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Design, semisynthesis and potent cytotoxic activity of novel 10-fluorocamptothecin derivatives

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Abstract

Fluorination is a well-known strategy for improving the bioavailability of bioactive molecules in the lead optimization phase of drug discovery projects. In an attempt to improve the antitumor activity of camptothecins (CPTs), novel 10-fluoro-CPT derivatives were designed, synthesized and evaluated for cytotoxicity against five human cancer cell lines (A-549, MDA-MB-231, KB, KB-VIN and MCF-7). All of the derivatives showed more potent in vitro cytotoxic activity than the clinical CPT-derived drug irinotecan against the tumor cell lines tested, and most of them showed comparable or superior potency to topotecan. Remarkably, compounds **16b** (IC₅₀, 67.0 nM) and **19b** (IC₅₀, 99.2 nM) displayed the highest cytotoxicity against the multidrug-resistant (MDR) KB-VIN cell line and merit further development as preclinical drug candidates for treating cancer, including MDR phenotype. Our study suggested that incorporation of a fluorine atom into position 10 of CPT is an effective method for discovering new potent CPT derivatives.

Graphical abstract

A novel type of 10-fluorocamptothecin derivatives with excellent cytotoxicity activity were designed and synthesized.

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Keywords

Camptothecin; Cytotoxic activity; Fluorination; Synthesis

Fluorine, a highly active element, is widely used in drugs with high electronegativity and small size.^{1,2} Fluorine incorporation often improves drug-like properties by blocking undesired metabolism at a specific site, increasing lipophilicity or binding affinity, or altering drug absorption, distribution, or excretion. The unique and complex physicochemical and biological properties provided by incorporation of fluorine atoms or fluorinated moieties into bioactive molecules have resulted in a wide application of fluorinated compounds for construction of new drugs.^{3,4} As a result, more than 20% of the currently marketed drugs contain at least one fluorine atom. Particularly, an increasing number of fluorinated antitumor agents such as fluorouracil derivatives, enzalutamide, teriflunomide, ponatinib, sorafenib, gefitinib and vandetanib are becoming available for cancer treatment.⁵ Therefore, derivatization of current pharmaceuticals to fluorinated analogues has become an increasingly attractive strategy to obtain more potent drugs for drug discovery.

Camptothecin (CPT, **1**), a naturally occurring quinoline alkaloid, exhibits significant antitumor activity against a broad spectrum of cancers via inhibition of DNA enzyme topoisomerase I (topo I).^{6,7} Extensive structural modifications on the A-, B-, E-ring or acylation of the 20-hydroxy group of CPT led to the successful identification and development of the antitumor drugs irinotecan (**2**), topotecan (**3**) and belotecan (**4**), as well as several candidates such as lurtotecan (**5**), gimatecan (**6**), rubitecan (**7**), and prothecan (**8**), which are in various stages of preclinical development.^{8–16} Significantly, newly emerging fluorinated derivatives, including exatecan (**9**) and BN-80915 (**10**), where a fluorine atom has been introduced on the A ring of CPT, have shown excellent antitumor activities both in vitro and in vivo and are currently undergoing clinical trials.^{17,18} Recently, Zhang et al reported the synthesis of **11** via a new strategy to improve the metabolic stability of CPT's lactone by replacing it with an α -fluoro ether as a lactone bioisostere.¹⁹ The clinical successes and accumulated SAR studies have stimulated great interest in further exploration of CPT-derivatives with better antitumor activity.

In our continuous efforts to produce CPT-derived antitumor agents, we recently found that a series of novel 20-sulfonylamidine-CPT derivatives displayed potent antitumor activity with significantly different drug-resistance profiles from those of CPT. Among them, YQL-9a (**12**) showed excellent antitumor activities both in vitro and in vivo, indicating it to be a promising antitumor lead.^{20,21} In addition, in prior SAR studies on CPT, addition of an electronegative substituent at C-10 has always resulted in improved cytotoxicity. Based on these facts, we postulated that the addition of fluorine at the C-10 positions of CPT and **12** would be an efficient way to increase the cytotoxicity of both compounds (Fig. 2). Also, as mentioned earlier, fluorination has the potential to improve the pharmacological profile of drugs. Therefore, herein, we report our synthesis of C-10 fluorinated CPT and **12** derivatives, as well as evaluation of their cytotoxic activity.

The synthetic routes to target compounds **16a–b** and **19a–b** are outlined in Scheme 1. Briefly, 10-(trifluoromethanesulfonyloxy)camptothecins **14a** and **14b** were prepared from **13a** and **13b** by a classical synthetic method using *N*-phenylbis(trifluoromethanesulfonimide) and triethylamine in DMF. Next, both **14a** and **14b** were converted to 10-(tributylstannyl)camptothecins (**15a** and **15b**) in excellent yields by reaction with bis(tri-*n*-butyltin) in the presence of tetrakis(triphenylphosphine)palladium/lithium chloride.^{22,23} Treatment of **15a** and **15b** with 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octanebis(hexafluorophosphate) (F-TEDA-PF₆) and silver triflate (AgOTf) in dry acetone solution at 23 °C afforded the desired compounds **16a–b** in moderate yields.²⁴ Furthermore, the 20-hydroxy groups of **16a–b** were converted to *N*-Boc protected glycine esters (**17a–b**) using a combination of DIPIC (*N,N'*-diisopropylcarbodiimide) and DMAP (4-dimethylaminopyridine). Deprotection of **17a** and **17b** with TFA/CH₂Cl₂ afforded the TFA salts (**18a–b**). Subsequently, the key intermediates (**18a–b**) were successfully reacted with 4-ethynylanisole and *p*-toluenesulfonyl azide in a Cu-catalyzed three-component reaction to produce the corresponding target compounds (**19a–b**) in good yields.²⁵

The 10-fluorinated derivatives of CPT and **12** were evaluated for in vitro cytotoxic activity against five tumor cell lines, A-549 (lung carcinoma), MDA-MB-231 (triple-negative breast carcinoma), KB (originally isolated from epidermoid carcinoma of the nasopharynx), KB-VIN (MDR KB subline) and MCF-7 (breast adenocarcinoma) by using a sulforhodamine B colorimetric assay.^{26,27} Irinotecan (**2**) and topotecan (**3**) were used as the positive controls. 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 8.72 to 649 nM, and they exhibited more potent in vitro cytotoxic activity than **2**, while most of the new derivatives also showed comparable or superior potency to **3**. The data 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 derivatives of CPT.²⁸ Remarkably, all of the compounds were more potent than **2** (IC₅₀ >20,000 nM) against the MDR KB-VIN cell line, with **16b** (IC₅₀ 67.0 nM) and **19b** (IC₅₀ 99.2 nM) showing the greatest cytotoxicity against this cell line. They also showed increased cytotoxic potency against the triple-negative breast

cancer (MDA-MB-231) cell line compared with **2** or **3**. This result implied that the introduction of a fluorine atom at C-10 position or a sulfonylamidine group at C-20 might combat the tumor MDR phenotype caused by P-glycoprotein overexpression. Compared with **16a** and **19a**, the corresponding 7-ethyl derivatives **16b** and **19b** exhibited greater in vitro cytotoxic activity against the five tested tumor cell lines. Thus, the introduction of a C-7 ethyl group contributed to improved cytotoxicity.

It is well known that therapeutic use of unmodified CPT is hindered by its poor solubility, high toxicity, and rapid inactivation through lactone ring hydrolysis in vivo. 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. Therefore, approaches that stabilize the lactone ring prior to entry into the site of action are a critical feature in order to maximize therapeutic activity and minimize toxicity. It is widely known that fluorine incorporation is an efficient strategy for improving the bioavailability of bioactive molecules in the lead optimization phase. Additionally, early efforts by clinicians and research scientists to reduce the side effects of CPT and improve the metabolic stability of CPT's lactone were directed at synthesis of modified esters at position 20. It is interesting to note from Table 1 that, in general, the introduction of an ester moiety at the 20 position of CPT (compounds **19a**, **19b**) caused a loss of cytotoxic activity, which is a common feature of prodrugs. Also, these target compounds may improve the bioavailability of CPT by such modified approaches.

In summary, CPT and our previous lead compound **12** are effective cytotoxic agents that merit considerable attention. In response to the need to design novel CPT-derived antitumor agents with improved activity, we incorporated fluorine, an atom with unique properties, into CPT and **12**. In a preliminary in vitro cytotoxicity assay, the four new fluorinated compounds showed potent cytotoxicity (IC_{50} 8.72–649 nM) and were more effective than **2**. Notably, 7-ethyl 10-fluoro compounds **16b** (IC_{50} 67.0 nM) and **19b** (IC_{50} 99.2 nM) showed the greatest cytotoxicity against the MDR KB-VIN cell line cell line (cf, **2**, IC_{50} >20,000 nM) and were selected as lead molecules for further development. The results in this study provide the first convincing evidence that the introduction of fluorine at the C-10 position of CPT is favorable for enhanced cytotoxic activity. Meanwhile, the mechanism of action of these new derivatives is unknown, and further investigation will be required to understand the effect of fluorine. A better understanding of its role could provide meaningful insights for optimization with fluorine in further drug design. With a concise synthesis and potent cytotoxic profiles, we hope that 10-fluorinated CPT-derivatives will be promising templates for further preparation of improved anticancer agents in the future. Continuing studies to substantiate and improve activity profiles are underway in our laboratories and will be reported in due course.

Acknowledgments

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24. *Synthesis of compounds 16a, 16b.* Silver triflate (0.1 mmol) was added to a solution of the appropriate 10-(tributylstannyl)camptothecin (**15a** or **15b**)^{22,23} (0.1 mmol) in acetone at 23 °C. The reaction mixture was stirred at the same temperature for 15 min. Then 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo [2.2.2]octane bis(hexafluorophosphate) (F-TEDA-PF6) (0.120 mmol) was added to the reaction mixture, and stirred for 30 min at 23 °C. The reaction was completed as determined by thin layer chromatography (TLC) and then concentrated in vacuo. The residue was

purified by chromatography on silica gel eluting with CH₂Cl₂/MeOH 20:1 (v/v) to afford the target compound (**16a** or **16b**) as a light yellow solid. *Compound 16a*. Yield 43%; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 8.68 (s, 1H, C7-H), 8.26 (d, 1H, J=8Hz, C9-H), 7.98 (d, 1H, J=8Hz, C12-H), 7.80 (t, 1H, J=8Hz, C11-H), 7.34 (s, 1H, C14-H), 6.55 (s, 1H, C20-OH), 5.43 (s, 2H, C17-H), 5.30 (s, 2H, C5-H), 1.93-1.82 (m, 2H, C19-H), 0.89 (t, 3H, J=8Hz, C18-H); MS-ESI *m/z*: 389.4 [M+Na]⁺. *Compound 16b*. Yield: 47%; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 8.25 (d, 1H, J=8Hz, C9-H), 8.07 (d, 1H, J=8Hz, C12-H), 7.78 (t, 1H, J=8Hz, C11-H), 7.33 (s, 1H, C14-H), 6.54 (s, 1H, C20-OH), 5.44 (s, 2H, C17-H), 5.33 (s, 2H, C5-H), 3.24-3.17 (m, 2H, C7-CH₂-CH₃), 1.91-1.84 (m, 2H, C19-H), 1.32 (t, 3H, J=8Hz, C7-CH₂-CH₃), 0.89 (t, 3H, J=8Hz, C18-H); MS-ESI *m/z*: 417.3 [M+Na]⁺.

25. *Synthesis of compounds 19a, 19b*. Triethylamine (1.2 mmol) was added slowly to a suspension of the TFA salt (**18a** or **18b**) (0.5 mmol) in CH₂Cl₂, and this mixture was stirred for 10 min until a clear solution was obtained.²⁰ Under an N₂ atmosphere, 4-ethynylanisole (0.5 mmol), *p*-toluenesulfonyl azide (0.6 mmol), and CuI (0.05 mmol) were added. The reaction mixture was stirred for 2–6 h at 25 °C. After the reaction was completed, as judged by TLC, the reaction mixture was diluted with CH₂Cl₂ (4 mL) and aqueous NH₄Cl solution (6 mL). The mixture was stirred for an additional 30 min, and two layers were separated. The aqueous layer was extracted with CH₂Cl₂ (3 mL × 3). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. The crude residue was purified by chromatography on silica gel with CH₂Cl₂/MeOH to give the target compound (**19a** or **19b**). *Compound 19a*. Yield: 67%; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 8.90 (br, 1H, NH), 8.67 (s, 1H, C-7), 8.19 (d, 1H, J=8Hz, C9-H), 7.97 (d, 1H, J=8Hz, C12-H), 7.76 (t, 1H, J=8Hz, C11-H), 7.51-7.49 (m, 2H, Ar-H), 7.20-7.18 (m, 2H, Ar-H), 7.10 (s, 1H, C14-H), 7.03-7.01 (m, 2H, Ar-H), 6.78-6.76 (m, 2H, Ar-H), 5.48 (s, 2H, C17-H), 5.30 (s, 2H, C5-H), 4.39-4.33 (m, 1H, -COCH₂NH-), 4.27-4.21 (m, 1H, -COCH₂NH-), 4.01 (s, 2H, -CH₂-Ar), 3.66 (s, 3H, -OCH₃), 2.21 (s, 3H, CH₃-Ar), 2.13-2.09 (m, 2H, C19-H), 0.85 (t, 3H, J=8Hz, C18-H); MS-ESI *m/z*: 747.8 [M+Na]⁺. *Compound 19b*. Yield: 70%; ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 8.90 (br, 1H, NH), 8.67 (s, 1H, C-7), 8.19 (d, 1H, J=8Hz, C9-H), 8.07 (d, 1H, J=8Hz, C12-H), 7.77 (t, 1H, J=8Hz, C11-H), 7.52-7.50 (m, 2H, Ar-H), 7.19-7.17 (m, 2H, Ar-H), 7.08 (s, 1H, C14-H), 7.01-6.99 (m, 2H, Ar-H), 6.78-6.75 (m, 2H, Ar-H), 5.50 (s, 2H, C17-H), 5.33 (s, 2H, C5-H), 4.35-4.33 (m, 1H, -COCH₂NH-), 4.26-4.24 (m, 1H, -COCH₂NH-), 4.00 (s, 2H, -CH₂-Ar), 3.65 (s, 3H, -OCH₃), 3.21-3.19 (m, 2H, C7-CH₂-CH₃), 2.19 (s, 3H, CH₃-Ar), 2.13-2.09 (m, 2H, C19-H), 1.28 (t, 3H, J=8Hz, C7-CH₂-CH₃), 0.86 (t, 3H, J=8Hz, C18-H); MS-ESI *m/z*: 775.7 [M+Na]⁺.
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27. Cytotoxic activity was determined by the sulforhodamine B (SRB) colorimetric assay. In brief, the cells (3–5 × 10³ cells/well) were seeded in 96-well plates filled with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) containing various concentrations of samples, and incubated for 72 h. At the end of the exposure period, the attached cells were fixed with cold 50% trichloroacetic acid for 30 min followed by staining with 0.04% SRB (Sigma Chemical Co.) for 30 min. The bound SRB was solubilized in 10 mM Tris-base and the absorbance was measured at 515 nm on a Microplate Reader ELx800 (Bio-Tek Instruments, Winooski, VT) with a Gen5 software. All results were representative of three or more experiments and IC₅₀ is expressed as the average with standard deviation.
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Fig. 1.
Structures of CPT-derivatives

Fig. 2.
Design strategy for 10-fluorocamptothecin derivatives.

Scheme 1.

General synthetic procedure for 10-fluorocamptothecin derivatives **16a–b** and **19a–b**.

Table 1

In vitro cytotoxicity data for compounds **16a–b** and **19a–b** against five tumor cell lines.

Entry	IC ₅₀ (nM)				
	A-549	MDA-MB-231	KB	KB-VIN	MCF-7
16a	58.6 ± 0.23	579 ± 2.41	185 ± 0.02	2954 ± 20.9	431 ± 67.7
16b	9.91 ± 0.36	319 ± 107	69.6 ± 2.37	67.0 ± 0.77	76.9 ± 5.11
19a	62.2 ± 1.36	601 ± 57.0	280 ± 50.6	497 ± 37.6	649 ± 71.8
19b	8.72 ± 0.03	238 ± 28.3	47.0 ± 6.20	99.2 ± 17.7	80.8 ± 3.48
1	8.63 ± 0.66	307 ± 118	50.0 ± 9.7	15.7 ± 1.1	87.5 ± 6.4
2	8,300 ± 280	15,600 ± 302	7,990 ± 312	>20,000	11,300 ± 249
3	112 ± 28.4	773 ± 36.0	102 ± 8.1	287 ± 25.3	666 ± 26.7