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A Novel Competitive Class of α-Glucosidase Inhibitors: (*E*)-1-Phenyl-3-(4-Styrylphenyl)Urea Derivatives

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Competitive glycosidase inhibitors are generally sugar mimics that are costly and tedious to obtain because they require challenging and elongated chemical synthesis, which must be stereo- and regiocontrolled. Here, we show that readily accessible achiral (*E*)-1-phenyl-3-(4-strylphenyl)ureas are potent competitive α-glucosidase inhibitors. A systematic synthesis study shows that the 1-phenyl moiety on the urea is critical for ensuring competitive inhibition, and substituents on both terminal phenyl groups contribute to inhibition potency. The most potent inhibitor, compound **12** ($IC_{50}=8.4$ μM, $K_i=3.2$ μM), manifested a simple slow-binding inhibition profile for α-glucosidase with the kinetic parameters $k_3=0.005256$ μM^{-1} min^{-1}, $k_4=0.003024$ min^{-1}, and $K_i^{app}=0.5753$ μM.

Introduction

Owing to the involvement of specific glycosidases in virtually all carbohydrate cleavage processes in humans, plants and animals, selective inhibition of glycosidases enacting a particular pathway offers high therapeutic promise.[1] Glycosidase inhibitors have proven useful for reducing postprandial hyperglycaemia by suppressing the absorption of glucose, and are thus effective for the treatment of type II diabetes and obesity.[2–4] On the other hand, glucosidase I and II sequentially trim N-linked oligosaccharides at the Glc(1→2)Glc and Glc(1→3)Glc linkages within the Glc3Man9GlcNAc2 glycoprotein, respectively, liberating the three terminal glucose residues.[5] Thus, these enzymes are critical for ensuring correct processing and hence proper function of glycoproteins. Glycosidases are accordingly excellent therapeutic targets for a diverse range of diseases including lysosomal storage disorders,[6] cancer,[7] and special attention has been given to the treatment of HIV.[8]

Among the various types of glucosidase inhibitors, iminosugars,[9] carbasugars[10] and thiosugars[11] have received the most attention because of their potent inhibitory activity and well established inhibition mechanisms, which are mainly competitive. Some carbocyclic glucosidase inhibitors include potent HIV inhibitors, such as conduritol epoxides and aminoconduritols, as well as conduritol A analogues, which modulate the release of insulin. Thiosugars, either synthesized or isolated from natural sources, have also been investigated as glucosidase inhibitors and have widened the structural diversity of compounds available from natural sources. The great potency and specificity of iminosugars is related to their ability to mimic the transition state for hydrolysis of natural glucosidase substrates. Competitive inhibition is observed with many of these inhibitors, suggesting that both conformational (shape) and electrostatic (charge) influences could be important in binding the active site. Amino sugars have structural features similar to the target enzyme's sugar substrate or the hypothetical reactive intermediate closest in energy to the transition state (oxocarbenium ion). This information provides a detailed rational basis for design of selective and potent inhibitors and the interpretation of subtle differences in reaction mechanism.[12]

Until recently, the structural design of highly potent, competitive glucosidase inhibitors was mostly based on carbohydrate-derived natural products possessing several stereogenic centres. This is because of their high potency and well-understood structure–activity relationships. However, these all require tedious multistep synthesis from carbohydrate and non-carbohydrate sources.[13,14] Synthesis routes are further complicated by the need to control/install numerous stereocentres. These reactions can be highly substrate dependent and hence not amenable to the synthesis of numerous analogues from common starting materials, which complicates SAR. For these reasons, compounds with no obvious structural similarity to carbohydrates are a new class of inhibitors. Importantly these species can be simple in structure (potentially having no stereocentres) and thus their synthesis is simple and readily amenable to analogue production. However, as these compounds are not constrained to bind at the active site, their mode of action can be complex and hard to model. Thus, nonsugar

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derived inhibitors with well-defined enzyme binding modes are of utmost interest.

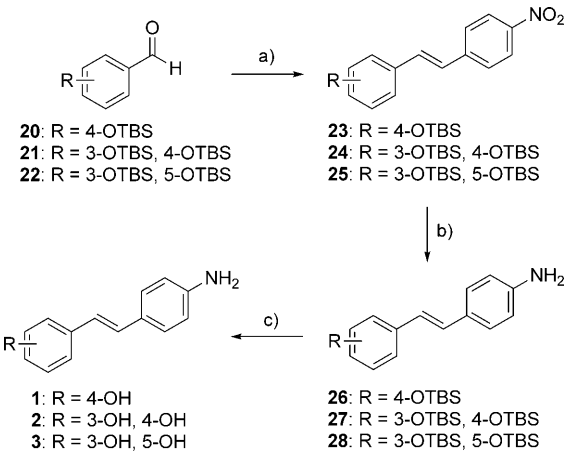
We have reported a new class of sulfonamide chalcone-derived noncompetitive α-glucosidase inhibitors,[15] and shown their antitumour activities in hepatocytes[16] and trans-sialidase inhibitory activity in vitro.[17] We were, however, concerned by the possibility of off-target effects, and hence were keen to find equally chemically simple species that are competitive glucosidase inhibitors as we believed that this could simplify SAR, deconvolute binding modes and ultimately lead to higher selectivity through rational design. The focus of our efforts was on the stilbene skeleton. This is for three principal reasons: 1) stilbene polyphenols are abundant in edible plants and have been shown to display a diverse array of pharmacological activities including antitumour and anti-inflammatory properties;[18,19] 2) resveratrol (also a stilbene derivative) is a noncompetitive inhibitor of α-glucosidase with an IC_{50} of 18.5 μM;[20] 3) our unpublished results disclosed that aminated stilbenes can act as a new class of highly potent competitive α-glucosidase inhibitors. These are achiral compounds that can be obtained with simple steps economically. We thus present the most pertinent results of an extensive initiative aimed at developing new competitive glycosidase inhibitors.

We screened inhibitory activity using maltase, an α-glucosidase (E.C. 3.2.1.20), from *Saccharomyces cerevisiae*. This protein is encoded by the MAL 12 gene and belongs to the glycoside hydrolase (GH) 13 family, to which human pancreatic α-amylase also belongs. It catalyzes the hydrolysis of nonreducing 1,4-linked α-D-glucose residues with the release of α-D-glucose and retention of configuration at the anomeric carbon. It can also catalyze transglycosylation for the synthesis of oligosaccharides and alkylglycosides.[21] In our present study, we report the first nonsugar-derived competitive α-glucosidase inhibitors with micromolar IC_{50} values.

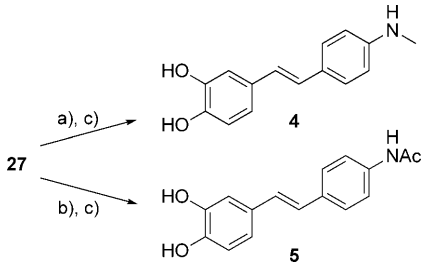
Results and Discussion

As outlined above, development of a novel series of α-glucosidase inhibitors began with the stilbene skeleton because of its structural similarity to resveratrol. To determine the structural requirements for the inhibitory activity of α-glucosidase, our initial studies focused on aminostilbenes. This is because amines are easily converted to other functionalities with a wide range of chemical properties.

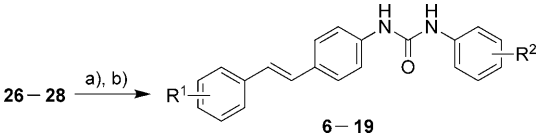
A general route for the preparation of aminostilbenes was developed as outlined in Scheme 1. Hydroxybenzaldehyde derivatives were treated with TBSCl to form the silylated hydroxybenzaldehydes **20**–**22**. The coupling reaction of protected aldehydes **20**–**22** with diethyl (4-nitrobenzyl)phosphate was achieved with the Wadsworth–Emmons reaction. The coupling reagent, diethyl (4-nitrobenzyl)phosphate was obtained through Arbusov reaction of 4-nitrobenzylbromide and triethylphosphite. The stereoselectivity of the C–C coupling reaction favoured the formation of the *E* isomer (*E*/*Z* > 7:1) as generally observed. The geometrical isomer selectivity was determined for a number of bases: NaOH, *n*BuLi and *t*BuOK; *t*BuOK gave the best selectivity. Assignment of the *E* configuration is based upon analysis of coupling constants ($^3J = 16.2$ Hz) in ^{1}H NMR spectra. Both *E*/*Z* isomers have sufficiently different R_f values to be separated by silica gel chromatography. Subsequent reduction of the nitro group to the primary amine was achieved by using Fe powder in 5% acetic acid in EtOAc to give the parent compounds **26**–**28** in quantitative yield. Deprotection of TBS groups by using TBAF eventually led to free alcohols **1**–**3**. Treatment of compound **2** with iodomethane and deprotection of TBS group with TBAF gave N-methylated compound **4**. *N*-Acetate **5** was easily prepared by treatment of **2** with acetylchloride (Scheme 2). Stilbene urea analogues in Table 2 were obtained by addition of the corresponding isocyanates to TBS ethers **2** and ensuing desilylation of the TBS functions. For example, treatment of compound **2** with 2,4-difluorophenyl isocyanate in CH_2Cl_2 and subsequent desilylation with TBAF provided stilbene urea **12** (Scheme 3).



Scheme 1. Reagents and conditions: a) diethyl (4-nitrobenzyl)phosphonate, *t*BuOK, DMF, room temperature, 2 h; b) Fe powder, 5% acetic acid, 95 °C, 1 h; c) TBAF 1 M, THF, room temperature, 3 h.

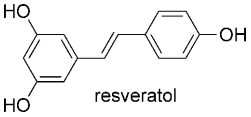


Scheme 2. Reagents and conditions: a) methyl iodide, CH_2Cl_2, room temperature, 2 h; b) acetyl chloride, CH_2Cl_2, room temperature, 2 h; c) TBAF (1 M), THF, room temperature, 3 h.



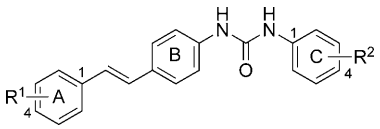
Scheme 3. Reagents and conditions: a) isocyanate, CH_2Cl_2, room temperature, 2 h; b) TBAF (1 M), THF, room temperature, 3 h.

Table 1. Inhibitory effects of stilbene derivatives **1**–**6** on α-glucosidase activity.[a]

Compound	IC_{50} [μM]	Inhibition type (K_i [μM])
1	>200	NT[b]
2	46.4	noncompetitive (35.1)
3	102.4	NT
4	>200	NT
5	>200	NT
6	42.1	competitive (10.6)
 resveratol	26.4	noncompetitive (21.5)

[a] Effect of compounds **1**–**6** on the α-glucosidase catalyzed hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside; [b] NT: not tested.

Table 2. Inhibitory effects of stilbene derivatives **6**–**19** on α-glucosidase activity.[a]



Compound	R^1	R^2	IC_{50} [μM]	Inhibition type (K_i [μM])
6	3-OH, 4-OH	H	42.1±1.1	competitive (10.6±0.5)
7	3-OH, 4-OH	4-OCH_3	19.5±0.3	competitive (10.5±0.2)
8	3-OH, 4-OH	4-NH_2	68.5±2.2	NT[b]
9	3-OH, 4-OH	4-CH_3	63.6±1.8	NT
10	3-OH, 4-OH	4-CF_3	28.8±0.4	competitive (12.1±0.3)
11	3-OH, 4-OH	4-F	19.8±0.1	competitive (7.2±0.4)
12	3-OH, 4-OH	2-F, 4-F	8.4±0.4	competitive (3.2±0.3)
13	3-OH, 4-OH	4-Cl	14.3±0.2	competitive (4.6±0.3)
14	4-OH	H	29.1±0.3	competitive (17.0±1.3)
15	4-OH	4-OCH_3	60.1±1.5	NT
16	4-OH	2-F, 4-F	18.5±1.2	competitive (9.4±0.4)
17	3-OH, 5-OH	H	58.8±0.8	NT
18	3-OH, 5-OH	4-OCH_3	104.7±2.4	NT
19	3-OH, 5-OH	2-F, 4-F	64.3±1.3	NT
DNJ			39.5±0.5	competitive (18.0±1.2)

[a] Effect of compounds **6**–**19** on the α-glucosidase catalyzed hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside; [b] NT: not tested.

Stilbenes **1**–**6** in Table 1 were thus synthesized and subsequently tested for their inhibitory activity as documented in the Experimental Section. Compounds showing significant inhibition at 42.1–46.4 μM were further investigated and their IC_{50} and K_i values were calculated. Taken as an ensemble, the following general SAR features can be deduced from the initial compounds tested (Table 1, **1**–**6**). The presence of two free hydroxyl groups in the A ring appears to be important for activity, with a 3,4-arrangement giving the best results. For example, **1** (bearing only one OH group, IC_{50} >200 μM) and **3** (3,5-dihydroxy-substituted derivative, IC_{50} = 102.4 μM) versus **2** (3,4-dihydroxy-substituted derivative, IC_{50} = 46.4 μM). Next, we investigated the effect of derivatizing the amino group. Alkylation (**4**) and acetylation (**5**) resulted in considerable losses of potency, indicating that H-bond donor ability and number can be important. Further screening showed that enhanced α-glucosidase inhibitory activity was obtained by replacing the amino group with a urea function, an excellent H-bond donor. Surprisingly, introduction of the aryl urea moiety led to competitive inhibition as shown for compound **6**.

This phenomenon was observed in all active ureas in Table 2. As shown in Table 2, all stilbene ureas **6**–**19** exhibited a dose dependent inhibitory effect on the hydrolysis activity of α-glucosidase (IC_{50} 8.4–104.7 μM). To determine the specificity of compounds **6**–**19** for α-glucosidase, their inhibitory activities against the related enzymes β-glucosidase, α-galactosidase, α-mannosidase and α-amylase were determined. None of the inhibitors showed any activity against any of these hydrolases at concentrations up to 200 μM. The inhibition of compound **12** is demonstrated in Figure 1 representatively. Once

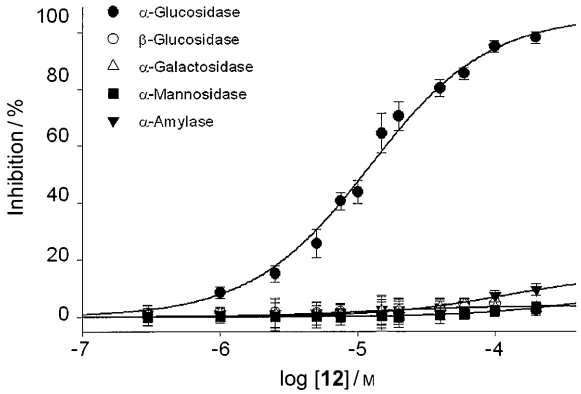


Figure 1. Selectivity of inhibitor **12** with related hydrolases. Inhibitors were tested on glycosidases (α-glucosidase, β-glucosidase, α-galactosidase, α-mannosidase, and α-amylase). Data represent the results of three independent experiments performed with triplicates of each sample.

again, the activity was significantly influenced by position and number of hydroxy groups on both the stilbene and the aryl moieties. Direct comparison of inhibitory potencies of **12** (IC_{50} = 8.4 μM), **16** (IC_{50} = 18.5 μM) and **19** (IC_{50} = 64.3 μM), which have the same 2,4-difluoro substitution on the C ring but differ in the substitution pattern in the stilbene moiety, implies that a 4-hydroxyl group on the A phenyl ring is the most important. Maximal activity was generally obtained with a 3,4-dihydroxy motif in the A ring. The effects of varying substitution patterns and chemotypes on the C ring while holding substitution on the A ring invariant was also evaluated. 2,4-Difluoride **12** was found to display an eightfold increase in activity, relative to amino-bearing **8** and methyl-bearing **9**. In line with this analysis, the second and third most potent analogues in this series bore 4-chloro (**13**, IC_{50} = 14.3 μM) and 4-methoxy (**7**, IC_{50} = 19.5 μM) groups. This trend seems to follow neither steric size (there might be a small preference for smaller groups) nor electron density on the aromatic C ring. These substituents might thus be interacting with H-bond donor species on the protein otherwise there could be a polarity effect,

the difluoro species being more polar than the chloro derivative and the methoxy analogue.

All inhibitors manifested a similar relationship between enzyme activity and enzyme concentration. The inhibition of α-glucosidase by compound **12**, the most potent inhibitor ($K_i = 3.2$ μM), is illustrated in Figure 2, representatively. Raising the concentrations of the inhibitors drastically lowered residual enzyme activity. Plots of residual enzyme activity versus enzyme concentration at different concentrations of compound **12** gave a family of straight lines with a *y*-axis intercept of 0; this indicates that **12** is a reversible inhibitor (see the Supporting Information).

We then further characterized the inhibitory mechanism of the synthesized stilbene derivatives. This was done by using both Lineweaver–Burk and Dixon plots. As shown representatively in Figure 2B, the kinetic plot shows that compound **12** is a competitive inhibitor. This is because increasing concentration of **12** resulted in a family of lines with a common intercept on the 1/*V* axis but with different gradients (Figure 2A). The K_i value of the most potent inhibitor **12** was determined to be 3.2 μM (Table 2) by using a Dixon plot (see the Supporting Information).

To further investigate the inhibition mechanism, time dependence of the inhibition of the hydrolysis of *p*-nitrophenol-α-D-glucopyranoside by these inhibitors was subsequently probed. The enzyme showed no loss in activity over this time range: *this indicates that the enzyme is stable and steady state is maintained throughout*. This was achieved by measuring initial velocities of substrate hydrolysis as a function of preincubation time of the enzyme with the inhibitor. As a decrease in residual activity was seen as a function of preincubation time, compound **12** emerged to be a slow-binding inhibitor at 12.5 μM (Figure 2B). Increasing concentrations of compound **12** led to a decrease in both the initial velocity (v_i) and the steady-state rate (v_s; Figure 2B, insert). Figure 2C shows representatively that residual enzyme activity decreases as a function enzyme/inhibitor incubation time. This relationship is typical for slow binding inhibitors: time dependence of the rate of hydrolysis by α-glucosidase was measured in the presence of different concentrations of **12** (0, 5, 7.5, 10, 12.5, 15, and 20 μM). As shown in Figure 2B and C, the time dependent residual activities obtained by using the differing concentrations of the inhibitors were fitted to Equations (1) and (2) to determine k_{obs}:

$$\frac{[P]_t}{[E]} = v_s t + \frac{(v_i - v_s)}{k_{obs}}(1 - e^{-k_{obs}t}) \quad (1)$$

$$\frac{v}{v_0} = e^{-k_{obs}t} \quad (2)$$

where $[P]_t$ is the concentration of product formed, [E] is the total enzyme concentration, v_i is the initial velocity, v_s is the steady state velocity, *t* is time, k_{obs} is the exponential rate constant for equilibration.[22]

Then, we identified which of the four possible inhibition modes (uninhibited, simple reversible slow binding, enzyme

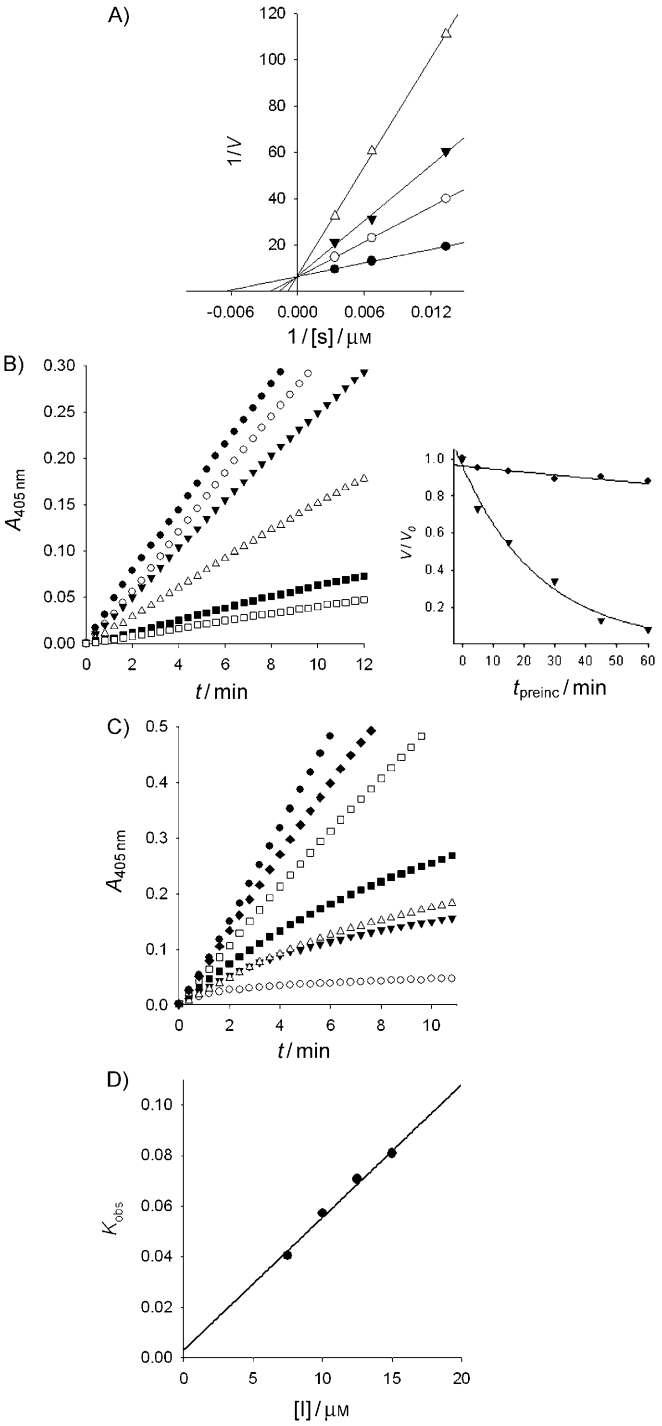


Figure 2. A) Lineweaver–Burk plots for the inhibition of the hydrolysis activity of α-glucosidase by compound **12** (●: 0, ○: 7.5, ▼: 10, △: 15 μM). Conditions were as follows: 75, 150, 225 μM *p*-nitrophenyl-α-D-glucopyranoside, 0.05 units of α-glucosidase, 0.05 M potassium phosphate buffer (pH 6.8) at 37 °C. B) Inhibition as a function of preincubation time (●: 0, ○: 5, ▼: 15, △: 30, ■: 45, □: 60 min) for active compound **12** at 12.5 μM [Eq. (2)] Inset: ●: 0, ▼: 12.5 μM. C) Time course for slow-binding inhibition of compound **12** (●: 0, ◆: 5, □: 7.5, ■: 10, △: 12.5, ▼: 15, ○: 20 μM). The k_{obs} values at each inhibition concentration were determined by fitting the data to Equation (1). D) Plot of k_{obs} as a function of inhibitor **12** concentration for a slow-binding inhibitor fitted by Equations (3) and (4).

isomerization, and mechanism-based inhibition) that could give this kinetic profile was at play. Slow binding inhibition mechanisms can be investigated by preincubation of the enzyme with inhibitor followed by measurement of residual active enzyme as a function of incubation time. The parameters k_3, k_4 and K_i^{appt} can be fitted to Equations (3) and (4).[22]

$$k_{obs} = k_4 + \frac{k_4[I]}{K_i^{appt}} \quad (3)$$

$$K_i = \frac{k_4}{k_3} \quad (4)$$

We established that the progress curves yielded the following: $k_3 = 0.005256\ \mu M^{-1}\,min^{-1}$, $k_4 = 0.003024\ min^{-1}$, and $K_i^{appt} = 0.5753\ \mu M$. This is a characteristic feature of **12**, which indicates simple reversible slow binding mode (Figure 2B–D). A plot of k_{obs} versus inhibitor concentration displays a linear dependence (Figure 2D), which is consistent with the single-step mechanism:

$$Enz + I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Enz\text{-}I$$

Conclusions

In summary, we have been able to develop a range of highly potent competitive inhibitors of α-glucosidase. These are some of the first nonsugar-derived competitive α-glucosidase inhibitors with very low (< 10) micromolar IC_{50} values. SAR indicates favourable substituent arrangements on both the A and C rings and that the urea chemotype is required to ensure competitive inhibition. We believe that these inhibitors are important lead compounds as their synthesis is simple and a wide range can be made for developing a series of simple and highly potent inhibitors of α-glucosidase.

Experimental Section

General: All the reagent-grade chemicals were purchased from Sigma–Aldrich. All solvents used for isolation were of analytical grade.

General procedure for the coupling reaction (23–25): *t*BuOK (0.31 g, 2.76 mmol) was added to a solution of diethyl (4-nitrobenzyl)phosphonate (0.58 g, 0.5 mmol) in DMF (10 mL), and this mixture was stirred under a nitrogen atmosphere for 20 min. The solution of aldehyde **20**–**22** (2.33 mmol) in cosolvent of DMF/THF (2:1) was added to the reaction mixture and stirred for 2 h. The mixture was diluted with water (100 mL) and extracted with EtOAc (3× 100 mL). The combined organic phase was washed with water and dried (Na_2SO_4), and the solvent was evaporated. The product was purified by silica chromatography by using hexane/EtOAc (4:1) as eluent to give 70% yields of nitrostilbenes (**23**–**25**) as *E* isomer.

(*E*)-*tert*-Butyldimethyl(4-(4-nitrostyryl)phenoxy)silane (23): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.07$ (s, 6H), 0.83 (s, 9H), 6.69 (d, J= 8.4 Hz, 2H), 6.84 (d, J= 16.2 Hz, 1H), 7.05 (d, J= 16.2 Hz, 1H), 7.27 (d, J= 8.4 Hz, 2H), 7.42 (d, J= 8.7 Hz, 2H), 8.03 ppm (d, J= 8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 161.1$, 148.8, 137.5, 134.0, 132.9, 131.0, 128.8, 128.7, 125.1, 125.0, 30.2, 22.8, 0.1 ppm; EIMS *m/z*: 355.

(*E*)-(5-(4-Nitrostyryl)-1,3-phenylene)bis(oxy)bis(*tert*-butyldimethylsilane) (24): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.24$ (s, 12H), 1.02 (s, 18H), 6.84 (d, J= 4.2 Hz, 1H), 6.67 (d, J= 2.1 Hz, 2H), 7.06 (d, J= 16.2 Hz, 1H), 7.15 (d, J= 16.2 Hz, 1H), 7.63 (d, J= 9.0 Hz, 2H), 8.22 ppm (d, J= 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 149.2$, 147.3, 145.7, 144.4, 132.7, 129.5, 125.6, 124.3, 124.0, 121.1, 119.8, 118.9, 26.9, 18.5, −4.01 ppm; EIMS *m/z*: 485.

(*E*)-(4-(4-Nitrostyryl)-1,2-phenylene)bis(oxy)bis(*tert*-butyldimethylsilane) (25): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.22$ (s, 12H), 1.01(s, 18H), 6.84 (d, J= 9.0 Hz, 1H), 6.92 (d, J= 16.2 Hz, 1H), 7.04 (m, 2H), 7.14 (d, J= 16.2 Hz, 1H), 7.59 (d, J= 8.7 Hz, 2H), 8.19 ppm (d, J= 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 148.1$, 147.2, 146.4, 144.3, 133.2, 129.9, 126.5, 124.3, 124.1, 121.3, 120.8, 119.6, 25.7, 18.2, −4.20 ppm; EIMS *m/z*: 485.

General procedure for the reduction of the nitro group (26–28): Fe powder (0.22 g, 4.0 mmol) in 5% acetic acid (15 mL) was stirred at 95 °C for 30 min and cooled to 50 °C. The solution of nitrostilbene (0.39 g, 0.8 mmol) in EtOAc (5 mL) was added to the reaction mixture. The mixture was stirred at 95 °C for an additional 1 h. After being cooled to room temperature, the mixture was filtered through Celite to remove Fe powder. The filtrate was quenched with saturated $NaHCO_3$ (30 mL) and extracted with EtOAc (3× 100 mL). The combined organic phase was washed with brine and dried (Na_2SO_4), and the solvent was evaporated. The product was purified by silica chromatography by using hexane/EtOAc (2:1) as eluent to give 87% yields of aminostilbenes (**27**–**29**).

(*E*)-4-(4-(*tert*-Butyldimethylsilyloxy)styryl)benzenamine (26): ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 0.21$ (s, 6H), 1.01 (s, 9H), 5.30 (br s, 2H), 6.58 (d, J= 8.4 Hz, 2H), 6.84 (d, J= 8.7 Hz, 2H), 6.95 (d, J= 16.5 Hz, 2H), 7.26 (d, J= 8.4 Hz, 2H), 7.41 ppm (d, J= 8.7 Hz, 2H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 153.8$, 148.4, 131.4, 127.3, 126.9, 124.9, 122.3, 119.98, 113.8, 25.5, 17.9, −4.5 ppm; EIMS *m/z*: 325.

(*E*)-4-(3,4-Bis(*tert*-butyldimethylsilyloxy)styryl)benzenamine (27): ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 0.02$ (s, 12H), 0.77 (s, 18H), 5.07 (s, 2H), 6.35 (d, J= 8.4 Hz, 2H), 6.61 (m, 3H), 6.75 (m, 2H), 6.81 (dd, J= 8.4, 1.8 Hz, 1H), 7.05 ppm (d, J= 8.4 Hz, 2H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 148.4$, 146.1, 145.0, 131.35, 127.3, 124.8, 122.5, 120.9, 119.1, 118.3 113.8, 25.7, 18.1212, 25.7, 18.1, −4.22 ppm; EIMS *m/z*: 455.

(*E*)-4-(3,5-Bis(*tert*-butyldimethylsilyloxy)styryl)benzenamine (28): ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 0.24$ (s, 12H), 1.02 (s, 18H), 5.34 (s, 2H), 6.12 (d, J= 3.9 Hz, 1H), 6.54 (d, J= 8.4 Hz, 2H), 6.61 (d, J= 2.1 Hz, 2H), 6.79 (d, J= 16.2 Hz, 1H), 6.96 (d, J= 16.2 Hz, 1H), 7.27 ppm (d, J= 8.4 Hz, 2H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta =$ 156.1, 148.8, 140.2, 129.5, 127.8, 124.4, 122.4, 113.73, 110.8, 109.8, 25.5, 17.96, −4.50 ppm.

General procedure for deprotection of the TBS group: Bu_4NF (1.0 M in THF, 1.5 mL, 1.5 mmol) was added to a solution of TBS-protected stilbene (0.36 g, 0.7 mmol) in THF (5 mL). After being stirred for 4 h at room temperature, the mixture was quenched with water (25 mL) and extracted with EtOAc (3×30 mL). The combined organic phase was washed with brine and dried (Na_2SO_4), and the solvent was evaporated. The product was purified by silica chromatography by using hexane/EtOAc (1:3) as eluent to give 95% yields of aminostilbenes (**1**–**3**).

(*E*)-4-(4-Aminostyryl)phenol (1): M.p. 119–121 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 5.16$ (s, 2H; NH_2), 6.51 (d, J= 8.4 Hz, 2H), 6.70 (d, J= 8.7 Hz, 2H), 6.78 (d, 2H), 7.18 (d, J= 8.4 Hz, 2H), 7.29 (d, J= 8.7 Hz, 2H), 9.38 ppm (s, 1H; OH); ^{13}C NMR (75 MHz, [D_6]DMSO):

δ = 156.8, 148.6, 129.5, 127.6, 127.5, 126.4, 125.7, 123.4, 115.9, 114.4 ppm; HREIMS calcd for $C_{14}H_{13}NO$: 211.0997 $[M]^+$; found 211.0999.

(*E*)-5-(4-Aminostyryl)benzene-1,3-diol (2): M.p. 208–210 °C; ^{1}H NMR (300 MHz, CD_3OD): δ = 6.12 (t, 1H), 6.41 (d, *J* = 2.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 16.2 Hz, 1H), 6.91 (d, *J* = 16.2 Hz, 1H), 7.26 ppm (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.6, 148.7, 141.6, 129.9, 128.8, 128.6,125.7, 116.5, 105.7, 102.4 ppm; HREIMS calcd for $C_{14}H_{13}NO_2$: 227.0946 $[M]^+$; found 227.0946.

(*E*)-4-(4-Aminostyryl)benzene-1,2-diol (3): M.p. 140–142 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 5.19 (s, 2H, NH_2), 6.50 (d, *J* = 8.1 Hz, 2H), 6.65 (m, 4H), 6.87 (s, 1H), 7.17 (d, *J* = 8.1 Hz, 2H), 8.84 (s, 1H), 8.91 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 148.1, 145.3, 144.6, 129.6, 127.1, 125.8, 125.3, 123.3, 117.7, 115.7, 113.9, 112.7 ppm; HREIMS calcd for $C_{14}H_{13}NO_2$: 227.0946 $[M]^+$; found 227.0944.

General procedure for phenylureas: A solution of isocyanate (0.12 g, 0.75 mmol) in CH_2Cl_2 (5 mL) was added to a solution of aminostilbene (0.34 g, 0.75 mmol) in CH_2Cl_2 (20 mL) at room temperature. The reaction mixture was stirred for 2 h. The product was removed from the reaction by filtration and used crude in the next step. Bu_4NF (1 M, in THF, 1.5 mL, 1.5 mmol) was added to a solution of TBS urea in THF (10 mL) at room temperature. The mixture was quenched with water (25 mL) and extracted with EtOAc (3× 50 mL). The combined organic phase was washed with brine and dried (Na_2SO_4) and the solvent was evaporated. The product was purified by silica chromatography by using hexane/EtOAc (1:3) as eluent to give 85% yields of phenylureas (**6**–**19**).

(*E*)-1-(4-(3,4-Dihydroxystyryl)phenyl)-3-phenylurea (6): M.p. 139–141 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.69 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 16.2 Hz, 2H), 6.74 (m, 3H), 7.26 (t, 2H), 7.43 (m, 6H), 8.65 (s, 1H), 8.70 (s, 1H), 9.0 ppm (br s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.4, 145.4, 145.2, 139.6, 138.6, 131.3, 128.9, 128.7, 126.8, 126.5, 124.7, 121.8, 118.2, 118.2, 115.7, 113.1 ppm; HREIMS calcd for $C_{21}H_{18}N_2O_3$: 346.1317 $[M]^+$; found 346.1315.

(*E*)-1-(4-(3,4-Dihydroxystyryl)phenyl)-3-(4-methoxyphenyl)urea (7): M.p. 143–145 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.69 (s, 3H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.86 (m, 5H), 6.94 (d, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.41 (m, 4H), 8.48 (s, 1H), 8.64 (s, 1H), 8.96 ppm (br s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 154.4, 152.6, 145.4, 145.2, 138.9, 132.7, 131.0, 128.9, 126.7, 126.5, 124.8, 120.0, 118.2, 118.1, 115.7, 113.9, 113.0, 55.1 ppm; HREIMS calcd for $C_{22}H_{20}N_2O_4$: 376.1423 $[M]^+$; found 376.1421.

(*E*)-1-(4-Aminophenyl)-3-(4-(3,4-dihydroxystyryl)phenyl)urea (8): M.p. 220–221 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 4.79 (s, 2H), 6.49 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 17.1 Hz, 2H), 6.93 (s, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 7.42 (m, 4H), 8.13 (s, 1H), 8.53 (s, 1H), 8.89 (s, 1H), 9.02 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.7, 145.4, 145.2, 143.9, 139.1, 130.8, 128.98, 128.5, 126.5, 124.8, 120.7, 118.2, 117.9, 115.7, 114.2, 113.0 ppm; HREIMS calcd for $C_{22}H_{19}N_3O_3$: 361.1426 $[M]^+$; found 361.1424.

(*E*)-1-(4-(3,4-Dihydroxystyryl)phenyl)-3-*p*-tolylurea (9): M.p. 164–166 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.22 (s, 3H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.82 (m, 2H), 6.92 (d, *J* = 14.7 Hz, 2H), 7.06 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.41 (m, 4H), 8.55 (s, 1H), 8.66 (s, 1H), 8.90 (s, 1H), 9.02 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.4. 145.3, 145.2, 138.7, 136.98, 131.1, 130.6, 129.1, 128.9, 126.7, 126.5, 124.7, 118.2, 118.1, 115.6, 112.98, 20.2 ppm; HREIMS calcd for $C_{22}H_{20}N_2O_3$: 360.1474 $[M]^+$; found 360.1472.

(*E*)-1-(4-(3,4-Dihydroxystyryl)phenyl)-3-(4-trifluoromethylphenyl)urea (10): M.p. 168–170 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.71(d, *J*=8.1 Hz, 1H), 6.84 (m, 2H), 6.92 (d, *J* = 16.8 Hz, 2H), 7.13 (m, 2H), 7.48 (m, 6H), 8.73 (s, 2H), 9.00 ppm (br s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 113.6, 116.2, 118.3, 118.8, 119.0, 125.1, 126.5, 126.6, 127.0, 127.5, 129.4, 132.1, 138.7, 143.9, 145.8, 145.9, 152.7 ppm; HREIMS calcd for $C_{22}H_{17}F_3N_2O_3$: 414.1189 $[M]^+$; found 414.1189.

(*E*)-1-(4-(3,4-Dihydroxystyryl)phenyl)-3-(4-fluorophenyl)urea (11): M.p. 156–158 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.74 (d, *J* = 8.1 Hz, 1H), 6.86 (m, 2H), 6.98 (d, *J* = 16.8 Hz, 2H), 7.13 (m, 2H), 7.48 (m, 6H), 8.73 (s, 2H), 9.00 ppm (br s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.5, 145.3, 138.6, 136.0, 131.3, 128.9, 126.8, 126.5, 124.7, 120.0, 118.3, 118.2, 115.7, 115.4, 115.1, 113.1 ppm; HREIMS calcd for $C_{21}H_{17}FN_2O_3$: 364.1223 $[M]^+$; found 364.1219.

(*E*)-1-(2,4-Difluorophenyl)-3-(4-(3,4-dihydroxystyryl)phenyl)urea (12): M.p. 210–212 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.75(d, *J*=8.1 Hz, 1H), 6.88 (m, 2H), 7.00 (d, *J* = 16.2 Hz, 2H), 7.05 (m, 1H), 7.35 (m, 1H), 7.45 (m, 4H), 8.13 (m, 1H), 8.55 (s, 1H), 8.96 (s, 1H), 9.11 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.2, 145.3, 138.3, 131.5, 128.9, 126.9, 126.6, 124.7, 124.1, 123.9, 122.0, 121. 9, 118.2, 115.7, 113.1, 111.1, 110.8 ppm; HREIMS calcd for $C_{21}H_{16}F_2N_2O_3$: 382.1129 $[M]^+$; found 382.1127.

(*E*)-1-(4-Chlorophenyl)-3-(4-(3,4-dihydroxystyryl)phenyl)urea (13): M.p. 216–218 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.74 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 16.5 Hz, 2H), 7.00 (m, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.50 (m, 6H), 8.78 (s, 1H), 8.84 (s, 1H), 9.01 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 152.2, 145.3, 138.3, 131.5, 128.9, 126.9, 126.6, 124.7, 124.1, 123.9, 122.0, 121.9, 118.2, 115.7, 113.1, 111.1, 110.8 ppm; HREIMS calcd for $C_{21}H_{17}ClN_2O_3$: 380.0928 $[M]^+$; found 380.0926.

(*E*)-1-(4-(4-Hydroxystyryl)phenyl)-3-phenylurea (14): M.p. 242–244 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.74 (d, *J* = 8.4 Hz, 1H), 6.89–6.94 (m, 3H), 7.26 (m, 2H), 7.35–7.45 (m, 8H), 8.65 (s, 1H), 8.70 (s, 1H), 9.50 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 156.95, 152.4, 139.6, 138.7, 131.3, 128.8, 128.4, 127.5, 126.5, 126.4, 124.9, 121.8, 118.2, 118.2, 115.5 ppm; HREIMS calcd for $C_{21}H_{18}N_2O_2$: 330.1368 $[M]^+$; found 330.1366.

(*E*)-1-(4-(4-Hydroxystyryl)phenyl)-3-(4-methoxyphenyl)urea (15): M.p. 241–243 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.69 (s, 3H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.96 (d, *J* = 16.5 Hz, 2H), 7.35–7.44 (m, 8H), 8.65 (s, 1H), 8.46 (s, 1H), 8.62 (s, 1H), 9.50 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 156.9, 154.5, 152.6, 138.9, 132.6, 131.1, 128.4, 127.5, 126.5, 126.3, 124.9, 120.0, 118.1, 1155, 113.96, 55.1 ppm; HREIMS calcd for $C_{22}H_{20}N_2O_3$: 360.1474 $[M]^+$; found 360.1479.

(*E*)-1-(2,4-Difluorophenyl)-3-(4-(4-hydroxystyryl)phenyl)urea (16): M.p. 238–240 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.73 (d, *J* = 8.1 Hz, 2H), 6.89–7.06 (m, 3H), 7.26–7.46 (m, 7H), 8.08 (m, 1H), 8.50 (s, 1H), 9.06 (s, 1H), 9.50 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 156.98, 152.1, 138.3, 131.5, 128.3, 127.5, 126.6, 124.8, 124.1, 123.9, 121.9, 118.2, 115.5 ppm; HREIMS calcd for $C_{21}H_{16}F_2N_2O_2$: 366.1180 $[M]^+$; found 366.1183.

(*E*)-1-(4-(3,5-Dihydroxystyryl)phenyl)-3-phenylurea (17): M.p. 230–232 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 6.12 (s, 1H), 6.40 (s, 2H), 6.87–6.94 (m, 2H), 6.98 (d, *J* = 16.5 Hz, 1H), 7.24–7.29 (m, 2H), 7.43–7.50 (m, 6H), 8.66 (s, 1H), 8.73 (s, 1H), 9.20 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 158.5, 152.4, 139.6, 139.2, 139.0, 130.7, 128.8, 127.6, 127.0, 126.9, 121.8, 118.2, 118.2, 104.4,

101.97 ppm; HREIMS calcd for $C_{21}H_{18}N_2O_3$: 346.1317 $[M]^+$; found 346.1315.

(*E*)-1-(4-(3,5-Dihydroxystyryl)phenyl)-3-(4-methoxyphenyl)urea (18): M.p. 223–245 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.70$ (s, 3H), 6.11 (s, 1H), 6.39 (d, $J = 1.5$ Hz, 2H), 6.85 (d, $J = 9.0$ Hz, 2H), 6.92 (d, $J = 16.2$ Hz, 2H), 7.33 (d, $J = 9.0$ Hz, 2H), 7.40–7.48 (m, 4H), 8.46 (s, 1H), 8.65 (s, 1H), 9.20 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 158.5$, 154.5, 152.5, 139.4, 139.0, 132.6, 130.5, 127.6, 127.0, 126.7, 120.0, 118.0, 113.96, 104.4, 101.9, 55.1 ppm; HREIMS calcd for $C_{22}H_{20}N_2O_4$: 376.1423 $[M]^+$; found 376.1418.

(*E*)-1-(2,4-Difluorophenyl)-3-(4-(3,5-dihydroxystyryl)phenyl)urea (19): M.p. 218–220 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 6.12$ (t, 1H), 6.39 (d, $J = 1.8$ Hz, 2H), 6.93 (d, $J = 16.5$ Hz, 2H), 7.00–7.07 (m, 1H), 7.27–7.33 (m, 1H), 7.41–7.51 (m, 4H), 8.08 (m, 1H), 8.50 (s, 1H), 9.08 (s, 1H), 9.21 ppm (s, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 158.5$, 158.3, 153.9, 138.9, 138.8, 130.9, 127.5, 127.1, 127.0, 118.1, 104.4, 104.1, 102.0 ppm; HREIMS calcd for $C_{21}H_{16}F_2N_2O_2$: 382.1129 $[M]^+$; found 382.1124.

Assay of glycosidase activity: α-Glucosidase (E.C. 3.2.1.20), β-glucosidase (E.C. 3.2.1.21), α-mannosidase (E.C. 3.2.1.24), α-galactosidase (E.C. 3.2.1.22) and α-amylase (E.C. 3.2.1.1) inhibitory activities were calculated by using standard experimental procedures with some modifications. All enzyme activities were determined by using the appropriate substrates (*p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *o*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, and starch, respectively) at the optimum pH of each enzyme. The reaction was stopped by adding NaOH (2 M). The glycosidases were assayed according to standard procedures either by following the hydrolysis of nitrophenyl glycosides spectrophotometrically[23,24] or by evaluating the reducing sugar formed in amylase assays.[15] The inhibitory effects of the tested compounds were expressed as the concentrations that inhibited 50% of the enzyme activity (IC_{50}). Kinetic parameters were determined by using the Lineweaver–Burk double-reciprocal-plot method with increasing concentrations of substrates and inhibitors. The inhibitors deoxynorijirimycin (Sigma–Aldrich) and resveratrol (ChromaDex, Irvine, CA, USA) were used in the assays for comparison. All parameter values were then calculated by using SigmaPlot (Systat Software Inc.).

Progress curve determination and time-dependent assays: Time-dependent assays and progress curves were carried out by using 0.05 unit mL^{-1} α-glucosidase, and *p*-nitrophenyl-α-D-glucopyranoside as substrates in potassium phosphate buffer (0.05 M; pH 6.8) at 37 °C. Enzyme activities were measured spectrophotometrically continuously for 12 min. To determine the kinetic parameters associated with time-dependent inhibition of α-glucosidase, progress curves for 30 min were obtained at several inhibitor concentrations by using fixed substrate concentrations. The data were analyzed by using the a nonlinear regression program (Sigma Plot, SPCC Inc., Chicago, IL, USA) to give the individual parameters for each curve; v_i (initial velocity), v_s (steady-state velocity), k_{obs} (apparent first-order rate constant for the transition from v_i to v_s), and K_i^{app} (apparent K_i) according to Equations (3) and (4).[22]

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