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Design and one-pot synthesis of new 7-acyl camptothecin derivatives as potent cytotoxic agents

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ABSTRACT

New 7-acyl camptothecin derivatives were designed and synthesized from camptothecin in a one-pot reaction through a Minisci type-reaction and were evaluated for cytotoxicity against four tumor cell lines, A-549, DU-145, KB, and KB-vin. All of the new compounds showed significant inhibition of human tumor cell growth, with IC_{50} values ranging from 0.01538 to 13.342 μM. Most of the derivatives were more cytotoxic than irinotecan, and the (**7a**) and 7-propionyl (**7b**) analogs exhibited the highest cytotoxic activity against the tumor cell lines tested. This compound class merits further development as anticancer clinical trial candidates.

Camptothecin (CPT, **1**, Fig. 1), a cytotoxic natural alkaloid isolated from *Camptotheca acuminata*, acts as a selective poison of the nuclear enzyme topoisomerase I by forming a ternary complex with topoisomerase I and DNA.[1–4] Elucidation of the mechanism and extensive structural modifications led to the successful identification and development of the antitumor agents topotecan (**2**) and irinotecan (**3**), as well as other CPT analogs that are currently in clinical trials.[5,6]

From structure-activity relationship (SAR) investigations, modifications at the 7- or 10-position or both appear to be the most efficient approach to increase the antitumor potency.[7] Many studies[8–11] have shown that appropriate substitutions at either or both of these positions can decrease or abolish the high-affinity binding of the inactive 'carboxylate form' of **1**'s E-ring to human serum albumin (HSA), and thus, shift the hydrolysis equilibrium in human blood to favor the active 'lactone-closed form'. Furthermore, a binding model of **1** with biological macromolecules indicated that the 7-position can accommodate various substituents without steric problems.[12–14] Therefore, most structural modifications of **1** have focused on positions 7 and 10. In particular, various substituents, such as ethyl, alkylsilyl, oxyiminoalkyl, and alkylsilylalkyl, were introduced at the 7-position of **1** to provide potent antitumor agents. Indeed, most of the second-generation **1**-derivatives that have currently reached preclinical or clinical development studies are 7-substituted compounds, for example, gimatecan (**4**)[15], CKD-602 (**5**),[16] and BNP-1350 (**6**),[17] which all bear highly lipophilic substituents intended to increase lactone stability and antitumor activity. These successful examples imply that C-7 substitution plays an important role in the activity profiles of **1**-analogs and that optimization of this compound class through rational C-7 modification is quite feasible. On this basis, we designed a series of new **1**-derivatives with different substituents at position-7. Herein, we report an efficient one-pot synthesis of the new 7-acyl **1**-derivatives as potent cytotoxic agents.

As shown in Scheme 1, the new 7-acyl camptothecin derivatives (**7a–l**) were obtained by Minisci free radical acylation of **1** with the appropriate aldehydes in sulfuric acid in the presence of *t*-BuOOH and ferrous sulfate. The structures of the target molecules were characterized through ^{1}H NMR, ^{13}C NMR, IR and HR-MS analyses.

Target compounds **7a–l** were evaluated for in vitro cytotoxic activity against four different tumor cell lines, KB (nasopharyngeal), A549 (lung), DU-145 (prostate), and KBvin (an MDR KB subline), using a sulforhodamine B colorimetric assay,[18] in triplicate experiments, with **1** and **3** used as positive controls. The screening results are shown in Table 1. All of the new compounds exhibited significant in vitro cytotoxic activity against the four tested tumor cell lines, with IC_{50} values ranging from 0.01538 to 13.342 μM, and were as or more potent than **3**. Among the new derivatives, **7a** and **7b**, with 7-acetyl and 7-propionyl groups, respectively, exhibited

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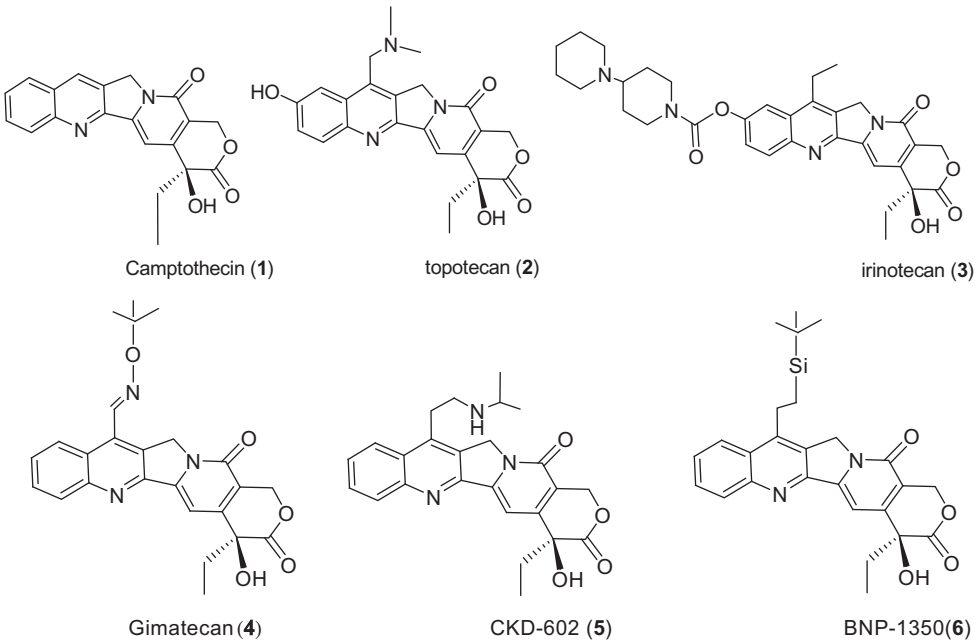
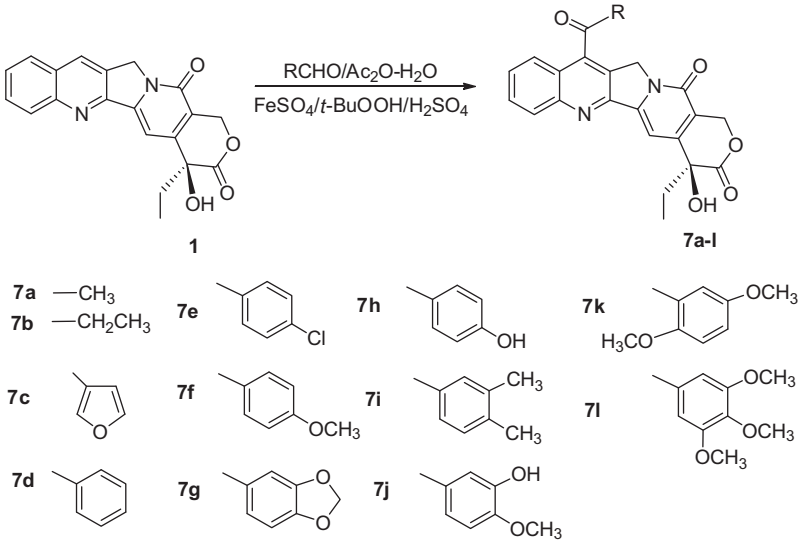


Figure 1. Structures of camptothecin (**1**), topotecan (**2**), irinotecan (**3**), gimatecan (**4**), CKD-602 (**5**), and BNP-1350 (**6**).



Scheme 1. Synthesis of target compounds **7a–l**.

the highest potency. However, except for **7a** and **7b**, the new 7-acyl derivatives showed substantially reduced cytotoxic potency relative to **1**, which exhibited remarkable cytotoxic effects against the four tumor cell lines. The 7-carbonyl would provide an additional hydrogen bond receptor and affect the conjugation in the molecule. Either of these effects could influence the binding with the enzyme and might result in reduced activity. An alternate explanation for the reduced activity is steric hindrance resulting from the more rigid and larger aromatic systems in **7c**–**7l**.

To assist in further identification of more efficient 7-acyl **1**-derivatives, preliminary SAR correlations were formulated. As the length of the 7-acyl group increased from acetyl to propionyl with **7a** and **7b**, the corresponding cytotoxic activity was generally reduced. For example, the IC_{50} value of compound **7a** was 0.0402 μM against the DU-145 cancer cell line, while that of compound **7b** was 0.0898 μM. In addition, the 7-alkylcarbonyl substituted derivatives (compounds **7a** and **7b**) were more potent than the 7-arylcarbonyl substituted compounds (compounds **7c–7l**) against all tested tumor cell lines. This result indicated that an aryl group in the 7-acyl moiety is much less favorable than a short aliphatic group. The conjugation of the aromatic group with the carbonyl group might disturb the electronic properties of ring B to a greater extent than alkyl groups. For those analogs bearing a 7-benzoyl group (compounds **7d**–**7l**), substituents on the aromatic ring resulted in substantially increased cytotoxic activity compared to the unsubstituted derivative **7d**. This effect was generally independent of the number, electronic, and steric characteristics of the groups, particularly against the A-549 cancer cell line. Compounds **7e**, **7f**, and **7h**–**7l**, with 4-chloro, 4-methoxy, 3,4-methylenedioxy, 3,4-dimethyl, 3-hydroxy-4-methoxy, 2,5-dimethoxy,

Table 1
In vitro cytotoxicity results for **7a**–**7l** against four cancer cell lines

Cmpd	IC_{50}(μM)			
	A-549	DU-145	KB	KB-vin
7a	0.0154 ± 0.0002	0.0402 ± 0.0056	0.0233 ± 0.0038	0.131 ± 0.156
7b	0.0317 ± 0.0146	0.0898± 0.0049	0.0592 ± 0.0059	0.0287 ± 0.0032
7c	2.02 ± 0.2037	2.01 ± 0.146	2.73 ± 0.145	1.80 ± 0.181
7d	11.0 ± 0.952	11.0 ± 0.0422	13.3 ± 0.563	11.9 ± 0.527
7e	0.949 ± 0.0366	1.68 ± 0.0786	3.07 ± 0.391	1.12 ± 0.0345
7f	0.911 ± 0.0502	1.61 ± 0.0302	4.78 ± 0.318	3.14 ± 0.278
7g	1.13 ± 0.0054	2.60 ± 0.192	11.8 ± 0.530	1.77 ± 0.150
7h	2.51 ± 0.268	2.99 ± 0.399	12.5± 0.141	1.79 ± 0.0192
7i	1.00 ± 0.0616	1.70 ± 0.0618	2.49 ± 0.0275	4.620 ± 0.268
7j	1.02 ± 0.0295	1.543 ± 0.0644	1.73 ± 0.0214	1.14 ± 0.106
7k	1.01 ± 0.0707	2.061 ± 0.155	2.92 ± 0.200	2.25 ± 0.257
7l	1.00 ± 0.042	2.11 ± 0.202	3.14 ± 0.0715	1.17 ± 0.0183
1	0.0158 ± 0.0005	0.0287 ± 0.0025	0.121 ± 0.0091	0.0371 ± 0.0031
3	9.48 ± 0.106	9.30 ± 0.613	9.83 ± 0.481	>20

and 3,4,5-trimethoxy substitution, showed similar cytotoxicity against A-549 cells. Compound **7h**, with 4-hydroxy substitution, was somewhat less potent than most of the 7-substituted benzoyl derivative, except against the KB-vin cell line. These results indicated that the electronegativity of the substituted groups and their positions on the phenyl ring affected the cytotoxicity only slightly. Consequently, we plan to synthesize additional 7-acyl derivatives of **1** based on our preliminary results with the current 7-acyl compounds in order to produce compounds with greater potency.

In summary, 12 new 7-acyl camptothecin derivatives (**7a**–**l**) were designed, synthesized, and evaluated for cytotoxicity against four tumor cell lines (A-549, DU-145, KB, and KB-vin) in a sulforhodamine B colorimetric assay. Several derivatives, particularly compounds **7a** and **7b**, showed impressive cytotoxicity compared with irinotecan. Certain variations in the acyl groups at the 7-position of **1** markedly affected the activity profiles of this compound class. Interestingly, with the two most potent compounds, **7a** showed a five-fold decrease in potency against KB-vin (drug-resistant) compared with the KB (normal) cell line, while **7b** showed a two-fold increase in potency against the same two cell lines. Overall, important SAR information has been achieved that will assist the design and development of new **1**-derivatives as cytotoxic agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.002.

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