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Original article

Synthesis and α-glucosidase inhibitory activity of chrysin, diosmetin, apigenin, and luteolin derivatives

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ABSTRACT

Several derivatives have been synthesized from chrysin, diosmetin, apigenin, and luteolin, which were isolated from diverse natural plants. The α-glucosidase inhibitory activity of these compounds was evaluated. The glucosidase inhibitory activity of all derivatives ($IC_{50} < 24.396$ μmol/L) was higher compared with that of the reference drug, acarbose ($IC_{50} = 563.601 \pm 40.492$ μmol/L), and 1-deoxynojirimycin ($IC_{50} = 226.912 \pm 12.573$ μmol/L). $O^{3'}$,O^{7}-Hexyl diosmetin ($IC_{50} = 2.406 \pm 0.101$ μmol/L) was the most potent inhibitor identified. These compounds showed a higher inhibitory ability compared with their precursors except the luteolin derivatives. In general, the inhibitory activity of the synthetic derivatives was enhanced with long alkyl chains at positions 3′, 4′ and 7 of the flavonoid.

1. Introduction

Diabetes mellitus is a serious worldwide health problem. Each year, the number of patients diagnosed with diabetes mellitus increases. Diabetes mellitus is known to be a lifestyle-related disease, which can cause numerous complications, such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases. Diabetes mellitus is also one of the leading causes of patient mortality. The International Diabetes Federation reported that about 366 million people were diagnosed with diabetes in 2011, and this number is expected to increase to up to 552 million by 2030 [1]. Type II diabetes mellitus is more prevalent in developed countries is characterized by reduced insulin sensitivity and impaired insulin secretion [2–6]. Type II diabetes mellitus can be effectively treated by α-glucosidase inhibitors, which have the ability to delay and reduce postprandial blood glucose spike [7–9].

α-Glucosidase is involved in carbohydrate metabolism and has a crucial function in diabetes, viral infection, and cancer. α-Glucosidase has diverse bioactivities and is considered an attractive drug target. At present, a number of α-glucosidase inhibitors have been discovered and studied. Anti-diabetic agents that are used in clinical practice, such as acarbose [10], voglibose, and miglitol [11], competitively inhibit α-glucosidase in the brush border of the small intestine, which consequently delay the hydrolysis of carbohydrates and alleviate postprandial hyperglycemia. However, the continuous administration of these agents may cause several adverse effects, such as diarrhea, abdominal discomfort, flatulence [12–14], and hepatotoxicity [15]. Therefore, developing novel α-glucosidase inhibitors lacking these liabilities is necessary given the therapeutic challenge of type II diabetes mellitus [16–22]. Flavonoids are widely distributed in plants, for example, vegetables and fruits. Researchers had focused their efforts on a few pharmacological activities of flavonoids, such as, scavenging free radicals and protecting against lipid peroxidation [23,24]. Recently, researchers showed more interest in some specific flavonoids because of modulating endothelial nitric oxide metabolism and NADPH oxidase activity of them [25–31]. The results from mechanistic research suggested that flavonoids may also alleviate hyperglycemia, increase insulin secretion, and improve insulin sensitivity [32]. Flavonoids, such as baicalein, luteolin, kaempferol, apigenin, and chrysin, have the ability to inhibit α-glucosidase activity [33,34]. The structure of the A, B, and C rings are related to the activity of α-glucosidase and α-amylase inhibition. The unsaturated C ring, linkage of the B ring at the 3′ position, 3-OH, 4-CO, and the hydroxyl substitution on

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the B ring have been reported to increase the inhibitory activity against α-glucosidase and α-amylase [35]. In addition, Hakamata et al. [36] have shown that catechin derivatives showed potent antioxidant activity and α-glucosidase inhibitory activity with alkyl side chains of various lengths, whereas Shin et al. [37] have shown that a series of alkyl or acetyl derivatives of chrysin have hypoglycemic effects. Based on the previous studies, we synthesized a series of alkylated flavonoids such as chrysin, diosmetin, apigenin, and luteolin, and studied their α-glucosidase inhibitory activity.

In this study, chrysin, diosmetin, apigenin, and luteolin derivatives were synthesized from the corresponding naturally occurring flavonoids, and their yeast α-glucosidase inhibitory activity were evaluated. We attempted to study the relationship between the structure of the derivatives and their inhibitory effect.

2. Experimental

2.1. Reagents and instruments

All solvents used in this research were of analytical grade, and the chemicals used for synthesizing the derivatives were of reagent grade and commercially available. Chrysin, diosmetin, apigenin and luteolin (>98% by HPLC) were purchased from Shanxi Huike Co., Ltd., Jiangsu, China, and were used without further purification. Biotech-grade PNPG (4-nitrophenyl-α-D-glucopyranoside), α-glucosidase from baker's yeast, 1-deoxynojirimycin and acarbose were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA), and L-glutathione (reduced) was obtained from Roche, Switzerland. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H on a Bruker Avance 400 spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as an internal standard (chemical shift in ppm, δ). *J* values are reported in Hertz. Molecular mass was determined by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF MS) using a Bruker Aupoflex-III mass spectrometer. Elemental analysis (C, H) of the targeted compounds was measured using an elementary Vario EL III analyser. Melting points (mp) of derivatives were determined on a Shanghai SHENGUANG WRS-1B digital melting-point apparatus, China. The absorbance of samples was obtained using a Mapada UV-1600 spectrophotometer, Shanghai, China.

2.2. Synthesis

Chrysin (1.3 g, 5 mmol) in 120 mL of acetone was added to anhydrous potassium carbonate (0.7 g, 5 mmol). The mixture was stirred at reflux for 30 min, bromoethane (1.2 mL, 15 mmol) was added dropwise, followed by refluxing for 24 h. The mixture was cooled to room temperature, filtered, and was concentrated *in vacuo*. The residue was purified with a silica gel column eluting with a mixed solvent ($EtOAc/CH_2Cl_2$ = 1:10) to obtain O^7-ethyl chrysin (**5**). Yellowish powder; yield: 68.5%; mp: 163.2–163.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.71 (s, 1H, 5-OH), 7.88 (d, 2H, *J* = 7.9 Hz, aromatic $H_{2',6'}$), 7.53 (d, 3H, *J* = 7.4 Hz, aromatic $H_{3',4',5'}$), 6.66 (s, 1H, aromatic H_8), 6.49 (s, 1H, aromatic H_6), 6.36 (s, 1H, aromatic H_3), 4.11 (q, 2H, *J* = 7.0 Hz, $-OCH_2-$), 1.46 (t, 3H, *J* = 7.0 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 283 ($[M+H]^+$); Anal. calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; found: C, 72.10; H, 4.99.

Compounds **6** and **7** were obtained according to the method for compound **5** [38].

O^7-Butyl chrysin (**6**): Yellowish powder; yield: 74.5%; mp: 145.9–148.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.70 (s, 1H, 5-OH), 7.89 (d, 2H, *J* = 7.7 Hz, aromatic $H_{2',6'}$), 7.53 (d, 3H, *J* = 7.4 Hz, aromatic $H_{3',4',5'}$), 6.66 (s, 1H, aromatic H_8), 6.50 (s, 1H, aromatic H_3), 6.37 (s, 1H, aromatic H_6), 4.05 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 1.94–1.68 (m, 2H, $-CH_2-$), 1.50 (dt, 2H, *J* = 14.7, 7.4 Hz, $-CH_2-$), 1.00 (t, 3H, *J* = 7.4 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 311 ($[M+H]^+$); Anal. calcd. for $C_{19}H_{18}O_4$: C, 73.53; H, 5.85; found: C, 73.42; H, 5.82.

O^7-Hexyl chrysin (**7**): Yellowish powder; yield: 89.7%; mp: 143.3–143.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.70 (s, 1H, 5-OH), 7.89 (d, 2H, *J* = 6.7 Hz, aromatic $H_{2',6'}$), 7.53 (d, 3H, *J* = 7.2 Hz, aromatic $H_{3',4',5'}$), 6.66 (s, 1H, aromatic H_8), 6.50 (s, 1H, aromatic H_3), 6.37 (s, 1H, aromatic H_6), 4.04 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 1.94–1.71 (m, 2H, $-CH_2-$), 1.71–1.32 (m, 2H, $-CH_2-$), 1.26 (m, 4H, $-CH_2CH_2-$), 0.92 (t, 3H, *J* = 6.4 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 339 ($[M+H]^+$); Anal. calcd. for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55; found: C, 74.24; H, 6.41.

General procedures for the synthesis of compounds **8**–**13** based on the method used to obtain chrysin alkyl derivatives.

$O^{4'}$,O^7-Diethyl apigenin (**8**): Yellowish powder; yield: 62.3%; mp: 159.3–159.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.80 (s, 1H, 5-OH), 7.82 (d, 2H, *J* = 8.9 Hz, aromatic $H_{2',6'}$), 7.00 (d, 2H, *J* = 8.9 Hz, aromatic $H_{3',5'}$), 6.56 (s, 1H, aromatic H_8), 6.47 (s, 1H, aromatic H_3), 6.34 (s, 1H, aromatic H_6), 4.11 (dd, 4H, *J* = 6.9, 4.1 Hz, $-OCH_2-$), 1.45 (t, 6H, *J* = 6.9 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 327 ($[M+H]^+$); Anal. calcd. for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56; found: C, 69.73; H, 5.49.

$O^{4'}$,O^7-Dibutyl apigenin (**9**): Yellowish powder; yield: 70.6%; mp: 130.9–131.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.80 (s, 1H, 5-OH), 7.82 (d, 2H, *J* = 8.8 Hz, aromatic $H_{2',6'}$), 7.00 (d, 2H, *J* = 8.8 Hz, aromatic $H_{3',5'}$), 6.56 (s, 1H, aromatic H_8), 6.47 (s, 1H, aromatic H_3), 6.35 (s, 1H, aromatic H_6), 4.04 (t, 4H, *J* = 6.4 Hz, $-OCH_2$), 2.02–1.68 (m, 4H, $-CH_2-$), 1.68–1.21 (m, 4H, $-CH_2-$), 0.99 (t, 6H, *J* = 7.3 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 383 ($[M+H]^+$); Anal. calcd. for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85; found: C, 72.14; H, 6.83.

$O^{4'}$,O^7-Dihexyl apigenin (**10**): Yellowish powder; yield: 78.4%; mp: 88.5–88.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.80 (s, 1H, 5-OH), 7.82 (d, 2H, *J* = 8.8 Hz, aromatic $H_{2',6'}$), 7.00 (d, 2H, *J* = 8.8 Hz, aromatic $H_{3',5'}$), 6.56 (s, 1H, aromatic H_8), 6.47 (s, 1H, aromatic H_3), 6.35 (s, 1H, aromatic H_6), 4.17–3.93 (m, 4H, $-OCH_2-$), 2.02–1.66 (m, 4H, $-CH_2-$), 1.42 (dd, 4H, *J* = 48.2, 3.8 Hz, $-CH_2-$), 1.25 (s, 8H, $-CH_2CH_2-$), 0.92 (t, 6H, *J* = 6.7 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 439 ($[M+H]^+$); Anal. calcd. for $C_{27}H_{34}O_5$: C, 73.94; H, 7.81; found: C, 73.79; H, 7.79.

$O^{3'}$,O^7-Diethyl diosmetin (**11**): Yellow powder; yield: 55.6%; mp: 191.9–192.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.78 (s, 1H, 5-OH), 7.51 (d, 1H, *J* = 10.4 Hz, aromatic $H_{6'}$), 7.34 (s, 1H, aromatic $H_{2'}$), 6.98 (d, 1H, *J* = 8.5 Hz, aromatic $H_{5'}$), 6.57 (s, 1H, aromatic H_8), 6.47 (s, 1H, aromatic H_3), 6.35 (s, 1H, aromatic H_6), 4.15 (dt, 4H, *J* = 30.8, 6.9 Hz, $-OCH_2-$), 3.96 (s, 3H, $-OCH_3$), 1.49 (dt, 6H, *J* = 27.5, 7.0 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 357 ($[M+H]^+$); Anal. calcd. for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66; found: C, 67.21; H, 5.56.

$O^{3'}$,O^7-Dibutyl diosmetin (**12**): Yellow powder; yield: 66.7%; mp: 111.6–112.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.78 (s, 1H, 5-OH), 7.49 (d, 1H, *J* = 8.4 Hz, aromatic $H_{6'}$), 7.34 (s, 1H, aromatic $H_{2'}$), 6.96 (d, 1H, *J* = 8.4 Hz, aromatic $H_{5'}$), 6.56 (s, 1H, aromatic H_8), 6.48 (s, 1H, aromatic H_3), 6.35 (s, 1H, aromatic H_6), 4.07 (dt, 4H, *J* = 23.8, 6.3 Hz, $-OCH_2-$), 3.94 (s, 4H, $-OCH_3$), 1.98–1.71 (m, 4H, $-CH_2-$), 1.66–1.38 (m, 4H, $-CH_2-$), 1.12–0.93 (t, 6H, $-CH_3$); MALDI-TOF: *m*/*z* 413 ($[M+H]^+$); Anal. calcd. for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84; found: C, 69.58; H, 6.62.

$O^{3'}$,O^7-Dihexyl diosmetin (**13**): Yellow powder; yield: 75.9%; mp: 87.4–87.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.78 (s, 1H, 5-OH), 7.50 (d, 1H, *J* = 6.7 Hz, aromatic $H_{6'}$), 7.34 (s, 1H, aromatic $H_{2'}$), 6.97 (d, 1H, *J* = 8.5 Hz, aromatic $H_{5'}$), 6.56 (s, 1H, aromatic H_8), 6.48 (s, 1H, aromatic H_3), 6.35 (s, 1H, aromatic H_6), 4.06 (dt, 4H, *J* = 23.7, 6.6 Hz, $-OCH_2-$), 3.94 (s, 3H, $-OCH_3$), 1.97–1.74 (m, 4H, $-CH_2-$), 1.67–1.38 (m, 4H, $-CH_2-$), 1.36 (m, 8H, *J* = 6.8, 3.5 Hz, $-CH_2CH_2-$), 0.92 (t, 6H, *J* = 1.4 Hz, $-CH_3$); MALDI-TOF: *m*/*z* 469 ($[M+H]^+$); Anal. calcd. for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74; found: C, 71.42; H, 7.55.

General procedures for the synthesis of $O^{3'}$,$O^{4'}$-ethylidene luteolin [30]: Firstly, to a mixture of luteolin (1.5 g, 5 mmol) and K_2CO_3 (0.4 g, 2.5 mmol) in 30 mL of DMSO was added 1,

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2-dibromoethane dropwise, followed by heating at 70 °C for 1 h. The mixture was poured into ice water, filtered, washed with water, the precipitates were dried *in vacuo*. The residue was chromatographed on silica gel to afford compound **14**. Yellow powder; yield: 32.4%; mp: 307.3–308.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.90 (s, 1H, 5-OH), 10.87 (s, 1H, 7-OH), 7.60–7.57 (m, 2H, aromatic $H_{2',6'}$), 7.04–7.02 (d, 1H, J = 8 Hz, aromatic $H_{5'}$), 6.87 (s, 1H, aromatic H_8), 6.51 (s, 1H, J = 1.7 Hz, aromatic H_3), 6.20–6.19 (s, 1H, J = 4 Hz, aromatic H_6), 4.33–4.32 (d, 4H, J = 4 Hz, –OCH_2CH_2O–); MALDI-TOF: m/z 313 ([M+H]$^+$).

General procedures for synthesis of compounds **15**–**17** based on the method used to obtain chrysin alkyl derivatives.

O^7-Ethyl-$O^{3'}$,$O^{4'}$-ethylidene luteolin (**15**): Yellow powder; yield: 70.2%; mp: 174.1–174.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.37 (m, 2H, aromatic $H_{2',6'}$), 6.98–6.96 (d, 1H, J = 8 Hz, aromatic $H_{5'}$), 6.54 (s, 1H, aromatic H_8), 6.45 (s, 1H, aromatic H_3), 6.34 (s, 1H, aromatic H_6), 4.33–4.32 (d, 4H, J = 4 Hz, –CH_2CH_2–), 4.13–4.08 (q, 2H, J = 6.7 Hz, –CH_2–), 1.47–1.43 (t, 3H, J = 8 Hz, –CH_3); MALDI-TOF: m/z 341 ([M+H]$^+$); Anal. calcd.Anal. calcd. for $C_{19}H_{10}O_6$: C, 67.05; H, 4.74; found: C, 67.25; H, 4.79.

O^7-Butyl-$O^{3'}$,$O^{4'}$-ethylidene luteolin (**16**): Yellow powder; yield: 80.4%; mp: 153.9–154.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.37 (m, 2H, aromatic $H_{2',6'}$), 6.98–6.96 (d, 1H, J = 8 Hz, aromatic $H_{5'}$), 6.54 (s, 1H, aromatic H_8), 6.45 (s, 1H, aromatic H_3), 6.34 (s, 1H, aromatic H_6), 4.33–4.32 (d, 4H, J = 4 Hz, –CH_2CH_2–), 4.04–4.01 (t, 2H, J = 6.2 Hz, –CH_2O–), 1.81–1.78 (t, 2H, J = 7 Hz, –CH_2–), 1.53–1.47 (q, 2H, J = 7.2 Hz, –CH_2–), 1.01–0.97 (t, 3H, J = 7.2 Hz, –CH_3); MALDI-TOF: m/z 369 ([M+H]$^+$); Anal. calcd. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47; found: C, 68.35; H, 5.50.

O^7-Hexyl-$O^{3'}$,$O^{4'}$-ethylidene luteolin (**17**): Yellow powder; yield: 75.9%; mp: 125.6–125.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.36 (m, 2H, aromatic $H_{2',6'}$), 6.97–6.95 (d, 1H, J = 8 Hz, aromatic $H_{5'}$), 6.52 (s, 1H, aromatic H_8), 6.44 (s, 1H, aromatic H_3), 6.33 (s, 1H, aromatic H_6), 4.32–4.31 (d, 4H, J = 4 Hz, –CH_2CH_2–), 4.03–4.00 (t, 2H, J = 6.5 Hz, –OCH_2–), 1.82–1.78 (t, 2H, J = 8 Hz, –CH_2–), 1.46–1.34 (m, 6H, –$CH_2CH_2CH_2$–), 0.91 (s, 3H, –CH_3); MALDI-TOF: m/z 397 ([M+H]$^+$); Anal. calcd. for $C_{23}H_{24}O_6$: C, 69.68; H, 6.10; found: C, 69.45; H, 6.15.

2.3. α-Glucosidase inhibitory activity

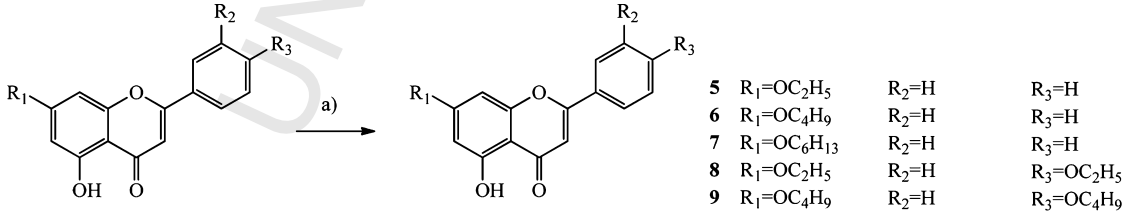
An improved methodology used in the assessment of α-glucosidase inhibitory activity has been reported in previous studies [39–42]. *p*NP-α-Glu was hydrolyzed into *p*NP by α-glucosidase. The absorption of *p*NP at 400 nm was determined using a spectrophotometer, which indicated the activity of α-glucosidase. Sodium phosphate buffer (pH 6.8), 50 μL of the samples in various concentrations, and α-glucosidase (200 μL, 0.3 U/mL) were mixed at 37 °C and the mixture was allowed to stir for 10 min. The reaction was started with the addition of 4-nitrophenyl-α-D-glucopyranoside (500 μL, 10 mmol/L) at 37 °C and reaction lasted for 20 min. An aliquot (200 μL) was added to a sodium carbonate solution (9.8 mL, 100 mmol/L) to stop the reaction. The glucosidase activity was detected using a silica dish, and the absorbance was determined at 400 nm (for *p*NP). The inhibitory degree (%) of the targeted compounds upon α-glucosidase addition was calculated using $[(1 - \Delta A_{test}/\Delta A_{control}) \times 100]$, where ΔA indicates the absorbance increase in 20 min. The 50% inhibitory concentration values (IC_{50}) were expressed as mean ± SD (n = 3), which were obtained using the Sigmaplot 12.0 software. All the tests were performed in triplicate. Acarbose and 1-deoxynojirimycin were used as positive controls.

3. Results and discussion

The synthesis of derivatives **5**–**13** was achieved by coupling various bromoalkanes with their respective precursors in anhydrous acetone (Scheme 1). Chrysin derivatives **5**–**7** were derived at the C-7 position, diosmetin derivatives **8**–**10** were derived at the C-7 and C-3′ positions, and apigenin derivatives **11**–**13** were obtained by alkylating the C-7 and C-4′ positions. Luteolin derivatives **15**–**17** were conveniently prepared using the method described in literature (Scheme 2). The structures of all the derivatives were characterized by ^{1}H NMR, MALDI-TOF, and elemental analyses.

The α-glucosidase inhibition effect of the precursors and their derivatives were determined using similar protocols described in literature [39,43,44]. 1-Deoxynojirimycin (a clinically used α-glucosidase inhibitor) and acarbose were used as reference drugs. The IC_{50} values of all compounds are shown in Table 1, which shows that the precursors **1**–**4** and their derivatives **5**–**13** and **15**–**17** were more active against yeast α-glucosidase than 1-deoxynojirimycin (IC_{50} = 226.912 ± 12.573 μmol/L) and acarbose (IC_{50} = 563.601 ± 40.492 μmol/L). The α-glucosidase inhibition of chrysin **1** (IC_{50} = 77.730 ± 3.490 μmol/L) was weaker compared with its derivatives **5**–**7** (IC_{50} < 24.396 μmol/L). Similarly, apigenin derivatives **8**–**10** (IC_{50} < 7.350 μmol/L) and diosmetin derivatives **11**–**13** (IC_{50} < 22.051 μmol/L) are more potent that the parent apigenin **2** (IC_{50} = 16.780 ± 0.257 μmol/L) and diosmetin **3** (IC_{50} = 22.764 ± 3.503 μmol/L). The inhibitory activity of luteolin derivatives **15**–**17** did not exceed that of luteolin **4**. But increasing the hydrophobicity or the alkyl chain length led to more potent analogs (IC_{50}: **15** > **16** > **17**).

Transition states of the reactions catalyzed by glycosidase involve covalent intermediates [45,46]. Hydrogen bonding is a crucial factor for the interactions between the enzyme and its substrates and the conformation and orientations of the inhibitors in the active site [47]. Previous studies have reported that the hydroxyl group has an important function in α-glucosidase inhibition at position 5 of the flavonoids [48,49], and the hydroxyl substitution on the B ring by particular groups could enhance inhibitory activity [35]. Compared with that of chrysin **1** (IC_{50} = 26.417 ± 0.485 μmol/L), the inhibitory effect of



1 R_1=OH R_2=H R_3=H
2 R_1=OH R_2=H R_3=OH
3 R_1=OH R_2=OH R_3=OCH_3

5 R_1=OC_2H_5 R_2=H R_3=H
6 R_1=OC_4H_9 R_2=H R_3=H
7 R_1=OC_6H_{13} R_2=H R_3=H
8 R_1=OC_2H_5 R_2=H R_3=OC_2H_5
9 R_1=OC_4H_9 R_2=H R_3=OC_4H_9
10 R_1=OC_6H_{13} R_2=H R_3=OC_6H_{13}
11 R_1=OC_2H_5 R_2=OC_2H_5 R_3=OCH_3
12 R_1=OC_4H_9 R_2=OC_4H_9 R_3=OCH_3
13 R_1=OC_6H_{13} R_2=OC_6H_{13} R_3=OCH_3

Scheme 1. Preparation of derivatives **5**–**13**, reagents and conditions: (a) bromoalkane, K_2CO_3, acetone, reflux.

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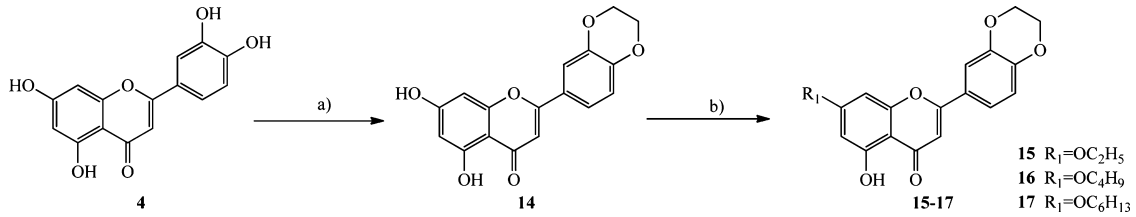


Scheme 2. Preparation of derivatives **15–16**, reagents and conditions: (a) dibromoethane, K_2CO_3, DMSO, 70 °C; (b) bromoalkane, K_2CO_3, acetone, reflux.

the 7-methylated compound **5** (IC_{50} = 24.396 ± 0.665 μmol/L) was minimally increased, whereas butylated and hexylated analogs **6** and **7** (**6**: IC_{50} = 7.040 ± 1.634 μmol/L; **7**: IC_{50} = 4.476 ± 1.109 μmol/L) showed excellent activity against the yeast α-glucosidase (Fig. 1). Therefore, α-glucosidase inhibitory activity was improved when the hydrophobicity of chrysin was enhanced. To further study the effect of flavone hydrophobicity on inhibitory activity, we analyzed the inhibitory activity of apigenin derivatives (IC_{50}: **8** > **9** > **10**) and diosmetin derivatives (IC_{50}: **11** > **12** > **13**) from Fig. 1. The results indicated that hydrophobicity has an important function in increasing inhibitory activity. Inhibitory activity was dramatically enhanced when the alkyl chain was longer than or equal to two carbons. In addition, We found that the OH groups at positions 3′ and 4′ of benzopyran that were alkylated by ethylidene did not improve α-glucosidase inhibitory activity; the inhibitory activity of luteolin derivatives **15–17** did not exceed that of luteolin **4**. But increasing the hydrophobicity or the elongation of alkyl chains improved the inhibitory activity (IC_{50}: **15** > **16** > **17**).

Kumar et al. [49] reported that replacement of the OH group at positions 3′, 4′ and 7 of benzopyran by any electron-withdrawing group (NO_2, OCH_3 *etc.*) favors α-glucosidase inhibitory activity.

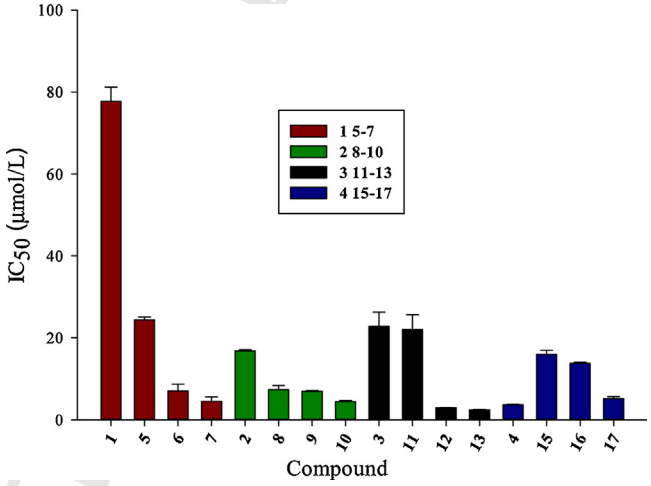
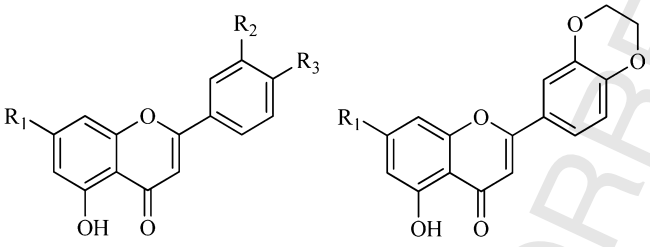


Fig. 1. Effect of compounds on α-glucosidase activity *in vitro*.

Table 1
Structures and *in vitro* α-glucosidase inhibitory activity of targeted compounds.



Compound	R_1	R_2	R_3	IC_{50} (μmol/L)[a]
1 (Chrysin)	OH	H	H	77.730 ± 3.490
2 (Apigenin)	OH	H	OH	16.780 ± 0.257
3 (Diosmetin)	OH	OH	OCH_3	22.764 ± 3.503
4 (Luteolin)	OH	OH	OH	3.652 ± 0.077
5	OC_2H_5	H	H	24.396 ± 0.665
6	OC_4H_9	H	H	7.040 ± 1.634
7	OC_6H_{13}	H	H	4.476 ± 1.109
8	OC_2H_5	H	OC_2H_5	7.350 ± 0.998
9	OC_4H_9	H	OC_4H_9	6.897 ± 0.263
10	OC_6H_{13}	H	OC_6H_{13}	4.426 ± 0.281
11	OC_2H_5	OC_2H_5	OCH_3	22.051 ± 3.596
12	OC_4H_9	OC_4H_9	OCH_3	2.940 ± 0.051
13	OC_6H_{13}	OC_6H_{13}	OCH_3	2.406 ± 0.101
15	OC_2H_5	–	–	15.952 ± 1.013
16	OC_4H_9	–	–	13.733 ± 0.299
17	OC_6H_{13}	–	–	5.180 ± 0.451
Acarbose[b]				563.601 ± 40.492
1-Deoxynojirimycin[b]				226.912 ± 12.573

[a] Data represents means ± SD of triplicate samples obtained from the dose inhibition curve.
[b] This compound was used as a positive control.

Therefore, the substitution of the hydrogen of OH by alkyls at position 7 of benzopyran would enhance the inhibitory activity, which is dependent on the alkyl chain lengths. The lengths were found to be positively correlated with the IC_{50} values of derivatives (IC_{50}: **5** > **6** > **7**, **8** > **9** > **10**, **11** > **12** > **13**, **15** > **16** > **17**). To develop novel α-glucosidase inhibitor, the results of this study could provide a series of potential compounds.

4. Conclusion

In summary, twelve chrysin, diosmetin, apigenin, and luteolin alkyl derivatives were synthesized as novel inhibitors of yeast α-glucosidase. All compounds were verified by ^{1}H NMR, MALDI-TOF, and elemental analyses. The glucosidase inhibitory activity of all derivatives is higher compared with those of the positive control drugs, acarbose, and 1-deoxynojirimycin. O^7-Hexyl chrysin **7**, $O^{4'}$,O^7-hexyl apigenin **10**, $O^{3'}$,O^7-hexyl diosmetin **13**, O^7-hexyl-$O^{3'}$,$O^{4'}$-ethylidene luteolin **17** were the most effective among the inhomogeneous derivatives. From a structural–activity relationship perspective, replacing the hydrogen of OH group at positions 3′, 4′, and 7 of benzopyran with various alkyl chains was the critical factor in altering the inhibition activity of flavonoids. To develop novel α-glucosidase inhibitor, the results of this study could provide a series of potential compounds.

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