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

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Authors’ contributions

This work was carried out in collaboration between all authors. Author JC designed the study, performed the syntheses and the analyses and wrote the first draft of the manuscript. Author MA performed the syntheses and the measurements, wrote the protocol and managed the literature searches. Authors TT and YH supervised and managed the analyses of the study. All authors read and approved the final manuscript.

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 Kᵢ 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; gluconooimidinylsulfone.

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 glycosidase inhibitors [3,9]. A mechanism-based general strategy is transition-state analogue of the enzymatic hydrolysis process. Shape and charge are the key points to mimic the enzymatic process. One of the successful inhibitors designed in this way is gluconoamidinylsulfone (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 [10-12].

We recently reported a chemoselective reaction between thioamides and sulfonyl azides to yield sulfonyl amidines without side reaction even under the coexistence of hydroxy, amino, and carboxy 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-thiothiopiperidone, with methyl and phenyl sulfonyl azides showed good reactivity in EtOH or H2O (Scheme 1b). Inspired by the structural similarity of 2-thiothiopiperidone with iminosugars, we considered that commercially available D-glucosithiolactam would afford sulfonyl amidine derivatives of D-glucosithiolactam, namely gluconooimidinylsulfones (Fig. 1d), by the coupling reaction with sulfonyl azides. Here we report simple synthesis of gluconoimidinylsulfones 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.

![Chemical structures of (a) 1-deoxynojirimycin, miglitol, and miglustat, (b) a glycosyl oxocarbenium intermediate, (c) a gluconooimidinylsulfone (protonation form under physiological conditions), and (d) a gluconoimidinylsulfone (protonation form under physiological conditions)](image)
2. MATERIALS AND METHODS

2.1 General

\(^1\)H 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 Thermo LTQ Orbitrap XL ETD mass spectrometer.

2.2 Materials

D-Gluconothiolactam 3 is commercially available from FCH Group (order number: FCH3937573) but takes long time around 8 weeks 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 (Wako Pure Chemical Industries, Ltd. and Tokyo Chemical Industry Co., Ltd.).

2.3 Synthetic Procedures

2.3.1 N-Sulfonylphenyl D-gluconoamidine 1

A mixture of 3 (6.1 mg, 0.03 mmol) and phenyl sulfonyl azide (30.1 mg, 0.15 mmol) in distilled water (0.5 mL) was vigorously stirred at 50°C 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\(_2\)O, TSP): \(\delta\) 7.96 (dd, J = 1.4, 7.6 Hz, 2 H), 7.74 (tt, J = 1.4, 7.6 Hz, 1 H), 7.64 (t, J = 7.6 Hz, 2 H), 4.14 (d, J = 9.6 Hz, 1 H), 3.96 (dd, J = 3.2, 11.6 Hz, 1 H), 3.73 (t [dd], J = 9.6 Hz, 1 H), 3.64 (t [dd], J = 7.2, 11.6 Hz, 1 H), 3.50 ppm (dd, J = 3.2, 7.2, 9.6 Hz, 1 H). \(^{13}\)C NMR (100 MHz, D\(_2\)O, TSP): \(\delta\) 169.0, 142.5, 136.5, 132.3, 128.9, 75.6, 74.0, 70.3, 63.8, 61.8 ppm. ESI-HRMS (m/z) calcd for MH\(^+\), C\(_{12}\)H\(_{17}\)N\(_2\)O\(_5\): 317.0807; found 317.0801, and calcd for MNa\(^+\), C\(_{12}\)H\(_{16}\)N\(_2\)O\(_5\)Na: 339.0627; found 339.0620.

2.3.2 N-Sulfonylmethyl D-gluconoamidine 2

A mixture of 3 (5.8 mg, 0.03 mmol) and mesyl azide (17.8 mg, 0.15 mmol) in distilled water (1 mL) was vigorously 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\(_2\)O, TSP): \(\delta\) 4.18 (d, J = 9.2 Hz, 1 H), 3.96 (dd, J = 2.8, 12.0 Hz, 1 H), 3.80 (t [dd], J = 9.6 Hz, 1 H), 3.72 (t [dd], J = 9.6 Hz, 1 H), 3.69 (dd, J = 6.4, 12.0 Hz, 1 H), 3.53 (ddd, J = 2.8, 6.4, 9.2 Hz, 1 H), 3.13 ppm (s, 3 H). \(^{13}\)C NMR (100 MHz, D\(_2\)O, TSP): \(\delta\) 168.8, 75.6, 73.8, 70.2, 63.6, 61.8, 43.9 ppm. ESI-HRMS (m/z) calcd for MH\(^+\), C\(_{7}\)H\(_{13}\)N\(_2\)O\(_5\): 255.0651; found 255.0644, and calcd for MNa\(^+\), C\(_{7}\)H\(_{12}\)N\(_2\)O\(_5\)Na: 277.0470; found 277.0463.

2.3.3 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°C 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\(_2\)O (3x15 mL). The combined organic phase was washed...
with saturated NaCl aqueous solution (5x15 mL) and then dried over MgSO₄. After removal of the solvent, the residue was chromatographed (SiO₂; eluent, hexane:EtOAc = 5:1) to give 6 (352 mg, 51%) as a colorless solid. ¹H NMR (400 MHz, CDCl₃, TMS): δ 7.54 (brs, 1 H), 3.96–3.98 (m, 1 H), 3.86 (dd, J = 1.6, 3.2 Hz, 1 H), 3.80 (dd, J = 3.2, 10.0 Hz, 1 H), 3.69–3.75 (m, 1 H), 3.60 (dt, J = 1.6, 6.4 Hz, 1 H) 3.55 (dd, J = 7.6, 10.0 Hz, 1 H), 0.892 (s, 9 H), 0.887 (s, 18 H), 0.86 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H), 0.11 (s, 6 H), 0.10 (s, 3 H), 0.07 (s, 9 H), 0.889 (s, 9 H), 0.886 (s, 9 H), 0.869 (s, 9 H), 0.866 (s, 9 H), 0.18 (s, 3 H), 0.17 (s, 3 H), 0.16 (s, 3 H), 0.12 (s, 3 H), 0.10 (s, 3 H), 0.09 (s, 3 H), 0.07 (s, 3 H), 0.06 ppm (s, 6 H). ESI-HRMS (m/z) calcd for MN⁺, C₃₀H₄₀NO₅Si₄Na: 656.3994; found 656.3982.

2.3.4 2,3,4,6-Tetra-O-(tert-butyldimethylsilyl)-D-glucothiolactam 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 (SiO₂; eluent, hexane:EtOAc = 20:1) to give 7 (288 mg, 80%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃, TMS): δ 7.90 (brs, 1 H), 4.50–4.52 (m, 1 H), 3.86–3.90 (m, 2 H), 3.74–3.80 (m, 1 H), 3.56–3.63 (m, 2 H), 0.90 (s, 9 H), 0.898 (s, 9 H), 0.886 (s, 9 H), 0.86 (s, 9 H), 0.18 (s, 3 H), 0.17 (s, 3 H), 0.16 (s, 3 H), 0.12 (s, 3 H), 0.10 (s, 3 H), 0.09 (s, 3 H), 0.07 (s, 3 H), 0.06 ppm (s, 6 H). ESI-HRMS (m/z) calcd for MH⁺, C₃₀H₄₀NO₅Si₄: 650.3946; found 650.3954.

2.3.5 D-Gluconoethiolactam 3

AcCl (221 mg, 2.82 mmol) was added slowly and dropwise to a MeOH (6 mL) solution of 7 (153 mg, 0.235 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. ¹H NMR (400 MHz, D₂O, TSP): δ 3.98 (d, J = 9.6 Hz, 1 H), 3.86 (dd, J = 2.8, 12.4 Hz, 1 H), 3.83 (t [dd], J = 9.6 Hz, 1 H), 3.76 (dd, J = 4.0, 12.4 Hz, 1 H), 3.65 (t [dd], J = 9.6 Hz, 1 H), 3.42 ppm (dd, J = 2.8, 4.0, 9.6 Hz, 1 H). ¹³C NMR (100 MHz, D₂O, TSP): δ 206.2, 77.4, 75.6, 70.2, 64.8, 62.5 ppm. ESI-MS (m/z) calcd for MH⁺, C₃₀H₄₀NO₅S: 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°C using 0.01 M KH₂PO₄/K₂HPO₄ buffer solution (pH 6.8). As a substrate, 4-nitrophenyl-α-glucopyranoside (α-NPG) toward α-glucosidase from yeast Saccharomyces cerevisiae or 4-nitrophenyl-β-glucopyranoside (β-NPG) 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-NPG or β-NPG 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™). 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) 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 gluconoaminidylsulfones 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 gluconoaminidylsulfones 1 and 2 in 68% and 86% isolated yields, respectively. These compounds were characterized by means of ¹H NMR, ¹³C NMR and ESI-HRMS (electrospray ionization high resolution mass spectrometry) (Fig. 2). Because regio- and stereoselective sugar derivatization generally tends to be a complicated multistep synthesis involving protection and deprotection steps, it is notable that, by using this coupling reaction, one-step synthesis in a protection-free manner generated a new class of potential inhibitors directly.
Scheme 2. Protection-free, one-step synthesis of gluconoamidinylsulfones 1 and 2 by the coupling reaction

Fig. 2. $^1$H NMR (A and B: 400 MHz in D$_2$O with TSP), $^{13}$C NMR (C and D: 100 MHz in D$_2$O with TSP), and ESI-HRMS (E and F: under the data collection time of 1 min after mass calibration with polyethylene glycol) spectra of gluconoamidinylsulfones 1 (left side) and 2

D-glucodontiolactam 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 TBDMSCI 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-gluconoamidinylsulfone 3 quantitatively.

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 constants of 24.5 μM for α-glucosidase and 28.1 μM for β-glucosidase (Table 1). Although multiple Kᵢ values have been reported for 1-deoxynojirimycin toward the glucosidases under the different measurement setup [1,8], the obtained Kᵢ values in this study fall within the range of these 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 Kᵢ 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.

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

![Scheme 3. Synthesis of D-gluconoamidinylsulfone](image)

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

| Inhibitors                      | Kᵢ for α-glucosidas (µM) | Kᵢ for β-glucosidas (µM) |
|---------------------------------|--------------------------|--------------------------|
| 1                               | 13.9                     | 8.2                      |
| 2                               | >1000                    | 764                      |
| 1-deoxynojirimycin              | 24.5                     | 28.1                     |

a: α-glucosidase from yeast S. cereviceae, b: β-glucosidase from sweet almonds.
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 gluconoamide skeleton at the glycon subsite, 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 glucosidases 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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