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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF HEXACYCLIC CAMPTOTHECIN ANALOGUES

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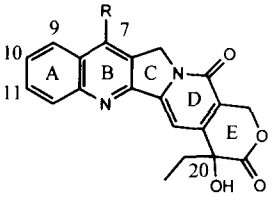
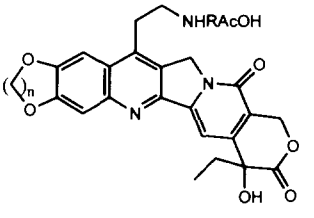
Abstract: A series of C(7)-*N*-alkylaminoethyl-C(10), C(11)-methylenedioxy- and ethylenedioxy-camptothecin (**3a-g, 4a-h**) were prepared. Their syntheses and *in vitro* cytotoxicity were reported. Among 15 derivatives, **3a** and **3b** showed more potent cytotoxicity than Camptothecin, especially in CAOV-3 cell line.

Camptothecin (CPT, C(20)(*S*)-**1**) is an alkaloid which was first isolated by Wani and co-workers from a Chinese tree, *Camptotheca acuminata* in 1966.[1] CPT has been widely studied as a potent antitumor agent which has a novel mechanism of action associated with the inhibition of Topoisomerase I (Topo I).[2] The drug development of CPT itself, which has a broad spectrum of antitumor activity, was discontinued by unpredicted toxicity in clinical trial,[3] but the less toxicity of semi-synthetic analogues led to commercial antitumor drugs, Irinotecan[4] and Topotecan.[5] It has been possible to study the structure-activity relationship (SAR) owing to the developed total synthesis for CPT.[6] According to the accumulated SAR studies,[2,6,8] the introduction of polar groups at C(7), C(9), C(10), and C(11) on CPT generally showed enhanced cytotoxicity and less toxicity which was associated with the higher water solubility.[5,7] Recently, we reported that C(7)-*N*-isopropylaminoethyl-CPT (**2**)[8] showed enhanced cytotoxicity and lower toxicity in comparison with C(20)(*S*)-**1** and Topotecan.[9] The additional polar groups at C(9), C(10), and C(11) on **2** might lead even more potent analogues which have lower toxicity. Based on the viewpoint, we designed a series of C(10), C(11)-methylenedioxy or ethylenedioxy-C(7)-*N*-alkylaminoethyl-CPT analogues (**3, 4**). We expected the additional polar groups could maximize the antitumor activity of **2**. In this paper the syntheses and *in vitro* cytotoxicity of **3** and **4** are reported.



1 R = H
2 R = -$(CH_2)_2$NHCH$(CH_3)_2$AcOH



3 R = alkyl, n = 1
4 R = alkyl, n = 2

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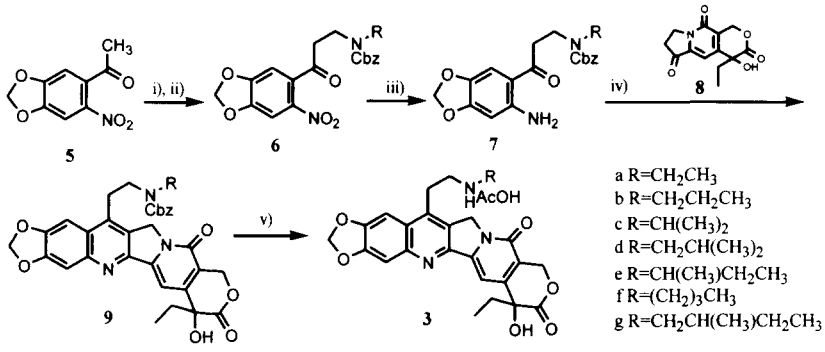
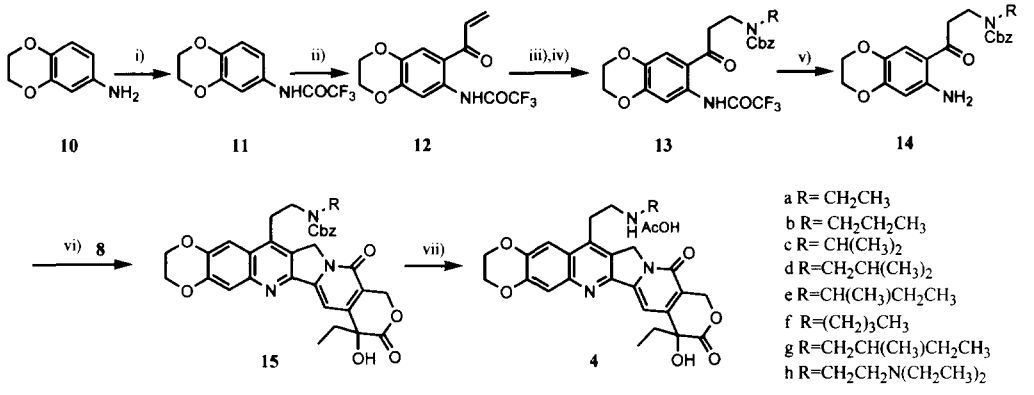
Scheme 1



Conditions and reagents: i) RNH_2HCl, $(CH_2O)_n$, EtOH, *c*-HCl, reflux, ii) CbzCl, Et_3N, CH_2Cl_2, rt (**6a**, 60%; **6b**, 50%; **6c**, 14%; **6d**, 30%; **6e**, 10%; **6f**, 25%; **6g**, 45%), iii) $Na_2S_2O_4$, EtOH, reflux (**7a**, 42%; **7b**, 63%; **7c**, 58%; **7d**, 35%; **7e**, 86%; 7f, 62%; 7g, 75%), iv) **8**, *p*-TsOH (cat.), toluene, reflux (**9a**, 26%; **9b**, 76%; **9c**, 78%; **9d**, 65%; **9e**, 71%; **9f**, 37%; **9g**, 54%), v) H_2, 10 % Pd on C, AcOH, rt (**3a**, 38%; **3b**, 40%; **3c**, 59%; **3d**, 20%; **3e**, 20%; **3f**, 50%; **3g**, 54%).

Scheme 2



Conditions and reagents: i) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, rt (94%), ii) 3-bromopropionyl chloride, $AlCl_3$, CH_2Cl_2, rt (88%), iii) RNH_2, CH_2Cl_2, rt, iv) CbzCl, Et_3N, CH_2Cl_2, rt (**13a**, 7%; **13b**, 20%; **13c**, 33%; **13d**, 44%; **13e**, 35%; **13f**, 31%; **13g**, 30%; **13h**, 44%), v) LiOH, THF/H_2O, rt (**14a**, 32%; **14b**, 53%; **14c**, 86%; **14d**, 80%; **14e**, 85%; **14f**, 100%; **14g**, 85%; **14h**, 70%), vi) **8**, *p*-TsOH (cat.), toluene, reflux (**15a**, 73%; **15b**, 47%; **15c**, 65%; **15d**, 41%; **15e**, 59%; **15f**, 18%; **15g**, 25%; **15h**, 16%), vii) H_2, 10 % Pd on C, AcOH, rt (**4a**, 44%; **4b**, 13%; **4c**, 37%; **4d**, 21%; **4e**, 90%; **4f**, 18%; **4g**, 84%; **4h**, 66%).

The syntheses of **3a-g** were accomplished in five steps starting from **5** which is commercially available (Scheme 1). Mannich reaction of **5** with the corresponding alkylamine gave **6a-g**, which were reduced to **7a-g** by sodium dithionite, respectively. The Friedlander condensation of **7a-g** with **8**[8] followed by hydrogenation in acetic acid gave **3a-g**.[10] **4a-h** were prepared in 7 steps from **10** (Scheme 2). The protected amine (**11**), obtained by the treatment of amine (**10**) with trifluoroacetic anhydride in basic condition was subjected to Friedel-Crafts

acylation to give α,β-unsaturated ketone (**12**). Michael addition of **12** with the corresponding alkylamine, followed by *N*-protection using benzyl chloroformate gave **13a-h**, respectively. The hydrolysis of amide **13a-h** afforded **14a-h**. Finally the Friedlander condensation of **14a-h** and **8** followed by hydrogenation in acetic acid produced **4a-h**.[10]

Table 1. *In vitro* Cytotoxicity[11] of CPT Analogues(**3**, **4**) against Human Tumor Cell Lines[12](IC_{50}, μM).

	analogues 3					analogues 4				
compd.	A549	DLD-1	HEC-1-B	CAOV-3	KATO-III	A549	DLD-1	HEC-1-B	CAOV-3	KATO-III
a	9.4×10^{-4}	6.1×10^{-3}	6.06	3.3×10^{-5}	2.9×10^{-2}	5.0×10^{-3}	—[a]	0.19	1.4×10^{-1}	2.9×10^{-1}
b	1.1×10^{-4}	2.6×10^{-2}	2.11	6.3×10^{-5}	0.11	1.4×10^{-2}	>100	>100	>100	2.10
c	2.0×10^{-4}	8.9×10^{-2}	0.67	1.7×10^{-3}	4.5×10^{-2}	8.4×10^{-2}	0.85	12.83	2.32	2.9×10^{-2}
d	9.96	0.65	10.05	21.89	0.11	2.85	4.04	3.65	7.12	1.38
e	7.02	3.12	14.83	1.51	2.38	0.12	0.57	3.29	2.0×10^{-2}	7.4×10^{-2}
f	1.42	1.13	4.68	62.03	0.36	2.37	4.04	3.65	7.12	1.38
g	5.59	1.13	2.71	6.57	0.19	0.76	1.81	3.44	3.5×10^{-2}	1.05
h						>100	>100	12.12	>100	>100
2	2.6×10^{-4}	7.3×10^{-3}	—[a]	4.0×10^{-3}	1.6×10^{-3}	2.6×10^{-4}	7.3×10^{-3}	—[a]	4.0×10^{-3}	1.6×10^{-3}
C(20)(*S*)-1	3.3×10^{-4}	1.4×10^{-2}	0.06	2.9×10^{-3}	4.6×10^{-2}	3.3×10^{-4}	1.4×10^{-2}	0.06	2.9×10^{-3}	4.6×10^{-2}

[a] The test was not performed.

In vitro cytotoxic activities against five human tumor cell lines for the above CPT analogues (**3a-g**, **4a-h**) along with C(20)(*S*)-**1** and **2** are listed in Table 1. **3a-c** (R; Et, *n*-Pr, *i*-Pr) and **4a-c** (R; Et, *n*-Pr, *i*-Pr) showed 10 to 10^4 times more potency than **3d-g** (R; *n*-Bu, *i*-Bu, *sec*-Bu, 2-methylbutyl) and **4d-h** (R; *n*-Bu, *i*-Bu, *sec*-Bu, 2-methylbutyl, *N,N*-diethylaminoethyl), respectively. Therefore, the more bulky alkyl groups at C(7) in both series of **3a-g** and **4a-h** showed the less cytotoxicity. Generally, methylenedioxy derivatives (**3a-c**) showed higher cytotoxicity than the corresponding ethylenedioxy derivatives (**4a-c**). It is thought that the ethylenedioxy group cannot hold the coplanarity with A, B, C–ring of CPT which is required to interact with DNA and Topo I complex. **3a-c** showed comparable or more potent cytotoxicity than C(20)(*S*)-**1** and **2** depending on cell lines. Especially, 50-100 times more potent cytotoxicity of **3a** and **3b** in comparsion with C(20)(*S*)-**1** and **2** in CAOV-3 cell line gives us possible chance to develop specifically effective antitumor agent against ovarian cancer. Considering that **3a** and **3b** were racemate, the optical active **3a** and **3b** would show even higher cytotoxicity. The study of chiral **3a** and **3b** is currently being investigated.

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