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Synthesis and antitumor activity of the hexacyclic camptothecin derivatives

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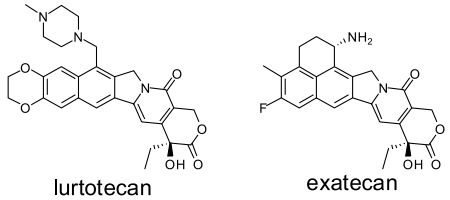
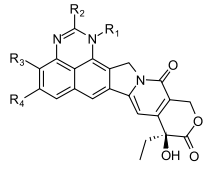
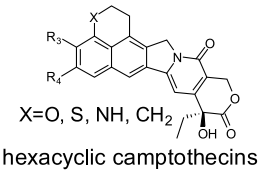
Abstract—A series of hexacyclic camptothecin derivatives were synthesized to test for antitumor activity as topoisomerase I inhibitor. The strategy of synthesis was used for the formation of additional furan and dihydrofuran rings fused with 9- and 10-positions of camptothecin. All of the hexacyclic camptothecins were assayed for cytotoxicity against four human tumor cell lines, HL60, BEL-7402, HCT-116, and HeLa, and showed very impressive cytotoxicity activity in vitro. Enzyme activity of the hexacyclic camptothecins was evaluated, being equal or superior to that of SN-38. The stability of four compounds was assessed in human plasma. Two of these compounds were chosen to test for antitumor activity in vivo against Sarcoma-180. The results suggested that additional furan and dihydrofuran rings could improve the antitumor activity in vitro and vivo, though the stability of the lactone ring did not increase.

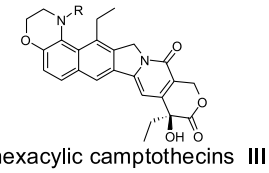
Camptothecins, as antitumor agents, are the most important topoisomerase I (Top I) inhibitors. Their antitumor activities are believed to impact on replication, transcription, and the repair of DNA and cause cancer-cell death by interfering with the catalytic cycle of DNA Top I and stabilizing the DNA–Top I binary complex.[1,2] Since elucidation of their mechanism of action, many derivatives have been synthesized and some of them are in various stages of preclinical and clinical development. Among them, two derivatives, topotecan (Hycamtin) and irinotecan (Camptosar), are the only a Top I inhibitors to be used in clinical practice so far.[3]

According to the previous modifications of camptothecin, the SAR had summarized that the natural *S*-configuration at position 20 and the stability of the ring-E lactone of the camptothecins are both essential for their antitumor activity, that substitutions at the 7-, 9-, or 10-positions of most camptothecin analogs enhance their antitumor activity, and that substitutions at the 11- or 5-positions lead to activity decrease.[4] Especially, the derivatives with an additional ring combined with the 10- and 11-positions, 9- and 10-positions, or 7- and 9-positions were prepared, and many of them showed potent antitumor activity superior to those of original pentacyclic camptothecins, such as lurtotecan,[5] exatecan,[6] and other hexacyclic camptothecins I,[7] II,[8] and III.[9]









Keywords: Camptothecin; Topoisomerase I; Antitumor activity.
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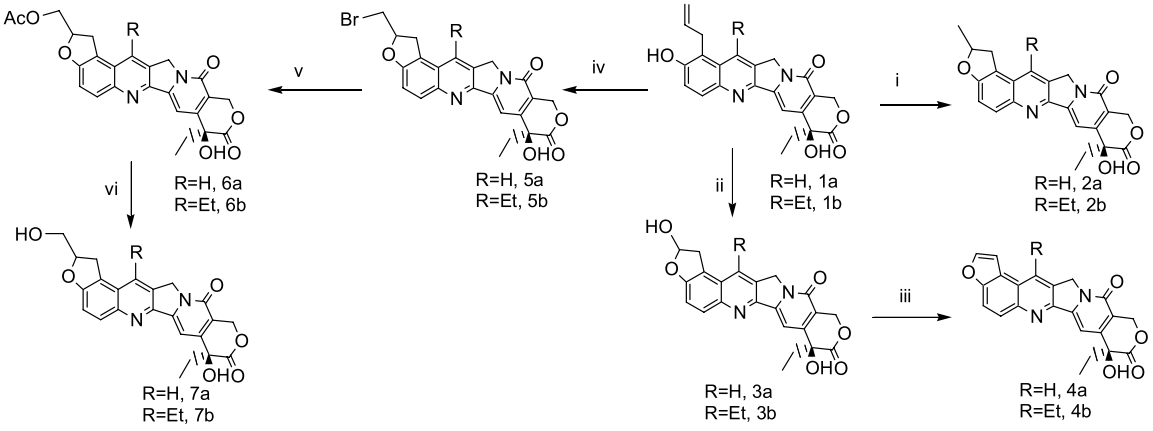
Among the hexacyclic camptothecins, lurtotecan and exatecan are, respectively, studied in the clinical phase II and phase III stages.[10] Based on the results, we developed a novel series of hexacyclic camptothecins which have a furan or dihydrofuran ring combined with positions 9 and 10 of the ring A. These new hexacyclic structures likely embodied the alkyls at the 9-position and the hydroxyl in the direction of the position 10. The abovementioned structure characteristics are believed to benefit the improvement of antitumor activity and the stability of the lactone ring.[11,12]

All the compounds synthesized are reported in Scheme 1. The two starting materials, **1a** and **1b**, were synthesized according to the strategy reported in our recent papers,[12] the Claisen rearrangement. The compounds **1a** and **1b** were treated with concentrated hydrochloride acid to give the compounds **2a** and **2b**, respectively. Compounds **3a** and **3b** were produced by the treatment of **1a** and **1b** with $OsO_4/NaIO_4$ in 1,4-dioxane and water. The conversion of **3a** and **3b** to **4a** and **4b** was completed with the acid catalysis dehydration in 48% hydrobromic acid. The treatment of **1a** and **1b** with NBS in glacial acetic acid produced intermediate **5a** and **5b**. Subsequent nucleophilic replacement reaction with anhydrous potassium acetate in DMF generated **6a** and **6b**, which were hydrolyzed to produce **7a** and **7b**.

The series of hexacyclic camptothecins were assayed against four human cancer-cell lines: leukemia HL60, liver cancer BEL-7402, colon cancer HCT-116, and cervix cancer HeLa, using SN-38 as the reference drug. The results of cytotoxicity studies are shown in Table 1. Against the four cell lines, all of the hexacyclic camptothecins exhibited the impressive cytotoxicity activity in the range of IC_{50} values of 0.28–25 nM. The in vitro activities of most of them were equal or even superior to that of SN-38. Against HL60, BEL-7402, and HCT-116 cell lines, most of the hexacyclic camptothecins showed several nanomolar IC_{50} values. In particular, compounds **4b** and compound **6b** achieved IC_{50} values of 0.28 and 0.52 nM, respectively, correspondingly against HL60 and HCT-116. Against HeLa cell line, the other compounds, excluding compound **4b**, displayed similar cytotoxicity activity to SN-38, achieving 10–25 nM IC_{50} values. The cytotoxicity of these hexacyclic camptothecins was very remarkable, probably due to the additional furan or dihydrofuran ring. The additional ring changed the planarity of ring system and, in some sense, still possessed alkyl group in the 9-position of campothecin. The cytotoxicity of **3a**, **3b**, **7a**, and **7b** was not above that of other compounds, and the result indicated that the hydroxyl possessed in the direction of 10-position did not bring about the cytotoxicity increase. Of course, the most impressive cytotoxicity of **4a** and **4b** may be ascribed to conjugation with the additional ring and the original pentacyclic ring of camptothecin. The conjugation effect changed the electricity of the whole ring system. The result suggested that the furan and dihydrofuran rings fused with 9- and



Scheme 1. Reagents and conditions: (i) concentrated hydrochloride acid, refluxed; (ii) $OsO_4/NaIO_4$, 1,4-dioxane, H_2O, rt; (iii) 48% HBr, refluxed; (iv) NBS, glacial acetic acid, rt; (v) AcOK, DMF; (vi) NaOH, H_2O, rt.

Table 1. Assay of cytotoxic activity against human tumor cell lines HL60, BEL-7402, HCT-116, and HeLa

Cell lines	IC_{50} (nM)[a]										
	SN-38	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**6a**	**6b**	**7a**	**7b**
HL60	19	3.1	4.9	8.4	4.2	3.0	0.28	11	9.1	8.7	7.1
BEL-7402	13	7.4	7.5	2.6	4.4	1.6	2.6	15	11	19	7.8
HCT-116	3.3	4.9	2.6	3.2	1.4	1.6	1.7	4.3	0.52	2.6	3.8
HELA	22	10	19	—[b]	20	15	2.3	11	14	25	23

[a] In vitro antitumor activities of the hexacyclic camptothecins against three cell lines, BEL-7402, HCT-116, and HELA, were measured by the MTT assay after 3 days of incubation and expressed as the doses required to inhibit the growth of 50% of the cells cultivated (IC_{50}, nM) and against HL60 by SRB assay.

[b] No test.

10-positions of camptothecin could still possess the activity in vitro.

As shown in Figure 1, all the novel compounds were tested for inhibition of Top I and exhibited very remarkable enzyme activity, similar to those of three reference drugs, camptothecin, 10-hydroxycamptothecin, and SN-38.[13] All the hexacyclic camptothecins including three reference drugs clearly exhibited the strong stability of Top I–DNA–drug ternary complexes. In particular, almost only ternary complexes remained when compounds **6b** and **2a** were incubated with Top I and supercoiled DNA. In conclusion, the enzyme activity almost paralleled the cytotoxicity activity. The result suggested that the additional rings did not change the activity of inhibiting Top I.

Compounds **3a**, **3b**, **4a**, and **4b** were chosen to assay the stability of lactone in human plasma because the structure of compounds **3a** and **3b** still possessed 9-alkyl and hydroxyl in the direction of 10-position of camptothecin, and the structure of **4a** and **4b** formed a new large conjugated ring system. We wanted to know whether the structure change would bring about an alteration in the stability of lactone. As shown in Figure 2,[14] four hexacyclic camptothecins, **3a**, **3b**, **4a**, and **4b**, incubated in human plasma achieved 0.82, 3.6, 6.9, and 8.8% remaining lactone percentage, respectively. The stability of lactone of **3a** and **3b** was inferior to that of 10-hydroxycamptothecin (8.6%), while compounds **4a** and **4b** showed merely similar stability to that of 10-hydroxycamptothecin, much less than that of 10-hydroxy-9-allylcamptothecin (17.5%). The result indicated us that additional furan or dihydrofuran rings did not bring about the stability increase though their structure reserved the 9-alkyl and hydroxyl in the direction of

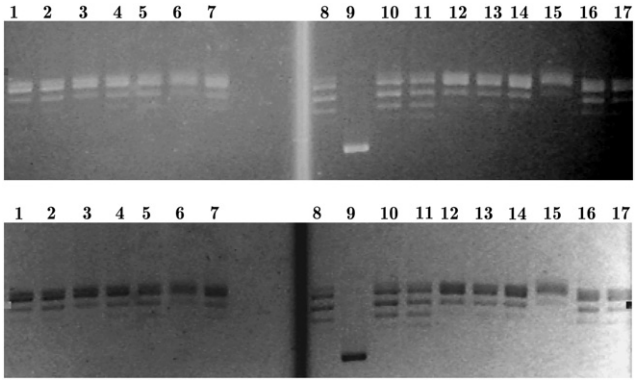


Figure 1. Top I-mediated supercoiled DNA relaxation was inhibited by hexacyclic camptothecins. In this experiment, the concentration of all compounds, including the reference drugs camptothecin, 10-hydroxycamptothecin, and SN-38, was 100 μM. The reaction buffer contained 0.25 μg supercoiled DNA and 10 units of Top I in a total volume of 20 μL. Relaxation was induced at 37 °C for 30 min and terminated by the addition of 10% sodium dodecyl sulfate. The mixtures were then treated with proteinase K, incubated at 37 °C for 30 min, and analyzed on a 1% agarose gel. Lanes 1–7, **7a**, **6a**, **2b**, **3b**, **4b**, **6b**, and **7b**, respectively. Lane 8, Top I and supercoiled DNA. Lane 9, only supercoiled DNA. Lane 10, Top I, supercoiled DNA and 0.4 μL DMSO. Lane 11, DNA marker. Lanes 12–17, camptothecin, 10-hydroxycamptothecin, SN-38, **2a**, **3a**, and **4a**, respectively.

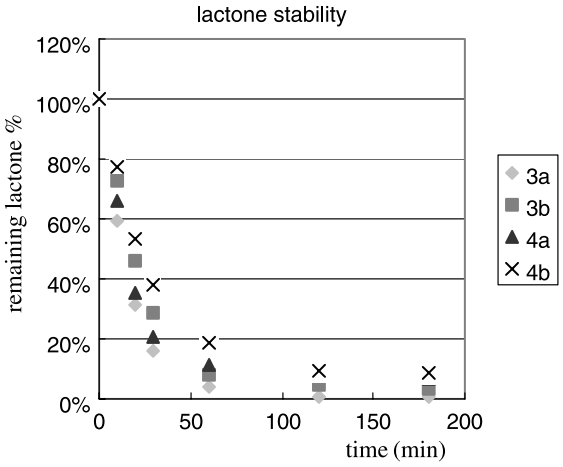


Figure 2. The stability of compounds **3a**, **3b**, **4a**, and **4b** in human plasma. Stability profiles were determined using HPLC methods with a fluorescence detector. Drug concentrations of 1 μM were used, and drug samples were incubated at 37 °C in human plasma. Each data point represents the average of three determinations with an uncertainty of 10% or less.

10-position in some sense or formed a new conjugated larger ring. The conclusion also demonstrated that the activity in vitro and enzyme activity were not completely consistent with the stability of lactone.[12]

Most of the hexacyclic compounds possessing ethyl in 7-position did not exhibit much increase of the cytotoxicity activity and enzyme activity in vitro, relative to those possessing no ethyl in 7-position. Thus, we evaluated the in vivo antitumor activity of compounds **3a** and **4a**, according to the chemical synthesis easiness, against the mouse tumor model: Sarcoma-180 tumor with irinotecan (CPT-11) and 5-fluorouracil (5-FU) used as the reference drugs (Table 2). Compounds **3a** and **4a**, administered once daily for consecutive 7 days, exhibited very impressive antitumor efficacy in vivo. Compounds **3a** and **4a**, respectively, at doses of 5 and 1 mg/kg, achieved tumor-weight inhibitions (TWI) of 97 and 98%, almost equivalent to the TWI of 99.4% achieved with CPT-11 at a dose of 40 mg/kg. In particular, compound **4a** still achieved a TWI of 85% at a dose of 0.5 mg/kg. When the doses of **3a** and **4a** decreased from 5 to 2.5 mg/kg, or from 1 to 0.5 mg/kg, the corresponding TWIs did not decrease to a half and, at the same time, the mouse weight clearly increased.

In summary, the furan and dihydrofuran rings, fused with 9- and 10-positions of camptothecin, possessed the antitumor activity in vitro and in vivo, though they did not enhance the stability of the lactone ring. All the hexacyclic camptothecins exhibited excellent cytotoxic activity and encouraging activity in inhibiting Top I, being equal or superior to that of SN-38 in the two aspects. Among them, **3a** and **4a** showed impressive activity in vivo, being promising hexacyclic camptothecins for preclinical development. The results encourage us to continue the study of the hexacyclic camptothecins.

Table 2. Assessment of antitumor efficacy of compounds **3a** and **4a** against mouse tumor Sarcoma-180 using intraperitoneal (ip) injection

Compound	Dose (mg/kg)	Lethal[a] toxicity	BWC[b] (%)	TW[c]	TWI[d] (%)	*P*
NS	—	0/20	+64	1.54 ± 0.52	—	<0.01
3a	5.0	0/8	−31	0.05 ± 0.06	97	<0.01
3a	2.5	0/8	+5	0.45 ± 0.34	70	<0.01
4a	1.0	0/8	−34	0.03 ± 0.03	98	<0.01
4a	0.5	0/8	+26	0.23 ± 0.16	85	<0.01
CPT-11	40	0/8	−28	0.01 ± 0.01	99.4	<0.01
5-FU	75	0/10	+31	0.22 ± 0.20	86	<0.01

[a] Number of dead mice/total number of mice.
[b] Percentage of mouse body-weight change (BWC) after drug treatment: BWC% = (mean BWfinal day/BWfirst day × 100) − 100; "+" means body-weight increase and "−" means body-weight decrease.
[c] Average tumor weight after drug treatment.
[d] Percentage of tumor-weight inhibition (TWI) versus control mice.

Acknowledgment

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