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Synthesis and antitumor activity of novel 20s-camptothecin analogues

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ABSTRACT

In an effort to decrease the toxicity and improve the stability of labile lactone ring of camptothecin, nitrogenous heterocyclic aromatic groups were introduced into 20-position of camptothecin and seventeen new 20s-camptothecin derivatives were obtained in quantitative yield. The cytotoxicity in vitro on three cancer cell lines and the stability of the lactone in phosphate-buffered solution (PBS) of these derivatives were evaluated. Most of these tested derivatives possessed better cytotoxicity than topotecan. Analogues **6, 12** exhibited the best antitumor activity in vivo in all derivatives we prepared. The results suggested that introduction of pyrazole in 10- or 20-position of camptothecin could promote antitumor activity in vitro and in vivo, simultaneously bring much increase of the stability of lactone.

20(S)-Camptothecin (CPT, **1**), that targets the nuclear enzyme topoisomerase I, is a naturally occurring cytotoxic alkaloid isolated from Chinese plant *Camptotheca acuminata*.[1–4] However, clinical use of CPT had been severely hindered by toxicity stemming in part from the instability of its E-lactone ring, resulting in the formation of the inactive but toxic carboxylate species.[5,6] In addition, extremely poor water solubility and severe toxicity have plagued the development of CPT as a therapeutic agent. These results prompted the synthesis of many CPT derivatives, such as topotecan (TPT, **2**) and irinotecan (**3**) (Fig. 1) have been approved as antineoplastic agents and a number of other analogues of CPT are currently in clinical trials.[7–10] Ever since E-ring plays a key role in supporting both efficient topoisomerase I inhibition and potency in vivo, 20-OH of E-ring modifications can either eliminate the intramolecular hydrogen bonding or increase the steric hindrance of carbonyl group.[11] The compounds which have a 20-OH substituted groups can increase the stability of the drugs–DNA–Topo I ternary complex,[12] decrease the toxicity and weak the opening of the lactone ring.[13]

In previous studies, we have introduced nitrogenous heterocyclic aromatic groups in the 10-postion of camptothecin and obtained a series of 10-substituted camptothecin analogues, some of which showed good water solubility and potent antitumor activity in vitro and in vivo. These nitrogenous heterocyclic aromatic groups introduced in 10-postion can indeed enhance stability of lactone and antitumor activity of CPT.[14,15] In order to investigate the influence of nitrogenous heterocyclic aromatic substituted groups on activity and stability of CPT, they were introduced into the molecule of CPT at its 20-hydroxyl. Based on the structure–activity relationship (SAR), more stability and less toxicity of these novel analogues are expected to be achieved.

In our experiments, camptothecin was firstly converted into camptothecin-20-*O*-chloroacetate (**4**) in high yield (97.5%) in the presence of a double agent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and a catalyst 4-dimethylaminopyridine (DMAP) at room temperature.[11] The CPT derivatives (**6–11, 18–22**) were prepared in proper yields by treatment of **4** with heterocyclic aromatic compounds in dimethylsulfoxide (DMSO) at 60 °C (Scheme 1). Compound **11** was a byproduct from most of these reactions. 2-Chloropropionic acid was also attached to 20-OH of CPT to obtain camptothecin-20-*O*-2-chloropropionate (**5**), and the derivatives **12–17** were synthesized by compound **5** and corresponding heterocyclic aromatic compounds. The yield (98.1%) of compound **5** was also high, but the yields of its related derivatives **12–17** (17.8–45.0%) were lower than that of compounds **6–11** (27.3–86.5%). This may relate to the electron-donating effect of methyl and the high sterical hindrance of the side-chain linked to compound **5**. Compounds **18–22** were pyridine quaternary salt derivatives and they were all obtained in low yields from the reactions of **4** with corresponding pyridine heterocyclic aromatic groups. We also tried to synthesize pyridine quaternary salt derivatives of **5**, but the yields of these products were too low to get pure products. The ^{1}H NMR, ESI-MS, IR, and HR-MS spectra of these novel camptothecin derivatives were consistent with their structures and were listed in Supporting Information.

To determine the stability of these new derivatives prepared, some compounds were also subjected to PBS (pH 7.4) stability studies.[15] The analogues were incubated in 0.1 mol/L PBS at 37 °C, aliquots were taken at different time points and examined

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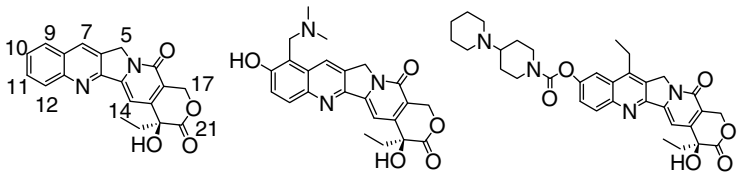
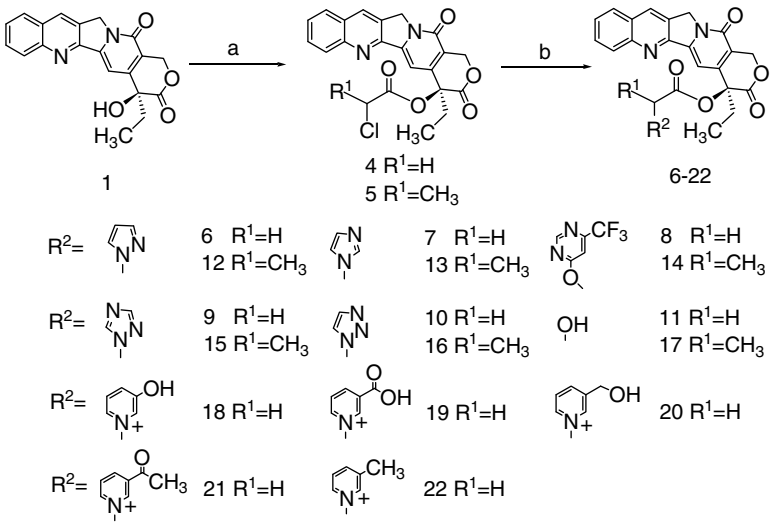


Figure 1. Structures of camptothecin (**1**), topotecan (**2**), and irinotecan (**3**).



Scheme 1. Reagents and conditions: (a) $ClCH_2COOH$ or $CH_3ClCHCOOH$, EDCI, DMAP, CH_2Cl_2, rt; (b) R^2H and pyridines derivatives, DMSO, 60 °C.

by HPLC. The results were shown in Table 1. Compounds **6** and **11, 12** revealed complete buffer stability for the length of the experiment (>48 h). Compound **9** were also stable in buffer with a half-life of around 45 h. The compounds (**6, 12**) introduced pyrazole at 20-position of CPT showed good stability, which was surprisingly consistent with the previous research,[15] pyrazole also improved stability of lactone when it was introduced at 10-position of CPT.

Table 1
Cytotoxicity and buffer half-life of CPT derivatives

Compound	In vitro cytotoxicity (IC_{50}, μM)			Half-life (h)
	MCF-7[b]	HCT-8	PC-3	
TPT	5.55	3.66	3.99	—[a]
6	0.12	0.61	0.16	>48
7	0.90	0.60	0.21	24
8	1.79	1.51	1.34	14
9	0.69	0.74	0.15	45
10	1.20	0.38	0.16	24
11	10.37	3.00	0.03	>48
12	0.81	0.23	0.13	>48
13	0.11	0.59	0.38	18
14	0.27	1.11	0.32	9
15	0.65	0.51	0.44	14
16	0.16	0.48	0.59	13
17	0.56	0.82	0.50	—
18	3.80	2.43	0.11	—
19	2.12	0.76	0.55	—
20	2.57	2.09	0.80	—
21	3.45	2.58	0.54	—
22	3.76	3.78	0.60	—

[a] —, not tested.
[b] MCF-7, human breast cancer; HCT-8, human colon cancer; PC-3, human prostate cancer.

Cytotoxicity of these derivatives were evaluated on three different human cancer cell lines (MCF-7, HCT-8 and PC-3) using MTT assay.[16] TPT was used as the reference drug. The results of the cytotoxicity studies were shown in Table 1. Most of the derivatives displayed potent or similar cytotoxic activity in vitro compared with TPT. All the analogues showed 10–30 times more active than TPT against PC-3 cell line, with IC_{50} value (defined as the concentrations corresponding to 50% growth inhibition) ranging from 0.03 to 0.80 μM. Compounds **6, 13, 16** had more efficacy on MCF-7 cell line and compound **12** showed best activity on HCT-8 cell line. The compounds **6** and **12**, which contained pyrazole substituent,

Table 2
Antitumor activity of the analogues (q3d × 3) against HCT-8 xenografted model

Compound	Dose (mg/kg)	Lethal[a] toxicity	BWC%[b]	TIR[c](%)
Control	—	0/8	+35	—
TPT	5	0/8	−3	62.0**
	10	3/8	−29	92.5**
6	10	0/8	+7	35.3*
	20	0/8	+8	75.6**
7	10	0/8	+6	27.8
	20	0/8	+1	46.2*
8	10	0/8	+36	25.4
	20	0/8	+12	49.3*
12	10	0/8	−2	77.1**
	20	1/8	−5	92.9**

Student's *t*-test was used.
* *P* value < 0.05.
** *P* value < 0.01, versus the control group.
[a] Number of the dead mice/total number of mice.
[b] Percentage of mice body-weight change (BWC) after drug treatment: BWC% = (mean BW final day/mean BW first day × 100) − 100; "+" means body-weight increase; "−" means body-weight decrease.
[c] Tumor inhibitory rate.

revealed obviously potent cytotoxicity. The results also indicated these CPT analogues which were modified by nitrogenous heterocyclic aromatic groups exhibited good cytotoxicity.

The preliminary antitumor activity studies in vivo of these compounds were evaluated against mouse sarcoma S180, compared with that of TPT. The pyridine quaternary salt derivatives **18–22** all showed low antitumor activity and high toxicity. The further antitumor activity in vivo of the promising analogues **6, 7, 8, 12** were evaluated with HCT-8 xenografted model on male BALB/c nu/nu mice using intraperitoneal (ip) injection compared with TPT (Table 2). Compound **12** possessed the best antitumor activity among these compounds, achieved a tumor inhibitory rate (TIR) of 92.9% at a dose of 20 mg/kg (similar to the TIR of TPT at a dose of 10 mg/kg) and showed better dose-efficacy relationship. Compound **6** also achieved a TIR of 75.6% at a dose of 20 mg/kg. Other derivatives did not show efficient antitumor activities in vivo. Compounds **6**, **12** possessed lower toxicity than TPT from the change of the mice body-weight before and after administration. Interestingly, substituent on derivatives **6, 12** were also pyrazole.

In summary, seventeen new CPT derivatives modified by nitrogenous heterocyclic aromatic groups were prepared, and these CPT analogues showed better cytotoxicity than TPT, the stability of the lactone in PBS was increased mostly. Compound **12** exhibited the best antitumor activity among these compounds in vivo. Especially compound **12, 6** modified by pyrazole showed notable antitumor activity and stability of the lactone, which were consistent with that of our previous studies.[14] The antitumor activity was not only related to the substituted positions but also to substituent. Further biological studies about these derivatives are still underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.031.

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