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Perspectives on Biologically Active Camptothecin Derivatives

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Abstract

Camptothecins (CPTs) are cytotoxic natural alkaloids that specifically target DNA topoisomerase I. Research on CPTs has undergone a significant evolution from the initial discovery of CPT in the late 1960s through the study of synthetic small molecule derivatives to investigation of macromolecular constructs and formulations. Over the past years, intensive medicinal chemistry efforts have generated numerous CPT derivatives. Three derivatives, topotecan, irinotecan, and belotecan, are currently prescribed as anticancer drugs, and several related compounds are now in clinical trials. Interest in other biological effects, besides anticancer activity, of CPTs is also growing exponentially, as indicated by the large number of publications on the subject during the last decades. Therefore, the main focus of the present review is to provide an ample but condensed overview on various biological activities of CPT derivatives, in addition to continued up-to-date coverage of anticancer effects.

Keywords

Camptothecins; DNA topoisomerase I; biological activities; structure-activity relationship

INTRODUCTION

Camptothecin (CPT, **1**, Fig. **1**) is a pentacyclic alkaloid isolated by Wall *et al.*[1] in the early 1960s from the Chinese tree *Camptotheca acuminata*. This compound attracted immediate interest as a potential cancer chemotherapeutic agent due to its impressive activity against

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leukemias and various solid tumors in experimental systems. Due to CPT's negligible water solubility, clinical trials were initiated using the water-soluble sodium salt (**2**) of CPT in the early 1970s. [2] The trials were suspended in the 1970s due to lower efficacy of **2**, accompanied by unpredictable and severe levels of toxicity, including hemorrhagic cystitis and myelotoxicity. Interest in CPT then subsided for over a decade.[3,4] Revived attention resulted from the breakthrough discovery of DNA topoisomerase I (Topo I) as a therapeutic target for CPT. This discovery put CPT back on the frontlines of anticancer drug development in the late 1980s.[5-7] Accordingly, CPT's total synthesis, mechanism of action, structure–activity relationship (SAR), analog synthesis as well as pharmacology, formulation, drug delivery, preclinical studies and clinical trials have been studied extensively. Recent interesting research approaches include using prodrug concepts and drug delivery systems for CPT.[8]

As the result of these renewed research efforts, three CPT analogues, topotecan (TPT, **3**),[9] irinotecan (CPT-11, **4**),[10] and belotecan (CKD-602, **5**),[11] received governmental approval for the clinical treatment of ovarian, small-cell lung, and refractory colorectal cancers. Three additional water-soluble analogues, exatecan (DX-8951f, **6**)[12-14] lurtotecan (GG-211, **7**),[15,16] and sinotecan (**8**),[17,18] are currently under clinical evaluation. Moreover, preclinical and clinical studies of non-water soluble CPT analogues, rubitecan (9-nitrocamptothecin, **9**),[19,20] 9-aminocamptothecin (9-AC, **10**),[21] gimatecan (**11**),[22] karenitecin (BNP-1350, **12**),[23] and DB-67 (**13**),[24] are also ongoing. Interestingly, newly emerging homocamptothecin (hCPT) derivatives, BN-80915 (**14**, diflomotecan) and BN-80927 (**15**),[25,26] with a stabilized 7-membered hydroxylactone ring are currently undergoing clinical trials (**Fig. 2**). More recently, a CPT prodrug (**16**) and delivery systems (**17-19**) are also currently in clinical trials (**Fig. 3.**).[8,27-29] Today, with three successful compounds in clinical practice (**3–5**) and 14 compounds in clinical development, CPT analogues have become highly relevant clinical anticancer compounds.

Between 1966 and 2012, over 5,000 publications (journal articles and patents) on CPT were recorded. This dramatic number of publications reflects the research intensity in this field, as well as the interplay of enthusiasm and setbacks encountered during almost 50 years. Some excellent reviews on CPT derivatives from a historical point of view are available.[30-35] Recently, several reviews on the distribution, sources, applications, total synthesis, and SAR correlations of CPT have been published,[36-41] which cover the literature up to early 2005. However, since then, significant studies on new CPT derivatives have been carried out and published. A more comprehensive and up-to-date review is needed to describe such continued studies on anticancer and other biological activities, as well as rapid developments in the CPT field. This review presents more coverage not only in regard to structures and anticancer activities, but also other biological (antiviral, pesticidal, antiparasitic, antipsoriatic) effects of CPT-related derivatives.

2. Biological activity of CPT and related derivatives

2.1 Antitumor Activity

Following its isolation and structural elucidation in 1966, the naturally occurring CPT attracted considerable attention in the clinical community on the basis of its promising

antitumor activity in many in vitro and in vivo studies. In 1985, it was discovered that CPT inhibited the nuclear protein Topo I by a unique mechanism,[42] which stimulated revived interest in CPT as an important lead compound and led to active and clinically useful anticancer drugs, such as the approved TPT (**3**) and CPT-11 (**4**). Their clinical success and intriguing mechanism of action stimulated great interest in further exploration of CPT derivatives with better antitumor activity; among them, several derivatives are now undergoing preclinical evaluation. Published reviews have covered the literature concerning CPT until the early part of 2003. Here, we present continued up-to-date coverage of CPT in regard to structure and anticancer activity from 2004 to 2012.

2.1.1 A and B ring modified CPT derivatives—Numerous SAR studies have shown that substitutions in positions 7, 9, 10 are tolerated or can substantially increase anticancer activity. In particular, both TPT (**3**) and SN-38 (7-ethyl-10-hydroxy-camptothecin), the hydrolysis product and active metabolite of irinotecin (**4**) in which the carbamate group has been removed, have an OH substituent at position 10, which seems to be important either to increase water solubility or decrease unwanted stabilization of the open hydroxyacid form by human albumin. A recent X-ray crystallographic analysis of a ternary complex between a topo I construct, a DNA oligonucleotide and **3** indicated that modifications at the 7- and 9-positions of CPT would not interfere with drug–protein interactions. Furthermore, substitution at positions 7–10 and fusion of an additional ring on the A/B ring have led to potent compounds now in clinical studies, such as gimatecan, silatecan, lurtotecan, and exatecan. These successful examples imply that these positions can tolerate a large group and a wide possibility for structural modification. Accordingly, CPTs with lipophilic moieties at position 7 have been synthesized, including compounds with variously substituted C=N groups linked to the CPT scaffold via iminomethyl or oxyiminomethyl moieties. With one exception (**20**), several oxyiminomethyl substituted compounds (**21-23**) exhibited potent cytotoxic activity in vitro and in vivo comparable or superior to TPT (**3**).[43]

In a further study, Dallavalle *et al.*[44] synthesized a series of imines (**24-40**) derived from camptothecin-7-aldehyde and variously substituted aromatic amines and evaluated their cytotoxicity against tumor cell line H460. All of the prepared 7-aryliminomethyl CPT imines exhibited potent cytotoxic activity superior to that of **3** under the same conditions (**Table 1**).

Subsequently, a series of 9-substituted CPTs (**41-52**) derived from 9-formylcamptothecin were synthesized by the same group;[45] most of the new compounds showed higher cytotoxic activity than **3** (Table 2). Moreover, these compounds induced comparable DNA damage comparable to that of the reference compound SN-38. A molecular docking study suggested that the small polar 9-substituents interacted favorably with the topo I–DNA complex, which is consistent with their higher activity relative to corresponding 7-substituted CPTs.

You *et al.*[46] reported a series of 7-cycloalkylcamptothecin derivatives (**53-66**). As shown in **Table 3**, many of the compounds exhibited IC_{50} values in the low μM to nM level and were up to 40-fold more potent than **3**, the reference compound.

The authors' laboratories also designed and synthesized a series of 7-acyl CPT derivatives.[47] All of the new compounds showed significant inhibition of human tumor cell (A-549, DU-145, KB, and KBvin) growth, with IC_{50} values ranging from 0.0154 to 13.3 μM. Interestingly, while compound **67** showed a five-fold decrease in potency against KB-vin (IC_{50} = 0.13 μmol/L) compared with the KB cell line (IC_{50} = 0.023 μmol/L), compound **68** showed a two-fold increase in potency against the former cell line.[47]

Several 7-alkynyl CPT derivatives (**69–74**) were recently prepared via copper-free Sonogashira coupling by Xiao *et al.*[48] As shown in **Table 4**, most of the compounds were less cytotoxic than SN-38, but were generally more potent than **3**. Some of the new compounds showed almost equal cytotoxicity to SN-38 against Eca-109 and SGC7901 cells.[48]

Eighteen novel water soluble derivatives were designed based on the structures of **4** SN-38. Most of the new compounds possessed lower cytotoxicity compared with CPT. However, compound **75** exhibited potent cytotoxicity similar to CPT with IC_{50} less than 0.01 nM against KB and HCT-8 cancer cell lines, and compounds **76-78** showed similar or superior cytotoxic activity to **3**.[49]

Seventeen 10-arylcamptothecins were synthesized by Suzuki cross-coupling chemistry.[50] Some of the derivatives showed very potent cytotoxicity in preliminary in vitro cytotoxicity testing with IC_{50} values on the order of 9 nM. 10-(4-Pyridyl)camptothecin (**79**) and its water soluble hydrochloride **80** displayed comparable potency to **3** in various assays. Mechanistic studies indicated that **79** and typical CPT derivatives had similar pharmacological profiles in topo I inhibitory and cell cycle arrest assays.

Gao *et al.*[51] reported a new synthetic strategy using a Claisen rearrangement reaction to modify 10-allyloxy-7-ethylcamptothecin and generatc a series of 7-ethyl-9-alkyl derivatives (**81-86**) of CPT. As shown in **Table 5**, all of the new compounds exhibited significant in vitro cytotoxic activity against four tested tumor cell lines with IC_{50} values ranging from 0.012 to 3.84 μM, and were as or more potent than **3**. The biological results suggested that the small alkyl groups at the both 7- and 9-positions of CPT could promote liposolubility, as well as antitumor activity in vitro and in vivo, but did not increase stability of the lactone.[51]

Recently, a novel series of A-ring modified hexacyclic CPT derivatives containing a 1,3-oxazine ring were first designed and synthesized by Wang and coworkers.[52] All of the new compounds were assayed for in vitro cytotoxicity against nine human cancer cell lines and most of them showed impressive cytotoxicity. Compounds **87** and **88** showed the highest potency against several cell lines. Moreover, compound **88** (IC_{50} = 0.01 μM) was about 13-fold more potent than CPT (IC_{50} = 0.13 μM) and about 6-fold more potent than **3** (IC_{50} = 0.06 μM) against HEPG-2.[52]

Subsequently, several hexacyclic CPT analogs were designed by Niizuma *et al.*[53] based on the proposed structure of the topo I/DNA/CPT ternary complex. Remarkably, compound **89** exhibited *in vivo* antitumor activities superior to **4** in human cancer xenograft models in

mice at maximum tolerated doses, although its *in vitro* antiproliferative activity was comparable to SN-38 against corresponding cell lines.[53]

Glucuronide prodrugs are useful in antibody-directed enzyme prodrug therapy (ADEPT), because extracellular β-glucuronidase in tumor cells can be targeted by administration of antibody–β-glucuronide conjugates. Recently, a β-glucuronidase activated prodrug approach was applied to 9-aminocamptothecin and 10-hydroxycamptothecin. Compound **90**, a glucuronide derivative of 9-aminocamptothecin (**9**). is a promising β-glucuronidase-cleavable prodrug. It was less toxic than **9** against human tumor cell lines, but upon enzyme activation, displayed similar cytotoxicity to that of the parent drug. Furthermore, compound **90** showed promising in vivo prodrug properties and activity. Therefore, the same approach was applied to 10-hydroxycamptothecin, and the resulting compound (**91**) was 80 times more soluble than 10-hydroxycamptothecin in aqueous solution at pH 4.0 and stable in human plasma. Prodrug **91** was 10- to 15-fold less toxic than the parent drug against HepG2, Colo205, HT29, and H928 cell lines with IC_{50} values 56.5, 94.2, 97.8, 91.1 nM, respectively. Molecular modeling studies predicted that compound **91** would have a higher binding affinity to human β-glucuronidase than compound **90**. [54]

2.1.2 C and D ring modified CPT analogues—Historically, SAR efforts have largely focused on the A, B, and E rings of CPT. Relatively few D ring analogues have been investigated. Two early examples, 14-chloro and 14-nitro derivatives, were much less cytotoxic than the parent CPT, suggesting a lack of tolerance for substitution at that position.[55-57] Recently, Hecht and coworkers[58] synthesized a water-soluble 14-aza CPT (**92**), which is a hybrid between luotonin A and CPT. Compound **92** stabilized the topo I-DNA complex at the same sites as CPT and was cytotoxic with a similar but somewhat higher IC_{50} value then CPT. Further, the new compound mediated inhibition of DNA relaxation more effectively than CPT and possessed a faster off-rate from the ternary complex than CPT. It appeared that replacing the C14-H group with N augments the ability to form the ternary complex while concomitantly reducing the lifetime of the formed complex, thus reducing the cytotoxic effects of the resulting analogue.[58] Therefore, water-soluble 14-aza CPT represents an attractive core structure toward the development of a CPT analogue with useful therapeutic properties.

The synthesis and biological evaluation of the CPT thiopyridone isostere, thiocamptothecin (TCPT, **93**) were accomplished by using Lawesson's reagent. Significantly, TCPT was more potent than the parent compound against H460, HT29, and IGROV-1 cell lines. The increased cytotoxic potency of **93** versus CPT was even more evident against HT29 colon carcinoma cells and the subline HT29/mit. Also, compound **93** caused slightly more DNA damage to that observed for CPT, but an identical DNA cleavage pattern.[59]

More recently, Duan *et al.*[60] synthesized 14-aminocamptothecins **94** and **95**. These two compounds exhibited excellent cytotoxic potency against human tumor cell lines *in vitro*, and were not substrates for any of the major clinically relevant efflux pumps (MDR1, MRP1, and BCRP). Compounds **94** and **95** showed similar cytotoxicity against human and mouse bone marrow progenitor cells. In contrast, many CPT analogues are substrates for efflux pumps and are dramatically more toxic to human relative to murine marrow cells.

Compounds **94** and **95** demonstrated significant brain penetration when dosed orally in mice. Compound **94** showed significantly better efficacy relative to **3** when dosed orally in three ectopic xenograft models, H460, HT29, and PC-3.[60]

So far, few studies have featured C-ring synthetic modifications, probably because of the low anticancer activity observed with certain reported analogues, including 5-hydroxy, -alkoxy, -acyloxy, -hydroxymethyl, and -amino substituted derivatives,[61-63] as well as contradictory studies on 5-substituted CPT derivatives. For example, 5-propionyl and 5-ethoxy derivatives bearing a hydroxy, methoxy, or fluoro group at the β-position showed good antitumor activity. One of these analogues, DRF-1042, is currently in phase II clinical trials.[64] These findings prompted other studies to investigate the role of modification at the C-5 position. In 2009, using an enolate chemical protocol, in addition to C5-alkylated derivatives, new C5-fluorinated (**96**) and -aminated analogues (**97**), along with the first six-membered ring CPT derivative **(98)** were synthesized and tested for antiproliferative activity against human non-small-cell lung cancer carcinoma NCI-H460. The new compound was less active than **3** and SN-38, but caused similar DNA patterns to that of SN-38.[65]

2.1.3 E ring modified CPT analogues—Recent work on CPT has focused on construction of ester prodrugs at the C-20 position particularly aimed at new forms of administration (especially on a nanoscale) to optimize drug delivery. For example, Deshmukh *et al.*[66] recently synthesized a series of α-amino acid ester prodrugs of CPT. A successful example from this work is afeletecan (CPT glycoconjugate, **99**), a C-20 glycoconjugated, clinical prodrug. Compound **99** was the best candidate for a passively targeted sustained release lung delivery system.

In addition, a series of nitrogen-based 20(*S*)-hydroxyl CPT ester derivatives were prepared by Wang *et al.*[67] These ester compounds showed comparable or superior cytotoxic activity to **3**, but most of them were less cytotoxic compared with CPT. As shown in **Table 6**, 3-aminopropionates (**100**–**110**) were more cytotoxic in vitro against several human tumor cell lines than 3-amidopropionates (**111**, **112**). The 3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinin-2-yl)propionate ester **110** showed the best antitumor activity in vivo and in vitro among all compounds prepared.

Other nitrogenous heterocyclic groups have been introduced at CPT's 20-position to increase water solubility and improve activity. For example, compounds **113** and **114** modified with pyrazole acetates showed notable antitumor activity and lactone stability. Compound **114** achieved a tumor inhibitory rate (TIR) of 92.9% at a dose of 20 mg/kg (similar to the TIR of CPT at a dose of 10 mg/kg) and showed a better dose-efficacy relationship. Compound **113** achieved a TIR of 75.6% at a dose of 20 mg/kg. Compounds **113** and **114** were less toxic in mice than CPT based on change of body weight before and after administration.[68]

Based on previous work on nitroxyl free radicals as well as the fact that *L*-amino acids are actively transplanted into mammalian tissue, have good water solubility, and are often used as carrier vehicles for some drugs, the authors linked a nitroxyl radical moiety (1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxylic acid) at the 20-hydroxyl of CPT via different hydrophilic amino acid spacers to synthesize a series of novel spin-labeled CPT derivatives

(**115-119**). Compounds **115-119** showed similar or better *in vitro* cytotoxicity than the parent drug CPT and the clinically available drug **4** against human bladder cancer T-24.[69]

Recently, Hecht *et al.*[70] reported the effects of replacing the C-20 and C-21 O-atoms with S-atoms (**120**, **121**). 20-Mercapto CPT (**120**) stabilized the topo I-DNA covalent binary complex at the same site as CPT, but was less cytotoxic than CPT. The dimercapto CPT analog **121** had only a very slight effect on stabilization of the topo I-DNA complex and was not cytotoxic.

2.1.4 A, B, and E ring modified CPT analogues—The β-hydroxy-ε-lactone E-ring of hCPTs is generally more stable and hydrolyses less rapidly than the classical α-hydroxy-ö-lactone of CPTs.[71] In addition, the resulting ring-opened compound is an isolable product that does not spontaneously recyclize. Subsequent investigation of various A- or B-ring derivatives showed that some hCPTs are more potent topo I poisons than CPT and exhibit greater antiproliferative activity than CPT in both parental and CPT-resistant cell lines, particularly prostate cell lines. One highlight of these first structural studies was diflomotecan (**14**), a 10.11-difluoro-hCPT, which showed high in vitro cytotoxicity, promising in vivo efficacy, and was the first hCPT to enter clinical trials.[72,73]

As described previously, substituted C=N bonds increased antitumor activity in aryliminomethyl and oxyiminomethyl CPT analogues. Therefore, a series of novel 9-benzylideneamino derivatives of hCPT were designed with various substituents, including -Me, -NMe_2, and -OMe, on the benzylidenamino residue. The resulting derivatives showed excellent cytotoxic and topo I inhibitory activities. Compared with **3**, compounds **122–125** showed greater growth inhibition **(**IC_{50} 2.3–9.8 nM) against breast cancer cells.[74]

Subsequently, the same research group also published a bioisosteric series of compounds (**126-141**) bearing a C=C bond as a linker. From the results shown in **Table 7**, several hCPT compounds with an alkenyl or an (alkoxycarbonyl)ethenyl group exhibited good cytotoxic activity comparable or superior to **3** against A-549 cancer cells.[75]

The high cytotoxicity of hCPT derivatives prompted Zhang *et al.*[76] to investigate a series of water-soluble 10-phosphate esters. Most of the resulting compounds displayed moderate cytotoxicity against three tested cell lines (**142**–**149**, **Table 8**). From a structure-activity relationship (SAR) viewpoint, the cytotoxicity of the phosphodiesters was lower than that of the phosphotriesters and the length of the phosphate alkyl chain affected the potency. Compound **146** with dibutyl phosphate group showed greater tumor inhibitory activity than **4** in a A549 xenograft model, potent activity in a DNA cleavage assay at a concentration of 100 μM, and good stability at both pH 7.4 and 3.0. [76]

Seven new 7-trifluoromethylated hCPT derivatives were prepared by Zhu *et al.*[77] Three derivatives (**150-152**) possessed higher *in vitro* antitumor activity than **3**. Further in vitro and in vivo results provided convincing evidence that the 7-position of hCPT is a favorable site for introduction of a trifluoromethyl group.

To further promote lactone stability, Lu *et al.*[78] recently synthesized a series of hCPTs bearing a hydroxy or acetoxy group at the α-position of the E-ring β-hydroxy-ε-lactone

(**153-160**). Compared with 10-hydroxycamptothecin (10-HCPT) and CPT, most of the new compounds exhibited similar or better cytotoxic activity against HCT116 and A549 cells. When assayed for topo I inhibition, some compounds displayed potent activity at 100 or 10 μM, equal or superior to the activity of CPT (**1**), SN-38, and 10-HCPT. Moreover, compound **159** retained 90% and 60% of the lactone form after incubation at 37 °C for 8 h in phosphate buffered saline (PBS) at pH 7.4 and in PBS containing human serum albumin (HSA), respectively, while CPT retained only 30% and 18%, respectively. Therefore, the introduction of a second oxygenated substituent in the seven-membered E-ring is feasible and produces effective agents for the treatment of human cancer.[78]

Based on accumulated SAR studies and critical modeling clues, modifications at the 7-position appear to be the most efficient approach to increase the antitumor potency of CPTs. Furthermore, the crystal structures of topo I-DNA in complex with CPT revealed that C-7 substituents extended into the major groove of DNA, which could reinforce the stability of the inhibitor-topo I-DNA covalent complex. Recently Liu *et al.* presented a postulated binding mode of the hCPT compound class with the DNA-Topo I complex. In this model, they found a large space around the C-7 position of hCPT that allowed the introduction of substituted acyl groups with preservation of two key hydrogen bonds. Accordingly, they designed and synthesized a series of novel 7-acyl derivatives of hCPT. Compounds **161-163** showed highly potent cancer cell growth inhibitory activity with the IC_{50} values in the range of 1 nM to 2.2 nM against A549, MDA-MB-435, and HCT116 tumor cell lines.[79]

Li *et al* recently synthesized a series of five-membered E-ring CPT derivatives. Consistent with previous observations that five-membered E-ring analogs were inactive with respect to Topo I inhibition, the new racemic analogs generally exhibited markedly reduced cytotoxic activity against tested tumor cell lines. With IC_{50} values of 1.28 and 2.03 μM against A549 and HT-29 cancer cell lines, only compound **163** showed similar cytotoxic activity to **3**.[80]

Recently, due to the important role of fluorine substitution in drug design, a series of (20*S*, 21*S*)-21-fluorocamptothecins were designed and synthesized. All of these analogues showed potent in vitro antitumor activities and were potent Topo I inhibitors with increased hydrolytic stability. Among them, 7-cyclohexyl-21-fluorocamptothecin (**165**) exhibited the best antiproliferative activity against all three tested cancer cell lines (IC_{50} range: 0.71−0.07 μM), which was two-fold (A549) and six-fold (HCT116) more active than CPT. This compound represents a promising lead for further optimization.[81]

In continuing these efforts, our group recently reported that a series of 20-sulfonylamidine CPT derivatives displayed potent antitumor activity with significantly different drug-resistance profiles from those of CPT. Among them, compound **166** was more active than **4** against the growth of A549, DU-145, KB, and KBvin with IC_{50} values of 0.031, 0.050, 0.14, and 0.026 μM, respectively. Mechanistically, **166** induced significant DNA damage by selectively inhibiting Topo I and activating the ATM/Chk related DNA damage-response pathway. In mouse xenograft models, **166** demonstrated significant activity without overt adverse effects at 5 and 10 mg/kg, with two and three mice, respectively, among groups of eight undergoing complete regression. Notably, **166** at 300 mg/kg (i.p.) showed no overt acute toxicity in contrast to CPT (LD_{50} 56.2 mg/kg, i.p.) and **4** (LD_{50} 177.5 mg/33 kg, i.p.).

Thus, **166** is attractive as a potential candidate for anticancer chemotherapy and the modification with sulfonylamidine-substituted side chains may overcome some limitations of CPT.[82]

2.2 Antiviral activity

Besides anticancer activity, antiviral activity is another outstanding property of CPT and many of its derivatives. Topoisomerase I activity has been associated with various retroviruses including Rous sarcoma virus, [83,84] Molone murine leukemia virus,[85] equine infectious anemia virus, and human immunodeficiency virus (HIV).[86] Therefore, several studies have been conducted with CPT in order to elucidate the role of topo I in various retroviral functions.[87] Horwitz *et al.*[89] showed that CPT inhibited DNA and RNA synthesis in HeLa cells and induced DNA degradation, and then designed an experiment to determine whether it would inhibit growth of viruses that replicate in the cytoplasm of a host cell. Results indicated that CPT inhibited DNA synthesis in HeLa cells at a concentration of 10 μM. Inhibition of viral DNA synthesis was observed when CPT was added at 1 or 2 hours after infection.[89] Priel *et al.*[90] reported that non-cytotoxic doses of CPT inhibited HIV replication in acute infection of H9 cells at a high efficacy (>90%). Moreover, CPT inhibited EIAV replication in chronically infected Cf2Th cells. Continuous exposure of these cells to CPT for 52 days revealed 85 to 92% inhibition of virus production. Cell viability and growth rate were not affected.[91]

CPT inhibited HIV-1 LTR activity and viral production in cultures of human cells expressing HIV-1 LTR activity and cells chronically infected with HIV-1, respectively.[92] The mechanism of HIV-1 infection and the progression of immunosuppression were associated with activation of latent virus, which in turn was regulated by the long terminal repeat (LTR) in viral (proviral) DNA.[93] Based on these results, CPT may interact with a protein that either binds to the LTR or is involved in the posttranscriptional process.

Furthermore, some CPT analogues have been evaluated for antiviral activity. TPT (**3**) was efficient in treatment of AIDS-related progressive multifocal leukoencephalopathy.[94] Sadaie *et al.*[95] reported that 9-nitrocamptothecin (**9**) inhibited HIV-1 replication in freshly infected U937 monocytoid cells. Subsequently, the authors also investigated in vitro anti-HIV activities (including HIV-1 and HIV-2) of 10-hydroxy-CPT (**167**) and 7-hydroxymethyl-CPT (**168**).[96] The results demonstrated that **168** showed more potent anti-HIV activity than CPT, while **167** was less active. Taken together, the above studies indicate that CPT can serve as a valuable lead compound for developing a new anti-retroviral drug.

A few studies have demonstrated that CPT is a potent inhibitor of replication, transcription, and packaging of double-stranded DNA-containing adenoviruses, papovaviruses, and single stranded DNA-containing autonomous parvoviruses. These findings indicated that CPT analogues could be developed for use as potent drugs against DNA viruses. Some researchers have reviewed the literature focusing on antiviral potential.[97,98]

Yamada and coworkers[99] showed that CPT is active against human HSV-2 due to drug inhibition of host cell Topo I, which was apparently involved in the process of transcription, but not in the elongation step of HSV-2 DNA synthesis. In addition, mappicine ketone

(MPK, **169**), a decarboxylated E-ring CPT analogue, has recently been identified as an antiviral agent with selective activities against HSV-1, HSV-2, and human cytomagalovirus (HCMV) with PR_{50} values of 2.9, 0.5, and 13.2 μM, respectively. MPK appears to be herpesvirus-selective; it does not inhibit other DNA or RNA viruses. Although its mechanism of action has not been determined, MPK functions differently from acyclovir (ACV) as demonstrated by the observation that ACV-resistant HSV-1 and HSV-2 are inhibited by MPK and that MPK resistant mutants are sensitive to ACV at HSV-1 wild-type virus levels.[100,101] More recently, the authors assessed the *in vitro* antiviral efficacies of CPT analogues against herpes simplex virus type 2 (HSV-2) in Vero cells.[103] Several compounds exhibited similar or better antiviral activity than ACV against HSV-2 in vitro. Among them, compound **170** was the most potent with an IC_{50} value of 1.3 *μ*g/mL and selectivity index (SI) of 27.04.

2.3 Pesticidal activity

In ancient China, the crude extract of *C. acuminate* containing CPT has been used traditionally to control insect pests for centuries, and it was reported to be a potent chemosterilant against the housefly and cabbage caterpillar.[103,104] It also exhibited significant inhibitory activities against several agricultural pests like *Empoasca vitis*, *Mythimna separate* Walker, *Brevicoryne brassicae*, *Nilaparvata lugens*, and *Chilo suppressalis*, supporting its potential use as a field pesticide.[105-108] Additionally, a recent study showed that CPT could cause visible changes in the midguts from the lepidopteran pests *Trichoplusia ni* and *Spodoptera exigua*, such as losing the single layer of epithelial cells and disrupting the peritrophicmembrane.[109] Investigations by Zhong *et al.*[110] also demonstrated that CPT-induced apoptosis in SL-1 cells and midgut cells of *S. litura*. Consistent with these results, Zhang *et al.*[111] recently revealed that CPT caused Sf21 and IOZCAS-Spex-II cell apoptosis via a mitochondrial-dependent apoptosis signal transduction pathway, suggesting that its mode of action may be related to apoptosis. Moreover, pretreatment with CPT led to reduction in both the enzymatic activity and the steady accumulation of the Topo I protein in IOZCAS-Spex-II cells despite up-regulation of its mRNA expression in response to the treatment.[112] In connection with these efforts, in order to find new CPT-derived insecticides with improved profiles and to clarify the structure–activity relationships of campothecin analogues, a number of CPT derivatives modified in the different positions have been synthesized and evaluated their insecticidal activity against some important insect pests by our group;[113-117] among which, some compounds exhibited insecticidal activity equal to or higher than that of CPT. For example, we synthesized a series of spin-labeled CPT derivatives by esterifying the 20-hydroxyl of CPT with *L*-amino acids containing a nitroxyl radical moiety and evaluated their antifeedant and insecticidal effects against third-instar larvae of *Mythimna separate*. In the antifeedant bioassays, the spin-labeled compounds were less potent than CPT.[113] All of the derivatives showed delayed insecticidal activity, which was different from traditional neurotoxic insecticides. Furthermore, eight spin-labeled CPT analogues (**172-179**) were synthesized based on 5-(2'-hydroxythoxy)-20(S)-camptothecin (**171**). When tested against fifth-instar larvae of *Brontispa longissima*, compounds **171** and **172** showed promising insecticidal activity with corrected mortality rates of 69.55% and 74.07%, respectively.[115]

Recently, a series of CPT derivatives via alkylation, oxidation, and esterification at the 5-, 7-, and 20-positions were synthesized. Among them, compounds 7-CH_2OH-CPT, 7-COOH-CPT, and 10-OCH_3-CPT displayed higher antifeedant activity (>90%) than CPT against third-instar larvae of *Spodoptera litura* at 24 h and 48 h. In addition, several compounds, including 7-$CH_2C_6H_5$-CPT, 7-CHO-CPT, 7-$CH_2OCOC_6H_5$-CPT, 10-O-$CH_2OCOC_6H_5$-CPT, 20-$CH_2OCOC_6H_5$-CPT, and 20-F-CPT exhibited more potent nematocidal activity (LC_{50} 2.28, 2.21, 1.37, 1.68, 0.13, and 1.71 mg/L, respectively) against *Bursaphelenchus xylophilus* than CPT (LC_{50} 12.18 mg/L) after 24 h.[116,117]

More recently, we also investigated a large variety of CPT analogues for antifeedant activity against *Spodoptera litura*. Several compounds, including 20-SH-CPT,21-*N*-amino-CPT lactam, and 7-acetyl-CPT, displayed more potent antifeedant activity than CPT (**Table 10**).[118]

On the other hand, plant-derived CPTs can be used to deter and eliminate termites, particularly subterranean species. Pure or raw water-insoluble CPT and its analogs may be useful in termite control strategies as barriers at higher concentrations (>50 ppm) by preventing termites from colonizing or feeding on particular substrates and structures or as toxicants in termiticides and baits particularly at lower concentrations.[119]

2.4 Antiparasitic activity

African trypanosomes (*Trypanosoma brucei* species) are parasitic protozoa that cause lethal diseases in humans and cattle. Studies showed that CPT was cytotoxic to African trypanosomes and related pathogenic hemoflagellates.[120] CPT generated covalent DNA–topoisomerase complexes with both nuclear and kinetoplastic preparations of DNA from trypanosomes, leishmanias,[121] and other protozoan parasites of medical importance. Bodley *et al.*[122] showed that CPT inhibited the nuclear and mitochondrial topo I of *T. brucei* thus blocking DNA replication and inducing the death of bloodstream trypomastigotes (IC_{50} = 1.6 μM). In SAR studies, Bodley *et al.*[123] also tested a series of CPT analogues for their in vitro effectiveness against African trypanosomes and found that their cytotoxicity was closely correlated to the ability to promote the formation of covalent protein–DNA complexes. This finding indicated that the sole cellular target of these agents is the parasite's topo I. Significantly, antiparasitic activity was increased by addition of substituents to the parent ring system (e.g. 10.11-methylenedioxy or ethylenedioxy groups, 7-alkyl groups, or 9-amino or 9-chloro substituents) (**Table 11**). Among them, 9-substituted-10,11-methylenedioxy derivatives were significantly more active than CPT against *T. brucei* bloodstream trypomastigotes. For example, 9-chloro-10,11-methylenedioxy-CPT (IC_{50} 0.041 μM) was 40 times more potent than CPT (IC_{50} 1.6 μM). Although these CPT derivatives were still more toxic against mammalian cells than trypanosomes, selective toxicity might be achievable using this lead compound as a starting point.

Recently, Werbovetz *et al.*[124] further examined CPT and four 10,11-methylenedioxy analogues the against pathogenic protozoan *Leishmania donovani* in vitro. Compared with CPT, 10,11-methylenedioxy-CPT (**180**) exhibited 90-fold greater antileishmanial activity (EC_{50} 0.064 mM). Introduction of difluoro substitution at the methylene position (**181**)

reduced the activity, while introduction of a chloro (**182**) or amino (**183**) group at position 9 resulted in two-fold greater activity than **180**.

Furthermore, Proulx *et al.*[125] 3xplored tested the efficacy of free and liposome-loaded CPT against leishmaniasis, the parasite burden was significantly reduced when infected mice were treated with 2.5 mg/kg body weight CPT via intraperitoneal injections of free and liposomal CPT. Its excellent index suggested that a liposomal delivery may be exploited as a potential strategy against visceral leishmaniasis.

2.5 Antipsoriasis activity

Psoriasis is a chronic inflammatory skin disease characterized by epidermal keratinocyte hyperproliferation, abnormal differentiation, and inflammatory infiltration. Since the early 1970s, several reports of topical preparations of *Camptotheca* or CPT in treatment of psoriasis have been published.[126-129] these early studies showed that, as a topoisomerase inhibitor, CPT is effective in psoriasis therapy. Recently Wang *et al.*[130] also reported that 10-hydroxy-CPT exhibited significant curative efficacy in treatment of psoriasis. Subsequently, Lin *et al.*[131,132] revealed that CPT and iso-CPT inhibited the growth of cultured normal human adult keratinocytes *in vitro* by inducing apoptosis. Furthermore, study by Lin *et al.*[133,134] demonstrated that the therapeutic mechanism of CPT in psoriasis may be associated with its antiproliferative activity and apoptosis of keratinocytes through down-regulation of telomerase activity.

2.6 Antifungal/ Antimicrobial activity

Del Poeta *et al.*[135] evaluated antifungal activity of CPT and its analogues in *vitro* against an isogenic series of *S. cerevisiae* strains including wild-type, △*erg6* permeable mutant, △*erg6* △*top1* double mutant, and △*erg6*△*top1* mutant strains expressing *C. neoformans* topo I. They found that some derivatives were extremely active antifungal agents with minimum inhibitory concentrations (MICs) of less than 0.09 μg/mL when the strain contained overexpressed cryptococcal topo I. Particularly, a 10,11-methylenedioxy system in ring A (**184** and **185**), increased interaction with the fungal topo I. These findings suggested that CPT and certain derivatives can efficiently target the *C. neoformans* topo I enzyme to produce antifungal activity. Considering CPT's potent antifungal activity, Li *et al.*[136] evaluated CPT against *Alternaria alternata*, *Epicoccum nigrum*, *Pestalotia guepinii*, *Drechslera* sp., and *Fusarium avenaceum*. CPT inhibited mycelial growth by approximately 50% (EC_{50}) at 10–30 μg/mL and fully inhibited growth at 75–125 μg/mL. Recently, Zhang *et al.*[137] also reported that CPT was effective against *Rhizoctonia solani, Sphaerotheca fuliginea*, and *Pseudoperonospora cubensis* under greenhouse and field conditions. Under greenhouse conditions, the IC_{50} and IC_{90} values of CPT against the three plant pathogens were 41.96 mg/L, 40.49 mg/L, 27.48 mg/L, and 756.77 mg/L, 247.02 mg/L, 341.81 mg/L respectively. Under field conditions, CPT also showed high inhibitory effects against the *P. cubensis* pathogen; the IC_{50} and IC_{90} values were l1.22 mg/L and 69.12 mg/L, respectively.

More recently, Alaghaz *et al.*[138] synthesized a series of 10-substituted CPT phosphorothioate analogues and evaluated their antifungal /antimicrobial activity against fungal strains *Aspergillus niger, Aspergillus flavus (molds), S. cerevisiae, C. albicans, T.*

longifucus, *A. flavus*, *M. canis*, *F. solani*, and *C. glaberata (yeasts)*. However, none of the tested compounds affected fungal growth significantly.

2.7. HIF-1 inhibitory activity

Hypoxia hypoxia-inducible factor 1(HIF-1) has attracted considerable attention as a molecular target for new antitumor agents because of its involvement in various aspects of cancer cell biology. In 2002, researchers at the National Cancer Institute screened 2000 diverse compounds for functional inhibition of hypoxia-inducible factor 1 (HIF-1), a master regulator of a cancer cell's ability to survive under oxygen deprivation. Active compounds included three CPT analogues [TPT (**3**), CPT 20-ester (*S*) (**186**), and 9-glycineamido-20(*S*)-CPT HCl (**187**)]. [139]

The best characterized compound (**3**) inhibited both hypoxia (1% O_2)- and iron chelator (desferoxime; DFO)-induced HIF-1 activation at submicromolar concentrations. Compound **3** inhibited HIF-1 by decreasing HIF-1α protein translation in a topo I-dependent, oxygen-independent manner.[140] The HIF-1 inhibitory activity of **3** was reversible and schedule-dependent *in vitro* (U251 cells), and required a daily (not intermittent) administration schedule *in vivo* (U251 tumor xenograft model).[139] Hence, these drugs may have other desirable activities against solid tumors that are independent of topo I poisoning. The therapeutic potential of TPT (**3**) as a HIF-1 inhibitor has been reviewed recently.[142] This critical clue also prompted Jones and co-workers[143] to synthesize several 5-substituted CPT analogs through an efficient microwave-mediated procedure. Among the newly synthesized compounds, a 5-fluoroethyl CPT analog showed potent HIF-1a inhibitory activity with an inhibitory rate of 88% at 10 μM.

More recently, Klausmeyer *et al.*[144] isolated and identified four bioactive CPTs from an extract of *Ophiorrhiza trichocarpon*, which reduced hypoxia induction to 22% of control in U251-HRE cells and exhibited an EC_{50} of 0.21 μg/mL with minimal effect on U251-pGL3 cells.

2.8. Other biological activities

A study by Clements *et al.*[145] showed CPT was not only capable of inhibiting endothelial cell growth in a non-toxic manner, but also it inhibited angiogenic growth. In a study to investigate the antiangiogenic and antitumor effects of oral ST1481 (gimatecan) in human tumor xenografts, Petrangolini *et al.*[146] suggested that the antiangiogenic properties of ST1481 could possibly contribute to its antitumor potential and that this effect might be enhanced by continuous low-dose treatment. Recent results have shown that CPT-11 is an effective inhibitor of angiogenesis and provide strong implications for wider clinical application of this drug for colon cancer.[147] This observation demonstrates that, besides the tumoricidal activity, CPT may have indirect antitumor activity due its anti-angiogenic activity and, thus, may have clinical relevance in treating other conditions such as restenosis and psoriasis.

In addition, CPT (10^{-8} M to 10^{-6} M) significantly inhibited secretion of newly synthesized collagenous proteins into conditioned media by 50%. CPT (10^{-8} M to 10^{-6} M) caused a

significant dose-dependent inhibition of COL1A2 mRNA levels and COL1A2 promoter activity by as much as 60%. The inhibitory effect of CPT on collagen production by fibroblasts from patients with systemic sclerosis suggests that CPT may be effective in limiting fibrosis in such patients.[148] Furthermore, CPT has also proved effective in the treatment of rheumatoid arthritis because it reduces the activation of the complement system.[149, 150]

Other biological activities of CPT are also receiving increased interest, it has been reported that CPT-11 (**4**) inhibited acetylcholinesterase activity.[151] 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.

More recently, the neurotoxic activity of CPT in cultured cerebellar granule neurons has been investigated. The screening results showed that CPT-induced neurotoxicity may be due to induction of protein–DNA cross-links and other unknown drug-related interactions rather than inhibition of topo I activity alone. [152]

The variety of biological activities and medicinal applications exhibited by CPT analogues are impressive. Additional systemic investigations of the biological activities utilizing numerous compounds should further uncover new physiological information and medicinal uses for CPT analogues.

3. Conclusion

Almost five decades after CPT's first isolation, CPT-based drugs remain appealing to many researchers worldwide and more CPT analogues are emerging as promising chemotherapeutic agents. The discovery of Topo I as CPT's therapeutic target opened a new area for anticancer drug development. The tremendous efforts in this field included the total syntheses or semisyntheses of CPTs, which have been crucial to make new anticancer drugs of this family possible. This review has summarized up-to-date coverage of CPTs in regard to structure modification and anticancer activities from 2004 to 2012. However, the expectation and value of CPT as a lead compound go beyond the development of anticancer agents, as additional new biological activities have been discovered. Based on such various biological activities, CPT will continue to attract tremendous attention and long lasting interest from both the academic community and the pharmaceutical industry. On the other hand, continued studies on the CPT–DNA–topo I interaction may suggest new directions in the development of CPT-related biological molecules. Interest in CPTs will undoubtedly remain high based both on the current pharmaceutical potential and the further discovery of new and better drugs based on varied biological activities.

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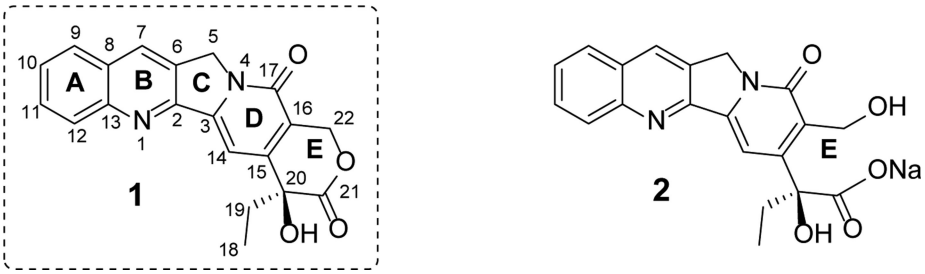
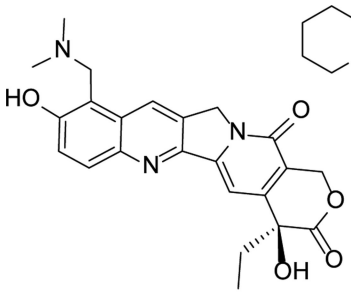
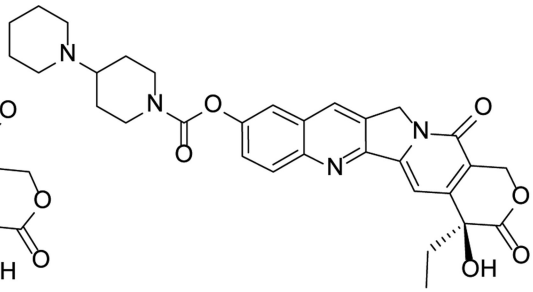


Fig 1.
Structures of camptothecin (**1**) and camptothecin sodium salt (**2**).



Topotecan (**3**)



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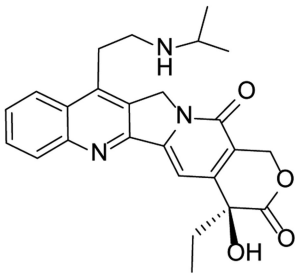


Belotecan (**5**)

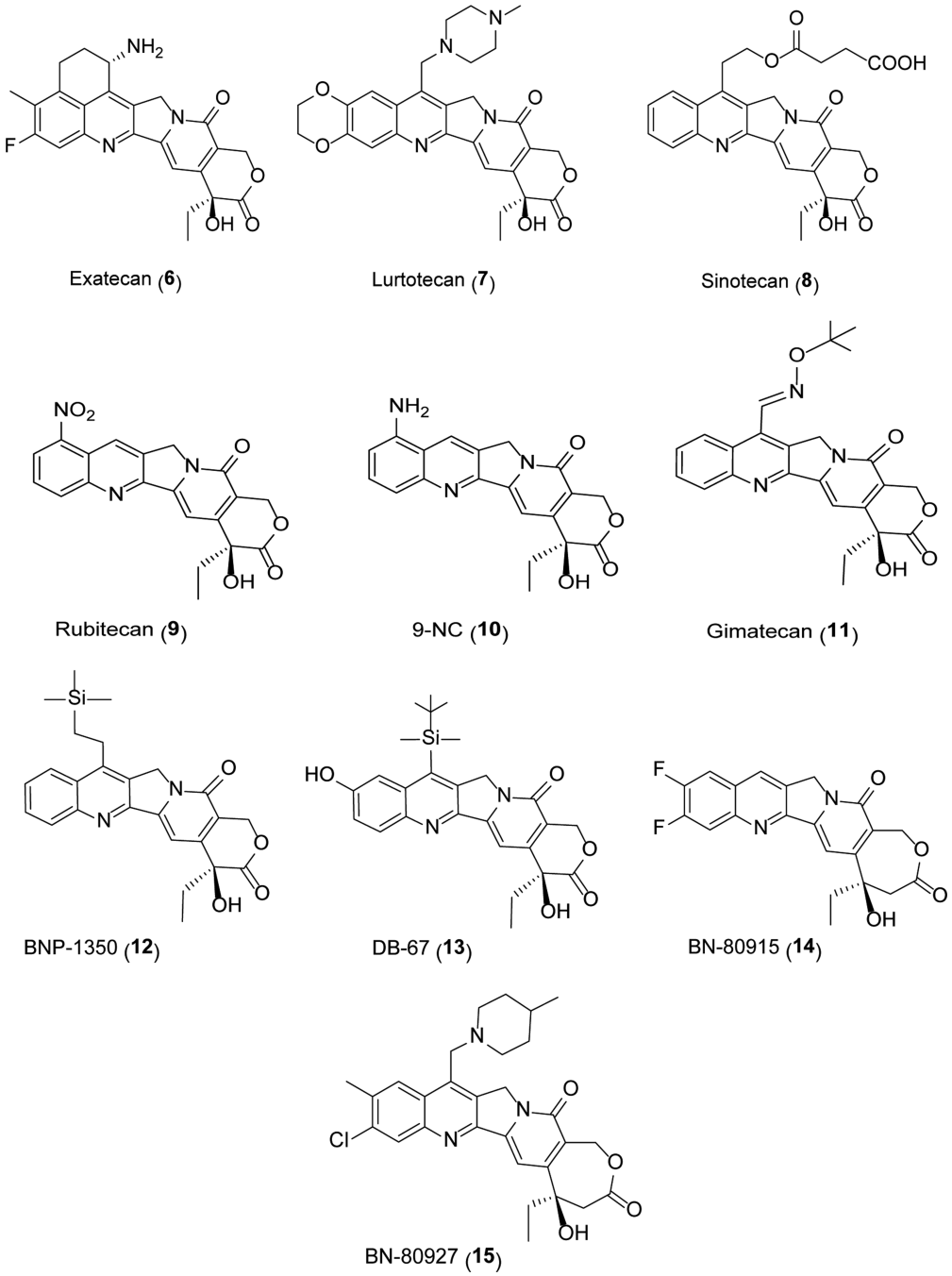


Fig. 2.
Structures of CPT analogs in clinical practice or clinical trials.

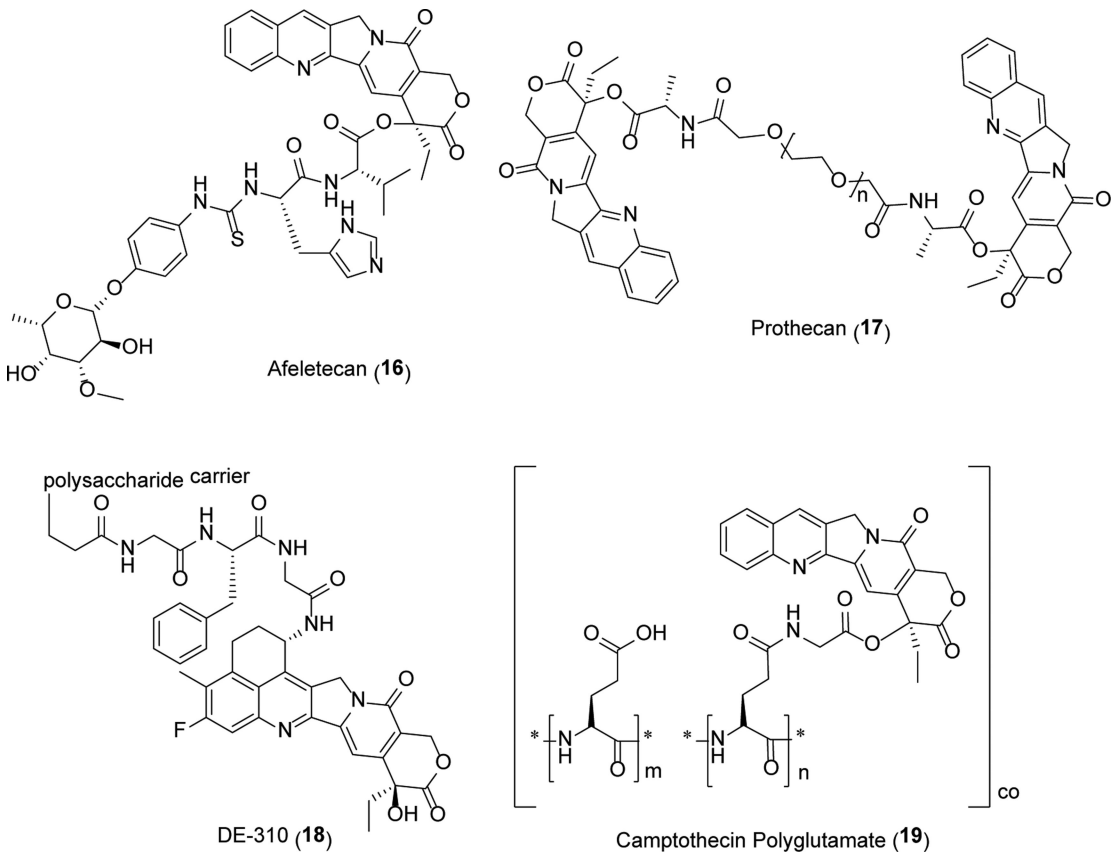


Fig. 3.
CPT prodrugs and delivery systems currently in clinical trials.

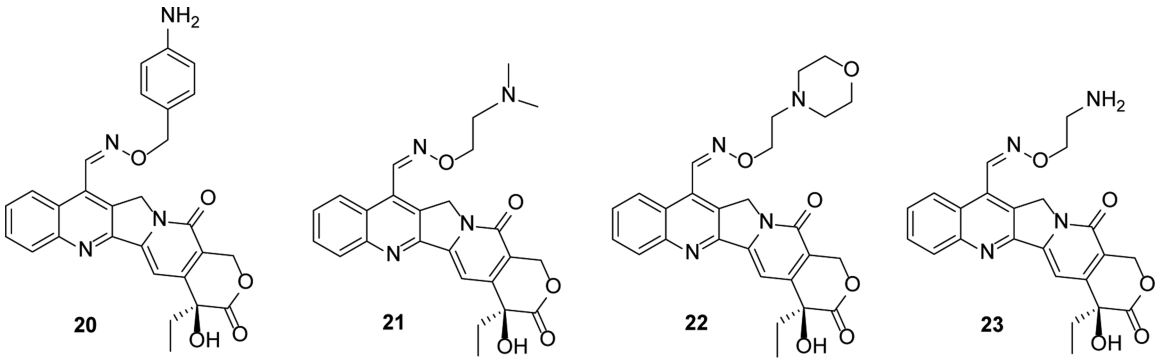


Fig. 4.
Structures of 7-substituted lipophilic CPTs (**20**-**23**).

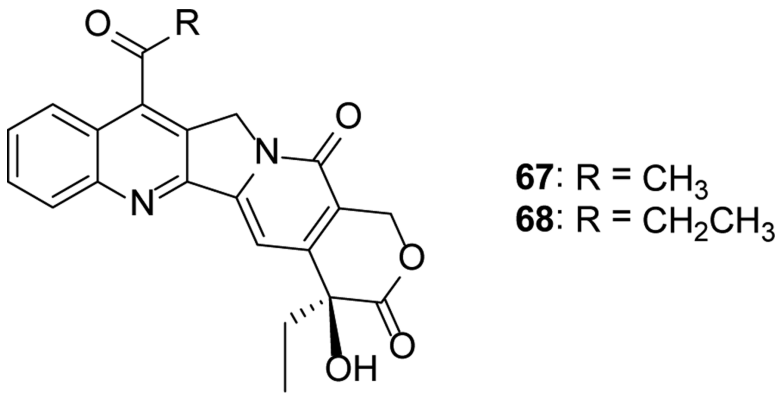


Fig. 5.
Structures of 7-acyl-CPT derivatives (**67**,**68**).

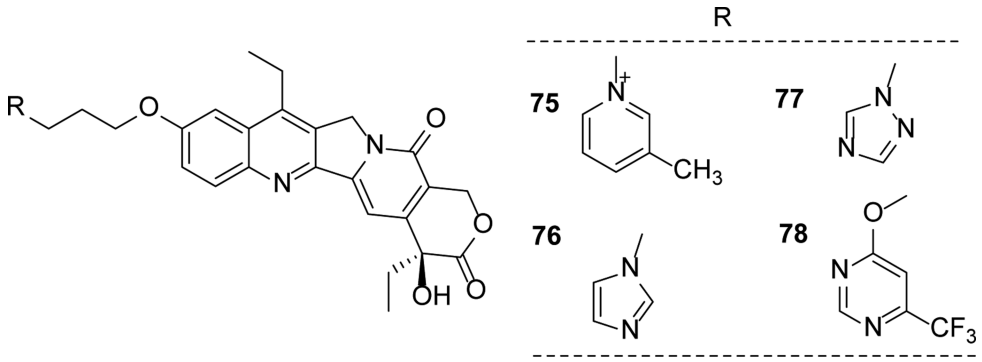
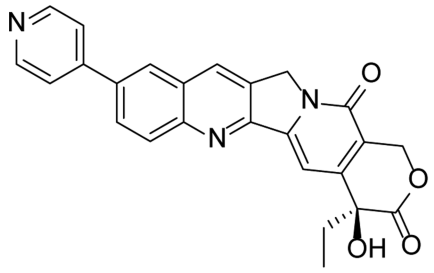


Fig. 6.
Structures of 10-position substituted heterocyclic derivatives (**74**-**77**).



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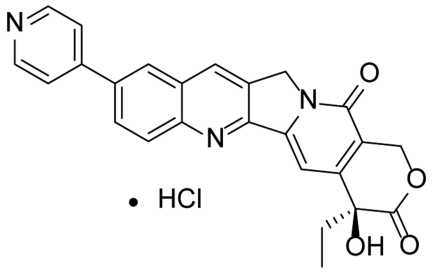

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Fig. 7.
Structures of 10-arylcamptothecins (**79,80**).

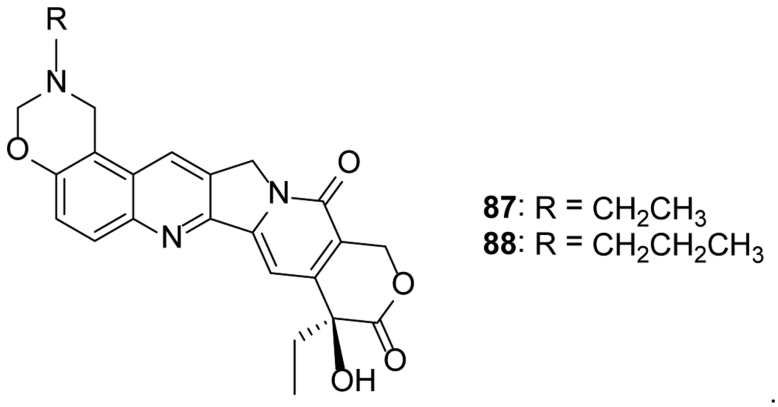


Fig. 8.
Structures of derivatives containing 1,3-oxazine ring (**87**, **88**).

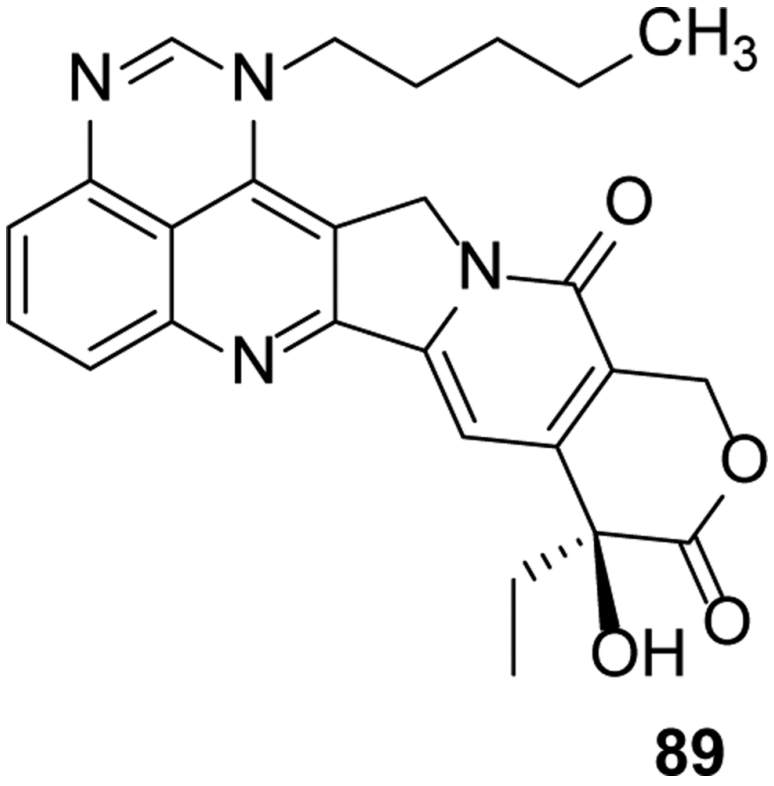


Fig. 9.
Structure of compound **89**.

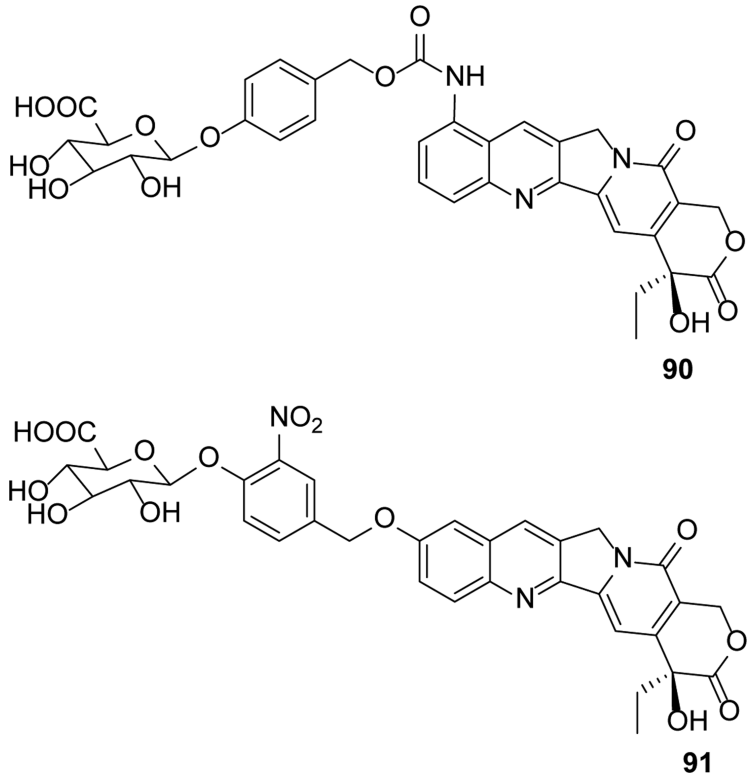


Fig. 10.
Structures of glucuronide derivatives **90** and **91**.

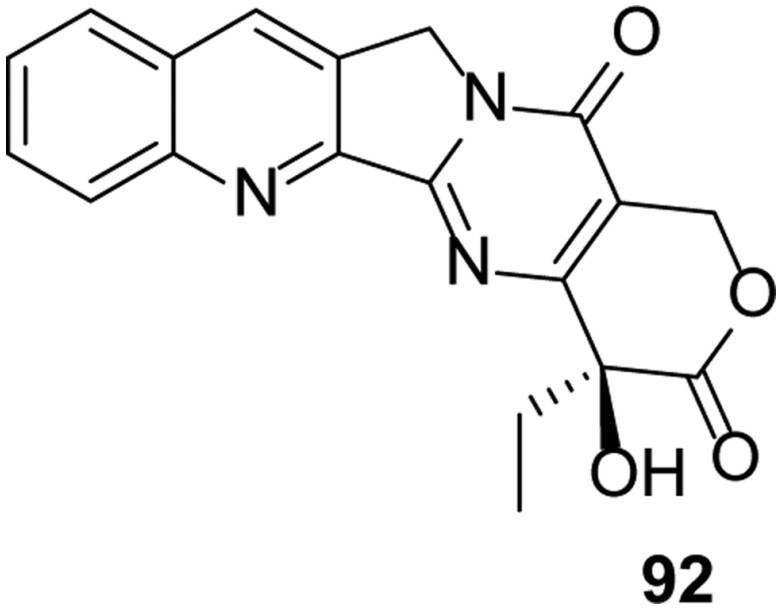


Fig. 11.
Structure of 14-aza-CPT (**92**).

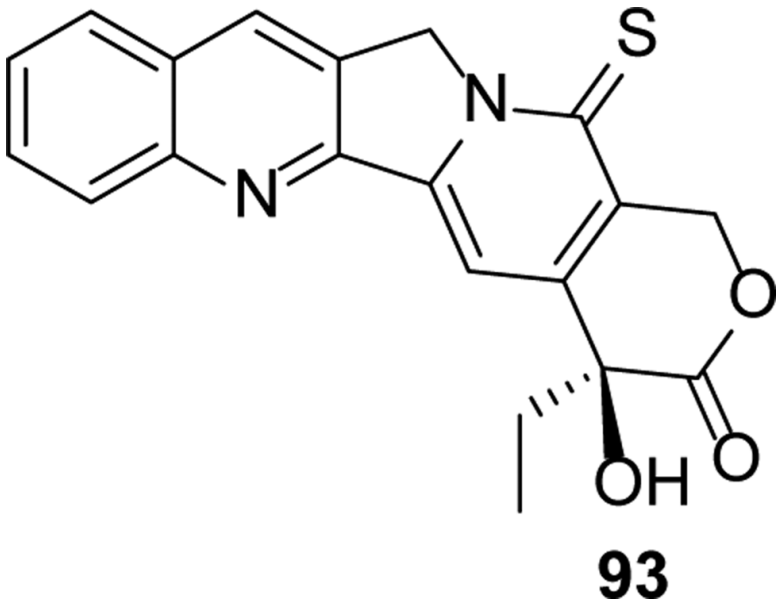


Fig. 12.
Structure of thio-CPT **93**.

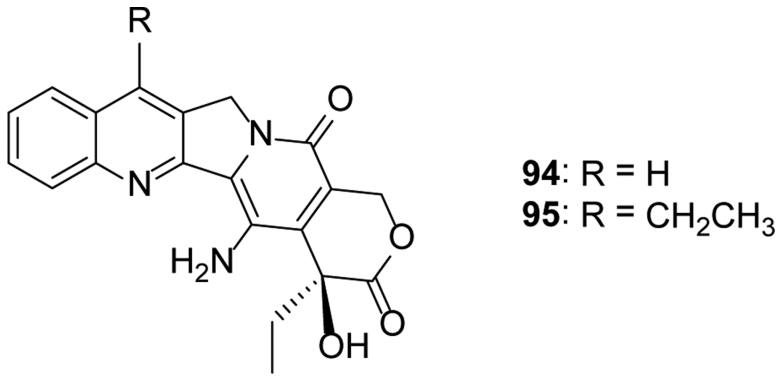


Fig. 13.
Structures of 14-amino-CPTs **94** and **95**.

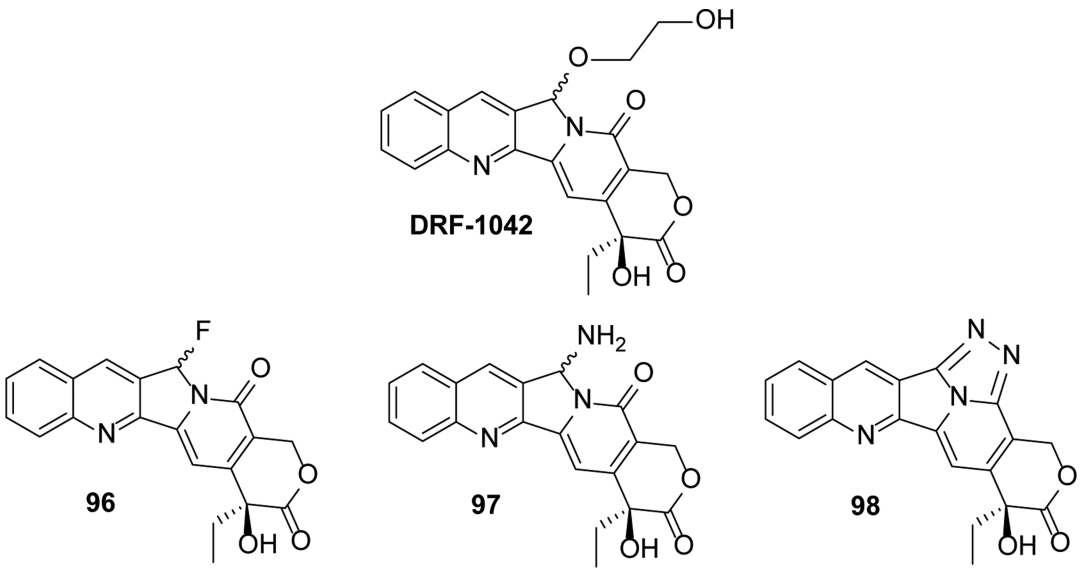


Fig. 14.
Structures of C5-substituted analogues (**96**-**98**).

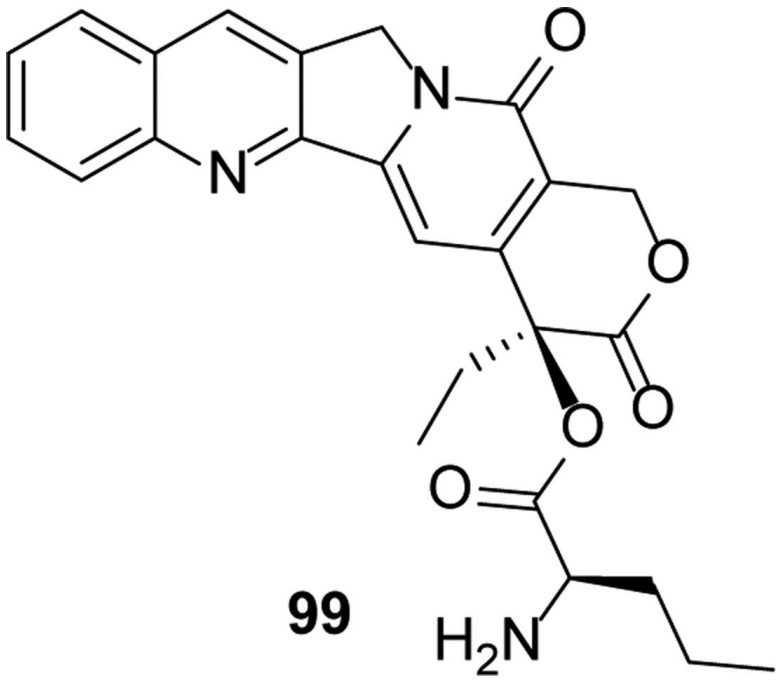


Fig. 15.
Structure of compound **99**.

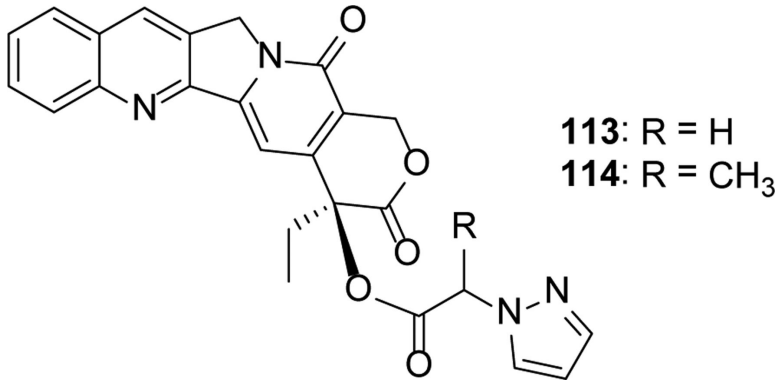


Fig. 16.
Structures of compounds **113** and **114**.

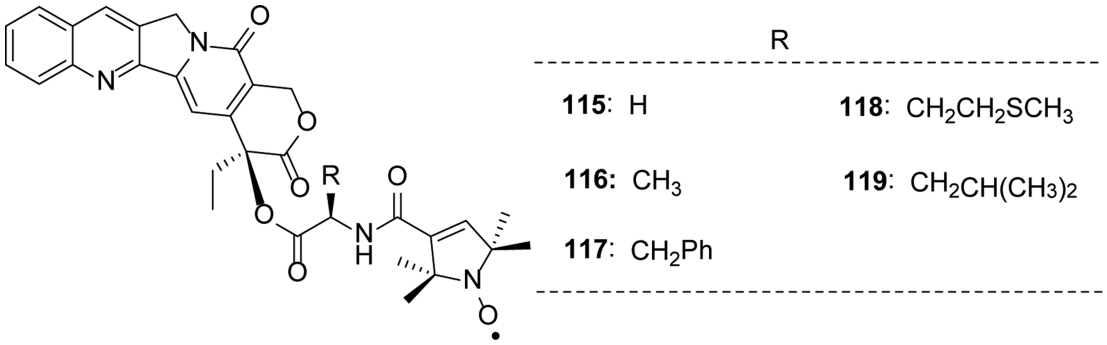
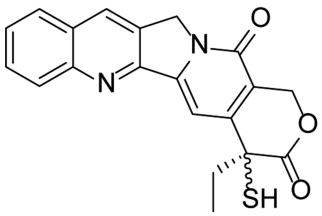
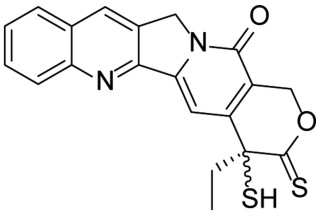


Fig. 17.
Structures of spin-labeled CPTs (**115-119)**.



120



121

Fig. 18.

Structures of compounds **120** and **121**.

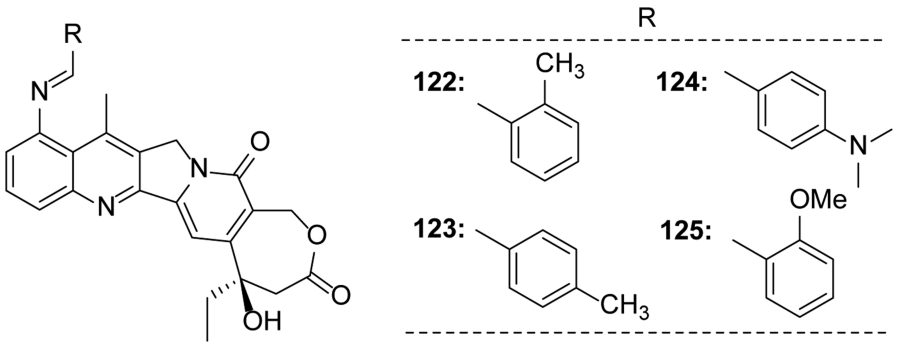


Fig. 19.
Structures of 9-benzylideneamino hCPT derivatives (**122-125)**.

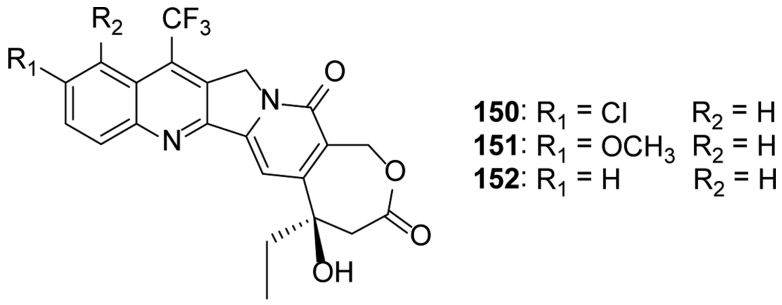


Fig. 20.
Structures of 7-trifluoromethylated homocamptothecin derivatives (**150-152)**.

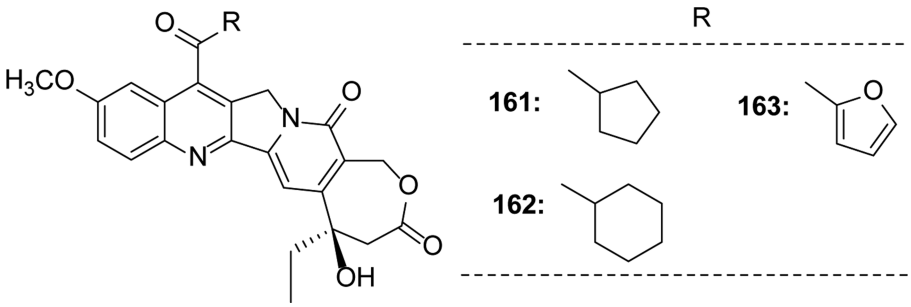


Fig. 21.
Structures of 7-acyl homocamptothecin derivatives (**161-163)**.

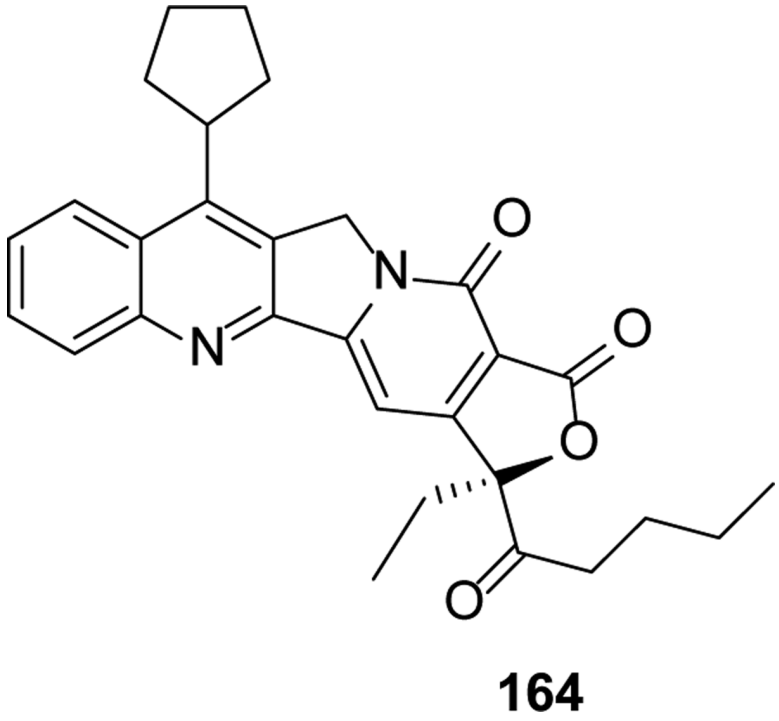
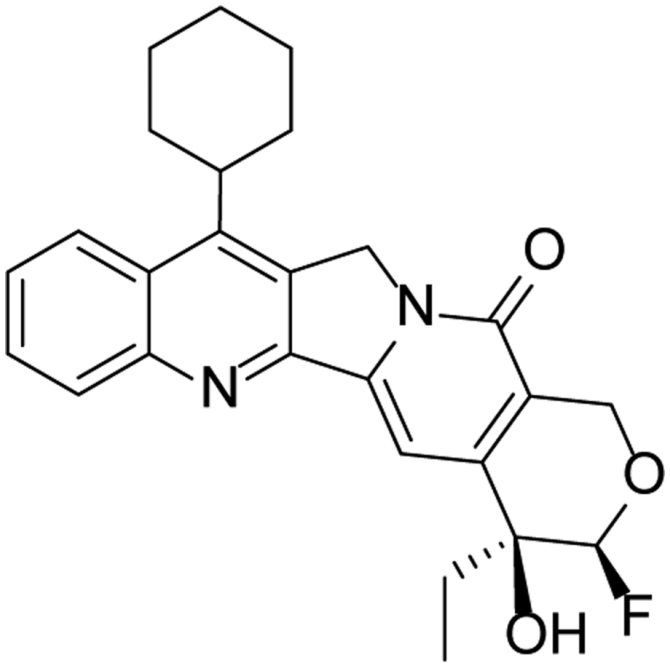
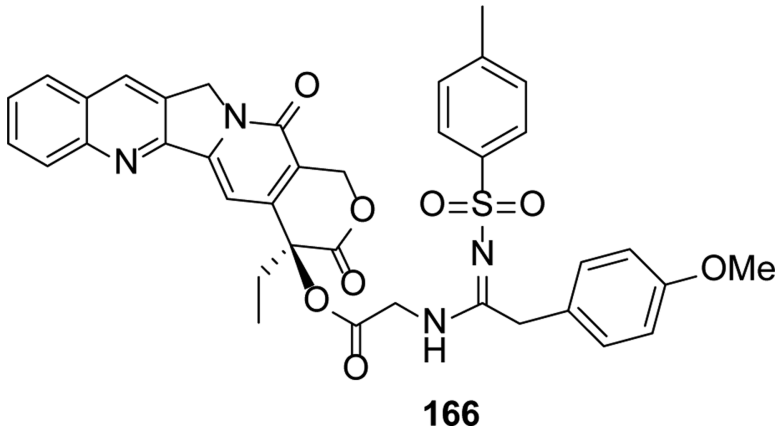


Fig. 22.
Structure of compound **164**.



165

Fig. 23.
Structure of compound **165**.



166

Fig. 24.
Structure of compound **166**.

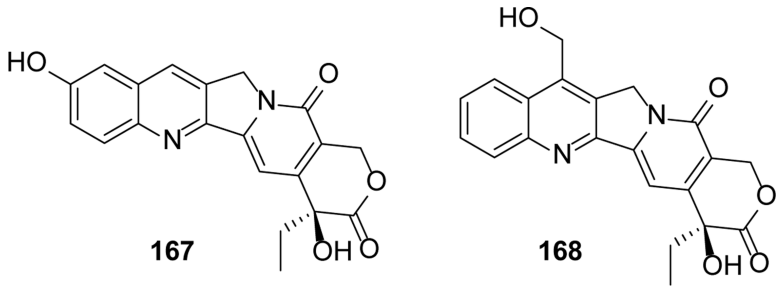


Fig. 25.
Structures of compounds **167** and **168**.

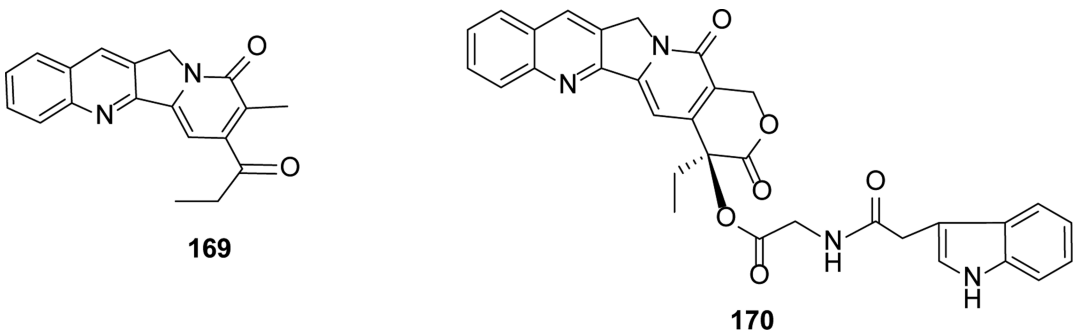


Fig. 26.
Structures of compounds **169** and **170**.

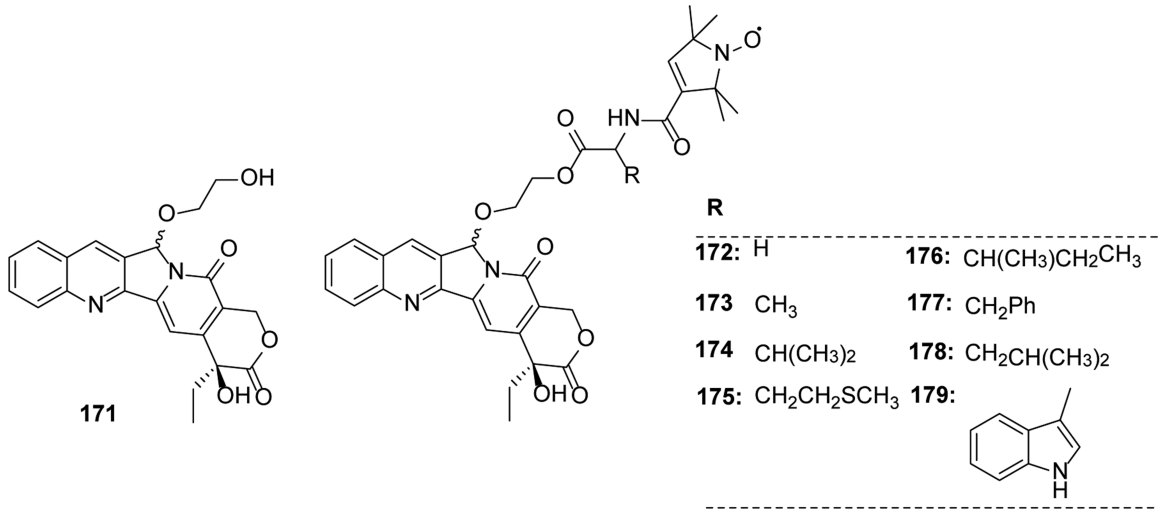
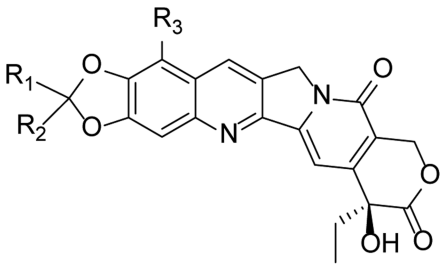


Fig. 27.
Structures of compounds **171-179**.



	R_1	R_2	R_3
180	H	H	H
181	F	F	H
182	H	H	Cl
183	H	H	NH_2

Fig. 28.
Structures of compounds **180–183**.

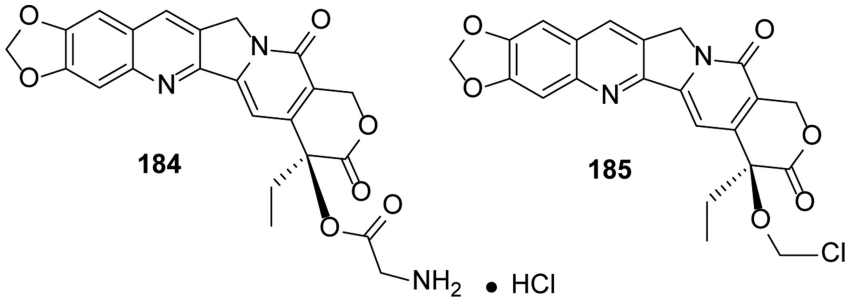


Fig. 29.
Structures of compounds **184** and **185**.

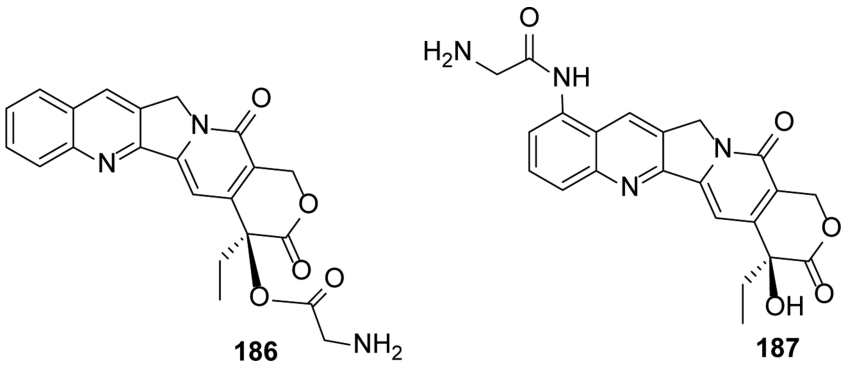
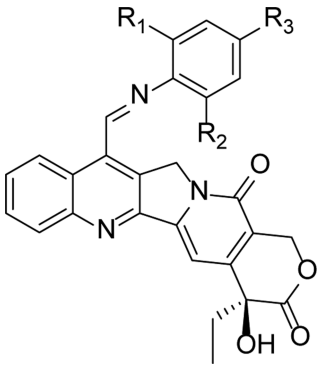


Fig. 30.
Structures of compounds **186** and **187**.

Table 1

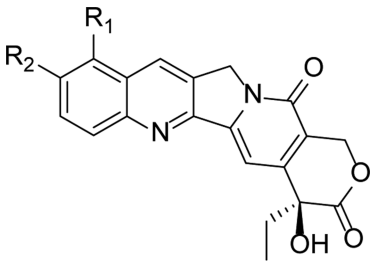
Cytotoxic Activity (IC_{50}, μM) of 7-(Aryliminomethyl)-CPT Derivatives 24–40.



Compd	R_1	R_2	R_3	H460/IC_{50} (μM)
24	CH_3	H	H	0.11
25	Cl	H	H	0.075
26	OCH_3	H	H	0.058
27	SCH_3	H	H	0.066
28	$C(CH_3)_3$	H	H	0.065
29	OH	H	H	0.032
30	CH_3	CH_3	H	0.156
31	S-S-*o*-NH_2-Ph	H	H	0.049
32	H	H	CH_3	0.18
33	H	H	Cl	0.086
34	H	H	OCH_3	0.166
35	H	H	SCH_3	0.074
36	H	H	$C(CH_3)_3$	0.09
37	H	H	OH	0.22
38	H	H	S-*p*-NH_2-Ph	0.24
39	H	H	S-S-*p*-NH_2-Ph	0.387
40	H	H	NO_2	0.28
TPT (3)	----	----		1.38

Table 2

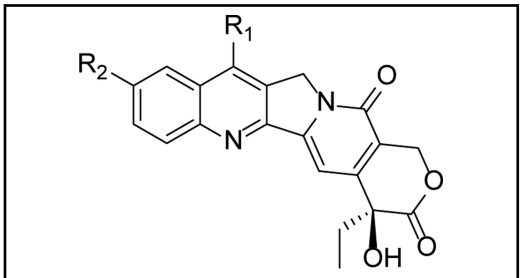
Cytotoxic Activity (IC_{50}, μM) of Compounds 41–52.



Compd	R_1	R_2	H460/IC_{50} (μM)
41	CH_2OH	H	0.21
42	CHO	H	0.058
43	CHO	OH	6.96
44	CHO	OCH_3	6.00
45	CN	H	0.428
46	CN	OH	9.86
47	CN	OCH_3	0.67
48	$CH{=}NOC(CH_3)_3$	H	0.23
49	$CH{=}NOC(CH_3)_3$	OH	0.36
50	$CH{=}NOC(CH_3)_3$	OCH_3	0.32
51	$CH{=}NOCH_2CH_2NH_2$	H	2.53
52	CH=NOH	H	0.23
TPT (3)	CH_2-$N(CH_3)_2$	OH	1.18

Table 3

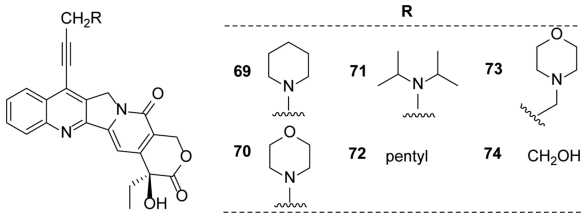
Cytotoxic Activity (IC_{50}, μM) of 7-Cycloalkyl-CPT Derivatives 53–66.



Compd	R_1	R_2	IC_{50} (μM)	
			A549/ATCC	HT-29
53	cyclopropyl	H	0.12	0.12
54	cyclobutyl	H	0.05	0.61
55	cyclopentyl	H	0.066	0.094
56	cyclohexyl	H	0.024	0.02
57	cycloheptyl	H	0.015	0.14
58	cyclooctyl	H	0.36	0.45
59	H	OH	0.11	0.21
60	cyclopentyl	OH	0.071	0.058
61	cycloheptyl	OH	0.003	0.012
62	H	OMe	0.04	0.076
63	cyclobutyl	OMe	0.011	0.041
64	cyclopentyl	OMe	0.031	0.031
65	cyclohexyl	OMe	0.039	0.035
66	cycloheptyl	OMe	0.056	0.025
CPT	H	H	0.047	0.12
TPT (3)	----	----	0.36	0.41

Table 4

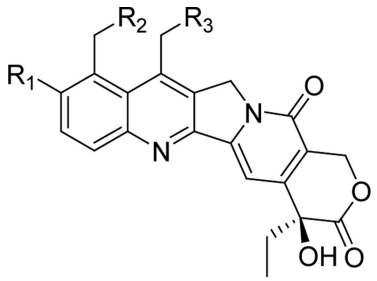
Cytotoxic Activity (IC_{50}, μM) of 7-Alkynyl CPT Derivatives 69–74.



Compd	IC_{50} (μM)			
	Esophageal (Eca-109)	Leukemia (K562)	Bladder (5637)	Gastric (SGC7901)
69	54.8	0.383	1.792	45.4
70	63.9	0.013	0.001	39.2
71	6.8	0.159	0.469	12.0
72	31.3	0.652	2.708	44.7
73	65.2	0.202	0.78	35.2
74	108.6	0.088	0.233	2558.7
SN-38	67.1	0.001	0.001	23.6
TPT (3)	389.5	0.383	1.189	152.0

Table 5

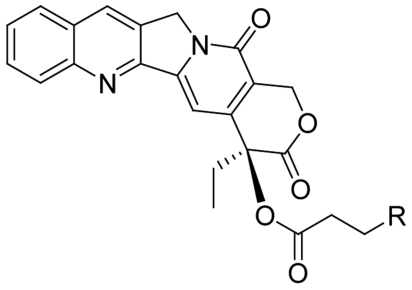
Cytotoxic Activity (IC_{50}, μM) of 7-Ethyl-9-alkyl-CPT Derivatives 81–86.



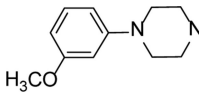
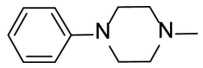
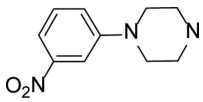
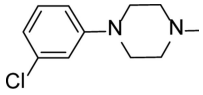
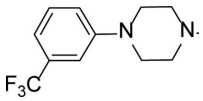
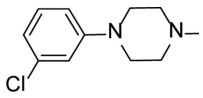
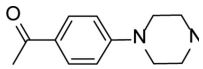
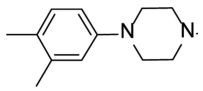
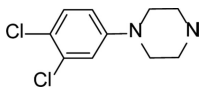
Compd	R_1	R_2	R_3	IC_{50} (μM)	
				Bel-7402	HCT-116
81	OH	$CH_2CH{=}CH_2$	CH_2CH_3	2.75	0.014
82	CH_2OH	$CH_2CH{=}CH_2$	CH_2CH_3	2.16	0.068
83	OH	$CH_2CH_2CH_3$	CH_2CH_3	3.01	0.012
84	CH_2OH	$CH_2CH_2CH_3$	CH_2CH_3	3.84	0.153
85	CH_2OH	CH_2CH_3	CH_2CH_3	2.40	0.061
86	OH	CH_2CH_3	CH_2CH_3	2.01	0.023
SN-8	OH	H	CH_2CH_3	3.19	0.007
HCPT	OH	H	H	2.47	0.148
TPT (3)	----	----	----	3.98	0.100

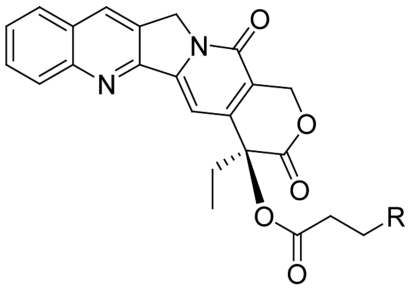
Table 6

Cytotoxic activity (IC_{50}, μM) of nitrogen-based CPT ester derivatives **100–112**.



100-112

Compd	R	IC_{50} (μM)					
		KB	KB/VCR	A2780	A549	HCT-8	Bel7402
100		0.043	0.100	0.056	0.051	0.042	0.009
101		0.013	0.080	0.027	0.010	0.023	0.008
102		0.054	0.384	0.067	0.068	0.089	0.059
103		0.067	0.092	0.069	0.091	0.076	0.064
104		0.007	0.179	0.016	0.046	0.052	0.018
105		0.043	0.084	0.056	0.055	0.008	0.007
106		0.065	0.129	0.089	0.088	0.066	0.065
107		0.056	0.098	0.092	0.094	0.055	0.051
108		0.071	0.297	0.291	0.255	0.077	0.077



100-112

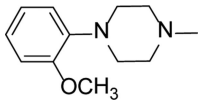
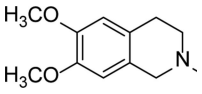
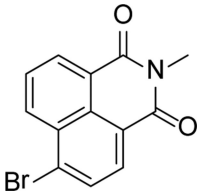
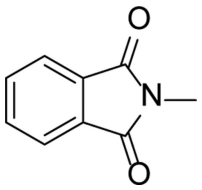
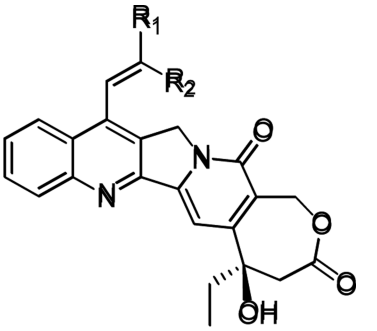
Compd	R	IC_{50} (μM)					
		KB	KB/VCR	A2780	A549	HCT-8	Bel7402
109		0.055	0.177	0.080	0.280	0.065	0.047
110		0.009	0.086	0.083	0.088	0.009	0.009
111		0.673	>1.0	>1.0	>1.0	0.862	0.783
112		0.716	>1.0	>1.0	>1.0	0.817	0.802
CPT	----	0.009	0.009	0.007	0.008	0.007	0.007
TPT (3)	----	0.062	0.339	0.058	0.087	0.074	0.078

Table 7

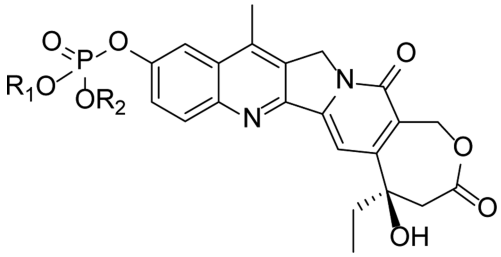
Cytotoxic activity (IC_{50}, μM) of 7-alkenyl-hCPTs 126–141.



Compd	R_1	R_2	IC_{50} (μM)
			A-549
126	H	4-NO_2-C_6H_4	0.259
127	H	Ph	0.493
128	H	2-Br-C_6H_4	3.25
129	H	4-NC-C_6H_4	2.62
130	H	3-F-C_6H_4	0.431
131	H	2-F-C_6H_4	4.38
132	H	C_6F_5	4.68
133	H	4-Me-C_6H_4	3.35
134	H	2-O-C_6H_4	2.65
135	H	3-O-C_6H_4	3.15
136	H	2-CF_3-C_6H_4	0.387
137	H	4-CF_3-C_6H_4	20.6
138	H	t-BuOOC	0.811
139	H	EtOOC	2.54
140	H	MeOOC	0.486
141	Me	EtOOC	5.29
TPT (3)	----	----	2.64

Table 8

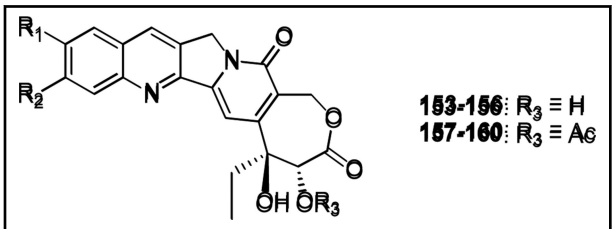
Cytotoxic Activity (IC50, μM) of Phosphodiester- and Phosphotriester-hCPTs 142–149.



Compd	R_1	R_2	IC_{50} (μM)		
			A549	**MDA-MB-435**	**LOVO**
142	H	Et	11.40	5.26	7.24
143	H	$C_6H_5CH_2$	29.35	10.77	9.31
144	Et	Et	8.05	0.79	1.26
145	iso-Pr	iso-Pr	11.39	3.40	5.56
146	Bu	Bu	2.84	0.51	0.91
147	pentyl	pentyl	2.30	0.72	1.19
148	cetyl	cetyl	>100	>100	>100
149	CF_3CH_2	CF_3CH_2	15.51	0.22	5.42
TPT (3)	----	----	0.04	<0.001	0.02
CPT-11 (4)	----	----	4.61	1.14	4.99

Table 9

Cytotoxic Activity (IC_{50}, μM) of Compounds 153–160.



Compd	R_1	R_2	IC_{50} (μM)	
			HCT-116	A549
153	H	H	14.2	49.2
154	OCH_3	H	4.4	4.7
155	OCH_2O	OCH_2O	2.1	1.2
156	OCH_2CH_2O	OCH_2CH_2O	4.9	4.6
157	H	H	49.3	16.8
158	OCH_3	H	10.6	4.8
159	OCH_2O	OCH_2O	3.1	3.1
160	OCH_2CH_2O	OCH_2CH_2O	3.4	2.9
10-HCPT	----	----	31.2	33.6
CPT	----	----	22.9	33.8

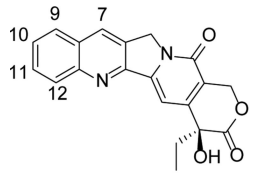
Table 10

Antifeedant activity of CPT analogues against third-instar larvae of *Spodoptera litura* (Test Concentration = 1000 μL).

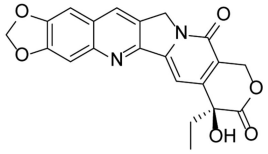
Compd	Antifeedant activity (%)	
	24 h	48 h
CPT	81.73±4.32	81.22±2.43
9-NO_2-CPT	52.65±4.86	35.56±4.27
16a-S-CPT	71.47±2.78	60.45±4.39
20-SH-CPT	85.98±2.61	79.91±5.89
21-*N*-Amino-CPT lactam	92.58±2.11	89.57±2.88
7-Me-CPT	74.40±4.87	68.66±6.97
7-Acetyl-CPT	93.20±2.43	90.63±2.38
7-Methylenedioxybenzoyl-CPT	94.75±3.72	88.82±3.30
5-OH-CPT	67.00±1.56	64.25±6.44
12-NO_2-CPT	55.41±1.50	27.97±3.75
10-OH-CPT	75.07±1.54	66.16±2.31
Toosendanin	95.6±72.19	93.89±0.65

Table 11

Antitrypanosomal activity of CPT derivatives that retain the parent ring system.



a



b

Compd	EC_{50} (μM)	
	(a) CPT	(b) 10,11-methylenedioxy-CPT
7-Me	------	0.044
7-Et	0.63	0.060
7-Propyl	0.80	------
7-Et-9-NH_2	0.86	0.057
7-Et-9-NO_2	2.7	0.17
7-Et-10-NH_2	0.62	------
7-Et-10-NO_2	0.60	--------
9-Cl	0.81	0.041
9-NH_2	0.84	0.074
9-NO_2	1.6	0.40
10-Me	2.3	------
10-Cl	1.5	-------
10-NH_2	1.2	------
10-NO_2	2.1	-------
10,11-Dimethoxy	>100.0	-------
11-NH_2	18	--------
12-NH_2	12	--------
7-Me-10,11-ethylenedioxy	0.070	---------
CPT	1.6	0.16