs against a wide variety of diseases and cancer is certainly no exception. Amongst podpophyllotoxin (PDT) **90**, aryltetralin lactone is a bioactive lignan isolated from the plant *Podophyllum peltatum* and *Podophyllum emodi* and has been the focus of extensive chemical modification leading to clinically useful anticancer drugs [168-195]. The semisynthetic derivatives of podophyllotoxin such as etoposide (VP-16) **91**, teniposide (VM-26) **92** and etopophos **93** (Fig. **14**), are widely used as anticancer drugs and shows good clinical effects against several types of neoplasms [168, 196, 197]. However, several limitations such as myelosuppression, development of drug resistance and cytotoxicity towards normal cells, still exist. Most of the lignans are known to inhibit

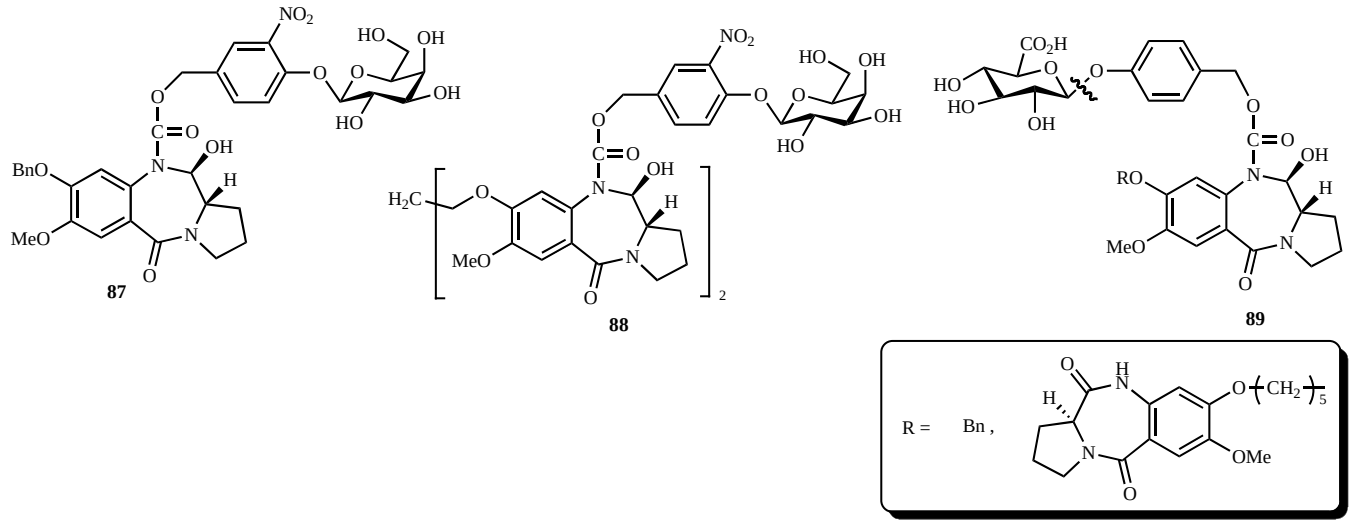


Fig. (13).

Table 3. Cytotoxicity of the Prodrugs

	A375 cell line			Hep G2 cell line		
Prodrug	**IC_{50} (μmol)**	**IC_{50} (μmol) in the presence of β-galactosidase**	**IC_{50} (μmol) of parent PBD**	**IC_{50} (μmol)**	**IC_{50} (μmol) in the presence of β-galactosidase**	**IC_{50} (μmol)of parent PBD**
87	422.9	1.2	0.37	14.11	5.52	8.56
88	9.74	0.09	0.06	>200	0.87	0.94

the tubulin polymerization and DNA topoisomerase II enzyme [198-203]. Studies on Structure-Activity Relationship (SAR) have shown that podophyllotoxin like compounds preferentially inhibit tubulin polymerization, which leads to arrest of the cell cycle in the metaphase. However, etoposide like compounds are potent irreversible inhibitors of DNA topoisomerase II and their action is based on the formation of nucleic acid-drug-enzyme complex, which induces single- and double-stranded DNA breaks, eventually lead to cell death.

Structure Activity Relationship Studies of Podophyllotoxin

Recent developments on podophyllotoxin based compounds have provided insights into the Structure-Activity Relationships (SARs), which have assisted in the design and synthesis of new podophyllotoxin derivatives with potential antitumor activity. The SARs for etoposide analogues have been recently reviewed [204, 205] and some of the modifications have been carried out by various researchers to explain the mechanism of action [206-220]. The studies conducted

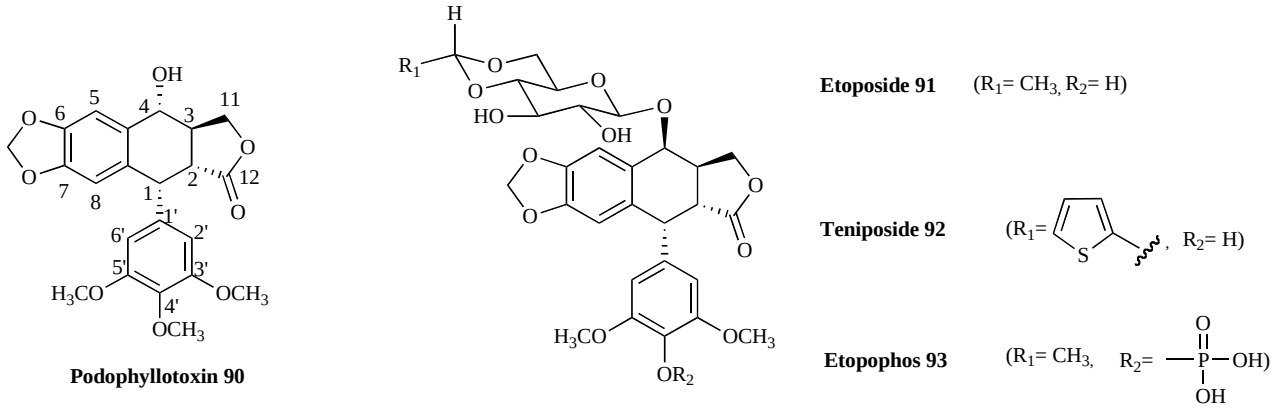
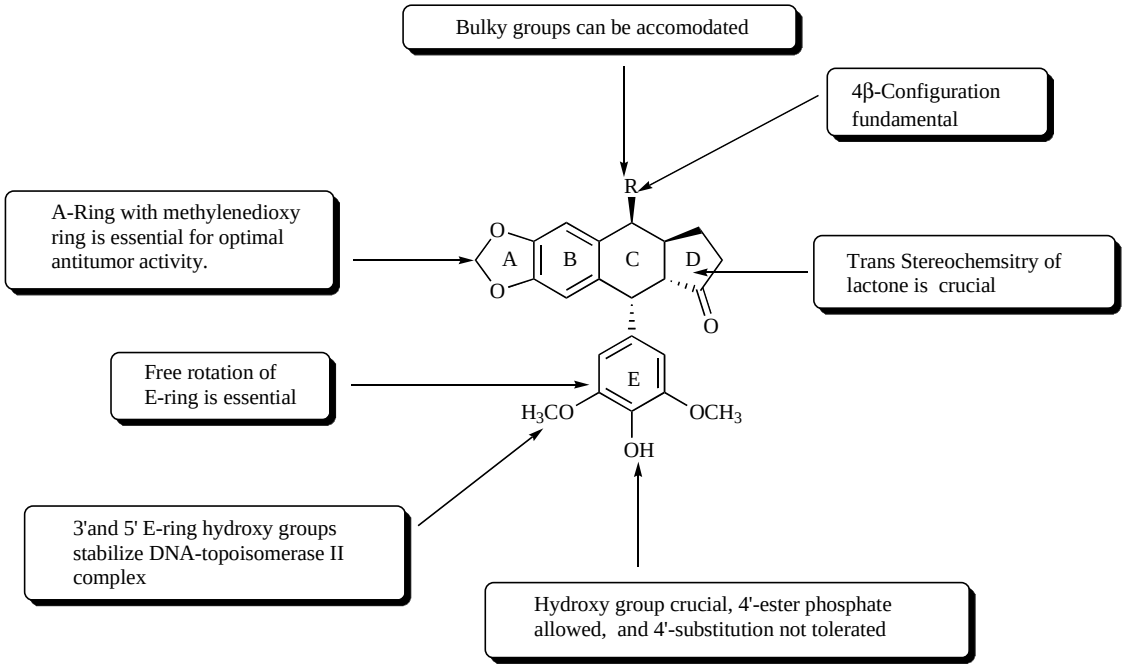


Fig. (14).



Structure activity relationship studies of podophyllotoxin

with regard to the inhibition of DNA topoisomerase II have shown the following relationships between structure and anticancer activity. The epimerisation at 4-position is considered important as it increases the biological activity in such a manner that the compounds with β -configuration are more potent than that of α -configuration. It has been observed that *trans* lactone ring is crucial for exhibiting biological activity. The methylenedioxy group is important to exhibit optimal antitumor activity and whereas, the free rotation of E ring is necessary for antitumor activity. Further, demethylation at 4-position of E-ring appears to be essential for DNA breakage activity. Based on the SAR studies various modifications on podophyllotoxin have been carried out and discussed below.

A-Ring Modifications

This includes derivatives in which the methylenedioxy group has been cleaved to produce two free phenolic groups and further transformed into other groups or oxygenated rings with or without substituents (**94-96**). These compounds have exhibited potent immunosuppressive activity. In few cases A-ring has been modified into phenazine ring and all these compounds have shown improved cytotoxic profile compared to that of etoposide (Fig. **15**) [221-225].

B-Ring Modification

There are very few reports on the B-ring modifications. Thurston and coworkers [226] have synthesized α-peltatin esters and ethers including its glycosidic ethylidene, ethenylidene cyclic acetals **97**, and have found these compounds to be more cytotoxic than etoposide but less active in their inhibitory action against DNA topoisomerase II (Fig. **16**).

C-Ring Modifications

The C-ring modification has gained much attention among all the podophyllotoxin modifications to improve the cytotoxicity profile. In this context, several C-ring aromatized analogues **98**, epipodophyllotoxin derivatives with an unsaturation side chain **99**, benzodioxole lactones analogues **100**, and 4-oxa/thia-2-azapodophyllotoxin **101** have been

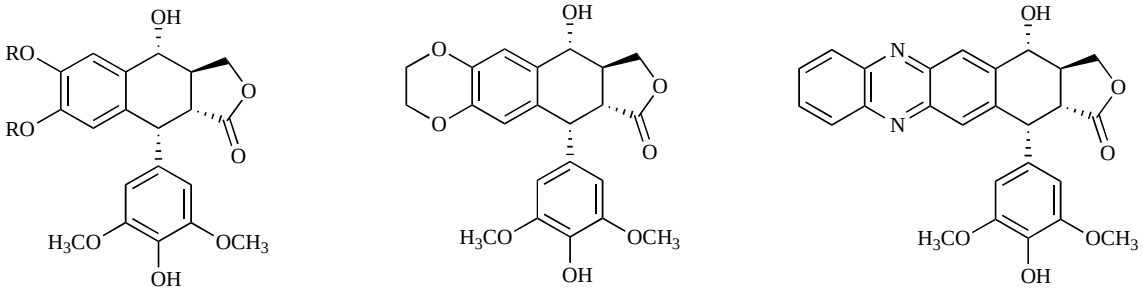
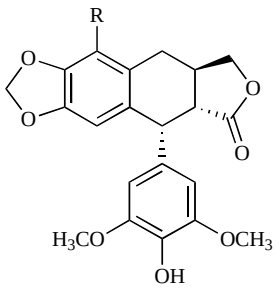


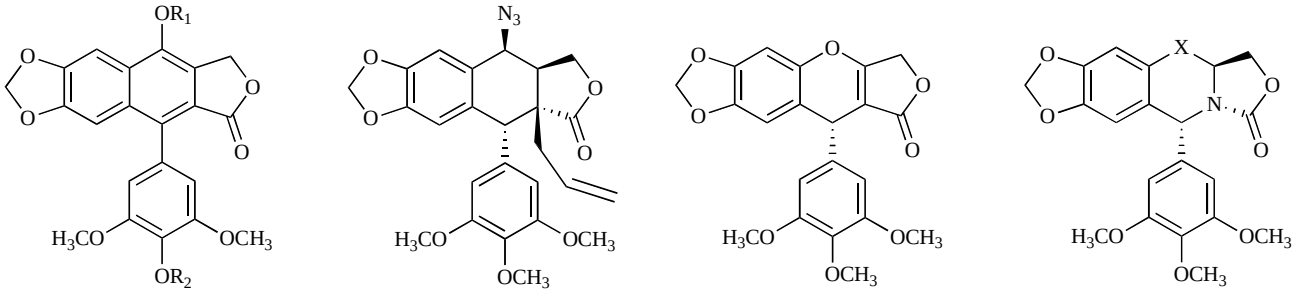
Fig. (15).



R= $COOCH_2C_6H_5$
R= $COCH_3$
R= $COCH_2CH_2COOH$
R= $COCH_2CH_3$
R= $COCH_2CH_2CH_3$
R= $COCH_2C_6H_5$
R= CH_3

97

Fig. (16).



98 **99** **100** **101** X= O, S

R_1 = H; R_2 = Me
R_1 = R_2 = H
R_1 = Me; R_2 = H
R_1 = Et; R_2 = H
R_1 = n-Pr; R_2 = Me

Fig. (17).

synthesized and tested against human DNA topoisomerase II for antitumor activity [227, 228]. Laatsch and coworkers [229] prepared some C-ring expansion products of podophyllotoxin, obtained from the appropriate azide *via* a photochemical nitrene rearrangement. Jurd and Hitatsuyanagi have synthesized benzodioxole lactones analogues of podophyllotoxin, and 4-oxa/thia-2-azapodophyllotoxin **101** respectively [230-232]. These compounds have been tested for their biological activity and some of them have shown significant activity against different cancer cell lines (Fig. **17**).

Modifications at C4 Position of C-Ring

The C4-*N*-substituted podophyllotoxin congeners occupy a significant position in the recent development of podophyllotoxin. Lee and co-workers [233] have carried out very extensive work in this area for the development of more potent podophyllotoxin based compounds. They have synthesized a number of C4-*N*-substituted podophyllotoxin derivatives **102** and substituted aniline-PDT derivatives **103** by replacing the C4-hydroxyl group by an amino group with promising DNA topoisomerase II inhibitory activities [234, 235]. Another synthesized series of novel 4β-amino derivatives **104** of etoposide have demonstrated excellent activity against MDR and topoisomerase II resistant cell lines [236]. The podophyllotoxin-glutamate diethyl ester analogues **105** have been synthesized and evaluated with encouraging cytotoxicity [237]. Similarly, they have synthesized new benzimidazole substituted analogues **106** of podophyllotoxin, and they have been more active than etoposide on biological evaluation (Fig. **18**) [238]. Interestingly, this group has also synthesized camptothecin- epipodophyllotoxin, and taxiod-epipodophyllotoxin conjugates with low and enhanced activity, respectively [239, 240]. Tian and coworkers have synthesized 4β-*N*-substituted-5-FU-4'-demethylepipodophyllotoxin derivatives with significant cytotoxic activity against HL-60 and A-549 cell line [241].

During the investigations on the modification of podophyllotoxin ring system have led to novel podophyllotoxin dimers with promising cell growth inhibition in human cell line assays. Most of the new podophyllotoxin dimers are more cytotoxic in comparison to etoposide (Fig. **19**) [242]. Recently, in this laboratory some new benzophenone-PDT, 4β-amido (**107a-d**) and sulfonamido–PDT conjugates (**108a-d**) with interesting DNA topoisomerase II inhibition and promising antitumor activity (Fig. **19**) have been synthesized [243]. Further, several series of compounds based on PDT have been synthesized and evaluated activity against several cancer cell lines [244]. Some of them have displayed encouraging results against these cell lines, and detailed mechanistic studies are under process.

D-Ring Modifications

The *trans* lactone on D-ring of PDT is essential for antiproliferative activity. However compounds with methylene

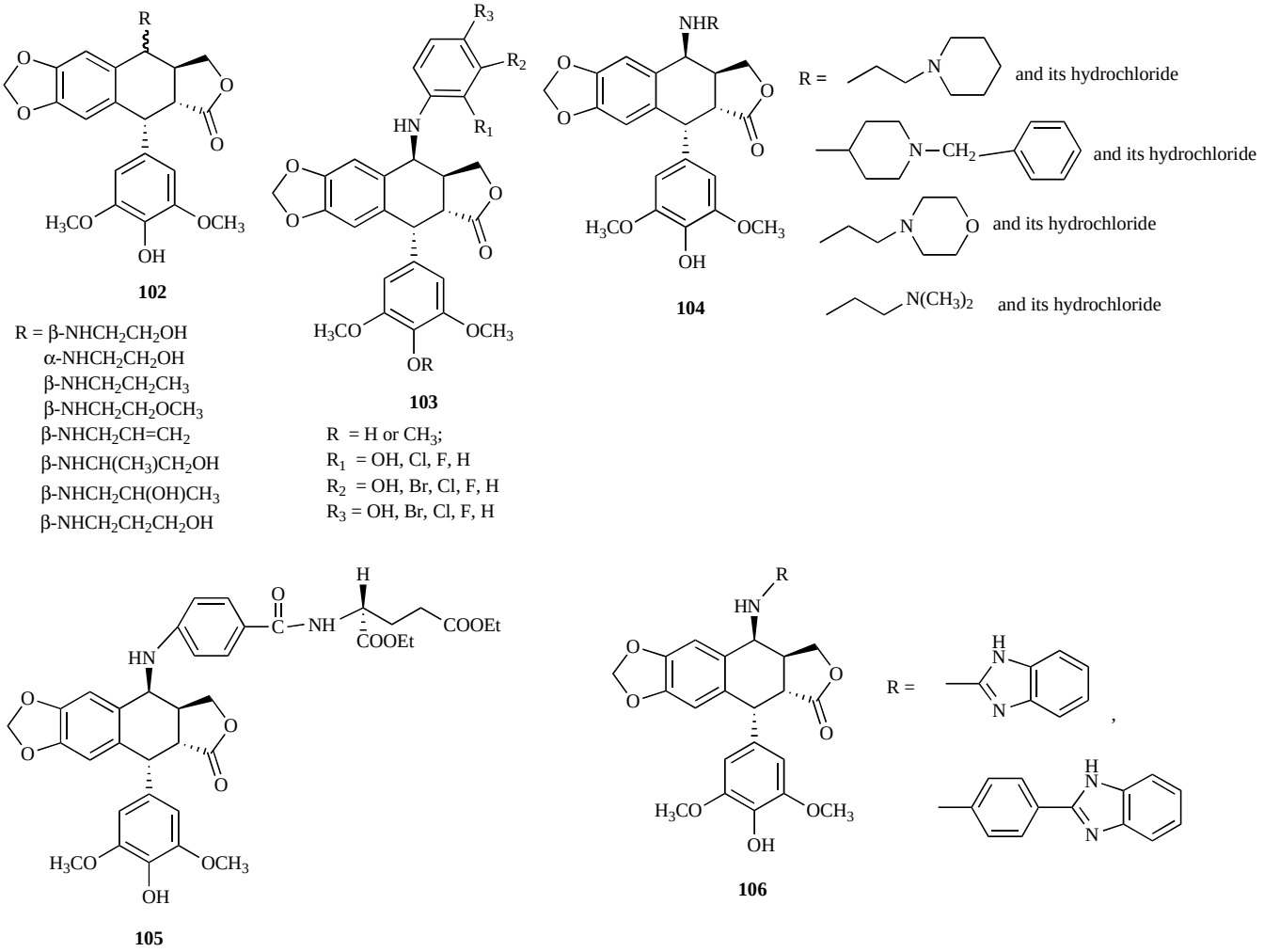


Fig. (18).

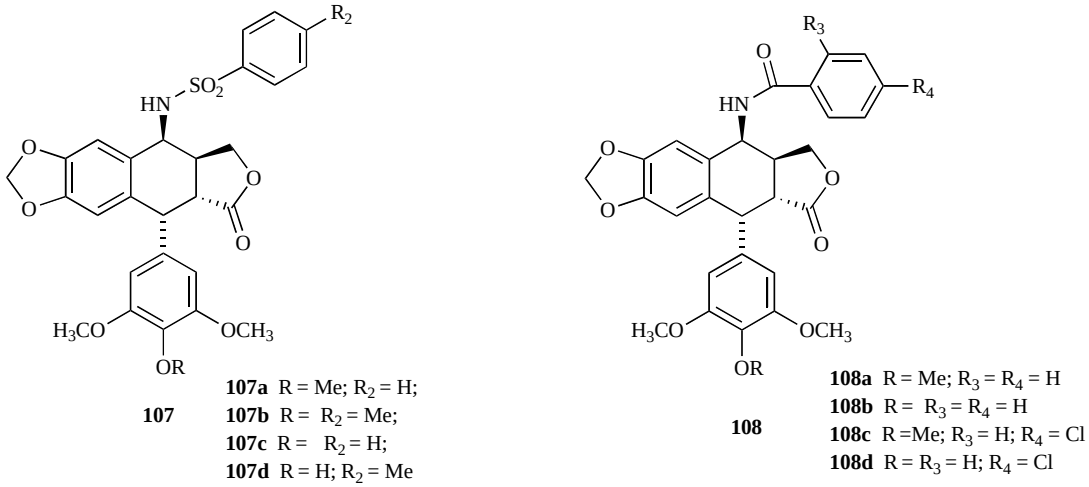


Fig. (19).

group have been synthesized by the replacement of lactone group and evaluated to possess moderate activity [245, 246]. Wang and coworkers [247] have synthesized a novel spin labeled derivative of PDT, *N*'-podophyllic acid-*N*[3-(2,2,5,5-tetramethylpyrrolinenyloxy)]semicarbazide **109** (GP-11) and tested its antitumor activity. Similarly, D-ring modified etoposide lactam **110** and cyclosulfite analogue of PDT **111** by expanding lactone ring have been synthesized. The compound **111** has significant cytotoxic activity against human

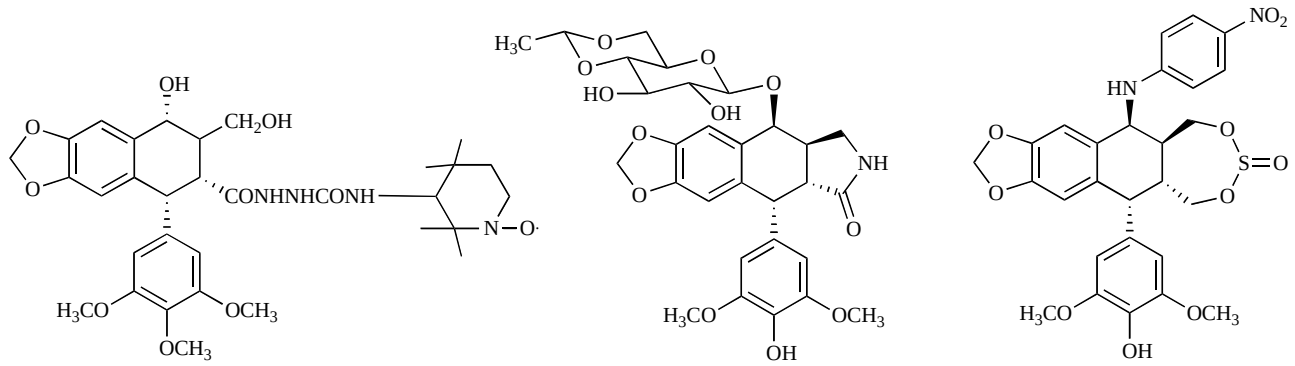


Fig. (20).

tumor cell lines, but showed no *in vitro* inhibition against human DNA topoisomerase II (Fig. **20**) [248, 249].

E-Ring Modifications

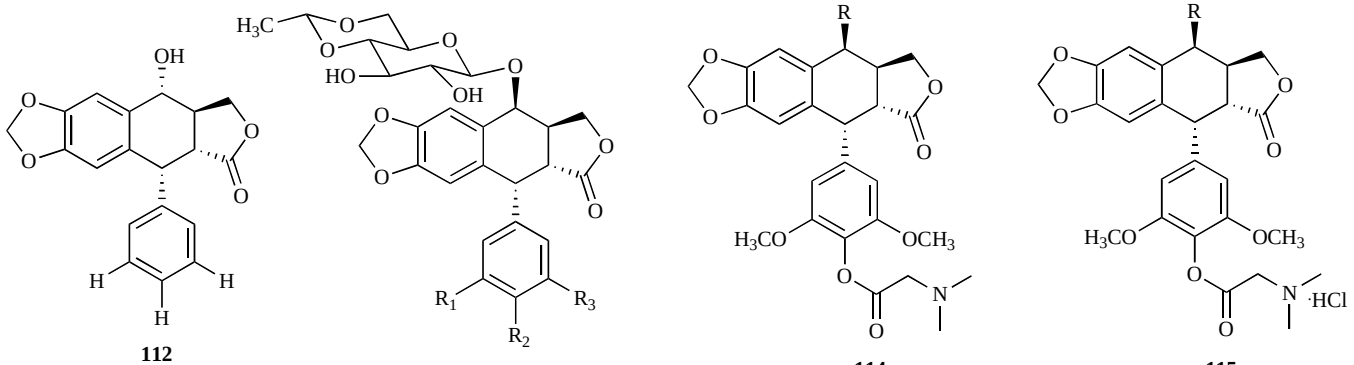
The modification of E-ring on PDT is related to lignans metabolism and its inactivation and, is perhaps the least modified part. However, some transformations have been carried out, which includes demethylations, oxidation to the *o*-quinone and the introduction of nitrogen radicals. Saulnier and coworkers have synthesized the deoxy E-ring analogues of etoposide (**112**, **113**) with weak cytotoxic activity [250]. Furthermore, modified E-ring PDTs without 3',4',5'-trimethoxy groups and novel ester analogues (**114**, **115**) have shown better activity compared to etoposide (Fig. **21**) [251, 253].

Prodrugs of Etoposide

Although etoposide (VP-16) is widely used in therapy, it presents several limitations, such as moderate potency, poor water solubility, development of drug resistance, metabolic inactivation, and toxic effects which is due to lack of selectivity. A glucuronide-based, prodrug of etoposide (**116-118**) has been synthesized for prodrug monotherapy of solid tumors by Schmidt and Monneret with an aim to selectively liberate the active compound by β-D-glucuronidase, an enzyme that is present in necrotic tumors [254]. Senter and coworkers found that etoposide phosphate prodrug **119** was 100-fold less active than the parent drug at *in vitro* conditions, and that immuno-specific activation of the prodrug could be accomplished using an L6-AP mAb-enzyme conjugate (Fig. **22**) [255].

BENZOTHIADIAZINE 1,1-DIOXIDE AND RELATED COMPOUNDS

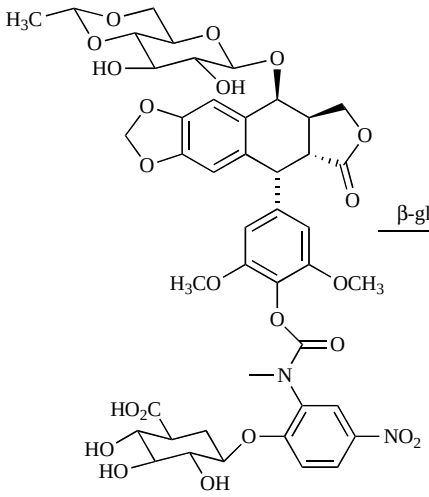
The versatile and synthetically accessible scaffolds have provided the inspiration for the discovery of a number of new antitumor agents with unusual mechanisms of action in recent years [256]. Benzothiadiazine 1,1-dioxides, the benzenesulfonamide derivatives constitute an important class of therapeutic agents in medicinal chemistry and in recent years have attracted considerable attention as anticancer agents [257, 258]. This class of compounds (**120-126**) exerts their biological effect by acting on the inhibition of DNA, RNA or protein synthesis, which illustrate important targets for the development of anticancer agents [259, 260]. 1,2,4-Benzothiadiazine1,1-dioxide ring system 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-*b*][1,2,4]benzothiadiazine 6,6-dioxides that possess a built in sulfonylhydroxyguanidine



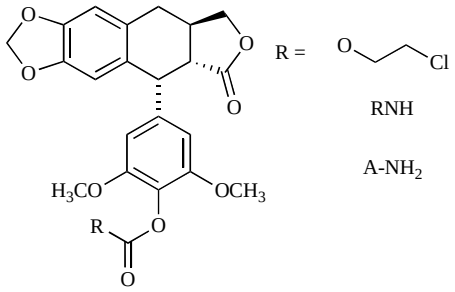
$R_1 = OCH_3$; $R_2 = H$; $R_3 = OH$
$R_1 = R_2 = OCH_3$; $R_3 = H$
$R_1 = R_2 = H$; $R_3 = OH$

R = 4-NO_2 C_6H_4,
= 4-F C_6H_4

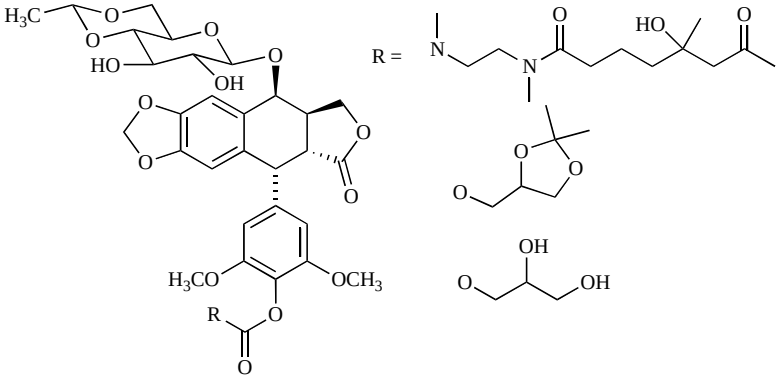
Fig. (21).



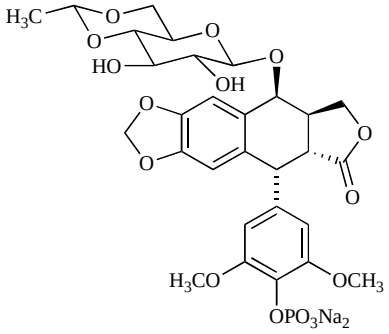
Etoposide β-glucuronide prodrug **116**



Prodrugs of 4'-demethyl-4-deoxypodophyllotoxin **118**

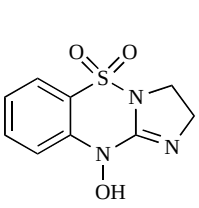


Glucuronide-based prodrug of etoposide **117**



Etoposide phosphate prodrug **119**

Fig. (22).



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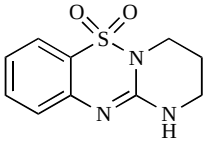

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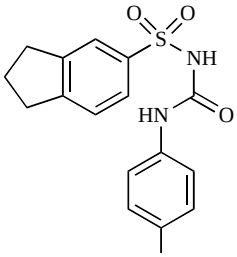

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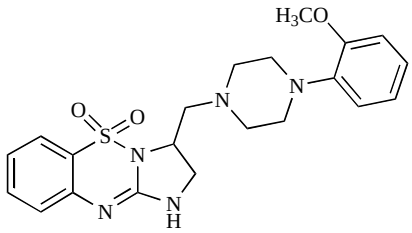

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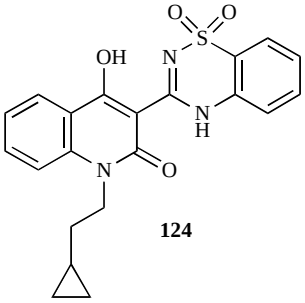

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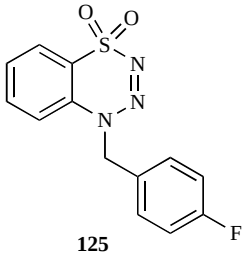
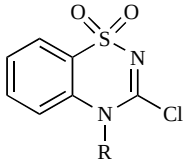


125



R = CH_3, C_2H_5

126

Fig. (23).

moiety **122** exhibits anti-proliferative activity by inhibition of ribonucleotide reductase [260, 261]. Recently Lin and coworkers reported 1,2,4-triazole-3,5-diamine analogues as potent antitumor agents by inhibition of cyclin dependent kinases (Fig. **23**) [262]. Moreover, some other analogues are reported to display antiproliferative activity by inhibiting cyclin-dependent kinase and tubulin polymerization [263, 264]. However, the exact mechanism for antitumor activity is yet to be established.

Brzozowski and co-workers [265] have reported the synthesis of 7-substituted 8-chloro-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazines **127** as potent antitumor agents with a high degree of selectivity against leukemia HL-60 cells. Similarly, the benzodithiazine-aryl sulfonamide conjugates (**128**, log GI_{50} = < -8.00 for the leukemia SR cell line) and a novel series of 6-chloro-1,4,2-benzodithiazine 1,1-dioxide derivatives (**129** GI_{50} <10 nM for leukemia CCRF-CEM cell line) with alkyl, aryl or heteroaryl substituents at position 3 have also displayed remarkable activity on the leukemia cell lines with moderate activity against the other human tumor cell lines derived from nine different cancer type [266, 267]. The 1,2,4-benzodithiazine 1,1-dioxide derivatives (**130, 131**) and cyclic sulfonamide derivatives of 8-chloro-5,5-dioxoimidazo[1,2-*b*][1,4,2]benzodithiazine **132** have been reported to possess interesting anticancer properties [268]. Based on these findings, Pomarnacka and co-workers [269, 270] have reported triazolobenzodithiadiazines as promising anticancer agents and benzothiadiazine derivatives (Fig. **24**) have also shown potent activity against several cancer cell lines with ED_{50} up to 1.1 μg/mL [271]. Further, Chern and co-workers have reported fused 1,2,4-benzothiadiazine 1,1-dioxides as potential anticancer agents [272, 273].

From this laboratory structurally well characterized novel [1,2,4]triazolo[1,5-*b*][1,2,4]benzothiadiazine-benzothiazole conjugates have been reported as antitumor agents [274]. On evaluation of cytotoxicity against 60 human tumor cell lines screen, the compound **133** displayed significant growth inhibition against almost all the 60 cell lines. Further, another series of meracapto triazolo-benzothiadiazine linked amino-benzothiazole hybrids have synthesized and evaluated against selected cancer cell lines [275]. Interestingly, one of the synthesized compounds **134** exhibited GI_{50} values of 1.4 and 2.1 μM against RPMI-8226 (leukemia) and HOP-62 (lungs) cell lines respectively. However, further structural modifications are required to assess the SAR studies as well as mechanism of anticancer activity.

NAPHTHALIMIDES

The search for novel chemotherapeutic agents and approaches to cancer treatment is an active research field stimulated by the discovery of new biological targets, eventually to develop new drugs without serious side effects [276]. The binding of small ligands to the DNA has attracted an enormous amount of study in the field of molecular pharmacology, medicinal chemistry, and carcinogenesis [277-282]. In this perspective, a series of compounds with naphthalimide chromophore (benz[*de*]isoquinoline-1,3-dione) has been discovered as potential antitumor agents. Brana and co-workers have shown that 3-nitronaphthalene monoimides with cationic substituents exhibit significant antitumor activity by interaction with DNA [283, 284]. Similarly, Yen and co-workers have found that naphthalene monoimides **135** and diimides **136** having a variety of cationic substituents bind to DNA by intercalation [285]. A series of naphthalimides and diimides with varying length of spacers have been synthesized and the viscometric titrations indi-

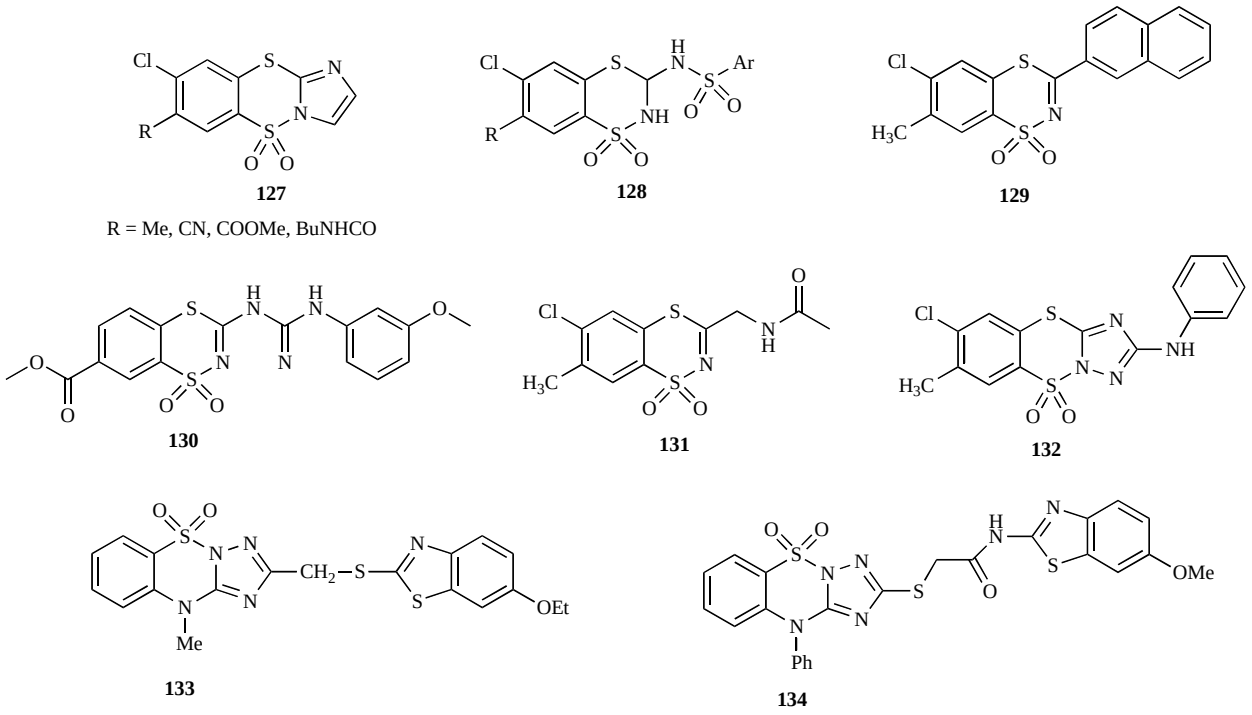


Fig. (24).

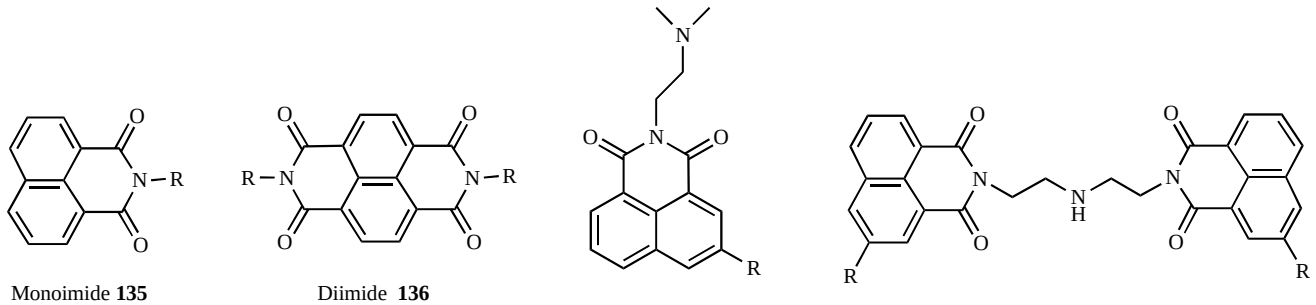


Fig. (25).

cated that these compounds can interact strongly with DNA by the formation of intercalating complexes. The diimides bind atleast 10-fold more strongly to DNA than monoimides with their corresponding substituents. The naphthalimide, mitonafide **137** have been evaluated in phase II clinical trials as potential anticancer agents [286]. Mitonafide is an effective antitumor agent and it binds to DNA by intercalation with an association constant of 1.5 x 10^5 M^{-1} at 0.01 M ionic strength [287]. Unfortunately, mitonafide has shown inappropriate central nervous system toxicity and overall produced a limited clinical activity [288]. Similarly, the related amonafide **138** has also been assessed extensively in clinical trials with limited success [289, 290].

Brana and co-workers have attempted to improve the activity of lead molecules by increasing its binding capability to the DNA [291]. In this context, a series of *bis*-intercalating agents have been designed using the structural features of the lead molecules. They bind to DNA as bis-intercalators and show higher binding affinity with greater cytotoxic activity than their parent mono-intercalators. Particularly, elinafide (**139**, LU-79553) displayed potent cellular cytotoxicity, marked *in vivo* activity against several tumor xenograft models [292, 293]. It intercalates in the major groove of DNA helix and has been evaluated in clinical trials against solid tumors. The other dinitro bis-naphthalimide DMP-840 **140** (bisnafide) shows a wide spectrum of activity similar to that of elinafide, and is also under clinical trials (Fig. **25**) [294].

Recently efforts have been made to enhance the potency of mononaphthalimides and *bis*-naphthalimides, by incorporating an anthracene moiety rather than the simpler naphthalene chromophore. These compounds include azonafide **141** and bibenoline that shows an interesting antitumor profile. Similarly, many naphthalimides as well as bis-naphthalimides have also been synthesized by fusing imidazole, pyrazine, furan and thiophene rings (**142-145**) to the naphthalene moiety have shown an interesting antitumor profile [295-298]. Some of these compounds have shown more potent cytotoxicity than elinafide against human colon carcinoma cells (HT-29). Tumiatti and coworkers have synthesized a series of naphthalimide (NI) and 1,4,5,8-naphthalentetracarboxylic diimide (NDI) derivatives (**146a-i**) and evaluated for their anti-proliferative activity in breast cancer and leukemia cell lines. Interestingly, *bis*-substituted derivatives are more cytotoxic than the corresponding NI derivatives in the range of 0.2-1.7 μM [299].

To overcome the side effects of amonafide, Kiss and coworkers have designed, synthesized a library of new molecules and evaluated their antiproliferative activity [300]. Among them the compound (UNBS3157) **147** has displayed a 3-4 folds higher *in vitro* antitumor activity (IC_{50} range of 0.8-1.8 μM) than amonafide with a distinct mechanism of action, notably inducing autophagy and senescence in cancer cell lines. It also displays higher *in vivo* activity in a range of cancer models and higher tolerance. Continuing on the success of platinum anticancer drugs, Ranninger and coworkers reported two Pt-*bis*-(naphthalimide) complexes (**148, 149**) which demonstrated promising cytotoxic activity due to combined effect of platinum and intercalation [301]. Moreover, non-platinum complexes such as gold (I) phosphine complex [*N*-(*N'*, *N*'-dimethyl aminoethyl)-1,8-naphthalimide-4-sulfide] **150** have strong antiproliferative effects by induction of apoptosis *via* mitochondrial pathways (Fig. **26**) [302].

As a part of development of new anticancer agents, from this laboratory several series of coumarin-naphthalimido conjugates, naphthalimido-dihydropyrimidinone hybrids have been synthesized and evaluated for antitumor activity [303, 304]. Amongst them, coumarin linked compound **151** has been the most active (IC_{50} 0.19-31 μM) against all the six cell lines, while most of these hybrids displayed moderate activity due to high polarity. Recently, another series of benzimidazole-naphthalimide conjugates have been synthesized and evaluated for activity against 60 anti-tumor cell line screen. Most of them have shown significant activity and the conjugate **152** have shown potent activity (Log GI_{50} = -5.62) against human tumor cell lines [305]. Moreover, efforts are under progress to develop new molecules based on naphthalimides scaffolds as potential antiproliferative agents.

Naphthalimide Prodrugs

The efficacy of clinically used anticancer therapeutics is limited, since they are not selective for cancer cells causing side effects such as damages in bone marrow and gut epithelia [306]. To fulfill this need, one strategy is the development of tumor activated prodrugs (TAP), which are relatively non-toxic and can be selectively activated in tumor tissue [307, 308]. In this perspective, Xu and coworkers have recently

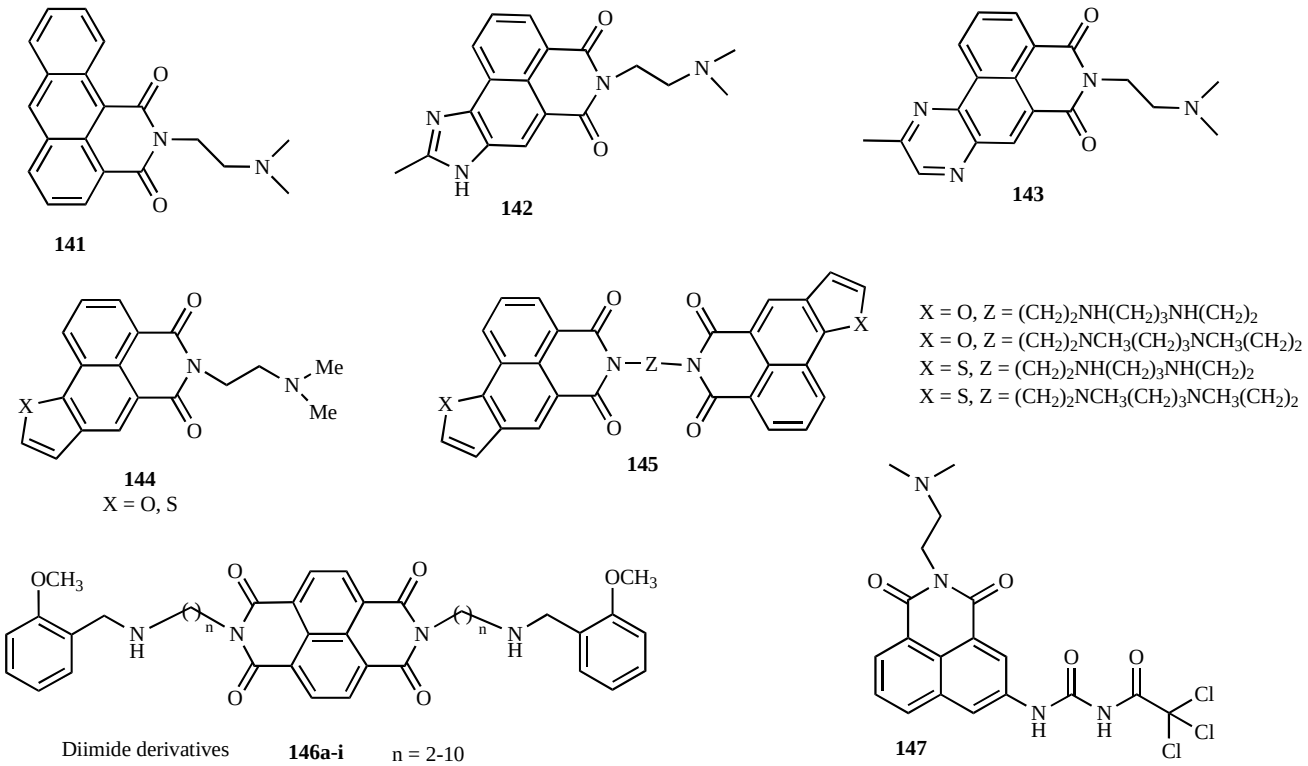

141
142
143
144
X = O, S
145
X = O, Z = $(CH_2)_2NH(CH_2)_3NH(CH_2)_2$
X = O, Z = $(CH_2)_2NCH_3(CH_2)_3NCH_3(CH_2)_2$
X = S, Z = $(CH_2)_2NH(CH_2)_3NH(CH_2)_2$
X = S, Z = $(CH_2)_2NCH_3(CH_2)_3NCH_3(CH_2)_2$
Diimide derivatives
146a-i
n = 2-10
147

Fig. (26).

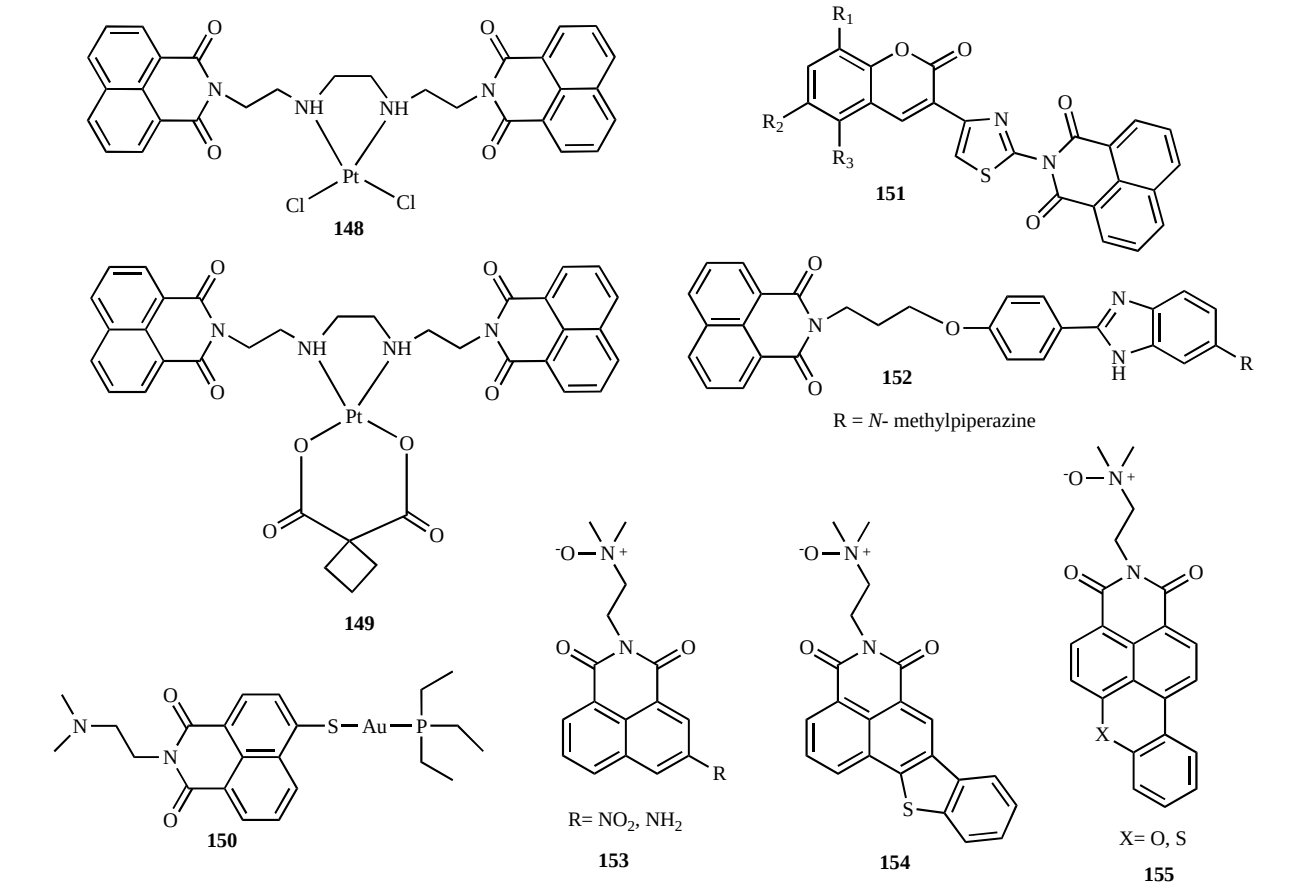

148
149
150
151
152
R = *N*- methylpiperazine
153
$R= NO_2, NH_2$
154
155
X= O, S

Fig. (27).

designed and synthesized novel tertiary amine *N*- oxides of naphthalimide prodrugs (**153-155**) as potential anticancer agents as depicted in Fig. (**27**) [309]. These *N*-oxides have shown less cytotoxicity compared to corresponding naphthalimides in oxic A375 cell cultures. However, all these have displayed more *in vitro* cytotoxicity against hypoxic A375 cells and might be used as interesting candidates of prodrug leads in hypoxic solid tumor cells.

MONASTROL AND ITS ANALOGUES

The cell permeable small molecules that perturb specific proteins associated with mitotic process are potential leads for development of anti-proliferative agents as well as valuable tools for understanding dynamic cellular processes. In 1999, a phenotype screening based on post transitional modification and visualization of microtubules and chromatin has led to identification of compounds that affect mitosis. From the library of 16,320 compounds, one of the compound with structurally simple dihydropyrimidone scaffold has been found to arrest mitotic cell division by specifically inhibiting the motility of kinesin specific protein (KSP, Eg5) required for spindle bipolarity and has been named as monastrol **156** [310]. Thus, monastrol is the first Eg5 inhibitor to be identified with an IC_{50} value of 14 μM causing a specific and reversible cell cycle block. In contrast to anticancer drugs like taxanes, it does not display any neurotoxicity and is in fact reported to enhance axonal growth [311-313]. The antimitotic activity of monastrol itself is not very high and this does not warrant it as a drug candidate. Therefore in recent years, the development of more potent, specific and cell permeable monastrol analogues has been carried out. Few reports are available in literature on the derivatization of monastrol and the subsequent determination of kinesin Eg5 inhibition activity.

Giannis and co-workers have reported the screening of nearly 40 compounds for their ability to inhibit Eg5 by using an *in vitro* steady-state ATPase assay [314]. Most of the compounds are found to be less potent compared to monastrol, however, three conformationally rigid bicyclic compounds are significantly more potent Eg5 inhibitory activity. Enastron **157** (IC_{50} = 2μM), dimethylenastron **158** (IC_{50} = 200 nM) and enastrol **159** (IC_{50} = 2 μM) obtained by the cyclization of the side chain have exhibited 10-100 times more potency than monastrol. Moreover, these compounds have also arrested mitosis in cultured cells. Recently, Lebeau and coworkers have designed and synthesized a series of monastrol derivatives and evaluated for their Eg5 inhibitory activity [315]. The compound **160** has appeared to be more potent than monastrol by a five-fold factor, whereas the compounds (**161-163**) have shown potent Eg5 inhibitory activity. Gheber and co-workers have described the differential effects of monastrol on AGS and HT-29 cell lines in comparison with taxol [316]. At 50 μM, monastrol has inhibited AGS cell growth, while HT29 cells have been completely inhibited at a concentration as high as 150 μM. Russowsky and coworkers have reported the synthesis and anti-proliferative activity of monastrol, oxo-monastrol **164** and other eight oxygenated derivatives on seven human cancer cell lines [317]. For all evaluated cell lines, monastrol was shown to be more active than its oxo-analogue, suggesting the importance of the sulfur atom for the antiproliferative activity. The thio-derivatives (**165-167**) have displayed relevant antiproliferative properties with 3,4-methylenedioxy derivative **167** being approximately more than 30 times more potent than monastrol against colon cancer (HT-29) cell line (Fig. **28**).

In this laboratory, efforts are in progress exploring the SAR studies of monastrol based scaffold. A library of structural variants of monastrol have been designed and synthesized with a view to enhance the kinesin Eg5 inhibitory and cytotoxicity, which will be communicated shortly [318].

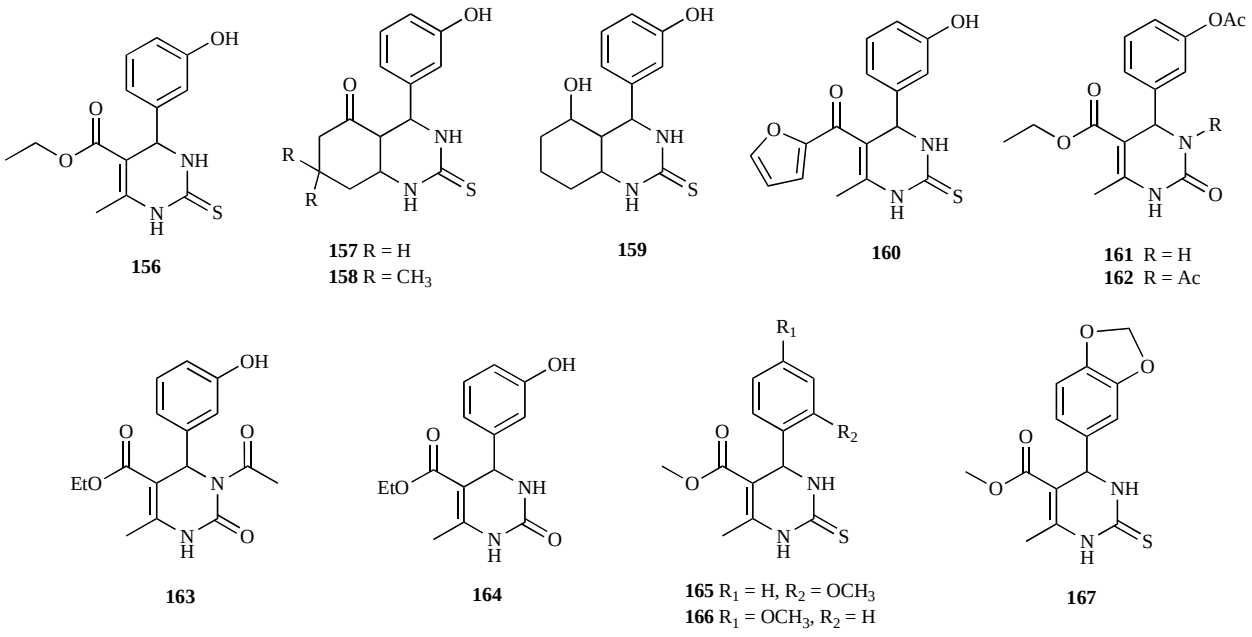


Fig. (28). Compounds based on structurally modification of monastrol.

CONCLUSION

Despite the availability of anticancer agents derived from natural products and their semisynthetic derivatives, the development of a safe and site-specific anticancer drug still remains a challenge. The major obstacles for this challenge are the association of toxicity with drugs which is due to lack of specificity, as these agents kill healthy cells and the drug resistance which have arisen in recent years. The combination therapy employing different chemotherapeutic agents has been used to combat this problem with some success. However, the possibility of the development of drug resistance still remains. Keeping pace with these challenges, around the world and from this laboratory a good number of diverse molecules with a novel mode of action have been developed based on pyrrolo[2,1-*c*][1,4]benzodiazepines, podophyllotoxins, benzothiadiazine1,1-dioxides, naphthalimides, monastrol etc. Now efforts are being focused towards the intervention of site-specific anticancer agents eventually to develop effective chemotherapeutics for the treatment of human malignancies.

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