Bioorganic & Medicinal Chemistry 22 (2014) 6453–6458

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design and synthesis of novel spin-labeled camptothecin derivatives as potent cytotoxic agents

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ARTICLE INFO

Article history:
Received 29 July 2014
Revised 10 September 2014
Accepted 16 September 2014
Available online 28 September 2014

Keywords:
Camptothecin
C-20 position
Cytotoxic activity
Spin-labeled

ABSTRACT

In our continuing search for natural product-based spin-labeled antitumor drugs, 20 novel spin-labeled camptothecin derivatives were synthesized via a Cu-catalyzed one pot reaction and evaluated for cytotoxicity against four human tumor cell lines (A-549, MDA-MB-231, KB, and KBvin). Eighteen of the target compounds (**9a**, **9b**, **9d**–**9k**, **9m**–**9t**) exhibited significant in vitro antiproliferative activity against these four tested tumor cell lines. Compounds **9e** and **9j** (IC_{50} 0.057 and 0.072 μM, respectively) displayed the greatest cytotoxicity against the multidrug-resistant (MDR) KBvin cell line and merit further development into preclinical and clinical drug candidates for treating cancer including MDR phenotype.

1. Introduction

Camptothecin (CPT, **1**, Fig. 1), a pentacyclic alkaloid isolated from *Camptotheca acuminata* by Wall et al., showed excellent antitumor activity against a broad spectrum of tumor cell lines by inhibiting topoisomerase I (Topo I).[1–5] Two semisynthetic derivatives, topotecan (**2**) and irinotecan (**3**), are widely used clinically for treating ovarian and small-cell lung cancers, respectively. Several other drug candidates, such as gimatecan (**4**), CKD-602 (**5**), and BNP-1350 (**6**), are the subject of ongoing preclinical or clinical evaluation.[6–9]

Although CPT analogues remain a promising class of antitumor agents, their therapeutic use has been severely hindered by toxicity issues and delivery problems, due to poor water solubility, as well as intrinsic instability of the highly electrophilic α-hydroxylactone of the E ring, due to preferential binding of the opened carboxylate to serum albumin.[10,11] This chemical feature diminished efficacy of various CPT derivatives in vivo compared to the spectacular results often obtained from in vitro studies and xenograft models. Thus, several promising strategies to overcome this challenge have been developed. These approaches include the development of prodrugs (conjugates and polymer bound CPTs), new formulations (liposomes or microparticulate carriers), and synthetic lipophilic CPTs.[12–14] Most of these strategies aimed to maintain the active closed-lactone form in the plasma compartment, and encouraging results have been obtained. Additionally, extensive structure–activity relationship (SAR) investigations have suggested that the intact lactone ring E of CPT is the most critical structural feature with respect to antitumor activity. A free 20-hydroxy group favors lactone ring-opening due to formation of intra-molecular hydrogen bonding, while acylation of this group should render the lactone moiety more stable toward ring opening.[15,16] Many studies have been focused on highly efficient semisynthetic methodologies paving the way for development of new potent C-20-modified CPT analogues. Indeed, our own results,[17–19] as well as those of others with 20(*S*)-*O*-acyl esters,[20,21] 20(*S*)-*O*-carbonate linked tripeptide conjugates,[22] and 20(*S*)-*O*-linked glycoconjugates,[23] have supported the importance of esterified CPT derivatives for potent cytotoxic and antitumor activity. Esterification of the 20-hydroxyl group also enhances plasma stability compared with unmodified CPT, as well as augments in vivo superior antitumor activity without notable toxicities in liver, lung, kidney, and spleen.[19]

Furthermore, novel nitroxide-derived spin-labeling of antitumor drugs is a promising direction in anticancer chemotherapy, not only because these compounds exhibit superior cytotoxic activity, but also because they can be monitored by electron paramagnetic resonance (EPR) in pharmacological experiments. Based

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http://dx.doi.org/10.1016/j.bmc.2014.09.035
0968-0896/

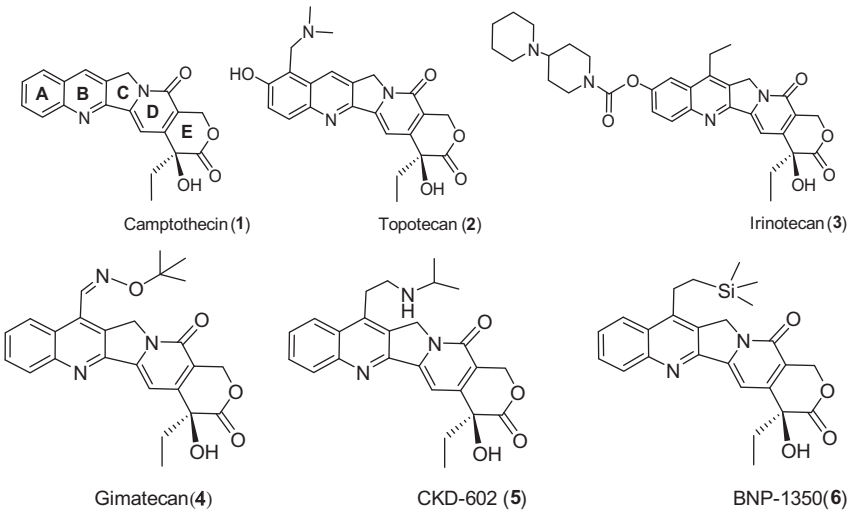

Figure 1. Structures of camptothecin (**1**), topotecan (**2**), irinotecan (**3**), gimatecan (**4**), CKD-602 (**5**), and BNP-1350 (**6**).

on current knowledge, the introduction of a stable nitroxyl radical into pharmaceutical molecules can reduce toxicity and potentiate antitumor effects to a certain degree. Some studies have shown that the introduction of a nitroxyl moiety can lead to fast decomposition, higher alkylating and lower carbamoylating activity, better anti-melanomic activity, lower general toxicity, and the ability to transport molecules through cell membranes, while the nitroxyl free radicals themselves possess low toxicity and are not mutagenic or carcinogenic.[24–28] In our prior studies, we successfully prepared a number of spin-labeled derivatives of known antitumor agents, such as podophyllotoxin,[29–36] CPT,[17] rotenone,[37] glycyrrhetinic acid,[38] and combretastatin,[39] resulting in compounds with superior pharmacological properties compared to those of the parent compounds. Inspired by this prior work, we herein report the design, synthesis, and in vitro cytotoxicity of a series of novel 20-modified spin-labeled CPT derivatives as part of our continuing search for promising natural product-derived anticancer agents.

2. Results and discussion

2.1. Chemistry

The synthetic routes to target compounds are outlined in Scheme 1. Briefly, the 20-hydroxyl of CPT was esterified with various *N*-Boc-amino acid derivatives (**7**) in suitable yields by a simple modification of the carbodiimide method using a combination of *N*,*N*′-diisopropylcarbodiimide (DIPC) and 4-dimethylaminopyridine (DMAP). Removal of the *N*-Boc group of **7** with trifluoroacetic acid (TFA) in CH_2Cl_2 (1:1) formed the key intermediate TFA salts **8**. Subsequently, these key precursors were successfully combined with sulfonylazides and alkynes in a Cu-catalyzed three-component reaction[40] to afford the target compounds **9a–9t** in 59–75% yields. All newly synthesized compounds were purified by column chromatography and their structures were characterized by ESI-MS, EPR, IR, and elemental analysis.

2.2. Cytotoxicity

Target compounds **9a–9t** were evaluated against a panel of human tumor cell lines, including A-549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (nasopharyngeal carcinoma), and KBvin (vincristine resistant KB subline), using a sulforhodamine B colorimetric (SRB) assay[41] with triplicate experiments. Topotecan (**2**) was used as a positive control and the antiproliferative activities of compounds are shown in Table 1. Except for compounds **9c** and **9l**, all target compounds exhibited significant in vitro antiproliferative activity against the four tested tumor cell lines, with IC_{50} values ranging from 0.055 to 0.84 μM, and were as or more potent than **2**. Remarkably, all of the compounds were more potent than **2** (IC_{50}, 0.40 μM) against the multidrug-resistant KBvin cell line, with **9e** (IC_{50}, 0.057 μM) and **9j** (IC_{50}, 0.072 μM) showing the greatest cytotoxicity against this cell line. Thus, spin-labeling of CPT at the C-20-hydroxyl position might overcome the MDR phenotype. As commonly found with prodrugs, esterification of the C-20-hydroxyl of CPT with different sulfonylamidine side chains led to somewhat decreased cytotoxic activity against A549, DU-145, and KB tumor cell lines in comparison to **2**.[19] This result is in agreement with our prior observations with C-20-substituted CPT derivatives as potent prodrugs.[17] 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.[20]

Furthermore, some preliminary SAR correlations were also observed for these spin-labeled 20-sulfonylamidine CPT derivatives. As shown in Scheme 1, the modified groups included R_1 on the amidine carbon, R_2 on the sulfonyl moiety, and R_3 as the nitroxide moiety. When the R_2 and R_3 substituents were kept constant and the R_1 group in the sulfonylamidine was varied, hydrogen (**9a**) and methyl (**9b**) gave the best results compared with the larger alkyl groups in **9c** (isopropyl), **9k** (isobutyl), and **9l** (*sec*-butyl), suggesting that small aliphatic chains appear to be the best R_1 substituents for greater cytotoxic potency. Moreover, when the R_1 group was fixed as hydrogen, R_3 was fixed as a piperidinyl moiety, and the R_2 group in the sulfonylamidine was varied, similar results were seen in the corresponding derivatives **9d–9j**, **9n**, and **9o**, indicating that the substituent's size is critical. Furthermore, the cytotoxicity of these compounds was distinctly correlated with the nitroxide moiety. Also, the ring size and degree of unsaturation did not obviously affect the potency of the target compounds against three (A-549, MDA-MB-231, KB) of the four tested tumor cell lines, which was consistent with the literature.[32] Overall, the results suggest that the cytotoxic potencies of our designed 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 **9e** and **9j**.