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# Design and Synthesis of Novel PEG-conjugated 20(*S*)-Camptothecin Sulfonylamidine Derivatives with Potent *in vitro* Antitumor Activity via Cu-Catalyzed Three-Component Reaction

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## Abstract

In our continuing search for camptothecin (CPT)-derived antitumor drugs, novel structurally diverse PEG-based 20(S)-CPT sulfonylamidine derivatives were designed, synthesized via a Cu-multicomponent reaction (MCR), and evaluated for 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, and were more potent than irinotecan. Significantly, these derivatives exhibited comparable cytotoxicity against KBvin, while irinotecan was less active against this cell line. With a concise efficient synthesis and potent cytotoxic profiles, especially significant activity towards KBvin, these compounds merit further development as a new generation of CPT-derived PEG-conjugated drug candidates.

## Graphical Abstract

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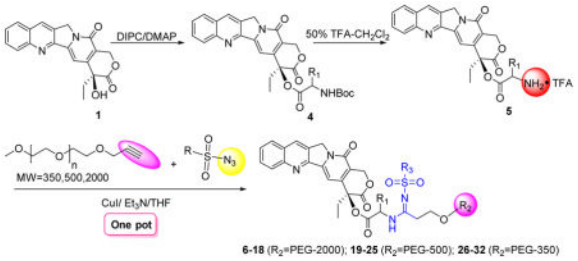


**Keywords**

Camptothecin; C-20 position; cytotoxic activity; poly(ethylene glycol) (PEG); sulfonylamidine

Multicomponent reactions (MCRs) have received great attention in combinatorial and medicinal chemistry because of their ability to generate diverse molecules economically in simple one-pot reactions.[1,2] Especially, the marriage of MCRs and combinatorial synthesis has innovated medicinal chemistry by boosting diversification of organic molecules to discover new drug candidates.[3,4] Meanwhile, MCRs have recently been introduced into macromolecular science. Highly efficient MCRs, such as the Passerini, Kabachnik–Fields, and Cu-catalyzed three-component reactions, have been employed to prepare condensed polymers, while the multiple reactants of MCRs provide new functionalities to polymer main chains.[5–13] Compared with the conventional step-by-step or post-modification approaches based on two component reactions, this conceptually new strategy offers a simple way to prepare functional polymers and greatly enriches the variety of functional polymers (both main chains and side groups). Despite fruitful possibilities of MCRs in polymer chemistry, the application of polymer-based MCRs in drug-conjugation has not yet been described in the literature, and more importantly, the use of MCRs for PEG-based camptothecin drugs as described in this communication is a completely new approach in the area of polymer-conjugated drugs.

Camptothecin (**1**, Figure 1) is a cytotoxic natural alkaloid with topoisomerase I inhibitory activity. However, therapeutic use of unmodified **1** is hindered by its very low solubility in aqueous media, high toxicity, and rapid inactivation through lactone ring hydrolysis in vivo. Lactone hydrolysis, which is reversible in acidic media, leads to a water soluble carboxylate. In addition, the carboxylate form readily binds to human serum albumin, making it less accessible for cellular uptake. This behavior gives rise to a drop in therapeutic efficacy, along with formulation difficulties.[14–17] To improve the therapeutic potential of **1** (i.e., reduce toxicity and improve antitumor activity), polymeric conjugation,[18,19] including poly(ethylene glycol) (PEG),[20,21] cyclodextrin copolymer,[22] poly(L-glutamic acid),[23] and phthalimide polymer,[24] have been investigated. Among the many biocompatible polymers, the PEG carrier system has well documented properties regarding water solubility, biocompatibility, and low immunogenicity, and has been granted FDA approval for human usage.[25–27] Accordingly, PEGylation can effectively improve the disadvantages of **1**, including increasing water solubility and lactone stabilization.[28,29] For example, linear PEG conjugation, as well as elegant architectures such as “bow-tie” dendrimers, has led to **1**-polymer therapeutic drugs and clinical candidates such as prothecan (**2**, Figure 1),[30,31]

which display increased water solubility, reduced side effects, and enhanced specificity due to the action of the enhanced permeability and retention (EPR) effect.

Inspired by the promising results applying CuMCR in macromolecular chemistry, in connection with an ongoing investigation of sulfonylamidine-derived antitumor agents, we successfully synthesized a large variety of 20-sulfonylamidine camptothecin derivatives.[32] Among them, some new compounds displayed potent antitumor activity with significantly different drug-resistance profiles from those of irinotecan (**3**, Figure 1), a clinically available anticancer drug. In addition, they also were effective in drug-sensitive and drug-resistant xenograft models at lower doses than **3**, demonstrating potential as drug candidates for anticancer chemotherapy. These encouraging results prompted us to further extend our investigation by exploring the three-component coupling reaction of alkyne, sulfonyl azides, and amines as reactants to prepare versatile PEG-based **1**-sulfonylamidine derivatives. In this study, we describe our design and synthesis of structurally diverse PEG-based 20(*S*)-**1**-sulfonylamidine derivatives via CuMCR and their cytotoxic activity.

As outlined in Scheme 1, various Boc-amino acids were initially added to the 20-hydroxyl group of **1** using a diisopropylcarbodiimide (DIPC) coupling reaction. Without isolation, the intermediate (**4**) was further reacted with trifluoracetic acid (TFA) in $CH_2Cl_2$ to give the key precursor TFA salts (**5**).[33] Subsequently, these key precursors were successfully employed as efficient reacting partners in a Cu-catalyzed three-component reaction with sulfonyl azides and PEG-alkynes to produce the corresponding target compounds **6**–**32** in moderate yields. In contrast to our previous study, under the optimized conditions, a wide range of sulfonyl azide counterparts, including aliphatic, aryl, and heterocyclic types, were all efficiently coupled to furnish the corresponding amidines. Moreover, various types of alkynes were likewise incorporated with almost the same efficiency. The coupling reaction has a wide substrate scope, a high tolerance to various functional groups, and very mild reaction conditions. All newly synthesized compounds were purified by column chromatography and their structures were confirmed by $^{1}$H-NMR and $^{13}$C-NMR analysis.

Target compounds **6**–**32** were evaluated for cytotoxicity against four human tumor cell lines, A-549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (nasopharyngeal carcinoma), and KBvin (MDR KB subline), using a sulforhodamine B colorimetric (SRB) assay with triplicate experiments.[34] The positive controls were **1** and **3**, 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 0.0623 to 0.9784 μM, and were more active than **3**, a clinically used CPT-derived chemotherapeutic drug. Remarkably, the new compounds exhibited comparable cytotoxicity against the parental KB cell line and MDR KB subline KBvin, while **3** was inactive against KBvin. The encouraging results suggested that these new derivatives could overcome the MDR phenotype overexpressing P-glycoprotein. In general, the $IC_{50}$ values also revealed that the A-549 cell line was more sensitive than the other three cell lines to these compounds, which is consistent with the clinical behavior of other CPT derivatives. Notably, the four most promising compounds **15**, **24**, **27** and **31** showed broad in vitro antitumor spectra and were

about 10- to 150-fold more potent than **3**. Furthermore, similar to our previous results,[32] the cytotoxic potencies of these derivatives were dual controlled by altering the length of the sulfonylamidine arm as well as the size of the substituent group. The best antiproliferative activity was achieved only with an appropriate balance between flexibility and size, such as in **15**, **24**, **27**, and **31**.

In summary, application of PEG-conjugated drugs has become increasingly frequent in medicinal chemistry studies during recent years. Compared with the conventional step-by-step PEG-modification approaches based on two component reactions, this MCR strategy offers a simple way to prepare functional polymers and greatly enriches the variety of PEG-based **1**-related drugs. As an extension to our studies on **1**-derived antitumor drugs, we designed and synthesized a series of novel PEG-based 20(*S*)-**1** sulfonylamidine derivatives via CuMCR, which were then evaluated for antiproliferative activities against four tumor cell lines (A-549, MDA-MB-231, KB, and KBvin) by using a sulforhodamine B colorimetric assay. All synthesized compounds were more potent than **3** in the cytotoxicity assays. Significantly, the new compounds exhibited comparable cytotoxicity against the parental KB cell line and MDR KB subline KBvin, while **3** was inactive against KBvin. SAR analysis indicated that the size, electron density, and distribution of the substituents within the sulfonylamidine side chain are critical to the derivatives' activity. These findings support our further optimization of **1** to develop potential **1**-derived anticancer drug candidates. Continuing studies to substantiate and improve activity profiles are underway in our laboratory and will be reported in due course.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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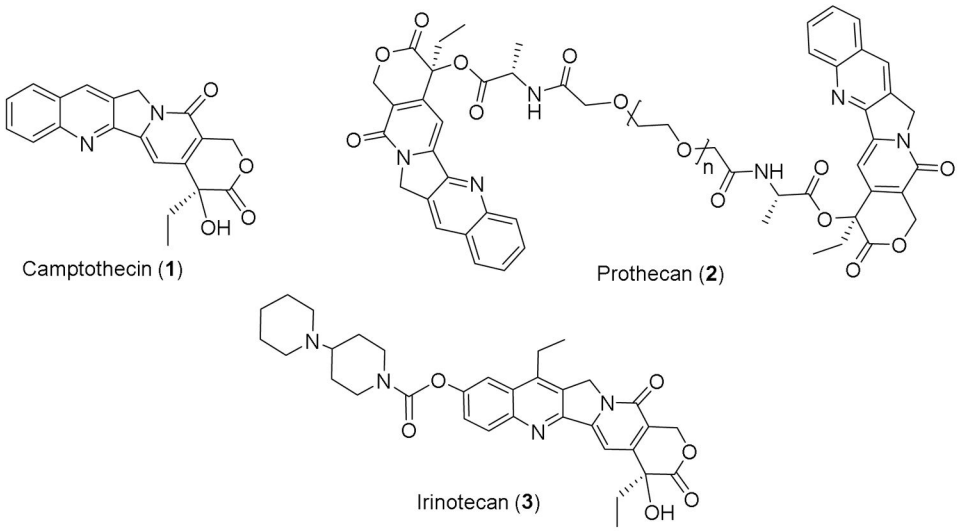
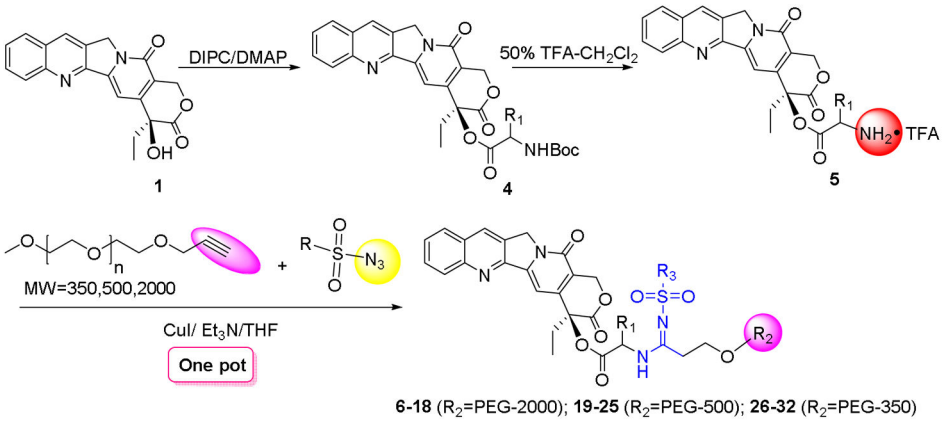



**Figure 1.**
Structures of camptothecin derivatives




| | R1 | R3 | | R1 | R3 |
|---|---|---|---|---|---|
| 6 | H | | 14 | H | |
| 7 | $CH_3$ | | 15 | H | |
| 8 | $(CH_3)_2CHCH_2$ | | 16 | H | |
| 9 | | | 17 | H | |
| 10 | H | $CH_3$ | 18 | H | |
| 11 | H | $CH_3CH_2CH_2CH_2$ | | | |
| 12 | H | | | | |
| 13 | H | | | | |

| | R1 | R3 | | R1 | R3 |
|---|---|---|---|---|---|
| 19 | H | | 26 | H | |
| 20 | H | $CH_3$ | 27 | H | $CH_3$ |
| 21 | H | $(CH_3)_2CHCH_2$ | 28 | H | $CH_3CH_2CH_2CH_2$ |
| 22 | H | | 29 | H | |
| 23 | H | | 30 | H | |
| 24 | H | | 31 | H | |
| 25 | $CH_3$ | | 32 | $CH_3$ | |

**Scheme 1.**
General synthetic procedure for target compounds **6–32**.

**Table 1**

Cytotoxicity results against four tumor cell lines.

| compound | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | **A-549** | **MDA-MB-231** | **KB** | **KBvin** |
| **6** | 0.1065 ± 0.0019 | 0.5331 ± 0.0081 | 0.5750 ± 0.0214 | 0.1260 ± 0.0263 |
| **7** | 0.5047 ± 0.0071 | 0.6809 ± 0.0158 | 0.6849 ± 0.0155 | 0.4335 ± 0.1168 |
| **8** | 0.6728 ± 0.0091 | 0.9784 ± 0.0059 | 0.9115 ± 0.0222 | 0.8714 ± 0.0520 |
| **9** | 0.1098 ± 0.0052 | 0.5004 ± 0.0199 | 0.5387 ± 0.0062 | 0.1277 ± 0.0064 |
| **10** | 0.0841± 0.0023 | 0.3319 ± 0.0117 | 0.1670 ± 0.0044 | 0.1124 ± 0.0161 |
| **11** | 0.1032 ± 0.0056 | 0.4248 ± 0.0139 | 0.4622 ± 0.0156 | 0.1179 ± 0.0024 |
| **12** | 0.1748 ± 0.0122 | 0.4580 ± 0.0143 | 0.3959 ± 0.0769 | 0.1055 ± 0.0044 |
| **13** | 0.0766 ± 0.0459 | 0.3992 ± 0.2238 | 0.1278 ± 0.0593 | 0.1099 ± 0.0073 |
| **14** | 0.1039 ± 0.0023 | 0.4427 ± 0.0470 | 0.3408 ± 0.0571 | 0.3029 ± 0.2155 |
| **15** | 0.0637 ± 0.0146 | 0.2705 ± 0.1891 | 0.1283 ± 0.0678 | 0.0955 ± 0.0295 |
| **16** | 0.0806 ± 0.0013 | 0.1921 ± 0.0134 | 0.1390 ± 0.0614 | 0.0996 ± 0.0049 |
| **17** | 0.0803 ± 0.0218 | 0.3432 ± 0.1853 | 0.3223 ± 0.2861 | 0.2227 ± 0.1677 |
| **18** | 0.0837 ± 0.0024 | 0.1756 ± 0.0412 | 0.1042 ± 0.0141 | 0.0884 ± 0.0038 |
| **19** | 0.1194 ± 0.0037 | 0.3025 ± 0.0159 | 0.4943 ± 0.0446 | 0.1355 ± 0.0018 |
| **20** | 0.0992 ± 0.0297 | 0.4468 ± 0.2307 | 0.3262 ± 0.2713 | 0.1176 ± 0.0203 |
| **21** | 0.1290 ± 0.0603 | 0.6625 ± 0.0081 | 0.5592 ± 0.1508 | 0.2702 ± 0.1207 |
| **22** | 0.0969 ± 0.0003 | 0.1781 ± 0.0093 | 0.1396 ± 0.0139 | 0.1007 ± 0.0049 |
| **23** | 0.0886 ± 0.0283 | 0.1899 ± 0.0031 | 0.1491 ± 0.0557 | 0.1026 ± 0.0156 |
| **24** | 0.0654 ± 0.0238 | 0.1391 ± 0.0229 | 0.0873 ± 0.0192 | 0.1039 ± 0.0292 |
| **25** | 0.4864 ± 0.0102 | 0.6033 ± 0.0025 | 0.5941 ± 0.0122 | 0.3556 ± 0.0350 |
| **26** | 0.1117 ± 0.0359 | 0.3810 ± 0.1858 | 0.3503 ± 0.2528 | 0.2495 ± 0.1561 |
| **27** | 0.0642 ± 0.0265 | 0.1393 ± 0.0035 | 0.0998 ± 0.0320 | 0.0996 ± 0.0284 |
| **28** | 0.3120 ± 0.2834 | 0.7377 ± 0.0309 | 0.5782 ± 0.1777 | 0.4226 ± 0.2258 |
| **29** | 0.2586 ± 0.2508 | 0.5842 ± 0.0246 | 0.4598 ± 0.1659 | 0.3315 ± 0.1568 |
| **30** | 0.1960 ± 0.1539 | 0.5850 ± 0.0969 | 0.4347 ± 0.2726 | 0.3257 ± 0.1703 |
| **31** | 0.0623 ± 0.0195 | 0.1143 ± 0.0215 | 0.0803 ± 0.0281 | 0.0862 ± 0.0279 |
| **32** | 0.4746 ± 0.0013 | 0.6238 ± 0.0203 | 0.5885 ± 0.0376 | 0.3359 ± 0.0011 |
| **1** | 0.0086 ± 0.0007 | 0.3066 ± 0.1180 | 0.0500 ± 0.0097 | 0.0157 ± 0.0011 |
| **3** | 9.480 ± 0.106 | 14.2820 ± 4.3003 | 9.828 ± 0.481 | >20 |