Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 8, pp. 853-856, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00127-1

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

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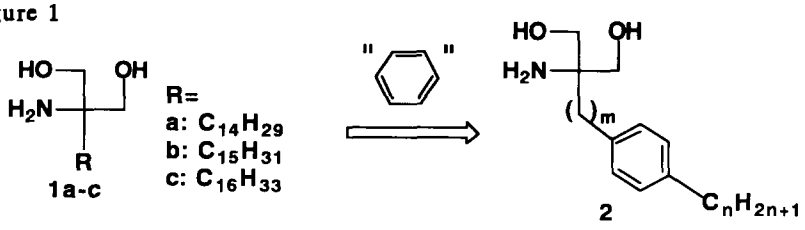
Abstract: FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol hydrochloride), a novel synthetic immunosuppressant led by modification of ISP-I (myriocin, thermozymocidin) displayed potent immunosuppressive activity both *in vitro* and *in vivo*.

As reported in the preceding communication[1], 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.

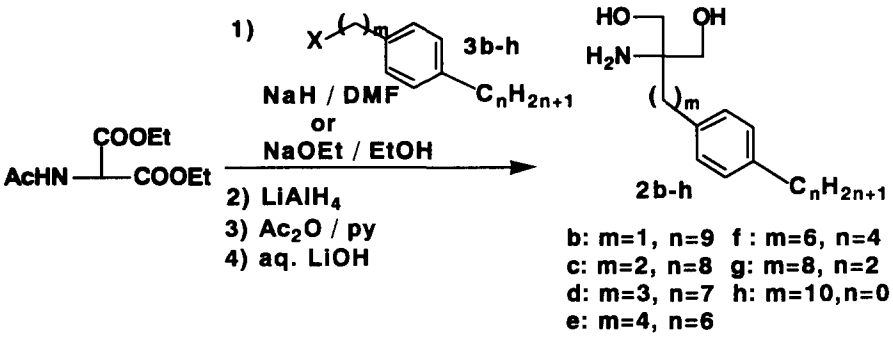
Figure 1



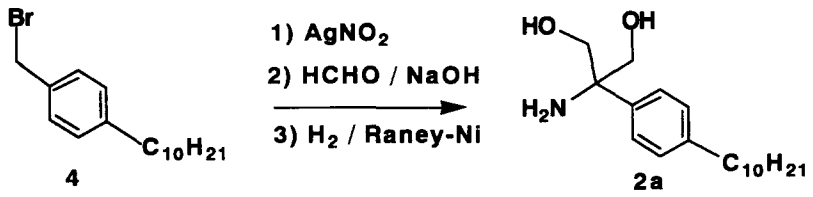
Design: Each of 2-amino-1,3-propanediols (**1a-c**) consists of both a hydrophilic part (amino alcohol) and a lipophilic part (hydrocarbon chain). The amphiphilicity should be one of the most important features of these compounds. The lipophilic side chain contains a number of rotatable bonds. The activity 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 molecules[2], 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 paper[1] 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 ethanol[5] 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].

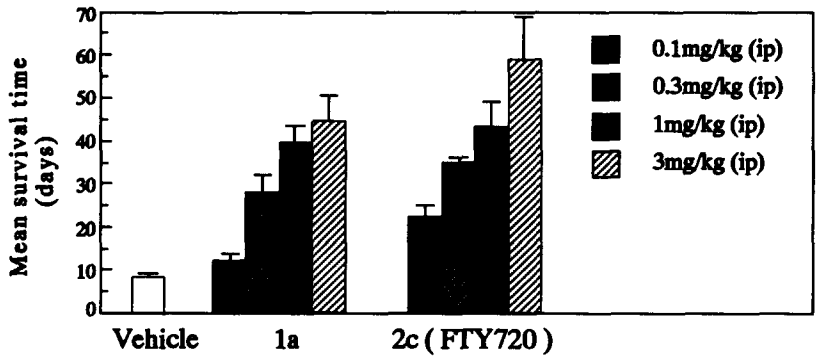
Table 1. Effect of 2a-h on mouse allogeneic MLR

	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 **1a**[7] (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 toxic[9] 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 responces by selective depletion of mature T-cells probably caused by lymphocyte migration and apotosis (data not shown here[12]).

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 immunosuppressant[13] 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 administrated 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 (IC_{50}=8.5nM) corresponding to **1b**. It exhibited a slight decrease in potency compared with **1b** (IC_{50}=2.9 nM).
8. The effect of the compounds on rat skin allograft was examined by the method described in the preceding paper[1].
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.

(*Received in Japan* 20 *February* 1995; *accepted* 6 *March* 1995)