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Synthesis of novel 10-hydroxycamptothecin derivatives utilizing topotecan hydrochloride as *ortho*-quinonemethide precursor

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ABSTRACT

A series of 9-(alkylthiomethyl)-10-hydroxycamptothecins and pyrano-fused camptothecin derivatives were synthesized via the reaction of topotecan hydrochloride with various thiols and alkyl vinyl ethers respectively. In the reactions, topotecan hydrochloride was utilized as *ortho*-quinonemethide (*o*-QM) precursor. The configuration of **19** was determined by ^{1}H NMR and NOESY spectra as *syn*-isomers, suggesting that the cycloaddition of topotecan with alkyl vinyl ethers could undergo a hetero Diels–Alder reaction. All the synthesized compounds were screened on cancer cell lines HepG2, KB, HCT-8 and SGC7901. Some compounds were selected to assess their inhibitory activity against Topo I via Topo I mediated DNA cleavage assays. The results showed that among those tested 9-(alkylthiomethyl)-10-hydroxycamptothecins, the compounds with bulkier hydrophobic side chains at 9-position have better bioactivities. As well as all pyrano-fused camptothecins possess antiproliferative activity against the tested cancer cell lines. Docking studies suggested that there are more interactions between the novel analogues and the binding site of Topo I.

1. Introduction

Camptothecin (CPT, **1**, Fig. 1) is a pentacyclic alkaloid isolated from *Camptotheca acuminata* by Wall and Wani in 1966.[1] Even though this compound and its natural analogues showed prominent inhibitory activity against a broad spectrum of tumors, few relevant studies had been carried out due to their poor water solubility and severe toxicity.[2] Since the action mechanism of camptothecin as an inhibitor of topoisomerase I was discovered in 1985, this compound regained worldwide interest and intensive efforts have been devoted to design and synthesize novel camptothecin derivatives in order to overcome the disadvantages.[3a–c] Now a great number of camptothecins are undergoing investigation,[4a–e] in particular structure modifications at positions 7, 9 and 10.[5a–e] Up to date, a few semi-synthetic camptothecins such as 10-hydroxycamptothecin (HCPT, **2**),[6] Topotecan (**3**),[7a,b] Irinotecan (**4**)[8a,b] and Belotecan (**5**)[9] have been widely used in clinic for treatment against several human cancers. Among them, topotecan (**3**) is one of the water-soluble derivatives which was synthesized via Mannich reaction to introduce a dimethylaminomethyl motif at 9-position of 10-hydroxycamptothecin.[3a]

In our previous studies,[10] we found that when topotecan was dissolved in methanol, it could partially convert into 9-(methoxymethyl)-10-hydroxycamptothecin (**6**) even at room temperature. Kingsbury and co-workers[3a] had obtained 9-(ethoxylmethyl)-10-hydroxycamptothecin under similar condition, however, they did not investigate the reaction mechanism particularly. We proposed that this specific chemical behavior of topotecan (**3**) was attributed to the formation of *ortho*-quinonemethide (*o*-QM) as reaction intermediate (Fig. 2). Consequently, a series of 10-hydroxycamptothecin derivatives with different alkyloxymethyls at 9-position were obtained using similar protocol. These derivatives also exhibited similar activities as topotecan to inhibit the proliferation of several cancer cell lines in preliminary bioassays.

In order to investigate the reactivity of this intermediate in Figure 2 and discover novel camptothecin derivatives with better bioactivities and physicochemical properties, herein two kinds of reactions, including the nucleophilic addition and [4+2] cycloaddition of the *o*-QM produced from topotecan (**3**), were conducted and expanded. In this paper, owing to their stronger nucleophilicity than that of alcohols, thiols were expanded as nucleophiles to provide a series of 9-(alkylthiomethyl)-10-hydroxycamptothecins.

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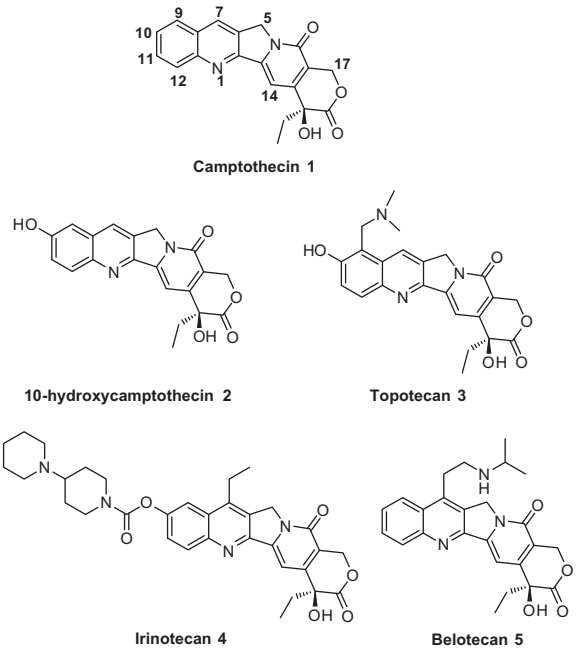


Figure 1. Structures of camptothecin and its derivatives used in clinic.

Additionally, a number of novel pyrano-fused camptothecin derivatives were prepared via [4+2] cycloaddition reaction of *o*-QM with various alkyl vinyl ethers.

2. Chemistry

2.1. Synthesis of 9-(alkylthiomethyl)-10-hydroxycamptothecins

9-(Alkylthiomethyl)-10-hydroxycamptothecins (**7**–**14**) were synthesized as described in Scheme 1. Instead of alcohols, a series of thiols, including methanethiol (prepared from 20% aqueous $NaSCH_3$ in acetic acid), ethanethiol, propane-1-thiol, *iso*-propanethiol, *tert*-butanethiol, 2-(diethylamino)ethylthiol, 3-mercaptopropanoic acid and methyl 2-mercaptoacetate were employed as nucleophiles, respectively. Considering that the protonated amino-group has better leaving ability than its neutral form, commercially available topotecan hydrochloride was chosen as *ortho*-quinonemethide precursor instead of its free alkali. After several trials, DMSO or $CHCl_3$ was used as solvent because of the poor solubility of topotecan hydrochloride in thiols. Most reactions could afford the products with acceptable yields from 25% to 50% due to the equilibrium between the substrate and the products. However, in the reactions of topotecan hydrochloride with 20% $NaSCH_3$ in acetic acid and 3-mercaptopropanoic acid, the yields were below 10%. It is presumed that the acidic medium could block the formation of quinone and reduce the nucleophilicity of the thiols. On the contrary, the yield of the reaction of 2-(diethylamino)ethanethiol achieved 67%. This is likely due to the hydrogen bond that formed between the nitrogen atom and the sulfhydryl group of this thiol, improved its nucleophilicity.

2.2. Synthesis of pyrano-fused camptothecins

As shown in Scheme 2, pyrano-fused camptothecin derivatives (**15**–**20**) were obtained through [4+2] cycloaddition reaction of *o*-QM from topotecan hydrochloride with various alkyl vinyl ethers. In these reactions, a mixed-solvent of water and DMF (3:2) was used and water could improve the leaving ability of dimethylamino-group.[11] The yields ranged from 22% to 55% depending on the size of alkyl vinyl ethers. Comparing to previous reactions, the substrate topotecan hydrochloride disappeared thoroughly rather than in reaction equilibria. Pyrano-fused derivatives were expected to be more stable in solutions because they were no longer *ortho*-quinonemethide precursors (*o*-QMPs). Under acidic condition, pyrano-fused camptothecins **15**, **16** and **17** could be hydrolyzed to provide the hemiacetal compound (**20**).

Furthermore, some other kinds of olefins, such as styrene or methyl acrylate, were also treated as dienophiles to react with topotecan hydrochloride under similar conditions. However, there were no products detected even under microwave condition, which suggested that such [4+2] cycloaddition reactions were difficult to occur for alkenes without electron-donating groups.

2.3. Configuration determination of 19

Compounds **18** and **19** are heptacyclic compounds carrying hydrogenated furo[2,3-*b*]pyran and pyrano[2,3-*b*]pyran moieties, respectively. We carried out NOESY analysis of **19** (Fig. 3A) in order to determine the configuration of α and β carbons. And this

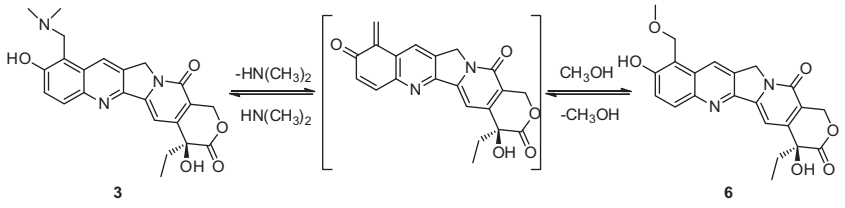
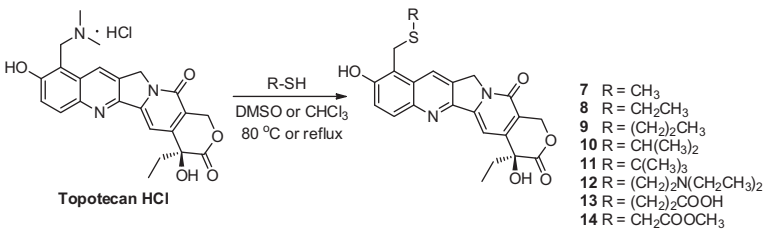


Figure 2. Formation of 9-(methoxymethyl)-10-hydroxycamptothecin via *ortho*-quinonemethide.



Scheme 1. Synthesis of 9-(alkylthiomethyl)-10-hydroxy-camptothecins (**7**–**14**).