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Review

Camptothecin: current perspectives

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Abstract—This review provides a detailed discussion of recent advances in the medicinal chemistry of camptothecin, a potent antitumor antibiotic. Two camptothecin analogues are presently approved for use in the clinic as antitumor agents and several others are in clinical trials. Camptothecin possesses a novel mechanism of action involving the inhibition of DNA relaxation by DNA topoisomerase I, and more specifically the stabilization of a covalent binary complex formed between topoisomerase I and DNA. This review summarizes the current status of studies of the mechanism of action of camptothecin, including topoisomerase I inhibition and additional cellular responses. Modern synthetic approaches to camptothecin and several of the semi-synthetic methods are also discussed. Finally, a systematic evaluation of novel and important analogues of camptothecin and their contribution to the current structure–activity profile are considered.
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1. Introduction

Camptothecin (CPT) (**1**) (Fig. 1) is a potent antitumor antibiotic isolated by Monroe E. Wall and Mansukh C. Wani in 1958 from extracts of *Camptotheca acuminata*, a tree native to China and Tibet which has been extensively used in traditional Chinese medicine.[1] The structure was determined to be that of a pentacyclic alkaloid and was first reported in 1966.[2] The success of CPT in preclinical studies led to clinical investigations. Due to the negligible water solubility of CPT, these trials were initiated using the water-soluble sodium salt (**2**). The lesser efficacy of **2**, accompanied by unpredictable and severe levels of toxicity associated with treatment, including hemorrhagic cystitis and myelotoxicity, resulted in suspension of the trials.[3]

The discovery that the primary cellular target of CPT is type I DNA topoisomerase (topo) created renewed interest in the drug.[4] Advances in the medicinal chemistry of CPT resulted in the semi-synthetic, more water-soluble analogues topotecan (Hycamtin, **3**) and irinotecan (Camptosar, **4**), which are used clinically (Fig. 2) for the treatment of colon and ovarian cancers, respectively.[5] Additional CPT analogues are under investigation, and are also of interest in combination regimens as radiation sensitizers.[6] The clinical application of the CPTs has been discussed in several excellent reviews.[7]

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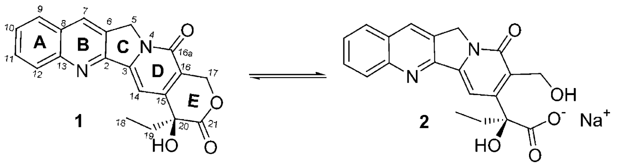


Figure 1. Structures of camptothecin (**1**) and water soluble sodium salt (**2**).

Here, we describe the status of the present understanding of the mechanism of action of CPT, several of the synthetic efforts focused on the elaboration of CPT (analogues), as well as a number of novel CPT derivatives and their influence on the study and development of this important medicinal agent.

2. Topoisomerase I–DNA interaction

Early reports detailing the life prolongation and inhibition of solid tumor growth in animal tumor models by CPT incited a flurry of research designed to ascertain the mechanism of action. It was shown that CPT is capable of inhibiting DNA synthesis via strand scission, thus causing cell death during the S-phase of the cell cycle.[8] The finding that treatment of cultured cells with CPT led to protein-associated DNA strand breaks provided the key clue that led to the identification of a DNA–protein complex as the target for CPT.[4] The S-phase cytotoxicity is attributed to cessation of DNA synthesis and double-strand breakage when the replication fork encounters the covalently bound DNA–topo I site.[9]

Topo I and topo II catalyze the relaxation of supercoiled chromosomal DNA during DNA replication.[10] The relaxation of DNA by topo II involves the transient double-strand breakage of DNA, followed by strand passage and religation of the DNA strands. Topo II relaxation of DNA requires ATP and results in a change in linking number by (multiples of) two. In contrast, the mechanism of DNA relaxation by topo I involves the transient *single*-strand cleavage of duplex DNA, unwinding and religation.[11] Specifically, topo I-mediated DNA strand scission involves a nucleophilic attack by the active site tyrosine OH group on the DNA phosphodiester at the site of cleavage (Fig. 3). This attack results in breakage of the DNA phosphodiester backbone and formation of a phosphorotyrosine bond between the enzyme and DNA; the resulting intermediate is termed the 'covalent binary complex.' Following DNA relaxation via passage of the broken DNA strand around the unbroken strand, the phosphodiester backbone is reformed by religation with concomitant release of the enzyme. Inhibitors of both topo I and topo II are characterized by their ability to stabilize the covalent binary complex (Fig. 3), thus diminishing religation and ultimately DNA synthesis and cell viability.

Medicinal agents believed to function at the locus of topo II include a number of clinically used anti-neoplastic agents such as doxorubcin and mitoxantrone.[12] Numerous topo I inhibitors have been characterized, including rebeccamycin, indolocarbazole, nitidine and 2-phenylbenzimidazole.[13] The CPT family, however, remains the most extensively studied class of agents that target topo I.

A few different lines of evidence support topo I–DNA interaction as the locus of action of CPT. For example, a number of CPT resistant cell lines have been studied, and these are characterized by specific mutations within topo I.[14] Further, deletion of the gene for topo I from *Saccharomyces cerevisiae* resulted in viable cells that were fully resistant to CPT.[15] Re-expression of the yeast or human enzymes in *S. cerevisiae* restored sensitivity to CPT.

Topo I cleaves DNA at multiple sites; the highest efficiency cleavage sites exhibit significant sequence homology. Topo I cleaves DNA preferentially following thymidine (cf Fig. 3), but has less preference for the nucleobase immediately downstream from the site of cleavage. Sites of cleavage stabilized by CPT, however, exhibit a strong preference for guanosine at the +1 position, while thymidine remains the preferred nucleobase at the −1 position (Fig. 3).

The exact mechanism by which CPT stabilizes the DNA–topo I covalent binary complex is not fully understood. CPT has been shown not to exhibit a significant degree of binding to either DNA or topo I alone,[16] although it has been reported more recently that topotecan (**3**), which should be protonated at physiological pH, does bind to DNA at high concentrations.[17] 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 E-ring of CPT and the topo I–DNA covalent binary complex has been suggested,[18] and would not be altogether surprising

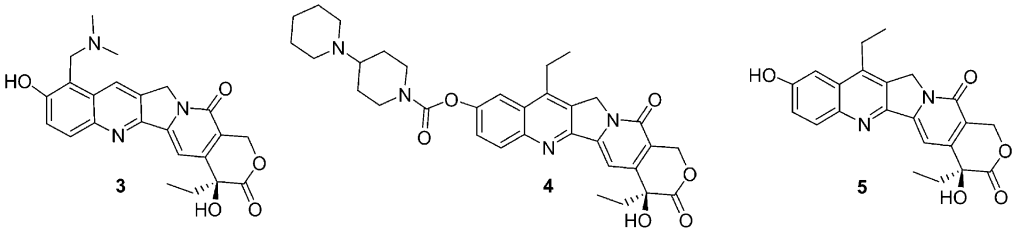


Figure 2. Structures of topotecan (**3**), irinotecan (**4**) and SN-38 (**5**).

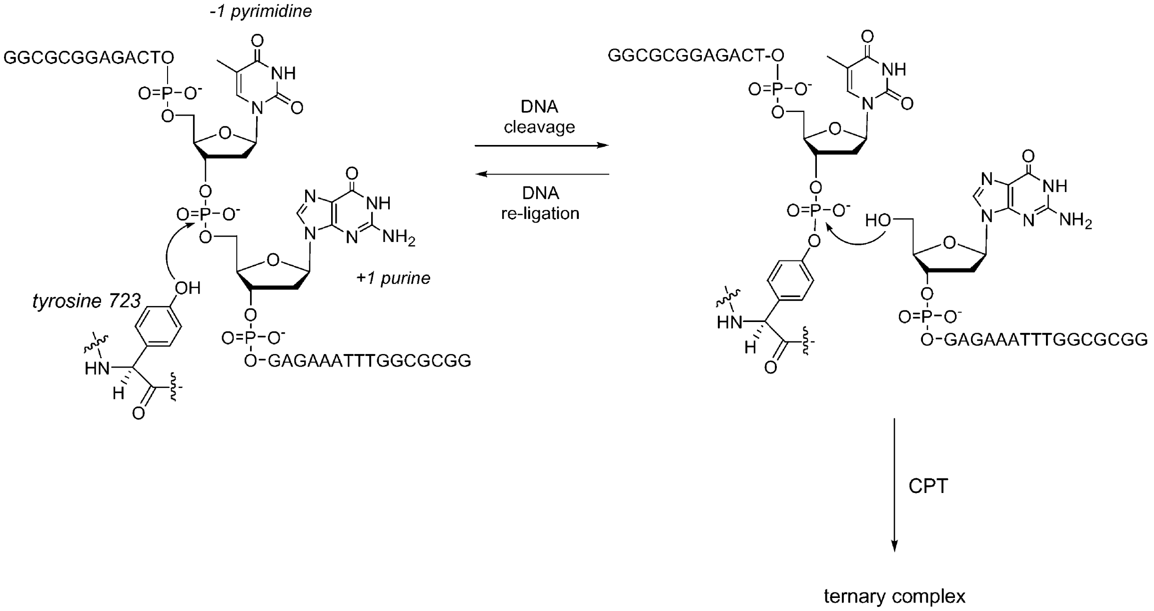


Figure 3. Mechanism of DNA relaxation by topoisomerase I.

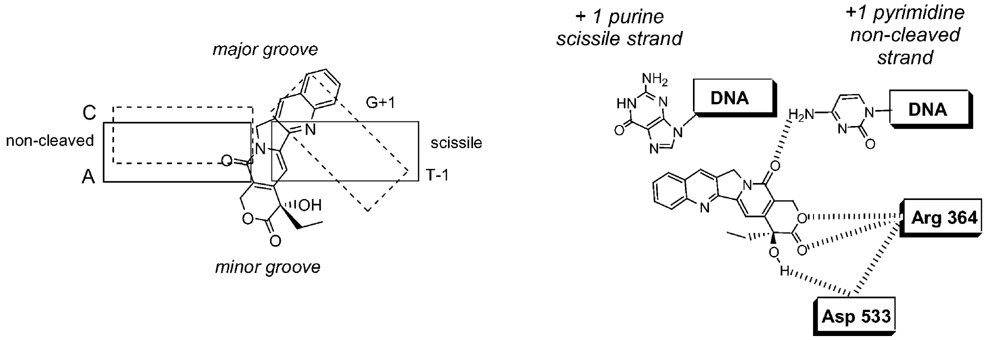
given the unusual chemistry of the lactone ring,[19] there is no direct evidence for the formation of a covalent bond. Further, the finding that triplex-forming oligonucleotides containing CPTs attached in different orientations[20] could all stabilize DNA duplexes cleaved by topo I argues against the need for covalent attachment of CPT to the topo I–DNA covalent binary complex.

A number of studies have provided compelling evidence supporting the interaction between CPT and the covalent binary complex comprised of topo I and DNA. Hertzberg and coworkers demonstrated that a CPT analogue containing a bromoacetamide functionality at the 10-position initially bound reversibly to the covalent binary complex with the same selectivity as CPT itself. Upon prolonged incubation, 10-bromoacetamidoCPT formed a time-dependent drug–enzyme crosslink.[21] Pommier and coworkers subsequently showed that a 7-chlorinated CPT analogue alkylated N3 of guanosine at the + 1 position, that is, immediately adjacent to the site of DNA cleavage.[22] These studies demonstrated convincingly that CPT is capable of interacting with the covalent binary complex at or near the point of the topo I–DNA interface.

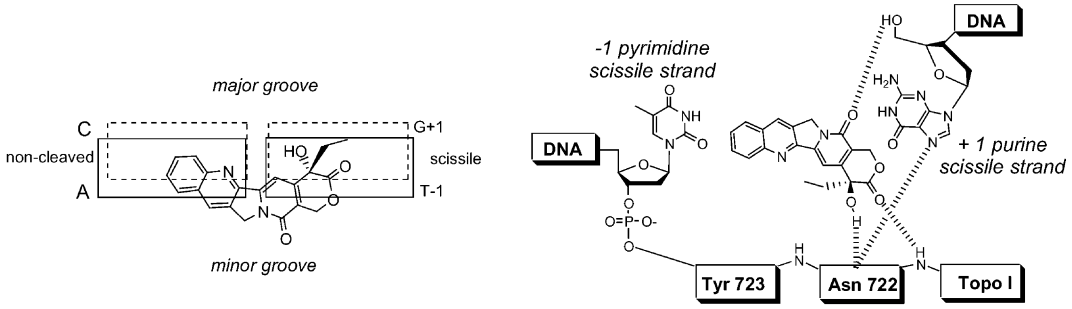
Several structural models have been suggested for the interaction of CPT with the covalent binary complex. The recent binding models were greatly facilitated by X-ray crystal structures of topo I interacting both covalently and noncovalently with DNA.[23] Accompanying the reported crystal structures were predicted binding interactions for CPT within the covalent binary complex (Fig. 4). Models reported from the Pommier and Pilch laboratories present alternative binding arrangements (Fig. 4).[24]

In the Hol model,[23] CPT and the + 1 purine are shown in a stacking arrangement following rotation of the nucleobase out of the helix and a 180° flip of the base relative to the canonical Watson–Crick orientation. Pommier argued that CPT interacts with the covalent binary complex via intercalation in a manner capable of maximizing both stacking interactions and hydrogen bonding to specific amino acid and DNA residues. Kerrigan and Pilch[24b] have suggested an intercalative model for the drug, placing the E-ring lactone within the minor groove where it is potentially capable of additional hydrogen bonding interactions with the covalent binary complex. Pommier and coworkers have also reported a computational model for the DNA interaction of 7-ethyl-10-hydroxyCPT (**5**), a potent topo I inhibitor (Fig. 5).[25] The docking arrangement requires the rotation of the + 1 base out of the DNA helix based upon the orientation of benzo[*a*]pyrene-containing DNA duplexes that inhibited religation. The position of this analogue relative to the covalent binary complex involves an intercalation of CPT where the E-ring lactone is positioned in the minor groove, and the quinoline ring in the major groove, with the 20-hydroxyl group facing the non-scissile strand. It may be noted that this orientation was acknowledged to differ from that implied by an earlier crosslinking experiment from the same laboratory that employed an electrophilic CPT derivative.[22]

Each of these reports further defined a H-bonding profile for the drug–DNA–enzyme ternary complex (Figs 4 and 5). The analysis of several CPT resistant cell lines containing topo I mutations has provided a detailed description of the amino acid residues that play a decisive role in supporting CPT binding.[26–28] The D533G enzyme mutation results in CPT resistance which is suggestive of a potential interaction, possibly a hydrogen bond, between Asp533 and CPT. Mutational analysis of Arg364, Asn722, and Lys532 has shown that each of these amino acid residues also plays a role in supporting CPT binding.[27] It is important to note, Hol Model



Pommier Model



Pilch Model

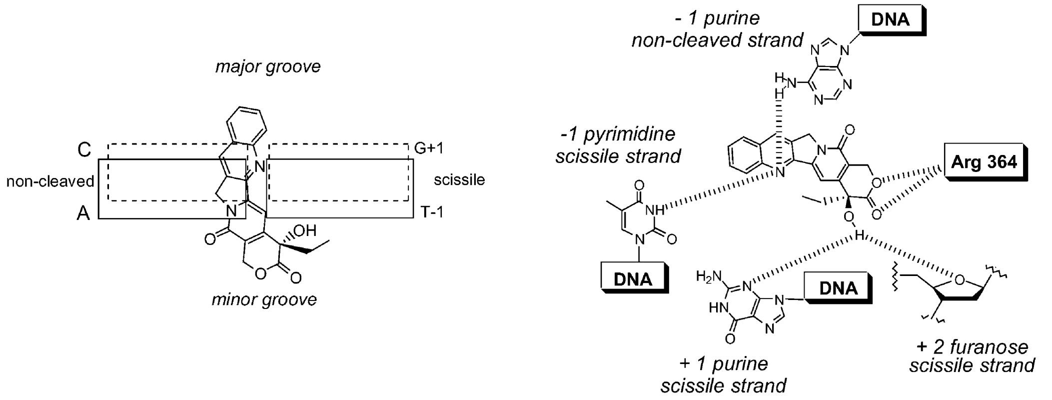


Figure 4. Two-dimensional representation of selected computational docking models of CPT within the covalent binary complex. Rectangles represent DNA base pairs; those below CPT are shown in dotted lines.

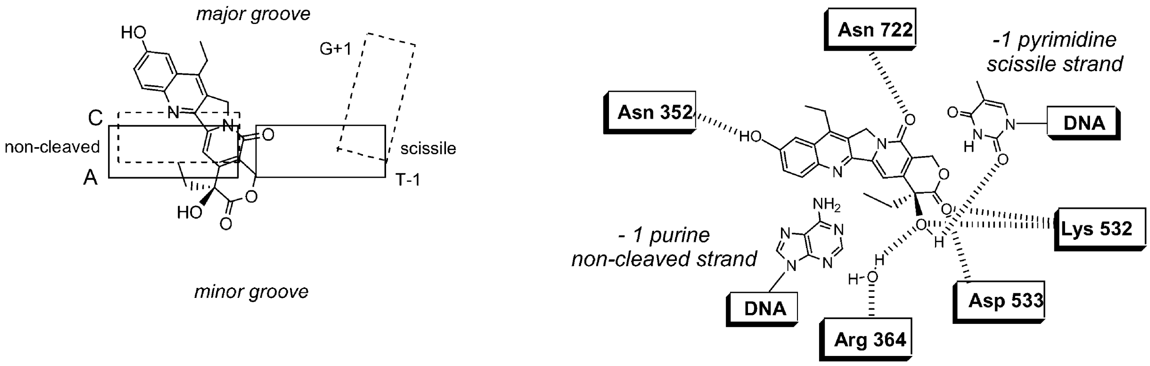


Figure 5. Two-dimensional representation of docking model of SN-38 within the covalent binary complex.

though, that alteration of these key residues may bring about CPT resistance through the disruption of the innate binding region of CPT rather than the elimination of required hydrogen bonds or van der Waals interactions. The existing CPT docking models attempt to simultaneously produce an energetically minimized interface with the covalent binary complex while incorporating the maximum degree of appropriate interactions with key amino acid residues.

Recently, Staker et al. reported the X-ray crystal structure of a ternary complex containing a human topo I construct covalently attached to a DNA duplex containing a 5′-bridging phosphothioate in the presence of topotecan.[28] The X-ray structure showed topotecan interacting by way of full intercalation into the duplex DNA within the covalent binary complex (Fig. 6). Important features are base stacking interaction with both the upstream and downstream base pairs and utilization of only Asp 533 in a direct hydrogen bond with topotecan. The positioning of carbons 7, 9 and 10 in a manner that situates them in the vicinity of the major groove is in agreement with many SAR studies of CPT analogues which have found that even fairly large substituents at these sites did not disrupt function. Interestingly, on the basis of residual electron density in the crystallographic data, the report also proposes that the open carboxylate form can bind within the intercalation pocket in a fashion similar to the lactone and suggests a number of potential water-mediated hydrogen bonds between the carboxylate form and key amino acid residues (Fig. 6). Since the carboxylate form of CPT apparently does not bind to the covalent binary complex in solution,[19b,29] its appearance in the X-ray crystal structure is an interesting observation, particularly from the perspective of microscopic reversibility. While the suggested model allows for the existence of the open carboxylate form, it does not show evidence of a direct covalent bond between CPT and the enzyme. It is important to note that differences between the structure determined by X-ray crystallography and those suggested in the preceding models could reflect differences in the docking arrangements of topotecan and CPT with DNA, consistent with the apparently unique ability of topotecan to bind to DNA in the absence of topo I.[17]

3. Other biochemical effects of CPT

The ability of CPT to convert topo I into a cellular poison is believed to be contingent upon the trapping of the covalent binary complex, which results in DNA damage during the S-phase of the cell cycle.[9] It has been reported that elevated levels of topo I exist in tumors of the colon, ovary and prostate, which could well result in enhanced sensitivity toward CPT.[30] Also suggested as a source of selectivity has been deficiencies in metabolic pathways within cancer cells that result in the impairment of normal DNA repair mechanisms.[31] The exact mechanism(s) by which campothecin produces a relatively high degree of cancer cell specific cytotoxicity clearly requires further study.

While the interaction between CPT and topo I may be the sole determinant of CPT-induced cytotoxicity, other cellular responses to CPT have been noted. Further, in addition to relaxation of supercoiled chromosomal DNA, topo I participates in other cellular functions that are sensitive to CPT.[11a]

It has been reported that CPT induced rapid conjugation of ubiquitin and small ubiquitin modifiers (SUMO) to topo I, resulting in subsequent protein degradation via the 26*S* proteasome complex.[32] CPT, therefore, activates a cellular response capable of degrading, and thus potentially resolving, the trapped covalent binary complex. However, it has also been suggsted that other cellular processes may also be affected by CPT, leading to modulation of the effects of CPT.[33]

Tazi et al. have demonstrated that topo I possesses activity as a phosphorylating enzyme for SR proteins and, by extension, influences the splicing activity of SR proteins.[34] The kinase activity of topo I utilizes ATP as

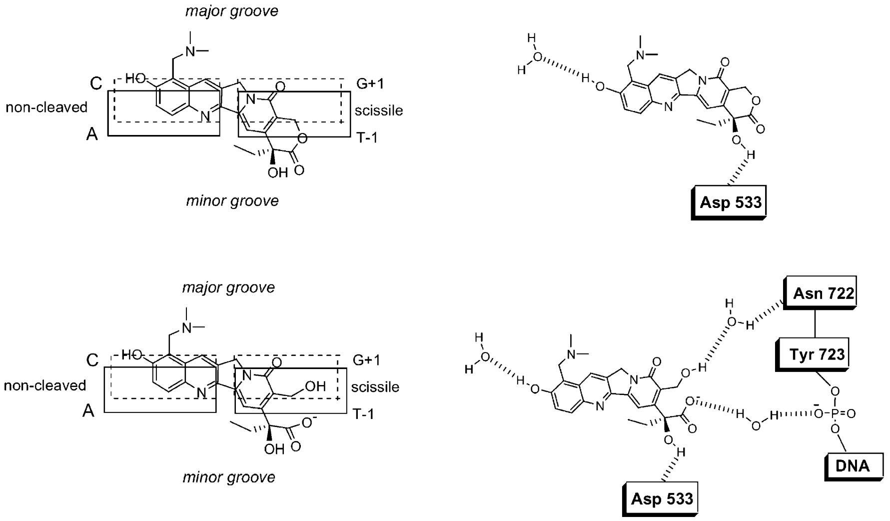


Figure 6. Two-dimensional representation of X-ray crystal structure of topotecan within the covalent binary complex.

the phosphate donor and has been shown to be separate from its DNA relaxation function through mutational studies.[35] Despite this separation of activities, the kinase function of topo I is inhibited by CPT in the presence of DNA. Interestingly, the splicing factor *SF2/ASF*, a phosphorylation substrate for the kinase activity of topo I, was shown to be capable of inhibiting CPT-induced DNA cleavage in a cell free system.[36]

The identification of a series of *topoisomerase I related function proteins* (*TRFp*) has been reported by Christman et al.[37] Analysis shows that one member of this family, *TRF4p*, plays a critical role during the S phase of the cell cycle. Specifically, in conjunction with topo I, *TRF4p* is involved in mitotic chromosome condensation.[37,38] Additionally, *TRF4p* is associated with the DNA binding protein *Smc1p* during chromosomal condensation.[38] Importantly, reports detailing mutations to *TRF4p* have produced cell lines with surprising hypersensitivity toward CPT, suggesting that *TRF4p* may be important for repairing DNA damage caused by CPT.[39] The *TRF4* locus has recently been shown to encode a novel DNA polymerase,[37b] presently denoted polymerase σ.[40] The effects of inhibition of this enzyme on cellular sensitivity to CPT seem important to establish.

In addition to its DNA relaxation activity and the other functions described above, topo I is able to regulate transcription,[41] recognize and cleave mismatched nucleotides at intrinsic cleavage sites[42] and associate with numerous proteins in vivo.[11a] Topoisomerase I is also implicated in non-homologous recombination,[43] which could well be linked to the process of tumorigenesis.[44] It has also been reported that irinotecan inhibits acetylcholinesterase activity.[45] Inhibition of acetylcholinesterase could obviously constitute a dose-limiting factor for utilization of the drug. While the reports described above represent a solid foundation, a thorough understanding of the manner by which CPT may affect cellular function at loci other than topo I is still in its infancy.

4. Synthetic studies

Analogues of CPT are of great interest for understanding the mechanism of topo I interaction in greater detail, and should ultimately lead to CPTs having improved therapeutic properties. There are now several synthetic approaches to CPT and analogues. Additionally, there are semi-synthetic approaches for the production of derivatives of CPT.

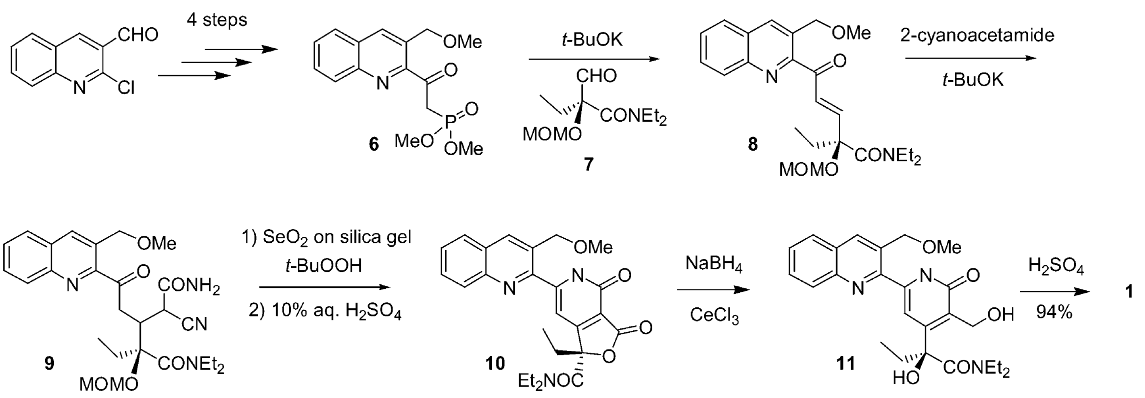
Following the initial description of the isolation of CPT, numerous synthetic efforts were reported utilizing a multitude of approaches.[46] It was not until 1971, however, that the first successful total synthesis of the racemic form of the molecule was reported by Stork and Schultz.[47] Numerous syntheses of 20(*RS*)-CPT have since been published.[48]

Corey et al. were the first to report a successful resolution via quinine of a chiral intermediate that could be employed for the synthesis of 20(*S*)-CPT.[49] The successful resolution of the *R* and *S* enantiomers of CPT using (*R*)-(+)-α-methylbenzylamine has also been reported.[50] Recently, Imura et al. described the first asymmetric synthesis of a key chiral intermediate using enzyme-mediated resolution.[51]

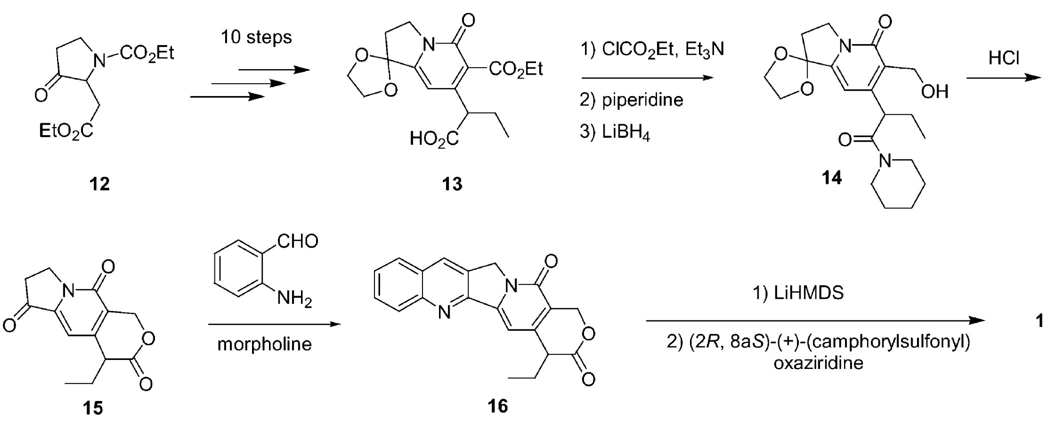
The first asymmetric synthesis of 20(*S*)-CPT was reported by Tagawa et al. in 1989 utilizing an *N*-tosyl-*R*-proline derivative as a chiral auxiliary to induce the stereocontrolled assembly of the lone chiral center.[52] Fang et al. and Jew et al. used the chiral catalyst $(DHQD)_2$-pyr for the stereocontrolled oxidation of carbon 20 in greater than 90% enantiomeric excess as part of the total synthesis of 20(*S*)-CPT.[53] Comins et al. have utilized chiral auxilaries to establish the correct stereochemistry for the 20(*S*)-hydroxyl group.[54] Refinement of their method has culminated in the asymmetric synthesis of 20(*S*)-CPT in only six steps from commercially available starting materials.[55] Many of these synthetic strategies have been reviewed previously.[56] Although not intended as an exhaustive review, representative total syntheses are described below in some detail.

Ciufolini and Roschangar reported a synthesis of 20(*S*)-CPT utilizing an esterase to establish the correct C-20 stereochemistry (Scheme 1).[57] Phosphonate **6** was prepared in four steps and in 59% overall yield from commercially available 2-chloroquinoline-3-carboxaldehyde. To control absolute stereochemistry at C-20 of CPT the strategy utilized aldehyde **7** which was obtained by an enzymatic desymmetrization of the corresponding malonate with pig liver esterase.[57] Condensation of **6** with aldehyde **7** afforded enone **8** in 81% yield. Conjugate addition with the anion derived from 2-cyanoacetamide provided intermediate **9** in quantative yield. Selenium dioxide oxidation of **9** followed by acidic deprotection of the MOM protecting group not only afforded the pyridone functionality but further resulted in lactone formation to yield intermediate **10** in 68% yield. Reduction of the lactone was achieved by modification of a published procedure[58] to give diol **11** in quantative yield. Acid treatment of diol **11** effected lactonization to furnish 20(*S*)-CPT.

Nagao et al. have described a novel synthetic strategy leading to 20(*S*)-CPT from racemic ethyl 1-ethoxycarbonyl-3-oxopyrrolidin-2-ylacetate (**12**) (Scheme 2).[59] Ketal **13** was synthesized from **12** by a 10 step protection/condensation/deprotection sequence in 28% overall yield. Treatment of ketal **13** with ethyl chloroformate in the presence of triethylamine, followed by treatment with piperidine and $LiBH_4$ reduction, gave alcohol **14** in 39% overall yield. Exposure of alcohol **14** to 6N HCl stimulated lactonization and thus provided δ-lactone **15** in 60% yield. Condensation of **15** with 2-aminobenzaldehyde in the presence of morpholine afforded racemic 20-deoxyCPT (**16**) in 64% yield. This synthesis was completed via asymmetric hydroxylation onto C_{20} of racemic 20-deoxyCPT (**16**) employing a chiral Davis reagent, ((2*R*, 8a*S*)-(+)-(camphoryl)sulfonyl)oxaziridine. This strategy is important in that it should be



Scheme 1.



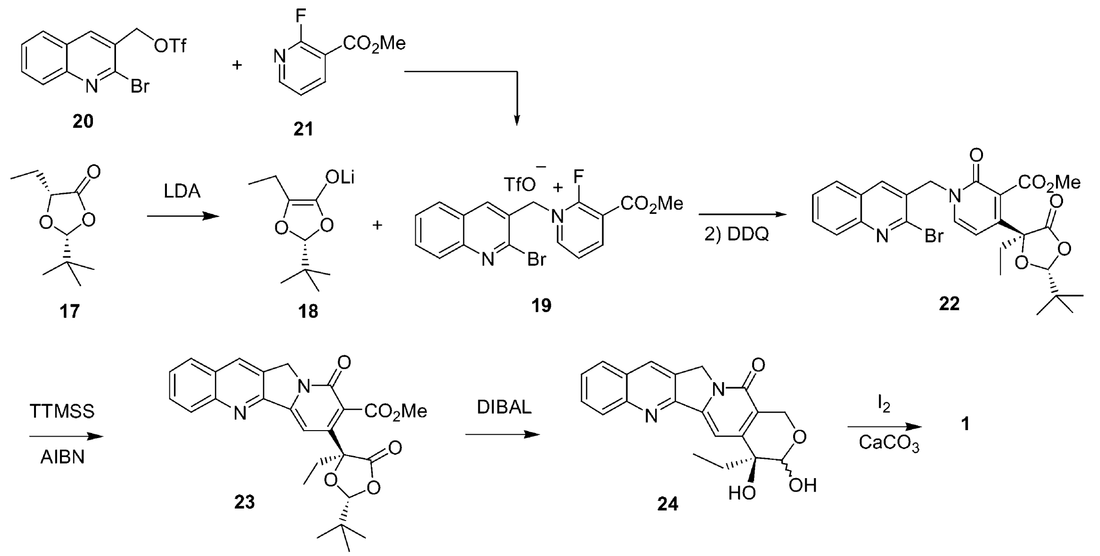
Scheme 2.

useful for the synthesis of C-20 functionalized CPT analogues.

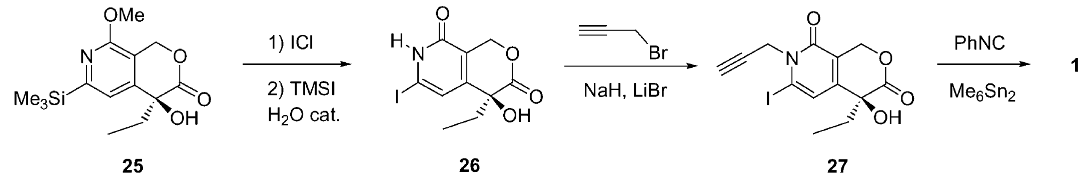
A recent strategy employed by Bennasar et al. made use of a (2*R*,5*R*)-2-*tert*-butyl-5-ethyl-1,3-dioxolan-4-one (**17**) to establish C_{20} asymmetry and thereby afford access to 20(*S*)-CPT.[60] A key step involved the synthesis of pyridinium triflate **19**, obtained by alkylation of 2-fluoropyridine **21** with quinoline triflate **20** (Scheme 3). The C_{20} stereocenter was introduced by reaction of the enolate of **18** with pyridinium triflate **19** followed by DDQ oxidation to give pyridone **22** as a single diastereomer in 20% overall yield. Intramolecular arylation of the pyridone moiety in **22** was accomplished by treatment with tris(trimethylsilyl)silane (TTMSS)-AIBN to afford the tetracyclic intermediate **23** in 65% yield. DIBAL reduction of the ester of **23** followed by ring closure provided α-hydroxylactol **24** in 45% yield. Lactol **24** was converted to (*S*)-CPT by oxidation with iodine in the presence of $CaCO_3$ in 60% yield.

Curran et al. have reported a synthesis of (*S*)-CPT based upon an interesting 4+1 radical cascade annulation.[61] Lactone **25** was obtained in a fashion similar to the synthesis developed by Fang et al.[53a] Exchange of the TMS group in **25** for iodine, followed by demethylation, provided **26** in 33% yield (Scheme 4). *N*-propargylation of lactone **26** provided alkyne **27** in 88% yield. The reaction of phenyl isonitrile with the enantiopure alkyne derivative **27** under irradiation conditions provided 20(*S*)-CPT in 63% yield. Curran et al. have reported improvements in the synthesis of the enantiopure DE ring precursor **26** using a samarium catalyst.[62] Radical methods of this type have been shown to tolerate A- and B-ring substituents and have, therefore, been utilized for the synthesis of numerous CPT analogues.[63]

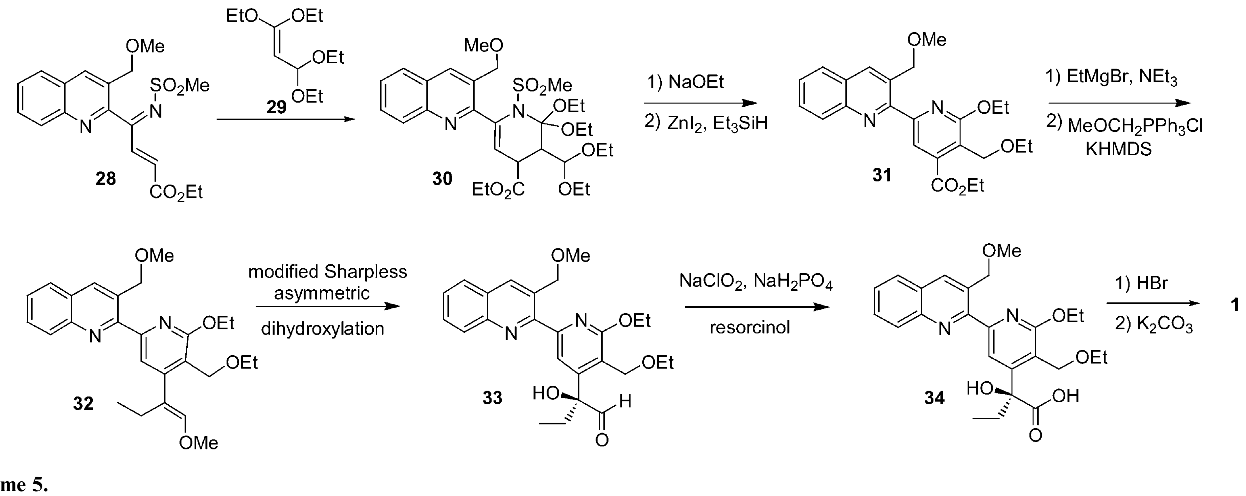
Blagg and Boger recently described a new synthesis of 20(*S*)-CPT based on a Diels–Alder cycloaddition of the electron deficient diene *N*-sulfonyl-1-aza-1,3-butadiene **28** with the electron rich dienophile **29** for the assembly of **30**, a precursor to the A, B and D rings (Scheme 5)[64] Treatment of **30** with sodium ethoxide followed by addition of zinc iodide and triethylsilane resulted in the sequential elimination of elements of methanesulfinic acid and ethanol, and the conversion of the diethyl acetal to the ethyl ether to yield quinoline derivative **31**. Ethylation of **31** with EtMgBr in presence of Et_3N and subsequent Wittig olefination furnished the separable *cis* and *trans* isomers of quinoline **32**. The C-20 tertiary alcohol was introduced through a Sharpless asymmetric dihydroxylation reaction upon *E*-**32** to yield aldehyde **33** in 84% yield (86% ee). Oxidation of aldehyde **33** to the corresponding carboxylic acid **34** was accomplished using sodium chlorite and resorcinol. Cleavage of the aryl ethyl ether and the two benzylic ethers was accomplished using HBr, and intramolecular ring closure was catalysed by the subsequent addition of potassium carbonate to provide 20(*S*)-CPT.



Scheme 3.



Scheme 4.

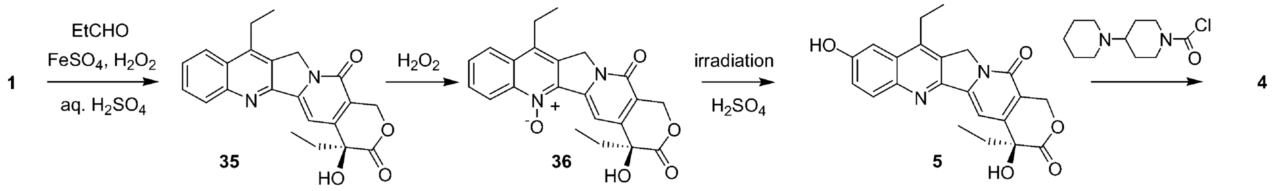


Scheme 5.

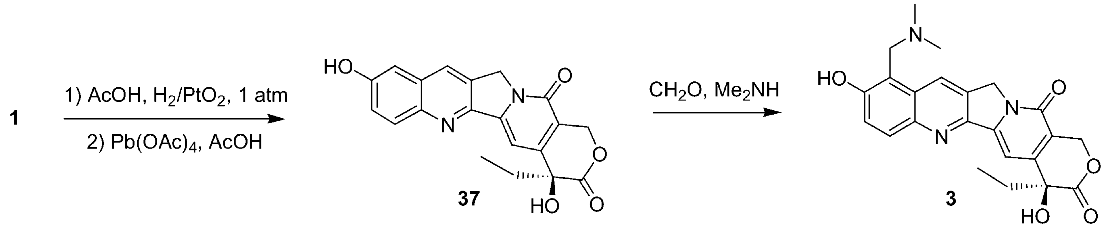
In addition to numerous total syntheses of CPT, many synthetic efforts are aimed at producing analogues. The majority of these efforts have involved the semi-synthetic manipulation of CPT. Among the most important efforts have been the semi-synthetic approaches to irinotecan, topotecan and homocamptothecin.

By means of semi-synthetic methods, Sawada et al. reported the synthesis of irinotecan in 1991.[65] Hydrogen peroxide was added to a solution of 20(*S*)-CPT in aqueous sulfuric acid in presence of ferrous sulfate and propionaldehyde to afford 7-ethylCPT (**35**) in 77% yield (Scheme 6). 7-EthylCPT (**35**) was converted into the corresponding *N*-oxide (**36**) using hydrogen peroxide in acetic acid. Irradiation of **36** in acidic media furnished the active metabolite of irinotecan (SN-38) (**5**) in 49% yield. Treatment of SN-38 (**5**) with 4-(1-piperidino)-1-(piperidino)-chlorocarbamate provided irinotecan (**4**) in 80% yield.

The second currently marketed CPT derivative, topotecan, was synthesized in 1991 by Kingsbury et al. in two steps starting from 20(*S*)-CPT.[66] The conversion of 20(*S*)-CPT to 10-hydroxyCPT (**37**) was accomplished through a reduction-oxidation sequence in 71% yield (Scheme 7). Treatment of **37** with dimethylamine in



Scheme 6.



Scheme 7.

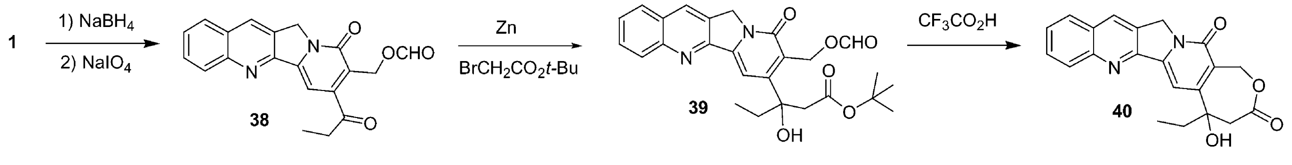
aqueous formaldehyde and acetic acid provided topotecan (**3**) in 62% yield.

The parent compound of a new class of CPT derivatives, homoCPT (**40**), was first prepared in racemic fashion by Bigg et al. utilizing a four-step semi-synthetic sequence (Scheme 8).[67] Reduction of 20(*S*)-CPT was accomplished with sodium borohydride to provide the corresponding hydroxylactol which was oxidatively cleaved with periodic acid to give formyloxymappicin ketone (**38**) in 84% overall yield from CPT. A Reformatsky reaction with *tert*-butyl bromoacetate and **38** gave hydroxyester **39** in 31% yield. Treatment of **39** with trifluoroacetic acid afforded homoCPT (BN 80245) (**40**) in 73% yield. An asymmetric total synthesis of 20(*S*)-homoCPT was recently reported by Curran et al. using the previously discussed 4+1 radical cascade annulations as a key step.[68]

5. Structure–activity relationships

The development of these synthetic and semi-synthetic strategies has facilitated the study of CPT mechanism, as well as the identification of analogues with improved properties. Likewise, the availability of topo I–DNA and topotecan-topo I–DNA crystal structures and computational docking models provide insight regarding the mechanism of action of CPT, although a number of questions remain unanswered. The latter include an understanding of the exact mode and uniqueness of CPT binding to the enzyme–DNA covalent binary complex and the specific roles of the various structural features of CPT in this critical interaction. Further, it is important to gain an understanding of how systematic modifications within the CPT structure may enhance or suppress the effect of the drug in a biological context. Numerous studies exploring the structure–activity relationships (SARs) of CPT have provided novel insights and contributed to the clinical successes already realized. Recent studies have explored lactone stabilization, solubility and drug transport mechanisms, tumor cell recognition and enhancement of DNA sequence specificity. The present discussion of CPT derivatives focuses individually upon substitutions, additions and deletions of the quinoline ring system (A/B rings), the C and D rings, and the E ring of the pentacyclic structure (Fig. 1). A separate section addresses the properties of key CPT conjugates.

At present, the design of novel CPT analogues rests upon a few important working assumptions. The first is that the conjugation and planarity of the A, B, C and D rings are required for in vitro and in vivo activity of the CPTs. Second, it has been thought that the E ring lactone is a necessary structural feature, as the corresponding sodium salt (**2**) exhibits much lower activity; finally, the *S* configuration at carbon 20 is believed to be essential for activity, as racemic forms of CPT (analogues) have repeatedly been found to have lesser potency than the optically pure forms.[69] However, as described below, recently reported results require a reevaluation of the belief that the E-ring lactone is essential.



Scheme 8.

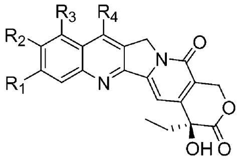
5.1. Quinoline (A/B) ring

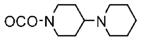
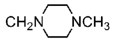
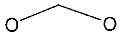
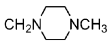
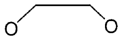
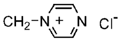
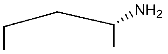
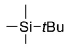
To date, the only CPT analogues approved for clinical use, that is, topotecan (**3**) and irinotecan (**4**) (Fig. 2), are derivatives with substitutions within the quinoline ring. Indeed, the A and B rings of CPT have proven to be the most amenable to modification without perturbation of activity. Modifications can involve additions to the quinoline ring or the complete replacement of the quinoline ring with an alternative ring system. Reports detailing analogues that replace the quinoline ring system with alternative conjugated, planar heterocycles suggest that some maintain moderate to good activities.[70] However, none of these derivatives surpasses CPT in potency of action. Consequently, the vast majority of studies have focused upon additions to the quinoline ring and the effectiveness of substitutions at each of the carbon atoms of the quinoline ring (Table 1).

In seminal work, Wani et al. described CPT analogues that afforded a preliminary understanding of the SAR profile of CPT. Included in this foundation was the finding that substitution at carbons 11 and 12 was generally unfavorable to biological activity.[71] Notable exceptions are analogues containing the 10,11-(methylenedioxy) or 10,11-(ethylenedioxy) functional groups, and also 11-fluoro and 11-cyano derivatives, which enhanced topo I inhibition.[69,72] Numerous substitutions of amino, halogeno and hydoxyl groups at positions 9 and 10 were observed to have beneficial effects on antitumor activity and life prolongation of mice implanted with L1210 leukemia.[69] Additionally, key studies by Kingsbury et al. produced a number of analogues with substitutions at carbons 7, 9 and 10 that maintained good levels of activity.[66] Also of importance were the initial reports of water insoluble analogues 9-aminoCPT (**41**) and 9-nitroCPT (rubitecan) (**42**), both of which were reported to possess levels of in vivo activity sufficient to merit clinical evaluation. Rubitecan (**42**)[73] serves as a metabolic precursor to 9-aminoCPT and is currently in phase III clinical trials for the treatment of pancreatic cancer. Among the derivatives reported by Kingsbury et al. for the first time were 9-((dimethylamino)methyl)-10-hydroxyCPT (topotecan) (**3**) and 10-hydroxy-9-((*N*-methylpiperazinyl)methyl)CPT (**43**), both of which displayed enhanced water solubility.[66] These analogues illustrated successful strategies for substitution of the quinoline ring system and have been discussed in excellent reviews.[74]

The demonstration that positions 7, 9, 10 and sometimes 11 of the quinoline ring system could be altered

Table 1. Selected A and B ring modified camptothecin analogues



Analogue	R_1	R_2	R_3	R_4	IC_{50}[a] μM (Topo I)	IC_{50}[b] μM (proliferation)
1 (camptothecin)	H	H	H	H	0.6–1.4	23(L1210) 0.046(HT-29) 0.33(H460)
3 (topotecan)	H	OH	$CH_2N(CH_3)_2$	H	1.1	56(L1210) 1.38(H460)
4 (irinotecan)	H		H	Ethyl	> 100	1200 (L1210)
5 (SN-38)	H	OH	H	Ethyl	1.1	8.9(L1210) 0.025(HT-29)
41	H	H	NH_2	H	0.9	12 (L1210)
42 (rubitecan)	H	H	NO_2	H	NA	NA
43	H	OH		H	0.5	72 (L1210)
44 (lurtotecan)			H		0.42	0.006 (HT-29)
45			H		0.18	0.016 (HT-29)
46 (exatecan)	F	Me			NA	0.008 (OVCAR-3)
47 (DB-67)	H	OH	H		NA	NA
48	H	H	H	$CH{=}NOC(CH_3)_3$	NA	0.01 (H460)

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

[b] Concentration that inhibited the proliferation of L1210 cells, HT-29 cells, P388 cells, and H460 cells by 50% upon continuous exposure. Taken from refs 6a, 70b, 71, 72, and 80c.

without diminishing topo I inhibitory activity and antineoplastic activity allowed researchers to examine a broader repertoire of derivatives. In recent years, efforts have been made to increase the water solubility, and conversely the lipophilicity, of CPT, to activate CPT as a prodrug and to produce conjugates with improved properties. A further effort has focused on manipulation of the lactone-carboxylate equilibrium (i.e., between **1** and **2**), specifically in human plasma. The stability of the E ring lactone in human plasma can be affected by derivatization of the quinoline ring and numerous SAR studies reflect this fact.

Considerable evidence supports the idea that small substituents at carbon 10 have little effect upon the ability of CPT to stabilize the enzyme–DNA binary complex, while larger groups can inhibit the ability of CPT to stabilize the covalent binary complex. For example, irinotecan (**4**), which has a large 4-(1-piperidino)-1-piperidinocarbamate group attached at carbon 10, does not stabilize the topo I–DNA binary complex, yet exhibits good clinical activity.[72] Significant plasma concentrations of 7-ethyl-10-hydroxyCPT (SN-38) (**5**) in irinotecan treated mice bearing implanted L1210 tumor cells prompted researchers to suggest that irinotecan functions as a prodrug.[75] It has since been determined that irinotecan is indeed metabolized in vivo into SN-38. Human hepatic carboxylesterases have demonstrated high efficiencies in this transformation.[75,76] Further, irinotecan exhibited nearly 20-fold enhancement of lactone ring stability in human plasma relative to CPT.

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. Some of the most successful water soluble derivatives have resulted from the use of aliphatic and aromatic amine substitutions at carbon 7 of 10,11-(ethylenedioxy)CPT. Initial reports of 10,11-(methylenedioxy)-7-((*N*-methylpiperazino)methyl)CPT (lurtotecan) (**44**) highlighted the merit of this strategy and the compound has been in clinical trials for breast, colorectal and small cell lung cancers.[77] Further development led to the use of aromatic quarternary ammonium salts as water solubilizing functional groups. Derivatives such as 10,11-(ethylenedioxy)-7-pyraziniumylmethylCPT chloride (**45**) have been reported to possess both superior water solubility and enhanced tumor growth suppression.[78] Another derivative that has received substantial attention is the water soluble exatecan (**46**). The potency of exatecan has been reported to be up to 28-fold greater than that of topotecan in the treatment of various human malignant cells; the compound is also a poor substrate for the protein BCRP which has been implicated in drug resistance.[79]

Some workers have employed a different approach while endeavoring to establish novel CPT analogues. The need for an intact E-ring lactone in supporting the antitumor effects CPT was noted to be in conflict with the strong preference of human serum albumin for binding the carboxylate form of CPT, thus creating a potential obstacle in delivering the active form of the drug to tumor cells.[80] Further, a quantitative SAR analysis of the National Cancer Institute drug database by Fan et al.[81] indicated that increased lipophilicity was highly advantageous and Burke et al. showed that the lactone was stabilized by passage through lipid bilayer.[82] Hence, Curran et al. have prepared a number of silicon-containing CPT analogues (silatecans) that display increased lipophilicity.[83]

In addition to the preparation of novel lipophilic CPT analogues, lipid bilayer vesicles have been utilized successfully in the binding the lactone form of CPT, offering an additional approach to drug delivery.[84] Moreover, the demonstration of the successful encapsulation of topotecan and irinotecan within such lipid carrier systems suggested that the technique might well be applicable to a broad range of CPT derivatives.[85] Silatecans, such as 7-(*tert*-butyldimethylsilyl)-10-hydroxyCPT (DB-67) (**47**), are more lipophilic than topotecan and are capable of elevated levels of liposomal core-loading. Importantly, the silatecans have also demonstrated levels of cytotoxicicity similar to that of CPT and elevated levels of in vivo activity; selected derivatives have entered into clinical trials.[86]

Zunino et al. have demonstrated that the use of appropriate substituents on the quinoline ring, including aminomethyl, iminomethyl and oxyiminomethyl groups, are an alternative method by which the lipophilicity of CPT can be increased.[87] Particularly successful were the oxyiminomethyl derivatives substituted at carbon 7. Specifically, the alkylated oxyiminomethyl derivatives with large lipophilic groups, such as *t*-Bu, produced highly active CPT analogues.[88] Conversely, the addition of ionizable groups, such as carboxylic acids and amines, conferred unfavorable results. Like the silatecans, these novel C-7 substituted CPTs have demonstrated elevated lactone stability and shown superior antitumor efficacy in terms of tumor growth inhibition and rate of response. Among the most successful of these derivatives is the 7-(*tert*-butoxyiminomethyl)CPT (**48**), which caused complete tumor regression in mice bearing lung carcinoma LX-1.[88]

5.1.1. C/D Rings.

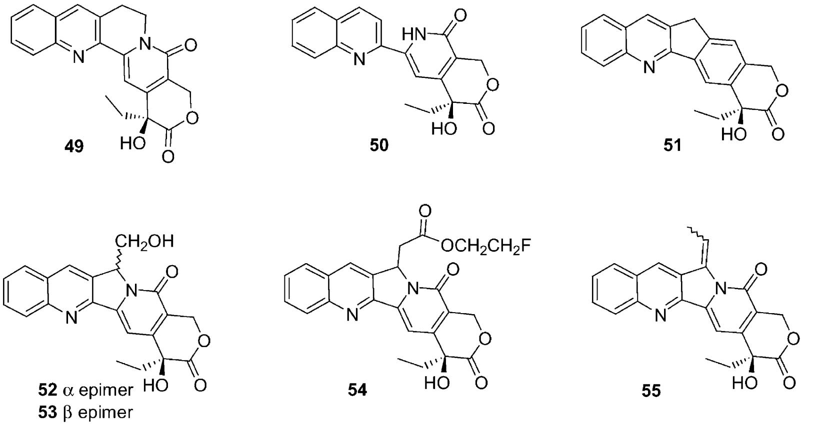
Few studies have focused on modification of the C and D rings of campothecin (Fig. 7). These have generally resulted in loss of activity as illustrated by homologated CPT derivative **49** and *C*-nor-4,6-secoCPT **50**.[89] The inability of these analogues to stabilize the covalent binary complex was attributed to the disruption of planarity. The deaza analogue **51**, that should maintain quite similar shape and planarity relative to CPT, was found to be approximately 60-fold less efficient as a topo I inhibitor.[90] This finding presumably reflects the importance of the pyridone carbonyl of CPT in stabilizing the enzyme–DNA–CPT ternary complex.

Reports of C/D substituents have also been limited, presumably due both to the paucity of accessible carbons for substitution and more arduous synthetic routes leading to potential analogues. Indeed, carbon 5 of the C ring and carbon 14 of the D ring are the only sites available for substituents. Crothers and Crow described the activity of a series of C14 methyl ester derivatives of a number of CPT synthetic precursors.[91] The activities of these analogues were demonstrated to be greatly reduced in comparison to campothecin.

Substitutions at C5 of the C ring have also been carried out. Initial studies by Sawada et al. involved CPT analogues having a series of 5-hydroxy, 5-alkoxy and 5-acetoxy substituents.[92] These analogues were reported to confer no antitumor activity in mice bearing L1210 leukemia. Wang et al. synthesized and tested the 5α and 5β epimers of hydroxymethylCPT (**52** and **53**) and the 5,5-bis-hydroxymethyl CPT and racemic 5-trimethylbenzoyl CPT.[93] These analogues showed no significant antineoplastic activity in any of the numerous cell lines employed and exhibited diminished activity with increasing steric bulk. A series of 5-aminoCPT analogues reported by Subrahmanyam et al. exhibited a similar loss of potency as the size of the amine substituent increased.[94] In other studies it was shown that additions of 5-alkoxy and several other functional groups to C5 were tolerated reasonably well when accompanied by additions of hydroxyl or nitro groups to the A ring.[95] Interestingly, good in vitro activities were demonstrated by the C5 substituted 2-fluoroethyl ester derivative (**54**), which contained no additional functionality within the quinoline ring.[95a] Terasawa et al. reported the synthesis and activity of 5-ethylideneCPT (**55**) which maintained potency equivalent to CPT against P388 cells.[96] The success of **55** suggests that CPT can more easily accept additions upon the C ring when planarity is unperturbed.

5.1.2. E Ring. E ring substitutions have underscored the importance of certain structural features to the maintenance of the activity of CPT. Foremost is the importance of the α-hydroxylactone in supporting both efficient topoisomerase I inhibition and in vivo potency. Unfortunately, under physiological conditions, the presence of the α-OH group results in an equilibrium that favors the (inactive) open carboxylate over the (active) ring-closed lactone form.[80] Hertzberg et al. replaced the lactone functionality with the more hydrolytically stable lactam, thereby reducing the tendency of the E ring to open.[19b] However, the resulting CPT lactam **56** (Fig. 8) was ineffective as a topoisomerase I inhibitor.[19b,90] Other derivatives described in this report,[19b] including the carbinol lactam **57**, the thiolactone **58** and imide **59** (Fig. 8) were also essentially inactive.

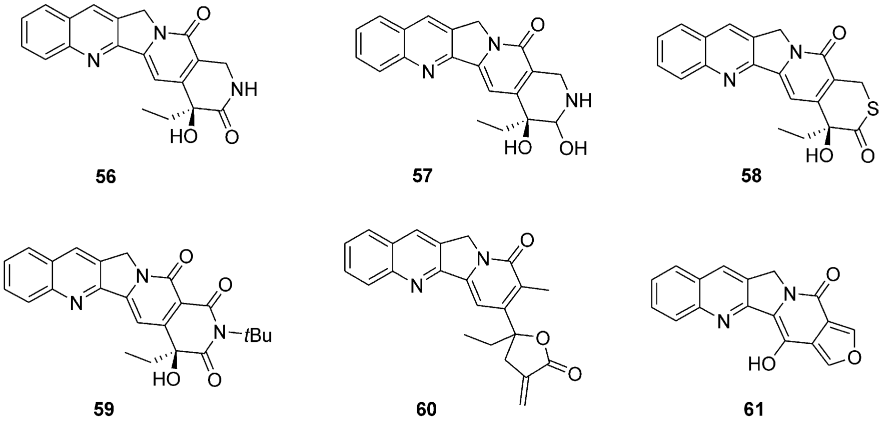
Interestingly, Hertzberg et al. also reported on the synthetically derived methylene lactone analogue **60** and a furanyl derivative **61**, each of which possessed pronounced cytotoxicity (Fig. 8).[19b] While these analogues



49 **50** **51**

52 α epimer
53 β epimer
54 **55**

Figure 7. CPT analogues modified in rings C/D.



56 **57** **58**

59 **60** **61**

Figure 8. CPT analogues modified in ring E.

were revealed to be effective DNA damaging agents, neither had activity as a topo I poison and their cytotoxicities were determined to be unrelated to topo I inhibition.

The stereochemistry at carbon 20 of CPT is apparently crucial, as the analogue having a 20 (*R*)-OH group is inactive.[69] Further, the hydroxyl group itself is presumed to participate in one or more essential hydrogen bonding interactions. The ability to inhibit topo I-mediated ligation and mediate cytotoxicity was retained by 20-aminoCPT (**62**) (Fig. 9), an analogue capable of mimicking the hydrogen bond donor ability of the 20-OH group in CPT.[90,97] It has been suggested that an intramolecular hydrogen bond between the 20-hydroxyl group and the lactone carbonyl results in the activation of the lactone, furthering its susceptibility to nucleophilic attack.[98] At physiological pH, the amine functionality in analogue **62** would presumably be protonated and capable of the same intramolecular hydrogen bond donation as the 20-OH group normally present in CPT. Thus, the diminished activity of **62** demands reassessment of the role of the 20-OH group. In this context, it may be noted that at physiological pH the 20-NH_2 group would be incapable of acting as a hydrogen bond *acceptor* while a free OH could still participate in such a hydrogen bond. This could plausibly explain the decreased activity. The rapid rate of disassociation of **62** from the covalent binary complex, as demonstrated by NaCl induced disassociation experiments, is consistent with the diminished level of activity of **62**, but does not provide a firm molecular basis for the reduced level of activity.[97b]

The 20-deoxy species (**63**) (Fig. 9), which is incapable of hydrogen bonding in any fashion, was also shown to have significantly diminished topo I inhibition and no cytotoxicity, although it did inhibit a topoisomerase I-induced rearrangement of DNA structure.[19b,90,97b] The substantial potencies of the homoCPT derivatives (see below) and the 20-chloro and 20-bromoCPTs (**64** and **65**) (Fig. 9) argue that an intramolecular hydrogen bond to the lactone carbonyl is not essential.[84,97b] However, it is noteworthy that the halogenated CPTs (**64** and **65**) are incapable of acting as hydrogen bond donors, yet capable of acting as hydrogen bond acceptors. Thus, the behaviors of **64** and **65** are altogether consistent with that of 20-amino CPT (**62**).

Replacement of the α-hydroxylactone moiety of CPT with a β-hydroxylactone was first reported by Lavergne et al.[67a] HomoCPT (**66**) (Fig. 9) was reported to exert potent inhibition of topo I and elevated levels of cytotoxicity. Further, the β-hydroxylactone was reportedly stable after 24 h at physiological pH. The demonstration that the seven membered β-hydroxylactone moiety did not diminish CPT activity has prompted the synthesis and evaluation of numerous derivatives.[67,99] Among the most promising of the homoCPT derivatives is 10,11-difluorohomoCPT (BN-80915) (**67**) which exhibited strong antiproliferative activity against numerous cell lines and is currently in phase I clinical trials.[100] Curran et al. have also introduced silicon into homoCPT derivatives, resulting in analogues having increased lipophilicity.[101] The 7-(*tert*-butyldimethylsilyl)homoCPT derivative (**68**) (Fig. 9) is reported to display the highest level of lactone stability in both buffer systems and plasma. Interestingly, Du1441 (**69**) (Fig. 9), an analogue similar to homoCPT, contains an α-hydroxyketo ether in place of the β-hydroxylactone, was inactive as a topo I inhibitor and in inhibiting cellular proliferation.[102] The inactivity of Du1441 again supported the thesis that an intact lactone ring is a structural feature essential for CPT function.

Pommier et al. have provided a model to accommodate the wide range of observed activities.[18b] The inactivity of the 20 (*R*)-hydroxyl derivative argues for a remarkably specific interaction between the hydroxyl group and the topo I–DNA covalent binary complex. Further, the presumed absence of *intra*molecular hydrogen bonding for the halogeno species **64** and **65**, as well as the homoCPTs suggest that activation of the lactone leading to a nucleophilic reaction between the enzyme and lactone moiety is not essential.

In spite of several lines of evidence that the E-ring lactone of CPT is essential to its function as a topoisomerase I inhibitor, two recent reports have described novel E-ring non-lactone analogues that strongly inhibit

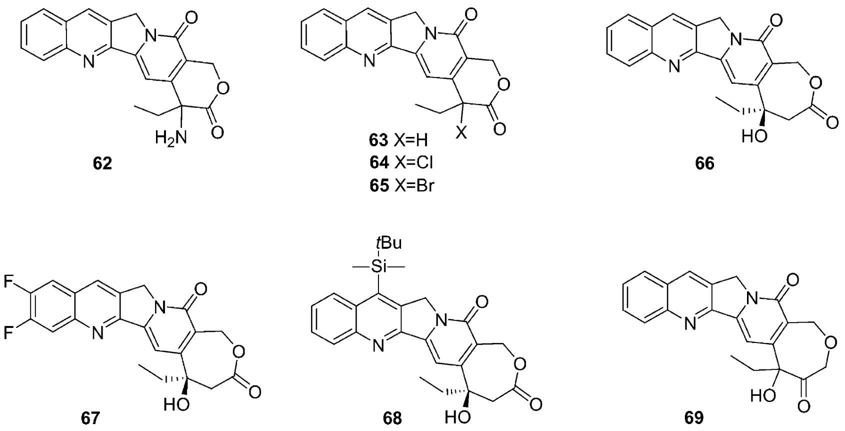


Figure 9. CPT analogues modified in ring E.

topoisomerase I function and exhibit cytotoxicity.[103,104] These findings necessitate a reevaluation of the role of the E-ring lactone moiety.

5.2. Conjugated analogues

The use of conjugates has emerged as a frequent strategy in efforts to optimize therapeutically beneficial properties of CPT, including lactone stability, solubility/lipophilicity, tumor cell recognition and sequence specificity of DNA damage (Fig. 10). Two predominant methodologies have been utilized for the synthetic preparation of conjugates. The first relies on the utilization of the 20(*S*)-hydroxyl group as the site for conjugation. The second involves the exploitation of reactive functional groups, for example, amino, hydroxyl and carboxylic acid groups, on modified CPT analogues. Most of these groups have been part of the quinoline (A/B) ring system.

Perhaps the simplest of these derivatives have been obtained by esterification of the 20(*S*)-hydroxyl group. Giovanella et al. reported the synthesis and biological evaluation of a series of alkyl esters of varying size.[105] Specifically, the 20(*S*)-*O*-ethyl ester (**70**) was reported to possess reduced toxicities while demonstrating enhanced lactone stability and pronounced in vivo activities against human tumor xenografts in nude mice. Studies utilizing 20(*S*)-*O*-acyl esters resulted in CPT analogues with excellent in vitro cytotoxicity and antitumor activity equivalent to those of CPT.[106] The utilization of 20(*S*)-*O*-amino acid analogues as prodrugs has also been studied.[107] Interestingly, the isolation of 20(*S*)-*O*-β-glucopyranosyl CPT from *Mostuea brunonis* has been reported, suggesting that the 20(*S*)-hydroxyl group acts as a point of attachment for natural bioconjugates.[108] A biosynthetic precursor of CPT glycosylated at the 20-position has also been reported.[109] These studies have demonstrated the feasibility of using the 20(*S*)-hydroxyl group to modify CPT. The use of 20(*S*)-*O*-esters, amides, carbonates and carbamates with a variety of linkers have been employed in recent studies in the preparation of novel CPT conjugates.

The antitumor activity, biodistribution and lactone stabilization have been reported for a series of polymeric conjugates of CPT, including poly(ethylene glycol) (PEG) and poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) conjugates, which were designed to be CPT delivery vehicles.[110] The polymeric PEG–CPT conjugate **71** was reported to produce improved levels of tumor regression in HT-29 xenografts and decreased toxicity relative to CPT.[105] Fiebig et al. reported a 20(*S*)-*O*-linked glycoconjugate of CPT designed to improved cellular uptake of the drug.[111] Utilizing previously described[112] fucoside residues to establish multivalent interactions between the drug–glycoconjuate and cell surfaces resulted in derivatives with excellent in vitro activity against HT29 tumor cells. This study also demonstrated the importance of the specific peptide linker in rendering such conjugates stable and biologically active. Specifically, the fucoside glycoconjugate analogue containing a valine/histidine linker (**72**) was reported to inhibit tumor growth >96% in breast cancer xenograft MX-1 models and has consequently been selected for clinical trials.

In a recent study, Firestone et al. have reported the synthesis and initial biological profile of a CPT immunoconjugate designed to target tumor cells specifically.[113] Utilizing the tumor-recognizing antibody BR96 attached to CPT through a 20(*S*)-*O*-carbonate linker cleavable by cathespin B, the resulting immunoconjugate (**73**) was intended to eliminate the dose-limiting side effects while retaining the antitumor capabilities of

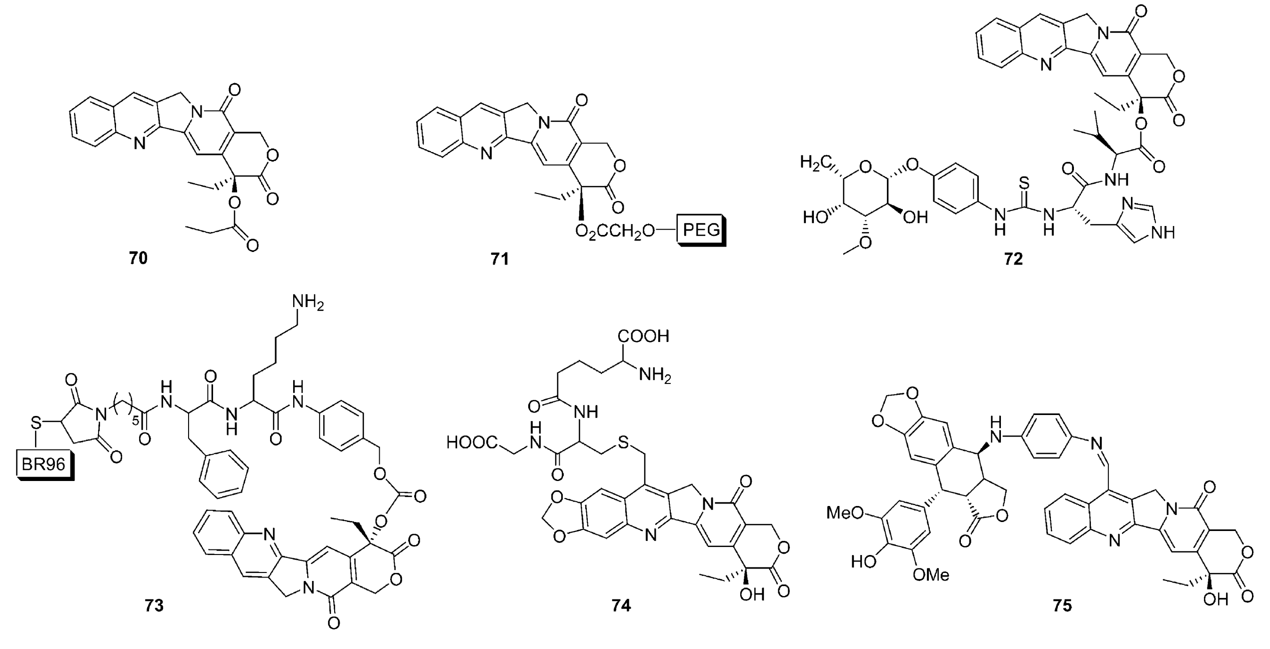


Figure 10. CPT conjugates.

the parent drug. The in vitro activity of the BR96 CPT conjugate was demonstrated to be superior to that of CPT alone.

The demonstration that substituents are well tolerated at carbons 7, 9 and 10 of the quinoline ring (*vide supra*) has prompted the use of these positions for the preparation of campthecin conjugates. The successful conjugation of glucuronic acid to 9-aminoCPT through an aromatic carbamate linker by Chern et al. exemplifies this approach.[114]

It has been reported that buthionine sulfoximine resulted in the cellular depletion of the ubiquitous antioxidant glutathione.[115] It was further demonstrated that the depletion of glutathione potentiated CPT-induced cytotoxicity. Interestingly, a glutathione–CPT conjugate, 7-(glutathionylmethyl)-10,11-methylenedioxyCPT (**74**) showed enhanced stability of the derived ternary complex.[115] Analogue **74** was shown to inhibit topo I and exhibit potent antineoplastic activity against U937 and P388 leukemia cell lines.

The observation that some CPT resistant cell lines concurrently developed resistance toward the topoisomerase II inhibitor etoposide, prompted Chang et al. to develop a CPT conjugate incorporating the 4β-amino-4′-*O*-demethylepipodophyllotoxin moiety of etoposide.[116] Two bioconjugates were synthesized differing only through the *ortho* (W2) and *para* (W1) phenylenediamine linkers and were shown to exert their cytotoxic effects primarily through topo I inhibition. Further, W1 (**75**) was demonstrated to be a more effective antineoplastic agent against human prostate cancer cells than either CPT or etoposide.

Human topoisomerase I exhibits sequence selectivity for thymidine in the −1 position during DNA cleavage; the extent of cleavage is enhanced by CPT when there is also a guanosine in the +1 position.[24a] In an effort to target specific DNA sequences, CPT has been conjugated to DNA oligonucleotides. Conjugates involving triplex-forming oligonucleotides (TFOs) covalently attached to campothecin through amide linkages with various aliphatic spacers have been demonstrated to be of utility in amplifying topo I-mediated cleavage at specific sites in DNA.[117] Dervan et al. and Arimondo et al. have also utilized this approach with CPT conjugates of minor and major groove binding ligands.[118]

6. Conclusions

Camptothecin and its analogues exhibit a broad spectrum of antitumor activity and are a very promising class of agents. Efforts devoted to understanding the mechanisms of action of CPT are yielding novel insights into this potent topo I inhibitor. Innovations in the synthetic preparation of CPT and the synthetic and semi-synthetic construction of novel CPT analogues are of great current interest. Enormous progress has been made in defining the biochemical loci at which CPT acts, and in optimizing those structural elements required for the expression of antitumor activity. These efforts have led to the approval for clinical use of topotecan (Hycamtin) (**3**) and irinotecan (Camptosar) (**4**) and to several novel CPT derivatives that are currently in various stages of clinical trials. The apparent function of the CPTs at multiple biochemical loci argues that additional progress can be made in the identification of CPT analogues that display improved properties as antitumor agents. High expectations surround the next generation of CPTs, and research continues on several important fronts.

Acknowledgements

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