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Synthesis, cleavage, and antifungal activity of a number of novel, water-soluble ester prodrugs of antifungal triazole CS-758

Yoshiko Kagoshima [a], Makoto Mori [b], Eiko Suzuki [c], Takahiro Shibayama [c], Tamako Iida [d], Yasuki Kamai [d], Toshiyuki Konosu [a,*]

[a] Medicinal Chemistry Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[b] Medicinal Chemistry Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[c] Drug Metabolism and Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[d] Biological Research Laboratories IV, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

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ABSTRACT

In this study, the synthesis and evaluation of a number of esters of CS-758 as injectable prodrugs are described. Phosphoryl ester **1a** was soluble in water (>30 mg/mL) and was converted to CS-758 in human liver microsome. It was also converted to CS-758 in rats after iv administration, wherein the bioavailability of CS-758 was 53%. Compound **1a** (iv) reduced the viable cell counts in kidneys in a murine systemic *Candida albicans* infection model, wherein the effect was comparable to or slightly superior to that of CS-758 (*po*). The prodrug **1a** proved to be a promising injectable antifungal agent whose further evaluation is warranted.

There is a great medical need for an injectable antifungal agent with a broad spectrum for the treatment of severe deep mycoses of hospitalized patients. Currently, fluconazole (or fosfluconazole) and amphotericin B are available for parenteral use, but they have limitations in terms of antifungal spectra and safety, respectively.[1] Most of the azoles under development have a broader spectrum but cannot be administered parenterally without modification because of low water-solubility.[2,3] There have been some efforts to overcome this problem by using a prodrug approach.[4]

Previously, we identified CS-758[5] (Fig. 1), which has a broad antifungal spectrum covering *Aspergillus* spp., fluconazole-resistant *Candida* spp. and has a good safety profile including low drug–drug interaction. Since the water solubility of CS-758 was, however, too low for parenteral formulation, we conducted a study on a new prodrug of CS-758, which should have sufficient water-solubility and efficient bioconversion.

CS-758 has two obvious functional groups, namely the tertiary hydroxy and the triazole groups, with the possibility of being linked to a pro-moiety. It is known that water-soluble prodrugs can be readily accessed by alkylating a triazole ring with halomethyl acetate derivatives to give a quarternary ammonium salt prodrug.[4d,6] However, the prodrugs in this class liberate an equivalent amount of formaldehyde or acetaldehyde in vivo when cleaved. Fosfluconazole,[1d,e] the phosphoric acid ester prodrug of fluconazole, has improved water-solubility, but its antifungal spectrum is limited only to that of fluconazole. Consequently, we focused our efforts on identifying a suitable polar or charged group with which we could functionalize CS-758 on the tertiary hydroxy group. In this paper, we describe the synthesis and the characteristics of such prodrugs of CS-758.

For water-soluble prodrugs, we first planned to prepare compounds **1a**–**c** in order to check their solubility in water and ability to release CS-758. Then, compounds **2a** and **2b**, derivatives of **1b**, were investigated. Similarly, compounds **3a**–**c**, derivatives of **1c**, were investigated (Scheme 1–5).

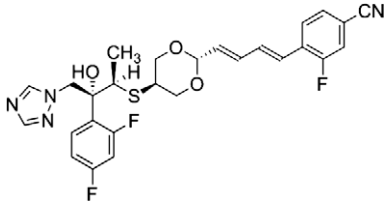

Figure 1. Structural formula of CS-758.

* Corresponding author. Tel.: +81 3 3680 0151; fax: +81 3 5496 8344.

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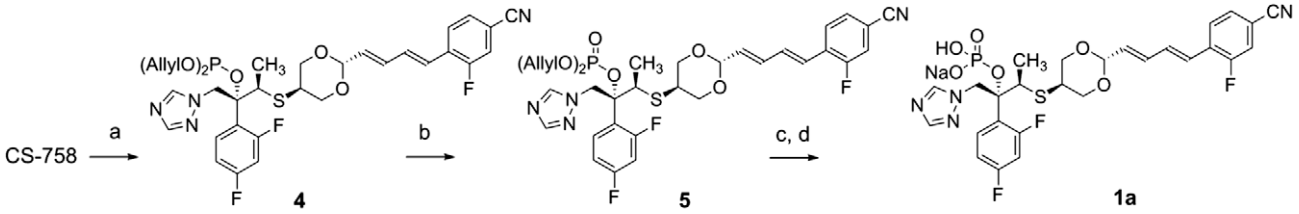
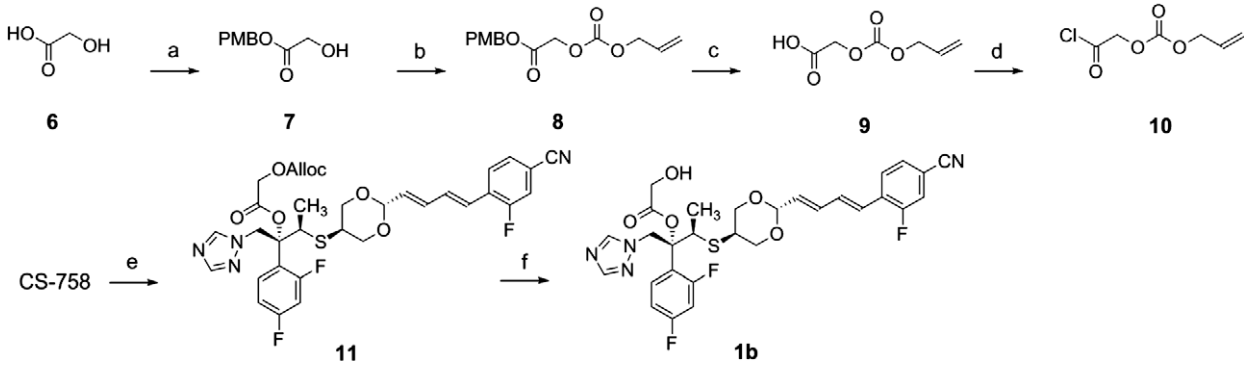
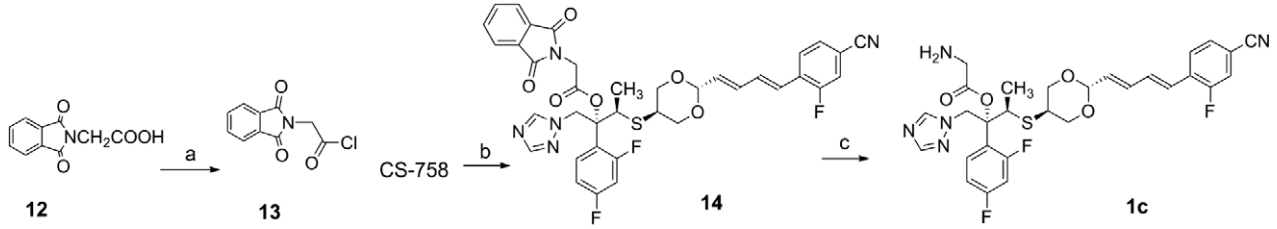


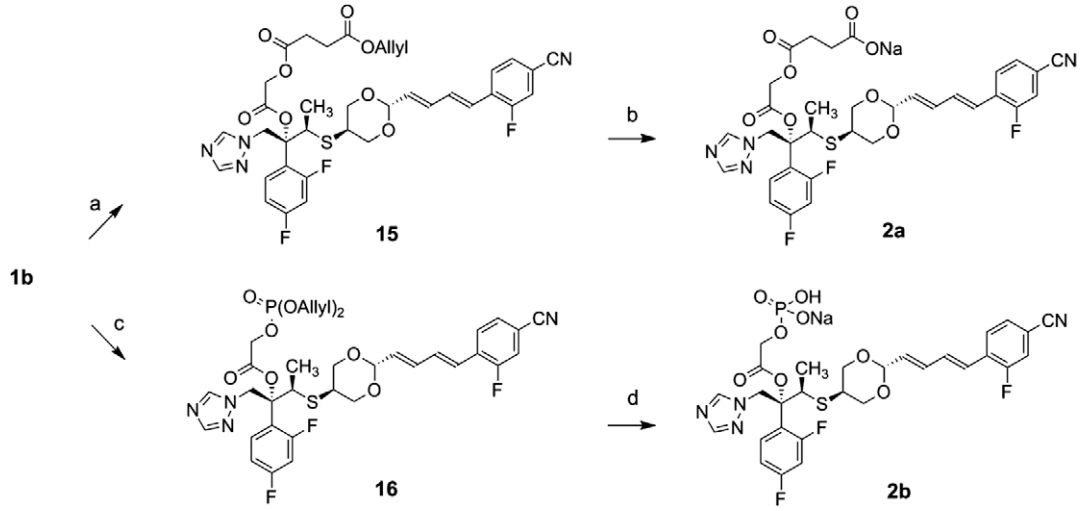
Scheme 1. Synthesis of **1a**. Reagents and conditions: (a) *i*-$Pr_2NP(OAllyl)_2$, 1*H*-tetrazole, CH_2Cl_2, 89%; (b) *t*-BuOOH, CH_2Cl_2, 82%; (c) $PdCl_2(PPh_3)_2$, *n*-Bu_3SnH, CH_2Cl_2; (d) $NaHCO_3$ aq, then C-18 reverse-phase column chromatography, 45%, two steps.



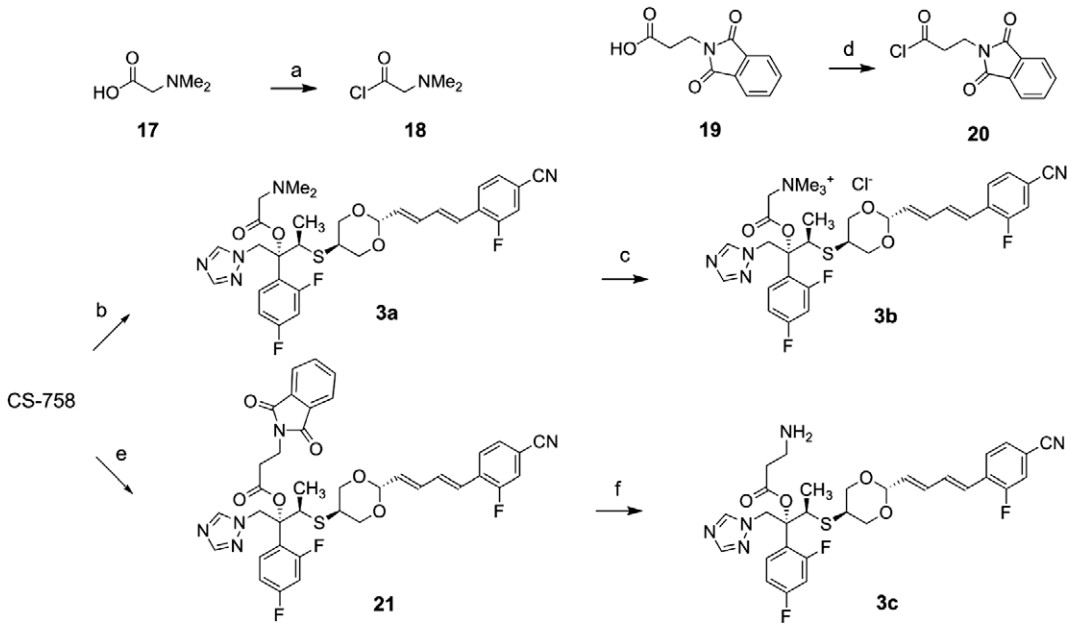
Scheme 2. Synthesis of **1b**. Reagents and conditions: (a) 1-$NaHCO_3$, H_2O, 2-4-(MeO)BnCl, 60%; (b) allyl chloroformate, DMAP, CH_2Cl_2, 86%; (c) anisole, TFA, quant.; (d) $(COCl)_2$, cat. DMF, THF, 90%; (e) 1-NaH, THF; 2–**10**, 16%; (f) $PdCl_2(PPh_3)_2$, *n*-Bu_3SnH, CH_2Cl_2, quant.



Scheme 3. Synthesis of **1c**. Reagents and conditions: (a) $(COCl)_2$, cat. DMF, CH_2Cl_2; (b) NaH, **13**, 26%; (c) CH_3NHNH_2, CH_2Cl_2, 85%.



Scheme 4. Synthesis of compound **1b**'s derivatives **2a** and **2b**. Reagents and conditions: (a) monoallyl succinic acid chloride, NEt_3, DMAP, CH_2Cl_2, 77%; (b) 1-$PdCl_2(PPh_3)_2$, *n*-Bu_3SnH, CH_2Cl_2, 2-$NaHCO_3$ aq, then C-18 reverse phase column, 34%; (c) 1-*i*-$Pr_2NP(OAllyl)_2$, 1*H*-tetrazole, CH_2Cl_2; 2-*t*-BuOOH, CH_2Cl_2, 84%; (d) $PdCl_2(PPh_3)_2$, *n*-Bu_3SnH, CH_2Cl_2 then $NaHCO_3$ aq, 52%, two steps.



Scheme 5. Synthesis of compound **1c**'s derivatives **3a**–**c**. (a) $(COCl)_2$, cat. DMF, THF; (b) NaH, **18**, THF, 37%; (c) 1-MeOTf, *i*-Pr_2NEt, CH_2Cl_2, 2-DOWEX1 × 4 (Cl form), 32%; (d) $(COCl)_2$, cat. DMF, CH_2Cl_2; (e) NaH, **20**, THF, 10%; (f) $MeNHNH_2$, CH_2Cl_2, 61%.

Table 1
Solubility in water and conversion to CS-758 (in human plasma and liver microsome)

Compd	Solubility in water (mg/mL)	In vitro (human)[a]			
		Plasma		Liver ms	
		30 min	120 min	30 min	120 min
1a	>30	–	–	+	+
1b	<1	±	±	–	–
2a	>10	–	–	–[b]	–[b]
2b	>10	–	–	–[b]	–[b]
1c	<1	–	±	–	–
3a	<1	–	±	–	–
3b	>10	+	++	–	–
3c	<1	–	–	–	–

[a] –; no detection of CS-758 (<10%), ±; low formation of CS-758 (10–30%), +; middle formation of CS-758 (30–60%), ++; high formation of CS-758 (>60%).
[b] Compound **1b** was detected.

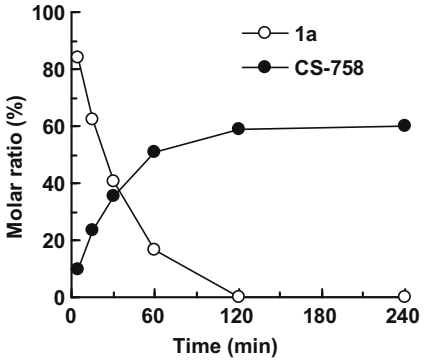


Figure 2. Conversion of **1a** (○) to CS-758 (●) in human liver microsome.

The synthesis of **1a** is shown in Scheme 1. Despite the steric hindrance of the tertiary hydroxy group of CS-758, compound **1a** could smoothly be synthesized from CS-758 using the phosphorylation procedure developed by Fraser-Reid.[7] Thus, CS-758 was treated with diallyl diisopropylphosphoramidite in the presence of 1*H*-tetrazole in dichloromethane to afford the diallyl phosphite **4**. Phosphite **4** was then oxidized by *t*-butyl hydroperoxide to give the corresponding phosphate **5**. Removal of the two allyl groups was accomplished by use of bis(triphenylphosphine)dichloropalladium and tri(*n*-butyl)tin hydride in dichloromethane. The sodium salt **1a** was prepared by treatment with sodium hydrogen carbonate and purified by C-18 reverse-phase column chromatography.

The syntheses of **1b** and **1c** are shown in Schemes 2 and 3. The OH group of glycolic acid **6** was protected by the allyloxycarbonyl group in three steps to give **9**. Treatment of **9** with oxalyl chloride in dichloromethane afforded corresponding acid chloride **10**. The usual methods reported for the preparation of esters from alcohols were not successful with CS-758 because of its steric hinderance. After many attempts to esterify this hydroxyl moiety, a direct esterification reaction of oxide anion, which was generated from CS-758 and NaH in THF, with acid chloride **10** at room temperature, provided a modest yield (8–10%) of the desired ester **11**. The allyloxycarbonyl group of **11** was removed by bis(triphenylphosphine)dichloropalladium and tri(*n*-buthyl)hydride to give compound **1b**.

In a similar manner, **1c** was synthesized from CS-758 and acid chloride **13** which was prepared from *N*-phthaloyl glycine **12**.

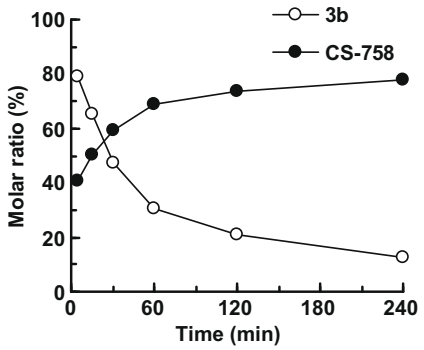


Figure 3. Conversion of **3b** (○) to CS-758 (●) in human plasma.

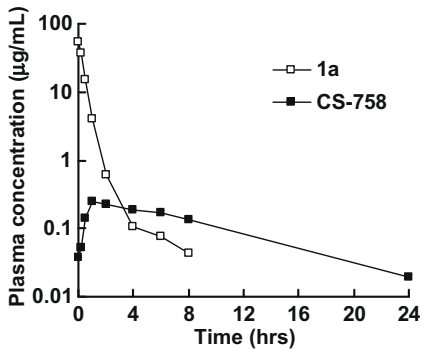


Figure 4. Plasma level of CS-758 after iv administration of **1a** to rats at a dose of 2 mg/kg (average of three rats).

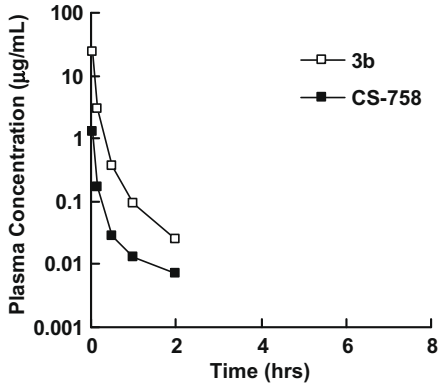


Figure 5. Plasma level of CS-758 after iv administration of **3b** to rats at a dose of 2 mg/kg (average of three rats).

Removal of the phthaloyl group in **14** was achieved by the use of methylhydrazine to afford the desired amine **1c**.

The syntheses of **2a** and **2b** are shown in Scheme 4. We attempted to modify the hydroxy group of **1b** with a hydrophilic function such as succinic acid or phosphoric acid groups. **1b** was allowed to react with mono allyl succinic acid chloride in dichloromethane to give allyl ester **15**. The allyl group in **15** was removed by the use of Pd(0) and tri(*n*-butyl)tin hydride. The sodium salt **2a** was prepared by treatment with sodium hydrogen carbonate and purified by C-18 reverse-phase column chromatography.

When compound **1b** was treated with diallyl diisopropylphosphoramidite in the presence of 1*H*-tetrazole in dichloromethane, a smooth conversion to the diallyl phosphite was achieved. This phosphite was oxidized successively with *t*-butyl hydroperoxide to give the corresponding phosphate **16**. Removal of the allyl groups was accomplished by use of bis(triphenylphosphine)dichloropalladium and tri(*n*-butyl)tin hydride in dichloromethane to afford **1c**.

The syntheses of **3a**–**c** are shown in Scheme 5. *N*,*N*-Dimethylaminoglycine ester **3a** was synthesized from CS-758 and **18**. **3a** was allowed to react with methyl trifluoromethanesulfonate in CH_2Cl_2 followed by anion exchange chromatography with DOWEX-1 to afford quaternary ammonium compound **3b**. β-Alanine ester **3c** was synthesized in a similar manner to that shown in Scheme 3.

The solubility in water and the conversion rate to CS-758 in human plasma and in human liver microsome (ms) are shown in Table 1. Compound **1a** has good water solubility (>30 mg/mL). **1a** was converted to CS-758 in human liver ms, but it was not converted to CS-758 in human plasma. The solubility of **1b** and **1c** was not sufficient. Though these compounds were not converted to CS-758 in human liver ms, they released CS-758 in human plasma moderately. Compounds **2a** and **2b** were sufficiently improved in water-solubility. These compounds were not converted to CS-758 in human plasma. When **2a** and **2b** were incubated with human liver ms, they were smoothly converted to intermediate **1b**. However, **1b** was not converted to CS-758 in human liver ms. Compounds **3a** and **3c** did not show any improvement in either water-solubility or conversion rates to CS-758, unfortunately. However, betaine ester **3b** has good water solubility. Furthermore, compound **3b** was quickly converted to CS-758 in human plasma.

The disappearance of **1a** and the formation of CS-758 after incubation of **1a** with human liver ms are shown in Figure 2, and the disappearance of **3b** and formation of CS-758 after incubation of **3b** with human plasma are shown in Figure 3.

The in vivo conversion of **1a** and **3b** to CS-758 upon iv administration to rats is shown in Figures 4 and 5, respectively. When **1a** was administrated to rats, CS-758 was gently formed and slowly eliminated with $t_{1/2}$ of 5.7 h. The bioavailability[8] of CS-758 was 53%. When **3b** was administrated to rats, CS-758 was quickly formed and a high concentration of CS-758 was observed (C_{max}, 1.3 μg/mL). But CS-758 was quickly eliminated and the bioavailability of CS-758 was only 3.3%. The reason for the low conversion rate of **3b** was unclear.

The in vivo efficacies of CS-758 (oral administration) and prodrug **1a** (iv administration) were evaluated in a systemic *C. albicans* infection model in mice (Fig. 6). The efficacy of iv-administered prodrug **1a** was comparable or slightly superior to the efficacy of orally administered CS-758.

Consequently, the phosphoryl ester prodrug **1a** proved to be a promising agent as an antifungal agent for parenteral use and its further preclinical evaluation is warranted.

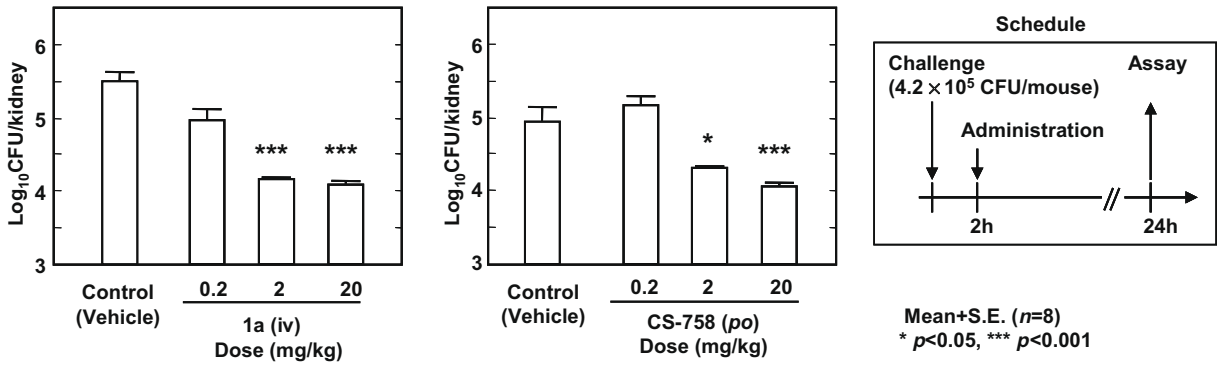


Figure 6. In vivo efficacy of compound **1a** (iv) against murine intravenous infection with *C. albicans* SANK 51486 compared with that of CS-758 (*po*).

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8. Bioavailability (BA) was calculated by the following. BA (%) = [(AUC of CS-758 after iv administration of the prodrug)/(AUC of CS-758 after iv administration of CS-758 dissolved in polyethylene glycol solution)] × 100.