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Histidines, histamines and imidazoles as glycosidase inhibitors

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This present study reports the ability of a range of derivatives of L-histidine, histamine and imidazole to act as inhibitors of sweet-almond β-glucosidase, yeast α-glucosidase, and *Escherichia coli* β-galactosidase. The addition of a hydrophobic group to the basic imidazole nucleus greatly enhances binding to both the α- and β-glucosidases. L-Histidine β-naphthylamide (K_i 17 μM) is a potent competitive inhibitor of sweet-almond β-glucosidase as is ω-*N*-acetylhistamine (K_i 35 μM), which inhibits the sweet-almond β-glucosidase at least 700 times more strongly than either yeast α-glucosidase or *Escherichia coli* β-galactosidase, and suggests potential for the development of selective reversible β-glucosidase inhibitors. A range of hydrophobic ω-*N*-acylhistamines were synthesized and shown to be among the most potent inhibitors of sweet-almond β-glucosidase reported to date.

INTRODUCTION

Glycosidases catalyse chemical transformations at the C-1 position of carbohydrates, and a number of metabolic processes rely on these enzymes for their efficiency, selectivity and control. Glycosidase inhibitors are of interest both in studies on the mechanism of enzyme-catalysed glycoside hydrolysis [1] and in medicinal chemistry [2,3]. For example, the potent glycosidase inhibitor 1-deoxynojirimycin, a 5-amino-1,5-dideoxy-D-glucopyranose derivative, has been shown to inhibit human immunodeficiency virus ('HIV') replication *in vitro* [4]. In addition, immobilized glycosidase inhibitors are becoming increasingly popular in affinity chromatography for the purification of a wide range of glycohydrolases and glycosyltransferases ([5], for instance).

A recurring feature in enzyme-catalysed reaction mechanisms is the use of proton-transfer processes to effect catalysis. The classic example of this phenomenon are the serine proteinases [6], such as chymotrypsin, where the nucleophilicity of the active-site serine hydroxy group is enhanced by indirect co-ordination to an anionic aspartate residue, mediated by a histidine-side-chain imidazole nucleus, the so-called 'catalytic triad'. Similar proton-transfer processes mediated by histidine have been proposed to account for the catalytic efficiency of lactate dehydrogenase [7] and also, recently, for certain lipases [8,9]. Lewis-acid-containing metalloenzymes, such as the zinc-based superoxide dismutase and thermolysin, may also effect proton transfer in a related manner [10]. The well-characterized general acid–base catalysis employed by glycosidases to bring about cleavage of the glycosidic linkage (for reviews of this topic, see [11] and [12] and references cited therein) led us to speculate that placement of an imidazole unit between the 'acid' and 'base' residues would result in shuttling of a proton between the two groups 'through' the imidazole nucleus.

The proposal outlined above is represented diagramatically in Scheme 1 for the interaction of the imidazole with the active site of sweet-almond β-glucosidase [13]. For other retaining glycosidases, alternative general acid groups have been implicated; however, one could reasonably expect interactions similar to those outlined above to occur for other potential acid catalysts such as histidine, tyrosine or Mg^{2+} [11].

It was envisaged that compounds bound to glycosidases in the proposed manner would act as potent inhibitors of enzymic glycoside hydrolysis. The present paper details preliminary studies carried out to investigate this proposal.

After our work was completed, Li & Byers [14] reported the potent inhibition of sweet-almond β-glucosidase by imidazole and 4-phenylimidazole.

MATERIALS AND METHODS

Reagents

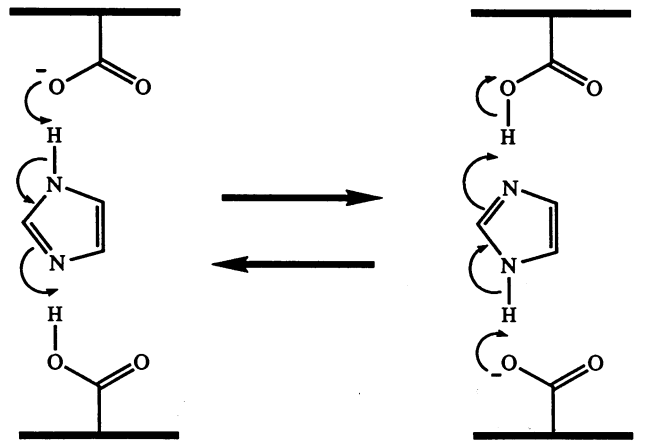
All chemicals were purchased from either Sigma Chemical Co. or Aldrich Chemical Co. and were the purest forms available.

General

Melting points were determined on a hot-stage microscope and are uncorrected. Proton n.m.r. spectra were recorded at 60 MHz on a JEOL PMX-60 spectrometer. Solutions in [$^2H_4$]-methanol were referenced to internal tetramethylsilane; those in [$^2H_6$]dimethyl sulphoxide were referenced to a sealed internal sample of the same standard.

Synthesis of ω-*N*-acylhistamines

ω-*N*-Acylhistamines were prepared by the procedure outlined below.



Scheme 1. Prospective mode of interaction of imidazole with the active-site catalytic residues of a glycosidase

Abbreviations used: α-PNP-Glc, *p*-nitrophenyl α-D-glucopyranoside; β-PNP-Glc, *p*-nitrophenyl β-D-glucopyranoside; β-PNP-Gal, *p*-nitrophenyl β-D-galactopyranoside; IC_{50}, concentration of inhibitor lowering the rate of the enzyme-catalysed reaction by 50%.

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To a stirred, ice-cooled solution of histamine (333 mg, 3 mmol) in dichloromethane (20 ml) were added 0.4 nm (4 Å) molecular sieves (0.5 g) and the flask was flushed with N_2. Triethylamine (1.25 ml, 9 mmol) was added, followed dropwise by a solution of the acyl chloride (3.3 mmol) in dichloromethane (10 ml) over 30 min. The solution was stored at room temperature for 24 h. Dilute aq. 2 M-NaOH (50 ml) was added, and the resulting solution was extracted with dichloromethane (3 × 30 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a solid. Recrystallization from ethyl acetate/methanol gave the analytically pure ω-*N*-acylhistamines (approx. 40%).

ω-*N*-Benzoylhistamine. M.p. 151–153 °C (lit. [15] m.p. 148.5–149.5 °C) (Found: C, 66.9; H, 6.1; N, 19.5; Calc. for $C_{12}H_{13}N_3O$: C, 67.0; H, 6.1; N, 19.5%); δ_H (p.p.m.) ([$^2H_4$]methanol) 2.76–3.88 (4H, AA′BB′ system, $-CH_2CH_2-$), 6.92 (1H, broad s, -CH=), 7.20–8.20 (6H, complex, aromatic-H and -CH=).

ω-*N*-(2-Naphthoyl)histamine. M.p. 174–175 °C (Found: 72.0; H, 5.8; N, 15.6. $C_{16}H_{15}N_3O$ requires C, 72.4; H, 5.7; N, 15.8%); δ_H (p.p.m.) ([$^2H_6$]dimethyl sulphoxide) 3.12–4.16 (4H, AA″BB″ system, $-CH_2CH_2-$), 7.24 (1H, s, -CH=), 7.88–8.56 (7H, complex, naphthoyl-H), 8.84 (1H, s, -CH=), 9.12 (1H, broad s, -NHCO-).

ω-*N*-(1-Adamantanecarbonyl)histamine. M.p. 213 °C (Found: C, 70.2; H, 8.7; N, 15.2. $C_{16}H_{23}N_3O$ requires C, 70.3; H, 8.5; N, 15.4%); δ_H (p.p.m.) ([$^2H_4$]methanol) 1.60–2.24 (15H, complex, adamantyl-H), 2.64–3.56 (4H, AA″BB″ system, $-CH_2CH_2-$), 6.80 (1H, s, -CH=), 7.56 (1H, s, -CH=).

ω-*N*-Phenylacetylhistamine. M.p. 174–175 °C (Found: C, 67.7; H, 6.5; N, 17.9. $C_{13}H_{15}N_3O$ requires C, 68.1; H, 6.6; N, 18.3%); δ_H (p.p.m.) ([$^2H_6$]dimethyl sulphoxide) 2.88–3.72 (4H, AA″BB″ system, $-CH_2CH_2-$), 3.76 (2H, s, $-CH_2Ar$), 7.12 (1H, s, -CH=), 7.64 (5H, broad s, aromatic-H), 7.88 (1H, s, -CH=), 8.42 (1H, broad s, -NHCO=).

To assess the inhibitory properties of acylhistamines against sweet-almond β-glucosidase, the hydrochloride salts of these compounds were prepared. The free bases were dissolved in the minimum volume of ethyl acetate and ethereal HCl solution (5%, w/v; 1.1 equiv.) added to give, after rotatory evaporation, the corresponding hydrochloride salts.

Enzymes and assay buffers

All enzymes used in this study were purchased from Sigma Chemical Co.; precise details are given below.

Enzymes were assayed at, or near, their respective pH optima. The pH of buffered solutions was adjusted by the addition of 5 M-HCl or 5 M-NaOH as appropriate. α-Glucosidase (type VI, from brewer's yeast) was assayed at pH 6.8 (10 mM-Pipes/20 mM-sodium acetate/0.1 mM-EDTA buffer) and at 30 °C with *p*-nitrophenyl α-D-glucopyranoside (α-PNP-Glc) as substrate ([S] = 500 μM; K_m = 200 μM) [16]. β-Glucosidase (type I, from sweet almonds) was assayed at pH 6.2 (10 mM-Pipes/20 mM-sodium acetate/0.1 mM-EDTA buffer) and at 27 °C with *p*-nitrophenyl β-D-glucopyranoside (β-PNP-Glc) as substrate ([S] = 5 mM; K_m = 2.5 mM) [17]. β-Galactosidase was assayed at pH 7.0 (30 mM-Pipes/145 mM-NaCl/1 mM-$MgCl_2$) and at 25 °C with *p*-nitrophenyl β-D-galactopyranoside (β-PNP-Gal) as substrate ([S] = 80 μM; K_m = 48 μM) [18].

In view of conflicting reports in the literature concerning the isoenzyme of sweet-almond β-glucosidase present in commercial samples [13,17,19], the enzyme used in the present study was subjected to further analysis. SDS/PAGE, performed under reducing and denaturing conditions [20], indicated an apparent monomeric molecular mass of 68 kDa, therefore showing that the commercial enzyme preparation used in the present study contains sweet-almond β-glucosidase isoenzyme A [21]. The sample was judged to be at least 90% pure by SDS/PAGE, with no other major contaminating proteins; the enzyme used in the present study is therefore effectively a single isoenzyme.

Kinetics

All assays were performed by monitoring the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycosides at 400 nm. Enzyme and substrate concentrations were chosen 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. Assays were initiated by the addition of enzyme to a thermally equilibrated mixture of the other reagents.

The concentrations of inhibitor required to lower the rate of the enzyme-catalysed reaction by 50% (IC_{50}) were determined from plots of $1/v$ against inhibitor concentration ([I]). At least five inhibitor concentrations were used in such determinations. Where such plots were linear, data were fitted to a straight line by using a least-squares method. Where such plots were non-linear, lower limits for the IC_{50} values were obtained by taking tangents to the plots as [I] tends to zero and extrapolating to 50% inhibition. For compounds giving such non-linear plots, Hill coefficients were determined according to the literature [21].

Enzyme–inhibitor dissociation constants, K_i, were determined by two methods. Firstly, K_i values were calculated from IC_{50} values, with the assumption of purely competitive inhibition, using eqn. (1) [22]:

$$IC_{50} = K_i[1 + ([S]/K_m)] \quad (1)$$

Secondly, K_i values were determined from the effect of inhibitor on K_m and $V_{max.}$ according to eqn. (2):

$$(K_m/V_{max.})_{apparent} = (K_m/V_{max.})[1 + ([I]/K_i)] \quad (2)$$

where $(K_m/V_{max.})_{apparent}$ is the ratio of K_m to $V_{max.}$ in the presence of inhibitor. K_m, $V_{max.}$, $K_{m,apparent}$, and $V_{max.,apparent}$ were estimated using the direct-linear-plot method of Eisenthal & Cornish-Bowden [23]. Competitive inhibition was also confirmed using this form of data analysis.

pK_a values for inhibitors were measured at 25 °C from the half-neutralization points of a titration curves. pH-independent K_i values for synthetic ω-*N*-acylhistamines were calculated using pK_a = 6.85 as measured for ω-*N*-acetylhistamine.

The estimated accuracy of IC_{50} and K_i values is ±10%.

RESULTS AND DISCUSSION

The only report dealing specifically with glycosidase inhibition by imidazole-based compounds is by Shinitzky and co-workers [24] (see [14] also), who investigated the inhibition of lysozyme by a variety of imidazole and histidine derivatives. Table 1 details IC_{50} values calculated from Shinitzky *et al.*'s [24] data and inhibition constants, K_i, obtained in the present study for the inhibition of yeast α-glucosidase and sweet-almond β-glucosidase by imidazole and imidazole derivatives.

From Table 1 it can be seen that imidazole, L-histidine methyl ester and histamine all inhibit β-glucosidase most strongly, α-glucosidase less strongly and lysozyme only weakly. Although inhibition data for histidinol with lysozyme and for L-histidine and α-*N*-acetyl-L-histidine with α-glucosidase are not available, the results with the two enzymes show the same general pattern in each case. The data for β-glucosidase show that compounds containing either acidic (histidine) or basic (histamine) groups do not bind to the enzyme as well as less-polar materials (histidine methyl ester and histidinol). A K_i of 520 μM for the inhibition of β-glucosidase by imidazole at pH 6.2 is a particularly significant

Table 1. Inhibition of glycosidases by imidazole-based compounds

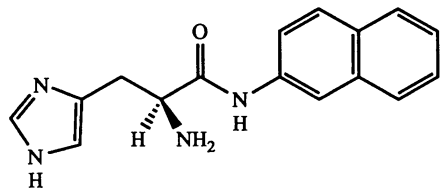
Inhibitor	Parameter...	IC_{50} (mM)	K_i (mM)	
	Glycosidase...	Lysozyme*	α-Glucosidase	β-Glucosidase
Imidazole		65	1.7	530 μM
L-Histidine		96	n.d.‡	7.3
L-Histidine methyl ester		32	2.4	670 μM
Histamine		16	2.7	2.1
Histidinol		n.d.	2.7	1.3
α-*N*-Acetyl-L-histidine		†	n.d.	27

* Data calculated from literature source [24].
† "Practically no inhibition was observed with α-*N*-acetylhistidine" [24].
‡ n.d., not determined.

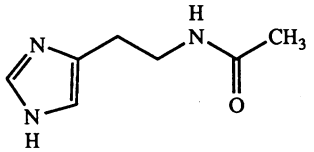
Table 2. Inhibition constants (K_i) for the inhibition of glycosidases by hydrophobic imidazole derivatives

Inhibitor	K_i		
	α-Glucosidase	β-Glucosidase	β-Galactosidase
L-Histidine benzyl ester	770 μM	220 μM	n.d.†
L-Histidine β-naphthylamide	260 μM	17 μM	*
α-*N*-Benzyl-L-histidine methyl ester	4.9 mM	11 mM	n.d.
Benzimidazole	n.d.	930 μM	n.d.
2-Aminobenzimidazole	260 μM	110 μM	8.6 mM
ω-*N*-Acetylhistamine	20 mM	35 μM	‡

* No detectable inhibition at 1.2 mM.
† n.d., not determined.
‡ No detectable inhibition at 3.1 mM.



L-Histidine β-naphthylamide



ω-*N*-Acetylhistamine

Fig. 1. Chemical structures of L-histidine β-naphthylamide and ω-*N*-acetylhistamine

result when one considers that, of the 150 or so inhibitors of this enzyme reported to date, less than a dozen inhibit the enzyme more strongly than this.

In the light of literature data indicating the importance of hydrophobic interactions in recognition processes between glycosidases and inhibitor or substrate [17,25], a range of hydrophobic imidazole-based compounds were investigated. Inhibition studies were conducted with the previously tested glucosidases and the compounds listed in Table 2 and, in addition, some of these compounds were tested against *Escherichia coli* β-galactosidase (Table 2).

The inhibition of β-galactosidase by the imidazole-based compounds is noticeably much weaker than that observed for either glucosidase and, with the exception of α-*N*-benzyl-L-histidine methyl ester, all of the compounds inhibited the β-glucosidase most strongly.

L-Histidine β-naphthylamide and ω-*N*-acetylhistamine (Fig. 1) both proved to be competitive inhibitors of the β-glucosidase with $K_i = 17$ μM and $K_i = 35$ μM respectively at pH 6.2. This corresponds to pH-independent K_i values of 2.3 μM for the naphthylamide (side-chain $pK_a = 7.00$) and 6.0 μM for the acylhistamine ($pK_a = 6.85$), assuming that only the unprotonated inhibitor is inhibitory, as has been shown to be the case for the inhibition of sweet-almond β-glucosidase by ω-*N*-acetylhistamine [26], imidazole and a number of its derivatives [14].

The finding that hydrophobic histidine and histamine derivatives are very effective β-glucosidase inhibitors is encouraging, since, with the exception of 4-phenylimidazole (pH-independent $K_i = 0.83$ μM) [14], all other potent inhibitors of this enzyme have a sugar-type ring in their structures. For comparison, previously reported potent carbohydrate-based inhibitors of sweet-almond β-glucosidase have the following K_i values at pH 6.2: 1-deoxynojirimycin, $K_i = 29$ μM; D-gluconolactam, $K_i = 37$ μM; D-gluconolactone, $K_i = 200$ μM [17].

Whereas hydrophobic derivatives of the carboxy group of L-histidine bind strongly to α- and β-glucosidase, substitution of the α-amino group, giving for instance α-*N*-benzyl-L-histidine methyl ester, actually causes a reduction in the affinity for both

Table 3. Inhibition of sweet-almond β-glucosidase by ω-*N*-acylhistamines

Acyl group	Parameter...	IC_{50} (μM)*	Hill coefficient	K_i (μM) †	K_i (μM) ‡
Acetyl		100	1.0	33	6.0
1-Adamantanecarbonyl		50	1.0	17	3.1
Benzoyl		1.8	0.85	0.6	0.11
2-Naphthoyl		14	0.70	4.7	0.86
Phenylacetyl		35	1.0	12	2.2

* Measured at pH 6.2
† Calculated from the corresponding IC_{50} values
‡ pH-independent K_i values calculated assuming only the unprotonated inhibitor is the inhibitory species.

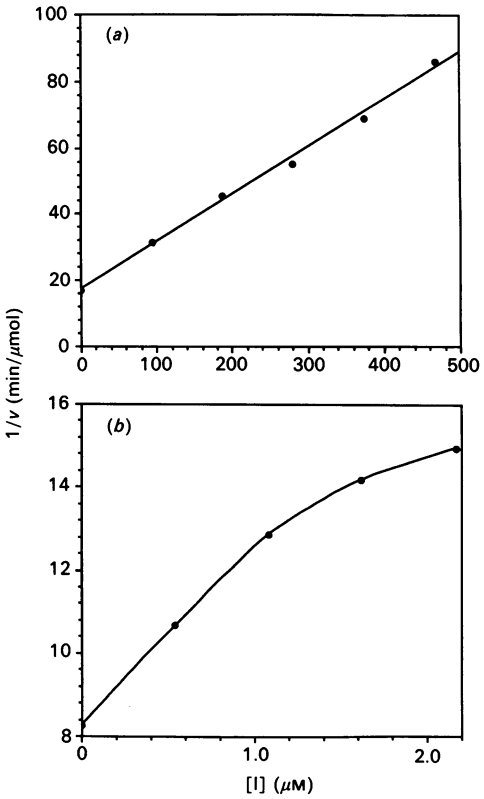


Fig. 2. Plots of reciprocal rate versus inhibitor concentration for the inhibition of sweet-almond β-glucosidase by (*a*) ω-*N*-acetylhistamine and (*b*) ω-*N*-benzoylhistamine

enzymes. In contrast, amino substitution of histamine to give ω-*N*-acetylhistamine enhances binding to β-glucosidase by a factor of about 60. It is also noteworthy that this latter compound inhibits β-glucosidase 700 times more strongly than either α-glucosidase or β-galactosidase (for β-galactosidase this assumes less than 4% inhibition at [I] = 3.1 mM; in fact no inhibition was detectable at this inhibitor concentration).

Substitution of benzimidazole at C-2 with an amino group brings about an increase in inhibition of the β-glucosidase by a factor of 8–9. It is not possible to say from current data whether this increase in affinity is due to a change in pK_a of the imidazole ring or due to the change in functionality brought about by this substitution.

Structure–activity relationships for inhibition of sweet-almond β-glucosidase by *C*-substituted methylamines reported in the literature [17] indicate that replacement of one hydrogen atom in the methyl group of methylamine by a naphthyl residue decreases the binding constant for the almond enzyme from 32 mM to 750 μM, a factor of 43. The prospect of achieving a similar reduction in the binding constant for ω-*N*-acetylhistamine (i.e. from 35 μM to approx. 0.8 μM) led us to synthesize a number of ω-*N*-acylhistamines bearing hydrophobic acyl groups. The synthesis of such compounds proved facile; acylation of histamine by treatment of the free base with triethylamine and an acyl chloride gave, on basic work-up, a solid which could be crystallized from ethyl acetate/methanol to give the ω-*N*-acylhistamines as white needles or prisms in reasonable yield (40–50%, unoptimized). Data obtained for the interaction of the hydrochloride salts of such compounds with sweet-almond β-glucosidase is given in Table 3, and representative examples of the $1/v$-versus-[I] plots obtained for these compounds are given in Fig. 2.

Replacement of the acetyl group of ω-*N*-acetylhistamine by a benzoyl group led to a 55-fold reduction in the inhibition constant for this compound with sweet-almond β-glucosidase. The pH-independent K_i for ω-*N*-benzoylhistamine (K_i for ω-*N*-benzoylhistamine (K_i = 0.11 μM) is the lowest value reported to date for an imidazole-based glycosidase inhibitor, and is lower than that of 4-phenylimidazole (pH-independent K_i = 0.83 μM) [14]. A pH-independent K_i value of 0.86 μM for ω-*N*-naphthoylhistamine indicates that the structure–activity relationship for the inhibition of sweet-almond β-glucosidase by substituted methylamines [17] is not shown by such aromatic ω-*N*-acylhistamines, possibly owing to electronic effects arising from interaction of the aromatic ring with the amide carbonyl group.

The relationships between $1/v$ and [I] for the two most potent acylhistamine inhibitors (i.e. the benzoyl and naphthoyl derivatives) are non-linear (see, e.g., Fig. 2*b*). The IC_{50} values for these two compounds were calculated from tangents to the curve as [I] tends to zero and are thus limiting values. Non-linear effects of this type have previously been reported for the inhibition of calf liver microsomal glucosidases by 1-deoxynojirimycin [5] and of pure sweet-almond β-glucosidase isoenzyme A by nojirimycin [21]. Similar results were also obtained for the inhibition of the almond enzyme by tetrahydropyranylmethanol [26]. It has been suggested such non-linear effects may be due to either unknown modes of binding of inhibitor at the enzyme active site [5] or slow formation and dissociation of the enzyme–inhibitor complex [21]. pH-dependent changes in the tryptophan fluorescence spectrum of sweet-almond β-glucosidase have been interpreted as resulting from a conformational change of the enzyme [27]. It is possible that such a conformational change may be important for effective glycoside hydrolysis and may also have a role in inhibitor binding [28]. For instance, one could imagine the formation of a weakly bound enzyme–inhibitor complex followed by a conformational change of the enzyme either to facilitate or to accommodate protonation of the inhibitor by the enzyme. This could result in a more tightly bound enzyme–inhibitor

complex. Such multi-step binding processes may give rise to 'slow-binding' inhibition and a non-linear dependence of K_i on inhibitor concentration.

CONCLUSIONS

The synthetic ω-*N*-acylhistamines reported in this study are among the most potent inhibitors of the sweet-almond β-glucosidase reported to date and are, with the exception of 4-phenylimidazole [14], the only non-carbohydrate inhibitors with K_i values for this enzyme of less than 100 μM. It appears that hydrophobic imidazole-based compounds show great potential as glycosidase inhibitors, potentially as selective inhibitors of β-glucosidase In view of the facile synthesis of ω-*N*-acylhistamines (with respect to amino-sugars), this class of compounds should prove of considerable interest in future glycosidase-inhibition studies.

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