Bioorganic & Medicinal Chemistry Letters 19 (2009) 4107–4109

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

7-Cycloalkylcamptothecin derivatives: Preparation and biological evaluation

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ARTICLE INFO

Article history:
Received 2 March 2009
Revised 26 May 2009
Accepted 2 June 2009
Available online 6 June 2009

Keywords:
Camptothecin
7-Cycloalkylcamptothecin derivatives
Anti-tumor
Synthesis

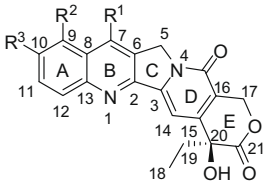
ABSTRACT

A series of 7-cycloalkylcamptothecin derivatives were synthesized from camptothecin with two methods. Their biological activities in vitro were evaluated with sulforhodamine-B (SRB) method on four types of human tumor cell lines A549/ATCC, HT29, NCI-H460 and HL60. Most of these camptothecin analogues show higher antitumor activity than the reference compounds SN-38 and Topotecan, with the IC_{50} values low to nM level. Structure–activity relationship studies of these compounds mostly match the conclusion we achieved before from quantitative structure–activity relationship (QSAR) research.

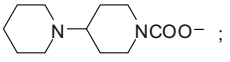
Camptothecin[1] (CPT, **1**, Fig. 1) is a famous efficient inhibitor of Topoisomerase I (**Top I**).[2] Thousands of its derivatives have been prepared and their antitumor activities were evaluated. Many of them have been developed in pre-clinic and clinic trials,[3] three compounds (Irinotecan[4] **2**, Topotecan[5] **3** and Belotecan[6] **4**) have been approved to use in clinic as anticancer drugs in many countries.

According to our investigation, modifications in A or B ring or both are the most efficient way to develop potential antitumor drugs. Most of the active derivatives of CPT are substituted in AB rings.[3] Structure–activity relationship (SAR) studies indicate that substitutions at 7 or 10-position or both could effectively affect the hydrolysis equilibrium (Scheme 1) of CPT E-ring in human blood to favor the active component 'lactone-closed form', by ablating high-affinity binding of the carboxylate form to human serum albumin (HSA).[7] In fact, several series of 7-substituted or 7- and 10-dual substituted CPT derivatives have been developed as effect antitumor candidate compounds.[4,6–8]

In our earlier work,[9] we introduced topological molecular descriptors to study the QSAR of two series of 7- and 10-substituted CPT analogues, and achieved a conclusion that more lipophilic substitutions at 7-position would lead to higher antitumor activity. This result showed good predictive ability. Further more, it was pointed out in a binding model of CPT with biological macromolecules that there is wide space for substitutions in 7-position of CPT without steric repulsion.[10] These investigations encouraged us to develop 7-substituted CPT analogues. In this Letter, the



1 Camptothecin (CPT): $R^1 = R^2 = R^3 = H$;

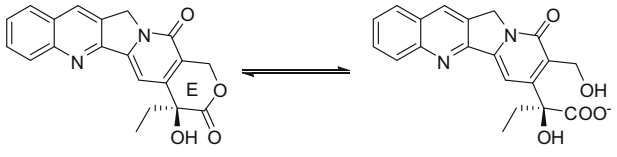
2 Irinotecan: $R^1 = Et$, $R^2 = H$, $R^3 =$  ;

3 Topotecan: $R^1 = H$, $R^2 = Me_2NCH_2$-, $R^3 = OH$;

4 Belotecan: $R^1 = Me_2CHNHCH_2CH_2$-, $R^2 = R^3 = H$;

5 SN-38: $R^1 = Et$, $R^2 = H$, $R^3 = OH$.

Figure 1. Structures of camptothecin and derivatives.



Scheme 1. Hydrolysis equilibrium of camptothecin E-ring in human blood.

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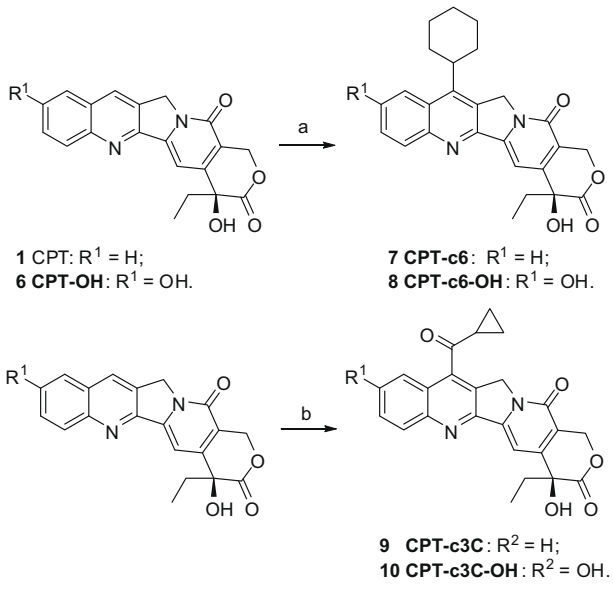
doi:10.1016/j.bmcl.2009.06.010

synthesis and biological evaluation of a series of 7-cycloalkylcamptothecin derivatives are reported.

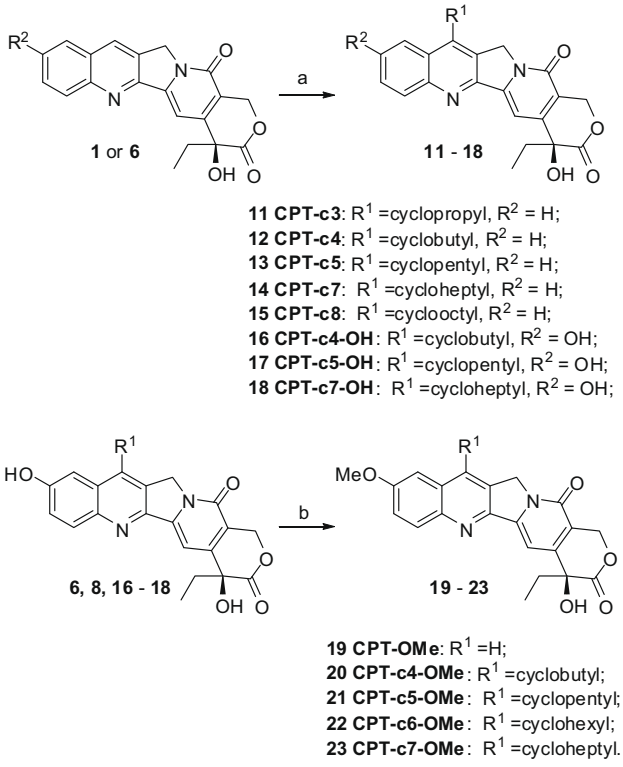
Our attempts to introduce the cycloalkyl groups at C-7 from camptothecin or 10-hydroxycamptothecin with the Sawada method[4,11,12] were unsuccessful except in the case of cyclohexyl derivatives (**CPT-c6** and **CPT-c6-OH**, Scheme 2). The reaction using cyclopropanecarbaldehyde as cyclopropyl radical source afforded the corresponding cyclopropylcabonyl derivative(**CPT-c3C** and **CPT-c3C-OH**, Scheme 2)[13] instead of the desired 7-cyclopropyl substituted camptothecin. We also tried to introduce cyclobutyl at C-7 by using cyclobutylmethanol, but only a complex mixture was recovered. Finally we found that cycloalkyl bromides could be employed as source of cycloalkyl radicals in the presence of diphenylsilane (Ph_2SiH_2) and *t*-butoxyperoxyde. Several 7-cycloalkyl derivatives were synthesized starting from camptothecin and 10-hydroxycamptothecin (Scheme 3)[13] in medial yields except 7-cyclopropyl and 7-cyclobutly CPT analogues which were gained with low yields due to the instability of cyclopropyl and cyclobutly radicals. The 10-hydroxycamptothecin analogs were methylated following standard conditions to give the corresponding 7-cycloalkyl-10-methoxy-camptothecins[14] (Scheme 3).

The antitumor activities of these synthetic compounds were tested on human lung cancer cell line A549/ATCC, human colorectal cancer cell line HT-29 (Table 1), human lung cancer cell line NCI-H460 and human leukemia cancer cell line HL60 (Table 2). Most of these compounds have higher antitumor activities than their parent compounds (without 7-substitution) and reference drugs SN-38 (the active component of irinotecan, Fig. 1) and Topotecan. The highest activities appear with 7-cyclopentyl, 7-cyclohexyl and 7-cycloheptyl CPT analogs. The IC_{50} values of tested compounds on NCI-H460 and HL60, the more sensitive cell lines to CPT analogues are all low to nM level, some of them even lower than nM, which is up to 40-folds more active than Topotecan.

However, activities of compounds with 7-cyclopropylcarbonyl (**CPT-c3C** and **CPT-c3C-OH**) and 7-cyclooctyl (**CPT-c8**) are obviously lower than other analogues'. A possible explanation for lower activity of **CPT-c3C** and **CPT-c3C-OH** is that 7-carbonyl would affect the conjugated system of CPT and provide an additional hydrogen bond receptor, and either of the evens would influence their



Scheme 2. Reagents and conditions: (a) cyclohexanecarbaldehyde, H_2O_2, AcOH–H_2O, H_2SO_4, 0–5 °C, 3 h, 49% (**CPT-c6**) and 19% (**CPT-c6-OH**); (b) cyclopropanecarbaldehyde, H_2O_2, AcOH–H_2O, H_2SO_4, 0–5 °C. 3 h, 22% (**CPT-c3C**) and 16% (**CPT-c3C-OH**).



Scheme 3. Reagents and conditions: (a) R^1Br, Ph_2SiH_2, *t*-BuOOH, AcOH-acetone H_2SO_4, 80 °C, 8–12 h, 15–62%; (b) MeI, K_2CO_3, acetone, refluxed, over night, 88–97%.

Table 1
Antitumor activity on human tumor cell lines A549/ATCC and HT-29

Compds	IC_{50}[a] (μM)		Compds	IC_{50} (μM)	
	A549/ATCC	HT-29		A549/ATCC	HT-29
CPT	0.047	0.12	**CPT-OH**	0.11	0.21
CPT-c3	0.12	0.12	**CPT-c5-OH**	0.071	0.058
CPT-c4	0.05	0.61	**CPT-c6-OH**	0.030	0.018
CPT-c5	0.066	0.094	**CPT-c7-OH**	0.003	0.012
CPT-c6	0.024	0.02	**CPT-c3C-OH**	1.28	0.57
CPT-c7	0.015	0.14	**CPT-OMe**	0.04	0.076
CPT-c8	0.36	0.45	**CPT-c4-OMe**	0.011	0.041
CPT-c3C	0.179	0.098	**CPT-c5-OMe**	0.031	0.031
SN-38	0.088	0.17	**CPT-c6-OMe**	0.039	0.035
Topotecan	0.36	0.41	**CPT-c7-OMe**	0.056	0.025

[a] Values are measured with SRB method.

Table 2
Antitumor activity on human tumor cell lines NCI-H460 and HL60

Compds	IC_{50} (μM)		Compds	IC_{50} (μM)	
	NCI-H460	HL60		NCI-H460	HL60
CPT-c5	<0.001	<0.001	**CPT-c6**	0.0023	0.0016
CPT-c5-OH	<0.001	<0.001	**CPT-c6-OH**	<0.001	<0.001
CPT-c5-OMe	<0.001	<0.001	**CPT-c5-OMe**	<0.001	<0.001
CPT-c3C	0.0045	0.0024	Topotecan	0.036	0.012
CPT-c3C-OH	0.028	0.0042			

[a] Values are measured with SRB method.

binding manners with enzyme and cause the reduction of activity. The exceptional case of **CPT-c8** is interesting, and the reason is not clear to our current knowledge. The space for substitutions at 7-position of CPT was proposed to be large enough, and the more bulky cyclooctyl substituting at 7-positon was expected to have higher activity than the less bulky one. However, the situation turned

up to be opposite. Perhaps, the 7-cycloalkyl CPT analogues would change their binding model to the enzyme at this point as the ring side increasing, and reduced its antitumor activity.

The relationship of structure–activity presents three situations. Activities of derivatives of 7-cycloalkyl-CPT (**CPT-c*n***, *n* = **3–7**) or 10-OH-CPT (**CPT-c*n*-OH**, *n* = **5–7**) on cell line A549/ATCC and that of **CPT-c*n*-OH** or 7-cycloalkyl-10-OMe-CPT derivatives (**CPT-c*n*-OMe**, *n* = **4–7**) on cell line HT-29 generally respectively follow the regularity that more lipophilic substitutions at 7-position would lead higher antitumor activity, match the QSAR conclusion we achieved before. However, activity of **CPT-c*n*** derivatives on cell line A549/ATCC displays unordered, and situation of **CPT-c*n*-OMe** derivatives act a contrary law to the QSAR conclusion on cell line A549/ATCC. Methoxyl at 10-position displays to reduce the activities when the groups at 7-position become larger.

In summary, we reported a series of 7-cycloalkyl-CPT analogues with their synthesis and biological evaluations. These analogues display high antitumor activities on four human tumor cell lines A549/ATCC, HT-29, NCI-H460 and HL60. The SAR studies mostly support our previous QSAR conclusion. The exceptional compounds might indicate differences of CPT-DNA-Top 1 complex model from others.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.010.

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12. *Synthesis of 7-cyclohexylcamptothecin and 7-cyclohexyl-10-hydroxylcamptothecin with Sawada method, general procedure*: To a stirred solution of CPT (35 mg, 0.1 mmol) in HoAc (1.5 ml), deionized water (1.5 ml) and concd H_2SO_4 (0.4 ml) in ice bath was add aldehyde (or cyclobutylmethanol) (0.3 mmol) and 30% H_2O_2 (0.3 mmol). The stirring was continued for 3 h or stop according to the TLC analysis. The reaction mixture was then diluted with ice water (6 ml) and extracted with CH_2Cl_2 (10 ml × 3). Combined organic layers were dried on Na_2SO_4, evaporated and purified on silica gel column chromatography (1:100 MeOH/$CHCl_3$).
13. *Synthesis of 7-alkyl-camptothecin and 7-alkyl-10-hydroxylcamptothecin with cycloalkyl bromides, general procedure*: To the suspension of CPT (17 mg 0.05 mmol) in HOAc (2 ml) and acetone (1 ml) was added concd H_2SO_4 (0.03 ml), cycloalkyl bromides (0.4 mmol), Ph_2SiH_2 (0.45 mmol) and *t*-BuOOH (1.0 mmol). The reaction mixture was heated on 80 °C oil bath and stirred for 8–12 h according to the TLC analysis. Then the reaction mixture was diluted with ice water (10 ml) and extracted with CH_2Cl_2 (6 ml × 3). The combined organic phase was washed with brine, dried on anhydrous sodium sulfate, evaporated and purified on silica gel column chromatography (1:100 MeOH/$CHCl_3$).
14. *Methylation of 10-hydroxycamptothecin derivatives, general procedure*: To the suspension of 10-hydroxy-camptothecin derivatives (**6**, **8**, **16**–**18**, 0.1 mmol) in anhydrous acetone (10 ml) was added anhydrous K_2CO_3 (0.5 mmol). After stirring for 10 min, MeI (0.5 mmol) was added. The reaction mixture was then stirred over night, cooled to room temperature, diluted with CH_2Cl_2 (20 ml), washed with water, brine, and dried on anhydrous sodium sulfate, evaporated and purified on silica gel column chromatography (1:100 MeOH/$CHCl_3$) to give compounds **19**–**23**.