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FULL PAPER

Synthesis and biological evaluation of 10-substituted camptothecin derivatives with improved water solubility and activity

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Abstract: Despite the remarkable clinical achievement, camptothecin (CPT) still suffers from poor solubility and severe toxicity. Therefore, it is necessary to redevelop CPT derivatives as supplementary antitumor agents with good water solubility and small side-effects. In this work, twenty-seven camptothecin derivatives were synthesized and screened for their cytotoxicity against A549 (lung) and HCT-116 (colon) cancer cell lines. Among them, compound **B7**, 7-ethyl-10-(2-oxo-2-(4-methylpiperidin-1-yl)ethoxy)camptothecin, was demonstrated in vitro to be a more potent antitumor agent than SN-38 by the comparison of their inhibitory activities against cell proliferation and colony formation and interference effect on process of cell cycle and cell apoptosis. Additionally, molecular docking model revealed that **B7** can interact with topoisomerase I-DNA complex, and the solubility of **B7** reached 5.73 μg/mL in water. Moreover, **B7** significantly inhibited the tumor growth in A549 xenograft model at the dosage of 0.4 and 2.0 mg/kg. And **B7** exhibited minimum lethal doses comparable to irinotecan. These results indicated that **B7**, with improved solubility, enhanced activity and acceptable acute toxicity, can be used as a lead compound for development of novel anti-cancer agent.

Introduction

The contribution of natural products (NPs) to drug discovery seems impressive.[1] NPs have constantly provided leads compounds or entered clinical-trials for human disease, particularly the cancer. [2]The therapeutic area of oncology has benefited from numerous NPs, and many of anti-tumor drugs currently in clinical use are NPs or designed using NP templates[3], such as vinblastine[4], paclitaxel[5] and homoharringtonine[6].

Camptothecin (CPT, Figure 1), a quinoline based cytotoxic alkaloid isolated from the bark of Chinese plant Camptotheca acuminata, shows significant anticancer activity, but early clinical trials of CPT were all terminated due to its poor solubility and stability as well as unpredictable severe toxicity.[7] It was not until 1985 that the field of camptothecin research was resurrected because the unique mechanism of CPT was characterized.[8] The mechanism investigation indicated that CPT can bind to the transient Topo I/DNA cleavage complex, causing Topo I-mediated DNA breakage, subsequently blocking intracellular DNA replication, transcription and repair, and eventually leading to cell death.[9] Thereafter, thousands of CPT analogues or derivatives were synthesized and activity evaluated. Most of the successful structural modifications are focused on the A-B rings, especially at 7-, 9-, 10-, and 11-position of CPT (Figure 1), intending to improve water solubility and antitumor potency.[10] To date, structural modification of natural CPT has generated two antitumor agents approved by FDA, namely topotecan[11] and irinotecan[12], which are now used clinically to treat ovarian, small cell lung and colon cancers.

SN-38, the active metabolite of irinotecan, displays potent anti-tumor activity in vitro, but its therapeutic application is hindered by its poor water solubility.[13] Moreover, intestinal inflammation caused by SN-38 is of serious concern, because 10-O-glucuronide SN38 (SN-38G), produced by SN-38 and glucuronic acid in the liver, converts back to the biologically active compound SN-38 in the presence of bacterial β-glucuronidase in the intestinal lumen. In this process, SN-38 remaining in the intestines causes intestinal inflammation, leading to diarrhea. In fact, the 10-O-substitution strategy was used to avoid the hepatic metabolism of 10-O-glucuronidation in the liver and its derivatives have been developed from the medicinal chemistry perspective to further increase the aqueous solubility and the antitumor activity.[14] Chimmitecan, a case of camptothecin 9-position modification, was reported in 2007.[15] The allyl substitution at the 9-position of CPT benefits chimmitecan a salient anti-MDR activity and oral availability.[16] 10-HCPT, another natural alkaloid, has been approved by CFDA because of its excellent anti-tumor activity. Nonetheless, side effects such as digestive tract

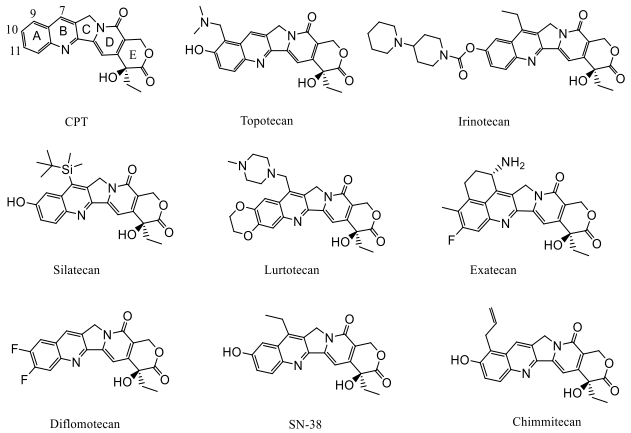

Figure 1. CPT and it's derivatives with multisubstitution at 7, 9, 10, and 11 positions.

reactions and bone marrow suppression largely circumscribe the clinical practicability of 10-HCPT.

Previous work from our group concluded that a hydrophobic moiety, especially a ring fragment, attached to the 10-position of CPT improved solubility and reduced side-effects.[17] In the present study, new hydrophobic groups were linked to the 10-position of 10-HCPT, SN-38 or chimmitecan, and twenty-seven new CPT derivatives were synthesized and evaluated for their antitumor activity. It's demonstrated that the most promising compound **B7** was a potent antitumor agent with improved solubility, acceptable acute toxicity and potent activity both in vitro and in vivo.

Results and Discussion

The synthetic routes for the target compounds are illustrated in Figures 2-3. As shown in Figure 2, 10-HCPT or SN-38 underwent one or two-step nucleophilic substitution reaction to generate compounds **A1**, **A8**, **B1** and **B5-B6**. Then **A1** was reduced to give **A2**. 10-HCPT or SN-38 was acylated with alkyl formic acid or benzoic acid in the presence of condensing agent 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to afford **A3-A7** and **B2-B4**. In the presence of anhydrous potassium carbonate, the reaction of SN-38 with ethyl bromoacetate in DMF gave intermediate **1**, which was subsequently converted to acid **2** via hydrolysis. In the presence of HATU, acid **2** reacted with 4-methylpiperidine to produce **B7** (Figure 2).

The intermediate produced by the nucleophilic substitution of 10-HCPT with allyl bromide was rearranged to produce chimmitecan. Then chimmitecan reacted with ethyl bromoacetate, ethyl 3-bromo-3-methylbutanoate or 1-bromo-2-methoxyethane to generate **C1-C3**, respectively. The reaction of hydrolysate of **C1** with piperidine or morpholine produced **C6-C7**. Subsequently **C1-C2** and **C6** were reduced by H_2 (catalyst, Pd/C) to give **C4-C5** and **C8**. SN-38 or chimmitecan reacted with the active carbonic acid ester which was generated from phenylmethanol and 4-nitrophenyl carbonochloridate to afford **B8** and **C9**. Similarly, compounds **C10-C11** were synthesized from 4-bromobenzyl alcohol and benzylamine respectively (Figure 3).

With target compounds in hand, we firstly evaluated their cytotoxic activity against human colon carcinoma cell line (HCT-

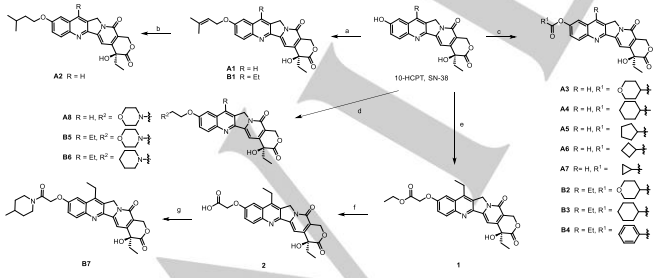

Figure 2. Synthetic pathways for preparation of compounds **A1-A8** and **B1-B7**. Reagents and reaction conditions: (a): 1-bromo-3-methyl-2-butene, K_2CO_3, acetone, reflux 3 h; (b): Pd/C, MeOH, rt, overnight; (c): substituted formic acid, HATU, DIPEA (*N*,*N*-diisopropylethylamine), DMF (*N*,*N*-dimethylformamide), rt, 2-5 h; (d): 1) 1,2-dibromoethane, K_2CO_3, DMF, rt, 3 h; 2) morpholine or piperidine, K_2CO_3, DMF, rt, 2-3 h; (e): ethyl bromoacetate, K_2CO_3, DMF, rt, 3 h; (f): K_2CO_3, DMF/ H_2O (1:1, volume ratio), rt, overnight; (g): 4-methylpiperidine, HATU, DIPEA, dichloromethane, rt, 3 h.

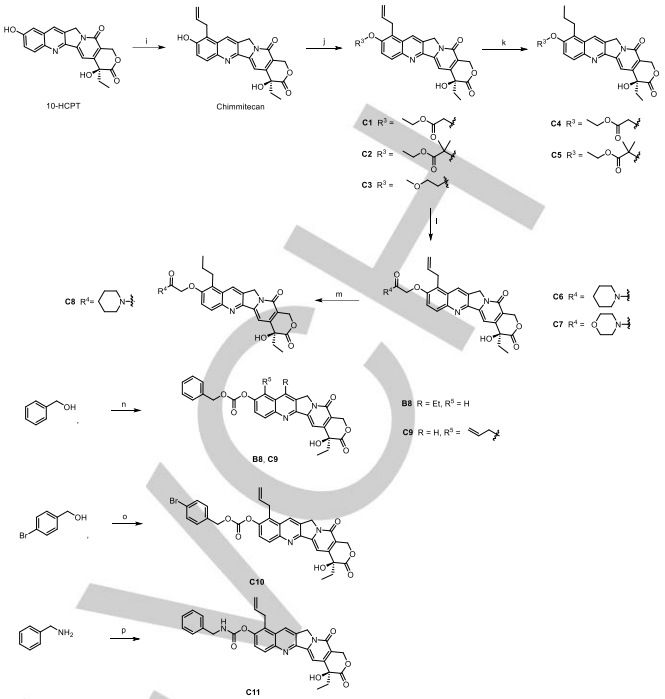

Figure 3. Synthetic pathways for preparation of compounds **B8**, **C1-C11**. Reagents and reaction conditions: (i): 1) allyl bromide, K_2CO_3, DMF, rt, 2 h, 2) acetic acid, Ar, reflux, 5 days; (j): ethyl bromoacetate or 1-bromo-2-methoxyethane, K_2CO_3, DMF, rt, 2-4 h; ethyl 2-bromoisobutyrate, K_2CO_3, acetonitrile, reflux, 5 h; (k): Pd/C, H_2, 1,4-dioxane, rt, overnight; (l): 1) K_2CO_3, DMF/ H_2O (1:1, volume ratio), rt, overnight; 2) morpholine or piperidine, HATU, DIPEA, DCM, rt, 3 h; (m): Pd/C, H_2, 1,4-dioxane, rt, overnight; (n): 1) 4-nitrophenyl chloroformate, DMAP (4-dimethylaminopyridine), dichloromethane, ice-bath, 3-5 h, 2) SN-38 or chimmitecan, TEA (triethylamine), DMF, rt, 2-3 h. (o): 1) 4-nitrophenyl chloroformate, DMAP (4-dimethylaminopyridine), DCM, ice-bath, 3-5 h, 2) chimmitecan, TEA (triethylamine), DMF, rt, 2-3 h. (p): 1) 4-nitrophenyl chloroformate, DMAP (4-dimethylaminopyridine), DCM, ice-bath, 3-5 h, 2) chimmitecan, TEA (triethylamine), DMF, rt, 2-3 h.

116) and human lung adenocarcinoma epithelial cell line (A549) by applying the MTT colorimetric assay (Table 1). 10-HCPT and SN-38 were selected as the positive controls. As illustrated in Table 1, all the tested compounds dominantly inhibited growth of HCT-116 and A549 cell lines. The different antiproliferative effect suggested that the activity of synthesized compounds was associated with the structure of substituted groups at the 10-position of CPT. Among the tested compounds, **A1-A7** and **B1-B4** showed activity comparable to that of their parent 10-HCPT and SN-38 respectively. The introduction of 2-methyl-2-butene fragment (**A1**, **B1**) or 2-methylbutane fragment (**B2**) into the 10-position of 10-HCPT or SN-38 had negligible effect on its activity. Moreover, **A3-A7** and **B2-B4,** the substituent of which at 10-position was changed to alkyl formyl or benzoyl, also displayed submicromolar inhibitory activity against the two cell lines. However, as prodrugs, the activity of **A3-A7**, **B2-B4** and their corresponding parent drug was nearly indistinguishable. Subsequently, we elongated the distance between 10-position oxygen atom of 10-HCPT or SN-38 and the cycloalkane by two CH_2 to obtain compounds **A8** and **B5-B6** which displayed reduced activity (IC_{50} > 1 μM on A549). In our previous work, we found that the acetamide fragment appended to 10-position of SN-38 would affect the activity, and the cyclic hydrophobic amine played a positive role. Therefore, we designed and synthesized compound **B7** which exhibited extremely strong antiproliferative activity against both A549 and HCT-116 cell lines (IC_{50} = 0.006, 0.004 μM,

Table 1. Cytotoxic activities of synthetic compounds against HCT-116 and A549 cell lines (n = 3, $\bar{x}$ ± SD).

Compds	IC_{50} (μM)	
	A549	HCT-116
A1	0.051 ± 0.008	0.056±0.011
A2	0.064 ± 0.010	0.058±0.062
A3	0.041 ± 0.023	0.024±0.002
A4	0.048 ± 0.009	0.043±0.002
A5	0.053 ± 0.013	0.064±0.003
A6	0.117 ± 0.086	0.059±0.009
A7	0.013 ± 0.005	0.032±0.010
A8	5.152 ± 1.101	0.524 ± 0.008
B1	0.057 ± 0.010	0.031 ± 0.007
B2	0.034 ± 0.006	0.018 ± 0.003
B3	0.043 ± 0.005	0.020 ± 0.001
B4	0.067 ± 0.011	0.036 ± 0.001
B5	1.332 ± 0.124	0.219 ± 0.064
B6	2.469 ± 0.632	0.299 ± 0.110
B7	0.006 ± 0.001	0.004 ± 0.001
B8	0.013 ± 0.010	0.006 ± 0.002
Chimmitecan	0.018 ± 0.004	0.007 ± 0.001
C1	0.630 ± 0.101	1.521 ± 0.675
C2	0.572 ± 0.212	1.167 ± 0.141
C3	0.050 ± 0.021	0.073 ± 0.011
C4	0.435 ± 0.119	0.372 ± 0.021
C5	0.803 ± 0.108	2.101 ± 0.367
C6	0.214 ± 0.034	0.278 ± 0.091
C7	0.608 ± 0.008	0.384 ± 0.085
C8	0.120 ± 0.013	0.329 ± 0.103
C9	0.028 ± 0.009	0.005 ± 0.001
C10	0.022 ± 0.005	0.010 ± 0.001
C11	0.011 ± 0.001	0.007 ± 0.003
10-HCPT	0.065 ± 0.005	0.041 ± 0.009
SN-38	0.024 ± 0.007	0.013 ± 0.002

respectively).

Next, chimmitecan was synthesized as parent compound and the introduction of an ester fragment at its 10-position significantly reduced its activity (**C1-C2**, IC_{50} > 1 μM on HCT-116). **C6** produced by the reaction of hydrolysate of **C1** and piperidine also showed poor activity. The same result was obtained when piperidine was replaced with morpholine (**C7**). Whether the allyl group at the 9th position of chimmitecan was reduced to a propyl group or not had almost no impact on the activity (**C4-C5**, **C8**). Substitution of 10-position of chimmitecan with methoxyethyl (**C3**) slightly diminished the activity despite the same order of activity magnitude as chimmitecan. These results indicated that the introduction of large substituents at the 10-position of chimmitecan was detrimental to the activity, which might be caused by the steric hindrance between the 9 and 10 positions. Subsequently, we synthesized several chimmitecan prodrugs through the linkage of carbonate or carbamate, all of which showed comparable activity to chimmitecan, and **C11** performed best (A549, IC_{50} = 0.011 μM; HCT-116, IC_{50} = 0.007 μM). Similarly, **B8**, as a prodrug of SN-38, also showed a satisfactory anti-proliferative effect.

As a derivative of SN-38, we anticipated that **B7** also has a broad-spectrum anti-tumor effect, so we determined the cytotoxic activities of **B7** against several different cancer cell lines. The results were presented in Table 2. As we expected, **B7** showed significantly stronger anti-proliferative activity than SN-38 against proliferation of Lovo, Colo205, HT-29 (human colon carcinoma cell lines) and HepG2 (human liver carcinoma cell line).

Table 2. Cytotoxic activities of **B7** against different cancer cell lines (n = 3).

Compds	IC_{50} (μM)			
	Lovo	Colo205	HT-29	HepG2
B7	0.098 ± 0.010	0.103 ± 0.003	0.022 ± 0.003	0.077 ± 0.003
SN-38	0.328 ± 0.091	0.295 ± 0.031	0.072 ± 0.010	0.185 ± 0.036

Due to their excellent anti-proliferative activities on HCT-116 and A549 cells, **B7** and **C11** were selected for solubility determination in different menstruum, and the results were listed in Table 3. Compared with SN-38, the solubility of **B7** in different solvents was greatly improved and even reached about 11.10 μg/mL in a buffer solution at pH 4.5. We infer that the introduction of a hydrophobic moiety to the 10-position of CPT may destroy the rigid planar structure and improve solubility. Tested by HPLC, **C11** showed enhanced solubility in water and pH 4.5 buffer, but its carbonate structure was hydrolyzed in pH7.4 buffer.

Based on cellular activity and solubility, we selected **B7** for further study. As shown in Figure 4, colony formation assay confirmed that **B7** could remarkably suppress the cell cloning of A549 cell line at concentrations as low as 0.001 nM, and that the formation of cell cluster colonies was almost completely inhibited at a concentration of 10 nM. Compared with the positive control

Table 3. The solubility of **B7** and **C11** in different menstruum (μg/mL).

	H_2O	Buffer solution	
		pH4.5	pH7.4
B7	5.73 ± 0.03	11.10 ± 0.04	17.32 ± 0.02
C11	2.11± 0.02	5.37± 0.16	—
SN-38	< 0.25	< 0.25	< 0.25

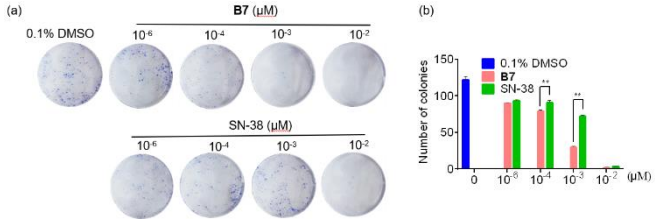

Figure 4. Colony formation assay. A549 cells were seeded on 6-well plate and treated with **B7** under indicated concentrations for 10 days and then the generating colonies were stained and counted. The colony formation was photographed (a) and the numbers of colonies were counted (b). Date are shown as the mean ± SD of three independent experiments (**P < 0.01 compared with SN-38).

SN-38, **B7** displayed more potent inhibitory effect on A549 clone formation at 0.1 nM and 1 nM, which was consistent with the cytotoxicity results.

To explore whether **B7** can affect cell cycle, we applied flow cytometry assay to determine A549 cell cycle after the treatment with **B7**. SN-38 was used as positive control. As shown in Figure 5, the results indicated that 10 nM of **B7** arrested A549 cells in the G2 phase.[18] In contrast, 100 nM and 1000 nM of **B7** blocked A549 cells in the S phase. And the blocking effect of **B7** was much stronger than SN-38 at same concentration (1 μM).

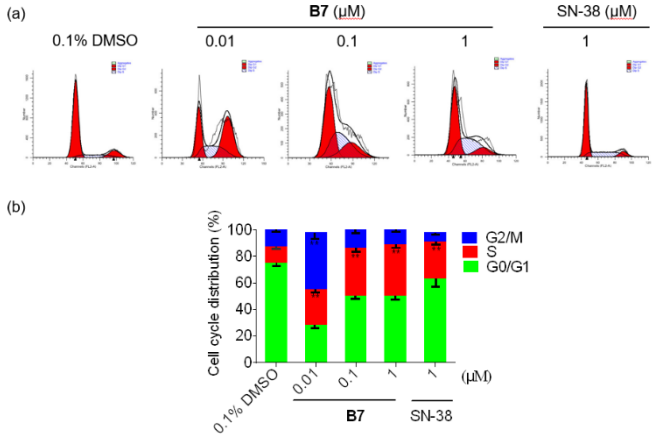

Figure 5. Effect of compound **B7** on cell cycle progression of A549 cell line. Cells were exposed to various concentrations of B7 for 24 h and the cell cycle was assessed by PI/RNase staining and analyzed by flow cytometry (a). The cell cycle distribution was shown on the histogram (b). Data shown are the mean ± SD of three independent experiments (**P < 0.01, significant difference compared with control)

To confirm whether **B7** can induce cell apoptosis, the annexinV/propidium iodide (PI) biparametric cyto-fluorimetric assay was applied on A549 cells. As depicted in Figure 6, **B7** could facilitate A549 cells apoptosis in a concentration-dependent manner. **B7** induced 13.4% and 16.16% of A549 cell line to undergo apoptosis at concentration of 0.1 and 1 μM, respectively. Meanwhile, **B7**-induced apoptosis rates of A549 cells are higher than the positive drug SN-38 at the concentrations of 0.1 and 1 μM.

The studies in vitro demonstrated that **B7** is a more potent antitumor agent than SN-38. To examine the antitumor efficacy of **B7** in vivo, a test using A549 xenograft model was performed. The results were shown in Figure 7. Compared with the solvent group,

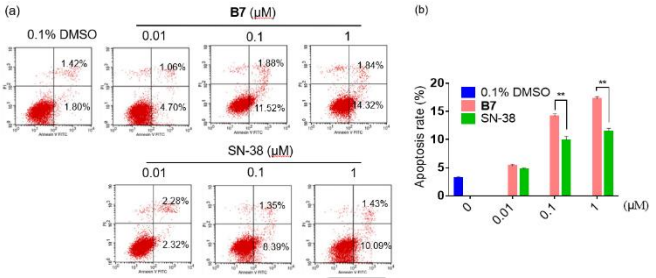

Figure 6. Compound **B7** induced apoptosis on A549 cells. Cells were treated with indicated concentrations for 24 h and the apoptosis rates were assessed by FITC Annexin V/PI staining and analyzed by flow cytometry (a). The apoptosis rates were show on the histogram (b). Data shown are the mean ± SD of three independent experiments (**P < 0.01, compared with SN-38).

B7 treatment at doses of 0.4 and 2.0 mg/kg led to a significant tumor growth inhibition. **B7**-induced inhibition rates of tumor growth at low and high dosage group reached 57.1% and 63.8%, respectively. Therefore, we could confirm that **B7** exerts a remarkable antitumor effect in vivo by intraperitoneal administration treatment.

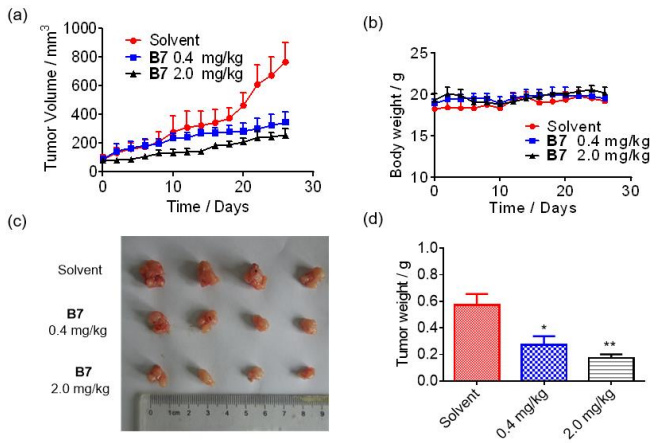

Figure 7. Antitumor efficacy of **B7** administered intraperitoneally in vivo. Balb/C nude mice bearing A549 cells xenografts were treated with B7 at doses of 0.4 and 2.0 mg/kg once the other day. Tumor size (a) and body weight (b) of the nude mice were measured every two days. On the last day, mice were sacrificed and tumors were removed, photographed (c) and weighed (d). (n = 4; *P < 0.05, **P < 0.01, significant difference compared with solvent group).

In order to investigate the acute toxicity, the minimum lethal dose of **B7** was measured (Table 4). The minimum lethal doses of **B7**, SN-38 and Irinotecan in mice determined by intravenous administration were 35, 40 and 40 mg/kg (or 0.066, 0.102 and 0.064 mmol/kg), respectively. The acute toxicity of **B7** was increased compared to SN-38, but was equivalent to Irinotecan. Moreover, the minimum lethal dose of **B7** was much higher than its inhibitory tumor dose of 0.4-2 mg/kg.

To explore the binding mode of **B7** and SN-38 with Topo I-DNA complex, molecular docking studies were performed. From the binding model, we noticed that compounds **B7** and SN-38 parallelly inserted into the hydrophobic region of DNA, and **B7** was more parallel to DNA. As Figure 8A showed, the oxygen atom in the lactone ring of **B7** formed a hydrogen bond interaction with Thr718 of Topo I, the hydroxyl at 20-position formed hydrogen bond interaction with Asp533, and nitrogen in the pyridine ring

Table 4. Minimum lethal dose of **B7** to mice (n = 6).

B7		SN-38		Irinotecan	
Dose (mg/kg)	mortality	Dose (mg/kg)	mortality	Dose (mg/kg)	mortality
25	0/6	25	0/6	30	0/6
35	1/6	40	1/6	40	1/6
50	3/6	50	5/6	50	4/6

formed hydrogen bond interaction with Arg364. Importantly, the 4-methylpiperidinyl in **B7** extended to the hydrophobic region of Topo I, which provided additional hydrophobic interaction with the binding site and enhanced the stability of **B7**-Topo I-DNA complex. Therefore, we speculated that **B7** was more parallel to DNA and the additional hydrophobic interaction brought by 4-methylpiperidine resulted in better activity than SN-38.

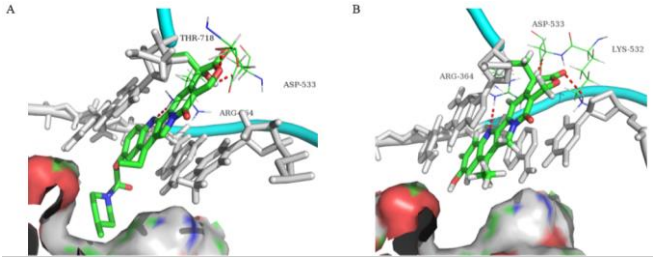

Figure 8. Docking of **B7** and SN-38 with Topo I-DNA complex (PDB: 1k4t). Hydrogen bonds are indicated as red dashed lines. The gray residue represents a piece of DNA.

Conclusion

In this work, twenty-seven new camptothecin derivatives were synthesized and screened for their cytotoxicity against A549 (lung) and HCT-116 (colon) cancer cell lines. Among them, compound **B7** was demonstrated in vitro to be a more potent antitumor agent than SN-38 by the comparison of their inhibitory activities on cell proliferation and colony formation and interference effect on the process of cell apoptosis and cell cycle. Moreover, the solubility of **B7** reached 5.73 μg/mL in water. Importantly, **B7** significantly inhibited the tumor growth in A549 xenograft model at the dosage of 0.4 and 2.0 mg/kg. And **B7** exhibited minimum lethal doses comparable to irinotecan. Additionally, molecular docking model revealed that **B7** can interact with topoisomerase I-DNA complex. These results indicated that **B7**, with improved solubility, enhanced activity and acceptable acute toxicity, can be used as a lead compound for development of novel anti-cancer agent.

Experimental Section

General methods. Unless specified otherwise, all the starting materials, reagents and solvents are commercially available. All the reactions were monitored by thin-layer chromatography on silica gel plates (GF254) and visualized with UV light (254 nm and 365 nm). NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). High resolution mass spectrum (HRMS) was obtained by electrospray ionization (positive mode) on an Ultra performance liquid chromatography- Quadrupole-time of flight Mass Spectrometer (WATERS I-Class VION IMS Q-TOF).

(*S*)-4-ethyl-4-hydroxy-9-((3-methylbut-2-en-1-yl)oxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (A1): To a round bottom flask were added 10-HCPT (50.0 mg, 0.14 mmol), K_2CO_3 (22.7 mg, 0.16 mmol), 1-bromo-3-methyl-2-butene (16 μL, 0.14 mmol) and acetone (10 mL). The solution was refluxed for 3h. Product **A1** was isolated using column chromatography (DCM:MeOH = 50:1). Pale yellow solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.05 (d, *J* = 9.2 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.28 (s, 1H), 6.52 (s, 1H), 5.54 (t, *J* = 6.7 Hz, 1H), 5.42 (s, 2H), 5.25 (s, 2H), 4.71 (d, *J* = 6.7 Hz, 2H), 1.93 – 1.81 (m, 2H), 1.79 (d, *J* = 2.7 Hz, 6H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 157.7, 157.3, 150.5, 150.5, 146.2, 144.4, 138.3, 130.9, 130.6, 130.3, 129.8, 123.7, 119.9, 118.8, 107.4, 96.5, 72.9, 65.7, 65.4, 50.6, 30.7, 25.9, 18.6, 8.2. HRMS (ESI): m/z calcd for C25H25N2O5: 433.17635 $[M+H]^+$; found: 433.17551.

(*S*)-4-ethyl-4-hydroxy-9-(isopentyloxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (A2): Compound **A1** (86.2 mg, 0.20 mmol) and Pd/C (8.0 mg, 10%) were mixed with MeOH (6 mL). The suspension was stirred under H_2 atmosphere at room temperature overnight. The mixture was filtered through celite, and the filtrate was purified by column chromatography (DCM:MeOH = 50:1). Pale yellow solid, 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.05 (d, *J* = 9.2 Hz, 1H), 7.53 (d, *J* = 2.6 Hz, 1H), 7.49 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.28 (s, 1H), 6.51 (s, 1H), 5.42 (s, 2H), 5.26 (s, 2H), 4.18 (t, *J* = 6.6 Hz, 2H), 1.89 – 1.84 (m, 2H), 1.78 (d, *J* = 2.7 Hz, 1H), 1.72 (q, *J* = 6.6 Hz, 2H), 0.98 (d, *J* = 6.6 Hz, 6H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 158.0, 157.3, 150.6, 150.5, 146.3, 144.4, 130.9, 130.7, 130.4, 129.9, 123.7, 118.8, 107.3, 96.5, 72.9, 67.0, 65.7, 50.7, 37.8, 30.7, 25.1, 22.9 (2C), 8.2. HRMS (ESI): m/z calcd for $C_{25}H_{27}N_2O_5$: 435.19200 $[M+H]^+$; found: 435.19309.

(*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl tetrahydro-2*H*-pyran-4-carboxylate (A3): To a round bottom flask were added **10-HCPT** (50.0 mg, 0.14 mmol), tetrahydropyran-4-yl-carboxylic acid (19.7 mg, 0.15 mmol), HATU (54.0 mg, 0.14 mmol), DIPEA (72 μL, 0.41 mmol) and DMF (1.5 mL). The resulted mixture was stirred at r.t. for 2 h. Add water (5 mL) to the reaction system, and the precipitated white solid was filtered off and recrystallized to obtain **A3.** Off-white solid, 87% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.17 (d, *J* = 9.1 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.63 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.32 (s, 1H), 6.54 (s, 1H), 5.42 (s, 2H), 5.25 (s, 2H), 3.92 (dd, *J* = 8.0, 3.3 Hz, 2H), 3.46 (td, *J* = 11.2, 1.8 Hz, 2H), 3.05 – 2.91 (m, 1H), 2.02 – 1.94 (m, 2H), 1.93 – 1.84 (m, 2H), 1.83 – 1.72 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3, 172.9, 157.2, 153.0, 150.4, 149.4, 146.3, 145.7, 131.6, 130.9, 130.8, 128.7, 126.4, 119.6, 119.6, 97.2, 72.8, 66.5 (2C), 65.7, 50.6, 30.8, 28.7 (3C), 8.2. HRMS (ESI): m/z calcd for C26H25N2O7: 477.16618 $[M+H]^+$; found: 477.16600.

(*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl cyclohexanecarboxylate (A4): Compound **A4** was prepared similarly as described for **A3**. Off-white solid, 92% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.21 (d, *J* = 9.1 Hz, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.65 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.35 (s, 1H), 6.54 (s, 1H), 5.44 (s, 2H), 5.30 (s, 2H), 2.77 – 2.64 (m, 1H), 2.11 – 1.98 (m, 2H), 1.96 – 1.81 (m, 2H), 1.82 – 1.71 (m, 2H), 1.71 – 1.49 (m, 3H), 1.45 – 1.23 (m, 3H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.3, 172.9, 157.2, 152.9, 150.4, 149.5, 146.3, 145.8, 131.6, 130.9, 130.8, 128.7, 126.4, 119.6, 119.6, 97.1, 72.8, 65.7, 50.6, 42.6, 30.8, 28.9 (2C), 25.8, 25.2 (2C), 8.2. HRMS (ESI): m/z calcd for C27H27N2O6: 475.18691 $[M+H]^+$; found: 475.18670.

(*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl cyclopentanecarboxylate (A5): Compound **A5** was prepared similarly as described for **A3**. Off-white solid, 87% yield. ^{1}H NMR (400 MHz, DMSO-

d$_6$) δ 8.61 (s, 1H), 8.15 (d, *J* = 9.1 Hz, 1H), 7.86 (d, *J* = 2.3 Hz, 1H), 7.61 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.32 (s, 1H), 6.53 (s, 1H), 5.42 (s, 2H), 5.24 (s, 2H), 3.11 (p, 1H), 2.06 – 1.98 (m, 2H), 1.95 – 1.84 (m, 4H), 1.74 – 1.61 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 175.1, 172.9, 157.2, 152.9, 150.4, 149.5, 146.3, 145.7, 131.5, 130.8, 130.7, 128.7, 126.4, 119.6, 119.5, 97.1, 72.8, 65.7, 50.6, 43.5, 30.8, 29.9 (2C), 25.9 (2C), 8.2. HRMS (ESI): m/z calcd for C26H25N2O6: 461.17126 [M+H]$^+$; found: 461.17077.

(*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl cyclobutanecarboxylate (A6): Compound **A6** was prepared similarly as described for **A3**. Off-white solid, 85% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.63 (s, 1H), 8.17 (d, *J* = 9.1 Hz, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.65 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.33 (s, 1H), 6.54 (s, 1H), 5.42 (s, 2H), 5.26 (s, 2H), 3.54 (p, *J* = 8.5 Hz, 1H), 2.42 – 2.30 (m, *J* = 13.8, 12.9, 7.2 Hz, 4H), 2.09 – 1.86 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.9, 172.9, 157.2, 152.9, 150.5, 149.5, 146.3, 145.8, 131.6, 130.8, 130.8, 128.8, 126.5, 119.6, 119.6, 97.1, 72.8, 65.7, 50.7, 37.7, 30.8, 25.2 (2C), 18.3, 8.2. HRMS (ESI): m/z calcd for C25H23N2O6: 447.15561 [M+H]$^+$; found: 447.15497.

(*S*)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl cyclopropanecarboxylate (A7): Compound **A7** was prepared similarly as described for **A3**. Off-white solid, 88% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.61 (s, 1H), 8.15 (d, *J* = 9.1 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.63 (dd, *J* = 9.1, 2.5 Hz, 1H), 7.32 (s, 1H), 6.53 (s, 1H), 5.42 (s, 2H), 5.24 (s, 2H), 2.02 – 1.95 (m, 1H), 1.93 – 1.83 (m, *J* = 13.6, 6.8 Hz, 2H), 1.18 – 1.08 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.4, 172.9, 157.2, 152.9, 150.4, 149.4, 146.3, 145.7, 131.6, 130.8, 130.7, 128.7, 126.4, 119.6, 119.6, 97.1, 72.8, 65.7, 50.6, 30.8, 13.1, 9.7 (2C), 8.2. HRMS (ESI): m/z calcd for C24H21N2O6: 433.13996 [M+H]$^+$; found: 433.13959.

(*S*)-4-ethyl-4-hydroxy-9-(2-morpholinoethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (A8): A flask was charged with 10-HCPT (50.0 mg, 0.14 mmol), 1,2-dibromoethane (236 μL, 2.7 mmol), K_2CO_3 (94.7 mg, 0.69 mmol) and DMF (1mL). The resulted mixture was stirred at 60℃ for 12h. When 10-HCPT was completely consumed, add water (5 mL) to the reaction system, then the precipitated white solid was filtered off for the next step. To a round bottom flask were added the precipitated white solid (20.0 mg, 0.04 mmol), morpholine (19 μL, 0.21 mmol), K_2CO_3 (29.4 mg, 0.21 mmol) and DMF (1.5 mL). The solution was stirred at room temperature for 2h in Argon atmosphere. The solvent was evaporated under vacuum, and the residue was isolated using column chromatography (DCM:MeOH = 10:1) to give target compound **A8.** Yellow solid, 76% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.52 (s, 1H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.55 (d, *J* = 2.6 Hz, 1H), 7.51 (d, *J* = 9.2 Hz, 1H), 7.28 (s, 1H), 6.51 (s, 1H), 5.43 (s, 2H), 5.26 (s, 2H), 4.28 (t, *J* = 5.7 Hz, 2H), 3.65 – 3.56 (m, 4H), 2.80 (t, *J* = 5.7 Hz, 2H), 2.53 (s, 4H), 1.94 – 1.78 (m, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 157.7, 157.2, 150.5, 150.4, 146.1, 144.3, 130.9, 130.4, 130.3, 129.7, 123.5, 118.8, 107.3, 96.5, 72.9, 66.7 (2C), 66.2, 65.7, 57.4, 54.1 (2C), 50.6, 30.7, 8.2. HRMS (ESI): m/z calcd for C26H28N3O6: 478.19781 [M+H]$^+$; found: 478.19680.

(*S*)-4,11-diethyl-4-hydroxy-9-((3-methylbut-2-en-1-yl)oxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (B1): Compound **B1** was prepared similarly as described for **A1**. Pale yellow solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.05 (d, *J* = 8.9 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.26 (s, 1H), 6.50 (s, 1H), 5.50 (t, *J* = 6.6 Hz, 1H), 5.42 (s, 2H), 5.27 (s, 2H), 4.77 (d, *J* = 6.6 Hz, 2H), 3.16 (q, *J* = 14.9, 7.3 Hz, 2H), 1.93 – 1.85 (m, 2H), 1.81 (d, *J* = 9.7 Hz, 6H), 1.30 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 157.8, 157.3, 150.5, 150.0, 146.8, 144.8, 144.2, 138.1, 131.8, 128.8, 128.3, 123.1, 120.1, 118.7, 103.7, 96.4, 72.9, 65.7, 65.4, 50.0, 30.7, 25.9, 22.7, 18.6, 13.9, 8.2. HRMS (ESI): m/z calcd for C27H29N2O5: 461.20765 [M+H]$^+$; found: 461.20733.

(*S*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl tetrahydro-2*H*-pyran-4-carboxylate (B2): Compound **B2** was prepared similarly as described for **A3**. Off-white solid, 81% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.17 (d, *J* = 9.1 Hz, 1H), 7.97 (d, *J* = 2.3 Hz, 1H), 7.63 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.31 (s, 1H), 6.54 (s, 1H), 5.44 (s, 2H), 5.29 (s, 2H), 3.93 (dd, *J* = 8.1, 3.3 Hz, 2H), 3.46 (qd, *J* = 11.3, 9.4 Hz, 2H), 3.17 (q, *J* = 7.4 Hz, 2H), 3.04 – 2.92 (m, 1H), 1.99 (d, *J* = 11.1 Hz, 2H), 1.94 – 1.73 (m, 4H), 1.29 (t, *J* = 7.6 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.4, 172.9, 157.2, 152.4, 150.5, 149.6, 146.9, 146.3, 145.7, 131.7, 129.0, 127.5, 126.1, 119.5, 115.6, 97.1, 72.8, 66.5 (2C), 65.7, 55.4, 50.0, 30.8, 28.7 (2C), 22.7, 14.3, 8.2. HRMS (ESI): m/z calcd for C28H29N2O7: 505.19748 [M+H]$^+$; found: 505.19703.

(*S*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl cyclohexanecarboxylate (B3): Compound **B3** was prepared similarly as described for **A3**. Pale yellow solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.17 (d, *J* = 9.1 Hz, 1H), 7.95 (d, *J* = 2.3 Hz, 1H), 7.61 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.32 (s, 1H), 6.53 (s, 1H), 5.44 (s, 2H), 5.30 (s, 2H), 3.17 (q, *J* = 7.2 Hz, 2H), 2.76 – 2.63 (m, 1H), 2.07 (d, *J* = 10.2 Hz, 2H), 1.94 – 1.83 (m, 2H), 1.82 – 1.74 (m, 2H), 1.70 – 1.48 (m, 3H), 1.45 – 1.34 (m, 2H), 1.29 (t, 4H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 174.3, 172.9, 157.3, 152.3, 150.5, 149.7, 146.9, 146.3, 145.7, 131.7, 129.0, 127.5, 126.1, 119.4, 115.6, 97.1, 72.9, 65.7, 55.4, 50.0, 42.6, 30.8, 28.9 (2C), 25.2 (2C), 22.7, 14.3, 8.2. HRMS (ESI): m/z calcd for C29H31N2O6: 503.21821 [M+H]$^+$; found: 503.21807.

(*S*)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl benzoate (B4): Compound **B4** was prepared similarly as described for **A3**. Pale yellow solid, 94% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.26 – 8.17 (m, 4H), 7.84 – 7.76 (m, 2H), 7.65 (t, *J* = 7.7 Hz, 2H), 7.34 (s, 1H), 6.54 (s, 1H), 5.44 (s, 2H), 5.30 (s, 2H), 3.26 – 3.12 (m, 2H), 1.99 – 1.80 (m, 2H), 1.30 (t, *J* = 7.5 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.9, 165.2, 157.3, 152.4, 150.5, 149.8, 147.1, 146.3, 145.9, 134.7, 131.8, 130.4 (2C), 129.5 (2C), 129.2, 129.0, 127.6, 126.1, 119.5, 116.0, 97.1, 72.8, 65.7, 50.0, 30.8, 22.7, 14.3, 8.2. HRMS (ESI): m/z calcd for C29H25N2O6: 497.17126 [M+H]$^+$; found: 497.17169.

(*S*)-4,11-diethyl-4-hydroxy-9-(2-morpholinoethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (B5): Compound **B5** was prepared similarly as described for **A8**. Gray solid, 62% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.04 (d, *J* = 9.9 Hz, 1H), 7.48 (dd, *J* = 4.8, 2.1 Hz, 2H), 7.25 (s, 1H), 6.51 (s, 1H), 5.42 (s, 2H), 5.25 (s, 2H), 4.31 (t, *J* = 5.6 Hz, 2H), 3.61 (t, 4H), 3.17 (dd, *J* = 14.7, 7.1 Hz, 2H), 2.79 (t, *J* = 5.6 Hz, 2H), 2.53 (s, 4H), 1.95 – 1.79 (m, 2H), 1.31 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 157.9, 157.3, 150.5, 150.1, 146.8, 144.9, 144.3, 131.9, 128.8, 128.3, 122.9, 118.7, 103.5, 96.5, 72.9, 66.7 (2C), 66.3, 65.7, 57.5, 54.2 (2C), 50.0, 30.7, 22.7, 14.0, 8.2. HRMS (ESI): m/z calcd for C28H32N3O6: 506.22911 [M+H]$^+$; found: 506.22906.

(*S*)-4,11-diethyl-4-hydroxy-9-(2-(piperidin-1-yl)ethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (B6): Compound **B6** was prepared similarly as described for **A8**. Gray solid, 70% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.01 (d, *J* = 9.5 Hz, 1H), 7.45 (dd, 2H), 7.24 (s, 1H), 6.50 (s, 1H), 5.42 (s, 2H), 5.20 (s, 2H), 4.26 (t, *J* = 5.8 Hz, 2H), 3.14 (dd, *J* = 14.7, 7.0 Hz, 2H), 2.73 (t, *J* = 5.8 Hz, 2H), 2.48 (s, 4H), 1.95 – 1.79 (m, 2H), 1.58 – 1.48 (m, 4H), 1.40 (d, *J* = 4.7 Hz, 2H), 1.30 (t, *J* = 7.5 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 157.9, 157.3, 150.5, 149.9, 146.7, 144.8, 144.2, 131.8, 128.6, 128.3, 122.9, 118.7, 103.4, 96.4, 72.9, 66.6, 65.7, 57.8, 55.0 (2C), 49.9, 30.8, 26.1 (2C), 24.4, 22.6, 13.9, 8.2. HRMS (ESI): m/z calcd for C29H34N3O5: 504.24985 [M+H]$^+$; found: 504.24928.

(*S*)-2-((4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)acetate (1): A round

bottom flask were charged with 10-HCPT (547.0 mg, 1.5 mmol), K_2CO_3 (415.0 mg, 3.0 mmol), ethyl bromoacetate (200 μL, 1.8 mmol) and DMF (10 mL). The resulted mixture was stirred at room temperature for 12 h. The solvent was evaporated by rotary evaporation, and the residue was added with water (10 mL). A large amount of yellow solid precipitated and then filtered with suction. The filter cake was washed 3 times with water, dried and separated by silica gel column chromatography (DCM:MeOH=100:1~ 40:1) to obtain compound **1**. Light yellow powder, 71% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.08 (d, *J* = 9.2 Hz, 1H), 7.53 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.47 (d, *J* = 2.7 Hz, 1H), 7.25 (s, 1H), 6.49 (s, 1H), 5.41 (s, 2H), 5.27 (s, 2H), 5.05 (s, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.16 (m, 2H), 1.87 (m, 2H), 1.26 (t, *J* = 7.5 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.86 (t, *J* = 7.3 Hz, 3H).

(*S*)-2-((4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)acetic acid (2): Compound **1** (336.0 mg, 0.75 mmol), K_2CO_3 (104.0 mg, 0.75 mmol), H_2O (15 mL) and DMF (15 mL) were mixed in a flask. The suspension was stirred at room temperature for 14 h. The solvent was evaporated by rotary evaporation, and the residue was added with water (10 mL). The system was adjusted to pH 2~3 with 1 M HCl and the precipitated solid was filtered and dried by suction to obtain intermediate **2**. Dark yellow, 91% yield. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.15 (s, 1H), 8.08 (d, *J* = 9.2 Hz, 1H), 7.53 (m, 1H), 7.47 (d, *J* = 2.5 Hz, 1H), 7.26 (s, 1H), 6.49 (s, 1H), 5.41 (s, 2H), 5.28 (s, 2H,), 4.95 (s, 2H), 3.16 (m, 2H), 1.85 (m, 2H), 1.27 (t, *J* = 7.5 Hz, 3H), 0.86 (t, *J* = 7.3 Hz, 3H).

(*S*)-4,11-diethyl-4-hydroxy-9-(2-(4-methylpiperidin-1-yl)-2-oxoethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (B7): Compound **2** (100.0 mg, 0.22 mmol), HATU (169.0 mg, 0.44 mmol), 4-methylpiperidine (52 μL, 0.44 mmol), DIPEA (77 μL, 0.44 mmol) and DCM (5 mL) were mixed in a flask. The mixture was stirred at room temperature for 3.5 h and then isolated using column chromatography (DCM:MeOH = 75:1~50:1) to produce compound **B7**. Yellow solid, 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 9.2 Hz, 1H), 7.53 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.43 (d, *J* = 2.7 Hz, 1H), 7.27 (s, 1H), 6.52 (s, 1H), 5.43 (s, 2H), 5.29 (s, 2H), 5.09 (q, *J* = 14.7 Hz, 2H), 4.32 (d, *J* = 13.0 Hz, 1H), 3.93 (d, *J* = 13.7 Hz, 1H), 3.15 (d, *J* = 7.4 Hz, 2H), 3.07 (t, *J* = 12.0 Hz, 1H), 2.61 (t, *J* = 11.8 Hz, 1H), 1.87 (q, 2H), 1.77 – 1.56 (m, 3H), 1.30 (t, *J* = 7.6 Hz, 3H), 1.16 (d, *J* = 12.3 Hz, 1H), 1.00 – 0.86 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 165.6, 157.4, 157.3, 157.0, 150.5, 150.1, 146.7, 144.9, 144.1, 131.8, 128.7, 128.0, 122.6, 118.7, 104.0, 96.5, 72.9, 66.8, 65.7, 49.9, 45.0, 42.1, 34.8, 34.0, 30.8, 22.7, 22.1, 13.9, 8.2. HRMS (ESI): m/z calcd for C30H34N3O6: 532.24476 $[M+H]^+$; found: 532.24422.

(*S*)-benzyl 4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) carbonate (B8): To a solution of benzyl alcohol (96 μL, 0.93 mmol) and DMAP (170.0 mg, 1.39 mmol) in DCM (15 mL) were added 4-nitrophenyl carbonochloridate (280.0 mg, 1.39 mmol) in DCM (10 mL) dropwise in an ice bath. After the addition was complete, the reaction system was stirred at room temperature for 2 h and then the solvent was evaporated. Subsequently, SN-38 (110.0 mg, 0.28 mmol), TEA (115 μL, 0.84 mmol) and DMF (3 mL) were added to the residue. The mixture was stirred at room temperature overnight and isolated using column chromatography (DCM:MeOH = 50:1) to obtain compound **B8**. White solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, *J* = 9.2 Hz, 1H), 8.15 (d, *J* = 2.4 Hz, 1H), 7.77 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.58 – 7.37 (m, 5H), 7.33 (s, 1H), 6.54 (s, 1H), 5.44 (s, 2H), 5.34 (s, 2H), 5.30 (s, 2H), 3.17 (q, *J* = 7.6 Hz, 2H), 1.89 (q, *J* = 7.3 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 157.2, 153.3, 152.6, 150.5, 149.8, 147.0, 146.3, 146.0, 135.4, 131.9, 129.1, 129.1(3C), 128.9(2C), 127.5, 125.4, 119.6, 115.5, 97.2, 72.8, 70.6, 65.7, 50.0, 30.8, 22.7, 14.3, 8.2. HRMS (ESI): m/z calcd for C30H27N2O7: 527.18183 $[M+H]^+$; found: 527.18308.

(*S*)-10-allyl-4-ethyl-4,9-dihydroxy-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (Chimmitecan): A flask was charged with 10-HCPT (50.0 mg, 0.14 mmol), allyl bromide (24 μL, 0.27 mmol), K_2CO_3 (37.9 mg, 0.27 mmol) and DMF (1.5 mL). The resulted mixture was heated in an oil bath at 60°C for 3 h, then poured into ice water, adjusted to pH = 5 with 1M HCl, and the precipitated solid was filtered and dried for direct use in the next step. The solid obtained by suction filtration was added to glacial acetic acid (7 mL) and refluxed for 4 days under an argon atmosphere. The mixture was isolated using column chromatography (DCM:MeOH = 20:1) to obtain chimmitecan. Brown solid, 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.61 (s, 1H), 7.95 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 9.1 Hz, 1H), 7.26 (s, 1H), 6.50 (s, 1H), 6.07 – 5.94 (m, 1H), 5.41 (s, 2H), 5.23 (s, 2H), 4.99 (d, *J* = 5.0 Hz, 1H), 4.97 (d, *J* = 13.5 Hz, 1H), 3.78 (d, *J* = 4.6 Hz, 2H), 1.94 – 1.79 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 157.3, 154.2, 150.5, 149.3, 146.4, 144.0, 136.8, 130.1, 129.3, 128.9, 126.8, 122.7, 118.5, 117.6, 115.8, 96.3, 72.9, 65.7, 50.8, 30.7, 29.0, 8.2. HRMS (ESI): m/z calcd for C23H19N2O5: 405.14505 $[M+H]^+$; found: 405.14515.

ethyl (*S*)-2-((10-allyl-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)acetate (C1): Compound **C1** was prepared similarly as described for compound **1**. Pale yellow solid, 53% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.05 (d, *J* = 9.3 Hz, 1H), 7.67 (d, *J* = 9.4 Hz, 1H), 7.28 (s, 1H), 6.51 (s, 1H), 6.09 – 5.97 (m, 1H), 5.41 (s, 2H), 5.21 (s, 2H), 5.08 – 4.97 (m, 4H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.88 (d, *J* = 5.6 Hz, 2H), 1.93 – 1.79 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 169.2, 157.3, 154.0, 150.8, 150.4, 146.0, 144.5, 136.6, 130.4, 129.5, 128.1, 127.6, 121.7, 119.0, 118.8, 116.2, 96.7, 72.9, 66.1, 65.7, 61.2, 50.8, 30.7, 29.1, 14.5, 8.2. HRMS (ESI): m/z calcd for C27H27N2O7: 491.18183 $[M+H]^+$; found: 491.18194.

ethyl (*S*)-2-((10-allyl-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-2-methylpropanoate (C2): To a flask were added chimmitecan (70.0 mg, 0.17 mmol), ethyl 2-bromo-2-methylpropanoate (67.6 mg, 0.35 mmol), K_2CO_3 (47.8 mg, 0.35 mmol) and CH_3CN (10 mL). The reaction mixture was heated at reflux for 5 h and isolated using column chromatography (DCM:MeOH = 75:1) to obtain compound **C2**. Yellow solid, 78% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.02 (d, *J* = 9.3 Hz, 1H), 7.36 (d, *J* = 9.3 Hz, 1H), 7.30 (s, 1H), 6.51 (s, 1H), 6.08 – 5.94 (m, 1H), 5.42 (s, 2H), 5.25 (s, 2H), 5.06 – 4.97 (m, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.87 (d, *J* = 5.3 Hz, 2H), 1.94 – 1.81 (m, *J* = 13.8, 6.8 Hz, 2H), 1.61 (s, 6H), 1.20 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 173.0, 157.3, 151.9, 151.1, 150.5, 146.0, 144.8, 136.6, 130.5, 129.1, 128.3, 128.0, 124.7, 122.5, 119.1, 116.3, 96.8, 80.1, 72.9, 65.7, 61.8, 50.9, 30.7, 29.6, 25.9, 25.8, 14.4, 8.2. HRMS (ESI): m/z calcd for C29H31N2O7: 519.21313 $[M+H]^+$; found: 519.21320.

(*S*)-10-allyl-4-ethyl-4-hydroxy-9-(2-methoxyethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (C3): Compound **C3** was prepared similarly as described for **C2**. Off-white solid, 49% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.07 (d, *J* = 9.3 Hz, 1H), 7.77 (d, *J* = 9.4 Hz, 1H), 7.28 (s, 1H), 6.51 (s, 1H), 6.05 – 5.95 (m, 1H), 5.41 (s, 2H), 5.22 (s, 2H), 5.02 (d, *J* = 6.2 Hz, 1H), 4.99 (s, 1H), 4.33 (t, 2H), 3.84 (d, *J* = 5.8 Hz, 2H), 3.73 (t, 2H), 3.35 (s, 3H), 1.94 – 1.78 (m, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 157.3, 154.9, 150.6, 150.5, 146.1, 144.4, 136.7, 130.3, 129.6, 128.1, 127.5, 121.6, 119.5, 119.0, 116.1, 96.6, 72.9, 71.1, 69.1, 65.7, 58.8, 50.8, 30.7, 29.1, 8.2. HRMS (ESI): m/z calcd for C26H27N2O6: 463.18691 $[M+H]^+$; found: 463.18633.

ethyl (*S*)-2-((4-ethyl-4-hydroxy-3,14-dioxo-10-propyl-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)acetate (C4): Compound **C4** was prepared similarly as described for **A2**. Pale yellow solid, 73% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.63 (d, *J* = 9.4 Hz, 1H), 7.28 (s, 1H), 6.50 (s, 1H), 5.41 (s, 2H), 5.22 (s, 2H), 5.03 (s, 2H), 4.19 (q, *J* = 7.1 Hz, 2H), 3.08 (t, 2H), 1.93 – 1.80 (m, 2H), 1.63 (dq, *J* = 15.0, 7.4 Hz, 2H), 1.23 (t, *J*

= 7.1 Hz, 3H), 1.01 (t, *J* = 7.3 Hz, 3H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 169.3, 157.3, 153.9, 150.7, 150.5, 146.1, 144.5, 130.3, 128.9, 128.1, 127.6, 124.6, 119.0, 118.5, 96.6, 72.9, 66.0, 65.7, 61.2, 50.8, 30.7, 27.0, 23.4, 14.6, 14.5, 8.2. HRMS (ESI): m/z calcd for C27H29N2O7: 493.19748 [M+H]$^+$; found: 493.19703.

ethyl (*S*)-2-((4-ethyl-4-hydroxy-3,14-dioxo-10-propyl-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)-2-methylpropanoate (C5): Compound **C5** was prepared similarly as described for **A2**. Off-white solid, 89% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.78 (s, 1H), 7.98 (d, *J* = 9.3 Hz, 1H), 7.32 (d, *J* = 9.4 Hz, 1H), 7.29 (s, 1H), 6.51 (s, 1H), 5.42 (s, 2H), 5.26 (s, 2H), 4.23 (q, *J* = 7.0 Hz, 2H), 3.06 (t, 2H), 1.95 – 1.79 (m, 2H), 1.63 (s, 8H), 1.18 (t, *J* = 7.1 Hz, 3H), 1.02 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.8, 173.0, 157.3, 151.7, 150.9, 150.5, 146.1, 144.8, 130.3, 128.5, 128.3, 127.9, 127.4, 122.1, 119.1, 96.7, 79.8, 72.6, 65.7, 61.8, 50.9, 30.7, 27.4, 25.9, 25.9, 23.6, 14.6, 14.3, 8.2. HRMS (ESI): m/z calcd for C29H33N2O7: 521.22878 [M+H]$^+$; found: 521.22858.

(*S*)-10-allyl-4-ethyl-4-hydroxy-9-(2-oxo-2-(piperidin-1-yl)ethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (C6): Compound **C6** was prepared similarly as described for **B7**. The hydrolysate of **C1** was not purified, and condensed with piperidine to obtain **C6**. Pale yellow solid, 69% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.68 (s, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.64 (d, *J* = 9.5 Hz, 1H), 7.28 (s, 1H), 6.50 (s, 1H), 6.12 – 5.95 (m, *J* = 11.7, 5.9 Hz, 1H), 5.41 (s, 2H), 5.21 (s, 2H), 5.09 (s, 2H), 5.03 (dd, *J* = 21.8, 5.8 Hz, 2H), 3.88 (d, *J* = 5.2 Hz, 2H), 3.44 (s, 4H), 1.94 – 1.79 (m, 2H), 1.59 (s, 4H), 1.46 (s, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 165.9, 157.3, 154.6, 150.5, 150.5, 150.0, 146.1, 144.3, 136.7, 130.3, 129.3, 128.1, 127.4, 121.0, 118.9, 116.1, 96.6, 72.9, 67.2, 65.7, 50.8, 45.5, 42.7, 30.7, 29.1, 26.4, 25.7, 24.4, 8.3. HRMS (ESI): m/z calcd for C30H32N3O6: 530.22911 [M+H]$^+$; found: 530.22888.

(*S*)-10-allyl-4-ethyl-4-hydroxy-9-(2-morpholino-2-oxoethoxy)-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (C7): Compound **C7** was prepared similarly as described for **B7**. White solid, 51% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.68 (s, 1H), 8.04 (d, *J* = 9.4 Hz, 1H), 7.66 (d, *J* = 9.5 Hz, 1H), 7.28 (s, 1H), 6.50 (s, 1H), 6.09 – 5.99 (m, *J* = 10.3, 6.0 Hz, 1H), 5.41 (s, 2H), 5.21 (s, 2H), 5.12 (s, 2H), 5.02 (t, 2H), 3.88 (d, *J* = 5.4 Hz, 2H), 3.62 (d, *J* = 19.4 Hz, 4H), 3.55 – 3.44 (m, 4H), 1.93 – 1.80 (m, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 166.5, 157.3, 154.6, 150.5, 150.5, 146.1, 144.4, 136.7, 130.3, 129.3, 128.1, 127.4, 121.1, 119.0, 119.0, 116.1, 96.6, 72.9, 67.0, 66.5, 66.5, 65.7, 50.8, 45.1, 42.1, 30.7, 29.1, 8.3. HRMS (ESI): m/z calcd for C29H30N3O7: 532.20838 [M+H]$^+$; found: 532.20819.

(*S*)-4-ethyl-4-hydroxy-9-(2-oxo-2-(piperidin-1-yl)ethoxy)-10-propyl-1,12-dihydro-14*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4*H*)-dione (C8): Compound **C8** was prepared similarly as described for **A2**. Off-white solid, 89% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.74 (s, 1H), 8.00 (d, *J* = 9.3 Hz, 1H), 7.59 (d, *J* = 9.4 Hz, 1H), 7.28 (s, 1H), 6.50 (s, 1H), 5.42 (s, 2H), 5.23 (s, 2H), 5.07 (s, 2H), 3.45 (s, 4H), 3.12 – 3.02 (m, 2H), 1.87 (m, 2H), 1.60 (d, *J* = 6.5 Hz, 6H), 1.47 (s, 2H), 1.01 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 173.0, 166.0, 157.3, 154.5, 150.5, 150.4, 146.2, 144.3, 130.2, 128.7, 128.1, 127.4, 124.0, 119.0, 118.7, 96.6, 72.9, 67.1, 65.7, 50.8, 45.6, 42.7, 30.7, 27.0, 26.5, 25.7, 24.4, 23.4, 14.7, 8.3. HRMS (ESI): m/z calcd for C30H34N3O6: 532.24476 [M+H]$^+$; found: 532.24440.

(*S*)-10-allyl-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl benzyl carbonate (C9): Compound **C9** was prepared similarly as described for **B8**. White solid, 69% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.87 (s, 1H), 8.12 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 7.52 – 7.37 (m, 5H), 7.34 (s, 1H), 6.54 (s, 1H), 5.93 (m, *J* = 10.3, 5.9 Hz, 1H), 5.43 (s, 2H), 5.33 (s, 2H), 5.26 (s, 2H), 5.02 – 4.88 (m, 2H), 3.80 (d, *J* = 5.5 Hz, 2H), 1.87 (q, *J* = 7.2 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.9, 157.2, 153.3, 152.8, 150.4, 147.6, 147.0, 145.7, 135.6, 135.4, 130.8, 129.7, 129.4, 129.1, 129.1, 128.9, 128.8, 128.7, 127.7, 127.3, 126.4, 119.7, 117.0, 97.2, 72.8, 70.7, 65.7, 50.9, 30.8, 29.6, 8.2. HRMS (ESI): m/z calcd for C31H27N2O7: 539.18183 [M+H]$^+$; found: 539.18168.

(*S*)-10-allyl-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (4-bromobenzyl) carbonate (C10): Compound **C10** was prepared similarly as described for **B8**. White solid, 61% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.87 (s, 1H), 8.13 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.34 (s, 1H), 6.54 (s, 1H), 5.93 (dq, *J* = 10.6, 5.9 Hz, 1H), 5.43 (s, 2H), 5.28 (d, *J* = 14.2 Hz, 4H), 4.96 (q, 2H), 3.80 (d, *J* = 5.1 Hz, 2H), 1.96 – 1.78 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.9, 157.2, 153.2, 152.8, 150.4, 147.5, 147.0, 145.7, 135.6, 134.9, 132.0(2C), 131.0(2C), 130.8, 129.7, 129.4, 127.7, 127.3, 126.3, 122.3, 119.7, 117.0, 97.2, 72.8, 69.8, 65.7, 50.9, 30.8, 29.6, 8.2. HRMS (ESI): m/z calcd for C31H26BrN2O7: 617.09234 [M+H]$^+$; found: 617.09197 (^{79}Br), 619. 090580 (^{81}Br).

(*S*)-10-allyl-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl benzylcarbamate (C11): Compound **C11** was prepared similarly as described for **B8**. Off-white solid, 52% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.83 (s, 1H), 8.56 (t, *J* = 6.1 Hz, 1H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.42 – 7.35 (m, 4H), 7.34 – 7.26 (m, 2H), 6.53 (s, 1H), 5.98 (dq, *J* = 10.5, 6.0 Hz, 1H), 5.43 (s, 2H), 5.27 (s, 2H), 5.05 (d, *J* = 9.9 Hz, 1H), 5.02 (s, 1H), 4.34 (d, *J* = 6.0 Hz, 2H), 3.83 (d, *J* = 5.5 Hz, 2H), 1.93 – 1.82 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 172.9, 157.3, 155.1, 152.2, 150.5, 148.0, 146.7, 145.9, 139.6, 136.0, 130.5, 129.1, 128.9(3C), 128.7, 127.8, 127.5(2C), 127.5(2C), 119.5, 117.0, 97.1, 72.9, 65.7, 50.9, 44.6, 30.8, 30.0, 8.3. HRMS (ESI): m/z calcd for C31H28N3O6: 538.19781 [M+H]$^+$; found: 538.19771.

Solubility determination. B7, **C11** or SN-38 was supersaturated in water, acetate buffer (pH 4.5), or phosphate buffer (pH 7.4) and equilibrated for 24 h at room temperature with shaking. Mixtures were centrifuged for 8 min at 13000 r/min. Supernatants were analyzed by HPLC and quantified using standard curves generated with pure **B7**, **C11** and SN-38. All samples underwent isometric elution on a 5 mm Kromasil 100-5-C18 column (4.6 × 150 mm) at 40℃. Eluent composed of 70% methanol and 30% water (0.1% acetic acid) was run at a flow rate of 1 mL/min. Then the outflows were detected by the ultraviolet detector at 254 nm.

Cell culture and MTT assay. The tested six human tumor cell lines including human colon carcinoma cell lines (HCT-116, Lovo, Colo205 and HT-29), human lung adenocarcinoma epithelial cell line (A549) and human liver carcinoma cell line (HepG2) were purchased from Shanghai institute of life sciences, Chinese Academy of Sciences. All cancer cells were maintained in DMEM medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and incubated in a humidified atmosphere containing 5% CO_2 at 37℃. 10-HCPT (Cool Pharm, Ltd., purity: >98%) and SN-38 (Energy Chemical, purity: 98%) were used as positive controls. Cellular chemosensitivity was determined by using a modified MTT as we described.[19] Cells were simultaneously treated with final concentrations of 1.6, 8, 40, 200, 1000, 5000 nM of tested compounds. The IC_{50} value, that is, the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture, was calculated according to the inhibition ratios.

Colony formation assay. A549 cells were seeded in a six-well plate (300 cells/well), incubated for 24 h and then treated with various concentrations of **B7** or SN-38 for 10 days until colonies were visible. The medium with drugs were discarded, cells were washed with PBS and then fixed with methanol for 30 min. Then cells were washed with double distilled water twice and then dyed with 0.1% crystal violet for 30 min. Afterwards, cells were washed with water to wipe off the residual stain. The colony formations were imaged and counted.

Cell cycle analysis. A549 cells were plated into 6-well plate (1×10^6 cells/well) and incubated for 24 h and then incubated with various concentrations of **B7** or SN-38 for 24 h. The controls were treated with vehicle (0.1% DMSO). The cells were then harvested and fixed with 70% ice-cold ethanol overnight at 4℃. After centrifugation, the cell pellets were treated with propidium iodide (5 mg/mL) and RNase A in PBS (100 mg/mL) in PBS for 15 min at room temperature. DNA was observed and analyzed with a flow cytometer. The independent experiment was repeated three times.

Apoptosis assay. A549 cells were seeded into 6-well plate (1×10^6 cells/well) and incubated for 24 h and then incubated with various concentrations of **B7** or SN-38 for 24 h. The controls were treated with vehicle (0.1% DMSO). The cells were harvested, washed with PBS and stained with Annexin V-FITC/PI according to the manufacturer's instruction. Then the cells were analyzed with a flow cytometer.

In vivo antitumor efficacy. B7 was dissolved in DMSO, diluted with tween 80 and 5% glucose injection (DMSO: tween 80: 5% glucose injection = 1: 2: 7, volume ratio) to prepare different test solutions. Four-week-old Balb/c female nude mice were purchased from Beijing Vitong Lihua Experimental Animal Technology Company (Beijing, China) and housed in the Animal Center of Xi'an Jiaotong University, Xi'an, China, in a 12:12-h light/dark cycle with food and water readily available. A549 cells (5×10^6 cells/mouse) were transplanted into the right flank of the mice. The mice were randomly allocated to **B7**-treated and vehicle-treated groups (intraperitoneally administered with **B7** or solvent once the other day) when the average tumor volume reached 80 mm^3. Tumor size was measured with a caliper and calculated as 0.5 × length × $width^2$. The mice were sacrificed on the second day of the last treatment, and the tumor masses were isolated and weighed.

$$TGI\ \% = [1-(V_{d26,\ B7}-V_{d0,\ B7})/(V_{d26,\ vehicle}-V_{d0,\ vehicle})] \times 100\%$$

$V_{d26,\ B7}$ and $V_{d0,\ B7}$ mean the tumor volume of **B7** group on d26 and d0, respectively. $V_{d26,\ vehicle}$ and $V_{d0,\ vehicle}$ mean the tumor volume of vehicle group on d26 and d0, respectively.

Acute toxicity test. Kunming species mice (20.0 ± 2.0 g) were purchased from Experiment Animal Center of Xi'an Jiaotong University and fed in the same place. DMSO/ tween 80/ 5% glucose (1: 2: 7, volume ratio) were used as mix solvents. Kunming species mice were injected intravenously with a volume of 5 mL/kg. **B7** was dissolved in the mix solvents to give test solutions of 5, 7, 10 mg/mL. Irinotecan was dissolved in the mix solvents to give test solutions of 6, 8, 10 mg/mL. The maximum solubility of SN-38 in the mix solvents is 5 mg/mL, thus, the maximum single dose of SN-38 was 25 mg/kg. When the dose is larger than this, the solution of SN-38 should be given once every 10 min until the final dose was reached. On the first day of drug administration, mice were observed for 10 days to determine the minimum lethal dose.

Molecular docking studies. To explore the binding mode of **B7** and SN-38 with Topo I-DNA complex (PDB code 1k4t), we utilized the C-DOCKER program within Discovery Studio 2.5 software package. The topotecan with human topoisomerase I/DNA protein-ligand complex crystal structure was chosen as the template. The reported compound was removed and **B7** was placed. After end of molecular docking, ten docking poses were scored and selected based on calculated C-DOCKER energy. The diagrams were prepared using PyMol 1.6 software.

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Conflict of interest

There is no interest conflict to declare.

Keywords: camptothecin derivatives • drug design • cytotoxicity • solubility • anti-tumor effect

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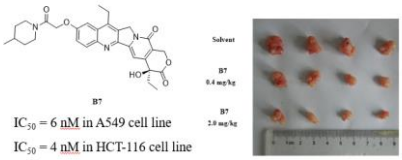

Twenty-seven camptothecin derivatives were synthesized and cytotoxicity screened. Compound **B7**, 7-ethyl-10-(2-oxo-2-(4-methylpiperidin-1-yl)ethoxy)camptothecin, was demonstrated in vitro and in vivo to be a potent antitumor agent. And **B7** exhibited minimum lethal doses comparable to irinotecan. Additionally, the solubility of **B7** reached 5.73 μg/mL in water. These results indicated that **B7**, with improved solubility, enhanced activity and acceptable acute toxicity, can be used as a lead compound for development of novel anti-cancer agent.