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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF (*RS*)-20-DESETHYL-20-SUBSTITUTED CAMPTOTHECIN ANALOGUES

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Abstract: Sixteen (*RS*)-20-desethyl-20-substituted camptothecin analogues were designed and prepared by total synthesis. These analogues were evaluated for cytotoxic activity against five tumor cell lines. The cytotoxic activity of compound **14f** was shown to be comparable to that of camptothecin.

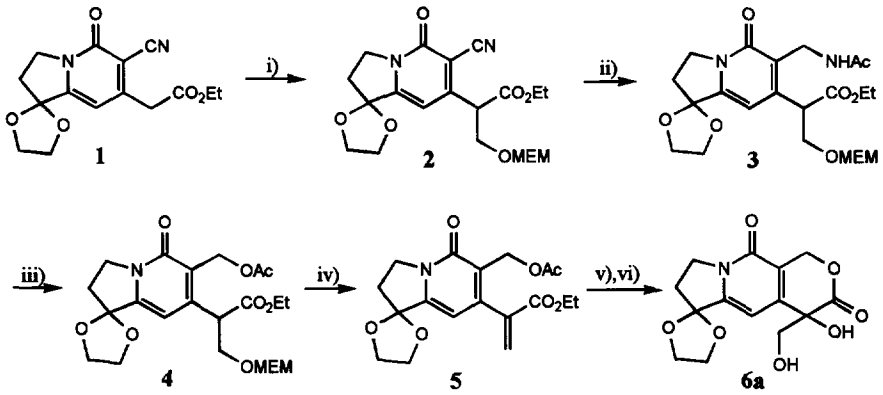
Camptothecin, a pentacyclic alkaloid isolated from *Camptotheca acuminata* by Wall, has potent antitumor activity[1]. The clinical utility of camptothecin as an anticancer agent was limited due to its toxicity and extremely poor solubility profile[2]. However, the recent discovery of camptothecin's mode of action, the inhibition of topoisomerase I[3], has awakened our great interest. A number of promising analogues having improved solubility, low overall toxicity, and considerable *in vivo* activity against certain solid tumors, have been reported[3].

In connection with the investigation of camptothecin analogues as potential antitumor candidates, we recently developed an efficient enantioselective synthesis of 20(*S*)-camptothecin and its analogues[4]. It was also worth noting that the 20-ethyl group could be replaced by an allyl group with no loss of *in vivo* activity[5]. We were interested in the effect of modification of the 20-ethyl group on antitumor effect. The 20-ethyl group was replaced with a polar substituent containing oxygen or nitrogen that would contribute to improve aqueous solubility. In this paper, we wish to report the synthesis and cytotoxic activity of (*RS*)-20-desethyl-20-substituted camptothecin analogues containing a polar substituent.

The key tricyclic intermediate **6a** was obtained in high yield by dihydroxylation of **5** followed by hydrolysis (Scheme I). The required unsaturated ester **5** was efficiently prepared by treating **4** with DBU. Conversion of known ester **1**[6] to diester **4** was performed by hydroxymethylation followed by sequential protection of the resulting alcohol, catalytic hydrogenation over Raney Ni in Ac_2O - AcOH, nitrosoation, and the final rearrangement. The other key intermediate **11a** was prepared starting from **7**, which was obtained by α–alkylation of ester **1** with $BrCH_2CH_2OTHP$. The alkylated compound **7** was transformed to diester **9** according

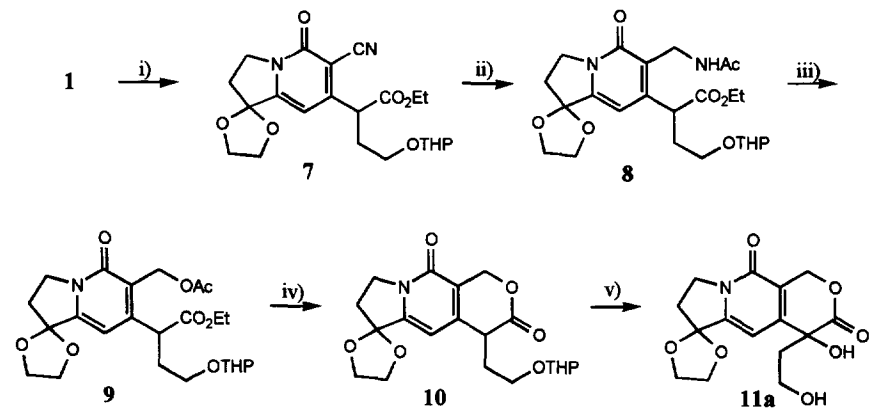
to the previous procedures[6]. α-Hydroxylation and subsequent deprotection of lactone **10** which was produced by hydrolysis of **9**, provided tricyclic ketal **11a** (Scheme II).

Scheme I



Reagents: i) 35% HCHO/1,4-dioxane/H_2O/EtOH, rt, 15h (47 %); then MEMCl/CH_2Cl_2/*i*-Pr_2NEt, 0°C to rt, 20h (80 %), ii) Raney Ni/Ac_2O/AcOH, 45°C, 3h (96 %), iii) $NaNO_2$/Ac_2O/AcOH, 0°C, 4h; then CCl_4, reflux, 15h (94 %), iv) DBU/benzene, rt, 3h (90 %), v) OsO_4/pyridine, rt, 4h (90 %), vi) LiOH/MeOH/H_2O, rt, 1h; then AcOH/CH_2Cl_2, rt, 10h (84 %).

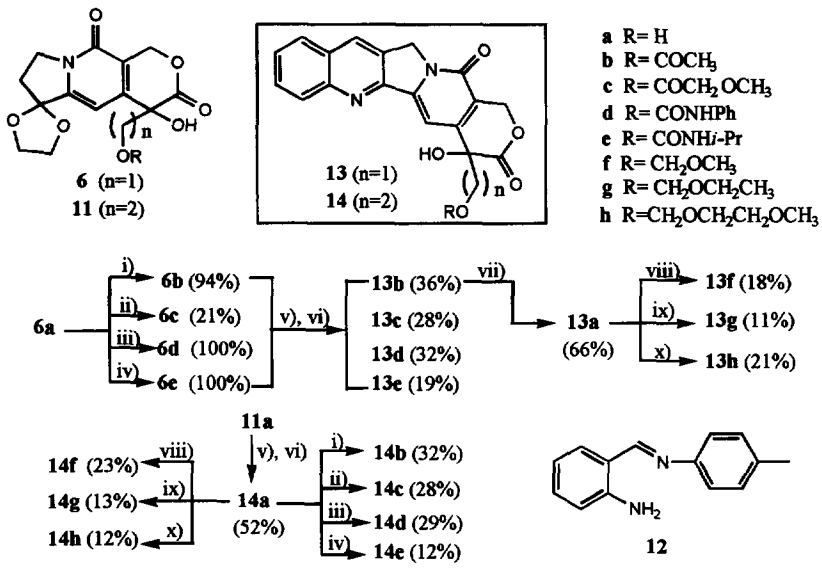
Scheme II



Reagents: i) KO^{t}Bu/BrCH_2CH_2OTHP/DMF, 50°C, 60h (88 %), ii) Raney Ni/Ac_2O/AcOH, 45°C, 3h (92 %), iii) $NaNO_2$/Ac_2O/AcOH, 0°C, 4h; then CCl_4, reflux, 12h (86 %), iv) LiOH/MeOH/H_2O, rt, 1h; then AcOH/CH_2Cl_2, rt, 14h (85 %), v) KO^{t}Bu/DMF/$(EtO)_3P/O_2$, 0°C, 3h (93 %); then cat.PPTS/EtOH, 55°C, 7h (73 %).

The target pentacyclic analogues were prepared from tricyclic ketal **6a** or **11a** as shown in Scheme III. Acylation or carbamoylation of **6a** followed by deketalization and Friedlander condensation with imine **12**[7], gave **13b - 13e**. The compound **13a** obtained from **13b** was converted to **13f - 13h**. The pentacyclic intermediate **14a**[8] which was prepared from **11a**, were also converted to **14b - 14h**.

Scheme III



Reagents: i) Ac_2O/CH_2Cl_2/pyridine, rt, ii) methoxyacetyl chloride/CH_2Cl_2/pyridine, 0°C to rt, iii) $PhNCO/CH_2Cl_2$/pyridine, rt, iv) *i*-PrNCO/CH_2Cl_2/Et_3N/cat. *n*-$Bu_2Sn(OAc)_2$, rt, v) 80 % TFA, rt, 3h - 5h (39-100 %), vi) **12**/*p*-TsOH/toluene, reflux, 4h, vii) LiOH/MeOH/H_2O, rt; then 1N HCl (pH = 3), viii) MOMCl/CH_2Cl_2/*i*-Pr_2NEt, 0°C to rt, ix) chloromethyl ethyl ether/CH_2Cl_2/*i*-Pr_2NEt, 0°C to rt, x) MEMCl/CH_2Cl_2/DMF/*i*-Pr_2NEt, 0°C to rt.

Table I. *In vitro* Cytotoxicity[9] of Camptothecin Analogues(**13**,**14**) against Human Tumor Cell Lines[10](IC_{50}, μM).

	analogues **13**					analogues **14**				
compd.	A172	DLD-1	CAOV-3	KATO-III	L1210	A172	DLD-1	CAOV-3	KATO-III	L1210
a	7.19	17.81	2.17	11.53	3.97	4.47	2.50	6.31	9.72	6.31
b	1.53	1.22	0.82	9.43	1.71	3.99	7.04	4.23	0.98	5.09
c	17.59	47.14	12.90	>100	13.61	6.99	31.80	6.39	3.05	24.54
d	2.77	4.24	1.58	13.06	2.77	0.06	1.20	3.72	5.38	6.87
e	11.23	23.42	22.48	72.29	5.97	5.25	10.95	1.42	46.46	9.41
f	8.39	2.41	4.00	23.25	1.65	2.62	1.86	0.56	0.32	0.44
g	11.14	17.09	17.58	40.74	3.40	1.37	1.07	1.85	19.27	1.42
h	4.38	25.82	6.00	83.50	22.19	4.04	15.12	1.92	52.99	4.97
(*S*)-CPT	0.14	0.21	0.03	1.17	0.18	0.14	0.21	0.03	1.17	0.18

In vitro cytotoxic activities against five tumor cell lines for all the camptothecin analogues[11] along with comparative data for camptothecin are listed in Table I. Cytotoxicity of analogues **14** (n=2) was better than that of analogues **13** (n=1). Although the compounds **14b** and **14d** having acyl or carbamate group, respectively,

showed potent cytotoxicity against specific tumor cell lines, the compounds **14f** and **14g** bearing ether functionality were generally more potent than the analogues bearing acyl or carbamate groups. Ether oxygen of the two compounds may improve the water solubility of camptothecin by acting as a hydrogen bond acceptor. Although all the analogues were 10 - 1000 fold less potent than camptothecin, the compound **14f** was shown to have cytotoxicity comparable to that of camptothecin in some cell lines. Considering that **14f** is racemic, higher activity of the optically active compound would be expected.

The present study suggests that the most potent analogue **14f** may be worth to be further developed. By combining the structural features of **14f** and the C-7 modified camptothecin analogues[12] bearing secondary amine, further developments of potential anticancer candidates are in progress.

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10. *In vitro* antiproliferative activities of the analogues against five tumor cell lines (A172, human CNS cancer; DLD-1, human colon cancer; CAOV-3, human ovarian cancer; KATO-III, human gastric cancer; L1210, mouse leukemia) were measured by SRB assay[9] after 3 days of incubation, and expressed as the doses required to inhibit the growth of 50% of the cells cultivated (IC_{50}, μM).
11. All new compounds gave satisfactory spectroscopic data consistent with the proposed structures.
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