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Synthesis and antitumor activity of biotinylated camptothecin derivatives as potent cytotoxic agents

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ABSTRACT

A series of biotinylated camptothecin derivatives were designed and synthesized. The key to the synthesis was achieved by employing an esterification reaction and click chemistry. All of the new derivatives were tested for cytotoxicity against five human tumor cell lines, including HL-60, SMMC-7721, A-549, MCF-7, and SW480 with IC_{50} values ranging from 0.13 to 21.53 μM. Most of the derivatives exhibited potent cytotoxicity, especially compound **17** (IC_{50} = 0.13–3.31 μM) and compound **18** (IC_{50} = 0.23–1.48 μM), which exhibited the highest potencies. The structure-activity relationships (SARs) of the biotinylated camptothecin derivatives were discussed for exploring novel anticancer agents.

Introduction

Camptothecin (CPT, **1**) is a natural quinolone alkaloid that was isolated from the Chinese tree *Camptotheca acuminate* in 1966 (Fig. 1), which showed potent antiproliferative activity against a broad spectrum of tumors.[1–3] The compound has a pentacyclic ring system with an asymmetrical center in ring E with a 20*S* configuration. The pentacyclic ring system includes apyrrolo[3,4-*b*]quinoline moiety (rings A, B, and C), a conjugated pyridone (ring D), and a six-membered lactone (ring E) with an *α*-hydroxyl group. In the early stages, clinical trials of the water-soluble sodium carboxylate salt of camptothecin were used because camptothecin itself has poor water solubility. However, this form of camptothecin was discontinued due to its severe side effects including neutropenia, thrombocytopenia, hemorrhagic cystitis, and *G*.I. symptoms with significant diarrhea.[4–6] Interest in CPT and its analogues as anticancer agents was revived by the finding that camptothecin inhibits topoisomerase I (Topo I).[7–10]

Topo I inhibition by CPT results from its capacity to stabilize the Topo I-DNA complex, which can be detected as DNA single-strand breaks by alkaline elution,[7,11,12] leading to the inhibition of DNA and RNA syntheses. The results show that the cytotoxicity of camptothecin arises from irreversible DNA damage triggering cell death.[13] Based on the mechanism and remarkable anticancer activity of CPT, a number of new syntheses and modification of the molecule have been reported in the literature. Among them, two groups of CPT have been developed leading to clinical trials: the water-soluble camptothecin analogues, consistings of toprtecan (Fig. 1, **5**)[14] and trinotecan (Fig. 1, **6**),[15] both of which are now injectable intravenously for human treatment;[16] and the water-insoluble camptothecin analogues, which have been tested in clinical trials, including camptothecin (**1**) and its semisynthetic derivatives, 9-nitrocamptothecin (9-NC, **7**)[17] and 9-aminocamptothecin (9-AC, **8**).[18]

Biotin (vitamin H or vitamin B_7) is a growth promoter at the cellular level and has been used as a suitable targeting agent in several studies.[19–22] Biotin receptors are overexpressed on the surface of cancer cells, such as leukemia (L1210FR), mastocytoma (P815), ovarian (OV 2008, ID8), colon (Colo-26), lung (M109), renal (RENCA, RD0995), and breast (4T1, JC, MMT06056) cancer cell lines.[22] Recently, several studies have shown that biotin-conjugated drug molecules were able to increase the uptake of anticancer drugs in tumor cells.[23–27]

Several CPT derivatives remain successful in the inhibition of Topo I and have offered important information that the unmodified lactone E ring moiety is necessary for the most critical structural feature with respect to antitumor activity. However, the *α*-hydroxylacetone-*δ*-

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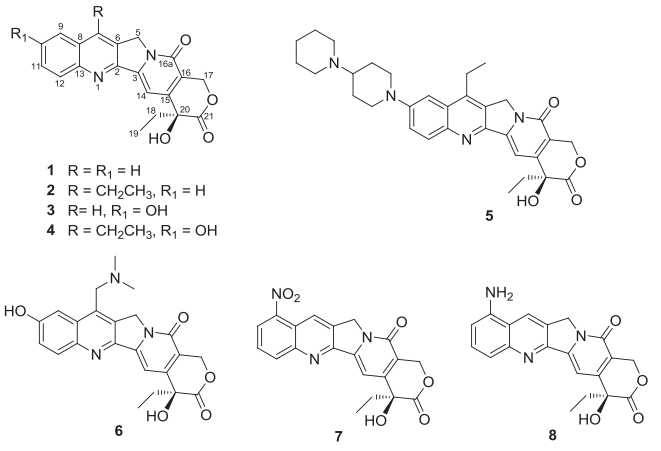


Figure 1. Structures of camptothecin (**1**), 7-ethylcamptotrecin (**2**), 10-hydroxycamptothecin (**3**), 7-ethyl-10-hydroxycamptothecin (**4**), irinotecan (**5**), topotecan (**6**), 9-nitrocamptothecin (**7**), and 9-aminocamptothecin (**8**).

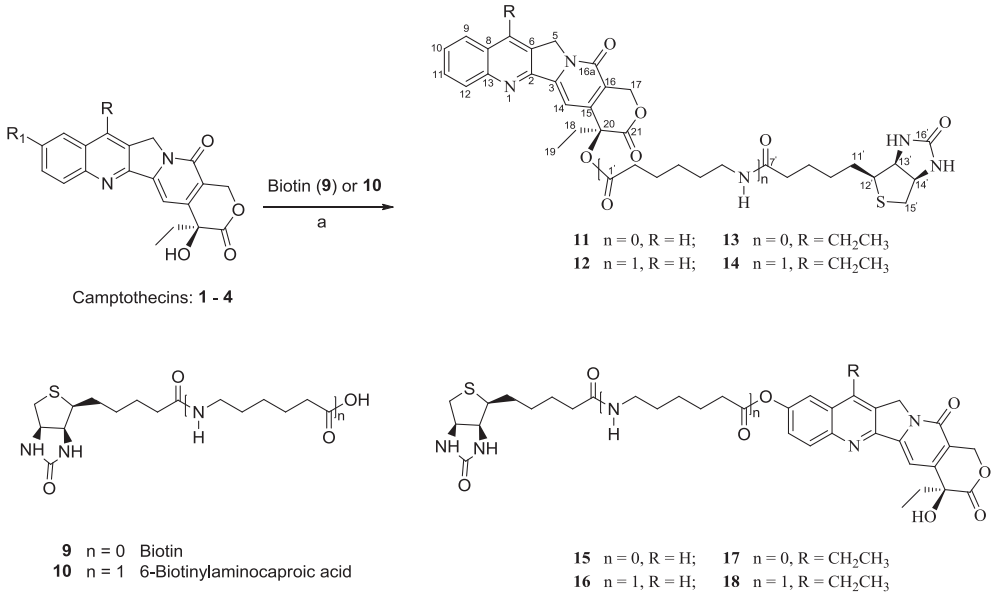
lactone ring rapidly opens to the carboxylate form at physiological pH.[28] Wall et al.[29] demonstrated that the lactone E ring moiety of the molecule is important in drug development. Thus, we propose the intact lactone ring would be better protected if camptothecins were transformed into the corresponding water-soluble esters. In addition, in order to improve the therapeutic potential of camptothecin analogues for cancer therapy, we decide to covalently link biotin to these molecules to synthesize biotin-camptothecin conjugates. Herein, we designed and synthesized a series of biotinylated camptothecin conjugates and assessed their anticancer activities against five human cancer cell lines, including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer).

The coupling between biotin (**9**)/6-biotinylaminocaproic acid (**10**) and camptothecins (**1** – **4**) via an ester bond provide camptothecin derivatives (**11** – **18**). As shown in Scheme 1, biotin (**9**)/6-biotinylaminocaproic acid (**10**) reacted with camptothecin analogues **1** – **4** in the presence of diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP) at room temperature to produce the target compounds **11** – **18** with 39% – 65% yields.[30] As illustrated in Scheme 2, biotinylated camptothecin derivatives **23** – **26** were synthesized by the cycloaddition reaction of *2-propyn-1-yl-camptothecin* **19** and *2-propyn-1-yl-7-ethylcamptothecin* **20** with the biotin-azide derivatives **21**,[31] and **22**.[32] Compounds **19** and **20** were prepared by the treatment of camptothecin (**1**) and 7-ethylcamptothecin (**2**) with sodium hydride and propargyl bromide with 65% and 70% yields. Then, the biotin-azide derivatives **21** and **22** were allowed to react with alkynes **19** and **20**, in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, in ^{t}BuOH/H_2O (1:1) at room temperature to yield selectively biotinylated camptothecin derivatives with 77%–89% yields.[33] All synthesized target compounds were purified by column chromatography, and their structures were characterized by ^{1}H NMR, ^{13}C NMR, electrospray ionization mass spectrometry (ESI-MS) and high-resolution mass spectrometry (HRESI-MS).

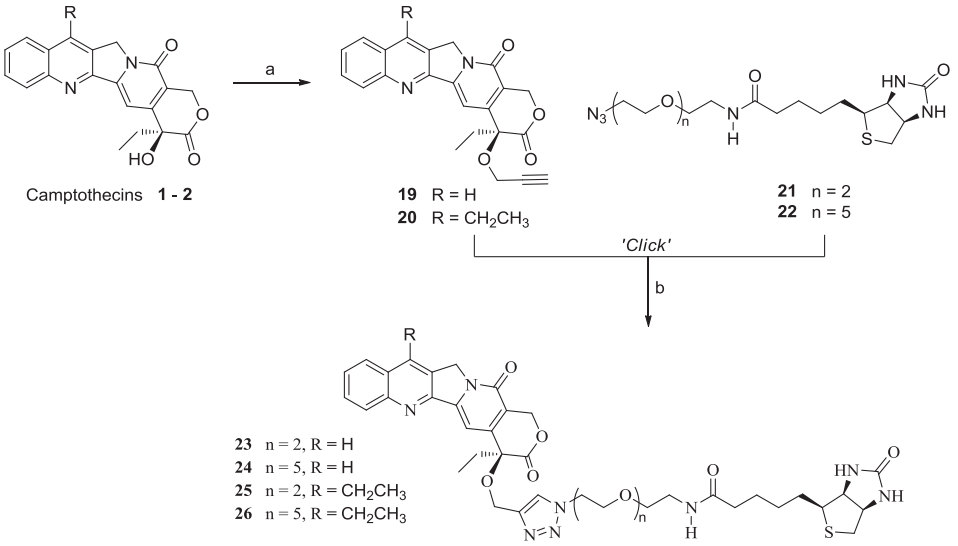
Target compounds **11** – **18** and **23** – **26** were evaluated for in vitro cytotoxicity against a panel of human tumor cell lines, including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer), using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[34] Camptothecin (**1**) and cisplatin were used as positive controls and the screening results are shown in Table 1.

As illustrated in Table 1, most of the new compounds exhibited significant in vitro cytotoxic activity against the five tested tumor cell lines, with IC_{50} values ranging from 0.13 to 21.53 μM. Compounds **24** and **25** showed weak activity (all having IC_{50} > 40 μM). Among them, compound **17** revealed the highest potency against all five cancer cell lines tested, with IC_{50} values ranging from 0.13 to 3.31 μM. Compound **15** (IC_{50} = 0.55–3.32 μM), **16** (IC_{50} = 0.34–1.85 μM), and **18** (IC_{50} = 0.40–1.48 μM) displayed relatively high potency. Compounds **16**, **17**, and **18** were the most potent compounds in the series and were also superior to **1** (IC_{50} = 0.064–0.68 μM) against the MCF-7 cell line, which was in general the most sensitive to these biotinylated camptothecin derivatives. Against the SW480 cell line, many compounds, including **15**, **16**, and **17** also showed better anticancer activity than **1**.

Structure-activity relationships (SARs) analysis results from the synthesized compounds showed that several structural properties could influence the in vitro anticancer activity of the biotinylated camptothecin derivatives. The length of the linking spacer between the biotin and the camptothecin analogues did not exhibit a uniform effect on the cytotoxic potency of these compounds. For example, compound **24** (IC_{50} > 40 μM) with the longest linking spacer (five ethylene glycol repeating units and a 1,2,3-triazole ring) showed weak activity, followed by **23** (IC_{50} = 0.72–6.05 μM) which had a shorter linking spacer (two ethylene glycol repeating units and a 1,2,3-triazole ring), but **26** (IC_{50} = 0.57–9.21 μM) had the same linking spacer as **24** and was more



Scheme 1. Synthesis of biotinylcamptothecins **11** – **18**. Reagents and conditions: a. DIC, DMAP, DMF, 24 h, rt. 39% – 65%.



Scheme 2. Synthesis of biotinylcamptothecins **23 – 26**. Reagentsand conditions: a. NaH, DMF, 0 °C, propargyl bromide, then, reflux, overnight, 65% and 70%; b. $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF, ^{t}BuOH: H_2O (1: 1), 4 h, rt, 77% – 89%.

Table 1
In vitro anticancer activity of biotinylated camptothecin derivatives **11 – 18**, and **23 – 26**[a].

Compounds	IC_{50} (μM)				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
11	2.30	7.69	3.47	18.17	12.52
12	6.57	15.26	19.86	21.53	> 40
13	1.81	3.28	1.14	4.26	8.73
14	0.84	12.23	3.08	1.94	16.25
15	3.19	3.32	1.23	2.30	0.55
16	1.14	1.85	0.75	0.34	0.45
17	3.31	1.55	0.52	0.80	0.13
18	0.76	1.14	0.23	0.40	1.48
23	0.72	4.77	3.63	3.47	6.05
24	> 40	> 40	> 40	> 40	> 40
25	> 40	> 40	> 40	> 40	> 40
26	4.77	4.10	5.18	9.21	0.57
CPT (1)	< 0.064	0.45	0.09	0.68	0.29
Cisplatin	1.17	6.43	9.24	15.86	13.42

[a] The in vitro cytotoxicity of the camptothecin derivatives against five cell lines, HL-60 (human leukemia), SMMC-7721 (human hepatoma), A-549 (human lung cancer), MCF-7 (human breast cancer), and SW480 (human colon cancer) was measured by the MTT assay and expressed as the half maximal inhibitory concentration (IC_{50}, μmol L^{-1}).

active than **24**. Compounds **14** and **16** with the linking spacer (6-aminocaproic acid) were less active than compounds **13** and **15**, which had no linking spacers. However, compound **17** with no linking spacer was more active than compound **18** (with a 6-aminocaproic acid linker and was the most active compound in those tested against the five cancer cell lines, with IC_{50} values ranging from 0.13 to 3.31 μM. Compound **23** (IC_{50} = 6.05 μM) with a 1,2,3-triazole ring exhibited higher activity than **12** (IC_{50} > 40 μM) with a linking spacer (6-aminocaproic acid) against the SW480 cell line. Similarly, compound **26** (IC_{50} = 4.10 μM, 0.57 μM) with a 1,2,3-triazole ring showed higher activity than **14** (IC_{50} = 12.23 μM, 16.25 μM) against the SMMC-7721 and SW480 cell lines. These results suggested that a 1,2,3-triazole ring is more favorable for better activity in some of the synthesized compounds. Previously, Wall et al.[29] reported that the 20-OH group in the E ring moiety of the molecule is important for the cytotoxic activity. The cytotoxic potency of compounds (**15**/**16**/**17**/**18**) displayed significantly higher activity than compounds (**11**/**12**/**13**/**14**), which is quite similar to previous studies. A group of compounds that displayed high potency were those bearing the 7-ethyl group (compounds **13**, **14**, **17**, and **18**). In most cases, the derivatives with a 7-ethyl group were more active than those with no 7-ethyl group in the molecule, while the length of linking spacer between the biotin moiety and camptothecin analogues did not affect the cytotoxic potency of these compounds (e.g., **13** vs **11**, **14** vs **12**, **17** vs **15**, and **18** vs **16**). Taken together, these results showed that the length of linker, the 1,2,3-triazole ring, the 20-OH group in the E ring, and the 7-ethyl group of the camptothecin analogues scaffold can affect the potency of the anticancer activity.

In summary, 12 novel biotinylated camptothecin derivatives were designed, synthesized, and evaluated for anticancer activities against a panel of five human cancer cell lines including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). Most of the new derivatives showed comparable activity to camptothecin (**1**). In particular, compound **17** (IC_{50} = 0.13–3.31 μM) and compound **18** (IC_{50} = 0.23–1.48 μM) were the most promising derivatives against the five human cancer cell lines. Furthermore, the SARs study indicated that the length of linker, the 1,2,3-triazole ring, the 20-OH group in the E ring, and the 7-ethyl group of the CPT analogues scaffold can affect the potency of the anticancer activity. Potentially, these findings may aid our further optimization and biological evaluation of biotinylated camptothecin derivatives as anticancer drug candidates, Studies are ongoing in our laboratory and results will be reported in due course.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.11.049.

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30. General synthetic procedure for target compounds **11** – **18**. To a mixture of Biotin or 6-biotinylaminocaproic acid (0.3 mmol), camptothecin analogue (0.1 mmol) in DMF (2.5 mL) were added 4-Dimethylaminopyridine (DMAP) (0.01 mmol) and N, N′-Diisopropylcarbodiimide) (DIC) (0.6 mmol) dropwise. The reaction mixture was stirred at room temperature for 2 days under N_2. Solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (methyl trichloride: methyl alcohol = 15:1→9:1) to afford the product. Representative analytical and spectroscopic data of Biotin-(20s)-camptothecin (**11**): Yellow amorphous powder, yield 60%; mp.173–176 °C (CH_2Cl_2); $[\alpha]_D^{22.9}$: –124.9 (c 0.15, Pyridine); 1H-NMR ($CDCl_3$, 400 MHz) δ 8.50 (s, 1H, $C^{7'}$-CH), 8.33 (d, 1H, J = 8.4 Hz, C^{9}-CH), 8.00 (d, 1H, J = 8.4 Hz, C^{12}-CH), 7.88 (t, 1H, J = 7.6 Hz, C^{10}-CH), 7.70 (t, 1H, J = 6.8 Hz), 7.43 (s, 1H, C^{14}-CH), 5.66 (d, 1H, J = 17.2 Hz), 5.38 (d, 1H, J = 17.2 Hz), 5.03 (s, 1H), 4.45–4.42 (m, 1H), 4.27–4.24 (m, 1H), 3.07–3.03 (m, 1H), 2.75 (dd, 1H, J = 4.8 Hz, 13.2 Hz), 2.66–2.63 (m, 1H), 2.53 (t, 2H, J = 7.6 Hz), 2.27–2.22 (m, 2H), 2.15–2.10 (m, 2H), 1.69–1.59 (m, 4H), 1.45–1.37 (m, 2H), 0.98 (t, 3H, J = 7.2 Hz, C^{19}-CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 172.8 (C-7′), 167.7 (C-21), 163.6 (C-16′), 162.5 (C-16$_a$), 157.2 (C-2), 151.5 (C-13), 146.1 (C-3), 145.1 (C-15), 132.6 (C-7), 131.5 (C-6), 128.8, 128.5, 128.3, 128.2 (2), 120.6 (C-16), 97.3 (C-14), 75.7 (C-20), 67.0 (C-17), 61.8 (C-13′), 60.2 (C-14′), 55.4 (C-12′), 50.0 (C-5), 40.4 (C-15′), 33.2, 31.7, 28.1, 27.9, 24.4, 7.6 (C-19); ESIMS: m/z 597 $[M + H]^+$, HRESIMS: calcd for $C_{30}H_{30}N_4O_6SH$ $[M + H]^+$ 575.1959, found 575.1938.
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33. General synthetic procedure for target compounds **23** – **26**. To a solution of **19**/**20** (0.1 mmol) and **21**/**22** (0.1 mmol) in ^{t}BtOH-H_2O (1mL, 1:1) and THF (1.0 mL) at room temperature were added Copper (II) acetate (0.01 mmol) and sodium ascorbate (1.0 M in H_2O, 3 drops). The reaction mixture was stirred at room temperature for 4 h. Then, the mixture was carefully diluted with water (20.0 mL) and extracted with ethyl acetate (3 × 20.0 mL), combined organic portions were dried Na_2SO_4. The solvent was evaporated and the residue was chromatographed on silica gel (methyl trichloride: methyl alcohol = 15:1) to afford the product. Representative analytical and spectroscopic data of [(N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-(biotin))-1,2,3-triazol-1-yl]-(20s)-camptothecin (**32**): Yellow amorphous powder; yield 89%; mp. 79–81 °C (CH_2Cl_2); $[\alpha]_D^{22.7}$: –23.6 (c 0.11, $CHCl_3$); 1H-NMR (CD_3OD, 600 MHz) δ 8.56 (s, 1H), 8.10 (d, 1H, J = 9.0 Hz, C^{9}-CH), 8.07 (d, 1H, J = 8.4 Hz, C^{12}-CH), 7.84 (t, 1H, J = 7.8 Hz, C^{10}-CH), 7.70 (t, 1H, J = 7.2 Hz, C^{11}-CH), 7.46 (s, 1H, C^{14}-CH), 7.28 (s, 1H), 5.67 (d, 1H, J = 16.2 Hz), 5.39 (d, 1H, J = 16.2 Hz), 4.68–4.65 (m, 1H), 4.58–4.56 (s, 1H), 4.26 (t, 2H, J = 5.4 Hz), 3.77 (dd, 1H, J = 3.0 Hz, 15.0 Hz), 3.55 (t, 2H, J = 4.2 Hz), 3.51 (dd, 1H, J = 2.4 Hz, 13.2 Hz), 3.42 (t, 2H, J = 5.4 Hz), 3.37–3.34 (m, 2H), 3.14–3.10 (m, 1H), 3.08–3.05 (m, 1H), 2.21 (t, 2H, J = 7.2 Hz), 1.97–1.92 (m, 2H), 1.65 (t, 2H, J = 7.2 Hz), 1.55–1.49 (m, 2H), 0.99 (t, 3H, J = 7.2 Hz, C^{19}-CH_3); ^{13}C-NMR (CD_3OD, 150 MHz) δ 176.0 (C-7′), 174.9 (C-21), 159.7 (C-16$_a$), 153.2 (C-2), 152.3 (C-15), 150.0 (C-13), 147.0 (C-3), 141.8, 133.7, 133.5, 132.0, 130.0, 129.9, 129.1, 125.1, 121.7 (C-16), 99.3 (C-14), 74.1 (C-20), 71.9, 71.2, 70.5, 70.2, 67.0, 63.6, 59.4 (C-13′), 58.3 (C-14′), 55.3 (C-12′), 51.1 (C-5), 40.3, 36.5, 32.2, 30.7, 28.1, 27.0, 26.8, 26.6, 26.4, 8.2 (C-19); ESIMS: m/z 787 $[M + H]^+$, HRESIMS: calcd for $C_{39}H_{46}N_8O_8SH$ $[M + H]^+$ 787.3232, found 787.3258.
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