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Synthesis and biological activities of a pH-dependently activated water-soluble prodrug of a novel hexacyclic camptothecin analog

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ABSTRACT

CH0793076 (**1**) is a novel hexacyclic camptothecin analog showing potent antitumor activity in various human caner xenograft models. To improve the water solubility of **1**, water-soluble prodrugs were designed to generate an active drug **1** nonenzymatically, thus expected to show less interpatient PK variability than CPT-11. Among the prodrugs synthesized, **4c** (TP300, hydrochloride) having a glycylsarcosyl ester at the C-20 position of **1** is highly water-soluble (>10 mg/ml), stable below pH 4 and rapidly generates **1** at physiological pH in vitro. The rapid (ca. <1 min) generation of **1** after incubation of TP300 with plasma (mouse, rat, dog and monkey) was also demonstrated. TP300 showed a broader antitumor spectrum and more potent antitumor activity than CPT-11 in various human cancer xenograft models.

Camptothecin analogs are potent topoisomerase I inhibitors showing strong antitumor activity both in vitro and in vivo.[1] However, most have poor water solubility. Two strategies had been taken to address this issue: (i) synthesis of camptothecin analogs with an amino functional group, for example, topotecan and Dx-8951, and (ii) synthesis of water-soluble prodrugs of lipophilic camptothecin analogs, for example, irinotecan (CPT-11). The compounds in the first category generally show reduced antitumor activity due to a lower tissue distribution profile. CPT-11 is a water-soluble prodrug of SN-38 and mainly used clinically for the treatment of gastrointestinal tumors (Fig. 1). Its clinical utility, however, is limited due to the drawbacks depicted in Figure 2: high interpatient variability in pharmacokinetics (due to poor bioconversion to the active drug SN-38 and SNPs of the metabolizing enzyme UGT1A1), severe toxicities (bone marrow and intestine), and the function of SN-38 as a substrate of the breast cancer resistant protein (BCRP) efflux pump.[2]

We previously reported a new hexacyclic camptothecin analog, CH0793076 (**1**),[3] that is not a substrate of BCRP and has higher in vivo antitumor activities than CPT-11, even though the poor water solubility of **1** remained to be improved for intravenous administration.

In this Letter, we report the design, synthesis and biological activities of a water-soluble prodrug of **1** (**4c**: named TP300) that can be pH-dependently converted to **1** and exhibited higher antitumor activity than CPT-11 in various human cancer xenograft models.

Design and synthesis. The bioconversion of CPT-11 to the active drug SN-38 by carboxyl esterase is reported to be very low in human, only 4–5%, and is one of the reasons for the wide interpatient PK variability of CPT-11. To simultaneously achieve greater water solubility, conversion efficiency of the prodrug, and tissue distribution of the active drug, we designed three types of new water-soluble prodrugs of **1**: (i) a prodrug (**2**) activated by peptidase such as membrane dipeptidase[4] overexpressed in gastro intestinal tumors (Fig. 3), (ii) prodrugs that can be nonenzymatically activated (Fig. 4). Namely, we designed prodrugs (**4a–f**) having a dipeptide ester group at the C-20 position of **1,** which were stable at low pH but rapidly converted to the active drug at physiological pH by intramolecular cyclization of the dipeptide moiety. Another design included sulfone prodrugs (**6a, 6b**) that can be activated by β-elimination triggered by an intramolecular deprotonation with a terminal amino group of the promoiety.

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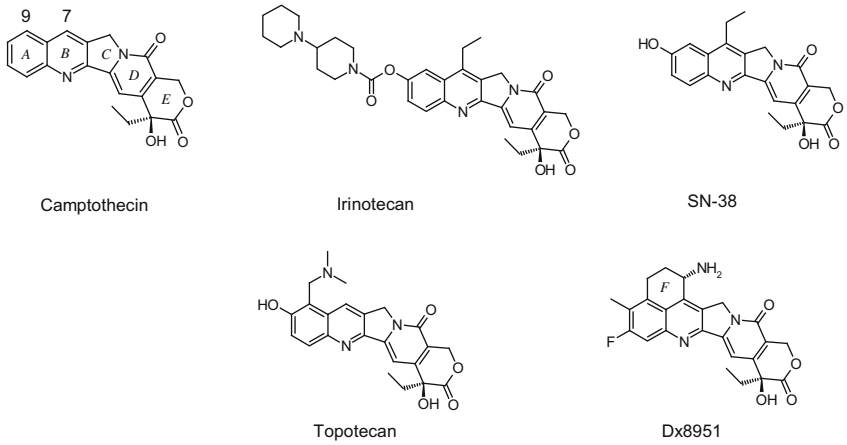

Figure 1. Camptothecin and representative analogs.

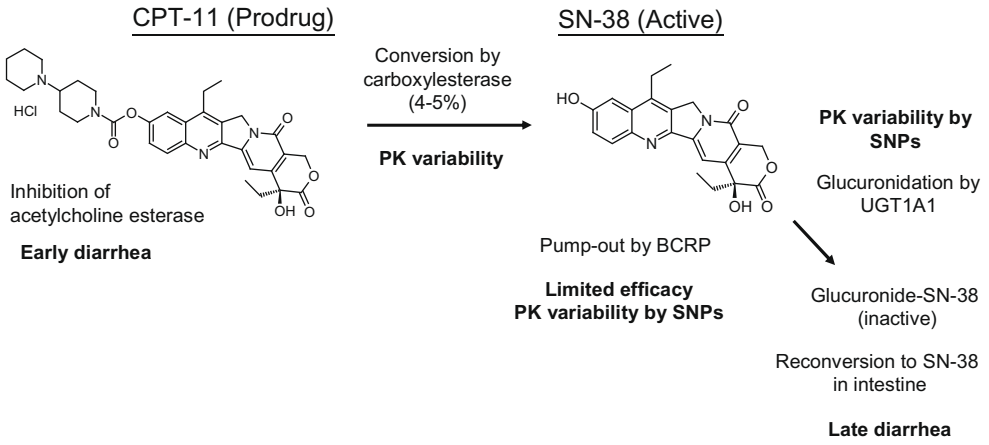

Figure 2. Major drawbacks of CPT-11.

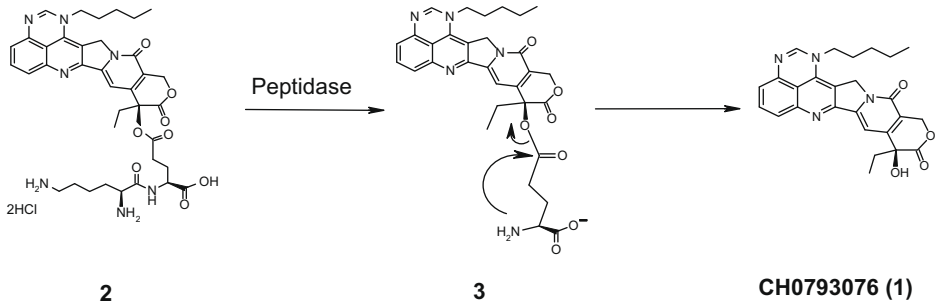

Figure 3. Design of a water-soluble prodrug activated by peptidase.

The synthesis of the prodrugs (**4a–f**, and **6a, 6b**) is shown in Figure 5. Prodrugs **4a–f** were prepared by esterification of **1** with *N*-Boc-dipeptide using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCI) in the presence of 4-(dimethylamino)pyridine at room temperature followed by removal of the Boc group by 1 N HCl in AcOH. **6a** was prepared in three steps from **1**. First, treatment of **1** with *p*-nitrophenyl chloroformate, diisopropylethylamine and 4-dimethylaminopyridine in methylene chloride gave the *p*-nitrophenylcarbonate derivative. Then the carbonate was reacted with 2-(2-*tert*-butoxycarbonylaminoethanesulfonyl)ethanol[5] to afford the sulfone derivative. Removal of the Boc group gave **6a** in high yield. **6b** was synthesized via the 2-bromoethylcarbonate derivative, derived from **1** and 2-bromoethylchloroformate. The 2-bromoethylcarbonate derivative was then reacted with *tert*-butoxycarbonyl-cysteine ethyl ester in the presence of potassium carbonate in acetonitrile to give the sulfide derivative. Oxidation of the sulfide with OXONE followed by removal of the Boc group by 1 N HCl in AcOH gave **6b** as a yellow powder. The final products were obtained as hydrochloride salts after lyophilization. The synthesis of the prodrug **2** from **1** is described in Ref. 4.

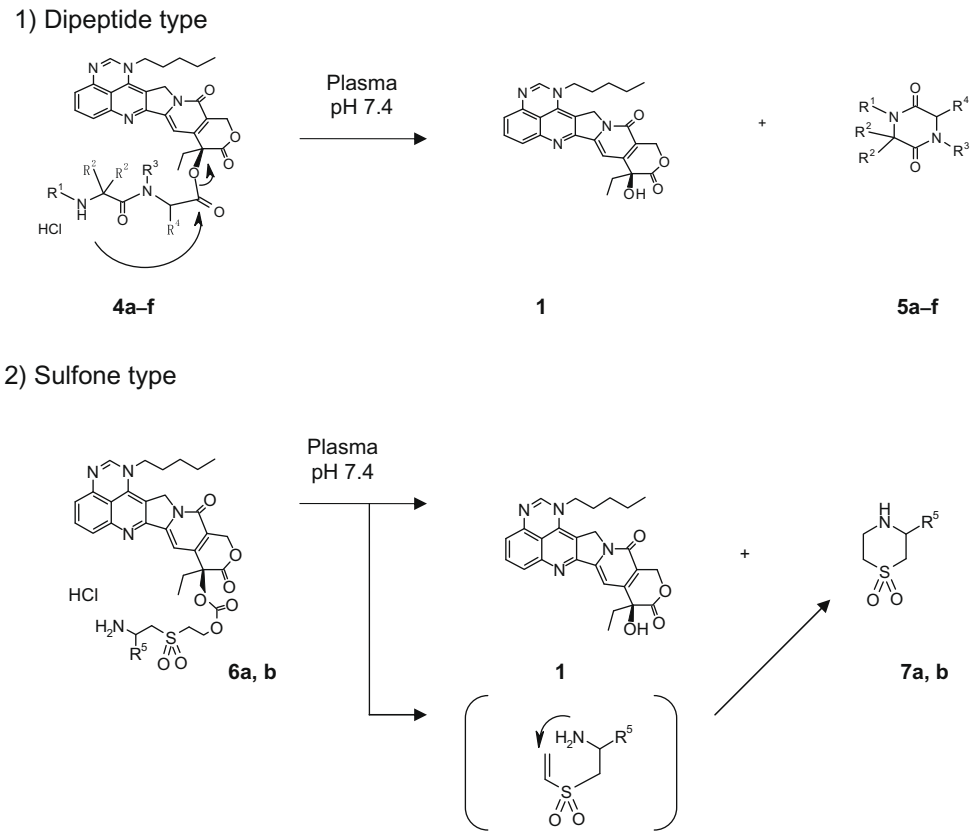

Figure 4. Design of water-soluble prodrugs that are pH-dependently activated (non-enzymatic process).

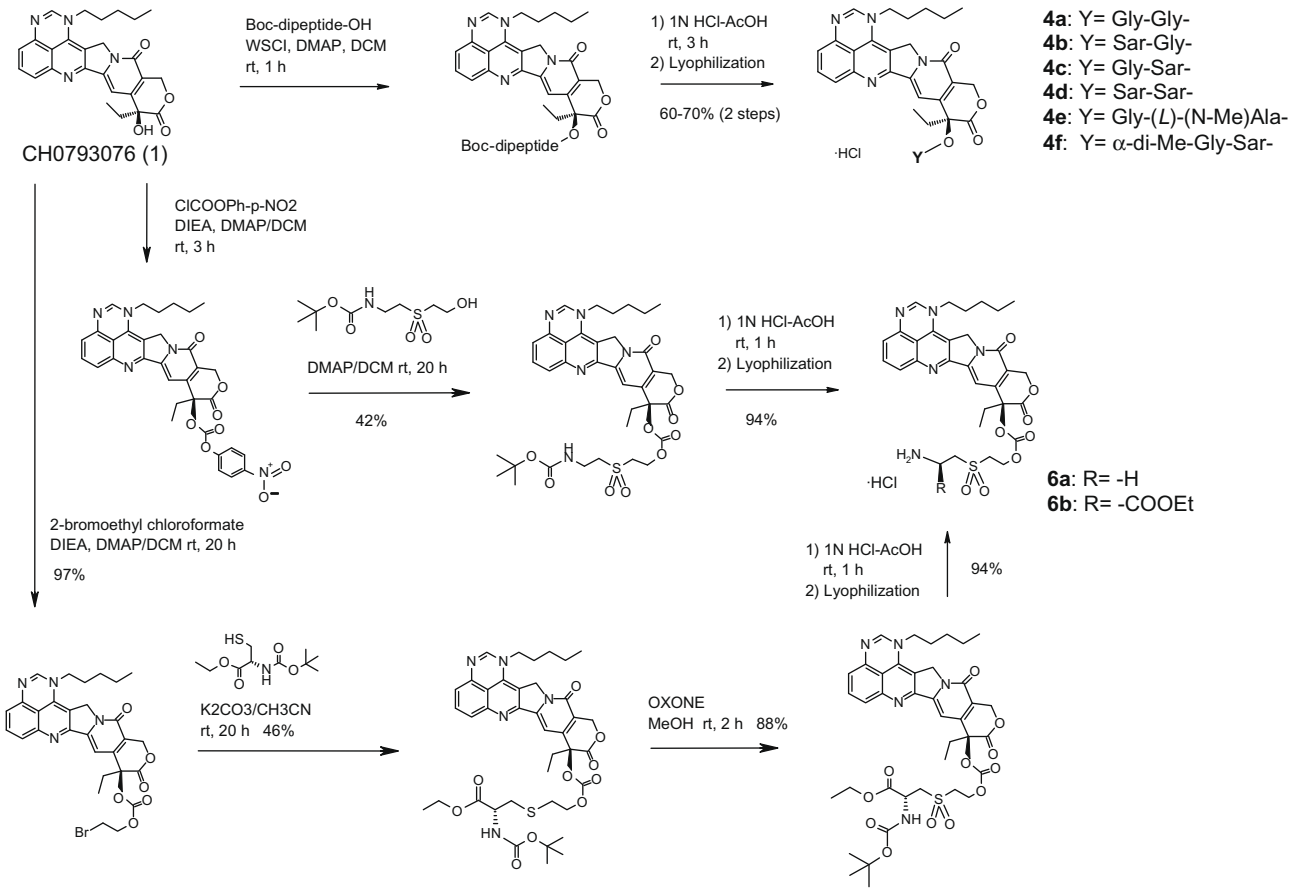

Figure 5. Synthesis of water-soluble prodrugs of CH0793076 (**1**).

Table 1
pH stability of prodrugs **4a–f**, **6a** and **6b**

Compound	R^1	R^2	R^3	R^4	R^5	pH stability Remaining % of the prodrug in phosphate buffer solution at rt after 3 h		
						pH 2	pH 5	pH 7.4
4a	H	H	H	H	—	N.D.	100	47
4b	Me	H	H	H	—	100	100	73
4c (TP300)	H	H	Me	H	—	100	83	0
4d	Me	H	Me	H	—	95	83	0
4e	H	H	Me	Me	—	99	47	0
4f	H	Me	Me	H	—	78	0	0
6a	—	—	—	—	H	100	96	4
6b	—	—	—	—	CO_2Et	100	68	43

The prodrug (**2**) was converted to the intermediate (**3**) smoothly after i.v. administration, but further conversion to the active drug (**1**) was too slow ($T_{1/2}$ = ~4 h).

The stability of the prodrugs (**4a–f**, **6a**, **6b**) at various pH conditions is summarized in Table 1. The non-N-methylated (R^3 = H) dipeptides (**4a, 4b**) were stable at pH 7.4 at room temperature for 3 h. In other words, their conversion to the active drug (**1**) was slow at physiological pH. On the other hand, the N-methylated (R^3 = Me) dipeptides (**4c–f**) were rapidly converted to **1** at pH 7.4. This rapid conversion can be explained by the contribution of an *s-cis* amide configuration that facilitates intramolecular cyclization. Among the N-methylated dipeptides, the gly-sar ester (**4c**: TP300) and the sar-sar ester (**4d**) were more stable at pH 5 than the gly-N-Me-(L)-ala ester (**4e**) and α,α-di-Me-gly-sar (**4f**). We selected TP300 (**4c**) as a clinical candidate since it had the highest stability at pH 2 as well. A sulfone prodrug (**6a**) also showed favorable conversion at pH 7.4 similar to **4c**.

The conversion of TP300 to **1** occurred very rapidly, in less than 1 min, in plasma from mouse, rat, dog, monkey and human (Fig. 6). TP300, a hydrochloride, is stable as an amorphous powder and has sufficient water solubility (>10 mg/ml) for i.v. formulation.

The mechanisms of releasing an active drug (**1**) from TP300 (**4c**) and **6a** were confirmed by ^{1}H NMR and MS analysis of the products (**5c** and **7a**′, respectively)[6] derived from the promoieties as depicted in Figure 7.

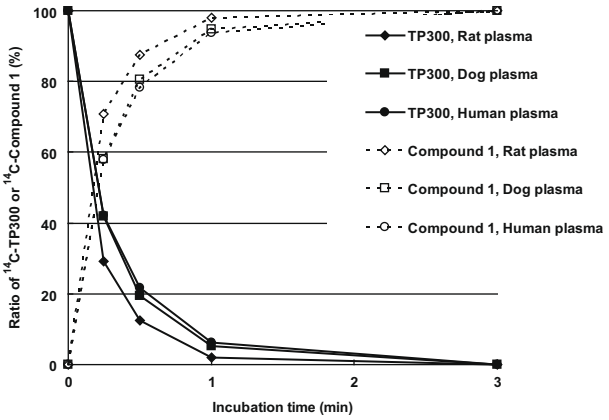

Figure 6. Conversion of ^{14}C-TP300 to ^{14}C-Compound **1** in rat, dog and human plasma.

Biological activities. The in vivo antitumor activities of TP300 were evaluated in human cancer xenograft models of colorectal cancer HCT116 (BCRP negative) and non-small cell lung cancer NCI-H460 (BCRP positive) and compared with CPT-11 at the maximum tolerated doses. The results are shown in Figure 8. TP300 exhibited higher efficacy than CPT-11 in human cancer xenografts models regardless

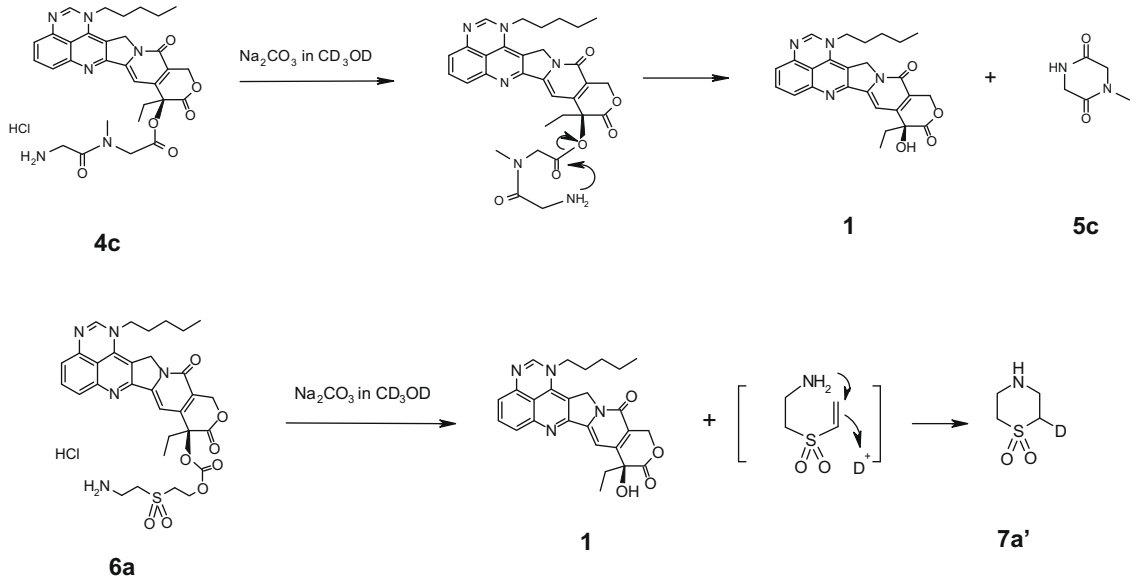

Figure 7. Confirmation of the mechanism of releasing active drug (**1**) from **4c** (TP300) and **6a** by ^{1}H NMR and MS.

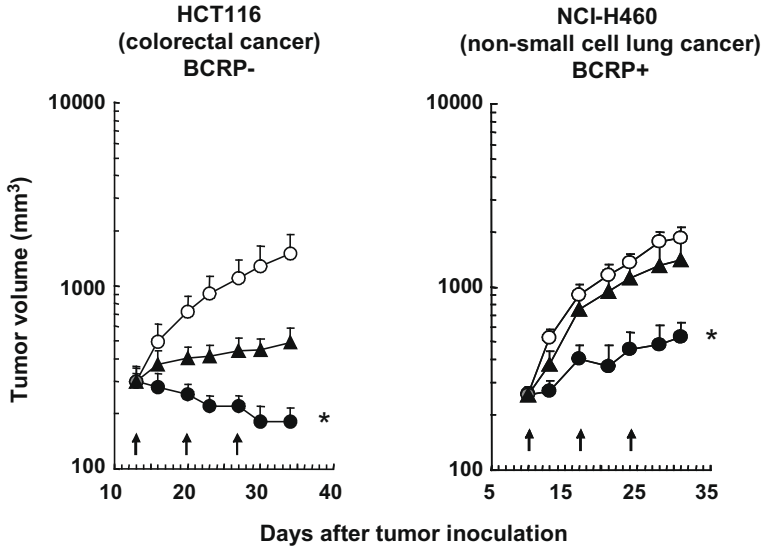

Figure 8. Antitumor effect of TP300 and CPT-11 in human cancer xenograft models. TP300 and CPT-11 were administered at the maximum tolerated dose (MTD) by bolus intravenous injection once per week for 3 weeks. The MTD was 47 mg/kg for TP300 and 100 mg/kg for CPT-11. Each group consisted of 7 mice. Values for tumor volume are given as the mean ± SD. ○: vehicle; ●: TP300; ▲: CPT-11. The BCRP protein was detected by Western blotting. *: statistically significantly different in mice treated with TP300 compared with mice treated with CPT-11 ($P < 0.05$).

of the level of BCRP expression. Data indicating additional antitumor efficacy of TP300 will be reported in a separate paper.[7]

In summary, we successfully designed and synthesized a water-soluble prodrug of **1** which can be pH-dependently converted to **1.** TP300 exhibited higher antitumor activity in various human cancer xenograft models than CPT-11. Because of the non-enzymatic activation of TP300, less interpatient PK variability than CPT-11 is expected in clinic. With the unique biological profile of the parent drug (**1**) together with improved solubility, TP300 will exhibit better efficacy and safety than CPT-11. A Phase 1 trial of TP300 is currently in progress.

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6. *Spectral data of* **5c**: ^{1}H NMR (270 MHz, CD_3OD) δ ppm 4.00 (2H, s), 3.93 (2H, s), 2.96 (3H, s); Spectral data of **7a′**: ^{1}H NMR (500 MHz, CD_3OD) δ ppm 3.22 (4H, m), 3.02 (3H, m), MS m/z 136 (M^+).
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