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Concise synthesis and antidiabetic activity of natural flavonoid glycosides, oroxins C and D, isolated from the seeds of *Oroxylum indium*

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Abstract

The first concise synthesis of natural flavonoid glycosides, oroxins C (**1**) and D (**2**), which were isolated from the seeds of *Oroxylum indicum*, was efficiently achieved by a convergent strategy. The synthesized natural products **1** and **2** were evaluated for their inhibitory activities against α-glucosidase, α-amylase, and lipase. Compound **1** showed strong α-amylase and lipase inhibition, with IC_{50} values of 210 and 190 μM, respectively, but exhibited no inhibitory activity against α-glucosidase. Compound **2** showed strong inhibition against α-glucosidase and lipase, with the respective IC_{50} values of 180 and 80 μM.

Keywords

antidiabetic activity, flavonoid glycosides, glycosylation, oroxin C, oroxin D

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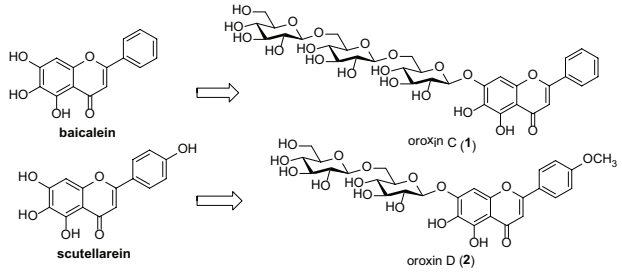
The first concise synthesis of natural flavonoid glycosides, oroxins C (**1**) and D (**2**), which were isolated from the seeds of *Oroxylum indicum*, was efficiently achieved by a convergent strategy. Preliminary pharmacological research exhibited positive response against α-glucosidase, α-amylase, and lipase. Notably, the flavonoid glycoside **2** showed stronger lipase inhibitory activity (IC_{50} = 80 μM), which was two times than positive control orlistat.



Introduction

Flavonoid glycosides, glycosylated secondary metabolites widely distributed in the plant kingdom (such as vegetables, fruits, and medicinal plants),[1–3] having tremendous structural diversity, exhibit a multitude of biological and physiological activities, such as antimicrobial,[4] antitumor,[5] anti-inflammatory,[6] antidiabetic,[7] and hepatoprotectant activities.[8] Compared with flavonoids, flavonoid glycosides possess similar stability, bioactivity, and better solubility and are often more efficacious than their aglycones in pharmaceutical studies.[9] Although flavonoid glycosides are widely distributed and have been demonstrated to possess important biological activities, access to this class of natural

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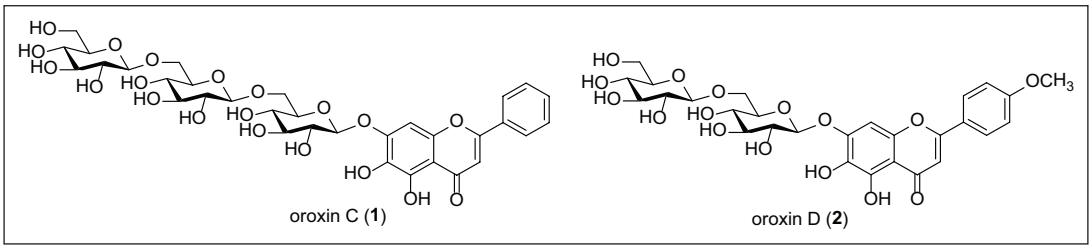


Figure 1. Chemical structure of flavonoid glycosides, oroxins C (**1**) and D (**2**).

products by separation and purification, especially in desirable amounts, presents a formidable task. This results in a great obstacle for further pharmacological studies of homogeneous flavonoid glycosides. Chemical synthesis could provide a feasible way to solve the problem, and many groups have made great efforts to provide sufficient amounts of flavonoid glycosides through chemical synthesis.[10–12]

Recently, Wu et al.[13] reported two novel flavonoid glycosides, oroxin C (baicalein 7-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, **1**) and oroxin D (scutellarein 4'-methyl ether 7-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, **2**), isolated from the seeds of *Oroxylum indicum* as a part of a program to search for potent and effective antidiabetic agents. However, the formidable task of isolating these two natural products turned out to be an obstacle for further pharmacological research, and so it is useful for syntheses of the two novel flavonoid glycosides **1** and **2** to be completed to enable further SAR investigation. In this article, we report the first, concise synthesis of oroxin C (1) and oroxin D (**2**) (Figure 1); the two flavonoid glycosides are also evaluated for in vitro inhibition activity against α-glucosidase, α-amylase, and lipase. These investigations are valuable for the design and preparation of stronger inhibitors and the elucidation of structure–activity relationships (SARs) for the treatment of type 2 diabetes.

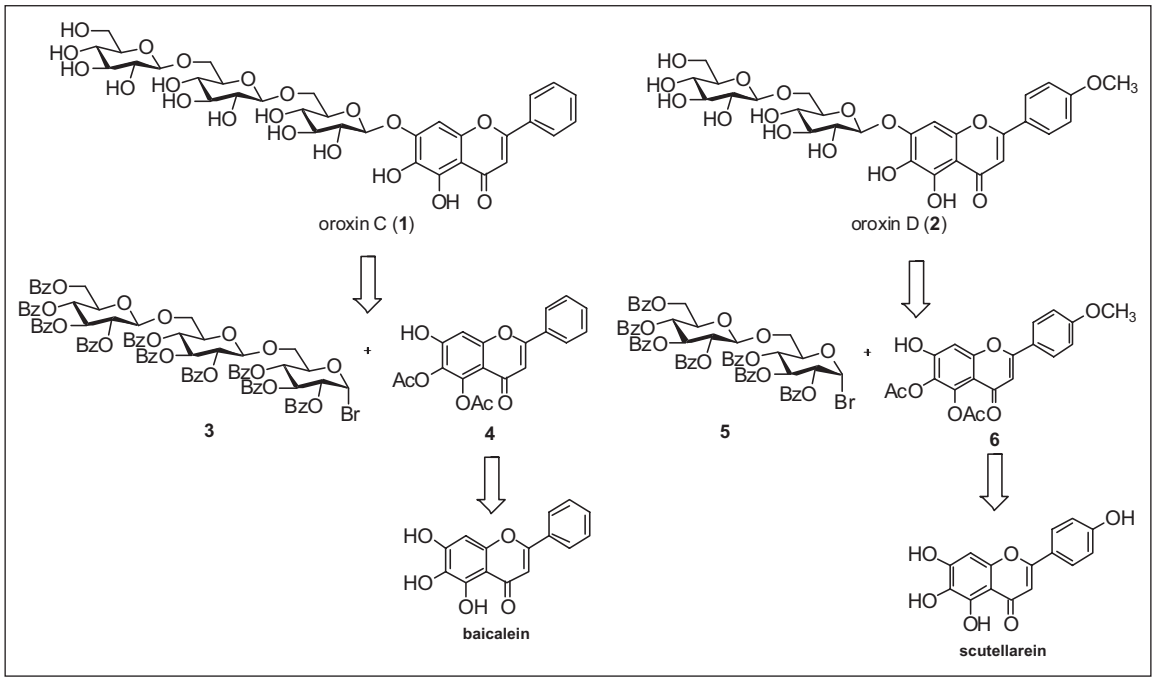
Results and discussion

In terms of their chemical structures, the glycoside residues of the target compounds **1** and **2** are attached to a phenolic hydroxyl group rather than to an alkyl hydroxyl group. Because of their reduced nucleophilicity, phenolic hydroxyl groups are known as low-yielding glycosylation acceptors compared to alkyl hydroxyl groups.[14] Glycosyl bromides were chosen as efficient donors for the construction of this kind of glycosydic bond, and glycosyl bromides were the first type of donor applied to flavonoid glycoside synthesis.[15] A bisglycosyl kaempferol was efficiently synthesized through a phase-transfer-catalyzed (PTC) protocol with glycosyl bromides as the donor by our group,[16] and the Zemplen-Farkas protocol and Koenigs–Knorr protocols have also been developed for this kind of donor.[17] Herein, the target molecules **1** and **2** were obtained through the condensation of the glycosyl bromides **3** and **5** with partially protected baicalein and scutellarein acceptors **15** and **20** as a key step (Scheme 1).

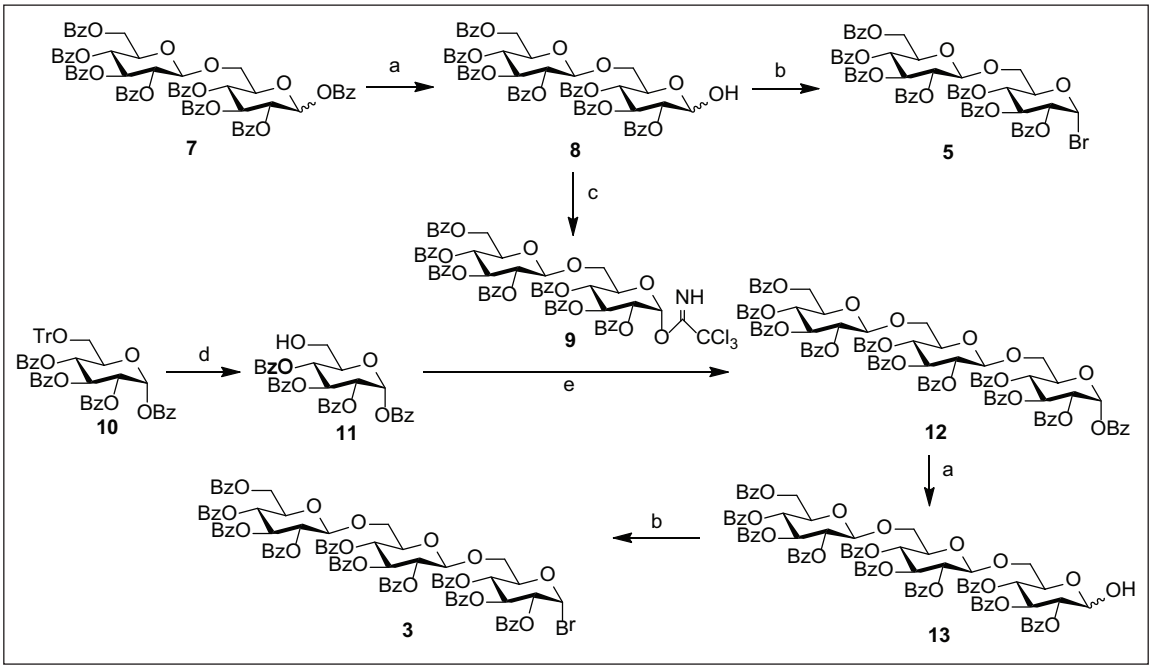
As shown in Scheme 2, disaccharide bromide **5** was readily prepared in a straightforward manner from compound **7** previously reported by our group[18] and was treated with NH_2NH_2·HOAc in dimethylformamide (DMF) at room temperature, to obtain 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-D-glucopyranoside **8**, followed by CBr_4 and PPh_3 in CH_2Cl_2 to provide the desired donor **5** in 90% yield. The preparation of donor **3** began with 1,2,3,4-tetra-*O*-benzoyl-6-*O*-trityl-D-glucopyranoisde **10**. The latter was treated with $FeCl_3$·$6H_2O$ in CH_2Cl_2 at room temperature to afford 1,2,3,4-tetra-*O*-benzoyl-D-glucopyranoisde **11**, which was coupled with 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **9** in anhydrous CH_2Cl_2 in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to obtain the trisaccharide **12** in 89% yield. Treatment of **12** with NH_2NH_2·HOAc in DMF, followed by CBr_4 and PPh_3 in CH_2Cl_2, provided the trisaccharide bromide donor **3** in 83% yield.

The partially protected baicalein and scutellarein acceptors **4** and **6** were readily prepared in a concise manner as depicted in Scheme 3. Baicalein was treated with Ac_2O and AcONa to obtain fully acetylated compound **14**, which was sequentially benzylated with BnBr and hydrogenolysed with $Pd(OH)_2$/C to provide acceptor **4** in 90% yield for the two steps.[11] The synthesis of the acceptor **6** was started with scutellarein, which was treated with dichlorodiphenylmethane in diphenyl ether to produce the desired compound **15** in 83% yield.[19] Subsequently, the $C_{4'}$-OH of compound **15** was selectively reacted with MeI to afford **16**,[20] and then the benzophenone ketal group was removed under the hydrolysis conditions with 80% AcOH in H_2O to obtain **17** in 95% yield.[20] Similar to the aforementioned route to the baicalein acceptor **4**, compound **17** was first treated with Ac_2O and AcONa to obtain **18**, followed by selective substitution of the most reactive C_7-OAc by a benzyl group using BnBr and KI to produce C_7-OBn scutellarein derivative, which was reduced with $Pd(OH)_2$/C and H_2 to remove the benzyl group affording the desired scutellarein acceptor **6** in 78% yield for the two steps.

With an effective synthetic access to the bromide donors (**3** and **5**) and baicalein and scutellarein acceptors (**4** and **6**), we then set about to assemble the target flavonoid glycosides **1** and **2**. According to Koenigs–Knorr protocol, coupling of baicalein acceptor **4** or scutellarein acceptor **6** with the bromide donor **3** or **5** was carried out in quinoline in the presence of a catalytic amount of Ag_2O at room temperature to afford the desired flavonoid derivatives **19** and **20** with β-isomeric glycosydic linkage formed highly stereoselectively. The benzoyl groups was then removed with $Mg(OMe)_2$ in MeOH-CH_2Cl_2 to provide the target flavonoid glycosides, oroxins C (**1**) and D (**2**) (Scheme 4). All the analytical data



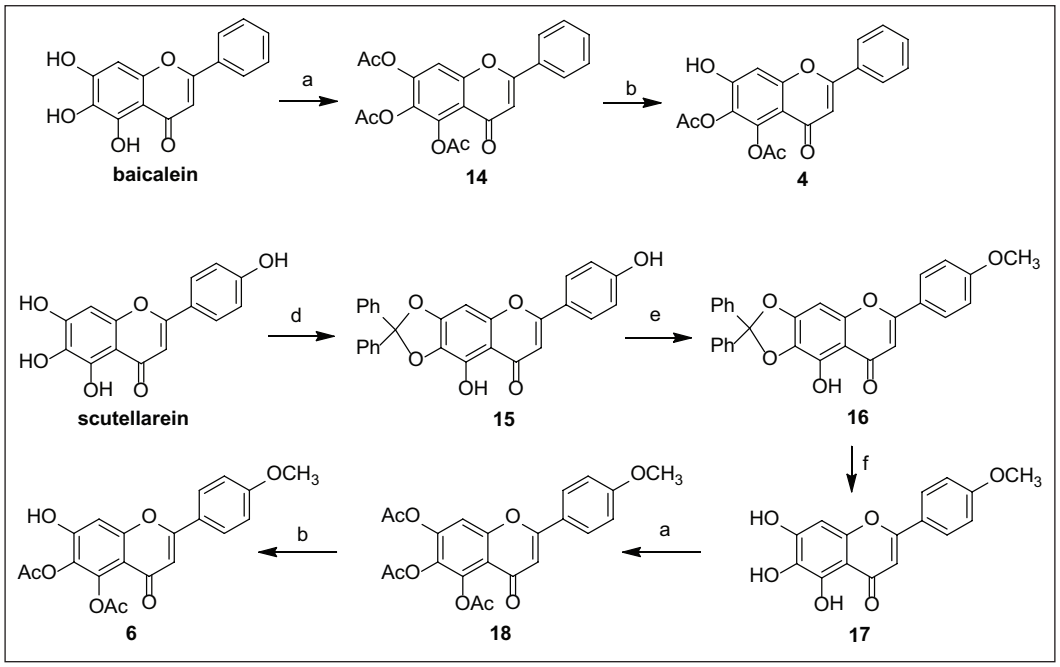
Scheme 1. Retrosynthesis of target flavonoid glycosides, oroxins C (**1**) and D (**2**).



Scheme 2. Reagents and conditions: (a) $NH_2NH_2{\cdot}HOAc$, DMF, 81% for **8**, 78% for **13**; (b) CBr_4, PPh_3, CH_2Cl_2, 90% for **5**, 83% for **3**; (c) $NC.CCl_3$, DBU, CH_2Cl_2, 88%; (d) $FeCl_3{\cdot}6H_2O$, CH_2Cl_2, 93%; (e) TMSOTf, CH_2Cl_2, 0 °C, 89%.

(see supplemental material) for the synthesized flavonoid glycosides **1** {$[\alpha]_D^{25} = -73.1$ (*c* 0.12, MeOH); ref: $[\alpha]_D^{20} = -72.0$ (*c* 0.1, MeOH)} and **2** {$[\alpha]_D^{25} = -168.2$ (*c* 0.11, MeOH); ref: $[\alpha]_D^{20} = -170.0$ (*c* 0.1, MeOH)} were identical in all respects to those reported in the literaure.[13]

To examine the potential antidiabetic ability of the natural flavonoid glycosides, oroxins C (**1**) and D (**2**), their in vitro inhibitory activities against α-glucosidase, α-amylase, and lipase were evaluated, and the results are summarized in Table 1. The data indicate that two flavonoid glycosides were active. Compound **1** showed strong α-amylase and lipase inhibition, with IC_{50} values of 210 and 190 μM, respectively, but it exhibited no inhibitory activity against α-glucosidase. Compound **2** showed strong inhibition against α-glucosidase and lipase, with the respective IC_{50} values of 180 and 80 μM. Notably, the lipase inhibitory activity of Compound **2** was twofold stronger than orlistat, a widely used clinically useful drug, used here as positive control. These results demonstrated that glycosyl fragment favorably enhances the lipase inhibitory activity.



Scheme 3. Reagents and conditions: (a) Ac_2O, AcONa, 80 °C, 90% for **14**, 91% for **18**; (b) BnBr, K_2CO_3, acetone, reflux; (c) $Pd(OH)_2/C$, H_2, THF, 80% for **4**, 78% for **6**; (d) dichlorodiphenylmethane, Ph_2O, 175 °C, 83%; (e) MeI, K_2CO_3, DMF, 91%; (f) 80% AcOH, reflux, 95%.

Table 1. Inhibitory activities of **1** and **2** for α-glucosidase, α-amylase, and lipase.

Compounds	IC_{50} (μM)[a]		
	α-Glucosidase	α-Amylase	Lipase
1	NA	210.3 ± 19.1	190.1 ± 18.2
2	180.4 ± 25.7	NA	80.0 ± 9.5
Acarbose	449.3 ± 38.9	579.2 ± 30.7	-[b]
Orlistat	-	-	158.4 ± 26.6

NA: not active.
[a]The IC_{50} values in μM were calculated from the dose response curve of six concentrations of each test compound in triplicate.
[b]"-": not determined.

Conclusion

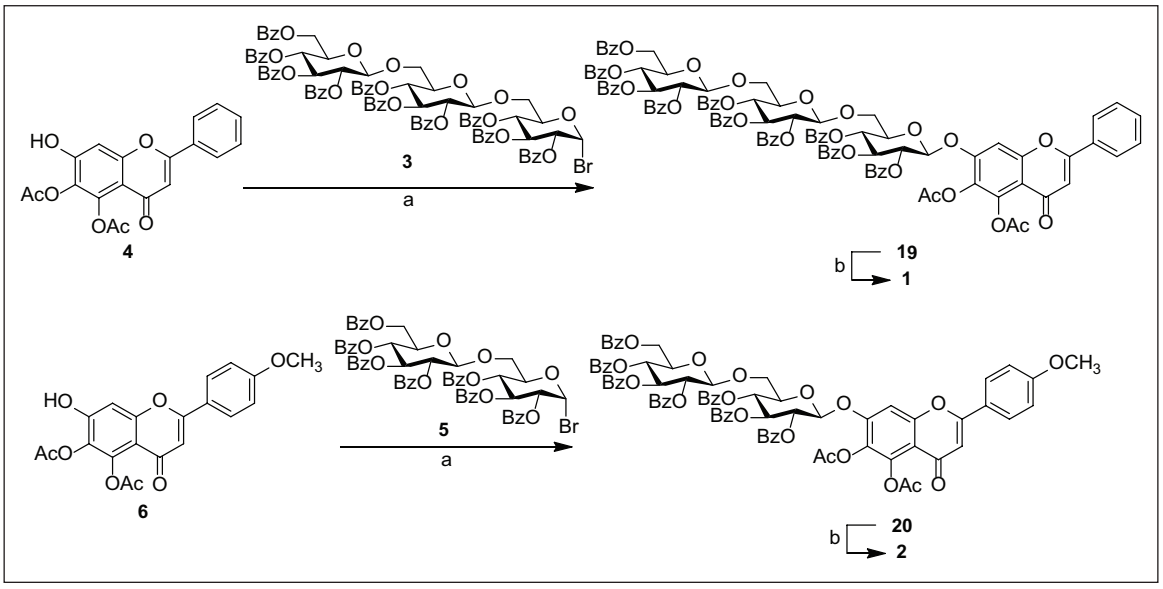
In conclusion, we have succeeded in the first concise synthesis of the natural flavonoid glycosides, oroxins C (**1**) and D (**2**) using a convergent strategy. Preliminary pharmacological research exhibited positive responses against α-glucosidase, α-amylase, and lipase. Notably, the flavonoid glycoside **2** showed stronger lipase inhibitory activity (IC_{50} = 80 μM), which was twice that of the positive control orlistat. Further study on preparation and bioactivity evaluation of analogs and derivatives of these compounds is in progress and will be reported in due course.

Experimental

Chemistry

Commercial reagents were used without further purification unless specialized. Solvents were dried and redistilled prior to use in the usual way. Thin-layer chromatography (TLC) was performed on precoated E. Merck Silica Gel 60 F254 plates. Flash column chromatography was performed on silica gel (200–300 mesh). Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. 1H NMR and 13C NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer with tetramethylsilane as the internal standard, and chemical shifts are recorded in δ values. Mass spectra were recorded on a Q-TOF Global mass spectrometer.

2,3,4,6-Tetra-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*d*-glucopyranosyl bromide (**5**). A solution of **8** (1.20 g, 1.12 mmol), triphenylphosphine (1.46 g, 5.60 mmol), and carbon tetrabromide (1.85 g, 5.60 mmol) in dichloromethane (20 mL) was stirred at room temperature for 3 h. The organic solution was diluted with ethyl acetate, and then washed with saturated sodium bicarbonate twice and brine in sequence, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 5:1) to obtain **5** (1.14 g, 90%) as a colorless syrup. $[\alpha]_D^{25} = +40.3$ (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20–8.03 (m, 35 H), 6.75 (d, *J* = 3.6 Hz,



Scheme 4. Reagents and conditions: (a) Ag_2O, quinoline, 79% for **21**, 85% for **22**; (b) $Mg(OMe)_2$, MeOH-CH_2Cl_2, 83% for **1**, 86% for **2**.

1 H, H-1), 6.24 (t, *J*=9.9 Hz, 1 H, H-3), 5.95 (t, *J*=9.6 Hz, 1 H, H-3'), 5.66 (t, *J*=9.6 Hz, 1 H, H-4'), 5.58 (dd, *J*=9.6, 7.8 Hz, 1 H, H-2'), 5.53 (t, *J*=9.9 Hz, 1 H, H-4), 5.43 (t, *J*=9.9, 3.6 Hz, 1 H, H-2), 5.07 (d, *J*=7.8 Hz, 1 H, H-1'), 4.63 (t, *J*=12.4, 3.2 Hz, 1 H, H-6'-1), 4.53 (m, 1 H, H-5), 4.51 (t, *J*=12.4, 5.3 Hz, 1 H, H-6'-2), 4.21 (m, 1 H, H-5'), 4.19 (dd, *J*=11.6, 1.8 Hz, 1 H, H-6-1), 3.91 (dd, *J*=11.6, 6.0 Hz, 1 H, H-6-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.9, 165.8, 165.7, 165.5, 165.3, 165.2, 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.1, 128.9, 128.3, 100.6 (C-1'), 90.6 (C-1), 72.9 (C-3'), 72.1 (C-5'), 71.9 (C-5), 71.7 (C-2'), 70.5 (C-2), 70.1 (C-3), 69.6 (C-4'), 68.6 (C-4), 67.1 (C-6), 63.0 (C-6'). HRMS (ESI) calcd for $C_{61}H_{49}O_{17}BrNa$ $[M+Na]^+$ 1155.2045, found 1155.2049.

2,3,4,6-Tetra-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-*d*-glucopyranosyl -(1→6)-2,3,4-tri-*O*-benzoyl-α-*d*-glucopyranoside (***12***). A solution of **11** (0.50 g, 0.84 mmol), disaccharide trichloroacetimidate 9 (1.53 g, 1.26 mmol, 1.5 eq), and 4 Å powdered molecular sieves were stirred for 30 min at room temperature in dry CH_2Cl_2 (20 mL). The mixture was cooled to 0 °C for 30 min, followed by the dropwise addition of TMSOTf (50 μL, 0.04 mmol, 0.05 eq). After 1 h, the reaction was quenched with triethylamine, filtered through Celite and evaporated. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 4:1) to obtain **12** (1.23 g, 89%) as a white solid. $[\alpha]_D^{25}=+61.8$ (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18–8.05 (m, 55 H), 6.79 (d, *J*=3.6 Hz, 1 H, H-1), 6.32 (t, *J*=9.9 Hz, 1 H, H-3), 5.99 (t, *J*=9.6 Hz, 1 H, H-3'), 5.87 (t, *J*=9.7 Hz, 1 H, H-3"), 5.66 (t, *J*=9.6 Hz, 1 H, H-4'), 5.61 (t, *J*=9.7 Hz, 1 H, H-4"), 5.58 (dd, *J*=9.6, 7.8 Hz, 1 H, H-2'), 5.55 (dd, *J*=9.7, 7.7 Hz, 1 H, H-2"), 5.51 (t, *J*=9.9 Hz, 1 H, H-4), 5.41 (t, *J*=9.9, 3.6 Hz, 1 H, H-2), 5.13 (d, *J*=7.8 Hz, 1 H, H-1'), 5.09 (d, *J*=7.7 Hz, 1 H, H-1"), 4.75 (t, *J*=12.3, 3.3 Hz, 1 H, H-6'-1), 4.63 (t, *J*=12.0, 3.2 Hz, 1 H, H-6"-1), 4.55 (m, 1 H, H-5), 4.51 (t, *J*=12.3, 5.5 Hz, 1 H, H-6'-2), 4.49 (t, *J*=12.0, 5.7 Hz, 1 H, H-6"-2), 4.23 (m, 2 H, H-5', H-5"), 4.17 (dd, *J*=11.9, 2.1 Hz, 1 H, H-6-1), 3.95 (dd, *J*=11.9, 5.8 Hz, 1 H, H-6-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.9, 165.8, 165.7, 165.6, 165.5, 165.3, 165.1, 164.7, 133.3, 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.3, 129.1, 128.9, 128.7, 128.3, 101.1 (C-1"), 100.5 (C-1'), 90.5 (C-1), 73.5 (C-3"), 72.9 (C-3'), 72.3 (C-5'), 72.1 (C-5"), 71.9 (C-5), 71.7 (C-2"), 71.5 (C-2'), 70.5 (C-2), 70.1 (C-3), 69.9 (C-4"), 69.6 (C-4'), 68.6 (C-4), 68.3 (C-6'), 67.1 (C-6), 63.0 (C-6"). HRMS (MALDI) calcd for $C_{95}H_{76}O_{27}Na$ $[M+Na]^+$ 1671.4466, found 1671.4461.

2,3,4,6-Tetra-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-*d*-glucopyranosyl -(1→6)-2,3,4-tri-*O*-benzoyl-α-*d*-glucopyranosyl bromide (***3***). A solution of **12** (0.80 g, 0.49 mmol) and NH_2NH_2·HOAc (45 mg, 0.49 mmol, 1.0 eq) in DMF (10 mL) were stirred for 6 h at room temperature. The reaction mixture was evaporated. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 3:1) to obtain **13**. To a solution of **13** in dichloromethane (15 mL), triphenylphosphine (0.64 g, 2.45 mmol) and carbon tetrabromide (0.81 g, 2.45 mmol) was added, and the reaction mixture was stirred at room temperature for 4 h. The organic solution was diluted with ethyl acetate, and then washed with saturated sodium bicarbonate twice and brine in sequence, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 4:1) to obtain **3** (512 mg, 65% for two steps) as a colorless syrup. $[\alpha]_D^{25}=+56.7$ (c=1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19–8.06 (m, 50 H), 6.73 (d, *J*=3.7 Hz, 1 H, H-1), 6.31 (t, *J*=9.9 Hz, 1 H, H-3), 5.95 (t, *J*=9.7 Hz, 1 H, H-3'), 5.89 (t, *J*=9.7 Hz, 1 H, H-3"), 5.67 (t, *J*=9.7 Hz, 1 H, H-4'), 5.63 (t, *J*=9.7 Hz, 1 H, H-4"), 5.58 (dd, *J*=9.7, 7.7 Hz, 1 H, H-2'), 5.54 (dd, *J*=9.7, 7.8 Hz, 1 H, H-2"), 5.51 (t, *J*=9.9 Hz, 1 H, H-4), 5.45 (t, *J*=9.9, 3.7 Hz, 1 H, H-2), 5.19 (d, *J*=7.7 Hz, 1 H, H-1'), 5.07 (d, *J*=7.8 Hz, 1 H, H-1"), 4.79 (t, *J*=12.5, 3.2 Hz, 1 H,

H-6'-1), 4.63 (t, *J* = 12.1, 3.3 Hz, 1 H, H-6"-1), 4.55 (m, 1 H, H-5), 4.50 (t, *J* = 12.5, 5.6 Hz, 1 H, H-6'-2), 4.49 (t, *J* = 12.0, 5.7 Hz, 1 H, H-6"-2), 4.29 (m, 2 H, H-5', H-5"), 4.17 (dd, *J* = 12.3, 2.5 Hz, 1 H, H-6-1), 3.99 (dd, *J* = 12.3, 5.1 Hz, 1 H, H-6-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.9, 165.8, 165.7, 165.6, 165.5, 165.3, 165.2, 164.9, 133.2, 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.3, 129.1, 128.9, 128.7, 128.5, 128.3, 101.3 (C-1"), 100.5 (C-1'), 90.3 (C-1), 73.5 (C-3"), 72.9 (C-3'), 72.6 (C-5'), 72.1 (C-5"), 71.7 (C-5), 71.6 (C-2"), 71.5 (C-2'), 70.7 (C-2), 70.1 (C-3), 69.8 (C-4"), 69.3 (C-4'), 68.5 (C-4), 68.3 (C-6'), 66.9 (C-6), 63.1 (C-6"). HRMS (MALDI) calcd for $C_{88}H_{71}O_{25}BrNa$ $[M+Na]^+$ 1629.3360, found 1629.3366.

5,6,7-Tri-*O*-acetyl-4'-*O*-methyl scutellarein (**18**). A solution of compound **17** (3.00 g, 10 mmol) and NaOAc (1.13 g, 14 mmol) in acetic anhydride (15 mL) was stirred at 75 °C until start material disappear. The reaction mixture was poured into ice water, and the precipitate was collected by filtration and washed by EtOH to give compound **18** (3.88 g, 91%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.5 Hz, 2 H, H-2', H-6'), 6.94 (d, *J* = 8.5 Hz, 2 H, H-3', H-5'), 6.79 (s, 1 H, H-8), 6.65 (s, 1 H, H-3), 3.86 (s, 3 H, OCH_3), 2.34, 2.36, 2.43 (s each, 3 H each, $3\times COCH_3$); HRMS (ESI) calcd for $C_{22}H_{18}O_9Na$ $[M+Na]^+$ 449.0843, found 449.0849.

5,6-Di-*O*-acetyl-4'-*O*-methyl scutellarein (**6**). A solution of compound **18** (2.13 g, 5 mmol), benzyl bromide (1.78 mL, 15 mmol), and anhydrous K_2CO_3 (2.76 g, 20 mmol) in acetone (120 mL) was refluxed for 24 h with stirring. The reaction mixture was cooled down to room temperature, filtered, and the solvent was evaporated under reduced pressure to give the crude compound as a yellow solid. A solution of above crude compound and $Pd(OH)_2$/C (1.12 g) in anhydrous THF (100 mL) was stirred at room temperature for 3 h until start material was disappeared. The precipitate was collected by filtration and purified by silica gel column chromatography (petroleum ether-EtOAc, 3:1) to obtain **6** (1.50 g, 78%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.6 Hz, 2 H, H-2', H-6'), 6.95 (d, *J* = 8.6 Hz, 2 H, H-3', H-5'), 6.75 (s, 1 H, H-8), 6.63 (s, 1 H, H-3), 3.87 (s, 3 H, OCH_3), 2.35, 2.43 (s each, 3 H each, $2\times COCH_3$); HRMS (ESI) calcd for $C_{20}H_{16}O_8Na$ $[M+Na]^+$ 407.0737, found 407.0731.

5,6-Di-*O*-acetyl-7-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*d*-glucopyranosyl] baicalein (**19**). To a solution of **4** (300 mg, 0.85 mmol), **3** (2.04 g, 1.27 mmol) and silver oxide (296 mg, 1.27 mmol) in dry quinoline (30 mL) at room temperature was added activated 4 Å MS (ca. 500 mg) under argon atmosphere. The mixture was stirred for 24 h, and then diluted with dichloromethane followed by filtration to remove insoluble substances. The solution was washed with 1 M HCl three times, saturated sodium bicarbonate and brine in sequence, dried with anhydrous Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether-EtOAc, 2:1) to obtain **19** (1.26 g, 79%) as a white solid. $[\alpha]_D^{25} = +80.9$ (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13–8.03 (m, 55 H), 6.91 (s, 1 H, H-8), 6.79 (d, *J* = 7.8 Hz, 1 H, H-1"), 6.61 (s, 1 H, H-3), 6.36 (t, *J* = 9.9 Hz, 1 H, H-3"), 5.95 (t, *J* = 9.6 Hz, 1 H, H-3'"), 5.88 (t, *J* = 9.7 Hz, 1 H, H-3'""), 5.71 (t, *J* = 9.6 Hz, 1 H, H-4'"), 5.65 (t, *J* = 9.6 Hz, 1 H, H-4""), 5.59 (dd, *J* = 9.6, 7.7 Hz, 1 H, H-2'"), 5.55 (dd, *J* = 9.6, 7.8 Hz, 1 H, H-2""), 5.52 (t, *J* = 9.6 Hz, 1 H, H-4"), 5.45 (t, *J* = 9.6, 7.8 Hz, 1 H, H-2"), 5.23 (d, *J* = 7.7 Hz, 1 H, H-1'"), 5.11 (d, *J* = 7.8 Hz, 1 H, H-1""), 4.85 (t, *J* = 12.1, 3.3 Hz, 1 H, H-6'"-1), 4.73 (t, *J* = 12.5, 3.6 Hz, 1 H, H-6""-1), 4.61 (m, 1 H, H-5"), 4.56 (t, *J* = 12.1, 5.7 Hz, 1 H, H-6'"-2), 4.49 (t, *J* = 12.5, 5.7 Hz, 1 H, H-6""-2), 4.33 (m, 2 H, H-5'", H-5""), 4.26 (dd, *J* = 12.0, 2.6 Hz, 1 H, H-6"-1), 3.97 (dd, *J* = 12.0, 5.1 Hz, 1 H, H-6-2"), 2.33, 2.43 (s each, 3 H each, $2\times COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5 (C-4), 166.0, 165.9, 165.8, 165.7, 165.6, 165.5, 165.3, 165.2, 164.6, 163.5 (C-2), 159.1 (C-7), 150.3 (C-5), 133.3, 133.2, 133.0, 130.1, 130.0, 129.9, 129.8, 129.7, 129.5, 129.4, 129.2, 128.9, 128.5 (C-6), 128.3, 104. 3 (C-3), 101.6 (C-1""), 100.1 (C-1'"), 90.9 (C-1"), 73.7 (C-3""), 72.8 (C-3'"), 72.5 (C-5'"), 72.3 (C-5""), 71.9 (C-5"), 71.7 (C-2""), 71.4 (C-2'"), 70.6 (C-2"), 70.3 (C-3"), 69.7 (C-4""), 69.5 (C-4'"), 68.4 (C-4"), 68.1 (C-6'"), 66.7 (C-6"), 63.1 (C-6""). HRMS (MALDI) calcd for $C_{107}H_{84}O_{32}Na$ $[M+Na]^+$ 1903.4838, found 1903.4833.

5,6-Di-*O*-acetyl-7-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-*d*-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*d*-glucopyranosyl]-4'-*O*-methyl scutellarein (**20**). Similar procedure as that used for the synthesis of **19** was used to get **20**. Thus, **6** (200 mg, 0.52 mmol) coupled with **5** (884 mg, 0.78 mmol) under the effect of silver oxide (181 mg, 0.78 mmol), after silica gel column chromatography (petroleum ether-EtOAc, 2:1) to afford **20** (635 mg, 85%) as a white solid. $[\alpha]_D^{25} = +56.5$ (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19–8.03 (m, 37 H), 6.94 (d, *J* = 8.6 Hz, 2 H, H-3', H-5'), 6.73 (s, 1 H, H-8), 6.69 (d, *J* = 7.7 Hz, 1 H, H-1"), 6.61 (s, 1 H, H-3), 6.25 (t, *J* = 9.6 Hz, 1 H, H-3"), 5.93 (t, *J* = 9.6 Hz, 1 H, H-3'"), 5.67 (t, *J* = 9.6 Hz, 1 H, H-4'"), 5.55 (dd, *J* = 9.6, 7.8 Hz, 1 H, H-2'"), 5.51 (t, *J* = 9.6 Hz, 1 H, H-4"), 5.40 (t, *J* = 9.6, 7.7 Hz, 1 H, H-2"), 5.05 (d, *J* = 7.8 Hz, 1 H, H-1'"), 4.61 (t, *J* = 12.0, 3.3 Hz, 1 H, H-6'"-1), 4.55 (m, 1 H, H-5"), 4.51 (t, *J* = 12.0, 5.5 Hz, 1 H, H-6'"-2), 4.23 (m, 1 H, H-5'"), 4.18 (dd, *J* = 12.1, 3.0 Hz, 1 H, H-6"-1), 3.91 (dd, *J* = 12.1, 5.8 Hz, 1 H, H-6"-2), 3.87 (s, 3 H, OCH_3), 2.35, 2.43 (s each, 3 H each, $2\times COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5 (C-4), 166.0, 165.9, 165.8, 165.7, 165.5, 165.3, 165.2, 163.6 (C-2), 159.7 (C-4'), 158.8 (C-7), 145.3 (C-5), 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.1, 128.9, 128.0 (C-6), 128.3, 114.3 (C-3'), 114.2(C-5'), 104. 3 (C-3), 100.9 (C-1'"), 91.7 (C-1"), 72.7 (C-3'"), 72.3 (C-5'"), 71.9 (C-5"), 71.6 (C-2'"), 70.7 (C-2"), 70.3 (C-3"), 69.5 (C-4'"), 68.3 (C-4"), 67.2 (C-6"), 63.0 (C-6'"), 55.9 (C_4-OCH_3). HRMS (MALDI) calcd for $C_{81}H_{64}O_{25}Na$ $[M+Na]^+$ 1459.3629, found 1459.3621.

Baicalein 7-*O*-β-*d*-glucopyranosyl-(1→6)-β-*d*-glucopyranosyl-(1→6)-β-*d*-glucopyranoside (**1**). To a solution of **19** (100 mg, 0.053 mmol) in methanol (2 mL) and CH_2Cl_2

(0.5 mL) was added 7% magnesium methoxide (1.2 mL). The solution was stirred at room temperature overnight. The solution was neutralized with the weakly acidic ion-exchange resin (amberlite IRC 76, H^+ form) and filtered. The filtrate was concentrated in vacuo and purified by RP-18 column chromatography (methanol–water, 1:2) to afford oroxin C (**1**) (33 mg, 83%) as a yellow solid. $[\alpha]_D^{25} = -73.1$ (c 0.12, MeOH); ^{1}H NMR (400 MHz, dimethyl sulfoxide (DMSO)-d_6) δ 12.09 (s, 1 H, C_5-OH), 8.58 (s, 1 H, C_6-OH), 8.09 (d, J=8.5 Hz, 2 H, H-2', H-6'), 7.63 (d, J=8.5 Hz, 2 H, H-3', H-5'), 7.62 (m, 1 H, H-4'), 7.08 (s, 1 H, H-8), 6.99 (s, 1 H, H-3), 5.01 (d, J=7.3 Hz, 1 H, H-1"), 4.23 (d, J=7.5 Hz, 1 H, H-1'"), 4.03 (dd, J=11.2, 1.9 Hz, 1 H, H-6"-1), 3.70 (t, J=9.1 Hz, 1 H, H-4'"), 3.68 (dd, J=11.2, 4.1 Hz, 1 H, H-6"-2), 3.66 (m, 3 H, H-6'"-1, H-6'"-2, H-4""), 3.51 (dd, J=11.2, 4.3 Hz, 1 H, H-6""-1), 3.45 (m, 2 H, H-2"", H-6""-2), 3.42 (t, J=9.0 Hz, 1 H, H-3"), 3.41 (t, J=9.1 Hz, 1 H, H-3"), 3.34 (m, 1 H, H-5"), 3.33 (m, 2 H, H-5'", H-3""), 3.23 (t, J=9.0 Hz, 1 H, H-4"), 3.17 (m, 2 H, H-2'", H-5""), 3.15 (dd, J=9.0, 7.2 Hz, 1 H, H-2"); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.6 (C-4), 163.8 (C-2), 151.6 (C-7), 149.2 (C-9), 146.4 (C-5), 132.1 (C-4'), 130.9 (C-1'), 130.5 (C-6), 129.3 (C-3', C-5'), 126.7 (C-2', C-6'), 106.3 (C-10), 104.5 (C-3), 103.9 (C-1'"), 101.0 (C-1"), 98.5 (C-1""), 94.3 (C-8), 76.8 (C-5""), 75.6 (C-5"), 75.5 (C-5'"), 75.0 (C-3""), 73.6 (C-3'"), 73.3 (C-3"), 73.2 (C-2""), 72.4 (C-2'"), 72.1 (C-2"), 70.2 (C-4"), 70.1 (C-4'"), 69.8 (C-4""), 69.2 (C-6"), 66.9 (C-6'"), 60.6 (C-6""). HRMS (ESI) calcd for $C_{33}H_{41}O_{20}$ $[M+H]^+$ 757.2186, found 757.2193.

Scutellarein 4'-methyl ether 7-*O*-β-*d*-glucopyranosyl-(1→6)-β-*d*-glucopyranoside (**2**). Similar procedure as that used for the synthesis of **1** from **19** was used to provide **2**, after RP-18 column chromatography (methanol–water, 1:2), (37 mg, 86%) as a yellow solid. $[\alpha]_D^{25} = -168.2$ (c 0.11, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1 H, C_5-OH), 8.55 (s, 1 H, C_6-OH), 8.07 (d, J=8.8 Hz, 2 H, H-2', H-6'), 7.19 (d, J=8.8 Hz, 2 H, H-3', H-5'), 7.13 (s, 1 H, H-8), 6.89 (s, 1 H, H-3), 5.05 (d, J=7.3 Hz, 1 H, H-1"), 4.21 (d, J=7.8 Hz, 1 H, H-1'"), 4.04 (dd, J=11.2, 2.2 Hz, 1 H, H-6"-1), 3.86 (s, 3 H, $C_{4'}$-OCH_3), 3.78 (m, 1 H, H-5"), 3.66 (m, 2 H, H-6"-2, H-6'"-1), 3.38 (dd, J=9.0, 7.3 Hz, 1 H, H-2"), 3.35 (dd, J=11.3, 4.1 Hz, 1 H, H-6'"-2), 3.31 (dd, J=9.3, 7.8 Hz, 1 H, H-2'"), 3.21 (t, J=9.5 Hz, 1 H, H-4'"), 3.18 (m, 2 H, H-3", H-5'"), 3.14 (t, J=9.4 Hz, 1 H, H-4"), 3.09 (t, J=9.5 Hz, 1 H, H-3'"); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4 (C-4), 163.8 (C-2), 162.4 (C-4'), 151.5 (C-7), 149.1 (C-9), 146.4 (C-5), 130.5 (C-6), 128.5 (C-2', C-6'), 122.9 (C-1'), 114.8 (C-3', C-5'), 106.0 (C-10), 103.0 (C-3), 103.9 (C-1'"), 101.0 (C-1"), 94.4 (C-8), 77.4 (C-5'"), 76.7 (C-3"), 75.8 (C-2'"), 75.6 (C-5"), 73.6 (C-3'"), 73.1 (C-2"), 70.2 (C-4"), 69.7 (C-4'"), 69.5 (C-6"), 61.2 (C-6'"). HRMS (ESI) calcd for $C_{28}H_{33}O_{16}$ $[M+H]^+$ 625.1763, found 625.1770.

Biological activities assay

Assay for α-glucosidase inhibitory activities. Inhibitory α-glucosidase activities were determined spectrophotometrically in a 96-well microtiter plate based on *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate. In brief, 20 μL of enzyme solution (0.8 U/mL α-glucosidase in 0.01 M potassium phosphate buffer (pH 6.8) containing 0.2% of bovine serum albumin (BSA)) and 120 μL of the synthetic compound in 0.5% DMSO of 0.01 M potassium phosphate buffer were mixed and was preincubated at 37 °C prior to the initiation of the reaction by adding the substrate. After 15 min of preincubation, PNPG solution (20 μL; 5.0 mM PNPG in 0.1 M potassium phosphate buffer (pH 6.8)) was added and then incubated together at 37 °C. After 15 min of incubation, 0.2 M Na_2CO_3 (80 μL) in 0.1 M potassium phosphate buffer was added to the test tube to stop the reaction. Acarbose was used as positive control. The increment in absorption at 410 nm due to the hydrolysis of PNPG by α-glucosidase was monitored continuously with an auto multi-functional microplate reader (BIORAD680).

Assay for α-amylase inhibitory activities. The α-amylase inhibitory activities were measured with the method reported by Xiao et al.[21] and Yoshikawa et al.[22] with slight modifications. Substrate was prepared by heating starch (250 mg) in 12 mL of 0.4 M NaOH solution for 5 min at 100 °C, and then cooled to 0 °C and adjusted to pH 7 with 2 M HCl. Sample solutions were prepared by dissolving each solution in acetate buffer (pH 6.5). The sample (20 μL) and the substrate (40 μL) were mixed in a microplate well. After preincubation at 37 °C for 15 min, 5 mg/mL α-amylase solution (20 mL) was added and the solution was incubated at 37 °C for 15 min. The reaction was stopped by adding 50 mL 1 M HCl, and then 50 mL iodine solution was added. The absorbances were measured at 650 nm by a microplate reader. Acarbose was used as positive control.

Assay for lipase inhibitory activities. Lipase inhibitory activities were measured according to the method of Han et al.[23] with slight modifications. Substrate was prepared by sonication of a mixture of glyceryl trioleate (80 mg), lecithin (10 mg), and sodium cholate (5 mg) suspended in 9 mL of 0.1 M TES buffer (pH 7.0). Samples were prepared by dissolving each sample in 0.1 M TES buffer. The sample (20 μL) and the substrate (20 μL) were mixed in microplate wells. After preincubated for 5 min, 10 μL of lipase solution (20 μg/mL) was added to each reaction mixture and incubated for 30 min at 37 °C. The amount of released fatty acid was measured at 405 nm. Inhibition of lipase activity was expressed as the percentage decrease in the absorbance when porcine pancreatic lipase was incubated with the test compounds. Orlistat was used as positive control.

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Supplemental material

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