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Camptothecins
A Review of Their Chemotherapeutic Potential

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Contents

Abstract

Camptothecin analogues and derivatives appear to exert their antitumour activity by binding to topoisomerase I and have shown significant activity against a broad range of tumours. In general, camptothecins are not substrates for either the multidrug-resistance P-glycoprotein or the multidrug-resistance–associated protein (MRP). Because of manageable toxicity and encouraging activity against solid tumours, camptothecins offer promise in the clinical management of human tumours. This review illustrates the proposed mechanism(s) of action of camptothecins and presents a concise overview of current camptothecin therapy, including irinotecan and topotecan, and novel analogues undergoing clinical trails, such as exatecan (DX-8951f), IDEC-132 (9-aminocamptothecin), rubitecan (9-nitrocamptothecin), lurtotecan (GI-147211C), and the recently developed homocamptothecins diflomotecan (BN-80915) and BN-80927.

1. Camptothecin, a Specific Topoisomerase (Topo) I Poison

The early 1960s discovery of camptothecin as an anticancer drug with a unique mode of action (i.e. inhibition of DNA topoisomerase I) has added an entirely new dimension to the field of chemotherapy. This naturally occurring alkaloid was first extracted from the stem wood of the Chinese tree *Camptotheca acuminata* during the screening of thousands of plants in a search for steroids. Testing of each extract for their antibacterial, antitumour

and antiviral activity further revealed that the extract from *C. acuminata* has a substantial antitumour activity in standard *in vivo* test systems as well as in mouse leukaemia cells (L1210).[1] These astonishing findings greatly increased interest in this natural product as a possible antitumour agent.

In the early 1970s, initial studies examining the mechanism of action of camptothecin suggested that cytotoxicity might result from its immediate and profound inhibition of DNA and RNA synthesis.[2-5] Inhibition of RNA and DNA synthesis was found to be reversible following brief exposures to camptothecin, but DNA inhibition progressively became irreversible with increasing concentration and exposure duration.[2,3,6] These studies also suggested that camptothecin is selectively cytotoxic to S-phase cells, arrests cells in the G_2 phase, and induces fragmentation of chromosomal DNA.[2,5]

After the approval of camptothecin by the US Food and Drug Administration (FDA) in the 1970s against colon carcinoma, it was evaluated as a possible agent in the treatment of human cancer in phase I and phase II studies.[7-10] Although camptothecin had shown strong antitumour activity among patients with gastrointestinal cancer, it also caused unpredictable and severe adverse effects including myelosuppression, vomiting, diarrhoea and severe haemorrhagic cystitis. These findings eventually resulted in the discontinuation of phase II trials in 1972.

In the late 1980s, topoisomerase I (topo I) was identified as the unambiguous site of action of camptothecin; and this finding renewed the interest in the development of topo I poisons as such drugs may be valuable additions to combination chemotherapeutic regimens.[11,12] In early clinical studies, administration of the water soluble camptothecin-Na^+ salt to the patients had revealed that the open lactone ring of camptothecin is a much less potent antitumour agent with severe adverse effects than the natural product camptothecin.[7] Therefore, in the late 1980s and early 1990s, several camptothecin derivatives with an intact lactone ring were synthesised based on the structure-activity relationship (SAR) studies.[13-18] Within these series of compounds, the water soluble analogues, irinotecan (CPT-11) and topotecan were found to be the most promising anticancer agents in phase I clinical studies[19,20] and currently being evaluated in phase II and phase III clinical trials. In addition, water insoluble analogues of camptothecin, such as IDEC-132 (9-aminocamptothecin; 9-AC), rubitecan (9-nitrocamptothecin; 9-NC) and 10,11-methylenedioxy camptothecin analogues (10-11 MDC) have shown strong antitumour activity against solid tumour xenografts;[21,22] and recently these compounds have been reformulated and introduced into clinical trials. Currently, several other camptothecin analogues and homologues (e.g. homocamptothecins; hCPT) receive consideration for clinical trials since they have been shown to have considerable cytotoxic activity in L1210 mouse leukaemia assays.[23] All these analogues have been shown to kill tumour cells effectively by inhibiting cellular DNA topo I by the same mechanism as camptothecin with similar or higher potency.

2. DNA Topo I

On the basis of the fundamental differences in their reaction mechanism, DNA topoisomerases are classified in two types, type I and type II.[24-26] Type I topoisomerases change the topological state of DNA via transient enzyme-linked single-strand breaks. In contrast, type II topoisomerases catalyse the strand passing reaction by transiently breaking both strands of duplex DNA to generate a gap for the passage of second duplex DNA prior to religation reaction.[27-30] (Characteristically, type I DNA topoisomerases change the linking number of closed circular DNA in steps of one, and type II DNA topoisomerases change in steps of two[25,27,31]). Since the biological functions of topoisomerases are profoundly embedded in the double helix structure of DNA, these enzymes engage in almost all biological transactions of DNA such as replication, transcription, recombination and repair, and most probably function to resolve topological problems generated during these processes.[32-35]

Mammalian DNA-topo I is a monomeric 100 kDa polypeptide encoded by a single copy gene on chromosome 20q12-13.2.[29,36-39] Topo I is a type I topoisomerase and, along with other topoisomerases, accomplishes the relaxation of torsionally strained supercoiled DNA by breaking and resealing DNA.[27,28,40-43] Unlike topoisomerase II, the relaxation reaction of topo I does not require energy in the form of adenosine triphosphate and can occur in the presence of EDTA (ethylene diamine tetraacetic acid).[32,44]

The primary function of topo I is most probably to remove excessive positive supercoils as well as negative supercoils arising during DNA replication and transcription.[32,45] In this reaction, DNA topoisomerase I binds noncovalently to superhelical DNA and cleaves one of the DNA strands via a nucleophilic attack of the phosphodiester bond in DNA, forming a covalent linkage between a tyrosine group at the active site of topo I and a 3′ phosphate group along the DNA backbone.[46-49] It is *this* normal reaction intermediate, covalently bound enzyme-DNA complex, termed the 'cleavable or covalent complex'.[41,43,50,51] Once the covalent complex has been formed, the relaxation of superhelical DNA occurs either by passing the intact strand through a gap in the cleaved strand of DNA (favoured for *Escherichia coli* topo I)[52] or by free rotation of the 3′ of the cleaved strand around the intact strand (favoured for vaccina viral topo I),[53] or through one or more cycles of controlled rotation (currently proposed model for human topo I).[54] Following the relaxation of torsionally strained DNA, the broken strand is rapidly religated with concomitant release of the active tyrosine (Tyr723 for human topo I) from the end of the DNA. Subsequently, topo I dissociates from the DNA molecule and undergoes another cycle of DNA binding and relaxation. As a result of the transient relaxation and unwinding of supercoiled DNA, the replication fork proceeds down the DNA strand and serves as a template for the synthesis of a new strand of DNA.[54]

While topo I appears to be an essential enzyme for DNA replication, the potential role of topo I in transcription is much less clear. During transcription, the rotation of the transcription ensemble relative to the DNA template generates positive supercoils in front of the RNA polymerase and negative supercoils behind it; and excessive positive supercoils may eventually lead to cessation of RNA polymerase movement.[32,55] Topo I presumably participates in transcription to relieve the transcription-associated torsional strain. In fact, studies demonstrating the ability of eukaryotic topo I to relax both negatively and positively supercoiled DNA with about equal efficiency and preferential association of topo I with actively transcribed genes[44,56] strongly supports the involvement of topo I in transcription processes.[33,57-59]

Several lines of evidence suggest that topo I may also participate in illegitimate recombination that is often found in the genomes of bacteriophage, bacteria and higher organisms. Illegitimate recombination occurs between nonhomologous DNA sequences and can lead to deletions, amplifications, insertions and translocations as well as the integration of foreign DNA. The involvement of vaccinia virus topo I in sequence specific illegitimate recombination in *E. coli* cells provides direct evidence that eukaryotic topo I is able to promote illegitimate recombination.[60,61] However, the role of DNA topoisomerases in recombination is less clearly understood in higher eukaryotes.[62]

While the potential role of topo I is to maintain the genomic stability, under certain conditions it can also lead to genomic instability. It is well known that topo I is the primary target for camptothecin and its analogues; and these compounds act to stabilise covalent complexes between topo I and DNA. Stabilisation of topo I on DNA covalent complexes leads to irreversible double strand DNA breaks and recombinogenic ends upon collision of replication machinery with the stabilised topo I-DNA complex,[63,64] or may stimulate chromosomal deletions and rearrangements along with sister chromatid exchange.[65] Thus, topo I poisons effectively convert the enzymes into nucleases.

3. The Cytotoxic Mechanisms of Topo I Poisons

The cytotoxic mechanism of topo I poisons is multifaceted but is most commonly explained by the replication collision model illustrated in figure 1.[66] In this model, camptothecin stabilises the normally transient cleavable DNA-topo I complex and forms an enzyme-drug-DNA ternary cleavable complex. As a consequence of the formation of a cleavable complex, both the initial cleavage reaction and religation steps are inhibited.[67] The collision of the replication fork with this cleaved strand of DNA causes an irreversible arrest of replication fork, double strand DNA breakage and conversion of reversible cleavable complex into an irreversible complex. One or more of these events eventually trigger other cellular responses which can lead to the cell cycle arrest in the G2 phase and to cell death.[63,66,68,69]

Whereas the replication collision model clearly explains the replication-dependent cytotoxic activity of camptothecin, it is unable to explain other

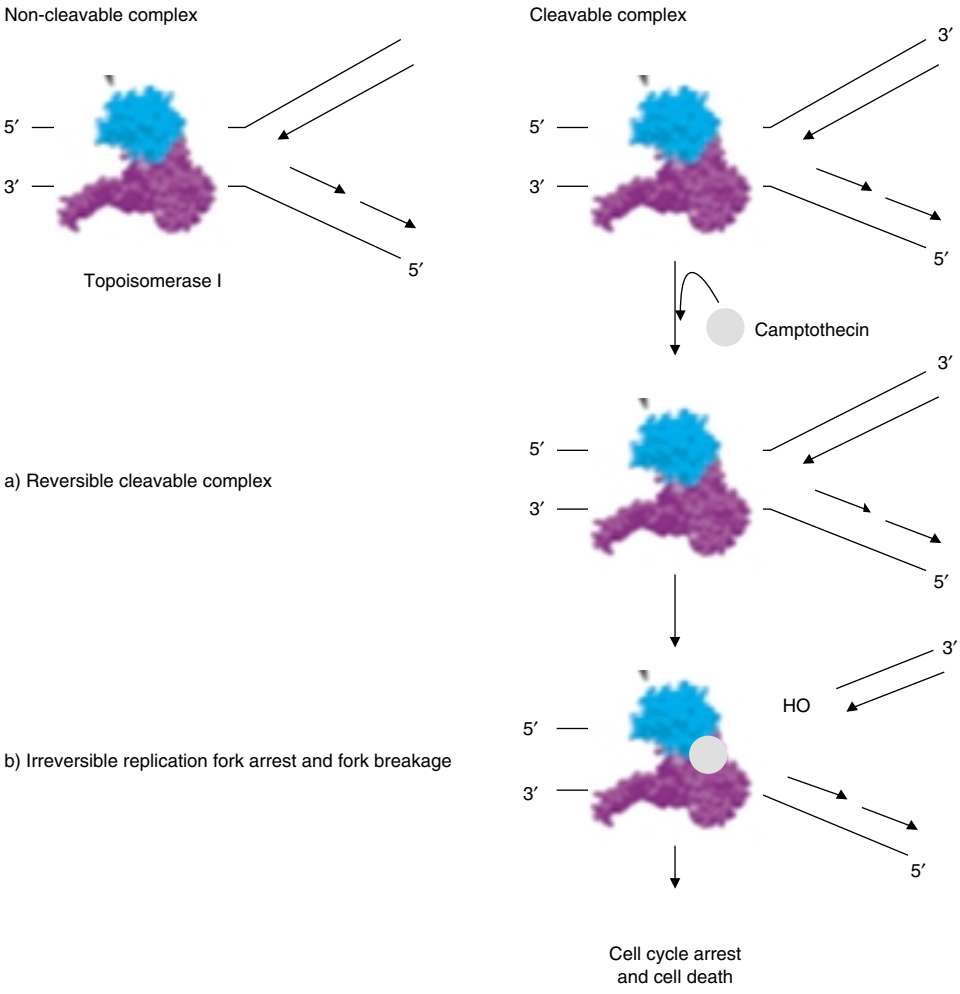


Fig. 1. The cytotoxic mechanism of camptothecin according to the 'Replication Collision Model'.

cellular effects of camptothecin independent from replication, such as inhibition of RNA synthesis, multi-ubiquitination and degradation of topo I, chromatin reorganisation and activation of signal transduction molecules.[70-74] All these activities are also believed to be involved in transformation of the reversible cleavable complex into irreversible strand breaks.

More recently, the transcription collision model has been proposed by Wu and Liu[74] to explain S-phase–independent cellular activity of camptothecin (figure 2). Their data suggest that collision between topo I-cleavable complexes located on the template strand and the elongating RNA polymerase results in transcription arrest and conversion of topo I cleavable complexes into 'irreversible' single strand breaks. These events ultimately terminate the RNA transcript at the arrested site, and cause potentially lethal double strand DNA breakage and cell death.

Finally, there is *in vivo* evidence for elevated topo I DNA complex formation in cells engaging in nucleotide excision repair.[35,75] DNA templating (polymerase reading of the DNA) is a key feature in common with replication, transcription and repair; thus, one might argue that the combination of topo I-DNA damage and polymerase readthrough has a greater cytotoxic impact for a given amount of topo I target.

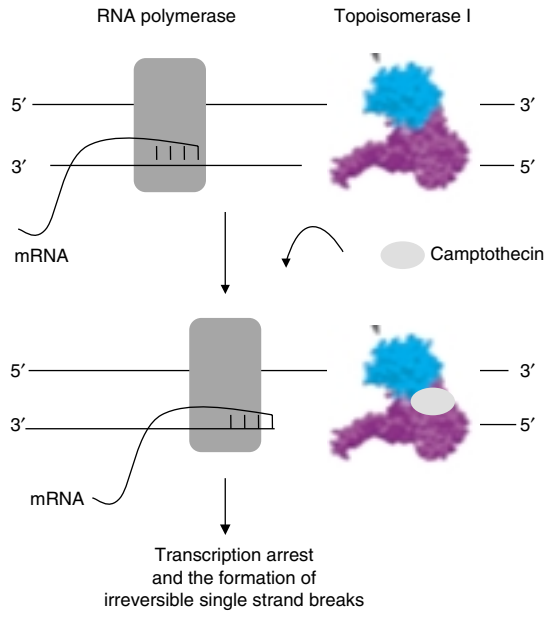
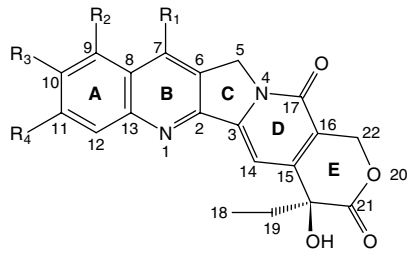


Fig. 2. The cytotoxic mechanism of camptothecin according to the 'Transcription Collision Model'.

4. Structure-Activity Relationships of Camptothecins

Initial studies examining the structure of camptothecin revealed that the naturally occurring alkaloid consists of a pentacyclic ring structure that includes a pyrrolo (3,4-β) quinoline moiety (figure 3; rings A, B, C) and one assymetric centre within the α-hydroxy lactone ring with 20 (S) configuration (ring E).[1] In this molecule, the planar pentacyclic ring system (ring A-E) was suggested to be one of the most important structural features in topo I inhibition since the omission of the A ring or A and B rings or A, B and C rings resulted in tetracyclic, tricyclic and bicyclic ring compounds that are devoid of activity (reviewed in[76]). Similarly, the saturation of the B ring resulted in compounds that show little activity *in vitro* even at higher concentrations (>100 μmol/L).[77] On the other hand, modification of pentacyclic ring structure with an addition of six-membered F ring yielded a hexacyclic ring compound that exhibited the same order of potency as camptothecin, suggesting that at least a pentacyclic ring system is required for the activity.[78]

Early SAR studies also suggested that the intact α-hydroxylactone group in the E ring is another structural requirement for *in vivo* and *in vitro* activity of camptothecins. However, this group is highly susceptible to facile ring opening by a nucleophilic hydrolysis reaction catalysed by OH^- ions. On acidification, the E ring opening can be readily reversed.[1] In early clinical trials, antitumour activity of the carboxylate form (camptothecin-Na^+ salt) was found to be negligible even at levels higher than the applied concentration of camptothecin lactone form.[7] Further *in vivo* and *in vitro* studies have supported the view that the



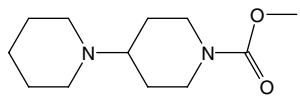
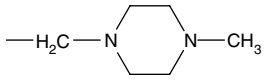
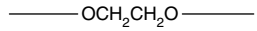
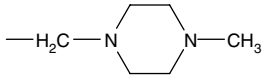
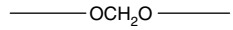
Name	R_1	R_2	R_3	R_4
Camptothecin	H	H	H	H
Topotecan	H	$CH_2NH(CH_3)_2$	OH	H
Irinotecan	C_2H_5	H		H
SN-38	C_2H_5	H	OH	H
IDEC-132	H	NH_2	H	H
Rubitecan	H	NO_2	H	H
Lurtotecan		H		
GI-149893		H		

Fig. 3. Chemical structures of camptothecin and camptothecin analogues that currently undergoing clinical trials.

open-ring carboxylate form possesses minimal antitumour activity compared with the closed-ring lactone form of camptothecin.[9,10] Consistent with these findings, modification of the oxygen of the lactone ring with sulfur or nitrogen (21-lactam derivative) abolished the activity of camptothecin.[79,80] Similarly, replacement of the α-hydroxyl group at position 20 by hydrogen[79,81] or fluorine or acetyl derivatives[79] resulted in complete loss of activity. It was also found that the 20 (S) form of camptothecin and its analogues exhibits greater potency (10- to 100-fold) than the corresponding 20 (R) form in *in vitro* and *in vivo* tumour cell models.[82] Collectively, these findings suggested that the hydroxyl group at position 20 on the E ring, as well as the correct specific stereochemistry at this position, are essential for the *in vivo* and *in vitro* activity of this compound.[22,80] Furthermore, the D ring pyridone group is also required for the antitumour activity of camptothecin since the replacement of the ring D pyridone group with the D ring benzo resulted in compounds that are almost inactive in cleavable complex assay and much less potent than the parent compound in other cytotoxicity assay.[83]

In general, modifications of the A and B ring have been shown to be well tolerated and in many

cases enhanced the potency of camptothecin both *in vitro* and *in vivo*[84] Substitution of the A ring with amino, nitro, bromo or chloro groups at position 9 or 10 and hydroxyl group at position 10 or 11 resulted in compounds with considerably greater *in vivo* activity than is found with the parent compound, whereas all the 12 substituted derivatives were found to be far less active.[85] The resulting compounds are relatively insoluble in aqueous solutions that make the formulation of them difficult for intravenous administration. Accordingly, more water soluble derivatives of 10 hydroxy camptothecin having such functional groups as glycosides, phosphates, sulfates and carbamates were prepared, and their antitumour activities were examined against L1210 leukaemia in mice (reviewed in Sawada et al.[85]). Among carbamate derivatives, substitution of the [4-(1-piperidino)-1-piperidino carbonyloxy] group at position 10 of the A ring with an ethyl group at position 7 of the B ring yielded a cytotoxic camptothecin analogue (irinotecan, CPT-11) with enhanced solubility in aqueous solution.[86,87] Studies of the metabolic pathway of irinotecan revealed that it is a prodrug and requires enzymatic hydrolysis of carbamate moiety to its active 10-hydroxy metabolite (SN-38) in order to exert its pharmacological activity.[88] Similarly, the combination of 10 hydroxy camptothecin with a positively charged dimethyl-aminomethyl group at position 9 (topotecan) enhanced aqueous solubility without substantially affecting activity (figure 3).[16] In addition to their water solubility and high potency, irinotecan and topotecan demonstrated broad-spectrum of activity in preclinical tumour models and are currently undergoing extensive clinical trials.

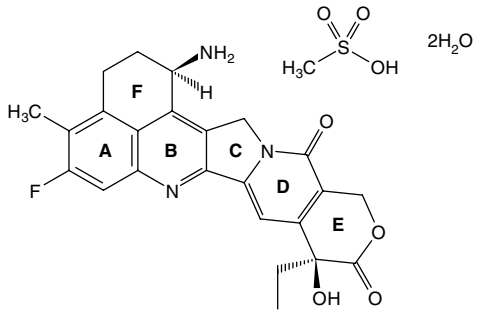
Recent SAR studies have shown that substitution of methylenedioxy or ethylenedioxy group at position 10 and 11 of the A ring results in a remarkable enhancement of both *in vivo* and *in vitro* activity of camptothecin.[22,80,89] The addition of methoxy groups at these positions (10,11 dimethoxy camptothecin), however, resulted in complete loss of activity.[80,90] A combination of 10, 11-methylenedioxy or ethylenedioxy analogues with substituents such as an amino or chloro group at 9 position or chloromethyl group at 7 position led to compounds that have greater cytotoxicity than the parent methylenedioxy or ethylenedioxy analogues, but the resulting compounds still have limited solubility in aqueous solution.[89] Substitution of the water solubilising groups (e.g. 4-methylpiperazinomethylene) at 7 position of 10, 11-methylenedioxy (GI149893) or 10, 11-ethylenedioxy (lurtotecan, GI-147211) yielded compounds that are approximately two times more soluble than topotecan (figure 3), and demonstrated marked topo I inhibitory activity in the cleavable complex assay and potent antitumour activity *in vivo* in xenograft models.[91,92] In general, the 10, 11-ethylenedioxy analogues were found to be less potent than the corresponding methylenedioxy analogues and that was attributed to the unfavourable steric interactions of ethylenedioxy analogues with the enzyme or enzyme-DNA complex.[76,89] Despite the lack of enhanced activity of ethylenedioxy analogues relative to methylenedioxy analogues, lurtotecan, a 7-(4-methylpiperazino methylene) substituted ethylene-dioxy analogue, is emerging as a potential candidate within this series for the treatment of human cancer.

More recently, interest greatly increased in hexacyclic derivatives of camptothecin having an additional six-membered F ring. Several compounds have been synthesised and display an enhanced stability as well as an increased topo I inhibition and *in vitro* antitumour activities.[93-95] Similar to pentacyclic derivatives, the antitumour activity of hexacyclic analogues results from inhibition of cellular topo I by the same mechanism as camptothecin with similar or higher potency. Modifications at position 5 (which corresponds to position 11 in the A ring of pentacyclic ring system) with electron-withdrawing groups such as hydroxy, methoxy, chloro or fluoro showed a distinct tendency to increase *in vitro* antitumour activity.[94] Addition of amino or methyl groups at position 4 leads to compounds that have superior activity to that of the parent hexacyclic analogue. Among

them, a non-prodrug, water soluble, 4-methyl-5-fluoro substituted hexacyclic compound, exatecan (DX-8951f; figure 4) was found to be the most potent of all and is currently undergoing clinical development.[96,97]

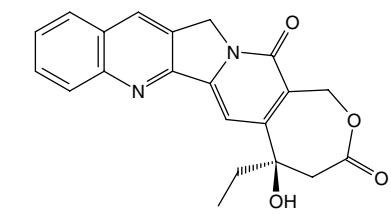
In clinical studies, one of the major drawbacks observed with camptothecin analogues is a marked loss of therapeutic activity due to their intrinsic instabilities resulting from the rapid hydrolysis of the lactone ring in the body. Thus, producing potent camptothecin analogues with a prolonged biological life in their active lactone form has become an important goal.

In recent studies, a modification of the crucial camptothecin lactone ring was proposed and studied to stabilise the lactone ring opening while maintaining its topo I inhibitory activity. As a result, an initially racemic compound, homologous to camptothecin (homocamptothecin; *dl*-hCPT) was synthesised by Lavergne and colleagues[98,99] by inserting a methylene group between the lactone and 20-hydroxy groups of camptothecin (figure 5). The *d*-enantiomer has the highest activity and subsequent studies were performed with pure *d*-hCPT. Surprisingly, in this new molecule, the seven membered β-hydroxylactone group remarkably enhanced the stability of the compound in buffer solutions as well as plasma, while maintaining strong topo I inhibitory activity.[23,98,100] This finding challenged the general acceptance of the naturally occurring six-membered α-hydroxylactone ring to be an essential structural requirement for both *in vivo* and *in vitro* antitumour activity of camptothecin. More surprisingly, the topo I cleavable complexes formed in the presence of hCPT appears to be more stable than those induced by camptothecin.[101] These results were highly attributed to two phenomena: (i) less-reactive lactone ring in hCPT compared with that of conventional camptothecin analogues; and (ii) significant

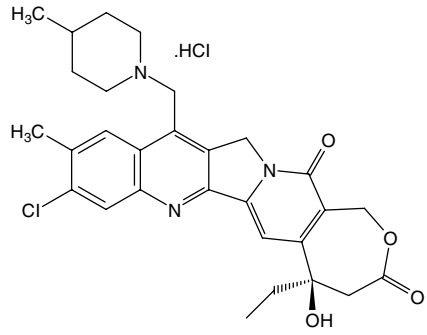


Exatecan (DX-8951f)

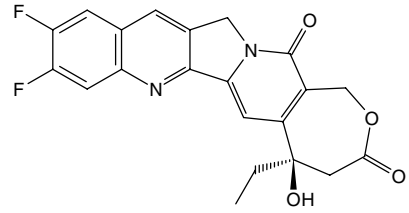
Fig. 4. Chemical structure of exatecan (DX-89951f), a hexacyclic analogue of camptothecin.



Homocamptothecin



BN-80927



Diflomotecan (BM-80915)

Fig. 5. Chemical structures of homocamptothecin and its analogues, BN-80927 and diflomotecan (BN-80915).

differences in DNA sequence specificity of hCPT relative to that of camptothecin (e.g. in the presence of hCPT, topo I cleaves DNA at additional sites undetected with camptothecin). It was suggested that the reduced reactivity of the lactone ring diminishes the intramolecular H bonding between the hydroxyl and the lactone group observed with camptothecin analogues and further enhances the interaction of the free hydroxyl group optimally with topo I.[100]

To date, several hCPT derivatives have been synthesised and tested in a variety of tumour cells *in vitro* and in xenograft models.[102] Among them, the A ring substituted derivatives were found to have a pronounced influence on biological activity. Within this series of compounds, the difluoro-substituted derivative, diflomotecan (BN-80915), and another promising analogue, BN-80927 (figure 5) have been selected for further development in preclinical and clinical trials as hCPT-based topo I poisons.[103,104]

In addition to hCPT analogues, 7 silylcamptothecin derivatives, 'silatecans[105] and homosilatecans'[106] were synthesised to optimise circulating lactone levels by enhancing lipophilicity and reducing the high affinity binding of camptothecin carboxylate for human albumin (figure 6). Among them, 7-tert-buthyldimethylsilyl-10 hydroxycamptothecin (DB-67) exhibited dramatically improved blood stability compared with clinically relevant camptothecin analogues. In addition to their improved stability, strong topo I inhibitory activity, and comparable *in vivo* and *in vitro* antitumour potencies relative to camptothecin suggested that 7-silylcamptothecin analogues might be potential candidates for the treatment of human cancer.

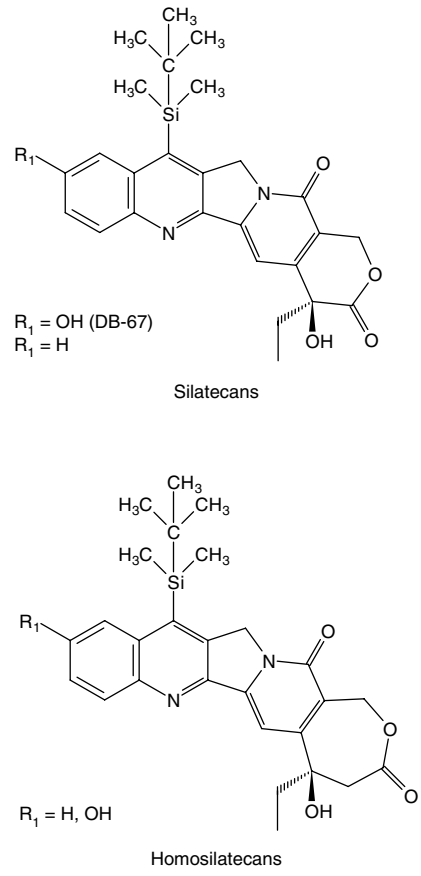


Fig. 6. Chemical structures of silatecans and homosilatecans.

5. Molecular Interaction of Camptothecin with Topo I-DNA Complexes

Initial attempts to simulate molecular interactions between camptothecins, DNA and topo I, were based on homology modelling techniques. Fan and colleagues[90] docked 18 camptothecin derivatives with different DNA cleavage potencies into a hypothetical cleavable complex binding site. The results suggest that a ternary cleavable complex might be stabilised by several hydrogen bonds in the binding site. In their proposed 'drug-stacking' model, camptothecin is pseudointercalated in the topo I-linked DNA cleavage site, and interacts with the protein near its catalytic tyrosine through hydrogen bonding and stacking. The structural model is consistent with experimental observations, such as: (i) the N3 position of the 5′ terminal purine of the cleaved DNA strand is readily alkylated by 7-chloromethyl 10,11- methylenedioxy camptothecin; (ii) camptothecin generally tolerates substituents at positions 7, 9, and 10 but is inactivated by additions at position 12; (iii) 10,11-methylenedioxy camptothecin is much more po-

tent than 10,11- dimethoxy camptothecin; (iv) the lactone portion of camptothecin is essential for topo I inhibitory activity; (v) 20S derivatives of camptothecin are much more potent than the 20R analogues; (vi) a catalytic tyrosine hydroxyl in topo I covalently links to the 3′ terminal base, T, of the cleaved DNA strand; and (vii) topo I mutation Asn722Ser leads to camptothecin resistance.[90]

On the basis of the observations obtained from the structure-activity studies and crystallographic data of topo I-DNA complexes, a hypothetical model for the camptothecin-topo I-DNA ternary complexes has been proposed by Redinbo and coworkers[107] (figure 7). In this model, the lactone moiety of the E ring, the 20 (S) hydroxyl group, the pyridone moiety of the D-ring, the tolerable modifications at 9,10, and 11 positions of the A ring and the 7 position of the B ring as well as the positions of amino acid residues that, when mutated, produce a camptothecin resistant enzyme were taken into account. This hypothetical model proposes that the active lactone form of camptothecin is stacked between the terminal +1 guanine nucleotide on the scissile strand of DNA and the side chain of Asn722. The A ring of camptothecin highly interacts with the planar conjugated side chain of Asn722 because mutation of Asn722 to serine, which lies in close proximity to active Tyr723, was associated with camptothecin resistance.[108,109] The B ring, particularly C-7 position was found to be in close proximity to the N3 nitrogen of the +1 guanine base since 7-chloromethyl substituted derivatives of camptothecin were shown to interact with N3 position and able to alkylate the nitrogen group after cleavage.[110] The double bonded lactone oxygen in the E ring and the hydroxy group at the 20 S chiral centre form a hydrogen bonding network with Arg364 and Asp533 side chains, respectively. The carbonyl oxygen group (C-17) of the D ring further stabilises the topo I-DNA covalent complexes by forming a H bond with the NH_2 group on the pyrimidine ring of the +1 cytosine.[107]

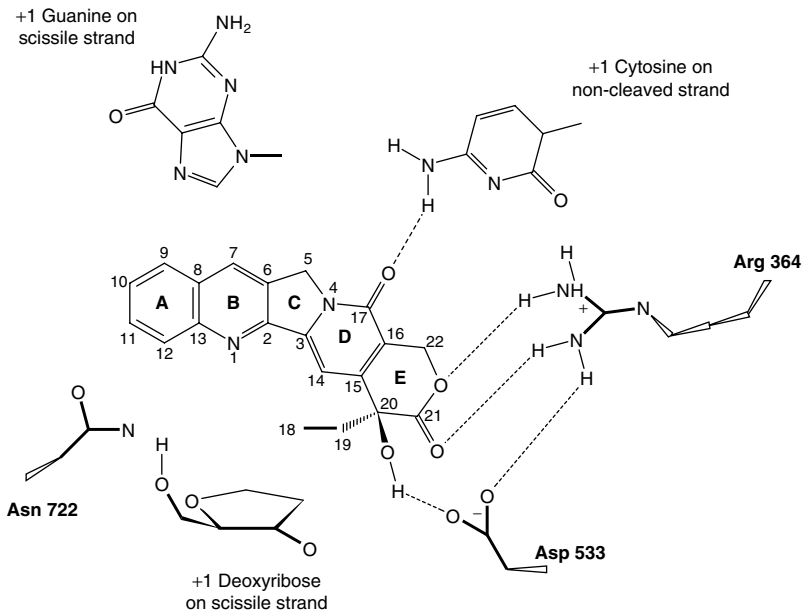


Fig. 7. Proposed model for the binding of camptothecin to topoisomerase I-DNA complexes.

More recently, Kerrigan and Pilch[111] used the crystal structure of the aforementioned cleavable complex[107] to develop a general model for the complex formed with camptothecin and its analogues. This model has the drug intercalated between the -1 and +1 base pairs, with the E ring pointing into the minor groove and the A ring directed toward the major groove. The ternary complex is stabilised by an array of hydrogen bonding and hydrophobic interactions between the drug, and both the enzyme and the DNA. Again, the proposed model is consistent with the current body of experimental mutation, cross-linking and structure-activity data. In addition, the model reveals potential sites of interaction that can provide a more rational basis than previous models for the design of next generation compounds as well as for *de novo* drug design.[107]

6. Clinical Development of Camptothecin Analogues

Among the water soluble camptothecin derivatives synthesised on the basis of structure activity studies, topotecan and irinotecan have now reached the clinic, and exatecan was found to be a promising anticancer agent.[112] Another water soluble derivative, lurtotecan has been recently introduced into preclinical and clinical studies. The water insoluble analogues such as IDEC-132, rubitecan, diflomotecan and BN-80927 are currently undergoing preclinical and clinical development. The development of the relatively new silyl derivatives of camptothecin 'silatecans' is still in a preclinical setting.

6.1 Irinotecan and Topotecan

In phase I and II clinical trials, a wide range of activity was documented for irinotecan and topotecan in non-small cell lung cancer,[19] ovarian cancer,[113] cervical cancer,[20] non-Hodgkin's lymphoma,[114] refractory or relapsed lymphoblastic leukaemia and acute leukaemia.[115] Substantial activity has been observed for irinotecan among previously treated patients with metastatic colorectal adenocarcinoma.[116] In 1996, topotecan was approved for use by the US FDA for previously treated patients with advanced ovarian cancer.[117] For these patients, topotecan provides another therapeutic option upon disease progression after initial platinum-based chemotherapy. Topotecan combination regimens with paclitaxel, etoposide, cisplatin, and cytarabine, and with other treatment modalities, such as radiation therapy, are in development. Irinotecan was approved by the FDA in 2000[118] as first-line treatment for advanced colorectal carcinoma.

Toxicity studies in phase II clinical trials have demonstrated that the toxicity caused by topotecan presents a less complex problem than toxicities induced by irinotecan. The dose limiting toxicities of irinotecan have been observed to be largely dependent on the treatment schedule.[119,120] Myelosuppression, significant neutropenia, thrombocytopenia or anaemia appeared when the drug was administered on a daily schedule.[19] An intermittent schedule with high single doses and continuous administration induced gastrointestinal toxicities. Among these gastrointestinal toxicities, the prevailing and most troublesome was the development of diarrhoea (any grade in 79 to 87% of patients).[19,121] Diarrhoea is now recognised as the dose limiting toxicity of this compound. Other common toxicities of irinotecan have included nausea, vomiting, fatigue and alopecia.[19,51,115]

Unlike irinotecan, neutropenia was the principal dose limiting toxicity of topotecan.[122] The gastrointestinal adverse effects of topotecan, including diarrhoea, nausea and vomiting, were successfully controlled with standard supportive care measures.[122,123] In clinical studies, irinotecan and topotecan-induced myelosuppression can be readily managed by granulocyte colony-stimulating factors (G-CSF), but irinotecan induced gastrointestinal toxicities do not respond well to conventional treatment. Although some antidiarrhoeal agents, such as loperamide and acetorphan, could offer some help in reducing irinotecan induced diarrhoea, this dose limiting toxicity still remains a clinical problem for patients receiving this drug.[124-126]

The elucidation of several aspects of these two drugs, including antitumour activities, pharmacology, pharmacokinetics and metabolism, now explains the disparity between irinotecan and topotecan associated gastrointestinal toxicities. For example, unlike topotecan, irinotecan acts as a prodrug that needs to be converted to its active metabolite SN-38, *in vivo*. Most of SN-38 undergoes subsequent conjugation in the liver to form SN-38 glucuronide. SN-38 glucuronide is excreted into the bile and further deconjugated in the intestinal microflora to form SN-38.[127] In addition to this complex metabolic pathway, the terminal half life of irinotecan and SN-38 (6.3 and 11.5 hours, respectively) is significantly longer than that of topotecan (3 hours).[128] Furthermore, biliary clearance is the major route of excretion for irinotecan and SN-38, but topotecan is mainly excreted in urine. Compared with irinotecan, a shorter drug exposure time and lower cellular accumulation of topotecan as a result of its strong affinity for the efflux transporter P-glycoprotein may further explain the lower incidence of gastrointestinal adverse effects observed with topotecan treatment.

Today, topotecan and irinotecan are the most advanced camptothecin analogues in clinical studies and used as second-line therapy for advanced epithelial ovarian cancer and first-line therapy for colon cancer, respectively. In ongoing phase II and phase III clinical studies, the combination therapies of irinotecan with various regimens such as irinotecan plus fluorouracil (5FU), irinotecan plus cisplatin (CDDP), and irinotecan plus etoposide (VP16) have been evaluated in metastatic colon cancer (reviewed in[129,130]). A number of combination studies with topotecan have also been reported, including with cisplatin,[131] paclitaxel,[132] etoposide[133,134] and cyclophosphamide.[135]

The collective data from these studies indicated that irinotecan/5-FU/folinic acid (leucovorin) combinations have stronger antitumour activity in stage III colon cancer and improve response rates and time to progression compared with 5-FU/folinic acid regimens alone.[136,137] Although the combination of irinotecan with 5-FU/folinic acid increased the likelihood of neutropenia, the incidence of febrile neutropenia and infection remained low. Other toxic effects were manageable, noncumulative and reversible.[138] Similarly, the combination of irinotecan and cisplatin was significantly active against small cell lung cancer,[139] and in untreated as well as previously treated patients with gastric or gastroesophageal junction carcinoma[140] with a manageable toxicity profile. However, the combination of irinotecan with etoposide showed intolerable overlapping myelotoxicity, severe diarrhoea and pulmonary toxicity with modest activity against non-small cell lung cancer.[130]

Similarly, administration of topotecan and etoposide in patients with advanced solid malignancies did not provide substantial evidence that this combination is more advantageous than either topotecan or etoposide alone.[141] On the other hand, the combination of topotecan cisplatin, paclitaxel and G-CSF support represented a well tolerated and active therapeutic approach in both pre-treated and untreated patients with ovarian cancer or small cell lung cancer.[142]

Currently, extensive clinical trials are continuing to better describe the spectra of clinical activity of topotecan and irinotecan, to determine the optimal dose, schedules and route of administration, and to define the use of these agents in combination with other chemotherapeutic agents.

6.2 Exatecan

Exatecan is a relatively new water-soluble hexacyclic analogue of camptothecin having an amino group at 1 position and a fluorine at 5 position (figure 4). *In vitro*, exatecan demonstrated stronger antitumour activity than did other clinically relevant camptothecin analogues, such as SN-38, topotecan and camptothecin itself.[143] The greater antitumour activity of exatecan was observed in a wide range of human tumour xenografts in nude mice, including gastric, pancreatic, colon, breast, ovarian and lung tumours.[143-145] Substantial antitumour activity has also been observed in

an intracranial xenograft of human rhabdomyosarcoma, murine lung and liver metastasis models.[96]

More recently, phase I clinical studies of exatecan have been completed in Japan, the US and Europe, and the results have been summarised by De Jager and coworkers.[96] Collective data have revealed that the major dose limiting toxicity encountered with this compound was myelosuppression, such as neutropenia and thrombocytopenia. Gastrointestinal adverse effects, such as vomiting and nausea, were moderate and can be easily controlled with standard antiemetic drugs. Unlike irinotecan, severe diarrhoea was not observed with exatecan.

The antitumour activity of exatecan has been observed in patients with non-small cell lung cancer, colorectal cancer, hepatocellular cancer and sarcoma, as well as irinotecan- and topotecan-resistant cancers. On the basis of the encouraging results obtained from phase I studies, exatecan has entered into the phase II clinical studies as a potent topo I poison.

6.3 IDEC-132

In *in vitro* studies, IDEC-132 demonstrated much stronger topo I inhibitory activity than topotecan or irinotecan.[16] In preclinical studies, the antitumour activity of IDEC-132 was established in a variety of human tumour xenografts, including colon cancer, melanoma,[146] acute leukaemia,[147] prostate,[148] breast,[149] ovarian[150] and bladder cancer.[151] In spite of its impressive preclinical activity, clinical development of IDEC-132 was hampered until 1993 because of its poor water solubility. In 1993, the clinical formulation of IDEC-132 was first prepared in dimethylacetamide (DMA) consisting of polyethylene glycol and phosphoric acid, and used in phase I clinical trials. Subsequently, the colloidal dispersion (CD) of IDEC-132 was formulated and introduced into clinical studies. Unfortunately, phase I and II clinical studies with IDEC-132 in either the DMA or CD formulation have failed to demonstrate its activity in patients with metastatic colorectal cancer or advanced squamous cell head and neck cancer,[152,153] while modest response rates have been reported in patients with ovarian cancer[154] and lymphoma.[155]

The major dose limiting toxicity was neutropenia and thrombocytopenia, which appeared when the drug was infused continuously for 72 hours every 2 or 3 weeks.[152,156,157] In some prolonged infusion schedules, gastrointestinal toxicities such as diarrhoea, vomiting and nausea were reported, but the diarrhoea was found to be much less severe than that was seen with irinotecan.[158]

Despite its remarkable antitumour activities in preclinical studies, almost all clinical studies suggested that IDEC-132 is much less clinically useful than its close camptothecin relatives, topotecan and irinotecan, and these findings resulted in discontinuation of phase II studies.

6.4 Rubitecan

Rubitecan is another water-insoluble camptothecin analogue that is metabolically converted *in vivo* into equally or more potent IDEC-132. Rubitecan has been shown to be more stable to handle and relatively inexpensive to prepare than IDEC-132.[159] Because of its limited solubility in aqueous solution, rubitecan has mainly been formulated as an oral agent.

In a phase II study, the oral dose of rubitecan was well tolerated in patients with heavily refractory ovarian, tubal or peritoneal cancer.[160] The principal toxicities were myelosuppression, including neutropenia and thrombocytopenia, and non-haematological toxicities, including nausea, vomiting, diarrhoea, weight loss, chemical cystitis and neutropenic sepsis.

More recently, liposomal formulations of rubitecan have been prepared and the antitumour activity was examined in various human cancer xenograft models in mice.[161] In these studies, intravenous and intramuscular administration of liposomal rubitecan enhanced the antitumour activity in a tumour-bearing athymic mouse model with human colon and breast carcinoma. In another study, administration of liposomal rubitecan in

aerosol demonstrated that aerosol therapy is much more effective in the treatment of murine melanoma and human osteosarcoma lung metastases in mice than those given by oral, intravenous or intramuscular routes.[162] On the basis of these findings, aerosol delivery of liposomal rubitecan has been introduced into phase I clinical studies and the available data up until now have demonstrated that administration of liposomal rubitecan on 5 consecutive days is well tolerated in patients with advanced malignancies in lungs, with moderate toxicity.[163]

6.5 Lurtotecan

Lurtotecan, a water soluble semisynthetic analogue of camptothecin, is currently being investigated for the treatment of cancer as a second generation topo I targeting drug. Compared with topotecan, lurtotecan was found to be approximately three times more potent in the cleavable complex assay and three to five times more potent in tumour cell cytotoxicity assays *in vitro*. In *in vivo* preclinical studies, lurtotecan has shown strong antitumour activity in HT-29 and SW48 human tumour xenograft models in nude mice.[91] Unlike topotecan, lurtotecan was able to induce regression of established tumours.

Phase I clinical and pharmacological studies revealed that the major dose limiting toxicity associated with this drug is myelosuppression, including severe neutropenia and thrombocytopenia. Other toxicities, including nausea, vomiting, fatigue, alopecia and anorexia, were mild to moderate.[164,165] In a phase II clinical study, the antitumour activity of lurtotecan was moderate in patients with breast cancer and minimal in patients with non-small cell lung cancer. No substantial activity was observed in patients with colorectal cancer.[166]

Recently, a liposomal formulation of lurtotecan (OSI-211; NX-211) has been prepared to enhance the delivery of drug to tumours and to increase antitumour efficacy.[167,168] Preclinical data have shown that the therapeutic index of the liposomal formulation of lurtotecan is increased approximately 3-fold over that of non-liposomal lurtotecan in both KB and ES-2 xenograft models, suggesting that the liposomal formulation could demonstrate increased therapeutic index in humans.[168]

6.6 Diflomotecan and BN-80927

Diflomotecan and BN-80927 are relatively new homocamptothecin family topo I poisons with unique features. Replacement of the six membered α-hydroxy lactone ring of camptothecin with a seven membered β-lactone ring enhances the plasma stability as well as potent topo I inhibitory activity of these compounds compared with the clinically advanced camptothecin analogues, irinotecan and topotecan.[23,98,100]

BN-80927 has shown substantial activity in resting cells as well as cell lines exhibiting multidrug-resistant phenotypes.[169] In addition to topo I inhibitory activity, BN-80927 inhibits topo II mediated DNA relaxation and DNA catenation.[170] Because of the dual topoisomerase inhibitory activity and considerable cytotoxicity in a panel of human tumour cell lines, BN-80927 has been selected for preclinical and clinical studies. In initial preclinical studies, impressive antitumour activity was established in HT-29 cell line, and PC-3 and DU-145 prostate tumours in mice with BN-80927 given by oral route.[170] On the basis of these promising findings, BN-80927 is currently receiving consideration for further clinical studies.

7. Conclusions

A renewed interest in camptothecins as potent anticancer chemotherapeutics has resulted directly from advances in understanding their mechanism of action. The identification of topo I as the primary cellular target, the recent crystallisation of a DNA-topo I complex and subsequent ternary complex models have provided valuable insights in the interactions at the molecular level. With this information in hand, research has now focused on the development of highly specific camptothecin analogues that bind to topo I and have more optimal physicochemical properties than the original camptothecin analogues, irinotecan and topotecan.

The resulting compounds may exert fewer adverse effects, which have hampered earlier clinical application of this class of chemotherapeutics. In fact, dose dependent adverse effects limited the therapeutic options of the early camptothecin analogues. Second generation camptothecins such as IDEC-132 and rubitecan, and lurtotecan, exert strong topo I inhibitory activity against a variety of tumour types in combination with a good toxicity profile in humans. Early preclinical and clinical trials are promising and we can expect to see a surge in clinical applications of novel camptothecin analogues as monotherapy as well in combination therapy with other antitumour compounds.

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