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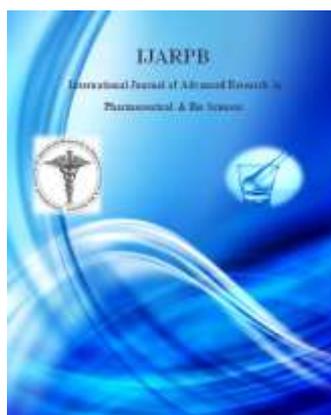
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Plant-Based Anticancer Molecules: A Chemical and Biological Profile of Some Important Leads

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ABSTRACT

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 the 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.

KEY WORDS – Cyclophosphamide, Chlorambucil, Melphalan, Ifosfamide (Ifex).

(Review Article)**INTRODUCTION**

Cancer occurs when cell division gets out of control. Usually, the timing of cell division is under strict constraint, involving a network of signals that work together to say when a cell can divide, how often it should happen and how errors can be fixed. Mutations in one or more of the nodes in this network can trigger cancer, be it through exposure to some environmental factor (e.g. tobacco smoke) or because of a genetic predisposition, or both. Usually, several cancer-promoting factors have to add up before a person will develop a malignant growth: with some exceptions, no one risk alone is sufficient¹.

The predominant mechanisms for the cancers featured here are

- (i) Impairment of a DNA repair pathway
- (ii) The transformation of a normal gene into an oncogene and
- (iii) The malfunction of a tumor suppressor gene.

TYPES OF CANCER

1	Breast and ovarian cancer
2	Burkitt lymphoma
3	Colon cancer
4	Leukemia, chronic myeloid
5	Lung carcinoma, small cell
6	Malignant melanoma
7	Multiple endocrine neoplasia
8	Neurofibromatosis
9	The p53 tumor suppressor protein

10	Pancreatic cancer
11	Polycystic kidney disease
12	Prostate cancer
13	Harvey Ras oncogene
14	Retinoblastoma
15	Tuberous sclerosis
16	Von Hippel-Lindau syndrome

Classification of the anticancer drugs**I. Alkylating agents****A. Nitrogen mustards**

1. Mechlorethamine hydrochloride (Mustargen, HN2, nitrogen mustard)
2. Cyclophosphamide (Cytosan)
3. Chlorambucil (Leukeran)
4. Melphalan (Alkeran, L-PAM, L-phenylalanine mustard)

5. Ifosfamide (Ifex)**B. Alkyl sulphonates**

1. Busulfan (Myleran)

C. Nitrosoureas

1. Carmustine (BCNU, BiCNU)
2. Lomustine (CCNU, CeeNU)
3. Semustine (methyl-CCNU)
4. Streptozocin (Zanosar, streptozotocin)

D. Ethylenimines

1. Thiotepa

E. Triazenes

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1. Dacarbazine (DTIC-Dome)
- II. Antimetabolites
 - A. Folate antagonist
 1. Methotrexate (Folex, Mexate)
 - B. Purine analogues
 1. Thioguanine(6-TG,6-thioguanine)
 2. Mercaptopurine (6-MP, Purinethol)
 3. Fludarabine(Fludara)
 4. Pentostatin(deoxycoformycin,Nipent)
 5. Cladribine(2-chloro-deoxyadenosine, Leustatin)
 - C. Pyrimidine analogues
 - 1.Cytarabine(cytosine arabinoside,Cytosar-U,ara-C)
 2. Fluorouracil (5-FU, 5-fluorouracil)
- III. Antibiotics
 - A. Anthracyclines
 1. Doxorubin hydrochloride (Adriamycin)
 2. Daunorubicin(daunomycin, Cerubidine)
 3. Idarrubicin (Idamycin)
 - B. Bleomycins
 1. Bleomycin sulphate (Blenoxane)
 - C. Mitomycin (mitomycin C, Mutamycins)
 - D. Dactinomycin (actinomycin D, Cosmegen)
 - E. Plicamycin (Mithracin)
- IV. Plant-derived products
 - A. Vinca alkaloids
 1. Vincristine (Oncovin)
 2. Vinblastin
 - B. Epipodophyllotoxins
 1. Etoposide (VP-16,Vepesid)
 2. Teniposide (VM-26,Vumon)
 - C. Taxanes: paclitaxel (Taxol)
 - V. Enzymes
 - A.L-Asparaginase (Elspar)
 - VI. Hormonal agents
 - A. Glucocorticoids
 - B.Estrogens, antiestrogens
 1. Tamoxifen citrate (Nolvadex)
 2. Estramustine phosphate sodium
 - C.Androgens,antiandrogens
 1. Flutamide (Eulexin)
 - D. Progestins
 - E. Luteinizing hormone-releasing hormone (LH-RH antagonists)
 1. Buserelin (Suprefact)
 2. Leuprolide (Lupron)
 - F. Octreotide acetate (Sandostatin)
 - VII. Miscellaneous agents
 - A.Hydroxyurea (Hydrea)
 - B.Procarbazine (N-methylhydrazine, Matulane, Natulane)
 - C.Mitotane (o, p-DDD, Lysodren)
 - D.Hexamethylamine (HMM)

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E. Cisplatin (cis-platinum II; Platinol)

F. Carboplatin (Paraplatin)

G. Mitoxantrone (Novantrone)

VIII. Monoclonal antibodies

IX. Immunomodulating agents

A. Levamisole (Ergamisol)

B. Interferon

1. Interferon alfa-2a (Roferon-A)

2. Interferon alfa-2b (Intron A)

C. Interleukins: aldesleukin (interleukin-2, IL-2, Proleukin)

X. Cellular growth factors

A. Filgrastim (G-CSF; Neupogen)

B. Sargramostim (GM-CSF, Leukine, Prokine)^{2, 3}.

Camptothecin

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 anti-tumour 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 anti-tumour 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 cancers⁴.

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 (3,4b) 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.

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 reported results show that the E-ring lactone is not essential for its activity⁵. However, this ring in the present lactone form with specific C-20 configuration is required for better activity. A brief description⁶ of its SAR is as follows:

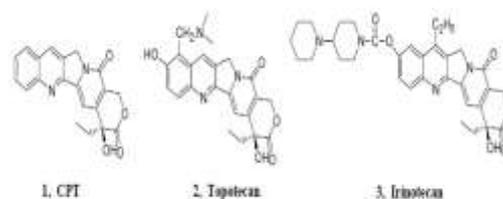
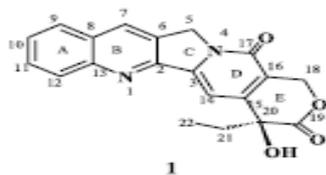


Figure 1. CPT and its analogues.

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Rings A–D is 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 activity⁶.

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 inactive⁷. 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 scientist.

BIOLOGICAL ACTIVITY

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

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^{7,8}.

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 involve the transient single strand cleavage of duplex DNA, followed by unwinding and relegation. Topoisomerase I cleaves DNA at multiple sites, and the highest efficiency cleavage sites exhibit significant sequence homology⁹.

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 anti-tumour activity among patients with gastrointestinal cancer, it also caused unpredictable and severe adverse effects including myelosuppression, vomiting, diarrhoea, and severe haemorrhagic cystitis^{10, 11}.

Synthetic analogues of CPT

CPT as such could not be used as a drug of choice due to its severe toxicity. Several groups

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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.

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 ring¹⁴. 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 irinotecan¹⁷. 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. 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.

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 camptothecin²⁰. 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.

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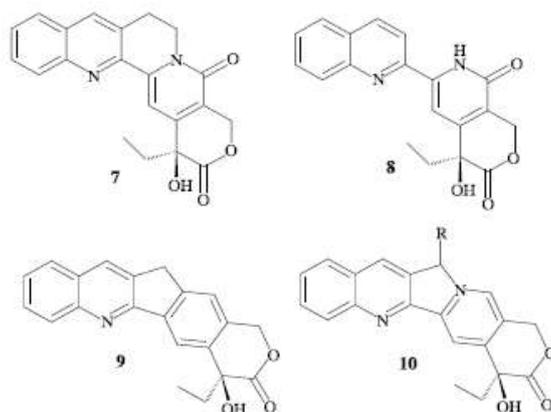
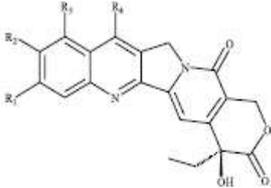
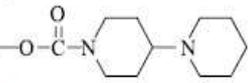


Figure 2. CPT analogues modified at C and D rings.

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-1)	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 ₂ N(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)

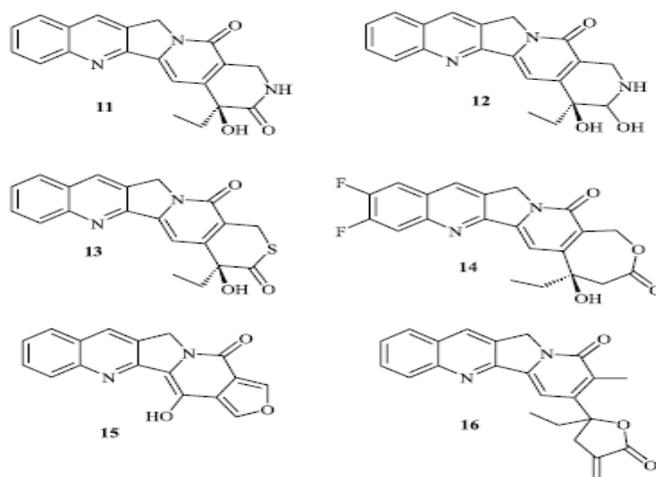


Figure 3. CPT analogues modified at ring E.

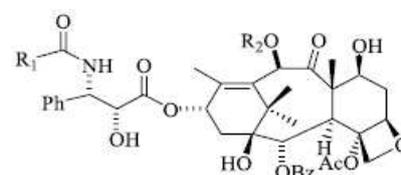
(Review Article)**Modifications in E ring**

The α -hydroxy lactonesystem 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.

Future prospects

CPT and its analogues exhibit a broad spectrum of anti-tumour 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 development²². 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. diterpenoid isolated from the pacific yew, *Taxus brevifolia*, by the same research group of Dr. Wall and Dr.Wani²³. 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).



17: R₁ = -C₆H₅, R₂ = Ac; (Taxol)

18: R₁ = , R₂ = H; (Taxotere)

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

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 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

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have to look towards nature for another diverse molecule with a novel mode of action to tackle this dreadful disease.

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