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Review

Plant-based anticancer molecules: A chemical and biological profile of some important leads ☆

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Abstract—A number of natural products, with diverse chemical structures, have been isolated as anticancer agents. Several potential lead molecules such as camptothecin, vincristine, vinblastine, taxol, podophyllotoxin, combretastatins, etc. have been isolated from plants and many of them have been modified to yield better analogues for activity, toxicity or solubility. Several successful molecules like topotecan, irinotecan, taxotere, etoposide, teniposide, etc. also have emerged as drugs upon modification of these natural leads and many more are yet to come. In this review, the authors have focused on four important anticancer leads, that is, camptothecin, taxol, combretastatin A-4 and podophyllotoxin. Their chemistry, structure and activity relationships, biological activities, modes of action, analogue synthesis and future prospects have been discussed.

Contents

Keywords: Anticancer; Camptothecin; Taxol; Combretastatin A4; Podophyllotoxin.
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1. Introduction

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of deaths after cardiovascular diseases. It is a disease characterized by unregulated proliferation of cells. The search for natural products as potential anticancer agents dates back, at least, to the Ebers papyrus in 1550 BC, but the scientific period of this search is much more recent, beginning with the investigations by Hartwell and co-workers in late 1960s on the application of podophyllotoxin and its derivatives as anticancer agents. A large number of plant, marine, and microbial sources have been tested as leads, and many compounds have survived the potential leads.

2. Camptothecin

2.1. Introduction

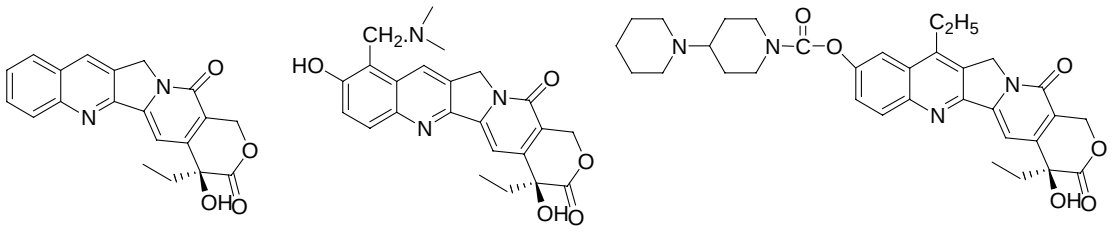
In the early sixties, the discovery of camptothecin (CPT, **1**) by Wall and Wani as an anticancer drug with a unique mode of action, that is, inhibition of DNA topoisomerase I, added an entirely new dimension to the field of chemotherapy. This naturally occurring alkaloid was first extracted[2,3] from the stem wood of the Chinese ornamental tree *Camptotheca acuminata* during the screening of thousands of plants in a search for steroids. Preliminary studies revealed a substantial antitumour activity in standard in vitro test system as well as in mouse leukaemia cells. These astonishing findings greatly increased interest in this natural product as a possible antitumour agent. The molecule became so important that during 1966–2004 over 3000 research papers appeared on it. Presently, the first generation analogues of CPT, hycamtin (**2**, topotecan) and camptosar (**3**, irinotecan, CPT-11), marketed by Glaxo-SmithKline and Pfizer, respectively, are used for the treatment of ovarian and colon cancers[4,5] (see Fig. 1).

2.2. Chemistry

Camptothecin was first isolated from the Chinese ornamental tree *Camptotheca acuminata*, also known as the 'tree of joy' and 'tree of love'. It has also been isolated from *Ophiorrhiza pumila* and *Mapia foetida*. It is a member of the quinolinoalkaloid group. It consists of a pentacyclic ring structure that includes a pyrrole (3,4β) quinoline moiety and one asymmetric centre within the α-hydroxy lactone ring with 20(*S*) configuration (ring E). Camptothecin occurs in different plant parts like the roots, twigs and leaves.

2.3. Structure and activity relationship of CPT

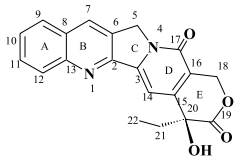
The planar pentacyclic ring structure (rings A–E) was suggested to be one of the most important structural features. Earlier, it was reported that the complete pentacyclic ring system is essential for its activity, but recently



1, CPT **2, Topotecan** **3, Irinotecan**

Figure 1. CPT and its analogues.

reported results show that the E-ring lactone is not essential for its activity. However, this ring in the present lactone form with specific C-20 configuration is required for better activity. A brief description[6] of its SAR is as follows:



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- Rings A–D are essential for in vitro and in vivo activity.
- Saturation of ring B: compounds show little activity.
- α-Hydroxy lactone ring is necessary for activity.
- Oxygen at 20 is essential for activity. Replacement of this oxygen with sulfur or nitrogen abolishes the activity of CPT.
- Conformation at C-20 is crucial for better activity as the 20(*S*) isomer is 10- to 100-fold more active than 20(*R*).
- D-ring pyridone is required for antitumour activity.
- Modifications in rings A and B are well tolerated and resulted in better activity than CPT in many cases.

2.3.1. The stereochemistry at C-20. The stereochemistry at C-20 of CPT is very crucial for its activity, as 20(*S*) hydroxyl is active while the corresponding 20(*R*) hydroxyl is inactive.[7] One of the major drawbacks observed in the use of CPT analogues in clinical studies was a marked loss of therapeutic activity due to their intrinsic instabilities resulting from the rapid hydrolysis of the lactone ring in the body. Thus, synthesizing an analogue with adequately long biological life/activity in its active lactone form has been an important task for scientists.

2.4. Biological activity

CPT is a potent cytotoxic agent. It shows anticancer activity mainly for solid tumours. It inhibits DNA topoisomerase I.[8,9] It shows anticancer activity mainly against colon and pancreatic cancer cells. But its analogues showed anticancer activity in breast, liver, prostate, etc.

2.5. Mode of action

In the early 1970s, initial studies examining the mechanism of action of CPT suggested that cytotoxicity might result from its immediate synthesis, which was found to be reversible following brief exposure to camptothecin, but DNA topoisomerase I inhibition progressively became irreversible with increasing concentration and exposure duration. These studies also suggested that camptothecin is selectively cytotoxic to S-phase cells, arrests cells in the G-2 phase and induces fragmentation of chromosomal DNA.

Topoisomerase I and topoisomerase II catalyze the relaxation of supercoiled chromosomal DNA during DNA replication. The relaxation of DNA by topoisomerase II involves the transient double strand breakage of DNA, followed by strand passage and relegation of the DNA strand. In contrast, topoisomerase I involves the transient single strand cleavage of duplex DNA, followed by unwinding and relegation.[10] Topoisomerase I cleaves DNA at multiple sites, and the highest efficiency cleavage sites exhibit significant sequence homology. CPT was approved by US Food and Drug Administration in the 1970s against colon carcinoma and thus it was evaluated[11a,11b] as a possible drug in the treatment of human cancer in phase I and phase II studies. Although camptothecin showed strong antitumour activity among patients with gastrointestinal cancer, it also caused unpredictable and severe adverse effects including myelosuppression, vomiting, diarrhoea, and severe haemorrhagic cystitis. These findings eventually resulted in the discontinuation of phase II trials in 1972.

2.6. Synthetic analogues of CPT

CPT as such could not be used as a drug of choice due to its severe toxicity. Several groups have tried to synthesize derivatives having lower toxicity. Thus, the development of these synthetic and semisynthetic strategies have facilitated the study of the CPT mechanism, as well as the identification of analogues with improved properties.

2.6.1. Modifications in quinoline A and B rings. The most successful derivatives of CPT have been obtained due to modifications of rings A and B. To date, the only CPT analogues approved for clinical use[12,13] are topotecan

(**2**) and irinotecan (**3**), which were obtained by modifications of these rings. Modifications can involve additions to the quinoline ring or the complete replacement of the quinoline ring with an alternative ring system. Several other heterocyclic ring systems have been found to have significant cytotoxicity on replacement of the quinoline ring.[14] But the quinoline ring system was found to be the most potent and hence, most of the modifications were done with retention of the quinoline ring system.

Within this series of compounds, the water-soluble analogues irinotecan (CPT-11, **3**), which is a prodrug of SN-38, and topotecan were found to be the most promising anticancer agents, and currently they are being marketed. In view of the clinical success of the water-soluble CPT derivatives topotecan and irinotecan, efforts to increase the water solubility of camptothecin have comprised a major research focus. The most successful derivative of this class is lurtotecan (**5**), that is, 10,11-(methyl ethylenedioxy)-7-((*N*-methylpiperazino) methyl) camptothecin. The compound is presently in clinical trials for breast, colorectal and small cell lung cancers.[15,16]

Water-insoluble analogues of CPT such as IDEC-132 (9-amino camptothecin or 9-AC, **6**) and 10,11-dimethylenedioxy camptothecin analogues (10,11-MDC) have shown strong antitumor activities against solid tumours. IDEC-132 showed much stronger topoisomerase I inhibitory activity than topotecan and irinotecan.[17] Unfortunately, in phase I and II clinical trials, it did not perform well and it was dropped afterwards. Rubitecan (9-nitro camptothecin, 9-NC) serves as a metabolic precursor to 9-amino CPT and is currently in phase III clinical trials for the treatment of pancreatic cancer.[18,19] Recently, several camptothecin analogues have shown a very promising cytotoxicity against L1210 mouse leukaemia cells (Table 1).

All these analogues of CPT have proved to be potent cytotoxic agents by inhibiting cellular DNA topoisomerase I by a mechanism similar to CPT with similar or better activity.

2.6.2. Modifications in C and D rings. In general, modifications at the C and D rings of camptothecin led to complete loss of cytotoxicity. If we see these rings, the only positions available for modifications are C-5, C-14 and C-17. Several derivatives have been reported either with less activity or with loss of activity. It might be because the CPT molecule loses its planarity on these modifications to some extent, which is presumed essential for enzyme–DNA–CPT ternary complex stabilization. This was further supported by deaza derivatives, which showed significant cytotoxicity due to their shape and planarity being quite close to camptothecin.[20] Reduction of 17-carbonyl leads to inactive molecules as the pyridine carbonyl is essential for receptor binding. The rest of the positions, that is, C-5 and C-14, yielded derivatives with very poor activity (Fig. 2).

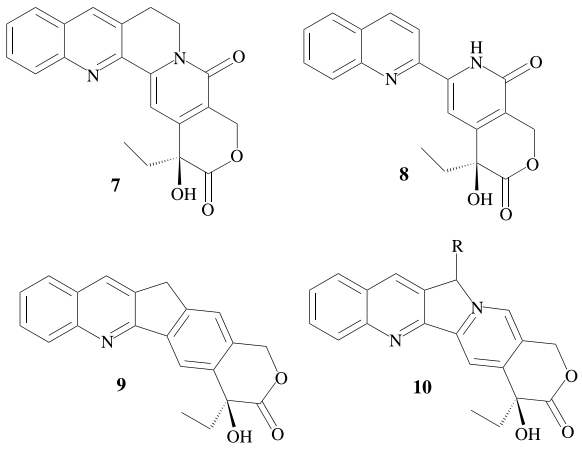
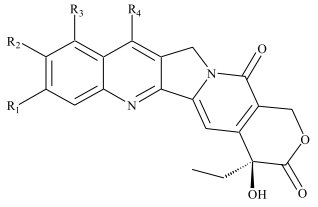
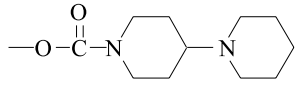
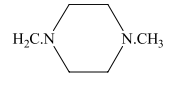


Figure 2. CPT analogues modified at C and D rings.

Table 1. Camptothecin analogues on A and B ring modifications



S. No.	Analogue	R_1	R_2	R_3	R_4	IC_{50} (μM) (Topo- I)	IC_{50} (μM) (Proliferation)
1.	CPT	H	H	H	H	0.6–1.4	23 (L1210) 0.046 (HT-29)
2.	Topotecan	H	OH	$CH_2N(CH_3)_2$	H	1.1	56 (L1210)
3.	Irinotecan	H		H	Ethyl	>100	1200 (L1210)
4.	Rubitecan	H	H	NO_2	H	NA	
5.	Lurtotecan			H		0.42	0.006 (HT-29)
6.	9-Amino CPT	H	H	NH_2	H	0.9	12 (L1210)

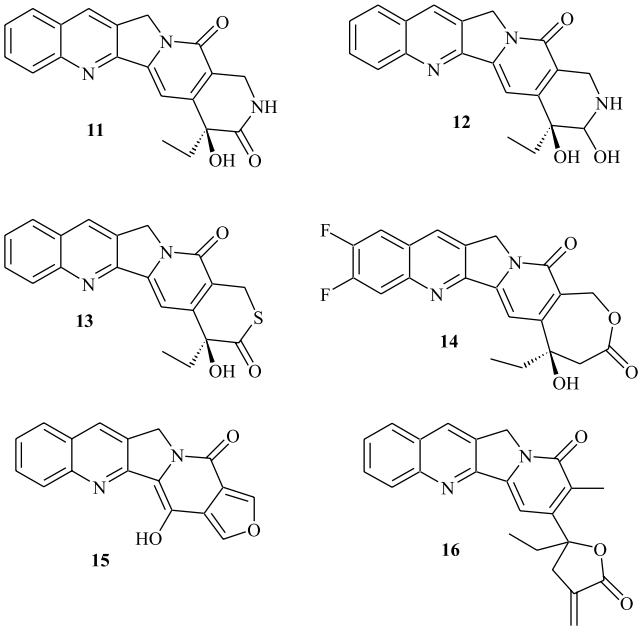


Figure 3. CPT analogues modified at ring E.

2.6.3. Modifications in E ring. The α-hydroxy lactone system of ring E has been found to be important for the inhibition of the topoisomerase enzyme as well as for in vivo potency. Modifications in ring E generally reduce or abolish the activity. Under physiological conditions, due to α-hydroxy group, the lactone ring is opened to inactive carboxylate group. Several stable derivatives have also been synthesized having a lactam group instead of a lactone, but the compounds were devoid of topoisomerase inhibitor activity. Several other derivatives having thiolactone, imide and carbinol lactam have also been reported without activity[21] (see Fig. 3).

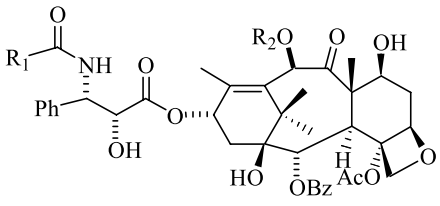
2.7. Future prospects

CPT and its analogues exhibit a broad spectrum of antitumour activity and represent a very promising class of agents. The discovery of topoisomerases as new targets for cancer chemotherapy and the mechanism of action of camptothecin put camptothecin back on the frontlines of anticancer drug development.[22] Two of the successful drugs, topotecan and CPT-11, have achieved nearly $750 million in annual sales. Camptothecin will continue to remain a target for new synthetic methods, which are certainly expected considering the fast development of modern organic synthesis. Continued studies on the camptothecin–DNA–topoisomerase I interaction in addition to its detailed mechanism of action may suggest new directions in the synthesis of new camptothecins.

3. Taxol

3.1. Introduction

Taxol (**17**, now known by the generic name paclitaxel and the trade name taxol) is a complex polyoxygenated



17: R_1= -C_6H_5, R_2=Ac; (Taxol)

18: R_1=  , R_2=H;(Taxotere)

Figure 4. Paclitaxel (taxol) and Docetaxel (taxotere).

diterpenoid isolated from the pacific yew, *Taxus brevifolia*, by the same research group of Dr. Wall and Dr. Wani.[23] It was discovered during extensive screening of different plant materials for antineoplastic agents in the late 1960s by a systematic research approach. Later on, it was isolated from several other species of *Taxus* including *Taxus wallichiana*, the Himalayan yew. So far, more than 300 taxoids have been isolated and characterized from different species of *Taxus*.[24] Taxol as a drug has been developed by the National Cancer Institute, USA. In 1992, Bristol–Myers–Squibb received approval to market taxol for the treatment of refractory ovarian cancer, metastatic breast and lung cancer and Kaposi's sarcoma. Taxotere (**18**), one of its semisynthetic derivatives, is now known as a better anticancer drug than taxol (see Fig. 4).

3.2. Chemistry

Taxol for the first time was isolated and characterized by Wall and Wani, National Cancer Institute, USA. It has a basic [9.3.1.$0^{3,8}$] pentadecane, tetracyclic ring system. It has a *N*-benzoyl-β-phenylisoserine side chain attached at the C-13 hydroxyl as an ester linkage.

3.3. Structure and activity relationship of taxol

The comprehensive SAR of taxol[25] is depicted in Figure 5.

3.3.1. SAR at the C-13 side chain. This side chain is essentially required in taxol for anticancer activity. The C-2′-hydroxyl is important for activity. When this hydroxyl is protected, activity is reduced to a great extent and if the protection is made with a labile group it shows similar activity in vivo, while no activity is shown in in vitro testing. It is because in vivo the protecting group is hydrolyzed due to its labile nature. Thus this type of modifications acts as prodrugs of taxol. A summary[26,27] of interesting features is shown in Figure 5. The studies reveal:

1. C-3′ aryl group is critical, while the amide's aryl group may be replaced by similar aryl or alkyl groups.
2. The C-3′ aryl group is required for better activity. On replacement with a methyl group, activity is reduced 19-fold.

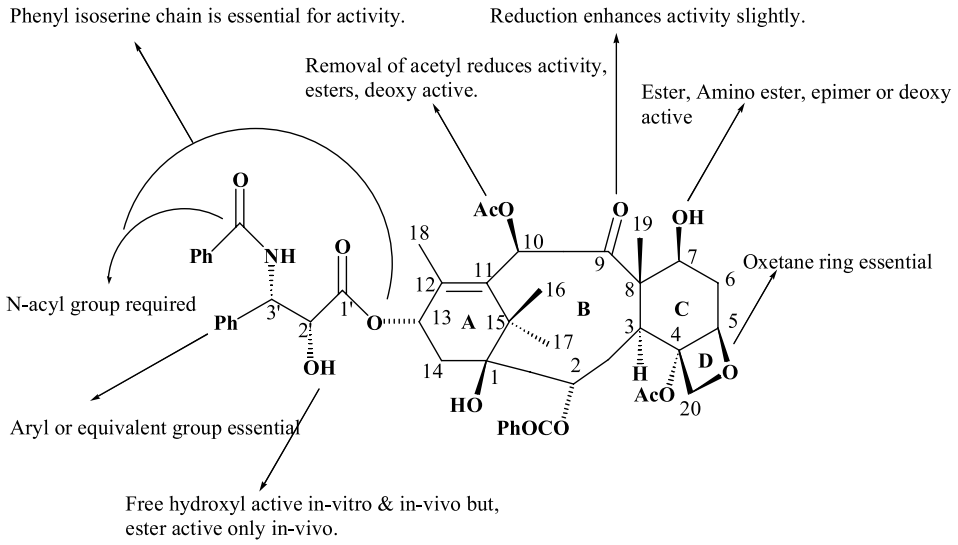


Figure 5. A brief description of SAR of taxol.

3. Replacement of the C-3′ bound nitrogen with an oxygen atom is acceptable without significant loss of activity.
4. Stereochemistry at C-2′ and C-3′ has a dramatic effect on activity. The (2′*R*,3′*S*) isomer is significantly less active than the natural (2′*R*,3′*S*) isomer, but the (2′*S*,3′*S*) and (2′*R*,3′*R*) isomers show comparable activity with the natural isomer.

The side chain of taxol as such is inactive but it plays a crucial role in the biological activity of taxol. Although it is not fully understood, it is expected that the taxol side chain orients through hydrogen bonding and may fit into a hydrophobic cleft on the taxane-binding site, which stabilizes the drug–tubulin interactions.

3.3.2. SAR at the diterpenoid moiety. Several diverse analogues of taxol have been prepared by Samaranayake et al.[28] Figure 5 shows the effect of different groups on the biological activity of taxol. Specifically, these workers have observed that modifications at the C-2, C-7 and C-10 positions have less effect on the biological action of the drug. The oxetane ring is crucial for its cytotoxicity. C-1 hydroxyl, C-2 benzoyloxy, and C-4 acetate are very important for maintaining cytotoxic activity. Overall, the structure of taxol may be divided into two hemispheres, that is, northern and southern hemispheres.

If we see overall modifications, we can summarize that the modifications in the northern hemisphere are allowed while the modifications in the southern hemisphere are strictly forbidden. It may be because the southern hemisphere plays a crucial role in microtubulin binding. Overall,

- The removal of C-1-hydroxyl reduces the activity.
- The C-2-benzoyloxy is essential for activity. However, some substituted benzoyloxy groups are also acceptable. Removal of the 4-acetyl group reduces the activity. The 4,5,20-oxetane ring is essential for activity.
- The derivatization of the C-7-hydroxyl or change of its stereochemistry has no significant effect on anticancer activity of the molecule.
- Reduction of 9-ketone slightly increases the activity.
- The 10-acetate has better activity in the case of taxol but in some analogues 10-hydroxyls have better activity.

3.4. Biological activity

Taxol showed promising results in phase I and phase II clinical trials in lung, ovarian and breast cancers and squamous cell carcinoma of the head and neck. Taxol was approved by US FDA in 1992 for the treatment of drug refractory metastatic ovarian cancer.[29] The approved dose of 135 mg/m^2 by continuous infusion over 24 h reflects the cautious recommendation of the FDA's oncology advisory committee. A major drawback of taxol is that it has poor bioavailability due to its poor solubility in water. The widely used technique to solubilize taxol is in a vehicle cremophore EL (polyethoxylated castor oil/ethanol).[30] The dose recommendations are generally in the range of 200–250 mg/m^2.

Recently, it was reported[31] that nanoparticle taxol in the form of a drug-eluting stent is likely to gain FDA approval for preventing restenosis following balloon angioplasty treatment of coronary arterial blockage.

3.5. Mode of action

Taxol exhibits a unique mode of action.[32] It acts as microtubulin stabilizing agent while the other anticancer agents destabilize this process.[33] Actually, tubulin polymerizes to microtubulin and again microtubulin converts into tubulin. In a normal case, this process is in equilibrium. Later on, fixed-size 24-nm microtubulin bundles are formed and the cell multiplication process takes place, whereas taxol makes stabler bundles of microtubulins of size 22 nm. Due to this, a defective polymerization process occurs and thus, these cells have

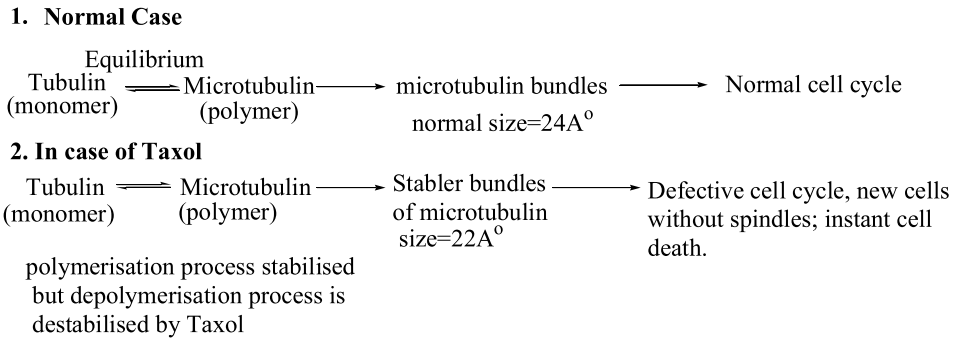


Figure 6. Tubulin polymerization in normal cell cycle and effect of taxol.

unnatural 'bundles' of microtubules and no mitotic spindle. The cancerous cells lack a check point to detect the absence of a spindle and attempt to continue the cell cycle, which leads to cell death. Because of this reason, taxol is sometimes also referred as a 'spindle poison'[34] (see Fig. 6).

3.5.1. Side effects. Taxol is thought to be tolerated by its recipients better than any other anticancer drug used today, but as most drugs do, it also has some side effects. Of the several side effects, the major ones include numbness, nausea (quite severe in some cases), tingling in toes and fingers, and a reduction in the infection-fighting white blood cells due to taxol's effect on bone marrow. However, if a growth factor, a protein called granulocyte colony-stimulating factor, is used, the bone marrow is protected. This means that white blood cells and platelets are maintained even when the dosage is increased. Taxol is a drug that is hard to obtain due to the reproduction and growth rates that characterize it. Another problem concerns direct infusion of the drug into the body, due to poor solubility, and is severe when dosages are higher.

3.6. Synthetic analogues

3.6.1. Taxotere. Taxotere (**18**, docetaxel) is a structurally related analogue of taxol. It shows potent anticancer activity better than taxol.[35,36] It also has better pharmacological properties such as improved water solubility, and acts at the microtubules. It enhances polymerization of tubulin into stable microtubule bundles leading to cell death. It is used for the treatment of patients with locally advanced metastatic breast cancer and nonsmall cell lung cancer.[37]The use of this drug is also associated with several side effects like bone marrow suppression, hypersensitivity reactions, vomiting, alopecia, etc (see Fig. 7).

3.6.2. Water-soluble prodrugs of taxol. Taxol is sparingly soluble in water, that is, 0.00025 mg/ml. In drug formulations it is given with a carrier cremophore EL (polyethoxylated castor oil or polysorbate 80). Several hypersensitive reactions have been reported due to these carriers. Hence, several water-soluble prodrugs of taxol have been synthesized. A number of derivatives at C-7, C-2′ or both hydroxyls have been synthesized, including carboxylic acid salts, basic moieties (quaternary ammonium salts) and other designed functionalities.[38] Most of

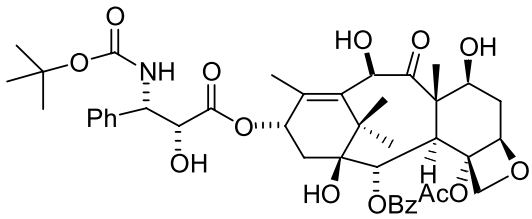


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Figure 7. Taxotere.

these derivatives had better water solubility and some of them showed better pharmacological profiles also.

Recently, a prodrug isotaxel (**19**) has been reported.[39] It is nearly 1800-fold more soluble than taxol. Its solubility

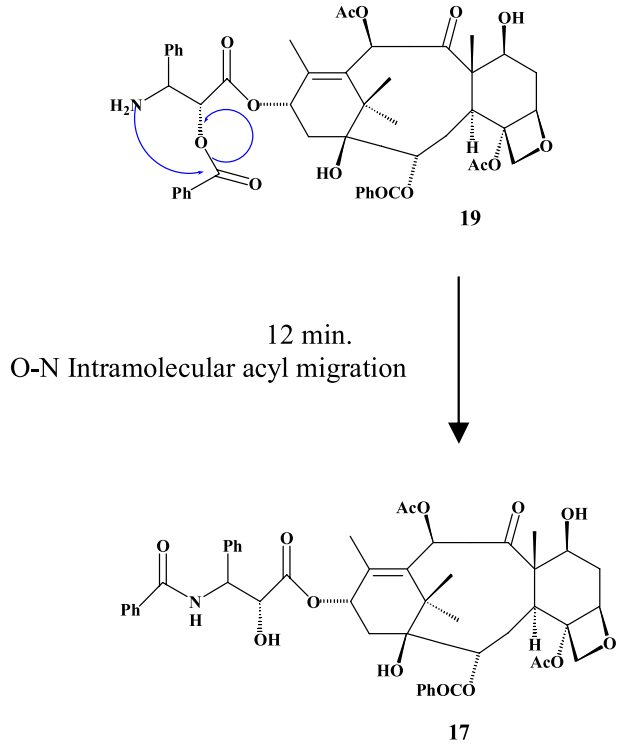


Figure 8. Conversion of isotaxel to taxol under physiological conditions.

in water is 0.45 ± 0.04 mg/ml. Isotaxel itself is an inactive molecule, but, at physiological pH, O–N intramolecular acyl migration takes place and within 12 min it converts into taxol, the active molecule.

3.6.3. Conversion of isotaxel to paclitaxel. In physiological solution an O–N intramolecular acyl migration takes place and isotaxel is converted into taxol. O–N intramolecular migration is a known side reaction of serine and threonine containing peptides (see Fig. 8).

3.6.4. Future prospects. Earlier, the availability of paclitaxel was a problem due to its poor abundance in the plant. Several research groups now have devised the total synthesis of taxol, but being a complex molecule it is not economic. The last decade has witnessed paclitaxel and related taxanes emerging as aggressive frontline therapies for advanced tumours including breast, lung and ovarian carcinomas, but their high toxicity and poor solubility may ultimately limit their utility.

4. Combretastatin A-4

4.1. Introduction

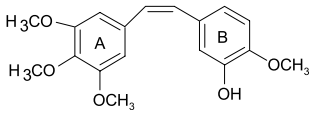
Combretastatins are mitotic agents isolated from the bark of the South African tree *Combretum caffrum*. The most potent combretastatin A-4 [**20**, *cis*-1-(3,4,5-trimethoxyphenyl)-2-(3′-hydroxy-4′-methoxy phenyl) ethene] is a simple stilbene that has been shown to compete with colchicines for binding sites on tubulin. It has been found to be a potent cytotoxic agent which strongly inhibits the polymerization of brain tubulin by binding to the colchicine site. CA-4 shows potent cytotoxicity against a wide variety of human cancer cell lines including MDR cancer cell lines. CA-4 is thus an attractive lead molecule for the development of anticancer drugs[40,41] (see Fig. 9).

4.2. Chemistry

In 1982 Pettit et al.[42–44] reported the isolation and structure of Combretastatin, the first member of a series of biologically active bibenzyls, stilbenes and phenantherenes from the bark of African willow tree *C. caffrum* at Arizona State University, USA. Combretastatins A-1 (**21**)[45] and A-4 (**20**)[46] were isolated by the same group in 1987 and 1989, respectively. Chemically, they are stilbene derivatives having two phenyl rings separated by a C-C double bond. Ring-A has three methoxy groups in 3,4,5-positions while in ring B one hydroxy group is at the C-3 position and one methoxy group at the C-4 position.

4.3. Structure and activity relationship

A number of studies have been reported on the structure and activity relationship of combretastatins.[47,48] For a minimal cytotoxic activity of such compounds, a diaryl system should be separated through a double bond along with a trimethoxy system in one of the rings.



Overall,

- Trimethoxy benzene moiety is essential for its activity.[49]
- The two aryl groups should be separated through a double bond and the *cis* (*Z*) isomer is preferred over the *trans* (*E*) as *cis* is much more active than *trans*.[50]

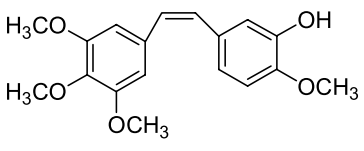
Cushman et al.[50] reported that the 3-hydroxyl group on ring B of CA-4 is not necessary for potent activity. However, a 4-methyl or 4-methoxy group is required in the ring B for strong cytotoxic activity.[51] An additional hydroxyl group at C-2 on ring B of CA-4 is less preferred as it decreases its activity as in CA-1.[52] Introduction of an amino group in ring B significantly decreased antimitotic activity but showed strong cytotoxicity, more potent than that of CA-4.[45]

4.4. Biological activity

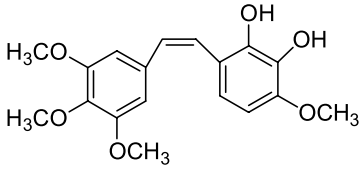
CA-4 is an investigation drug of the National Cancer Institute and the University of Arizona, USA. The compound is active against colon, lung and leukaemia cancers. It is stated about this molecule that it is the most cytotoxic phytomolecule isolated so far. CA-4 exhibited an LD_{50} value of 7 nm (0.007 μM) against murine L1210 leukaemia cell lines.[53,54]

4.5. Mode of action

In vitro studies have shown that CA-4 competes with colchicine for binding sites on tubulin. Hence, it is a member of the colchicine-like inhibitors of microtubulin assembly rather than the vinca alkaloid type compound (i.e., tubulin polymerization inhibitors).[55] McGown and Fox suggested that the trimethoxy benzene moiety in all



20, CA-4



21, CA-1

Figure 9. Combretastatins CA-4 and CA-1.

these compounds (colchicine, CA-4 and podophyllotoxin) probably provides a favourable binding site for tubulin.[43,56] Several in vivo experiments have been done in rats. Magnetic resonance imaging (MRI) experiments showed[57] that CA-4P (combretastatin-4, 3-O phosphate) significantly reduces blood flow to the tumour cells in a dose-dependent manner. Thus, it acted as an antivascular targeting agent,[58] which blocks tumour blood supply. Thus, it is one of a new class of anticancer therapies that act by attacking a tumour's blood supply. In animal tumours, it can cause the shutdown of blood flow[59] leading to extensive tumour necrosis. A sodium phosphate derivative of CA-4 induced a complete vascular shutdown within metastatic tumours at doses one-tenth of the maximum tolerated dose,[60] while the reduction in blood flow by CA-4 is up to 70%.

4.6. Synthetic analogues of CA-4

Varied modifications have been reported in the CA-4 molecule. In some analogues, only the functional groups have been modified but in several analogues the total aryl ring is either replaced or totally modified by some other groups. However, in almost all cases 3,4,5-trimethoxy aryl or ring A was kept intact, which is considered to be indispensable for cytotoxicity of the molecule.

4.6.1. Modifications in aromatic rings. Pinney et al.[61] synthesized several nitrogen-containing stilbene derivatives. In these derivatives, the nitrogen atom was present as a nitro, amino or azide group and some of the derivatives exhibited excellent activity against the NCI 60 human cancer cell line. Similarly, Lawrence et al.[62] synthesized mono/difluoro derivatives of CA-4 at the C-3 and/ C-5 positions of ring B. Melero et al.[63] replaced one of the aryl groups with a naphthalene group and synthesized naphthylcombretastatins, where they modified ring B of CA-4 to some quinoline and quinoxaline derivatives. All the compounds exhibited cytotoxicity comparable to or better than CA-4 and concluded that ring B in the present form is not essential for the cytotoxicity of CA-4.

Table 2. Cytotoxicities of sulfonate derivatives of CA-4

Compound	HCT-15 human colon carcinoma IC_{50} (nM)	NCI-H460 Human lung carcinoma IC_{50} (nM)
20, CA-4	1.7	3
17, Paclitaxel	450	15
22	3.3	3.1
23	4.1	2.7

Several phosphate esters of CA-4 have been reported at phenolic hydroxyl of ring B, but none of the analogues showed better activity than CA-4 or CA-4P by MTT assay.

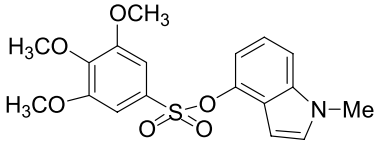
4.6.2. Modifications in linker alkene. From the SAR studies, it is concluded that the presence of an alkene is not necessary for activity. However, the restricted rotation of rings A and B of CA-4 can also be maintained by introducing suitable conformationally restricted arrangements. Sun et al.[64] reported 1,4-disubstituted azetidinone ring system having good cytotoxicity against MCF-7, CHO-K, and NCI-H69 cancer cell lines. Thiophene-based analogues of CA-4 have shown tubulin polymerization inhibition activity comparable to CA-4. Gwaltney et al.[65] used a sulfonate group between the aryl groups for restricted rotation, and compounds **22** and **23** showed cytotoxicity comparable to CA-4 (see Table 2; Fig. 10).

In the place of a stilbene arrangement of the two aryl rings, several benzophenone type analogues have also been synthesized. Few of the derivatives showed potent cytotoxicity, while phenastatin (**25**) showed much better activity than the CA-4 itself.[66] Recently, several benzophenones having an additional amino group and methoxy/chloro substitution also showed potent cytotoxicities[67] (see Fig. 11).

Ohsumi et al.[53] synthesized several modified stilbene derivatives in which ring B and the olefin group were substituted. Several compounds showed potent activity against colon-26 cancer cells and antitubulin activity. Pettit et al.[68] synthesized several asymmetric diols on the alkene part of CA-4 by using the well-known Sharpless reactions. But these compounds were found to be less potent than CA-4 or its disodium phosphate derivative (see Table 3; Fig. 12).

4.7. Future prospects

A large number of combretastatins has been synthesized and evaluated. Natural and synthetic compounds of diverse structures have been shown to inhibit tubulin polymerization through interaction with a protein at different binding sites. The colchicine, taxol and vinca alkaloid sites are the best known of them. Among ligands of the colchicine-binding site, combretastatins are highlighted as strongly cytotoxic and angiogenic agents. CA-4 itself has shown a new mode of action by targeting at vascular system. But the main problem associated with this class of compounds is their poor water solubility. Therefore, the main emphasis on com-



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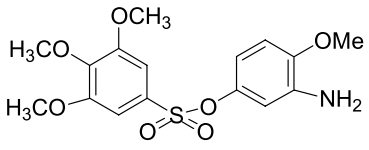


23

Figure 10. Novel sulfonate analogues of CA-4.

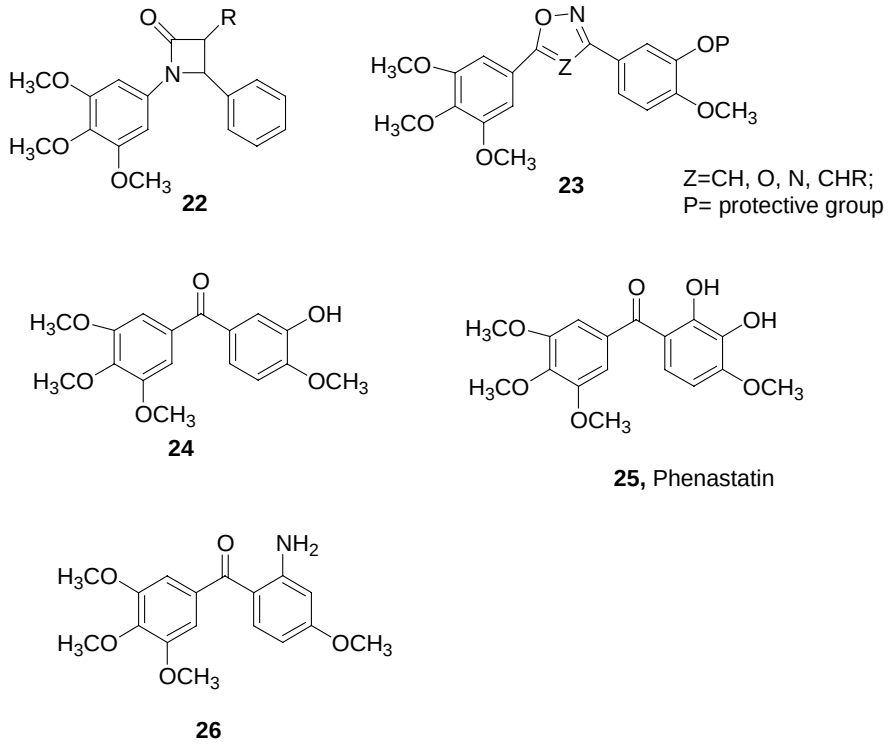


Figure 11. CA-4 analogues having different moieties; azetidinone, benzophenone, etc.

Table 3. Several modified stilbene derivatives with cyano substitution at alkene part

S. No.	Compound	Colon-26 IC_{50} (nM)	Antitubulin activity IC_{50} (μM)
1.	CA-4	18.0	4.0
2.	**27**	63.7	>20
3.	**28**	12.6	>20
4.	**29**	5.9	10
5.	**30**	36	8
6.	**31**	8.1	7

bretastatins research has been diverted towards water-soluble prodrugs.[69–75] One of the prodrugs, that is, CA-4 phosphate (**36**), is currently in phase II clinical trial in the UK and the USA (see Ref. 13).

Combretastatin or its analogues may come up as anticancer drugs of choice in near future.

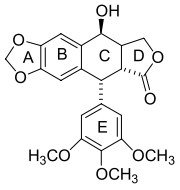
5. Podophyllotoxin

5.1. Introduction

Podophyllotoxin (PDT, **37**) and deoxypodophyllotoxin are two well-known naturally occurring aryltetralin lignans. Podophyllotoxin, a bioactive lignan, was first isolated by Podwyssotzki in 1880 from the North American plant *Podophyllum peltatum* Linnaeus (American podophyllum), commonly known as the American mandrake or May apple.[76] Later on, it was isolated from several other species like *P. emodi* Wall (Indian podophyllum, syn. *P. hexandrum* Royle) and *P. pleiantthum* (Taiwanese *podophyllum*). Other than these, 4-deoxypodophyllotoxin has also been isolated[77] from *Anthriscus sylvestris* and *Pulsatilla koreana*. It is a potent cytotoxic agent. Two of the semisynthetic derivatives of PDT, that is, etoposide and teniposide, are currently used in frontline cancer chemotherapy against various cancers[78] (see Fig. 14).

5.2. Chemistry

Chemically, it is an aryltetralin lignan, having a lactone ring.



5.3. Structure and activity relationship

Podophyllotoxin contains a five-ring system (i.e., A, B, C, D and E rings).

- Only the A and E rings are essential for its activity. Earlier it was reported that all the rings are essential for its activity, but now the statement is modified.
- D-ring in lactone form is preferred for better activity.
- Modifications at the C-4 position in ring C are mostly acceptable and bulky groups at this position enhance both anticancer and topoisomerase activities.

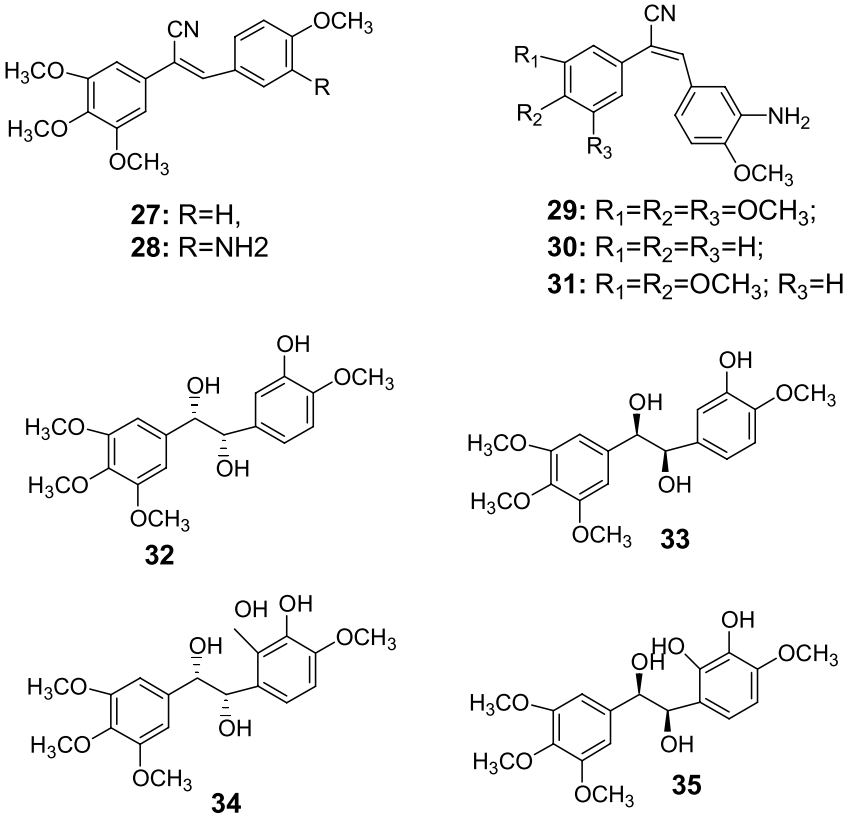


Figure 12. Several stilbene nitriles and asymmetric diols of CA-4.

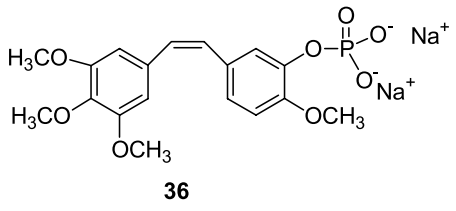


Figure 13. CA-4 disodium phosphate, a prodrug of combretastatin A4.

5.4. Biological activity

Podophyllotoxin shows strong cytotoxic activity against various cancer cell lines. It is effective in the treatment of Wilms tumours, various genital tumours and in non-Hodgkin's and other lymphomas and lung cancer.[79,80] The attempts to use PDT in the treatment of human neoplasia were mostly unsuccessful due to complicated side effects[81,82] such as nausea, vomiting, damage of normal tissues, etc. Because of this reason, PDT as such is not used as a drug. Extensive structure modifications were performed to obtain more potent and less toxic

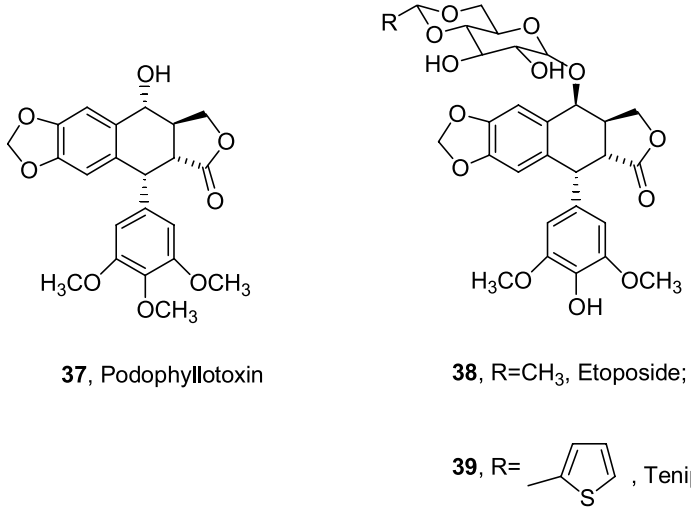


Figure 14. Podophyllotoxin and its two successful analogues.

anticancer agents, which resulted in two semisynthetic glucosidic cyclic acetals of epipodophyllotoxin, etoposide and teniposide. These are the most widely used derivatives for the treatment of lymphomas, acute leukaemia, testicular cancer, small cell lung cancer, ovarian, bladder, brain cancers, etc.[83]

5.5. Mode of action

Podophyllotoxin acts as an inhibitor of assembly of microtubules and arrests the cell cycle in metaphase.[84,85] These PDT lignans block the catalytic activity of DNA topoisomerase II by stabilizing a cleavage enzyme–DNA complex in which the DNA is cleaved and covalently linked to the enzyme.[86] It binds at the colchicine site of the tubulin.[87–89] From podophyllotoxin to etoposide/teniposide, some chemical modifications were made that also led to a change in the mechanism of action, from the inhibitor of microtubule formation by the parent compound PDT to DNA topoisomerase II inhibitor by etoposide and congeners.

Podophyllotoxin → Etoposide/Teniposide

Antimicrotubule agent DNA topoisomerase II inhibitor

5.6. Synthetic analogues of podophyllotoxin

Extensive structural modifications have been performed on PDT to obtain analogues with better activity and/or less toxicity. These modifications have been done mainly in rings C, D and E. Of these, the C-4 positions of ring C have been found to yield better derivatives. However, there are a few reports on ring A modifications. Castro et al.[90] synthesized podophyllotoxin derivatives lacking the methylenedioxy group or with different functionalizations of the A ring of the cyclolignan skeleton. Most of the derivatives showed cytotoxic activities on four neoplastic cell lines (P-388, A-549, HT-29 and MEL-28). Most of them maintained their cytotoxicity at the mM level.

5.6.1. Modifications at ring C of PDT. As stated earlier, the C-4 position is found to be most amenable to modifications, and it is also reported that the presence of a bulky group at this position enhances cytotoxic as well as topoisomerase inhibitor activities.

Several 4β-amino derivatives as hydrochloride salts, have been synthesized having 4′-demethylated PDT as in etoposide.[91] Having good water solubility and bioavailability, these derivatives were found to possess better pharmacological profiles. Both **42** and its hydrochloride salt **43** were found to be highly active towards etoposide-resistant KB cell lines (see Table 4; Fig. 15).

Cho et al.[92] synthesized several 4β-nitro aniline derivatives as potent inhibitors of topoisomerase II. Analogues **46** and **47** showed better activities than etoposide against KB and KB/7d (VP-16 resistant) cells (Fig. 16).

Table 4. Cytotoxicity and DNA topo II inhibition by 4β-amino derivatives and their corresponding hydrochloride salts of 4′-demethylepipodophyllotoxin

Compound	Cytotoxicity ID_{50} KB(μM)	Inhibition of DNA topoisomerase II ID_{50} (μM)
38, etoposide	0.2	50
40	1.4	25
41	1.6	50
42	0.027	100
43	0.021	50
44	0.18	25
45	0.74	25

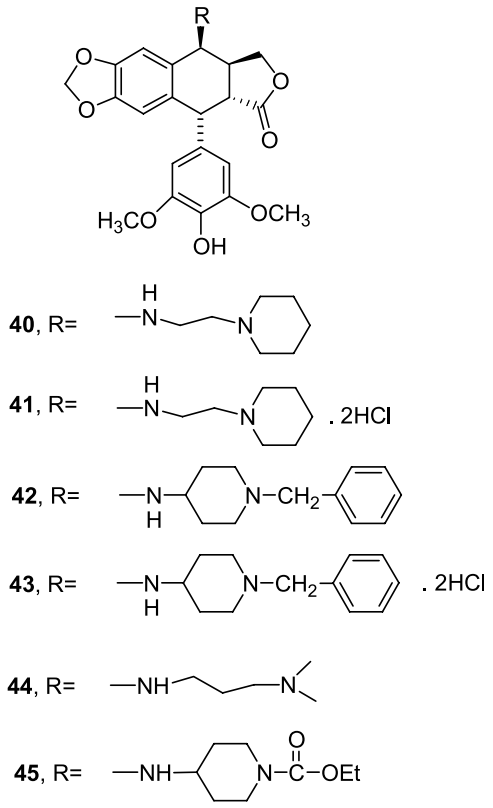


Figure 15. 4β-Amino derivatives and their corresponding hydrochloride salts of 4′-demethylepipodophyllotoxin.

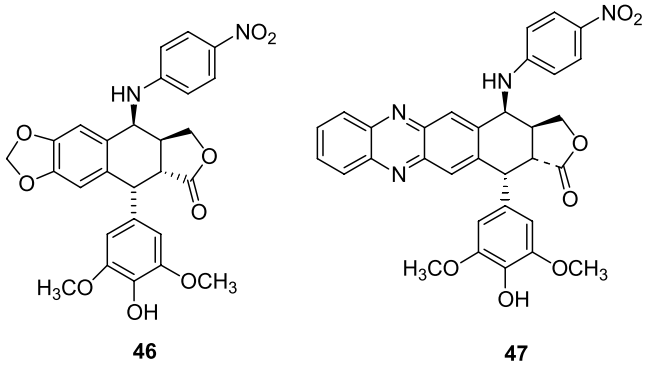


Figure 16. 4β-Nitro aniline derivatives of 4′-demethylepipodophyllotoxin.

Kamal et al.[93] have synthesized 4β-amido and sulfonamido derivatives of PDT having these A, B and C prototypes. Among these compounds, compounds **47**–**50** were found to be highly potent against all the six cancer cell lines given in Table 5, while compounds **51**–**54** have exhibited promising cytotoxicity (see Table 6; Fig. 17).

Several sulfonamide derivatives were also reported[94] in which the main nucleus was epipodophyllotoxin in the place of PDT. The main substitutions were methyl, propyl, 3-chloropropyl, azidopropyl, etc. These derivatives were found to possess cytotoxic activity better than etoposide against several cancer cell lines (see Fig. 18).

Table 5. IC_{50}(μM) drug concentration that inhibit 50% of the control in DMSO

Analogue	KB	KB/7d
38, Etoposide	0.164 ± 0.044	23.8 ± 1.5
46	0.032 ± 0.008	0.55 ± 0.26
47	0.11 ± 0.03	0.56 ± 0.13

Table 6. GI_{50} values of some of the 4β-amido and 4β-sulfonamido derivatives of podophyllotoxin

Compound	Cytotoxicity GI_{50} (μM)			
	DU145 (prostate)	HT29 (colon)	MCF7 (breast)	NCI H460 (lung)
Etoposide	0.8	59	4.3	1.1
48	0.02	0.004	0.03	0.02
49	2.3	1.7	5.0	4.3
50	27.2	28.9	46.2	30.8
52	0.16	0.03	0.05	0.08
53	2.7	1.8	3.5	3.7
54	0.05	0.019	0.12	0.03

Roulland et al.[95] reported several acetamido and formamido derivatives at the C-4 position. Compounds **56**, **57**, **58** and **59** showed high antiproliferative activity against L1210 cell lines. Compound **59** was the most active with an IC_{50} of 35 nM (see Table 7; Fig. 19).

5.6.2. Modifications in ring E. The modification in ring E was found to be crucial for the mode of action of PDT. PDT, where the ring E is a 3,4,5-trimethoxy aryl, shows antimicrotubule activity. But when the p-methoxy group is demethylated, that is, ring E is 3,5-dimethoxy, 4-hydroxy aryl, compounds generally show DNA topoisomerase II inhibition activity as in etoposide and teniposide.

Modifications in ring E are not much reported, but recently several fatty acid esters have been synthesized at the 4-hydroxyl group of ring E and most of these derivatives showed cytotoxic activity better than etoposide and DPT (deoxypodophyllotoxin),[77]. In in vitro assays shorter alkyl chain esters showed stronger cytotoxicities, while in animal models moderate alkyl chain esters had better activities (see Fig. 20).

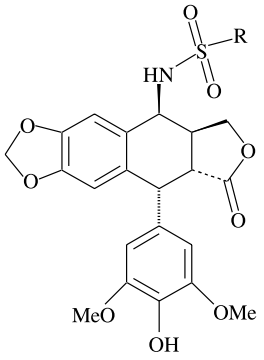
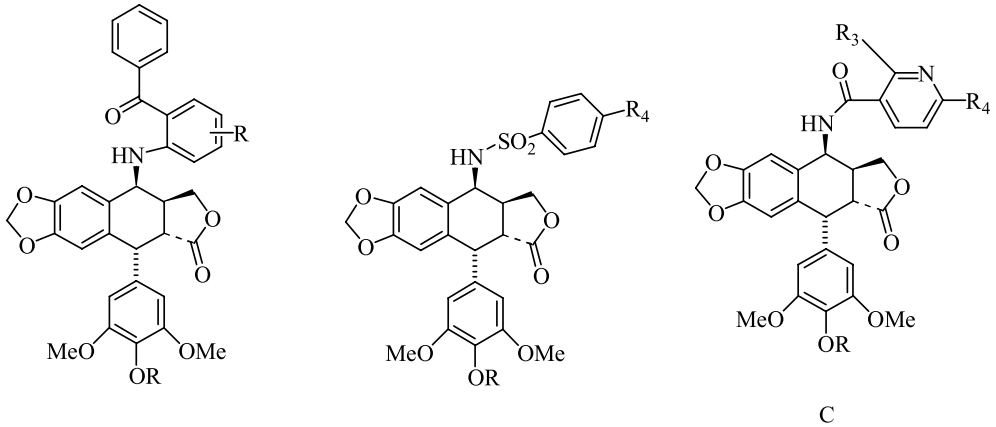


Figure 18. Basic unit of sulfonamide derivatives of epipodophyllotoxin.



47. R=Me, R_4=H;
48. R=Me, R_4=Me;
49. R=H, R_4=H;
50. R=H, R_4=Me

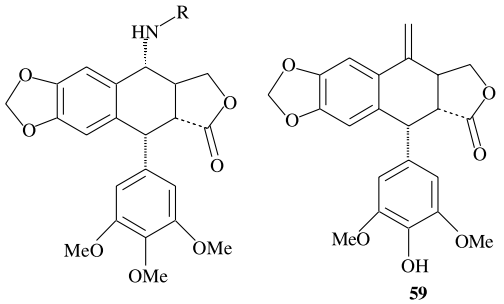
51. R=Me, R_3=Cl, R_4=H
52. R=H, R_3=Cl, R_4=H
53. R=Me, R_3=H, R_4=Cl
54. R=H, R_3=H, R_4=Cl.

Figure 17. Several 4β-amino benzophenone, 4β-amido and sulfonamido derivatives of PDT.

Table 7. IC_{50} values of active molecules as acetamido and formamido derivatives of PDT

Compound	L1210 IC_{50} (μM)	Inhibition of microtubule assembly IC_{50} (μM)	Topoisomerase I inhibition	Topoisomerase II inhibition
55	0.06	0.7	0	18
56	0.07	0.7	0	21
57	0.08	1.6	0	13
58	0.035	2.3	nd*	nd*

nd* = not determined.



56. R= CHO;
57. R=$COCH_3$
58. R=H

Figure 19. Several acetamido and formamido derivatives at C-4 position.

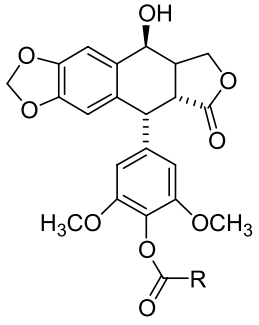


Figure 20. Several fatty acid esters at C-4″ phenolic hydroxyl of PDT on modification at E-ring.

5.7. Future prospects

Podophyllotoxin and their analogues are potent anticancer agents. But their toxicities limit the clinical use of these agents. Two of the semisynthetic derivatives have overcome these problems. Etoposide, a clinical drug, is also associated with the problems of drug, resistance and poor bioavailability.[96–98] Therefore, efforts towards the development of new etoposide analogues are very much required.

6. Conclusion

Over the years, a number of approaches have been developed for clinical use and a number of anticancer drugs have come out of these as a result. The main problem with these agents is the toxicity associated with them due to their lack of specificity, as these agents also kill healthy cells. Other than this, drug resistance is another problem which arises after some time. Nowadays, combination therapy is used[99] to combat this problem, which seems to be a temporary one. But this approach threatens the possibility of the development of drug resistance. Though a good number of anticancer agents have been developed from plants or their derived agents, development of a safe, economic and site-specific anticancer drug is still a challenge. Perhaps, scientists will have to look towards nature for another diverse molecule with a novel mode of action to tackle this dreadful disease.

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