

Review Article**CAMPTOTHECIN AND ITS ANALOGS ANTITUMOR ACTIVITY BY POISONING TOPOISOMERASE I, THEIR STRUCTURE ACTIVITY RELATIONSHIP AND CLINICAL DEVELOPMENT PERSPECTIVE OF ANALOGS****Muqet Wahid, Qudsia Bano**Department of Pharmacy, Bahauddin Zakariya University Multan, Pharmacy
Multan, Punjab, Pakistan**ABSTRACT**

Camptothecin or CPT and its analogs appear to employ anticancer action by poisoning topoisomerase I (topo I) enzyme. As a result topo I activity inhibited and have established important role against a wide range of tumors. CPT was extracted from bark of Chinese plant *Camptotheca acuminata* but now a large number of synthetic and semi-synthetics are identified. After activity, researchers organized evaluation of A, B and E-ring modified CPT reported analogs. This review illustrated a discussion of modern approaches in medicinal chemistry development of CPT, effective anticancer agent that targets topoisomerase I and also with their Clinical studies. This review summarizes the mode of action of CPT, the structure-activity relationship (SAR), a list of CPT analogs and their biological action with detail clinical development.

Keywords: Camptothecin, 9-Aminocamptothecin, Topoisomerase I inhibitor, Topotecan, Irinotecan, SN-38

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INTRODUCTION

There have been marvelous changes in the antitumor therapy from last several years with outcome of improvement in survival for the individuals with cancer. Modern cancer dealing strategies, with the recognition of chemotherapeutical agents with new mode of action, are requirements for betterment of incident free and long run survival of poor prognosis of neoplastic patients. The topoisomerase-I or topo I poisons interpret a assuring new classification or category of anti-cancer drugs for the handling of cancer.

Camptothecin (CPT) is the cytotoxic quinoline alkaloid with pentacyclic ring structure that are secluded by Wall ME and Wani MC in 1966 from the bark of *Camptotheca acuminata* a tree commonly in China, but detected that Camptothecin suffers from many restrictions admitting low stability and solubility [1]. The encouraging consequences for testing as an antitumor agent in animal models led to the assessment of CPT in the clinic. However, the CPT acquired more concern in the later eighties (80s) when molecular target of CPT was identified .i.e DNA topoisomerase I (topo I) is conceived for its biological activity. This discovery made more concern for researchers in this agent and push researchers to making more water soluble derivatives of CPT. In beginning of 20th century, the Food and Drug Administration (FDA) approved the two water-soluble camptothecin analogs for clinical use: 1) topotecan, got approval as a secondary agent for therapy of ovarian cancer or small cell lung cancer, and 2) irinotecan, got approval from FDA, for used as a therapy agent for colorectal carcinoma refractory and also approved for primary therapy in combination with 5-fluorouracil (5-FU) for metastatic colorectal cancer [2-4].

Structure of Camptothecin Molecule

The outstanding phytochemical elements are alkaloids , flavonoids and ellagic acid derivatives, of leaves of *C. acuminata* were identified using HPLC. Camptothecin a modified monoterpene indole alkaloid, made by *C. acuminata*, initially separate by Wall ME and his co-workers in 1966 [1].

Camptothecin ($C_{20}H_{16}N_2O_4$) has a five member cyclic rings structure (Fig. 1), having 3 rings of pyrrolo-(3,4- β)-quinoline part (A, B, and C), coupled with pyridone (ring D) and at position 20, one chiral center within the α -hydroxy lactone ring with (S) configuration (E ring). The E ring, which is believed to be the most chemically activated ring of the structure, experiences an balance reaction between an active part lactone ring and a less active part carboxylate form.[5, 6]

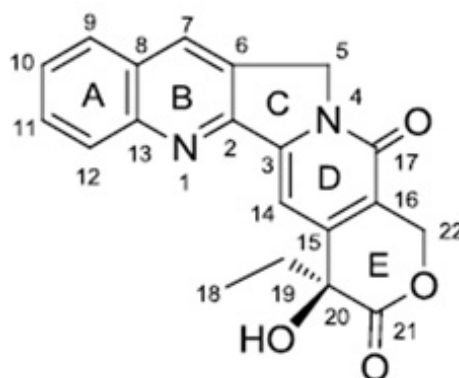


Fig. 1 Camptothecin (CPT)

Antitumor Activity of the Camptothecin

The anticancer activity of CPT and its derivatives is principally attributable its interaction with Topoisomerase-I (topo I), a enzyme requisite in the regulating of desoxyribonucleic acid (DNA) topology during replication, recombination and transcription. Topo I builds a phosphotyrosine bond with DNA, catalyzing a forward-reaction in which DNA is split to provide relaxing, and a inverse response in which DNA is re-ligated. CPT binds to enzyme topo I and DNA complex (covalent complex) leading in a ternary composite, this forbids DNA re-ligation and consequently causes DNA impairment which results in apoptosis. Hydrogen bonds are responsible for the adherences of CPT with both to the enzyme and DNA [3].

The utmost crucial portion of the CPT's body is the E-ring which interacts with the enzyme at three different places. The -OH group locating in twenty positioning causes H bond to the side-chain at 533 number aspartic acid in the enzyme's polypeptide chain. The lactone part of CPT's E- ring, bound with 2 H bonds to the arginine 364 of the enzyme. The D-ring of CPT interconnects with the +1 cytosine of non-cleaved chain and stabilizes covalent complex of the topo I and DNA, by making H bond. This H bond is between carbonylic group at position 17 of D -ring and amino group on pyrimidine ring of +1 cytosine [7]. It was hypothesized that CPT binding took place by displacement of the nucleotide that was at the 1+ base pair downstream from the cleavage site, allowing CPT to occupy the space.

A work performed with Topotecan discovered that the CPT analogue, playing as a noncompetitive inhibitor, introduced between the up-stream (-1) and down-stream (+1) of base pairs located at the DNA cleavage site. The intersperse induced in a alteration of base pairs and deracination of 5' -OH chain departed from phosphotyrosine bond hence halting re-legation. More reading of the interaction between the rings on the CPT structure and particular amino acids in the center of TOPO 1 may leading to advantageous changes of the CPT rings to improve binding and cell kill. One report manifested that the presence of the N²-ethyl-2-deoxyquanosine (N²-ethyl-dG) adducts raises the CPT interaction with the

Topo I-DNA complex, particularly when the adducts was positioned at the 1+ position. The O⁶-methylguanine (O⁶MG) molecule also gains cellular sensitivity to CPT. It is considered that N²-ethyl-dG and O⁶MG suppress the religation response by blocking the detachment of CPT from the cleavable complex [8, 9].

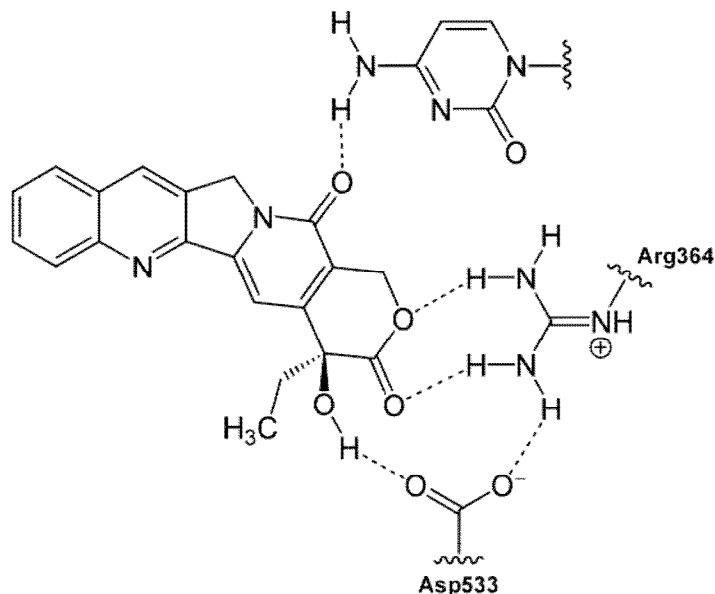


Fig.2 Binding of Camptothecin to Topoisomerase I and DNA

A study examining the occurrence of CPT-Topo I-DNA complexes in telomeric parts of DNA found out that TOPO 1 clung a particular G-rich nucleotide sequence, 5'GGGTT ↓ AGGGTT3', in the presence of CPT. The C-rich telomeres did not possess these identical sequences, nor do they undergo DNA segmentation by CPT. It was found that binding of CPT on the G-rich telomeres extended to apoptosis [10].

Camptothecin S Phase Apoptosis

CPT, which causes an extraordinary DNA impairment by trapping cellular topo I on chromosomal's DNA in the arrangement of cleavable complexes of drug-enzyme-DNA, as a result 1) blockage of DNA production and 2) abolishes S-phase cells. The halt of the replication forks by CPT caused topo I-DNA cleavable complexes is expected to be basic reason of CPT cytotoxicity. In fact it has been established that CPT-induced cell death does not take place in existence of aphidicolin, a DNA-polymerase inhibitor. Moreover, observation have evidenced that cleavable complex of CPT having characteristic to be bound with the complementary strand of DNA, for the possibly lethal crash to be occur [11].

Furthermore showed that the factor that best correlates with the variance in growth suppression between CPT-exposed cell lines is the formation of cleavable complexes; however, some cell lines exhibiting marked conflicts in growth suppression had minimum conflicts in cleavable complexes. This study indicated that CPT cell kill was depending on both the shaping of the cleavable complex and a downstream mechanism. Also the S phase base pathways, non S phase base pathways have been studied. One pathway is associated with interference of transcription. Upon exposure to CPT among the basic responses noted is rapid discontinuation of RNA synthesis. The halting of RNA synthesis is considered to occur downstream from the promotor region, only occur if the cleavable complex of CPT forms bound on the template strand [12, 13].

Camptothecin Analogs

Reports have demonstrated that substitution of the new groups at placement 7, 9, 10 and 11 can be result in positive effect on CPT activity and on physical characters, for example, metabolic stability of CPT and its potency. The lactone ring expansion by one -CH₂ group can raises CPT anti-tumor activities, as in homo-camptothecin or Homo - CPT. But CPT anti-tumor decline if substitution take place at position 12 and 14 as a result inactive derivative of CPT produced [14].

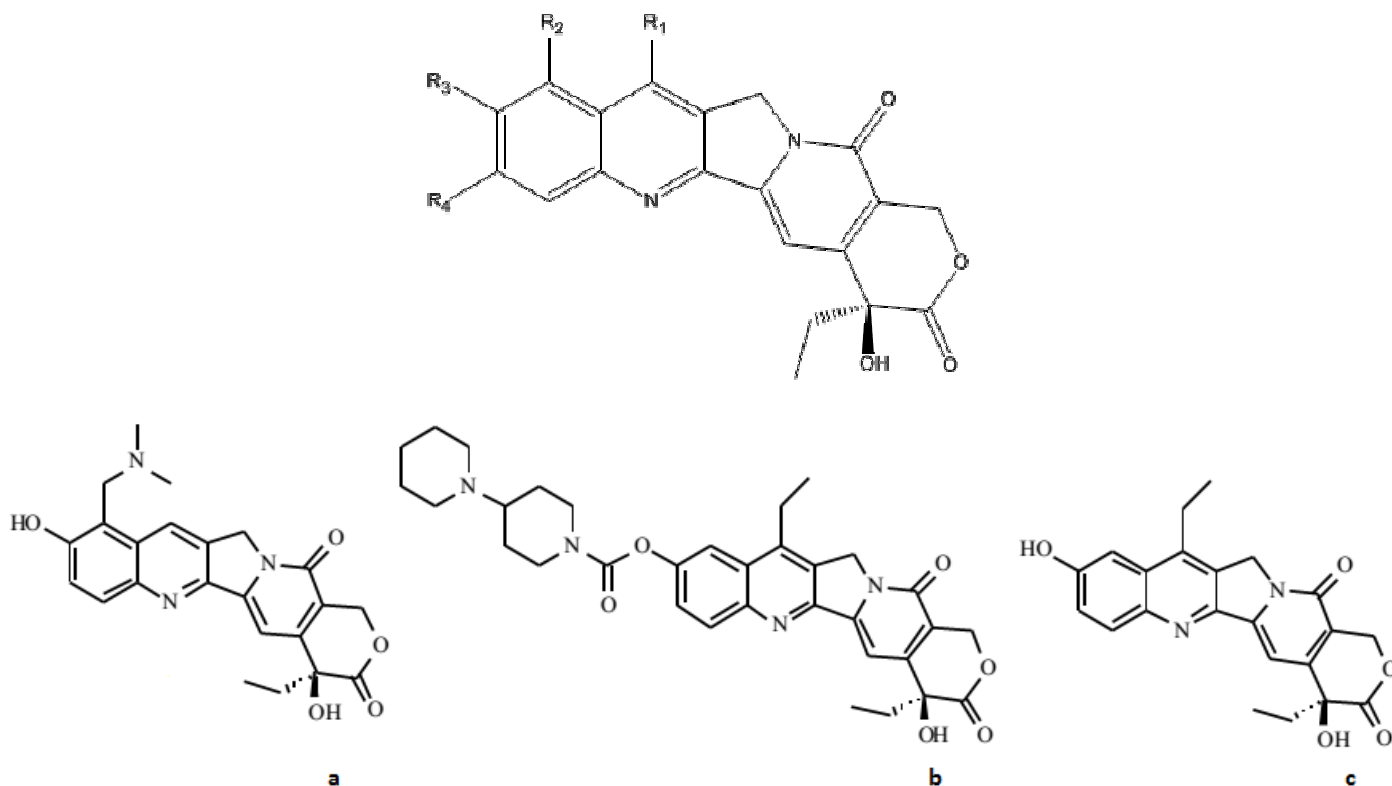
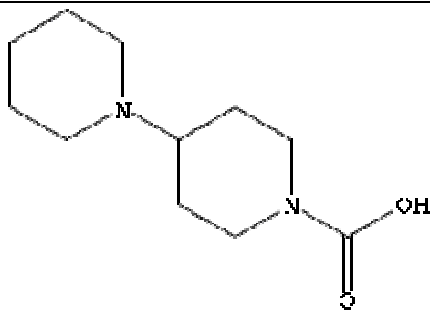
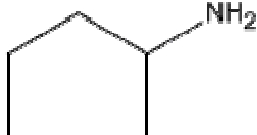
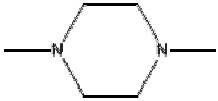



Fig.3 Structure of Structures of topotecan (a), irinotecan (c) and SN-38 (c).

Medicinal Chemistry of Camptothecin Analogs

The emerging of artificial and semi-synthetic approaches and analyses of CPT mechanisms had helped recognition of analogues with bettered characteristics, including 1) stabilization of lactone ring, 2) solubility of CPT 3) drug carrier and transport mechanisms, 4) identification of tumor cell and 5) improvement of DNA sequence specification. The above mentioned structural examples specify insight concerning the mechanism of CPT, and empathizing of methodical alterations within CPT structure may be raise and/or destroy the effects of drug in a biologic condition. A lot of readings regarding structure activity relationships (SAR) of CPT's structure provoked the production of many byproducts and analogues, comprising 1) pro-drugs, 2) new formulations, and 3) lipophilic (non-water soluble) or hydrophilic (water soluble) CPT. The current debate of CPT derivatives elaborates discretely upon substitutions, additions and deletions of groups in CPT pentacyclic rings: 1) quinoline ring (A/B-ring), 2) C and D rings, and 3) the E ring [14, 15].

Table 1 Modified Camptothecin Derivatives of Ring A & B

Analogue	R ₁	R ₂	R ₃	R ₄	Topoisomerase I Inhibition Activity*
CPT	H	H	H	H	+
Topotecan	-H	CH ₂ N(CH ₃) ₂	-OH	H	++
9-Aminocamptothecin	H	NH ₂	H	H	++
Irinotecan	CH ₂ CH ₃	H		H	+++
Rubitecan	H	NO ₂	H	H	NA
Exatecan		H	CH ₃	F	NA
Lurtotecan		H		H	+
ST 1481	CH=NOC(CH ₃) ₃	H	H	H	NA
CKD 602	CH ₂ CH ₂ NHCH(CH ₃) ₂	H	H	H	
SN-38	C ₂ H ₅	H	OH	H	++

[* Topoisomerase I Inhibition activity = It is Concentration that may produced 50% DNA cleavage in the existence of topoisomerase I.] [14].

Quinoline (A/B) ring modification

The studies on quinolone (A/B) ring of CPT reports that modification at position Carbon 7 (C-7), Carbon 9 (C-9), Carbon 10 (C-10), can raise effectiveness of CPT. Alkyl such as ethyl group (C₂H₅) or chloromethyl (CH₂Cl) group replacement at position C-7, expanded the cytotoxicity. These alkyl groups having capability to interact with DNA in occurrence of topo I, which makes it more anti neoplastic. It is reported that maximizing the carbon chain at position C-7 in quinolone ring, may leads to raise its more lipid soluble (lipophilicity), this property increase the CPT potency level and stability in plasma. Addition of an extra ring e.g methylenedioxy or ethylenedioxy group in CPT, to produce Hexacyclic CPT analogous. These analogues which has more solubility for water and increased strength of CPT. The scientists have discovered that methylenedioxy analogues are more effective than ethylenedioxy, They more investigated and found that less effect of ethylenedioxy analogues is due to negative steric interfaces with the enzyme. The addition of amino or chloro (-Cl) group at position 9 or chloro-methyl (-CH₂Cl) group at position 7 to

these hexa CPT analogues results increase the cytotoxicity but addition of these group loss the water solubility [14].

It is observed that derivatives with an extra ring combined at 1) C-10 and C-11, 2) C-9 and C-10, or 3) C-7 and C-9 were predicted to be more cytotoxic active than original pentacyclic CPT, such as exatecan and Lurtotecan [16].

C and D-ring modification

The addition or substitution or any modification of the C and D rings of CPT are less reported. The reason is behind that either replacement or substitution takes place in C/D-ring, CPT molecule loss its cytotoxic activity. Few investigators modified C/D-ring but unluckily, none of them was approved for clinical trials [17].

E-ring modification

E-ring does restrict many structural changes without losing CPT activity. The possible substitution is hydroxyl (-OH) group to Cl, F or Br because they enhance the polarity ability and also is enough to stable the enzyme complex. Supported on scheme, E ring changes had underlined the constancy of lactone. The study reported that substituted the Carbon 21 (C-21) with other atom i.e. N and S to produce lactam and thiolactone, as a result the opening tendency of the ring is loss however, resultant CPT lactam and thiolactone were inactive. The other possible modification is introduction of methylene group between hydroxyl group and lactone ring on the E ring generating a 7-membered β -hydroxylactone group, so called homocamptothecin or HomoCPT. The HomoCPT's hydroxyl group has low inductive effect on the carboxyl group as a result lactone become more reactive. It was reported HomoCPT substitution of two hydroxylactone moieties a and b of CPT with each other may exerts affected potency and constancy of lactone while it has incapability of intra-molecular H bond. HomoCPT is new formulation for cytotoxic activity against topo I and its numerous analogs such as 10,11-difluorohomoCPT (BN-809) have strong anti-proliferative activity against cancerous cell lines [18, 19].

Clinical Developments of Camptothecin Analogs

Topotecan

The Topotecan is listed in platinum based primary healing for non-small cell lung cancer (NSCLC) patients by modern American Society of Clinical Oncology strategies. After preclinical testing, topotecan (9-(dimethylamino)methyl-10-hydroxycamptothecin) shift in phase I trials in America and European Communities. In this trial short, medium and prolonged/ sustained infusion plans have been examined as well as: individual get IV injection each 21 days; 2) individual get 30min infusion of Topotecan on 5 back-to-back days each 21 to 28 days; 3) 24, 72, 96 and 120 hours continual IV infusions each 21 to 28 days; and 4) 21 day continual IV infusion each 28 days. In all analyses, myelosuppression reported but it was redose-limiting while outline of myelosuppression are different with different technique of administration. Discontinuous short and bolus infusion plans resulted in non-cumulative neutropenia which is main toxicity, while anaemia, neutropenia and thrombocytopenia were reported when sustained continual infusions were administrated. Non haematological harmful results were normally moderate comprised fatigue, nausea, alopecia, diarrhoea, nausea, elevations in hepatic enzymes, vomiting, mucositis, and skin rash [20, 21].

The pharmacokinetic findings show that drug quickly hydrolyzed to the open ring form in plasma after IV dose. The clearance of plasma was bi exponential with final half life 3 hours. The renal excretion of the drug show that 40% of it is excreted in urine within first 24 hours. There's a beneficial association between Area under Curve of total drug and the status of neutropenia in individuals with normal or dysfunctional renal and/or hepatic function. There is need of dose re-adjustment require for those individuals having decreased clearance of creatinine because topotecan may enhance chances of toxicity. However,

hyperbilirubinaemia does not change topotecan toxicity or disposal, so there is no need of dose modification in patients having serum bilirubin level as high as 170 μmol^{-1} [22].

Irinotecan (CPT-11)

In *in vitro* and *in vivo* studies, Irinotecan or (CPT-11) is active agent against a various cancerous cell lines. The chemical name of CPT-11 is 7-ethyl-10-(4-[1-piperidino]-1-piperidino) methyl-10-hydroxycamptothecin. It was first water-soluble analogues of CPT. CPT-11 is prodrug having controlled action. CPT-11 changed into SN-38 in plasma under de-esterification process. SN-38 is thousand times more potent and effectiveness than parent compound as more efficient inhibitor of Topo I and more toxic towards HT-29 human colon-carcinoma cells lines than CPT, 9-aminocamptothecin (9-amino CPT) and topotecan [48,49]. Phase I evaluation of CPT-11 were carried in Japan, America and European Community on numerous plans: 1) 30 mins infusion each week; 2) 30 to 90 mins infusion each day for 3 days; 3) 90 mins infusion each week; and 4) 120 hours continual IV infusion each thirty-four weeks, first 3 plans based on each 3 weeks. Dose limiting side effects of CPT-11 were slightly reliant on treatment plan. Dose limiting, neutropenia, leucopenia and diarrhoea were noticeable in single dose regimen, whereas GI toxicities prevailed with *c.i.v.* plans. In Diarrhoea, which is major GI toxicity, two mechanism are involved i.e. at muscarinic receptors, it inhibit acetylcholinesterase enzyme and in autonomic ganglion cells, it stimulate nicotinic receptors. Besides of these major Toxicities of CPT-11, other included transient elevation of liver function tests, thrombocytopenia, anaemia, eosinophilia, rash, fatigue, alopecia and mucositis [23].

The pharmacokinetic of CPT-11 are complex than other CPTs. The enzyme carboxylesterases quickly change prodrug CPT-11 into SN-38, in plasma. Moreover, both CPT-11 and SN-38 changed to lactone forms to carboxylate forms by pH dependent hydrolysis, whereas, hydrolysis is less than topotecan and 9-aminoCPT. Area under curve and peak plasma levels of CPT-11 associate with dose. The reported final half-life of CPT-11 is 5.2 to 9.3 hours. The excretion pathways of CPT-11 are biliary and urinary excretion paths. In humans, after 48 hours, 37 \pm 4% of the CPT-11 is found in urine. The glucuronic acid conjugation metabolism occurs for both CPT-11 and SN-38 as result they excreted in bile. The GI microflora enzyme β -Glucuronidase, can cleave glucuronide and free SN-38, these factors play role in late diarrhea [24].

9 - Aminocamptothecin (9 -aminoCPT)

Between numerous semi-synthetic or completely synthetic CPT derivatives tested, 9 -aminocamptothecin (9 -aminoCPT) was one who get designated for further screening and for clinical evolution due to capability complete reductions of human colonic adenocarcinoma & malignant melanoma cell-lines in mice. These cell lines having immunity against standard chemotherapy. Similar to topotecan, the pharmacokinetic and effectiveness trails of 9-aminoCPT advised that sustaining plasma concentration of lactone, over a threshold level, for an extended duration need the optimum therapy effects for management [25].

The inherent water in-solubility of 9-aminoCPT cause difficulty in developing an appropriate pharmaceutical formulation as a result late beginning of phase I clinical trials. The preclinical trials reported optimal antitumor activity occur when plasma concentration of lactone near 10 nM threshold concentration for minimum 72 hours. Early phase I clinical trials of 9-aminoCPT included administration by continual IV infusion for periods extending from twenty-four hours to twenty-one days. After, daily management, drug was given in short IV infusion on 5 successive days, each three weeks. Neutropenia, leucopenia, thrombocytopenia and diarrhea was dose-limiting toxicity in both tests [26].

The pharmacokinetic trials completed in clinical tests. It exposed that active form of 9 -aminoCPT lactone was <10% of total administered 9- aminoCPT, present in plasma. This significant difference is due to the pharmacokinetic performance of 9- aminoCPT in mice, undamaged lactone form and open ring carboxylate form of 9-aminoCPT in plasma levels were more alike after bolus IV injection, so 9-aminoCPT lactone

accomplished 62% of total drug, area under curve. However, phase I studies conducted in patients having malignant gliomas, showed that clearance of 9-aminoCPT was increased in individuals getting anticonvulsant therapy that induce enzymes CYP450. This behavior of 9- aminoCPT was surprising, because there was no previous evidence that it play significant role in hepatic metabolism. The primary excretion pathway is biliary excretion of drug excretion as suggested in pre-clinical reports, that 55% of the radioactivity from the injection of [3H]9- aminoCPT was found in feces of mice. The preclinical pharmacokinetic reports showed that urinary elimination accounts unchanged drug ,about one-third of the total dose [69] Other commonly found toxicities include mucositis, anemia, vomiting, fatigue, nausea and alopecia. There was correlation between steady state plasma concentration of 9- aminoCPT and quantity of myelosuppression, in phase I clinical trials [27, 28].

9-nitrocamptothecin (9- nitroCPT)

9-nitrocamptothecin (9- nitroCPT) a strong but similar to others CPT analogues poorly soluble, is established for oral form. While doubtless, 9- nitroCPT has intrinsically anti cancerous activity alike other CPTs by inhibiting topo I. The more significant about 9- nitroCPT that it experiences pH dependent metabolic change to 9-aminoCPT in plasma in *vitro*, maximum transition occur at pH 6.0. 9-nitroCPT established to be extraordinarily tough to evaluate in biological liquids. A pharmacokinetic test accomplish in healthy volunteers administered with oral 9- nitroCPT that 9- aminoCPT, plasma concentrations of total drug well above the supposed 10 nM therapeutic threshold concentration could be easily achieved. However, area under curve of 9-aminoCPT was only 12% of parent drug, meaning it is limitation of conversion of 9-nitroCPT to 9-aminoCPT in individuals was comparatively negligible [29].

Dose limiting toxicity when given on repetitive basis in order of 5-days per 2 days off weekly regimen, myelosuppression, hemorrhagic cystitis and diarrhea. The anti-cancerous activity has been reported in phase II clinical tests in individuals having pancreatic carcinoma healed with single agent 9-nitroCPT, admitting response in individuals having progressive disease notwithstanding preceding management with gemcitabine. A consequent phase II clinical trial utilising formal response standards document response rate of only 9% in pancreatic cancer individuals managed earlier. An challenging phase II trials to assess clinical efficiency of agent against wide range of tumors. It is difficult to comprehend that there is any important clinical superiority over oral 9-aminoCPT because similarities in the chemical structure, biological activities and physicochemical properties of 9-nitroCPT and 9-aminoCPT. , it is hard to notice that 9-nitroCPT have any significant clinical improvement over oral 9-aminoCPT [30] .

CONCLUSION

The CPT is the promising class of the antitumor agents have shown noteworthy clinical activity against wide range of tumors by poisoning the topoisomerase I enzyme. Understanding CPT mechanism and their activity lead to development of different analogs having modified and enhanced pharmacological activities. The medicinal chemistry of CPT well understand the structure activity relationship, due to which numerous analogs, two of them clinical approved and some are under clinical trials. Topotecan and CPT-11 are well established chemo-agents in chemotherapy management of different tumors. Topotecan with cisplatin is limited because of hematological toxicity. However, topotecan has greater role in administration of hematological malignancies.CPT-11 one of most promising clinically documented CPT analogues and approved for the colorectal cancer. For new developments a effective and enhance drug delivery and formulation policies may also require to achieve maximum antiproliferative therapeutic effect of the CPT.

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