Synthetic N-Alkylated Iminosugars as New Potential Immunosuppressive Agents

Guan-Nan Wang, Yulan Xiong, Jia Ye, Li-He Zhang, and Xin-Shan Ye*

State Key Laboratory of Natural and Biomimetic Drugs and School of Pharmaceutical Sciences, Peking University, Xue Yuan Road No. 38, Beijing 100191, China

Supporting Information

ABSTRACT: The new emerging immunosuppressive effects displayed by iminosugars have not been much investigated so far. Several new N-alkyl dideoxy iminoalditols were designed and synthesized to explore their immunosuppressive effects. These iminosugars inhibited the proliferation of mouse splenocytes and the secretion of both IFN-γ and IL-4, which are the hallmark cytokines of Th1 and Th2 cells, respectively. Some compounds exerted good inhibitory effects. More importantly, the synthetic iminosugars prolonged the allograft survival in the mouse skin transplantation experiment. Our results provide a lead for further elucidation of the structure—activity relationships and modifications of iminosugars for better immunosuppressive agents.

KEYWORDS: Iminosugar, immunosuppressive agent, mouse skin allograft, synthesis

The search to find better immunosuppressants has been stimulated by the need to improve transplant survival and to decrease the toxicity of current agents. The main clinically used immunosuppressive drugs, such as cyclosporin A (CsA), sirolimus, tacrolimus, and mycophenolate mofetil, have significant side effects including hypertension, dyslipidemia, hyperglycemia, and neurotoxicity and may lead to liver and kidney injury.1–3 So, more effective and safer immunosuppressants are in great demand.

Iminosugars, widespread in plants and microorganisms, are carbohydrate mimetics in which the endocyclic oxygen is replaced by nitrogen.4 These kinds of compounds raise many synthetic challenges, and as inhibitors or chaperones of carbohydrate-processing enzymes,5 they open the way to treat a wide range of diseases including diabetes,6 viral infections,7 tumor metastasis,8 and lysosomal storage disorders.9 However, the use of iminosugar derivatives as immunosuppressive agents is an area that is less explored. To date, only castanospermine, a naturally occurring indolizidine alkaloid (bicyclic polyhydroxylated iminosugar), has been found to exhibit some immunosuppressive activity.10,11 Our recent investigation disclosed that some synthetic iminosugars, especially N-alkylated derivatives, displayed immunosuppressive activity in vitro.12–14 On the basis of the previous studies in our group, in this report, new N-alkylated 1,5-dideoxy-l,5-iminopentitols and N-alkylated 1,4-dideoxy-l,4-imnotretitol have been synthesized. These iminosugars show inhibitory effects on proliferation of mouse splenocytes induced by concanavalin A (Con A) as well as the secretion of cytokines from the mouse splenocytes. More importantly, the inhibitory effects of these iminosugars have been confirmed in the mouse skin transplantation model.

Iminosugars continue to be of great synthetic interest because of the intrinsically pharmacological potential and the need for more potent and selective compounds.15 1,5-Dideoxy-l,5-iminopentitols 1–3 (Figure 1) were isolated from E. fortunei TURZ by Kusano and co-workers in 1995 and have been shown to be the active components of the extracts of this plant, which are traditionally used in Chinese and Japanese folk medicine as diuretic, antipyretic, emmenagogue, and antidiabetic agents.16 Since then, a number of new derivatives of compounds 1–3 have been synthesized and have been displayed to be good inhibitors of glycosidases.17–21

Previously, a one-pot tandem reaction to construct N-substituted δ-lactams was reported by us.22 On the basis of this reaction, a new and expeditious approach to the synthesis of N-alkylated dideoxy-iminoalditols was developed. As shown in Scheme 1, the exoglucal 4 was easily prepared from methyl α-D-glucopyranoside through short three steps23 or five steps in high overall yield (77%).24 The ozonolysis of compound 4 at −78 °C gave the methoxyl acetal lactone 5, which was then subjected to the one-pot amination and cyclization tandem reaction providing N-substituted δ-lactams in high yield. The N-substituted δ-lactams were subsequently reduced by BH₃·THF, which was followed by catalytic hydrogenolysis to afford N-alkylated 1,5-dideoxy-l,5-iminoyltolts 8a and 8b in excellent yield.

Figure 1. 1,5-Dideoxy-l,5-iminopentitols isolated from E. fortunei TURZ.

Received: April 17, 2011
Accepted: July 5, 2011
Published: July 05, 2011
Compounds 8a and 8b can be regarded as N-alkylated 5-de-(hydroxymethyl)-1-deoxyxojirimycin. Our synthetic strategy was then extended to prepare N-alkylated 5-de-(hydroxymethyl)-1-deoxygalactonojirimycin (12) and N-alkylated 5-de-(hydroxymethyl)-1-deoxymannonojirimycin (16) by a similar procedure starting from exogalactose and exomannose alkenes, respectively (Scheme 2). The reduction of lactams needs more explanation. It is known that lactams are generally reduced by reducing agents such as lithium aluminum hydride (LiAlH₄) and sodium borohydride. When LiAlH₄ was chosen as the reductant, it worked very well for the reduction of glucose type δ-lactams 6a and 6b; however, LiAlH₄ was not a good reductant for galactose type δ-lactam 10 and mannose type δ-lactam 14. The yield for the reduction of compound 10 was poor (20%), and LiAlH₄ did not reduce the compound 14 at all. Although the LiAlH₄ reductive reaction could be influenced by many factors, it is proposed that the steric hindrance around the carbonyl group and the chelation of cis-benzylether with aluminum preventing the delivery of hydride are possible reasons for this phenomenon, since there are axial bonds with benzyloxy groups on them in both compound 10 and compound 14. BH₃-THF was finally used to reduce different type lactams 10 and 14, and both worked very smoothly. Although it was reported that in some circumstances the adduct of BH₃ product was too tight to release the free iminosugar, 24 BH₃-iminosugar was successfully dissociated by 6 N HCl during the workup operation.

Besides N-alkylated 1,5-dideoxy-l,5-iminopentitols, the N-alkylated 1,4-dideoxy-l,4-iminotetritol 21 was also designed since these kinds of structures have been rarely reported on both chemistry and biological activity. Our one-pot tandem procedure provides an expeditious access to this type of compounds. As shown in Scheme 3, the exogalactyl 18 was prepared from the

Figure 2. N-Decyl lactams used in the immunosuppressive assay.
corresponding 5-iodo-ribofuranoside through a one-step elimination and benzylation process. Ozonolysis of compound followed by the tandem reaction gave N-decyl γ-lactam. The lactam was subsequently reduced by using BH₃·THF to yield compound, which was deprotected to afford the target compound. On the other hand, to make comparison, N-decyl 6-lactams 6a, 10, and 14 and N-decyl γ-lactam 19 were also deprotected to provide compounds 22, 23, 24, and 25, respectively (Figure 2).

With five iminoalditols 8a, 8b, 12, 16, and 21 and four lactams 22–25 in hand, their effects on mouse splenocyte proliferation induced by Con A were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measured the mitochondrial dehydrogenase activity of surviving cells (Figure 3). The mouse splenocytes were incubated with 2.5 μg/mL of Con A with 30 μM concentration of each compound at 37 °C and 5% CO₂ for 48 h. The assays were conducted using the Con A-treated splenocytes as the experimental control and CsA (1 μM, 58.3% inhibitory rate) as the positive control. None of the lactams 22–25 exhibited inhibitory effects of more than 40% on mouse splenocyte proliferation induced by Con A. However, most of the iminoalditols, as shown in Figure 3, displayed good inhibitory effects on splenocyte proliferation. Especially, the inhibitory rates of compounds 12 (57.2%), 16 (59.0%), and 21 (61.0%) were more than 50%. The inhibitory rate of compound 8a (45.9%) was a little lower than that of compounds 12, 16, and 21 but much better than compound 8b (27.1%). The only difference between compound 8a and compound 8b is the substituent group on the nitrogen atom, so it seems that N-decyl chain is much better than N-octyl chain on the behavior of inhibition. Moreover, the activity difference between lactams and iminoalditols indicates that some structural features are essential for inhibition, although there is a certain redundancy around the six- or five-membered iminosugars.

Next, we tested the effects of the N-alkylated dideoxy iminoalditols on the secretion of cytokines from mouse splenocytes. The spleen cells induced by 2.5 μg/mL of Con A were incubated with each compound at 37 °C and 5% CO₂ for 48 h. The amount of cytokines was measured with enzyme-linked immunosorbent assay. All of these five compounds showed inhibitory ability to the interleukin (IL)-4 secretion. As compared to the control, the levels of IL-4 secretion were reduced by 60.9, 8.7, 95.7, 85.6, and 88.6% when including 30 μM compounds 8a, 8b, 12, 16, and 21, respectively (Figure 4, 96.8% for CsA at 1 μM). It was found that among the five dideoxy iminoalditols, compound 12 displayed the strongest inhibitory effects on the release of the cytokine IL-4.

The assay on secretion of interferon (IFN)-γ from splenocytes was similar to the assay of IL-4. The supernatant of the spleen cells was detected by mice IFN-γ ELISA kit. All of these five compounds showed inhibition to the IFN-γ secretion. The levels of IFN-γ secretion were reduced by 89.1, 40.3, 78.1, 75.0, and 94.7% when including 30 μM compounds 8a, 8b, 12, 16, and 21, respectively (Figure 5, 97.1% for CsA at 1 μM). The inhibition efficiency of iminosugar 21 was the strongest out of the five compounds.

Figure 3. Effects of iminoalditols on Con A-induced mouse splenocytes proliferation were assessed by the MTT assay. Values are means ± SEMs; **p < 0.01, and ***p < 0.001 vs control.

Figure 4. Effects of iminoalditols on the secretion of IL-4 from mouse splenocytes induced by Con A. Values are means ± SEMs; ***p < 0.001 vs control.

Figure 5. Effects of iminoalditols on the secretion of IFN-γ from mouse splenocytes induced by Con A. Values are means ± SEMs; ***p < 0.001 vs control.
Table 1. Effects of Compounds 8a, 12, 16, and 21 on Mouse Skin Allograft* (MST ± SE, Days)

<table>
<thead>
<tr>
<th>compound</th>
<th>MST ± SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vel</td>
<td>11.25 ± 1.83</td>
</tr>
<tr>
<td>8a</td>
<td>14.71 ± 1.70**</td>
</tr>
<tr>
<td>12</td>
<td>14.14 ± 2.54*</td>
</tr>
<tr>
<td>16</td>
<td>15.20 ± 2.39**</td>
</tr>
<tr>
<td>21</td>
<td>15.60 ± 0.55**</td>
</tr>
</tbody>
</table>

*a Grafts were inspected daily and were considered to be rejected when no viable donor epidermis remained. **MST in days. Each group consisted of eight mice with half males and half females (body weight, 18–22 g)."P < 0.05, and ""P < 0.01 vs Vel group. The subcutaneous LD50 of compound 21 is 392.1 mg/kg in mouse.

On the basis of the results of the mouse skin allograft together with the splenocyte proliferation and cytokine assays, iminotritol 21 was better than the others, although it is hard to further tell the suppression difference between compounds 8a, 12, and 16. It seems that the long hydrophobic N-decyl group is more favored for the immunosuppressive activity, indicating that there might be a lipophilic pocket in the binding site of the target biomacromolecule. Moreover, the activity difference between lactams 22–25 and iminoalditols 8a, 12, 16, and 21 in proliferation assays indicates that dideoxy iminoalditol scaffolds are good for inhibition. These N-decyl dideoxy iminoalditols not only showed good inhibition in the splenocyte proliferation and cytokine assays but also improved the mouse skin allograft survival by 3–5 days.

Generally, iminosugars are potent inhibitors of many carbohydrate-processing enzymes. All cytokines are N-glycoproteins. The immunosuppressive activities by these dideoxy iminoalditols may come from the N-glycoprotein-processing inhibition effects on these glycoproteins. However, there are other possibilities for the immunosuppressive effects of these iminoalditols with a long hydrophobic chain. These N-alkylated iminoalditols might bind to some lipophilic sites of proteins by the alkyl group. The mechanism of this kind of iminosugars working on immune system needs to be further explored.

In summary, we report herein the synthesis of hitherto unknown N-alkylated 1,5-dideoxy-l,5-iminopentitols and N-alkylated 1,4-dideoxy-1,4-iminotritol by an expeditious strategy. These iminosugars inhibit the mouse splenocyte proliferation induced by Con A. Further studies revealed that the inhibitory effects on splenocyte proliferation may come from the suppression of both IFN-γ and IL-4 cytokines, which are the hallmark cytokines for Th1 and Th2 cells, respectively. Importantly, these iminosugars can prolong the allograft survival on the mouse skin transplantation model. Although the effects are not as good as CsA in mouse skin allograft at the current stage, to our knowledge, they are the first synthetic iminosugars that show immunosuppressive activities in animal model. It is a forward step for investigating immunosuppressive effects of iminosugars. Our results provide a lead for further elucidation of the structure—activity relationships on iminosugars and modifications for better immunosuppressive agents.

** ASSOCIATED CONTENT

Supporting Information. Full experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel: +(86)10-82805736. E-mail: xinshan@bjmu.edu.cn.

Funding Sources
This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 21072014 and 20732001).

ABBREVIATIONS
CsA, cyclosporin A; Con A, concanavalin A; Th, T helper cells; Tc, T cytotoxicity cell; MST, mean survival time; LD50, median lethal dose
REFERENCES


(9) Fan, J.-Q. Iminosugars as Active-Site-Specific Chaperones for the Treatment of Lysosomal Storage Disorders. In Iminosugars: From Synthesis to Therapeutic Applications; Compain, P., Martin, O. R., Eds.; John Wiley & Sons Ltd.: New York, 2007; pp 225/C0.


(30) The structure and potency of compounds described in our previous research have been brieﬂy summarized in the Supporting Information.