8252 J. Med. Chem. 2010, 53, 8252–8259
DOI: 10.1021/jm100757r

Journal of Medicinal Chemistry Article

Hydroxycoumarin Derivatives: Novel and Potent α-Glucosidase Inhibitors

Qiong Shen,*[,†,‡,§] Jialiang Shao,[‡] Quan Peng,[‡] Wanjin Zhang,[§] Lin Ma,*[,‡] Albert S. C. Chan,[§] and Lianquan Gu[§]

[†]*School of Pharmacy and Centre for Biomolecular Science, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.,* [‡]*School of Chemistry and Chemical Engineering, and* [§]*School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510275, P. R. China*

Received December 3, 2009

A novel class of hydroxycoumarin derivatives were found to be potent α-glucosidase inhibitors. Their syntheses were reported and the structure−activity relationship was established. Kinetic enzymatic assays indicated that compound **10** was a slow-binding and noncompetitive inhibitor with a K_i value of 589 nM, while compound **11** was a competitive inhibitor with a K_i value of 4.810 μM. Among all hydroxycoumarin derivatives studied, compounds **10** and **11** exhibited the highest activities, were specific inhibitors of α-glucosidase, and could be exploited as the lead compounds for the development of potent α-glucosidase inhibitors. Compounds **10** and **11** were also selected for further discussion for the mechanism of enzymatic inhibition.

1. Introduction

α-Glucosidases are ubiquitous in nature. They are involved in the synthesis and breakdown of α-linked di-, oligo-, and polysaccharides, playing a crucial role in metabolic processes such as food storage and utilization and in cellular communication through modification of the glycosylation state of proteins.[1,2] Inhibition of α-glucosidases and related enzymes play important roles in the treatment of diabetes, human immunodeficiency virus (HIV/AIDS), cancer, and other degenerative diseases.[3−6] Indeed several α-glucosidase inhibitors including nojirimycin, *N*-butyldeoxy nojirimycin, and castanospermine have been shown to inhibit HIV replication and HIV-mediated syncytium formation.[6−10]

Coumarin derivatives have been reported to possess some biological activities similar to those of α-glucosidase inhibitors such as anti-HIV activity,[11] HIV-1 protease inhibition,[12] antimutagenic,[13] lipoxygenase and cycloxygenase inhibition,[14,15] and antitumor metastasis.[16,17] Moreover, the structure similarity between hydroxycoumarin derivatives and a potent α-glucosidase inhibitor genistein[18] prompted us to investigate the α-glucosidase inhibitory activity of hydroxycoumarin derivatives.

Although coumarin derivatives have shown to be active against various biological targets, its α-glucosidase inhibition property has not been studied. In the course of looking for novel, easily accessible, and chemically stable α-glucosidase inhibitors, we found that hydroxycoumarin derivatives are good candidates. We propose to examine their α-glucosidase inhibition properties and to expand the application scope of this type of easily accessible compound. Herein we report the preparation and the α-glucosidase inhibition properties of a series of hydroxycoumarin derivatives and discuss thoroughly the mechanism of enzymatic inhibition for the first time.

2. Chemistry and Biological Assay

2.1. Synthesis of Compounds 1−11. The general synthesis is described in Schemes 1−4. Condensation reaction between benzene-1,2,3-triol and ethylacetonacetate gave 7,8-dihydroxy-4-methyl-2*H*-chromen-2-one (**1**) under Pechmann conditions.[19] Compounds **2** and **3** were prepared through reaction of *p*-quinone with acetic anhydride and subsequently treated with either ethyl acetonacetate[20] or (±)-malic acid, as indicated in Scheme 2. Treatment of resorcinol or 2-methylresorcinol with malonic acid using a modified method[21] yielded **4** and **5**, which were further reacted with *p*-quinone to give **10** and **11**, respectively. O-Methylation of compounds **4** and **5** with methyl iodide in acetone afforded **4′**, **4″**, and **5′**. Compound **6** was obtained by a method similar to that for compounds **4** and **5**. Resorcinol and ethylacetoacetate were treated under Pechmann conditions[19] to give substituted coumarin **7**. Scheme 3 illustrates the synthesis of compound **8** by Knoevenagel condensation of salicylaldehyde with glycine methyl ester in the presence of triethylamine to give an intermediate, which was subjected to hydrolysis with 38% sulfuric acid to give compound **8**.[22] Scheme 4 illustrates the synthesis of compound **9** by Wittig type reaction to give an intermediate, which was treated with boron tribromide in refluxing dichloromethane, leading to compound **9**.[23]

It has been shown previously that treatment of 1,4-benoquinone with hydroxycoumarins afforded 2,3-disubstituted but not 2,5-disubstituted 1,4-benoquinones in a region-selective fashion.[24] The X-ray structure of **10** confirmed it to be a 2,3-disubstituted 1,4-*p*-dihydroxyphenol (Figures 1 and 2).[25] However, a 1,4-benzoquinone structure was indicated for **11**, based on results from mass spectrometry and ^{1}H NMR in comparison with published data.[24]

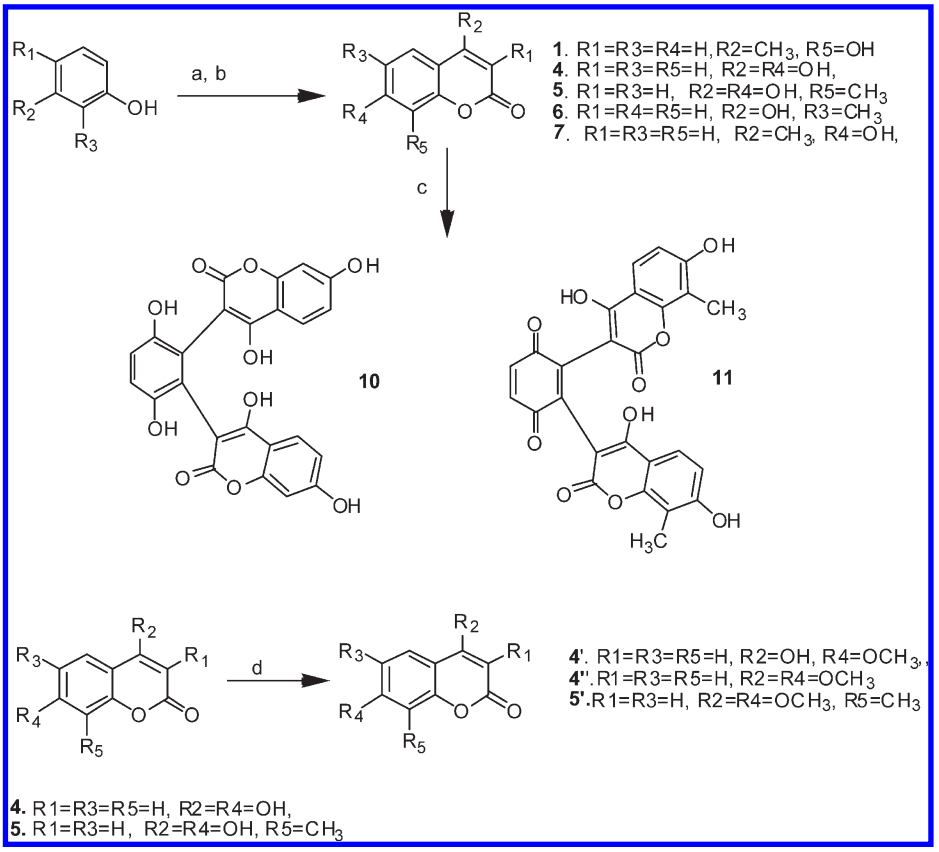
2.2. Biological Assay. Both *p*-nitrophenyl (PNP[a]) glycosides and α-glucosidase (from baker's yeast) were purchased from Sigma (St. Louis, MO). Compounds **12**, **13**, and **14**

*To whom correspondence should be addressed. For Q.S.: phone, 86-20-84110688; fax, 86-20-84112786; e-mail,nespq@mail.sysu.edu.cn. For L.M.: phone, 86-20-84113690; fax, 86-20-84112245; e-mail, cesmal@mail.sysu.edu.cn..

[a] Abbreviations: PNP, *p*-nitrophenyl; CD, circular dichroism, DMF, dimethylformamide; DMSO, dimethylsulfoxide; ESI, electrospray ionization; HPLC, high pressure liquid chromatography; LC, liquid chromatography; RT, room temperature; MS, mass spectrometry; NMR, nuclear magneticresonance; THF, tetrahydrofuran; TLC, thin layer chromatography; UV, ultraviolet; NAD^+, nicotinamide adenine dinucleotide coenzyme; TMS, tetramethylsilane; FAB, fast-atom bombardment; DNS, dinitrosalicyclic acid; PNPG, *p*-nitrophenyl-β-D-galactopyranoside; IC_{50}, concentration required for 50% of full inhibitory effect.

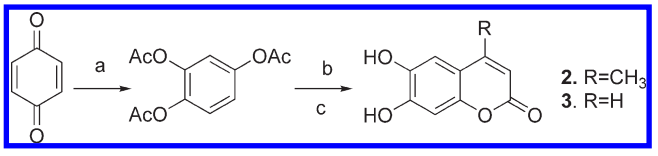
 Published on Web 11/05/2010

Scheme 1[a]



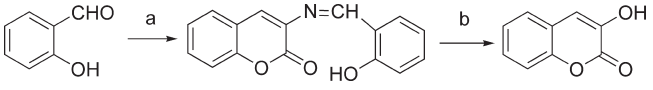
[a] Reagents and conditions: (a) ethyl acetonacetate, H_2SO_4, 0−5 °C; (b) malonic acid, $ZnCl_2$, $POCl_3$, 60−65 °C, 12 h; (c) **4** or **5**, *p*-quinone, 50% aq acetone, 50 °C, 10 h; (d) **4′**, **4″**, **5′**, anhydrous K_2CO_3, anhydrous acetone, iodomethane, reflux, 8 h.

Scheme 2[a]



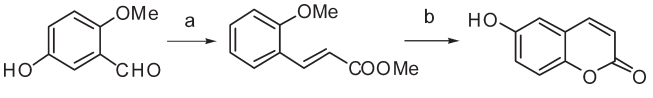
[a] Reagents and conditions: (a) acetic anhydride, concentrated sulfuric acid, 40−50 °C; (b) (±)-malic acid, 75% sulfuric acid, 80 °C; (c) ethyl acetoacetate, 75% sulfuric acid, 80 °C.

Scheme 3[a]



[a] Reagents and conditions: (a) glycine methyl ester, Et_3N; (b) hydrolysis, 38% sulfuric acid.

Scheme 4[a]



[a] Reagents and conditions: (a) $Ph_3P{=}CHCOOMe$, toluene, reflux; (b) BBr_3, CH_2Cl_2, reflux.

(Figure 3) were purchased from Aldrich-Sigma and used for a comparative study.

All compounds were tested for their ability to inhibit α-glucosidase using the reported procedures.[26,27] The inhibition constants (K_i) were generated by the Lineweaver−Burk plots.[28] Circular spectroscopy was used to examine the compounds affecting on the secondary structure of α-glucosidase protein.[29] Selected compounds were evaluated for *β*-glucosidase[30,31] and *β*-D-galactosidase inhibition.[32,33]

3. Results and Discussion

The compounds were tested in α-glucosidase enzymatic assay using yeast α-glucosidase as described in the Experimental Section. 5,7-Dihydroxy-4-methyl-2*H*-chromen-2-one (**12**), deoxynojirimycin (**13**), and genistein (**14**) (Figure 3) were used for a comparative study. The results are shown in Table 1. Compound **10** was the most potent α-glucosidase inhibitor with an IC_{50} value of 0.86 *μ*M, being ∼14 times more potent than genistein (**14**, IC_{50} = 12.36 *μ*M). Compound **11** also exhibited potent inhibitory activity with an IC_{50} value of 2.82 *μ*M, being more effective than genistein, a known α-glucosidase inhibitor. Hydroxycoumarin derivatives (**1**−**5**) had significantly reduced inhibitory activity with IC_{50} in the range 38−94 *μ*M. Nevertheless 4,7-dihydroxy-2*H*-chromen-2-one (**4**) and compound **8** gave modest activity with IC_{50} values of 38.85 and 35.30 *μ*M, having a potency similar to that of the reference inhibitor deoxynojirimycin (**13**). Compounds **4′**, **4″**, and **5′** showed weak inhibitory activity. Compound **7** has potency similar to that of compounds **1** and **5**. Compounds **3**, **6**, and **9** were not active at the highest concentrations tested.

Analysis of the structure−activity relationship indicated that the introduction of either a methyl or hydroxyl group at the 8-position of hydroxycoumarin (**4**) resulted in a decreased α-glucosidase inhibitory activity as shown by compounds **1**

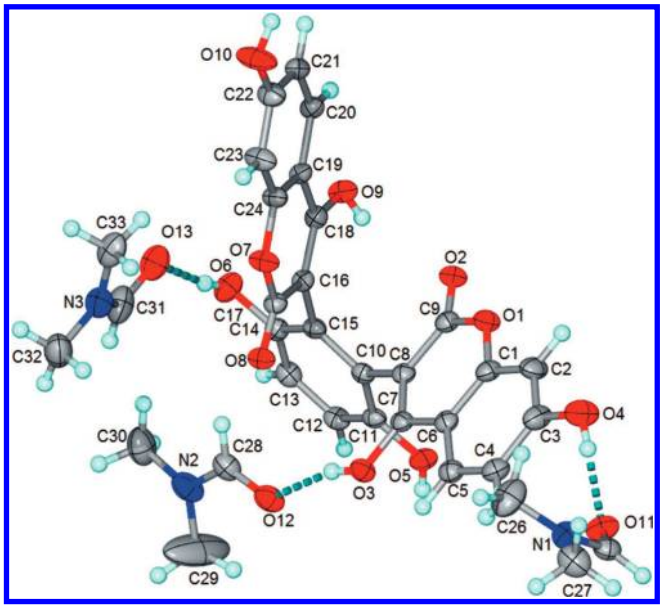


Figure 1. X-ray crystal structure of **10**:[25] asymmetric unit, with displacement ellipsoids drawn at the 50% probability level.

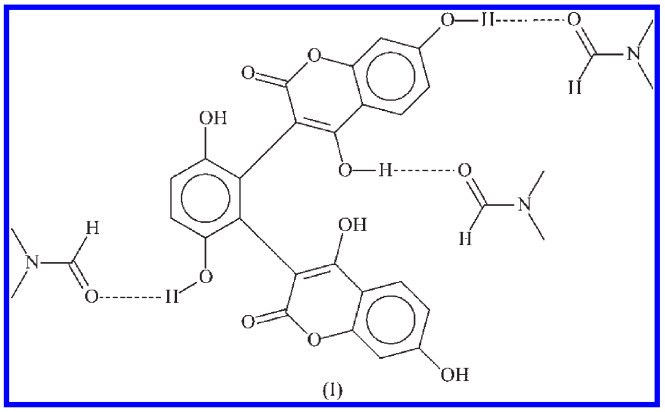


Figure 2. Compound **10**[25] crystallizes from dimethylformamide (DMF) as a trisolvate.

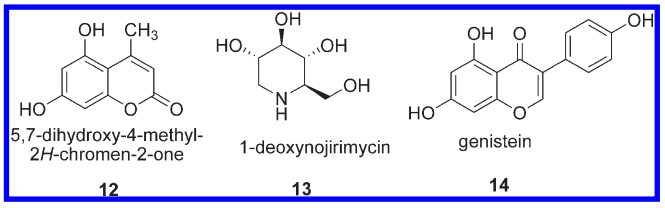


Figure 3. Structures of **12**−**14**.

and **5** in comparison with **4**. An additional hydroxyl or a methyl substituted at 6-position was not tolerated; thus, compounds **2**, **3**, **6**, and **9** almost completely abolished the activity. In contrast, additional hydroxyl substituted at 3-position played a major role in the inhibitory activity, so compound **8** showed modest activity. Introduction of a hydroxyl group at the 4-position of hydroxycoumarin (**4**) played a more major role in the inhibitory activity than that of methyl group at the 4-position of hydroxycoumarin (**7**). O-Methylation of 4-OH or/and 7-OH led to a large decrease in the activity as shown by compounds **4′**, **4″**, and **5′** in comparison with **4** and **5**. This indicates that 4-OH and 7-OH in compounds **4** and **5** were crucial to the activity, primarily as an H-bonding donor to interact with α-glucosidase, since the hydroxyl group is an H-bonding donor/acceptor, while the methoxy group can just act as an H-bonding acceptor. Compounds **10** and **11**, possessing excellent enzymatic inhibiting activity, were favorable for binding to the α-glucosidase. **10** was more potent than **11**, confirming further deactivation of the methyl group at the 8-position of hydroxycoumarin, as shown also in **5**. It is assumed that the hydroxyl groups at C-1 and C-4 of **10** may serve as hydrogen donors to form hydrogen bonds with the enzyme, while **11** may not be able to form such interactions.

We evaluated the selectivity of potent several α-glucosidase inhibitors for two sugar hydrolases (β-glucosidase and β-D-galactosidase) inhibitions (Tables 2 and 3). As indicated, compound **8** neither inhibited β-glucosidase nor β-D-galactosidase. Compounds **10** and **11** showed weak inhibition on β-glucosidase and β-D-galactosidase (IC_{50} > 200 μM). Compound **12** showed modest inhibitory activity against β-glucosidase and β-D-galactosidase. Compounds **4** and **5** did not inhibit the β-D-galactosidase. Compound **5** gave some inhibition against the β-glucosidase (IC_{50} = 87.6 μM), whereas compound **4** had potency similar to that of compounds **10** and **11**. Therefore, compounds **4**, **8**, **10**, and **11** could belong to specific α-glucosidase inhibitors.

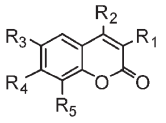
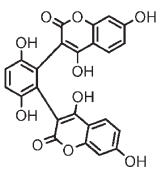
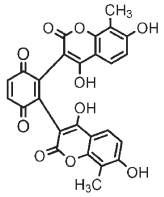
To further investigate the inhibitory effect of compounds **10** and **11** on the activity of α-glucosidase, we also performed dose-response experiments. These results (not shown) showed that the activity of α-glucosidase was decreased by compounds **10** and **11** in a dose-responsive manner, indicating that these two compounds have strong affinity toward α-glucosidase.

Slow binding, or slow onset of inhibition, is a widespread phenomenon among potent glycosidase inhibitors. In order to support our point, we investigated whether the IC_{50} values vary by preincubation of the inhibitor **10** with the enzyme. These results show that the IC_{50} value for the inhibition of α-glucosidase vary depending on the time of pretreatment with compound **10**. When the substrate and compound **10** were added simultaneously, the IC_{50} was 88.36 μM. This value decreased almost 100-fold when α-glucosidase was treated with compound **10** at 37 °C for 1 h before the initiation of enzyme reaction (not shown). The data indicate that the inhibition mode seems to be a slow-binding inhibitory mode.

The mechanism of the representative inhibitors binding to the α-glucosidase was further studied by analysis of the Lineweave−Burk plots.[28] The K_i value was calculated using the values of V_{max} obtained at 1.00 and 3.00 μM for compound **10** and the values of V_{max} obtained at 2.00 and 5.00 μM for compound **11**, respectively. It revealed that compound **10** was a noncompetitive inhibitor with a K_i value of 0.587 μM (Figure 4). In contrast, compound **11** was a competitive inhibitor with a K_i value of 4.81 μM.

The percentage of α-helix and loop (β-turn and random) and the special ratio of α/β within the secondary structure of the enzyme molecular of α/β structure relate to the native conformation.[34,35] The special functional domain may be important for preservation of enzymatic activity. To further elucidate the inhibitory mechanism, the effects of inhibitors **4**, **10**, **11** on the secondary structure of α-glucosidase were studied with circular dichroism spectra.[29,36] The results showed that the inhibitors caused a decline in the percent of α-helix in a dose dependent manner and changes in the ratios of α/β, as shown in Table 4. Generally, the helices are the most stable elements of secondary structure of protein.[37] This suggested that inhibitors caused some disturbance in the secondary structure and affected the folding of α-glucosidase.[29,34]

Table 1. Inhibition of α-Glucosidase[a,26,27]

Compound structure	Compound No	R1	R2	R3	R4	R5	IC_{50}, μM
	**1**	H	CH_3	H	OH	OH	52.70
	2	H	CH_3	OH	OH	H	94.25
	3	H	H	OH	OH	H	N/A
	4	H	OH	H	OH	H	38.85
	4'	H	OH	H	OCH	H	96.10
	4''	H	OCH_3	H	OCH_3	H	>100
	5	H	OH	H	OH	CH_3	50.53
	5'	H	OCH_3	H	OCH_3	CH_3	NA
	6	H	OH	CH_3	H	H	NA.
	7	H	CH_3	H	OH	H	56.2
	8	OH	H	H	H	H	35.3
	9	H	H	OH	H	H	NA
 **10**  **11**	**10**						0.86
	11						2.82
	12						64.61
	13						32.53
	14						12.36

[a] Values are the mean of at least three independent determinations. NA means no inhibition (less than 20% inhibition at 200 μM).

Table 2. Selection of Compounds for Inhibition of β-Glucosidase[a,30,31]

test sample	content (μmol/L)	maximum inhibition (%)	IC_{50} (μM)
4	400	36.7	>400
5	400		87.6
8	400	0	NA
10	200	23.0	>200
11	200	15.6	>200
12	400		45.4

[a] Values are the mean of at least three independent determinations. NA means no inhibition (less than 20% inhibition at 400 μM).

Table 3. Selection of Compounds for Inhibition of β-D-Galactosidase[a,32,33]

test sample	content (μmol/L)	maximum inhibition (%)	IC_{50} (μM)
4	400	0	NA
5	400	0	NA
8	400	0	NA
10	200	2.0	>200
11	200	31.7	>200
12	400	53.9	>200

[a] Values are the mean of at least three independent determinations. NA means no inhibition (less than 20% inhibition at 400 μM).

Apparently, the enzyme undergoes a series of inhibitor-induced conformational changes as shown in Figure 5 (conformational changes of the of α-glucosidase is most obvious when compound **11** interacted with the enzyme), but minor conformational changes may have influence on the potency of enzyme inhibition.[37] The inhibitor induced conformational changes of specific elements of the secondary structure in the enzyme molecule may be part of the mechanism of the inhibition required to prevent the hydration of the substrate binding site and also required to induce the cleft closure to avoid substrate entrance.[34,35,37] This may result in the failure of formation the active center, a decrease of the substrate binding, a misfolding of the polypeptide chain, and finally inactivation of the enzyme. These may have been caused by the cluster effect, and these results may contribute to a more complete understanding of the enzyme−inhibitor interaction.

Taken together, the reasons why the compounds could inhibit the activity of α-glucosidase and why the specific elements of the secondary structure in the enzyme molecule could have been changed, even why these two compounds could have strong affinity toward α-glucosidase, were mainly ascribed to the interaction (cluster effect) between the compounds and enzyme. Compounds **10** and **11** were selected for further detailed evaluation for the mechanism of enzymatic inhibition.

We know the crystal structure of **10** (Figures 3 and 4) and note that the 1-hydroxy group serves as a hydrogen-bond donor to a DMF molecule, whereas the substituent at the 3-position functions as a hydrogen-bond donor to two DMF molecules,[25] indicating that compound **10** could serve as a hydrogen-bond donor easily. Therefore, we proposed that the

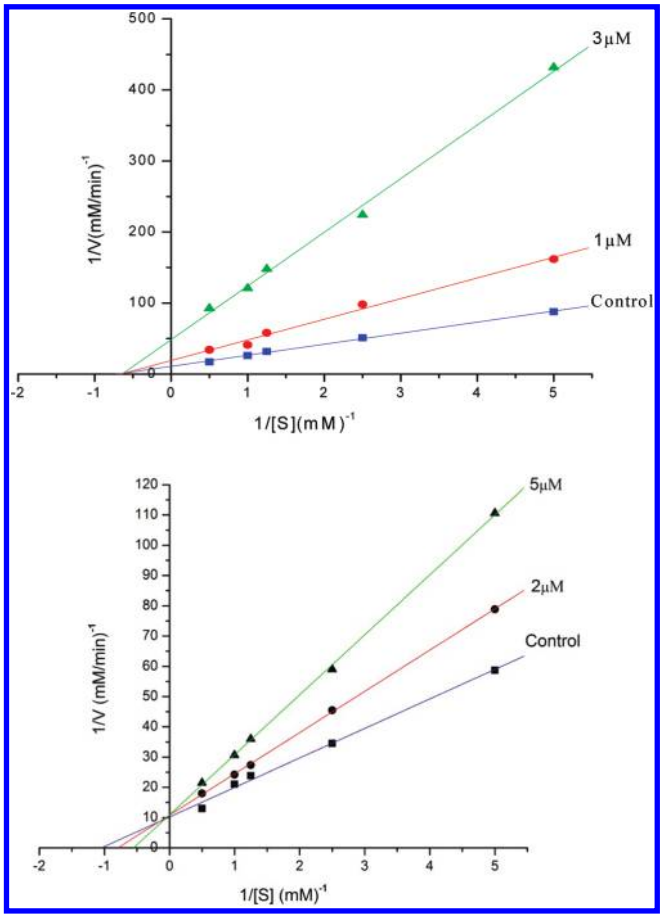


Figure 4. Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by compounds **10** (up) and **11** (down). α-Glucosidase (50 μL, 10 U/mL) was treated with **10** or **11** at 37 °C for 1 h, followed by varying concentrations of PNP.

Table 4. Effects of Hydroxycoumarin Inhibitors on the Secondary Structure of α-Glucosidase[a]

compd	α-helix (%)	β-sheet (%)	β-turn (%)	random (%)
without inhibitor	35.00	33.30	6.60	25.10
10 (0.5 μM)	32.70	30.00	9.20	28.10
10 (0.3 μM)	33.40	23.80	13.40	29.40
without inhibitor	32.60	27.60	10.90	28.90
11 (5.0 μM)	26.00	33.60	9.30	31.10
11 (8.0 μM)	25.40	34.30	8.20	32.00
without inhibitor	50.10	26.70	3.20	20.00
4 (30.0 μM)	37.10	7.40	21.40	34.10
4 (15.0 μM)	47.10	5.30	18.00	29.40

[a] Values are the mean of at least three independent determinations.

1-hydroxy group and the substituent at the 3-position functions may receive and donate the hydrogen bond from the side chain of α-glucosidase protein residues because residues are believed to play an important role in the catalytic mechanism as the corresponding residues in oligo-1,6-glucosidase.[38] The phenol moiety seems to be an effective chemical group for binding its catalytic activity.[38]

The multiple hydroxyl functions of **10** and **11** are critical for their inhibitory activity toward α-glucosidase. The X-ray crystal structure[34] of maltose in complex with α-glucosidase indicates that one of its glucose rings is bound by multiple hydrogen bonds to Asn-153, His-203, Asp-260, Asp-116, and Arg-263 while hydrophobically stacked between Val-117 and the β-nicotinamide ring of NAD^+. Another ring of maltose

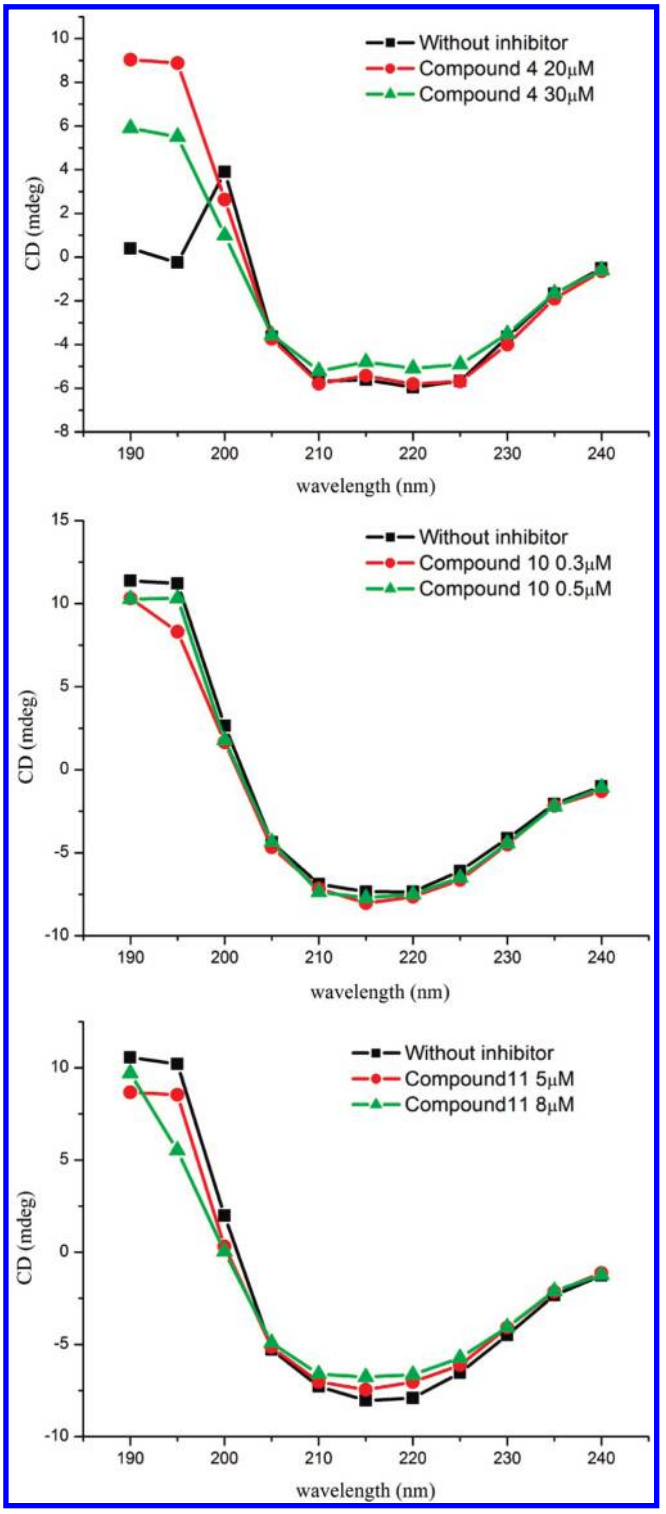


Figure 5. CD (circular dichroism) spectra of enzyme after enzyme interaction with compounds **4**, **10**, **11**. The CD spectra were recorded with 5 times accumulation.

formed a hydrophobic interaction with Ph-238 to stabilize the interactions. The structures of compounds **10** and **11** have some similarity to that of maltose (many hydroxy groups and six-member cyclic structure), indicating that a similar interaction might be formed between compounds and α-glucosidase. Serving as a hydrogen-bond, the hydroxy groups at the 1C-/4C-position of **10** donors may contribute to its greater potency in comparison to **11**. *p*-Dihydroxyphenol has been

displaced by *p*-benzoquinone,[25,29] and the number to form the hydrogen bond may decrease in the absence of some phenol moiety and then result in weakening of the hydrogen bond interaction. A strong hydrogen bond between the phenolic oxygen of chalcone derivatives and α-glucosidase protein residues in the active binding site has been suggested previously,[38] supporting the potential role of the phenol group as a surrogate for the terminal monosaccharide of the substrate.[38,39] Furthermore, the bis-chromen-2-one moieties (extended π-conjugated system) of **10** or **11** may facilitate the extensive hydrophobic interactions (π-stacking) within the active binding site.[38,40] The two interactions (mainly hydrogen bond and hydrophobic interactions) between the inhibitors and enzyme form a cluster effect to influence the conformation and then change the activity of the enzyme. The aforementioned results also strongly suggest that the hydroxyl functions make substantial contributions to the remarkably enhanced inhibitory activities as shown by compounds **4** and **5** in comparison with **4′**, **4″**, and **5′**. However, the detailed structural characteristics of the inhibitor in complex with α-glucosidase can only be fully elucidated by X-ray crystallography.

In summary we have prepared a class of novel α-glucosidase inhibitors bearing a coumarin skeleton. The lead compounds **10** and **11** have demonstrated excellent inhibitory activities against α-glucosidase with IC_{50} values in a range from low micromolar to subnanomolar and specific inhibitors of α-glucosidase. The mechanism studies indicated that the hydrogen bond and extensive hydrophobic interactions between the inhibitors and enzyme are in a cooperative fashion (cluster effect) to have influence on the conformation of the enzyme, to change the specific elements of the secondary structure, and finally to cause inactivation of the enzyme. Knowledge gained in this study could help future developments for searching for new α-glucosidase inhibitors.

4. Experimental Section

Chemistry. Melting points were measured using an Electrothermal 8103 apparatus and were uncorrected. IR spectra were recorded with Perkin-Elmer 398 and FT spectrophotometers. ^{1}H NMR spectra were recorded on a Varian Unity INOVA 500 MHz spectrometer with TMS as internal standard. The values of the chemical shift (δ) are given in ppm, and the coupling constants (*J*) are in Hz. All chemicals used were of analytical grade. Progress of the reactions was monitored by TLC on precoated 60 F_{254} silica gel plates (0.25 mm, Merck). ESI-MS spectra were performed on a Thermo Finigan LCQ DECA XP ion trap mass spectrometer. Elemental analysis was carried out on an Elementar Vario EL CHNS elemental analyzer. All compounds tested in vitro were determined to be of >95% purity by HPLC and EA. The purity of compound **10** is determined to be almost 100%, as it is a single crystal.

7,8-Dihydroxy-4-methyl-2*H*-chromen-2-one (1). A mixture of pyrogallol (12.6 g, 0.1 mol) and concentrated H_2SO_4 (100 mL) was stirred at −5 to 5 °C. Ethyl acetoacetate was added cautiously to the reaction mixture, and the solution was kept at −5 to 5 °C for 2 h with stirring. The mixture was then poured into cold water (1 L), and the precipitate was collected, washed with cold water, and crystallized from EtOH.

6,7-Dihydroxy-4-methyl-2*H*-chromen-2-one (2).[21] Hydroxydroquinone triacetate was prepared according to known methods. A mixture of ethyl acetoacetate (0.045 mol) and hydroxydroquinone triacetate (0.045 mol) was stirred for 20 min, and 75% sulfuric acid (45 mL) was added. The mixture became a homogeneous and gave a deep red solution. The red solution was heated on a water bath with occasional shaking and stirring until the temperature reached 80 °C. The mixture was maintained at this temperature for $1^1/_2$ h. It was then cooled to room temperature, filtered, and the precipitate was washed to give **2**.

6,7-Dihydroxynaphthalen-2(1*H*)-one (3). A similar procedure to that described above was employed.

4,7-Dihydroxy-2*H*-chromen-2-one (4). A mixture of resorcinol (11 g), malonic acid (12.3 g), anhydrous zinc chloride (44 g), and phosphorus oxychloride (33 mL) was heated with stirring at 60−65 °C for 12 h. The mixture was cooled and quenched with ice−water. The crude product was collected and dissolved in 5% sodium carbonate. An oily byproduct was removed and acidification of the remaining solution gave the residues which were recrystallized from EtOAc to yield **4**. Yield 62%, mp 244−246 °C. MS: *m*/*z* 179 $(M + H)^+$. ^{1}H NMR (acetone-d_6) δ: 7.68 (d, 1H, J = 9.0 Hz, 5-H), 6.82 (d d, 1H, J = 2.4 Hz, 6-H), 6.71 (d, 1H, J = 2.4 Hz, 8-H), 5.55 (d, 1H, J = 3.0 Hz, 3-H). Anal. Calcd for $C_9H_6O_4$: C, 60.68; H, 3.39. Found: C, 60.53; H, 3.48.

4-Methoxy-7-hydroxy-2*H*-chromen-2-one (4′). To a 25 mL round-bottom flask, compound **4** (4.4 mmol), iodomethane (4.8 mmol), K_2CO_3 (6.6 mmol), and anhydrous acetone (50 mL) were added. The mixture was refluxed for 8 h. After the mixture was cooled to RT, solvent was removed in vacuo, and the residue was dissolved in a 1:1 EtOAc/H_2O mixture. The product was extracted with EtOAc, washed with H_2O and brine, then dried over anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure. The residue was purified by column chromatography through silica gel (DCM/EtOAc) to get compound **4′**. Yield 25%, ESI-MS: 191 $(M - H)^-$. ^{1}H NMR (300 MHz, DMSO) δ: 3.80 (s, 3H), 5.99 (s,1H), 6.67 (dd, J = 9.39, J = 2.28, 1H), 6.75(dd, J = 9.39, J = 2.28, 1H), 7.95(d, J = 2.28,1H). Anal. Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.41; H, 4.26.

4,7-Dimethoxyy-2*H*-chromen-2-one (4″). **4″** was obtained by a similar method described above. Yield 32%. ESI-MS: *m*/*z* 205 $(M - H)^-$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.87 (s, 3H), 3.97 (s, 3H), 5.56 (s, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.83 (dd, J = 8.4 and 2.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H). Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 64.00; H, 4.92.

4,7-Dihydroxy-8-methyl-2*H*-chromen-2-one (5). **5** was obtained by the method described to get **4** above. Yield 75%, mp 269−271 °C. MS: *m*/*z* 205 $(M - H)^-$. ^{1}H NMR (acetone-d_6) δ: 7.54 (d, 1H, J = 9.0 Hz, 5-H), 6.88 (d, 1H, J = 9.0 Hz, 6-H), 5.51 (s, 1H, 3-H), 2.22 (s, 3H, 8-CH_3). Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 64.01; H, 4.95.

4-Methoxy-7-hydroxy-8-methyl-2*H*-chromen-2-one (5′). **5′** was obtained by a similar method described to get the compound **4′**. Yield 22%. ESI-MS: *m*/*z* 219 $(M - H)^-$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.29 (s, 3H), 3.91 (s, 3H), 3.96 (s, 3H), 5.56 (s, 1H), 6.81 (d, J = 8.7 Hz, 1H), 7.62 (d, J = 8.7 Hz, 1H). Anal. Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.42; H, 5.51

4-Hydroxy-6-methyl-2*H*-chromen-2-one (6). **6** was obtained by the method described to get compound **4**.

4-Methyl-7-hydroxycoumarin (7). Compound **7** was prepared based on a literature method.[19] Yield 80%. FAB-MS *m*/*z*: 177 ([M + H]), 154 ([M − OH]). These data are in agreement with the literature results.[41]

3-Hydroxy-2*H*-chromen-2-one (8). **8** was prepared based on a literature procedure.[22]

3,3′-(3,6-Dihydroxycyclohexa-1,4-diene-1,4-diyl)bis(4,7-dihydroxy-2*H*-chromen-2-one) (10). A solution of compound **4** and *p*-quinone in 50% aqueous acetone was heated at 50 °C for 10 h with stirring. Solvents were evaporated under reduced pressure. The residue was purified by column chromatography through silica gel (cyclohexane/acetone 1:4 v/v) to yield pure compound **10**. Yield: 15%, mp 140−142 °C. ESI-MS: 463 $(M + H)^+$. ^{1}H NMR (acetone-d_6) δ: 7.74−7.71 (dd, 2H, 5′-H), 6.91 (d, 2H, 2-H, 3-H), 6.84−6.80 (dd, 2H, 6′-H), 6.63−6.62 (m, 2H, 7′-OH). Anal. Calcd for $C_{24}H_{14}O_{10}$: C, 62.34; H, 3.04. Found: C, 62.21; H, 3.15.

2,5-Bis(4,7-dihydroxy-8-methyl-2-oxo-2*H*-chromen-3-yl)cyclohexa-2,5-diene-1,4-dione (11). **11** was prepared in a similar manner. Yield: 12%, mp 152−154 °C. ESI-MS: 487 $(M - H)^-$.

^{1}H NMR (DMSO-d_6) δ: 7.58, 7.40 (dd, 2H, J = 6.0 Hz, 5′-H), 6.83−6.72 (m, 4H, 2-H, 3-H, 6′-H), 2.08 (d, 6H, 8′-CH_3). Anal. Calcd for $C_{26}H_{16}O_{10}$: C, 63.94; H, 3.30. Found: C, 63.82; H, 3.37.

Compounds **12**, **13**, and **14** were purchased from Aldrich-Sigma Chemical Co. Ltd. and used for the comparative study.

Enzyme Assays. *p*-Nitrophenyl (PNP) glycosides and α-glucosidase were purchased from Sigma (St. Louis, MO). α-Glucosidase enzymatic assay was performed as described previously.[26,27]

β-Glucosidase activity was assayed[30,31] at 37 °C by following the increasing absorbance at 540 nm, accompanying the hydrolysis of the substrate (salicin). Enzyme (10 mL) was added to 1 mL of the activity assay system containing inhibitor, 5.0 mM salicin, 0.1 M NaAc−HAc buffer, pH 5.0. After reaction for 10 min at 40 °C, 1 mL of 3,5-dinitrosalicyclic acid (DNS) reagent[1] was added to the reaction mixture to stop the reaction. Then the mixture was heated for 5 min in boiling water for color development. The enzyme activity was calculated by the increased absorption of the reaction mixture at 540 nm, using glucose as a standard. The assay was performed in triplicate with five different concentrations around the IC_{50} values that were roughly estimated in the first round of experiments, and the mean values were adopted

Assay of *β*-Galactosidase. The activity of the *β*-galactosidase (9.4 unit/mg solid, from *Aspergillus oryzae*, Sigma Co.) was measured by the modified method[32,33] described.

Enzymatic Kinetics. The reaction was performed according to the above reaction conditions with inhibitors of various concentrations. Inhibition types for the inhibitors were determined by double-replot of slope versus the reciprocal of the substrate concentration. K_i values for each inhibitor were determined by measuring the rate PNP hydrolysis by α-glucosidase at varying inhibitor concentrations. Data were plotted in Lineweaver−Burk plots (1/rate vs 1/[substrate]), and K_i values were determined through a Dixon plot. The K_i reported was estimated by averaging the K_i values obtained from each of the different inhibitor concentrations.

Circular Dichroism Assays. Circular dichroism (CD) was carried out with a J-810 spectropolarimeter (Jasco) in the UV range (186−240 nm) at protein concentrations of 0.53−0.66 mg/mL, using 0.1 cm quartz cuvettes. The CD spectra were recorded with 5 times accumulation. The data of the secondary structure were dealt with by using the professional software Secondary Structure Estimation and Origin 7.0.

Acknowledgment. Q.S. expresses thanks for a Marie Curie Fellowship (Grant PIIF-GA-2008-221486) and The Hong Kong Polytechnic University ASD fund. The authors thank Prof. Kevin Shakesheff, Prof. Clive Roberts, Dr. French Elizabeth, Dr. Anthony Wilson, Dr. Wang Lei Lei, Dr. Zhang Lei, Dr. Chen Bin, and Bin Huang for their helpful assistance. The authors thank the anonymous reviewers for their constructive comments that have enriched our work.

Supporting Information Available: Experimental and spectroscopic details of compounds **1**−**3** and **6**−**9** and enzyme assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dove, A. Seeking sweet relief for diabetes. *Nat. Biotechnol.* **2002**, *20*, 977–981.
(2) Davies, G. J.; Wilson, K. S. Trapped in the act of catalysis. *Nat. Struct. Biol.* **1999**, *6*, 406–408.
(3) Braun, C.; Brayer, G. D.; Withers, S. G. Mechanism-based inhibition of yeast alpha-glucosidase and human pancreatic alpha-amylase by a new class of inhibitors. 2-Deoxy-2,2-difluoro-alpha-glycosides. *J. Biol. Chem.* **1995**, *270*, 26778–26781.
(4) Jacob, G. S. Glycosylation inhibitors in biology and medicine. *Curr. Opin. Struct. Biol.* **1995**, *5*, 605–611.
(5) Mehta, A.; Zitzmann, N.; Rudd, P. M.; Block, T. M.; Dwek, R. A. Alpha-glucosidase inhibitors as potential broad based anti-viral agents. *FEBS Lett.* **1998**, *430*, 17–22.
(6) Dwek, R. A.; Butters, T. D.; Platt, F. M.; Zitzmann, N. Targeting glycosylation as a therapeutic approach. *Nat. Rev. Drug Discovery* **2002**, *1*, 65–75.
(7) Winchester, B.; Fleet, G. W. Amino-sugar glycosidase inhibitors: versatile tools for glycobiologists. *Glycobiology* **1992**, *2*, 199–210.
(8) Fischer, P. B.; Karlsson, G. B.; Dwek, R. A.; Platt, F. M. *N*-Butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with impaired gp120 shedding and gp41 exposure. *J. Virol.* **1996**, *70*, 7153–7160.
(9) Fischer, P. B.; Karlsson, G. B.; Butters, T. D.; Dwek, R. A.; Platt, F. M. *N*-Butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with changes in antibody recognition of the V1/V2 region of gp120. *J. Virol.* **1996**, *70*, 7143–7152.
(10) Walker, B. D.; Kowalski, M.; Goh, W. C.; Kozarsky, K.; Krieger, M.; Rosen, C.; Rohrschneider, L.; Haseltine, W. A.; Sodroski, J. Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8120–8124.
(11) Trenor, S. R.; Shultz, A. R.; Love, B. J.; Long, T. E. Coumarins in polymers: from light harvesting to photo-cross-linkable tissue scaffolds. *Chem. Rev.* **2004**, *104*, 3059–3077.
(12) Kirkiacharian, S.; Thuy, D. T.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T. Structure−activity relationships of some 3-substituted-4-hydroxycoumarins as HIV-1 protease inhibitors. *Farmaco* **2002**, *57*, 703–708.
(13) Pillai, S. P.; Menon, S. R.; Mitscher, L. A.; Pillai, C. A.; Shankel, D. M. Umbelliferone analogues and their potential to inhibit benzo(*a*)pyrene- and hydrogen peroxide-induced mutations. *J. Nat. Prod.* **1999**, *62*, 1358–1362.
(14) Kimura, Y.; Okuda, H.; Arichi, S.; Baba, K.; Kozawa, M. Inhibition of the formation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid from arachidonic acid in polymorphonuclear leukocytes by various coumarins. *Biochim. Biophys. Acta* **1985**, *834*, 224–229.
(15) Hofmanova, J.; Kozubik, A.; Dusek, L.; Pachernik, J. Inhibitors of lipoxygenase metabolism exert synergistic effects with retinoic acid on differentiation of human leukemia HL-60 cells. *Eur. J. Pharmacol.* **1998**, *350*, 273–284.
(16) Sharma, S.; Stutzman, J. D.; Kelloff, G. J.; Steele, V. E. Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer Res.* **1994**, *54*, 5848–5855.
(17) Hayes, J. D.; Pulford, D. J.; Ellis, E. M.; McLeod, R.; James, R. F.; Seidegard, J.; Mosialou, E.; Jernstrom, B.; Neal, G. E. Regulation of rat glutathione *S*-transferase A5 by cancer chemopreventive agents: mechanisms of inducible resistance to aflatoxin B1. *Chem.-Biol. Interact.* **1998**, *111*−*112*, 51–67.
(18) Lee, D. S.; Lee, S. H. Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. *FEBS Lett.* **2001**, *501*, 84–86.
(19) Sugimoto, H.; Yamanishi, Y.; Iimura, Y.; Kawakami, Y. Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. *Curr. Med. Chem.* **2000**, *7*, 303–339.
(20) Vliet, E. B. 4-Methylesculetin. *Organic Syntheses*; Wiley & Sons: New York, 1941; Collect. Vol. 1, p 360; *Org. Synth.* **1925**, *4*, 45.
(21) Shah, V. R.; B., J. L; Shah, R. C. New synthesis of 4-hydroxycoumarins. *J. Org. Chem.* **1960**, *25*, 677–678.
(22) Kokotos, G.; Tzougraki, C. Synthesis and study of substituted coumarins—a facile preparation of D,L-*o*-tyrosine. *J. Heterocycl. Chem.* **1986**, *23*, 87–92.
(23) Dubuffet, T.; Loutz, A.; Lavielle, G. An efficient large scale synthesis of coumarins by a dealkylative boron-mediated ring closure of 3-(orthomethoxyaryl)propenoic esters. *Synth. Commun.* **1999**, *29*, 929–936.
(24) Zhang, S.-L.; Huang, Z.-S.; S., Y.-D.; L., Y.-M.; Y., J.-H.; H., M.; C., A. S. C.; G., L.-Q. The unique regioselectivity in the formation of disubstituted-1,4-benzoquinones generated from the reaction of 4-hydroxycoumarins with 1,4-benzoquinone. *Tetrahedron Lett.* **2006**, *47*, 6757–6760.
(25) Shen, Q. 3,3′-(3,6-Dihydroxy-*p*-phenylene)bis(4,7-dihydroxy-2*H*-chromen-2-one)dimethylformamide trisolvate. *Acta Crystallogr., Sect. E* **2006**, *62*, o4732–o4733.
(26) Tsujii, E.; Muroi, M.; Shiragami, N.; Takatsuki, A. Nectrisine is a potent inhibitor of alpha-glucosidases, demonstrating activities similarly at enzyme and cellular levels. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 459–466.
(27) Muroi, M.; Ando, O.; Bols, M.; Takatsuki, A. Processing glucosidase inhibition by 1-azafagomine. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 1103–1105.
(28) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; Wiley: New York, 1975.
(29) Raasch, C.; Armbrecht, M.; Streit, W.; Hocker, B.; Strater, N.; Liebl, W. Identification of residues important for NAD^+ binding

by the *Thermotoga maritima* alpha-glucosidase AglA, a member of glycoside hydrolase family 4. *FEBS Lett*. **2002**, *517*, 267–271.
(30) Chen, Q. X.; Zhang, Z.; Zhou, X. W.; Zhuang, Z. L. Kinetics of inhibition of beta-glucosidase from *Ampullarium crossean* by bromoacetic acid. *Int. J. Biochem. Cell Biol*. **2000**, *32*, 717–723.
(31) Bernfeld, P. *Methods in Enzymology*; Academic Press Inc.: New York, 1955; Vol. 1.
(32) Biswas, T. K. Cationic form of beta-galactosidase in the germinating seeds of *Vigna sinensis* (Linn) Savi. *Arch. Biochem. Biophys*. **1986**, *251*, 379–384.
(33) Biswas, T. K. Beta-galactosidase activity in the germinating seeds of *Vigna sinensis*. *Phytochemistry* **1985**, *24*, 2831–2833.
(34) Lodge, J. A.; Maier, T.; Liebl, W.; Hoffmann, V.; Strater, N. Crystal structure of *Thermotoga maritima* alpha-glucosidase AglA defines a new clan of NAD^+-dependent glycosidases. *J. Biol. Chem*. **2003**, *278*, 19151–19158.
(35) Lovering, A. L.; Lee, S. S.; Kim, Y. W.; Withers, S. G.; Strynadka, N. C. Mechanistic and structural analysis of a family 31 alpha-glycosidase and its glycosyl−enzyme intermediate. *J. Biol. Chem*. **2005**, *280*, 2105–2115.
(36) Greenfield, N.; Davidson, B.; Fasman, G. D. The use of computed optical rotatory dispersion curves for the evaluation of protein conformation. *Biochemistry* **1967**, *6*, 1630–1637.
(37) Marques, M. R.; Vaso, A.; Neto, J. R.; Fossey, M. A.; Oliveira, J. S.; Basso, L. A.; dos Santos, D. S.; de Azevedo Junior, W. F.; Palma, M. S. Dynamics of glyphosate-induced conformational changes of *Mycobacterium tuberculosis* 5-enolpyruvylshikimate-3-phosphate synthase (EC 2.5.1.19) determined by hydrogen−deuterium exchange and electrospray mass spectrometry. *Biochemistry* **2008**, *47*, 7509–7522.
(38) Park, H.; Hwang, K. Y.; Oh, K. H.; Kim, Y. H.; Lee, J. Y.; Kim, K. Discovery of novel alpha-glucosidase inhibitors based on the virtual screening with the homology-modeled protein structure. *Bioorg. Med. Chem*. **2008**, *16*, 284–292.
(39) Seo, W. D.; Kim, J. H.; Kang, J. E.; Ryu, H. W.; Curtis-Long, M. J.; Lee, H. S.; Yang, M. S.; Park, K. H. Sulfonamide chalcone as a new class of α-glucosidase inhibitors. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 5514–5516.
(40) Lo Piparo, E.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C. J. Flavonoids for controlling starch digestion: structural requirements for inhibiting human alpha-amylase. *J. Med. Chem*. **2008**, *51*, 3555–3561.
(41) Shen, Q.; Peng, Q.; Shao, J.; Liu, X.; Huang, Z.; Pu, X.; Ma, L.; Li, Y. M.; Chan, A. S.; Gu, L. Synthesis and biological evaluation of functionalized coumarins as acetylcholinesterase inhibitors. *Eur. J. Med. Chem*. **2005**, *40*, 1307–1315.