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Research Article

Thio-β-D-glucosides: Synthesis and Evaluation as Glycosidase Inhibitors and Activators

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Structurally simple 1-thio-β-D-glucopyranosides were synthesized and tested as potential inhibitors toward several fungal glycosidases from *Aspergillus oryzae* and *Penicillium canescens*. Significant selective inhibition was observed for α- and β-glucosidases, while a weak to moderate activation for α- and β-galactosidases.

1. Introduction

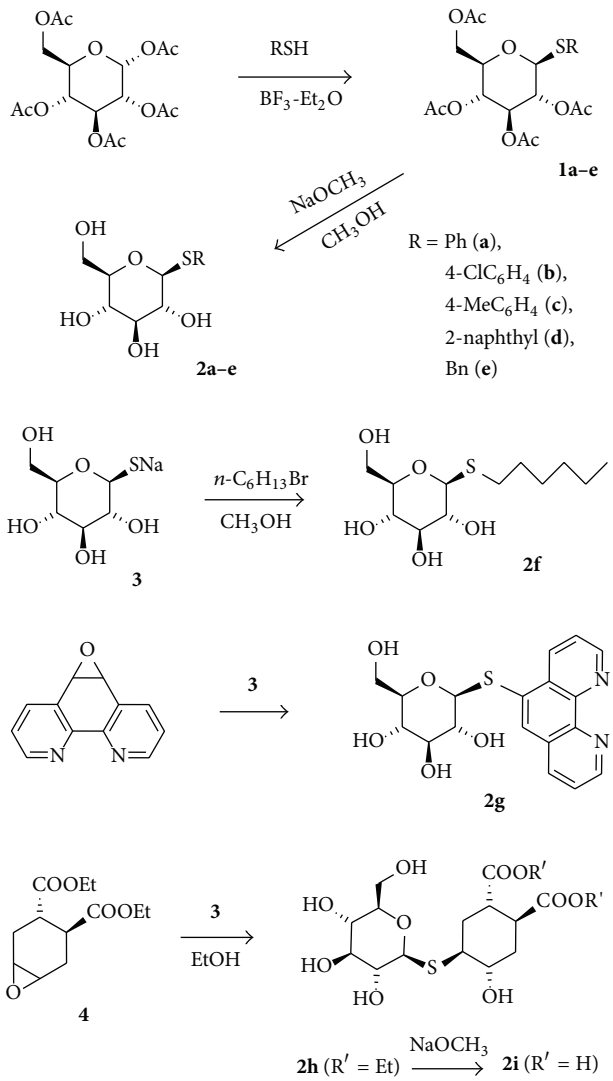
Thioglycosides are the hydrolysis- and metabolism-resistant synthetic *S*-analogs of natural *O*-glycosides. They attracted recently a rapidly increasing attention as competitive inhibitors of glycosidases and other enzymes involved in a variety of biochemical processes [1, 2] related in particular to metabolic disorders and diseases, such as diabetes [1, 3], to inflammations [2, 4] and viral or bacterial infections [5–17], including tuberculosis [9, 10], and to cancer [2, 18–20]. However, surprisingly little is known about the inhibition of glucosidases by 1-thio-β-D-glucosides so far [21–24] (see the discussion). In a search for simple, readily accessible, and efficient glycosidase inhibitors [25–27], we designed, prepared, and assayed a series of structurally simple 1-thio-β-D-glucosides.

2. Results and Discussion

2.1. Synthesis of 1-Thio-β-D-glucopyranosides. A series of aryl and alkyl 1-thio-β-D-glucopyranosides **2a–2i** was synthesized according to Scheme 1. In addition to the model compounds **2a–2f** with simple aryl and alkyl aglycones, we included the phenanthroline derivative **2g**, which was found to possess activity towards some glycosidases [27]. Compounds **2h–2i** were designed as disaccharide analogues in view of the significant inhibitory activity of the 1,2-cyclohexanedicarboxylic acid derivatives towards fungal glycosidases [25, 26].

The structures of all products and intermediates were determined from the data of ^{1}H NMR and ^{13}C NMR, including COSY and HMQC techniques. Thanks to a bias of the conformational equilibria towards the all-equatorial form of the pyranose ring, the configurational assignment was rather straightforward: large spin-spin coupling constants (9–11 Hz) indicated a *trans*-diaxial orientation of the corresponding vicinal protons, while all other relative positions (axial-equatorial and equatorial-equatorial) resulted in small couplings between them (2–4 Hz).

2.2. Glycosidase Inhibitory Activity. The synthesized compounds have been assayed for enzyme inhibitory activity against several glycosidases in multienzyme complexes isolated from fungi *Penicillium canescens* and *Aspergillus oryzae* [28–32]. The multienzyme complex from *P. canescens* contains α-D- and β-D-galactosidase, β-D-glucosidase, and α-L- and β-D-fucosidase [28–31]. The multienzyme complex from *A. oryzae* contains α-D- and β-D-galactosidase, α-D- and β-D-glucosidase, and α-L- and β-D-fucosidase [32]. All assays have been performed in a standard way [33] by



Scheme 1: Preparation of 1-thio-β-D-glucopyranosides **2a–2i**.

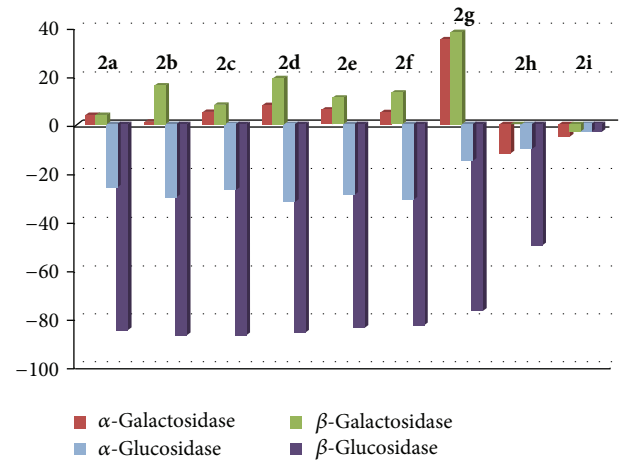


Figure 1: Effect of 1-thio-β-D-glucopyranosides (1 mM) on the activity of *A. oryzae* glycosidases.

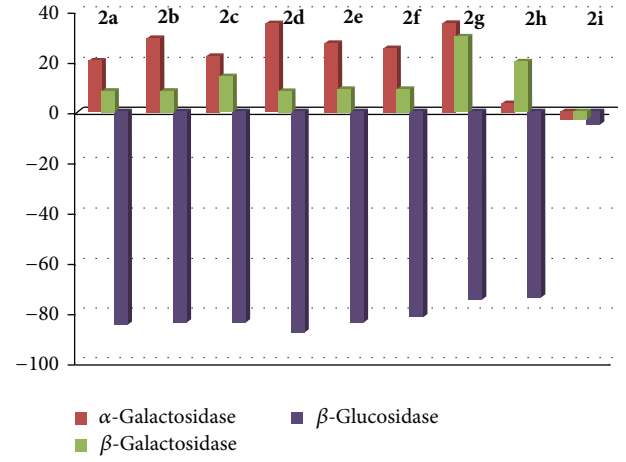


Figure 2: Effect of 1-thio-β-D-glucopyranosides (1 mM) on the activity of *P. canescens* glycosidases.

monitoring spectrophotometrically at 400 nm the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycosides. The inhibitory activity of studied compounds was found to depend on the structure of inhibitor, the type of enzyme, and the particular source of enzyme.

Usually, the structural analogues of carbohydrates with certain configuration are expected to influence activity of the corresponding enzymes ("like inhibits like"). Thus, 4-nitrophenyl 1-thio-β-D-glucoside was used to study binding to β-D-glucosidase from barley and rice and was shown to be a competitive inhibitor [22]. As another example, a series of alkyl/aryl 1-thio-β-D-galactosides was designed and studied as inhibitors of β-D-galactosidase from *E. coli* [17]. Similarly, we tried the 1-thio-β-D-glucosides **2a–2i** first of all as possible inhibitors against β-D-glucosidases. In the preliminary tests, all studied compounds demonstrated indeed a substantial inhibitory activity toward fungal β-D-glucosidases from both *P. canescens* and *A. oryzae* strains (Figures 1 and 2). It is worth mentioning that compounds **2a–2f** were 10-fold more potent in the case of β-D-glucosidase from *A. oryzae* than methyldeoxynojirimycin at 85% inhibition [34].

It was found previously that the inhibitors of β-D-glucosidase may also possess the α-D-glucosidase inhibitory activity [25, 26, 35]. For example, *N*-substituted aminomethyl-β-D-glucopyranosides inhibited yeast and rat intestinal α-D-glucosidases [35]. In this study, compounds **2a–2h** showed only weak inhibition of α-D-glucosidase from *A. oryzae* (Figure 1).

Quite unusually, 1-thio-β-D-glucosides revealed a weak to moderate ability to activate α- and β-D-galactosidases (Figures 1 and 2), especially α-D-galactosidase from *P. canescens* (Figure 2). The strongest activation was observed in the case of phenanthrolyl-1-thio-β-D-glucoside **2g** (Figures 1 and 2). Some derivatives of 1,10-phenanthroline were found earlier to activate β-D-galactosidases, while other derivatives acted as inhibitors [27]. Towards the fungal β-D-glucosidases, all 1,10-phenanthrolines were activators, with exception of thioglucoside **2g**, which was an inhibitor

Table 1: Inhibition of β-D-glucosidases at pH 4.2 and 30°C.

Inhibitor (R)	β-D-Glucosidase *A. oryzae* K_i, μM	β-D-Glucosidase *P. canescens* K_i, μM
2a (Ph)[a]	129	4.02
2b (p-ClC_6H_4)	183	5.56
2c (p-MeC_6H_4)	218	11.1
2d (2′-naphthyl)	159	4.53
2e ($PhCH_2$)	109	6.32
2f (*n*-C_6H_{13})	80.7	3.45
2g (5′-phen)	91.7	2.84
2h (diester)	223	12.3
2i (diacid)	1500	27.5

[a]K_i = 1530 μM (for β-D-glucosidase from fungus *Stachybotrys atra*) [21].

[27]. Noteworthy, the nitrogen analog of **2g**, *N*-(1,10-phenanthrolin-5-yl)-β-D-glucopyranosylamine was recently synthesized, but no data on enzyme inhibition or activation were obtained yet [36]. The mechanism of activation is currently unknown. It may be a result of an allosteric effect in these enzymes [37] or of a change in their hydration [38]. The activation of α/β-D-galactosidases may be interesting for treatment of certain diseases and merits further studies [37, 38].

The analysis of the Lineweaver-Burk plots indicated that compounds **2a–2i** inhibited the β-D-glucosidases competitively. Their inhibition parameters are presented in Table 1. The values of K_i are systematically larger for the enzyme from *A. oryzae* as compared to *P. canescens* (by factors 32–35 for **2a**, **2b**, **2d**, and **2g**; and by factors 17–20 for **2c**, **2e**, **2f**, and **2h**). Similar ratios were obtained earlier for the inhibition of these enzymes by derivatives of cyclohexane-1,2-dicarboxylic acids [25]. This is apparently due to structural differences of the enzymes, which result also in different values of Michaelis constant: K_M = 500 μM (*A. oryzae*) and K_M = 40 μM (*P. canescens*) [25]. Interestingly, higher values of K_M were found also for β-D-galactosidase from *A. oryzae* as compared to enzyme from *P. canescens* [39].

The previously available data on 1-thio-β-D-glucopyranosides as glycosidase inhibitors were limited to the derivatives with the following aglycones: phenyl (**2a**), K_i = 1530 μM (*Stachybotrys atra*) [21]; 4-nitrophenyl, K_i = 664 μM (rice) and K_i = 95 μM (barley) [22]; benzo-1,4-quinone, K_i = 60 μM (*A. faecalis*); chlorobenzo-1,4-quinone, K_i = 400 μM (*A. faecalis*) [23]; and 2-(3-methyl-3*H*-diazirine-3-yl)-ethyl, K_i = 1500 μM (sweet almond) [24]. Although thioglycosides are usually resistant to hydrolysis, it was found also that with some specific aglycones they may be hydrolysable and can serve as glycosidase substrates [40]. It is interesting to compare our results (Table 1) to the data for some known inhibitors of β-D-glucosidases: 1-deoxynojirimycin, K_i = 0.3–2.7 μM (*Aspergillus wentii*); K_i = 12 μM (*Agrobacterium faecalis*); *N*-methyl-1-deoxynojirimycin, K_i = 43 μM (sweet almond); and *N*-hexyl-1-deoxynojirimycin, K_i = 200 μM (calf liver cytosolic) and K_i = 69 μM (calf spleen lysosomal) [41]. From this comparison, compounds **2a–2h** are moderately strong β-D-glucosidase inhibitors.

The comparison of thioglucosides **2a–2i** allows a preliminary elucidation of the effect of aglycon upon their inhibitory activity. It is known that the presence of alkyl groups and other lipophilic moieties often increases the efficiency of glycosidase inhibitors. This effect, mostly towards β-glucosidases, was observed for derivatives of 1-deoxynojirimycin and other iminosugars, alkyl glycosides, carbasugars, and some other inhibitors. It was explained by additional stabilization of the alkyl chains or aromatic residues by the hydrophobic pocket or cleft within or in vicinity of the enzyme binding site (for the detailed discussion see [25, 42, 43] and references therein; in addition see [37, 44, 45]). The results of our study (Table 1) seem to confirm the general regularities observed earlier. Thus, the most potent inhibitors of β-D-glucosidases, compounds **2f** and **2g**, have large lipophilic aglycones, while the thioglucoside **2i** with the most polar aglycone in this series shows almost negligible inhibition. However, the dependence of inhibition on the aglycone's lipophilicity is not straightforward: despite their large aromatic aglycones, the thioglucosides **2a–2e** produced much weaker inhibition than **2f** and **2 g** (see also discussion in [25]). The influence of the aglycone shape remains also uncertain. The best results were achieved both for the narrow alkyl chain (**2f**) and for the wide polyaromatic moiety (**2g**).

3. Experimental Section

The chemicals used in this study were purchased from commercial sources (Sigma-Aldrich, TCI, and Across Organics) and used without additional purification. All solvents were purified by conventional techniques prior to use. Column chromatography was performed on silica gel (40–75 μm, Sorbent Technologies) and aluminum oxide (activated basic, 58Å, Aldrich). The reactions were monitored by TLC on silica gel plates, Analtech Inc (visualization by staining with sulfuric acid followed by heating or with I_2).

^{1}H NMR and ^{13}C NMR spectra were acquired on JEOL ECA-600 NMR-spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) with spinning at rt. ^{1}H-^{1}H-COSY and ^{1}H-^{13}C-HMQC techniques were used to assign the signals. High resolution mass spectra (HRMS) were obtained on a JEOL AccuTOF time-of-flight mass spectrometer (Peabody, MA) coupled with an Ionsense DART open-air ionization source (Saugus, MA). The instrument was tuned to a resolving power of 7,000 with reserpine directly infused into the electrospray ionization source; this provided a stable ion current to tune the time-of-flight parameters. Samples were introduced into the DART sample gap with a glass melting point capillary by first dipping the closed end of the capillary into the sample then immediately placing it into the helium metastable beam. The helium gas temperature was set to 250°C to aid in the desorption of the analyte from the capillary. The samples were held in the sample gap for 10–15 seconds to acquire several mass spectra to average for an accurate *m/z* assignment. Optical rotation was measured for solutions in a 5 cm cell

with an automatic polarimeter Autopol I. A Beckman Du-65 spectrometer was used for the enzymatic hydrolysis studies.

3.1. General Procedure for the Synthesis of 1-Thio-β-D-glucopyranoside Tetraacetates (Adapted from [46]). α-D-Glucose pentaacetate (0.5–2.1 g and 1.3–5.3 mmol) was dissolved in CH_2Cl_2 (25 mL), followed by addition of a thiol (1.5 mol eq). The reaction mixture was stirred for 10 minutes in an ice bath. Then $BF_3{\cdot}Et_2O$ (3 mol eq) was added slowly, and the mixture was allowed to come to room temperature and was stirred with gentle reflux overnight. The solvent was removed by evaporation and product was isolated by column chromatography (silica gel, hexane/EtOAc 5 : 1).

*3.1.1. Phenyl-1-thio-β-D-glucopyranoside Tetraacetate (***1a***).* The stirring overnight was done without reflux. Yield 1.01 g (45%) from 2.00 g (5.1 mmol) α-D-glucose pentaacetate. ^{1}H NMR ($CDCl_3$): δ 2.00 (s, CH_3), 2.02 (s, CH_3), 2.087 (s, CH_3), 2.094 (s, CH_3), 3.73 (ddd, J = 2.5, 5.1, 10.0 Hz; H5), 4.18 (dd, J = 2.5, 12.2 Hz; H6), 4.23 (dd, J = 5.1, 12.2 Hz; H6), 4.71 (d, J = 10.1 Hz; H1), 4.98 (dd, J = 9.3, 10.1 Hz; H2), 5.05 (dd, J = 9.6, 10.0 Hz; H4), 5.23 (t, J = 9.4 Hz; H3), 7.31–7.35 (m, 3H; Ph), 7.50 (m, 2H; Ph). ^{13}C NMR ($CDCl_3$): 20.60, 20.75 (CH_3, Ac), 62.09 (C6), 68.10 (C4), 69.86 (C2), 73.91 (C3), 75.74 (C5), 85.71 (C1), 128.41, 128.92, 131.59, 133.10 (Ph), 169.26, 169.40, 170.18, 170.58 (C=O); (Lit. ^{1}H, ^{13}C NMR [47]). HRMS: $C_{20}H_{24}O_9S$ requires m/z $[M+NH_4]^+$ 458.1485; observed m/z 458.1468.

3.1.2. p-Chlorophenyl-1-thio-β-D-glucopyranoside Tetraacetate (**1b***).* Yield 0.97 g (39%) from 2.07 g (5.3 mmol) α-D-glucose pentaacetate. ^{1}H NMR ($CDCl_3$): δ 1.99 (s, CH_3), 2.02 (s, CH_3), 2.09 (s, CH_3), 2.10 (s, CH_3), 3.72 (ddd, J = 2.6, 4.9, 10.1 Hz; H5), 4.18 (dd, J = 2.6, 12.3 Hz; H6), 4.22 (dd, J = 4.9, 12.3 Hz; H6), 4.65 (d, J = 10.1 Hz; H1), 4.94 (dd, J = 9.4, 10.1 Hz; H2), 5.02 (t, J = 9.8 Hz; H4), 5.22 (t, J = 9.4 Hz; H3), 7.30 (m, 2H; Ar), 7.44 (m, 2H; Ar). ^{13}C NMR ($CDCl_3$): 20.56, 20.72 (CH_3, Ac), 61.96 (C6), 67.99 (C4), 69.71 (C2), 73.79 (C3), 75.81 (C5), 85.16 (C1), 129.04, 129.38, 134.97 (Ar), 169.21, 169.35, 170.13, 170.51 (C=O); (Lit. ^{1}H, ^{13}C NMR [47]). HRMS: $C_{20}H_{23}ClO_9S$ requires m/z $[M+NH_4]^+$ 492.1095; observed m/z 492.1062.

*3.1.3. p-Tolyl-1-thio-β-D-glucopyranoside Tetraacetate (***1c***).* Yield 1.75 g (71%) from 2.05 g (5.3 mmol) α-D-glucose pentaacetate. ^{1}H NMR ($CDCl_3$): δ 1.99 (s, CH_3), 2.02 (s, CH_3), 2.09 (s, CH_3), 2.10 (s, CH_3), 2.35 (s, CH_3, *p*-tolyl), 3.70 (ddd, J = 2.6, 4.9, 10.1 Hz; H5), 4.18 (dd, J = 2.6, 12.2 Hz; H6), 4.22 (dd, J = 4.9, 12.2 Hz; H6), 4.64 (d, J = 10.1 Hz; H1), 4.94 (dd, J = 9.4, 10.0 Hz; H2), 5.03 (t, J = 9.8 Hz; H4), 5.21 (t, J = 9.4 Hz; H3), 7.13 (d, J = 7.8 Hz, 2H; Ar), 7.39 (d, J = 8.0 Hz, 2H; Ar). ^{13}C NMR ($CDCl_3$): 20.68, 20.70, 20.85, 20.87 (CH_3, Ac), 21.29 (CH_3, *p*-tolyl), 62.08 (C6), 68.12 (C4), 69.85 (C2), 73.97 (C3), 75.69 (C5), 85.79 (C1), 127.49, 129.65, 133.81, 138.79 (Ar), 169.23, 169.38, 170.19, 170.58 (C=O); (Lit. ^{1}H, ^{13}C NMR [47]). HRMS: $C_{21}H_{26}O_9S$ requires m/z $[M+NH_4]^+$ 472.1641; observed m/z 472.1590.

*3.1.4. (2-Naphthyl)-1-thio-β-D-glucopyranoside Tetraacetate (***1d***).* Yield 0.23 g (37%) from 0.50 g (1.3 mmol) α-D-glucose pentaacetate dissolved in 10 mL CH_2Cl_2. ^{1}H NMR ($CDCl_3$): δ 1.99 (s, CH_3), 2.02 (s, CH_3), 2.04 (s, CH_3), 2.12 (s, CH_3), 3.74 (ddd, J = 2.4, 5.1, 10.1 Hz; H5), 4.18 (dd, J = 2.4, 12.2 Hz; H6), 4.24 (dd, J = 5.2, 12.3 Hz; H6), 4.79 (d, J = 10.1 Hz; H1), 5.02 (dd, J = 9.3, 10.0 Hz; H2), 5.05 (dd, J = 9.5, 10.0 Hz; H4), 5.24 (t, J = 9.4 Hz; H3), 7.51 (m, 2H; Ar), 7.56 (dd, J = 1.8, 8.6 Hz, 1H; Ar), 7.79 (d, J = 8.5 Hz, 1H; Ar), 7.82 (m, 2H; Ar), 8.00 (d, J = 1.7 Hz, 1H; Ar). ^{13}C NMR ($CDCl_3$): 20.58, 20.70, 20.79 (CH_3, Ac), 62.10 (C6), 68.14 (C4), 69.98 (C2), 73.92 (C3), 75.82 (C5), 85.80 (C1), 126.65, 126.75, 127.68, 128.46, 128.70, 130.19, 132.74, 132.84, 133.39 (Ar), 169.29, 169.36, 170.18, 170.59 (C=O); (Lit. ^{1}H, ^{13}C NMR [47]). HRMS: $C_{24}H_{26}O_9S$ requires m/z $[M+NH_4]^+$ 508.1641; observed m/z 508.1583.

*3.1.5. Benzyl-1-thio-β-D-glucopyranoside Tetraacetate (***1e***).* Yield 1.09 g (46%) from 2.05 g (5.3 mmol) α-D-glucose pentaacetate. ^{1}H NMR ($CDCl_3$): δ 1.99 (s, CH_3), 2.01 (s, CH_3), 2.02 (s, CH_3), 2.12 (s, CH_3), 3.57 (ddd, J = 2.3, 5.1, 10.0 Hz; H5), 3.81 (d, J = 12.9 Hz, 1H; CH_2Ph), 3.91 (d, J = 12.9 Hz, 1H; CH_2Ph) 4.11 (dd, J = 2.3, 12.4 Hz; H6), 4.21 (dd, J = 5.1, 12.4 Hz; H6), 4.26 (d, J = 10.0 Hz; H1), 5.04 (dd, J = 9.3, 10.0 Hz; H2), 5.05 (dd, J = 9.3, 10.0 Hz; H4), 5.11 (t, J = 9.3 Hz; H3), 7.27–7.34 (m, 5H; Ph). ^{13}C NMR ($CDCl_3$): 20.56, 20.58, 20.64, 20.76 (CH_3, Ac), 33.77 ($\underline{C}H_2$Ph), 62.18 (C6), 68.31 (C4), 69.74 (C2), 73.78 (C3), 75.76 (C5), 81.89 (C1), 127.41, 128.59, 129.04, 136.76 (Ph), 169.40, 170.18, 170.63 (C=O); (Lit. ^{1}H, ^{13}C NMR [47]). HRMS: $C_{21}H_{26}O_9S$ requires m/z $[M+NH_4]^+$ 472.1641; observed m/z 472.1596.

3.2. General Procedure for the Synthesis of 1-Thio-β-D-glucopyranosides (Adapted from [46]). The respective 1-thio-β-D-glucopyranoside tetraacetate **1a–1e** (100 mg) was mixed with MeOH (2 mL) in a 5 mL flask. The freshly prepared MeONa solution was added slowly until the pH of the mixture was 9-10 as measured by pH paper. The reaction mixture was allowed to stir until all starting tetraacetate was consumed (TLC; silica gel, hexane/EtOAc 5 : 1). The basic solution was then neutralized with Dowex 50W X8 ion exchange resin, filtered, and evaporated to yield 95–99% of pure thioglucoside.

*3.2.1. Phenyl-1-thio-β-D-glucopyranoside (***2a***).* 100 mg (0.227 mmol) **1a** yielded 60 mg (97%) **2a** as a white solid, mp 131–133°C (Lit. 113°C [48]). ^{1}H NMR (CD_3OD): δ 3.21 (dd, J = 8.8, 9.8 Hz; H2), 3.28 (m, J = 8.5 Hz; H3), 3.32 (ddd, J = 2.3, 5.7, 9.7 Hz; H5), 3.38 (t, J = 8.6 Hz; H4), 3.66 (dd, J = 5.5, 12.1 Hz; H6), 3.86 (dd, J = 2.1, 12.1 Hz; H6), 4.59 (d, J = 9.8 Hz; H1), 7.22 (m, 1H; Ph), 7.29 (m, 2H; Ph), 7.55 (m, 2H; Ph). ^{13}C NMR (CD_3OD): 62.85 (C6), 71.32 (C4), 73.75 (C2), 79.68 (C3), 82.04 (C5), 89.41 (C1), 128.29, 129.87, 132.66, 135.32 (Ph); (Lit. ^{1}H, ^{13}C NMR [48]). HRMS: $C_{12}H_{16}O_5S$ requires m/z $[M+NH_4]^+$ 290.1062; observed m/z 290.0993.

*3.2.2. p-Chlorophenyl-1-thio-β-D-glucopyranoside (***2b***).* 100 mg (0.21 mmol) **1b** yielded 61 mg (94%) **2b** as a white solid, mp 169.5–171.5°C (Lit. 172–175°C [49]). ^{1}H NMR (CD_3OD):

δ 3.16 (dd, J = 8.8, 9.7 Hz; H2), 3.25 (dd, J = 8.9, 9.6 Hz; H3), 3.32 (ddd, J = 2.2, 5.7, 9.7 Hz; H5), 3.35 (t, J = 8.7 Hz; H4), 3.63 (dd, J = 5.7, 12.1 Hz; H6), 3.85 (dd, J = 2.1, 12.1 Hz; H6), 4.55 (d, J = 9.8 Hz; H1), 7.28 (m, 2H; Ar), 7.52 (m, 2H; Ar). ^{13}C NMR (CD_3OD): 62.81 (C6), 71.29(C4), 73.67 (C2), 79.63 (C3), 82.10 (C5), 89.11 (C1), 129.88, 134.04, 134.28, 134.41 (C, Ar). HRMS: $C_{12}H_{15}ClO_5S$ requires m/z $[M+NH_4]^+$ 324.0673; observed m/z 324.0619.

*3.2.3. p-Tolyl-1-thio-β-D-glucopyranoside (***2c***).* 100 mg (0.22 mmol) **1c** yielded 62 mg (98%) **2c** as a white solid, mp 139.5–142°C (Lit. 148°C [48]). ^{1}H NMR (CD_3OD): δ 2.30 (s, CH_3), 3.16 (dd, J = 8.8, 9.7 Hz; H2), 3.25 (t, J = 9.8 Hz; H3), 3.28 (ddd, J = 2.1, 5.5, 9.6 Hz; H5), 3.35 (t, J = 8.6 Hz; H4), 3.64 (dd, J = 5.4, 12.1 Hz; H6), 3.84 (dd, J = 1.9, 12.0 Hz; H6), 4.50 (d, J = 9.8 Hz; H1), 7.11 (m, 2H; Ar), 7.45 (m, 2H; Ar). ^{13}C NMR (CD_3OD): 21.10 (CH_3), 62.87 (C6), 71.35 (C4), 73.70 (C2), 79.68 (C3), 81.96 (C5), 89.64 (C1), 130.53, 131.21, 133.48, 138.74 (C, Ar); (Lit. ^{1}H, ^{13}C NMR [48]). HRMS: $C_{13}H_{18}O_5S$ requires m/z $[M+NH_4]^+$ 304.1219; observed m/z 304.1214.

*3.2.4. (2-Naphthyl)-1-thio-β-D-glucopyranoside (***2d***).* 100 mg (0.20 mmol) **1d** yielded 62 mg (94%) **2d** as a white solid, mp 122–123.5°C (Lit. 117–125°C [50]). ^{1}H NMR (CD_3OD): δ 3.26 (dd, J = 9.8, 8.8 Hz; H2), 3.29 (m, H3), 3.32 (ddd, J = 2.3, 6.0, 9.8 Hz; H5), 3.38 (t, J = 8.9 Hz; H4), 3.66 (dd, J = 6.0, 12.1 Hz; H6), 3.86 (dd, J = 2.2, 12.1 Hz; H6), 4.71 (d, J = 9.8 Hz; H1), 7.45 (m, 2H; Ar), 7.63 (m, 1H; Ar), 7.79 (m, 3H; Ar), 8.07 (m, 1H; Ar). ^{13}C NMR (CD_3OD): 62.89 (C6), 71.39 (C4), 73.82 (C2), 79.70 (C3), 82.16 (C5), 89.37 (C1), 127.18, 127.52, 128.53, 128.65, 129.23, 130.13, 131.16, 132.75, 133.81, 135.14 (Ar). HRMS: $C_{16}H_{18}O_5S$ requires m/z $[M+NH_4]^+$ 340.1219; observed m/z 340.1218.

*3.2.5. Benzyl-1-thio-β-D-glucopyranoside (***2e***).* 100 mg (0.22 mmol) **1c** yielded 61 mg (97%) **2c** as a clear oil. ^{1}H NMR (CD_3OD): δ 3.19–3.29 (m, 4H; H2+H3+H4+H5), 3.67 (dd, J = 6.2, 12.1 Hz; H6), 3.85 (d, J = 12.7 Hz, 1H; CH_2Ph), 3.89 (dd, J = 2.2, 12.1 Hz; H6), 4.03 (d, J = 12.7 Hz, 1H; CH_2Ph), 4.17 (d, J = 9.3 Hz; H1), 7.22 (t, J = 7.4 Hz, 1H; Ph), 7.29 (t, J = 7.3 Hz, 2H; Ph), 7.37 (m, 2H; Ph). ^{13}C NMR (CD_3OD): 32.98 ($\underline{C}H_2Ph$), 61.67 (C6), 70.27 (C4), 73.02 (C2), 78.35(C3), 80.68 (C5), 83.78 (C1), 126.66, 128.11, 128.97, 138.10 (Ph); (Lit. ^{1}H, ^{13}C NMR [17]). HRMS: $C_{13}H_{18}O_5S$ requires m/z $[M+NH_4]^+$ 304.1219; observed m/z 304.1202.

*3.3. Hexyl-1-thio-β-D-glucopyranoside (***2f***).* 1-Thio-β-D-glucopyranose sodium salt **3** (100 mg, 0.46 mmol) was mixed with methanol (2 mL) followed by the addition of bromohexane (75 μL, 0.6 mmol). The reaction mixture was stirred at room temperature overnight and then evaporated *in vacuo*, and the resulting white paste was rinsed with hexane and then redissolved in methanol. The resulting suspension was filtered, and the filtrate evaporated to yield clear oil **2f** (98 mg, 76%). ^{1}H NMR (CD_3OD): δ 0.90 (t, J = 11.4 Hz, 3H; CH_3) 1.31 (m, 4H; CH_2), 1.40 (m, 2H; CH_2), 1.62 (m, 2H; CH_2), 2.68 (dt, J = 12.5, 7.5 Hz, 1H; SCH_2), 2.73 (dt, J = 12.4, 7.3 Hz, 1H; SCH_2), 3.18 (dd, J = 8.7, 9.7 Hz; H2), 3.25 (ddd, J = 2.1, 5.5, 9.5 Hz; H5), 3.29 (m, H3), 3.33 (t, J = 8.9 Hz; H4), 3.64 (dd, J = 5.5, 12.0 Hz; H6), 3.84 (dd, J = 2.2, 12.0 Hz; H6), 4.33 (d, J = 9.8 Hz; H1). ^{13}C NMR (CD_3OD): 13.05 (CH_3), 22.30 (CH_2), 28.36 (CH_2), 29.52 (CH_2), 29.68 (CH_2), 31.26 (CH_2), 61.58 (C6), 70.17 (C4), 73.06 (C2), 78.27 (C3), 80.79 (C5), 85.81 (C1); (Lit. ^{1}H, ^{13}C NMR [51]). HRMS: $C_{12}H_{24}O_5S$ requires m/z $[M+NH_4]^+$ 298.1688; observed m/z 298.1661.

*3.4. (1,10-Phenanthrolin-5-yl)-1-thio-β-D-glucopyranoside (***2g***) [27].* 1-Thio-β-D-glucopyranose sodium salt **3** (65 mg, 0.30 mmol) in dry ethanol (2 mL) was added to a solution of 5,6-epoxy-5,6-dihydro-1,10-phenanthroline (50 mg, 0.255 mmol) in dry ethanol (2 mL) and stirred at room temperature for 36 h until complete conversion of the epoxide as monitored by TLC (silica gel, EtOAc/CH_3OH/NH_4OH 7 : 3 : 1). The precipitate was collected and recrystalized from EtOH affording a yellowish solid product (61 mg, 64%): mp 154-155°C; $[\alpha]_D^{24}$ −64.0° (c 0.38, DMSO). ^{1}H NMR (DMSO): 3.08 (t, J = 9.2 Hz; H4), 3.17 (dd, J = 9.6, 8.8 Hz; H2), 3.23 (t, J = 8.7 Hz; H3), 3.30 (ddd, J = 9.6, 6.5, 2.0 Hz; H5), 3.41 (dd, J = 11.8, 6.4 Hz; H6), 3.69 (dd, J = 11.8, 1.9 Hz; H6), 4.79 (d, J = 9.7 Hz; H1), 7.74 (dd, J = 8.1, 4.3 Hz; H8′), 7.80 (dd, J = 8.3, 4.2 Hz; H3′), 8.29 (s, H6′), 8.36 (dd, J = 8.1, 1.6 Hz; H4′), 8.79 (dd, J = 8.4, 1.6 Hz; H7′), 9.02 (dd, J = 4.3, 1.6 Hz; H9′), 9.09 (dd, J = 4.2, 1.6 Hz; H2′). ^{13}C NMR (DMSO): 61.57 (C6), 70.36 (C4), 73.17 (C2), 79.74 (C3), 81.75 (C5), 87.46 (C1), 123.91 (C3′), 124.29 (C8′), 128.73, 128.82 (C4a′/C6a′), 129.13 (C6′), 131.44 (C5′), 134.13 (C4′), 136.34 (C7′), 145.20, 145.86 (C10a′/C10b′), 150.48, 150.55 (C2′/C9′). HRMS: $C_{30}H_{26}N_4O_2S_2$ requires m/z $[M+H]^+$ 375.1015; observed m/z 375.1022.

*3.5. Diethyl 7-oxabicyclo[4.1.0]heptane-trans-3,4-dicarboxylate (***4***).* The epoxide **4** was prepared as described before [25] and isolated by column chromatography (Al_2O_3, gradient washing with hexane/EtOAc 8 : 2 → 7 : 3) as a colorless liquid (60%). ^{1}H NMR ($CDCl_3$): δ 1.21 (t, J = 7.1 Hz, 3H; CH_3), 1.22 (t, J = 7.1 Hz, 3H; CH_3), 1.87 (ddd, J = 14.9, 10.8, 2.1 Hz; H2), 2.04 (dd, J = 15.5, 10.9 Hz; H5), 2.30 (ddd, J = 15.5, 6.6, 4.8 Hz; H5), 2.45 (ddd, J = 14.9, 4.8, 1.8 Hz; H2), 2.58 (dt, J = 10.7, 6.7 Hz; H4), 2.80 (dt, J = 10.7, 4.9 Hz; H3), 3.17 (t, J = 4.3 Hz; H6), 3.24 (m, H1), 4.11 (m, 4H; OCH_2). ^{13}C NMR ($CDCl_3$): δ 14.20 (CH_3), 26.44 (C5), 27.30 (C2), 37.80 (C3), 40.14 (C4), 50.40 (C6), 52.00 (C1), 60.84 (OCH_2), 173.76, 174.82 (C=O). HRMS: $C_{12}H_{18}O_5$ requires $[M+H]^+$ m/z 243.1233; observed m/z 243.1240.

3.6. [(1S,2S*,4S*,5S*)-2-Hydroxy-4,5-bis(ethoxycarbonyl)-cyclohexyl]-1-thio-β-D-glucopyranoside (***2h***).* 1-Thio-β-D-glucopyranose sodium salt **3** (100 mg, 0.46 mmol) in 7 mL of ethanol was added to a suspension of epoxide **4** (80 mg, 0.33 mmol) in ethanol (2 mL) at room temperature. Reaction mixture was stirred at 40°C for 12 h until complete conversion of the epoxide as monitored by TLC (silica gel, EtOAc/MeOH 4 : 1). The solvent was evaporated under vacuum and residue was purified by column chromatography (silica gel, EtOAc/MeOH 7 : 3) affording product **2h** (a

mixture of diastereomers 1 : 1) as a white solid (55 mg, 38%). ^{1}H NMR (CD_3OD): δ 1.21 (t, J = 7.1 Hz, 6H; $2CH_3$), 1.89 (dd, J = 8.3, 3.4 Hz, 1H; H3′); 1.94–2.05 (m, 2H; H3′+H6′), 2.21, 2.24 (dt, J = 3.9, 11.6 Hz, 1H; H6′), 2.86, 2.89 (dt, J = 3.7, 11.3 Hz; H4′), 2.98 (m, H5′), 3.17 (t, J = 8.6 Hz; H2), 3.23–3.35 (m, 4H; H1′+H3+H4+H5), 3.62, 3.65 (dd, J = 12.1, 5.1 Hz, 1H; H6), 3.84 (dd, J = 12.1, 1.8 Hz, 1H; H6), 3.98, 4.02 (br dd, J = 6.4, 3.3 Hz; H2′), 4.06–4.14 (m, 4H; OCH_2), 4.41, 4.42 (d, J = 9.8 Hz; H1). ^{13}C NMR (CD_3OD): δ 13.14 (CH_3), 28.51, 28.92 (C6′), 30.29, 30.45 (C3′), 39.09, 39.14 (C5′), 40.22, 40.24 (C4′), 44.34, 44.89 (C1′), 60.53, 60.58 (OCH_2), 61.53 (C6), 67.73, 68.36 (C2′), 69.92, 70.01 (C4), 73.12, 73.18 (C2), 78.21 (C3), 80.77 (C5), 85.91, 86.00 (C1), 174.88, 175.18 (C=O). HRMS: $C_{18}H_{30}O_{10}S$ requires m/z $[M+NH_4]^+$ 456.1903, $[M+H]^+$ 439.1638; observed m/z 456.1967, 439.1654.

3.7. [(1S,2S*,4S*,5S*)-2-Hydroxy-4,5-bis (hydroxycarbonyl)-cyclohexyl]-1-thio-β-D-glucopyranoside (**2i**).* 1 mL of KOH (1 M) was added to a solution of diester **2h** (150 mg, 0.34 mmol) in 2 mL of methanol and mixture stirred for 72 h at room temperature. HCl (1 M) was added to reaction mixture till pH 6 and solvent was evaporated under vacuum. Residue was purified by column chromatography (silica gel, EtOAc/CH_3OH 1 : 4) affording product **2i** (one diastereomer) as a white solid (40 mg, 31%; mp > 240°C). ^{1}H NMR (CD_3OD): δ 1.85 (dd, J = 13.4, 3.8 Hz; H3′), 1.93–2.05 (m, 2H; H6′+H3′), 2.26 (m, 1H; H6′), 2.85 (m, H5′), 2.95 (m, H4′), 3.19 (t, J = 9.1 Hz; H2), 3.21–3.35 (m, 4H; H1′+H3+H4+H5), 3.64 (m, 1H; H6), 3.84 (m, 1H; H6), 3.95 (m, H2′), 4.43 (d, J = 9.8 Hz; H1). ^{13}C NMR (CD_3OD): δ 29.23 (C6′), 29.66 (C3′), 31.02 (C4′), 31.18 (C5′), 48.53 (C1′), 61.46 (C6), 69.05 (C2′), 69.98 (C4), 73.17 (C2), 78.24, 80.72 (C3/C5), 85.99 (C1), 179.73 (C=O). HRMS: $C_{14}H_{22}O_{10}S$ requires m/z $[M+NH_4]^+$ 400.1277; observed m/z 400.1289.

3.8. Procedures for the Glycosidase Inhibition and Activation Assay. The enzyme activities (α/β-D-galactosidases and α/β-D-glucosidases) were assayed using multienzyme complexes isolated from fungi *P. canescens* and *A. oryzae* as described before [25–27]. All assays were performed in a standard way by monitoring spectrophotometrically (with Beckman Du-65 spectrometer) the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycosides at 30°C. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per minute. Enzyme and substrate concentrations were selected so that the degree of hydrolysis was never more than 20% and in most cases was less than 10%, over the course of the assay. The method used to measure the rate of the reaction assumes that the amount of the substrate is high enough, such that the disappearance over a given period is insignificant; that is, the rate of the reaction is close to linear for the first stage of the reaction.

For the preliminary estimation, the enzyme solutions (100 μL with activities 750 ± 150 μU) were mixed with a set of inhibitor/activator solutions (100 μL, 10 mM) and then diluted with 700 μL of 0.2 M acetic buffer (pH 4.2), and the mixture was incubated for 1 h at 30°C. The reaction was initiated with addition of a proper substrate (100 μL of 20 mM *p*-nitrophenyl glycopyranoside), and aliquots were taken after 5 and 10 min. The reaction was terminated by addition of 1 mL of 1 M Na_2CO_3 to 0.5 mL of aliquot solution. The concentration of the released *p*-nitrophenol was determined at 400 nm using molar extinction coefficient 18.3 mM^{-1} cm^{-1}. The inhibition/activation was estimated as a loss/increase of enzymatic activity in % (Figures 1 and 2).

The same protocol was used for estimation of the kinetic parameters with the following changes: 1 mL of enzyme solution, 0.5 mL inhibitor solution, and 3.0 mL of 0.2 M acetic buffer (pH 4.2) were incubated for 1 h at 30°C before addition of 0.5 mL of substrate (20 mM). Six substrate solutions were prepared with various concentrations up to saturation point for each enzyme. The final substrate concentration after all additions varied from 0.01 mM to 2.00 mM. The final inhibitor concentrations were 330 μM for *A. oryzae* and 33 μM for *P. canescens*. Reaction rates were measured as described above. Control experiments with no enzyme were performed to exclude the errors due to the substrate spontaneous hydrolysis. Enzyme behavior (at pH 4.2 and 30°C) abided the Michaelis-Menten equation. Parameters K_M and $K_M{}^{\text{app}}$ were calculated from Lineweaver-Burk (double-reciprocal) plot [52, 53]. The K_M values were used to calculate K_i by the following equation:

$$K_i = \frac{K_M [I]}{\left(K_M{}^{\text{app}} - K_M\right)}. \quad (1)$$

Three independent trials using freshly prepared substrate and enzyme solutions were performed to obtain each parameter. Values for K_M were reproducible within ±5% and ±10%, respectively, and the standard error was ±15% for the K_i values (Table 1).

4. Conclusions

The results of our studies show that simple and readily available 1-thio-β-D-glucopyranosides can be potent inhibitors for α- and β-D-glucosidases. The efficiency of inhibition strongly depends on the structure of aglycon and generally increases for β-glucosidases with increase of its lipophilicity. Another interesting feature of the studied compounds is an activation of α- and β-galactosidases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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