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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, 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

\[
\text{HO} \quad \text{OH} \quad \text{R= a: C}_{14}\text{H}_{29} \quad \text{b: C}_{15}\text{H}_{31} \quad \text{c: C}_{16}\text{H}_{33} \\
\text{H}_2\text{N} \quad \text{R} \quad \text{1a-c} \quad \overset{\text{HO}}{\text{OH}} \quad \text{(m)} \quad \text{HO} \quad \text{OH} \\
\text{2} \quad \text{C}_n\text{H}_{2n+1} \\
\]

853
**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, 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. 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 were synthesized by a similar route to that described in the preceding paper 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 followed by reduction with Raney-nickel to afford the desired compound 2a.

**Scheme 1. Synthesis of 2b-h.**

1) LiAlH₄  
2) NaH / DMF or NaOEt / EtOH  
3) Ac₂O / py  
4) aq. LiOH

**Scheme 2. Synthesis of 2a.**

1) AgNO₂  
2) HCHO / NaOH  
3) H₂ / Raney-Ni
Results and discussion: Compounds 2a-h were evaluated for their ability to inhibit mouse allogeneic MLR (IC$_{50}$) in vitro (Table 1).  

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

<table>
<thead>
<tr>
<th></th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
<th>2f</th>
<th>2g</th>
<th>2h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ (nM)</td>
<td>13</td>
<td>70</td>
<td>6.1</td>
<td>350</td>
<td>19</td>
<td>100</td>
<td>32</td>
<td>54</td>
</tr>
</tbody>
</table>

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 (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). 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 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. 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 here).

**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 for organ transplantations and for the treatment of autoimmune diseases.

**References and Notes:**

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.
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.
9. The term "toxic" used here means that animals die at indicated doses.
12. Our study on mechanism of action of FTY720 (2c) will be reported in due course.

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