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Synthesis and antitumor activity of 7-ethyl-9-alkyl derivatives of camptothecin

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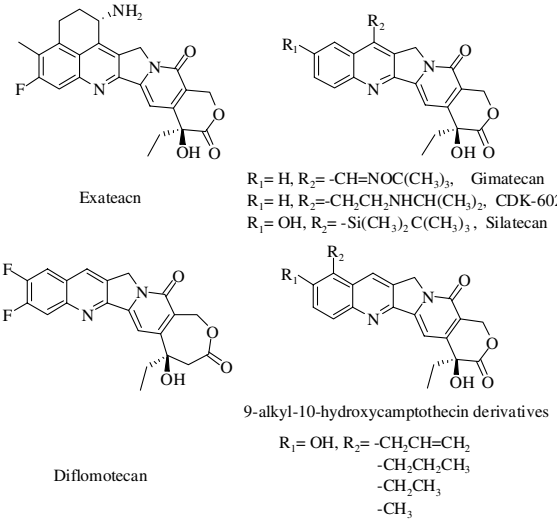
Abstract—A series of new camptothecin derivatives, as topoisomerase I inhibitor, were synthesized to identify potent antitumor agents. The synthesis method was based on the Claisen rearrangement of 10-allyloxy-7-ethylcamptothecin. All of the compounds were assayed for cytotoxicity against two human tumor cell lines, Bel7402, HCT116, and showed good potency in vitro. Compounds **2**, **4**, **9**, were assessed for the stability of lactone in human plasma. And then compound **2** was tested for antitumor activity in vitro against mouse tumor sarcoma-180. The results suggested that the small alkyl groups in the both 7- and 9-positions of camptothecin could promote liposolubility, antitumor activity in vitro and vivo, though did not bring much increase of the stability of lactone.

Camptothecins are the most important topoisomerase I (Top I) inhibitors. Their antitumor activity has been ascribed to its ability to interfere the catalytic cycle of DNA Top I, and stabilize the DNA–enzyme complex by forming the irreversible drug–enzyme–DNA ternary complex to prevent religation of single-strand DNA breaks induced by Top I. So camptothecin is believed to impact on replication, transcription, and the repair of DNA and cause the cancer cell death.[1,2] Since elucidation of the mechanism, many derivatives of camptothecin have been synthesized. Two of these, topotecan (Hycamtin) and irinotecan (Camptosar) are the only two Top I inhibitors used in clinic so far and some derivatives, such as exatecan,[3] gimatecan,[4] CKD-602,[5] diflomotecan,[6] and silatecan,[7] are in various stages of preclinical or clinical development.[8] Most structural modifications of camptothecin have focused on rings A and B, especially in positions 7, 9, and 10. In particular, various substitutions, such as ethyl, alkylsilyl, oxyiminoalkyl, and alkylsilylalkyl, were introduced at the 7-position of camptothecin and achieved many potent antitumor agents. So, it was believed that small lipophilic groups in the 7-position can indeed promoted stability of lactone and antitumor activity.



In our recent modification of camptothecin, we have introduced the small alkyl groups in the 9-position of camptothecin and found a serial of 9-alkyl-10-hydroxycamptothecin derivatives with potent antitumor activity in vitro and in vivo. These small alkyls introduced in the 9-position of camptothecin can indeed enhance the

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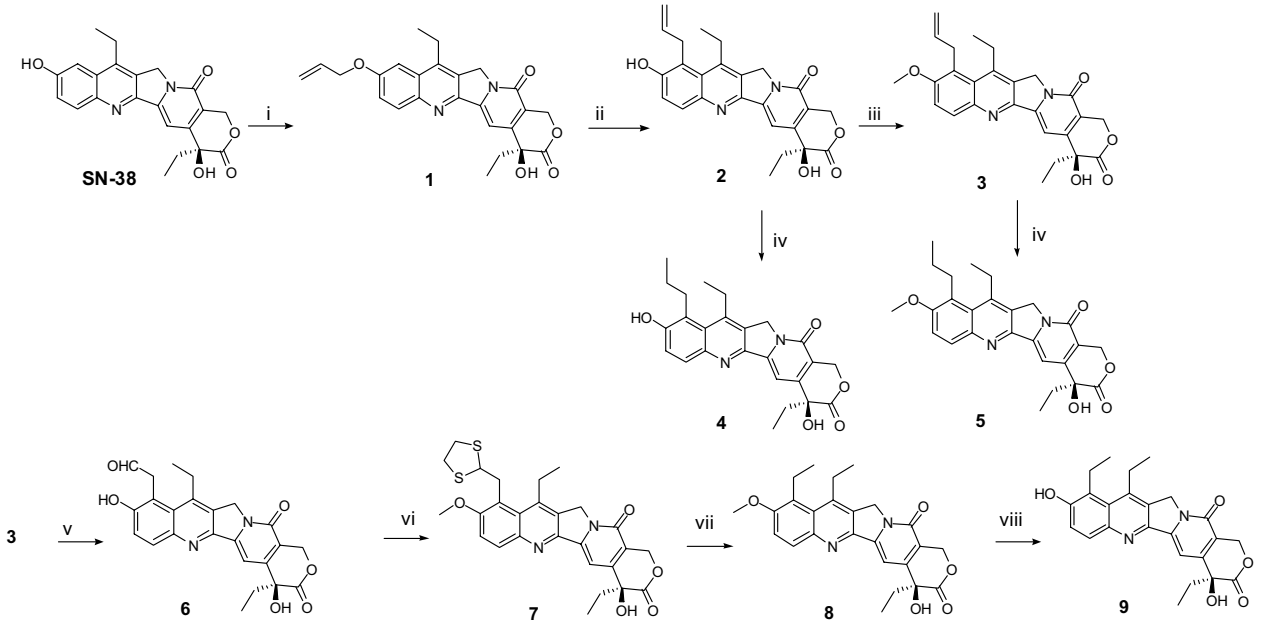

Scheme 1. Reagents and conditions: (i) allyl bromide, DMF, K_2CO_3, 80 °C; (ii) glacial acetic acid, refluxed; (iii) methyl iodide, DMF, K_2CO_3, 80 °C; (iv) H_2, Pd/C, THF, rt; (v) $OsO_4/NaIO_4$, 1,4-dioxane, H_2O, rt; (vi) ethylene disulfhydrate, TsOH, acetic acid, rt; (vii) Raney nickel, ethanol, refluxed; (viii) 48% HBr, refluxed.

liposolubility, stability of lactone, and antitumor activity.[9] The results encouraged us to introduce small alkyl groups in the positions both 7 and 9 in this study in order to identify the agents with more stability of lactone and greater antitumor activity.

All the compounds synthesized are reported in Scheme 1. 7-Ethyl-10-hydroxycamptothecin (SN-38), which already possesses a small moiety (ethyl) in the 7-position, was the starting material, and we only introduced small alkyl groups in the 9-position of it according to the strategy in our recent paper.[9] SN-38 was treated by etherization with allyl bromide in the presence of a base, usually K_2CO_3, and then with the Claisen rearrangement[10] to produce compound **2**. The rearrangement was performed in many kinds of polar solvents, including DMA, DMF, DMSO, *N*,*N*-dimethylaniline, *N*,*N*-diethylaniline, and glacial acetic acid. The optimal solvent was glacial acetic acid for the substrate **1**. Compound **3** was produced by the methylation of the hydroxy group at the 10-position of compound **2** with methyl iodide. The conversion of compounds **2** and **3** to compounds **4** and **5**, respectively, was completed with the hydrogenation of the double bond with Pd/C and H_2. Compound **3** was treated with OsO_4/$NaIO_4$ in 1,4-dioxane and water to produce the aldehyde **6**.[11] Subsequent treatment with ethylene disulfhydrate catalyzed by TsOH converted the aldehyde to acetal **7** with an almost 100% yield in the glacial acetic acid.[12] Without purification, **7** was hydrogenated to desulfurize with Raney nickel to produce **8**.[13] Demethylation of **8** with 48% concentrated hydrobromic acid produced **9**.[14]

The cytotoxicities of the new camptothecin derivatives were first assayed against the human liver Bel-7402 and colon cancer HCT-116 cell lines, using 10-hydroxycamptothecin (HCPT), topotecan (TPT), SN-38 as reference compounds. The results of the cytotoxicity studies are shown in Table 1.

Table 1. Cytotoxicity assay against Bel-7402, HCT-116 cell lines

Compound	IC_{50} (μM)[a]	
	Bel-7402	HCT-116
HCPT	2.47 (±0.12)	0.148 (±0.039)
Topotecan	3.98 (±0.21)	0.100 (±0.000)
SN-38	3.19 (±0.25)	0.007 (±0.001)
2	2.75 (±0.02)	0.014 (±0.004)
3	2.16 (±0.04)	0.068 (±0.008)
4	3.01 (±0.06)	0.012 (±0.001)
5	3.84 (±0.10)	0.153 (±0.025)
8	2.40 (±0.14)	0.061 (±0.010)
9	2.01 (±0.12)	0.023 (±0.006)

[a] IC_{50} values are means of three experiments, standard deviation is given in parentheses.

Against human liver cancer cell line Bel-7402, all the novel compounds showed such similar cytotoxicities as the three reference compounds, HCPT, TPT, SN-38. Their cytotoxicity activity IC_{50} values were in the range of several micromole. Against human colon cancer cell line HCT-116, most of the novel serial of compounds exhibited more cytotoxic activity than HCPT and topotecan. Cytotoxicity of compounds **2**, **4**, **9** were almost similar to that of SN-38, which might result from reservation of 10-hydroxyl. However, cytotoxicity of compounds **3**, **5**, **8**, 10-hydroxyl of which were etherized, relatively decreased. It may be that the 10-hydroxyl has elevated lactone form, which are usually active under physiological conditions.[7] But, the results may indicate that the

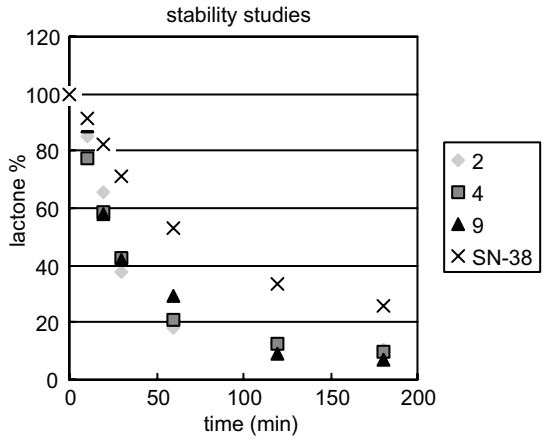

Figure 1. The stability of compounds **2**, **4**, **9** relative to that of SN-38. Stability profiles were determined using HPLC methods with a fluorescence detector. Drug concentrations of 1 μM were used, and drug samples were incubated at 37 °C in human plasma. Each data point represents the average of three determinations with an uncertainty of 10% or less.

introduction of small alkyl groups at the both 7- and 9-postions of 10-hydroxycamptothecin did not seem to bring expected much cytotoxicity enhancement compared with respective introduction of small alkyl goups at the 7-position or 9-position.

All the camptothecin derivatives contain a α-hydroxy-δ-lactone structure, and exist in two distinct forms: the lactone form, or the carboxylate form under physiological conditions at pH 7.4. The lactone form is active, but the carboxylate form is inactive. In human blood, most of the lactone form is converted to the biologically inactive carboxylate form. Therefore, the stability of the lactone of camptothecins in human blood has been a parameter of concern during the modifications of camptothecin.

Compounds **2**, **4**, **9** were chosen to test their stability of lactone in human plasma.[15] As shown in Figure 1, compounds **2**, **4**, **9** incubated in plasma at 37 °C were converted to inactive carboxylate form much faster than SN-38. From Table 2, after incubation for 3 h, the lactone percentage of compounds **2**, **4**, **9** remained, respectively, 10.61%, 9.65%, 7.24%, almost similar to that of HCPT (8.6%), and much less than that of SN-38

Table 2. Summary of the remnant lactone after incubation for 3 h in human plasma

Compound	2	4	9	SN-38
Lactone (%)[a]	10.61	9.65	7.24	25.64

[a] The remaining percentage of lactone after incubation for 3 h at 37 °C in human plasma, representing the right first points in Figure 1.

(25.64%) and 9-allyl-10-hydroxycamptothecin (17.5%).[9] The result suggested that the introduction of small alkyl groups at both 7- and 9-positions of 10-hydroxycamptothecin did not enhance the stability of lactone, relative to 10-hydroxycamptothecin. This may explain that though the lipophilicity increased, the cytotoxicity did not increase largely after introduction of small alkyl at both 7- and 9-positions.

The antitumor efficacy of the potent compound **2** was evaluated using intraperitoneal (i.p.) injection against mouse tumor model: sarcoma-180 tumor with SN-38 and 5-FU (5-fluorouracil) used as the reference drugs (Table 3). Compound **2**, administered once daily for executive 10 days, exhibited impressive antitumor potency in vivo as an antitumor agent at a dose of 2.5 mg/kg, achieving a tumor-weight inhibition (TWI) of 81.2%, which was as twofold much as that achieved with SN-38 at a dose of 5 mg/kg. Though the mouse weight both increased after administration of compound **2** and SN-38, SN-38 achieved a much greater body-weight change (BWC) than compound **2**. The result suggested that compound **2** showed more potent antitumor activity and larger toxicity than SN-38.

In summary, the small substitutions introduced at the both 9- and 7-positions of camptothecin could improve the antitumor activity in vitro and in vivo, though did not bring increase of stability of lactone ring. The results may indicate that the antitumor activity in vitro and vivo is not completely consistent with the stability of lactone, and that it should has something with the liposolubility and intracellular drug accumulation or partitioning in the lipid bilayer. So compound **2** still showed great potency in vivo in despite of decreased stability of lactone, compared with the derivatives with small alkyl groups, respectively, introduced in the 7- or 9-position of camptothecin, such as SN-38, 9-allyl-10-hydroxycamptothecin.

Table 3. Assessment of antitumor efficacy of compound **2** against mouse tumor sarcoma-180

Compounds	Dose (mg/kg)	Lethal[a] toxicity	BWC[b] (%)	TW[c]	TWI[d] (%)
NS	—	0/20	78	3.30 ± 1.34	—
2	2.5	0/10	9	0.62 ± 0.56	81.2
SN-38	5	0/10	45	1.93 ± 0.64	41.5
5-FU	75	0/10	74	1.52 ± 0.24	53.9

[a] Number of dead mice/total number of mice.

[b] Percentage of mouse body-weight change (BWC) after drug treatment: BMC% = (mean $BW_{final\ day}/BW_{first\ day} \times 100) - 100$; '+' means body-weight increase and '—' means body-weight decrease.

[c] Average tumor weight after drug treatment.

[d] Percentage tumor-weight inhibition (TWI) versus control mice.

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