Review Camptothecin: Current Perspectives

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Abstract: The review provides a detailed discussion of recent advances in the medicinal chemistry of camptothecin, a potent antitumor agent that targets topoisomerase I. Thousands of CPT derivatives have been synthesized. Two of them, Topotecan and Irinotecan, are commercially approved for use in clinic as antitumor agents while more are still in clinic trials. This review summarizes the current status of the modern synthetic approaches to CPT, the mechanism of action of CPT, the structure-activity relationship(SAR), a number of novel CPT analogs and their biologic activity. There is a systematic evaluation of A-, B- and E-ring- modified camptothecins reported recently.

Keyword: Camptothecin, Antitumor,Topo I inhibitor.

1. INTRODUCTION

20(S)-Camptothecin (CPT) (1) (Fig. **1**), a pentacyclic alkaloid, was first isolated by Wall and co-workers in 1966 from extracts of *Camptotheca acuminata*, a tree native to China [1]. The promising results for testing as an antitumor agent in animal models led to the evaluation of camptothecin in the clinic [2]. Therapeutic application of unmodified CPT is hindered by very low solubility in aqueous media, high toxicity, and rapid inactivation through lactone ring hydrolysis at physiological pH. Lactone hydrolysis, which is reversible in acidic media, leads to a water-soluble carboxylate (2) [3]. The latter is inactive and readily binds to human serum albumin, making it inaccessible for cellular uptake [4]. More unfortunately, the sodium salt (2) is cleared by the kidneys and causes hemorrhagic cystitis and myelotoxicity, which resulted in suspension of the trials [2, 5].

The discovery that the primary cellular target of CPT is DNA topoisomerase I (topo I) created renewed interest in this agent and led to synthesizing more water-soluble analogs [6]. Two of them, topotecan (Hycamtin, 3) [7] and irinotecan (Camptosar, 4) (Fig. **2**) [8], have received FDA approval for the clinical treatment of the ovarian cancer and small-cell lung cancer [9] and refractory colorectal cancers, respectively [10]. At least 10 additional CPT derivatives are in various stages of clinical trials. Here, we describe the current status of the modern synthetic approaches CPT (analogues), the mechanism of action of CPT and structure-activity relationship (SAR), as well as a number of novel CPT derivatives and their biologic activities will also be discussed.

2. SYNTHETIC STUDIES

Camptothecin and its analogs have provided a rich playing field for the development of convergent total synthesis strategies. There are now several synthetic approaches to CPT and its analogs. Additionally, there are some semi-synthetic approaches for the production of derivatives of CPT [11].

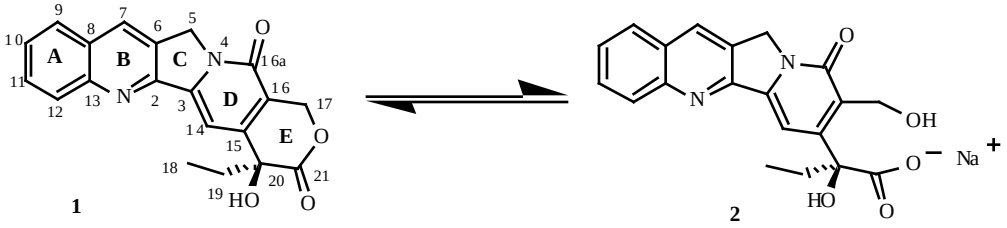

Fig. (1). Structures of camptothecin (1) and water soluble sodium salt (2).

Following the structure of CPT, numerous synthetic methods were reported utilizing a multitude of approaches [12]. The first successful total synthesis of the racemic form of the molecule was reported by Stork and Schultz in 1971 [13]. Since then more syntheses about 20(S)-CPT have been published.

The first asymmetric synthesis of 20(S)-CPT was reported by Tagawa *et al*. in 1989, utilizing an N-tosyl-R-proline derivative as the chiral auxiliary to induce the stereocontrolled assembly of the lone chiral center [14]. To date, the shortest asymmetric synthesis of CPT by Comins and Nolan involves the formation of the C-ring by connecting the A/B- and D/E-fragments via an N-alkylation and a key intramolecular Heck ring closure reaction (Fig. **3, A**), [15]. Curran *et al*. designed an interesting strategy in

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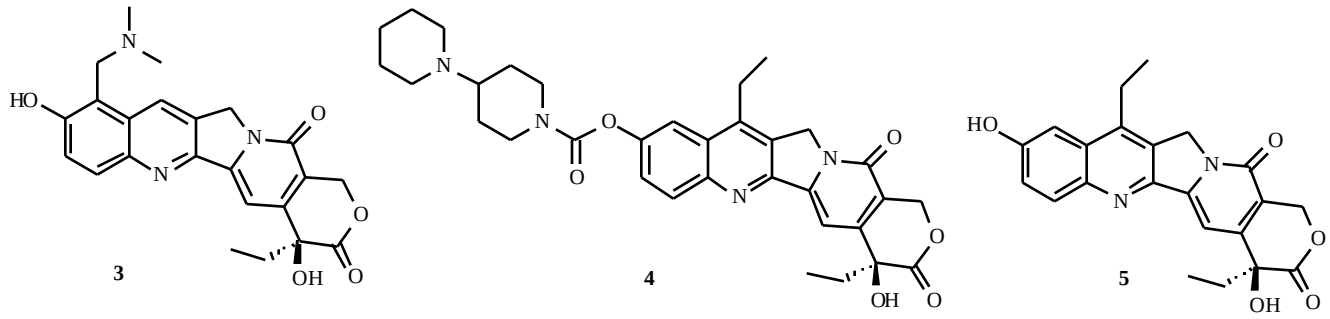


Fig. (2). Structures of topotecan (3), irinotecan (4) and SN-38 (5).

which the appropriately functionalized A- and D/E-fragments (Fig. **3**, **B**) participate in a free-radical cascade, which led to the formation of the B- and C-ring of CPT [16]. A different concomitant formation of the B- and C-rings was reported by Fortunak. They used an efficient intramolecular Diels-Alder reaction (Fig. **3, C**), which is now successful in an industrial scale [17]. In succession, Blagg and Boger described a new synthesis of 20(S)-CPT based on a Diels-Alder cycloaddition of the electron deficient diene N-sulfonyl-1-aza- 1,3-butadiene with the electron rich dienophile for the assembly of precursor to CPT (Fig. **3**, **D**) [18]. The key features of an inventive strategy [19] by Bosch consisted of an intramolecular radical cyclization to form the C-ring followed by asymmetric construction of the E-ring using enolate chemistry (Fig. **3**, **E**). Intermolecular [20] (Fig. **3**, **F**) and intramolecular [21] (Fig. **3**, **G**) Michael addition reactions by Ciufolini and Chavan, respectively, were utilized for the D-ring construction of CPT. Henegar *et al*. have described a novel synthetic strategy leading to 20(S)-CPT based on the classical Friedlander reaction of 2-aminobenzal-dehyde (A-ring) with the assembled C/D/E-ring framework (Fig. **3**, **H**) for B-ring of CPT [22]. Although not intended as an exhaustive review, recent representative total syntheses are described below in some detail.

Since several syntheses of 20(S)-CPT have been developed over the years, synthetic efforts now need to be directed to short, practical routes that are amenable to scale-

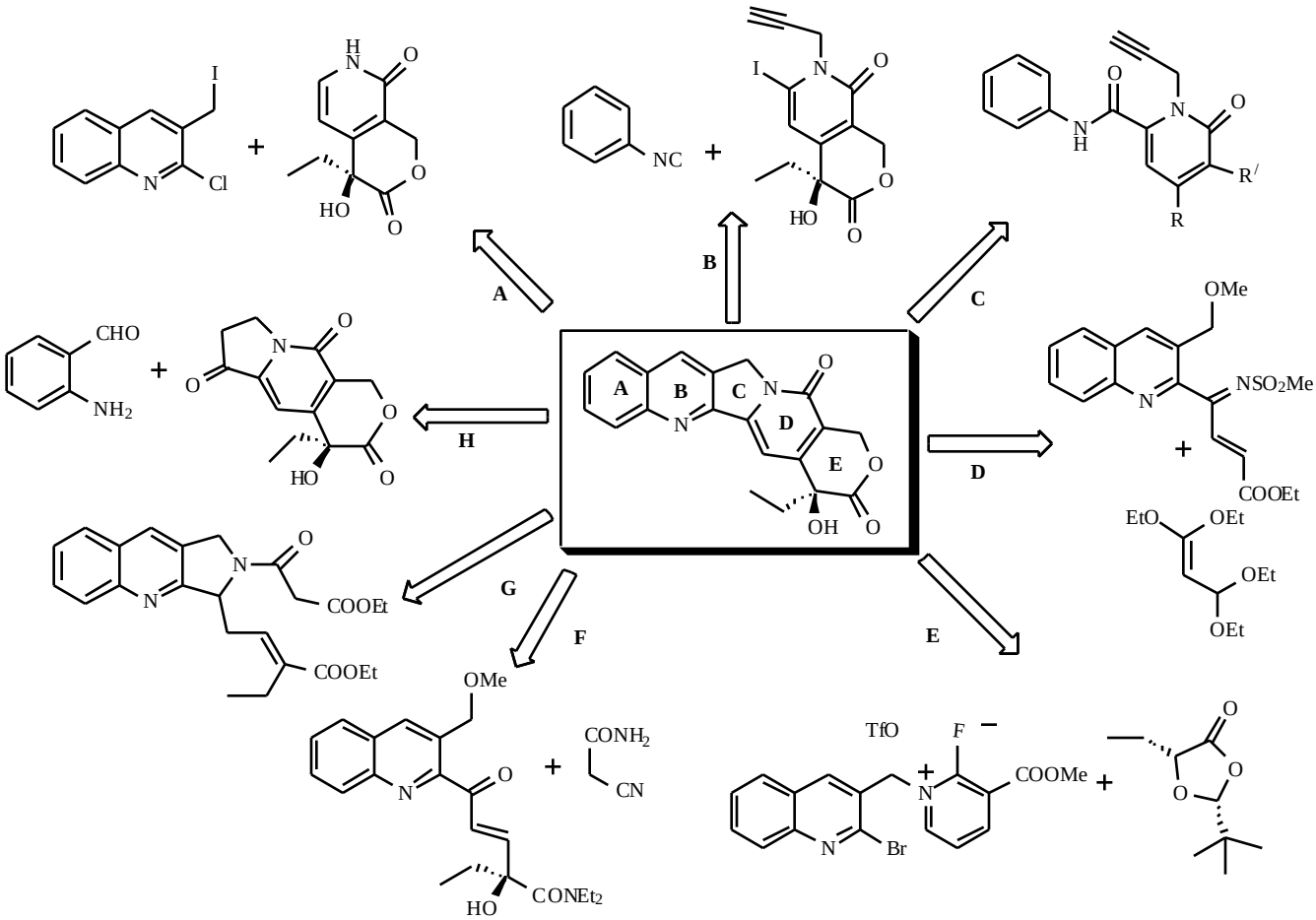
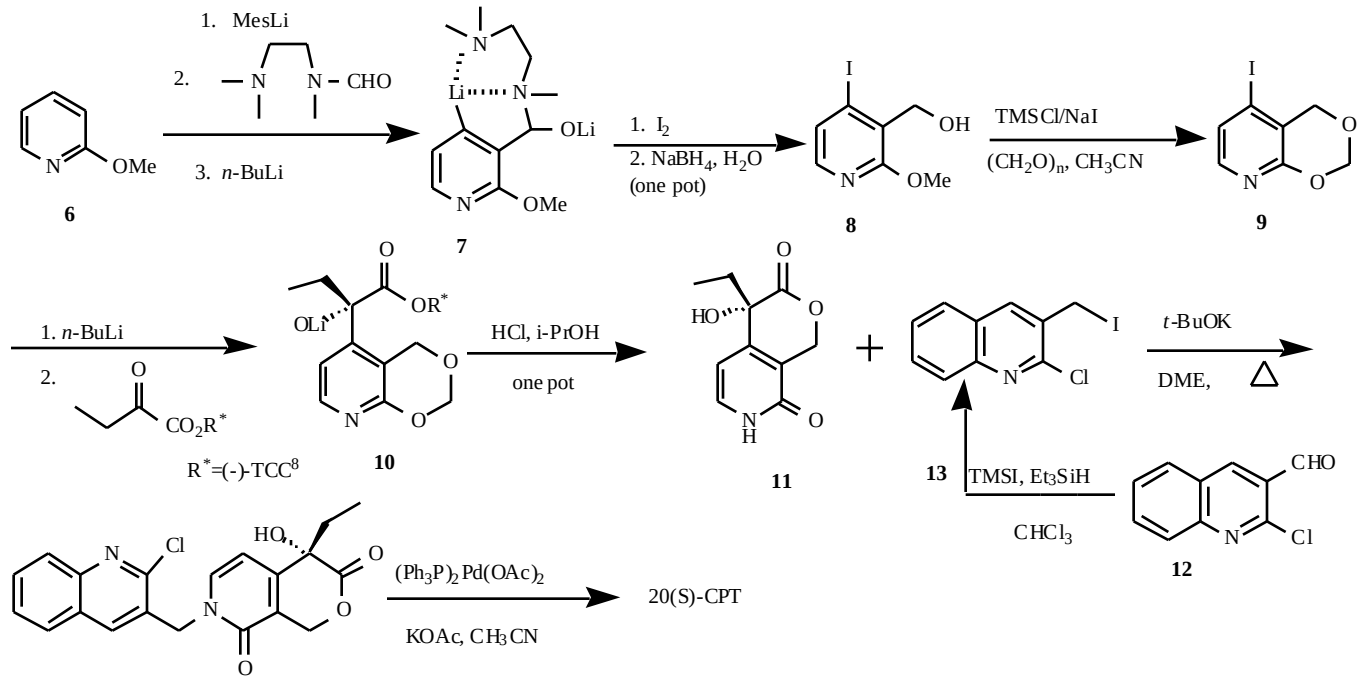
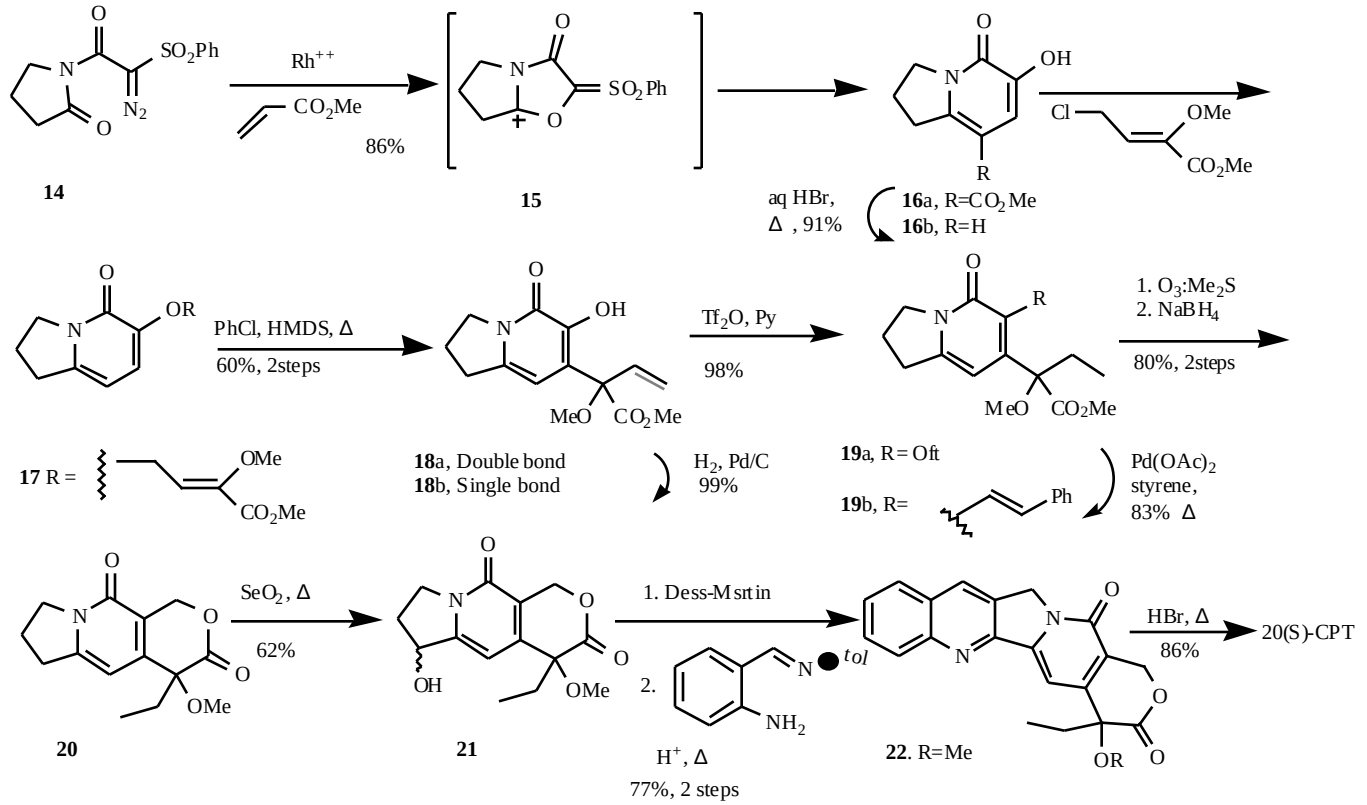


Fig. (3). Selected strategies for the synthesis of camptothecin.

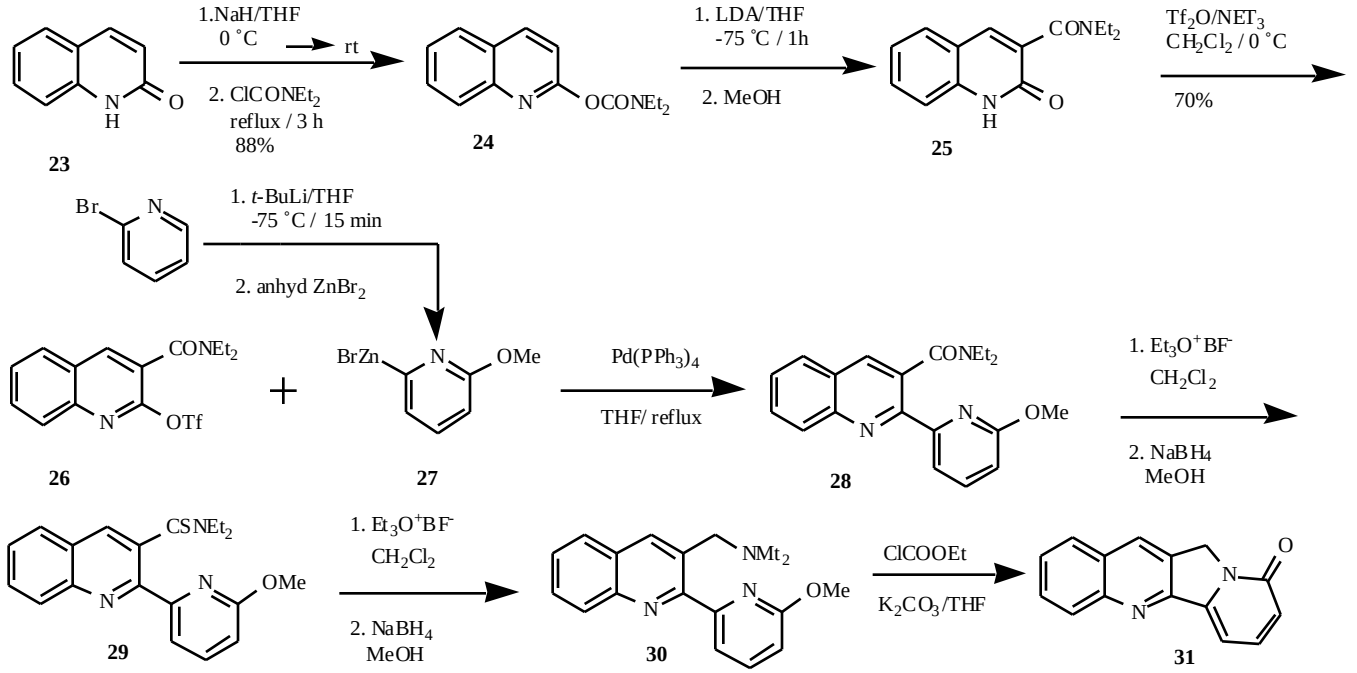


Scheme 1.

up for drug preparation. Comins and Nolan reported a synthesis of 20(S)-CPT accomplished in six steps (Scheme **1**), which started from two commercially available heterocycles 2-methoxy- pyridine (6) and 2-chloro-quinoline-3-carbaldehyde (12) [15]. For the six-step limitation, the A/B-ring precursor (13) had to be prepared in a single step and in 81% overall yield from quinoline derivative (12). The D/E-ring fragment (11) would have to be made *via* intermediate (9) in only three steps from facile material. This is the representative shortest total asymmetric synthesis through joining of the A/B-ring and the D/E-ring through construction of the C-ring, which advanced the industrial production of CPT [23].

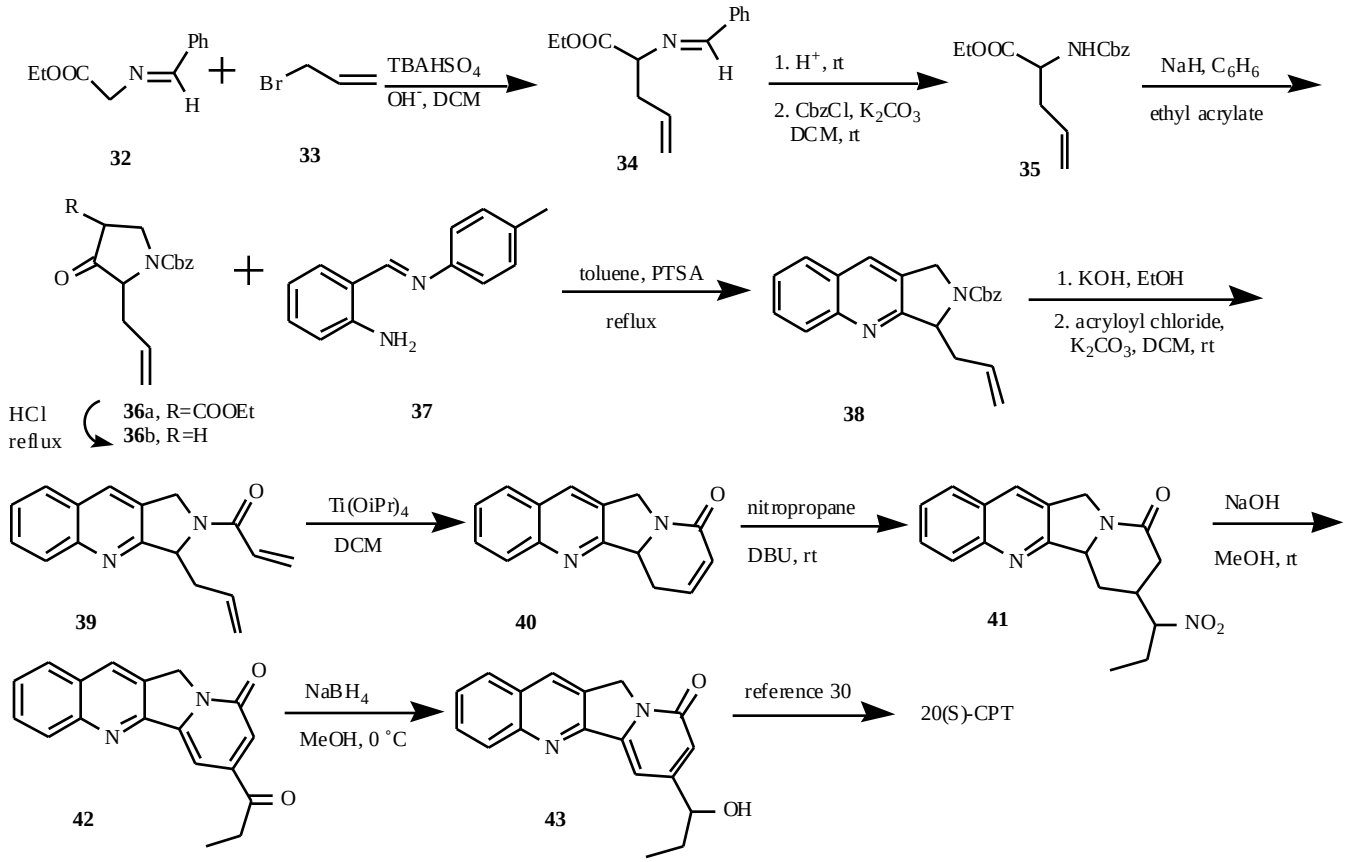


Scheme 2.



Scheme 3.

Anderson *et al*. have described a novel expeditious synthesis of racemic 20(S)-CPT (Scheme **2**) [24]. The synthesis began with etherification of hydroxy pyridone (16b), which provides from dizao imide (14)based on siomünchnone cycloadditions according to Padwa at two steps [25, 26], with (Z)-methyl4-chloro-2-methoxybut-2-enoate and cesium carbonate to give derivative (17), in preparation for a Claisen rearrangement to introduce a substituent in the β position and afford the hydroxy pyridone (18a). Compound (18b) could be provided by



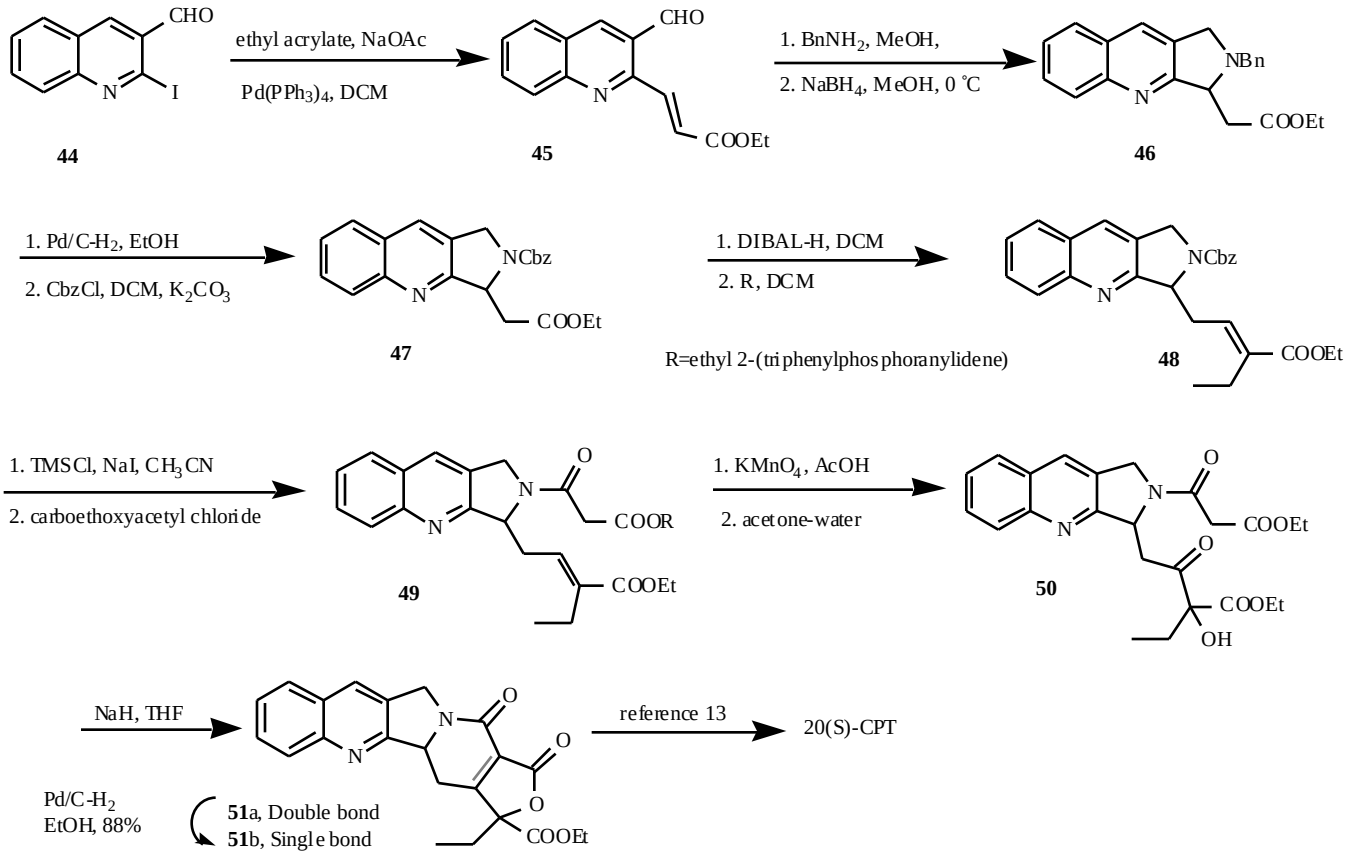
Scheme 4.

hydrogenation and then converted into 19a. Lactone construction was completed by treatment of 19b with ozone, followed by reduction of the resultant aldehyde with sodium borohydride, which provided lactone 20 in 80% overall yield. The reaction of lactone 20 with selenium dioxide afforded the hydroxy derivative 21 in 62% yield. The cycloaddition of B-ring was achieved by Friedländer condensation with an *o*-aminobenzaldehyde surrogate to give quinoline 22 in 77% yield at two steps [27]. In the hot aqueous HBr solution, the methyl protecting group in 22 was cleaved and 20(S)-CPT was smoothly produced in 86% yield.

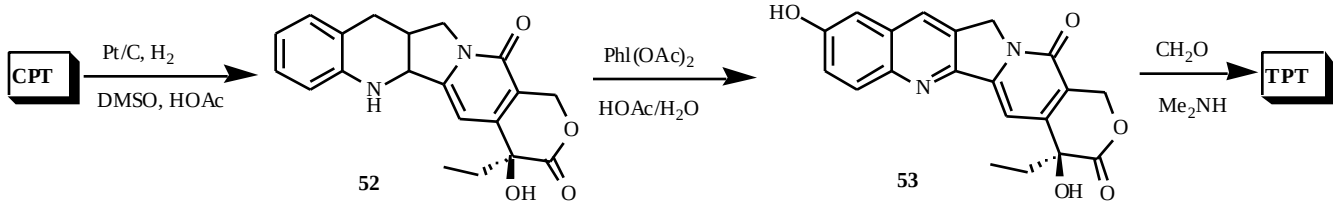
Tam *et al*. reported a new synthesis of the A/B/C/D-ring core (31) of 20(S)-CPT using a combined directed ortho-metalation (DoM)-transition metal catalyzed cross-coupling tactic (Scheme **3**) [23,28]. 2-quinolone (23) was first converted into the O-carbamate 24 at three steps in order to avoid the clean thermal 1,3-carbamaoyl rearrangement. The next reaction was carried out at low temperature to give the 3-amidoquinolone 25 in 61% yield. the quinolone 25 was transformed into the triflate 26 for completion of the A/B-fragment,. Organozinc species 27 prepared from 2-bromopyridine was subjected to the Pd^0-catalyzed cross-coupling procedure with triflate 28 to provide the biaryl 29 in 59% yield. Then triflate 28 was converted into the corresponding thioamide 29, which upon sequential ethylation with ethyl-Meerwein salt and reduction with NaBH, was transformed into the tertiary amine 30 in 83% yield. The final cyclization of tetracycle 31 was achieved in 62% yield *via* a modified von Braun reaction. The simplicity of both A/B- and D-ring fragments by Dom chemistry, and the potential further modification of ring D in 31 offered additional avenues for synthetic excursions in the camptothecin field.

Chavan *et al*. explored a new and efficient approach for the synthesis of the D-ring of CPT by intramolecular ring-closing metathesis (RCM) [29]. The key versatile tricyclic amine 38 was synthesized starting from a very simple Schiff's base 32 with allyl bromonide and another Schiff's base 37 at five steps in 72% yield (Scheme **4**). A consistent and higher yield of acrylamide 39 was obtained when the Cbz group in 38 was obtained under alkaline conditions. The important intermediate tetrahydro-pyridone 40 was provided from acrylamide 39 in the desired yield. It was observed that a mixture of DBU, nitropropane and 40 at room temperature afforded nitro compound 41 in a very high yield. Then the nitro compound 41 was converted into corresponding pyridone 42 on exposure to standard Nef conditions in 23% yield. Finally the reduction of the carbonyl group of 42 with $NaBH_4$ gave the desired hydroxy-pyridone 43 in nearly quantitative yield, which is the main intermediate in Murata's synthesis of camptothecin [30]. This synthetic strategy provided the advantage of high yield.

After exploited an intramolecular Michael addition strategy as a key step for the construction of ring D of CPT (Fig. **3**, **G**) [21], recently Chavan described a novel approach toward 20(S)-CPT [31]. The most important step is the implementation of an intramolecular aldol reaction of ketol 50 to construct the pyridone D-ring with functionality for manipulating the lactone E-ring of the title compounds as shown in (Scheme **5**). The ketol 50 was prepared from Meth-



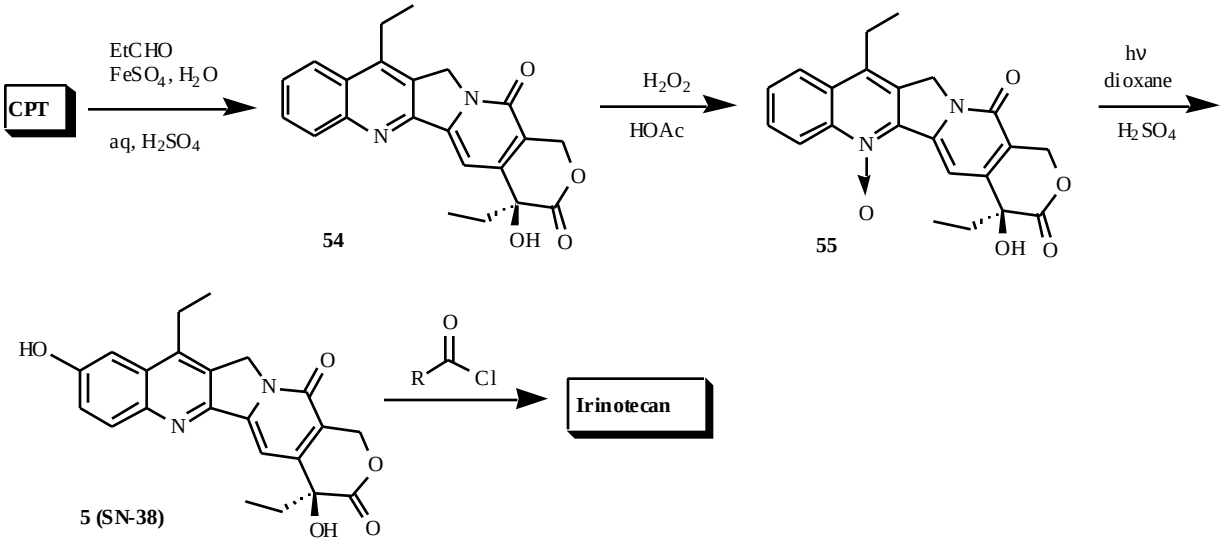
Scheme 5.



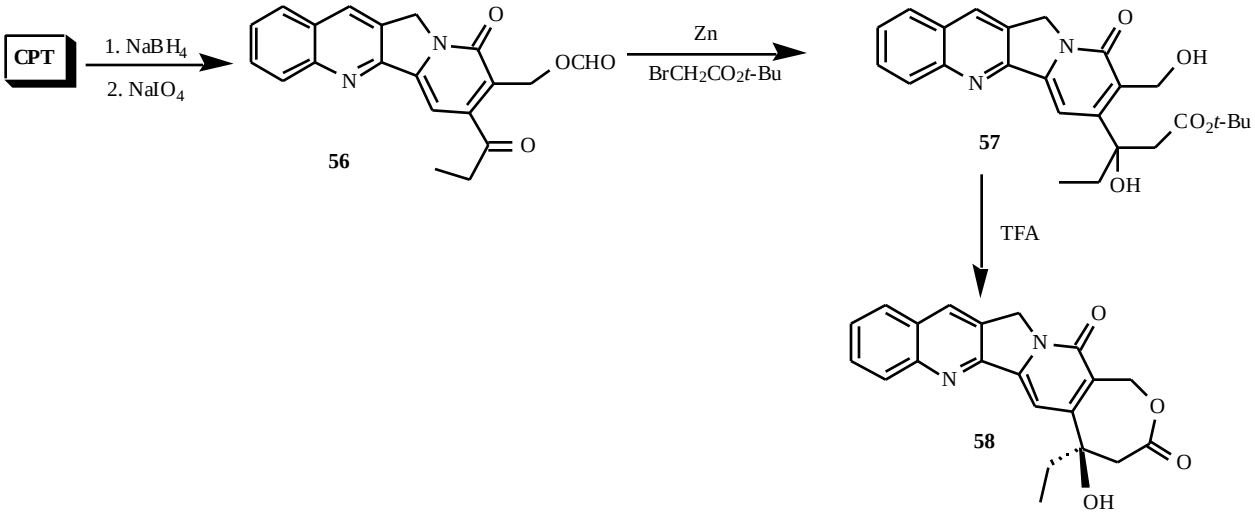
Scheme 6.

Cohn's aldehyde 44, which underwent facile olefination with ethyl acrylate to give olefin 45 in 74% yield. Condensation of 45 with benzylamine in methanol formed tricyclic amine 46 in 91% yield, which transformed into a secondary amine 47 under hydrogenation conditions. Reduction of this carbamate 47 with DIBAL-H resulted in the formation of an aldehyde, which was subjected to Wittig olefination to afford the α,β-unsaturated ester 48 in 80% yield [32]. Deprotection of the Cbz carbamate afforded amide 49, and then converted into ketol 50 *via* $KMnO_4$ oxidation. The dihydropyridone 51a was provided from the ketol 50 *via* an intramolecular reaction in 90% yield. At last, 51a was converted to the tetrahydro- pyridone 51b, which has already been converted to 20(S)-CPT by Stork and Schultz [13].

In addition to numerous total syntheses of CPT, much attention was paid to the syntheses of CPT analogs to overcome shortcomings of CPT. The representative cases are topotecan [33], irinotecan [34] and homocamptothecin (hCPT) [35]. While more complex analogs still rely on total synthesis, anticancer drugs topotecan, irinotecan, homocamptothecin and a number of drug candidates have been prepared by semi-synthesis, and are summarized here.



Scheme 7.



Scheme 8.

The semi-synthesis of topotecan is shown in (Scheme **6**) [7]. A reduction-oxidation sequence converts camptothecin to 10-hydroxycamptothecin 53. Platinum-catalyzed hydrogenation of camptothecin gave tetrahydroquinoline 52, which was oxidized with $PhI(OAc)_2$ in one pot to give 10-hydroxycamptothecin 53 in excellent yield. Condensation of 53 with formaldehyde and dimethyl amine yielded topotecan 3 in 62% yield.

Irinotecan, another currently marketed CPT derivative, was synthesized in 1991 by the Sawada group (Scheme **7**) [36]. Reaction of propanal in the presence of $FeSO_4$, H_2O_2 in aqueous acidic medium generates an ethyl radical, which adds to the 7-position of camptothecin to give 7-ethylcamptothecin (54). Oxidation of 55 to its N-oxide 56 followed by photo irradiation in the presence of acid gave the corresponding 7-ehyl-10-hydroxy camptothecin (5) (SN-38) in 49% yield. Treatment of SN-38 (5) with [1,4']bipiperidinyl-1'-carbonyl chloride provided irinotecan (4) in 80% yield.

Homocamptothecin was first prepared in racemic form by semi-synthesis (Scheme **8**) [37]. Reduction of camptothecin E-ring lactone gave a lactol. Subsequent oxidation resulted in C-C bond cleavage to give ketone (57) in 84% overall yield. A reformatsky reaction with *t*-butyl bromoacetate and 57 afforded hydroxy ester (58) in 31% yield, which was converted to homoCPT (58) in 73% yield.

3. MODE OF ACTION

According to the action mechanism of CPT, the compendious answer is reported as follows: CPTs act by binding to the topoisomerase I-DNA complex, leading to an accumulation of DNA strand breaks upon replication, ultimately causing cell death during the S-phase of the cell cycle. The complex is normally a transient intermediate which involved in DNA relaxation during a number of critical cellular processes, including replication, transcription, recombination, repair, chromatin assembly, and chromosome segregation, and generally rapidly reversible without CPT [38,39].

Topoisomerases are essential nuclear enzymes that modify the topological state of DNA through the introduction of transient breaks in the phosphodiester backbone of DNA [40,41]. They can relax torsional stress in supercoiled DNAs and resolve topologically complex DNA molecules via unknotting and decatenation. The topoisomerases have essential roles in the key cellular processes of replication and transcription [42, 43]. This is the basic reaction catalyzed by the topoisomerases. Initially, topoisomerases were simply classified as either a type I and type II enzyme depending on whether they catalyzed their reactions by making transient single strand DNA breaks (the type I enzyme) or transient double strand DNA breaks (the type II enzyme) as shown in (Fig. **4**). This mechanistic description is still correct but has become slightly more complicated with the recent discovery of new members of the topoisomerase family [44]. The type I enzymes have been further divided in two distinct subgroups with no sequence similarity, Type I A (including prokaryotic type I topoisomerases) and type I B (including eukaryotic type I topoisomerases) (Fig. **4**). Type I A enzymes require magnesium as a cofactor and attack a single-stranded stretch of DNA to produce a transient covalent complex where the

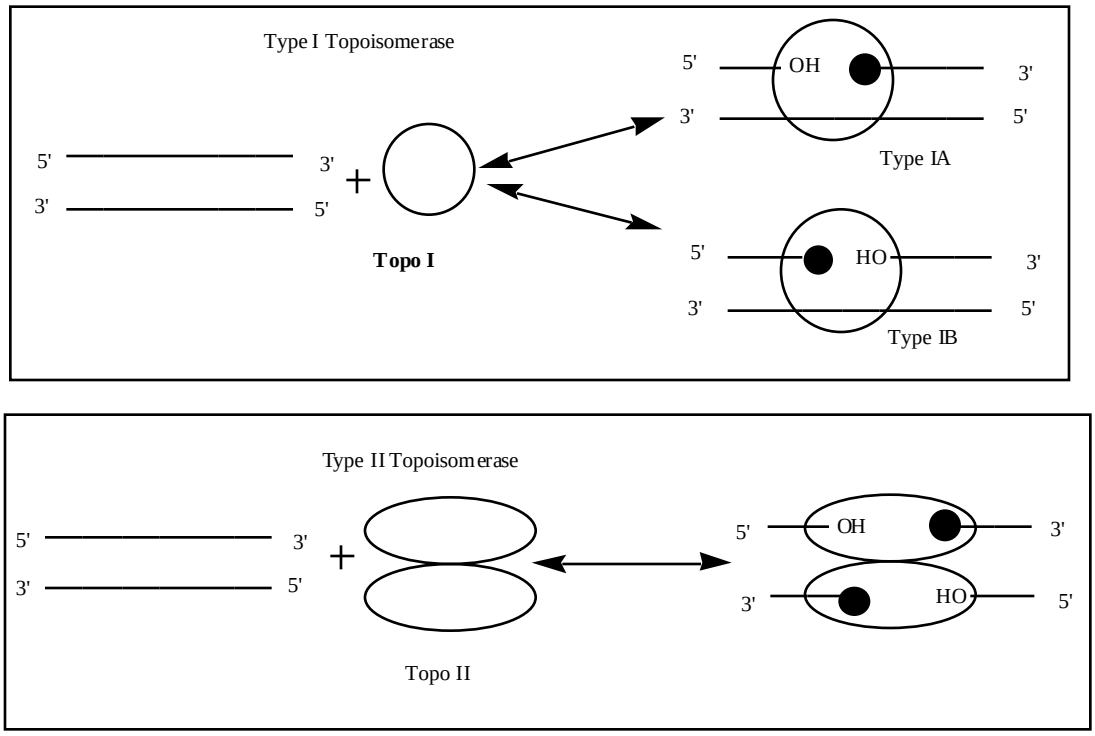


Fig. (4). Type I topoisomerases make single strand DNA cuts and type II topoisomerases make double strand DNA cuts. The topoisomerization reactions carried out by either the type I or typ II enzyme involve aY transient DNA break. During this reaction, the enzyme becomes covalently linked to the break site via a phosphotyrosine bond (from Ref. [44]).

enzyme is attached to the 5'-end of the nicked single strand and relax only negatively. On the contrary, topoisomerase I B requires no metal ions or single-stranded region for function to form a transient DNA-(3'-phospho-tyrosyl)-enzyme intermediate and is able to relax both positive and negative supercoils [45]. Additionally, all type II topoisomerases require ATP. Type I topoisomerases do not.

Because of the size of the eukaryotic chromosome, removal of these supercoils can only be accomplished locally by introducing breaks into the DNA helix. Topo I mediates DNA relaxation by creating a transient single-strand break in the DNA duplex (Fig. **5**). This transient nick allows the broken strand to rotate around its intact complement, effectively removing local supercoils. Strand nicking results from the transesterification of an active-site tyrosine (Tyr-723) at a DNA phosphodiester bond, forming a 3'-phosphotyrosine covalent enzyme–DNA complex. After DNA relaxation, the covalent intermediate is reversed when the released 5'-OH of the broken strand reattacks the phosphotyrosine intermediate in a second transesterification reaction. The rate of religation is normally much faster than the rate of cleavage, and this ensures that the steady-state concentration of the covalent 3'-phosphotyrosyl topo I–DNA complex remains low [46]. However, the CPTs have been shown to stabilize the covalent 3'-phosphotyrozyl intermediate. CPTs bind the covalent binary complex and specifically block DNA religation (Fig. **5**), thus converting topo I into a DNA-damaging agent [47]. All of these data support the interpretation that topo I is the sole intramolecular target of CPT, and that sensitivity of a cell to these drugs is dependent on elevated levels of topo I.

A number of CPT resistant cell lines have been studied to support topo I–DNA interaction as the locus of action of CPT, which are characterized by specific mutations within topo I. The enzyme mutations studies have become one of the most important measures to investigate the CPT-induced complexes. Others include study of structure-activity relationships, DNA base sequence analyses, and detailed examination of the drug's effects on different steps in the catalytic cycle [48].

The early studies reported that topo I cleaves DNA at multiple sites. The highest efficient cleavage sites exhibit significant sequence homology. Approximately 90% of topo I site have a tyrosine residue at position –1. However, sites of cleavage stabilized by CPT exhibit a strong preference for guanine at the +1 position, while T remains the preferred nucleobase at the –1 position [48, 49].

The exact mechanism by which CPT stabilizes the topo I–DNA covalent binary complex is not fully understood because the drug acts as an uncompetitive inhibitor and binds only the transient covalent binary complex [50, 51]. CPT has been shown to bond weakly to normal B-DNA under physiological conditions, and it does not bind to topo I alone [48]. Although it has been reported more recently that high concentrations of CPT and topotecan have been shown to bind to DNA and inhibit DNA relaxation at physiological pH by somehow hindering DNA rotation [52]. Despite the apparent lack of affinity of CPT for DNA or topo I alone, the binding of CPT to the covalent binary complex is suggested to be responsible for the observed stabilization. While the formation of a transient covalent bond between the open E-ring of CPT and the topo I–DNA covalent binary complex has been suggested by Pommier and co-workers, there is no direct evidence for the formation of a covalent bond. Thus, CPT and its derivatives bind to topo I–DNA covalent binary complex through uncovalent process at or near the site of DNA cleavage, which has been identified.

Some indirect evidence indicated that CPT probably forms a ternary complex with the enzyme and DNA. Additionally, camptothecin derivatives are currently being synthesized and evaluated. Some of these are able to stabilize the transient Topo I catalyzed DNA break to a greater brilliancy than either topotecan or irinotecan. Particularly exciting are camptothecin related compounds, which contain substituents at the 10, 11 and 7 positions

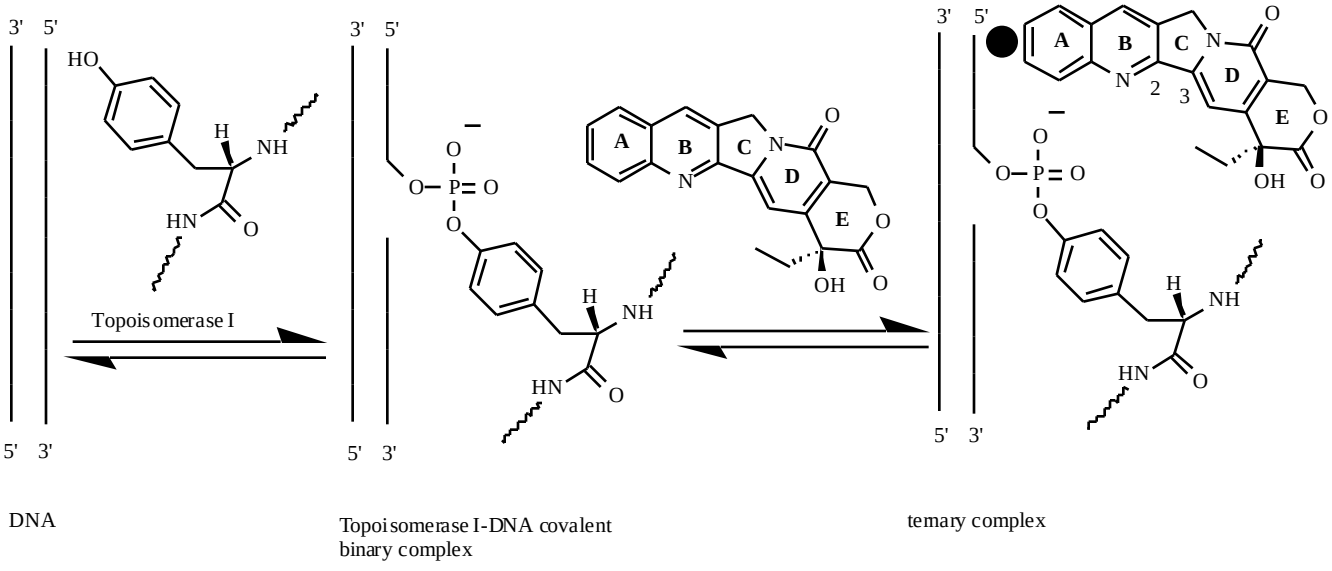


Fig. (5). Equlibria between DNA and the DNA-Topoisomerase I Covalent Binary Complex, and between the Enzyme-DNA Binary Complex and a Temary Complex Formed with Camptothecin (from Ref. [50]).

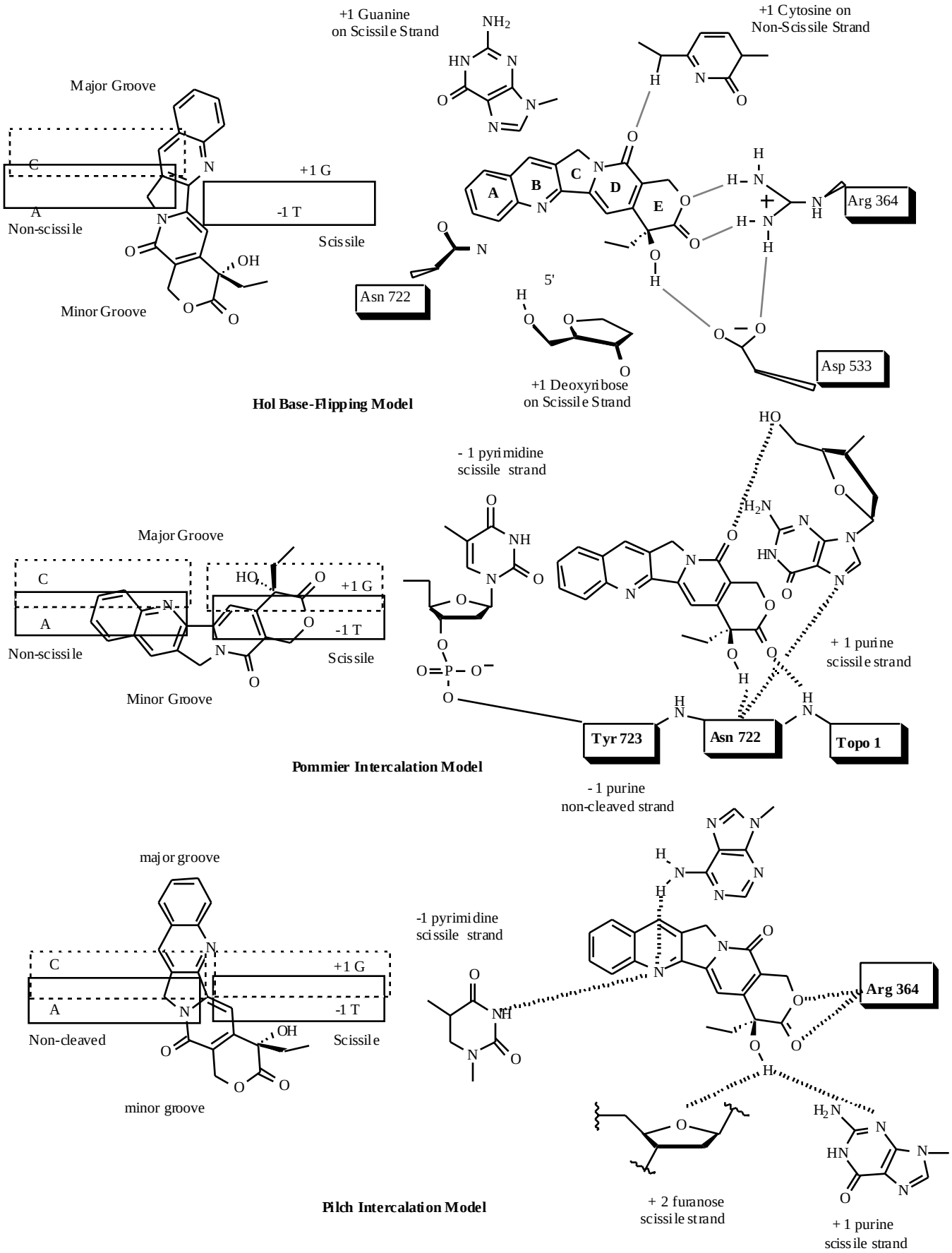


Fig. (6). Two-dimensional representation of selected computational docking models of CPT within the covalent binary complex.

such as 10,11-methylenedioxy-20(S)-CPT and 10-bromoacet-amido-20(S)-CPT. These compounds are more potent in producing Topo I induced strand breakage than camptothecin and this effect may be related to their ability to prolong the half life of Topo I mediated DNA break [53]. Treatment with these compounds could lead more tolerable side effects and a persistence of the DNA break in a tumor cell, which might increase the chance of tumor cell killing. Clinical trials with some of these newer agents will be necessary to determine how effective they are [44].

Several structural models have been suggested for the interaction of CPT with the covalent binary complex. The X-ray crystal structure of the human topoisomerase I-DNA cleavable complex was used to identify the general models for the ternary drug-DNA-Topo I cleavable complex formed with camptothecin and its analogs [51]. Although not intended as an exhaustive review, here recent representative models are described compendiously below.

The Hol group firstly reported a crystal structure of the binary human topo I-DNA cleavable complex [54]. The crystal structure affords the convenience of model studies of the ternary drug-DNA-Topo I via computer techniques. The Hol group proposed a model for the ternary cleavable complex in which the +1 guanine flips out of the DNA helix and stacks with the CPT molecule (Fig. **6**). In Pommier's proposed drug-stacking model, he argued that CPT was pseudointercalated in the topo I-DNA cleavable site and interacted with the protein near its catalytic tyrosine *via* stacking and hydrogen bonding to specific amino acid and DNA residues (Fig. **6**) [55]. Pommier and co-workers have also reported a computational model for the DNA interaction of 7-ethyl-10-hydroxy-CPT (5), a potent topo I inhibitor hydrolyzed from its prodrug irinotecan (Fig. **7**) [56]. The docking arrangement requires the rotation of the +1 guanine base out of the DNA helix, which can be propitious to docked by CPT. The 10-substituent of this CPT analogue, which was either an H-bond acceptor or a donor on the A-ring, does in fact exhibit more potent inhibitor activity than CPT via H-bond to Asn352 (Fig. **7**). Kerrigan and Pilch have suggested an intercalative model for the drug, placing E-ring into the minor groove where it is potentially capable of additional hydrogen bonding interactions with the covalent binary complex (Fig. **6**) [57].

In 2002, Staker and co-workers reported the X-ray crystal structure of the ternary complex containing human topo I covalently joined to a DNA duplex and bound to the clinical approved anticancer agent topotecan [52]. The X-ray structure reveals that topotecan mimics a DNA base pair in the DNA duplex and occupies the same space as the +1 base pair in structure without drug bound (Fig. **8**). The intercalation binding site is created *via* hydrogen bond including only one direct forming between the enzyme and Topotecan and conformational changes of the phosphodiester bond between the +1 and –1 base pairs of the uncleaved strand (Fig. **8**). It is generally believed that only the lactone form is active. However, there is an interesting observation that the open carboxylate form can bind within the same intercalation pocket. Pommier also speculated that when the CPT lactone enters the topo I–DNA active site, E-ring opening is activated [56]. Recently, Staker continued his study about the crystal structure of the ternary complex containing CPT displacing TPT [59]. This crystal structure reveals that CPT intercalates at the site of DNA cleavage and its binding mode is very similar to the binding mode of TPT, in which CPT mimics a DNA base pair and thereby forms stacking interactions with upstream and downstream base pairs. Compared to TPT ternary complex, CPT binds into the topo I–DNA covalent complex *via* two direct hydrogen bonds formed between the N-1 of CPT and Arg364 except for between the lactone ring of CPT and Asp533. Also, there is a light twist in the orientation of CPT relative to TPT along the vertical axis of the duplex DNA so that CPT can fit into the intercalation binding pocket (Fig. **9**). All these observations may result from steric interaction of the 7-C substituent of TPT with intact strand [59]. The position of C-7,9 and 10 in a manner that faces into the major groove is agreement with many SAR studies of CPT analogs within substituents at these sites do highly improve their activity.

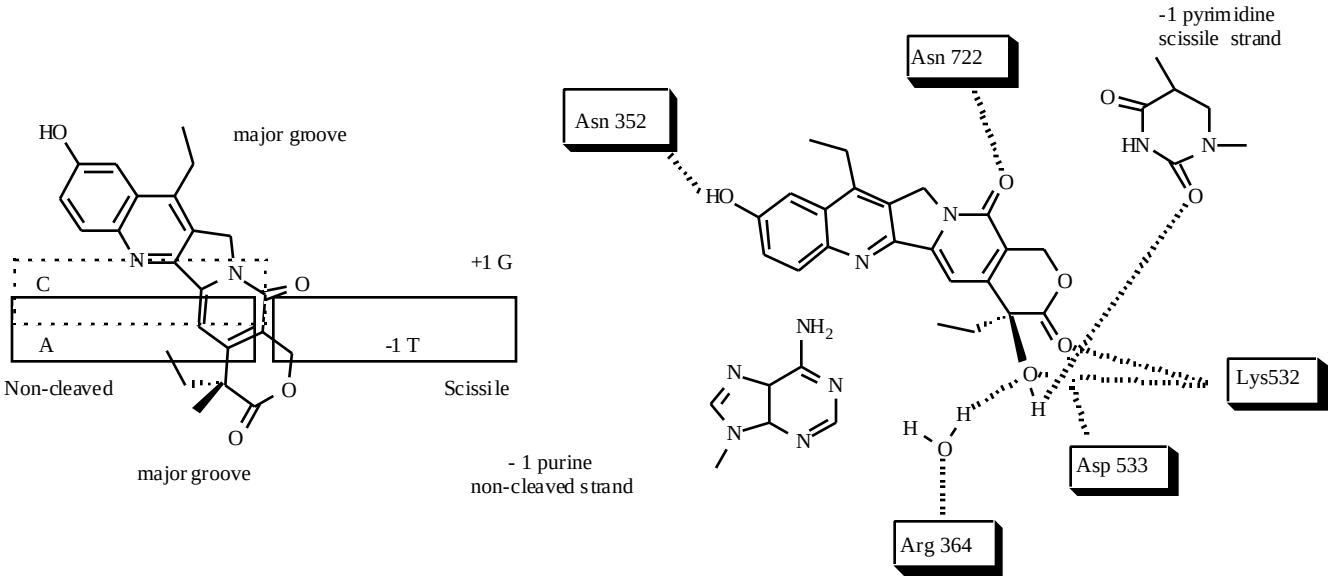


Fig. (7). Two-dimensional representation of selected computational docking models of SN-38 within the covalent binary complex.

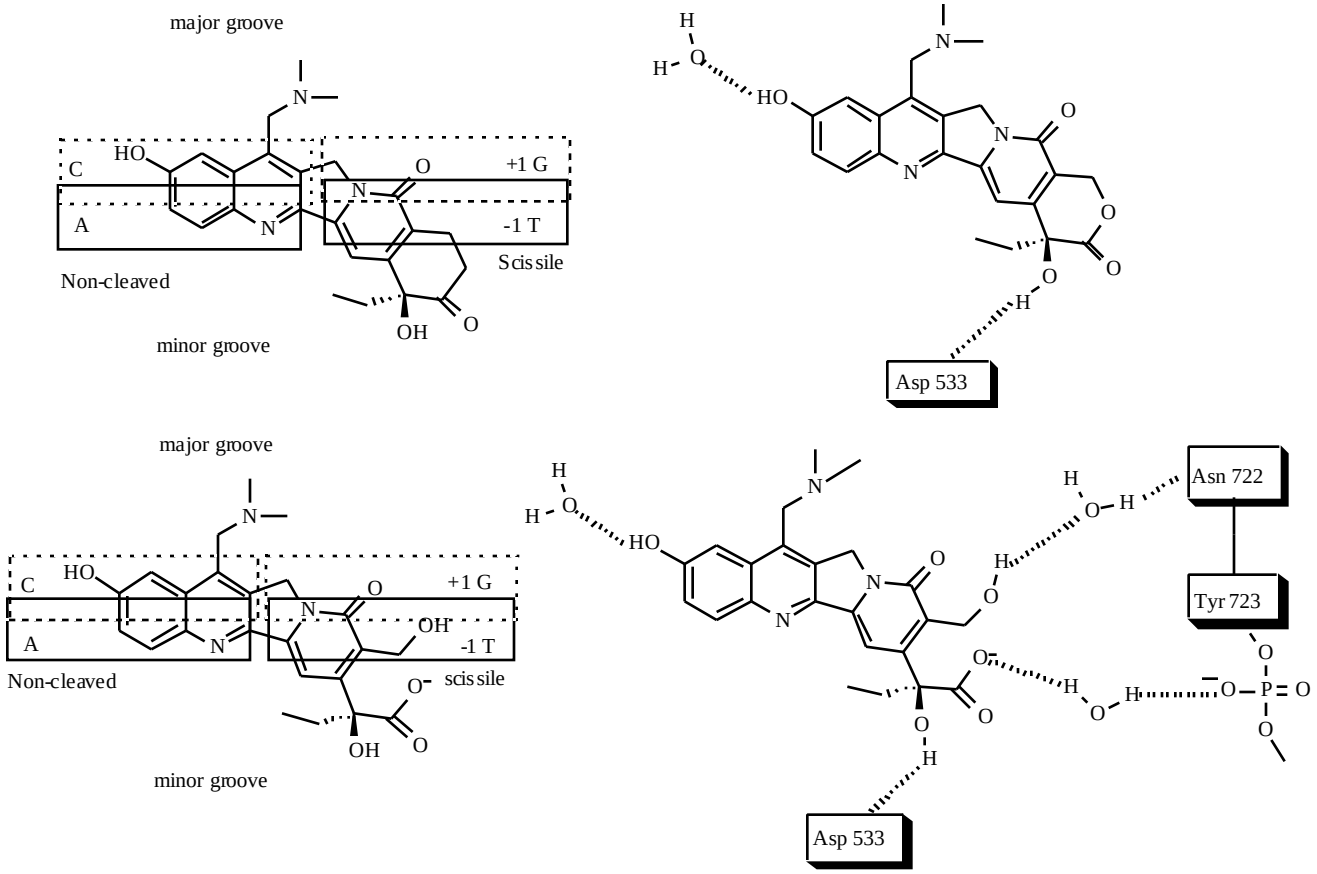


Fig. (8). Two-dimensional representation of X-ray crystal structure of topotecan within the covalent binary complex.

Each of these reports further emphasized the hydrogen bond, which played a key role during forming the drug-DNA-enzyme ternary complex. (Figs **6**, **7**, **8** and **9**). It is important to notice that many hydrogen bonds were formed via water as mediate. Especially, some amino acid residues Asp533, Arg364, Asn722, and Lys532 are important to support CPT binding through hydrogen bond with CPT. The existing CPT docking models always attempt to produce an energetically minimized interface with the covalent binary complex, while incorporating the maximum degree of appropriate interactions with important amino acid residues, aimed at stabilizing the cleavable ternary complex. Additionally, several CPT resistance (CPT^r) point mutations in human topo I are identified, including Asp533, Arg364, Asn722, Phe361, Gly363, Ala653, Glu418, Gly503 and Thr729. Especially, mutational analysis of residues Arg364, Asn722, Asp533 has shown that each of these amino acid residues also plays a key role in supporting CPT binding in each model. It is necessary to notice that differences between the structures determined by X-ray crystallography and those suggested in the preceding models could reflect differences in the docking arrangement of CPT and its analogs with DNA, such as unique binding site. In conclusion, these studies provide insight into a possible mechanism of topo I inhibition by CPT and its derivatives and suggest rational approaches for the design of new CPT drugs.

4. STRUCTURE-ACTIVITY RELATIONSHIPS

The development of synthetic and semi-synthetic strategies and the studies of CPT action mechanisms have

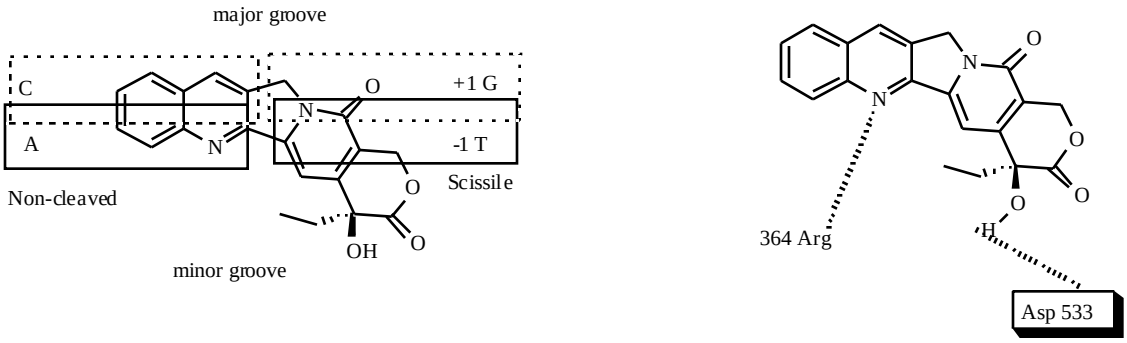
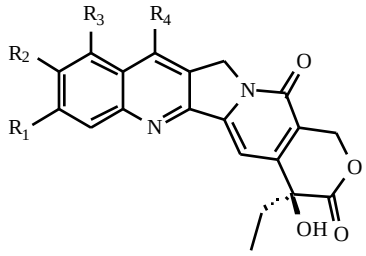
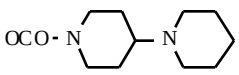
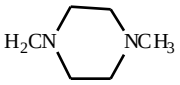
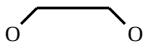
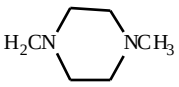
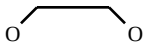
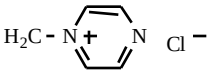
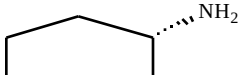
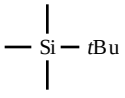


Fig. (9). Two-dimensional representation of X-ray crystal structure of topotecan within the covalent binary complex.

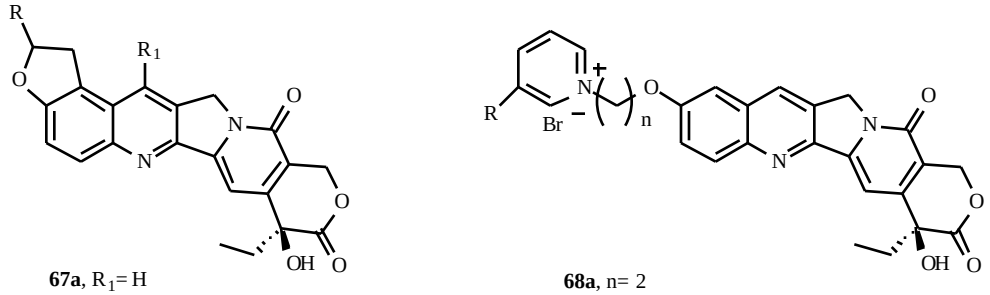
facilitated the identification of analogs with improved properties, including lactone stabilization, solubility and drug transport mechanisms, tumor cell recognition and enhancement of DNA sequence specificity. These above structural models provide insight regarding the mechanism of action of CPT, and understanding of how systematic modifications within the CPT structure may enhance or suppress the effect of the drug in a biological context. Numerous studies of the structure-activity relationships (SAR) of CPT prompted the synthesis of many derivatives and analogs, including prodrugs (conjugates and polymer-bound camptothecins), new formulations (liposomes or microparticulate carriers), and lipophilic and water-soluble camptothecins [60]. The present discussion of CPT derivatives focuses individually upon substitutions, additions and deletions of the quinoline ring (A/B-ring), the C and D rings, and the E ring of the pentacyclic structures [58].

Table 1. Selected A and B Ring Modified Camptothecin Derivatives



Analogue	R_1	R_2	R_3	R_4	Topo I inhibition[a]
1(CPT)	H	H	H	H	+
3(topotecan)	H	OH	$CH_2N(CH_4)_2$	H	++
4(irinotecan)	H		H	Ethyl	+++
5(SN—38)	H	OH	H	Ethyl	++
59	H	H	NH_2	H	++
60(rubitecan)	H	H	NO_2	H	NA
61	H	OH		H	+
62(lurtotecan)			H		+
63			H		—
64(exatecan)	F	Me			NA
65(DB—67)	H	OH	H		NA
66	H	H	H	$CH{=}NOC(CH_3)_3$	NA

[a]Concentration that produced 50% DNA cleavage in the presence of toposiomerase I.



67a, R_1= H
67b, R= Et

68a, n= 2
68b, n=3

Fig. (10). CPT analogues modified in quinoline ring.

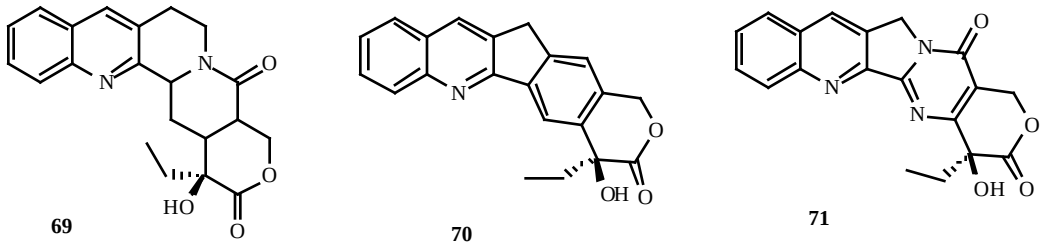
At present, the design of novel CPT derivatives rests on the following assumptions, including the conjugation and planarity of A, B, C and D rings, the E-ring lactone, and the S configuration at 20-C [61]. Correspondingly, the designed camptothecin analogs suitable for targeted enzymatic activation at tumor cells would possess four criteria: (1) improved water solubility, (2) stability in blood, (3) decreased cytotoxicity, and (4) susceptibility to defined enzymatic cleavage [62]. However, as described below, reporting recent results necessitate a reevaluation of the E-ring lactone function [52, 59].

4.1 Quinoline (A/B) Ring

Numerous reports indicated that CPT derivatives with substitutions at the quinoline ring are regarded to be of great interest. Most structural model studies referred to the modification at position C-7, C-9, C-10, which can enhance potency. However, CPT generally does not tolerate modification at position C-12. Modifications may involve substituents to the quinoline ring or the replacement of the quinoline ring with an alternative ring system. The fact was that replacing the quinoline ring system does not prompt CPT potency of action. Consequently, the most interest studies have focused upon additions or substituents to the quinoline ring, which results in two CPT analogs Topotecan (3) and irinotecan (4) (Fig. **2**) approved for clinical use. (Table **1**) shows some of representative derivatives substituted at the quinoline ring.

As shown in (Table **1**), these CPT analogs were in clinic use or in various stages of clinical trials. It's demonstrated that positions C-7, C-9, C-10 and sometimes C-11 of the quinoline ring system could be without diminishing topo I inhibitory activity and antineoplatic activity, which is consistent with SAR studies. In reflection of the clinical success of water soluble CPT derivatives Topotecan and irinotecan, efforts to increase the water solubility of CPT have comprised a major research focus. SN-38 (5), 9-aminoCPT (59), 9-nitroCPT (rubitecan) (60), 10-hydroxy-9-((N-methylpiperazinyl)methyl)CPT (61), and other derivatives (62, 63, 64) all displayed enhanced water solubility [63,64]. Additionally, attention has been paid to increase the lipophilicity of CTP (derivative 65 and 66) for keeping lactone stability [65]. These analogs illustrated successful strategies for substitution of the quinoline ring system and have been discussed in excellent reviews [58].

Some of the derivatives with an additional ring combined with the position C-10 and C-11, position C-9 and C-10, or position C-7 and C-9 were predicted to be superior antitumor activities to those original pentacyclic camptothecins, such as Lurtotecan (62) and exatecan (64), which are under studies in the clinical phase II and phase III stages [66], respectively. Based on this strategy, Gao and his co-workers [67] recently reported some new hexacyclic structural CPT analogs highlighted the merit of this strategy (Fig. **10**). Compound 67a and 67b, and their respectively analogs which R was substituted by hydroxy, methyl, hydroxymethyl, all possess both superior topo I inhibitory and antitumor activity *in vitro*. Antitumor activity evaluation of these CPT analogs *in vivo* showed similar or higher potency than irinotecan. However, their assay of the lactone stability in human plasma demonstrated that additional furan or dihydrofuran rings did not result in an increasing stability. The conclusion may be drawn from the new research that the open carboxylate can also stabilize the topo I–DNA covalent complex [52, 59]. Further development led to the use of aromatic quarternary ammonium salts as water solubilizing functional groups. Derivatives such as 10,11-(ethylenedioxy)-7-pyraziniumylmethyl CPT chloride (63) have been reported to possess both good water solubility and enhanced tumor growth suppression [64(d)]. Zu *et al*. have exploited a series of compounds which placed the several water- solubilizing groups in the position C-10 of CPT as aromatic quarternary



69 **70** **71**

Fig. (11). CPT analogues modified in C/D-ring.

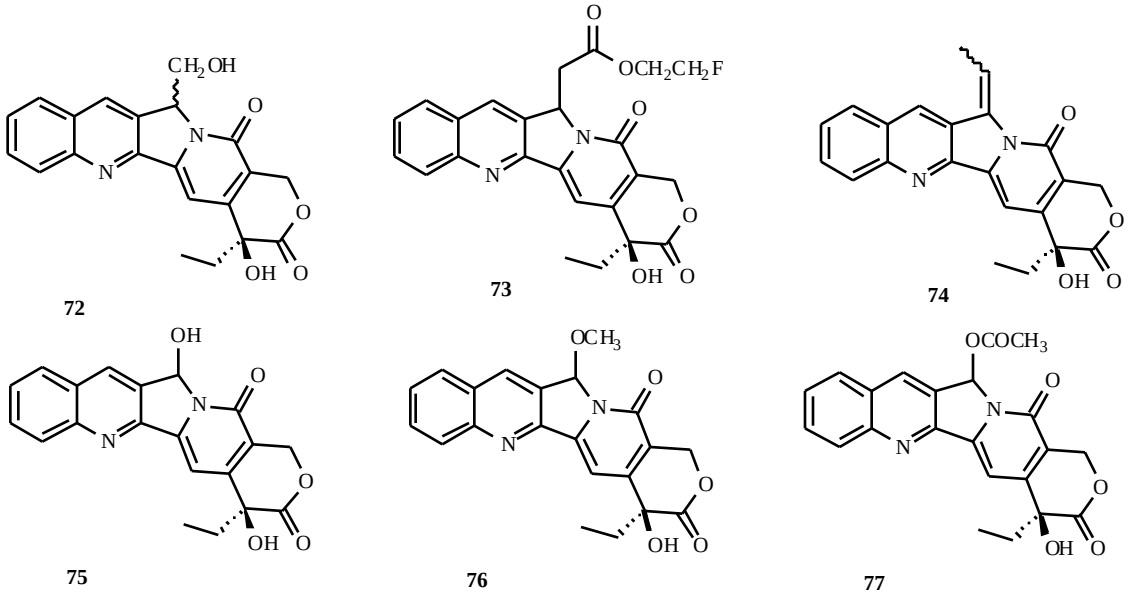


Fig. (12). CPT analogues substituted in C/D-ring.

ammonium salts (68) (Fig. **10**) [68]. These salts especially when R was substituted by hydroxy, methyl, hydroxymethyl, fluoro, carbaldehyde and so on, these salt showed specially lower cytotoxicity *in vitro* than CPT and impressive tumor inhibiting activities.

4.2 C/D Rings

There are few reports about modification of the C and D rings of camptothecin. It is generally suggested that either replacement or substitution at C/D-ring would loss the activity. Some researchers have exploited a few CPT analogs modified at C/D-ring (Fig. **11**). Unfortunately, none of them was approved for clinical trials for their poor topo I inhibility and antitumor activity [69,70]. These observations were reflected by studies of CPT action mechanism which show that it is the pyridone and carbonyl of CPT that stabilizes the DNA- topo I-drug ternary complex (see part 3). However, it was surprising to find that the 14-azacamptothecin (71) exhibited reasonable potency as a topoisomerase I poison and topoisomerase I dependent cytotoxic agent, and stabilized enzyme-linked DNA breaks with the same sequence selectivity as CPT itself [71, 72].

Reports of C/D-ring substituents are also limited, presumably due to the paucity of accessible carbons for substitution and more arduous synthetic routes leading to potential analogs [49]. There are only two available sites for substituents,C-5 and C-14 in the C/D-ring of CPT. Early, it was reported that substitutions at C-5 of the C-ring had been carried out (Fig. **12**) [73, 74]. Description of their activity did not display advantage in clinic experiment, which resulted in few interest in modifying C/D-rings of CPT from then on.

4.3. E-ring

E-ring plays a key role in supporting both efficient topo I inhibition and *in vivo* potency. Early studies regarded that, under physiological conditions, the presence of the α-OH

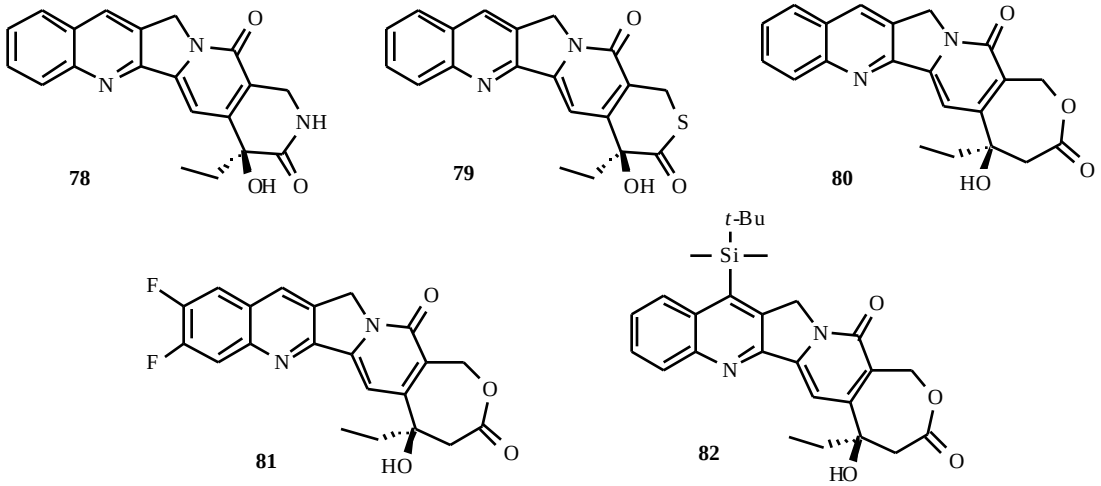
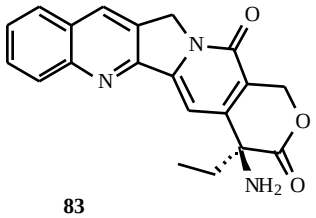


Fig. (13). CPT analogues modified in E-ring.



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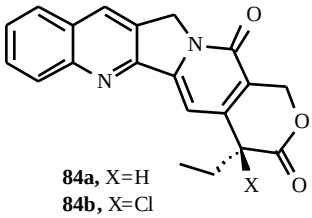


84a, X=H
84b, X=Cl
84c, X=Br

Fig. (14). CPT analogues modified in E-ring.

group results in an equilibrium, which favors the (inactive) open carboxylate over the (active) ring-closed lactone form [75]. On the basis of the experimental evidence, two possible reasons can be proposed for the importance of CPT 20-hydroxyl group in the S configuration for topo I inhibition: (1) the formation of a hydrogen bond between the hydroxyl group and the enzyme-DNA complex, (2) the presence of an intramolecular hydrogen bond with the lactone carbonyl of CPT (21 position). Both interactions may facilitate the possible E-ring opening reaction [76].

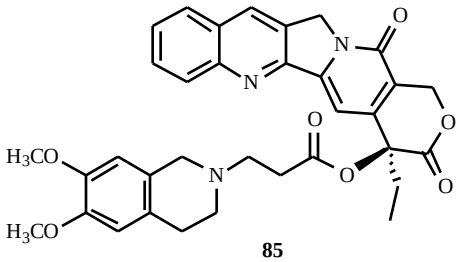
Based on this strategy, E-ring modifications have underscored the stability of lactone. Hertzberg *et al*. replaced the 21-C with other atom such as N and S to lactam (78) and thiolactone (79) (Fig. **13**), thereby reducing the tendency of the E-ring to open [77]. However, the resulting CPT lactam (78) and thiolactone (79) were essentially inactive. It was interesting to report that HomoCPT (80) by replacement of the α-hydroxylatone moiety of CPT with β hydroxylactone exerts impressed potency and stability of lactone [78, 79], although it is incapable of intramolecular hydrogen bond. HomoCPT has become a new formulation as topoisomerase I poison. Many analogs of HomoCPT have been exploited, such as 10, 11-difluorohomoCPT (BN-809) (81) which exhibited strong antiproliferative activity against numerous cell lines and is currently in phase I clinical trials [80]. The analogue (82), which was added *tert*-butyldimethylsilyl to B-ring of HomoCPT just like DB-67 (65), is reported to display the highest level of lactone stability in both buffer systems and plasma [81]. The replacement of 20-OH group has been exploited to afford 20-aminoCPT (83), 20-deoxyCPT (84a), and halogenated CPTs (84b, 84c) (Fig. **14**), which were all shown to have significantly diminished activity [82]. In HomoCPT (80) and halogenated CPTs (84b, 84c), the presumed hydrogen bond interaction between the C_{20}-OH and carbonyl groups in the E-ring of CPT can no longer be significant, which reduces the hydrolysis rate of the lactone.

Additional attention has been paid to esterification of 20-hydroxyl group, which can either eliminate the intramolecular hydrogen bonding or increase the steric hindrance of carbonyl group of E-ring, result in the stability of the lactone ring *in vivo* [83, 84]. Pan *et al*. [85] reported some 20-O-linked nitrogen-based camptothecin ester derivatives including ester (85) (Fig. **15**), which possesses both lower cytotoxic *in vitro* and better antitumor activity *in vitro* than topotecan. Rahier prepared four 20-O-phosphate and phosphonate analogs of CPT (86a, 86b, 86c, 86d) [86]. While these derivatives were less potent than CPT, stabilization was improved significantly. The experimental evidence revealed that esterification of 20-OH markedly reduced the toxicity of CPT analogs.

4.4. Conjugated Analogs

Besides structural modification, conjugation is another important strategy in efforts to optimize therapeutically beneficial properties of CPT, including lactone stability, solubility/lipophilic, tumor cell recognition and sequence specificity of DNA damage. The 20-OH group of CPT generates an intramolecular hydrogen bond with the carbonyl moiety of the lactone, which accelerates the hydrolysis of the otherwise stable lactone. Therefore, 20-OH group becomes the first site for conjugation.

The predominant methodology is esterification of the 20-OH group. It is necessary to note that differences between the conjugated esterification and the modified esterification depend on whether it is the prodrug or not. The ideal prodrug should be stable *in vivo*, far less toxic than its parent form, and activated specifically in or within the microenvironment of the tumor cells. Giovanella *et al*. reported the synthesis and biological evaluation of a series of alkyl esters of varying size [87]. The 20(S)-O-esters, 20(S)-O- amides, 20(S)-O-carbonates, and carbamates with a variety of linkers have been employed in recent studies for the preparation of novel CPT analogs (see review [58]). Furthermore, Such an ester prodrug can also be hydrolyzed by endogenous enzymes with esterase activity.



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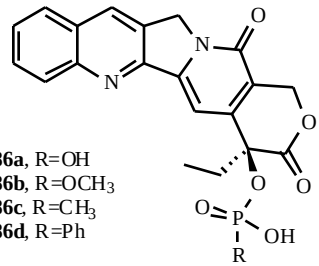


86a, R=OH
86b, R=OCH_3
86c, R=CH_3
86d, R=Ph

Fig. (15). CPT analogues modified in E-ring.

Shabat *et al*. reported two new prodrugs (Fig. **16**) with relative stability at physiological pH, lower toxicity and considerable solubility. These CPT prodrugs (87, 88) can be activated and hydrolysized by *Escherichia coli* penicillin-G amidase (PGA) or by catalytic antibody 38C2 [88].

A number of delivery systems are under development for targeted and controlled delivery of drugs. Early used polyethylene glycol (PEG) to prepare 20-ester prodrugs has led to the development of Prothecan (89) (Fig. **16**), which is currently in Phase II clinical trials [89]. Recently, Yokoyama *et al*. reported novel CPT-loaded polymeric micelles based on PEG named Poly(ethylene glycol)-poly(benzyl L-aspartate-69) block copolymer (PEG-P(Asp(Bz-69)) [90]. Interestingly, Greenwald *et al*. reported two PEG prodrugs

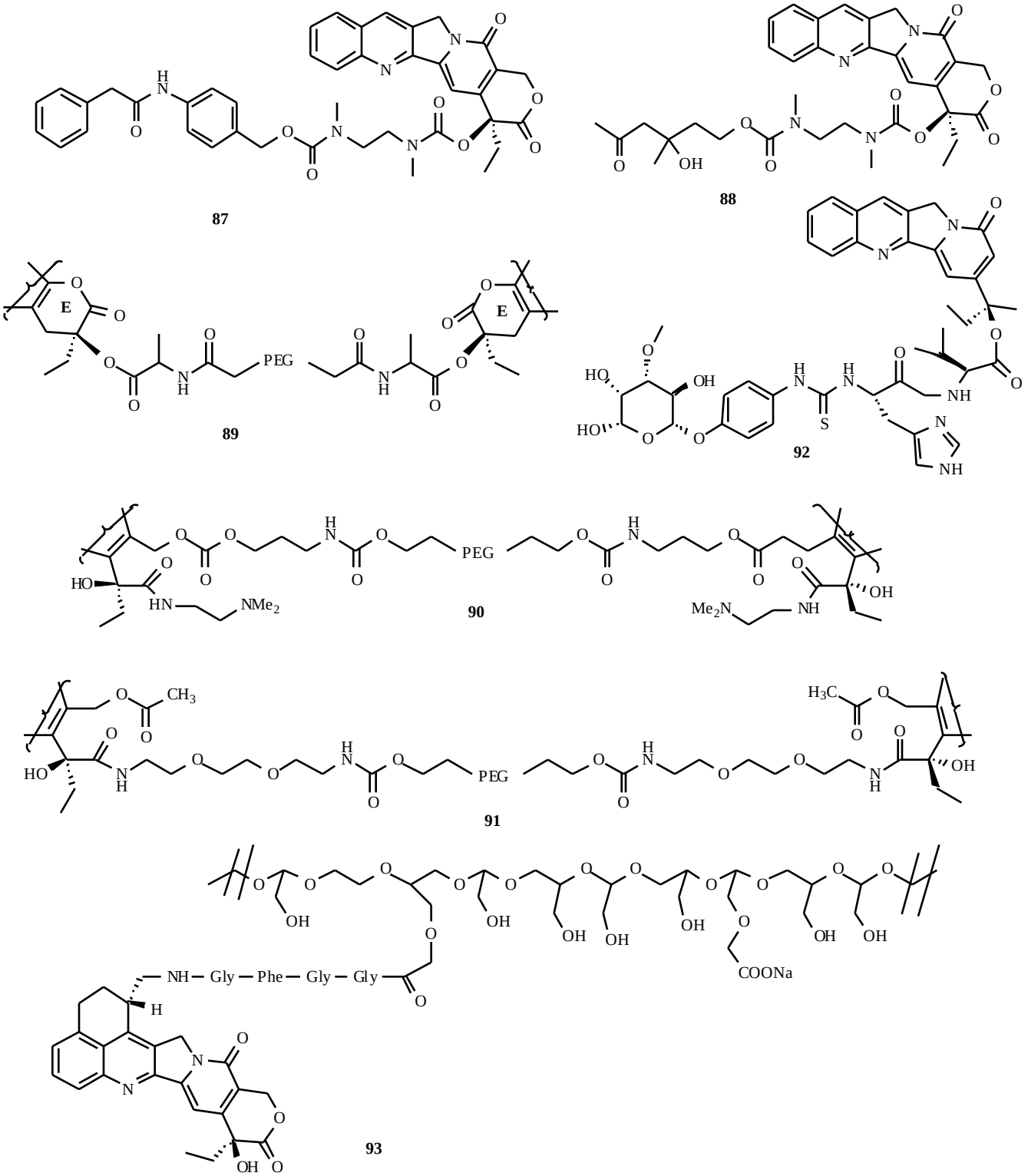
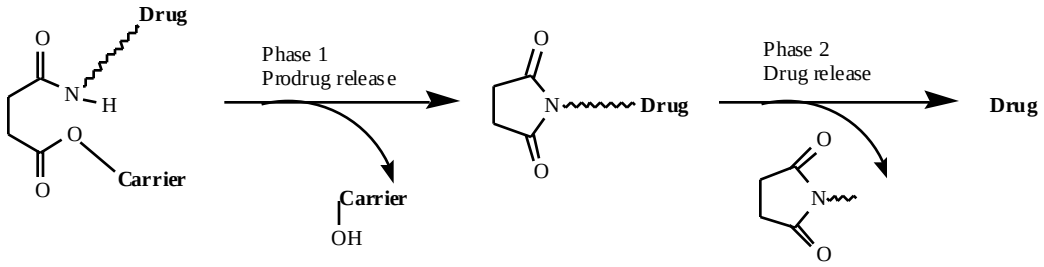


Fig. (16). CPT conjugates.



Scheme 9.

utilizing conjugation of PEG through the C-21 functionality as well as the C-17 OH group of CPT hydroxy-amide open forms (90, 91) [91]. Both of these open lactone tripartate prodrugs were shown to be water soluble and highly effective in MX-1 mouse xenograph studies. The specific peptide has been reported to link drug-glycoconjugate resulting in derivatives with excellent *in vitro* activity [92]. Especially, the fucoside glycoconjugate analogue containing a valine/histidine linker (92) was reported to inhibit tumor growth >96% in breast cancer xenograft and has consequently been selected for clinical trials.

Besides the 20-OH group, there is another exploitation of reactive functional groups including amino, hydroxyl and carboxylic acid groups on modified CPT analogs. Most of these groups have been part of the quinoline (A/B) ring system. DE-310 (93) is a novel macromolecular conjugate composed of DX-8951 (Fig. **16**), a camptothecin analogue, and a carboxymethyldex- tran polyalcohol carrier, which are covalently linked via a peptidyl spacer. The active moiety DX-8951 is released slowly from DE-310 and over an extended period, achieving the desired prolonged exposure to this topoisomerase I inhibitor. So far, DE-310 is under phase II clinical trials [93].

Recently a new approach for selective chemotherapy has been developed (Polymer Directed Enzyme Prodrug Therapy (PDEPT)) [94]. This method is a two-step antitumor approach in which both the prodrug and the enzyme are targeted to the tumor site with a polymer molecule. At the first step, a polymer-prodrug conjugate is administered and trapped in tumor tissues through the EPR (enhanced permeability and retention) effect. A conjugate of an HPMA-copolymer and catalytic antibody 38C2 has already been reported [95]. A water soluble macromolecular conjugate of camptothecin was successfully exploited by Papisov [96]. This new reduced toxicity conjugate involves a dual phase two-step drug release system as described as follows. The first stage is hydrolysis of the succinamido ester tether, which leads to drug cleavage from the polymer in the form of a cyclic succinimidoglycyl-CPT (Scheme **9**). The second stage is glycyl ester bond hydrolysis, which results in active drug release *in vivo*. The conjugate was assembled using poly(1- hydroxymethylethylene hydroxy-methyl formal) (PHF) as a backbone, which is a highly hydrophilic biodegardable polymer [97,98].

5. CONCLUSIONS

Camptothecin and its derivatives possess an excellent antitumor activity and are a very promising class of agents. Enormous progress promoted the straightforward synthetic strategies of CPT and the synthetic and semi-synthetic construction of novel CPT derivatives. Further studies about the mechanisms of CPT action identified the Topoisomerase I enzyme as the cellular target of camptothecin and its analogs. The structural models reveal that CPT noncovalently bond to Topo I–DNA binary complex. The structure-activity relationships provide insight into a possible mechanism of Topo I inhibition by CPT and its derivatives. These progress have opened a new area for anticancer drug development, including approval for clinical use of topotecan (Hycamtin) (3) and irinotecan (Camptosar) (4) and several analogs that are currently in various stages of clinical trials. Currently, high expectations still surround the next generation and further development of new and better antitumor CPTs.

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