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Design, synthesis, and cytotoxic activity of novel 7-substituted camptothecin derivatives incorporating piperazinyl-sulfonylamidine moieties

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Abstract

In our continuing search for camptothecin (CPT)-derived antitumor drugs, novel 7-substituted CPT derivatives incorporating piperazinyl-sulfonylamidine moieties were designed, synthesized and evaluated for cytotoxicity against five tumor cell lines (A-549, MDA-MB-231, MCF-7, KB, and KB-VIN). All of the derivatives showed promising in vitro cytotoxic activity against the tested tumor cell lines, and were more potent than irinotecan. Remarkably, most of the compounds exhibited comparable cytotoxicity against the multidrug-resistant (MDR) KB-VIN and parental KB tumor cell lines, while irinotecan lost activity completely against KB-VIN. Especially, compounds **13r** and **13p** (IC₅₀ 0.38 and 0.85 μ M, respectively) displayed the greatest cytotoxicity against the MDR KB-VIN cell line and merit further development into preclinical and clinical drug candidates for treating cancer, including MDR phenotype.

Keywords

Camptothecin; Cytotoxic activity; Piperazine; Sulfonylamidine; Synthesis

Camptothecin (CPT, **1**) (Fig. 1) is a potent cytotoxic natural alkaloid, which acts against a broad spectrum of cancers via inhibition of DNA enzyme topoisomerase I (topo I).^{1,2} Its three semisynthetic analogues, irinotecan (CPT-11, **2**), topotecan (TPT, **3**), and belotecan (CKD-602, **4**) (Fig. 1), received US government approval for the clinical treatment of ovarian, small-cell lung, and refractory colorectal cancers, while several other analogues are the subjects of ongoing preclinical or clinical evaluation.^{3–5}

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A. Supplementary data

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From published accumulated structure-activity relationship (SAR) studies, modifications at the 7- or 10-position or both appear to be the most efficient approach to improve the antitumor potency, as well as increase E-ring stability.^{6–9} Furthermore, several reports documented that the introduction of lipophilic sub-stituents at the 7-position provides favorable molecular interactions and improved pharmacological features that could have potential therapeutic advantages.^{10–15} A binding model of CPT with biological macromolecules also indicated that the C-7 molecular area could accommodate considerable structural diversity.¹⁶ Accordingly, various substituents, such as ethyl,¹⁷ arylmethyl,¹⁸ alkylsilyl,^{19,20} and cycloalkyl,²¹ were introduced at the 7-position of CPT leading to either enhanced or comparable activity. Some C-7 modified analogues exhibited superior pharmacological properties compared with **1**, and several clinical trial drug candidates, including gimatecan (**5**), BNP-1350 (**6**), lurtotecan (**7**) and sinotecan (**8**) (Fig. 1) emerged as alternatives to overcome the drawbacks of **1**.

In continuation of these efforts, we recently found that a series of 20-sulfonylamidine-CPT derivatives displayed potent antitumor activity with significantly different drug-resistance profiles from those of **1**.^{22,23} Among these derivatives, compound **9** is attractive as a potential candidate for anticancer chemotherapy and the modification with sulfonylamidine-substituted side chains may over-come some limitations of **1**. These encouraging results prompted us to further extend our investigation by synthesizing a novel series of 7-piperazinyl-sulfonylamidine-CPT derivatives. A piperazine group is commonly found in various drugs^{24–27} and its introduction can contribute to improved drug-like properties, such as bioavailability and metabolism. Therefore, in the present study, we proposed to incorporate the two privileged fragments, sulfonylamidine and piperazine, into the structure of **1** to design new derivatives.

The synthetic routes to target compounds **13a–r** are outlined in Scheme 1. Briefly, CPT was initially converted to 7-chloro-methyl-CPT (**10**) by using the Sawada method.¹⁷ Then, treatment of **10** with N-Boc protected piperazine in dry DMF solution furnished precursor **11** in 80% yield. Removal of the N-Boc group of **11** with TFA in CH₂Cl₂ (1:1) formed the TFA salts **12**. Subsequently, the key intermediate **12** was reacted with various sulfonyl azides and alkynes in a Cu-catalyzed three-component reaction²⁸ to produce the corresponding target compounds **13a–r** in 50–72% yields.

The newly synthesized compounds were evaluated for in vitro cytotoxic activity against five tumor cell lines, KB (nasopharyngeal carcinoma), A-549 (lung carcinoma), MDA-MB-231 (breast carcinoma), KB-VIN (MDR KB subline) and MCF-7 (breast adenocarcinoma) by using a sulforhodamine B colorimetric assay²⁹ with triplicate experiments. Irinotecan (**2**) was used as the positive control. The screening results are shown in Table 1.

As shown in Table 1, all target compounds exhibited significant in vitro cytotoxic activity against the five tested tumor cell lines, with IC₅₀ values ranging from 0.13 to 11.26 μM. All of the compounds were less potent than **3** in the assay, which is a common feature of prodrugs; whereas, except for **13q** (against KB), all new compounds exhibited comparable or superior cytotoxic activity to **2**. Significantly, all of the compounds were more potent than **2** (IC₅₀ >20 μM) against the MDR KB-VIN cell line, with **13p** (IC₅₀ 0.85 μM) and **13r** (IC₅₀

0.38 μM) showing the greatest cytotoxicity against this cell line. All of the tested compounds also showed increased cytotoxic potency against the triple-negative breast cancer (MDA-MB-231) cell line compared with **2**. This result implied that the introduction of a sulfonylamidine group at C-7 might combat the tumor MDR phenotype caused by P-glycoprotein overexpression. The IC_{50} values in Table 1 also revealed that the A-549 cell line was more sensitive than the other four cell lines to these compounds, which is consistent with the clinical behavior of other CPT derivatives.³⁰

Furthermore, some preliminary SAR correlations were also observed for these derivatives. For compounds **13a–g**, when the R_2 group was fixed as hydrogen and the R_1 group in the sulfonylamidines was varied, methyl (**13a**) and ethyl (**13b**) gave the best results compared with the aromatic groups in compounds **13c–g**, suggesting that small aliphatic chains appear to be the best R_1 sub-stituents for greater cytotoxic potency. For example, against the KB cell line, the rank order of cytotoxic potency was **13a** (methyl) > **13b** (ethyl) > **13d** (2-thienyl) > **13c** (phenyl) > **13f** (3-pyridinyl) **13e** (chlorophenyl) **13g** (2-naphthyl). In two other types of derivatives bearing a methoxy (**13h–13l**) or fluoro (**13m–13r**) R_2 group, the compounds with an ethyl R_1 group (**13h** and **13m**) were generally more potent than the remaining compounds in each series with aromatic R_1 groups. These results indicated that an aromatic group in R_1 substituents is much less favorable than a short aliphatic group. The conjugation of the aromatic (R_1) sub-stituents with the sulfonyl moiety might disturb the electronic properties of ring B to a greater extent than alkyl groups. Moreover, the potency of the compounds with the aromatic substituents (R_1) depended significantly upon the nature of the R_2 substituent on the aromatic ring; for example, compare **13p** (4-fluorophenyl) with **13n** (4-methylphenyl) or **13q** (4-nitrophenyl).

In summary, novel series of 7-substituted-CPT derivatives incorporating piperazinyl-sulfonylamidine moieties were designed, synthesized and evaluated for cytotoxicity against five tumor cell lines (A-549, MDA-MB-231, MCF-7, KB and KB-VIN) by using a sulforhodamine B colorimetric assay. All synthesized compounds showed comparable or superior cytotoxicity activity compared with **2**. Notably, all compounds were more potent than **2** against MDR KB-VIN cells. The SAR study found that the size, electron density, and distribution of the substituents within the sulfonylamidine side chain are critical to the derivatives' activity. With a concise synthesis and potent cytotoxic profiles, the new CPT-derivatives, especially compounds **13r** and **13p** with the greatest cytotoxicity against the MDR KB-VIN cell line, merit further development into preclinical and clinical drug candidates for treating cancer, including MDR phenotype. Further optimization and mechanism of action studies on **13r** and **13p** are ongoing in our laboratory, and the results will be reported in due course. In this paper, two privileged fragments, sulfonylamidine and piperazine, were successfully introduced into our prior modified **1**-analogue to produce a new derivative type with an improved cytotoxicity profile. We believe that such a strategy may be generally useful or, at least, shed light on other **1**-derived antitumor drug discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CKD-602	belotecan
CPT	camptothecin
CPT-11	irinotecan
DMF	dimethylformamide
MDR	multi-drug resistant
SAR	structure-activity relationship
TFA	trifluoroacetic acid
topo	topoisomerase
TPT	topotecan

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Fig. 1.
Structures of camptothecin derivatives.

Scheme 1. General synthetic procedure for target compounds **13a-r**.

Table 1

In vitro cytotoxicity data for **13a–r** against five tumor cell lines.

Entry	IC ₅₀ (μM)				
	A-549	MDA-MB-231	KB	MCF-7	KB-VIN
13a	0.65 ± 0.02	1.57 ± 0.46	0.13 ± 0.06	1.59 ± 0.47	3.82 ± 0.71
13b	0.73 ± 0.005	3.50 ± 0.12	0.49 ± 0.09	1.9 ± 0.18	4.29 ± 0.44
13c	0.65 ± 0.06	4.41 ± 0.11	0.63 ± 0.09	2.25 ± 0.64	1.77 ± 0.37
13d	0.65 ± 0.01	3.99 ± 0.61	0.59 ± 0.01	1.25 ± 0.19	1.27 ± 0.36
13e	0.64 ± 0.02	5.01 ± 1.36	0.74 ± 0.02	3.19 ± 0.31	1.55 ± 0.59
13f	0.76 ± 0.02	3.84 ± 0.41	0.72 ± 0.06	3.40 ± 0.49	2.09 ± 0.54
13g	3.15 ± 0.44	11.26 ± 1.54	2.11 ± 0.94	9.37 ± 1.04	0.89 ± 0.01
13h	0.71 ± 0.06	3.81 ± 0.19	0.47 ± 0.06	2.90 ± 0.53	2.73 ± 1.00
13i	0.74 ± 0.03	5.84 ± 0.66	0.83 ± 0.03	5.67 ± 0.43	3.08 ± 0.85
13j	2.93 ± 0.60	9.75 ± 1.09	1.06 ± 0.16	8.36 ± 0.74	0.97 ± 0.03
13k	0.80 ± 0.04	4.96 ± 0.27	0.88 ± 0.04	4.22 ± 1.04	2.38 ± 0.86
13l	0.74 ± 0.04	9.36 ± 0.07	0.84 ± 0.07	6.56 ± 0.55	3.66 ± 0.38
13m	0.50 ± 0.05	2.51 ± 0.47	0.33 ± 0.09	1.60 ± 0.28	0.97 ± 0.03
13n	1.77 ± 0.07	6.01 ± 0.69	1.07 ± 0.25	7.04 ± 0.59	2.77 ± 0.28
13o	2.59 ± 0.98	6.31 ± 1.42	6.76 ± 1.78	6.05 ± 0.95	5.82 ± 2.07
13p	0.63 ± 0.02	3.54 ± 0.47	4.43 ± 2.74	2.18 ± 0.40	0.85 ± 0.11
13q	4.31 ± 0.68	10.17 ± 0.81	10.12 ± 1.92	8.85 ± 0.05	1.81 ± 0.04
13r	0.60 ± 0.03	5.41 ± 0.46	4.72 ± 3.72	4.74 ± 0.49	0.38 ± 0.08
2	8.31 ± 0.28	15.56 ± 0.30	7.99 ± 0.31	11.32 ± 0.25	>20
3	0.11 ± 0.02	0.77 ± 0.03	0.101 ± 0.01	0.28 ± 0.02	0.66 ± 0.02