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SYNTHESIS AND ANTITUMOR ACTIVITY OF 7-SUBSTITUTED 20(*RS*)-CAMPTOTHECIN ANALOGUES

Sang-sup Jew[1]*, Hee-Jin Kim[1], Myoung Goo Kim[1], Eun-Young Roh[1], Youn-sang Cho[1], Joon-Kyum Kim[2], Kyung-Hoe Cha[2], Kang-Keun Lee[2], Hyun-Jung Han[2], Jae-Young Choi[3], Heesoon Lee[3]*

[1]*College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[2]*Chong Kun Dang Pharmaceutical Co. Seoul 152-070, Korea*
[3]*College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea*

Abstract: Novel water-soluble camptothecin analogues with excellent antitumor activity have been designed and synthesized by total synthesis. The analogues were evaluated for cytotoxic activity against five tumor cell lines. The most potent analogue **6c** exhibited significant antitumor activity at wider range of doses as compared with camptothecin (T/C 243 % at 70mg/kg; T/C > 150% at 8.75-140mg/kg). Copyright © 1996 Elsevier Science Ltd

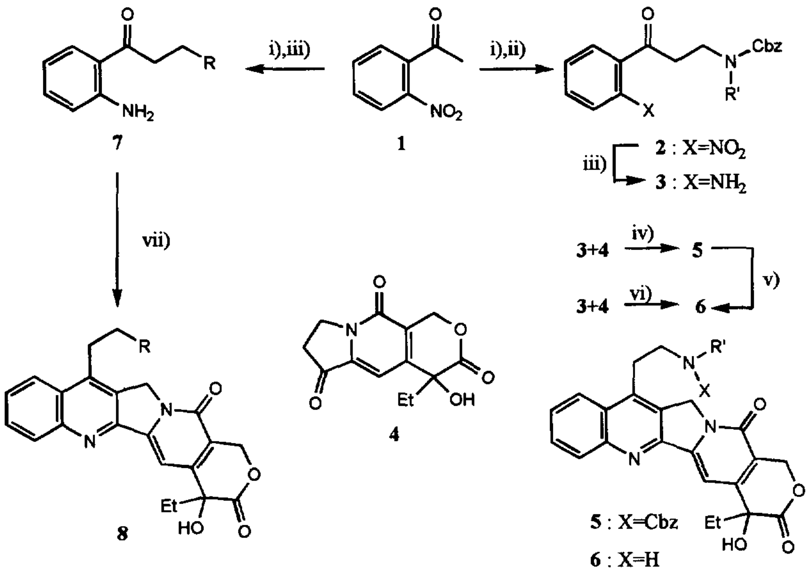
Camptothecin, a pentacyclic alkaloid natural product, was first reported by Wall and co-workers to have potent antitumor activity[1]. Despite its strong antitumor activity in experimental tumors including solid tumors, the development of camptothecin was stopped because of a variety of unacceptable side effects on humans[2]. However, the recent finding of the peculiar mechanism of action of camptothecin, the inhibition of topoisomerase I, has rekindled as a drug[3]. Numerous analogues have been synthesized and recent efforts have been directed toward the development of water-soluble analogues[3].

We recently reported an enantioselective synthesis of 20(*S*)-camptothecin using Sharpless catalytic asymmetric dihydroxylation[4] and turned our attention to the investigation of novel camptothecin analogues having better therapeutic efficacy without undesirable side effects. Our strategy is as follows: 1) incorporation of the side chain containing a basic nitrogen at 7-position of camptothecin, 2) replacement of C-20 ethyl group with other groups containing oxygen or nitrogen to explore the effect of incorporated heteroatoms 3) establishment of the optimum structures that would be obtained by combining the previous two approaches. We, herein, wish to report the first approach.

7-Substituted camptothecin analogues were prepared by total synthesis in analogy with the published methods (Scheme I)[5]. Mannich reaction of 2′-nitroacetophenone **1** with various alkyl amines (ethyl-, benzyl-, n-propyl-, *i*-propylamine and 2-aminoethanol) followed by subsequent protection of the resulting amines with benzyl chloroformate (CbzCl) afforded carbamates **2** (**2a**, 36%; **2b**, 31%; **2c**, 19%; **2e**, 28%; **2f**, 27%). **2** were transformed into amines **3** by treatment with sodium dithionite (**3a**, 58%; **3b**, 48%; **3c**, 43%; **3d**, 43%;

3f, 37%). Friedlander condensation of **3** with racemic tricyclic ketone **4**[6] was performed to give pentacyclic analogues **5** (**5a**, 63%; **5b**, 60%; **5c**, 30%; **5d**, 38%; **5e**, 12%). The camptothecin analogues **6** were obtained by deblocking the Cbz groups by catalytic hydrogenation in acetic acid (**6a**, 63%; **6b**, 53%; **6d**, 20%; **6e**, 30%). During Friedlander condensation of **3c** and **3f** with **4**, the Cbz group was gradually deprotected by excess *p*-toluenesulfonic acid to directly give **6c** and **6f** (**6c**, 76%; **6f**, 67%). Condensation of **7** with **4** immediately produced the camptothecin analogues **8** containing a tertiary amine (**8g**, 40%; **8h**, 27%; **8i**, 29%). The required aminoketones **7** were prepared by Mannich reaction of **1** with various dialkyl amines (pyrrolidine, piperidine and morpholine) followed by reduction of the nitro group with sodium dithionite (**7g**, 67%; **7i**, 89%).

Scheme I



	R
g	pyrrolidinyl
h	piperidinyl
i	morpholinyl

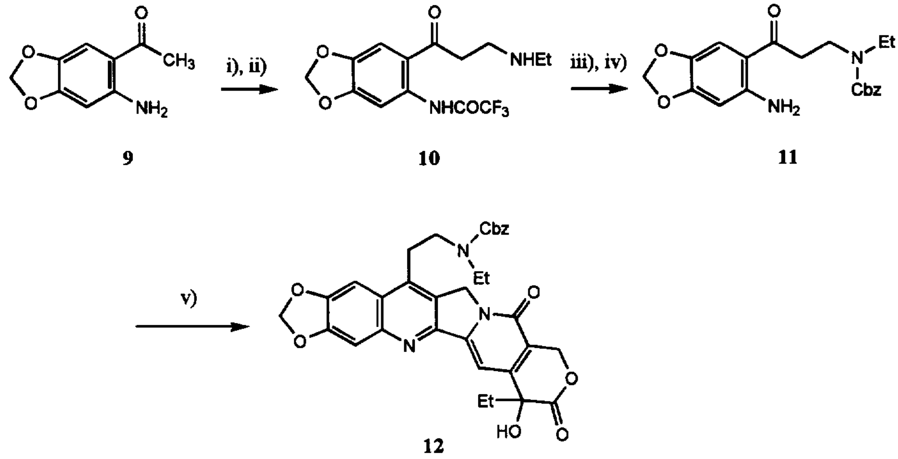
	R'
a	bn
b	n-Pr
c	*i*-Pr

	R'
d	CH_2CH_2OAc
e	CH_2CH_2OH
f	Et

Reagents: i) $R'NH_2$ or R_2NH, $(CH_2O)n$, EtOH, c-HCl, reflux, ii) CbzCl, Et_3N, CH_2Cl_2, rt, iii) $Na_2S_2O_4$, EtOH, reflux, iv) cat. *p*-TsOH, toluene, reflux, v) H_2, 10% Pd/C, AcOH, vi) excess *p*-TsOH, toluene, reflux, vii) **4**, cat. *p*-TsOH (or TFA), toluene, reflux.

Hexacyclic camptothecin analogue **12** was prepared according to the procedure similar to the previous approach[5](Scheme II). After protection of the amino group of the acetophenone **9** with trifluoroacetic anhydride, Mannich reaction with ethylamine was performed to give **10**. Protection of **10** with CbzCl (19%) followed by removal of the trifluoroacetamide group provided aminoketone **11** (31%). The hexacyclic analogue **12** was obtained by Friedlander condensation of **11** with **4** (40%).

Scheme II



Reagents: i) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, rt, ii) $EtNH_2 \cdot HCl$, $(CH_2O)n$, c-HCl, EtOH, reflux, iii) CbzCl, Et_3N, CH_2Cl_2, rt, iv) LiOH, THF-MeOH-H_2O, rt, v) **4**, cat. *p*-TsOH, toluene, reflux.

Table I. Cytotoxicity of Camptothecin Analogues against Human Tumor Cell Lines[7] (IC_{50}, μM)[8].

Compound	A172	DLD-1	CAOV-3	KATO-III	L1210
5a	1.18	1.06	1.97	13.96	0.70
5b	2.33	0.55	0.14	0.51	1.09
5c	0.95	1.83	0.041	1.85	1.29
5d	18.56	15.84	4.48	16.64	9.08
5e	1.92	9.72	1.14	13.81	2.90
6a	0.28	0.31	0.17	0.85	4.03
6c	0.11	0.11	0.041	0.040	0.13
6d	0.74	3.48	0.88	0.82	4.36
6e	23.31	8.79	4.63	47.76	13.03
6f	0.085	0.44	0.20	0.36	0.64
8g	>100	>100	>100	>100	>100
8h	51.11	>100	>100	>100	>100
8i	81.77	>100	42.20	82.03	>100
12	2.83	24.19	1.74	23.10	3.52
(*S*)-CPT	0.14	0.21	0.03	1.17	0.18

In vitro cytotoxic activities against human tumor cell lines for all of the camptothecin analogues[9] along with comparative data for camptothecin are listed in Table I. The analogues containing secondary amines were in general more potent than the tertiary amine analogues. Although the analogues (**5a-e**, **6d**, **6e**, **8g-i** and **12**) were less potent than camptothecin, the compounds **6a**, **6c** and **6f** showed comparable cytotoxicity to that of (*S*)-camptothecin.

The most potent analogue **6c** was evaluated for antitumor activity in mice bearing L1210 leukemia[10]. The compound **6c** showed T/Cs of 159, 180, 192, 243, and 210 % at doses of 8.75, 17.5, 35.0, 70.0, and 140.0

mg/kg, respectively, while camptothecin showed T/Cs of 155, 200, 79, and 59 % at doses of 5, 10, 50, and 100 mg/kg, respectively. It is noteworthy that the compound **6c** exhibited significant antitumor activity at wider range of doses as compared with camptothecin. Studies on the toxicity of **6c** and further development of the compounds related with **6c** as potential anticancer candidates are in progress.

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9. All new compounds gave satisfactory spectroscopic data consistent with the proposed structures.
10. L1210 cells (1 x 10^6) were transplanted intraperitoneally (i.p.) into BDF1 mice on day 0; compounds were administered i.p. on day 1; T/C = (median survival time of treated/control animals) x 100 %.

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