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Synthesis of new camptothecin analogs with improved antitumor activities

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ABSTRACT

Novel hexacyclic camptothecin analogs containing cyclic amidine, urea, or thiourea moiety were designed and synthesized based on the proposed 3D-structure of the topoisomerase I (Topo I)/DNA/camptothecin ternary complex. The analogs were prepared from 9-nitrocamptothecin via 7,9-diaminocamptothecin derivatives as a key intermediate. Among them, **7c** exhibited in vivo antitumor activities superior to CPT-11 in human cancer xenograft models in mice at their maximum tolerated doses though its in vitro antiproliferative activity was comparable to SN-38 against corresponding cell lines.

Tremendous efforts have been made over the years to discover more effective agents for the treatment of gastro-intestinal tumors, but 5-fluorouracil (5-FU)/leucovorine, an oral 5-FU prodrug (capecitabine) and a camptothecin derivative (irinotecan [CPT-11]) still remain the key drugs for the treatment of advanced gastric and/or colorectal cancer (CRC), in combination with other agents.[1,2]

CPT-11 is a prodrug of SN-38 with improved water-solubility. It is used for treatment of CRC in combination with 5-FU/leucovorine (named FOLFIRI) with or without an additional agent (e.g., bevacizumab). The clinical efficacy of CPT-11 is, however, limited due to the following drawbacks: high inter-patient variability in pharmacokinetics (from poor bioconversion to the active drug, SN-38, and SNPs of the metabolizing enzyme, UGT1A1) and severe toxicity in bone marrow and intestine. The camptothecin analogs with an amino group in the core structure for increasing solubility, for example, topotecan and Dx8951 (Fig. 1), showed limited clinical efficacy, likely because of insufficient tissue distribution in human.[3,4] In order to develop a new camptothecin analog with higher antitumor efficacy and broader spectrum by overcoming the drawbacks of CPT-11 mentioned above, we designed new lipophilic camptothecin analogs with high profile tissue distribution and their water-soluble prodrugs for intravenous application. In this paper, we describe the design, synthesis, and biological activities of the parent drug CH0793076 (**7c**) that exhibited higher antitumor activity than CPT-11 in various human cancer xenograft models.

We designed new camptothecin analogs (Fig. 2) based on the ternary complex of DNA-Topo I-camptothecin proposed by Redinbo et al.[5] In this model, we found a large space around the C-7 position of camptothecin that allowed the introduction of an additional F-ring and a hydrophobic side chain (R^1) (Fig. 3a). There

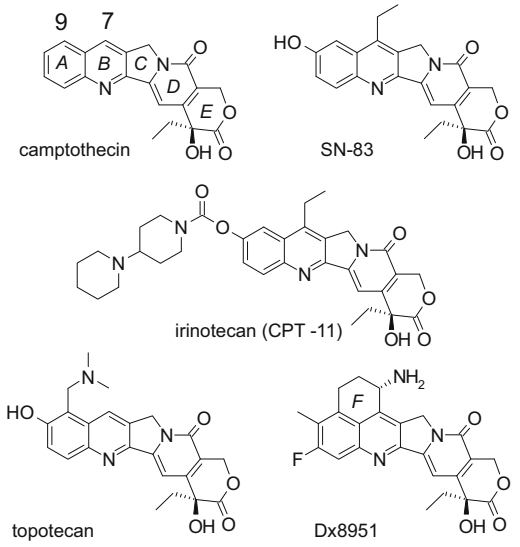

Figure 1. Camptothecin and representative analogs.

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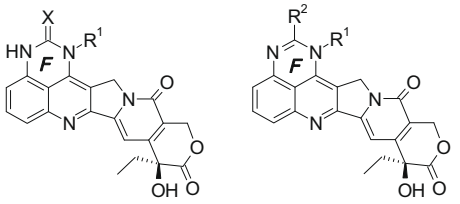


Figure 2. Newly designed camptothecin analogs.

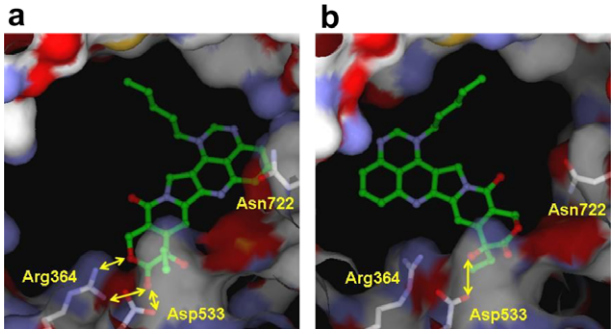


Figure 3. Presumed binding conformation of CH0793076 (**7c**) with DNA-Topo I complex. The presumed binding conformation (a) was made based on the model proposed by Redinbo et al. Originally, we designed our compounds based on the binding conformation (a). The presumed binding conformation (b) was made based on the crystal structure of DNA-Topo I-topotecan ternary complex (PDB accession number, 1K4T). Although the binding conformation (b) is different from the binding conformation (a), the additional F-ring and the hydrophobic side chain occupy the same binding pocket. DNA structures which intercalate with CH0793076 (**7c**) are omitted to be clarified.

is also a large space around the C-7 position of camptothecin in a new model based on the crystal structure of DNA-Topo I- topotecan ternary complex (Fig. 3b).[6]

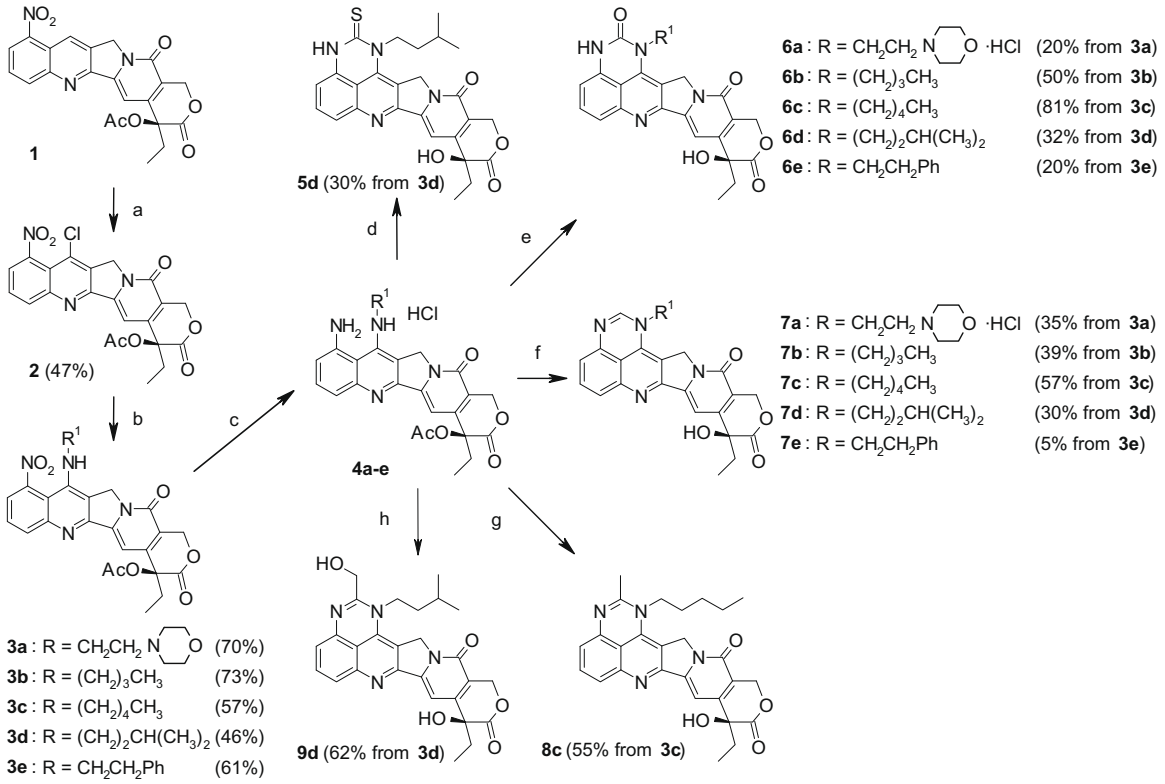
In order to synthesize various heterocyclic rings for the F-ring, the key intermediates, 7,9-diaminocamptothecins **4a–e**, were prepared from 9-nitrocamptothecin 20(*S*)-*O*-acetate (**1**) as illustrated in Scheme 1.[7] **1** was oxidized to the *N*-oxide using a hydrogen peroxide-urea complex, followed by treatment with oxalyl chloride to give a 7-chloro derivative (**2**).

A substituted amino group was introduced at C-7 by treatment of **2** with various amines in refluxing dioxane to give **3a–e** in modest to good yields. Derivatives **3a–e** were converted to the corresponding diamino derivatives **4a–e** by hydrogenation.

The resulting diamino derivatives **4a–e** were then transformed into various camptothecin analogs having an F-ring. Treatment of **4a–e** with triphosgene followed by the removal of the acetyl group at C-20 with hydrazine gave urea type analogs **6a–e**. In the same manner and by treating with thiocarbonyl 1,1′-diimidazole, **4d** was converted to the thiourea analog **5d**.

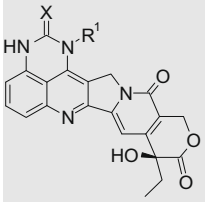
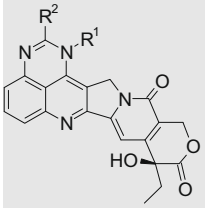
The syntheses of cyclic amidine analogs were carried out under two conditions: (1) **7a–e** and **8c** derivatives were obtained by treating **4** with methyl orthoformate or methyl orthoacetate in the presence of an acid catalyst followed by hydrazine treatment, and (2) the functionalized cyclic amidine analog **9d** was prepared by the reaction of **4d** with acetoxyacetyl chloride in the presence of diisopropylamine, followed by hydrolysis of the ester groups with hydrazine.

The in vitro antiproliferative activities of the new camptothecin analogs against human cancer cell lines, CRC (HCT116) and NSCLC (non-small cell lung carcinoma; QG56, NCI-H460), were evaluated (Table 1). The cyclic urea and amidine analogs having a morpholi-



Scheme 1. Reagents and conditions for camptothecin analogs: (a) (i) hydrogen peroxide-urea complex, TFA, rt, 5 h; (ii) oxalyl chloride, DMF, 15 °C, 1.5 h; (b) R^1NH_2, 1,4-dioxane, reflux; (c) Pd/C, H_2(g), MeOH/HCl, rt; (d) (i) thiocarbonyl 1,1′-diimidazole, diisopropylethylamine, DMAP, DCM, reflux, 6 h; (ii) hydrazine, MeOH, rt; (iii) 4 N HCl/MeOH; (e) (i) triphosgene, DCM, rt; (ii) hydrazine, MeOH, rt; (iii) 4 N HCl/MeOH; (f) (i) orthoformic acid methyl ester, TsOH; (ii) hydrazine, MeOH, rt; (iii) 4 N HCl/MeOH; (g) (i) orthoacetic acid methyl ester, TsOH, DCM, reflux, 3 h; (ii) hydrazine, MeOH, rt; (iii) 4 N HCl/MeOH; (h) (i) acetoxyacetyl chloride, diisopropylethylamine, DCM, rt, overnight; (ii) hydrazine, MeOH, rt; (iii) 4 N HCl/MeOH.

Table 1
In vitro antiproliferative activities of the derivatives against human cancer cell lines

Core structure	X	R^1	R^2	Compound	In vitro IC_{50} (nM)		
					HCT116 (CRC)	QG56 (NSCLC)	NCI-H460 (NSCLC)
	O	CH_2CH_2 N O ·HCl	—	**6a**	3.6	18	26
	O	$(CH_2)_3CH_3$	—	**6b**	0.40	1.2	0.99
	O	$(CH_2)_4CH_3$	—	**6c**	0.85	8.5	8.2
	O	$(CH_2)_2CH(CH_3)_2$	—	**6d**	0.05	0.58	0.72
	O	CH_2CH_2Ph	—	**6e**	0.25	1.2	0.38
	S	$(CH_2)_2CH(CH_3)_2$	—	**5d**	0.02	0.16	0.08
	—	CH_2CH_2 N O ·HCl	H	**7a**	2.2	9.4	7.2
	—	$(CH_2)_3CH_3$	H	**7b**	0.52	2.3	1.6
	—	$(CH_2)_4CH_3$	H	**7c**	0.36	2.3	2.3
	—	$(CH_2)_2CH(CH_3)_2$	H	**7d**	<0.04	0.25	0.11
	—	CH_2CH_2Ph	H	**7e**	0.36	2.1	0.91
	—	$(CH_2)_4CH_3$	Me	**8c**	0.12	1.8	0.66
	—	$(CH_2)_2CH(CH_3)_2$	CH_2OH	**9d**	0.44	1.8	0.52
SN-38					0.55	2.8	3.3

In vitro cytotoxic assay: The cells were exposed to test compounds for 3 days at 37 °C in an incubator containing 5% CO_2 in air. The concentration of a test compound producing 50% inhibition (IC_{50}) of cell growth was calculated.

noethyl group in the side chain (**6a, 7a**) showed weaker activity than SN-38, whereas the analogs having hydrophobic side chains (**6b–e**, **7b–e**) exhibited activities comparable to or stronger than SN-38. From the introduction of a methyl or hydroxymethyl group on the amidine carbon (**8c**, **9d**), the antiproliferative activities were retained. Among the analogs synthesized, **5d, 6d**, and **7d** with an isopentyl side chain exhibited most potent activities with IC_{50} values against human colon cancer cell line HCT116 of 0.02, 0.05, and <0.04 nM, respectively.

These analogs were further evaluated for their in vivo antitumor activity against human cancer xenograft HCT116 (CRC) as well as the effective dose range, defined as the range from the effective dose for 50% tumor growth inhibition (ED_{50}) to the maximum tol-

Table 2
Effect of BCRP on antiproliferative activities of SN-38 and **7c** in PC-6/BCRP and PC-6/pRC human small cell lung cancer cell lines

	IC_{50} (nM)[Mean ± S.E.]	
	SN-38	**7c**
PC-6/BCRP	5.1 ± 1.8	0.35 ± 0.04
PC-6/pRC	0.43 ± 0.07	0.18 ± 0.01
Ratio	12	2

The PC-6/BCRP and PC-6/pRC cells were established by transfecting the BCRP gene and vector, respectively, into PC-6 human small-cell lung cancer cells. The cells were exposed to test compounds for 6 days. The experiments were performed in triplicate.

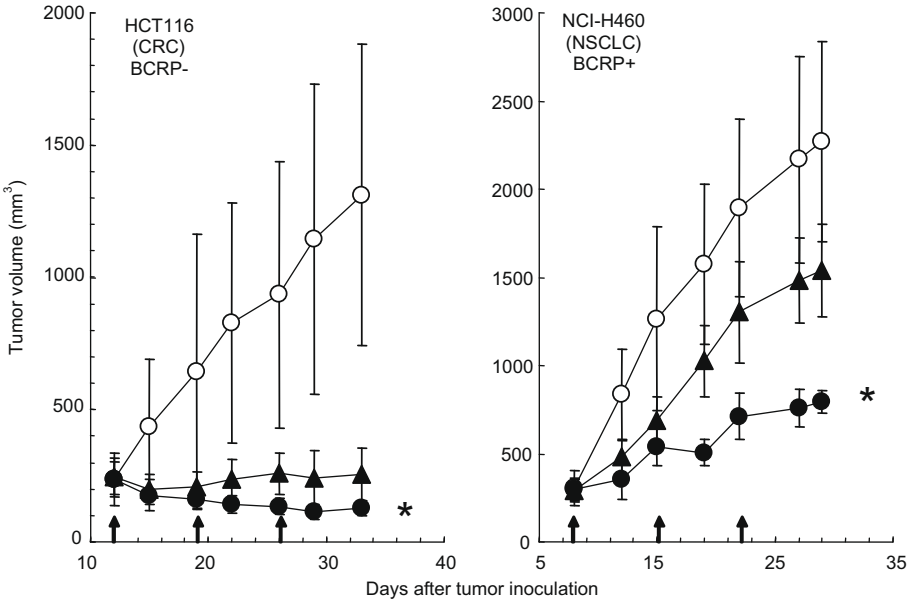


Figure 4. Antitumor effect of CH0793076 and CPT-11 in human cancer xenograft models. CH0793076 and CPT-11 were administered at the maximum tolerated dose (MTD) by bolus intravenous injection once per week for 3 weeks. The MTD was 40 mg/kg for CH0793076 and 100 mg/kg for CPT-11. Each group consisted of 4 to 5 mice. Values indicate the mean tumor volume with standard deviation. ○: vehicle, ▲: CPT-11, ●: CH0793076. BCRP protein was detected by Western blotting. *: Statistically significantly differences in mice treated with CH0793076 compared with mice treated with CPT-11 ($P < 0.05$).

erated dose (MTD). From the results, **7c** (CH0793076) was selected as a candidate for further development.

7c showed somewhat stronger inhibitory activity (IC_{50} = 2.3 μM) against human topoisomerase I than SN-38 (IC_{50} = 5.5 μM). It has been reported that CPT-11 is sensitive to the drug efflux pump BCRP (breast cancer resistant protein) that is overexpressed in several human cancer cell lines.[8] Although information on expression levels in the tumors in cancer patients is still limited, BCRP is thought to be one of the factors that limit the antitumor activity of CPT-11. We tested the effect of BCRP on the antiproliferative activity of SN-38 and **7c** in human small cell lung cancer cell line PC-6 that had been transfected with a BCRP gene (PC-6/BCRP) and with the vector alone (PC-6/pRC). The activity of SN-38 showed a decrease in the BCRP-gene transfected cells, but, in contrast, **7c** was only marginally influenced by the presence of BCRP (Table 2).

The antitumor activity of **7c** (one a week iv treatment × 3 at the MTD) was further evaluated in two cancer xenograft models: human colon cancer HCT116 (BCRP negative) and human non-small cell lung cancer NCI-H460 (BCRP positive).

The results are shown in Figure. 4. Compound **7c** exhibited significantly higher efficacy than CPT-11 in these two human cancer xenografts regardless of the level of BCRP expression.

In summary, we successfully designed and synthesized new hexacyclic camptothecin analogs based on the proposed structure of the Topo I/DNA/camptothecin ternary complex. CH0793076 (**7c**) is not a substrate of drug efflux pump BCRP and showed more potent antitumor activity in human cancer xenograft models than CPT-11, regardless of the level of BCRP expression. Thus, **7c** was selected as a parent drug for further development of the new water-soluble prodrug which enables intravenous administration.

We have developed a new water-soluble prodrug of **7c** that can be activated by a non-enzymatic process, namely pH-dependent activation. The results will be reported in a separate paper.

Acknowledgments

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