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DESIGN, SYNTHESIS, AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 2-SUBSTITUTED-2-AMINO-1,3-PROPANEDIOLS: DISCOVERY OF A NOVEL IMMUNOSUPPRESSANT, FTY720.

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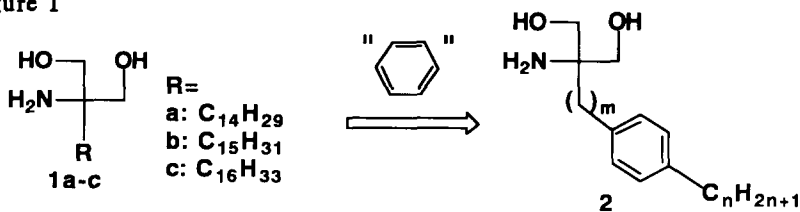
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Abstract: FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride), a novel synthetic immunosuppressant led by modification of ISP-I (myriocin, thermozymocidin) displayed potent immunosuppressive activity both *in vitro* and *in vivo*.

As reported in the preceding communication¹, simplification of the structure of ISP-I including removal of the side chain functionalities as well as elimination of chiral centers led to 2-alkyl-2-amino-1,3-propanediols such as **1a-c** (Figure 1). Some of them displayed more potent immunosuppressive activity than ciclosporin, which is currently used clinically. In addition to that, the toxicity of ISP-I was reduced to a considerable extent although it was still insufficient. Here, we displaced a part of the alkyl chain of **1a-c** with sterically equivalent 1,4-phenylene group in expectation of modifying their physicochemical, pharmacological, toxicological, or pharmacokinetical property. In this communication, we describe the design, synthesis, and structure-activity relationships of thus modified compounds.

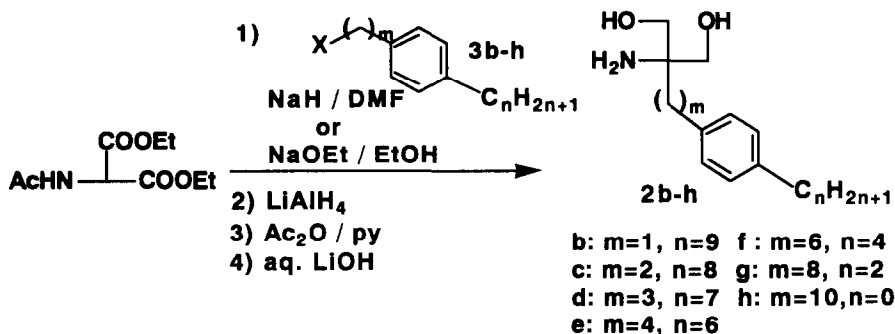
Figure 1



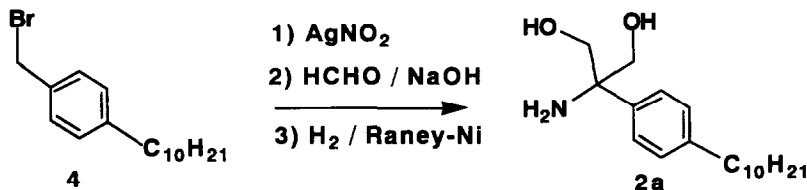
Design: Each of 2-amino-1,3-propanediols (**1a-c**) consists of both a hydrophilic part (amino alcohol) and a lipophilic part (hydrocarbon chain). The amphiphilicity should be one of the most important features of these compounds. The lipophilic side chain contains a number of rotatable bonds. The activity would be improved if conformation of the compound can be properly restricted. We planned to introduce a phenyl ring, which was considered an effective template for restricting the conformation of molecules², into the lipophilic side chain of **1** maintaining the total amphiphilicity of the molecule. We chose **1a** as a lead compound because **1a** had proved less toxic than **1b**³. We prepared a series of compounds possessing a phenyl ring on a variety of positions within the side chain keeping the chain length constant ($m+n=10$) (Figure 1).

Synthesis: Compounds **2b-h**⁴ were synthesized by a similar route to that described in the preceding paper¹ but (4-alkylphenyl)alkyl halides (**3b-h**; X=Br or I) were used instead of the simple alkyl bromides (Scheme 1). Compound **2a** was prepared in a totally different way (Scheme 2). 4-Decylbenzyl bromide (**4**) was nitrated with silver nitrate to give nitro compound, which was bishydroxymethylated using formalin and sodium hydroxide in ethanol⁵ followed by reduction with Raney-nickel to afford the desired compound **2a**.

Scheme 1. Synthesis of 2b-h.



Scheme 2. Synthesis of 2a.



Results and discussion: Compounds 2a-h were evaluated for their ability to inhibit mouse allogeneic MLR (IC_{50}) *in vitro* (Table 1)⁶.

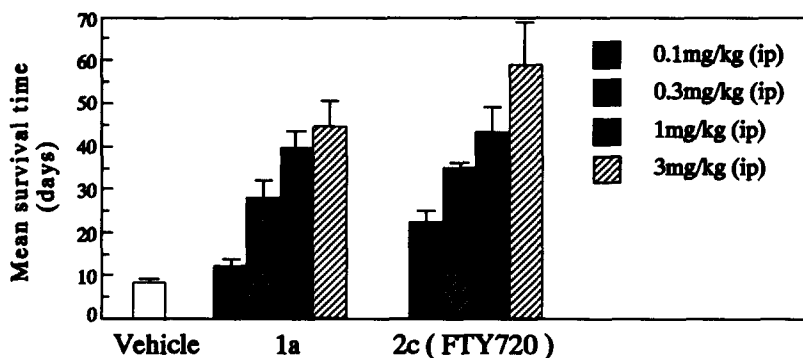
Table 1. Effect of 2a-h on mouse allogeneic MLR

	2a	2b	2c	2d	2e	2f	2g	2h
IC_{50} (nM)	13	70	6.1	350	19	100	32	54

All the compounds displayed moderate to potent inhibitory activity. Compound 2c (FTY720) was most potent among them and demonstrated comparable activity to the lead compound 1a⁷ (IC_{50} = 5.9nM). Moving the phenyl ring of 2c toward each direction by only one carbon (compounds 2b and 2d) resulted in a great loss in potency, suggesting that it is of critical importance for the potent activity where the phenyl ring is positioned within the side chain. Some "even-odd effect" was observed concerning the compounds 2a-2e. The compounds 2a, 2c, and 2e, the number of whose methylene units between the aminopropanediol terminus and the phenyl ring is even, were much more potent than the others (2b and 2d: odd number).

Compounds 1a and 2c were evaluated in rat skin allograft in combination with LEW donor and F344 recipient *in vivo* (Figure 2)⁸. FTY720 (2c) displayed remarkable immunosuppressive activity *in vivo* and prolonged rat skin allograft survival in a dose dependent manner. It was approximately 3-fold more potent than 1a.

Figure 2. Effect of 1a and 2c on rat skin allograft



Compound 2c also displayed excellent immunosuppressive activity in other administration routes *in vivo* (mean survival time: 24.8days/0.1mg/kg, 46.3days/3mg/kg, and 53.5days/10mg/kg, *iv*; 19.3days/0.1mg/kg, 41.0days/3mg/kg and 57.8days/30mg/kg, *po*). Moreover, 2c was not toxic in the rat skin allograft up to a dose of 10mg/kg, *iv*, while compounds 1a and 1b were toxic⁹ at a dose of 10 and 3mg/kg, *iv*, respectively. Our preliminary data show that the mechanism

of action of **2c** is different from that of ciclosporin and FK506^{10, 11}. Although **2c** did not inhibit the production of interleukin-2 unlike ciclosporin and FK506, it inhibited immune responses by selective depletion of mature T-cells probably caused by lymphocyte migration and apoptosis (data not shown here¹²).

Conclusion: We incorporated a phenyl ring into the side chain of the lead compound **1a** to obtain a novel immunosuppressant, FTY720 (**2c**), which displayed remarkable immunosuppressive activity both *in vitro* and *in vivo* as well as significant improvement in side effects. FTY720 (**2c**) is expected as a powerful candidate for safer immunosuppressant¹³ for organ transplantations and for the treatment of autoimmune diseases.

References and Notes:

1. Fujita, T.; Yoneta, M.; Hirose, R.; Sasaki, S.; Inoue, K.; Kiuchi, M.; Hirase, S.; Adachi, K.; Arita, M.; Chiba, K. *BioMed. Chem. Lett.*, preceding communication of this issue.
2. Moore, G.J. *TIPS*. **1994**, *15*, 124.
3. When intraperitoneally administered in the rat skin allograft, **1b** displayed intenser local irritation than **1a**.
4. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.
5. Feuer, H.; Nielsen, A. T.; Colwell, C. E. *Tetrahedron* **1963**, *19*, Suppl. 1, 57.
6. The effect of the compounds on mouse allogeneic MLR was examined by the method described in our previous paper: Fujita, T.; Inoue, K.; Yamamoto, S.; Ikumoto, T.; Sasaki, S.; Toyama, R.; Chiba, K.; Hoshino, Y.; Okumoto, T. *J. Antibiotics* **1994**, *47*, 208.
7. We also prepared 2-amino-2-[2-(4-nonylphenyl)ethyl]-1,3-propanediol (IC₅₀=8.5nM) corresponding to **1b**. It exhibited a slight decrease in potency compared with **1b** (IC₅₀=2.9 nM).
8. The effect of the compounds on rat skin allograft was examined by the method described in the preceding paper¹.
9. The term "toxic" used here means that animals die at indicated doses.
10. Mechanism of action : (a) Schreiber, S. L. *Science* **1991**, *251*, 283. (b) Liu, J.; Farmer, Jr., J. D.; Lane, W. S.; Friedman, J.; Weissmann, I.; Schreiber, S.L. *Cell* **1991**, *66*, 807. (c) Schreiber, S. L. *Cell* **1992**, *70*, 365.
11. FK506 : (a) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031. (b) Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics* **1987**, *40*, 1249.
12. Our study on mechanism of action of FTY720 (**2c**) will be reported in due course.
13. Ciclosporin and FK506 possess several adverse effects such as nephrotoxicity and neurotoxicity: (a) The U.S. Multicenter FK506 liver study group, *The New England Journal of Medicine* **1994**, *331*, 1110. (b) Japanese FK506 Study Group, *Transplantation Proceedings* **1993**, *25*, 649. (c) Japanese FK506 Study Group, *Transplantation Proceedings* **1991**, *23*, 3071.

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