Short Research Article

Simple Synthesis of Sulfonyl Amidine-Containing Glucosidase Inhibitors by a Chemoselective Coupling Reaction Between D-Gluconothiolactam and Sulfonyl Azides

ABSTRACT

In this report, we describe a simple synthesis of gluconoamidinylsulfones as a new class of potential inhibitors toward glycan processing enzymes. Gluconoamidinylsulfones have a glucose-based sulfonyl amidine skeleton, thus would form a distorted half-chair conformation with positive charge, which is analogous to transition state in the enzymatic process. A chemoselective coupling reaction between thioamide and sulfonyl azide enabled one-step synthesis of the iminosugar derivatives from commercially available D-gluconothiolactam in a protection-free manner. The phenyl-substituted gluconoamidinylsulfone displayed high inhibitory ability toward α- and β-glucosidases with K_i values of 13.9 and 8.2 μM, respectively, resulting that gluconoamidinylsulfones would be expected to entry in a new class of promising potential inhibitors toward various glycan-processing enzymes.

Keywords: inhibitor, glycosidase, iminosugar, coupling reaction, one-step synthesis, thioamide, sulfonyl azide, gluconoamidinylsulfone

1. INTRODUCTION

Iminosugars have been developed as small-molecule inhibitors for various types of glycan processing enzymes [1,2]. These inhibitors are crucial for detailed understanding of glycobiology as well as for development of new promising medicines prescribed in the treatment of diabetes, Gaucher's disease, influenza infection, HIV, hepatitis, cancer, etc., involving glycoside-bond cleavage pathway [3]. Chemical structures of glycosidase-inhibiting natural products afford valuable inspirations to design their synthetic analogues. Miglitol [4,5] and miglustat [6,7] are representative therapeutic iminosugars for diabetes and type-1 Gaucher's disease (GD1), respectively, derivatized from a natural glucosidase inhibitor, 1-deoxynojirimycin [8] (Fig. 1a). On the other hand, rationally designed iminosugars based on the mechanism of the enzymatic hydrolysis reaction have also been synthesized as candidates of lead compounds for glycosidase inhibition [3,9]. A mechanism-based general strategy is transition-state analogue of the enzymatic process. Shape and charge are the key points to mimic the transition state, that is, a partially planar structure in distorted half-chair conformations and delocalized positive charge around the anomeric center of the glycosyl oxocarbenium intermediates (Fig. 1b), the species considered close to the transition state of the hydrolysis process, are important common factors for designing potent glycosidase inhibitors. One of the successful inhibitors designed in this way is gluconoamidines (Fig. 1c) that have both a partially flattened geometry and positive charge at a basic amidine center under physiological conditions, which exhibited remarkable inhibition abilities toward glycosidases [10,11,12].

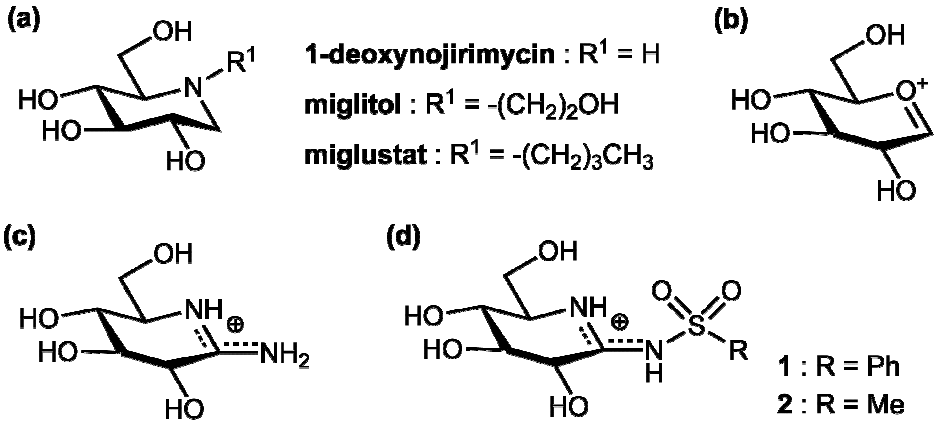
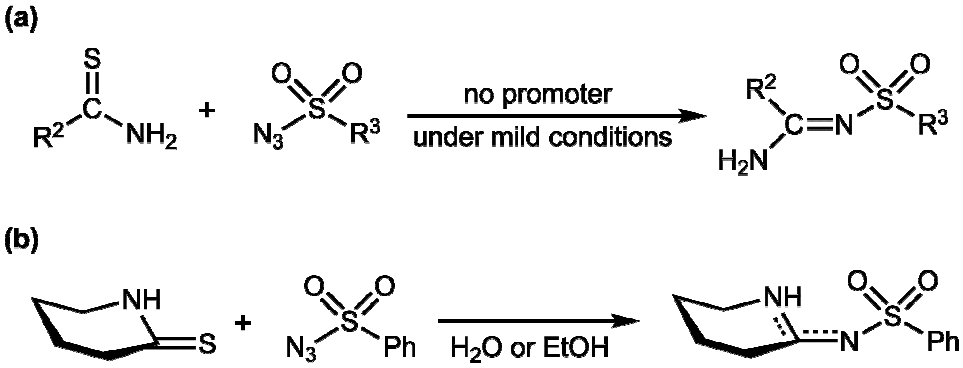


Fig. 1. Chemical structures of (a) 1-deoxynojirimycin, miglitol, and miglustat, (b) a glycosyl oxocarbenium intermediate, (c) a gluconoamidine (protonation form under physiological conditions), and (d) a gluconoamidinylsulfone (protonation form under physiological conditions)

We recently reported a chemoselective reaction between thioamides and sulfonyl azides to yield sulfonyl amidines without side reaction even under the coexistence of hydroxyl, amino, and carboxyl groups [13,14] (Scheme 1a). The reaction proceeds in various solvents without any activation additives. In the previous report [13], a six-membered cyclic thioamide, 2-thiopiperidone, with methyl and phenyl sulfonyl azides showed good reactivity in EtOH or H_2O (Scheme 1b). Inspired by the structural similarity of 2-thiopiperidone with iminosugars, we considered that commercially available D-gluconothiolactam would afford sulfonyl amidine derivatives of D-gluconolactam, namely gluconoamidinylsulfones (Figure 1d), by the coupling reaction with sulfonyl azides. Here we report simple synthesis of gluconoamidinylsulfones **1** and **2** as a new class of potential inhibitors for glycosidase. In addition, conventional inhibitory assay of these compounds toward α- and β-glucosidases is also described.



Scheme 1. Chemoselective coupling reaction between (a) thioamides and sulfonyl azides in general and (b) 2-thiopiperidone and phenyl sulfonyl azide

2. MATERIAL AND METHODS

2.1 General

^1H and ^{13}C NMR spectra were obtained at 400 and 100 MHz, respectively, on a JEOL ECX-400P spectrometer. ESI-HRMS analyses were conducted on a JEOL JMS-T100LC mass spectrometer or Thermo LTQ Orbitrap XL ETD.

2.2 Materials

D-Gluconothiolactam **3** is commercially available but takes long time to arrive, thus **3** was synthesized by simple procedures shown in Scheme 3. D-Gluconolactam **5** [15,16], phenyl sulfonyl azide [14], and mesyl azide [17] were prepared according to literature procedures. Other materials including dehydrate-grade solvents were all commercially available.

2.3 Synthetic procedures

***N*- Sulfonylphenyl D-gluconoamidine 1**

A 0.5 mL aqueous solution of **3** (6.1 mg, 0.03 mmol) and phenyl sulfonyl azide (30.1 mg, 0.15 mmol) was stirred at 50℃ for 24 h. After removal of the solvent, the residue was purified by silica gel PTLC (preparative thin layer chromatography) with a development solvent of $CHCl_3$:MeOH = 2:1 to afford **1** as a colorless solid (6.4 mg, 68%). ^{1}H NMR (400 MHz, D_2O): δ 7.82 (d, J = 4.0 Hz, 2 H), 7.61–7.48 (m, 3 H), 4.01 (d, J = 4.0 Hz, 1 H), 3.85 (dd, J = 12.0, 4.0 Hz, 1 H), 3.63 (t, J = 10.0 Hz, 1 H), 3.52–3.44 (m, 3 H), 3.42–3.34 ppm (m, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 166.7, 140.3, 134.3, 133.9, 130.1, 126.7, 126.4, 73.4, 71.7, 68.1, 61.5, 59.5 ppm. ESI-HRMS (*m/z*) calcd for MH^+, $C_{12}H_{16}N_2O_6S$: 317.0808; found 317.0812.

***N*-Sulfonylmethyl D-gluconoamidine 2**

A 1 mL aqueous solution of **3** (5.8 mg, 0.03 mmol) and mesyl azide (17.8 mg, 0.15 mmol) was stirred at room temperature for 72 h. After removal of the solvent, the residue was purified by silica gel PTLC with a development solvent of $CHCl_3$:MeOH = 2:1 to give **2** as a colorless solid (6.2 mg, 82%). ^{1}H NMR (400 MHz, D_2O): δ 4.06 (d, J = 8.0 Hz, 1 H), 3.85 (dd, J = 12.0, 4.0 Hz, 1 H), 3.69 (t, J = 8.0 Hz, 1 H), 3.63 (dd, J = 16.0, 4.0 Hz, 1 H), 3.57 (t, J = 6.0 Hz, 1 H), 3.42–3.34 ppm (m, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 166.6, 73.4, 71.6, 68.0, 61.3, 59.6, 41.7 ppm. ESI-HRMS (*m/z*) calcd for MH^+, $C_7H_{14}N_2O_6S$: 255.06516; found 255.0653.

2,3,4,6-Tetra-*O*-(*tert*-butyldimethylsilyl)-D-gluconolactam 6

A mixture of D-gluconolactam **5** (189 mg, 1.1 mmol), *tert*-butyldimethylsilyl chloride (TBDMSCl; 1.97 g, 12.8 mmol) and imidazole (1.76 g, 25.7 mmol) in anhydrous DMF (2 mL) was vigorously stirred at 0℃ for 30 min then at room temperature for 3 days under an argon atmosphere. The mixture was poured into ice water (10 mL) and extracted with Et_2O (3x15 mL). The combined organic phase was washed with saturated NaCl aqueous solutions (5x15 mL), dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed (SiO_2; eluent, hexane:EtOAc = 5:1) to give **6** (352 mg, 51%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (s, 1 H), 3.91 (bs, 1 H), 3.81 (d, J = 4.0 Hz, 1 H), 3.76 (dd, J = 20.0 Hz, 4.0 Hz, 1 H), 3.67 (bs, 1 H), 3.55–3.47 (m, 2 H), 0.87–0.80 (m, 35 H), 0.08–0.013 ppm (m, 25H). ESI-HRMS (*m/z*) calcd for MNa^+, $C_{30}H_{67}NO_5Si_4Na$: 656.3994; found 656.3982.

2,3,4,6-Tetra-*O*-(*tert*-butyldimethylsilyl)-D-gluconothiolactam 7

A mixture of **6** (352 mg, 0.55 mmol) and Lawesson's reagent (167 mg, 0.41 mmol) in toluene (2.5 mL) was refluxed for 3 h. After removal of the solvent *in vacuo*, the residue was chromatographed (SiO_2; eluent, hexane:EtOAc = 20:1) to give **7** (288 mg, 80%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1 H), 4.51 (bs, 1 H), 3.90 (dd, J = 12.0, 4.0 Hz, 2 H), 3.78 (bs, 1 H), 3.63–3.57 (m, 2 H), 0.96–0.86 (m, 36 H), 0.18–0.015 ppm (m, 24 H). ESI-HRMS (*m/z*) calcd for MH^+, $C_{30}H_{67}NO_4SSi_4$: 650.3947; found 650.3954.

D-Gluconothionolactam 3

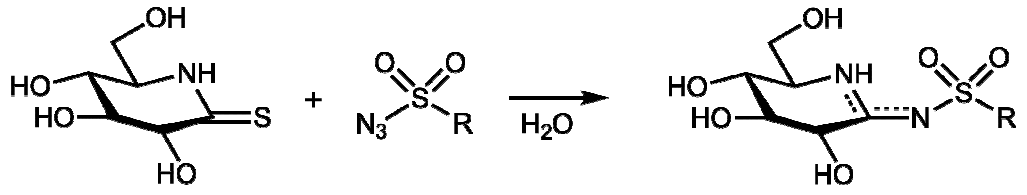
To a MeOH (6 mL) solution of **7** (153 mg, 0.235 mmol) was added AcCl (221 mg, 2.82 mmol) at room temperature and the reaction mixture was stirred for 6 h at that temperature. After removal of the solvent, the residue was dissolved in water. The aqueous solution was then flushed through a reverse-phase chromatograph cartridge (Mega BE 18C) to give **3** (46 mg, 98%) as a colorless solid. ^{1}H NMR (400 MHz, D_2O): δ 3.88 (d, J = 8.0 Hz, 1 H), 3.77 (dd, J = 12.0, 4.0 Hz, 1 H), 3.71 (d, J = 8.0 Hz, 1 H), 3.66 (dd, J = 12.0, 4.0 Hz, 1 H), 3.57 (t, J = 10 Hz, 1 H), 3.34–3.29 ppm (m, 1 H). ^{13}C NMR (100 MHz, D_2O): δ 204.0, 75.2, 73.4, 68.0, 62.6, 60.2 ppm. ESI-MS (*m/z*) calcd for MH^+, $C_6H_{11}NO_4S$: 194; found 194.

2.4 Enzymatic assays

Glucosidase inhibitory assays were carried out for **1**, **2**, and 1-deoxynojirimycin by means of a conventional spectrometric method [18] at 37℃ using 0.01 M KH_2PO_4/K_2HPO_4 buffer solution (pH 6.8). As a substrate, 4-nitrophenyl-α-glucopyranoside (α-NGP) toward α-glucosidase from yeast *Saccharomyces cereviceae* or 4-nitrophenyl-β-glucopyranoside (β-NGP) toward β–glucosidase from sweet almonds was selected. In advance to the assays, stock solutions of enzymes (2U/mL for α-glucosidase and 6U/mL for β-glucosidase), substrates (1 mM), and inhibitors (1, 5, 10, 25, 50, 100, and 1000 μM) were prepared by diluting with the buffer. Mixed solutions of an appropriate amount of α-NGP or β-NGP substrate with various concentrations of each inhibitor solution were poured in cells of a 96-well microplate. Addition of the enzyme solution to the cells immediately progressed the enzymatic reaction, affording 4-nitrophenolate anion as a cleavage product that can be monitored at 405 nm of absorbance by a microplate reader (FilterMax F5^{TM}). The absorption data were simultaneously collected from 1 to 25 min at 2 min intervals. In these assays, the final concentrations of the substrates were 16.7, 33.3, 50.0, 66.7, and 83.3 μM for α-glucosidase whereas 30.0, 60.0, 90.0, 120, and 150 μM in absence or presence of the inhibitors. The inhibition constants (K_i) were determined by using the slopes of Lineweaver–Burk plots and double reciprocal analysis. All experiments were conducted in duplicate and obtained data were averaged.

3. RESULTS AND DISCUSSION

One-step syntheses of gluconoamidinylsulfones **1** and **2** were performed by simply mixing of commercially available D-gluconothiolactam **3** with phenyl sulfonyl azide or mesyl azide in water (Scheme 2). The reaction mixture was vigorously stirred for an appropriate reaction time, affording gluconoamidinylsulfones **1** and **2** in 68% and 86% isolated yields, respectively. These compounds were characterized by means of ^{1}H NMR, ^{13}C NMR and ESI-HRMS (electrospray ionization high resolution mass spectrometry). Because regio- and stereoselective sugar derivatization generally tends to be a complicated multistep synthesis involving protection and deprotection steps, it is noteworthy that, by using this coupling reaction, one-step synthesis in a protection-free manner generated a new class of potential inhibitors directly.

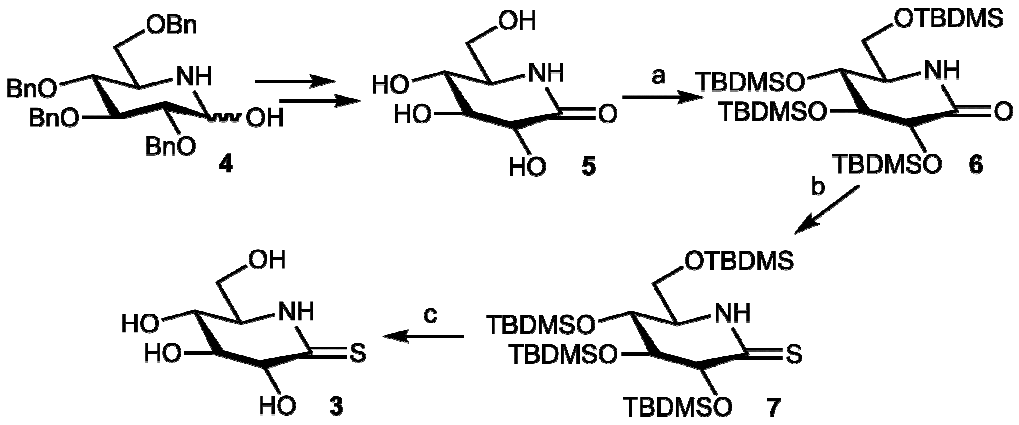


3 **1** : R = Ph, **2** : R = Me

Scheme 2. Protection-free, one-step synthesis of gluconoaminidylsulfones 1 and 2 by the coupling reaction

D-gluconothiolactam **3** is commercially available, however, it seems to take about two months to arrive. Thus, we prepared **3** separately according to literature procedures [10,15,16] with minor modification

(Scheme 3). Gluconolactam **5** was initially synthesized from a readily purchasable **4** in a manner similar to those reported [15,16]. Unfortunately, direct thioamidation of **5** by Lawesson's reagent (LR) was failed to produce **3**. Silylation of **5** with TBDMSCl followed by thioamidation with LR was a successful route, affording a thiolactam derivative **7**. Removal of the silyl-protection with AcCl in MeOH generated D-gluconothiolactam **3** quantitatively.

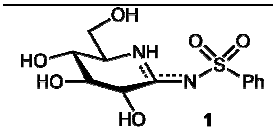
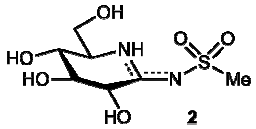


Scheme 3. Synthesis of D-gluconothiolactam

Reagents and reaction conditions: (a) TBDMSCl, imidazole, dry DMF, rt, 3 days, 51%, (b) Lawesson's reagent, toluene, reflux, 3 h, 80%, (c) AcCl, MeOH, rt, 6 h, 98%.

Next, enzymatic assay was performed for gluconoamidinylsulfones toward glucosidases by means of a conventional spectroscopic methodology with 4-nitrophenyl α- or β-glucopyranoside as a substrate. Table 1 shows the inhibitory ability of gluconoamidinylsulfones **1** and **2** against α-glucosidase from yeast *S. cereviceae* (E.C. 3.2.1.20) and β-glucosidase from almonds (E.C. 3.2.1.21). In advance, trial assays were conducted by using 1-deoxynojirimycin, which displayed the inhibition constant K_i of 24.5 μM for α–glucosidase and 28.1 μM for β–glucosidase. Although multiple K_i values have been reported for 1-deoxynojirimycin toward the glycosidases under the different measurement setup [1,8], the obtained K_i values in this study fall within the range of values, indicating that our assay condition and analysis procedure would be appropriate and reliable. Phenyl-substituted **1** exhibited strong inhibition against both α- and β-glucosidases with K_i of 13.9 and 8.2 μM, respectively, being more high inhibitory ability than that of 1-deoxynojirimycin. On the other hand, methyl-substitution showed weak inhibition against both α- and β-glucosidases.

Table 1. Inhibition constants of 1 and 2 toward α- and β-glucosidases

Inhibitors	K_i for α-glucosidas[a] (μM)	K_i for β-glucosidas[b] (μM)
 **1**	13.9	8.2
 **2**	>1000	764

a: *α-glucosidase from yeast S. cereviceae, b: β-glucosidase from sweet almonds.*

Glycosyl hydrases have several subsites in their binding pocket such as glycon and aglycon binding sites [18]. The aglycon subsites are usually made up of several hydrophobic residues such as phenylalanine, tyrosine, and tryptophan surrounding a ligand saccharide. Therefore, as an old trick, connecting a hydrophobic glycon analogue to aglycon mimics like iminosugars has been used for glycol-modification [19,20]. The strong glucosidase inhibition of the gluconoamidinylsulfone **1** seemly caused by the aglycon phenyl-group that might fit with the aglycon subsite of α- and β-glucosidases. From the analysis of amino acid sequence and three-dimensional structure, there are several phenylalanine residues around aglycon subsite of glucosidases [21]. In addition to the geometrically and electrostatically well-fitting of the gluconoamidine skeleton at the glycon subusite, hydrophobic and π-π interactions at the aglycon site might affect effectively, at least in part, to the inhibition activity of phenyl-substituted **1**, while the methyl group in **2** exhibited weak interaction with the subsite. Although only two gluconoamidinylsulfones and glycosidases were used in this report, the results indicate that further study may reveal the potential of this skeleton as a new class of inhibitors toward various glycan processing enzymes such as mannosidases, galactosidases, and transferases, by taking advantage of the simple synthetic approach.

4. CONCLUSION

The chemoselective coupling reaction of D-gluconothiolactam and sulfonyl azides successfully generated gluconoamidinylsulfones in a simple synthetic manner. The phenyl-substituted gluconoamidinylsulfone showed high inhibitory ability toward α- and β-glucosidases so that gluconoamidinylsulfones would be expected to entry in a new class of promising potential inhibitors toward various glycosidases. Making the best use of the synthetic advantage, expansion of the compound library of gluconoamidinylsulfones and the following enzyme assays toward wide variety of glycosyl hydrases and transferases are currently in progress.

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