Full Paper

Synthesis and Biological Evaluation of Novel Homocamptothecins Conjugating with Dihydropyrimidine Derivatives as Potent Topoisomerase I Inhibitors

Lingjian Zhu[1], Pengfei Cheng[1], Ning Lei[2], Jianzhong Yao[1], Chunquan Sheng[1], Chunlin Zhuang[1], Wei Guo[1], Wenfeng Liu[1], Yongqiang Zhang[1], Guoqiang Dong[1], Shengzhang Wang[1], Zhenyuan Miao[1], and Wannian Zhang[1]

[1] School of Pharmacy, Second Military Medical University, Shanghai, P. R. China
[2] Department of Quality Assurance, Lian Yun Gang JARI Pharmaceutical Co., Ltd., Lianyungang, P. R. China

Homocamptothecin (hCPT) is a camptothecin (CPT) homologue with the insertion of a methylene ($-CH_2-$) spacer between the alcohol moiety and carbonyl group of the classical six-membered α-hydroxylactone ring. This modification provides higher lactone stability and did not impair its activity against topoisomerase I (Topo I), but rather appears to improve it compared to CPT. In an attempt to improve the antitumor activity of homocamptothecins, a series of novel hCPT derivatives conjugating with dihydropyrimidine (DHPM) derivatives was designed and synthesized based on a synthetic route which couples 7-formylhomocamptothecin with different dihydropyrimidine derivates. Most of the synthesized compounds exhibited good antiproliferative activity on tumor cell lines A549, MDA-MB-435 and HCT116. Furthermore, this class of compounds showed superior Topo I inhibition activity comparable to or higher than CPT.

Keywords: Biological activity / Camptothecin / Dihydropyrimidine / Homocamptothecin

Received: December 28, 2010; Revised: February 28, 2011; Accepted: March 2, 2011

DOI 10.1002/ardp.201000402

Introduction

(20*S*)-Camptothecin (CPT) [1], a cytotoxic quinoline alkaloid which inhibits topoisomerase I (Topo I), is a potent anticancer agent that was isolated from the Chinese tree *Camptotheca acumunata* by Wani and Wall in 1966. It has already been discovered that the cytotoxicity of camptothecin is due to a novel mechanism of action involving selective inhibition of DNA topoisomerase I (Topo I) [2, 3]. Its two water-soluble analogs, topotecan and irinotecan, are used in clinics as anticancer agents (Fig. 1).

However, the severe toxicity, the instability of the lactone and other defects of CPTs [4] have promoted intensive efforts to increase the efficacy of these agents, reduce their toxicity in patients, and overcome the tumor drug resistance for this class of compounds. The most important discovery would be to modify the metabolically liable camptothecin lactone ring from a six-membered α-hydroxylactone to a seven-membered β-hydroxylactone ring, which leads to the development of the homocamptothecin (hCPT) family of compounds [5]. Homocamptothecin represents a promising prototype for the next generation of topoisomerase I targeting agents. Upgrading the highly reactive six-membered lactone to a more stable seven-membered ring is achieved without compromising any antitumor activity: hCPT not only retains superior antitumor activity, it also has a reinforced capacity to inhibit topoisomerase I compared with CPT [6]. According to these encouraging results, many hCPT analogues have been synthesized and evaluated *in vitro* and *in vivo* as the antitumor agents [7–9]. Among them, diflomotecan (BN80915) and elomotecan (BN80927) are representative ones and have been in clinical trial, and encouraging results have been obtained (Fig. 2) [10, 11]. Furthermore, a series of novel homocamptothecin derivatives have been synthesized in our

Correspondence: W. N. Zhang, Z. Y. Miao, C. Q. Sheng, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, P. R. China.
E-mail: zhangwnk@hotmail.com; miaozhenyuan@hotmail.com; shengcq@hotmail.com
Fax: +86-21-81871243

These authors contributed equally to this work:
L.-J. Zhu, lj_zhu@163.com; P.-F. Cheng, chengpf@163.com

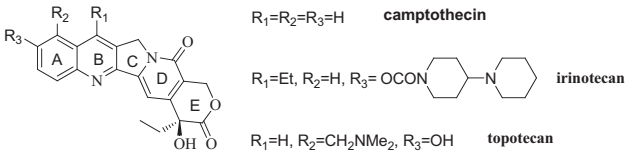


Figure 1. Camptothecin and representative analogs.

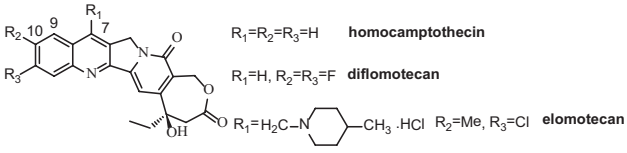


Figure 2. Homocamptothecin and representative analogs.

laboratory and several compounds that showed superior activity had been chosen to conduct preclinical studies [12–14]. Such findings encouraged us to develop novel and semisynthetic analogues of hCPT with improved pharmacological and pharmaceutical properties while maintaining (or even increasing) the antitumor activity of the parent compound.

The high stability of hCPT is with no doubt directly attributed to its expanded lactone E-ring but the activity and topoisomerase I inhibition activity can be enhanced further by adding a substituent to the native scaffold. In recent years, the use of conjugates has emerged as a frequent strategy in efforts to optimize therapeutically beneficial properties of CPT, including lactone stability, solubility/lipophilicity, tumor cell recognition and sequence specificity of DNA damage [15–18]. Dihydropyrimidine (DHPM) and their derivatives of the Biginelli-type have occupied an important place in natural and synthetic organic chemistry mainly due to their wide range of biological activities [19], notably as calcium channel blockers [20]. Additionally, their particular structure has been found in natural marine alkaloid batzalladines which are the first low molecular weight natural products reported in the literature to inhibit the binding of HIV gp-120 to $CD4^+$ cell, so disclosing new vistas towards the development of AIDS therapy [21, 22]. Meaningfully, the structurally related derivative monastrol was identified as a novel low molecular weight cell-permeable molecule for the development of potentially new anticancer drugs [23], and several screening assays indicated that this type of compounds exhibited effective antiproliferative activity against several cancer cell lines [24]. Furthermore, the past researches revealed that there was a potential correlation between cancer and hypertension which is actually a significant risk factor for cancer [25, 26]. Therefore, we supposed that introducing the organic molecules with hypotensive effect into antitumor molecules may be an effective measure to improve therapeutic effect of antitumor agents.

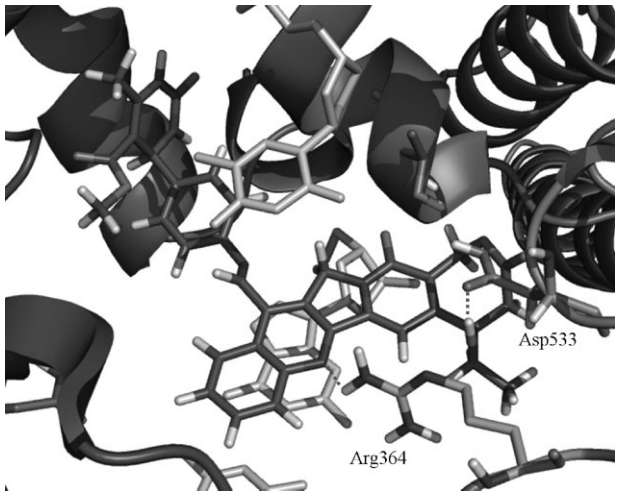


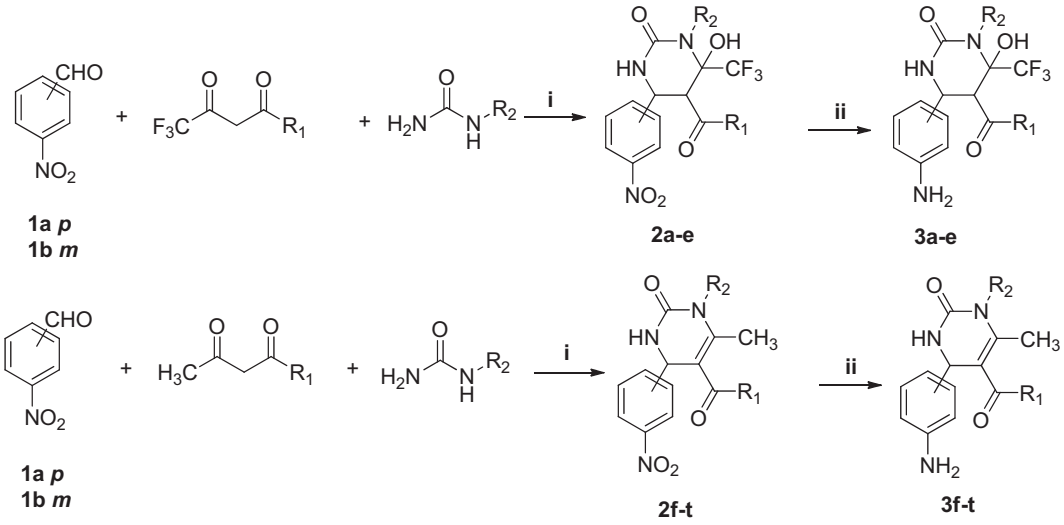
Figure 3. Hypothetical model for the binding of homocamptothecin-DHP conjugate (**4h**) with DNA–Topo I complex. The figure was prepared from PDB entry: 1T8I, using PyMol (http://pymol.souceforge.net/).

For above-mentioned pharmacological potency of the DHPM scaffold, we were interested in studying the incorporation of a dihydropyrimidine unit into the homocamptothecin skeleton in order that we could discover novel hCPT analogues with higher Topo I inhibition efficacy, better therapeutic effect and lower toxicity. Although the volume of dihydropyrimidine derivatives is relatively big, based on the ternary complex of DNA–Topo I-CPT [27], we presented a reasonable binding mode of this class of 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 DHPM derivatives, and two key hydrogen bonds were reserved (Fig. 3).

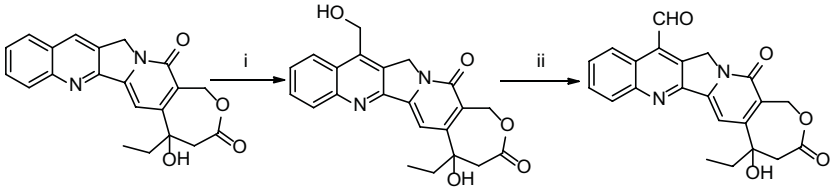
Result and discussion

Chemistry

To test the hypothesis concerning the effect of DHPM derivatives on position 7 of hCPT, we prepared twenty hCPT-DHPM derivatives in racemic mixtures. The chemical synthesis of the targeted compounds was based on two key intermediates, dihydropyrimidine derivatives and 7-formylhomocamptothecin. We employed classical Biginelli reaction [28] to synthesize the nitro substituted DHPM derivatives **2a–j**, then reduced to get amino substituted DHPM derivatives **3a–j** (Scheme 1). 7-Formylhomocamptothecin was prepared from hCPT according to the method reported by Sawada et al. [29]. Due to the acetoxymethyl hCPT as the main by-product, SeO_2 was used as the oxidant instead of previous acetic acid in this reaction (Scheme 2). Scheme 3 shows the synthetic strategy for our targeted compounds **4a–t** based on the coupling of



Scheme 1. Reagents and conditions: (i) TMSCl, DMF, r.t.; (ii) H_2/Pd-C, EtOH, r.t.



Scheme 2. Reagents and conditions: (i) 75% H_2SO_4, $FeSO_4 \cdot 7\ H_2O$, 30% H_2O_2, CH_3OH, H_2O, 12h, 79%; (ii) SeO_2/dioxane, 100°C, 8 h, 85%.

various DHPM derivatives and 7-formylhomocamptothecin. The bond C=N was selected as a linker, which was successfully adopted in our previous drug design [13].

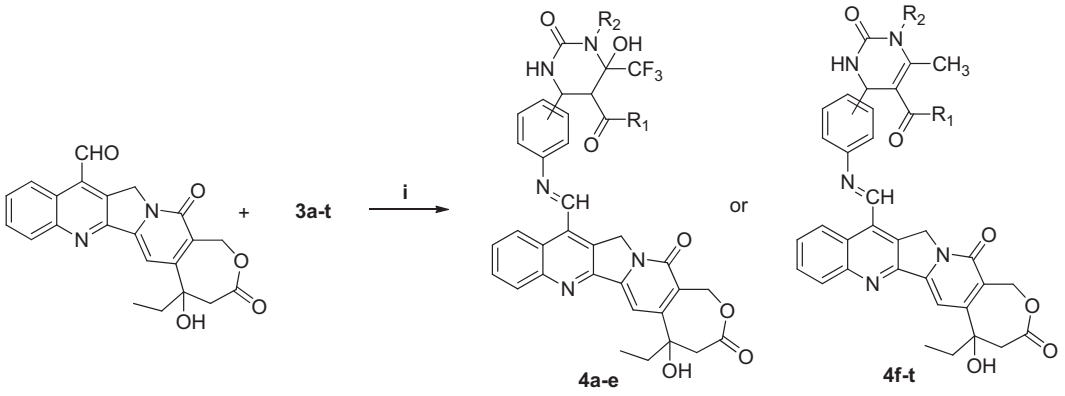
In-vitro antitumor activity

The *in-vitro* antitumor activities of these DHPM-hCPT derivatives against solid tumor cell lines, A549 (for non-small cell lung cancer), MDA-MB-435 (for breast cancer), and HCT116 (for colon cancer), were evaluated. Topotecan was used as a positive compound since it is a clinically effective CPT and does not require metabolic activation.

Table 1 indicated that these DHPM derivatives on position 7 of hCPT could promote antitumor activity of hCPTs as expected, which indicates the advantages of this strategy. The results also revealed that these compounds were more sensitive against MDA-MB-435 and HCT116 cell lines than against A549 cell line. For A549 cell line, most of the tested compounds showed increased cytotoxic potency compared with TPT. With regard to MDA-MB-435 cell line, with few exceptions (**4e**, **4q**, and **4s**), the tested compounds exhibited higher antitumor activities than TPT. According to HCT116 cell line, compounds **4a–4o** also showed higher *in-vitro* inhibitory activities than TPT. Especially, the IC_{50} values of compounds, **4b**, **4k**, and **4m**, reached to 78 nM, 48 nM, and 81 nM, respectively. Importantly, compared with hCPT, most of the synthesized compounds showed enhanced antitumor activity regardless of whether against A549, MDA-MB-435 and HCT116 cell lines. The bulky substitution conferred by DHPM and the diverse pharmacological properties of DHPM are the two main considerations for our original design idea, which was well proved by the research results. Furthermore, four compounds, **4b**, **4h**, **4k**, and **4m**, possessed high growth inhibitory effect at the concentration of 0.01, 0.1, 1, 10, and 100 μg/mL, while relatively low activity of TPT was observed at the same concentration (Fig. 4). In particular, the activities of the most promising compounds, **4b** and **4m**, showed broad *in-vitro* antitumor spectrum.

Topoisomerase I inhibition activity assays

Generally, camptothecin and its clinically used derivatives act by stabilizing a covalent topoisomerase I–DNA complex called the cleavable complex. Therefore, topoisomerase I-mediated DNA cleavage assay was used to investigate the ability of the prepared compounds to inhibit Topo I. Cleavable complexes were revealed by the appearance of short DNA fragments when the samples were analyzed by gel electrophoresis under denaturing conditions. The presented results (Fig. 5) indicated that compounds **4b**, **4h**, **4k**,



	R_1	R_2			R_1	R_2			R_1	R_2	
4a	CH_3CH_2O—	H	*p*	**4h**	CH_3O—	H	*p*	**4o**	CH_3O—	C_2H_5–	*p*
4b	CH_3CH_2O—	H	*m*	**4i**	H_3C—	H	*p*	**4p**	H_3C—	H_3C—	*p*
4c	CH_3CH_2O—	H_3C—	*p*	**4j**	CH_3CH_2O—	C_2H_5–	*p*	**4q**	$(CH_3)_3CO$—	C_2H_5–	*p*
4d	CH_3CH_2O—	Ph—	*p*	**4k**	CH_3CH_2O—	H_3C—	*p*	**4r**	$(CH_3)_2CHCO$—	H_3C—	*p*
4e	CH_3CH_2O—	C_2H_5–	*p*	**4l**	CH_3CH_2O—	Ph—	*p*	**4s**	$(CH_3)_3CO$—	Ph—	*p*
4f	CH_3CH_2O—	H	*p*	**4m**	$(CH_3)_3CO$—	H	*p*	**4t**	H_3C—	C_2H_5–	*p*
4g	CH_3CH_2O—	H	*m*	**4n**	$(CH_3)_2CHCO$—	H	*p*				

Scheme 3. Reagents and conditions: (i) $Yb(OTf)_3$, $CHCl_3$, r.t.

4l, **4m**, **4n**, and **4t** as cytotoxic agents were also very potent as topoisomerase I inhibitors. The results also showed that the addition of increasing concentrations of **CPT**, **4b**, or **4m** is accompanied by a dose-dependent increase in the level of cleavable complexes. Importantly, even in low concentration (1 μM), **4b** and **4m** showed obvious Topo I inhibition activity, while CPT exhibited no inhibition activity (Fig. 6).

Conclusion

In conclusion, twenty hCPT-DHPM conjugates were synthesized based on a semi-synthetic route. Most of the synthesized compounds exhibited good antiproliferative activity on tumor cell lines A549, MDA-MB-435 and HCT116. Especially, compounds **4b**, **4k**, and **4m** showed higher *in-vitro* inhibitory activities against HCT116 than TPT with the IC_{50} of 78 nM, 48 nM and 81 nM, respectively. It is likely that the improvement of cytotoxic activity is the result of multiple favorable events, including DHPM's biological diversity and increased drug uptake (as expected on the basis of increased lipophilicity). The results presented in this study also indicate that hCPT-DHPM conjugates as effective cytotoxic agents also showed superior Topo I inhibition activity. A better understanding of this feature could provide meaningful insights for the development of more effective topoisomerase I inhibitors.

Experimental

Chemistry

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 $CDCl_3$ or 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 200–300 mesh. Anhydrous solvent and reagents were all analytically pure and dried through routine protocols. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware was oven-dried and/or flame-dried.

Table 1. *In-vitro* antitumor activity of hCPT–DHPM derivative conjugates [a]

Compounds	IC_{50} (μM)		
	A549	**MDA-MB-435**	**HCT116**
4a	1.905	0.328	0.371
4b	0.891	0.365	0.0785
4c	2.469	0.0795	0.155
4d	1.798	0.213	0.148
4e	3.935	1.550	0.244
4f	1.808	0.695	0.200
4g	1.947	0.420	0.310
4h	1.974	0.270	0.206
4i	2.236	0.452	0.307
4j	1.837	0.450	0.240
4k	1.770	0.210	0.0488
4l	1.618	0.374	0.215
4m	0.158	0.349	0.0813
4n	2.162	0.164	0.160
4o	4.099	0.505	0.258
4p	6.434	0.879	0.977
4q	2.389	1.129	1.398
4r	1.988	0.306	0.940
4s	3.821	1.384	1.984
4t	1.860	0.167	0.741
hCPT	2.980	1.035	0.944
TPT	3.159	1.10	0.448

[a] Cancer cells: A549: lung cancer cell; HCT116: colon cancer cell; MDA-MB-435: breast cancer cell.

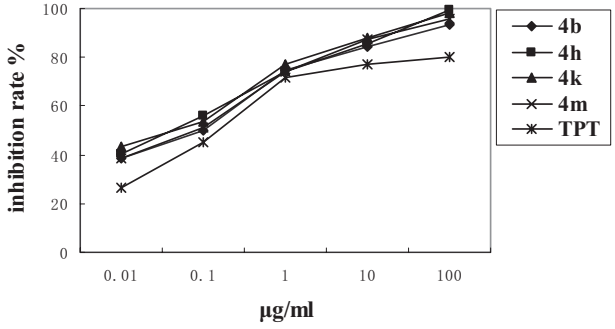


Figure 4. Comparison of the inhibition ratio of compounds **4b**, **4h**, **4k**, **4m**, and TPT against cancer cell line HCT116.

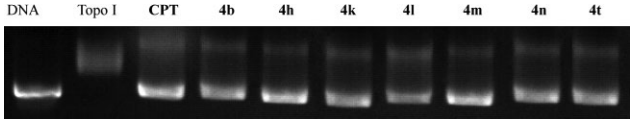


Figure 5. Effect of the selected compounds on Topoisomerase-I mediated DNA relaxation in single concentration. Topo I was Topoisomerase I and DNA without drugs. The samples were reacted with 100 μM drugs at 37°C for 15 min. Reactions were then stopped by adding 0.5% SDS. Gels were photographed under a UV transilluminator.

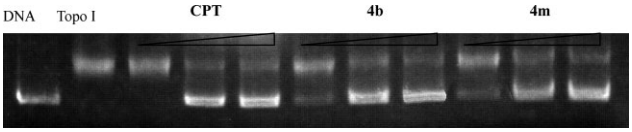


Figure 6. Effect of compounds CPT, **4b**, and **4m** on Topoisomerase-I mediated DNA relaxation in concentration gradient. Topo I was topoisomerase I and DNA without drugs; the samples were reacted with 1, 50, and 100 μM drugs at 37°C for 15 min. Reactions were then stopped by adding 0.5% SDS. Gels were photographed under a UV transilluminator.

*Typical experimental procedure for the synthesis of **2a–f***

A solution of aldehyde (5 mmol), an appropriate urea (7.5 mmol), 1,3-dione (7.5 mmol), and TMSCl (20 mmol) in dried DMF (20 mL) was stirred at room temperature until TLC showed complete disappearance of aldehyde. Reaction mixture was poured into cold water (25 mL) and stirred for 10 min. The precipitates formed were filtered and washed with cold water (2 × 10 mL) and then with 90% ethanol (10 mL). Recrystallization of the residues from an appropriate solvent yielded target compounds **2** as white solids.

*Typical experimental procedure for the synthesis of **3a–f***

Compound **2** (0.5g) was dissolved in ethanol (15 mL), then dry 10% palladium on activated carbon (20 mg) was carefully added to the solution and the dissolved oxygen was removed under vacuum. Then a balloon of hydrogen was mounted and the mixture was stirred vigorously for 12 h. The catalyst was removed by filtration of the reaction mixture through a pad of Celite. The filtrate was concentrated under reduced pressure to give crude compounds **3** without further purification.

*General experimental procedure for the synthesis of **4a–f***

A mixture of **3** (100 mg) and 7-formylhomocamptothecin (100 mg) was suspended in anhydrous $CHCl_3$ containing 4 Å MS. After 10 min, $Yb(OTf)_3$ (10 mg) was added to the solution. The resulting mixture was stirred at room temperature until the reaction was complete. After filtering the sieves, the solvent was evaporated to dryness and purified by flash chromatography (eluent: CH_2Cl_2/MeOH, 98:2) on silica gel to get target compound **4**.

7-Formylhomocamptothecin

A yellow powder; mp > 300°C; IR (KBr) *v*: 3371, 3057, 2960, 2927, 1730, 1692, 1658, 1607, 1537, 1503, 1481, 1462, 1381, 1308, 1286, 1212, 1044, 839, 775. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.87 (q, 2H, J = 7.3 Hz, CH_3CH_2), 3.04–3.49 (q, 2H, J = 11.6 Hz, CH_2CO), 5.43–5.55 (q, 2H, J = 15.3 Hz, OCH_2), 5.52 (s, 2H, NCH_2), 6.06 (s, 1H, OH), 7.46 (s, 1H, C_{14}-H), 7.92 (t, 1H, J = 7.5 Hz, C_{10}-H), 7.98 (t, 1H, J = 7.7 Hz, C_{11}-H), 8.30 (d, 1H, J = 8.5 Hz, C_{12}-H), 9.04 (d, 1H, J = 8.6 Hz, C_9-H). MS (ESI): 389 (M−H); Anal. calcd. for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18. Found: C, 67.57; H, 4.66; N, 7.15.

4a: A yellow powder; yield (77%); mp >300°C; IR (KBr) *v*: 3263, 3118, 2968, 2924, 2852, 1742, 1682, 1658, 1594, 1536, 1486, 1468, 1443, 1376, 1343, 1305, 1280, 1244, 1200, 1184, 842, 761, 721, 647. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 0.92 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.87

(q, 2H, J = 8.4 Hz, CH_3CH_2), 3.04–3.49 (q, 2H, J = 13.7 Hz, CH_2CO), 3.06 (s, 1H, COCH), 3.86 (q, 2H, J = 6.9 Hz, CH_3CH_2O), 4.89 (d, 1H, J = 11.4 Hz, NHC*H*), 5.43–5.59 (q, 2H, J = 11.9 Hz, OCH_2), 5.59 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.30 (s, 1H, C_{14}-H), 7.45 (s, 2H, NH), 7.50–7.60 (dd, 4H, J = 8.3 Hz, Ar-H), 7.69 (s, 1H, OH), 7.83 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.99 (d, 1H, J = 8.5 Hz, C_9-H), 9.72 (s, 1H, N = CH). ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 8.65, 14.05, 29.43, 36.69, 42.80, 51.18, 53.03, 53.32, 60.82, 61.74, 73.56, 99.96, 122.29, 123.15, 124.56, 126.42, 128.86, 129.12, 129.55, 130.22, 130.86, 134.00, 138.32, 144.44, 148.05, 149.53, 153.58, 154.14, 156.18, 156.90, 159.42, 167.43, 172.27. MS (ESI): 718 (M−H); Anal. calcd. for $C_{36}H_{33}F_3N_5O_8$: C, 60.08; H, 4.48; N, 9.73. Found: C, 60.18; H, 4.47; N, 9.71.

4b: A yellow powder; yield (90%); mp >300°C; IR (KBr) *v*: 3267, 3132, 2970, 2929, 1742, 1689, 1656, 1594, 1535, 1482, 1468, 1443, 1375, 1343, 1306, 1277, 1246, 1199, 1057, 1026, 851, 763, 698, 636. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.3 Hz, CH_3CH_2), 0.91 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.88 (q, 2H, J = 7.4 Hz, CH_3CH_2), 3.08–3.49 (q, 2H, J = 13.7 Hz, CH_2CO), 3.10 (s, 1H, COCH), 3.87 (q, 2H, J = 7.0 Hz, CH_3CH_2O), 4.94 (d, 1H, J = 11.4 Hz, NHC*H*), 5.40–5.55 (q, 2H, J = 14.95 Hz, OCH_2), 5.56 (s, 2H, NCH_2), 6.06 (s, 1H, OH), 7.35 (1H, Ar-H), 7.37 (s, 1H, C_{14}-H), 7.45 (s, 2H, NH), 7.48–7.59 (m, 3H, Ar-H), 7.73 (s, 1H, OH), 7.84 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.93 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.25 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.94 (d, 1H, J = 8.5 Hz, C_9-H), 9.68 (s, 1H, N=CH). ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 8.65, 14.04, 29.43, 36.69, 40.91, 43.22, 51.11, 53.03, 53.59, 60.75, 61.75, 73.56, 99.99, 121.98, 122.24, 123.17, 124.58, 125.89, 127.12, 128.90, 129.13, 129.83, 130.04, 130.87, 133.95, 140.26, 144.41, 149.53, 151.11, 153.58, 154.15, 156.19, 157.02, 159.42, 167.49, 172.27. MS (ESI): 718 (M−H); Anal. calcd. for $C_{36}H_{33}F_3N_5O_8$: C, 60.08; H, 4.48; N, 9.73. Found: C, 60.22; H, 4.47; N, 9.71.

4c: A yellow powder; yield (83%); mp >300°C; IR (KBr) *v*: 3252, 3093, 2978, 2933, 1742, 1660, 1596, 1537, 1470, 1376, 1341, 1301, 1274, 1237, 1188, 1140, 1058, 847, 766. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 0.88 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.87 (q, 2H, J = 8.4 Hz, CH_3CH_2), 2.9 (s, 3H, NCH_3), 3.07–3.49 (q, 2H, J = 13.8 Hz, CH_2CO), 3.22 (d, 1H, J = 11.2 Hz, COCH), 3.84 (q, 2H, J = 6.9 Hz, CH_3CH_2O), 4.77 (d, 1H, J = 11.1 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 11.8 Hz, OCH_2), 5.60 (s, 2H, NCH_2), 6.05 (s, 1H, OH), 7.46 (s, 1H, C_{14}-H), 7.49 (s, 1H, OH), 7.50–7.61 (dd, 4H, J = 8.3 Hz, Ar-H), 7.81 (s, 1H, NH), 7.84 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.96 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.27 (d, 1H, J = 8.4 Hz, C_{12}-H), 9.01 (d, 1H, J = 8.5 Hz, C_9-H), 9.72 (s, 1H, N = CH). MS (ESI): 732 (M−H); Anal. calcd. for $C_{37}H_{34}F_3N_5O_8$: C, 60.57; H, 4.67; N, 9.55. Found: C, 60.67; H, 4.68; N, 9.57.

4d: A yellow powder; yield (77%); mp >300°C; IR (KBr) *v*: 3338, 3059, 2966, 2923, 1744, 1655, 1597, 1498, 1440, 1371, 1312, 1277, 1223, 1208, 1053, 831, 759, 693, 632. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 0.92 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.87 (q, 2H, J = 8.4 Hz, CH_3CH_2), 3.07–3.48 (q, 2H, J = 13.7 Hz, CH_2CO), 3.86 (q, 2H, J = 6.9 Hz, CH_3CH_2O), 4.38 (s, 1H, CHCO), 4.39 (s, 1H, NHCH), 5.40–5.56 (q, 2H, J = 11.9 Hz, OCH_2), 5.60 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 6.68 (t, 1H, Ar-H), 6.90 (t, 1H, Ar-H), 7.23 (t, 2H, Ar-H), 7.41 (d, 1H, Ar-H), 7.43 (s, 1H, OH), 7.45 (s, 1H, C_{14}-H), 7.46–7.58 (dd, 4H, J = 8.3 Hz, Ar-H), 7.84 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.60 (s, 1H, NH), 9.00 (d, 1H, J = 8.5 Hz, C_9-H), 9.72 (s, 1H, N=CH). MS (ESI): 794 (M−H); Anal. calcd. for $C_{42}H_{36}F_3N_5O_8$: C, 63.39; H, 4.56; N, 8.80. Found: C, 63.48; H, 4.55; N, 8.78.

4e: A yellow powder; yield (69%); mp >300°C; IR (KBr) *v*: 3311, 3080, 2978, 2923, 2851, 1739, 1656, 1612, 1537, 1500, 1480, 1439, 1376, 1338, 1282, 1249, 1186, 1057, 930, 906, 846, 767, 722. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, 3H, J = 7.3 Hz, CH_3CH_2), 0.89 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.15 (t, 3H, J = 6.8 Hz, CH_3CH_2N), 1.87 (q, 2H, J = 8.4 Hz, CH_3CH_2), 3.07–3.49 (q, 2H, J = 13.4 Hz, CH_2CO), 3.06 (d, 1H, J = 11.0 Hz, COCH), 3.39–3.48 (d q, 2H, J = 6.7 Hz, CH_3CH_2N), 3.83 (q, 2H, J = 7.1 Hz, CH_3CH_2O), 4.76 (d, 1H, J = 11.0 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 14.9 Hz, OCH_2), 5.60 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.44 (s, 1H, C_{14}-H), 7.46 (s, 1H, OH), 7.48–7.61 (dd, 4H, J = 8.2 Hz, Ar-H), 7.75 (s, 1H, NH), 7.85 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.94 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.27 (d, 1H, J = 8.4 Hz, C_{12}-H), 9.01 (d, 1H, J = 8.5 Hz, C_9-H), 9.72 (s, 1H, N=CH). MS (ESI): 746 (M−H); Anal. calcd. for $C_{38}H_{36}F_3N_5O_8$: C, 61.04; H, 4.85; N, 9.37. Found: C, 61.19; H, 4.86; N, 9.35.

4f: A yellow powder; yield (80%); mp >300°C; IR (KBr) *v*: 3310, 3151, 2972, 2930, 1742, 1701, 1654, 1594, 1535, 1508, 1441, 1370, 1311, 1277, 1227, 1085, 830, 796, 760, 721, 650. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.16 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 1.86 (q, 2H, J = 7.9 Hz, CH_3CH_2), 2.28 (s, 3H, C = C-CH_3), 3.06–3.48 (q, 2H, J = 13.8 Hz, CH_2CO), 4.03 (q, 2H, J = 7.0 Hz, CH_3CH_2O), 5.23 (d, 1H, J = 3.1 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.4 Hz, OCH_2), 5.57 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.38–7.54 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (s, 1H, NH), 7.84 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.25 (s, 1H, NH), 9.69 (s, 1H, N = CH). ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 8.65, 14.62, 18.28, 29.41, 36.69, 42.80, 53.01, 54.06, 59.75, 61.75, 73.56, 99.66, 99.96, 122.28, 123.14, 124.85, 125.69, 127.69, 128.91, 130.21, 131.05, 133.97, 144.44, 144.66, 149.00, 149.51, 150.78, 152.60, 153.55, 156.18, 156.97, 159.42, 165.80, 172.28. MS (ESI): 646 (M−H); Anal. calcd. for $C_{36}H_{33}N_5O_7$: C, 66.76; H, 5.14; N, 10.81. Found: C, 66.63; H, 5.15; N, 10.83.

4g: A yellow powder; yield (79%); mp >300°C; IR (KBr) *v*: 3239, 3111, 2970, 2933, 1737, 1702, 1654, 1616, 1594, 1535, 1506, 1481, 1442, 1369, 1311, 1281, 1223, 1087, 1055, 829, 761, 722, 634. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.15 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 2.28 (q, 2H, J = 7.5 Hz, CH_3CH_2), 2.36 (s, 3H, C = C-CH_3), 3.04–3.48 (q, 2H, J = 13.7 Hz, CH_2CO), 4.03 (q, 2H, J = 6.9 Hz, CH_3CH_2O), 5.28 (s, 1H, NHC*H*), 5.53–5.58 (q, 2H, J = 11.8 Hz, OCH_2), 5.58 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.35 (1H, Ar-H), 7.39 (1H, Ar-H), 7.46 (s, 1H, C_{14}-H), 7.48–7.50 (2H, Ar-H), 7.84 (s, 1H, NH), 7.85 (t, 1H, J = 7.9 Hz, C_{10}-H), 8.03 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.27 (d, 1H, J = 8.3 Hz, C_{12}-H), 8.96 (d, 1H, J = 8.4 Hz, C_9-H), 9.25 (s, 1H, NH), 9.67 (s, 1H, N = CH). ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 8.65, 14.62, 18.28, 36.69, 42.80, 53.00, 54.31, 59.75, 61.72, 73.55, 99.46, 99.95, 119.58, 121.68, 123.14, 124.89, 125.01, 125.57, 128.90, 129.15, 130.09, 130.26. 130.89, 133.14, 144.44, 146.60, 149.14, 149.87, 151.23, 152.54, 153.62, 156.42, 157.26, 159.42, 165.79, 172.25. MS (ESI): 646 (M−H); Anal. calcd. for $C_{36}H_{33}N_5O_7$: C, 66.76; H, 5.14; N, 10.81. Found: C, 66.65; H, 5.16; N, 10.85.

4h: A yellow powder; yield (87%); mp >300°C; IR (KBr) *v*: 3315, 2926, 1743, 1699, 1658, 1594, 1537, 1515, 1435, 1371, 1345, 1278, 1237, 1078, 1053, 972, 829, 760, 722. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.86 (q, 2H, J = 7.7 Hz, CH_3CH_2), 2.29 (s, 3H, C=C–CH_3), 3.07–3.48 (q, 2H, J = 13.9 Hz, CH_2CO), 3.57 (s, 3H, CH_3O), 5.23 (d, 1H, J = 3.4 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 14.9 Hz, OCH_2), 5.57

(s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.38–7.54 (dd, 4H, J = 8.4 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.81 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.82 (s, 1H, NH), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 7.7 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.27 (s, 1H, NH), 9.68 (s, 1H, N=CH). MS (ESI): 632 (M−H); Anal. calcd. for $C_{35}H_{31}N_5O_7$: C, 66.34; H, 4.93; N, 11.05. Found: C, 66.47; H, 4.92; N, 11.07.

4i: A yellow powder; yield (78%); mp >300°C; IR (KBr) *v*: 3276, 2926, 1743, 1702, 1655, 1597, 1535, 1509, 1439, 1381, 1366, 1329, 1315, 1278, 1236, 1174, 1136, 1110, 1055, 849, 830, 763, 722, 579. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.87 (q, 2H, J = 7.5 Hz, CH$_3$CH_2), 2.16 (s, 3H, OCCH$_3$), 2.24 (s, 3H, C = C-CH$_3$), 3.06–3.48 (q, 2H, J = 13.6 Hz, CH$_2$CO), 5.35 (d, 1H, J = 3.2 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.0 Hz, OCH$_2$), 5.57 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.39–7.53 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (t, 1H, J = 8.0 Hz, C_{10}-H), 7.91 (s, 1H, NH), 7.95 (t, 1H, J = 8.1 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.24 (s, 1H, NH), 9.68 (s, 1H, N = CH). MS (ESI): 616 (M−H); Anal. calcd. for $C_{35}H_{31}N_5O_6$: C, 68.06; H, 5.06; N, 11.34. Found: C, 68.17; H, 5.05; N, 11.32.

4j: A yellow powder; yield (76%); mp >300°C; IR (KBr) *v*: 3346, 3095, 2979, 2932, 1751, 1680, 1659, 1608, 1532, 1509, 1464, 1441, 1378, 1314, 1277, 1229, 1171, 1082, 1054, 854, 759. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.3 Hz, CH_3CH$_2$), 1.12 (t, 3H, J = 6.9 Hz, CH_3CH$_2$N), 1.17 (t, 3H, J = 7.0 Hz, CH_3CH$_2$O), 1.87 (q, 2H, J = 7.8 Hz, CH$_3$CH_2), 2.54 (s, 3H, C=C–CH$_3$), 3.07–3.48 (q, 2H, J = 13.9 Hz, CH$_2$CO), 4.01–4.05 (d q, 2H, J = 7.0 Hz, CH$_3$CH_2N), 4.06 (q, 2H, J = 7.0 Hz, CH$_3$CH_2O), 5.22 (d, 1H, J = 3.5 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.4 Hz, OCH$_2$), 5.57 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.36–7.54 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 7.97 (s, 1H, NH), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.69 (s, 1H, N=CH). MS (ESI): 674 (M−H); Anal. calcd. for $C_{38}H_{37}N_5O_7$: C, 67.54; H, 5.52; N, 10.36. Found: C, 67.65; H, 5.53; N, 10.33.

4k: A yellow powder; yield (67%); mp >300°C; IR (KBr) *v*: 3307, 2960, 2925, 2852, 1746, 1674, 1659, 1593, 1534, 1507, 1458, 1443, 1383, 1349, 1302, 1269, 1246, 1173, 1075, 1053, 972, 857, 829, 806, 759, 821, 629. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.3 Hz, CH_3CH$_2$), 1.18 (t, 3H, J = 7.0 Hz, CH_3CH$_2$O), 1.87 (q, 2H, J = 7.8 Hz, CH$_3$CH_2), 2.52 (s, 3H, C=C–CH$_3$), 3.06–3.48 (q, 2H, J = 13.8 Hz, CH$_2$CO), 3.28 (s, 3H, NCH_3), 4.07 (q, 2H, J = 7.0 Hz, CH$_3$CH_2O), 5.24 (d, 1H, J = 3.8 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.4 Hz, OCH$_2$), 5.57 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.36–7.53 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.04 (d, 1H, J = 3.8 Hz, NH), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.69 (s, 1H, N = CH). MS (ESI): 660 (M−H); Anal. calcd. for $C_{37}H_{35}N_5O_7$: C, 67.16; H, 5.33; N, 10.58. Found: C, 67.26; H, 5.32; N, 10.55.

4l: A yellow powder; yield (70%); mp >300°C; IR (KBr) *v*: 3344, 3095, 3066, 2974, 2934, 1745, 1707, 1686, 1655, 1618, 1593, 1535, 1497, 1438, 1372, 1343, 1311, 1268, 1210, 1079, 973, 858, 829, 761, 699, 632. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH$_2$), 1.19 (t, 3H, J = 7.0 Hz, CH_3CH$_2$O), 1.88 (q, 2H, J = 7.8 Hz, CH$_3$CH_2), 2.07 (s, 3H, C=C–CH$_3$), 3.07–3.46 (q, 2H, J = 13.8 Hz, CH$_2$CO), 3.28 (s, 3H, NCH_3), 4.09 (q, 2H, J = 7.0 Hz, CH$_3$CH_2O), 5.37 (d, 1H, J = 3.5 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.4 Hz, OCH$_2$), 5.58 (s, 2H, NCH_2), 6.05 (s, 1H, OH), 7.26 (d, 2H, Ar-H), 7.41–7.54 (m, 3H, Ar-H), 7.46 (s, 1H, C_{14}-H), 7.55–7.63 (dd, 4H, J = 8.3 Hz, Ar-H), 7.82 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.24 (d, 1H, J = 3.5 Hz, NH), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.72 (s, 1H, N = CH). MS (ESI): 722 (M−H); Anal. calcd. for $C_{42}H_{37}N_5O_7$: C, 69.70; H, 5.15; N, 9.68. Found: C, 69.84; H, 5.14; N, 9.66.

4m: A yellow powder; yield (88%); mp >300°C; IR (KBr) *v*: 3352, 3253, 3132, 2973, 2931, 1746, 1698, 1653, 1595, 1533, 1510, 1444, 1368, 1343, 1315, 1278, 1235, 1169, 1090, 1057, 850, 760. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.36 (s, 9H, OC(CH$_3$)$_3$), 1.87 (q, 2H, J = 7.9 Hz, CH$_3$CH_2), 2.25 (s, 3H, C=C–CH$_3$), 3.07–3.49 (q, 2H, J = 13.9 Hz, CH$_2$CO), 4.03 (q, 2H, J = 7.0 Hz, CH$_3$CH_2O), 5.18 (d, 1H, J = 3.4 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.0 Hz, OCH$_2$), 5.58 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.38–7.56 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.74 (s, 1H, NH), 7.83 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.94 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.99 (d, 1H, J = 8.5 Hz, C_9-H), 9.12 (s, 1H, NH), 9.70 (s, 1H, N=CH). MS (ESI): 674 (M−H); Anal. calcd. for $C_{38}H_{37}N_5O_7$: C, 67.54; H, 5.52; N, 10.36. Found: C, 67.68; H, 5.51; N, 10.33.

4n: A yellow powder; yield (87%); mp >300°C; IR (KBr) *v*: 3310, 2960, 2931, 2873, 1742, 1705, 1682, 1651, 1594, 1537, 1510, 1440, 1396, 1377, 1312, 1276, 1234, 1081, 976, 829, 799, 760, 721, 631. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.81–0.83 (d d, 6H, J = 5.3 Hz, CH(CH_3)$_2$), 0.88 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.16 (t, 3H, J = 7.0 Hz, CH_3CH$_2$O), 1.85 (m, 1H, C*H*(CH$_3$)$_2$), 1.86 (q, 2H, J = 7.9 Hz, CH$_3$CH_2), 2.31 (s, 3H, C=C–CH$_3$), 3.07–3.45 (q, 2H, J = 13.8 Hz, CH$_2$CO), 3.78 (d, 1H, J = 6.0 Hz, OCH), 5.24 (d, 1H, J = 3.0 Hz, NHC*H*), 5.40–5.57 (q, 2H, J = 15.4 Hz, OCH$_2$), 5.58 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.38–7.54 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.81 (s, 1H, NH), 7.84 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.26 (s, 1H, NH), 9.68 (s, 1H, N=CH). MS (ESI): 674 (M−H); Anal. calcd. for $C_{38}H_{37}N_5O_7$: C, 67.54; H, 5.52; N, 10.36. Found: C, 67.69; H, 5.51; N, 10.38.

4o: A yellow powder; yield (85%); mp >300°C; IR (KBr) *v*: 3400, 3230, 3116, 2963, 2933, 2879, 1745, 1717, 1682, 1655, 1594, 1535, 1508, 1460, 1432, 1391, 1375, 1282, 1234, 1078, 1053, 997, 853, 800, 761, 719, 631. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.12 (t, 3H, J = 7.0 Hz, CH_3CH$_2$N), 1.87 (q, 2H, J = 7.6 Hz, CH$_3$CH_2), 2.54 (s, 3H, C=C–CH$_3$), 3.07–3.48 (q, 2H, J = 13.9 Hz, CH$_2$CO), 3.61 (s, 3H, OCH_3), 3.63–3.85 (d q, 2H, J = 7.0 Hz, CH$_3$CH_2N), 5.22 (d, 1H, J = 3.5 Hz, NHC*H*), 5.39–5.55 (q, 2H, J = 15.4 Hz, OCH$_2$), 5.54 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.36–7.53 (dd, 4H, J = 8.3 Hz, Ar-H), 7.44 (s, 1H, C_{14}-H), 7.81 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.93 (t, 1H, J = 7.9 Hz, C_{11}-H), 7.98 (d, 1H, J = 3.7 Hz, N*H*CH), 8.23 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.95 (d, 1H, J = 8.5 Hz, C_9-H), 9.66 (s, 1H, N=CH). ^{13}C-NMR (500 MHz, DMSO-d_6) δ: 8.54, 15.21, 15.98, 37.35, 38.15, 42.78, 51.49, 52.54, 52.87, 61.68, 73.48, 99.88, 102.96, 122.26, 123.08, 125.78, 126.14, 127.43, 128.75, 128.95, 130.13, 130.75, 133.97, 143.75, 144.36, 149.61, 150.22, 152.89, 153.50, 156.11, 156.93, 159.36, 166.48, 172.13. MS (ESI): 660 (M−H); Anal. calcd. for $C_{37}H_{35}N_5O_7$: C, 67.16; H, 5.33; N, 10.58. Found: C, 67.26; H, 5.32; N, 10.60.

4p: A yellow powder; yield (77%); mp >300°C; IR (KBr) *v*: 3351, 1968, 2929, 2883, 1744, 1687, 1655, 1593, 1535, 1508, 1439, 1421, 1381, 1348, 1270, 1243, 1206, 1072, 1056, 933, 852, 830, 760, 721. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.87 (q, 2H, J = 7.5 Hz, CH$_3$CH_2), 2.17 (s, 3H, OCCH$_3$), 2.50 (s, 3H, C=C–CH$_3$), 3.06–3.48 (q, 2H, J = 13.6 Hz, CH$_2$CO), 3.12 (s, 3H, NCH_3), 5.30 (d, 1H, J = 3.7 Hz, NHC*H*), 5.39–5.52 (q, 2H,

J = 15.0 Hz, OCH_2), 5.54 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.39–7.52 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.81 (t, 1H, J = 8.0 Hz, C_{10}-H), 7.94 (t, 1H, J = 8.1 Hz, C_{11}-H), 8.13 (d, 1H, J = 3.9 Hz, NH), 8.25 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.96 (d, 1H, J = 8.5 Hz, C_9-H), 9.67 (s, 1H, N=CH). MS (ESI): 630 (M−H); Anal. calcd. for $C_{36}H_{33}N_5O_6$: C, 68.45; H, 5.27; N, 11.09. Found: C, 68.59; H, 5.28; N, 11.11.

4q: A yellow powder; yield (88%); mp >300°C; IR (KBr) *v*: 3334, 2972, 2930, 2883, 1746, 1674, 1597, 1536, 1510, 1435, 1389, 1369, 1344, 1283, 1232, 1140, 1080, 1053, 950, 851, 759, 821, 630. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.11 (t, 3H, J = 6.9 Hz, CH_3CH_2N), 1.37 (s, 9H, $C(CH_3)_3$), 1.87 (q, 2H, J = 7.8 Hz, CH_3CH_2), 2.54 (s, 3H, C=C–CH_3), 3.07–3.48 (q, 2H, J = 13.9 Hz, CH_2CO), 3.60–3.86 (d q, 2H, J = 7.0 Hz, CH_3CH_2N), 5.17 (d, 1H, J = 3.3 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 14.9 Hz, OCH_2), 5.58 (s, 2H, NCH_2), 6.03 (s, 1H, OH), 7.36–7.57 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.88 (d, 1H, J = 3.6 Hz, N*H*CH), 7.94 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.99 (d, 1H, J = 8.5 Hz, C_9-H), 9.70 (s, 1H, N=CH). MS (ESI): 702 (M−H); Anal. calcd. for $C_{40}H_{41}N_5O_7$: C, 68.26; H, 5.87; N, 9.95. Found: C, 68.39; H, 5.88; N, 9.92.

4r: A yellow powder; yield (78%); mp >300°C; IR (KBr) *v*: 3339, 2961, 2929, 2873, 1746, 1677, 1659, 1597, 1535, 1511, 1462, 1443, 1380, 1270, 1246, 1073, 1051, 829, 760, 721, 629. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.83 (d d, 6H, J = 6.5 Hz, $CH(CH_3)_2$), 0.88 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.83 (m, 1H, $CH(CH_3)_2$), 1.87 (q, 2H, J = 7.8 Hz, CH_3CH_2), 2.50 (s, 3H, C=C–CH_3), 3.08–3.48 (q, 2H, J = 13.8 Hz, CH_2CO), 3.14 (s, 3H, NCH_3), 3.83 (d, 2H, J = 6.3 Hz, OCH_2), 5.26 (d, 1H, J = 3.7 Hz, NHC*H*), 5.39–5.55 (q, 2H, J = 15.4 Hz, OCH_2), 5.46 (d, 2H, J = 3.9 Hz, NCH_2), 6.05 (s, 1H, OH), 7.37–7.52 (dd, 4H, J = 8.3 Hz, Ar-H), 7.42 (s, 1H, C_{14}-H), 7.71 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.90 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.05 (d, 1H, J = 3.8 Hz, NH), 8.20 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.89 (d, 1H, J = 8.5 Hz, C_9-H), 9.61 (s, 1H, N=CH). MS (ESI): 660 (M−H); Anal. calcd. for $C_{39}H_{39}N_5O_7$: C, 67.91; H, 5.70; N, 10.15. Found: C, 67.85; H, 5.69; N, 10.13.

4s: A yellow powder; yield (78%); mp >300°C; IR (KBr) *v*: 3314, 3219, 2974, 2921, 2850, 1740, 1701, 1655, 1692, 1551, 1498, 1448, 1355, 1158, 854, 752, 697, 621. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.89 (t, 3H, J = 7.3 Hz, CH_3CH_2), 1.39 (s, 9H, $OC(CH_3)_3$), 1.88 (q, 2H, J = 7.8 Hz, CH_3CH_2), 2.02 (s, 3H, C=C–CH_3), 3.07–3.49 (q, 2H, J = 13.8 Hz, CH_2CO), 5.31 (d, 1H, J = 3.2 Hz, NHC*H*), 5.44–5.57 (q, 2H, J = 15.4 Hz, OCH_2), 5.61 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 6.87 (t, 2H, Ar-H), 7.41–7.54 (t, 2H, Ar-H), 7.38 (d, 1H, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.53–7.65 (dd, 4H, J = 8.3 Hz, Ar-H), 7.82 (t, 1H, J = 7.9 Hz, C_{10}-H), 7.95 (t, 1H, J = 7.9 Hz, C_{11}-H), 8.16 (d, 1H, J = 3.5 Hz, NH), 8.27 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.75 (s, 1H, N=CH). MS (ESI): 750 (M−H); Anal. calcd. for $C_{44}H_{41}N_5O_7$: C, 70.29; H, 5.50; N, 9.32. Found: C, 70.43; H, 5.51; N, 9.35.

4t: A yellow powder; yield (65%); mp >300°C; IR (KBr) *v*: 3348, 3061, 2971, 2924, 2851, 1744, 1676, 1655, 1591, 1535, 1509, 1387, 1373, 1279, 1226, 1170, 1030, 850, 831, 760, 721, 638. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.11 (t, 3H, J = 6.7 Hz, NCH_2CH_3), 1.87 (q, 2H, J = 7.5 Hz, CH_3CH_2), 2.16 (s, 3H, $OCCH_3$), 2.24 (s, 3H, C=C–CH_3), 3.06–3.48 (q, 2H, J = 13.6 Hz, CH_2CO), 3.62–3.85 (d q, 2H, J = 7.0 Hz, NCH_2CH_3), 5.28 (d, 1H, J = 3.5 Hz, NHC*H*), 5.40–5.56 (q, 2H, J = 15.0 Hz, OCH_2), 5.56 (s, 2H, NCH_2), 6.04 (s, 1H, OH), 7.39–7.55 (dd, 4H, J = 8.3 Hz, Ar-H), 7.45 (s, 1H, C_{14}-H), 7.82 (t, 1H, J = 8.0 Hz, C_{10}-H), 7.94 (t, 1H, J = 8.1 Hz, C_{11}-H), 8.05 (d, 1H, J = 3.3 Hz, N*H*CH), 8.26 (d, 1H, J = 8.4 Hz, C_{12}-H), 8.98 (d, 1H, J = 8.5 Hz, C_9-H), 9.69 (s, 1H, N = CH). MS (ESI): 644 (M−H); Anal. calcd. for $C_{37}H_{35}N_5O_6$: C, 68.82; H, 5.46; N, 10.85. Found: C, 68.97; H, 5.45; N, 10.83.

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.

Topoisomerase I inhibition 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 reaction was incubated at 37°C for 15 min. Reactions were 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, Bromophenol 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.

This research was supported in part by the National Natural Science Foundation of China (No. 30371689), the Key Project of Science and Technology of Shanghai (No. 09431901700), Major Special Project for the Creation of New Drugs (Grant Nos. 2009ZX09301-011).

The authors have declared no conflict of interest.

References

[1] M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, G. A. Sim, *J. Am. Chem. Soc.* **1966**, *88*, 3888–3890.

[2] Y. H. Hsiang, R. Hertzberg, S. Hecht, L. F. Liu, *J. Biol. Chem.* **1985**, *260*, 14873–14878.

[3] Y. H. Hsiang, M. G. Lihou, L. F. Liu, *Cancer Res.* **1989**, *49*, 5077–5082.

[4] H. Ulukan, P. W. Swaan, *Drugs* **2002**, *62*, 2039–2057.

[5] O. Lavergne, L. Lesueur-Ginot, F. P. Rodas, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2235–2238.

[6] C. Bailly, A. Lansiaux, L. Dassonneville, D. Demarquay, H. Coulomb, M. Huchet, O. Lavergne, D. C. Bigg, *Biochemistry* **1999**, *38*, 15556–15563.

[7] O. Lavergne, L. Lesueur-Ginot, R. F. Pla, P. G. Kasprzyk, J. Pommier, D. Demarquay, G. Prevost, G. Ulibarri, A. Rolland, A. M. Schiano-Liberatore, J. Harnett, D. Pons, J. Camara, D. C. Bigg, *J. Med. Chem.* **1998**, *41*, 5410–5419.

[8] O. Lavergne, D. Demarquay, C. Bailly, C. Lanco, A. Rolland, M. Huchet, H. Coulomb, N. Muller, N. Baroqqi, J. Camara, C. Le Breton, E. Manqinot, J. B. Cazaux, D. C. Bigg, *J. Med. Chem.* **2000**, *43*, 2285–2289.

[9] R. S. Tangirala, S. Antony, K. Agama, Y. Pommier, B. D. Anderson, R. Bevins, D. P. Curran, *Bioorg. Med. Chem.* **2006**, *14*, 6202–6212.

[10] H. Gelderblom, R. Salazar, J. Verweij, G. Pentheroudakis, M. J. de Jonge, M. Devlin, C. van Hooije, F. Seguy, R. Obach, J. Prunonosa, P. Principe, C. Twelves, *Clin. Cancer Res.* **2003**, *9*, 4101–4107.

[11] D. Demarquay, M. Huchet, H. Coulomb, L. Lesueur-Ginot, O. Lavergne, J. Camara, P. G. Kasprzyk, G. Prevost, D. C. Bigg, *Cancer Res.* **2004**, *64*, 4942–4949.

[12] L. J. Zhu, Z. Y. Miao, C. Q. Sheng, W. Guo, J. Z. Yao, W. F. Liu, X. Y. Che, W. Y. Wang, P. F. Cheng, W. N. Zhang, *Eur. J. Med. Chem.* **2010**, *45*, 2726–2732.

[13] Z. Y. Miao, C. Q. Sheng, W. N. Zhang, H. T. Ji, J. Zhang, L. C. Shao, L. You, M. Zhang, J. Z. Yao, X. Y. Che, *Bioorg. Med. Chem.* **2008**, *16*, 1493–1510.

[14] Z. Y. Miao, J. Zhang, L. You, J. Wang, C. Q. Sheng, J. Z. Yao, W. N. Zhang, H. Feng, W. Guo, L. Zhou, W. F. Liu, L. J. Zhu, P. F. Cheng, X. Y. Che, W. Y. Wang, C. Luo, Y. L. Xu, G. Q. Dong, *Bioorg. Med. Chem.* **2010**, *18*, 3140–3146.

[15] F. M. H. de Groot, G. F. Busscher, R. W. M. Aben, H. W. Scheeren, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2371–2376.

[16] V. R. Caiolfa, M. Zamai, A. Fiorino, E. Frigerio, C. Pellizzoni, R. d'Argy, A. Ghiglieri, M. G. Castelli, M. Farao, E. Pesenti, M. Gigli, R. Angelucci, A. Suarato, *J. Controlled Release* **2000**, *65*, 105–119.

[17] M. A. Walker, G. M. Dubowchik, S. J. Hofstead, P. A. Trail, R. A. Firestone, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 217–219.

[18] J.-Y. Chang, X. Guo, H.-X. Chen, Z. Jiang, Q. Fu, H.-K. Wang, K. F. Bastow, X.-K. Zhu, J. Guan, K.-H. Lee, Y.-C. Cheng, *Biochem. Pharmacol.* **2000**, *59*, 497–508.

[19] C. O. Kappe, *Acc. Chem. Res.* **2000**, *33*, 879–888; and references cited therein.

[20] B. Jauk, T. Pernat, C. O. Kappe, *Molecules* **2000**, *5*, 227–239.

[21] A. D. Patil, N. V. Kumar, W. C. Kokke, M. F. Bean, A. J. Freyer, C. Debrossi, S. Mai, A. Truneh, D. J. Faulkner, B. Carte, A. L. Breen, R. P. Hertzberg, R. K. Johnson, J. W. Westley, B. C. M. Potts, *J. Org. Chem.* **1995**, *60*, 1182–1188.

[22] B. B. Snider, J. Chen, A. D. Patil, A. Freyer, *Tetrahedron Lett.* **1996**, *37*, 6977–6980.

[23] T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. I. Schreiber, T. J. Mitchison, *Science* **1999**, *268*, 971–974.

[24] D. Russowsky, R. F. S. Canto, S. A. A. Sanches, M. G. M. DOca, A. de Fatima, R. A. Pilli, L. K. Kohn, M. A. Antonio, J. E. de Carvalho, *Bioorg. Chem.* **2006**, *34*, 173–182.

[25] P. Hamet, *Hypertension* **1996**, *28*, 321–324.

[26] E. Grossman, F. H. Messerli, V. Boyko, U. Goldbourt, *Am. J. Med.* **2002**, *112*, 479–486.

[27] B. L. Staker, M. D. Feese, M. Cushman, Y. Pommier, D. Zembower, L. Stewart, A. B. Burgin, *J. Med. Chem.* **2005**, *48*, 2336–2345.

[28] S. V. Ryabukhin, A. S. Plaskon, E. N. Ostapchuk, D. M. Volochnyuk, O. V. Shishkin, A. A. Tolmachev, *J. Fluorine Chem.* **2008**, *129*, 625–631.

[29] S. Sawada, K. Nokata, T. Furuta, T. Yokokura, T. Miyasaka, *Chem. Pharm. Bull.* **1991**, *39*, 2574–2580.

