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Cancer Chemotherapy: A SN-38 (7-Ethyl-10-hydroxycamptothecin) Glucuronide Prodrug for Treatment by a PMT (Prodrug MonoTherapy) Strategy

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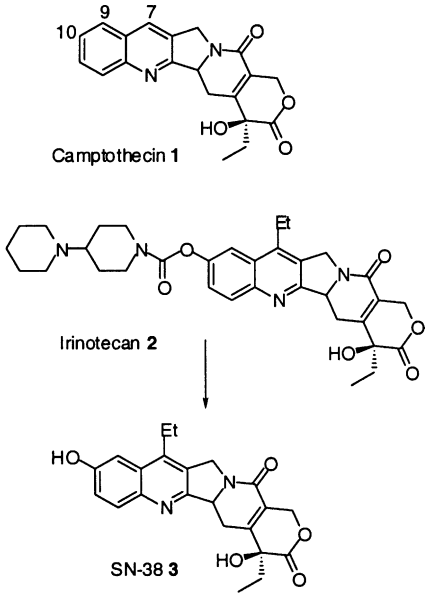
Abstract—A glucuronide-based prodrug of SN-38 (7-ethyl-10-hydroxycamptothecin) has been synthesized for use in a Prodrug MonoTherapy Strategy (PMT). Since this prodrug is significantly less cytotoxic than SN-38 itself and efficiently releases the drug in vitro in the presence of β-D-glucuronidase, it can be considered as an appropriate candidate for cancer treatment by a PMT strategy.

Introduction

20(*S*)-Camptothecin **1**, an antitumor alkaloid first isolated by Wall and co-workers in 1966,[1] inhibits the activity of topoisomerase I[2,3] and displays antitumor activity in various experimental tumor models.[4] The clinical tests of camptothecin as an anticancer agent were aborted due to its non-mechanism related toxicity and an extremely poor solubility profile. In an attempt to circumvent these problems, researchers discovered new derivatives of camptothecin. Among them, Irinotecan (CPT-11 or Campto®) **2** and Topotecan (Hycamtin®) were the first derivatives used in several indications including colon or ovarian cancer. Moreover, CPT-11 has also been shown to reduce angiogenesis.[5] Newer analogues are also in clinical development in order to find compounds with improved cytotoxicity and to solve selectivity problems.

In fact, Irinotecan[3,6–8] is a prodrug which is activated by esterases[9] to generate the more potent metabolite, SN-38 **3**, which is in turn, extensively conjugated by UDP-glucuronosyltransferase 1A1 into the inactive SN-38 glucuronide (SN-38G) (Scheme 1).



Scheme 1. Structures of compounds **1–3**.

In view of the narrow therapeutic index of CPT-11, we decided to prepare prodrugs of SN-38 able to release the active compound selectively at the tumor site. For this targeting strategy, we planned to use human β-D-glucuronidase as the enzyme for the cleavage. In normal

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tissues, this glycosidase is localized in the lysosome compartment of the cell and found at only low concentration in the blood. In contrast, it has been shown by enzyme histochemistry that it is specifically liberated into the extracellular matrix of solid tumors, mainly in necrotic areas. Based on these data, a new therapeutic procedure called Prodrug MonoTherapy[10–12] was proposed. By applying this strategy to SN-38, we expected to get enhanced solubility, sufficient hydrolytic and proteolytic stability, and overall improvement in tumor selectivity.

We report here the synthesis and in vitro biological tests of a new prodrug of SN-38. Glucuronide prodrugs of 9-aminocamptothecin were recently described.[13] As expected, these prodrugs were more soluble than 9-aminocamptothecin itself, were well detoxified and then released the drug by enzymatic hydrolysis.[14] But, to our knowledge, no prodrug of SN-38 able to liberate the active compound in a selective manner has been described.

Following a proposal from Katzenellenbogen for prodrug design,[15] self-immolative prodrugs have been proposed[16] for activation by tumor enzymes in targeting strategies. Indeed, superior enzymatic hydrolysis[17] of such prodrugs is one of the advantages of self-immolation.

Chemistry

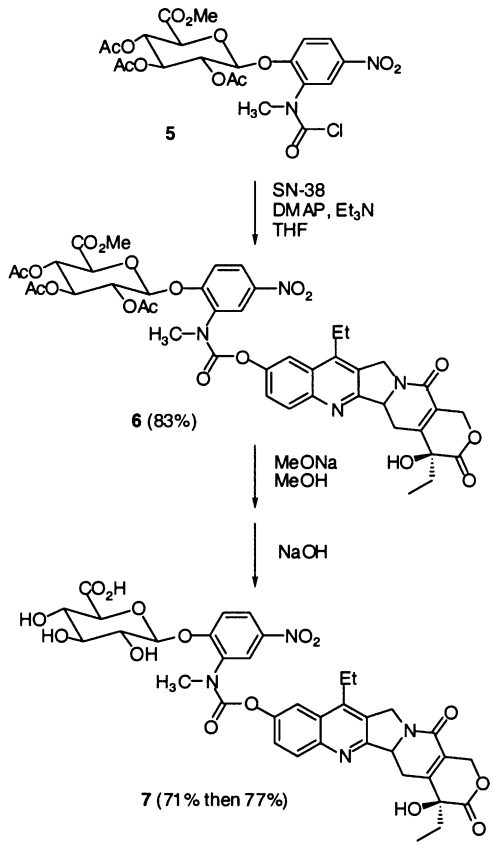
SN-38 was synthesized according to the publication of Sawada et al.[6] An ethylation of camptothecin, followed by a *N*-oxidation and a photochemical rearrangement afforded the drug **3** with a overall yield of 27%.

The glucuronic-spacer moiety **5** was obtained starting from D-glucurono-3, 6-lactone and 2-amino-4-nitrophenol, following our procedure.[18,19]

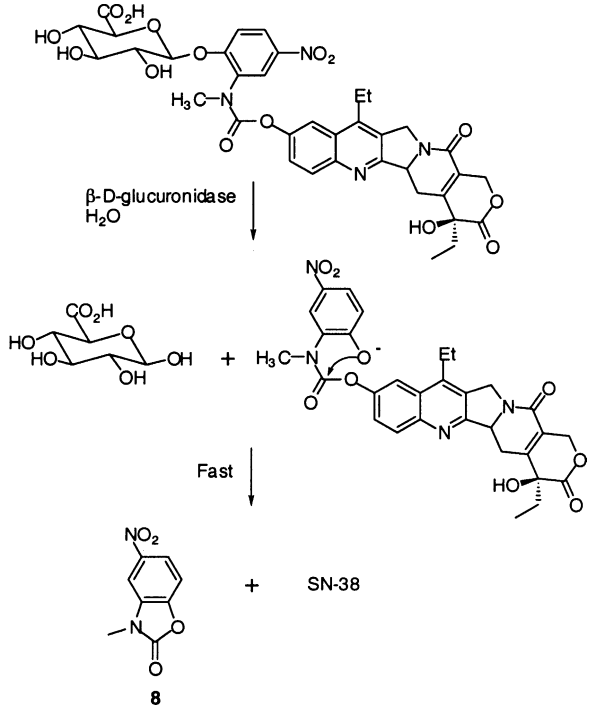
The carbamoyle chloride **5** was put into reaction with SN-38 and an excess of DMAP and triethylamine to give the coupling compound **6** in 83% yield. With pyridine instead of DMAP, the yield dropped to 59%. Finally, a two-step deprotection, with sodium methylate in methanol for the cleavage of acetate groups, and with sodium hydroxide for the methyl ester of the glucuronic moiety followed by a neutralization gave the final prodrug **7** in its lactone form (Scheme 2).

This prodrug **7** was planned to liberate the active SN-38 following Scheme 3.

After enzymatic cleavage, the carbamate function of **7** reacts through an intramolecular cyclisation[20] with spontaneous liberation of the drug and formation of the benzoxazolone **8**.



Scheme 2. Synthesis of prodrug **7**.



Scheme 3. Liberation of SN-38 from prodrug **7**.

Biological Results

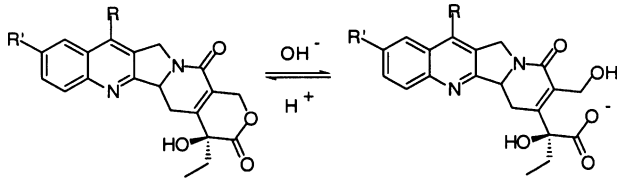
First of all, the cytotoxicity of prodrug **7** was evaluated against HT-29 (human colon cancer cells) cells and compared to that of free SN-38 in the absence or in the presence of β-D-glucuronidase (100 μg/mL). The measurements were done using the microculture tetrazolium assay (MTA)[21] during 48-h periods.

Table 1. Cytotoxicity of compounds 3 and 7

Compd	Cytotoxicity IC_{50} (nM)	Cytotoxicity IC_{50} (nM) in presence of β-D-glucuronidase
3	27.3	
7	1981	26

The prodrug 7 was 70 times less cytotoxic than the drug, but in the presence of the enzyme the cytotoxicity of the free drug was completely restored (Table 1).

Another important point to determine was the stability of the prodrug in solution. To test this, an in vitro stability study of 7 in a pH 7.2 phosphate buffer was undertaken over a 2-day period and followed by HPLC.[22] During that time, except an equilibrium between the lactone ring and the inactive carboxylate form of camptothecin[23] which is pH-dependent with predominance of the former form in acidic medium and of the open form in basic medium according to Scheme 4, the prodrug 7 displayed high stability.



Scheme 4. Opening of the lactone.

Finally, the cleavage of the prodrug 7 by *Escherichia coli* β-D-glucuronidase was measured by HPLC in a pH 7.2 phosphate buffer at 37 °C (Fig. 1).[24] In order to avoid the multiplication of peaks due to the coexistence of the open and closed forms, we chose basic (pH 9) HPLC conditions. The peaks were identified by comparison with samples of the prodrug 7, the drug 3, and the cyclised spacer 8. Unfortunately, as the prodrug 7 and drug 3 were mainly but not completely opened, we needed to add both open and close forms for the interpretation. After addition of the enzyme, the prodrug peaks decreased whereas those corresponding to SN-38 and cyclised spacer increased. For a prodrug concentration of 300 μM and an enzyme concentration of 10 units/mL, the half-life of 7 was 12.7 min.

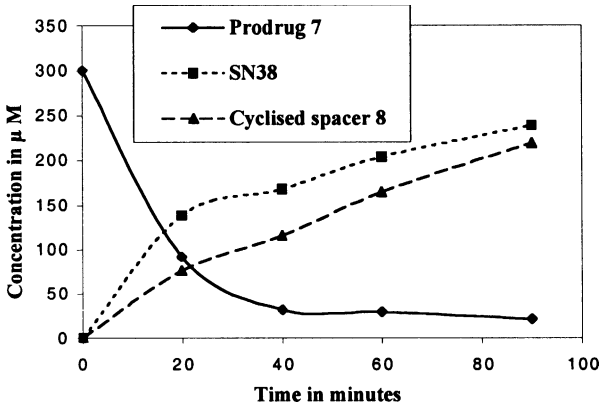


Figure 1. Kinetics of cleavage of prodrug 7.

Conclusion

As the data of prodrug 7 (cytotoxicity, stability and kinetics) are compatible for a PMT strategy, this compound has been selected for in vivo tests. Other spacers will be reported in a full paper.

Acknowledgements

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22. For the stability measurements, a solution of 250 μg/mL of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C. Aliquots (40 μL) were taken at various times and analyzed by HPLC after dilution with eluent (120 μL). HPLC conditions: analysis was carried out on a reverse-phase column (Spherisorb C18) using isocratic conditions (1 mL/min) of 60% phosphate buffer (0.02 M, pH 3) and 40% acetonitrile with UV detection at 265 nm.
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24. For the kinetic measurements, a solution of 300 μM of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C in the presence of 10 units/mL of β-D-glucuronidase (*E. coli*). Aliquots (40 μL) were taken at various times and analyzed by HPLC after dilution with eluent (120 μL). HPLC conditions: analysis was carried out on a reverse-phase column (WATERS X-terra, 250/4.6 mm) using isocratic conditions (1 mL/min) of 70% triethylammonium acetate buffer (0.1%, pH 9) and 30% acetonitrile with UV detection at 265 nm (extracted from PDA 3-D spectra).