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Synthesis and cytotoxic activity of new 9-substituted camptothecins

Sabrina Dallavalle,[a,*] Daniela Granza Rocchetta,[a] Loana Musso,[a] Lucio Merlini,[a] Gabriella Morini,[b] Sergio Penco,[a] Stella Tinelli,[c] Giovanni Luca Beretta[c] and Franco Zunino[c]

[a]*Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria 2, 20133 Milano, Italy*
[b]*Università di Scienze Gastronomiche, Pollenzo, Italy*
[c]*Unità di Chemioterapia e Farmacologia Antitumorale Preclinica, Dipartimento di Oncologia Sperimentale e Laboratori, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milano, Italy*

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Abstract—A series of novel 9-substituted camptothecins derived from 9-formylcamptothecin were synthesized. The aldehyde was obtained from 10-hydroxycamptothecin or, better, by total synthesis. The compounds showed antiproliferative activity higher than that of the reference compound topotecan. Modelling suggested the possibility of a favourable interaction of small and polar 9-substituents with the topoisomerase I–DNA complex, which is consistent with the higher activity of these derivatives with respect to the corresponding 7-substituted camptothecins.

The natural product camptothecin (CPT)[1] and its synthetic analogues are among the most promising new agents for the treatment of human cancers. CPT is a pentacyclic alkaloid, which was first isolated in 1966 from the extracts of a Chinese plant, *Camptotheca acuminata*, by Wall et al.[2] Although CPT is a potent cytotoxic agent, early clinical studies with this compound in the 1970s were unsuccessful as a consequence of severe and unpredictable toxicities. Interest in CPT derivatives was revitalized in 1985 by the discovery that CPT exhibits a unique mechanism of action because it targets the nuclear enzyme topoisomerase I (Topo I). [3] CPT forms a ternary complex with topoisomerase I and DNA, and the stabilization of this complex results in DNA breaks by preventing DNA religation.[4]

Intensive efforts in medicinal chemistry over the past decades have provided a large number of CPT analogues, of which topotecan and irinotecan (prodrug of SN-38) (Chart 1) are those so far approved for the clinical therapy.[5]

SAR studies on the numerous compounds prepared have shown that substitutions in positions 7, 9, 10, 11 (and in some cases also 12) are tolerated or give substantial increase of the activity.[6] In particular, both topotecan and SN-38 have an OH substituent in position 10, which seems to be important either to decrease the unwanted stabilization of the open hydroxyacid form by human albumin[7] or to increase the solubility. A recent X-ray crystallographic analysis of a ternary complex between a topoisomerase I construct, a DNA oligonucleotide and topotecan indicated that modifications at the 7- and 9-position of CPT would not interfere with drug–protein interactions.[8] To date most of the second-generation CPT that have reached preclinical or clinical development studies are 7-substituted derivatives, for example, silatecans, karenitecins, gimatecan, and belotecan, all bearing highly lipophilic substituents, deemed to increase the stability of the lactone form in ring E of CPT with respect to the open hydroxyacid form (Chart 2).[7,9]

In our laboratory a series of camptothecins substituted in position 7 with lipophilic moieties have been synthesized using as a key intermediate CPT-7-aldehyde. Various groups were linked to the CPT scaffold via carbon-carbon double bonds, iminomethyl or oxyiminomethyl moieties. These compounds exhibited potent cytotoxic activity in vitro and in vivo comparable or superior to topotecan.[10–12] The highest activity was shown by oxyiminomethyl derivatives: gimatecan (Chart 2), the most

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* Corresponding author. Tel.: +39 2 50316816.; e-mail: sabrina.dallavalle@unimi.it

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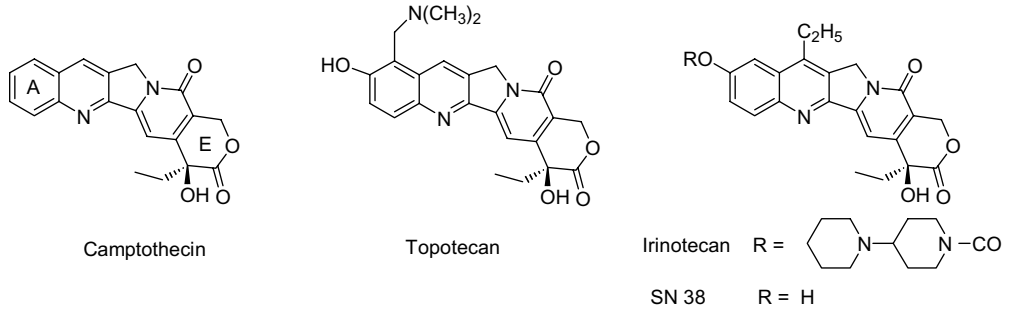


Chart 1.

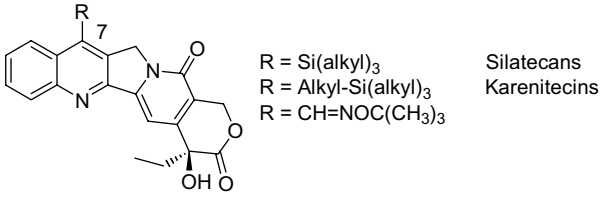


Chart 2.

active compound of the series, is in phase I–II of clinical studies in patients with advanced solid tumours no more sensitive to standard therapies.[13]

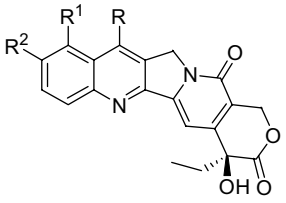
While most researches have focused on the synthesis of CPT derivatives bearing substituents in position 7, the literature reports only a few examples of camptothecins substituted in position 9, most relevant being 10-hydroxy-9-dimethylaminomethylcamptothecin (topotecan), a drug in current clinical use, 10-hydroxy-9-allyl-CPT (chimmitecan),[14] 9-nitrocamptothecin (rubitecan) and 9-aminocamptothecin, which have been submitted to preclinical and clinical studies.[5]

One of the reasons for the limited number of 9-substituted CPT studied may be the scarce accessibility of this position. In fact, in accordance with the reactivity of the quinoline nucleus, substitution of CPT in position 7 occurs easily via radical reactions.[1] On the contrary, the functionalization of position 9, that requires an electrophilic substitution on a deactivated position, usually occurs with low yields and gives mixtures with 12-substituted isomers.[1] This difficulty has been overcome, in most cases, starting from the naturally occurring 10-hydroxy-CPT, where the OH group confers increased reactivity to the A ring.[15–17]

The high cytotoxic activity of the few 9-substituted CPT reported in the literature prompted us to investigate the introduction of other moieties in this quite unexplored position. In particular, the goal of the present study was to synthesize analogues in position 9 of the most active examples of 7-substituted CPT (**1a**–**n**) previously obtained by us (Table 1).

Prior to starting the synthesis, we modelled the binding of the designed compounds to the Topo I covalent com-

Table 1. Cytotoxic activity (IC_{50} (μM) on H460 cell line)



Entry		R or R^1	R^2	IC_{50} (μM)	
7-Substituted	9-Substituted			7-Substituted (R^1 = H)	9-Substituted (R = H)
	Topotecan	CH_2–$N(CH_3)_2$	OH		1.18 ± 0.24
SN-38		CH_2CH_3	OH	0.22 ± 0.013	
1a	**16**	CH_2OH	H	0.49 ± 0.093	0.21 ± 0.074
1b	**7**	CHO	H	0.388 ± 0.29	0.058 ± 0.029
1c	**3**	CHO	OH	1.78 ± 0.5	6.96 ± 3.13
1d	**5**	CHO	OCH_3	0.18 ± 0.05	6.00 ± 1.49
1e	**18**	CN	H	1.04 ± 0.5	0.428 ± 0.149
1f	**4b**	CN	OH	5.65 ± 2.5	9.86 ± 0.18
1g	**8b**	CN	OCH_3	0.66 ± 0.02	0.67 ± 0.025
1h	**17c**	CH = $NOC(CH_3)_3$	H	0.015 ± 0.006	0.23 ± 0.11
1i	**4a**	CH = $NOC(CH_3)_3$	OH	0.13 ± 0.03	0.36 ± 0.015
1l	**8a**	CH = $NOC(CH_3)_3$	OCH_3	0.07 ± 0.004	0.32 ± 0.044
1m	**17b**	CH = NOCH $_2CH_2NH_2$	H	0.51 ± 0.15	2.53 ± 0.97
1n	**17a**	CH = NOH	H	0.03 ± 0.0076	0.23 ± 0.143

plex with DNA. Thus we compared the intermolecular interaction energy of all the couples of 7- and 9-substituted compounds with the model of topoisomerase I cleavage site. The results indicated intermolecular interaction energies of the same order of magnitude for the 7- and 9-substituted corresponding compounds, suggesting not only that there is space for substitution in position 9 of CPT, in accordance with Staker's crystallographic data and modelling,[8] but also that their binding activity should be quite strong, being comparable to that of 7-substituted CPT.

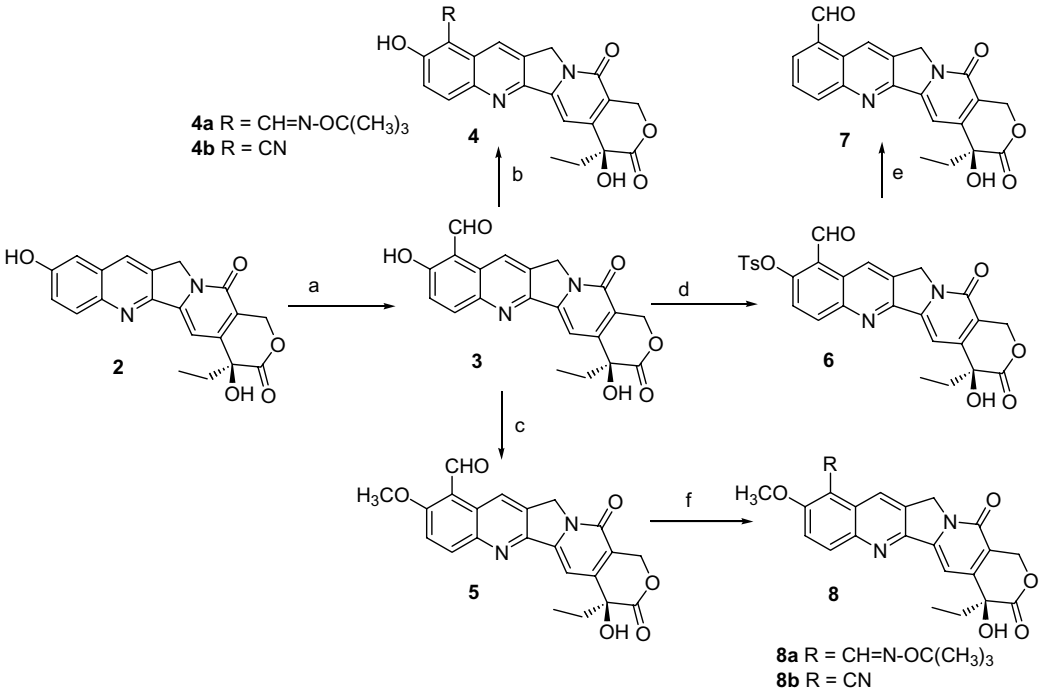
Our synthetic plan required CPT-9-aldehyde as a key intermediate. As a first approach to the synthesis, we decided to exploit the activating effect of the hydroxy group, starting from 10-hydroxy-CPT (**2**). The formylation of 10-hydroxy-CPT based upon Duff reaction has already been reported.[15] However, in our hands, the formyl derivative was obtained with erratic yields that never exceeded 26%, despite numerous attempts of optimization. Several other approaches to introduce the aldehyde group at position 9 of 10-hydroxy-CPT, including reaction with NaOH in chloroform, formaldehyde and $SnCl_4$ in toluene and with α,α-dichloromethylmethylether and $TiCl_4$ in dichloromethane, failed. Thus, in spite of the low yield, 9-formyl-10-hydroxycamptothecin (**3**) was synthesized via Duff reaction and then converted into 9-formyl-10-methoxycamptothecin (**5**) by methylation with diazomethane. Both compounds **3** and **5** were easily converted into oximes **4a** and **8a** and cyano derivatives **4b** and **8b** (Scheme 1).

At this stage removal of the 10-hydroxy group was necessary to obtain the desired 9-substituted camptothecins.

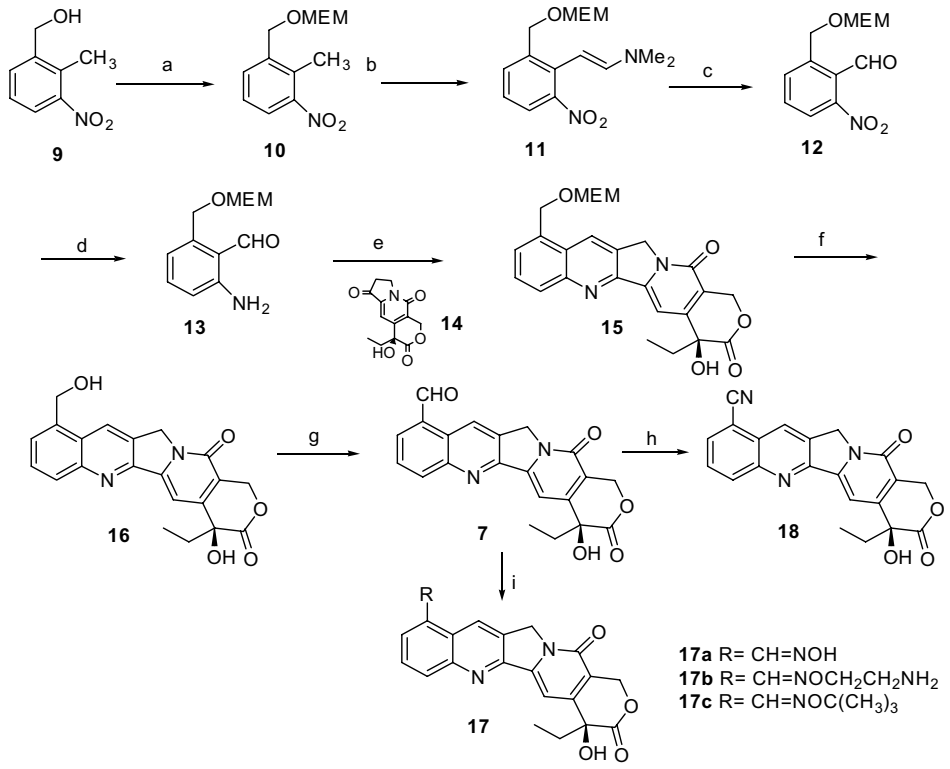
Attempts to obtain 9-formylcamptothecin by Pd-catalyzed deoxygenation of 9-formyl-10-hydroxycamptothecin triflate or tosylate[17] were hampered by the instability of the sulfonyl derivatives which partially reverted to the starting compound during the workup of their synthesis. The subsequent reaction with Et_3SiH catalyzed by $Pd(OAc)_2$ led to the desired 9-formylcamptothecin only in an unacceptably low yield (Scheme 1).

These difficulties led us to devise an alternative procedure based upon a total synthesis of 9-formylcamptothecin via Friedländer condensation of the chiral tricyclic ketone **14**[18] with an appropriately substituted *o*-aminobenzaldehyde. The structure of the final product required that this intermediate contained a protected or a potential second aldehyde group. After several attempts, the synthetic sequence of Scheme 2 gave satisfactory results. Commercially available (2-methyl-3-nitrophenyl)methanol **9** was protected by treatment with MEMCl in basic conditions to give **10**,[19] which was converted into enamine **11** by treatment with dimethylformamide dimethylacetal and pyrrolidine in DMF. Oxidation with sodium periodate followed by catalytic hydrogenation gave *o*-aminoaldehyde **13** in quantitative yield (Scheme 2).

The Friedländer condensation of **13** with ketone **14** catalyzed by acetic acid or PTSA led only to degradation products, whereas treatment with $ZnCl_2$ in ethanol[20]



Scheme 1. Reagents and conditions: (a) HMTA, TFA, N_2, 10 h, 26%; (b) for **4a**: *t*-$BuONH_2$·HCl, NaOH, EtOH, rt, 48 h, 57% for **4b**: NH_2OH·HCl, HCOONa, HCOOH, reflux, 11 h, 77%; (c) CH_2N_2, MeOH, rt, 3 h, 90%; (d) tosyl chloride, TEA, DMAP, CH_2Cl_2, N_2, 0 °C, then rt 40 min, 34%; (e) $Pd(OAc)_2$, dppp, Et_3SiH, DMF, N_2, 60 °C, 1 h, 20%; (f) for **8a**: *t*-$BuONH_2$·HCl, NaOH, EtOH, rt, 72 h, 62%; for **8b**: NH_2OH·HCl, HCOONa, HCOOH, reflux, 9 h, 65%.



Scheme 2. Reagents and conditions: (a) MEMCl, DIEA, CH_2Cl_2, 0 °C 10 min, then rt 12 h, 88%; (b) DMFDMA, pyrrolidine, DMF, N_2, 135 °C, 12 h, 95%; (c) $NaIO_4$, THF/H_2O 1:1, rt, 3 h, quantitative; (d) H_2, Pd/C 10%, EtOH, 2 h, quantitative; (e) $ZnCl_2$, molecular sieves, EtOH, N_2, 70 °C, 5 h, 40%; (f) TFA, CH_2Cl_2, rt, 24 h, 70%; (g) MnO_2, $CHCl_3$, rt, 12 h, 64%; (h) $NH_2OH{\cdot}HCl$, HCOONa, HCOOH, reflux, 6 h, 47%; (i) $RONH_2$, EtOH, Py, reflux, 2–4 h.

afforded 9-(2-methoxyethoxymethoxymethyl)-camptothecin **15** in good yield. The desired CPT-9-aldehyde **7** was obtained by the deprotection of **15** and subsequent oxidation of alcohol **16**. The availability of **7** allowed us to synthesize the three derivatives **17a**–**c** , and nitrile **18**.

The prepared compounds were tested for their growth inhibitory activity against the human non-small cell lung carcinoma cell line H460[21] (Table 1), a cell model selected for its sensitivity to Topo I inhibitors.[22] Topoisomerase I-mediated DNA cleavage assays with purified human topoisomerase I were used to investigate the ability of some derivatives to stimulate the DNA damage.[23] All the tested CPT revealed an intensity of DNA damage comparable to that of the reference compound SN-38 (Fig. 1). Moreover, DNA cleavage patterns were found identical to those observed in the presence of SN-38. Since the drug interaction with the DNA–enzyme complex is expected to be reversible, the persistence of the cleavable complex was evaluated after the addition of high salt concentration (0.6 M NaCl), which favours the dissociation of the ternary drug–enzyme–DNA complex. As already observed in our previous work,[12] such a stability was particularly evident for **1h**, which exhibited a DNA damage persistence around 90% after 10 min (Fig. 2). All the three 9-substituted derivatives tested (**7**, **17b**, **17c**) appreciably reduced the persistence of DNA damage as compared to the corresponding 7-substituted analogues.

From the data reported in Table 1, it appears that the 9-substituted CPT showed potent cytotoxic activity, in most cases higher than that of the reference compound topotecan. However, all the 9-oximinomethyl derivatives (**17a**–**c**) are of an order of magnitude less potent in growth inhibition than the corresponding 7-counterparts (**1n**, **1m**, **1h**). On the contrary, the presence of a single, small polar group, such as CH_2OH (**16**), CHO (**7**) and CN (**18**), in 9-position resulted in an enhancement of the activity with respect to the corresponding 7-analogues (**1a**, **1b**, **1e**), aldehyde **7** reaching the level of the most potent camptothecins so far prepared. This could be related to more strict steric requirements for the 9-substitution, or to a favourable effect of a H-accepting group in this position. Modelling experiments on compound 16 support this second hypothesis, showing the formation of two hydrogen bonds between the OH group in position 9 and the carbonyl group of the side chain of Asn 352 and the base C + 1 (Fig. 3). Among the compounds tested in the DNA cleavage assay (Fig. 1), no precise correlations could be found between Topo I inhibition and antiproliferative activity. This observation is not surprising, because the cytotoxic effect reflects not only target inhibition, but a number of events, including cellular pharmacokinetic behaviour. Indeed the most hydrophobic derivatives (e.g., **1h** and **17c**), which are expected to have favourable cellular accumulation, were more potent than the hydrophilic derivatives **1m** and **17b**. However the substantially reduced cytotoxic potency of **17b** reflected also a reduced stabilization (Fig. 1) and persistence (Fig. 2) of the cleavable complex. The contribution to the cytotoxic potency of factors other than Topo I inhibition is clearly

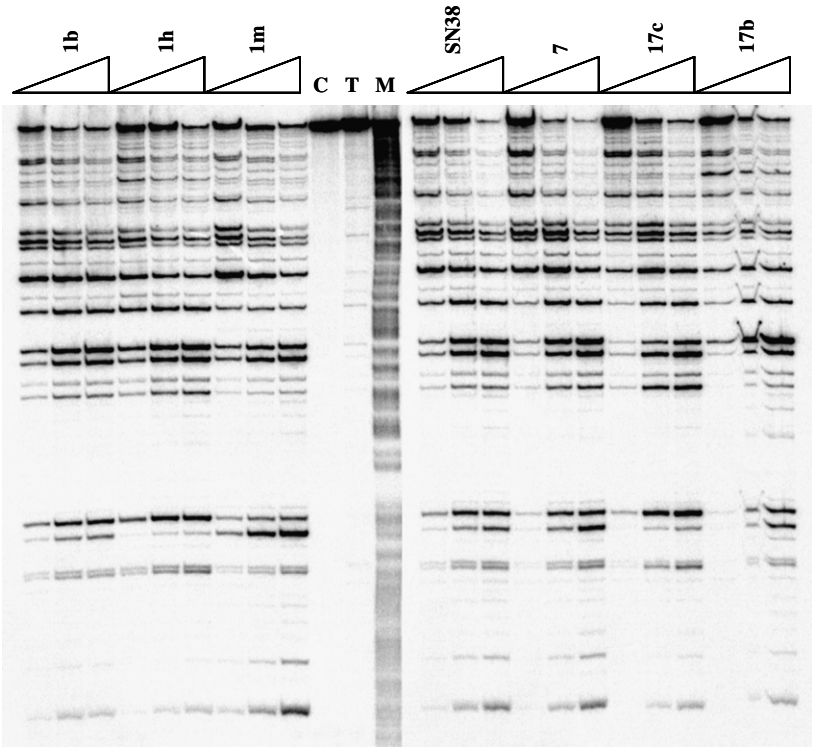


Figure 1. Topoisomerase I-mediated DNA cleavage assays. Samples were reacted with 1, 10 and 50 μM drug at 37 °C for 30 min. Reaction was then stopped by adding 1% SDS, 0.3 mg/ml of proteinase K and incubating for 45 min at 42 °C before loading on a denaturing 8% polyacrylamide gel. C, control DNA; T, reaction without drug; M, purine markers. The experiment was repeated three times and the results of a representative value are reported.

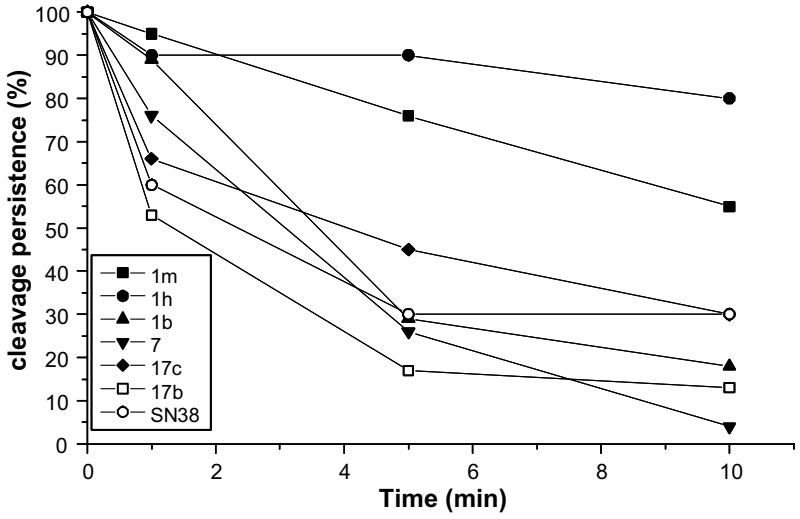


Figure 2. Cleavage persistence. The samples were reacted for 30 min with 10 μM drug. DNA cleavage was then reversed by adding 0.6 M NaCl. The 100% value is referred to the extent of DNA cleavage at 30 min of incubation. Each value was obtained by densitometric analysis. The experiment was repeated three times and the results of a representative value are reported.

documented by the quite different cytotoxicity of **1h** and **1m**, in spite of a comparable ability of these compounds in stabilizing DNA cleavage.

The introduction of an OH substituent in position 10 of 9-substituted CPT was expected to have a detrimental effect on the cytotoxicity, as observed in the 7-series,[12] most likely due to the increased hydrophilicity of the 10-OH derivatives.[7] This decrease of activity was stronger in the 9-series than in the 7-series in the presence of small substituents (cfr. **3/7**, **4b/18**, vs **1c/1b**, **1f/1e**). Methylation of the 10-hydroxy group restored the cytotoxic

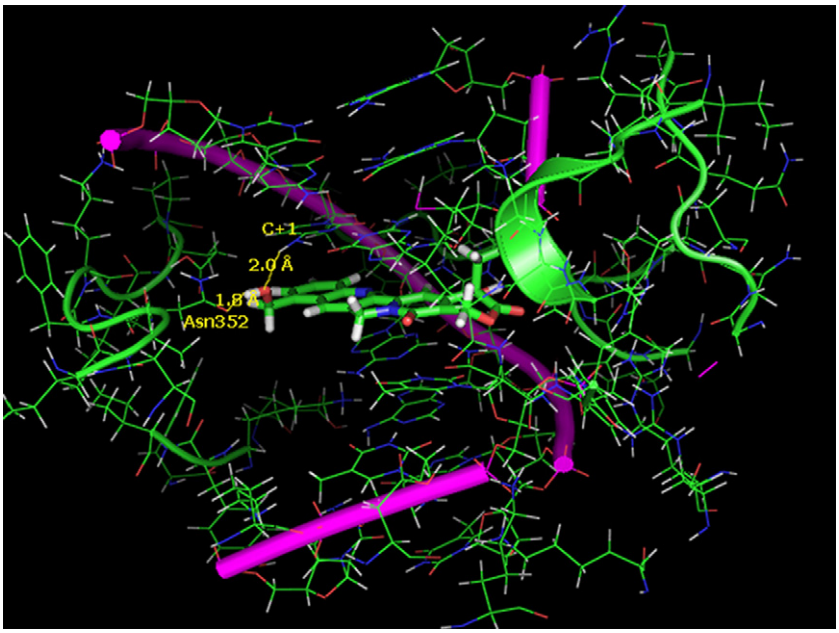


Figure 3. Structure of compound **16** in the ternary complex model. For the sake of clarity all the base pairs and only some amino acids are shown.

potency in the 7-substituted series (cfr. **1d** vs **1c**, **1g** vs **1f** and **1l** vs **1i**).[12] In the 9-substituted series a restoring effect of the methylation was observed for the 9-cyano derivative (**8b** vs **4b**), whereas in the case of 9-formyl-CPT **5** and oxyiminoderivative **8a** the drop of activity remained. These results suggest that the presence of substituents in position 10 is detrimental for the activity of 9-substituted CPT derivatives.

In conclusion, we have developed a synthetic method suitable for a convenient preparation of 9-substituted CPT derivatives using 9-formyl CPT as a versatile intermediate. By means of this synthetic pathway we have synthesized a series of novel camptothecins endowed with potent cytotoxic activity. The results show that the highest potency is obtained when a small polar, possibly H-acceptor group, is present in position 9.

Acknowledgments

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Supplementary data

Experimental: Synthesis and analytical data of compounds and docking procedures. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.016.

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