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

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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** (IC₅₀ 0.057 and 0.072 μ M, 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.

Keywords

Camptothecin; C-20 position; cytotoxic activity; spin-labeled

1. Introduction

Camptothecin (CPT, **1**, Figure 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**),

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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,^{17,18,19} 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 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,³⁷ rotenone,³⁸ glycyrrhetic acid,³⁹ and combretastatin,⁴⁰ 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 CH₂Cl₂ (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 reaction⁴¹ to afford the target compounds **9a–t** 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–t** 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 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 IC₅₀ 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** (IC₅₀, 0.40 μM) against the multidrug-resistant KBvin cell line, with **9e** (IC₅₀, 0.057 μM) and **9j** (IC₅₀, 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.³⁷ The IC₅₀ 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 R₁ on the amidine carbon, R₂ on the sulfonyl moiety, and R₃ as the nitroxide moiety. When the R₂ and R₃ substituents were kept constant and the R₁ 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 R₁ substituents for greater cytotoxic potency. Moreover, when the R₁ group was fixed as hydrogen, R₃ was fixed as a piperidinyll moiety, and the R₂ group in the sulfonylamidine was varied, similar results were seen in the corresponding derivatives **9d–j**, **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**.

3. Conclusion

In summary, application of free radical compounds of the stable nitroxide type has become increasingly frequent in medicinal chemistry studies during recent years. In particular, design and synthesis of spin-labeled antitumor drugs using nitroxide groups as biological response modifiers have elicited widespread interest in cancer chemotherapy. As an extension to our studies on spin-labeled antitumor drugs, we designed and synthesized a series of novel 20-modified spin-labeled CPT derivatives, which were then evaluated for antiproliferative activities against four tumor cell lines (A-549, MDA-MB-231, KB, KBvin) by using a sulforhodamine B colorimetric assay. The cytotoxic results showed that most of the new spin-labeled compounds exhibited significant antiproliferative activities against four tumor cell lines. Notably, except for compounds **9c**, **9k**, and **9l**, all compounds were more potent than **2** against multidrug-resistant KBvin cells. 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 CPT to develop potential spin-labeled CPT-derived anticancer drug candidates. Continuing studies to substantiate and improve activity profiles are underway in our laboratory and will be reported in due course.

4. Experimental section

4.1. General

All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd.). Melting points were taken on a Kofler melting point apparatus and are uncorrected. IR spectra were measured on a Nicolet 380 FT-IR spectrometer on neat samples placed between KBr plates. The EPR spectra were obtained with a Bruker A300 X-band EPR spectrometer. MS analyses were performed on ZAB-HS and Bruker Daltonics APEXII49e instruments. The starting CPT was isolated from the Chinese medicinal plant *C. acuminata*, and was purified before being used.³ The intermediate CPT-20-esters of *N*-Boc-amino acid derivatives **7** and their TFA salts **8** were synthesized according to our previous procedures.³⁷

4.2. General synthetic procedure for compounds **9a–9t**

Triethylamine (1.2 mmol) was added slowly to a suspension of the various TFA salts **8** (0.5 mmol) in CH₂Cl₂ (35mL), and this mixture was stirred for 10 min when a clear solution was obtained. Under an N₂ atmosphere, alkyne (0.5 mmol), sulfonylazide (0.6 mmol), and CuI

(0.05 mmol) were added into this reaction mixture at room temperature. After the reaction was completed, as monitored by TLC, the reaction mixture was diluted by adding 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 flash column chromatography with an appropriate eluting solvent system.

Compound **9a**. Yield 70%; yellow orange solid; mp 133–134 °C; IR (KBr) ν (cm⁻¹): 3432, 2978, 2939, 1753, 1664, 1618, 1560, 1449, 1402, 1385 (NO⁻), 1273, 1147, 1094, 881, 761, 669, 616, 556; Anal. Calcd for C₄₁H₄₆N₅O₉S: C, 62.74; H, 5.91; N, 8.92. Found: C, 62.70; H, 6.01; N, 8.87; EPR: $g_0=2.0064$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 785.3 [M+H]⁺.

Compound **9b**. Yield 67%; yellow orange solid; mp 118–121 °C; IR (KBr) ν (cm⁻¹): 3433, 2977, 2938, 1752, 1664, 1618, 1545, 1458, 1403, 1384 (NO⁻), 1273, 1145, 1090, 881, 787, 724, 616, 555; Anal. Calcd for C₄₂H₄₈N₅O₉S: C, 63.14; H, 6.06; N, 8.77. Found: C, 63.10; H, 6.01; N, 8.90; EPR: $g_0=2.0060$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 800.2 [M+2H]⁺.

Compound **9c**. Yield 71%; yellow orange solid; mp 128–130 °C; IR (KBr) ν (cm⁻¹): 3448, 2975, 2930, 2873, 1751, 1666, 1618, 1561, 1546, 1439, 1402, 1385 (NO⁻), 1272, 1145, 1091, 881, 670, 616, 555; Anal. Calcd for C₄₄H₅₂N₅O₉S: C, 63.90; H, 6.34; N, 8.47. Found: C, 64.03; H, 6.31; N, 8.56; EPR: $g_0=2.0061$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 828.3 [M+2H]⁺.

Compound **9d**. Yield 70%; yellow orange solid; mp 125–127 °C; IR (KBr) ν (cm⁻¹): 3448, 2974, 2929, 1752, 1655, 1618, 1560, 1508, 1402, 1385 (NO⁻) 1272, 1122, 881, 788, 670, 617, 474; Anal. Calcd for C₃₅H₄₂N₅O₉S: C, 59.31; H, 5.97; N, 9.88. Found: C, 59.43; H, 6.01; N, 8.79; EPR: $g_0=2.0066$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 731.3 [M+Na]⁺.

Compound **9e**. Yield 64%; yellow orange solid; mp 124–126 °C; IR (KBr) ν (cm⁻¹): 3431, 2974, 2929, 1753, 1655, 1618, 1561, 1508, 1439, 1403, 1384 (NO⁻), 1270, 1233, 1118, 881, 787, 758, 616, 475; Anal. Calcd for C₃₆H₄₄N₅O₉S: C, 59.82; H, 6.14; N, 9.69. Found: C, 59.73; H, 6.08; N, 9.58; EPR: $g_0=2.0064$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 745.4 [M+Na]⁺.

Compound **9f**. Yield 66%; yellow orange solid; mp 123–125 °C; IR (KBr) ν (cm⁻¹): 3423, 2973, 2926, 2870, 1758, 1663, 1618, 1561, 1458, 1404, 1384 (NO⁻), 1267, 1234, 1181, 1119, 881, 788, 763, 724, 669, 616; Anal. Calcd for C₃₈H₄₈N₅O₉S: C, 60.78; H, 6.44; N, 9.33. Found: C, 60.89; H, 6.38; N, 9.50; EPR: $g_0=2.0060$, An=14.62Gs (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 773.4 [M+Na]⁺.

Compound **9g**. Yield 68%; yellow orange solid; mp 142–144 °C; IR (KBr) ν (cm⁻¹): 3423, 2975, 2931, 1753, 1664, 1618, 1560, 1499, 1458, 1403, 1384 (NO⁻), 1257, 1146, 1094, 881, 854, 805, 762, 724, 696, 614, 555; Anal. Calcd for C₄₁H₄₆N₅O₁₀S: C, 61.49; H, 5.79; N,

8.74. Found: C, 61.54; H, 5.86; N, 8.67; EPR: $g_0=2.0062$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 823.2 $[\text{M}+\text{Na}]^+$.

Compound **9h**. Yield 73%; yellow orange solid; mp 144–146 °C; IR (KBr) ν (cm^{-1}): 3423, 2975, 2934, 1753, 1666, 1618, 1561, 1508, 1458, 1402, 1385 (NO^-), 1272, 1234, 1149, 1095, 881, 762, 726, 618, 483; Anal. Calcd for $\text{C}_{40}\text{H}_{43}\text{ClN}_5\text{O}_9\text{S}$: C, 59.66; H, 5.38; N, 8.70. Found: C, 59.70; H, 5.45; N, 8.57; EPR: $g_0=2.0064$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 806.3 $[\text{M}+2\text{H}]^+$.

Compound **9i**. Yield 64%; yellow orange solid; mp 105–107 °C; IR (KBr) ν (cm^{-1}): 3422, 2975, 2930, 2871, 1753, 1663, 1593, 1560, 1496, 1458, 1403, 1383 (NO^-), 1272, 1236, 1147, 1091, 1050, 880, 839, 764, 669, 616, 555; Anal. Calcd for $\text{C}_{40}\text{H}_{43}\text{FN}_5\text{O}_9\text{S}$: C, 60.90; H, 5.49; N, 8.88. Found: C, 61.01; H, 5.55; N, 8.49; EPR: $g_0=2.0066$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 790.3 $[\text{M}+2\text{H}]^+$.

Compound **9j**. Yield 68%; yellow orange solid; mp 135–137 °C; IR (KBr) ν (cm^{-1}): 3393, 2973, 2901, 1757, 1663, 1606, 1560, 1529, 1450, 1404, 1384 (NO^-), 1351, 1223, 1150, 1081, 1050, 880, 747, 617, 464; Anal. Calcd for $\text{C}_{40}\text{H}_{43}\text{N}_6\text{O}_{11}\text{S}$: C, 58.89; H, 5.31; N, 10.30. Found: C, 58.94; H, 5.45; N, 10.25; EPR: $g_0=2.0060$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 817.4 $[\text{M}+2\text{H}]^+$.

Compound **9k**. Yield 62%; yellow orange solid; mp 120–122 °C; IR (KBr) ν (cm^{-1}): 3385, 2973, 2900, 1750, 1663, 1618, 1549, 1452, 1404, 1384 (NO^-), 1272, 1234, 1146, 1087, 1050, 880, 814, 763, 689, 618, 556; Anal. Calcd for $\text{C}_{45}\text{H}_{54}\text{N}_5\text{O}_9\text{S}$: C, 64.27; H, 6.47; N, 8.33. Found: C, 64.38; H, 6.56; N, 8.21; EPR: $g_0=2.0062$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 842.4 $[\text{M}+2\text{H}]^+$.

Compound **9l**. Yield 70%; yellow orange solid; mp 122–124 °C; IR (KBr) ν (cm^{-1}): 3401, 2973, 2935, 2873, 1751, 1664, 1604, 1545, 1458, 1403, 1384 (NO^-), 1273, 1147, 1091, 1051, 881, 815, 669, 617, 556; Anal. Calcd for $\text{C}_{45}\text{H}_{54}\text{N}_5\text{O}_9\text{S}$: C, 64.27; H, 6.47; N, 8.33. Found: C, 64.38; H, 6.56; N, 8.21; EPR: $g_0=2.0064$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 842.4 $[\text{M}+2\text{H}]^+$.

Compound **9m**. Yield 68%; yellow orange solid; mp 112–114 °C; IR (KBr) ν (cm^{-1}): 3402, 2974, 2901, 1751, 1655, 1618, 1560, 1544, 1450, 1405, 1384 (NO^-), 1250, 1079, 1066, 1050, 881, 701, 617; Anal. Calcd for $\text{C}_{48}\text{H}_{52}\text{N}_5\text{O}_9\text{S}$: C, 65.89; H, 5.99; N, 8.00. Found: C, 65.98; H, 6.12; N, 8.06; EPR: $g_0=2.0066$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 876.4 $[\text{M}+2\text{H}]^+$.

Compound **9n**. Yield 59%; yellow orange solid; mp 141–143 °C; IR (KBr) ν (cm^{-1}): 3411, 2974, 2932, 1754, 1662, 1604, 1560, 1458, 1403, 1384 (NO^-), 1272, 1236, 1127, 1082, 1050, 880, 787, 724, 688, 619, 555, 480; Anal. Calcd for $\text{C}_{44}\text{H}_{46}\text{N}_5\text{O}_9\text{S}$: C, 64.37; H, 5.65; N, 8.53. Found: C, 64.30; H, 5.54; N, 8.46; EPR: $g_0=2.0062$, $A_n=14.62\text{Gs}$ (triplet peak in $1\times 10^{-4}\text{ M}$, DMF); MS-ESI m/z : 822.4 $[\text{M}+2\text{H}]^+$.

Compound **9o**. Yield 67%; yellow orange solid; mp 136–138 °C; IR (KBr) ν (cm^{-1}): 3423, 2975, 2938, 1753, 1663, 1617, 1560, 1458, 1404, 1384 (NO^-), 1292, 1234, 1143, 1093,

1015, 881, 762, 724, 669, 618, 592; Anal. Calcd for $C_{38}H_{42}N_5O_9S_2$: C, 58.75; H, 5.45; N, 9.01. Found: C, 58.65; H, 5.38; N, 9.21; EPR: $g_0=2.0066$, $An=14.62Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 878.3 $[M+2H]^+$.

Compound **9p**. Yield 75%; yellow orange solid; mp 105–107 °C; IR (KBr) ν (cm^{-1}): 3423, 2974, 2928, 1756, 1663, 1601, 1560, 1457, 1403, 1384 (NO $\cdot$), 1273, 1236, 1177, 1147, 1094, 1050, 920, 881, 818, 763, 695, 615, 556, 480; Anal. Calcd for $C_{42}H_{48}N_5O_9S$: C, 63.14; H, 6.06; N, 8.77. Found: C, 63.04; H, 6.19; N, 8.92; EPR: $g_0=2.0060$, $An=14.62Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 800.4 $[M+2H]^+$.

Compound **9q**. Yield 65%; light yellow solid; mp 121–123 °C; IR (KBr) ν (cm^{-1}): 3415, 2973, 2931, 2871, 1757, 1663, 1603, 1561, 1442, 1402, 1384 (NO $\cdot$), 1256, 1190, 1120, 1084, 1049, 977, 881, 787, 724, 613, 562, 475; Anal. Calcd for $C_{35}H_{40}N_5O_9S$: C, 59.48; H, 5.70; N, 9.91. Found: C, 59.56; H, 5.63; N, 10.02; EPR: $g_0=2.0058$, $An=14.76Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 729.3 $[M+Na]^+$;

Compound **9r**. Yield 61%; light yellow solid; mp 110–112 °C; IR (KBr) ν (cm^{-1}): 3422, 2974, 2929, 1752, 1655, 1603, 1560, 1458, 1439, 1384 (NO $\cdot$), 1273, 1147, 1093, 1050, 881, 724, 695, 616, 556; Anal. Calcd for $C_{41}H_{44}N_5O_9S$: C, 62.90; H, 5.66; N, 8.95. Found: C, 63.00; H, 5.53; N, 9.01; EPR: $g_0=2.0055$, $An=14.76Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 805.4 $[M+Na]^+$.

Compound **9s**. Yield 70%; light yellow solid; mp 120–122 °C; IR (KBr) ν (cm^{-1}): 3415, 2974, 2929, 1756, 1664, 1598, 1560, 1499, 1458, 1403, 1384 (NO $\cdot$), 1258, 1146, 1094, 1051, 881, 836, 805, 725, 697, 614, 567; Anal. Calcd for $C_{41}H_{44}N_5O_{10}S$: C, 61.64; H, 5.55; N, 8.77. Found: C, 61.81; H, 5.42; N, 8.85; EPR: $g_0=2.0058$, $An=14.76Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 800.3 $[M+2H]^+$.

Compound **9t**. Yield 69%; light yellow solid; mp 103–105 °C; IR (KBr) ν (cm^{-1}): 3423, 2974, 2927, 1753, 1663, 1592, 1560, 1495, 1458, 1403, 1384 (NO $\cdot$), 1279, 1234, 1186, 1148, 1091, 880, 839, 763, 724, 699, 613, 554, 481; Anal. Calcd for $C_{40}H_{41}FN_5O_9S$: C, 61.06; H, 5.25; N, 8.90. Found: C, 61.21; H, 5.15; N, 9.02; EPR: $g_0=2.0055$, $An=14.76Gs$ (triplet peak in 1×10^{-4} M, DMF); MS-ESI m/z : 809.4 $[M+Na]^+$.

4.3. Cytotoxicity assays

The human tumor cell lines, A549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (originally isolated from epidermoid carcinoma of the nasopharynx), and KBvin (vincristine-resistant KB subline), were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) or from ATCC (Manassas, VA), except KBvin, which was a generous gift of Professor Y.-C. Cheng (Yale University). All cell lines were maintained and assayed in RPMI-1640 medium containing 2 mM L-glutamine and 25 mM HEPES (HyClone), supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 100 $\mu g/mL$ streptomycin, 100 IU/mL penicillin, and 0.25 $\mu g/mL$ amphotericin B (Cellgro) in a humidified atmosphere containing 5% CO $_2$ in air. Cytotoxic activity was determined by the sulforhodamine B (SRB) colorimetric assay as previously described.³⁷ In brief, the cells ($4.0\text{--}7.5 \times 10^3$ cells/well) were seeded in 96-well plates filled with various concentrations of

samples in culture medium, and cultured for 72 h at 37 °C with 5% CO₂ in air. At the end of the exposure period, the surviving cells were fixed in 10% trichloroacetic acid for 30 min at room temperature followed by staining with 0.04% SRB (Sigma Chemical Co.) for 30 min. The protein-bound SRB was solubilized with 10 mM Tris-base and the absorbance of SRB was measured at 515 nm on a ELx800 Microplate Reader (Bio-Tek Instruments, Winooski, VT) operated by Gen5 software. All results were representative of three or more experiments and expressed as an average with standard deviation (SD).

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

	R ₁	R ₂	R ₃		R ₁	R ₂	R ₃
9a	H			9k	CH ₂ CH(CH ₃) ₂		
9b	CH ₃			9l	CH(CH ₃)CH ₂ CH ₃		
9c	CH(CH ₃) ₂			9m	PhCH ₂		
9d	H	CH ₃		9n	H		
9e	H	CH ₂ CH ₃		9o	H		
9f	H	CH ₂ (CH ₂) ₃		9p	H		
9g	H	H ₃ CO-		9q	H	CH ₃	
9h	H	Cl-		9r	H		
9i	H	F-		9s	H	H ₃ CO-	
9j	H	O ₂ N-		9t	H	F-	

Scheme 1.
Synthesis of target compounds **9a-9t**.

Table 1*In vitro* cytotoxicity of compounds **9a–t** against four human tumor cell lines.^a

Entry	IC ₅₀ (μ M) (Average $\pm$ SD) ^b			
	A-549	MDA-MB-231	KB	KBvin
9a	0.076 $\pm$ 0.026	0.45 $\pm$ 0.10	0.21 $\pm$ 0.016	0.11 $\pm$ 0.010
9b	0.45 $\pm$ 0.0067	0.53 $\pm$ 0.0099	0.56 $\pm$ 0.0086	0.25 $\pm$ 0.080
9c	>1	>1	>1	>1
9d	0.059 $\pm$ 0.021	0.11 $\pm$ 0.024	0.071 $\pm$ 0.030	0.090 $\pm$ 0.037
9e	0.055 $\pm$ 0.013	0.080 $\pm$ 0.0077	0.059 $\pm$ 0.0015	0.057 $\pm$ 0.014
9f	0.062 $\pm$ 0.015	0.23 $\pm$ 0.12	0.075 $\pm$ 0.011	0.095 $\pm$ 0.0094
9g	0.081 $\pm$ 0.010	0.27 $\pm$ 0.12	0.11 $\pm$ 0.0011	0.096 $\pm$ 0.0056
9h	0.063 $\pm$ 0.015	0.19 $\pm$ 0.076	0.073 $\pm$ 0.0080	0.084 $\pm$ 0.013
9i	0.086 $\pm$ 0.0065	0.27 $\pm$ 0.090	0.12 $\pm$ 0.028	0.10 $\pm$ 0.0035
9j	0.058 $\pm$ 0.012	0.10 $\pm$ 0.0024	0.059 $\pm$ 0.020	0.072 $\pm$ 0.014
9k	0.61 $\pm$ 0.0057	0.84 $\pm$ 0.0016	0.70 $\pm$ 0.021	0.74 $\pm$ 0.031
9l	>1	>1	>1	>1
9m	0.17 $\pm$ 0.087	0.50 $\pm$ 0.097	0.31 $\pm$ 0.18	0.10 $\pm$ 0.0016
9n	0.48 $\pm$ 0.022	0.61 $\pm$ 0.0066	0.58 $\pm$ 0.013	0.33 $\pm$ 0.066
9o	0.063 $\pm$ 0.0021	0.11 $\pm$ 0.010	0.063 $\pm$ 0.026	0.083 $\pm$ 0.022
9p	0.062 $\pm$ 0.0038	0.091 $\pm$ 0.0025	0.080 $\pm$ 0.020	0.084 $\pm$ 0.011
9q	0.0629 $\pm$ 0.0061	0.1027 $\pm$ 0.0027	0.0823 $\pm$ 0.0327	0.0834 $\pm$ 0.0207
9r	0.074 $\pm$ 0.0054	0.12 $\pm$ 0.0029	0.091 $\pm$ 0.013	0.094 $\pm$ 0.0021
9s	0.090 $\pm$ 0.0040	0.18 $\pm$ 0.0013	0.13 $\pm$ 0.038	0.15 $\pm$ 0.0059
9t	0.077 $\pm$ 0.0006	0.14 $\pm$ 0.0033	0.12 $\pm$ 0.026	0.095 $\pm$ 0.0013
2	0.045 $\pm$ 0.0004	0.10 $\pm$ 0.0055	0.063 $\pm$ 0.0042	0.40 $\pm$ 0.021

^a A549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), KB (originally isolated from epidermoid carcinoma of the nasopharynx), KBvin (vincristine-resistant KB subline).

^b Each assay was performed in triplicate with duplicated sample, and averaged IC₅₀ (μ M) values were expressed with standard deviation (SD).