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Synthesis and antitumor activity of 20-*O*-linked nitrogen-based camptothecin ester derivatives

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Abstract—A series of nitrogen-based 20*S*-hydroxyl camptothecin ester derivatives were prepared. 3-Aminopropionate of camptothecin was found more cytotoxic in vitro on several human tumor cell lines than 3-amidopropionate of camptothecin. Ester **16** showed best antitumor activity in vivo and in vitro in all esters we prepared.

1. Introduction

Camptothecin (CPT, **1**) derivatives are a class of promising antitumor agents. Irinotecan and topotecan (Fig. 1) are A-, B-ring modified analogues of camptothecin that are now being marketed in many countries, and a number of other analogues are the subject of ongoing clinical or preclinical evaluation.[1]

The camptothecins act by binding to the topoisomerase I-DNA complex, leading to an accumulation of DNA strand breaks upon replication, ultimately causing cell death.[2,3] The closed lactone E-ring is essential for activity based on the established mechanism of action. For camptothecins, a major problem is the opening of the lactone E-ring and the formation of an equilibrium between the ring closed lactone form and the open carboxylate form. Particularly, human serum albumin (HSA) preferentially binds to the carboxylate form of camptothecin derivatives, shifting the equilibrium in favor of the carboxylate.[4] There is an intramolecular hydrogen bonding in the E-ring of CPT molecule, it would not only activate the lactone but also diminish the interaction with the enzyme. However, the esterification of 20-hydroxyl group can either eliminate the intramolecular hydrogen bonding and increase the steric hindrance of carbonyl group of E-ring, so lactone ring stability was improved in vivo.

In previous studies[5] we prepared many CPT oxyacetic acid ester derivatives. These derivatives of 20-hydroxyl ester possessed excellent anticancer activity and low

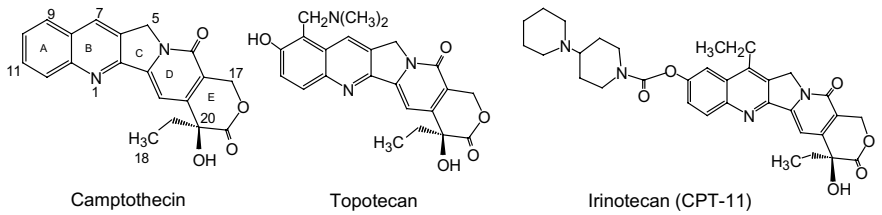

Figure 1. Structure of CPT, topotecan, and irinotecan.

Keywords: Camptothecin; Synthesis; Antitumor activity; 3-Aminopropionic acid.
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toxicity in vivo. Vishnuvajjala and Garzon-Aburbeh[6] have also prepared some glycin esters of CPT. The prodrug, (*N*,*N*-diethyl)glycinate of CPT, showed better antitumor activity in vivo than CPT. How is the antitumor activity of those derivatives, which oxygen atom of oxyacetic acid was replaced with nitrogen atom. We hypothesized that the opening rate of the intact lactone ring E could be remarkably reduced if the (20*S*)-hydroxyl group is esterified with an amino acid, so that the toxicity of these newly modified CPT derivatives could be drastically decreased and the water solubility could be improved, but their antitumor activity could be markedly increased. In this paper, a series of nitrogen-based (20*S*) CPT esters (**6**–**18**) were synthesized and tested for their in vitro and in vivo antitumor activity.

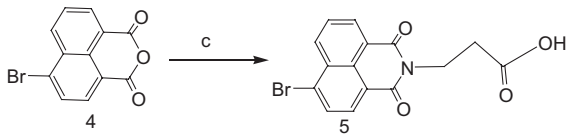
2. Results and discussion

2.1. Chemistry

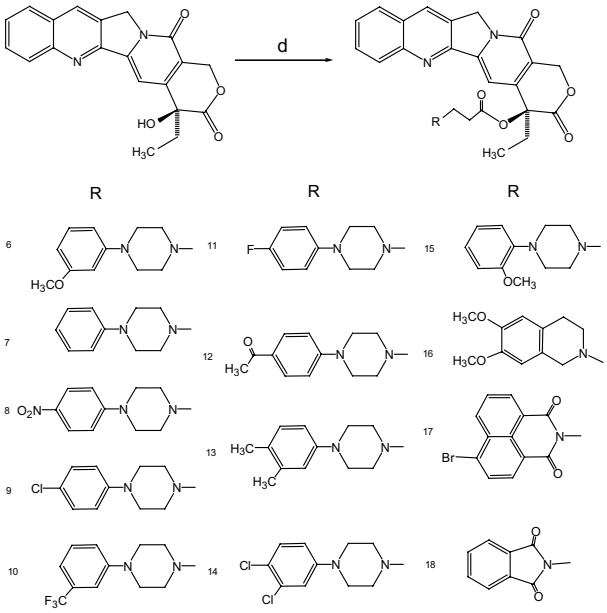
All 3-(substituted-phenyl)-1-piperazinylpropionic acids were prepared according to the literature.[7] 3-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)propionic acid was prepared in good yield with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and ethyl 3-bromopropionate according to known method (Scheme 1). 3-(4-Bromo-1,8-naphthalimido)propionic acid was synthesized with 4-bromo-1,8-naphthalic anhydride with β-alanine according to the method[8] (Scheme 2). The target esters of nitrogen-based CPT were prepared in proper yields by the straightforward acylation of CPT with the corresponding 3-amino- or 3-amido- propionic acid in the presence of a doubling agent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and a catalyst 4-dimethylaminopyridine (DMAP) at room temperature (Scheme 3). EDCI can reserve the configuration of the chrial reactants to a greater extent in the esterifying reaction.[9] We tried to substitute 2-(1-piperazinyl)acetic acid for 3-(1-piperazinyl)propionic acid, but no target compound was obtained. This may relate to the high steric hindrance of aryl-piperazinyl on CPT's structure. The ^{1}H NMR and mass spectra of these compounds were consistent with their structures.

2.2. Cytotoxicity studies

Cytotoxicities of nitrogen-based camptothecin ester derivatives were evaluated on six human cancer cell lines (KB, KB/VCR, A549, HCT-8, Bel7402, and A2780) using MTT assay. Topotecan and CPT were used as reference compounds. CPT possessed strong cytotoxic activity on all cancer cells. These ester compounds (Table 1) showed comparable or superior cytotoxic activity to topotecan. Most of them showed less cytotoxic compared with CPT. Two piperzinylpropionate **7** and **11** had more efficacy on two kinds of cancer cell lines, as same as CPT's. Compound **16** showed best cytotoxic activity in all esters, its IC_{50} value is in nanomolar on three cell lines. Ester **17** and **18** with 3-amidopropionate were shown to be 10–100 times less active than CPT in the assay of cytotoxic activity. Antitumor activity in vitro of 3-aminopropionate (**6**–**16**) of CPT was better than that of 3-amidopropionate (**17**, **18**). This result is likely due to that nitrogen atom of 3-aminopropionate possesses higher electronic cloud density than that of 3-amidopropionate, and forms more easily hydrogen bonding with receptor.

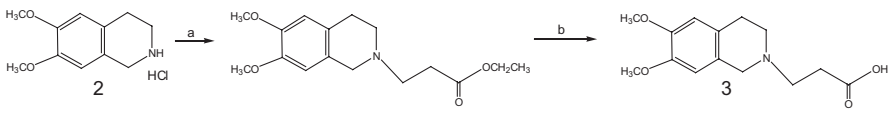


Scheme 2. Reagents: (c) β-alanine/DMAP/ethanol.



Scheme 3. Reagents: (d) RCH_2CH_2COOH/EDCI/DMAP/CH_2Cl_2.

2.2.1. In vivo toxicity and antitumor efficacy studies. In vivo preliminary toxicity study of ester **7**, **11**, and **16** showed that the MTD (maximum tolerance dose) values (over 60 mg/kg) of new camptothecin derivatives were higher than CPT and topotecan, indicating that these



Scheme 1. Reagents: (a) ethyl 3-bromopropionate/$NaHCO_3$/acetone; (b) 5% NaOH/dioxane, HCl.

Table 1. Cytotoxicity of camptothecin esters against six human cancer cell lines

Compound	$IC_{50}/\mu mol\,L^{-1}$					
	KB	KB/VCR	A2780	A549	HCT-8	Bel7402
Topotecan	0.062	0.339	0.058	0.087	0.074	0.078
CPT	0.009	0.009	0.007	0.008	0.007	0.007
6	0.043	0.100	0.056	0.051	0.042	0.009
7	0.013	0.080	0.027	0.010	0.023	0.008
8	0.054	0.384	0.067	0.068	0.089	0.059
9	0.067	0.092	0.069	0.091	0.076	0.064
10	0.007	0.179	0.016	0.046	0.052	0.018
11	0.043	0.084	0.056	0.055	0.008	0.007
12	0.065	0.129	0.089	0.088	0.066	0.065
13	0.056	0.098	0.092	0.094	0.055	0.051
14	0.071	0.297	0.291	0.255	0.077	0.077
15	0.055	0.177	0.080	0.280	0.065	0.047
16	0.009	0.086	0.083	0.088	0.009	0.009
17	0.673	>1.0	>1.0	>1.0	0.862	0.783
18	0.716	>1.0	>1.0	>1.0	0.817	0.802

KB: human epidermoid carcinoma of the nasopharynx; KB/VCR: vincristine-resistant KB; A549: human lung cancer; HCT-8: human colon cancer; Bel7402: human liver cancer; A2780: human ovarian cancer.

Table 2. Inhibitory effect of camptothecin esters on H_{22} transplanted mice liver tumor

Group	Mice number		Body weight (g)		TIR[a] (%)	*P* value	Dose (mg/kg)	MTD[b] (mg/kg)
	Begin	End	Begin	End				
Control	10	10	18.8	25.5	—			
Topotecan	10	10	18.3	18.6	70	<0.01	5×2	13
CPT	10	10	19.0	22.8	54	<0.01	12×1	12
7	10	10	19.0	26.6	47	<0.05	30×3	60
11	10	10	18.6	25.8	57	<0.01	30×3	60
16	10	10	18.7	25.5	72	<0.01	30×3	60

[a] Tumor inhibitory rate.
[b] MTD is approximate value.

esters were less toxic to normal mice than the parent drug, CPT. In vivo primary evaluation of antitumor activity of ester **7**, **11**, and **16** were performed on mice bearing mouse liver adenocarcinoma (H_{22}). Considering the character of CPT derivatives, we chosen a dose of 30 mg/kg (about half of MTD) as treating dose of mice, and mice was administered for three times. As a result, the best antitumor result of compound **16** was obtained. The data in Table 2 showed that the esterifying of 20-hydroxyl group significantly reduced the toxicity of CPT analogues. This result was consistent with our SAR hypothesis of camptothecin.[5] Topotecan was treated at a dose of 5 mg/kg of body weight against mice liver tumor because it has higher toxicity. The tumor inhibitory rate (TIR) of ester **16** was higher obviously than that of CPT, higher slightly than that of topotecan at dose of 30 mg/kg. Although 3-piperazinylpropionate **7** and **11** had lower IC_{50} value than topotecan, they showed poorer antitumor activity in vivo. Ester **16** possessed both lower IC_{50} value in vitro and better antitumor activity in vivo as compared with topotecan. These results suggested this kind of compound had promising developing perspective.

3. Conclusions

Thirteen nitrogen-based CPT esters were prepared according to known method. Their in vitro and in vivo antitumor activity was evaluated. All these esters showed lower toxicity in vivo than CPT and topotecan. 3-Aminopropionate of camptothecin possessed more efficacy than 3-amidopropionate in vitro. Compound **16** showed best antitumor activity in vitro and in vivo in all esters we prepared.

3.1. Experimental

Melting points were determined in Yanaco melting point apparatus and are uncorrected. Flash column chromatography was carried out on gel 100–200 mesh. TLC analysis was carried out on silica gel plates GF_{254}. IR spectra were recorded at NICOLET FT instrument. ^1H NMR spectra were recorded at 300 or 600 MHz with a Bruker instrument. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. MS (EI) was performed on a VG ZAB-2f 220C spectrometer. HR-MS (EI or ESI) was recorded on ZAB-HS instrument.

3.1.1. 3-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)propionic acid (3). The reaction mixture 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (995 mg, 4.3 mmol), ethyl 3-bromopropionate (1000 mg, 5.5 mmol), sodium bicarbonate (1000 mg, 11.9 mmol) and ethanol (30 mL) was refluxed for 7 h till the amine

disappeared completely. The mixture was filtered, the resulting solid was dissolved in 7 mL dioxane and 20 mL 5% sodium hydroxide solution. After the mixture was stirred for 4 h at room temperature, it was acidified to pH 4.0 with concentrated hydrochloride acid. Solid was filtered and washed with water, and then dried to give 3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)propionic acid(750 mg, 65.8%), mp 186 °C; IR (KBr) ν 2939, 2696, 2605, 1724, 1522, 1228, 1124, 999, 856, 795, 660 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 6.80 (s, 1H, Ar–H), 6.74 (s, 1H, Ar–H), 4.27 (s, 2H, NCH_2Ar), 3.72 (d, $J = 3$ Hz, 6H, OCH_3), 3.40 (m, 4H, NCH_2CH_2Ar), 2.93 (d, $J = 7.2$ Hz, 4H, NCH_2CH_2CO). MS (ESI): m/z 266 $(M+H)^+$; HRMS (ESI): m/z calcd for $C_{14}H_{19}NO_4$+H 266.1387, found 266.1385.

3.1.2. 3-(4-Bromo-1,8-naphthalimide)propionic acid (5). The reaction mixture of 4-bromo-1,8-naphthalic anhydride (277 mg, 1.0 mmol), β-alanine (120 mg, 1.3 mmol), DMAP (10 mg, 0.1 mmol), and ethanol (15 mL) was refluxed for 4 h. The mixture was filtered, and the solid was washed with ethanol, and then dried in the oven to give 3-(4-bromo-1,8-naphthalimide)propionic acid (300 mg, 86.5%) as a gray solid, mp 223–225 °C, IR (KBr) ν 2906, 2630, 1699, 1651, 1589, 1344, 1230, 783, 648, 484, 424 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 8.58 (t, $J = 6.6$ Hz, 2H, Ar–H), 8.35 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.24 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.01 (t, $J = 8.4$ Hz, 1H, Ar–H), 4.25 (t, $J = 7.8$ Hz, 2H, NCH_2), 2.59 (t, $J = 7.8$ Hz, 2H, $COCH_2$); MS (ESI): m/z 348 $(M+H)^+$; HRMS (ESI): m/z calcd for $C_{15}H_{10}BrNO_4$+H 347.9866, found 347.9866.

3.1.3. Camptothecin-20-*O*-3-[4-(3-methoxyphenyl)-1-piperazinyl]propionate (6). A mixture of camptothecin (30 mg, 0.087 mmol), 3-[4-(3-methoxyphenyl)-1-piperazinyl]propionic acid (46 mg, 0.17 mmol), EDCI (80 mg, 0.42 mmol), DMAP (6 mg, 0.056 mmol), and dichloromethane (5 mL) was stirred at room temperature for 7 h. Then chloroform (30 mL) was added, organic phase was washed with water (20 mL), saturated sodium bicarbonate aqueous solution (10 mL), and brine (20 mL), and then dried over magnesium sulfate. After the solvent was removed under reduced pressure, the residue was taken up in chloroform and chromatographed (eluent: $CHCl_3$–CH_3OH 97:3) to gave **6** as a pale yellow solid (44 mg, 85.1%), mp 180–182 °C, IR (KBr) ν 3435, 2943, 2829, 1751, 1662, 1603, 1496, 1454, 1171, 1047, 762 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.35 (s, 1H, Ar–H), 8.07 (d, $J = 9.0$ Hz, 1H, Ar–H), 7.90 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.71 (m, 1H, Ar–H), 7.64 (m, 1H, Ar–H), 7.31 (s, 1H, Ar–H), 7.05 (s, 1H, Ar–H), 6.40 (m, 3H, Ar–H), 5.70 (d, $J = 17.4$ Hz, 1H, H17), 5.40 (d, $J = 16.8$ Hz, 1H, H17), 5.28 (s, 2H, H15), 3.76 (d, $J = 36.6$ Hz, 4H, $OOCCH_2CH_2$), 3.20 (s, 3H, OCH_3), 2.71 (m, 8H, NCH_2CH_2N), 2.17 (dm, 2H, 19-CH_2), 1.00 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 594 (M^+), 402, 330, 302, 287, 263, 205, 192, 150; HRMS (EI): m/z calcd for $C_{34}H_{34}N_4O_6$ 594.2478, found 594.2490.

3.1.4. Camptothecin-20-*O*-3-(4-phenyl-1-piperazinyl)propionate (7). The titled compound was prepared from CPT and 3-(4-phenyl-1-piperazinyl)propionic acid according to the method of compound **6**, yield: 77.6%, mp 200–202 °C, IR (KBr): ν 3456, 2935, 2821, 1757, 1670, 1624, 1500, 1232, 1132, 764 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.38 (s, 1H, Ar–H), 8.07 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.91 (d, $J = 7.5$ Hz, 1H, Ar–H), 7.65 (m, 2H, Ar–H), 7.29 (d, $J = 11.1$ Hz, 1H, Ar–H), 7.13 (t, $J = 7.5$ Hz, 2H, Ar–H), 6.80 (m, 3H, Ar–H), 5.72 (d, $J = 17.1$ Hz, 1H, H17), 5.42 (d, $J = 17.7$ Hz, 1H, H17), 5.25 (s, 2H, H15), 3.19 (m, 4H, $OOCCH_2CH_2N$), 2.71 (m, 8H, NCH_2CH_2N), 2.19 (dm, 2H, 19-CH_2), 0.99 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 564 (M^+), 330, 302, 287, 233, 175, 120, 105; HRMS (EI): m/z calcd for $C_{33}H_{32}N_4O_5$ 564.2373, found 564.2360.

3.1.5. Camptothecin-20-*O*-3-[4-(4-nitrophenyl)-1-piperazinyl]propionate (8). The titled compound was prepared from CPT and 3-[4-(4-nitrophenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 77.4%, mp 255–257 °C, IR (KBr) ν 3467, 2976, 1745, 1618, 1595, 1489, 1321, 1240, 1113, 933, 827, 727 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.34 (s, 1H, Ar–H), 7.91 (m, 4H, Ar–H), 7.62 (m, 2H, Ar–H), 7.30 (s, 1H, Ar–H), 6.55 (d, $J = 9.6$ Hz, 2H, Ar–H), 5.70 (d, $J = 16.8$ Hz, 1H, H17), 5.41 (d, $J = 16.8$ Hz, 1H, H17), 5.28 (s, 2H, H15), 3.39 (dm, 4H, $OOCCH_2CH_2N$), 2.64 (m, 8H, NCH_2CH_2N), 2.19 (dm, 2H, 19-CH_2), 0.99 (t, $J = 7.8$ Hz, 3H, 18-CH_3); MS (EI): m/z 609 (M^+), 592, 402, 330, 302, 287, 279, 220, 165; HRMS (EI): m/z calcd for $C_{33}H_{31}N_5O_7$ 609.2223, found 609.2210.

3.1.6. Camptothecin-20-*O*-3-[4-(4-chlorophenyl)-1-piperazinyl]propionate (9). The titled compound was prepared from CPT and 3-[4-(4-chlorophenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 76.9%, mp 217–219 °C, IR (KBr) ν 3465, 2939, 2821, 1755, 1662, 1616, 1496, 1232, 1132, 816, 760, 723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.33 (s, 1H, Ar–H), 7.97 (m, 2H, Ar–H), 7.66 (m, 2H, Ar–H), 7.28 (d, $J = 7.5$ Hz, 1H, Ar–H), 7.01 (d, $J = 9.3$ Hz, 2H, Ar–H), 6.60 (d, $J = 8.7$ Hz, 2H, Ar–H), 5.71 (d, $J = 17.7$ Hz, 1H, H17), 5.43 (d, $J = 17.1$ Hz, 1H, H17), 5.25 (s, 2H, H15), 3.11 (s, 4H, $OOCCH_2CH_2N$), 2.79 (d, $J = 16.5$ Hz, 8H, NCH_2CH_2N), 2.16 (dm, 2H, 19-CH_2), 0.98 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 598 (M^+), 402, 330, 302, 268, 209, 196, 154, 139; HRMS (EI): m/z calcd for $C_{33}H_{31}ClN_4O_5$ 598.1983, found 598.1991.

3.1.7. Camptothecin-20-*O*-3-[4-(3-trifluoromethylphenyl)-1-piperazinyl]propionate (10). The titled compound was prepared from CPT and 3-[4-(3-trifluoromethylphenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 61.8%, mp 87–90 °C, IR (KBr) ν 3456, 2821, 1721, 1747, 1664, 1608, 1450, 1319, 1234, 1165, 1124, 787 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.34 (s, 1H, Ar–H), 8.00 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.89 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.62 (m, 2H, Ar–H), 7.30 (d,

$J = 24$ Hz, 1H, Ar–H), 6.97 (m, 3H, Ar–H), 5.68 (d, $J = 16.8$ Hz, 1H, H17), 5.42 (d, $J = 16.8$ Hz, 1H, H17), 5.27 (s, 2H, H15), 3.20 (m, 4H, $COCH_2CH_2N$), 2.71 (m, 8H, NCH_2CH_2N), 2.17 (dm, 2H, 19-CH_2), 0.99 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 632 (M^+), 330, 302, 287, 243, 173; HRMS (EI): m/z calcd for $C_{34}H_{31}F_3N_4O_5$ 632.2247, found 632.2225.

3.1.8. Camptothecin-20-*O*-3-[4-(4-fluorophenyl)-1-piperazinyl]propionate (11). The titled compound was prepared from CPT and 3-[4-(4-fluorophenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 95.2%, mp 162–165 °C, IR (KBr) v 3448, 2819, 1751, 1664, 1616, 1510, 1232, 1163, 816, 762, 723 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.36 (s, 1H, Ar–H), 8.04 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.92 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.71 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.66 (d, $J = 7.8$ Hz, 1H, Ar–H), 7.28 (d, $J = 12.6$ Hz, 1H, Ar–H), 6.80 (m, 2H, Ar–H), 6.66 (m, 2H, Ar–H), 5.71 (d, $J = 16.8$ Hz, 1H, H17), 5.42 (d, $J = 16.8$ Hz, 1H, H17), 5.27 (s, 2H, H15), 3.11 (s, 4H, $OOCCH_2CH_2N$), 2.63 (m, 8H, NCH_2CH_2N), 2.27 (dm, 2H, 19-CH_2), 0.99 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 582 (M^+), 402, 330, 302, 251, 193, 138, 123; HRMS (EI): m/z calcd for $C_{33}H_{31}FN_4O_5$ 582.2279, found 582.2286.

3.1.9. Camptothecin-20-*O*-3-[4-(4-acetylphenyl)-1-piperazinyl]propionate (12). The titled compound was prepared from CPT and 3-[4-(4-acetylphenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 75.9%, mp 224–227 °C, IR (KBr) v 3481, 2831, 1747, 1664, 1597, 1361, 1236, 1051, 825, 762 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.34 (s, 1H, Ar–H), 7.88 (dd, 2H, Ar–H), 7.72 (dd, 2H, Ar–H), 7.30 (m, 3H, Ar–H), 6.66 (d, $J = 7.8$ Hz, 2H, Ar–H), 5.68 (d, $J = 16.8$ Hz, 1H, H17), 5.42 (d, $J = 16.8$ Hz, 1H, H17), 5.27 (s, 2H, H15), 3.35 (d, $J = 10.2$ Hz, 4H, $OOCCH_2CH_2N$), 2.64 (m, 8H, NCH_2CH_2N), 2.50 (s, 3H, $ArCOCH_3$), 2.17 (dm, 2H, 19-CH_2), 1.00 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 606 (M^+), 402, 330, 302, 276, 217, 204, 162, 132; HRMS (EI): m/z calcd for $C_{35}H_{34}N_4O_6$ 606.2478, found 606.2467.

3.1.10. Camptothecin-20-*O*-3-[4-(3,4-dimethylphenyl)-1-piperazinyl]propionate (13). The titled compound was prepared from CPT and 3-[4-(3,4-dimethylphenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 85.3%, mp 190–192 °C, IR (KBr) v 3475, 2925, 2817, 1751, 1662, 1232, 1157, 762, 723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.39 (s, 1H, Ar–H), 8.25 (d, $J = 9.3$ Hz, 1H, Ar–H), 8.08 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.68 (dt, 2H, Ar–H), 7.28 (d, $J = 9.3$ Hz, 1H, Ar–H), 6.89 (d, $J = 8.4$ Hz, 1H, Ar–H), 6.60 (s, 1H, Ar–H), 6.50 (m, 1H, Ar–H), 5.72 (d, $J = 18$ Hz, 1H, H17), 5.42 (d, $J = 16.2$ Hz, 1H, H17), 5.26 (s, 2H, H15), 3.14 (s, 4H, $OOCCH_2CH_2N$), 2.80 (d, $J = 41.4$ Hz, 8H, NCH_2CH_2N), 2.18 (m, 2H, 19-CH_2), 2.13 (s, 6H, Ar-CH_3), 0.98 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 592 (M^+), 402, 330, 302, 287, 261, 203, 190, 148, 133; HRMS (EI): m/z calcd for $C_{35}H_{36}N_4O_5$ 592.2686, found 592.2692.

3.1.11. Camptothecin-20-*O*-3-[4-(3,4-dichlorophenyl)-1-piperazinyl]propionate (14). The titled compound was prepared from CPT and 3-[4-(3,4-dichlorophenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 80.0%, mp 201–203 °C, IR (KBr) v 2945, 2827, 1757, 1672, 1622, 1483, 1232, 1130, 1051, 955, 808, 756, 721 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.34 (s, 1H, Ar–H), 7.96 (m, 2H, Ar–H), 7.69 (m, 2H, Ar–H), 7.27 (d, $J = 6.0$ Hz, 1H, Ar–H), 7.02 (d, $J = 9.3$ Hz, 1H, Ar–H), 6.64 (s, 1H, Ar–H), 6.46 (dd, 1H, Ar–H), 5.72 (d, $J = 17.1$ Hz, 1H, H17), 5.43 (d, $J = 17.1$ Hz, 1H, H17), 5.27 (s, 2H, H15), 3.09 (s, 4H, $OOCCH_2CH_2N$), 2.77 (m, 8H, NCH_2CH_2N), 2.25 (dm, 2H, 19-CH_2), 0.98 (t, $J = 7.5$ Hz, 3H, 18-CH_3); MS (EI): m/z 632 (M^+), 402, 332, 302, 287, 245, 243, 190, 188, 128; HRMS (EI): m/z calcd for $C_{33}H_{30}Cl_2N_4O_5$ 632.1593, found 632.1612.

3.1.12. Camptothecin-20-*O*-3-[4-(2-methoxyphenyl)-1-piperazinyl]propionate (15). The titled compound was prepared from CPT and 3-[4-(2-methoxyphenyl)-1-piperazinyl]propionic acid according to the method of compound **6**, yield: 81.2%, mp 98–100 °C, IR (KBr) v 3485, 2939, 2821, 1743, 1662, 1616, 1500, 1238, 1157, 760, 723, 592 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.36 (s, 1H, Ar–H), 8.11 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.93 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.73 (t, $J = 7.5$ Hz, 1H, Ar–H), 7.63 (m, 1H, Ar–H), 7.29 (d, $J = 8.1$ Hz, 1H, Ar–H), 6.93 (m, 1H, Ar–H), 6.75 (m, 3H, Ar–H), 5.72 (d, $J = 17.7$ Hz, 1H, H17), 5.43 (d, $J = 17.7$ Hz, 1H, H17), 5.27 (s, 2H, H15), 3.79 (s, 3H, OCH_3), 3.08 (s, 4H, $OOCCH_2CH_2N$), 2.79 (m, 8H, NCH_2CH_2N), 2.19 (dm, 2H, 19-CH_2), 0.99 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 594(M^+), 402, 330, 332, 302, 287, 263, 205, 192, 150, 133; HRMS (EI): m/z calcd for $C_{34}H_{34}N_4O_6$ 594.2478, found 594.2462.

3.1.13. Camptothecin-20-*O*-3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinin-2-yl)propionate (16). The titled compound was prepared from CPT and 3-(6,7-dimethoxy-2-tetrahydroisoquininyl)propionic acid according to the method of compound **6**, yield: 86.9%, mp 170–172 °C, IR (KBr) v 3429, 2922, 1743, 1658, 1603, 1456, 1232, 1157, 764, 725, 592 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.35 (s, 1H, Ar–H), 7.94 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.83 (m, 1H, Ar–H), 7.66 (m, 1H, Ar–H), 7.28 (d, $J = 12.6$ Hz, 1H, Ar–H), 6.39 (s, 1H, Ar–H), 6.32 (s, 1H, Ar–H), 5.69 (d, $J = 16.8$ Hz, 1H, H17), 5.43 (d, $J = 16.8$ Hz, 1H, H17), 5.22 (dd, 2H, H15), 3.72 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.60 (dd, 2H, NCH_2Ar), 2.90 (m, 2H, CH_2N), 2.79 (m, 6H, $OOCCH_2$, NCH_2CH_2Ar), 2.17 (dm, 2H, 19-CH_2), 0.96 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 402, 330, 302, 164; HRMS (ESI): m/z calcd for $C_{34}H_{33}N_3O_7$+H 596.2391, found 596.2397.

3.1.14. Camptothecin-20-*O*-3-(4-bromo-1,8-naphthalimido)propionate (17). The titled compound was prepared from CPT and 3-(4-bromo-1,8-naphthalimido)propionic acid according to the method of compound **6**, yield: 76.3%, mp 250–252 °C, IR (KBr) ν 3446, 1749, 1664, 1601, 1361, 1232, 1155, 1047, 785, 723 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 8.61 (d, $J = 6.6$ Hz, 1H, Ar–H), 8.49 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.38 (m, 2H, Ar–H), 8.19 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.95 (m, 1H, Ar–H), 7.76 (m, 3H, Ar–H), 7.29 (d, $J = 20.4$ Hz, 1H, Ar–H), 5.67 (d, $J = 16.8$ Hz, 1H, H17), 5.42 (d, $J = 16.8$ Hz, 1H, H17), 5.30 (m, 2H, H15), 4.50 (t, $J = 7.8$ Hz, 2H, CH_2NCO), 3.02 (m, 2H, $OOCCH_2$), 2.30 (dm, 2H, 19-CH_2), 0.94 (t, $J = 7.2$ Hz, 3H, 18-CH_3); MS (EI): m/z 677(M^+), 635, 349, 330, 302, 287, 275; HRMS (EI): m/z calcd for $C_{35}H_{24}BrN_3O_7$ 677.0798, found 677.0800.

3.1.15. Camptothecin-20-*O*-3-phthalimidopropionate (18). The titled compound was prepared from CPT and 3-phthalimidopropionic acid according to the method of compound **6**, yield: 97.5%, mp 158–161 °C, IR (KBr) ν 3429, 1751, 1712, 1664, 1608, 1402, 1377, 1151, 723, 530 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.40 (s, 1H, Ar–H), 8.25 (d, $J = 8.4$ Hz, 1H, Ar–H), 7.95 (d, $J = 8.7$ Hz, 1H, Ar–H), 7.86 (m, 3H, Ar–H), 7.69 (m, 3H, Ar–H), 7.28 (d, $J = 7.5$ Hz, 1H, Ar–H), 5.67 (d, $J = 17.1$ Hz, 1H, H17), 5.41 (d, $J = 17.7$ Hz, 1H, H17), 5.27 (S, 2H, H15), 3.98 (t, $J = 7.8$ Hz, 2H, CH_2N), 2.98 (m, 2H, $OOCCH_2$), 2.21 (dm, 2H, 19-CH_2), 0.94 (t, $J = 7.5$ Hz, 3H, 18-CH_3); MS (EI): m/z 549(M^+), 330, 302, 287, 173, 160; HRMS (EI): m/z calcd for $C_{31}H_{23}N_3O_7$ 549.1576, found 549.1552.

3.2. MTT assay

One thousand two hundred cells per well were plated in 96-well plates. After culturing for 24 h, test compounds were added onto triplicate wells with different concentration, and 0.1% DMSO for control. After four days of incubation, 10 μL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution (5 mg mL^{-1}) was added to each well, and after shaking for 1 min the plate was incubated further for 4 h. Formazan crystals were dissolved with 100 μL DMSO. The absorbance (OD) was quantitated with microplate spectrophotometer at 570 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.

3.3. In vivo antitumor activity

Antitumor activity screens of CPT esters were conducted in KM mice bearing H_{22} mouse liver tumor. Briefly, female mice weighing 18–22 g were inoculated s.c. at the left flank with a tumor cell suspension (1×10^6) in 0.2 mL of PBS. The mice were divided in experimental groups after 24 h of inoculation. Groups ($n = 10$) consisted of control, topotecan, and the esters. Mice received treatments, i.p., once per two days. Tumor inhibitory rate (%) was calculated when the control group's median tumor weight reached 2.0–2.4 g and compared with the treatment group's median tumor weight at that time.

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