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New homocamptothecins: Synthesis, antitumor activity, and molecular modeling

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Abstract—Homocamptothecins (hCPTs) represent a class of new emerging antitumor agents, which contains a seven-membered β-hydroxylactone in place of the conventional six-membered α-hydroxylactone ring (E ring) of camptothecins. Some novel 7-substituted hCPTs were designed and synthesized based on a newly developed synthetic route which couples ring A with ring C, E and D. Most of the synthesized compounds exhibit very high cytotoxic activity on tumor cell line A549. Some compounds, such as **9b**, **9l**, and **9y**, show broad in vitro antitumor spectrum and are more potent than topotecan. Three-dimensional quantitative structure–activity relationship (3D-QSAR) methods, CoMFA and CoMSIA, were applied to explain the structure–activity relationship (SAR) of the synthesized compounds. Furthermore, molecular docking was used to clarify the binding mode of the synthesized compounds to human DNA topoisomerase I. The important hydrophobic, base-pair stacking, and hydrogen-bonding interactions were observed between the hCPT derivatives and their receptor. The results from molecular modeling will guide the design of novel hCPTs with higher antitumor activity.

1. Introduction

Camptothecin (CPT) was first isolated from the Chinese tree *Camptotheca acuminata* by Wall et al. in 1966.[1] The compound exhibits potent antitumor activity and has been evaluated in clinic.[2] However, the therapeutic application of unmodified CPT is hindered due to its poor aqueous solubility and high in vivo hepatotoxicity. Subsequently, the structural modification of natural CPT has generated many new CPT derivatives with improved pharmacological or pharmacokinetic profile.[3–5] Among those, topotecan[6] and irinotecan[7] (Chart 1) have entered clinical use and several drug candidates, such as rubitecan,[8] lurtotecan,[9] and exatecan,[10] are currently in different stages of the clinical trials.

Human DNA topoisomerase I (Topo I) is the molecular target of CPT derivatives.[11] These compounds act by binding to a transient Topo I–DNA covalent complex, leading to an accumulation of DNA strand breaks upon replication, ultimately causing cell death during the S phase of the cell cycle.[12–14] Although CPT analogues remain a promising class of antitumor agents, the intrinsic instability of the highly electrophilic α-hydroxylactone of the E ring undergoes rapid hydrolysis to the biologically inactive carboxylate form under physiological conditions.[15] This chemical feature diminishes efficacy of various CPT derivatives in vivo compared to the spectacular results often obtained from in vitro studies and xenograft models.[16] Thus, extensive efforts have been put to synthesize new CPT analogues with a prolonged biological life maintaining its active lactonic form. In 1997, Lavergne et al. reported the insertion of a methylene spacer between alcohol and carboxyl functions of CPT to obtain homocamptothecin (hCPT).[17] The replacement of the six-membered α-hydroxylactone with a seven-membered β-hydroxylactone reduces considerably the electrophilicity of the lactone and hence decreases the rate of lactone hydrolysis to the

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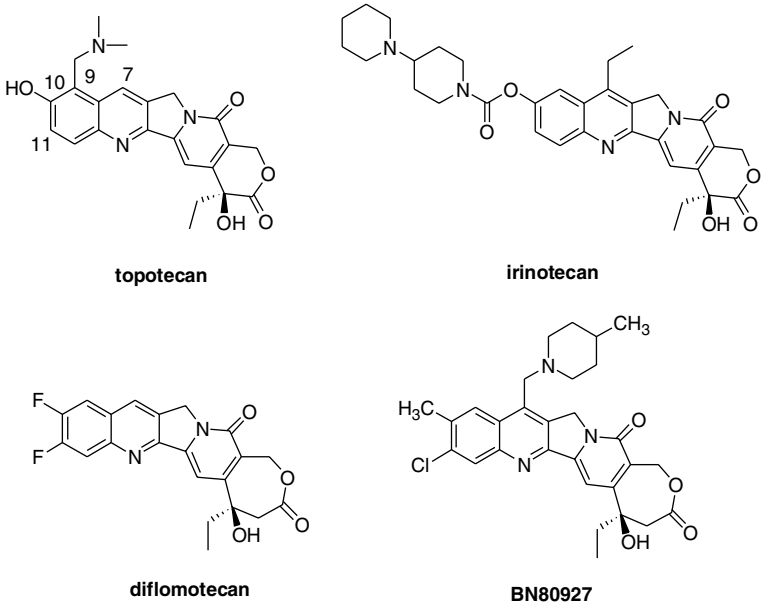


Chart 1.

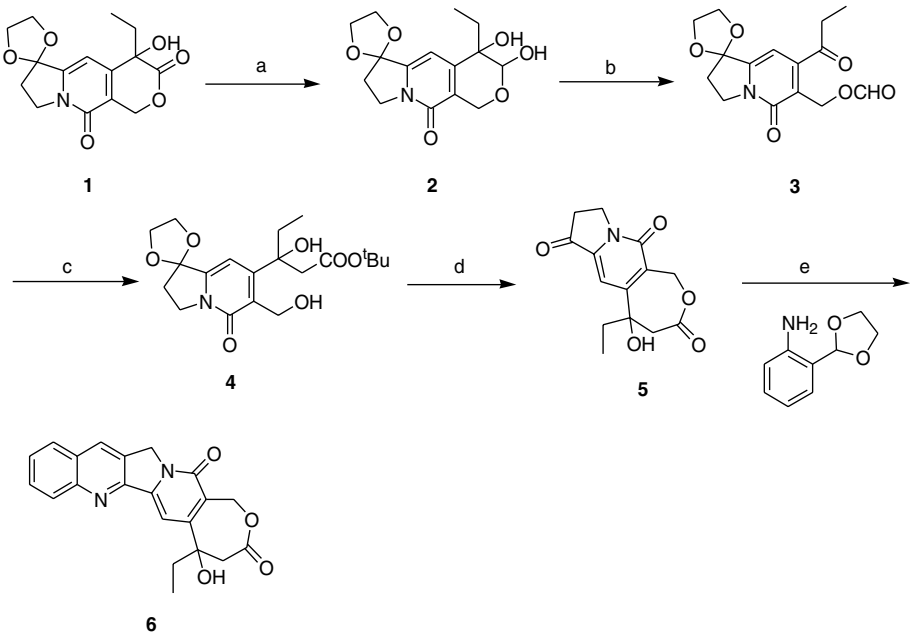
corresponding inactive carboxylate form. Moreover, hCPT is found to be much more stable than CPT in plasma because the affinity of hCPTs to human serum albumin is lower than that of the corresponding CPTs. As compared to CPT, hCPT exhibits better biological and pharmacological properties.[18] For example, hCPT conserves Topo I-mediated activity and was found to promote additional DNA breaks which are not seen with CPT.[19] It proves to be much more cytotoxic (up to 10 times) than CPT against a variety of tumor cells. Furthermore, the in vivo evaluation showed that hCPT exhibited superior antitumor activities compared to CPT in the HT-29 xenograft model.[20] On the basis of these encouraging results, several hCPT analogues with the modified A and/or B rings have been synthesized and evaluated in vitro and in vivo as the antitumor agents.[21–25] Two of them, BN80915 (Diflomotecan)[26] and BN80927[27] (Chart 1) are in clinical trial and encouraging results have been obtained. The exploration of new hCPT derivatives has recently emerged as a promising field to find better antitumor agents.

Some 7-substituted hCPT derivatives were designed in the present study. A new synthetic route was developed to synthesize the designed hCPT derivatives. Three-dimensional quantitative structure–activity relationship (3D-QSAR) methods were applied to explain the structure–activity relationship (SAR) of the synthesized compounds. Furthermore, molecular docking was used to clarify the binding mode of the synthesized compounds to human DNA topoisomerase I. The obtained information is useful in the future structural optimization.

2. Chemistry

hCPT was first synthesized by a semi-synthetic approach.[17] Starting from CPT, a four-step route, including reduction, oxidative cleavage, reformatsky reaction, and ring closure, was used to synthesize the racemic hCPT. However, the semi-synthetic approach was not appropriate for the preparation of diversely substituted hCPT analogues. Therefore, the total synthesis of hCPT analogues was more practical for the structural optimization. The first total synthesis of hCPT was reported by Lavergne et al. in 1998.[21] This route was based on the coupling of a DE heterobicycle with an AB quinoline. Another synthetic route of hCPT was developed by Curran and co-workers in 2002,[28] which was based on the coupling of an A ring with a DE heterobicycle by using the cascade radical annulation approach. However, the overall yield of these two synthetic routes is relatively low and some expensive or dangerous reagents have to be used. Therefore, the development of a new total synthesis route with mild reaction condition and high yield is particularly urgent.

Scheme 1 shows a new synthetic strategy for hCPTs based on the coupling of ring A with ring CDE. The key intermediate CDE ring **1** was synthesized according to the reported route[29] and the synthetic process was optimized in our previous studies.[30,31] Compound **1** was reduced by KBH_4 in anhydrous methanol to afford 1,2-diol **2**, which was oxidatively cleaved by $NaIO_4$ to give formyloxy ketone **3**. Reformatsky reaction of **3** with *tert*-butyl bromoacetate and Zn gave β-hydroxy ester **4** in a 97% yield and then the treatment with trifluoroacetic acid provided a key tricyclic intermediate **5**. Friedlander condensation of the tricyclic **5** with *N*-(*o*-aminobenzylidene)-*p*-toluidine in the presence of an acid catalyst gave the known homocamptothecin **6**. The overall yield of the present route is 12.8%, which is higher than those of the other two methods mentioned above.[21,28] Moreover, this route is more appropriate for the synthesis of diversely A, B ring-modified new hCPT derivatives.



Scheme 1. Reagents and conditions: (a) KBH_4, CH_3OH, rt, 20 min, 80%; (b) CH_3COOH, $NaIO_4$, rt, 30 min, 93%; (c) Zn, $BrCH_2COO^tBu$, THF, 75 °C, 3 h, 97%; (d) CF_3COOH, rt, 10 h, 94%; (e) toluene, *p*-TSA, 130 °C, 1.5 h, 80%.

7-Hydroxymethylhomocamptothecin **7** could be obtained by the reaction of **6** with hydrogen peroxide and methanol. The hydroxymethyl group was oxidized to form 7-formylhomocamptothecin **8**, which was allowed to react with different substituted anilines to generate the corresponding derivatives **9a**–**y** (Scheme 2).

Various 1-(2-amino substituted phenyl)-2-chloro-ethanones **11a**–**n** were obtained by reacting substituted anilines **10a**–**n** with chloroacetonitrile under the catalysis of BCl_3, which were treated with the intermediate **5** to give the 7-chloromethylhomocamptothecin A ring substituted derivatives **13a**–**n** (Scheme 3). Compounds **13a**–**n** reacted with the substituted pyridines to afford quaternary-salt derivatives **13a**–**n** and **14a**–**d**.

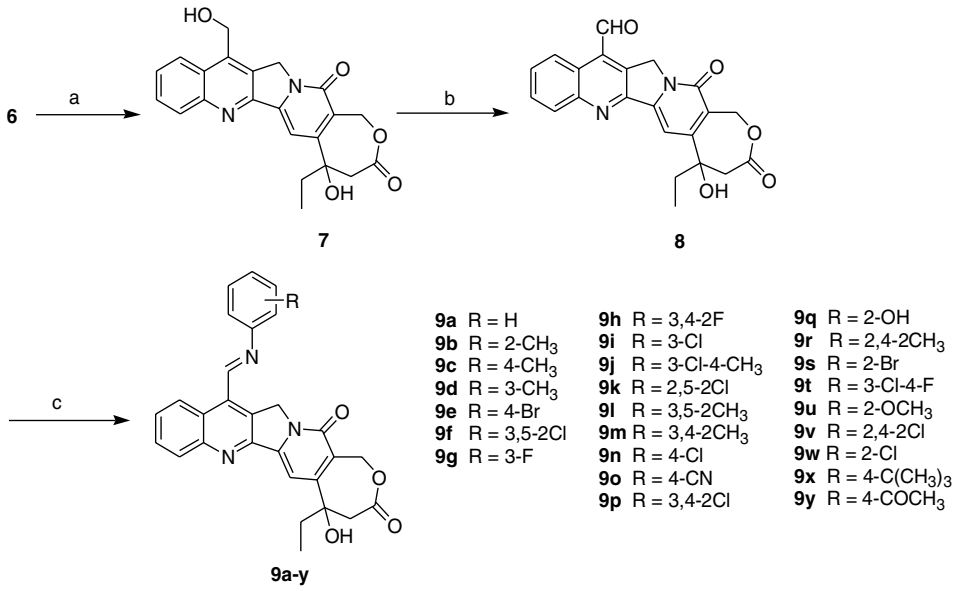
3. Results and discussion

3.1. 7-Aryliminomethyl hCPT derivatives

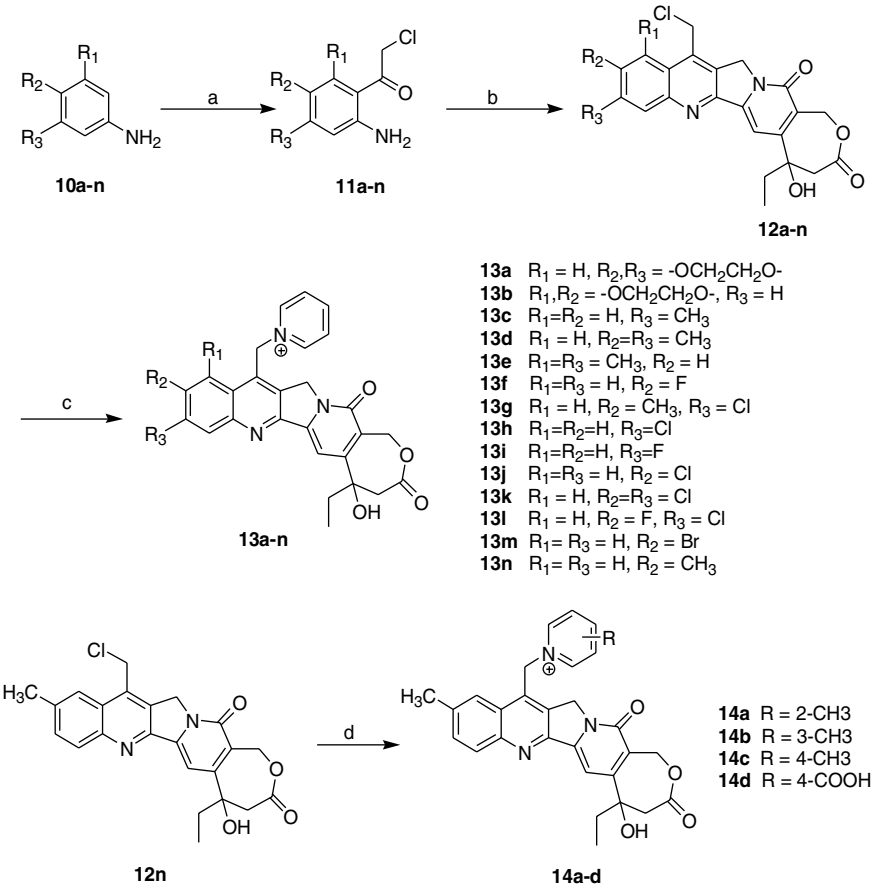
Numerous reports indicated that the CPT derivatives with substitutions at quinoline (A/B) ring,[3] especially at C-7, C-9, and C-10, are of great interest and several good drug candidates (such as exatecan, lurtotecan, and silatecan) are under clinical trials. The importance of the lipophilic groups linked to position 7 of camptothecin has been demonstrated to increase inhibitor intracellular uptake and cytotoxic activities.[32] The crystal structures of Topo I–DNA in complex with topotecan and CPT[33,34] also revealed that the substituents on C-7 were located into the major groove of DNA and they could reinforce the stability of the inhibitor-Topo I–DNA covalent complex. Moreover, some 7-aryliminomethyl CPT analogues have shown good in vitro antitumor activity.[35] Therefore, we designed and synthesized a series of 7-aryliminomethyl hCPT derivatives. The solid tumor cell lines, A-549 (for non-small cell lung cancer), LOVO (for colon cancer), and MCF-7 (for breast cancer), were chosen for testing the in vitro antitumor activities of the 7-aryliminomethyl hCPT derivatives. Topotecan and irinotecan were used as the reference compounds. The inhibitory activities suggested that the arylimino-methyl group in the 7-position can promote antitumor activity. These results revealed that the compounds were more sensitive against A-549 and LOVO cell lines than against MCF-7 cell line (Table 1), which is consistent with the clinical behavior of CPT derivatives.[35] For A-549 cell line, most compounds showed high inhibitory activities with the IC_{50} values lower than 0.2 nM, which were more potent than topotecan and irinotecan. Many compounds also showed better inhibitory activity against LOVO cell line than topotecan. For example, the IC_{50} values of **9l** and **9y** were lower than 0.2 nM. In particular, the activity of the most promising compounds, **9b**, **9l**, and **9y**, showed broad in vitro antitumor spectrum and are more potent than topotecan and irinotecan. Further pharmacological and toxicological evaluation of these promising compounds is in progress. The detailed explanation of SAR would be discussed in the following 3D-QSAR studies.

3.2. Water soluble hCPT derivatives

The clinical success of the water soluble CPT derivatives topotecan and irinotecan highlights the importance of new water soluble CPT or hCPT analogues, which has become a major research focus at present.[36,37] We designed and synthesized a series of ring A and/or ring B-modified 7-substituted pyridine quaternary-salt derivatives in this study. The in vitro cytotoxicity evaluations indicated that the quaternary salt hCPTs were less active than the above synthesized



Scheme 2. Reagents and conditions: (a) H_2O_2, CH_3OH, $FeSO_4{\cdot}7H_2O$, rt, 14 h, 64.7%; (b) CH_3COOH, 130 °C, 5 h, 52.7%[4]; (c) substituted aniline, rt, 10–24 h, 14.5–30.7%.



Scheme 3. Reagents: (a) BCl_3; (b) *p*-TSA; (c) pyridine, DMSO; (d) substituted pyridine, DMSO.

7-iminomethyl hCPTs (Table 2). Most compounds are more sensitive against A549 cell line than LOVO and MCF-7 with their IC_{50} values in the range of 19.6 μM to 7 nM. Only **13n** is more potent than topotecan with the IC_{50} value against A549 lower than 0.2 nM. For A549 cell line, the antitumor activity was increased when A-ring was substituted with a methyl group. However, the pyridyl group of C-7 is suitable to be unsubstituted because the incorporation of the methyl or carboxyl group could lead to a decrease

Table 1. In vitro antitumor activity of 7-aryliminomethyl hCPT derivatives

Compound	IC_{50} (μM)		
	A-549	LOVO	MCF-7
7	0.017	0.050	4.33
8	0.026	0.024	3.55
9a	0.002	0.014	0.488
9b	<0.0002	0.0008	0.517
9c	0.038	0.035	5.882
9d	0.093	0.125	20.855
9e	0.002	0.011	2.324
9f	0.828	0.533	9.291
9g	0.0008	0.014	9.245
9h	0.003	0.023	0.441
9i	5.822	5.192	>20
9j	0.013	0.020	1.344
9k	0.226	0.381	6.621
9l	<0.0002	<0.0002	0.003
9m	0.0006	0.007	0.559
9n	<0.0002	0.0004	2.000
9o	0.538	0.368	25.994
9p	0.0009	0.032	6.847
9q	<0.0002	0.002	20.768
9r	13.926	10.729	20.259
9s	<0.0002	0.018	0.035
9t	<0.0002	0.040	0.492
9u	<0.0002	0.015	2.763
9v	0.0009	0.060	3.943
9w	<0.0002	0.003	1.154
9x	0.002	0.021	0.853
9y	<0.0002	<0.0002	0.852
Topotecan	0.005	0.036	0.488
Irinotecan	6.528	9.015	17.403

of antitumor activity. Although the in vitro cytotoxicities of these water soluble hCPTs are not very satisfying, we expect that the in vivo studies of these compounds can provide some hints for the future inhibitor design.

3.3. In vivo antitumor activity

For most antitumor agents, their in vivo activity usually does not correlate well with their in vitro data. Thus, a preliminary in vivo screening of the synthesized compounds against C26 colon cancer model was performed (data not shown). Compounds **9j** and **13g** were found to be good candidates, which were selected for further detail in vivo studies. Dose–effect relationship studies revealed that the tumor growth inhibition rate of compounds **9j** and **13g** was increased with the addition of their dose. At the dose of 4 mg/kg, compounds **9j** and **13g** achieved tumor growth inhibition rate of 30.99% and 36.21%, respectively, which was almost equivalent to that of TPT at a dose of 1 mg/kg (Table 3). It is worth noting that when the dose of TPT was added to 1.5 mg/kg, all the mice in the group died after the treatment, while all the mice in the group of compounds **9j** and **13g** at the dose of 4 mg/kg remained alive. According to this, compounds **9j** and **13g** may be safer than TPT, although they were less effective than TPT at the same dosage. Further pharmacological and toxicological evaluation of these two compounds is in progress.

Table 2. In vitro antitumor activity of 7-substituted pyridine quaternary-salt hCPT derivatives

Compound	IC_{50} (μM)		
	A-549	LOVO	MCF-7
13a	1.122	>19.51	>19.51
13b	2.017	>19.51	>19.51
13c	2.391	>21.34	>21.34
13d	0.313	>20.72	>20.72
13e	0.290	>20.72	>20.72
13f	0.381	>21.16	>21.16
13g	0.007	7.08	>19.88
13h	18.282	>20.45	>20.45
13i	2.850	>21.16	>21.16
13j	3.49	9.039	>20.45
13k	19.608	>19.11	>19.11
13l	>19.73	>19.73	>19.73
13m	0.729	7.912	>18.75
13n	<0.0002	3.543	6.937
14a	5.015	9.318	>20.72
14b	2.072	>20.72	>20.72
14c	2.114	>20.72	>20.72
14d	7.316	>19.51	>19.51
Topotecan	0.005	0.036	0.488
Irinotecan	6.528	9.015	17.403

3.4. 3D-QSAR models

A data set of the above synthesized hCPTs was used to perform the comparative molecular field analysis (CoMFA)[38] and the comparative molecular similarity indices analysis (CoMSIA)[39] studies. In an attempt to build robust 3D-QSAR models, variation of the parameters, such as grid spacing and attenuation factor, was considered during the 3D-QSAR studies. However, the best results were obtained from the parameters with the default values. It has been discussed that five different descriptor fields are not totally independent of each other and such dependencies of individual fields usually decrease the statistical significance of the models.[40,41] A fast algorithm SAMPLS was used to evaluate which of these five CoMSIA fields was actually important for a predictive model by the combination of all possible descriptor fields[42] (Fig. 2). The first five models, using a single CoMSIA field, indicated that the steric field, the electrostatic field, and the hydrophobic field were more important than the other two fields, the hydrogen-bond donor and the hydrogen acceptor. However, the addition of hydrogen-bond acceptor field generated the best CoMSIA model ($q^2_{SAMPLS} = 0.717$). The correlation coefficients of r^2 of 0.905 and 0.938, and the cross-validated coefficients of q^2 of 0.703 and 0.717 were obtained for the best CoMFA and CoMSIA models, respectively. The final CoMFA and CoMSIA models were satisfactory from the viewpoint of the statistical significance (Table 4). The predictive ability of the training set and test set wad evaluated which indicated that the predictive IC_{50} values of the compounds correlated well with the experimental values (Table 5 and Fig. 3). A real external compound set of six previously synthesized 7-ester hCPT derivatives (compounds **15a**–**f**, Fig. 1) was tested to evaluate the reliability of their 3D-QSAR models. The results revealed that the biological activities of these compounds were also predicted with good accuracy.

To visualize the information content of the derived 3D-QSAR models, the CoMFA and CoMSIA contour maps were generated. The field energies at each lattice point were calculated as the scalar results of the coefficient and the standard deviation associated with a particular column of the data table ('stdev * coeff'), which was always plotted as the percentage of the contribution to the CoMFA or CoMSIA equation. Because the CoMFA and CoMSIA contour maps of steric field represented similar results, only the CoMFA contour map is displayed in Figure 4a. The green contours represent the regions of high steric tolerance, while the yellow contours represent regions of unfavorable steric effects. The sterically favored regions are mainly around the aromatic ring attached to the iminomethyl group of the C7-side chain, which indicates that larger substituents on the iminomethyl group are favorable for high cytotoxicity, for example, most of the compounds with substituted aromatic ring on the iminomethyl group are more potent than compounds with small substituents on C-7 (compounds **7** and **8**). Moreover, the addition of two methyl groups on the *meta* and *para* position of the phenyl group of **9a** (IC_{50} = 0.002 μM) led to a increase of the antitumor activity (compound **9m**, IC_{50} = 0.0006 μM). However, the length of C7-side chain cannot be too long because there is a yellow region above the *para* position of the pyridyl group of **13n** (IC_{50} < 0.0002 μM). When a methyl group was added to the *para* position of the pyridyl group of **13n**, the antitumor activity was decreased to a large extent (compound **14c**, IC_{50} = 2.114 μM). There are a green region and a yellow region around the C-10 and C-11 of the A ring, respectively, which is consistent with the SAR analysis of **13a**–**n**. For example, the addition of a methyl group to the C-10 of **13c** (IC_{50} = 2.391 μM) generates **13d** (IC_{50} = 0.313 μM) and its antitumor activity is improved accordingly. When the 9,10-ethylenedioxy group of **13a** (IC_{50} = 1.122 μM) was replaced by the 10,11-ethylenedioxy group (compound **13b**, IC_{50} = 2.107 μM), the antitumor activity was decreased much because a larger substituent on C-11 was prohibited.

From the CoMFA model, the contribution of electrostatic field (0.272) was lower than that of steric field (0.728). Negative charge favored regions (the red contour in Fig. 4b) were found around the C-2 and C-5 of the phenyl ring of the 7-iminomethyl hCPTs and the positive charge favored regions (the blue contour in Fig. 4b) were around the N atom of the imino group and the C-4 of the phenyl ring. These regions support the observation that the imino group is important for the antitumor activity. Moreover, when a hydroxy group was added to C-2 of the phenyl ring of **9a** (IC_{50} = 0.002 μM), the cytotoxicity was improved to a large extent (compound **9q**, IC_{50} < 0.0002 μM). The similar results were obtained when a chlorine atom was introduced to the C-5 of the phenyl ring of **9i**.

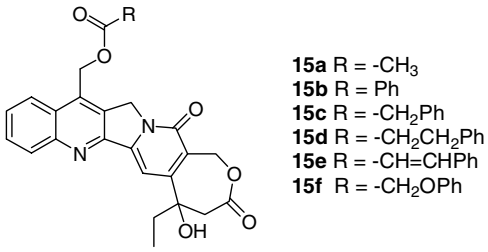
Furthermore, CoMSIA reveals three other contour maps that are not shown in CoMFA. The yellow regions in Figure 4c indicate the areas where the hydrophobic substitutions are preferred and the purple regions represent areas where hydrophobic substitutions are disfavored. The hydrophobic favored regions are around the phenyl ring of the C7-side chain. Except the *meta* position, the *ortho* and *para* positions of the phenyl ring are suitable to be substituted with hydrophobic groups. For example, compounds **9n** (IC_{50} < 0.0002 μM) and **9w** (IC_{50} < 0.0002 μM) are more potent than **9a** (IC_{50} = 0.002 μM). On the contrary, the addition of a hydrophobic chlorine atom to the *meta* position of the phenyl ring of **9a** led to a decrease of antitumor activity (compounds **9f** and **9i**). As shown in Figure 4d, the purple regions of hydrogen-bond acceptor field indicate where the hydrogen-bond acceptors are favored and it is at the *ortho* position of the phenyl ring, while hydrogen-bond acceptor disfavored area in the red color is at the *para* position. It was supported by the fact that the addition of a hydroxyl group to the *ortho* position of the phenyl group of **9a** led to a increase of antitumor activity (compound **9q**, IC_{50} < 0.0002 μM) and when the hydroxyl group was methylated (compound **9u**, IC_{50} < 0.0002 μM) the activity was unchanged. On the contrary, if a hydrogen-bond acceptor was added to the *para* position of the phenyl group, the antitumor activity was decreased (e.g., compound **9o**, IC_{50} = 0.538 μM).

3.5. The binding mode of the hCPTs

Compound **9l**, one of the most active compounds, was used to investigate the binding mode of the synthesized hCPT derivatives. InsightII/Affinity was used in the present docking study because its effectiveness in clarifying the interactions of hCPTs with Topo I has been proved by previous docking studies.[43,44] Moreover, the crystal structures of apo- and ternary complex of Topo I revealed the flexibility of the active site. Because the flexibility of both the active site of the target enzyme and the inhibitor is taken into account in the Affinity method, it should be a powerful tool to study the mode of action of the newly synthesized hCPT derivatives. A comparison between the docking model of **9l** and the ternary complex structures of CPT reveals only minor differences in the overall orientation of the heterocyclic ring. Compound **9l** intercalates at the site of DNA cleavage, forming base-stacking interactions with both the −1 (upstream) and +1 (downstream) base pairs (Fig. 5). The orientation of **9l** in the active site cavity is perpendicular to the main axis of the DNA and parallel to the bases. Previous studies indicated that hydrogen-bonding interaction played an important role during the formation of drug–DNA–enzyme ternary complex.[33,34] In the docking model, compound **9l** bound into Topo I–DNA covalent complex via three direct hydrogen bonds. The N1 of compound **9l** formed a hydrogen bond with Arg 364 and the β-hydroxyl group of E-ring formed another hydrogen bond with Asp 533, which is consistent with crystal structure of CPT–DNA–enzyme ternary complex.[34] The third hydrogen bond was observed between the lactone carbonyl group of E-ring and Thr 718. The C-7 side chain was faced into the major groove and formed hydrophobic interaction with the surrounding hydrophobic residues lined with Ala 351, Met 428, and Pro 431. The addition of the hydrophobic groups (such as a methyl group) on the

Table 3. The in vivo antitumor activity of compounds **9j** and **13g** against C26 colon cancer model

Sample	Dose (mg/kg)	Mice number		Body weight (g)		Tumor weight (g)	Tumor growth inhibition %
		Begin	End	Begin	End	$\bar{x} \pm SD$	
Control	25	18	18	18.85 ± 1.84	17.31 ± 2.32	1.54 ± 0.19	
TPT	1	10	10	16.88 ± 2.09	16.06 ± 1.97	0.82 ± 0.19	37.61
9j	1	10	10	17.85 ± 1.22	16.64 ± 1.11	1.21 ± 0.34	21.29
9j	2	10	10	18.36 ± 1.31	17.27 ± 1.73	1.09 ± 0.59	28.98
9j	4	10	10	17.98 ± 1.28	16.92 ± 1.30	1.06 ± 0.43	30.99
13g	1	6	6	21.26 ± 1.73	19.22 ± 2.15	2.04 ± 0.32	14.60
13g	2	6	6	22.10 ± 1.75	20.09 ± 1.82	2.01 ± 0.35	15.79
13g	4	6	6	21.66 ± 2.48	20.14 ± 2.58	1.52 ± 0.25	36.21



15a R = -CH_3
15b R = Ph
15c R = -CH_2Ph
15d R = -CH_2CH_2Ph
15e R = -CH=CHPh
15f R = -CH_2OPh

Figure 1. Structures of the 10-ester of derivatives of hCPT as external test set in 3D-QSAR studies.

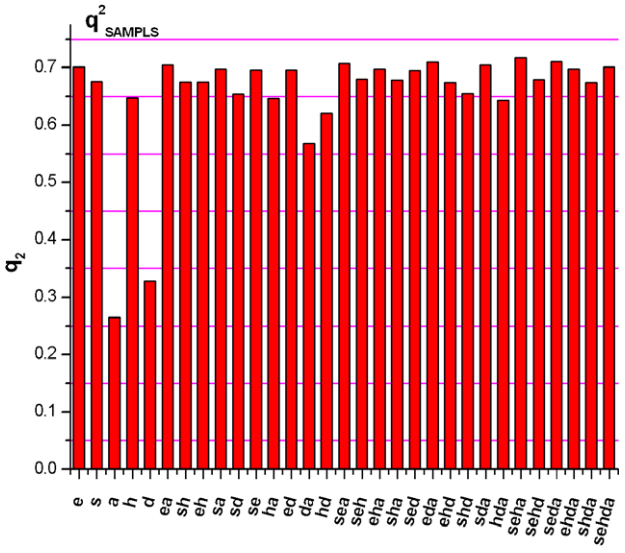


Figure 2. Results of 31 possible CoMSIA field combinations using q^2_{SAMPLS}. e: electrosteric field; s: steric field; a: hydrogen-bond field; h: hydrophobic field; d: hydrogen-bond donor field.

phenyl ring can reinforce the hydrophobic and van der Waals interactions with the residues in the binding site. However, the space at the *para* position of the phenyl ring is limited and the bulky group at this position would clash with Ala 351. The *ortho* position of the phenyl ring is near the side chain of Asn 352, a hydrogen-bond acceptor at this position is favorable to form the hydrogen-bonding interaction with this residue (for example, compound **9q**).

4. Conclusions

In response to the emergent need for novel antitumor agents with improved activity, a series of 7-substituted hCPTs were designed and synthesized. A new synthetic route was developed based on the coupling of ring A with ring CDE to effectively synthesize diversely substituted hCPT analogues. The in vitro antitumor assay revealed that most of the compounds exhibited good cytotoxic activities against the A549 cell line and several compounds are more potent than topotecan. In general, the 7-iminomethyl hCPT derivatives were more potent than 7-substituted pyridine quaternary-salt hCPTs. The SAR analysis of the synthesized compounds was explained in detail by the 3D-QSAR studies. The best derived CoMFA and CoMSIA models showed a predictive q^2 value of 0.703 and 0.717, and the activities of compounds in both training set and test set were predicted with high accuracy. Molecular docking was further used to clarify the binding mode of the new hCPTs. They intercalate at the DNA cleavage site and formed base-stacking interactions with the base pairs. Three hydrogen bonds are formed between the heterocyclic ring of hCPT and the active site of Topo I. The C7-side chain is extended into the major groove of DNA and mainly forms hydrophobic interaction with the active site residues. The addition of small hydrophobic groups or hydrogen-bond acceptors on the proper position of the phenyl ring of C-7 side chain can improve the hydrophobic or hydrogen-bonding interaction with the enzyme. The results obtained from 3D-QSAR studies show good correlation with the docking models, which allows for the rational design of more potent hCPT derivatives.

Table 4. The statistical parameters for the final CoMFA and CoMSIA models

	q^2	r^2	Standard error	F	n	Fraction			
						Steric	Electrostatic	Hydrophobic	HB acceptor
CoMFA	0.703	0.905	0.395	56.599	5	0.728	0.272		
CoMSIA	0.717	0.938	0.299	63.818	5	0.196	0.150	0.379	0.276

Table 5. Experimental and calculated antitumor activity ($-\lg IC_{50}$, μM) of compounds in training set and test set (marked with *)

Compound	Obsd	CoMFA calcd	CoMSIA calcd
7	1.7696	1.4003	2.0563
8	1.5850	1.5786	1.6033
9a	2.6990	2.8781	2.9643
9b	3.6990	3.8753	3.7073
9c	1.4202	2.3827	2.2391
9d	1.0315	2.9200	2.6737
9e	2.6990	2.2313	2.8366
9f*	0.0820	0.2434	0.1687
9g	3.0969	2.7289	2.6188
9h	2.5229	2.8640	2.5817
9i*	−0.7651	−0.7070	−0.8518
9j	1.8861	2.4187	2.0102
9k	0.6459	1.9663	1.1745
9l	3.6990	2.8683	2.6510
9m	3.2218	2.4931	2.2087
9n	3.6990	2.4180	2.8484
9o	0.2692	0.9453	0.4856
9p	2.0458	2.4134	2.6151
9q	3.6990	3.2362	3.6117
9r*	−1.1438	−1.0707	−1.0228
9s	3.6990	3.3716	3.5376
9t	3.6990	2.5638	2.8334
9u	3.6990	3.9991	3.7288
9v	3.0458	2.8658	3.3798
9w	3.6990	3.2248	3.2356
9x	2.6990	2.1994	2.6543
9y	3.6990	3.7429	3.9463
13a	−0.0500	0.2911	−0.0198
13b	−0.3237	−0.3447	−0.3606
13c	−0.3786	−0.6573	−0.0400
13d	0.5045	0.4774	0.2047
13e	0.5376	0.5418	0.4443
13f	0.4191	−0.4265	0.0424
13g*	2.1549	2.1168	2.1247
13h	−1.2620	−1.2113	−1.3005
13i	−0.4548	−0.4752	−0.2600
13j	−0.5428	−0.1803	−0.1469
13k	−1.2803	−1.1207	−1.1745
13l	−1.3010	−1.4313	−1.2879
13m	0.1373	0.1682	0.1112
13n*	3.6990	3.7292	3.6672
14a	−0.7003	−0.8010	−0.6421
14b	−0.3164	−0.1812	−0.2731
14c	−0.3251	−0.0801	−0.4118
14d	−0.8643	−0.5512	−0.7739
15a*	3.6990	4.1004	3.7337
15b*	3.6990	3.5579	6.6609
15c*	3.6990	3.4966	3.4747
15d*	3.6990	3.7823	4.1249
15e*	2.6990	2.6084	2.7511
15f*	1.1675	1.3937	0.7911

5. Experimental

5.1. General procedures

Melting points were measured on an electrically heated XT4A instrument and are uncorrected. The ^1H spectra were recorded at 500 MHz with a Bruker instrument, and reported with TMS as internal standard and CDCl3 or DMSO-d_6 as solvents. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI mass spectra were performed on an API-3000 LC–MS spectrometer. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within ±0.4%. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Flash column chromatography was carried out on silica gel 300–400 mesh. Anhydrous solvent and reagents were all analytically pure and dried through routine protocols.

5.1.1. 1,1-(Ethylenedioxy)-8-ethyl-7,8-dihydroxy-2,3,7,8-tetrahydro-5H-6-oxa-3a-aza-cyclopenta[*b*]naphthalene-1,4-dione (2). Potassium borohydride (1.0 g, 18.6 mmol) was added in portions, during 10 min, to a solution of 1,1-(ethylenedioxy)-8-Ethyl-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-aza-cyclopenta[*b*]naphthal-ene-1,4,7-trione (**1**) (8.0 g, 26.1 mmol) in anhydrous methanol (170 mL). The reaction mixture was stirred for 20 min at 25 °C.The solution was concentrated in vacuo, diluted with water, and brought to pH 4 by addition of 2 M HCl. The solid was filtered off to give **2** as a white solid: yield 6.4 g (79.5%). ^1H NMR (DMSO-d_6): 0.92 (t, 3H, J = 7.6 Hz), 1.75 (q, 2H, J = 7.5 Hz), 2.41 (t, 2H, J = 6.8 Hz), 2.72 (s, 1H), 3.31 (s, 1H), 4.11–4.25 (m, 4H), 4.19 (d, 2H, J = 7.0 Hz), 4.47–4.69 (q, 2H, J = 16.8 Hz), 5.06 (s, 1H) and 6.71 (s, 1H). MS (ESI): m/z 310 $(M+H)^+$. Anal. Calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.27; H, 6.20; N, 4.51.

5.1.2. Formic acid-1,1-(ethylenedioxy)-1,5-dioxo-7-propionyl-1,2,3,5-tetrahydro-indolizin-6-yl-methyl ester (3). To a suspension of **2** (4.0 g, 12.9 mmol) in glacial acetic acid (200 mL) was added dropwise a solution of $NaIO_4$ (4.0 g, 18.7 mmol) in H_2O (40 mL). The resulting mixture was stirred at room temperature for 30 min, at which time ethylene glycol (4 mL) was added. Water (400 mL) was slowly added and extracted with dichloromethane (3× 100 mL). The combined dichloromethane extracts were dried over sodium sulfate and concentrated under reduced pressure, and the residue was crystallized from THF yielding **3** as a white solid: 3.7 g (93.1%). ^1H NMR ($CDCl_3$): 1.16 (t, 3 H, J = 7.2 Hz), 2.45 (t, 2 H, J = 6.8 Hz), 2.89 (q, 2H, J = 7.1 Hz), 4.10–4.23 (m, 6H), 5.13 (s, 2H), 6.57 (s, 1H), 8.07 (s, 1H). MS (ESI): 308 $(M+H)^+$. Anal. Calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.47; H, 5.61; N, 4.58.

5.1.3. 1,1-(Ethylenedioxy)-3-hydroxy-3-(6-hydroxymethyl-1,5-dioxo-1,2,3,5-tetrahydro-indolizin-7-yl)-pentanoic acid *tert*-butyl ester (4). A suspension of zinc (9.0 g, 137.6 mmol) was stirred in anhydrous THF (120 mL) under N_2 and then heated to reflux. The heating bath was removed and *tert*-butyl bromoacetate (20 mL, 133 mmol) was added dropwise at a reflux-maintaining rate. Then a solution of **3** (5.5 g, 17.9 mmol) in anhydrous THF (50 mL) was added and continued for 0.5 h further. The reaction mixture was allowed to cool to room temperature, quenched with saturated ammonium chloride (100 mL), and extracted with dichloromethane (3× 100 mL). The combined dichloromethane extracts were dried over sodium sulfate and

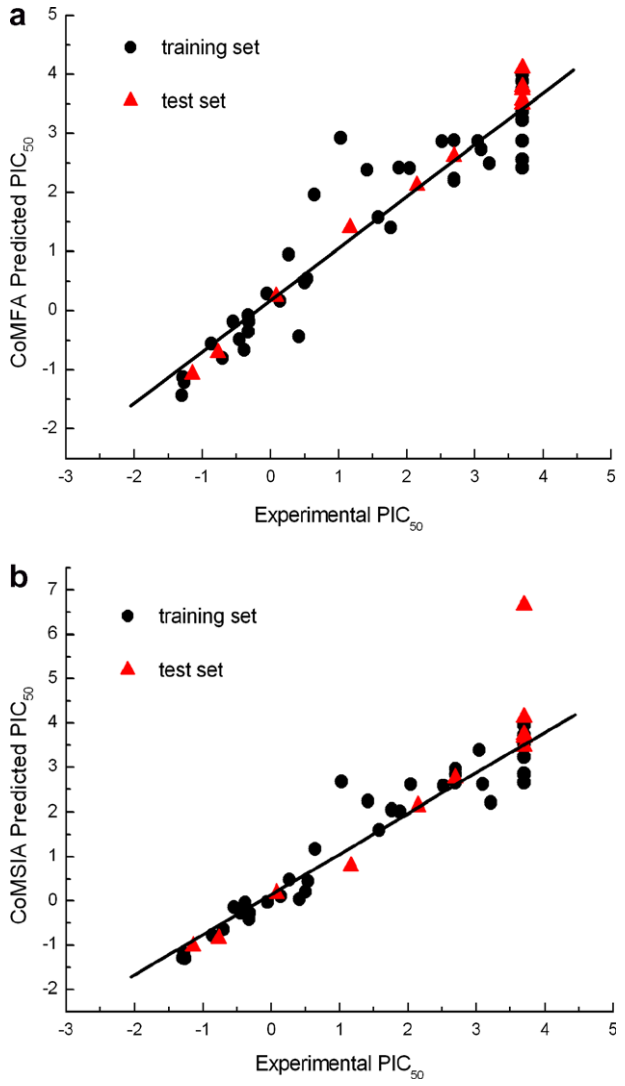


Figure 3. Plots of the experimental and calculated pIC_{50} ($-lgIC_{50}$, μM) values for the CoMFA (a) and CoMSIA (b) analysis of the training set and test set.

concentrated under reduced pressure, and the residue was purified by chromatography over silica gel (2%MeOH/CH_2Cl_2) to give ester **4** as a pale-yellow solid: 6.9 g (97.5%). ^1H NMR ($CDCl_3$): 0.85 (t, 3H, J = 7.4 Hz), 1.30 (s, 9H), 1.85–1.98 (m, 2H), 2.40 (t, 2H, J = 7.0 Hz), 2.84 (d, 2H, J = 16.0 Hz), 4.06–4.20 (m, 6H), 4.20 (s, 1H), 4.80 (s, 1H), 4.88 (q, 2H, J = 13.0 Hz), 6.52 (s, 1H). MS (ESI): 396 $(M+H)^+$. Anal. Calcd for $C_{20}H_{29}NO_7$: C, 60.74; H, 7.39; N, 3.54; Found: C 60.59, H 7.42, N 3.55.

5.1.4. 9-Ethyl-9-hydroxy-2,3,8,9-tetrahydro-5H-6-oxa-3a-aza-cyclohepta[*f*]indene-1,4,7-trione (5). Ester **4** (4.6 g, 11.6 mmol) was dissolved in trifluoroacetic acid (28 mL) and stirred at room temperature for 10 h. The solution was concentrated under reduced pressure and the residue was crystallized from acetone/water yielding **5** as a white solid: 3.0 g (93.0%). ^1H NMR ($CDCl_3$): 0.90 (t, 3 H, J = 7.5 Hz), 1.78–1.88 (m, 2H), 2.97 (t, 2H, J = 6.7 Hz), 3.31 (q, 2H, J = 13.9 Hz), 4.26 (t, 2H, J = 6.7 Hz), 5.45 (q, 2H, J = 15.7 Hz), 7.04 (s, 1H). MS (ESI): 278 $(M+H)^+$. Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.66; H, 5.43; N, 5.02.

5.1.5. 5-Ethyl-1,4,5,13-tetrahydro-5-hydroxy-3H,15H-oxepino-[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,15-dione (6). A solution of 2-[1,3]dioxolan-2-yl-phenyl-amine (0.8 g, 4.8 mmol) and tricyclic ketone **5** (1.0 g, 3.6 mmol) in toluene (800 mL) was refluxed using a Dean–Stark trap for 30 min. *p*-Toluenesulfonic acid (0.1 g) was then added and refluxing was continued for an additional 1 h. The solution was allowed to cool to room temperature and the solid was filtered off and washed by acetone (20 mL) and methanol (20 mL) to give homocamptothecin **6** as yellow solid: yield 0.98 g (75.5%). ^1H NMR (DMSO-d_6): 0.87 (t, 3 H, J = 7.2 Hz), 1.86 (q, 2H, J = 7.5 Hz), 3.07–3.48 (q, 2H, J = 14.0 Hz), 5.28 (s, 2H), 5.42–5.54 (q, 2H, J = 15.0 Hz), 6.03 (s, 1H), 7.42 (s, 1H), 7.72 (t, 1H, J = 7.3 Hz), 7.87 (t, 1H, J = 7.2 Hz), 8.15 (q, 2H, J = 7.9 Hz), 8.69 (s, 1H). MS (ESI): 363 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N 7.73. Found C, 69.37; H, 5.05; N, 7.77.

5.1.6. 7-Hydroxymethylhomocamptothecin (7). To a suspension of **6** (0.98 g, 2.7 mmol) in a mixture of MeOH (30 mL) and H_2O (25 mL), 75% H_2SO_4 (25 mL) was added dropwise, and then $FeSO_4{\cdot}7H_2O$ (0.80 g, 2.9 mmol) was added. To the ice-cold mixture, 30% H_2O_2 (5 mL, 2.2 mmol) was added dropwise and stirred at room temperature for 14 h and then diluted with H_2O. The solid was filtered and purified by chromatography over silica gel (4% MeOH/CH_2Cl_2) to give **7** as a yellow solid: 0.61 g (64.7%). ^1H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.4 Hz), 1.87 (q, 2 H, J = 7.4 Hz), 3.06–3.48 (q, 2H, J = 13.8 Hz), 5.27 (d, 2H, J = 5.3 Hz), 5.41 (s, 2H), 5.41–5.55 (q, 2H, J = 14.7 Hz), 5.80 (t, 1H, J = 5.6 Hz), 6.03 (s, 1H), 7.40 (s, 1H), 7.71 (t, 1H, J = 7.4 Hz), 7.86 (t, 1H, J = 7.4 Hz), 8.16 (d, 1H, J = 8.3 Hz), 8.20 (d, 1H, J = 8.3 Hz). MS (ESI):393 $(M+H)^+$. Anal. Calcd for $C_{22}H_{20}N_2O_5$: C 67.34, H 5.14, N 7.14. Found C, 67.12; H, 5.17; N, 7.19.

5.1.7. 7-Formylhomocamptothecin (8). A suspension of **7** (0.61 g, 1.6 mmol) in AcOH (200 mL) was heated under reflux for 6 h. The mixture was evaporated to dryness and the residue was purified by chromatography over silica gel (4% MeOH/CH_2Cl_2) to give **8** as a yellow solid: 0.32 g (52.7%). ^1H NMR (DMSO-d_6): 0.87 (t, J = 7.3 Hz, 3H), 1.88 (q, 2H, J = 7.4 Hz), 3.07–3.49 (q, 1H, J = 11.5 Hz), 5.42–5.55 (q, 1H, J = 15.3 Hz), 5.52 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.92 (t, 1H, J = 7.3 Hz), 7.98 (t, 1H, J = 7.4 Hz), 8.31 (d, 1H, J = 8.2 Hz), 9.04 (d, 1H, J = 8.2 Hz), 11.10 (s, 1H). MS (ESI): 391 $(M+H)^+$. Anal. Calcd for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18. Found C, 67.49; H, 4.67; N, 7.21.

5.1.8. 7-Phenyliminomethylhomocamptothecin (9a). To a suspension of $Yb(OTf)_3$ (16 mg, 0.03 mmol) in 15 mL CH_2Cl_2, **8** (50 mg, 0.13 mmol) and aniline (70 mg, 0.75 mmol) were added. The resulting mixture was stirred at room temperature for 10 h until the reaction was complete. The solvent was evaporated to dryness

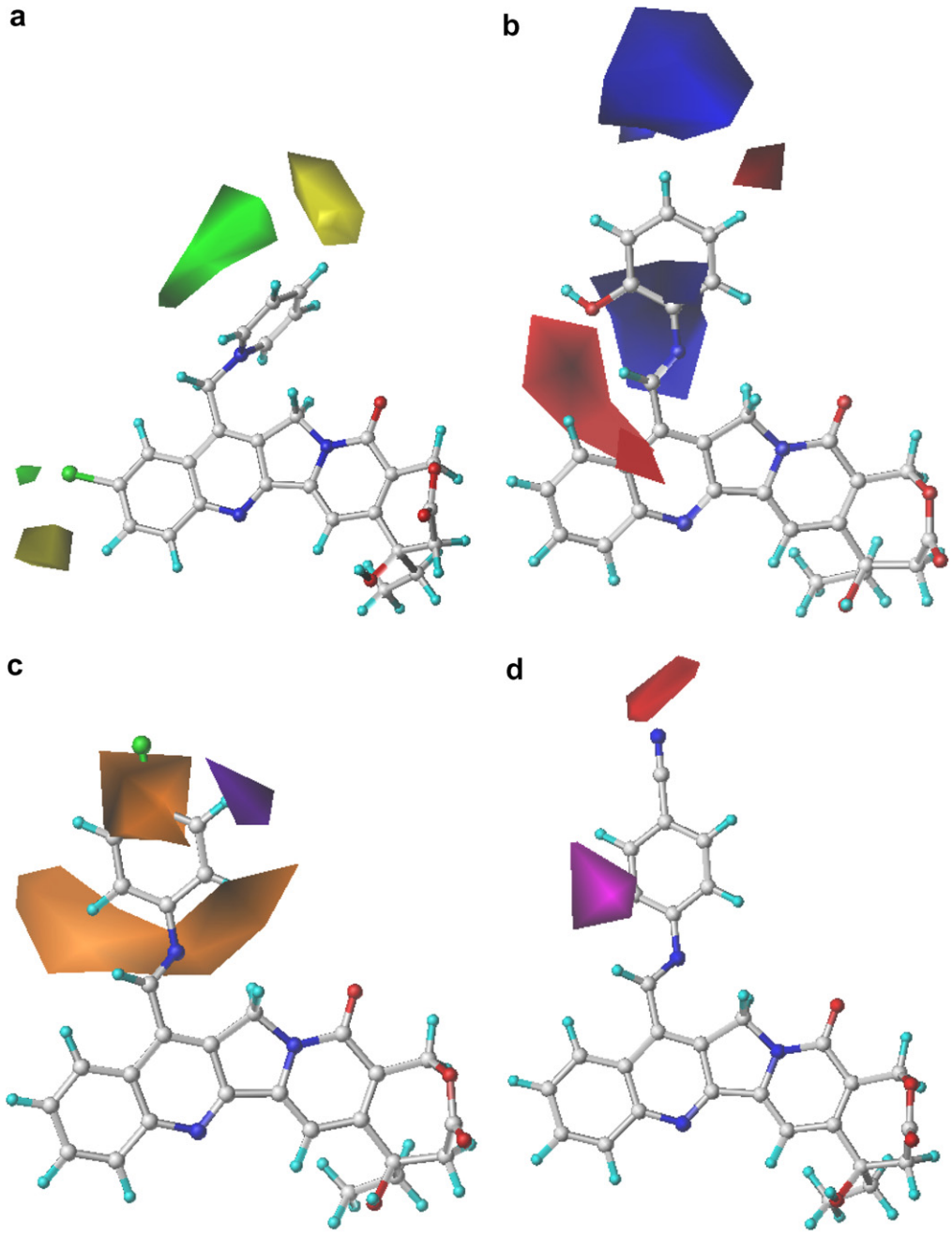


Figure 4. Contour plots of CoMFA steric fields (a, compound **13f** is shown in ball-and-stick mode), electrostatic fields (b, compound **9q** is shown in ball-and-stick mode), CoMSIA hydrophobic fields (c, compound **9n** is shown in ball-and-stick mode), and CoMSIA hydrogen-bonding acceptor fields (d, compound **9o** is shown in ball-and-stick mode).

and the residue was purified by chromatography over silica gel(2% MeOH/CH_2Cl_2) to give **9a** as a yellow solid: 14 mg (23.5%). ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.4 Hz), 1.88 (q, 2H, J = 7.4 Hz), 3.08–3.48 (q, 2H, J = 14.0 Hz), 5.42–5.55 (q, 2H, J = 15.1 Hz), 5.60 (s, 2H), 6.05 (s, 1H), 7.41 (t, 2H, J = 7.2 Hz), 7.46 (s, 1H), 7.54 (d, 2H, J = 7.5 Hz), 7.59 (m, 1H), 7.84 (t, 1H, J = 7.3 Hz), 7.96 (t, 1H, J = 7.3 Hz), 8.27 (d, 1H, J = 8.1 Hz), 9.01 (d, 1H, J = 8.1 Hz), 9.72 (s, 1H). MS (ESI): 466 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}N_3O_4$: C, 72.24; H, 4.98; N, 9.03. Found C, 72.36; H, 4.97; N, 9.01.

5.1.9. 7-(2-Methylphenyl)iminomethylhomocamptothecin (9b). The titled compound was prepared from **8** and 2-methylaniline according to the method of compound **9a**, yield: 24.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.1 Hz), 2.50 (s, 3H), 3.08–3.48 (q, 2H, J = 14.0 Hz), 5.42–5.54 (q, 2H, J = 15.0 Hz), 5.59 (s, 2H), 6.05 (s, 1H), 7.29 (t, 1H, J = 6.7 Hz), 7.35 (t, 1H, J = 6.7 Hz), 7.38 (d, 1H, J = 7.4 Hz), 7.46 (s, 1H), 7.50 (d, 1H, J = 7.4 Hz), 7.83 (t, 1H, J = 7.5 Hz), 7.95 (t, 1H, J = 7.5 Hz), 8.26 (d, 1H, J = 8.5 Hz), 8.99 (d, 1H J = 8.5 Hz), 9.66 (s, 1H). MS (ESI): 480 $(M+H)^+$. Anal. Calcd for $C_{29}H_{25}N_3O_4$:

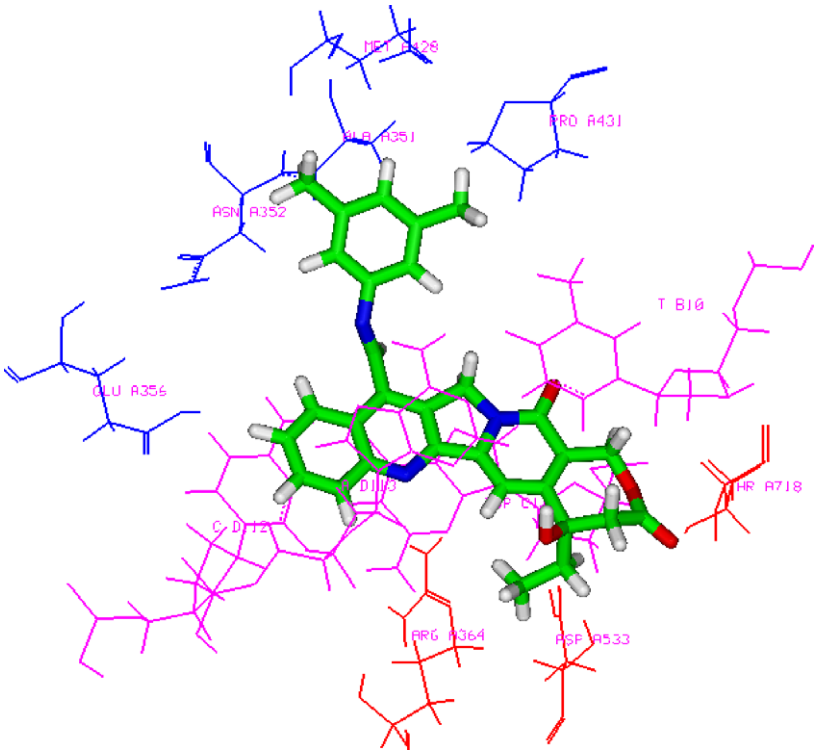


Figure 5. Stereoview of the binding mode of **9l** in the active site of Topo I. The base pairs forming stack interaction with **9l** are colored purple and residues forming hydrogen-bonding interaction with **9l** are colored red. The hydrogen bonds are displayed in dotted lines.

C, 72.64; H, 5.25; N, 8.76. Found C, 72.39; H, 5.27; N, 8.77.

5.1.10. 7-(4-Methylphenyl)iminomethylhomocamptothecin (9c). The titled compound was prepared from **8** and 4-methylaniline according to the method of compound **9a**, yield: 26.0%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.2 Hz), 1.87 (q, 2H, J = 8.5 Hz), 2.40 (s, 3H), 3.08–3.48 (q, 2H, J = 13.7 Hz), 5.42–5.55 (q, 2H, J = 15.1 Hz), 5.59 (s, 2H), 6.05 (s, 1H), 7.36 (d, 2H, J = 8.0 Hz), 7.46 (s, 1H), 7.53 (d, 2H, J = 8.0 Hz), 7.84 (t, 1H, J = 7.5 Hz), 7.94 (t, 1H, J = 7.5 Hz), 8.26 (d, 1H, J = 8.5 Hz), 9.00 (d, 1H, J = 8.5 Hz), 9.72 (s, 1H). MS (ESI): 480 (M+H)$^+$. Anal. Calcd for $C_{29}H_{25}N_3O_4$: C, 72.64; H, 5.25; N, 8.76. Found C, 72.41; H, 5.27; N, 8.78.

5.1.11. 7-(3-Methylphenyl)iminomethylhomocamptothecin (9d). The titled compound was prepared from **8** and 3-methylaniline according to the method of compound **9a**, yield: 24.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.2 Hz), 2.44 (s, 3H), 3.09–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.55 (q, 2H, J = 15.0 Hz), 5.60 (s,2H), 6.04 (s, 1H), 7.22 (t, 1H, J = 7.1 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.41 (s, 1H), 7.43 (d, 1H, J = 8.0 Hz), 7.46 (s, 1H), 7.84 (t, 1H, J = 7.4 Hz), 7.96 (t, 1H, J = 7.4 Hz), 8.27 (d, 1H, J = 8.6 Hz), 9.00 (d, 1H, J = 8.6 Hz), 9.71 (s, 1H). MS (ESI): 480 (M+H)$^+$. Anal. Calcd for $C_{29}H_{25}N_3O_4$: C, 72.64; H, 5.25; N, 8.76. Found C, 72.83; H, 5.23; N, 8.75.

5.1.12. 7-(4-Bromophenyl)iminomethylhomocamptothecin (9e). The titled compound was prepared from **8** and 4-bromoaniline according to the method of compound **9a**, yield: 20.1%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 7.3 Hz), 3.08–3.48 (q, 2H, J = 14.0 Hz), 5.42–5.55 (q, 2H, J = 15.1 Hz), 5.58 (s, 2H), 6.05 (s, 1H), 7.46 (s, 1H), 7.56 (d, 2H, J = 8.6 Hz), 7.74 (d, 2H, J = 8.6 Hz), 7.84 (t, 1H, J = 7.5 Hz), 7.95 (t, 1H, J = 7.5 Hz), 8.27 (d, 1H, J = 8.5 Hz), 8.99 (d, 1H, J = 8.5 Hz), 9.72 (s, 1H). MS (ESI): 545 (M+H)$^+$. Anal. Calcd for $C_{28}H_{22}BrN_3O_4$: C, 61.77; H, 4.07; N, 7.72. Found C, 61.88; H, 4.05; N, 7.71.

5.1.13. 7-(3,5-Dichlorophenyl)iminomethylhomocamptothecin (9f). The titled compound was prepared from **8** and 3,5-dichloroaniline according to the method of compound **9a**, yield: 30.7%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.0 Hz), 1.89 (q, 2H, J = 7.1 Hz), 3.08–3.47 (q, 2H, J = 13.9 Hz), 5.42–5.56 (q, 2H, J = 15.1 Hz), 5.59 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.63 (s, 1H), 7.68 (s, 2H), 7.84 (t, 1H, J = 7.9 Hz), 7.96 (t, 1H, J = 7.9 Hz), 8.27 (d, 1H, J = 8.5 Hz), 9.01 (d, 1H, J = 8.5 Hz), 9.74 (s, 1H). MS (ESI): 535 (M+H)$^+$. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4$: C, 62.93; H, 3.96; N, 7.86. Found C, 62.80; H, 3.98; N, 7.87.

5.1.14. 7-(3-Fluorophenyl)iminomethylhomocamptothecin (9g). The titled compound was prepared from **8** and 3-fluoroaniline according to the method of compound **9a**, yield: 14.5%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.87 (q, 2H, J = 8.4 Hz), 3.07–3.47 (q, 2H, J = 13.8 Hz), 5.42–5.55 (q, 2H, J = 15.1 Hz), 5.52 (s, 2H), 6.05 (s, 1H), 7.25 (d, 1H, J = 8.1 Hz), 7.46 (s, 1H), 7.49 (d, 1H, J = 8.1 Hz), 7.53 (t, 1H, J = 7.2 Hz),

7.89 (s, 1H), 7.91 (t, 1H, J = 7.5 Hz), 7.98 (t, 1H, J = 7.5 Hz), 8.30 (d, 1H, J = 8.4 Hz), 9.03 (d, 1H, J = 8.4 Hz), 9.73 (s, 1H). MS (ESI): 484 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}FN_3O_4$: C, 69.56; H, 4.59; N, 8.69. Found C, 69.77; H, 4.58; N, 8.69.

5.1.15. 7-(3,4-Difluorophenyl)iminomethylhomocamptothecin (9h). The titled compound was prepared from **8** and 3,4-difluoroaniline according to the method of compound **9a**, yield: 17.1%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.1 Hz), 1.88 (q, 2H, J = 7.4 Hz), 3.09–3.48 (q, 2H, J = 13.8 Hz), 5.42–5.55 (q, 2H, J = 15.0 Hz), 5.58 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.47 (s, 1H), 7.62 (d, 1H, J = 8.1 Hz), 7.83 (d, 1H, J = 8.1 Hz), 7.85 (t, 1H, J = 7.5 Hz), 7.96 (t, 1H, J = 7.5 Hz), 8.28 (d, 1H, J = 8.6 Hz), 9.01 (d, 1H, J = 8.6 Hz), 9.73 (s, 1H). MS (ESI): 502 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}F_2N_3O_4$: C, 67.06; H, 4.22; N, 8.38. Found C, 67.23; H, 4.21; N, 8.35.

5.1.16. 7-(3-Chlorophenyl)iminomethylhomocamptothecin (9i). The titled compound was prepared from **8** and 3-chloroaniline according to the method of compound **9a**, yield: 23.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.3 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.55 (q, 2H, J = 15.1 Hz), 5.59 (s, 2H), 6.05 (s, 1H), 7.43 (d, 1H, J = 8.2 Hz), 7.46 (s, 1H), 7.52 (d, 1H, J = 8.2 Hz), 7.57 (t, 1H, J = 7.4 Hz), 7.71 (s, 1H), 7.83 (t, 1H, J = 7.6 Hz), 7.95 (t, 1H, J = 7.6 Hz), 8.27 (d, 1H, J = 8.5 Hz), 9.02 (d, 1H, J = 8.5 Hz), 9.73 (s, 1H). MS (ESI): 501 $(M+H)^+$. Anal. Calcd for $C_{28}H_{22}ClN_3O_4$: C, 67.27; H, 4.44; N, 8.40. Found C, 67.14; H, 4.45; N, 8.43.

5.1.17. 7-(3-Chloro-4-methylphenyl)iminomethylhomocamptothecin (9j). The titled compound was prepared from **8** and 3-chloro-4-methylaniline according to the method of compound **9a**, yield: 19.7%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 7.4 Hz), 2.36 (s, 3H), 3.08–3.48 (q, 2H, J = 13.8 Hz), 5.42–5.55 (q, 2H, J = 15.2 Hz), 5.58 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.50 (d, 1H, J = 8.1 Hz), 7.52 (d, 1H, J = 8.1 Hz), 7.75 (s, 1H), 7.84 (t, 1H, J = 7.4 Hz), 7.95 (t, 1H, J = 7.4 Hz), 8.26 (d, 1H, J = 8.6 Hz), 9.02 (d, 1H, J = 8.6 Hz), 9.74 (s, 1H). ^{13}C NMR (DMSO-d_6): 8.56 (1C), 19.66 (1C), 29.35 (1 C), 36.61 (1C), 42.73 (1C), 52.92 (1C), 61.66 (1C), 73.47 (1C), 99.89 (1C), 121.84 (1C), 123.07 (1C), 125.05 (1C), 126.15 (1C), 128.75 (1C), 129.03 (1C), 130.09 (1C), 130.77 (1C), 132.13 (1C), 133.86 (1C), 134.30 (1C), 135.10 (1C), 144.31 (1C), 149.44 (1C), 149.79 (1C), 153.45 (1C), 156.10 (1C), 157.45 (1C), 159.34 (1C), 172.18 (1C). MS (ESI): 515 $(M+H)^+$. Anal. Calcd for $C_{29}H_{24}ClN_3O_4$: C, 67.77; H, 4.71; N, 8.18. Found C, 67.58; H, 4.72; N, 8.20.

5.1.18. 7-(2,5-Dichlorophenyl)iminomethylhomocamptothecin (9k). The titled compound was prepared from **8** and 2,5-dichloroaniline according to the method of compound **9a**, yield: 24.8%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.2 Hz), 1.88 (q, 2H, J = 8.1 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.54 (q, 2H, J = 14.8 Hz), 5.63 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.48 (d, 1H, J = 8.1 Hz), 7.69 (d, 1H, J = 8.1 Hz), 7.86 (t, 1H, J = 7.5 Hz), 7.95 (t, 1H, J = 7.5 Hz), 7.97 (s, 1H), 8.29 (d, 1H, J = 8.5 Hz), 9.06 (d, 1H, J = 8.5 Hz), 9.78 (s, 1H). MS (ESI): 535 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4$: C, 62.93; H, 3.96; N, 7.86. Found C, 62.88; H, 3.97; N, 7.88.

5.1.19. 7-(3,5-Dimethylphenyl)iminomethylhomocamptothecin (9l). The titled compound was prepared from **8** and 3,5-dimethylaniline according to the method of compound **9a**, yield: 23.7%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.2 Hz), 1.89 (q, 2H, J = 8.2 Hz), 2.39 (s, 6H), 3.08–3.47 (q, 2H, J = 14.0 Hz), 5.42–5.56 (q, 2H, J = 15.0 Hz), 5.58 (s, 2H), 6.07 (s, 1H), 7.04 (s, 1H), 7.32 (s, 2H), 7.46 (s, 1H), 7.84 (t, 1H, J = 7.7 Hz), 7.96 (t, 1H, J = 7.7 Hz), 8.27 (d, 1H, J = 8.1 Hz), 8.99 (d, 1H, J = 8.1 Hz), 9.69 (s, 1H). ^{13}C NMR (DMSO-d_6): 8.63 (1C), 21.31 (2C), 29.42 (1C), 36.68 (1C), 42.79 (1C), 53.00 (1C), 61.73 (1C), 73.54 (1C), 99.93 (1C), 119.84 (2C), 123.10 (1C), 124.98 (1C), 126.23 (1C), 128.76 (1 C), 129.48 (1C), 130.18 (1C), 130.79 (1C), 134.09 (1C), 138.98 (1 C), 144.43 (1C), 149.51 (1C), 150.90 (1C), 153.52 (1C), 156.17 (1 C), 159.41 (1C), 161.31 (1C), 170.87 (1C), 172.24 (1C). MS (ESI): 494 $(M+H)^+$. Anal. Calcd for $C_{30}H_{27}N_3O_4$: C, 73.01; H, 5.51; N, 8.51. Found C, 73.21; H, 5.51; N, 8.50.

5.1.20. 7-(3,4-Dimethylphenyl)iminomethylhomocamptothecin (9m). The titled compound was prepared from **8** and 3,4-dimethylaniline according to the method of compound **9a**, yield: 25.3%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.2 Hz), 2.31 (s, 3H), 2.34 (s, 3H), 3.09–3.48 (q, 2H, J = 13.8 Hz), 5.42–5.55 (q, 2H, J = 15.0 Hz), 5.58 (s, 2H), 6.05 (s, 1H), 7.30 (d, 1H, J = 8.3 Hz), 7.36 (d, 1H, J = 8.3 Hz), 7.43 (s, 1H), 7.46 (s, 1H), 7.83 (t, 1H, J = 7.4 Hz), 7.95 (t, 1H, J = 7.4 Hz), 8.26 (d, 1H, J = 8.6 Hz), 9.00 (d, 1H, J = 8.6 Hz), 9.72 (s, 1H). MS (ESI): 494 $(M+H)^+$. Anal. Calcd for $C_{30}H_{27}N_3O_4$: C, 73.01; H, 5.51; N, 8.51. Found C, 73.19; H, 5.49; N, 8.51.

5.1.21. 7-(4-Chlorophenyl)iminomethylhomocamptothecin (9n). The titled compound was prepared from **8** and 4-chloroaniline according to the method of compound **9a**, yield: 23.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.2 Hz), 1.88 (q, 2H, J = 8.3 Hz), 3.09–3.48 (q, 2H, J = 13.8 Hz), 5.42–5.52 (q, 2H, J = 15.0 Hz), 5.47 (s, 2H), 6.05 (s, 1H), 7.44 (s, 1H), 7.59 (d, 2H, J = 8.2 Hz), 7.61 (d, 2H, J = 8.2 Hz), 7.81 (t, 1H, J = 7.5 Hz), 7.93 (t, 1H, J = 7.5 Hz), 8.23 (d, 1H, J = 8.6 Hz), 8.95 (d, 1H, J = 8.6 Hz), 9.68 (s, 1H). MS (ESI): 501 $(M+H)^+$. Anal. Calcd for $C_{28}H_{22}ClN_3O_4$: C, 67.27; H, 4.44; N, 8.40. Found C, 67.17; H, 4.44; N, 8.42.

5.1.22. 7-(4-Cyanophenyl)iminomethylhomocamptothecin (9o). The titled compound was prepared from **8** and 4-aminobenzonitrile according to the method of compound **9a**, yield: 15.9%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.0 Hz), 3.08–3.48 (q, 2H, J = 13.8 Hz), 5.42–5.55 (q, 2H, J = 15.2 Hz), 5.59 (s, 2H), 6.06 (s, 1H), 7.47 (s, 1H), 7.68 (d, 2H, J = 8.1 Hz), 7.85 (t, 1H, J = 7.5 Hz), 7.97 (t, 1H, J = 7.5 Hz), 8.02 (d, 2H, J = 8.1 Hz), 8.28 (d, 1H, J = 8.5 Hz), 8.98 (d, 1H,

J = 8.5 Hz), 9.71 (s, 1H). MS (ESI): 491 $(M+H)^+$. Anal. Calcd for $C_{29}H_{22}N_4O_4$: C, 71.01; H, 4.52; N, 11.42. Found C, 71.22; H, 4.51; N, 11.38.

5.1.23. 7-(3,4-Dichlorophenyl)iminomethylhomocamptothecin (9p). The titled compound was prepared from **8** and 3,4-dichloroaniline according to the method of compound **9a**, yield: 24.8%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 7.4 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.54 (q, 2H, J = 14.6 Hz), 5.60 (s, 2H), 6.07 (s, 1H), 7.47 (s, 1H), 7.58 (d, 1H, J = 8.2 Hz), 7.80 (d, 1H, J = 8.2 Hz), 7.85 (t, 1H, J = 7.5 Hz), 7.95 (s, 1H), 7.96 (t, 1H, J = 7.5 Hz), 8.28 (d, 1H, J = 8.6 Hz), 9.01 (d, 1H, J = 8.6 Hz), 9.75 (s, 1H). MS (ESI): 535 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4$: C, 62.93; H, 3.96; N, 7.86. Found C, 63.11; H, 3.95; N, 7.86.

5.1.24. 7-(2-Hydroxyphenyl)iminomethylhomocamptothecin (9q). The titled compound was prepared from **8** and 2-aminophenol according to the method of compound **9a**, yield: 16.2%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.5 Hz), 1.88 (q, 2H, J = 8.3 Hz), 3.08–3.48 (q, 2H, J = 14.0 Hz), 5.42–5.55 (q, 2H, J = 15.4 Hz), 5.67 (s, 2H), 6.05 (s, 1H), 6.95 (t, 1H, J = 7.1 Hz), 6.96 (d, 1H, J = 8.6 Hz), 7.01 (d, 1H, J = 8.6 Hz), 7.20 (t, 1H, J = 7.1 Hz), 7.46 (s, 1H), 7.83 (t, 1H, J = 7.5 Hz), 7.96 (t, 1H, J = 7.5 Hz), 8.27 (d, 1H, J = 8.5 Hz), 8.93 (d, 1H, J = 8.5 Hz), 9.49 (s, 1H), 9.66 (s, 1H). MS (ESI): 482 $(M+H)^+$. Anal. Calcd for $C_{28}H_{23}N_3O_5$: C, 69.84; H, 4.81; N, 8.73. Found C, 70.00; H, 4.80; N, 8.71.

5.1.25. 7-(2,4-Dimethylphenyl)iminomethylhomocamptothecin (9r). The titled compound was prepared from **8** and 2,4-dimethylaniline according to the method of compound **9a**, yield: 25.3%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.1 Hz), 2.30 (s, 3H), 2.35 (s, 3H), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.54 (q, 2H, J = 15.0 Hz), 5.57 (s, 2H), 6.06 (s, 1H), 7.16 (d, 1H, J = 8.2 Hz), 7.20 (s, 1H), 7.45 (s, 1H), 7.49 (d, 1H, J = 8.2 Hz), 7.82 (t, 1H, J = 7.3 Hz), 7.94 (t, 1H, J = 7.3 Hz), 8.25 (d, 1H, J = 8.6 Hz), 8.99 (d, 1H, J = 8.6 Hz), 9.69 (s, 1H). MS (ESI): 494 $(M+H)^+$. Anal. Calcd for $C_{30}H_{27}N_3O_4$: C, 73.01; H, 5.51; N, 8.51. Found C, 73.17; H, 5.50; N, 8.49.

5.1.26. 7-(2-Bromophenyl)iminomethylhomocamptothecin (9s). The titled compound was prepared from **8** and 2-bromoaniline according to the method of compound **9a**, yield: 18.6%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.6 Hz), 1.88 (q, 2H, J = 8.1 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.54 (q, 2H, J = 15.2 Hz), 5.70 (s, 2H), 6.05 (s, 1H), 7.33 (t, 1H, J = 7.4 Hz), 7.46 (s, 1H), 7.57 (t, 1H, J = 7.4 Hz), 7.72 (d, 1H, J = 8.6 Hz), 7.84 (d, 1H, J = 8.6 Hz), 7.86 (t, 1H, J = 7.5 Hz), 7.96 (t, 1H, J = 7.5 Hz), 8.28 (d, 1H, J = 8.5 Hz), 9.02 (d, 1H, J = 8.5 Hz), 9.74 (s, 1H). MS (ESI): 545 $(M+H)^+$. Anal. Calcd for $C_{28}H_{22}BrN_3O_4$: C, 61.77; H, 4.07; N, 7.72. Found C, 61.59; H, 4.08; N, 7.74.

5.1.27. 7-(3-Chloro-4-fluorophenyl)iminomethylhomocamptothecin (9t). The titled compound was prepared from **8** and 3-chloro-4-fluoroaniline according to the method of compound **9a**, yield: 22.6%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.4 Hz), 1.88 (q, 2H, J = 8.0 Hz), 3.09–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.55 (q, 2H, J = 15.0 Hz), 5.57 (s, 2H), 6.05 (s, 1H), 7.45 (s, 1H), 7.58 (d, 1H, J = 8.3 Hz), 7.61 (d, 1H, J = 8.3 Hz), 7.84 (t, 1H, J = 7.4 Hz), 7.94 (t, 1H, J = 7.4 Hz), 7.95 (s, 1H), 8.26 (d, 1H, J = 8.5 Hz), 9.01 (d, 1H, J = 8.5 Hz), 9.74 (s, 1H). MS (ESI): 519 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}FClN_3O_4$: C, 64.93; H, 4.09; N, 8.11. Found C, 65.11; H, 4.08; N, 8.10.

5.1.28. 7-(2-Methoxyphenyl)iminomethylhomocamptothecin (9u). The titled compound was prepared from **8** and 2-methoxyaniline according to the method of compound **9a**, yield: 25.2%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.4 Hz), 1.88 (q, 2H, J = 8.3 Hz), 3.09–3.48 (q, 2H, J = 13.8 Hz), 3.92 (s, 3H), 5.42–5.54 (q, 2H, J = 15.1 Hz), 5.57 (s, 2H), 6.04 (s, 1H), 7.09 (t, 1H, J = 7.1 Hz), 7.19 (d, 1H, J = 8.0 Hz), 7.35 (t, 1H, J = 7.1 Hz), 7.46 (s, 1H), 7.47 (d, 1H, J = 8.0 Hz), 7.83 (t, 1H, J = 7.6 Hz), 7.95 (t, 1H, J = 7.6 Hz), 8.26 (d, 1H, J = 8.5 Hz), 8.94 (d, 1H, J = 8.5 Hz), 9.67 (s, 1H). MS (ESI): 496 $(M+H)^+$. Anal. Calcd for $C_{29}H_{25}N_3O_5$: C, 70.29; H, 5.09; N, 8.48. Found C, 70.42; H, 5.08; N, 8.46.

5.1.29. 7-(2,4-Dichlorophenyl)iminomethylhomocamptothecin (9v). The titled compound was prepared from **8** and 2,4-dichloroaniline according to the method of compound **9a**, yield: 24.8%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.1 Hz), 1.88 (q, 2H, J = 8.3 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.54 (q, 2H, J = 14.6 Hz), 5.60 (s, 2H), 6.07 (s, 1H), 7.47 (s, 1H), 7.58 (d, 1H, J = 8.2 Hz), 7.80 (d, 1H, J = 8.2 Hz), 7.85 (t, 1H, J = 7.4 Hz), 7.95 (s, 1H), 7.96 (t, 1H, J = 7.4 Hz), 8.28 (d, 1H, J = 8.6 Hz), 9.01 (d, 1H, J = 8.6 Hz), 9.75 (s, 1H). MS (ESI): 535 $(M+H)^+$. Anal. Calcd for $C_{28}H_{21}Cl_2N_3O_4$: C, 62.93; H, 3.96; N, 7.86. Found C, 63.10; H, 3.96; N, 7.84.

5.1.30. 7-(2-Chlorophenyl)iminomethylhomocamptothecin (9w). The titled compound was prepared from **8** and 2-chloroaniline according to the method of compound **9a**, yield: 23.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.2 Hz), 3.08–3.48 (q, 2H, J = 13.9 Hz), 5.42–5.55 (q, 2H, J = 15.2 Hz), 5.64 (s, 2H), 6.05 (s, 1H), 7.41 (t, 1H, J = 7.5 Hz),7.46 (s, 1H), 7.52 (t, 1H, J = 7.5 Hz), 7.67 (d, 1H, J = 8.1 Hz), 7.75 (d, 1H, J = 8.1 Hz), 7.85 (t, 1H, J = 7.4 Hz), 7.95 (t, 1H, J = 7.4 Hz), 8.28 (d, 1H, J = 8.5 Hz), 9.03 (d, 1H, J = 8.5 Hz), 9.75 (s, 1H). MS(ESI): 501 $(M+H)^+$. Anal. Calcd for $C_{28}H_{22}ClN_3O_4$: C, 67.27; H, 4.44; N, 8.40. Found C, 67.10; H, 4.45; N, 8.42.

5.1.31. 7-(4-*tert*-Butylphenyl)iminomethylhomocamptothecin (9x). The titled compound was prepared from **8** and 4-*tert*-butylaniline according to the method of compound **9a**, yield: 18.0%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.4 Hz), 1.35 (s, 9H), 1.88 (q, 2H, J = 8.5 Hz), 3.08–3.48 (q, 2H, J = 14.0 Hz), 5.42–5.55 (q, 2H, J = 14.9 Hz), 5.59 (s, 2H), 6.05 (s, 1H), 7.46 (s, 1H), 7.54 (d, 2H, J = 8.3 Hz), 7.56 (d, 2H, J = 8.3 Hz), 7.83 (t, 1H, J = 7.4 Hz), 7.95 (t, 1H, J = 7.4 Hz), 8.27 (d,

1H, J = 8.5 Hz), 9.00 (d, 1H, J = 8.5 Hz), 9.71 (s, 1H). MS (ESI): 522 $(M+H)^+$. Anal. Calcd for $C_{32}H_{31}N_3O_4$: C, 73.68; H, 5.99; N, 8.06. Found C, 73.81; H, 5.98; N, 8.03.

5.1.32. 7-(4-Acetylphenyl)iminomethylhomocamptothecin (9y). The titled compound was prepared from **8** and 1-(4-amino-phenyl)-ethanone according to the method of compound **9a**, yield: 15.4%. ^{1}H NMR (DMSO-d_6): 0.89 (t, 3H, J = 7.3 Hz), 1.88 (q, 2H, J = 8.2 Hz), 2.65 (s, 9H), 3.08–3.48 (q, 2H, J = 12.4 Hz), 5.42–5.55 (q, 2H, J = 15.2 Hz), 5.61 (s, 2H), 6.06 (s, 1H), 7.46 (s, 1H), 7.65 (d, 2H, J = 8.0 Hz), 7.86 (t, 1H, J = 7.4 Hz), 7.91 (t, 1H, J = 7.4 Hz), 8.13 (d, 2H, J = 8.0 Hz), 8.30 (d, 1H, J = 8.5 Hz), 9.02 (d, 1H, J = 8.5 Hz), 9.74 (s, 1H). MS (ESI): 508 $(M+H)^+$. Anal. Calcd for $C_{30}H_{25}N_3O_6$: C, 68.82; H, 4.81; N, 8.03. Found C, 68.99; H, 4.81; N, 8.00.

5.1.33. 10,11-(Methylenedioxy)-7-(pyridiniumylmethyl) homocamptothecin chloride (13a). A 1 L flask was charged with dry methylene chloride (50 mL) and 1,4-benzodioxan-6-amine (1.5 g, 10 mmol) and was cooled to 0 °C followed by slow addition of a 1 M solution of boron trichloride in methylene chloride solution (40 mL) while maintaining an internal temperature at or below 10 °C under N_2. Aluminum chloride (1.3 g, 10 mmol) was added quickly in three portions followed by addition of chloroacetonitrile (0. 7 mL, 11 mmol). The reaction mixture was stirred for 30 min at 0 °C and then heated to reflux for 16 h. The reaction mixture was allowed to cool to room temperature and then was quenched into a mixture of ice (100 g)/2 N HC1 (50 mL). The aqueous layer was extracted by methylene chloride (100 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure, and the residue was purified by chromatography over silica gel (2% MeOH/CH_2Cl_2) to give 2′-amino-2-chloro-4′,5′-(ethylenedioxy)acetophenone (**11a**) as a yellow solid: 0.45 g (35.2%).^{1}H NMR (DMSO-d_6): 4.22–4.24 (m, 2H), 4.34–4.36 (m, 2H), 4.75 (s, 2H), 6.22 (s, 1H), 6.87 (s, 1H). MS (ESI): 228 $(M+H)^+$.

A solution of **11a** (0.25 g, 1.1 mmol) and tricyclic ketone **5** (0.28 g, 1.0 mmol) in toluene (85 mL) was refluxed using a Dean–Stark trap for 30 mins. *p*-Toluenesulfonic acid (0.03 g) was then added and refluxing was continued for an additional 1 h. The solution was allowed to cool to room temperature and the solid was filtered off and washed by acetone (20 mL) and methanol (20 mL) to give 10,11-(methylenedioxy)-7-(chloromethyl)-homocamptothecin (**12a**) as yellow solid: yield 0.38 g (80.6%).^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.0 Hz), 1.90 (q, 2H, J = 8.1 Hz), 3.06–3.46 (q, 2H, J = 14.0 Hz), 4.45–4.47 (m, 4H), 5.28–5.37 (m, 4H), 5.40–5.54 (q, 2H, J = 15.4 Hz), 6.04 (s, 1H), 7.33 (s, 1H), 7.57 (s, 1H), 7.75 (s, 1H). MS (ESI): 470 $(M+H)^+$.

10,11-(Methylenedioxy)-7-(chloromethyl)-homocamptothecin **12a** (47 mg, 0.1 mmol) was added to anhydrous pyridine (1 mL) and DMSO (2 mL) at 50 °C. This was stirred for 12 h before the addition of diethyl ether (1 mL) which precipitated the desired product. The yellow solid was filtered off and washed once with ethanol (2 mL) and twice with diethyl ether (5 mL) to afford **13a** as a yellow solid: yield 19 mg (34.7%). ^{1}H NMR (DMSO-d_6): 0.86 (t, 3 H, J = 7.1 Hz), 1.86 (q, 2H, J = 8.1 Hz), 3.05–3.51 (q, 2H, J = 13.8 Hz), 4.25–4.41 (m, 4H), 5.36 (s, 2H), 5.38–5.52 (q, 2H, J = 14.8 Hz), 6.04 (s, 1H), 6.45 (q, 2H, J = 14.9 Hz), 7.39 (s, 1H), 7.57 (s, 1H), 7.65 (s, 1H), 8.13 (t, 2H, J = 7.1 Hz), 8.62 (t, 1H, J = 7.5 Hz), 9.06 (d, 2H, J = 5.9 Hz). MS (ESI): 512 (M^+). Anal. Calcd for $C_{29}H_{26}ClN_3O_6$: C, 63.56; H, 4.78; N, 7.67. Found C, 63.79; H, 4.77; N, 7.65.

5.1.34. 9,10-(Methylenedioxy)-7-(pyridiniumylmethyl)-homocamptothecin chloride (13b). The titled compound was prepared according to the method of compound **13a**, yield: 39.0%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.3 Hz), 1.87 (q, 2H, J = 8.1 Hz), 3.06–3.52 (q, 2H, J = 13.8 Hz), 4.14–4.26 (m, 4H), 5.41–5.51 (q, 2H, J = 15.1 Hz), 5.51 (s, 2H), 6.07 (s, 1H), 6.41 (q, 2H, J = 14.7 Hz), 7.41 (s, 1H), 7.50 (d, 1H, J = 7.5 Hz), 7.78 (d, 1H, J = 7.5 Hz), 8.07 (t, 2H, J = 7.2 Hz), 8.59 (t, 1H, J = 7.5 Hz), 9.03 (d, 2H, J = 6.0 Hz). MS (ESI): 512 (M^+). Anal. Calcd for $C_{29}H_{26}ClN_3O_6$: C, 63.56; H, 4.78; N, 7.67. Found C, 63.75; H, 4.78; N, 7.66.

5.1.35. 11-Methyl-7-(pyridiniumylmethyl)-homocamptothecin chloride (13c). The titled compound was prepared according to the method of compound **13a**, yield: 40.4%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.2 Hz), 1.87 (q, 2H, J = 8.1 Hz), 2.56 (s, 3H), 3.07-3.51 (q, 2H, J = 13.9 Hz), 5.38–5.53 (q, 2H, J = 15.3 Hz), 5.41 (s, 2H), 6.08 (s, 1H), 6.55 (q, 2H, J = 14.6 Hz), 7.45 (s, 1H), 7.59 (d, 1H, J = 7.3 Hz), 8.03 (d, 1H, J = 7.5 Hz), 8.05 (s, 2H), 8.12 (t, 2H, J = 7.4 Hz), 8.62 (t, 1H, J = 7.4 Hz), 9.09 (d, 2H, J = 6.0 Hz). MS (ESI): 468 (M^+). Anal. Calcd for $C_{28}H_{26}ClN_3O_4$: C, 66.73; H, 5.20; N, 8.34. Found C, 66.55; H, 5.21; N, 8.36.

5.1.36. 10,11-Dimethyl-7-(pyridiniumylmethyl)-homocamptothecin chloride (13d). The titled compound was prepared according to the method of compound **13a**, yield: 45.6%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.87 (q, 2H, J = 8.1 Hz), 2.43 (s, 3H), 2.47 (s, 3 H), 3.06–3.51 (q, 2H, J = 13.7 Hz), 5.38 (s, 2H), 5.40–5.52 (q, 2H, J = 15.1 Hz), 6.07 (s, 1H), 6.52 (q, 2H, J = 14.7 Hz), 7.43 (s, 1H), 7.94 (s, 1H), 8.04 (s, 1H), 8.13 (t, 2H, J = 7.3 Hz), 8.62 (t, 1H, J = 7.4 Hz), 9.10 (d, 2H, J = 6.1 Hz). MS (ESI): 482 (M^+). Anal. Calcd for $C_{29}H_{28}ClN_3O_4$: C, 67.24; H, 5.45; N, 8.11. Found C, 67.43; H, 5.44; N, 8.13.

5.1.37. 9,11-Dimethyl-7-(pyridiniumylmethyl)-homocamptothecin chloride (13e). The titled compound was prepared according to the method of compound **13a**, yield: 43.5%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.87 (q, 2H, J = 8.3 Hz), 2.51 (s, 3H), 2.58 (s, 3H), 3.05–3.51 (q, 2H, J = 13.7 Hz), 5.27 (s, 2H), 5.40–5.52 (q, 2H, J = 15.1 Hz), 6.07 (s, 1H), 6.59 (q, 2H, J = 14.8 Hz), 7.43 (s, 1H), 7.96 (s, 1H), 8.14 (t, 2H, J = 7.5 Hz), 8.64 (t, 1H, J = 7.4 Hz), 8.93 (d, 2H, J = 6.2 Hz). MS (ESI): 482 (M^+). Anal. Calcd for

$C_{29}H_{28}ClN_3O_4$: C, 67.24; H, 5.45; N, 8.11. Found C, 67.41; H, 5.44; N, 8.12.

5.1.38. 10-Fluoro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13f). The titled compound was prepared according to the method of compound **13a**, yield: 33.8%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.3 Hz), 1.87 (q, 2H, J = 8.3 Hz), 3.07–3.51 (q, 2H, J = 13.8 Hz), 5.40–5.53 (q, 2H, J = 15.2 Hz), 5.42 (s, 2H), 6.09 (s, 1H), 6.55 (q, 2H, J = 14.7 Hz), 7.47 (s, 1H), 7.87 (d, 1H, J = 7.3 Hz), 8.06 (d, 1H, J = 7.2 Hz), 8.13 (t, 2H, J = 7.4 Hz), 8.36 (s, 1H), 8.60 (t, 1H, J = 7.4 Hz), 9.08 (d, 2H, J = 6.2 Hz). MS (ESI): 472 (M^+). Anal. Calcd for $C_{27}H_{23}FClN_3O_4$: C, 63.84; H, 4.56; N, 8.27. Found C, 63.99; H, 4.55; N, 8.27.

5.1.39. 10-Methyl-11-chloro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13g). The titled compound was prepared according to the method of compound **13a**, yield: 31.8%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.3 Hz), 1.87 (q, 2H, J = 8.0 Hz), 2.53 (s, 3H), 3.06–3.51 (q, 2H, J = 13.8 Hz), 5.38 (s, 2H), 5.40–5.53 (q, 2H, J = 15.0 Hz), 6.09 (s, 1H), 6.54 (q, 2H, J = 14.6 Hz), 7.45 (s, 1H), 8.12 (t, 2H, J = 7.2 Hz), 8.14 (s, 1H), 8.35 (s, 1H), 8.63 (t, 1H, J = 7.4 Hz), 9.07 (d, 2H, J = 6.3 Hz). MS (ESI): 503 (M^+). Anal. Calcd for $C_{28}H_{25}Cl_2N_3O_4$: C, 62.46; H, 4.68; N, 7.80. Found C, 62.23; H, 4.69; N, 7.82.

5.1.40. 11-Chloro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13h). The titled compound was prepared according to the method of compound **13a**, yield: 22.5%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.3 Hz), 1.86 (q, 2H, J = 8.1 Hz), 3.06–3.52 (q, 2H, J = 14.2 Hz), 5.40 (s, 2H), 5.41–5.54 (q, 2H, J = 16.1 Hz), 6.10 (s, 1H), 6.56 (q, 2H, J = 14.3 Hz), 7.47 (s, 1H), 7.81 (d, 1H, J = 7.5 Hz), 8.13 (t, 2H, J = 7.3 Hz), 8.20 (d, 1H, J = 7.5 Hz), 8.35 (s, 1H), 8.63 (t, 1H, J = 7.3 Hz), 9.06 (d, 2H, J = 6.0 Hz). MS (ESI): 489 (M^+). Anal. Calcd for $C_{27}H_{23}Cl_2N_3O_4$: C, 61.84; H, 4.42; N, 8.01. Found C, 62.01; H, 4.41; N, 8.00.

5.1.41. 11-Fluoro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13i). The titled compound was prepared according to the method of compound **13a**, yield: 25.3%. ^{1}H NMR (DMSO-d_6): 0.86 (t, 3H, J = 7.3 Hz), 1.86 (q, 2H, J = 8.3 Hz), 3.06–3.50 (q, 2H, J = 14.1 Hz), 5.41 (s, 2H), 5.42–5.52 (q, 2H, J = 16.0 Hz), 6.08 (s, 1H), 6.51 (q, 2H, J = 14.2 Hz), 7.43 (s, 1H), 7.80 (d, 1H, J = 7.3 Hz), 8.12 (t, 2H, J = 7.5 Hz), 8.21 (d, 1H, J = 7.3 Hz), 8.32 (s, 1H), 8.60 (t, 1H, J = 7.3 Hz), 9.01 (d, 2H, J = 6.0 Hz). MS (ESI): 472 (M^+). Anal. Calcd for $C_{27}H_{23}FClN_3O_4$: C, 63.84; H, 4.56; N, 8.27. Found C, 63.97; H, 4.56; N, 8.25.

5.1.42. 10-Chloro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13j). The titled compound was prepared according to the method of compound **13a**, yield: 23.7%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.86 (q, 2H, J = 8.3 Hz), 3.08–3.51 (q, 2H, J = 13.9 Hz), 5.41 (s, 2H), 5.41–5.52 (q, 2H, J = 15.0 Hz), 6.07 (s, 1H), 6.54 (q, 2H, J = 14.8 Hz), 7.47 (s, 1H), 7.88 (d, 1H, J = 7.5 Hz), 8.07 (d, 1H, J = 7.5 Hz), 8.11 (t, 2H, J = 7.4 Hz), 8.35 (s, 1H), 8.62 (t, 1H, J = 7.4 Hz), 9.02 (d, 2H, J = 6.1 Hz). MS (ESI): 489 (M^+). Anal. Calcd for $C_{27}H_{23}Cl_2N_3O_4$: C, 61.84; H, 4.42; N, 8.01. Found C, 61.66; H, 4.43; N, 8.04.

5.1.43. 10,11-Dichloro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13k). The titled compound was prepared according to the method of compound **13a**, yield: 21.0%. ^{1}H NMR (DMSO-d_6): 0.86 (t, 3H, J = 7.3 Hz), 1.87 (q, 2H, J = 8.3 Hz), 3.08–3.51 (q, 2H, J = 13.9 Hz), 5.39 (s, 2H), 5.41–5.53 (q, 2H, J = 15.2 Hz), 6.07 (s, 1H), 6.54 (q, 2H, J = 14.7 Hz), 7.41 (s, 1H), 7.95 (s, 1H), 8.03 (s, 1H), 8.12 (t, 2H, J = 7.3 Hz), 8.61 (t, 1H, J = 7.4 Hz), 9.08 (d, 2H, J = 6.0 Hz). MS (ESI): 523 (M^+). Anal. Calcd for $C_{27}H_{22}Cl_2N_3O_4$: C, 58.03; H, 3.97; N, 7.52. Found C, 57.91; H, 3.98; N, 7.55.

5.1.44. 11-Fluoro-10-chloro-7-(pyridiniumylmethyl)-homocamptothecin chloride (13l). The titled compound was prepared according to the method of compound **13a**, yield: 23.7%.^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.87 (q, 2H, J = 8.2 Hz), 3.07–3.52 (q, 2H, J = 13. 8 Hz), 5.41 (s, 2H), 5.42–5.51 (q, 2H, J = 15.1 Hz), 6.09 (s, 1H), 6.56 (q, 2H, J = 14.7 Hz), 7.42 (s, 1H), 7.94 (s, 1H), 8.05 (s, 1H), 8.13 (t, 2H, J = 7.1 Hz), 8.62 (t, 1H, J = 7.4 Hz), 9.07 (d, 2H, J = 6.1 Hz). MS (ESI): 507 (M^+). Anal Calcd for $C_{27}H_{22}FClN_3O_4$: C, 59.79; H, 4.09; N, 7.75. Found C, 59.98; H, 4.08; N, 7.73.

5.1.45. 10-Bromo-7-(pyridiniumylmethyl)-homocamptothecin chloride (13m). The titled compound was prepared according to the method of compound **13a**, yield: 33.7%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.2 Hz), 1.87 (q, 2H, J = 8.1 Hz), 3.06–3.51 (q, 2H, J = 13.9 Hz), 5.37–5.53 (q, 2H, J = 15.4 Hz), 5.41 (s, 2H), 6.07 (s, 1H), 6.56 (q, 2H, J = 14.9 Hz), 7.48 (s, 1H), 8.06 (d, 1H, J = 7.5 Hz), 8.15 (t, 2H, J = 7.4 Hz), 8.20 (d, 1H, J = 7.5 Hz), 8.48 (s, 1H), 8.64 (t, 1H, J = 7.3 Hz), 9.06 (d, 2H, J = 6.1 Hz). MS (ESI): 533 (M^+). Anal. Calcd for $C_{27}H_{22}BrClN_3O_4$: C, 57.01; H, 4.08; N, 7.39. Found C, 57.22; H, 4.08; N, 7.37.

5.1.46. 10-Methyl-7-(pyridiniumylmethyl)-homocamptothecin chloride (13n). The titled compound was prepared according to the method of compound **13a**, yield: 25.3%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.4 Hz), 1.87 (q, 2H, J = 8.2 Hz), 2.50 (s, 3H), 3.06–3.51 (q, 2H, J = 13.9 Hz), 5.40 (s, 2H), 5.43–5.53 (q, 2H, J = 15.1 Hz), 6.06 (s, 1H), 6.54 (q, 2H, J = 14.5 Hz), 7.45 (s, 1H), 7.76 (d, 1H, J = 7.3 Hz), 7.97 (s, 1H), 8.13 (t, 2H, J = 7.4 Hz), 8.17 (d, 1H, J = 7.3 Hz), 8.63 (t, 1H, J = 7.5 Hz), 9.11 (d, 2H, J = 6.1 Hz). ^{13}C NMR (DMSO-d_6): 8.67 (1C), 21.96 (1C), 36.78 (1C), 42.39 (1C), 50.85 (1C), 57.42 (1C), 61.61 (1C), 73.61 (1C), 100.06 (1C), 122.68 (1C), 123.12 (1C), 125.08 (1C), 128.82 (2C), 130.37 (1C), 131.60 (1 C), 133.22 (1C), 139.56 (1C), 144.87 (1C), 145.42 (2C), 146.81 (1 C), 147.76 (1C), 149.91 (1C), 150.85 (1C), 156.33 (1C), 159.67 (1 C), 172.20 (1C). MS (ESI): 468 (M^+). Anal. Calcd for $C_{28}H_{26}ClN_3O_4$: C, 66.73; H, 5.20; N, 8.34. Found C, 66.95; H, 5.20; N, 8.31.

5.1.47. 10-Methyl-7-[(2′-methyl)pyridiniumylmethyl]-homocamptothecin chloride (14a). The titled compound was prepared from 2-methylpyridine according to the method of compound **13a**, yield: 33.1%. ^{1}H NMR (DMSO-d_6): 0.86 (t, 3H, J = 7.5 Hz), 1.86 (q, 2H, J = 8.2 Hz), 2.53 (s, 3H), 3.04–3.49 (q, 2H, J = 14.0 Hz), 3.05 (s, 3H), 5.35 (s, 2H), 5.37–5.48 (q, 2H, J = 15.0 Hz), 6.05 (s, 1H), 6.37 (q, 2H, J = 14.7 Hz), 7.45 (s, 1H), 7.78 (d, 1H, J = 7.1 Hz), 7.79 (t, 1H, J = 7.4 Hz), 7.95 (s, 1H), 8.19 (d, 1H, J = 7.1 Hz), 8.22 (d, 1H, J = 7.5 Hz), 8.38 (d, 1H, J = 7.3 Hz), 8.55 (s, 1H). MS (ESI): 482 (M^+). Anal. Calcd for $C_{29}H_{28}ClN_3O_4$: C, 67.24; H, 5.45; N, 8.11. Found C, 67.05; H, 5.46; N, 8.12.

5.1.48. 10-Methyl-7-[(3′-methyl)pyridiniumylmethyl]-homocamptothecin chloride (14b). The titled compound was prepared from 3-methylpyridine according to the method of compound **13a**, yield: 31.1%. ^{1}H NMR (DMSO-d_6): 0.88 (t, 3H, J = 7.5 Hz), 1.88 (q, 2H, J = 8.1 Hz), 2.44 (s, 3H), 3.06–3.51 (q, 2H, J = 13.7 Hz), 3.32 (s, 3H), 5.36 (s, 2H), 5.39–5.53 (q, 2H, J = 15.1 Hz), 6.06 (s, 1H), 6.47 (q, 2H, J = 14.6 Hz), 7.45 (s, 1H), 7.76 (d, 1H, J = 7.3 Hz), 7.94 (s, 1H), 8.06 (t, 1H, J = 7.1 Hz), 8.16 (d, 1H, J = 7.3 Hz), 8.47 (d, 1H, J = 7.4 Hz), 8.89 (s, 1H), 9.02 (d, 1H, J = 7.3 Hz). MS (ESI): 482 (M^+). Anal. Calcd for $C_{29}H_{28}ClN_3O_4$: C, 67.24; H, 5.45; N, 8.11. Found C, 67.01; H, 5.45; N, 8.14.

5.1.49. 10-Methyl-7-[(4′-methyl)pyridiniumylmethyl]-homocamptothecin chloride (14c). The titled compound was prepared from 4-methylpyridine according to the method of compound **13a**, yield: 26.9%. ^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.6 Hz), 1.88 (q, 2H, J = 8.0 Hz), 2.60 (s, 3H), 3.06–3.51 (q, 2H, J = 14.2 Hz), 3.32 (s, 3H), 5.40–5.54 (q, 2H, J = 15.1 Hz), 5.41 (s, 2H), 6.06 (s, 1H), 6.44 (q, 2H, J = 14.7 Hz), 7.45 (s, 1H), 7.76 (d, 1H, J = 7.2 Hz), 7.91 (s, 1H), 7.94 (d, 2H, J = 7.1 Hz), 8.16 (d, 1H, J = 7.2 Hz), 8.95 (d, 2H, J = 7.4 Hz). MS (ESI): 482 (M^+). Anal. Calcd for $C_{29}H_{28}ClN_3O_4$: C, 67.24; H, 5.45; N, 8.11. Found C, 67.45; H, 5.44; N, 8.09.

5.1.50. 10-Methyl-7-[(3′-carboxy)pyridiniumylmethyl]-homocamptothecin chloride (14d). The titled compound was prepared from nicotinic acid according to the method of compound **13a**, yield: 30.6%.^{1}H NMR (DMSO-d_6): 0.87 (t, 3H, J = 7.5 Hz), 1.87 (q, 2H, J = 8.3 Hz), 2.53 (s, 3H), 3.05–3.50 (q, 2H, J = 14.0 Hz), 5.37 (s, 2H), 5.39–5.52 (q, 2H, J = 15.1 Hz), 6.05 (s, 1H), 6.59 (q, 2H, J = 14.6 Hz), 7.44 (s, 1H), 7.78 (d, 1H, J = 7.1 Hz), 8.04 (s, 1H), 8.05 (t, 1H, J = 7.4 Hz), 8.17 (d, 1H, J = 7.1 Hz), 8.86 (d, 1H, J = 7.4 Hz), 8.92 (d, 1H, J = 7.3 Hz), 9.75 (s, 1H). MS (ESI): 512 (M^+). Anal. Calcd for $C_{29}H_{26}ClN_3O_6$: C, 63.56; H, 4.78; N, 7.67. Found C, 63.77; H, 4.77; N, 7.65.

5.2. MTT assay

One thousand two hundred cells per well were plated in 96-well plates. After culturing for 24 h, test compounds were added onto triplicate wells with different concentration, and 0.1% DMSO for control. After three days of incubation, 20 μL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) solution (5 mg mL^{-1}) was added to each well, and after shaking for 1 min the plate was incubated further for 4 h. Formazan crystals were dissolved with 100 μL DMSO. The absorbance (OD) was quantitated with microplate spectrophotometer at 570 μM. Wells containing no drugs were used as blanks for the spectrophotometer. The survival of the cells was expressed as percentage of untreated control wells.

5.3. In vivo antitumor activity

The in vivo antitumor activity of compounds **9j** and **13g** was evaluated and TPT was used as reference drug. C57BL/6 strain male mice (Certificate SCXK-2002-0010, weighing 18–20 g) were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences. C26 colon cancer cell suspensions were implanted subcutaneously into the right axilla region of mice. Daily treatment with drugs or normal saline commenced 3 days after implantation of cells. Mice were administered by sc injection once daily for 5 consecutive days. All the mice were euthanized and the tumors were weighed on day 14 after implantation of cells. The rate of inhibition of tumor growth in vivo was calculated using the following formula (ATWCG = Average Tumor Weight of Control Group, ATWTG = Average Tumor Weight of Test Group):

$$\text{Growth inhibition} = \frac{\text{ATWCG} - \text{ATWTG}}{\text{ATWTG}} \times 100\%$$

5.4. Computational details

5.4.1. General. The crystallographic coordinates of the ternary complex of CPT–DNA–Topo I (3.0 Å resolution, R_{cryst} = 0.244) were obtained from the Brookheaven Protein Databank as entries 1T8I. All calculations were performed with commercially available SYBYL 6.9[45] software package and InsightII 2000[46] software package. All calculations were performed on an Origin 300 Server.

5.4.2. Data set. A total of 45 hCPT derivatives were used as a data set in the following 3D-QSAR analysis. The selection of training set and test set is based on structural diversity and frequency of distribution of biological data by doing principal component analysis. As a result, five compounds were selected as a test set, which represented a range of inhibitory activity similar to that of a training set and was used to evaluate the predictive power of the resulting models. In order to reinforce the reliability of their 3D-QSAR models, six previously synthesized 7-ester hCPT derivatives (compound **15a**–**15f**)[47] were selected as real external compounds (Fig. 2) and were added into the test set. The biological activity of each compound was expressed as inhibitory concentrations (IC_{50}) against A549 cell line and $-\lg(IC_{50})$ was used for the 3D-QSAR analysis.

In the 3D-QSAR studies, pharmacophoric conformation and aliguMent rule are two major critical steps to

get meaningful results. Because the crystal structure of hCPT–DNA–Topo I ternary complex has not been reported, molecular docking was used to simulate the pharmacophoric conformation. As a result, the docked conformation of compound **9l** was used as the template to construct the structures of the remaining compounds in the data set. Energy minimization was performed using the Tripos force field, Powell optimization method, and MAXIMIN2 minimizer with a convergence criterion of 0.001 kcal/mol Å. Charges were calculated by the Gasteiger–Hückel method. Simulated annealing was then performed. The system was heated to 1000 K for 1.0 ps and then annealed to 250 K for 1.5 ps. The annealing function was exponential; 50 such cycles of annealing were run and the resulting 50 conformers were optimized using methods described above. The lowest energy conformation was selected. Compounds were aligned by RMS fitting of the atoms to the most active compound, compound **9l**, based on the heavy atoms in the five-membered ring (Fig. 3). The superimposing of all the compounds is shown in Figure 4.

5.4.3. CoMFA and CoMSIA Setup. In deriving the CoMFA and CoMSIA descriptor fields, a 3D cubic lattice with grid spacing of 2 Å and extending 4 Å units beyond the aligned molecules in all directions was created to encompass the aligned molecules. The charges were calculated using the MOPAC AM1 Hamiltonian semi-empirical method.[48] The CoMFA descriptors, steric (Lennard–Jones 6–12 potential) and electrostatic (Coulombic potential) field energies, were calculated by the following parameters: an sp3 carbon probe atom with +1 charge and a van der Waals radius of 1.52 Å, and energy cutoff of 30 kcal/mol. CoMSIA similarity indices descriptors (steric, electrostatic, hydrophobic, H-bond donor, and H-bond acceptor fields) were calculated using a C1+ probe atom with a radius of 1.0 Å placed at various grid spacing from 1 to 3 Å. CoMSIA similarity indices (A_F) for a molecule j with atoms i at a grid point q are calculated by the following equation:

$$A^q_{F,K}(j) = -\sum \omega_{\text{probe}'k}\omega_{ik}\mathrm{e}^{-\alpha r^2_{iq}} \qquad (1)$$

where k represents the following physicochemical properties: steric, electrostatic, hydrophobic, H-bond donor, and H-bond acceptor. A Gaussian type distance dependence was used between the grid point q and each atom i of the molecule. Here, steric indices are related to the third power of the atomic radii, electrostatic descriptors are derived from atomic partial charges, hydrophobic fields are derived from atom-based parameters, and H-bond donor and acceptor indices are obtained by a rule-based method based on experimental results. The attenuation factor (α) was initially set to the default value of 0.3.

The CoMFA and CoMSIA descriptors were used as independent variables, and $p\mathrm{IC}_{50}$ values were used as dependent variables in partial least squares (PLS) regression analyses to derive 3D-QSAR models using the standard implementation in the SYBYL package. To obtain the optimal number of principle components, the leave-one-out (LOO) cross-validation was utilized, which is used to be a measure of how good the model represents the data in the training set. The cross-validated coefficient, q^2, was calculated using the following equation:

$$q^2 = 1 - \frac{\sum(Y_{\text{predicted}} - Y_{\text{observed}})^2}{\sum(Y_{\text{observed}} - Y_{\text{mean}})^2} \qquad (2)$$

where Y_{pred}, Y_{actual}, and Y_{mean} are predicted, actual, and mean values of the target property (pMIC_{80}), respectively. To maintain the optimum number of PLS components and minimize the tendency to overfit the data, the number of components corresponding to the lowest PRESS value was used to derive the final PLS regression models. Conventional correlation coefficient r^2 and its standard error(s) were also computed for the final PLS models. To graphically interpret the 3D-QSAR results in terms of field contributions, isocontour maps were generated using the field type 'stdev * coeff' and the contour levels were set to default values.

5.4.4. Docking analysis. Compound **9l** was manually docked into the active site of Topo I on the basis of the binding mode of CPT. Then, the flexible ligand docking procedure in the Affinity module within InsightII was used to define the lowest energy position for the substrate using a Monte Carlo docking protocol. All the atoms within a defined radius (6 Å) of the inhibitor were allowed to move. The solvation grid supplied with the affinity Program was used. If the resulting inhibitor/enzyme system was within a predefined energy tolerance of the previous structure, the system was subjected to minimization. The resulting structure was accepted on the basis of energy check, which used the Metropolis criterion, and also a check of rms distance of the new structure versus the structure found so far. The final conformation was obtained through a simulation annealing procedure from 500 to 300 K, and then 5000 rounds of energy minimization were performed to reach a convergence, where the resulting interaction energy values were used to define a rank order.

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