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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2,2-DISUBSTITUTED 2-AMINOETHANOLS: ANALOGUES OF FTY720

Masatoshi Kiuchi,* Kunitomo Adachi, Toshiyuki Kohara, Koji Teshima, Yumi Masubuchi,
Tadashi Mishina and Tetsuro Fujita[a]

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 7-25 Koyata 3-chome,
Iruma-shi, Saitama 358, Japan
[a]*Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge, Hirakata-shi, Osaka*
573-01, Japan

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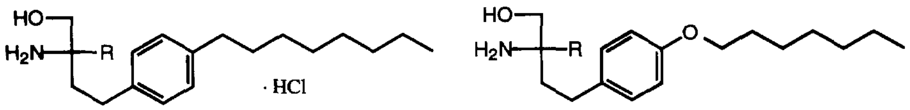
Abstract: Desymmerization of symmetric FTY720 by substitution of different alkyl groups for one of the prochiral hydroxymethyl groups was performed. The size of the alkyl groups and the absolute configuration at quaternary carbon were important on immunosuppressive activity.

2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720[1], **1a**) (Figure 1) is a novel synthetic immunosuppressant structurally simplified from myriocin[2] (thermozymocidin,[3] ISP-I[4]). The *in vivo* administration of FTY720 to rats and dogs resulted in remarkable decrease in the number of peripheral lymphocytes, especially T cells, at doses enough to show the immunosuppressive activity.[5] FTY720 has a symmetric structure and contains a pair of prochiral hydroxymethyl groups. We have already reported that the 2-substituted 2-aminoethanol moiety is the minimum key structure for the activity. For example, 2-amino- 4-(4-octylphenyl)butanol (**1b**) that lacks one of the prochiral hydroxymethyl groups of FTY720 showed similar activity on mouse

Figure 1



1a $R = CH_2OH$ (FTY720)
1b $R = H$

2a $R = CH_2OH$ **2d** $R = CH(CH_3)_2$
2b $R = CH_3$ **2e** $R = CH_2CH_2CH_3$
2c $R = CH_2CH_3$ **2f** $R = CH_2CH_2CH_2CH_3$

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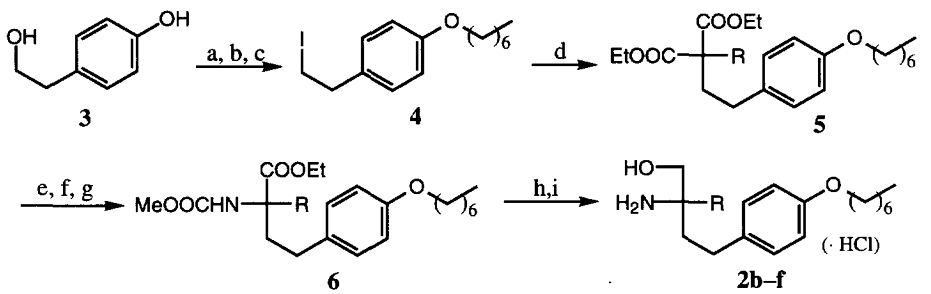
allogeneic mixed lymphocyte reaction (MLR)[6] in vitro. However, the in vivo immunosuppressive activity of the analogue was weak (Table 1). We presumed that compound **1b** can be easily metabolized due to lack of a quaternary carbon atom at the C-2 position. Therefore, we designed some compounds (**2a–f**) possessing a quaternary carbon. We first synthesized compound **2a** that has heptyloxy group instead of octyl group of FTY720 to investigate how introduction of an oxygen atom affects the activity. Next we synthesized the derivatives (**2b–f**) (Figure 1) having low alkyl substituents instead of one of the hydroxymethyl group to evaluate their in vivo activity. As for **2b** and **2e** optical resolution was done to investigate which hydroxymethyl group of FTY720 is important for the activity.

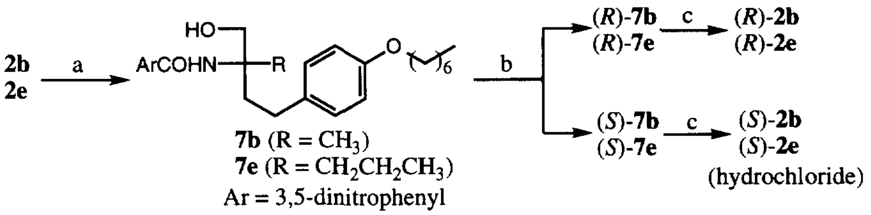
Chemistry: Compound **2a**[7] was synthesized via a similar synthetic route to that reported previously.[1b] The synthesis of compounds **2b–f**[7] was carried out as shown in Scheme 1. Diethyl

Scheme 1



Reagents and conditions: (a) heptyl bromide, NaOEt, EtOH, reflux (89%); (b) MsCl, Et_3N, CH_2Cl_2, rt; (c) NaI, 2-butanone, reflux (93%, 2 steps); (d) diethyl 2-alkylmalonate, NaH, DMF, rt (46–93%); (e) KOH, EtOH, 50 °C to reflux, then HCl; (f) ClCOOEt, Et_3N, THF, –10 °C, then NaN_3, 0 °C; (g) benzene, reflux, then benzene/MeOH, reflux (24–55%, 3 steps); (h) $LiBH_4$, THF, reflux; (i) 5 N KOH, THF, MeOH, reflux, then HCl (only **2b** and **2c**) (18–78%, 2 or 3 steps).

Scheme 2



Reagents and conditions: (a) 3,5-dinitrobenzoyl chloride, $KHCO_3$, $EtOAc/H_2O$, rt; (b) HPLC (columun: CHIRALCEL OD, mobile phase: hexane/EtOH = 2/3); (c) 2 M LiOH, THF, MeOH, then HCl.

2-alkylmalonates were alkylated with iodide (**4**) to give **5**. Diesters (**5**) were treated with potassium hydroxide in ethanol to give half esters, which were subsequently converted to acyl azides. The acyl azides underwent Curtius rearrangement in refluxing benzene, and the resulting isocyanates were trapped with methanol to give methylcarbamates (**6**), which were reduced with lithium borohydride and hydrolyzed with aqueous potassium hydroxide to give the target compounds **2b–f**.

We prepared the enantiomers of **2b** and **2e** as shown in Scheme 2. The racemates **2b** and **2e** were treated with 3,5-dinitrobenzoyl chloride under Schotten-Baumann condition. The resulting

Table 1. Effects of FTY720 (**1**), **1b**, **2a–2f** and cyclosporin A[8] on HvGR inhibition and decrease in the number of T cells.

Compound	ID_{50} values (mg/kg, po, n = 4)	
	HvGR	T cells
FTY720 (**1a**)	0.20 (0.052–0.65)	0.024 (0.016–0.035)
1b	>10	>10
2a	0.049 (0.019–0.15)	0.014 (0.012–0.018)
2b	0.031 (0.018–0.053)	0.016 (0.013–0.021)
(*R*)-**2b**	0.088 (0.032–0.22)	0.0092 (0.0068–0.010)
(*S*)-**2b**	>1	>1
2c	0.059 (0.029–0.11)	0.044 (0.039–0.050)
2d	2.7 (1.5–4.7)	4.5 (3.8–5.6)
2e	3.1 (1.6–5.7)	7.3 (4.6–12)
(*R*)-**2e**	0.63 (0.14–1.9)	4.4 (3.6–5.7)
(*S*)-**2e**	>10	>10
2f	>10	>10
cyclosporin A	2.8 (1.2–6.5)	>30

The 95% confidence limits are given in parentheses.

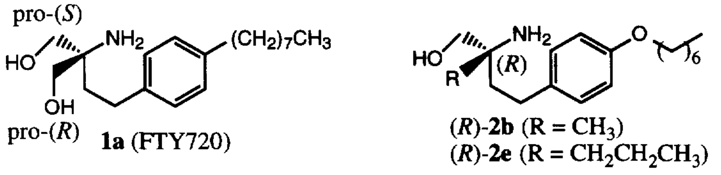
amides **7b** and **7e** were separated by HPLC on a chiral column.[9] Those were hydrolyzed with aqueous lithium hydroxide to afford the enantiomers of **2b** and **2e**.[10] The absolute configuration of (*S*)-**2e** was determined by a single crystal X-ray analysis.[11] The absolute configurations of **2b** were assumed from the similarity of the HPLC retention times and the optical rotations between **7b** and **7e**.[9]

Biological Assay: The *in vivo* immunosuppressive effect of compounds on host versus graft reaction (HvGR)[12] were evaluated by using popliteal lymph node gain assay in rats. Spleen cells (5 × 10^6 cells) of WKAH rats ($RT1^k$) were injected into the footpad of LEW rats ($RT1^l$) to induce an enlargement of the draining popliteal lymph node. Compounds were orally administrated for four days from the day of immunization, and the weight of the popliteal lymph node was measured four days after the injection of WKAH spleen cells. The number of T cells in peripheral blood was determined by flow cytometry using fluorescein isothiocyanate-conjugated anti-CD3 (clone:1F4) monoclonal antibody. The results were expressed as ID_{50} versus vehicle-treated group (Table 1).

Results and Discussion: Compound **2a** showed comparable activities to FTY720 on HvGR inhibition and T cells depletion, indicating that replacement of the methylene on the benzyl position at the side chain by an oxygen atom does not affect the activities. We found that both the two hydroxyl groups of FTY720 are not always necessary for the biological activities because compounds **2b** and **2c** both of which have small R (R = methyl and ethyl, respectively) also displayed remarkable activities. However, the activities decreased as the size of R group increased (compounds **2d–f**), which suggests that the size of the substituent R is important on the activities.

As for **2b**, (*R*)-**2b** had more than ten-fold potent immunosuppressive activity compared to (*S*)-**2b**. The similar result was observed for compound **2e**. Consequently, it appears that between the two hydroxymethyl groups of FTY720, only pro-(*S*) hydroxymethyl group is of critical importance for the potent activity (Figure 2).

Figure 2



1a (FTY720)

(*R*)-**2b** (R = CH_3)
(*R*)-**2e** (R = $CH_2CH_2CH_3$)

In summary, several analogues having alkyl groups instead of one of the prochiral hydroxymethyl groups of FTY720 were synthesized and evaluated for their effects on HvGR inhibition and decrease in the number of T cells in peripheral blood. A couple of the compounds showed comparable activities to FTY720, and the size of substituent R (Figure 1) was affected the activities. On selected compounds these activities were confirmed only for the (*R*)-configuration enantiomers.

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7. Compounds **2a-2f** gave satisfactory analytical and spectroscopic data in accord with their

assigned structures.

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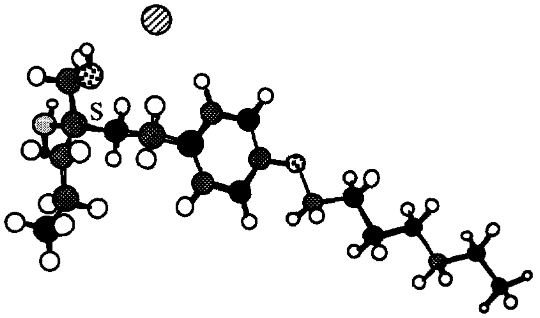
9. (*R*)-**7b**: shorter retention time than (*S*)-**7b** on CHIRALCEL OD (hexane/EtOH); mp 150–151 °C; $[\alpha]^{24}_{D}$ −18.0° (*c* 0.83, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.31 (m, 8H), 1.50 (s, 3H), 1.69 (quintet, *J* = 7.3 Hz, 2H), 2.02–2.09 (m, 1H), 2.29–2.37 (m, 1H), 2.65–2.72 (m, 1H), 2.75–2.80 (m, 1H), 3.49 (t, *J* = 6.3 Hz, 1H), 3.73–3.78 (m, 2H), 3.76 (dd, *J* = 11.2 Hz, 6.3 Hz, 1H), 3.88 (dd, *J* = 11.2 Hz, 6.3 Hz, 1H), 6.11 (s, 1H), 6.70 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.7 Hz, 2H), 8.64 (d, *J* = 1.9 Hz, 2H), 9.12 (t, *J* = 1.9 Hz, 1H). (*S*)-**7b**: mp 150–151 °C; $[\alpha]^{24}_{D}$ +17.2° (*c* 1.15, CHCl$_3$). (*R*)-**7e**: shorter retention time than (*S*)-**7e** on CHIRALCEL OD (hexane/EtOH); mp 115–116 °C; $[\alpha]^{24}_{D}$ −20.7° (*c* 0.98, CHCl$_3$). (*S*)-**7e**: mp 114–115 °C; $[\alpha]^{24}_{D}$ +18.2° (*c* 0.71, CHCl$_3$).
10. (*R*)-**2b**: $[\alpha]^{25}_{D}$ +3.30° (*c* 0.42, CHCl$_3$); ^{1}H NMR (400 MHz, CD$_3$OD) δ 0.90 (t, *J* = 7.1 Hz, 3H), 1.32 (s, 3H), 1.32–1.46 (m, 8H), 1.70–1.77 (m, 2H), 1.82–1.93 (m, 2H), 2.56–2.63 (m, 2H), 3.52 (d, *J* = 11.5 Hz, 1H), 3.61 (d, *J* = 11.5 Hz, 1H), 3.92 (t, *J* = 6.4 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H). (*S*)-**2b**: $[\alpha]^{25}_{D}$ −3.61° (*c* 0.31, CHCl$_3$). (*R*)-**2e**: mp 89–90 °C; $[\alpha]^{24}_{D}$ +1.68° (*c* 0.51, EtOH). (*S*)-**2e**: mp 90–91 °C; $[\alpha]^{26}_{D}$ −0.45° (*c* 0.51, EtOH).
11. Crystals of (*S*)-**2e**·HCl were grown from diethyl ether. Crystal data: $C_{20}H_{35}NO_2 \cdot HCl$, FW = 357.96, monoclinic, space group P2$_1$, *a* = 18.961(3), *b* = 8.299(1), *c* = 6.868(1) Å, β = 92.60(1) deg., *V* = 1079.6(3) Å^3, *Z* = 2, D_x = 1.101 g cm^{-3}, μ = 1.646mm^{-1}, λ = 1.5418 Å, *F*(000) = 392, 1. Intensities were collected for a crystal of dimensions 0.25 × 0.25 × 0.25 mm on a Enraf- Nonius CAD4 diffractometer. The final agreement factors were *R* 0.0435 and *wR* 0.0504 for 362 parameters and 1680 observed reflections.

   Crystal structure of compound (*S*)-**2e**·HCl.



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