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Synthesis of new cytotoxic E-ring modified camptothecins

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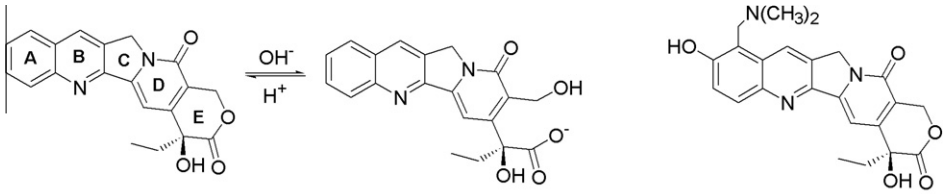
ABSTRACT

In an effort to decrease the toxicity and improve the stability of the E-ring of camptothecin, new analogues with an 'inverted' lactone ring were designed and synthesized. The compounds retained a good cytotoxic activity on human non-small lung cancer cells H-460.

Camptothecin (**1**, CPT) is a naturally occurring pentacyclic alkaloid first isolated in 1966 from the Chinese tree *Camptotheca acuminata* by Wall et al.[1] CPT and its derivatives selectively inhibit the nuclear enzyme Topoisomerase I (TopoI),[2] thus representing a very promising class of anticancer agents. The family of CPT derivatives has been enlarged rapidly during the last decades.[3] Among CPT analogues, Topotecan[4] and Irinotecan[5] have been approved as chemotherapeutic drugs in clinical treatment of human cancer and several other derivatives are in different phases of clinical trials.[6]

Unfortunately, under physiological conditions, the presence of the α-hydroxylactone group results in an equilibrium that favours the inactive but toxic open carboxylate over the active ring-closed



1 Camptothecin (CPT) **Camptothecin carboxylate** (inactive) **Topotecan**

One of the major limitations of CPT and analogues is the severe toxicity, stemming in part from the instability of the α-hydroxylactone E-ring. This ring plays a key role in supporting both efficient lactone form.[8] Moreover, the CPT carboxylate binds tightly to serum albumin, which limits the fraction of drug in the active lactone form.[9]

In early research, a number of E-ring modified CPT analogues were prepared. These studies showed that any change in the E-ring, such as deletion of the hydroxy group at C_{20} position, ring contraction to a five-membered lactone, replacement of the

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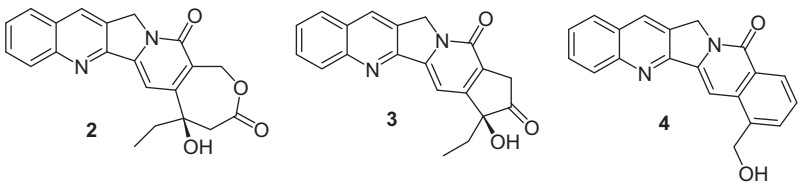
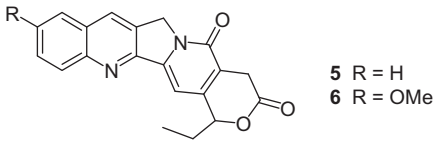


Scheme 1. Examples of E-ring modified CPT analogues.



Scheme 2. E-ring modified CPT analogues with an 'inverted' lactone ring.

lactone by a lactam, a thiolactone or an imide,[10] reduction of the lactone to lactol,[11] synthesis of six-member ring[12] or five-member ring ethers[10,13] gave essentially inactive compounds. These results led to the conclusion that the intact six-membered ring was indispensable for antitumor activity.

However, further investigations demonstrated that other E-ring modified CPT analogues showed comparable or even better antitumor activity than the parent compound. The first successful approach was to expand the α-hydroxylactone ring by an additional methylene, thereby generating seven-membered α-hydroxylactone E-ring analogues, which are referred to as homocamptothecins (**2**),[14] expectedly less prone to ring opening. HomoCPTs were reported to exert potent inhibition of TopoI and elevated levels of cytotoxicity, while showing enhanced stability and decreased protein binding in human plasma.[15]

Their dehydrated conjugated ene-lactone analogues were only modestly active in the TopoI cleavage test.[13] Other successful approaches to the synthesis of stabile E-ring CPT derivatives were recently reported. Five-membered lactone-free CPT analogues (**3**), appeared to be potent inhibitors of TopoI and showed interesting cytotoxic activity.[16] Compound **4**[17]and the natural pyrroloquinazolinoquinoline alkaloid luotonin A,[18] with an aromatic system as E-ring, were also found to have significant in vitro cytotoxic activity (Scheme 1).

These results raise the possibility that novel E-ring modified analogues of CPT might retain or even possess enhanced antitumour activity.

The present study was undertaken to explore a new structural change at the E-ring, while keeping a lactone moiety in this portion of the molecule. Thus, we designed derivatives with an 'inverted' lactone ring. (compounds **5** and **6**, Scheme 2). In these compounds the lactone ring is expected to be more stable, because the carbonyl group, lacking the activating α-hydroxy substituent typical of camptothecins, should be a weaker electrophile than the CPT α-hydroxylactone moiety (compare the HomoCPTs,[14] see above).

Molecular modelling studies were performed to explore the binding mode for the planned compounds to the covalent TopoI–DNA complex. This study was based on the X-ray crystal structure

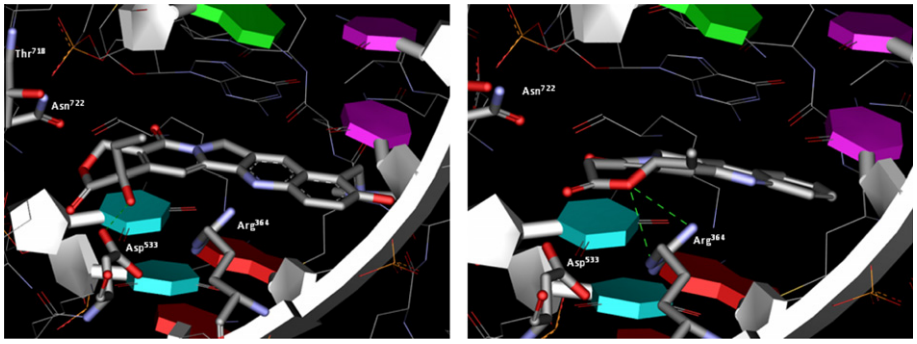


Figure 1. Schematic representation of the proposed binding mode for Topotecan (left) and compound **5** (right) in the TopoI–DNA complex. The side chains of aminoacids relevant to the discussion are labeled and rendered in stick. Hydrogen bonds are shown as green dotted lines.

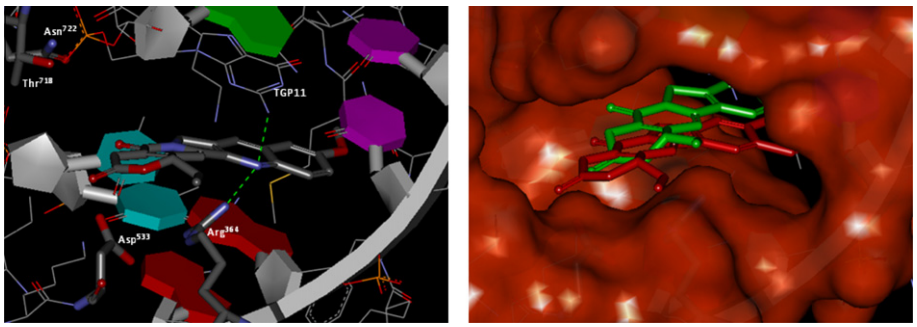


Figure 2. (left) Representation of the proposed top-score binding mode for compound **6** in the TopoI–DNA complex. The side chains of aminoacids relevant to the discussion are labeled and rendered in stick, and hydrogen bonds are shown as green dotted lines. (right) Superimposition of the top-score binding conformations for compounds **5** (green) and **6** (red) inside the TopoI–DNA complex. Ligands are shown in stick, TopoI–DNA complex is rendered by using its solvent accessible surface (SAS) representation.

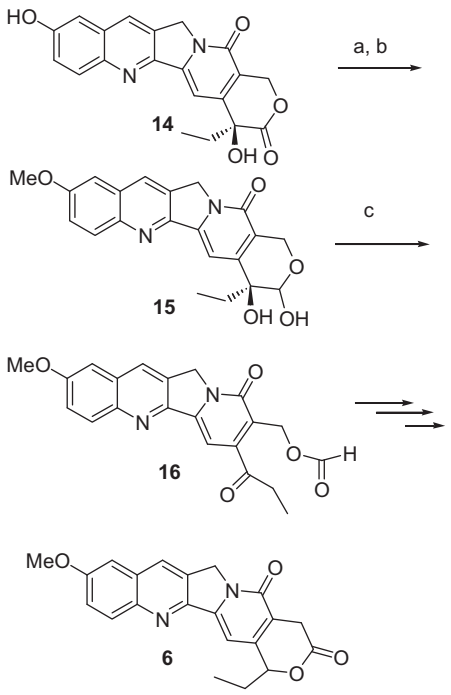
of a ternary complex between a human TopoI construct covalently attached to a DNA duplex with bound Topotecan.[7] For comparison purposes, the same docking protocol used to study the complex containing Topotecan was applied in the present work.

The intercalation binding site was created by conformational changes of the phosphodiester bond between the +1 (upstream) and −1 (downstream) base pairs of the uncleaved strand, which effectively 'open' the DNA duplex, with the evidence of only one direct hydrogen bond between the Asp^{533} residue of the enzyme and Topotecan in both the carboxylate and lactone models. The comparative analysis of the best poses obtained for compound **5** revealed that the molecule formed base-stacking interactions with both the −1 and +1 base pairs, with a strong π–π stacking between the C-ring and guanosine at position +1 of the cleaved strand (+1G). The interactions between compound **5** and TopoI–DNA complex are given in Figure 1 (together with the experimental Topotecan conformation), where the interactions listed are hydrogen bonds.

For compound **5**, the hydrogen bond between the Asp^{533} residue and Topotecan is replaced by two strong hydrogen bond interactions between the lactone ring oxygen atom and both the NH_1 and NH_2 hydrogen atoms of Arg^{364} (close to Asp^{533}), equal to 2.85 Å and 3.00 Å, respectively. Thus, compound **5** appears to make use of distinct sets of interactions to stabilize the binding mode at the intercalation site in TopoI–DNA complex. The promising binding provides structural support to synthesis of **5** and analogues. Among them, compound **6**, bearing a methoxy group in position 10, showed a calculated free energy (−9.12 kcal/mol) close to the value calculated for compound **5** (−9.81 kcal/mol).

As shown in Figure 2, the ligand is placed in the TopoI–DNA complex in almost the same orientation as Topotecan and compound **5**, but the molecule is shifted towards the entrance of the binding site. Consequently, the strong π–π stacking interaction is now between the B-ring and +1G. Due to the new position of the ligand, compound **6** is connected to the TopoI–DNA complex through two new hydrogen bonds between the heterocyclic nitrogen of B-ring and NH_1Arg^{364} (2.93 Å) and N2 of guanosine +1 (2.78 Å).

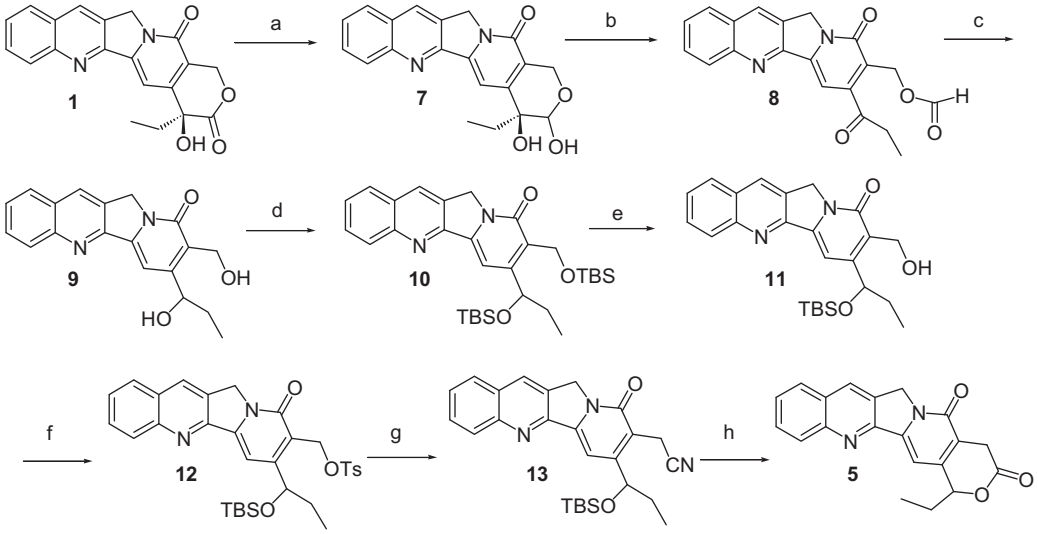
The synthesis of compound **5** was performed using natural camptothecin as a starting material. CPT was readily reduced with sodium borohydride to the corresponding lactol **7**, whose 1,2-diol moiety was oxidatively cleaved with periodic acid to give compound **8**.[12] Attempts to catalytically reduce the keto group failed,



Scheme 4. Synthesis of compound **6** from 10-hydroxycamptothecin. Reagents and conditions: (a) CH_2N_2, MeOH/dioxane 1:1, rt, 2 h, 100%; (b) $NaBH_4$, MeOH, rt, 2 h, 90%; (c) silica-gel supported $NaIO_4$, DCM, rt, 24 h, 60%.

while treatment with sodium borohydride in DCM/methanol gave compound **9** in good yield. The diol was disilylated using TBDMSCl in DCM and selective primary desilylation was successfully achieved using catalytic PTSA in methanol.[19] To install the carboxylic acid moiety in the right position for the subsequent lactonization, one-carbon homologation of the side chain at position 8 of compound **11** was required. Thus, compound **11** was converted into the corresponding tosylate, that was immediately treated with sodium cyanide to furnish compound **13**. Finally, deprotection of the hydroxyl group and simultaneous hydrolysis and cyclization were achieved using HCl in ethanol (Scheme 3).

The synthesis of the corresponding 10-methoxy derivative was achieved following the same pathway described for compound **13**,



Scheme 3. Synthesis of compound **5** from natural camptothecin. Reagents and conditions: (a) $NaBH_4$, MeOH, rt, overnight, 90%; (b) $NaIO_4$, AcOH, rt, overnight, 78%; (c) $NaBH_4$, DCM/MeOH 8:2, rt, 40 min, 87%; (d) TBDMSCl, imidazole, DMF, rt, 24 h, 70%; (e) PTSA, DCM/MeOH 1:1, 0 °C then rt, 1 h, 68%; (f) TsCl, DMAP, DCM, 0 °C, 24 h; (g) NaCN, DMSO, 70 °C, 2 h, 54%; (h) HCl, EtOH, 90 °C, 1.5 h, 29%.

starting from natural 10-OH-CPT. In this case the oxidative cleavage with sodium periodate appeared troublesome. Several attempts were performed but they all resulted unsuccessful, giving complex and unseparable mixtures of products. Finally, it was found that treatment of a DCM solution of compound **15** with silica-gel supported $NaIO_4$ afforded compound **16** in a satisfactory yield. The remaining steps to get compound **6** were the same as described previously for compound **5** (Scheme 4).

Compounds **5** and **6**[20] were tested for their antiproliferative activity on human non-small lung cancer cells H-460 (1 h exposure), using Topotecan as a reference compound (IC_{50} = 1.38 ± 0.95 μM). Both analogues **5** and **6**, even if still in the racemic form, maintained a good cytotoxic activity, with IC_{50} less than 10 μM (IC_{50} 8.73 and 7.45 μM, respectively). The results suggest that the introduction of an 'inverted lactone' moiety in ring E gives compounds with retained antitumour activity, making them worthy of further investigation. Work is currently in progress to evaluate the effect of substituents and to study the Topoisomerase I inhibitory activity of these new camptothecin analogues.

Acknowledgment

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20. Characterization of compound **5**. Mp 320 °C dec ^{1}H NMR 300 MHz (DMSO-d_6) δ: 8.70 (s, 1*H*), 8.10–8.20 (m, 2*H*), 7.86 (m, 1*H*), 7.70 (m, 1*H*), 7.30 (s, 1*H*), 5.58 (m, 1*H*), 5.31 (s, 2*H*), 3.68 (m, 2*H*), 2.10 (m, 2*H*), 1.10 (t, *J* 7.4 Hz, 3*H*). HRMS (ESI$^+$) calcd for $C_{20}H_{16}N_2O_3Na$ [M+Na]$^+$ 355.10531; found 355.10612. Characterization of compound **6**. mp 278 °C dec ^{1}H NMR 300 MHz ($CDCl_3$) δ: 8.28 (s, 1*H*), 8.10 (d, *J* 8.46 Hz, 1*H*), 7.50 (dd, *J* 8.46, 1.47 Hz, 1*H*), 7.19 (d, *J* 1.47 Hz, 1*H*), 7.10 (s, 1*H*), 5.41 (m, 1*H*), 5.31 (s, 2*H*), 4.00 (s, 3*H*), 3.89 (AB, 1*H*), 3.62 (AB, 1*H*), 2.10 (m, 2*H*), 1.10 (t, *J* 7.3 Hz, 3*H*). HRMS (ESI$^+$) calcd for $C_{21}H_{19}N_2O_4$ [M+H]$^+$ 363.13393; found 363.13426; HRMS (ESI$^+$) calcd for $C_{21}H_{18}N_2O_4Na$ [M+Na]$^+$ 385.15500; found 385.11626.