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Original article

Synthesis and biological evaluation of novel 7-acyl homocamptothecins as Topoisomerase I inhibitors

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ABSTRACT

A series of novel 7-acyl derivatives of homocamptothecin (hCPT) were designed and synthesized with the purpose to improve antitumor activity of hCPT, via Minisci free–radical reaction from 10-methoxyhomocamptothecin. All the compounds were evaluated for *in vitro* cytotoxicity against three cancer cell lines (A549, MDA-MB-435 and HCT116). For MDA-MB-435 cell line, compounds, **6a**, **6b**, **6k** and all of 7-alkylcabonyl homocamptothecin derivatives showed higher *in vitro* inhibitory activities than topotecan (TPT). Furthermore, compounds **6d**, **6e**, and **6k** showed highly potent inhibitory activities with the IC_{50} values from less than 1 nM to 2.2 nM. In Topoisomerase I (Topo I)-induced DNA cleavage assay, compounds **6a**, **6d**, and **6k**, as compared to CPT, revealed higher Topo I inhibitory activity.

1. Introduction

20(S)-Camptothecin (CPT) [1], a naturally occurring quinoline alkaloid, was first isolated from the leaves of the *Camptotheca acuminata* tree by Wall and Wani in 1966. The molecular target of CPT is DNA-Topo I, a nuclear enzyme which is required for topological manipulation of DNA during cellular events such as replication, transcription and repair. CPT has remarkable antitumoral and antileukemic activity, and thus became one of the prominent lead compounds in anticancer drug development. Two of its water-soluble derivatives, topotecan (Hycamtin) [2] and irinotecan (Camptosar) [3,4] have been approved by the Food and Drug Administration (FDA) to treat ovarian cancers and small-cell lung cancers (SCLC), respectively, and several other analogs are currently under clinical development at various stages [5]. Although CPT analogs 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 [6]. Considering that it is the OH group adjacent to the cyclic carboxylic ester function which weakens the intrinsic stability of the lactone ring, a novel family of CPT analogs in which a methylene spacer was inserted between the carboxylic and alcoholic functions of the E-ring was designed and synthesized by Lavergne et al in 1997 [7]. This new family of very active seven-member hydroxylactone derivatives represents a new promising class of Topo I inhibitors. Among these hCPT derivatives, 9,10-difluoro analog (diflomotecan) is one of the most potent Topo I inhibitors and was the first hCPT to be selected for clinical trials (Fig. 1) [8].

Most structural modification of CPT have focused on quinoline (A/B) ring [9] and achieved many potent antitumor agents of 7-substituted, 9-substituted and 10-substituted CPT derivatives. From the structure–activity relationship (SAR) of CPT [10], it appears that substituent on position 7 is very important to the activity of the CPT analogs. The previous studies have also demonstrated that there is a wide space for substitutions on position 7 of CPT without steric clash [11]. A number of analogs with substituents on position 7 show enhanced biological profiles [12].

In our laboratory, a series of analogs with substituent on position 7 of hCPT derivatives have been synthesized and many compounds showed superior activity comparable to the marketed CPTs [13,14].

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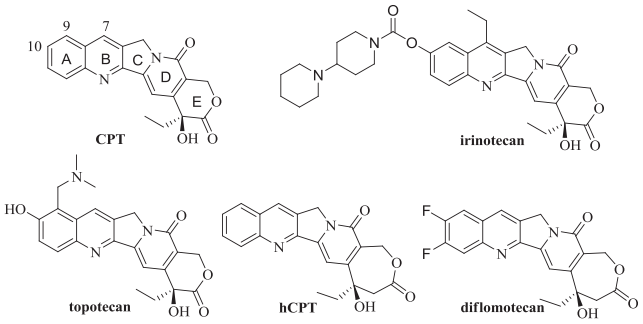


Fig. 1. Camptothecin, homocamptothecin and representative analogs.

Furthermore, there are many CPT or hCPT derivatives with electron-donating groups on position 7 such as silatecan and BN80927 (Fig. 2), which have remarkable antitumor activity [15,16]. These results indicate that CPT or hCPT with a proper electron-donating substituent on position 7 may have therapeutic advantages [17]. However, Li and co-workers reported that 7-cyclopropylcabonyl-camptothecin exhibited higher antitumor activities than TPT [18], which reveals that a proper electron-withdrawing substituent on position 7 might also achieve high activities. Based on the ternary complex of DNA-Topo I-CPT [19], we presented a presumed binding mode of this class compounds with DNA-Topo I complex. In this model, we found a large space around the C-7 position of hCPT that allowed the introduction of substituted acyl groups, and two key hydrogen bonds were reserved (Fig. 3). Therefore, we recently synthesized a series of racemic 7-acyl homocamptothecin analogs, hoping that these derivatives might have remarkable antitumor activity.

2. Chemistry

Detailed synthetic strategy to 7-substituted homocamptothecin derivatives **6a**–**6t** is illustrated in Scheme 1. According to published procedures [20], 2-amino-5-methoxybenzaldehyde **3** was prepared from 5-hydroxy-2-nitro-benzaldehyde **1** by methylation and hydrogenation. The key intermediate CDE ring **4** was synthesized according to the previously reported method [13], and the synthetic process was optimized in our present studies [21]. Reaction of 2-amino-5-methoxybenzaldehyde **3** with intermediate **4** in the presence of *p*-methylbenzenesulfonic acid (P-TSA) yielded the 10-methoxyhomocamptothecin **5**. Treatment of compound **5** and commercially available aldehyde via Minisci free–radical reaction led to the desired 7-substituted homocamptothecin **6a**–**6t** with the yield of 22%–36%. In Minisci free–radical reaction, there is a little different between 7-cycloalkyl and 7-acyl derivatives, which use hydrogen peroxide and tert-butyl hydroperoxide as oxidant, respectively.

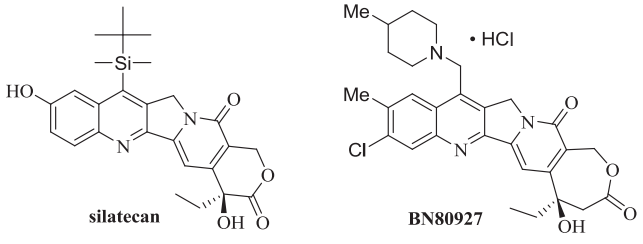


Fig. 2. Structures of silatecan and BN80927.

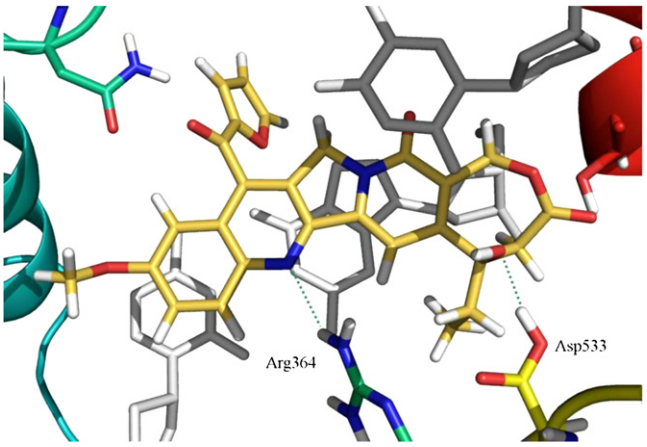
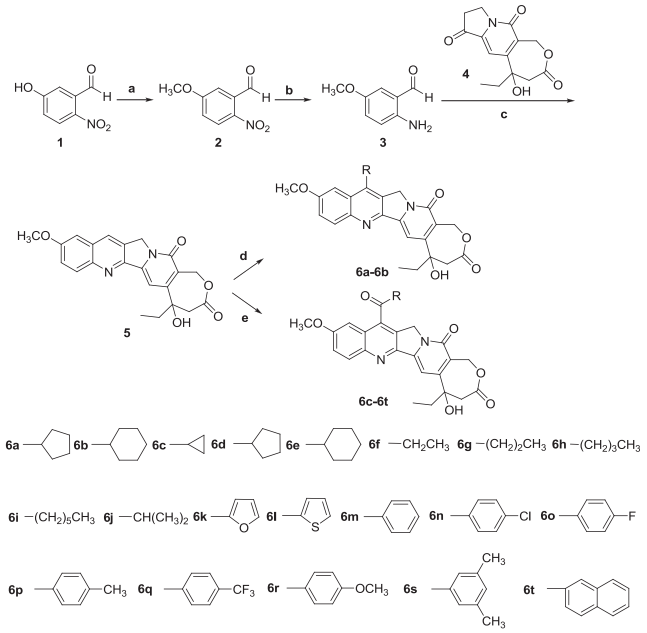


Fig. 3. Hypothetical model for the binding of 7-C=O linked homocamptothecin (**6k**) with DNA-Topo I complex. The figure was prepared from PDB entry: 1T8I, Using PyMol (http://pymol.souceforge,net/).

3. Results and discussion

3.1. In vitro antitumor activities

The antitumor activities of these synthetic compounds **6a**–**6t** were tested on human lung cancer (A549), breast cancer (MDA-MB-435) and colon cancer (HCT116) cell lines. To obtain more meaningful comparisons of relative potencies, TPT was tested as a positive control. From the data reported in Table 1, it appears that the alkyl and alkylcarbonyl groups on position **7** could promote the antitumor activity of hCPTs, however, the aroyl groups on position 7 did not get the same results, which indicates that it is not favorable to introduce the aroyl groups on position 7. It may be due to that aromatic groups conjugating with carbonyl group disturb the electronic factor in ring B more obviously than alkyl groups. The results also revealed that



Scheme 1. Reagents and conditions: (a) CH_3I, CH_3CN, reflux, 92%; (b) H_2, Pd–C, ethyl acetate, 30 °C, 6 h, 70%; (c) P-TSA, toluene, reflux, 6 h, 51%; (d) aldehyde derivatives, H_2O_2, AcOH–H_2O, H_2SO_4, $FeSO_4 \cdot 7H_2O$, 0–5 °C, 3 h, 22–30%; and (e) aldehyde derivatives, *t*-BuOOH, AcOH-H_2O, H_2SO_4, $FeSO_4 \cdot 7H_2O$, 0–5 °C, 3 h, 22–36%.

Table 1
In vitro antitumor activity of 7-substituted-10-methoxyhomocamptothecin.

Compounds	IC_{50}[a] (μM)		
	A549	MDA-MB-435	HCT116
6a	0.730	0.0415	0.0714
6b	0.657	0.0761	0.118
6c	2.48	0.0482	0.00593
6d	0.430	<0.001	0.00217
6e	0.669	<0.001	0.446
6f	8.96	0.0633	0.0560
6g	14.1	0.198	0.268
6h	7.03	0.198	0.277
6i	0.478	0.133	0.317
6j	2.27	0.0216	0.404
6k	<0.001	0.00218	0.294
6l	0.607	1.61	2.19
6m	1.77	2.21	2.94
6n	0.665	2.09	3.02
6o	7.79	15.9	13.1
6p	3.39	7.17	8.17
6q	0.625	1.48	1.91
6r	9.25	16.3	2.07
6s	48.0	>100	26.3
6t	>100	>100	22.8
Topotecan	3.16	0.448	1.10

[a] Values were measured with MTT method.

these compounds were more sensitive against MDA-MB-435 cell line than against A549 and HCT116 cell lines. For MDA-MB-435 cell line, **6a**, **6b**, **6k** and all of 7-alkylcabonyl homocamptothecin derivatives showed higher *in vitro* inhibitory activities than TPT. Especially, compounds **6d**, **6e**, and **6k** showed very high inhibitory activities with the IC_{50} values from less than 1 nM to 2.2 nM. According to HCT116 cell line, eleven compounds showed higher inhibitory activities than TPT. Among them, the antitumor activities of compounds **6c** and **6d** reached nM level. The IC_{50} values were 5.93 and 2.17 nM respectively. With regard to A549 cell line, thirteen compounds showed good inhibitory activities comparable to or higher than TPT. The IC_{50} of **6k** was lower than 1 nM. In particular, the activity of the most promising compounds, **6d** and **6k**, showed broad *in vitro* antitumor spectrum and are more potent than TPT. Further pharmacological and toxicological evaluation of these promising compounds is in progress. The further SAR analysis of the synthesized compounds revealed that the length of alkyl chain was important to *in vitro* cytotoxic activity. For example, with regard to HCT116 cancer cell, the antitumor activity of compound, **6f**, **6g**, **6h**, and **6i**, showed a decreasing tendency with the increase of the alkyl chain length. For the compounds with aromatic groups from **6m** to **6r**, the SAR analysis showed that compounds with electron-withdrawing groups at position 4 of phenyl ring exhibited a distinct tendency to increase *in vitro* antitumor activity, while compound **6o** with fluorine substitution revealed relatively low cytotoxic activity. To our surprise, compounds **6s** and **6t** showed weak antiproliferative activity, which may be accounted for by the changed molecule-binding model to the targeted enzyme due to their relatively bigger substituents.

3.2. Topoisomerase I mediated DNA cleavage

The ability of selected hCPT derivatives **6a**, **6d**, **6i**, **6k**, **6l** and **6q** to inhibit Topo I was investigated in the cleavable complex assay. The results presented in Fig. 4 indicated that all compounds as cytotoxic agents were also very potent as Topo I poisons, and compounds **6a**, **6d**, and **6k** were more potent as Topo I poisons than CPT, which was consistent with *in vitro* antitumor activity. Furthermore, the increasing concentrations of **CPT**, **6d**, or **6k** are

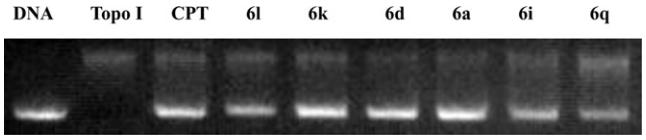


Fig. 4. Effect of the selected compounds on Topoisomerase I mediated DNA relaxation in single concentration. Topo I was Topoisomerase I and DNA without drugs. The samples were reacted with 10 μM drugs at 37 °C for 15 min. Reactions were then stopped by adding 0.5% SDS. Gels were photographed under UV transilluminator.

accompanied by a dose-dependent increase in the level of cleavable complexes. Importantly, **6d** and **6k** showed lower intensity of the migrating band (corresponding to nicked DNA) than **CPT** regardless of low concentration (1 μM) or high concentration (50 μM). The relative potency between the three compounds, as indicated by the remaining amount of intact DNA, is **CPT**, **6d**, and **6k** in increasing order (Fig. 5).

4. Conclusion

A series of novel 7-acyl and 7-cycloalkyl derivatives of hCPT have been obtained based on our synthetic route. Based on the results (discussed above), all compounds indicated potent growth inhibitory effect and most compounds showed more effective antitumor activity than TPT. Several compounds, such as **6a**, **6d**, **6i**, **6k**, **6l** and **6q**, were also very potent as Topo I poisons, and compound **6k** was found to exhibit more reasonably strong Topo I-dependent cytotoxic activity than CPT. In conclusion, this study provides evidence that a proper electron-withdrawing substituent on position 7 of hCPT skeleton might also improve antitumor activity of hCPT. A better understanding of this feature could provide meaningful insights for the development of more effective Topoisomerase I inhibitors.

5. Experimental protocols

5.1. General

All reagents and solvents were reagent grade or were purified by standard methods before use. The melting points were determined using an electrothermal apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz with a Bruker instrument, and reported with TMS as internal standard and DMSO-d_6 as solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. 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 analytical pure and dried through routine protocols.

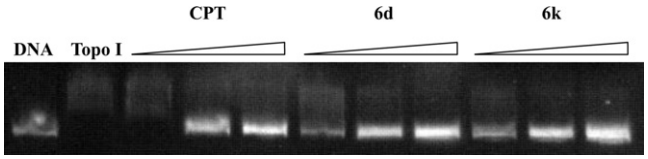


Fig. 5. Effect of compounds CPT, **6d**, and **6k** on Topoisomerase I mediated DNA relaxation in concentration gradient. Topo I was Topoisomerase I and DNA without drugs; the samples were reacted with 1, 10, and 50 μM drugs at 37 °C for 15 min. Reactions were then stopped by adding 0.5% SDS. Gels were photographed under UV transilluminator.

5.2. 10-Methoxyhomocamptothecin (**5**)

A solution of the tricyclic ketone **4** (6.1 g, 21.9 mmol) and 2-Amino-5-methoxybenzaldehyde 3 (5.3 g, 35.1 mmol) in toluene was refluxed using a Dean–Stark trap for 30 min. P-TSA (1.3 g, 6.6 mmol) was then added and refluxing was continued for an additional 3 h. The solvent was removed and the crude product was washed by acetone (100 mL) and methanol (100 mL) to give **5** as yellow solid (4.38 g, 51%), mp >250 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.5 Hz), 3.04–3.48 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.95 (s, 3H, $-OCH_3$), 5.25 (s, 2H, 5-CH_2) 5.37–5.54 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.00 (s, 1H, 20-OH), 7.35 (s, 1H, 14H), 7.50 (d, 1H, 9H, J = 2.8 Hz), 7.51 (d, 1H, 11H, J = 8.6 Hz), 8.05 (d, 1H, 12H, J = 10.7 Hz), 8.54 (s, 1H, 7H). ESI-MS: m/z, 391.40 [M − H]$^-$. Anal. calcd. for $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.14; N, 7.14. Found: C, 67.22; H, 5.15; N, 7.14.

5.3. General procedure for the synthesis of 7-cycloalkyl homocamptothecin derivatives (**6a**–**6b**)

To a suspension of 10-methoxyhomocamptothecin (100 mg, 0.25 mmol) and $FeSO_4 \cdot 7H_2O$ (142 mg, 0.5 mmol) in 50% aqueous acetic acid (9 mL) was added dropwise 0.9 mL of concentrated H_2SO_4 and different aldehyde (1.3 mmol). After cooling at 0 °C, 30% H_2O_2 (108 mg, 3.2 mmol) were added, and the mixture was stirred overnight at room temperature. Dilution with water, filtration of the precipitate, extraction with dichloromethane, and chromatography of the extract (CH_2Cl_2/MeOH 98/2) gave the product.

5.3.1. 7-Cyclopentyl-10-methoxyhomocamptothecin (**6a**)

The general synthetic method described above afforded **6a** as yellow solid (28%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.85 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.83–2.24 (m, 10H), 3.03–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.92 (t, 1H, $-CH(CH_2)_4$, J = 9.1 Hz), 3.97 (s, 3H, $-OCH_3$), 5.36 (s, 2H, 5-CH_2), 5.37–5.54 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.01 (s, 1H, 20-OH), 7.33 (s, 1H, 14H), 7.51 (dd, 1H, 11H, J_o = 2.6 Hz, J_m = 9.2 Hz), 7.55 (d, 1H, 9H, J = 2.4 Hz), 8.07 (d, 1H, 12H, J = 9.2 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.66, 26.70, 31.77, 36.79, 40.89, 42.84, 50.90, 56.10, 61,72, 73.59, 99.00, 103.69, 122.08, 122.37, 128.01, 128.48, 131.96, 144.93, 145.35, 145.92, 150.40, 156.23, 158.26, 159.37, 172.31. ESI-MS: m/z, 459.66 [M − H]$^-$. Anal. calcd. for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.30; H, 6.14; N, 6.09.

5.3.2. 7-Cyclohexyl-10-methoxyhomocamptothecin (**6b**)

The general synthetic method described above afforded **6b** as yellow solid (30%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.85 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.61–1.94 (m, 12H), 3.03–3.49 (q, 2H, 21-CH_2, J = 13.7 Hz), 3.99 (s, 3H, $-OCH_3$), 5.38–5.54 (q, 2H, 17-CH_2, J = 15.1 Hz), 5.41 (s, 2H, 5-CH_2), 6.01 (s, 1H, 20-OH), 7.33 (s, 1H, 14H), 7.54 (dd, 1H, 11H, J_o = 2.6 Hz, J_m = 9.2 Hz), 7.58 (d, 1H, 9H, J = 1.2 Hz), 8.07 (d, 1H, 12H, J = 9.2 Hz). ESI-MS: m/z, 473.37 [M − H]$^-$. Anal. calcd. for $C_{28}H_{30}N_2O_5$: C, 70.87; H, 6.37; N, 5.90. Found: C, 70.99; H, 6.36; N, 5.88.

5.4. General procedure for the synthesis of 7-acyl homocamptothecin derivatives (**6c**–**6t**)

To a suspension of 10-methoxyhomocamptothecin (100 mg, 0.25 mmol) and $FeSO_4 \cdot 7H_2O$(142 mg, 0.5 mmol) in 50% aqueous acetic acid (9 mL) was added dropwise 0.9 mL of concentrated H_2SO_4 and different aldehyde (1.3 mmol). After cooling at 0 °C, 80% t-BuOOH (57 mg, 0.5 mmol) were added, and the mixture was stirred overnight at room temperature. Dilution with water, filtration of the precipitate, extraction with dichloromethane, and chromatography of the extract (CH_2Cl_2/MeOH 98/2) gave the product.

5.4.1. 7-Cyclopropylcabonyl-10-methoxyhomocamptothecin (**6c**)

The general synthetic method described above afforded **6c** as yellow solid (30%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.33–1.46 (m, 4H), 1.86 (q, 2H, 19-CH_2, J = 7.3 Hz), 2.75 (t, 1H, $-CH(CH_2)_2$, J = 4.2 Hz), 3.05–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.94 (s, 3H, $-OCH_3$), 5.33 (s, 2H, 5-CH_2), 5.38–5.54 (q, 2H, 17-CH_2, J = 15.3 Hz), 6.03 (s, 1H, 20-OH), 7.38 (s, 1H, 14H), 7.48 (d, 1H, 9H, J = 2.7 Hz), 7.61 (dd, 1H, 11H, J_o = 2.6 Hz, J_m = 9.3 Hz), 8.17 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 459.35 [M − H]$^-$. Anal. calcd. for $C_{26}H_{24}N_2O_6$: C, 67.82; H, 5.25; N, 6.08. Found: C, 67.89; H, 5.24; N, 6.09.

5.4.2. 7-Cyclopentylcabonyl-10-methoxyhomocamptothecin (**6d**)

The general synthetic method described above afforded **6d** as yellow solid (35%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.63–1.93 (m, 10H), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.74 (t, 1H, $-CH(CH_2)_4$, J = 3.9 Hz), 3.92 (s, 3H, $-OCH_3$), 5.28 (s, 2H, 5-CH_2), 5.37–5.53 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.15 (d, 1H, 9H, J = 2.7 Hz), 7.37 (s, 1H, 14H), 7.60 (dd, 1H, 11H, J_o = 2.7 Hz, J_m = 9.3 Hz), 8.15 (d, 1H, 12H, J = 9.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.67, 26.07, 29.14, 36.77, 42.83, 50.57, 51.73, 56.18, 61,66, 73.59, 99.53, 103.71, 122.72, 123.48, 125.23, 126.80, 131.74, 140.21, 144.17, 144.60, 145.06, 150.88, 156.23, 159.34, 172.26, 207.60. ESI-MS: m/z, 487.44 [M − H]$^-$. Anal. calcd. for $C_{28}H_{28}N_2O_6$: C, 68.84; H, 5.78; N, 5.73. Found: C, 68.96; H, 5.77; N, 5.71.

5.4.3. 7-Cyclohexylcabonyl-10-methoxyhomocamptothecin (**6e**)

The general synthetic method described above afforded **6e** as yellow solid (36%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, 3H, 18-CH_3, J = 7.5 Hz), 1.24–1.95 (m, 12H), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.20 (t, 1H, $-CH(CH_2)_5$, J = 3.9 Hz), 3.92 (s, 3H, $-OCH_3$), 5.26 (s, 2H, 5-CH_2), 5.37–5.53 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.02 (s, 1H, 20-OH), 7.10 (d, 1H, 9H, J = 2.7 Hz), 7.37 (s, 1H, 14H), 7.59 (dd, 1H, 11H, J_o = 2.7 Hz, J_m = 9.3 Hz), 8.15 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 501.39 [M − H]$^-$. Anal. calcd. for $C_{29}H_{30}N_2O_6$: C, 69.31; H, 6.02; N, 5.57. Found: C, 69.22; H, 6.01; N, 5.58.

5.4.4. 7-Propionyl-10-methoxyhomocamptothecin (**6f**)

The general synthetic method described above afforded **6f** as yellow solid (36%), mp > 200 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.23 (t, 3H, $-CH_2CH_3$, J = 7.0 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.7 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.7 Hz), 3.16 (q, 2H, $-CH_2CH_3$, J = 7.1 Hz), 3.94 (s, 3H, $-OCH_3$), 5.32 (s, 2H, 5-CH_2), 5.38–5.54 (q, 2H, 17-CH_2, J = 15.3 Hz), 6.02 (s, 1H, 20-OH), 7.30 (d, 1H, 9H, J = 2.6 Hz), 7.37 (s, 1H, 14H), 7.60 (dd, 1H, 11H, J_o = 2.4 Hz, J_m = 9.2 Hz), 8.15 (d, 1H, 12H, J = 9.1 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.21, 8.65, 36.74, 37.06, 42.81, 50.70, 56.22, 61.67, 73.58, 99.50, 103.94, 122.69, 123.46, 125.03, 127.01, 131.63, 139.68, 144.55, 145.13, 150.74, 156.24, 159.32, 159.37, 172.26, 205.53. ESI-MS: m/z, 447.38 [M − H]$^-$. Anal. calcd. for $C_{25}H_{24}N_2O_6$: C, 66.95; H, 5.39; N, 6.25. Found: C, 66.83; H, 5.40; N, 6.27.

5.4.5. 7-Butyryl-10-methoxyhomocamptothecin (**6g**)

The general synthetic method described above afforded **6g** as yellow solid (26%), mp > 200 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.01 (t, 3H, $-CH_3$, J = 7.4 Hz), 1.79 (q, 2H, $-CH_2CH_3$, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.5 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.14 (t, 2H, $-CH_2CH_2CH_3$, J = 7.1 Hz), 3.93 (s, 3H, $-OCH_3$), 5.31 (s, 2H, 5-CH_2), 5.38–5.54 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.27 (d, 1H, 9H, J = 2.7 Hz), 7.37 (s, 1H, 14H), 7.59 (dd, 1H, 11H, J_o = 2.7 Hz, J_m = 9.3 Hz), 8.15 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 461.48 [M − H]$^-$. Anal. calcd. for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.63; H, 5.68; N, 6.03.

*5.4.6. 7-Pentanoyl-10-methoxyhomocamptothecin (**6h**)*

The general synthetic method described above afforded **6h** as yellow solid (30%), mp > 200 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, 3H, 18-CH_3, J = 7.3 Hz), 0.93 (t, 3H, −CH_3, J = 7.3 Hz), 1.42 (q, 2H, −CH_2CH_3, J = 7.5 Hz), 1.74 (t, 2H, −$CH_2CH_2CH_3$, J = 7.4 Hz), 1.84 (q, 2H, 19-CH_2, J = 7.4 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.15 (t, 2H, −$CH_2CH_2CH_2CH_3$, J = 7.2 Hz), 3.93 (s, 3H, −OCH_3), 5.31 (s, 2H, 5-CH_2), 5.37–5.53 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.28 (d, 1H, 9H, J = 2.6 Hz), 7.37 (s, 1H, 14H), 7.60 (dd, 1H, 11H, J_o = 2.6 Hz, J_m = 9.2 Hz), 8.15 (d, 1H, 12H, J = 9.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.64, 14.29, 22.22, 25.84, 36.74, 42.81, 43.34, 50.61, 56.19, 61.66, 73.57, 99.51, 103.78, 122.69, 123.45, 124.95, 126.91, 131.67, 139.75, 144.21, 144.54, 145.13, 150.78, 156.23, 159.31, 172.25, 205.17. ESI-MS: m/z, 475.50 [M − H]$^-$. Anal. calcd. for $C_{27}H_{28}N_2O_6$: C, 68.05; H, 5.92; N, 5.88. Found: C, 68.17; H, 5.92; N, 5.90.

*5.4.7. 7-Heptanoyl-10-methoxyhomocamptothecin (**6i**)*

The general synthetic method described above afforded **6i** as yellow solid (30%), mp > 200 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (m, 6H, 18-CH_3, −CH_3), 1.25 (q, 2H, −CH_2CH_3, J = 5.6 Hz), 1.30 (t, 2H, −$CH_2CH_2CH_3$, J = 6.0 Hz), 1.39 (t, 2H, −$CH_2CH_2CH_2CH_3$, J = 6.5 Hz), 1.76 (t, 2H, −$CH_2CH_2CH_2CH_2CH_3$, J = 7.4 Hz), 1.85 (q, 2H, 19-CH_2, J = 7.3 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.7 Hz), 3.14 (t, 2H, −$CH_2CH_2CH_2CH_2CH_2CH_3$, J = 7.2 Hz), 3.93 (s, 3H, −OCH_3), 5.31 (s, 2H, 5-CH_2), 5.37–5.53 (q, 2H, 17-CH_2, J = 15.0 Hz), 6.03 (s, 1H, 20-OH), 7.28 (d, 1H, 9H, J = 2.6 Hz), 7.37 (s, 1H, 14H), 7.60 (dd, 1H, 11H, J_o = 2.6 Hz, J_m = 9.4 Hz), 8.15 (d, 1H, 12H, J = 9.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.65, 14.33, 22.43, 23.68, 28.71, 31.56, 36.75, 42.81, 43.60, 50.62, 56.18, 61.66, 73.58, 99.51, 103.78, 122.70, 123.48, 124.96, 126.92, 131.68, 139.77, 144.22, 144.55, 145.15, 150.80, 156.23, 159.32, 172.25, 205.20. ESI-MS: m/z, 503.55 [M − H]$^-$. Anal. calcd. for $C_{29}H_{32}N_2O_6$: C, 69.03; H, 6.39; N, 5.55. Found: C, 68.95; H, 6.38; N, 5.58.

*5.4.8. 7-Isobutyryl-10-methoxyhomocamptothecin (**6j**)*

The general synthetic method described above afforded **6j** as yellow solid (22%), mp > 200 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.21 (d, 6H, −$CH(CH_3)_2$, J = 6.9 Hz), 1.85 (q, 2H, 19-CH_2, J = 7.4 Hz), 3.05–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.46–3.48 (m, 1H, −$CH(CH_3)_2$), 3.93 (s, 3H, −OCH_3), 5.26 (s, 2H, 5-CH_2), 5.37–5.53 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.04 (s, 1H, 20-OH), 7.10 (d, 1H, 9H, J = 2.7 Hz), 7.37 (s, 1H, 14H), 7.59 (dd, 1H, 11H, J_o = 2.7 Hz, J_m = 9.2 Hz), 8.14 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 461.54 [M − H]$^-$. Anal. calcd. for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.63; H, 5.66; N, 6.04.

*5.4.9. 7-(2-Furoyl)-10-methoxyhomocamptothecin (**6k**)*

The general synthetic method described above afforded **6k** as yellow solid (31%), mp > 260 °C (dec). 1H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.6 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.83 (s, 3H, −OCH_3), 5.09 (d, 2H, 5-CH_2, J = 4.1 Hz), 5.35–5.49 (q, 2H, 17-CH_2, J = 15.0 Hz), 6.03 (s, 1H, 20-OH), 6.85 (d, 1H, 4′-H, J = 2.0 Hz), 7.12 (d, 1H, 9H, J = 2.6 Hz), 7.40 (s, 1H, 14H), 7.55 (d, 1H, 3′-H, J = 3.6 Hz), 7.60 (dd, 1H, 11H, J_o = 2.4 Hz, J_m = 9.0 Hz), 8.19 (d, 1H, 12H, J = 9.3 Hz), 8.26 (s, 1H, 5′-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.68, 36.78, 42.80, 50.46, 56.15, 61.62, 73.60, 99.60, 103.62, 114.16, 122.72, 123.70, 124.84, 126.12, 128.07, 131.66, 137.13, 144.65, 145.05, 150.57, 150.90, 151.24, 151.36, 156.29, 159.29, 172.26, 180.40. ESI-MS: m/z, 485.41 [M − H]$^-$. Anal. calcd. for $C_{27}H_{22}N_2O_7$: C, 66.66; H, 4.56; N, 5.76. Found: C, 66.57; H, 4.57; N, 5.77.

*5.4.10. 7-(2-Thenoyl)-10-methoxyhomocamptothecin (**6l**)*

The general synthetic method described above afforded **6l** as yellow solid (30%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.3 Hz), 3.04–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.82 (s, 3H, −OCH_3), 5.04 (d, 2H, 5-CH_2, J = 5.2 Hz), 5.34–5.50 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.11 (d, 1H, 9H, J = 2.8 Hz), 7.27 (t, 1H, 4′-H, J = 4.4 Hz), 7.41 (s, 1H, 14H), 7.61 (dd, 1H, 11H, J_o = 2.8 Hz, J_m = 9.3 Hz), 7.73 (d, 1H, 3′-H, J = 3.7 Hz), 8.20 (d, 1H, 12H, J = 9.3 Hz), 8.35 (d, 1H, 5′-H, J = 4.9 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.67, 36.76, 42.81, 50.47, 56.11, 61.64, 73.59, 99.64, 103.71, 122.69, 123.57, 125.93, 127.63, 129.72, 130.04, 131.66, 137.80, 138.66, 139.29, 142.54, 144.68, 145.02, 150.90, 156.32, 159.27, 172.26, 186.33. ESI-MS: m/z, 501.54 [M − H]$^-$. Anal. calcd. for $C_{27}H_{22}N_2O_6S$: C, 64.53; H, 4.41; N, 5.57. Found: C, 64.42; H, 4.42; N, 5.58.

*5.4.11. 7-Benzoyl-10-methoxyhomocamptothecin (**6m**)*

The general synthetic method described above afforded **6m** as yellow solid (36%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.4 Hz), 3.03–3.48 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.75 (s, 3H, −OCH_3), 4.93 (d, 2H, 5-CH_2, J = 8.2 Hz), 5.33–5.48 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.03 (d, 1H, 9H, J = 2.7 Hz), 7.41 (s, 1H, 14H), 7.59–7.64 (m, 3H, 11, 3′-H, 5′-H), 7.80 (t, 1H, 4′-H, J = 7.4 Hz), 7.91 (d, 2H, 2′-H, 6′-H, J = 8.3 Hz), 8.20 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 495.33 [M − H]$^-$. Anal. calcd. For $C_{29}H_{24}N_2O_6$: C, 70.15; H, 4.87; N, 5.64. Found: C, 70.29; H, 4.86; N, 5.63.

*5.4.12. 7-(4-Chlorbenzoyl)-10-methoxyhomocamptothecin (**6n**)*

The general synthetic method described above afforded **6n** as yellow solid (36%), mp > 300 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.1 Hz), 3.03–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.76 (s, 3H, −OCH_3), 4.95 (d, 2H, 5-CH_2, J = 8.2 Hz), 5.33–5.49 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.03 (s, 1H, 9H), 7.41 (s, 1H, 14H), 7.60 (dd, 1H, 11H, J_o = 2.1 Hz, J_m = 9.0 Hz), 7.67 (d, 2H, 3′-H, 5′-H, J = 8.4 Hz), 7.92 (d, 2H, 2′-H, 6′-H, J = 8.5 Hz), 8.20 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 529.40 [M − H]$^-$. Anal. calcd. for $C_{29}H_{23}ClN_2O_6$: C, 65.60; H, 4.37; N, 5.28. Found: C, 65.78; H, 4.36; N, 5.27.

*5.4.13. 7-(4-Fluorobenzoyl)-10-methoxyhomocamptothecin (**6o**)*

The general synthetic method described above afforded **6o** as yellow solid (35%), mp > 300 °C (dec). 1H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.2 Hz), 3.03–3.49 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.76 (s, 3H, −OCH_3), 4.95 (d, 2H, 5-CH_2, J = 8.3 Hz), 5.33–5.49 (q, 2H, 17-CH_2, J = 15.0 Hz), 6.03 (s, 1H, 20-OH), 7.01 (d, 1H, 9H, J = 2.5 Hz), 7.41 (s, 1H, 14H), 7.42 (d, 2H, 3′-H, 5′-H, J = 8.8 Hz), 7.60 (dd, 1H, 11H, J_o = 2.5 Hz, J_m = 9.1 Hz), 8.00 (dd, 2H, 2′-H, 6′-H, J_o = 5.5 Hz, J_m = 8.6 Hz), 8.20 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 513.32 [M − H]$^-$. Anal. calcd. for $C_{29}H_{23}FN_2O_6$: C, 67.70; H, 4.51; N, 5.44. Found: C, 67.58; H, 4.51; N, 5.46.

*5.4.14. 7-(4-Toluyl)-10-methoxyhomocamptothecin (**6p**)*

The general synthetic method described above afforded **6p** as yellow solid (36%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.1 Hz), 2.43 (s, 3H, −CH_3), 3.03–3.48 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.76 (s, 3H, −OCH_3), 4.90 (d, 2H, 5-CH_2, J = 6.9 Hz), 5.33–5.48 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.02 (s, 1H, 20-OH), 7.02 (d, 1H, 9H, J = 2.7 Hz), 7.40 (s, 1H, 14H), 7.41 (d, 2H, 3′-H, 5′-H, J = 7.7 Hz), 7.59 (dd, 1H, 11H, J_o = 2.7 Hz, J_m = 9.3 Hz), 7.82 (d, 2H, 2′-H, 6′-H, J = 8.2 Hz), 8.19 (d, 1H, 12H, J = 9.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.64, 21.85, 36.73, 42.79, 50.37, 56.03, 61.61, 73.56, 99.63, 103.92, 122.64, 123.48, 126.23, 127.82, 130.45, 130.51, 131.67, 133.46, 138.19, 144.65, 145.04, 146.46, 150.78, 156.28, 159.17, 159.24, 172.23, 194.25. ESI-MS: m/z, 509.38 [M − H]$^-$. Anal. calcd. for $C_{30}H_{26}N_2O_6$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.61; H, 5.13; N, 5.47.

5.4.15. 7-(4-Triflouromethylbenzoyl)-10-methoxyhomocamptothecin (**6q**)

The general synthetic method described above afforded **6q** as yellow solid (35%), mp > 300 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.2 Hz), 3.03–3.49 (q, 2H, 21-CH_2, J = 13.8 Hz), 3.74 (s, 3H, $-OCH_3$), 4.97 (d, 2H, 5-CH_2, J = 10.3Hz), 5.33–5.48 (q, 2H, 17-CH_2, J = 15.3 Hz), 6.03 (s, 1H, 20-OH), 7.04 (d, 1H, 9H, J = 2.6 Hz), 7.41 (s, 1H, 14H), 7.61 (dd, 1H, 11H, J_o = 2.8 Hz, J_m = 9.2 Hz), 7.96 (d, 2H, 3′-H, 5′-H, J = 8.3 Hz), 8.09 (d, 2H, 2′-H, 6′-H, J = 8.4 Hz), 8.21 (d, 1H, 12H, J = 9.3 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.67, 36.77, 42.81, 50.50, 56.06, 61.60, 73.60, 99.67, 104.15, 122.76, 123.67, 126.05, 126.76, 128.58, 130.54, 131.21, 131.77, 134.14, 134.39, 136.95, 139.22, 144.57, 145.14, 150.95, 156.28, 159.25, 172.23, 194.32. ESI-MS: m/z, 563.30 $[M-H]^-$. Anal. calcd. for $C_{30}H_{23}F_3N_2O_6$: C, 63.83; H, 4.11; N, 4.96. Found: C, 63.92; H, 4.10; N, 4.98.

5.4.16. 7-(4-Methoxylbenzoyl)-10-methoxyhomocamptothecin (**6r**)

The general synthetic method described above afforded **6r** as yellow solid (36%), mp > 300 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, 18-CH_3, J = 7.4 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.4 Hz), 3.03–3.48 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.77 (s, 3H, 10-OCH_3), 3.88 (s, 3H, $-OCH_3$), 4.92 (d, 2H, 5-CH_2, J = 6.5 Hz), 5.33–5.48 (q, 2H, 17-CH_2, J = 15.0 Hz), 6.03 (s, 1H, 20-OH), 7.02 (d, 1H, 9H, J = 2.7 Hz), 7.10 (d, 2H, 3′-H, 5′-H, J = 9.0 Hz), 7.40 (s, 1H, 14H), 7.59 (dd, 1H, 11H, J_o = 2.8 Hz, J_m = 9.3 Hz), 7.89 (d, 2H, 2′-H, 6′-H, J = 8.9 Hz), 8.19 (d, 1H, 12H, J = 9.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.66, 36.75, 42.80, 50.35, 56.05, 56.27, 61.61, 73.58, 99.63, 103.94, 115.21, 122.64, 123.48, 126.30, 126.69, 128.71, 131.68, 132.99, 138.55, 144.73, 145.03, 150.81, 156.28, 159.14, 159.28, 165.13, 172.23, 192.85. ESI-MS: m/z, 525.32 $[M-H]^-$. Anal. calcd. for $C_{30}H_{26}N_2O_7$: C, 68.43; H, 4.98; N, 5.32. Found: C, 68.32; H, 4.97; N, 5.34.

5.4.17. 7-(3,5-Dimethylbenzoyl)-10-methoxyhomocamptothecin (**6s**)

The general synthetic method described above afforded **6s** as yellow solid (36%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.86 (q, 2H, 19-CH_2, J = 7.4 Hz), 2.33 (s, 6H, -CH_3), 3.04–3.47 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.78 (s, 3H, $-OCH_3$), 4.87 (d, 2H, 5-CH_2, J = 5.9 Hz), 5.33–5.48 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.01 (s, 1H, 20-OH), 7.03 (d, 1H, 9H, J = 2.7 Hz), 7.40 (s, 1H, 14H), 7.44 (s, 1H, 4′-H), 7.55 (s, 2H, 2′-H, 6′-H), 7.59 (dd, 1H, 11H, J_o = 2.8 Hz, J_m = 9.3 Hz), 8.18 (d, 1H, 12H, J = 9.3 Hz). ESI-MS: m/z, 523.33 $[M-H]^-$. Anal. calcd. for $C_{31}H_{28}N_2O_6$: C, 70.98; H, 5.38; N, 5.34. Found: C, 71.10; H, 5.37; N, 5.32.

5.4.18. 7-(2-Naphthoyl)-10-methoxyhomocamptothecin (**6t**)

The general synthetic method described above afforded **6t** as yellow solid (32%), mp > 260 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, 18-CH_3, J = 7.3 Hz), 1.87 (q, 2H, 19-CH_2, J = 7.4 Hz), 3.03–3.48 (q, 2H, 21-CH_2, J = 13.9 Hz), 3.74 (s, 3H, $-OCH_3$), 4.94 (d, 2H, 5-CH_2, J = 8.1 Hz), 5.31–5.45 (q, 2H, 17-CH_2, J = 15.1 Hz), 6.03 (s, 1H, 20-OH), 7.10 (d, 1H, 9H, J = 2.6 Hz), 7.44 (s, 1H, 14H), 7.60–7.74 (m, 3H, 11H, 6′-H, 7′-H), 8.02–8.18 (m, 4H, 3′-H, 4′-H, 5′-H, 8′-H), 8.23 (d, 1H, 12H, J = 9.3 Hz), 8.46 (s, 1H, 1′-H). ESI-MS: m/z, 545.38 $[M-H]^-$. Anal. calcd. for $C_{33}H_{26}N_2O_6$: C, 72.52; H, 4.79; N, 5.13. Found: C, 72.62; H, 4.78; N, 5.12.

5.5. Cytotoxicity

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) 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 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.

5.6. DNA Topoisomerase I activity assays

Camptothecin was obtained from the company of Tianzunzezhong in China. Topo I (calf thymus), buffer, BSA, loading buffer and supercoiled DNA pBR322 were all from TaKaRa Biotechnology CO., Ltd.

All reactions were carried out in 20 μL volumes (16 μL double distilled water, 2 μL DNA-Topo I buffer, 2 μL 0.1% BSA) including 0.25 μg supercoiled DNA, 0.5 U Topo I with or without drug. The reactions were incubated at 37 °C for 15 min and then stopped by adding SDS (0.5% final concentration). To the reaction mixtures, 3.5 μL, 6 × loading buffer (0.1 mM EDTA, 7% Glycerol, 0.01% Xylene Cyanol FF, Bromopenol Blue 0.01%) was added. The mixtures were electrophoresed in 0.8% agarose gel in TAE buffer for 40 min at 120 V. The gel was stained with ethidium bromide at room temperature and photographed with UV transilluminator.

5.7. Computational protocols

The crystallographic coordinates of the ternary complex of CPT-DNA-Topo I (3.0 Å resolution, R_{cryst} = 0.244) were obtained from the Brookhaven Protein Databank entry 1T8I. All crystallographic water molecules were removed from the coordinate set. The binding region was defined as all the atoms that are 15 Å around the centroid of CPT in the crystal structure. The ligands were extracted from the X-ray complexes, which were subsequently docked into their corresponding proteins by means of Gold 3.0.1.

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