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Semi-synthesis and biological activity of γ-lactones analogs of camptothecin

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ABSTRACT

A series of E-ring γ-lactone camptothecin derivatives were synthesized by semi-synthesis via a three-step domino reaction. Their biological activity was evaluated on two types of human tumor cell lines A549 and HT-29 with sulforhodamine-B (SRB) method. The antitumor activity of these compounds was lower than SN-38, only compound **12c** was found to be close to the activity of Topotecan. The structure–activity relationship (SAR) of these analogs was studied and discussed.

Camptothecin (CPT, **1**, Fig. 1),[1] an alkaloid firstly isolated by Wall and Wani in 1966 from Chinese tree *Camptotheca acuminata*, is one of the most famous lead compounds of antitumor agents. Attributed to the discovery of being a selective inhibitor against Topoisomerase I (Topo I) in the late 1980s,[2] the family of CPT derivatives has been enlarged rapidly during the last two decades. Among these thousands of CPT analogs, Topotecan[3] and Irinotecan[4] have been approved as chemotherapeutic drugs in clinical treatment on human cancers, and several other CPT derivatives are in different phases of clinical trials.[5]

In early research, a number of E-ring modified CPT analogs, including ring opened hydroxyl acid **2a** or its sodium salt **2b**,[6] CPT lactol **3**,[7] lactam **4a**, thiolactone **4b**,[8] α-halo, α-azide, α-amino-lactone **5a–c**[9,10] and α-hydroxy ketoether **6**[11] were reported. All these compounds were found to be either inactive or less active than CPT, and once led to a conclusion that the intact six-membered α-hydroxy lactone E-ring of CPT was indispensable for antitumor activity.[12]

However, further investigations demonstrated that many other E-ring modified CPT analogs, such as expanded seven-membered β-hydroxy lactone homo-camptothecin (hCPT) **7**[13], opened lactone form hydroxy-amide **8a** and ester-amide **8b**, opened lactone hydroxy-amide with conjugation of polyethylene glycol (PEG) through the C-17 hydroxyl **8c** as well as through C-21 carboxylic acid **8d**, were proved to have comparable or even better antitumor activity than CPT.[6b,14,15]

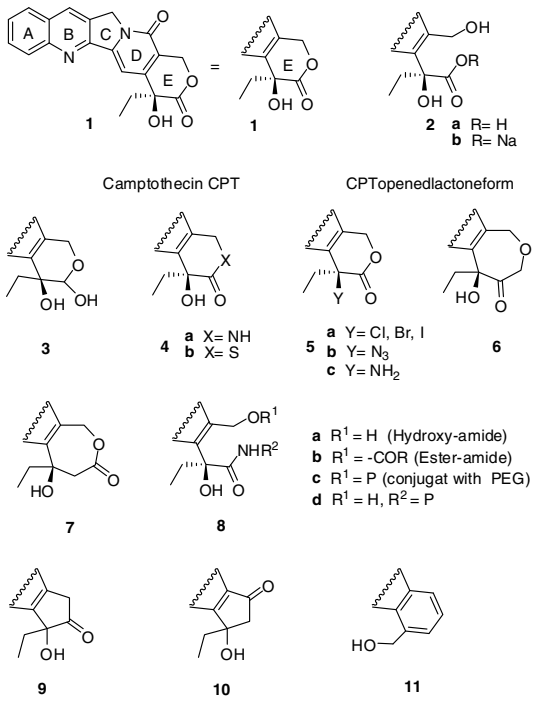

Figure 1. Structures of camptothecin and some analogs.

Recently, some lactone-free CPT analogs with five-membered ketone E-ring **9** and **10** were reported. Compound **9** revealed to

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be a potent inhibitor of Topoisomerase I and showed interesting cytotoxic activitie.[16] Another lactone-free CPT analog, 22-hydroxyacuminatine **11**, a CPT structurally-like compound with a substituted six-membered aromatic system as E-ring, was also found to have significant cytotoxic activity in vitro on the murine leukemia P-388 and KB.[17]

These observations raise the possibility that novel E-ring modified analogs of CPT might retain or even enhance antitumor activity. To study this possibility, we synthesized a series of analogs bearing a five-membered lactone E-ring with branching *N*-substituted amide at γ-position (compound **12**, Fig. 2) by semi-synthesis from CPT. We hoped that these compounds could maintain or enhance the cytotoxic activity.

To increase the aqueous solubility and enhance the activity, several *N*-substituted amides (substituted with terminal hydroxyl ethyl or propyl and terminal tertiary amino ethyl) analogs **16a–b** and **19a–n** were prepared and studied, since in our previous work, *N*-propyl morpholine and other terminal tertiary amino groups were once introduced to a series of hydroxy-amides and proved to remarkably improve the activity as well as water-solubility.[15]

The synthesis of lactones **12a–i**, **16a–b** and **17** is summarized in Scheme 1. CPTs[18] reacted with amine to open the lactone E-ring to give hydroxy-amide **13**, which was unstable in solvents and would slowly close back to lactone at neutral or acidic condition as reported before,[8] but stable as solid after being precipitated out with ether or petroleum ether. Compound **13** was then converted to the five-membered lactone **12** in the presence of pyridinium dichromate (PDC) in dichloromethane at room temperature. Similar work was once carried out by Hertzberg and his co-workers.[8] They used manganese dioxide as oxidizer to give imide in low yield. According to ESI-MS, IR ^{1}H and ^{13}C NMR spectra and compared to that of imide, product was identified to be γ-amide lactone **12**.[19] We deduced that this process underwent a three-step tandem reaction involving an oxidation in aldehyde of **13** following by an intramolecular acetalisation to give semi-acetal **15**,[20] which in turn, was immediately oxidized in the presence of PDC to afford lactone **12** (Scheme 1).

The same strategy failed to prepare a more hydrosoluble compound **12i** from **13i** (Scheme 1) with a terminal tertiary amine on amide substituent. In this case, the presence of tertiary amine group, also susceptible to the oxidation, can explain the formation of a complex mixture. To avoid this problem a different sequence was applied. Thus, we firstly converted **12f** to the corresponding aldehyde **18** which was then transformed to the desired amine **19** by reductive amination, in the presence of sodium triacetoxyborohydride. A series of analogs of compound **19a–n** were then prepared as described in Scheme 2.

The antitumor activity of these synthetic compounds was tested on two human tumor cell lines A549 and HT-29 with SRB method using SN-38 and Topotecan as references. The result was listed in Table 1. As can be seen, the resulting five-membered lactone analogs showed lower cytotoxic activity than SN-38, with IC_{50} values higher than 1 μM.

The SAR study from this series γ-lactone compounds indicated that cycloalkyls substituted at the 7-position resulted in significantly increasing activity when the amide was substituted with

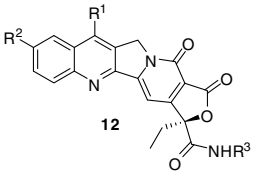
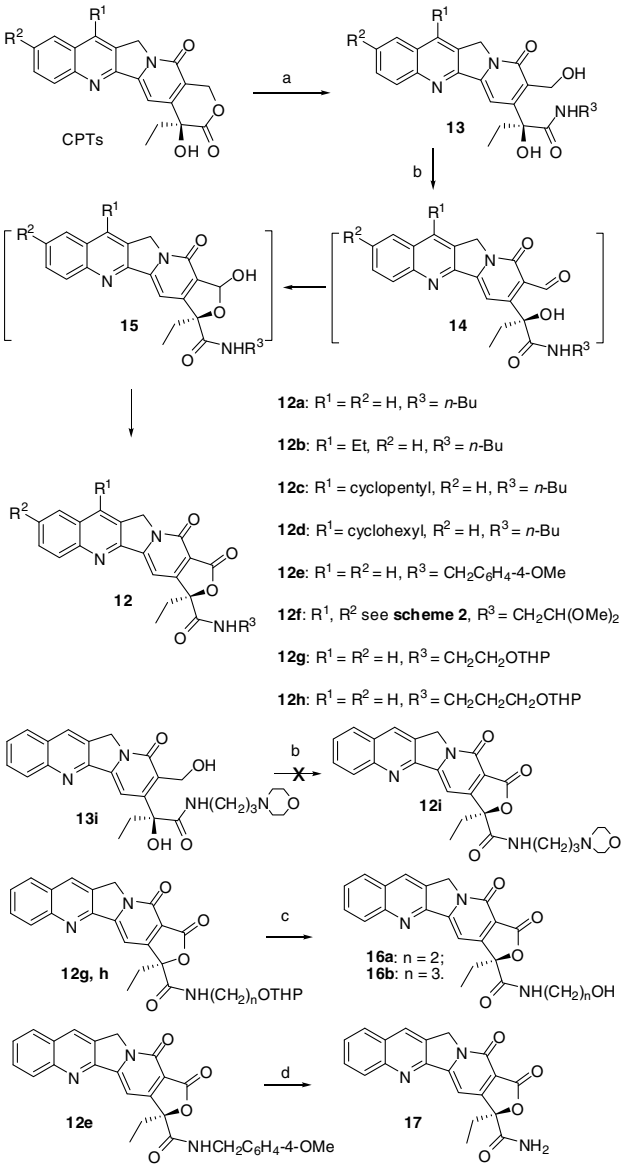


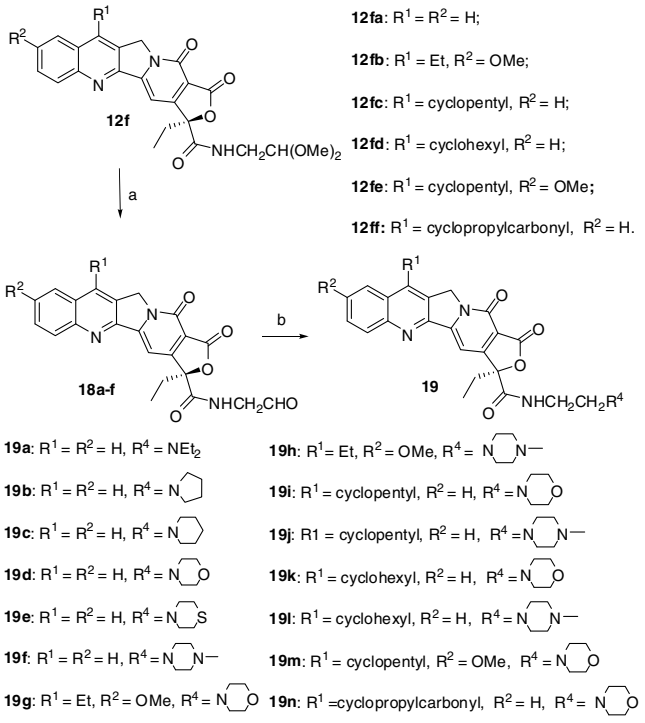
Figure 2. Structures of compounds **12**.



Scheme 1. Reagents and conditions: (a) R^3NH_2, neat, 70 °C, 0.5–1.5 h; (b) PDC, CH_2Cl_2, rt, 2 h (61–78%, two steps); (c) PPTS 15% equiv, ethanol, 60 °C, over night (89%); (d) CAN, CH_3CN:H_2O = 10/1, 20 h (65%).

N-(*n*-butyl), **12c** was the most active compound with IC_{50} value close to Topotecan. Amides **19a–n** substituted with *N*-(tertiary amino ethyl), also brought some benefits compared with the unsubstituted amide **17**. Compounds **19d** and **19e** which beared a terminal morpholinyl or 4-methylpiperazinyl showed more active than other congeners.

Several early SAR studies on AB ring modified CPT derivatives have shown that alkyl substituted at 7-position and hydroxy or methoxy substituted at 10-position of CPT would significantly enhance their activities.[21] So we combined this AB ring substituents with amide *N*-substituents and hypothesized that the activity of resulting modified compounds **19g–n** could be enhanced. However, to our disappointment, no combined positive correlation effect were observed from the results of these in vitro tests (**19g**, **19i**, **19k**, **19m**, **19n** vs **19d**; **19h**, **19j**, **19l** vs **19f**). The only exception was **19j** which was found to be slightly more active than **19f**. So it seemed that 7- and 10-position substituents in these compounds acted the opposite effect comparing to what we have learned.



Scheme 2. Reagents and conditions: (a) PPTS, acetone, refluxed for over night (76–91%); (b) R^4H, $NaBH(OAc)_3$, CH_2Cl_2, rt, 0.5–1 h (39–76%).

Table 1
Cytotoxicity activity on human tumor cell lines A549 and HT-29.

Compound	IC_{50}[a] (μM)		Compound	IC_{50}[a] (μM)	
	A549	HT-29		A549	HT-29
12a	70.1	68.3	**19d**	25.6	39.9
12b	Na	Na	**19e**	Na	Na
12c	1.28	2.03	**19f**	53.1	7.25
12d	25.5	28.1	**19g**	Na	Na
16a	Na	Na	**19h**	Na	31.6
16b	Na	Na	**19i**	Na	Na
17	Na	Na	**19j**	11.0	16.9
18c	Na	18.8	**19k**	Na	Na
19a	65.3	31.6	**19l**	75.7	31.6
19b	94.3	31.6	**19m**	96.5	62.9
19c	74.9	31.6	**19n**	Na	Na
SN-38	0.08	0.12	Topotecan	0.48	0.375

[a] Values are measured with SRB method (Na, not active up to 100 μM). Values of SN-38 and Topotecan are the average of two tests.

In summary, we have synthesized a series of five-membered-γ-amide-lactone from CPT with a key step via a domino reaction. These compounds were found to be obviously less active than SN-38 and only compound **12c** has the activity close to Topotecan. The SAR study from these compounds displayed two different situations: cycloalkyls substituted at the 7-position would significantly increase the activity when the amide was substituted with *N*-(*n*-butyl). While the amide was substituted with *N*-(2-tertiary amino ethyl) such as morpholinyl and 4-methylpiperazinyl, the substituents at 10-position or 7-position or both, became unfavorable for their antitumor activity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.074.

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18. CPTs are 7- and 10-substituted CPT analogs with R^1 and R^2 listed in compounds **12a**–**i** in Scheme 1 and **12fa**–**f** in Scheme 2. 7-Et–10-MeO–CPT was obtained from SN-38, 7-substituents were induced to CPT or 10-MeO-CPT with the method of Sawada.[3]
19. *Structure identification of compound **12a**:* (a) Data of compound **12a**: ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 0.91 (3H, t, J = 7.20 Hz), 0.99 (3H, t, J = 7.20 Hz), 1.34 (2H, m), 1.47 (2H, m), 2.15 (1H, m), 2.50 (1H, m), 3.20 (1H, m), 3.34 (1H, m), 5.37 (2H, s), 6.66 (1H, br), 7.72 (1H, t, J = 7.20 Hz), 7.82 (1H, s), 7.87 (1H, t, J = 7.20 Hz), 7.97 (1H, d, J = 7.80 Hz), 8.27 (1H, d, J = 8.40 Hz), 8.44 (1H, s); ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ 8.0, 13.7, 20.1, 31.0, 31.5, 39.6, 50.6, 87.9, 96.0, 111.1, 128.3, 128.7, 128.9, 129.8, 130.3, 131.0, 131.4, 149.3, 151.4, 153.6, 156.1, 166.4, 167.3, 167.7; IR (KBr, cm^{-1}): 1550, 1672, 1778, 3434; ESI-Ms: m/z = 418.2 (M+1). (b) The signal of ^{1}H NMR at 6.66 ppm as a broad peak (like triplet) is the proton of amide NH, which was not observed in Hertzberg's work; Infra-Red absorption band at 1778 cm^{-1} displayed that it was a lactone carbonyl, not imide carbonyl, so the product was identified as γ-amide lactone.
20. Experiments were once designed to capture intermediate **14** or **15**. Some intermediates were detected from TLC in deed, but after being purified by chromatography on silica gel, only CPT and product **12** were collected.
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