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Novel hexacyclic camptothecin derivatives. Part 1: Synthesis and cytotoxicity of camptothecins with an A-ring fused 1,3-oxazine ring

Sheng Wang [a], Yuyan Li [a], Yonghui Liu [a], Aijun Lu [b,*], Qidong You [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing, Jiangsu 210009, PR China
[b] JiangSu Simcere Pharmaceutical R&D Co., Ltd, Nanjing, Jiangsu 210042, PR China

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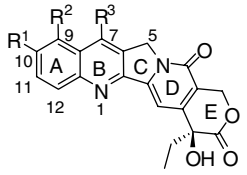
ABSTRACT

A novel series of A-ring modified hexacyclic camptothecin derivatives containing a 1,3-oxazine ring were first designed and synthesized. All of the hexacyclic camptothecins were assayed for in vitro cytotoxicity against nine human cancer cell lines. Among these compounds, **9b** and **9c** showed most potent cytotoxicity against several cell lines. Particularly, **9c** was about 13-fold more potent than camptothecin, and about sixfold more potent than topotecan toward HEPG-2. Furthermore, it was also found that the *N*-alkyl substituted derivatives were more potent than the *N*-aryl and *N*-benzyl substituted compounds against most cell lines.

Camptothecin (CPT, **1**), a pentacyclic alkaloid isolated by Wall in 1966 from the Chinese tree *Camptotheca cuminate* (Nyssaceae), was reported to possess excellent antitumor activity.[1] Clinical trials of camptothecin derivatives were discontinued in the early 1970s because of severe and unpredictable toxicity, in particular hemorrhagic cystitis.[2] In 1985, it was reported by Liu et al. that the cytotoxic activity of CPT was attributed to a novel mechanism of action involving the nuclear enzyme topoisomerase I (Topo I).[3] It was believed that camptothecin impacted upon replication, transcription, and the repair of DNA and caused cancer cell death by interfering with the catalytic cycle of DNA Top I and stabilizing the DNA–Topo I binary complex. Since elucidation of the unique 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[4] (TPT, **2**) and irinotecan[5] (**3**) (Fig. 1), have successfully entered into the market and are used as Top I inhibitors in clinical practice.[6]

Structure–activity relationship (SAR) studies suggested that substitution at the 7-, 9-, or 10-positions of most camptothecin derivatives enhances their antitumor activity, but at the 11- or 5-position usually leads to activity decrease.[7] In particular, many derivatives with an additional ring combined with the 10- and 11-positions, 7- and 9-positions, or 9- and 10-positions showed potent antitumor activity superior to those of original pentacyclic camptothecins, such as lurtotecan (**4**),[8] exatecan (**5**),[9] and other hexacyclic derivatives **6** and **7**,[10,11] probably due to the extended planarity exerted by an additional ring (Fig. 2). Among these hexacyclic camptothecins, **4** and **5** are, respectively, studied in the clinical phase II and phase III stages.

In order to further explore the potential of hexacyclic camptothecins, we designed a novel series of hexacyclic camptothecin derivatives containing a 1,3-oxazine ring fused with positions 9 and 10. We hypothesized that the novel hexacyclic camptothecins could exhibit potent antitumor activity for the two following reasons. First, it has a relatively rigid structure which could, in some sense, be regarded as the closed ring structure of TPT, **2** (Fig. 3). The flexible group at position 9 of TPTs molecule is fixed, which may be favorable to the interaction with the binary DNA–Top I complex. Second, their structure is very close to the known high antitumor activity compounds, hexacyclic derivatives **6**. Meanwhile, compared with **6**, our designed structure of hexacyclic camptothecins, in some sense, possesses an alkyl group in position



Camptothecin **1:** $R^1 = R^2 = R^3 = H$

Topotecan **2:** $R^1 = OH$, $R^2 = CH_2NMe_2$, $R^3 = H$

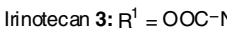
Irinotecan **3:** $R^1 =$  , $R^2 = H$, $R^3 = Et$

Figure 1. Structures of CPT, TPT, and irinotecan.

* Corresponding authors. Tel./fax: +86 25 83271351 (Q. You).
E-mail addresses: youqidong@gmail.com, youqidongcpu@gmail.com (Q. You).

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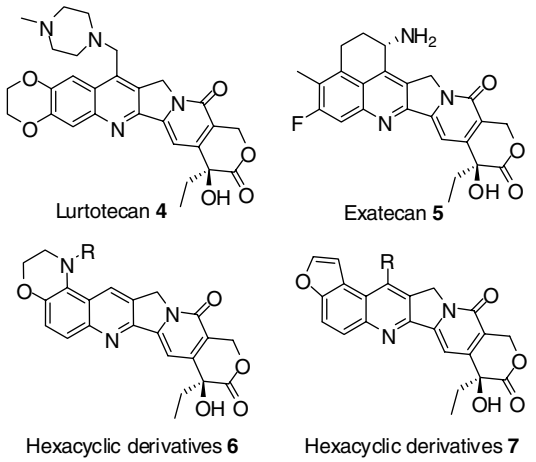


Figure 2. Structures of lurtotecan, exatecan, and hexacyclic derivatives **6** and **7**.

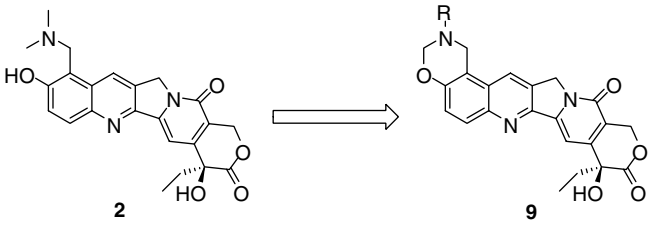
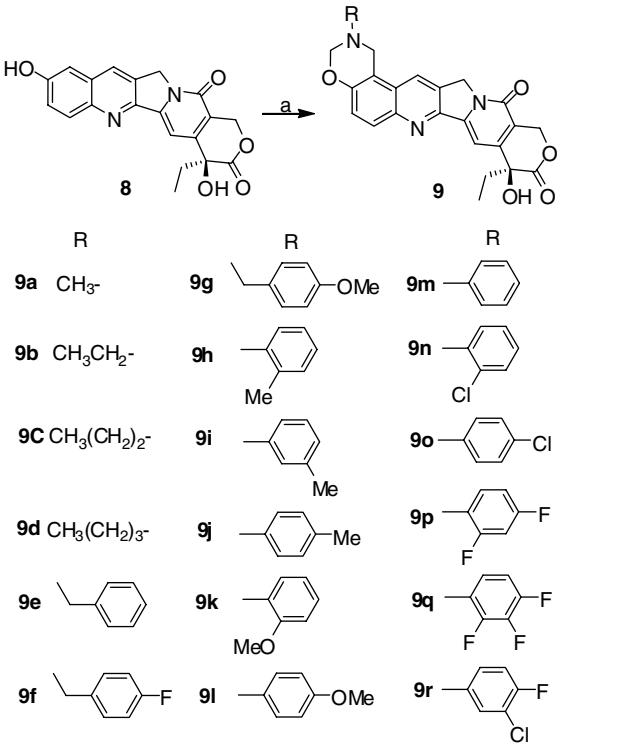


Figure 3. The design of hexacyclic camptothecins **9**.



Scheme 1. Reagents and conditions: (a) 1—primary amine or aniline derivatives, paraformaldehyde, 1,4-dioxane, 2 h; 2—**8**, 6–36 h.

9 which can enhance the liposolubility, antitumor activity, and stability of the lactone of camptothecins according to the literature.[12] Moreover, our reported compounds could be synthesized in one step with good yields from 10-hydroxycamptothecin, while the hexacyclic derivatives **6** were synthesized in relatively low yields in five steps from 10-hydroxycamptothecin. In this letter the synthesis and in vitro cytotoxicity of the novel series of hexacyclic camptothecins are reported.

The target hexacyclic camptothecins **9a–9r** were prepared from a Mannich-type reaction as shown in Scheme 1. Various primary amines were treated with excess paraformaldehyde in 1,4-dioxane at 70–105 °C under nitrogen for 2 h. Then 10-hydroxycamptothecin was added to the mixture and heated for another 6–36 h to give compounds **9a–9r**. It was noteworthy that the solvent played a key role in the reaction. The reaction did not take place when using acetic acid or CH_2Cl_2 as a solvent and only trace product was afforded in ethanol or DMF. However, good yields (60–85%) were achieved using the optimal solvent 1,4-dioxane.

Three types of primary amines (alkyl, benzyl or phenyl) were used as starting materials to give the corresponding hexacyclic camptothecins in good yields (71–85%) except for the aniline derivatives with electron-withdrawing substituted groups (compounds **9n–9r**, 60–67%), particularly, only trace product being obtained using 4-nitroaniline. This was probably because of the lower nucleophilicity of the aniline, which had a critical effect on the formation of imine from paraformaldehyde and amine in the Mannich-type reaction. The IR, ^{1}H NMR, ESI–MS, and HR–MS spectra of these novel hexacyclic camptothecin analogs were consistent with their structures and are listed in Supporting Information.

Cytotoxicity of these novel hexacyclic camptothecins was evaluated on nine human cancer cell lines (BXPC-3, NCI-446, MCF-7, HEPG-2, A549, A2780, Bel7402, HT-29, and KB) using MTT assay.[10] CPT and TPT were used as reference compounds. The results of the cytotoxicity studies are shown in Table 1.

Most of novel hexacyclic camptothecins showed comparable or superior cytotoxic activities to TPT, and five compounds (**9b**, **9c**, **9e**, **9m**, and **9q**) exhibited comparable cytotoxicities compared with CPT against several cell lines. Among the four *N*-alkyl substituted hexacyclic camptothecins, **9b** and **9c** showed more potent cytotoxicities than CPT and TPT against BXPC-3, HEPG-2, and A2708 cell lines. Particularly, **9c** was about 13-fold more potent than CPT, and about sixfold than TPT toward HEPG-2 cell line. This result indicated that the relatively smaller substituted groups at N atom of 1,3-oxazine ring could enhance cytotoxicity and it was consistent with the SAR of CPT that large substituted groups at C-9 and C-10 positions reduce the cytotoxicity of CPT.[7] However, compound **9a** with an *N*-methyl showed less cytotoxicity. This was probably due to its poor solubility in the solvents of DMSO and culture medium used in MTT assay. Cytotoxic activities of compounds **9b** and **9c** were comparable to the reported hexacyclic camptothecin derivatives **6** with a small alkyl group at N atom of 1,4-oxazine ring against A549 cell line, which also conformed to our primary hypothesis of the similar antitumor activity based on their close structures. The benzyl or phenyl with electron-withdrawing or electron-donating substituted groups at N atom of 1,3-oxazine ring showed similar cytotoxicity. For instance, compound **9f** showed similar cytotoxicity to **9g** and cytotoxic activity of compound **9j** was comparable to **9o** against most cell lines. The phenyl with *o*-, *m*-, or *p*-substituted groups also exhibited similar cytotoxic activity such as compounds **9i**, **9j**, and **9k**. These results indicated the substituted groups' electronegativity and positions at benzyl or phenyl groups slightly affected the cytotoxicity. Preliminary study of SAR of this novel series of hexacyclic camptothecins indicated that antitumor activities of *N*-alkyl substituted compounds (**9b and 9c**) were better than those of *N*-benzyl and *N*-aryl substituted compounds (**9e–9g and 9h–9r**) against most cell lines. This result was probably a result of the relatively smaller and more flexible substituted groups at N atom of 1,3-oxazine ring, which could

Table 1
Cytotoxicity of hexacyclic camptothecins against nine human cancer cell lines[a]

Compound	In vitro cytotoxicity (IC_{50}, μmol L^{-1})								
	BXPC-3	NCI-446	MCF-7	HEPG-2	A549	A2780	Bel7402	HT-29	KB
CPT	0.24	3.28	0.20	0.13	0.01	0.20	0.17	0.09	0.003
TPT	0.62	10.10	1.20	0.06	0.22	3.35	3.13	0.88	0.22
9a	0.48	37.33	4.52	0.64	0.41	0.53	1.67	0.69	0.67
9b	0.19	0.23	0.21	0.05	0.16	0.12	0.81	0.14	0.12
9c	0.15	3.90	—[b]	0.01	0.11	0.09	1.43	0.18	0.67
9d	0.97	10.10	0.55	0.35	0.20	3.19	4.01	0.13	0.09
9e	0.21	6.23	1.60	0.11	0.08	0.91	1.58	0.10	0.04
9f	1.73	43.23	0.65	0.14	0.12	11.62	5.22	1.19	0.06
9g	1.24	56.83	—	1.47	0.03	17.58	9.58	1.68	0.08
9h	0.53	1.14	—	0.18	1.21	4.26	6.00	0.22	0.06
9i	0.58	7.03	—	0.93	0.42	0.85	5.43	0.34	0.04
9j	2.05	9.39	1.64	—	0.57	2.16	3.86	0.53	0.34
9k	3.34	—	1.40	0.13	0.72	2.99	2.23	0.31	0.29
9l	0.42	14.45	—	0.12	0.27	1.12	4.99	0.31	0.23
9m	0.19	—	2.75	0.03	0.77	3.43	7.13	0.48	0.67
9n	0.89	19.22	2.00	—	0.45	4.10	15.17	1.05	0.33
9o	0.51	—	5.00	0.17	0.31	0.82	16.27	0.21	0.23
9p	1.57	—	4.48	0.55	0.39	2.07	4.70	0.62	0.66
9q	0.12	—	2.59	—	0.28	1.07	13.08	0.62	0.28
9r	0.92	—	9.51	0.18	0.56	3.10	3.06	1.13	0.62

[a] In vitro cytotoxicity of the hexacyclic camptothecins against nine cell lines, BXPC-3 (human pancreatic cancer), NCI-446 (human lung cancer), MCF-7 (human breast cancer), HEPG-2 (human liver cancer), A549 (human lung cancer), A2780 (human ovarian cancer), Bel7402 (human liver cancer), HT-29 (human colon cancer), and KB (human epidermoid carcinoma of the nasopharynx), were measured by the MTT assay after 4 days of incubation and expressed as the doses required to inhibit the growth of 50% of the cells cultivated (IC_{50}, μmol L^{-1}).
[b] Not tested.

be more favorable to the interaction with the binary DNA–Top I complex in three-dimension, and may also more readily make the N atom form a hydrogen bond with receptor.

In summary, 18 novel hexacyclic camptothecin derivatives containing a 1,3-oxazine ring were first designed and synthesized. The in vitro cytotoxicity was preliminarily evaluated. Several derivatives showed very impressive cytotoxicity, especially compounds **9b** and **9c**, which showed great potency against several cell lines. Further biological evaluation of compounds **9b** and **9c** is currently underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.103.

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