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

Jun Ohwada, Sawako Ozawa, Masami Kohchi, Hiroshi Fukuda, Chikako Murasaki, Hitomi Suda, Takeshi Murata, Satoshi Niizuma, Masao Tsukazaki, Kazutomo Ori, Kiyoshi Yoshinari, Yoshiko Itezono, Mika Endo, Masako Ura, Hiromi Tanimura, Yoko Miyazaki, Akira Kawashima, Shunsuke Nagao, Eitarou Namba, Koutarou Ogawa, Kazuko Kobayashi, Hisafumi Okabe, Isao Umeda, Nobuo Shimma *

Research Division, Chugai Pharmaceutical Co., Ltd, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan

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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.

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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.

* Corresponding author. Tel.: +81 467 47 2280; fax: +81 467 45 6824.
E-mail address: shinmanbo@chugai-pharm.co.jp (N. Shimma).

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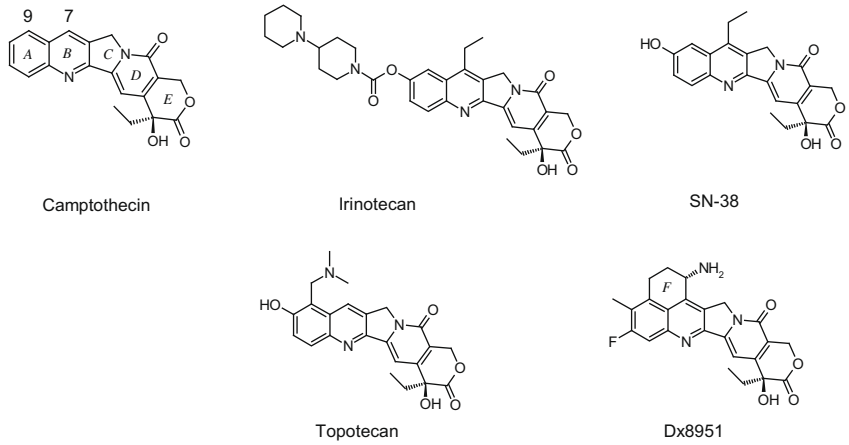

Figure 1. Camptothecin and representative analogs.

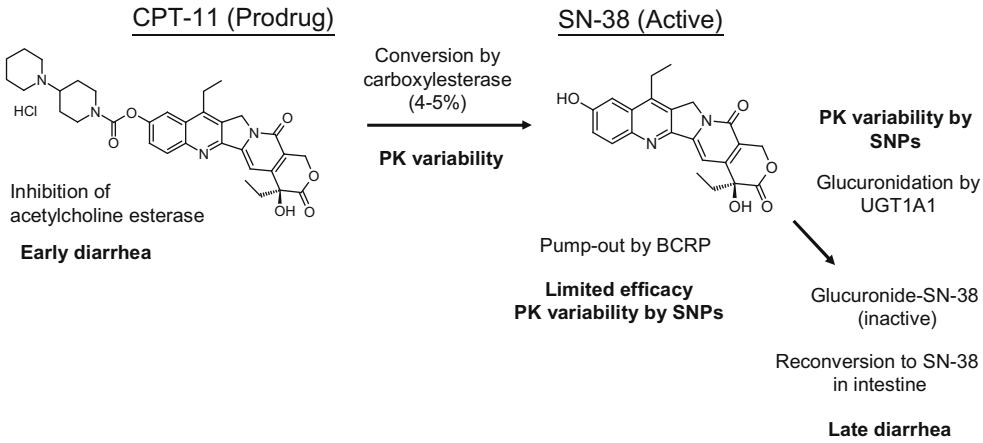

Figure 2. Major drawbacks of CPT-11.

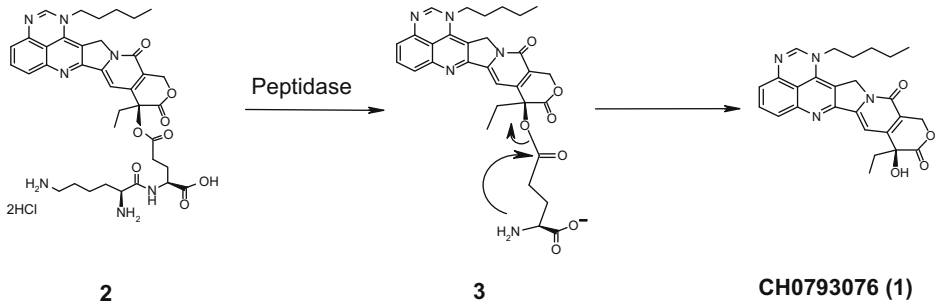

Figure 3. Design of a water-soluble prodrug activated by peptidase.

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.

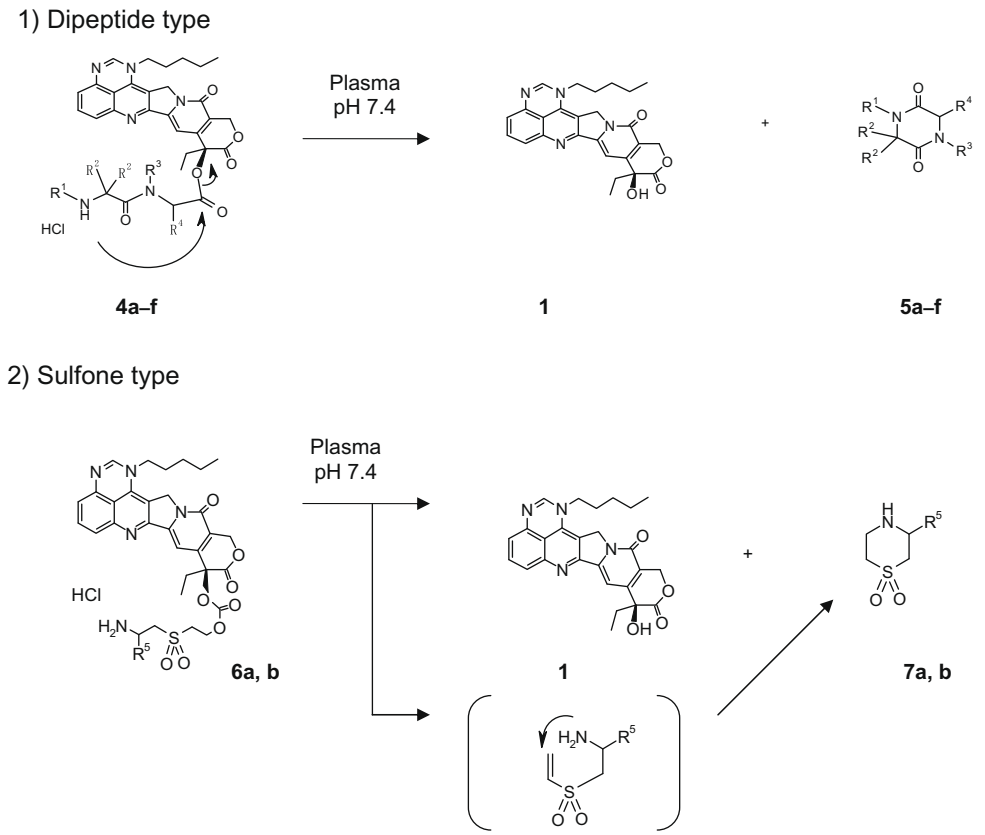

Figure 4. Design of water-soluble prodrugs that are pH-dependently activated (non-enzymatic process).

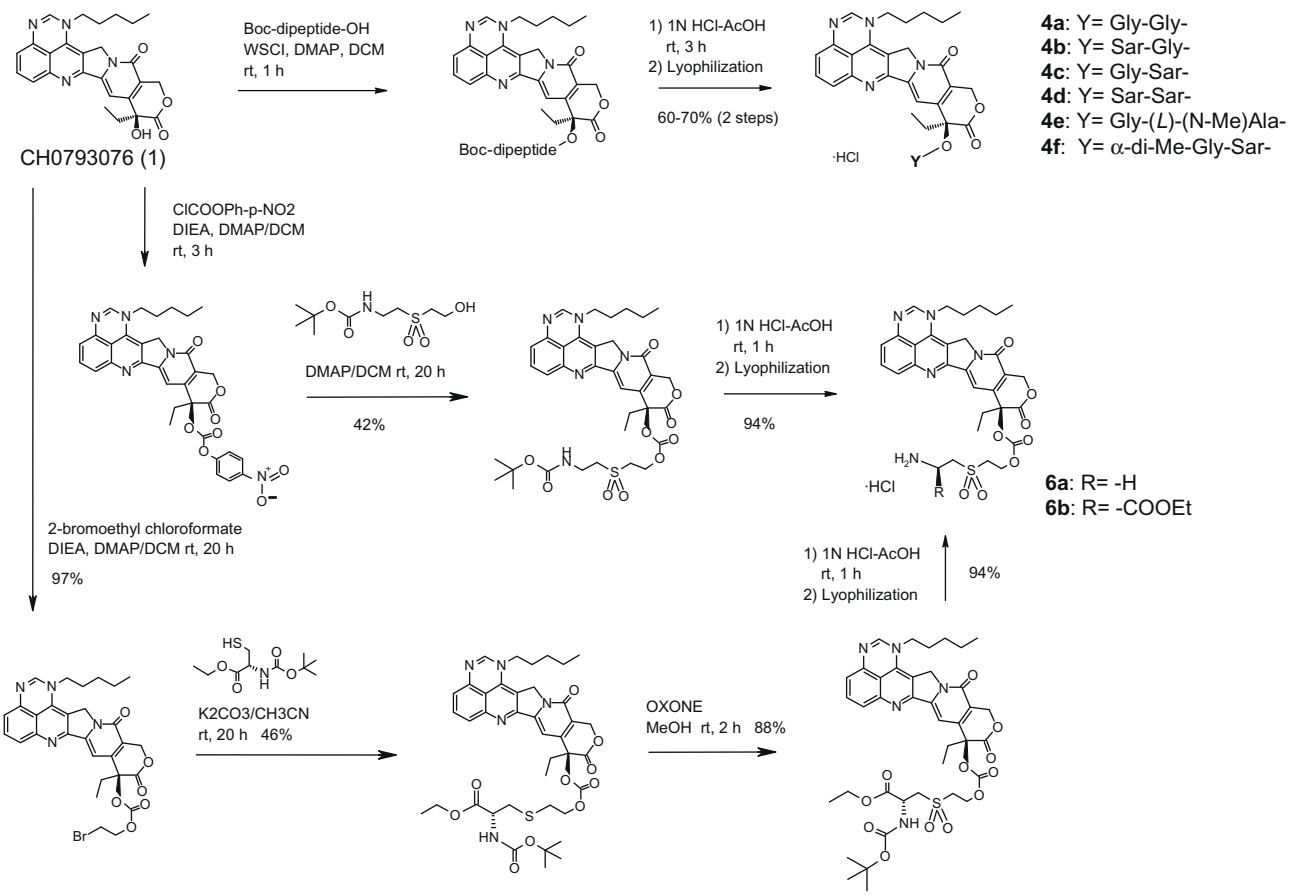

Figure 5. Synthesis of water-soluble prodrugs of CH0793076 (**1**).

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.

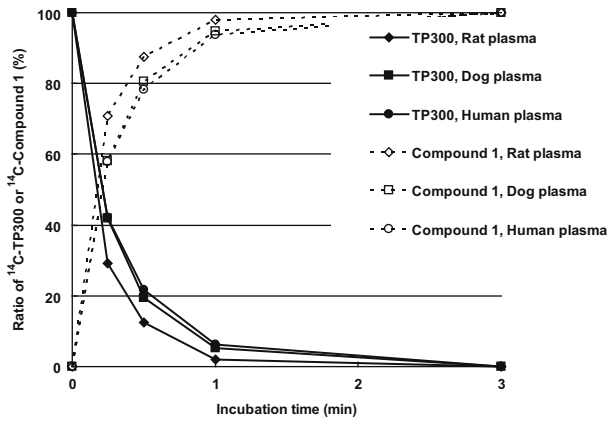

Figure 6. Conversion of ^{14}C-TP300 to ^{14}C-Compound **1** in rat, dog and human plasma.

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

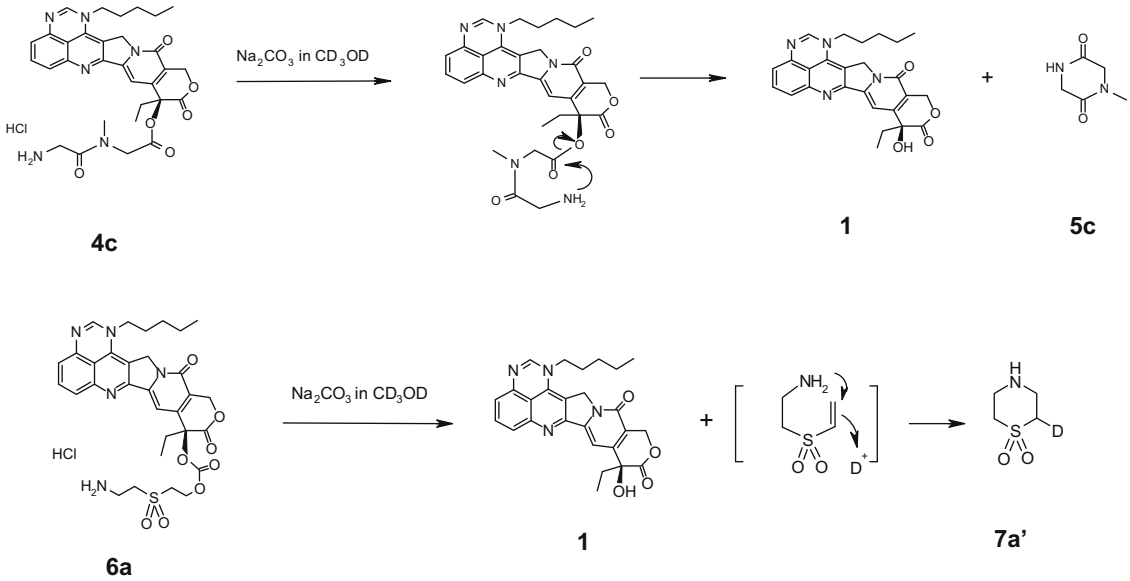

Figure 7. Confirmation of the mechanism of releasing active drug (**1**) from **4c** (TP300) and **6a** by ^{1}H NMR and MS.

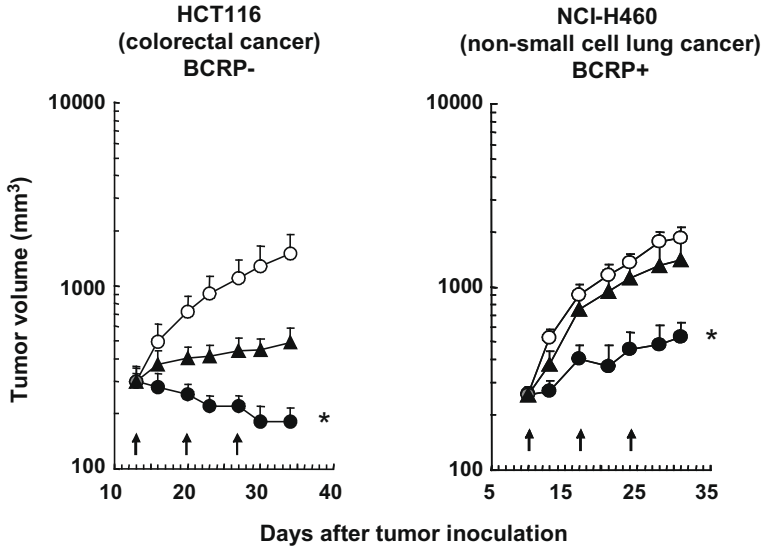

Figure 8. Antitumor effect of TP300 and CPT-11 in human cancer xenograft models. TP300 and CPT-11 were administered at the maximum tolerated dose (MTD) by bolus intravenous injection once per week for 3 weeks. The MTD was 47 mg/kg for TP300 and 100 mg/kg for CPT-11. Each group consisted of 7 mice. Values 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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