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Design and synthesis of new 7-(*N*-substituted-methyl)-camptothecin derivatives as potent cytotoxic agents

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ABSTRACT

A series of novel 7-(*N*-substituted-methyl)-camptothecin derivatives was designed, synthesized, and evaluated for in vitro cytotoxicity against four human tumor cell lines, A-549, MDA-MB-231, KB, and KBvin. All of the derivatives showed promising in vitro cytotoxic activity against the tested tumor cell lines, with IC_{50} values ranging from 0.0023 to 1.11 μM, and were as or more potent than topotecan. Compounds **9d**, **9e**, and **9r** exhibited the highest antiproliferative activity among all prepared derivatives. Furthermore, all of the compounds were more potent than paclitaxel against the multidrug-resistant (MDR) KBvin subline. With a concise efficient synthesis and potent cytotoxic profiles, especially significant activity towards KBvin, compounds **9d**, **9e**, and **9r** merit further development as a new generation of camptothecin-derived anticancer clinical trial candidates.

Camptothecin (CPT, **1**, Fig. 1), a natural quinoline alkaloid isolated by Wall and Wani from the Chinese tree *Camptotheca acuminata*, showed potent antiproliferative activity against a broad spectrum of tumors.[1–3] Its antitumor activity is induced by directly binding to topoisomerase I (Topo I), which results in interference with the catalytic cycle of DNA-Topo I and stabilization of the DNA-Topo I binary complex.[4–6] Based on CPT's remarkable anticancer activity and unique cytotoxic mechanism, numerous potent CPT analogs have been developed. Among them, two Topo I inhibitors, topotecan (**2**) and irinotecan (**3**), have been used successfully in the clinic as anticancer drugs, while several other analogs are the subjects of ongoing preclinical or clinical evaluation.[7–10]

Although CPT derivatives remain a promising class of antitumor agents, the highly electrophilic α-hydroxylacetone of the E ring is intrinsically unstable and undergoes rapid hydrolysis to the biologically inactive carboxylate form under physiological conditions.[11,12] This chemical feature diminishes the efficacy of various CPT derivatives in vivo compared to the impressive results often obtained from in vitro studies. Thus, several synthetic strategies to overcome this challenge have been developed, resulting in a logical mapping of the structure–activity relationship (SAR) of CPT derivatives.[13,14] Substitutions at the 11- or 5-position are not well tolerated, whereas various substituents at the 7-, 9-, or 10-position can improve the antitumor activity, as well as increase E-ring stability.[11,15,16] In particular, previous studies documented that the introduction of lipophilic substituents at the 7-position provides favorable molecular interactions and improved pharmacological features that could have potential therapeutic advantages.[17] A binding model of CPT with biological macromolecules also indicated that the C-7 molecular area could accommodate considerable structural diversity.[18–20] On the basis of these critical clues, various substitutions, such as ethyl, alkylsilyl, oxyiminoalkyl, and alkylsilylalkyl, were introduced at the 7-position of CPT to produce potent antitumor agents. To date, 7-substituted compounds constitute most of the second-generation CPT analogs that have reached preclinical or clinical development studies. Examples include gimatecan (**4**),[21] CKD-602 (**5**),[22] and BNP-1350 (**6**),[23] which contain highly lipophilic substituents intended to increase antitumor activity. These successful examples indicate the important role played by various C-7 substitutions in the activity profiles of CPT analogs and the feasibility of optimizing this compound class through rational C-7 modification.

In our continuing studies on the chemistry of CPT,[24–30] we recently reported a series of 7-ketone camptothecin derivatives with potent antitumor activity and significantly different drug-resistance profiles from those of the parent compound.[30] Some of

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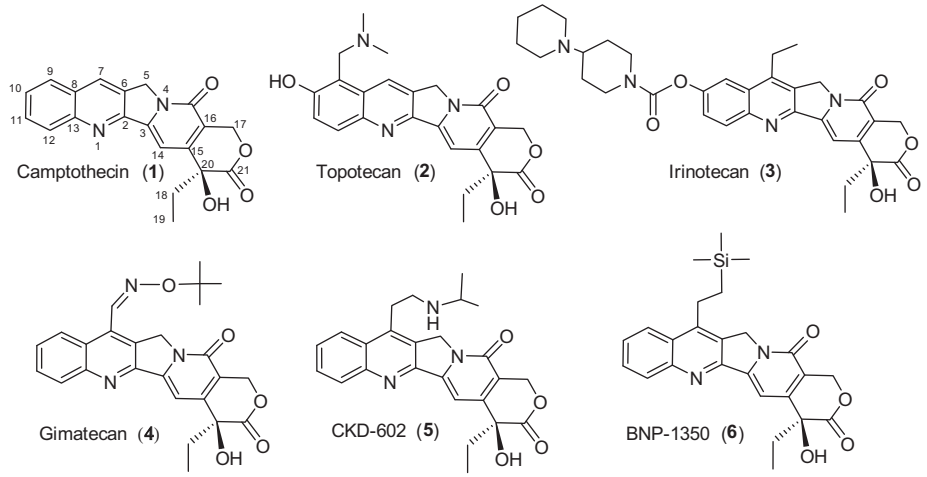


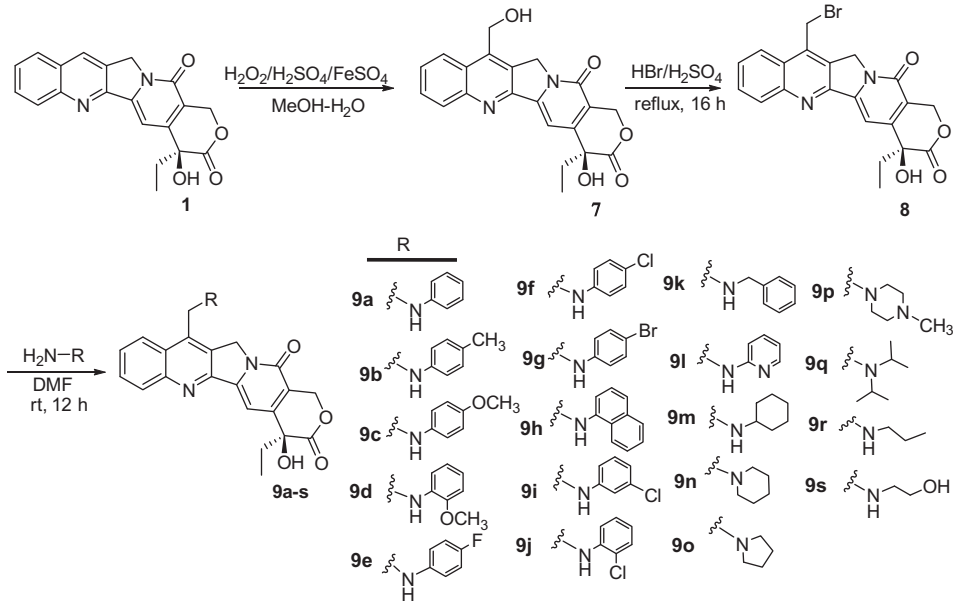
Figure 1. Structures of camptothecin (**1**), topotecan (**2**), irinotecan (**3**), gimatecan (**4**), CKD-602 (**5**), and BNP-1350 (**6**).

the new compounds exhibited activity comparable to that of marketed CPTs, such as **2** and **3**. Notably, some compounds displayed promising cytotoxicity against KBvin cells, while **3** lost activity completely. The encouraging preliminary results have prompted us to extend our investigation by synthesizing a novel series of 7-(*N*-substituted-methyl)-camptothecin derivatives. Herein, we describe our introduction of different nitrogen substituted groups into the 7-position of CPT via a coupling reaction and cytotoxic activity studies on the resulting compounds.

The synthetic route to target CPT derivatives **9a**–**s** is depicted in Scheme 1. Briefly, treatment of **1** with hydrogen peroxide and ferrous sulfate in an aqueous methanol–sulfuric acid solution furnished 7-hydroxymethylcamptothecin (**7**) in 80% yield.[31] Precursor **7** was converted into the key intermediate 7-bromomethylcamptothecin (**8**) in 66% yield by heating in hydrobromic acid.[32] Intermediate **8** was coupled with various substituted amines in dry DMF to afford the desired derivatives **9a**–**s** in 21–46% yields.[33] All synthesized target compounds were purified by column chromatography, and their structures were characterized by ^{1}H NMR, MS, and elemental analysis.

Target compounds **9a**–**s** were evaluated for in vitro cytotoxicity against a panel of human tumor cell lines, including A-549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (originally isolated from nasopharyngeal carcinoma), and KBvin (MDR KB subline), using a sulforhodamine B colorimetric (SRB) assay with triplicate experiments.[34,35] Paclitaxel and **2** were used as positive controls and the screening results are shown in Table 1.

As illustrated in Table 1, all new compounds exhibited significant in vitro cytotoxic activity against the four tested tumor cell lines, with IC_{50} values ranging from 0.0023 to 1.11 μM, and except for **9k**, **9q**, and **9s**, were mostly more active than **2**, a clinically used CPT-derived chemotherapeutic drug. Compounds **9d**, **9e**, and **9r** were the most potent compounds in the series and were also superior to paclitaxel against the A-549 cell line, which was generally most sensitive to these CPT derivatives. Against the same cell line, many compounds, including **9d**–**9f**, **9h**, **9j**, **9m**–**9o**, and **9r**, also showed better antiproliferative activity than **2**. With few exceptions (i.e., **9k**, **9q**, and **9s**), the tested compounds showed increased cytotoxic potency against the triple-negative breast cancer (MDA-MB-231) cell line compared with **2**. With regard to the KB



Scheme 1. Synthesis of target compounds **9a**–**s**.

Table 1
Antiproliferative activity of **9a–s** against four human tumor cell lines

Compd	IC_{50} (μM) with SD			
	A-549	MDA-MB-231	KB	KBvin
9a	0.0532 ± 0.0226	0.0781 ± 0.0178	0.0783 ± 0.0272	0.0479 ± 0.0126
9b	0.0587 ± 0.0262	0.0927 ± 0.0187	0.0817 ± 0.0201	0.0795 ± 0.0411
9c	0.0470 ± 0.0191	0.0842 ± 0.0062	0.0758 ± 0.0107	0.0773 ± 0.0255
9d	0.0023 ± 0.0024	0.0264 ± 0.0125	0.0046 ± 0.0032	0.0149 ± 0.0121
9e	0.0063 ± 0.0027	0.0300 ± 0.0163	0.0050 ± 0.0013	0.0132 ± 0.0055
9f	0.0391 ± 0.0173	0.0644 ± 0.0306	0.0397 ± 0.0015	0.0129 ± 0.0066
9g	0.0592 ± 0.0252	0.114 ± 0.0464	0.0821 ± 0.0093	0.0633 ± 0.0177
9h	0.0362 ± 0.0211	0.0640 ± 0.0025	0.0615 ± 0.0119	0.0780 ± 0.0853
9i	0.0504 ± 0.0206	0.0961 ± 0.0102	0.0713 ± 0.0139	0.0688 ± 0.0315
9j	0.0170 ± 0.0115	0.0576 ± 0.0514	0.0371 ± 0.0031	0.0128 ± 0.0081
9k	0.122 ± 0.0406	0.337 ± 0.0114	0.180 ± 0.0662	0.117 ± 0.0177
9l	0.0514 ± 0.0211	0.108 ± 0.0197	0.0786 ± 0.0058	0.0706 ± 0.0217
9m	0.0188 ± 0.0154	0.0532 ± 0.0006	0.0362 ± 0.0153	0.0165 ± 0.0149
9n	0.0219 ± 0.0107	0.0699 ± 0.0043	0.0499 ± 0.0074	0.0205 ± 0.0141
9o	0.0155 ± 0.0059	0.0609 ± 0.0096	0.0371 ± 0.0018	0.0208 ± 0.0199
9p	0.0622 ± 0.0168	0.110 ± 0.0177	0.0895 ± 0.0231	0.274 ± 0.1592
9q	0.534 ± 0.0701	1.11 ± 0.0059	0.813 ± 0.0556	1.06 ± 0.0115
9r	0.0048 ± 0.0042	0.0316 ± 0.0026	0.0150 ± 0.0102	0.0217 ± 0.0157
9s	0.150 ± 0.0275	0.378 ± 0.0082	0.208 ± 0.0209	0.183 ± 0.0219
2	0.0452 ± 0.0004	0.102 ± 0.0055	0.0625 ± 0.0042	0.396 ± 0.0207
Paclitaxel	0.0057 ± 0.0016	0.0066 ± 0.0018	0.0039 ± 0.0010	1.44 ± 0.137

cell line, seven compounds exhibited significant cytotoxic activity comparable to that of **2**. Remarkably, except for **9q**, all of the compounds (IC_{50} 0.0128–0.274 μM) were more potent than paclitaxel (IC_{50} 1.44 μM) and **2** (IC_{50} 0.396 μM) against the KBvin subline. These encouraging results suggested that these new derivatives could overcome the MDR phenotype overexpressing P-glycoprotein. Notably, the three most promising compounds **9d**, **9e**, and **9r** showed broad in vitro antitumor spectra and were about 7- to 20-fold more potent than **2**. Further pharmacological, toxicological evaluations and binding affinity analysis with DNA-Topo I target in the modeling of these promising compounds are in progress.

SAR analysis of the results from the synthesized compounds revealed several structural properties that could influence the in vitro cytotoxicity of the new CPT derivatives. Regarding compounds **9a** to **9j** with substituted (phenylamino)methyl groups at C-7, derivatives with *m*-/*p*-chlorophenyl rings (**9f** and **9i**) exhibited lower activity than their *o*-substituted isomer **9j**. Similarly, the relocation of an electron-donating methoxy group from the *o*-position (**9d**, IC_{50} 0.0023 μM) to the *p*-position (**9c**, IC_{50} 0.0470 μM) also decreased the cytotoxicity remarkably. These results suggested that an *o*-substituted phenyl group is more favorable than *m*-/*p*-substituted rings for better activity. Moreover **9e** and **9f** with *p*-fluoro and *p*-chloro substitution, respectively, on the phenyl ring were more potent against A-549 tumor cells than **9g** with *p*-bromo substitution, indicating that a substituent's size, in addition to position, could affect the activity. However, the presence of a pyridine-2-yl rather than phenyl group on the amine did not substantially change the cytotoxic activity (**9l** vs **9a**). Among compounds **9m–9s** with non-aromatic amino substituents, the SAR analysis showed that the alkyl variant had an important effect on the in vitro cytotoxic activity. For example, the cytotoxic activity of **9r** with a (propylamino)methyl group at C-7 was significantly greater than that of compounds with a (cyclohexylamino)methyl (**9m**) or (diisopropylamino)methyl (**9q**) moiety. These data suggested that the bulkier substituents in the two latter compounds might produce steric hindrance at the target level and thus lower the cytotoxic activity. The polarity and electron density in the C-7 side chain were also important, as changing the propylamino group in **9r** to a 2-hydroxyethylamino group in **9s** greatly decreased the cytotoxic activity (e.g., IC_{50} 0.0048 μM for **9r** vs 0.15 μM for **9s** against A-549). Taken together, these results showed that both the identity and substitution pattern in the R group at C-7 could greatly influence the cytotoxicity of the new CPT derivatives.

In summary, 19 novel 7-(*N*-substituted)-methyl-camptothecin derivatives were designed, synthesized, and evaluated for antiproliferative activity against four human tumor cell lines (A-549, MDA-MB-231, KB and KBvin) by using a sulforhodamine B colorimetric assay. Most of the new derivatives showed comparable or superior antiproliferative activity compared with **2**. In particular, compounds **9d**, **9e**, and **9r** were the most promising derivatives with 7- to 20-fold greater potency than **2** against the A-549 cell line and were selected as lead molecules for further development. Notably, with IC_{50} values ranging from 0.0128 to 1.06 μM, all of the compounds also were more potent than paclitaxel (IC_{50} 1.44 μM) against KBvin cells. Furthermore, SAR study indicated that both *N*-aromatic and *N*-aliphatic substituents at C-7 can produce potent activity, while selected variation of these substituents can greatly affect the activity. These findings support our further optimization of CPT to develop potential anticancer drug candidates. Continuing studies to substantiate and improve activity profiles are underway in our laboratories and will be reported in due course.

Acknowledgements

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Supplementary data

Supplementary data (analytical and spectroscopic data for all target compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.060.

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32. Synthesis of key intermediate 7-bromomethylcamptothecin (**8**). To a solution of 7-hydroxymethylcamptothecin (**7**) (300 mg, 0.80 mmol) in HBr (40%, 40 mL), 98% H_2SO_4 (0.1 mL) was added and the mixture was heated at reflux for 16 h. After completion of the reaction, the solvent was evaporated under vacuum and the residue recrystallized from MeOH to provide 7-bromomethylcamptothecin (**8**) as a light brown solid (234 mg, 66% yield). ^{1}H NMR (400 MHz, DMS0-d_6) δ: 0.87 (t, J = 7.2 Hz, 3H, 19-H), 1.84–1.90 (m, 2H, 18-H), 5.26 (s, 2H, $-CH_2-$), 5.28 (s, 2H, 5-H), 5.45 (s, 2H, 17-H), 6.50 (s, 1H, 20-OH), 7.33 (s, 1H, 14-H), 7.76 (t, J = 7.2 Hz, 1H, 11-H), 7.89 (t, J = 7.2 Hz, 1H, 10-H), 8.21 (d, J = 8.4 Hz, 1H, 12-H), 8.42 (d, J = 8.4 Hz, 1H, 9-H); MS-ESI m/z: 441.4 $[M+H]^+$; Anal. Calc. For $C_{21}H_{17}N_2O_4Br$: C 57.16%, H 3.88%, N 6.35%. Found: C 57.17%, H 3.88%, N 6.34%.
33. General synthetic procedure for target compounds **9a–s**. To a solution of 7-bromomethylcamptothecin (0.1 mmol) in dry DMF (15 mL), different amines (0.15 mmol) dissolved in toluene (5 mL) were added and the reaction mixture was stirred at room temperature for 12 h. After the reaction was completed, the mixture was evaporated to dryness and the residue was purified by chromatography on silica gel using $CHCl_3$/MeOH as eluant to give **9a–s**. Representative analytical and spectroscopic data of 7-(*N*-(2-methoxyphenylamino)methyl)-(20*S*)-camptothecin (**9d**). Yield 39%; mp 239–241 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.2 Hz, 3H, 19-H), 1.79–1.86 (m, 2H, 18-H), 3.85 (s, 3H, $-OCH_3$), 5.01 (d, J = 4.8 Hz, 2H, $-CH_2-$), 5.21 (s, 2H, 5-H), 5.43 (s, 2H, 17-H), 5.90 (m, 1H, NH), 6.35 (d, J = 7.6 Hz, 1H, Ar-H), 6.52 (s, 1H, 20-OH), 6.53–6.57 (m,1H, Ar-H), 6.61-6.65 (m, 1H, Ar-H), 6.87 (d, J = 8.0 Hz, 1H, Ar-H), 7.30 (s, 1H, 14-H), 7.72 (t, J = 7.2 Hz, 1H, 11-H), 7.86 (t, J = 7.2 Hz, 1H, 10-H), 8.17 (d, J = 8.4 Hz, 1H, 12-H), 8.49 (d, J = 8.4 Hz, 1H, 9-H); MS-ESI m/z: 484.9 $[M+H]^+$; Anal. Calc. For $C_{28}H_{25}N_3O_5$: C 69.55%, H 5.21%, N 8.69%. Found: C 69.56%, H 5.23%, N 8.67%.
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35. Cytotoxic activity was determined by the sulforhodamine B (SRB) colorimetric assay as previously described.[34] In brief, the cells (4000–7500 cells/well) were seeded with various concentrations of samples in 96-well plates filled with RPMI-1640 medium containing 10 mM HEPES and 2 mM L-glutamine (HyClone) supplemented with 10% fetal bovine serum (HyClone), 100 μg/mL streptomycin, 100 IU/mL penicillin, and 0.25 μg/mL amphotericin B (Cellgro). After 72 h incubation at 37 °C with 5% CO_2 in air, the living cells were fixed in 10% trichloroacetic acid for 30 min followed by staining with 0.04% SRB (Sigma Chemical Co.) for 30 min. The bound SRB was solubilized with 10 mM Tris-base and the absorbance was measured at 515 nm using a Microplate Reader ELx800 (Bio-Tek Instruments, Winooski, VT) operated by a Gen5 software. All results were representative of three or more experiments and IC_{50} is expressed as the average with standard deviation (SD).