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Synthesis and antitumor activity of novel 10-substituted camptothecin analogues

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Abstract—In an attempt to improve the antitumor activity and decrease the cytotoxicity of camptothecin, 18 new 10-substituted camptothecin derivatives were prepared. The cytotoxicity in vitro on cancer cell lines and antitumor activity in vivo, and inhibitory properties of topoisomerase I of these derivatives were evaluated. Most of these derivatives possessed lower cytotoxicities than CPT, and the compounds **13**, **21**, **22**, **23**, and **24** showed similar topoisomerase I inhibitory activity to CPT. Analogues **13** exhibited the best antitumor activity in vivo among all derivatives we prepared.

1. Introduction

20(*S*)-Camptothecin (CPT, **1**), an antitumor alkaloid, was first isolated by Wall and Wani in 1966 from *Camptotheca acuminata* a tree native to China.[1,2] Interest for its clinic application as an anticancer agent declined due to its severe side effect, extremely poor water solubility, and rapid inactivation through lactone ring hydrolysis at physiological pH, until its action mechanism was revealed that the functional target is topoisomerase I,[3] a nuclear enzyme that is required for topological manipulation of DNA during cellular events such as replication, transcription, and repair.[4] Camptothecin, topoisomerase I, and DNA form a 'so-called cleavable complex,'[5] leading to an accumulation of DNA strand breaks upon replication, transcription, ultimately causing cell death.[6]

These results prompted the synthesis of many CPT derivatives, such as topotecan (**2**) and irinotecan (**3**) that have been approved for marketing as clinical antitumor agents (Fig. 1). More analogues are in various stages of clinical trials. It was reported that the designed camptothecin analogues suitable for targeted enzymatic activation at tumor cells would possess four criteria: improved water solubility; stability in blood; decreased cytotoxicity; and susceptibility to defined enzymatic cleavage.[7]

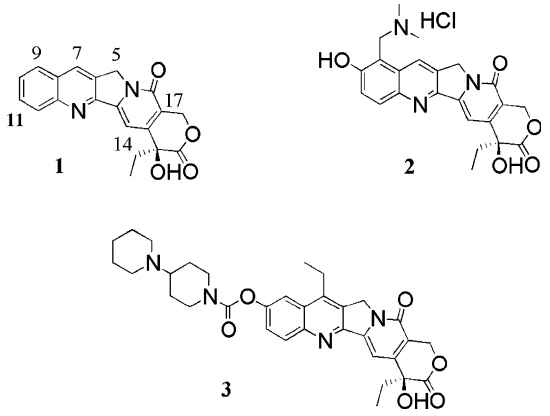

Figure 1. Structures of camptothecin (**1**), topotecan (**2**), and irinotecan (**3**).

Recently, we have reported a new synthetic methodology to transform 10-hydroxy-camptothecin (**4**) to camptothecin quaternary salts,[8] which placed the several water-solubilizing groups in the 10-position of CPT. These salts showed good water solubility and different cytotoxicities in vitro. In order to establish generality of this method, we have carried out this transformation with other heterocyclic aromatic compounds. According to the SAR studies,[9] the above-mentioned structure

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characteristics are believed to show that the opening rate of the intact lactone ring E could be remarkably reduced, so that the toxicity of these newly modified CPT derivatives could be greatly decreased and the water solubility could be improved, but their topo I inhibitory activity and antineoplastic activity could be without getting diminished.

2. Results and discussion

2.1. Chemistry

In our experiments, 10-hydroxy-camptothecin (**4**) was initially converted into 10-(2-bromo-ethoxy)-camptothecin (**5**) in good yields in the presence of 1,2-dibromo-ethane and a catalyst K_2CO_3 according to Kim et al.[10] The target CPT derivatives were synthesized in proper yields by the reaction of **5** with heterocyclic aromatic compounds in the presence of a agent dimethylsulfoxide (DMSO) and a catalyst K_2CO_3 at 60 °C (Scheme 1). Interestingly, we got the byproduct (**23**) from most of the reaction products. We also tried to substitute 1,3-dibromo-propane for 1,2-dibromo-ethane and obtained 10-(3-bromo-propoxy)-camptothecin (**6**), byproduct (**24**), and corresponding CPT derivatives. The yields of 10-(3-bromo-propoxy)-camptothecin (**6**) and its related derivatives were higher than those of 10-(2-bromo-ethoxy)-camptothecin (**5**) and its corresponding analogues. This may relate to the high steric clash on CPT's structure. It is necessary to note that the link sites of compounds **11** and **12** were different from our expected structure. The ^1H and ^{13}C NMR spectra of these novel camptothecin derivatives showed the right characteristic proton peaks for their different substituents. The mass spectra of all the compounds were consistent with their molecular weight.

2.1.1. Assays for lactone ring opening. CPT derivatives prepared were also subjected to phosphate buffer (pH 7.4) stability studies. Some of the results are shown in Table 2. The analogues were incubated in 0.1 mol/L phosphate buffer at 25 °C and aliquots were taken at different time points and examined by HPLC. Compounds **13**, **21**, **23**, and **24** showed (Table 2) complete buffer stability for the length of the experiment (48 h). Compound **22** was less stable in buffer with a half-life of around 18 h.

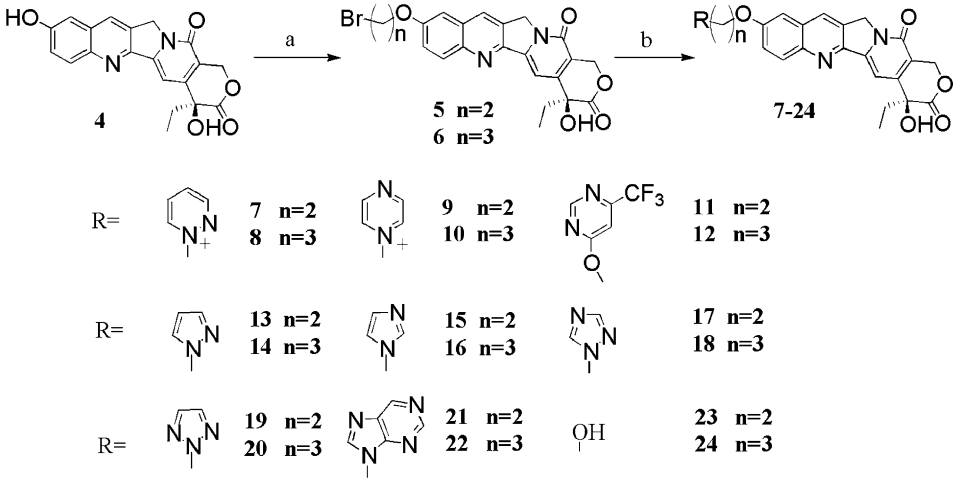
2.2. Biological results

2.2.1. Cell-growth inhibition. The cytotoxicities of the CPT derivatives (Table 1) were measured on three different human cancer cell lines (MCF-7, HCT-8, and BEL-7402) using MTT assay[11] in vitro. Topotecan and CPT were used as reference compounds. Compared with the

Table 1. Cytotoxicity and Topo I inhibition of CPT derivatives

Compound	In vitro cytotoxicity (IC_{50}, nmol/mL)			Topo I cleavage[c]
	MCF-7[b]	HCT-8	Bel-7402	
CPT	0.02	0.01	0.48	1
Topotecan	0.14	0.12	0.62	—[a]
7	0.34	0.09	0.43	0.032
8	1.16	0.21	1.1	0.053
9	0.1	0.11	1.79	0.560
10	0.61	0.08	0.26	0.098
11	0.24	0.15	1.28	0.638
12	0.18	0.18	0.9	0.742
13	0.04	0.07	1.48	0.974
14	0.03	0.07	0.16	0.242
15	0.71	0.1	0.09	0.103
16	0.38	0.98	1.06	0.307
17	0.62	1.02	1.55	0.312
18	0.62	0.9	0.40	0.025
19	2.92	2.36	19.18	0.261
20	0.06	0.12	0.53	0.150
21	0.87	0.20	0.50	1.384
22	2.09	1.81	4.02	1.228
23	0.75	0.22	0.86	1.405
24	0.07	0.19	0.18	1.044

[a] —, not tested.
[b] MCF-7, human breast cancer cells; HCT-8, human colon cancer; Bel-7402, human hepatocellular cancer cell.
[c] Topo I cleavage, Topo I-mediated DNA cleavage. The comparison between nicked band intensities of each compound and CPT's (50 μM).



Scheme 1. Reagents: (a) $Br(CH_2)_2Br$ (**5**) or $Br(CH_2)_3Br$ (**6**)/K_2CO_3/DMSO; (b) R/DMSO (**7**–**10**) or R/K_2CO_3/DMSO (**11**–**24**).

Table 2. Antitumor activity in nude mice bearing HCT-8 and buffer half-life of analogues and control compounds

Group	Dose (mg/kg)	Mice number		MTW (mm^3)		RTV $x \pm SD$	TW (g) $x \pm SD$	TIR[a] (%)	MTD[b] (mg/kg)	Half-life (h)
		Begin	End	Begin	End					
Control	—	8	8	132 ± 29.6	1354 ± 410.3	4.52 ± 3.111	1.15 ± 0.436	—	—	—
TPT	10	8	7	124 ± 36.5	218 ± 136.5	0.78 ± 0.410**	0.18 ± 0.141**	84.3	13	Na[c]
13	1.3	8	8	144 ± 30.5	574 ± 326.6	1.77 ± 1.088*	0.43 ± 0.273**	62.6	4	Stable[d]
23	6.2	8	8	142 ± 20.7	697 ± 367.1	2.17 ± 1.373	0.575 ± 0.331*	50.0	13	Stable
24	3.9	8	8	140 ± 21.3	595 ± 272.1	1.90 ± 1.204*	0.56 ± 0.264**	51.3	10	Stable

Student's *t*-test was used to compare tumor volumes of treated mice. *P values < 0.05 were considered significant, **P values < 0.01 were considered very significant.

[a] Tumor inhibitory rate.

[b] MTD is approximate value.

[c] Not tested.

[d] Experiment carried out for 48 h.

strong cytotoxicity of CPT on all cancer cells, most of the derivatives showed less cytotoxic activity in vitro. As shown in Table 1, analogues **19** and **22** showed 10–100 times less activity than CPT in the assay of cytotoxic activity, whereas derivatives **10**, **13**, **14**, **15**, **20**, and **24** showed similar or superior cytotoxic activity to topotecan. And compounds **13**, **14**, **20** and **24** had more efficacy on two kinds of cancer cell lines, MCF-7 and HCT-8, and are as same as CPT's.

2.2.2. Topoisomerase I inhibition. The topoisomerase I inhibitory properties of these CPT derivatives were evaluated using a DNA relaxation assay.[12] Negative supercoiled plasmid pBR322 was incubated with human topoisomerase I in the presence of 25 μM salt derivative. Parallel experiment was performed with CPT. Samples were treated with 2.5% SDS to remove any covalently bound protein, then resolved in 1% agarose gel containing ethidium bromide. The nicked band intensities of these derivatives in agarose gel, corresponding to the DNA–topoisomerase I -CPT derivatives cleavable complex,[13] were quantified and compared with the nicked band intensity of CPT.[11] The results in Table 1 indicate that all the tested derivatives varied significantly in their abilities to stabilize the topoisomerase I–DNA covalent binary complex. The most efficient analogues in this regard were compounds **13**, **21**, **22**, **23**, and **24**, whose topo I inhibitory activities are similar to CPT's. The compounds **9**, **11**, and **12** showed lower topo I inhibitory activity than that of CPT. Weaker stabilization was obtained in the presence of compounds **14**, **15**, **16**, **17**, **19**, and **20**. The remaining derivatives, compounds **7**, **8**, **10**, and **18** afforded little stabilization.

2.2.3. In vivo toxicity and antitumor studies. Since compounds **9**, **13**, **21**, **22**, **23**, and **24** showed the most brilliant topo I inhibitory activity, the preliminary antitumor activity studies in vivo of these compounds were evaluated against mouse sarcoma S180,[14] compared with that of topotecan. The MTD (maximum to tolerance dose) values indicated that camptothecin derivative **13** was more toxic to mice than topotecan whereas compound **24** provided less toxicity. The compound **23** exhibited similar MTD values to topotecan. Compounds **13**, **23**, and **24** were used tofurther antitumor activity studies on nude mice bearing HCT-8 in vivo. One proper dose for each compound was chosen according to preliminary in vivo antitumor activity study results against mouse sarcoma S180, and was repeated four times on a q4d × 4 schedule. As a result (Table 2), the best value of tumor inhibitory rate (TIR) at chosen dose was 62.6% in compound **13** treated group. TIR of compounds **24** and **23** was 51.3% and 50.0%, respectively. In addition, the other parameters used to evaluate antitumor activity in vivo were also right to the results above. The chosen dose of compound **13** (1.3 mg/kg) was nearly 1/8 of that of topotecan (10 mg/kg), and the TIR of compound **13** was a little lower than that of topotecan. In conclusion, compound **13** possessed better anticancer activity than other analogues and had promising perspective.

3. Conclusions

On the basis of work about CPT quaternary salt derivatives,[8] 18 new CPT derivatives were prepared. Preliminary biological studies of these CPT analogues including inhibitory Topo I-mediated DNA cleavage reactions, cytotoxicity in vitro and in vivo antitumor activity assay exhibit that most derivatives possess lower cytotoxicities in vitro than CPT, and compounds **13**, **21**, **22**, **23** and **24** show similar topo I inhibitory activity to CPT. The results of in vivo antitumor activity assay indicate that compound **13** has the best tumor inhibitory activity against HCT-8.

4. Experimental

4.1. Chemistry

The melting points were determined using an electrothermal apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at either 300 MHz or 500 MHz with a Bruker instrument, and reported with TMS as internal standard and $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz. ESI mass spectra were performed on a API-3000 LC–MS–MS spectrometer. HR-MS (EI or ESI) was recorded on ZAB-HS instrument. IR spectra were obtained at Magna-560 FT instrument. TLC analysis was carried out on silica gel plates GF_{254}. Flash column

chromatography was carried out on silica gel 300–400 mesh. HPLC analysis was performed on a Waters Alliance 2690 instrument with UV detection at 360 nm: column: Waters Symmetry-C_{18}. Anhydrous solvent and reagents were all analytical reagents and dried through routine method.

4.1.1. 10-(2-Bromo-ethoxy)-(20*S*)-camptothecin (5). A mixture of 10-hydroxy-CPT (182.2 mg, 0.5 mmol), and DMSO (1.5 mL) was stirred at room temperature until dissoluted, and then 1,2-dibromo-ethane (0.86 mL, 10.0 mmol) and anhydrous K_2CO_3 (345.94 mg, 2.5 mmol) were added. TLC was carried out to detect the reaction throughout. After the mixture was stirred under N_2 atmosphere at 60 °C for 72 h, it was added to certain water. The mixture was filtered and the solid was washed with water and acetone, and then dried. After the solvent was removed under reduced pressure, the residue was taken up in the mixed solution (eluent: $CHCl_3/CH_3OH$ 8:2,) and purified by column chromatography (eluent: $CHCl_3/CH_3OH$ 100:1–100:3) to give **5** as a yellow solid, yield: 68.0%, mp 235–237 °C; IR (KBr) υ 3421, 2970, 2925, 1747, 1662, 1605, 1558, 1505, 1429, 1370, 1237, 1151, 1105, 1049, 1015, 832 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 0.87 (3H, t, J = 7.2 Hz, H-18), 1.85 (2H, m, H-19), 3.91 (2H, t, J = 5.4 Hz, –CH_2Br), 4.51 (2H, t, J = 5.4 Hz, –OCH_2–), 5.25 (2H, s, H-5), 5.41 (2H, s, H-17), 6.51 (1H, s, OH-20), 7.27 (1H, s, H-14), 7.54 (1H, d, J = 9.3 Hz, H-11), 7.55 (1H, s, H-9), 8.08 (1H, d, J = 9.3 Hz, H-12), 8.52 (1H, s, H-7); MS (ESI): m/z 472 (MH^+). HRMS (ESI): m/z calcd for $C_{22}H_{19}BrN_2O_5$+H 471.0587, found 471.0589.

4.1.2. 10-(3-Bromo-propoxy)-(20*S*)-camptothecin (6). The titled compound was prepared from 10-OH CPT and 1,3-dibromo-propane according to the method of compound **5**, yield: 85.7%, mp 224–226 °C; IR (KBr) υ 3398, 2985, 2925, 1747, 1660, 1595, 1556, 1508, 1429, 1370, 1237, 1147, 1105, 1049, 1015, 832 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 0.88 (3H, t, J = 7.4 Hz, H-18), 1.86 (2H, m, H-19), 2.36 (2H, t, J = 5.6 Hz, CH_2), 3.74 (2H, t, J = 5.6 Hz, CH_2), 4.26 (2H, t, J = 5.6 Hz, CH_2), 5.25 (2H, s, H-5), 5.41 (2H, s, H-17), 6.53 (1H, s, 20-OH), 7.27 (1H, s, H-14), 7.50 (1H, d, J = 10 Hz, H-11), 7.55 (1H, s, H-9), 8.05 (1H, d, J = 10 Hz, H-12), 8.53 (1H, s, H-7); MS (ESI): m/z 485 (M^+). HRMS (ESI): m/z calcd for $C_{23}H_{21}BrN_2O_5$ 484.0664, found 484.0665.

4.1.3. 10-(2-Pyridaziniumyl-ethoxy)-(20*S*)-camptothecin bromide (7). The reaction mixture of compound **5** (94.0 mg, 0.2 mmol), superfluous pyridazine (80 mg, 1 mmol) and DMSO (1.5 mL) was stirred at 50 °C for 12 h. TLC was carried out to detect reaction throughout, and R_f of the product was zero in CH_3OH. After $CHCl_3$ was added to the solution, a yellow deposition precipitated immediately. The mixture was filtered, and the solid was washed with $CHCl_3$, and the dried at 60 °C in the oven to give compound **7** as a yellow powder, yield: 36.0 mg (32.6%), mp >209.5 °C, IR (KBr) υ 3418, 3103, 2981, 1743, 1656, 1593, 1505, 1373, 1240, 1049, 837, 725 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 0.88 (3H, t, J = 7.5 Hz, H-18), 1.86 (2H, m, H-19), 4.82 (2H, s, CH_2), 5.20 (2H, s, H-5), 5.39 (2H, s, CH_2), 5.40 (2H, s, H-17), 6.54 (1H, s, 20-OH), 7.24 (1H, s, H-14), 7.42 (1H, d, J = 9.2 Hz, H-11), 7.59 (1H, s, H-9), 8.02 (1H, d, J = 9.3 Hz, H-12), 8.52 (1H, s, H-7), 8.72 (1H, t), 8.84 (1H, t), 9.72 (1H, d), 10.15 (1H, d); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.74, 50.69, 63.21, 65.73, 65.95, 72.88, 96.58, 107.46, 118.90, 123.31, 129.74, 130.47, 130.71, 130.99, 136.40, 137.32, 144.49, 146.12, 150.50, 150.80, 155.02, 157.26, 173.02; MS (ESI) m/z 471.5 (M^+); HRMS (ESI): m/z calcd for $C_{26}H_{23}N_4O_5$ 471.1751, found 471.1751.

4.1.4. 10-(3-Pyridaziniumyl-propoxy)-(20*S*)-camptothecin bromide (8). The titled compound was prepared from compound **6** and pyridazine according to the method of compound **7**, yield: 40.9%, mp >224.9 °C, IR (KBr) υ 3419, 3102, 2915, 1743, 1658, 1597, 1505, 1373, 1242, 1161, 1047, 989, 835 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.2 Hz, H-18), 1.86 (2H, m, H-19), 2.62 (2H, t, CH_2), 4.34 (2H, t, CH_2), 5.09 (2H, t, CH_2), 5.28 (2H, s, H-5), 5.43 (2H, s, H-17), 6.54 (1H, s, 20-OH), 7.26 (1H, s, H-14), 7.28 (1H, d, J = 9.1 Hz, H-11), 7.49 (1H, s, H-9), 8.05 (1H, d, J = 9.2 Hz, H-12), 8.54 (1H, s, H-7), 8.66 (1H, t), 8.76 (1H, t), 9.66 (1H, d), 10.04 (1H, d); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 29.29, 30.74, 50.68, 63.07, 65.73, 65.99, 72.88, 96.58, 107.46, 118.90, 123.28, 129.73, 130.47, 130.70, 130.99, 136.41, 137.32, 144.47, 146.12, 150.50, 150.79, 154.96, 157.26, 173.02; MS (ESI) m/z 485.4 (M^+); HRMS (ESI): m/z calcd for $C_{27}H_{25}N_4O_5$ 485.1847, found 485.1849.

4.1.5. 10-(2-Pyraziniumyl-ethoxy)-(20*S*)-camptothecin bromide (9). The titled compound was prepared from compound **5** and pyrazine according to the method of compound **7**, yield: 32.6%, mp 206.8 °C, IR (KBr) υ 3418, 3012, 2910, 1743, 1656, 1593, 1505, 1373, 1239, 1160, 1049, 832 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 7.2 Hz, H-18), 1.85 (2H, m, H-19), 4.75 (2H, s, CH_2), 5.22 (2H, s, H-5), 5.25 (2H, s, CH_2), 5.40 (2H, s, H-17), 7.25 (1H, s, H-14), 7.51 (1H, d, J = 9.0 Hz, H-11), 7.56 (1H, s, H-9), 8.05 (1H, d, J = 9.0 Hz, H-12), 8.55 (1H, s, H-7), 9.41 (2H, d), 9.64 (2H, d); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.72, 50.70, 65.73, 65.90, 72.88, 96.58, 107.49, 118.92, 123.19, 129.73, 130.48, 130.73, 131.05, 138.06, 144.48, 146.12, 150.51, 150.80, 151.23, 157.17, 157.30, 173,03; MS (ESI) m/z 485.3 (M^+); MS (ESI) m/z 471.4 (M^+); HRMS (ESI): m/z calcd for $C_{26}H_{23}N_4O_5$ 471.1752, found 471.1751.

4.1.6. 10-(3-Pyraziniumyl-propoxy)-(20*S*)-camptothecin bromide (10). The titled compound was prepared from compound **6** and pyrazine according to the method of compound **7**, yield: 47.0%, mp >194.3 °C, IR (KBr) υ 3418, 2981, 1743, 1656, 1597, 1505, 1373, 1242, 1162, 1047, 835 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, J = 6.9 Hz, H-18), 1.85 (2H, m, H-19), 2.59 (2H, s, CH_2), 4.33 (2H, s, CH_2), 4.94 (2H, s, CH_2), 5.26 (2H, s, H-5), 5.42 (2H, s, H-17), 6.53 (1H, s, 20-OH), 7.26 (1H, s, H-14), 7.27 (1H, d, J = 9.5 Hz, H-11), 7.48 (1H, s, H-9), 8.05 (1H, d, J = 9.6 Hz, H-

12), 8.53 (1H, s, H-7), 9.37 (2H, d), 9.59 (2H, d); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.00, 30.72, 50.71, 65.73, 65.86, 72.88, 96.59, 107.51, 118.93, 123.15, 129.74, 130.50, 130.76, 131.05, 138.05, 144.49, 146.12, 150.51, 150.81, 151.23, 157.17, 157.29, 173,03; MS (ESI) *m*/*z* 485.3 (M^+); MS (ESI) *m*/*z* 485.4 (M^+); HRMS (ESI): *m*/*z* calcd for $C_{27}H_{25}N_4O_5$ 485.1847, found 485.1848.

4.1.7. 10-[2-(6-Trifluoromethyl-pyrimidin-4-yloxy)-ethoxy]-(20*S*)-camptothecin (11). The targeted compound was prepared from compound **5** and 6-(trifluoromethyl)-4-pyrimidinol according to the method of compound **5**, yield: 59.1%, mp 216.5 °C, IR (KBr) υ 3419, 2898, 1749, 1693, 1602, 1505, 1238, 1154, 1049, 937, 832 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.3 Hz, H-18), 1.87 (2H, m, H-19), 4.46 (4H, br, 2CH$_2$), 5.25 (2H, s, H-5), 5.41 (2H, s, H-17), 6.50 (1H, s, 20-OH), 7.01 (1H, s), 7.28 (1H, s, H-14), 7.48 (1H, d, *J* = 9.3 Hz, H-11), 7.57 (1H, s, H-9), 8.06 (1H, d, *J* = 9.3 Hz, H-12), 8.52 (1H, s, H-7), 8.80 (1H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.25, 30.73, 46.35, 50.63, 65.35, 65.70, 72.87, 96.56, 107.71, 113.48, 118.88, 123.27, 129.64, 130.46, 130.65, 131.05, 144.53, 146.06, 150.48, 150.79, 155.43, 157.06, 157.22, 160.27, 173.01; MS (ESI) *m*/*z* 555.2 $(MH)^+$; HRMS (ESI): *m*/*z* calcd for $C_{27}H_{21}F_3N_4O_6$+H 555.1432, found 555.1431.

4.1.8. 10-[3-(6-Trifluoromethyl-pyrimidin-4-yloxy)-propoxy]-(20*S*)-camptothecin (12). The titled compound was prepared from compound **6** and 6-(trifluoromethyl)-4-pyrimidinol according to the method of compound **5**, yield: 61.8%, mp 198.5 °C, IR (KBr) υ 3423, 2975, 1746, 1689, 1659, 1598, 1505, 1372, 1240, 1047, 948, 833 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.3 Hz, H-18), 1.86 (2H, m, H-19), 2.35 (2H, t, CH$_2$), 4.23 (4H, br, 2CH$_2$), 5.27 (2H, s, H-5), 5.42 (2H, s, H-17), 6.55 (1H, s, 20-OH), 6.97 (1H, s), 7.29 (1H, s, H-14), 7.35 (1H, d, *J* = 9.1 Hz, H-11), 7.49 (1H, s, H-9), 8.06 (1H, d, *J* = 9.1 Hz, H-12), 8.53 (1H, s, H-7), 8.75 (1H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.25, 30.01, 30.72, 46.33, 50.62, 65.35, 65.72, 72.86, 96.58, 107.70, 113.48, 118.88, 123.27, 129.64, 130.46, 130.65, 131.03, 144.53, 146.16, 150.49, 150.79, 155.33, 157.06, 157.22, 160.27, 173.02; MS (ESI) *m*/*z* 569.3 $(MH)^+$; HRMS (ESI): *m*/*z* calcd for $C_{28}H_{23}F_3N_4O_6$+H 569.1625, found 569.1625.

4.1.9. 10-(2-Pyrazolyl-ethoxy)-(20*S*)-camptothecin (13). The reaction mixture of compound **5** (94.0 mg, 0.2 mmol), superfluous pyrazole-1*H* (68 mg, 1 mmol), and DMSO (1.5 mL) was stirred at 50 °C for 1 week. TLC was carried out to detect reaction throughout. Then chloroform and anhydrous ether were added ordinarily to the solution, a straw yellow deposition precipitated immediately. The mixture was filtered, and the solid was washed with anhydrous ether. The crude product was separated and purified by column chromatography (eluent: $CHCl_3$/CH_3OH 100:5), and then dried at 60 °C under vacuum to give **13**, yield: 46.4%, mp >246.3 °C, IR (KBr) υ 3388, 2925, 2853, 1747, 1663, 1624, 1504, 1240, 1160, 830, 760 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.86 (2H, m, H-19), 4.53 (2H, t, CH$_2$), 4.60 (2H, t, CH$_2$), 5.25 (2H, s, H-5), 5.40 (2H, s, H-17), 6.26 (1H, s), 6.48 (1H, s, 20-OH), 7.28 (1H, s, H-14), 7.44 (1H, d, *J* = 9.5 Hz, H-11), 7.46 (1H, s), 7.51 (1H, s, H-9), 7.83 (1H, s), 8.04 (1H, d, *J* = 9.5 Hz, H-12), 8.51 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.74, 48.39, 50.69, 65.73, 72.89, 96.57, 105.55, 107.45, 118.86, 123.61, 129.85, 130.47, 130.71, 130.99, 139.20, 144.47, 146.19, 150.52, 150.67, 157.31, 157.68, 173.02; MS (ESI) *m*/*z*, 459.2 $(MH)^+$; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{22}N_4O_5$ +H 459.1619, found 459.1618.

4.1.10. 10-(3-Pyrazolyl-propoxy)-(20*S*)-camptothecin (14). The targeted compound was prepared from compound **6** and pyrazole-1*H* according to the method of compound **13**, yield: 51.4%, mp >251.3 °C, IR (KBr) υ 3417, 2900, 1750, 1659, 1600, 1505, 1239, 1156, 1046, 831, 759 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.86 (2H, m, H-19), 2.32 (2H, t, CH$_2$), 4.12 (2H, t, CH$_2$), 4.34 (2H, t, CH$_2$), 5.24 (2H, s, H-5), 5.40 (2H, s, H-17), 6.24 (1H, s), 6.48 (1H, s, 20-OH), 7.28 (1H, s, H-14), 7.44 (1H, d, *J* = 9.5 Hz, H-11), 7.48 (1H, s, H-9), 7.50 (1H, s), 7.75 (1H, s), 8.05 (1H, d, *J* = 9.5 Hz, H-12), 8.50 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.07, 30.74, 48.43, 50.70, 65.74, 72.89, 96.53, 105.54, 107.35, 118.85, 123.64, 129.86, 130.47, 130.70, 131.00, 139.18, 144.49, 146.23, 150.53, 150.67, 157.31, 157.80, 173.01; MS (ESI) *m*/*z*, 473.1 $(MH)^+$; HRMS (ESI): *m*/*z* calcd for $C_{26}H_{24}N_4O_5$ 473.1732, found 473.1732.

4.1.11. 10-(2-Imidazolyl-ethoxy)-(20*S*)-camptothecin (15). The targeted compound was prepared from compound **5** and imidazole-1*H* according to the method of compound **13**, yield: 43.2%, mp >197.9 °C, IR (KBr) υ 3404, 2925, 2853, 1747, 1657, 1596, 1505, 1239, 1159, 1049, 831, 731 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.86 (2H, m, H-19), 4.43 (2H, t, CH$_2$), 4.46 (2H, t, CH$_2$), 5.25 (2H, s, H-5), 5.41 (2H, s, H-17), 6.49 (1H, s, 20-OH), 6.91 (1H, s), 7.28 (1H, s, H-14), 7.30 (1H, s), 7.49 (1H, d, *J* = 9.0 Hz, H-11), 7.50 (1H, s, H-9), 7.74 (1H, s), 8.05 (1H, d, *J* = 9.0 Hz, H-12), 8.51 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.74, 43.51, 50.67, 65.57, 65.73, 72.88, 96.56, 107.44, 118.80, 119.95, 122.05, 123.65, 128.86, 129.80, 130.47, 130.98, 137.81, 144.47, 146.13, 150.51, 150.75, 157.26, 157.69, 173.02; MS (ESI) *m*/*z*, 459.3 $(MH)^+$; HRMS (ESI): *m*/*z* calcd for $C_{25}H_{22}N_4O_5$+H 459.1619, found 459.1619.

4.1.12. 10-(3-Imidazolyl-propoxy)-(20*S*)-camptothecin (16). The titled compound was prepared from compound **6** and imidazole-1*H* according to the method of compound **13**, yield: 48.7%, mp 193.6 °C, IR (KBr) υ 3282, 2925, 1744, 1657, 1601, 1504, 1240, 1160, 1045, 834, 664 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.5 Hz, H-18), 1.86 (2H, m, H-19), 2.27 (2H, t, CH$_2$), 4.09 (2H, t, CH$_2$), 4.21 (2 H, t, CH$_2$), 5.21 (2H, s, H-5), 5.41 (2H, s, H-17), 6.48 (1H, s, 20-OH), 6.92 (1H, s), 7.24 (1H, s), 7.26 (1H, s, H-14), 7.45 (1H, s, H-9), 7.47 (1H, d, *J* = 9.0 Hz, H-11), 7.69 (1H, s), 8.04 (1H, d, *J* = 9.0 Hz, H-12), 8.49 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.53,

30.75, 43.51, 50.65, 65.57, 65.72, 72.89, 96.54, 107.34, 118.83, 119.94, 122.05, 123.55, 128.82, 129.79, 130.45, 130.97, 137.84, 144.47, 146.17, 150.52, 150.62, 157.27, 157.69, 173.02; MS (ESI) *m/z*, 473.3 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{26}H_{24}N_4O_5$ 473.1732, found 473.1732.

4.1.13. 10-[1-(1,2,4-Triazolyl)-ethoxy]-(20*S*)-camptothecin (17). The targeted compound was prepared from compound **5** and 1,2,4-1*H*-triazole according to the method of compound **13**, yield: 44.8%, mp 247.3 °C, IR (KBr) υ 3417, 2925, 1746, 1657, 1623, 1598, 1505, 1399, 1242, 1047, 835 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (3H, t, *J* = 6.9 Hz, H-18), 1.85 (2H, m, H-19), 4.45 (2H, t, CH_2), 4.54 (2H, t, CH_2), 5.24 (2H, s, H-5), 5.40 (2H, s, H-17), 6.49 (1H, s, 20-OH), 7.26 (1H, s, H-14), 7.50 (1H, d, *J* = 9.0 Hz, H-11), 7.52 (1H, s, H-9), 8.05 (1H, d, *J* = 9.0 Hz, H-12), 8.51 (1H, s, H-7), 8.62 (2H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.75, 46.35, 50.69, 57.72, 65.73, 72.89, 96.55, 107.40, 118.86, 123.58, 123.70, 129.91, 130.47, 130.70, 130.98, 144.47, 146.18, 150.50, 151.93, 157.27, 157.70, 158.06, 173.02; MS (ESI) *m/z*, 460.3 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{24}H_{21}N_5O_5$+H 460.1510, found 460.1611.

4.1.14. 10-[1-(1,2,4-Triazolyl)-propoxy]-(20*S*)-camptothecin (18). The titled compound was prepared from compound **6** and 1,2,4-1*H*-triazole according to the method of compound **13**, yield: 52.1%, mp >232.8 °C, IR (KBr) υ 3417, 3125, 1746, 1650, 1623, 1585, 1505, 1380, 1240, 1045, 835 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (3H, t, *J* = 7.2 Hz, H-18), 1.85 (2H, m, H-19), 2.29 (2H, t, CH_2), 4.12 (2H, t, CH_2), 4.27 (2H, t, CH_2), 5.23 (2H, s, H-5), 5.40 (2H, s, H-17), 6.49 (1H, s, 20-OH), 7.26 (1H, s, H-14), 7.43 (1H, d, *J* = 9.0 Hz, H-11), 7.47 (1H, s, H-9), 8.04 (1H, d, *J* = 9.0 Hz, H-12), 8.49 (1H, s, H-7), 8.57 (2H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 29.44, 30.73, 46.21, 50.70, 57.72, 65.72, 72.89, 96.53, 107.34, 118.86, 123.60, 123.71, 129.89, 130.48, 130.71, 130.94, 144.60, 146.22, 150.60, 151.98, 157.31, 157.71, 158.06, 173.01; MS (ESI) *m/z*, 474.1 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{25}H_{23}N_5O_5$ 474.1727, found 474.1727.

4.1.15. 10-[1-(1,2,3-Triazolyl)-ethoxy]-(20*S*)-camptothecin (19). The targeted compound was prepared from compound **5** and 1,2,3-1*H*-triazole according to the method of compound **13**, yield: 35.0%, mp >263.7 °C, IR (KBr) υ 3422, 2925, 1747, 1655, 1623, 1506, 1240, 1159, 1047, 832, 668 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.86 (2H, m, H-19), 4.60 (2H, s, CH_2), 4.90 (2H, s, CH_2), 5.25 (2H, s, H-5), 5.41 (2H, s, H-17), 6.50 (1H, s, 20-OH), 7.27 (1H, s, H-14), 7.47 (1H, s, H-9), 7.53 (1H, d, *J* = 9.5 Hz, H-11), 7.77 (1H, s), 8.06 (1H, d, *J* = 9.5 Hz, H-12), 8.26 (1H, s), 8.51 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.74, 46.72, 50.69, 57.73, 64.89, 72.89, 96.57, 107.51, 118.82, 123.57, 127.68, 129.71, 130.47, 130.71, 130.99, 144.47, 146.28, 150.50, 151.79, 157.31, 157.70, 173.02; MS (ESI) *m/z*, 460.3 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{24}H_{21}N_5O_5$+H 460.1510, found 460.1610.

4.1.16. 10-[1-(1,2,3-Triazolyl)-propoxy]-(20*S*)-camptothecin (20). The targeted compound was prepared from compound **6** and 1,2,3-1*H*-triazole according to the method of compound **13**, yield: 42.9%, mp >270.0 °C, IR (KBr) υ 3423, 2925, 2853, 1747, 1658, 1597, 1505, 1464, 1242, 1161, 1047, 836, 723 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.86 (2H, m, H-19), 2.39 (2H, t, CH_2), 4.16 (2H, t, CH_2), 4.63 (2H, t, CH_2), 5.26 (2H, s, H-5), 5.41 (2H, s, H-17), 6.50 (1H, s, 20-OH), 7.28 (1H, s, H-14), 7.49 (1H, d, *J* = 9.0 Hz, H-11), 7.50 (1H, s, H-9), 7.75 (1H, s), 8.06 (1H, d, *J* = 9.0 Hz, H-12), 8.20 (1H, s), 8.52 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 29.40, 30.75, 46.71, 50.71, 57.72, 65.01, 72.88, 96.58, 107.56, 118.85, 123.67, 127.70, 129.72, 130.47, 130.70, 130.99, 144.47, 146.22, 150.50, 151.81, 157.26, 157.70, 173.02; MS (ESI) *m/z*, 474.2 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{25}H_{23}N_5O_5$ 474.1727, found 474.1727.

4.1.17. 10-(2-Purinyl-ethoxy)-(20*S*)-camptothecin (21). The targeted compound was prepared from compound **5** and purine according to the method of compound **13**, yield: 50.6%, mp 226.0 °C, IR (KBr) υ 3405, 2926, 1743, 1655, 1591, 1505, 1241, 1161, 1048, 834 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.87 (3H, t, *J* = 6.5 Hz, H-18), 1.85 (2H, m, H-19), 4.73 (2H, t, CH_2), 5.17 (2H, t, CH_2), 5.22 (2H, s, H-5), 5.40 (2H, s, H-17), 6.50 (1H, s, 20-OH), 7.25 (1H, s, H-14), 7.53 (1H, d, *J* = 9.0 Hz, H-11), 7.54 (1H, s, H-9), 8.05 (1H, d, *J* = 9.0 Hz, H-12), 8.54 (1H, s, H-7), 9.15 (1H, s), 9.62 (1H, s), 9.96 (1H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.72, 46.71, 50.77, 64.57, 65.72, 72.89, 96.54, 107.34, 118.89, 123.49, 124.95, 128.76, 129.81, 130.35, 130.88, 141.89, 144.47, 144.96, 146.17, 149.93, 150.50, 150.79, 151.90, 157.26, 157.71, 173.01; MS (ESI) *m/z* 511.1 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{27}H_{22}N_6O_5$ +H 551.1772, found 551.1770.

4.1.18. 10-(3-Purinyl-propoxy)-(20*S*)-camptothecin (22). The targeted compound was prepared from compound **6** and purine according to the method of compound **13**, yield: 54.2%, mp 263.9 °C, IR (KBr) υ 3418, 2928, 1746, 1657, 1600, 1505, 1373, 1241, 1160, 1048, 835 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, *J* = 7.0 Hz, H-18), 1.85 (2H, m, H-19), 2.45 (2H, t, CH_2), 4.16 (2H, t, CH_2), 4.64 (2H, t, CH_2), 5.25 (2H, s, H-5), 5.42 (2H, s, H-17), 6.53 (1H, s, 20-OH), 7.28 (1H, s, H-14), 7.43 (1H, d, *J* = 9.0 Hz, H-11), 7.44 (1H, s, H-9), 8.05 (1H, d, *J* = 9.0 Hz, H-12), 8.49 (1H, s, H-7), 8.78 (1H, s), 8.96 (1H, s), 9.27 (1H, s); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.42, 30.74, 46.83, 50.69, 64.55, 65.73, 72.89, 96.56, 107.41, 118.89, 123.50, 125.00, 128.83, 129.83, 130.35, 130.78, 141.88, 144.47, 144.98, 146.14, 149.95, 150.50, 150.80, 151.91, 157.27, 157.76, 173.02; MS (ESI) *m/z* 525.4 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{28}H_{24}N_6O_5$ +H 525.1867, found 525.1866.

4.1.19. 10-(2-Hydroxy-ethoxy)-(20*S*)-camptothecin (23). The titled compound was prepared from compound **5** and purine according to the method of compound **13**, yield 34.1%, mp 263.9 °C, IR (KBr) υ 3391, 2923, 2853, 1736, 1657, 1623, 1590, 1559, 1504, 1462, 1371,

1240, 1156, 1048, 905, 828 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, $J = 7.0$ Hz, H-18), 1.87 (2H, m, H-19), 3.82 (2H, t, CH_2), 4.18 (1H, t, OH), 4.52 (2H, t, CH_2), 5.26 (2H, s, H-5), 5.45 (2H, s, H-17), 6.48 (1H, s, 20-OH), 7.30 (1H, s, H-14), 7.53 (1H, d, $J = 9.0$ Hz, H-11), 7.56 (1H, s, H-9), 8.09 (1H, d, $J = 9.0$ Hz, H-12), 8.53 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.75, 46.75, 59.98, 65.55, 65.90, 72.89, 96.57, 107.72, 118.90, 123.28, 129.74, 130.46, 130.72, 131.23, 144.50, 146.10, 150.48, 150.79, 155.73, 157.52, 173.02; MS (ESI) *m/z* 409.4 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{22}H_{20}N_2O_6$+H 409.1310, found 409.1309.

4.1.20. 10-(3-Hydroxy-propoxy)-(20*S*)-camptothecin (24). The titled compound was prepared from compound **6** and purine according to the method of compound **13**, yield 35.6%, mp 248.9 °C, IR (KBr) υ 3389, 2923, 2853, 1738, 1657, 1623, 1590, 1559, 1504, 1462, 1370, 1240, 1156, 1048, 905, 828 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.88 (3H, t, $J = 7.3$ Hz, H-18), 1.86 (2H, m, H-19), 1.98 (2H, t, CH_2), 3.63 (2H, t, CH_2), 4.21 (2H, t, CH_2), 4.64 (1H, t, OH), 5.26 (2H, s, H-5), 5.42 (2H, s, H-17), 6.53 (1H, s, 20-OH), 7.26 (1H, s, H-14), 7.50 (1H, d, $J = 9.0$ Hz, H-11), 7.52 (1H, s, H-9), 8.05 (1H, d, $J = 9.0$ Hz, H-12), 8.53 (1H, s, H-7); ^{13}C NMR (300 MHz, DMSO-d_6): δ 8.26, 30.08, 30.79, 46.85, 59.99, 65.58, 65.89, 72.89, 96.58, 107.62, 118.90, 123.28, 129.64, 130.43, 130.72, 131.33, 144.48, 146.13, 150.51, 150.84, 155.73, 157.49, 173.02; MS (ESI) *m/z* 423.4 $(MH)^+$; HRMS (ESI): *m/z* calcd for $C_{23}H_{22}N_2O_6$+H 423.1510, found 423.1510.

4.2. Hydrolytic analysis of lactone

One hundred microliters of 1.0 mM test compound in DMSO was added to 1 mL phosphate-buffered solution (PBS) (pH 7.4). The mixture was incubated at 25 ± 1 °C, and 100 μL aliquots were taken at 2, 4, 6, 8, 24, and 48 h. For the analysis, 5 μL of the sample was injected into a Waters Symmetry-C_{18} reversed-phase column, 250 × 4.6 mm, 10 μm particle size proceeded by a matching C_{18} guard column. The mobile phase consisted of 35% acetonitrile and 65% H_2O. The flow rate was maintained at 1 mL/min. The percent of lactone was determined by the ratio of lactone levels measured at different time points to the lactone level measured at the starting time point ($t = 0$ h).

4.3. Topo I-mediated DNA cleavage reactions

The cleavable complex formation assay was performed as previously described with some modification.[12] All reactions were carried out in reaction buffer containing 50 mM Tris–HCl at pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, and 0.1 mM Spermidine. Each reaction mixture (20 μL total volume) contained 0.5 μg supercoiled plasmid DNA (pBR332), 10 U topo I, and 2 μL 50 μM test compound. The reaction mixture was incubated at 37 °C for 1 h and terminated by the addition of stop buffer/loading dye (2.5% SDS, 15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol) 3 μL. The samples were added to 1% agarose gels and run on electrophoresis for 1.5 h at 125 V. The buffer and gels were stained with EB (ethidium bromide, 0.5 μg/ml). The result was afforded by ImageMaster VDS-CL apparatus and then analyzed with Image Qunat TL analysis system.

4.4. In vitro cytotoxicity

The cytotoxicity was determined by the MTT-microtiter plate tetrazolinium cytotoxicity assay. The human breast cancer cells MCF-7, colonic cancer cell HCT-8, and hepatocellular cancer cell Bel7402 were provided by Academy of Medicinal Sciences. Exponentially growing tumor cells at a density of 1×10^4 cells/mL were incubated in a 96-well microtiter plate for 24 h (37 °C, 5% CO_2). For determination of IC_{50}, cells were exposed continuously for 5 days to various concentrations of test drug (10, 1, 0.1, and 0.01 nmol/mL). The media then were removed (when necessary, acentric method was used.) and replaced by media with MTT 100 μL/well (concentration 0.04%). Following further 4 h of incubation, the media also were removed (when necessary, acentric method was used). The residue in each well was dissolved with DMSO 150 μL. After mixing equally, the absorbance was quantitated at 540 nm. Wells containing no drugs were used as blanks for the spectrophotometer. The survival of the cells was expressed as percentage of untreated control wells. The IC_{50} values were defined as the concentration of compounds that produced a 50% reduction of surviving cells and calculated using Logit-method. The whole assay was repeated thrice.

4.5. HCT-8 xenograft model

HCT-8 was xenografted sc into BALB/c nu/nu mice and maintained by serial sc transplantation of 2–3 mm^3 fragments 3–4 pieces into the right subaxillary region of nude mice. Mice bearing the tumor xenograft of HCT-8 were randomized into treated and control groups, with 8 mice being used. Treatment was initiated at approximately 3 weeks after transplantation, when each tumor reached a volume of 100–200 mm^3. Compounds **13**, **23**, **24** and topotecan were first dissolved in Tween 80 and then suspended in 0.9% NaCl solution, these drugs were given ip on a q4d × 4 schedule at a dose of 1.3 mg/kg, 6 mg/kg, and 4 mg/kg per injection, respectively (previously determined as the MTD and the experiment experiences). Tumors were measured with Vernier calipers, and tumor volume (V) was calculated weekly using the equation $V = 1/2 \times a \times b^2$, where a and b represent the length and width (in millimeter). On day 28 after treatment, the mean tumor weight (MTW), relative tumor volume (RTG), Tumor weight (TW) and inhibition rate (TIR) were determined. RTG was calculated by dividing the tumor volume on day 28 (MTW28) by that on day 0 (MTW0). TIR was obtained from the equation (1-TW in treated/TW in control) × 100%. Student's t-test was used to compare tumor volumes of treated mice. P values < 0.05 were

considered significant, P values < 0.01 were considered very significant.

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