Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 8, pp. 847-852, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00126-3

SIMPLE COMPOUNDS, 2-ALKYL-2-AMINO-1,3-PROPANEDIOLS HAVE POTENT IMMUNOSUPPRESSIVE ACTIVITY.

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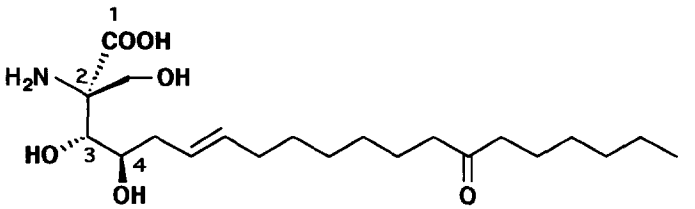
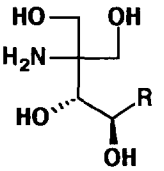
Abstract: ISP-I (myriocin, thermozymocidin) was structurally simplified to give 2-amino-2-alkyl-1,3-propanediols that were also potent immunosuppressants. Among the series, 2-amino-2-pentadecyl-1,3-propanediol most actively prolonged rat skin allograft survival and was more effective than ciclosporin.

Recently, our discovery of ISP-I[1] (**1**: myriocin[2] ,themozymocidin[3]) (Figure 1) as a potent immunosuppressant from the culture broth of *Isaria sinclairii* (ATCC 24400) has attracted attention of many synthetic chemists for the asymmetric total synthesis[4] of **1**. Because the structure of **1** resembles that of a biosynthetic precursor of sphingosine, some biochemists examine the compound in the area of biochemistry. Very recently, it was revealed that **1** inhibited enzyme reaction of serine palmitoyl transferase in IL-2-dependent mouse cytotoxic T cell line, CTLL-2[5].

Figure 1



1: ISP-I (myriocin, thermozymocidin)

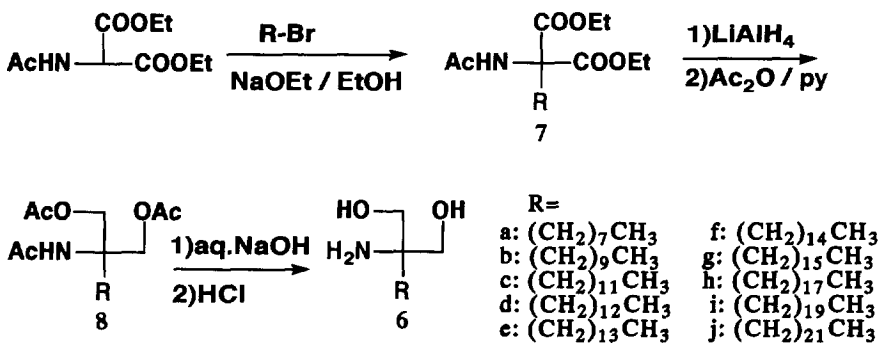


2: R= $CH_2CH=CH(CH_2)_6CHOH(CH_2)_5CH_3$
3: R= $CH_2CH=CH(CH_2)_{12}CH_3$
4: R= $(CH_2)_9CHOH(CH_2)_5CH_3$
5: R= $(CH_2)_{15}CH_3$

We have previously reported[6] that the side chain functionalities such as ketone and double bond were not always neccessary for the biological activities including suppression of lymphocyte proliferation in mouse allogeneic mixed lymphocyte reaction (MLR)[1b]. Furthermore, 4-deoxy compounds[7] such as mycestericins D (4-deoxy-ISP-I) and E (4-deoxy-3-*epi*-ISP-I), both of which are minor components of ISP-I producing fungus *Myceria sterilia*, have showed similar activity to that of **1** on the mouse allogeneic MLR, suggesting that the 4-hydroxyl group is not essential and that the configuration of 2-hydroxyl group plays no important role to develop the biological activities. Therefore, our aim was focused on further search of the fundamental unit of **1** for the biological activities and for the developement of a new clinical medicine as an immunosuppressant. We describe here that simple and symmetric compounds, 2-amino-2-alkyl-1,3-propanediols have a fundamental feature to express the biological activities as an immunosuppressant.

Synthesis: Semi-synthetic products[8] (**2-5**: Figure 1) were synthesized from **1**. Other simple analogs, 2-alkyl-2-amino-1,3-propanediols (**6a-j**) were synthesized by the routes outlined in Scheme 1. Diethyl 2-acetamidomalonate was alkylated in the presence of sodium ethoxide in ethanol to afford diethyl 2-alkylmalonates (**7a-j**) . Two successive treatments of **7a-j** (reduction with lithium aluminium hydride, then acetylation with acetic anhydride in pyridine) gave triacetates (**8a-j**). Hydrolysis of **8a-j** with aqueous sodium hydroxide afforded the target compounds **6a-j**.

Scheme 1



Results and discussion: Compounds **1-5**, **6a-j** were evaluated for their ability to inhibit mouse allogeneic MLR (IC_{50}, Table 1)[9]. Compound **2**, which was led by replacement of carboxy and ketone of **1** with hydroxymethyl and alcohol, respectively, possessed comparable activity to **1**, suggesting that the carboxyl group was not essential to the activity. Further simplification of the side chain decreased the activity (compounds **3-5**, Table 1). It is, however, interesting that the simplest analog **5** still retained the activity and was 5-fold more potent than **4**. Furthermore, **1** and the 4-deoxy derivatives, mycestericins D and E, showed similar activity[6] and racemic α-aminoicosanoic acid[10] (IC_{50}: 130 nM) still possessed the activity. Mycestericins D and E are epimeric isomers to each other at the position 3 and the α-aminoicosanoic acid lacks hydroxyl group at the position 3 in the molecule. Consequently, it appears that the hydroxyl groups at the positions 3 and 4 in **1** play no essential role on the activity.

Table 1. Effect of 1-5, 6a-j, and ciclosporin on mouse allogeneic MLR

	IC_{50}(nM)		IC_{50}(nM)
1	**3**	**6e**	**5.9**
2	**4.7**	**6f**	**2.9**
3	**56**	**6g**	**10**
4	**1630**	**6h**	**12**
5	**320**	**6i**	**190**
6a	**3700**	**6j**	**1600**
6b	**440**		
6c	**270**	**ciclosporin**	**14**
6d	**12**		

These results indicate that 2-octadecyl-2-amino-1,3-propanediol is the basic structure of ISP-I derivatives for the biological activities. This concept promoted us to examine a series of 2-alkyl-2-amino-1,3-propanediols (**6a-6j**) with no asymmetric centers. As was expected, the octadecyl analog **6h** having the same length of the side chain as **1** displayed potent activity although 4-fold decrease in potency was observed. Because a simple and symmetric compound **6h** possessed potent immunosuppressive activity, further refinement of the side chain length was carried out. Its requirement for optimal activity was quite specific (Figure 2). Five compounds **6d-h** were more potent than ciclosporin[11]. The pentadecyl analog **6f** was most potent among them and was 5-fold more potent than ciclosporin.

Next, compounds **6b-j** were also evaluated in rat skin allograft in combination with LEW donor and F344 recipient *in vivo* (Figure 2 and Table 2)[12]. We found apparent correlations between *in vitro* and *in vivo* activities. Compound **6f** displayed the most potent activities and demonstrated superior effectiveness to ciclosporin both *in vitro* and *in vivo*. The toxicity of **1** was also reduced to a considerable extent by structure simplification: **6f** was approximately 10-fold less toxic than **1**.

Figure 2. Effect of 6b-j on rat skin allograft and mouse allogeneic MLR.

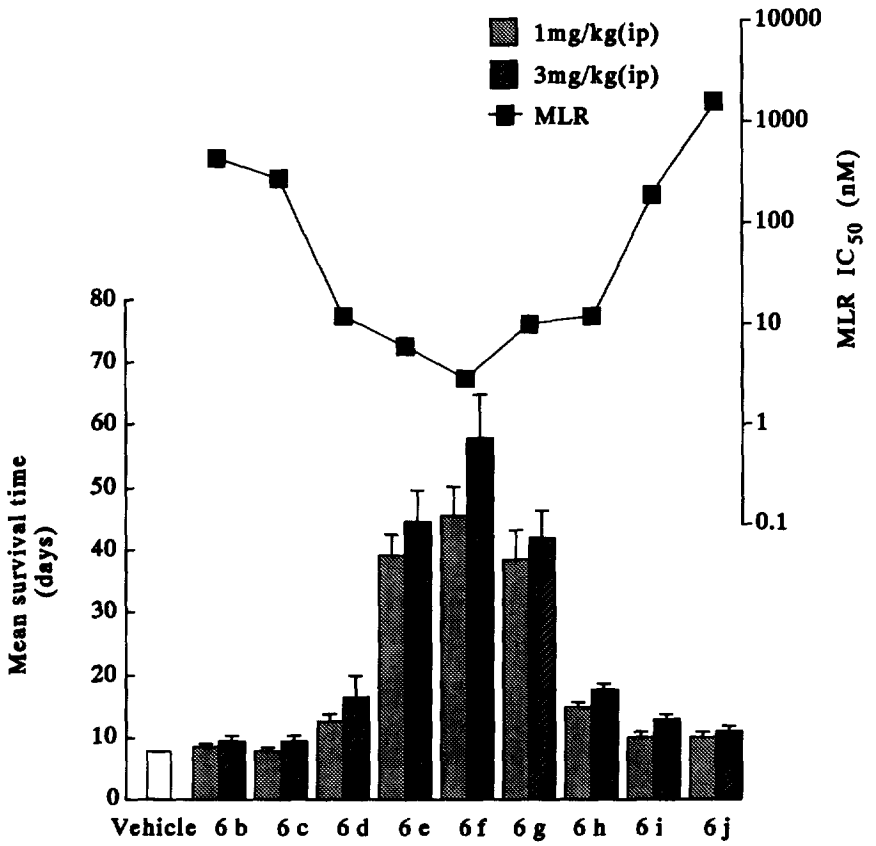


Table 2. Effect of 1, 6f, and ciclosporin on rat skin allograft.

	Mean survival time (days)							
	0.03	0.1	0.3	1	3	10	30	100 (mg/kg, ip)
ISP-I (1)	7.8	9.2	11.2	toxic*				
6f	10.8	14.0	33.5	45.3	57.8	toxic*		
ciclosporin				7.3	10.8	15.2	19.2	toxic*

* Animals died

Conclusion: 2-Alkyl-2-amino-1,3-propanediol skelton was confirmed to be minimally essential for the immunosuppressive activity of **1**. Compounds **6e-g** having such a partial structure were more effective than ciclosporin with the test on skin allograft survival in rats. These would be promising as lead compounds in the discovery of an immunosuppressant effective for organ transplantations and for the treatment of autoimmune diseases.

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8. All new compounds in this communication gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.
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12. The effect of compounds on rat skin allograft was examined as follows. The dorsal skin of LEW rats ($RT1^l$, male, 4 weeks old) was transplanted to the lateral thorax of F344 rats ($RT1^{lv1}$, male, 4 weeks old). The compounds were dissolved in 20% hydroxypropyl-β-cyclodextrin and intraperitoneally administrated daily for 10 days beginning on the day of transplantation.

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