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In vitro and *in vivo* evaluation of the antidiabetic activity of ursolic acid derivatives

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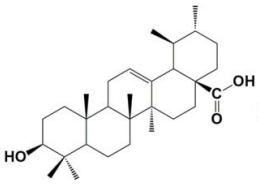
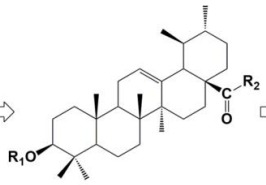


Compound **1** (ursolic acid)

IC_{50}=5.04 ± 0.80 μM

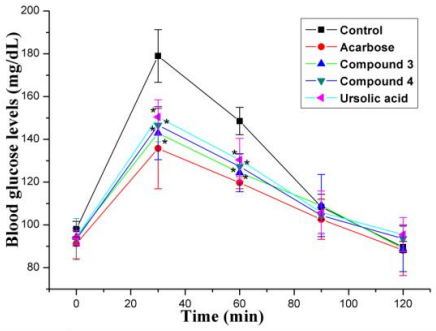


3 R_1=C(=O)CH_2CH_3, R_2=OH IC_{50}=2.66 ± 0. 40 μM

4 R_1=C(=O)$(CH_2)_2CH_3$, R_2=OH IC_{50}=1.01 ± 0.14 μM

5 R_1=C(=O)$CH_2(CH_3)_2$, R_2=OH IC_{50}=3.26 ± 0.52 μM

8 R_1=C(=O)CH_2CH_3, R_2=HN–  –Cl IC_{50}=3.24 ± 0.21 μM



Time (min)	Delta area under the curve			
	Acarbose	Compound **3**	Compound **4**	Ursolic acid
0-30	676.2	628.17	631.08	488.4
30-60	1006.2	933.9	879.9	700.4
60-90	518.7	351.6	378.6	309.2
90-120	108	0	0	-50.4
Total	2309.1	1913.67	1889.58	1447.5

Highlights

- A series of ursolic acid derivatives were synthesized and spectrally characterized.
- Compounds **3**-**5** and **8** are more potent *in vitro* than acarbose and **UA**.
- The kinetic study of compounds **3**-**5** and **8** was carried out.
- Both compounds **3** and **4** have hypoglycemic effect *in vivo*.

In vitro and *in vivo* evaluation of the antidiabetic activity of ursolic acid derivatives

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Authors' contributions

Pan-Pan Wu performed the experimental work and drafted the manuscript. Kun Zhang was the project coordinator. Yu-Jing Lu performed the ^{1}H NMR and ^{13}C NMR analysis. Ping He participated in the synthesis and testing work. Su-Qing Zhao was the thesis director and designer. All authors read and approved the final manuscript.

Abstract

In this study, a series of ursolic acid derivatives were synthesized, and their structures were confirmed. The activity of the synthesized compounds against α-glucosidase was determined *in vitro*. The results

suggested that all compounds have significant inhibitory activity, especially compounds **3**-**5** and **8**, the IC_{50} values of which were 2.66 ± 0.84, 1.01 ± 0.44, 3.26 ± 0.22, and 3.24 ± 0.21 μM. These compounds were more potent than acarbose (positive control) against α-glucosidase. Kinetic studies were performed to determine the mechanism of inhibition by compounds **3**-**5** and **8**. The kinetic inhibition studies indicated that compound **3** was a non-competitive inhibitor, and the inhibition constant K_i was calculated to be 2.67 ± 0.19 μM. Moreover, the kinetic inhibition studies of compounds **4**, **5** and **8** demonstrated that they were mixed-type inhibitors. Furthermore, the actual pharmacological potentials of synthesized compounds **3** and **4** were demonstrated by the reduction of postprandial blood glucose levels in normal Kunming mice. The hypoglycemic effects of these compounds were more evident 30 and 60 min after maltose ingestion ($P<0.05$), which was similar to the effect displayed by the positive control, acarbose.

Keywords: Ursolic acid derivatives; Synthesis; α-glucosidase inhibition; Anti-diabetic

1. Introduction

Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce sufficient insulin or when the body cannot use it effectively, thereby leading to an increased blood glucose concentration [1]. Over the last century, the ever-changing lifestyle of the population

has resulted in a dramatic increase in the incidence of diabetes. The chief form of the disease, type 2 diabetes, is associated with 'diabesity' and 'metabolic syndrome'. In conjunction with genetic susceptibility in certain ethnic groups, type 2 diabetes is influenced by environmental and other factors such as a sedentary lifestyle, overly rich nutrition and obesity [2]. Treatment of diabetes involves lowering blood glucose through different mechanisms, including insulin secretion, glucose absorption, and metabolism adjustment [3,4].

Pentacyclic triterpenes and their derivatives are ubiquitous in the plant kingdom and possess interesting bioactivities, such as antitumor [5,6,7,8], anti-HIV [9], antibacterial [10], antimalarial [11], protein tyrosine phosphatase 1B inhibition [4,12], etc. Ursolic acid (**UA**, 3β-hydroxy-urs-12-en-28-oic acid, **1**) is a well-known pentacyclic triterpene that has been reported to possess a wide range of biological activities. It serves as one of the major effective components of many traditional Chinese herbal medicines [13]. Studies have shown that **UA** has a positive effect on lowing blood glucose levels and curing diabetic complications in diabetic mice [14,15,16,17]. However, **UA** has a very low water solubility, which limits its bioavailability and therapeutic applications in clinical medicine [18]. To improve its activity and bioavailability, chemical modification of **UA** at the 3-OH or 17-COOH positions has recently been widely investigated. It has been reported that

UA and its derivatives esterified at the C-3 and/or C-28 positions exhibit significant cytotoxicity against several tumor cell lines [5,19,20]. Moreover, a series of **UA** derivatives were synthesized by condensation of **UA** and 1,4-bis(3-aminopropyl) piperazine pharmacophore, resulting in promising *in vitro* anti-malarial activity [12,21]. However, there are few studies focused on the anti-diabetic properties of **UA** derivatives.

In an attempt to explore the activity and bioavailability of **UA** and its derivatives, and study their structure-activity relationships and mechanisms, structural modifications were made to the 3-OH or 17-COOH positions of **UA**. A series of **UA** derivatives, particularly new halogen-containing derivatives were synthesized using previously reported methods [5,19,20] with slight modifications and different reactants. The hypoglycemic activity of **UA** and its derivatives was investigated *in vitro* and *in vivo*. In the present study, the results of experiments measuring the activity of **UA** and its derivatives toward inhibiting alpha-glucosidase and lowering blood glucose in a normal mice model were reported.

2. Results and discussion

2.1. Chemistry

To obtain a series of **UA** derivatives, structural modifications were made at the 3-OH or 17-COOH positions, beginning with **UA** as the parent compound. The synthetic routes are shown in Scheme 1 and

Scheme 2.

Ursolic acid (**1**) was esterified in anhydrous pyridine with acetic anhydride to give its 3-*O*-acetate (**2**). Compounds **3**-**5** were synthesized following the same procedure used to prepare the 3-*O*-acetate, but with different anhydrides (Scheme 1). Compound **2** was treated with oxalyl chloride to give the intermediate 28-acyl chloride [7,20], which is highly reactive and was coupled with small molecules containing reactive amine groups to produce compounds **6**-**12** (Scheme 2). The target compounds were purified by column chromatography using petroleum ether/ethyl acetate and/or chloroform/methanol as the eluent. Their structures were confirmed by electrospray ionization mass spectrometry (ESI-MS), high-resolution mass spectrometry (HRMS), ^{1}H NMR and ^{13}C NMR.

Scheme 1. Synthesis of compounds **2**-**5** from ursolic acid. Reagents and conditions: (a) anhydride/Pyr/DMAP, r.t.

Scheme 2. Synthesis of compounds **6**-**12** from compound **2**. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, r.t.; (b) CH_2Cl_2, Et_3N, RNH_2, r.t.

2.2. In vitro α-glucosidase inhibition assay

To investigate the inhibitory ability of each synthesized **UA** derivative, an *in vitro* experiment using α-glucosidase from baker's yeast, which is widely used in screens for compounds with anti-diabetic activity, was conducted [22]. A stock solution of each derivative (dissolved in DMSO at concentrations of 0.1 μM to 1 mM) was diluted with 0.1 M phosphate

buffer solution (pH=6.8) containing an appropriate concentration of enzyme solution (0.1 U/mL). After a 10 min pre-incubation at 37 ℃, the reactions were initiated by adding the substrate (1 mM *p*-nitrophenyl-α-D-glucopyranoside) and incubated at 37 ℃ for 30 min. The reactions were then terminated by adding 1 M Na_2CO_3, and their optical density values were measured using a Multimodel Plate Reader (Infinite 200). Table 1 displays the preliminary results. The inhibited enzyme activity of all compounds was firstly evaluated at two concentrations (5 and 10 μM), which was a guidance for future selection of inhibitor concentrations. Table 1 clearly shows that the inhibition effects were more obvious at higher concentrations.

To determine the IC_{50} value of each derivative, the enzyme activity was measured at a fixed substrate concentration, in which a series of different inhibitor concentrations were tested. The IC_{50} value was calculated based on the curve fit to the series of concentrations versus the corresponding inhibition activities (data not shown). All tested compounds had lower IC_{50} values than both positive control and **UA** against α-glucosidase from baker's yeast except compound **11** which had a low solubility and could not disperse well at higher concentrations.

Table 1

Inhibitory effects of compounds **1**-**12** on α-glucosidase from baker's yeast.

Each experiment was performed in quadruplicate. The data reported represent the means (n=4) ±

SD.

[a] Percent inhibition of α-glucosidase treated with a 5 μM concentration of each compound.

[b] Percent inhibition of α-glucosidase treated with a 10 μM concentration of each compound.

[c] IC_{50} value representing the concentration that caused a 50% loss of activity.

[d] Acarbose, positive control.

To obtain insight into the mechanism by which the derivatives inhibited α-glucosidase, kinetic studies was carried out. Lineweaver-Burk plots of the initial velocity versus the enzyme concentrations in the presence of different concentrations of the synthesized compounds gave a series of straight lines. As illustrated in Figs. 1-4, compound **3** was determined to be a non-competitive inhibitor because increasing substrate concentrations resulted in a series of lines with a common intercept on the -[I] axis, but different slopes (Fig. 1). The equilibrium constant for inhibitor binding, K_i, was obtained from the -[I] value at the intersection of the four straight lines. The family of lines for compounds **4** and **5** intersected in the second quadrant (Figs. 2-3), while the family of lines for compound **8** intersected in the third quadrant (Fig. 4). The inhibition constant K_i and K_i' values for compounds **3-5** and **8** were calculated using the appropriate equations, and the results are presented in Table 2. It was implied that compounds **4**, **5** and **8** inhibit α-glucosidase in two different ways: compounds **4**, **5** and **8** could retard enzyme function by not only directly binding to α-glucosidase (EI) but also interfering the formation of α-glucosidase-PNPG (ES) intermediate through forming an

α-glucosidase-PNPG-inhibitor (ESI) complex in a noncompetitive manner [23]. According to the inhibition type of tested compounds, it could not dismiss the possibility that an α-glucosidase inhibitor attaches to a wide region including the binding site of α-glucosidase, or attaches to a different region incurring structural modification [24].

Figure 1. Compound **3**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound concentrations were: 0 μM ■; 2.0 μM ●; 5.0 μM ▲; 9.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

Figure 2. Compound **4**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound concentrations were: 0 μM ■; 2.85 μM ●; 4.0 μM ▲; 6.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

Figure 3. Compound **5**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound concentrations were: 0 μM ■; 1.6 μM ●; 2.8 μM ▲; 4.5 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

Figure 4. Compound **8**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound concentrations were: 0 μM ■; 2.5 μM ●; 5.0 μM ▲; 8.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

Scheme 3. The linear mixed-type inhibition of compounds **3-5** and **8**. When the constant K_i=K_i', the model describes non-competitive inhibition.

Table 2

Inhibition constants (K_i and K_i') of compound **3-5** and **8** for α-glucosidase. The data shown represent the mean K_i and K_i' values ± S.E.M.

2.3 Hypoglycemic Effect in Normal Mice

To investigate the pharmacological potential of the most active α-glucosidase inhibitors among the synthesized **UA** derivatives as lead compounds for the development of new drugs to treat diabetes, compounds **3** and **4** were synthesized on a larger scale to obtain enough to perform assays of their hypoglycemic activity in normal mouse. With oral administration at a 50 mg/kg dose, acarbose, **UA**, compounds **3** and **4** were able to reduce glucose uptake in normal mice fed with maltose. The results were presented in Figure 5 and Table 3. It could be concluded that the hypoglycemic effect was more distinct at 30 and 60 min ($P<0.05$) after maltose ingestion (Figure 5). Compounds **3**, **4**, **UA** and acarbose had hypoglycemic effects, and postprandial blood glucose levels were all lower than those in control. The delta area under the curve of the evaluated compounds in figure 5 which could reflect their hypoglycemic effects was calculated in Table 3. It revealed that acarbose (positive drug) had the best potential to postprandial blood glucose levels. Moreover, compound **3** and **4** had similar effects according to the delta area under the curve in Table 3.

Figure 5. Effect of compounds **3**, **4** and acarbose after a single oral administration of 3 g/kg maltose in normal mice with a 50 mg/kg dose of each compound. After 5 min, a 3 g/kg maltose solution was administered to each mouse. The control group was administered the same volume of

maltose solution without an inhibitor. Blood samples were collected and immediately subjected to blood glucose level assays using a disposable glucose sensor at 0, 30, 60, 90 and 120 min. Data were calculated as the means (mg/dL of plasma) ± SD. Statistical significances were evaluated using Student's *t*-test ($n=5$, (*) $P < 0.05$).

Table 3

Delta area under the curve of evaluated compounds in figure 5.

[a] Delta area under the curve $\Delta S = S_{control}$ (The area under the curve of control) - S_{sample} (The area under the curve of the sample).

3. Conclusions

We have designed and synthesized a series of **UA** derivatives and have demonstrated their potential activity for inhibiting α-glucosidase. All tested compounds exhibited greater potency than acarbose in the α-glucosidase inhibition assay, except compound **11**, which is observed because compound **11** had a low solubility under the assay conditions and could not disperse well at higher concentrations. From the IC_{50} values of compounds **2**, **3**, **4** and **5**, it can be concluded that, the longer the ester group that is conjugated to the 3-OH group, the more active the inhibitor. These results also demonstrated that side chains on the ester group could decrease activity. Compounds containing a free carboxyl in this position might be the most active. The replacement of aniline in the *p*-position possessed higher potency than in the *o*-position. Furthermore, as elaborated in the section detailing the *in vitro* enzyme activity studies,

compound **3** exhibited non-competitive inhibition mechanism; compound **4**, **5** and **8** presented mixed-type inhibition mechanism. Moreover, postprandial glucose levels could be reduced by compounds **3** and **4**, with especially distinct effects at 30 and 60 min after administration ($P<0.05$). Compounds **3** and **4** might interfere with or delay the absorption of dietary carbohydrates in small intestine, leading to a suppression of plasma glucose increase after a meal. It is worth noting that the modification of **UA** was performed at the 3-OH and/or 17-COOH positions. This is the first report of **UA** derivatives with α-glucosidase inhibition activity. The introduction of straight-chain acyl groups at the 3-OH position preserved the α-glucosidase activity at the low micromolar level, and while the bioavailability is unknown, these compounds were found to reduce blood glucose *in vivo*. Further research examining the activities of these derivatives in Caco-2 cells for intestinal absorption study and in the streptozocin-induced diabetic rat model are now underway in our laboratory.

4. Experimental

4.1. General Remarks

Ursolic acid was purchased from Nanjing Zelang Medical Technology Co., Ltd., with over 98% purity. Silica gel (100-200 or 200-300 mesh) used in column chromatography was supplied by Tsingtao Marine Chemistry Co., Ltd. Further purification was carried out by column

chromatography using Sephadex columns (LH-20). Other reagents were purchased from commercial suppliers in their chemically or analytically pure forms.

^{1}H NMR spectra were recorded on Bruker AVANCE 400 or Mercury - Plus 300 NMR spectrometers using TMS as an internal standard in DMSO-d_6. Electrospray ionization (ESI) mass spectra were measured on an LC-MS-2010A or Thermo Fisher LCQ Fleet and are reported as m/z. High-resolution mass spectra of compounds **6-12** were measured on a Bruker maXis impact. The enzyme inhibition activity was measured using a Multimodel Plate Reader (Infinite 200).

4.2. Synthesis

*4.2.1. General procedure for the preparation of compounds (**2**-**5**)*

Ursolic acid (6 mmol) and 60 mg DMAP were added to 75 mL of anhydrous pyridine in a 125 mL flask and stirred at room temperature until they were completely dissolved. Next, the anhydride (24 mmol) was slowly added into the mixture dropwise. After stirring overnight, the reaction mixture was concentrated under reduced pressure, dispersed in 100 mL of distilled water and filtered. The filtration procedure was repeated several times. Next, the mixture was treated with 2 M HCl and filtered when the pH was 3-4. The solid crude product was washed until neutral and the pH remained constant. The resulting mixture was concentrated to dryness. The crude product was purified on a silica gel

column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to produce compounds **2**-**5**.

*4.2.1.1. (**2**)* According to the general procedure, **UA** was treated with acetic anhydride, and then purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **2** (R_f=0.55).

Yield: 81%; white powder; ^{1}H NMR (300 MHz, DMSO) δ 11.89 (s, 1H, -COO<u>H</u>), 5.12 (s, 1H), 4.40 (dd, *J* = 11.2, 4.5 Hz, 1H), 2.11 (d, *J* = 11.3 Hz, 1H), 2.00 (s, 3H, -OCOC<u>H</u>$_3$), 1.97 – 1.71 (m, 4H), 1.68 – 1.38 (m, 10H), 1.30 (t, *J* = 14.2 Hz, 4H), 1.10 – 0.96 (m, 5H), 0.91 (s, 7H), 0.84 (d, *J* = 13.2 Hz, 10H), 0.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 178.3, 170.1 (-O<u>C</u>OCH$_3$), 138.2, 124.4, 79.9, 54.5, 52.3, 46.8, 46.8, 41.6, 38.5, 38.4, 37.7, 37.2, 36.4, 36.3, 32.5, 30.2, 27.8, 27.5, 23.8, 23.2, 23.2, 22.8, 22.5 (-OCO<u>C</u>H$_3$), 21.1, 21.0, 17.8, 17.0, 16.9, 16.7, 15.1; ESI-MS m/z 497.77$(M-H)^-$.

*4.2.1.2. (**3**)* According to the general procedure, **UA** was treated with propionic anhydride, and then purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **3** (R_f=0.56).

Yield: 73%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 11.96 (s, 1H, -COO<u>H</u>), 5.13 (s, 1H), 4.42 (dd, *J* = 11.3, 4.5 Hz, 1H), 2.39 – 2.21 (m, 2H, -OCOC<u>H</u>$_2$CH$_3$), 2.11 (d, *J* = 11.2 Hz, 1H), 2.00 – 1.74 (m, 4H), 1.66

– 1.43 (m, 10H), 1.30 (dd, *J* = 22.7, 11.5 Hz, 4H), 1.04 (dd, *J* = 13.2, 5.6 Hz, 7H, -OCOCH$_2$C<u>H</u>$_3$), 0.91 (s, 7H), 0.88 – 0.79 (m, 11H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 178.3, 173.2 (-O<u>C</u>OCH$_2$CH$_3$), 138.2, 124.4, 79.7, 54.5, 52.3, 46.8, 46.8, 41.6, 38.5, 38.4, 37.7, 37.3, 36.4, 36.3, 32.5, 30.2, 27.8, 27.5, 27.2 (-OCO<u>C</u>H$_2$CH$_3$), 23.8, 23.2, 23.2, 22.8, 21.1, 21.1, 17.8, 17.0, 16.9, 16.7, 15.1, 9.2 (-OCOCH$_2$<u>C</u>H$_3$); ESI-MS m/z 511.80(M-H)$^-$.

*4.2.1.3. (**4**)* According to the general procedure, **UA** was treated with butyric anhydride, and then purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **4** (R_f=0.55).

Yield: 63%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 11.96 (s, 1H, -COOH), 5.13 (s, 1H), 4.42 (dd, *J* = 11.3, 4.6 Hz, 1H), 2.34 – 2.19 (m, 2H, -OCOC<u>H</u>$_2$CH$_2$CH$_3$), 2.11 (d, *J* = 11.2 Hz, 1H), 2.00 – 1.74 (m, 4H), 1.62 – 1.47 (m, 10H), 1.30 (dd, *J* = 22.8, 11.4 Hz, 4H), 1.08 – 0.97 (m, 5H), 0.97 – 0.85 (m, 12H, -OCOCH$_2$C<u>H</u>$_2$C<u>H</u>$_3$), 0.82 (s, 10H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 178.2, 172.4 (-O<u>C</u>OCH$_2$CH$_2$CH$_3$), 138.2, 124.4, 79.7, 54.5, 52.3, 46.8, 46.9, 41.6, 38.5, 38.4, 37.7, 37.3, 36.4, 36.3, 35.8 (-OCO<u>C</u>H$_2$CH$_2$CH$_3$), 32.5, 30.2, 27.8, 27.5, 23.8, 23.2, 23.2, 22.8, 21.0, 21.0, 18.1 (-OCOCH$_2$<u>C</u>H$_2$CH$_3$), 17.8, 17.0, 16.8, 16.7, 15.1, 13.4 (-OCOCH$_2$CH$_2$<u>C</u>H$_3$); ESI-MS m/z 525.83(M-H)$^-$.

*4.2.1.4. (**5**)* According to the general procedure, **UA** was treated with

isobutyl anhydride, and then purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **5** (R_f=0.57).

Yield: 61%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 11.96 (s, 1H, -COOH), 5.13 (s, 1H), 4.40 (dd, *J* = 11.4, 4.6 Hz, 1H), 2.11 (d, *J* = 11.2 Hz, 1H), 2.00 – 1.74 (m, 4H), 1.63 – 1.44 (m, 10H), 1.30 (dd, *J* = 23.0, 11.6 Hz, 4H), 1.09 (dd, *J* = 10.9, 7.0 Hz, 7H, -OCOC<u>H</u>(C<u>H</u>$_3$)$_2$), 1.06 – 0.97 (m, 5H), 0.91 (s, 7H), 0.82 (d, *J* = 8.0 Hz, 10H), 0.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 178.2, 175.6, (-O<u>C</u>OCH(CH$_3$)$_2$), 138.2, 124.4, 79.5, 54.5, 52.3, 46.8, 46.8, 41.6, 38.5, 38.4, 37.6, 37.4, 36.4, 36.3, 33.6 (-OCO<u>C</u>H(CH$_3$)$_2$), 32.5, 30.2, 27.8, 27.5, 23.8, 23.2, 23.2, 22.8, 21.0, 21.0, 19.0 (-OCOCH(<u>C</u>H$_3$)$_2$), 18.7 (-OCOCH(<u>C</u>H$_3$)$_2$), 17.7, 17.0, 16.9, 16.7, 15.1; ESI-MS m/z 525.82(M-H)$^-$.

*4.2.2. General procedure for the preparation of compounds (**6-12**)*

To a solution of compound **2** (0.5 g) in 50 mL of anhydrous CH_2Cl_2 in a 100 mL round-bottom flask was added oxalyl chloride (0.6 mL). The resulting solution was then stirred at room temperature for 36 h. The reaction was then concentrated to dryness under reduced pressure to obtain crude 3-*O*-acetylursolyl chloride. This intermediate was dissolved in anhydrous CH_2Cl_2. The mixture was stirred in the presence of triethylamine, and then treated with an equal amount of the desired amine. The reaction was stirred at room temperature for 6 h. Next, the CH_2Cl_2

was removed under vacuum to obtain the crude product, which was purified on a silica gel column with petroleum ether and ethyl acetate to yield a white powder. Further purification was performed via column chromatography with a Sephadex column (LH-20) using trichloromethane and methanol (v/v 1:1) as the eluent to obtain compounds **6-12**.

*4.2.2.1. (**6**)* According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of phenylamine was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **6** (R_f=0.67).

Yield: 76%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 8.86 (s, 1H), 7.53 (d, *J* = 7.7 Hz, 2H), 7.25 (t, *J* = 7.8 Hz, 2H), 7.00 (t, *J* = 7.2 Hz, 1H), 5.28 (s, 1H), 4.39 (d, *J* = 7.0 Hz, 1H), 2.37 (d, *J* = 10.9 Hz, 1H), 2.00 (s, 3H, -OCOC<u>H</u>$_3$), 1.76 (dd, *J* = 39.0, 11.3 Hz, 5H), 1.65 – 1.37 (m, 9H), 1.37 – 1.16 (m, 4H), 1.12 – 0.91 (m, 9H), 0.91 – 0.69 (m, 13H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 175.1 (-<u>C</u>O-NH-), 170.1 (-O<u>C</u>OCH$_3$), 139.3, 138.5, 128.3, 128.3, 124.5, 123.0, 120.4, 120.4, 79.9, 54.5, 51.6, 47.5, 46.8, 41.6, 38.8, 38.3, 37.7, 37.2, 36.4, 36.3, 32.4, 30.3, 27.8, 27.4, 23.4, 23.4, 23.2, 22.9, 21.1, 21.0, 17.7, 17.1, 16.6, 16.6, 16.6, 15.1; ESI-MS m/z 612.5$(M+K)^+$; HRMS (ESI) calcd for $C_{38}H_{55}NO_3$ $[M+H]^+$=574.4255, found: 574.4253.

*4.2.2.2. (**7**)* According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *p*-fluoroaniline was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent, followed by a Sephadex column (LH-20) with trichloromethane and methanol (v/v 1:1) as the eluent, to obtain compound **7** (R_f=0.43).

Yield: 51%; white powder; ^{1}H NMR (300 MHz, DMSO) δ 8.90 (s, 1H), 7.57 – 7.47 (m, 2H), 7.08 (t, *J* = 8.7 Hz, 2H), 5.74 (s, 1H), 5.27 (s, 1H), 4.38 (dd, *J* = 10.7, 4.6 Hz, 1H), 2.36 (d, *J* = 10.9 Hz, 1H), 1.99 (s, 3H), 1.90 – 1.65 (m, 5H), 1.63 – 1.38 (m, 9H), 1.27 (dd, *J* = 31.3, 11.4 Hz, 3H), 1.08 (s, 3H), 0.98 (d, *J* = 18.6 Hz, 6H), 0.84 (dd, *J* = 21.7, 5.1 Hz, 13H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO+$CDCl_3$) δ 176.3 (-CO-NH-), 172.9 (-OCOCH$_3$), 139.6, 138.3, 127.7, 127.7, 127.1, 127.1, 126.2, 124.5, 79.6, 54.6, 52.0, 46.8, 46.6, 42.2, 41.5, 38.8, 38.3, 37.6, 37.1, 36.9, 36.3, 32.4, 30.4, 27.7, 27.2, 27.2, 23.5, 23.1, 23.1, 22.7, 20.9, 17.6, 16.9, 16.5, 16.4, 15.0; ESI-MS m/z 630.5$(M+K)^+$; HRMS (ESI) calcd for $C_{38}H_{54}FNO_3$ $[M+H]^+$=592.4160, found: 592.4158.

*4.2.2.3. (**8**)* According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *p*-chloroaniline was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 10:1) as the eluent to obtain compound **8** (R_f=0.45).

Yield: 46%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 9.01 (s, 1H), 7.59 (d, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 8.9 Hz, 2H), 5.27 (s, 1H), 4.39 (dd, *J* = 11.3, 4.7 Hz, 1H), 2.36 (d, *J* = 10.9 Hz, 1H), 1.99 (s, 3H), 1.94 – 1.66 (m, 5H), 1.64 – 1.36 (m, 10H), 1.36 – 1.16 (m, 3H), 1.07 (s, 3H), 1.05 – 0.92 (m, 6H), 0.89 – 0.76 (m, 13H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 175.3 (-CO-NH-), 170.0 (-OCOCH$_3$), 138.4, 138.3, 128.2, 128.2, 126.6, 124.5, 121.7, 121.7, 79.8, 54.5, 51.6, 47.6, 46.7, 41.5, 38.7, 38.3, 37.7, 37.2, 36.3, 36.2, 32.3, 30.2, 27.7, 27.4, 23.4, 23.3, 23.2, 22.9, 21.0, 20.9, 17.7, 17.1, 16.6, 16.6, 16.5, 15.1; ESI-MS m/z 646.5(M+K)$^{+}$; HRMS (ESI) calcd for $C_{38}H_{54}ClNO_3$ [M+H]$^{+}$=608.3865, found: 608.3861.

*4.2.2.4. **(9)*** According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *p*-bromoaniline was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 20:1) as the eluent, followed by a Sephadex column (LH-20) with trichloromethane and methanol (v/v 1:1) as the eluent to obtain compound **9** (R_f=0.47).

Yield: 76%; white powder; ^{1}H NMR (300 MHz, DMSO) δ 8.99 (s, 1H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 5.26 (s, 1H), 4.38 (dd, *J* = 10.6, 4.6 Hz, 1H), 2.36 (d, *J* = 11.3 Hz, 1H), 1.99 (s, 3H), 1.76 (dd, *J* = 35.4, 12.2 Hz, 5H), 1.64 – 1.33 (m, 10H), 1.24 (dd, *J* = 20.6, 11.8 Hz, 3H), 1.12 – 0.91 (m, 9H), 0.86 (d, *J* = 9.9 Hz, 6H), 0.79 (d, *J* = 3.2

Hz, 7H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 175.4 (-CO-NH-), 170.1 (-OCOCH$_3$), 138.2, 126.3, 126.1, 125.9, 125.8, 125.7, 125.0, 124.1, 115.5, 115.3, 79.9, 54.5, 51.9, 47.7, 46.8, 41.7, 38.8, 38.3, 37.7, 37.2, 36.7, 36.4, 32.5, 30.3, 27.8, 27.4, 23.6, 23.2, 22.9, 21.0, 21.0, 17.7, 17.1, 16.6, 16.6, 15.1; ESI-MS m/z 692.5(M+K)$^+$; HRMS (ESI) calcd for $C_{38}H_{54}BrNO_3$ [M+H]$^+$=652.3360, found: 652.3356.

4.2.2.5. ***(10)*** According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *o*-fluoroaniline was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 20:1) as the eluent, followed by a Sephadex column (LH-20) with trichloromethane and methanol (v/v 1:1) as the eluent to obtain compound **10** (R_f=0.47).

Yield: 90%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 8.51 (s, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 7.0 Hz, 1H), 5.33 (s, 1H), 4.38 (dd, *J* = 10.9, 5.0 Hz, 1H), 2.21 (d, *J* = 10.7 Hz, 1H), 1.98 (s, 3H), 1.92 – 1.72 (m, 5H), 1.66 – 1.40 (m, 9H), 1.40 – 1.19 (m, 4H), 1.09 (s, 3H), 1.03 (d, *J* = 13.4 Hz, 3H), 0.94 (d, *J* = 6.0 Hz, 3H), 0.87 (d, *J* = 8.5 Hz, 6H), 0.80 (t, *J* = 8.0 Hz, 7H), 0.66 (s, 3H); ^{13}C NMR (100 MHz, DMSO+CDCl$_3$) δ 175.3 (-CO-NH-), 169.8 (-OCOCH$_3$), 137.8, 135.0, 129.0, 127.2, 125.6, 125.3, 125.2, 124.6, 124.3, 124.1, 79.8, 54.5, 52.3, 48.1, 46.7, 41.6, 38.2, 37.6, 37.1, 36.7, 36.3, 32.2, 30.3, 27.7, 27.2, 23.9, 23.2, 23.1, 22.7, 20.9, 20.8, 17.6, 16.9,

16.5, 16.5, 15.0; ESI-MS; m/z 630.5$(M+K)^+$; HRMS (ESI) calcd for $C_{38}H_{54}FNO_3$ $[M+H]^+$=592.4160, found: 592.4160.

4.2.2.6. ***(11)*** According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *o*-chloroaniline was added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 20:1) as the eluent, followed by a Sephadex column (LH-20) with trichloromethane and methanol (v/v 1:1) as the eluent to obtain compound **11** (R_f=0.52).

Yield: 87%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 8.29 (s, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.25 (t, *J* = 7.7 Hz, 1H), 6.98 (t, *J* = 7.7 Hz, 1H), 5.35 (s, 1H), 4.36 (dd, *J* = 9.6, 6.2 Hz, 1H), 2.15 (d, *J* = 10.6 Hz, 1H), 1.96 (s, 3H), 1.82 (dd, *J* = 40.4, 9.3 Hz, 4H), 1.66 – 1.38 (m, 10H), 1.38 – 1.18 (m, 4H), 1.11 – 0.96 (m, 6H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.89 – 0.82 (m, 6H), 0.78 (d, *J* = 8.2 Hz, 7H), 0.64 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 175.2 (-CO-NH-), 169.7 (-OCOCH$_3$), 137.6, 136.0, 132.0, 127.6, 125.9, 125.2, 123.6, 115.1, 79.8, 54.5, 52.6, 48.2, 46.7, 41.6, 39.0, 38.3, 37.6, 37.1, 36.7, 36.2, 32.1, 30.3, 27.6, 27.2, 24.0, 23.2, 23.0, 22.7, 20.8, 20.8, 17.6, 16.8, 16.5, 16.5, 16.3, 15.0; ESI-MS m/z 646.5$(M+K)^+$; HRMS (ESI) calcd for $C_{38}H_{54}ClNO_3$ $[M+H]^+$=608.3865, found: 608.3865.

4.2.2.7. ***(12)*** According to the general procedure, compound **2** was treated with oxalyl chloride, and then an equal amount of *o*-bromoaniline was

added. The product was purified on a silica gel column with petroleum ether/ethyl acetate (v/v 20:1) as the eluent, followed by a Sephadex column (LH-20) with trichloromethane and methanol (v/v 1:1) as the eluent to obtain compound **12** (R_f=0.55).

Yield: 80%; white powder; ^{1}H NMR (400 MHz, DMSO) δ 9.00 (s, 1H), 7.55 (d, *J* = 2.0 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.47 – 7.43 (m, 1H), 7.42 (d, *J* = 2.0 Hz, 1H), 5.27 (s, 1H), 4.38 (dd, *J* = 11.4, 4.7 Hz, 1H), 2.36 (d, *J* = 10.8 Hz, 1H), 1.99 (s, 3H), 1.94 – 1.66 (m, 5H), 1.66 – 1.35 (m, 10H), 1.35 – 1.17 (m, 3H), 1.07 (s, 3H), 1.04 – 0.92 (m, 6H), 0.89 – 0.82 (m, 6H), 0.79 (d, *J* = 5.4 Hz, 7H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 175.3 (-CO-NH-), 170.1 (-OCOCH$_3$), 138.7, 138.4, 131.1, 131.1, 124.5, 122.1, 122.1, 114.6, 79.9, 54.5, 51.6, 47.6, 46.7, 41.5, 38.7, 38.3, 37.7, 37.2, 36.3, 36.2, 32.3, 30.2, 27.7, 27.3, 23.4, 23.3, 23.2, 22.9, 21.0, 20.9, 17.6, 17.1, 16.6, 16.6, 16.5, 15.1; ESI-MS m/z 692.5$(M+K)^+$; HRMS (ESI) calcd for $C_{38}H_{54}BrNO_3$ $[M+H]^+$=652.3360, found: 652.3359.

4.3. α-glucosidase inhibitory activity

4.3.1. Baker's yeast α-glucosidase inhibitory activity

The α-glucosidase inhibition assay was performed according to the method of Worawalai et al. with slight modification [25]. The α-glucosidase enzyme (0.1 U/mL) and substrate (1 mM *p*-nitrophenyl-α-D-glucopyranoside) were dissolved in 0.1 M phosphate

buffer, pH 6.8. 10 μL of each synthesized compound (1 mg/mL in DMSO) was pre-incubated with 8 μL of α-glucosidase at 37 ℃ for 10 min. A 100 μL aliquot of substrate solution was then added to the reaction mixture, which was further incubated at 37 ℃ for 30 min. Then, the reaction was terminated by adding 100 μL of 1 M Na_2CO_3. Enzymatic activity was quantified by measuring the absorbance at 405 nm using a Multimodel Plate Reader (Infinite 200). The percentage of inhibition was calculated using $[(A_0-A_1)/A_0]\times100\%$, where A_0 was the absorbance without the compound, and A_1 was the absorbance with the compound. The IC_{50} value was determined from a plot of the percentage of inhibition versus the sample concentration. Acarbose was used as standard control and the experiment was performed in duplicate.

4.3.2. Measurement of kinetic constants [25]

For the kinetic analysis of α-glucosidase inhibition by the active compounds, the enzyme and active compounds were incubated with increasing concentrations of *p*-nitrophenyl-α-D-glucopyranoside (0-10 μM). The modes of inhibition for each compound were determined based on Lineweaver-Burk plots.

4.4. Hypoglycemic effect in normal mice [24,26]

Kunming mice (18-20 g body weight, 6-8 weeks old) were maintained under identical laboratory conditions. Animals were deprived of food for 16 h and allocated into three groups of five mice each by

plasma glucose level. Food was given 20 min after oral administration of 0.5% CMC (control) acarbose and the active compounds (50 mg/kg). Blood samples were collected by tail bleeding at 0, 30, 60, 90, and 120 min, and plasma glucose was assayed using a disposable glucose sensor (kit). Data are expressed as the means (mg/dL of plasma) ± standard error of the means (n=5).

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594
595 **Figure 1.** Compound **3**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound
596 concentrations were: 0 μM ■; 2.0 μM ●; 5.0 μM ▲; 9.0 μM ▼. All assays were performed in
597 quadruplicate. The data reported in the figure represent the means ± S.E.M.
598
599 **Figure 2.** Compound **4**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound
600 concentrations were: 0 μM ■; 2.85 μM ●; 4.0 μM ▲; 6.0 μM ▼. All assays were performed in
601 quadruplicate. The data reported in the figure represent the means ± S.E.M.
602
603 **Figure 3.** Compound **5**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound
604 concentrations were: 0 μM ■; 1.6 μM ●; 2.8 μM ▲; 4.5 μM ▼. All assays were performed in
605 quadruplicate. The data reported in the figure represent the means ± S.E.M.
606
607 **Figure 4.** Compound **8**. Double reciprocal plot (1/V versus 1/[S]) (A), the compound
608 concentrations were: 0 μM ■; 2.5 μM ●; 5.0 μM ▲; 8.0 μM ▼. All assays were performed in
609 quadruplicate. The data reported in the figure represent the means ± S.E.M.

610

611 **Figure 5.** Effect of compounds **3**, **4** and acarbose after a single oral administration of 3 g/kg
612 maltose in normal mice with a 50 mg/kg dose of each compound. After 5 min, a 3 g/kg maltose
613 solution was administered to each mouse. The control group was administered the same volume of
614 maltose solution without an inhibitor. Blood samples were collected and immediately subjected to
615 blood glucose level assays using a disposable glucose sensor at 0, 30, 60, 90 and 120 min. Data
616 were calculated as the means (mg/dL of plasma) ± SD. Statistical significances were evaluated
617 using Student's *t*-test ($n=5$, ($*$) $P < 0.05$).

618

Table 1

Inhibitory effects of compounds **1-12** on α-glucosidase from baker's yeast.

Compound	% Inhibition[a]	% Inhibition[b]	IC_{50} (μM)[c]
1 (UA)	48.65±6.85	87.55±10.43	5.04±0.80
2	48.85±5.66	75.62±11.53	5.27±0.35
3	68.89±3.58	86.51±6.89	2.66±0.40
4	99.84±5.09	99.79±5.00	1.01±0.14
5	69.31±3.91	90.83±3.20	3.26±0.52
6	45.38±2.48	67.38±7.82	5.64±1.12
7	45.28±5.09	66.71±10.57	6.53±1.33
8	64.52±2.38	82.03±11.94	3.24±0.21
9	28.31±11.36	52.97±8.50	9.33±4.36
10	16.85±7.55	37.89±9.04	14.18±3.66
11	6.83±3.51	13.66±2.44	>33
12	23.24±2.53	47.29±7.79	11.12±2.82
13[d]	<2	<5	579.15±20.58

Each experiment was performed in quadruplicate. The data reported represent the means (n=4) ± SD.

[a] Percent inhibition of α-glucosidase treated with a 5 μM concentration of each compound.

[b] Percent inhibition of α-glucosidase treated with a 10 μM concentration of each compound.

[c] IC_{50} value representing the concentration that caused a 50% loss of activity.

[d] Acarbose, positive control.

628 **Table 2**

Compound	K_i	K_i'	Inhibition type
3	2.67±0.19	-	Non-competitive inhibition
4	2.82±0.11	2.07±0.27	Liner mixed inhibition
5	3.15±0.16	4.62±0.17	Liner mixed inhibition
8	3.50±0.44	3.37±0.04	Liner mixed inhibition

629 Inhibition constants (K_i and K_i') of compound **3-5** and **8** for α-glucosidase. The data shown
630 represent the mean K_i and K_i' values ± S.E.M.
631

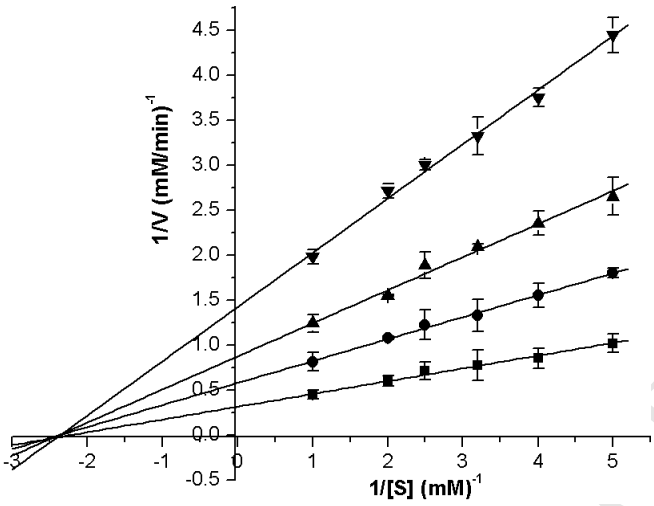
Table 3

Delta area under the curve of evaluated compounds in figure 5.

Time (min)	Delta area under the curve[a]			
	Acarbose	Compound **3**	Compound **4**	Ursolic acid
0-30	676.2	628.17	631.08	488.4
30-60	1006.2	933.9	879.9	700.4
60-90	518.7	351.6	378.6	309.2
90-120	108	0	0	-50.4
Total	2309.1	1913.67	1889.58	1447.5

[a] Delta area under the curve $\Delta S = S_{control}$ (The area under the curve of control) - S_{sample} (The area under the curve of the sample).

637 **Figure 1**



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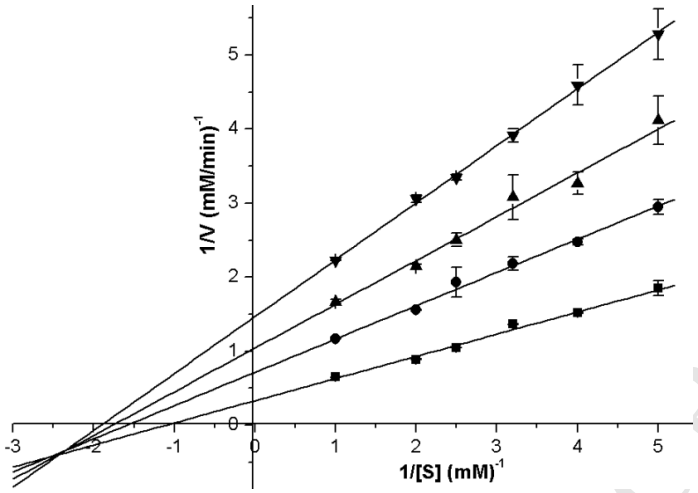
640 **Figure 2**



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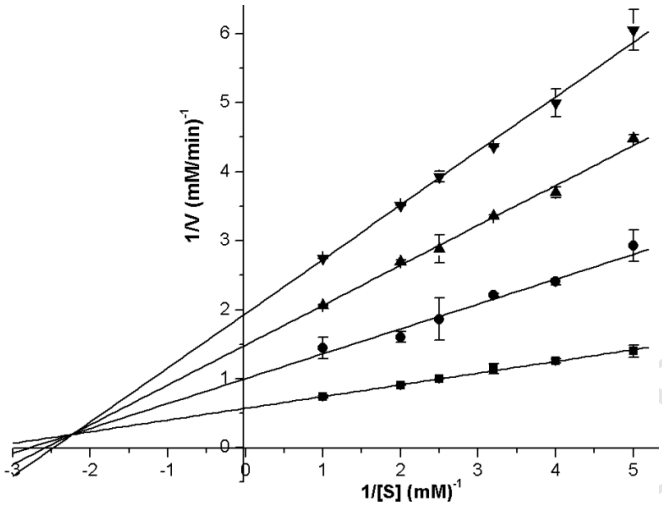
643 **Figure 3**



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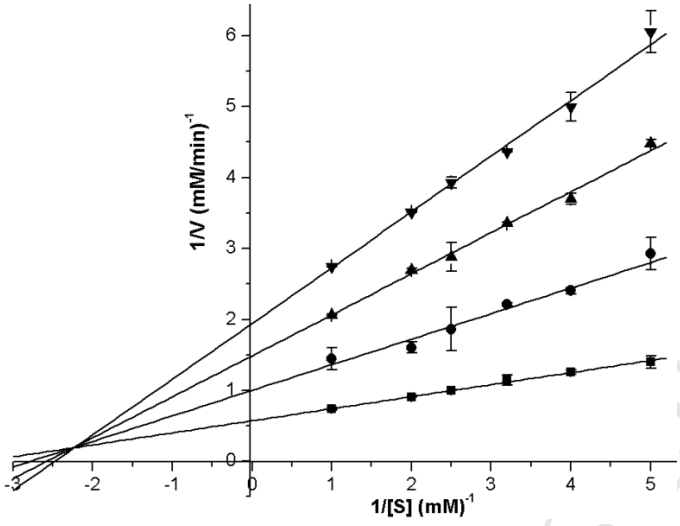
646 **Figure 4**



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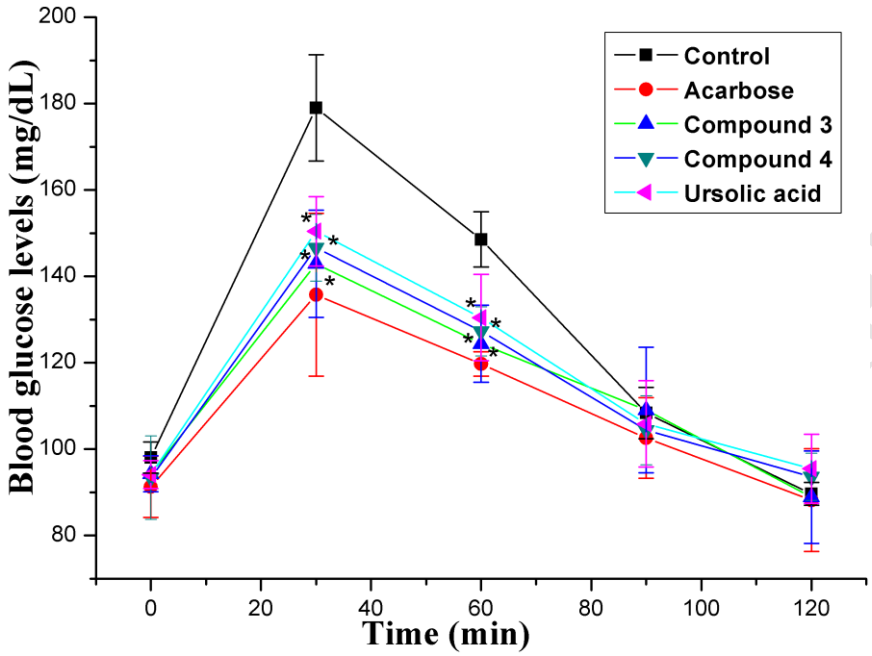
649 **Figure 5**



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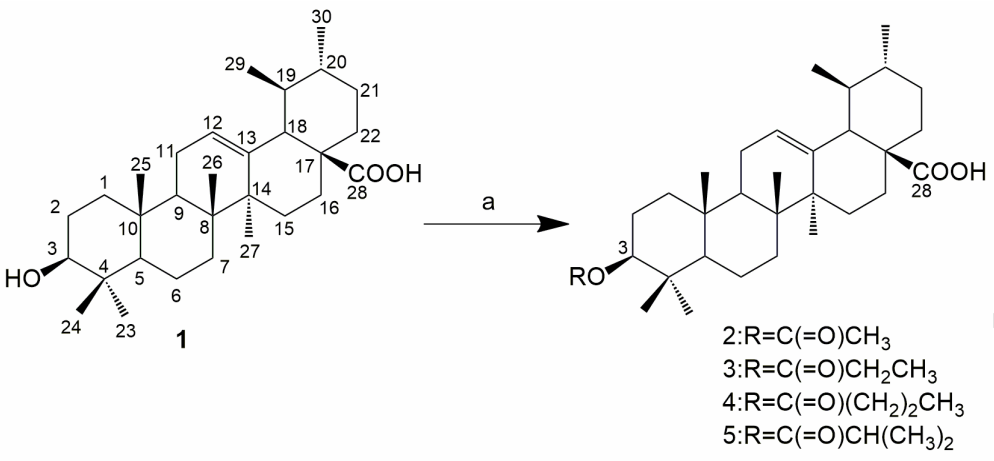
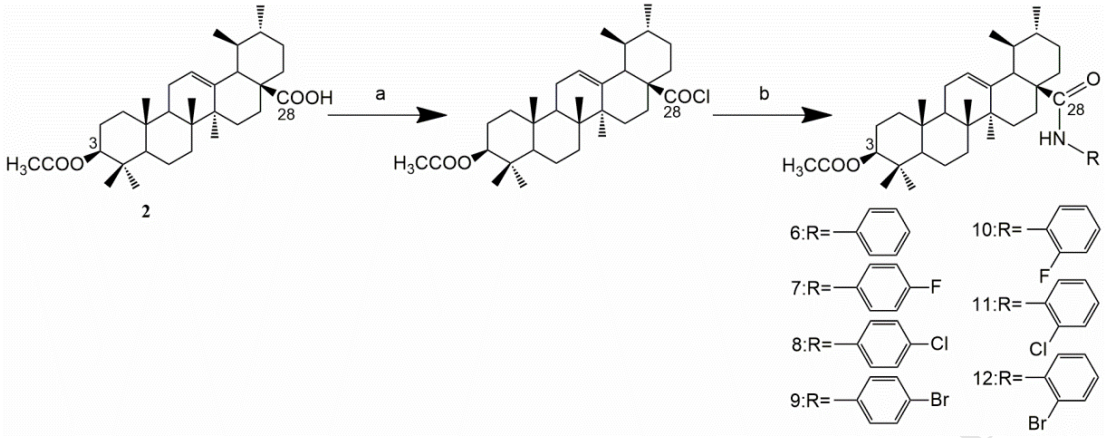


652

653 **Scheme 1.** Synthesis of compounds **2-5** from ursolic acid. Reagents and conditions: (a)

654 anhydride/Pyr/DMAP, r.t.

655

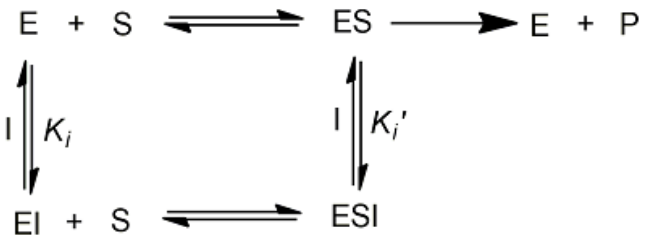


656

657 **Scheme 2.** Synthesis of compounds **6-12** from compound **2**. Reagents and conditions: (a) $(COCl)_2$,

658 CH_2Cl_2, r.t.; (b) CH_2Cl_2, Et_3N, RNH_2, r.t.

659



Scheme 3. The linear mixed-type inhibition of compounds **3**-**5** and **8**. When the constant K_i=K_i', the model describes non-competitive inhibition.

Table 1

Inhibitory effects of compounds **1-12** on α-glucosidase from baker's yeast.

Compound	% of Inhibition[a]	% of Inhibition[b]	IC_{50} (μM)[c]
1 (UA)	48.65±6.85	87.55±10.43	5.04±0.80
2	48.85±5.66	75.62±11.53	5.27±1.35
3	68.89±3.58	86.51±6.89	2.66±0.84
4	99.84±5.09	99.79±5.00	1.01±0.44
5	69.31±3.91	90.83±3.20	3.26±0.22
6	45.38±2.48	67.38±7.82	5.64±1.12
7	45.28±5.09	66.71±10.57	6.53±1.33
8	64.52±2.38	82.03±11.94	3.24±2.21
9	28.31±11.36	52.97±8.50	9.33±4.36
10	16.85±7.55	37.89±9.04	14.18±3.66
11	6.83±3.51	13.66±2.44	>33
12	23.24±2.53	47.29±7.79	11.12±2.82
13[d]	<2	<5	579.15±20.58

[a] Inhibitory percentages of α-glucosidase treated with 5 μM concentration of compound.

[b] Inhibitory percentages of α-glucosidase treated with 10 μM concentration of compound.

[c] IC_{50} value representing the concentration which caused 50% loss of activity.

[d] Acarbose, positive control.

Table 2

Inhibition constants (K_i and K_i') of compound **3-5** and **8** for α-glucosidase. The data shown represents the mean K_i or K_i' values ± S.E.M.

Compound	K_i	K_i'	Inhibition type
3	2.67±0.19	-	Non-competitive inhibition
4	2.82±0.11	2.07±0.27	Liner mixed inhibition
5	3.15±0.16	4.62±0.17	Liner mixed inhibition
8	3.50±0.44	3.37±0.04	Liner mixed inhibition

Table 3

Delta area under the curve of evaluated compounds in figure 5.

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[a] Delta area under the curve $\Delta S = S_{control}$ (The area under the curve of control) - S_{sample} (The area under the curve of the sample).

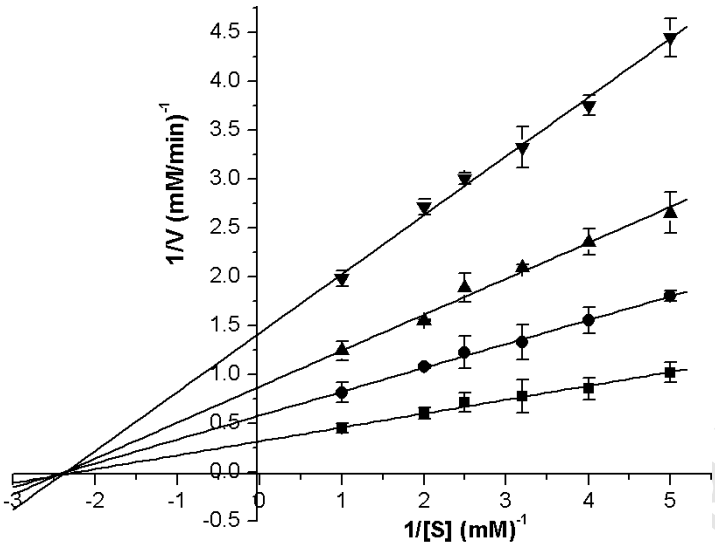

Figure 1. Compound **3**. Double reciprocal plot (1/V versus 1/[S]), the compound concentrations were: 0 μM ■; 2.0 μM ●; 5.0 μM ▲; 9.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

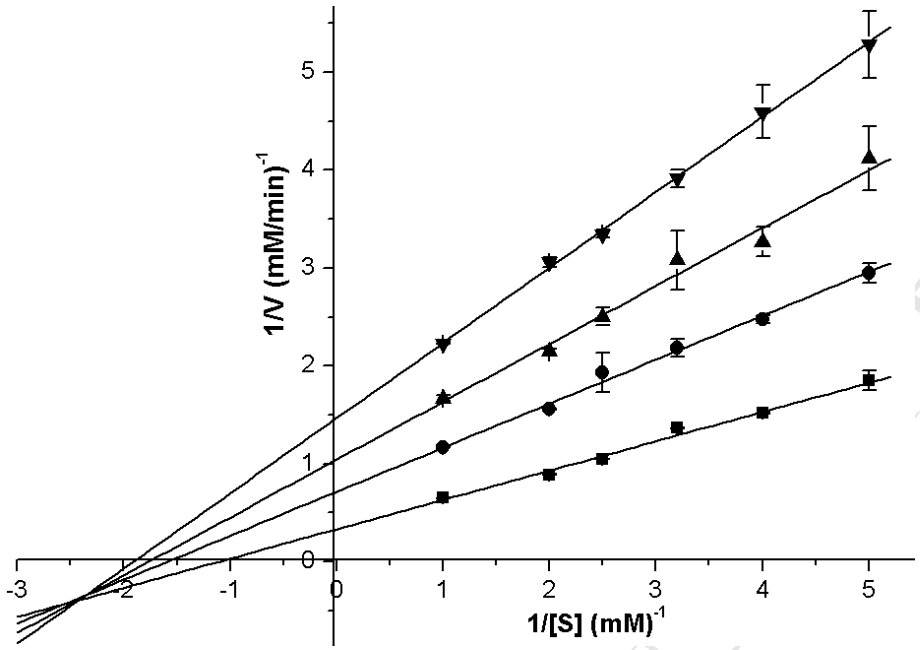

Figure 2. Compound **4**. Double reciprocal plot (1/V versus 1/[S]), the compound concentrations were: 0 μM ■; 2.85 μM ●; 4.0 μM ▲; 6.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

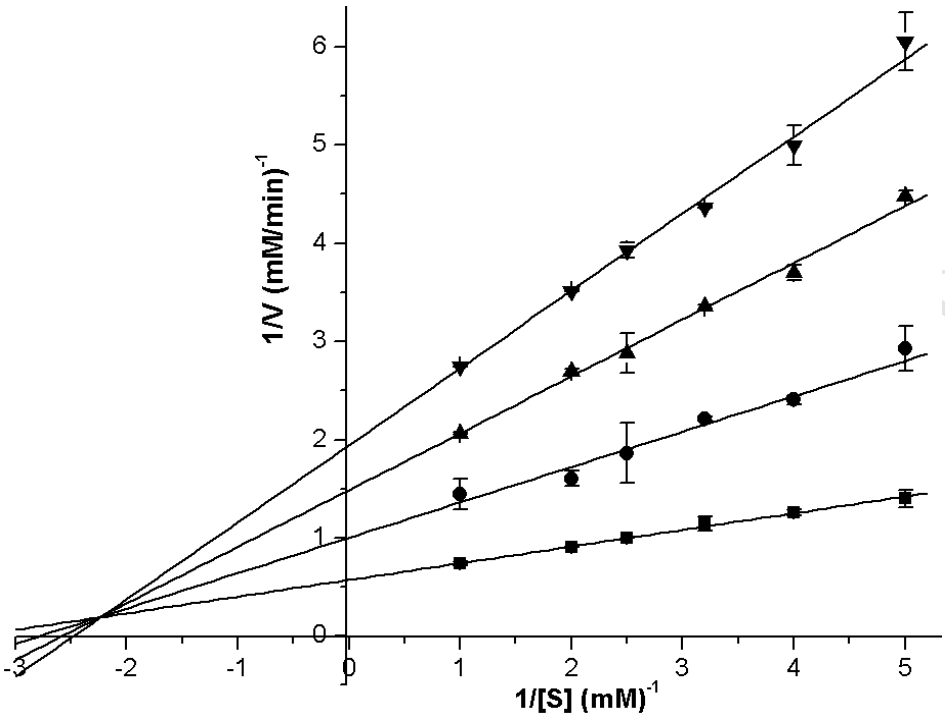

Figure 3. Compound **5**. Double reciprocal plot (1/V versus 1/[S]), the compound concentrations were: 0 μM ■; 1.6 μM ●; 2.8 μM ▲; 4.5 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

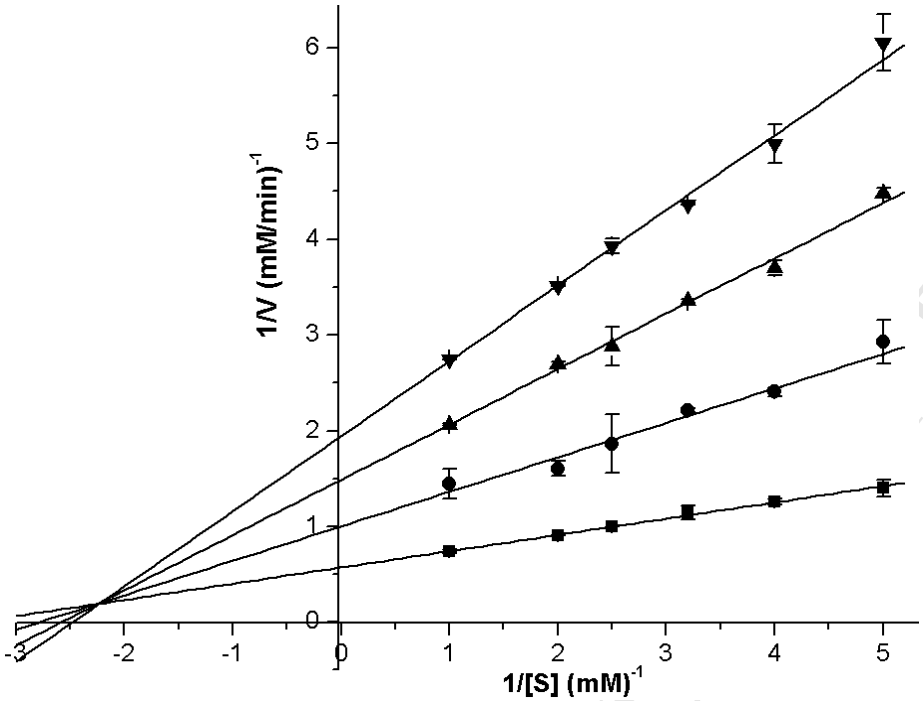

Figure 4. Compound **8**. Double reciprocal plot (1/V versus 1/[S]), the compound concentrations were: 0 μM ■; 2.5 μM ●; 5.0 μM ▲; 8.0 μM ▼. All assays were performed in quadruplicate. The data reported in the figure represent the means ± S.E.M.

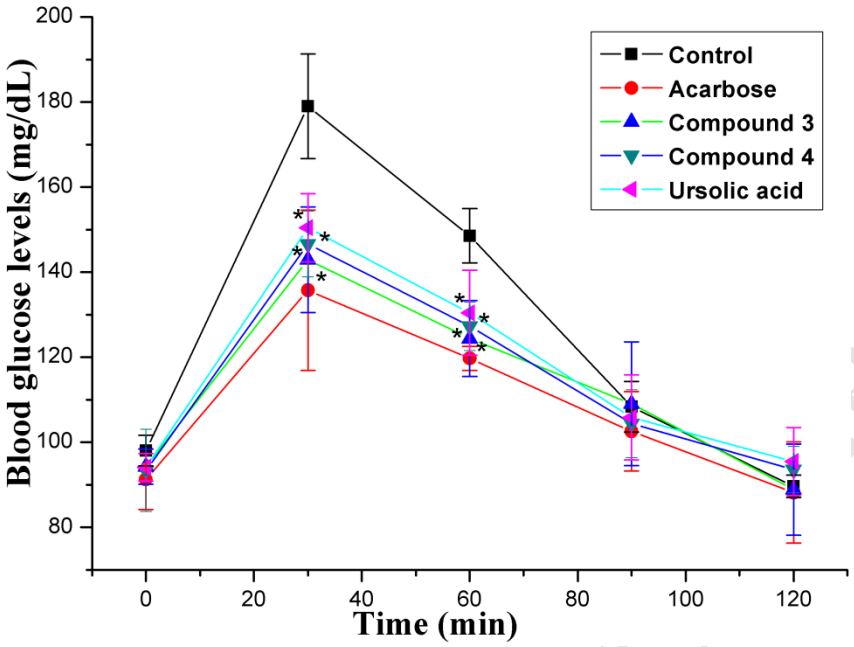

Figure 5. Effect of compounds **3**, **4** and acarbose after a single oral administration of 3 g/kg maltose in normal mice with a 50 mg/kg dose of each compound. After 5 min, a 3 g/kg maltose solution was administered to each mouse. The control group was administered the same volume of maltose solution without an inhibitor. Blood samples were collected and immediately subjected to blood glucose level assays using a disposable glucose sensor at 0, 30, 60, 90 and 120 min. Data were calculated as the means (mg/dL of plasma) ± SD. Statistical significances were evaluated using Student's *t*-test ($n=5$, (*) $P < 0.05$).