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LETTER

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# Synthesis of *C*-Pseudonucleosides Bearing Thiazolidin-4-one as a Novel Potential Immunostimulating Agent

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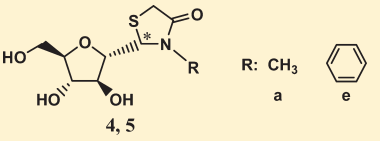
*Supporting Information*

**ABSTRACT:** Several novel *C*-pseudonucleosides bearing thiazolidin-4-one were synthesized by one-pot three-component condensation using unprotected sugar aldehyde at room temperature, and their effects on T cells, B cells, the cytokine secretion of IL-2, IL-4, and IFN-$\gamma$, T cell-associated molecules (CD3, CD4, CD8), and B cell-associated molecules (CD19) were first evaluated. The experimental data demonstrated that such thiazolidin-4-one *C*-pseudonucleosides hold potential as immunostimulating agents.




**KEYWORDS:** Immunostimulating agents, *C*-pseudonucleosides, thiazolidin-4-one

Immunostimulators, as adjuvant therapeutic agents for the treatment of many tumors and infectious diseases such as the AIDS epidemic, have aroused great attention due to their highly potential value to enhance the ability of the human immune system.[1] Many different compounds, such as biological macromolecules, including glycoproteins,[2] polynucleotides,[3] and polysaccharides,[4] and small molecules, including heterocycles,[5] nucleosides,[6] glycosylceramides (glycolipids),[7] and iminosugars,[8] have been demonstrated to possess immunostimulatory properties. Some of them have been applied in the clinic as immunoadjuvants or immunomodulating drugs, such as interferon,[2] $\beta$-glucan,[4] levamisole,[9] and pidotimod[10] (Figure 1A, B), and so on. However, the drawbacks, including side-effects, low titer, and high cost, limited their widely applications in clinical trails.[11] It is still of great interest and challenge to explore more effective and safe immunostimulants for treating immunologic disorders.

Nucleoside-based small molecule immunostimulants that affect the proliferation and potentiation of the cytokine-producing humoral cells have offered considerable promise in drug development.[6] Among them, the guanosine derivatives (e.g., Loxoribine, Figure 1D) have been extensively studied and proved to hold potential as immunostimulating agents.[12] Nonclassic base-modified nucleosides represent a large group of pseudonucleosides in which the natural pyrimidine or purine base was replaced by heterocycles such as thiazole, imidazole, triazole, triazine, etc.[13] However, such nucleoside analogues are scarcely explored for their immunomodulating activity, although they have been well documented as promising anti-infective and antitumor agents.[14,15]

The thiazolidin-4-one ring is a core substructure in various synthetic pharmaceuticals which are associated with diverse biological activities,[16] such as anticancer, antiviral, and anti-inflammatory, and some thiazolidine derivatives; for instance, levamisole, pidotimod, and CGP52608 (Figure 1C)[17] exhibited

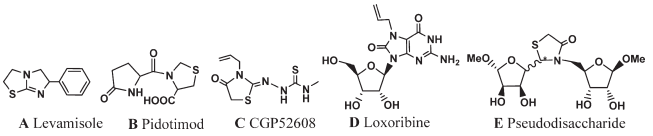



**Figure 1.** Some chemical small molecule immunostimulating agents.

strong immunostimulating activities. More recently, we have found that the thiazolidin-4-one linked pseudodisaccharides (Figure 1E) showed very good immunostimulatng activity,[18] which prompted us to develop the novel *C*-pseudonucleosides having a thiazolidin-4-one moiety for investigating their immune activity. Herein, as the continuation of our studies on functionalized nucleoside analogues,[19] we would like to report a simple and convenient synthesis of novel *C*-pseudonucleosides bearing thiazolidin-4-one (**4** and **5**) by one-pot, three-component condensation from the unprotected sugar aldehyde **1** (Scheme 1), and their biological effects on T cells, B cells, and the cytokine secretion of IL-2, IL-4, and IFN-$\gamma$ from mouse splenocytes. Furthermore, the effects on the membrane expression of T cell-associated molecules (CD3, CD4, CD8) and B cell-associated molecules (CD19) were also investigated.

The requisite sugar aldehyde **1** has been readily prepared in one step from D-glucosamine hydrochloride in the high yield of 85%.[20] The *C*-pseudonucleosides **4** and **5** were synthesized by one-pot, three-component condensation from the unprotected sugar aldehyde **1**, aliphatic primary amines or anilines **2a**−**e**, and mercaptoacetic acid **3** at room temperature, as shown in Scheme 1. The one-pot synthesis, following our reported precedure,[19] but without using the condensation reagent *N*,*N*′-dicyclohexylcarbodiimide

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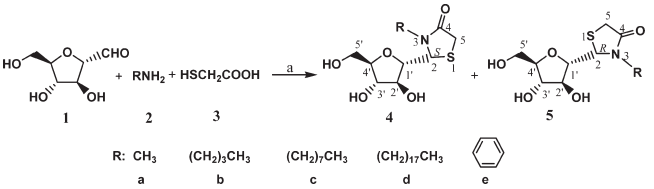
**Scheme 1. Reagents and Conditions: (a) 1 (1 mmol), 2 (1 equiv), 3 (2 equiv), dry EtOH (3 mL), rt, 1 h**



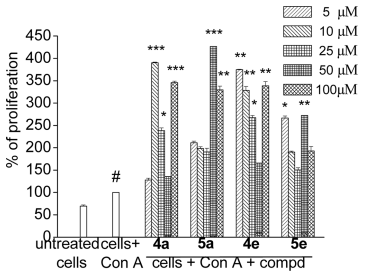




**Figure 2.** Effects of compounds **4a**, **5a**, **4e**, and **5e** on Con A-induced splenocyte proliferation. Data are means ± SEM of at least three independent experiments. $^{\#}p < 0.001$ with respect to the untreated cells; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the Con A-treated cells.

(DCC) and the promoter 4-dimethylaminopryidine (DMAP), directly afforded the diastereomeric products **4** (less polar) and **5** (more polar), respectively, in the overall yields of 36−43%, after simple working up and purification by reverse silica ($C_{18}$) gel column chromatography. This modified reaction provided a more direct, simple, and efficient access to the target *C*-pseudonucleosides containing thiazolidin-4-one using the unprotected sugar aldehyde, although the yields in the condensation step were moderate compared to those reported,[21,22] in which the protected sugars were used and a deprotection must be done to give the target molecules.

The structures of the *C*-pseudonucleosides **4** and **5** were assigned on the basis of the analyses of their spectral data of NMR and HRMS(ESI) (see the Supporting Information). The X-ray crystallographic analysis of compound **5a** indicated that the configuration of the new generated chiral carbon (C-2) is in the (*R*) form. Accordingly, its diastereomer **4a** should have the (*S*) form. The circular dichroism (CD) spectra of compounds **4** showed positive Cotton effects nearly at 230 nm and negative Cotton effects at 205 nm, while **5** showed opposite Cotton effects at the corresponding regions, providing a further support to the configurations of C-2 in the diastereomers **4** (*S*) and **5** (*R*).

The effects of the *C*-pseodonucleosides **4** and **5** on the concavalin A (Con A)-induced proliferation of mouse splenocyte were assessed by the MTT method. The cells were harvested after 72 h incubation at 37 °C, 5% $CO_2$. As shown in Figure 2, compounds **4a**, **5a**, **4e**, and **5e** promoted Con A-induced T cell proliferation significantly at certain concentrations. Compared to the Con A-treated sample, the treatment with the combination of Con A and **4a** (5, 10, 25, 50, and 100 $\mu$M) increased the proliferation by 28, 290, 138, 35, and 245%, respectively, and the concentration dependence was not obvious. Compound **5a** (5, 10, 25, 50, and 100 $\mu$M) also increased the Con A-induced cell proliferation irregularly by 111, 98, 91, 327, and 230%, respectively. Similarly concentration-independent phenomena were

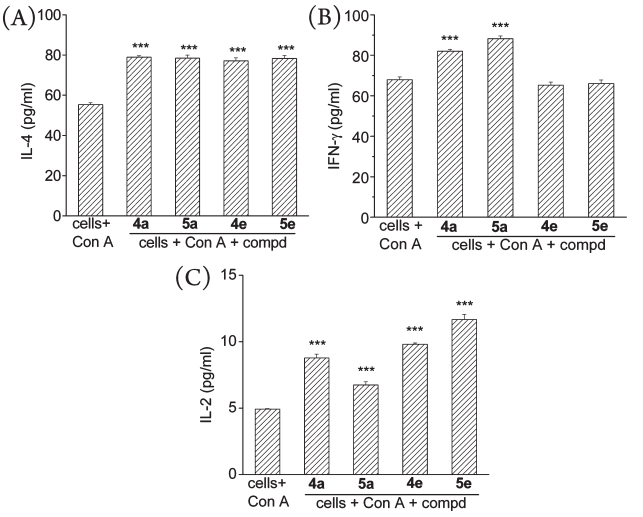



**Figure 3.** Effects of compounds **4a**, **5a**, **4e**, and **5e** on IL-4 (A), INF-$\gamma$ (B), and IL-2 (C) in mouse splenocyte cultures. The concentrations of compounds were 10, 50, 5, and 5 $\mu$M, respectively. Data are means ± SEM of at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

also found in compounds **4e** and **5e**, which showed the most pronounced effects on T cell proliferation, at 5 and at 50 $\mu$M, respectively. However, the other compounds, **4b**, **5b**, **4c**, **5c**, **4d**, and **5d**, did not express significant effects on T cells proliferation at the tested concentrations, and all the proliferation rates were below 50% (not shown). The results suggested that the immunostimulating activity decreased with the elongation of the alkyl chain on the *N*-3 position at the thiazolidin-4-one ring. In addition, the similar activity of the diastereomeric compounds **4a** and **5a** (or **4e** and **5e**) implied that the configurations in C-2 did not have significant effects on their immunostimulatory activity.

In the absence of Con A, compounds **4a**, **5a**, **4e**, and **5e** could not induce cell proliferation, implying that the compounds synergistically induced the immune response with Con A. Next we assayed the effects of these four compounds on the secretion of various cytokines in the culture of total splenocytes. Using a mice ELISA kit, the levels of the cytokines, such as interleukin-4 (IL-4), IL-2, and interferon-$\gamma$ (IFN-$\gamma$), were measured from the supernatant of cell cultures in the presence of Con A (20 $\mu$M) in combination with the compounds synthesized above. Compounds **4a**, **5a**, and **4e** at their optimized effective concentrations of 10, 50, and 5 $\mu$M were tested respectively. Since **5e** at 5 $\mu$M (166%) and 50 $\mu$M (171%) demonstrated similar effects on Con A-induced cell proliferation, 5 $\mu$M of **5e** were tested.

Compared with Con A alone, a combination of Con A and **4a**, **5a**, **4e**, or **5e** increased the levels of IL-4 secretion by 43, 42, 39, and 41% ($p < 0.001$), respectively (Figure 3 A), which indicated that the compounds had similar proliferation effects on IL-4. With regard to IFN-$\gamma$, compounds **4a** and **5a** obviously increased the secretion levels of this cytokine by 21 and 30% ($p < 0.001$), respectively, while compounds **4e** and **5e** had no effects on IFN-$\gamma$ secretion (Figure 3B). Compounds **4a**, **4e**, and **5e** also markedly induced the secretion levels of IL-2 by 78, 99, and 138% ($p < 0.001$), respectively, and compound **5a** increased slightly the IL-2 level by 37% (Figure 3C). Clearly, compounds **4e** and **5e** had more pronounced effects on IL-2 secretion than compounds **4a** and **5a**.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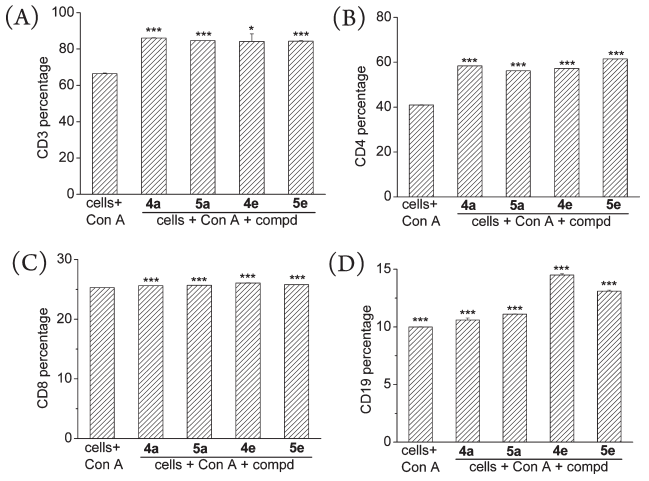


**Figure 4.** Proliferative effects on CD3+ (A), CD4+ (B), CD8+ (C), and CD19+ (D) cells of compounds **4a**, **5a**, **4e**, and **5e** with the concentration of 10, 50, 5, and 5 $\mu$M, respectively. Data are means ± SEM of at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

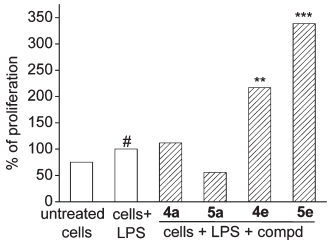


**Figure 5.** Effects of compounds **4a**, **5a**, **4e**, and **5e** on 10 $\mu$g/mL LPS-induced splenocyte proliferation. The concentrations of compounds were 10, 50, 5, and 5 $\mu$M, respectively. Data are means ± SEM of at least three independent experiments. $^{\#}p < 0.01$ with respect to the untreated cells; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to the LPS-treated cells.

The effects of the compounds on the T cells expressing CD3, CD4, and CD8 and on the B cells expressing CD19 were assayed by the flow cytometer (FCM).[23] Compared to the Con A-treated cells, the four compounds **4a**, **5a**, **4e**, and **5e** had proliferative effects on CD3+T cells and CD4+T cells (Figure 4A, B). However, they had no effects on CD8+T cells (Figure 4C). On the other hand, compounds **4e** and **5e** demonstrated stronger proliferative effects on the CD19+B cell population than compounds **4a** and **5a** did (Figure 4D), which indicated that compounds **4e** and **5e** preferred to promote B cell proliferation more than compounds **4a** and **5a** (Figure 5, compounds **4e** and **5e** at 5 $\mu$M increased the LPS-induced cell proliferation by 117 and 238%, respectively). These observations suggested that the *C*-nucleosides **4a** and **5a** had potentials in proliferative effects on the CD4+T cells, and compounds **4e** and **5e** specifically stimulated the proliferation of both CD4+T cells and B cells.

CD4+T cells which recognize the antigen peptide and MHC II complex include T helper type 1 and 2 ($T_{H1}$ and $T_{H2}$) cells. $T_{H1}$ cells secrete IFN-$\gamma$ and IL-2, while $T_{H2}$ cells secrete IL-4. IL-4, the hallmark cytokine of the $T_{H2}$ cells, stimulates B cells to secrete antibodies and improves the proliferation and differentiation of T-cells as a key regulator in humoral and adaptive immunity.[24] IL-2 secreted by the $T_{H1}$ cells is instrumental in the body's natural response to microbial infection. IFN-$\gamma$, the hallmark cytokine of the $T_{H1}$ cells, shows antiviral, immunoregulatory, and antitumor properties and is used to treat infectious diseases, although it may precipitate autoimmunity. The imbalance between $T_{H1}$ and $T_{H2}$ cytokines is closely related with the outcome of many diseases. $T_{H1}$ responses predominate in organ-specific autoimmune disorders, in acute allograft rejection, and in some chronic inflammatory disorders. In contrast, $T_{H2}$ responses predominate in Omann's syndrome, transplantation tolerance, chronic graft-versus-host disease, systemic sclerosis, and allergic diseases.

Compounds **4a** and **5a** (R = $CH_3$) had a significant capability to augment all of the secretions of IL-4, IFN-$\gamma$, and IL-2. Therefore, they might have immunopotentiating efficiency via both $T_{H1}$- and $T_{H2}$-mediated cellular as well as humoral immune activation. On the other hand, compounds **4e** and **5e** (R = phenyl) increased IL-4 and IL-2 secretions and inhibited IFN-$\gamma$, which indicated that they might have a bias via $T_{H2}$-mediated cellular immunity. Furthermore, compounds **4a** and **5a** specifically promoted CD3+ and CD4+T cell proliferation, while compounds **4e** and **5e** could enhance the proliferation of CD3+, CD4+T, and B cells. The results would suggeste that (1) such *C*-pseudonucleosides bearing thiazolidine-4-ones **4a**, **5a**, **4e**, and **5e** were good immunostimulators in controlling infection and triggering the response to antibodies and (2) the difference of the substituent at *N*-3 on the thiazolindin-4-one ring would lead to different mechanisms for the compounds to proliferate different cells, possibly due to the different structural regions.

In conclusion, we have reported a simple and convenient synthesis of *C*-pseudonucleosides bearing thiazolidin-4-one as base from the unprotected sugar aldehyde, aliphatic and alkyl primary amines, and mercaptoacetic acid at room temperature. Biological tests revealed that compounds **4a**, **5a**, **4e**, and **5e** had significant effects on T cell proliferation at certain concentrations. The preliminary structure−activity relationship (SAR) analysis indicated that the configuration difference in C-2 between **4** (*S*) and **5** (*R*) had little effect on their immunostimulating activity, and as the alkyl chain on the N-3 position at the thiazolindin-4-one ring lengthened, the immunostimulating activity reduced. Given that the compounds act synergistically with the mitogen Con A, they may be suitable adjuvants with immunboosting drugs and vaccines. The interesting findings may open a new avenue in the development of a new class of pseudonucleoside drugs possessing immunoregulatory activity.

## ■ ASSOCIATED CONTENT

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

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### Author Contributions

The manuscript was written through contributions of all authors.

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