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Graphical Abstract

Analogues of Xanthones——Chalcones and Bis-chalcones as α-Glucosidase Inhibitors and anti-Diabetes Candidates

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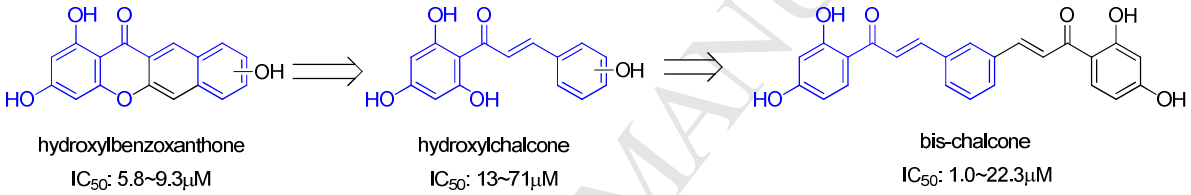

Two series of chalcones and bis-chalcones were synthesized and exhibited α-glucosidase inhibition *via* a non-competitive mechanism. Moreover, the compound **2g** could significantly reduce the glucose level in HepG-2 cells.

Analogues of Xanthones——Chalcones and Bis-chalcones as α-Glucosidase Inhibitors and Anti-Diabetes Candidates

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Abstract

Two series of compounds (chalcones and bis-chalcones) were designed, synthesized, and evaluated as α-glucosidase inhibitors (AGIs) with 1-deoxynojirimycin as positive control *in vitro*. Most of the compounds with two or four hydroxyl groups showed better inhibitory activities than 1-deoxynojirimycin towards α-glucosidase with noncompetitive mechanism. Moreover, most of the hydroxy bis-chalcones exhibit good α-glucosidase inhibitory activities in enzyme test. Inspiringly, bis-chalcones **2g (**at 1 μM concentration) has stronger effect than 1-deoxynojirimycin on reducing the glucose level in HepG-2 cells (human liver cancer cell line).

Keywords：α-glucosidase inhibitors; chalcones; bis-chalcones; noncompetitive.

1. Introduction

Diabetes mellitus (DM), a worldwide public health problem, is a group of metabolic diseases that feature with high blood sugar levels in patients. Thus, discovery of hypoglycemic agents with strong potency and weak side effect is highly desirable. Of all anti-diabetic drugs, α-glucosidase inhibitors seem to be the most effective in reducing post-prandial hyperglycemia [1]. The well-developed α-glucosidase inhibitors (AGIs), such as acarbose, miglitol and voglibose, have been clinically used. However, their adverse effects (e.g. gastrointestinal symptoms) [2-3] in some cases and newly discovered additive effects (like stabling carotid plaques, and reducing inflammation) [1] make the discovery of novel AGIs still attractive and challenging.

Our previous efforts on AGIs led to the discovery of that xanthones are potent α-glucosidase inhibitors and H-bond, extend π system, and flexibility are key factors influencing the inhibitory activities [4-6]. We reasonably speculated that chalcones, as the analogues of xanthones (Figure 1), would have better α-glucosidase inhibitory activity due to the flexibility of the framework of chalcones.

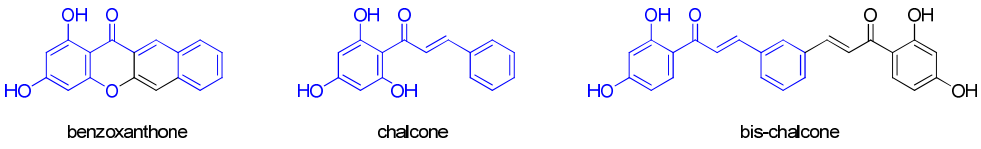


Figure 1 The structures of benzoxanthone, chalcone and bis-chalcone

Chalcones, also known as α-β-unsaturated ketones, display various biological activities, such as anti-cancer [7], anti-diabetic [8-9], anti-oxidant, anti-malarial, antimicrobial, anti-HIV, antifilarial [10] and anti-inflammatory effects [11]. Bis-chalcones also have many biological activities. As is reported, bis-chalcone analogues are the potent NO production inhibitors and cytotoxic agents against four human cancer cell lines (A549, DU145, KB, and KB-VIN) [12]. Biphenyl based bis-chalcones have anticancer activity against human breast cancer MCF-7 and MDA-MB-231 cell lines, HeLa cell line, and human embryonic kidney (HEK-293) cells [13]. Some bis-chalcones have appreciable antibacterial and antifungal activities [14]. However, the α-glucosidase inhibitory activities of bis-chalcones are still unknown. In this article, a series of chalcones and bis-chalcones (with extend π system) were synthesized and evaluated as α-glucosidase inhibitors, aimed at clarifying the structure-activity relationship and finding suitable anti-diabetes candidates.

2. Result and Discussion

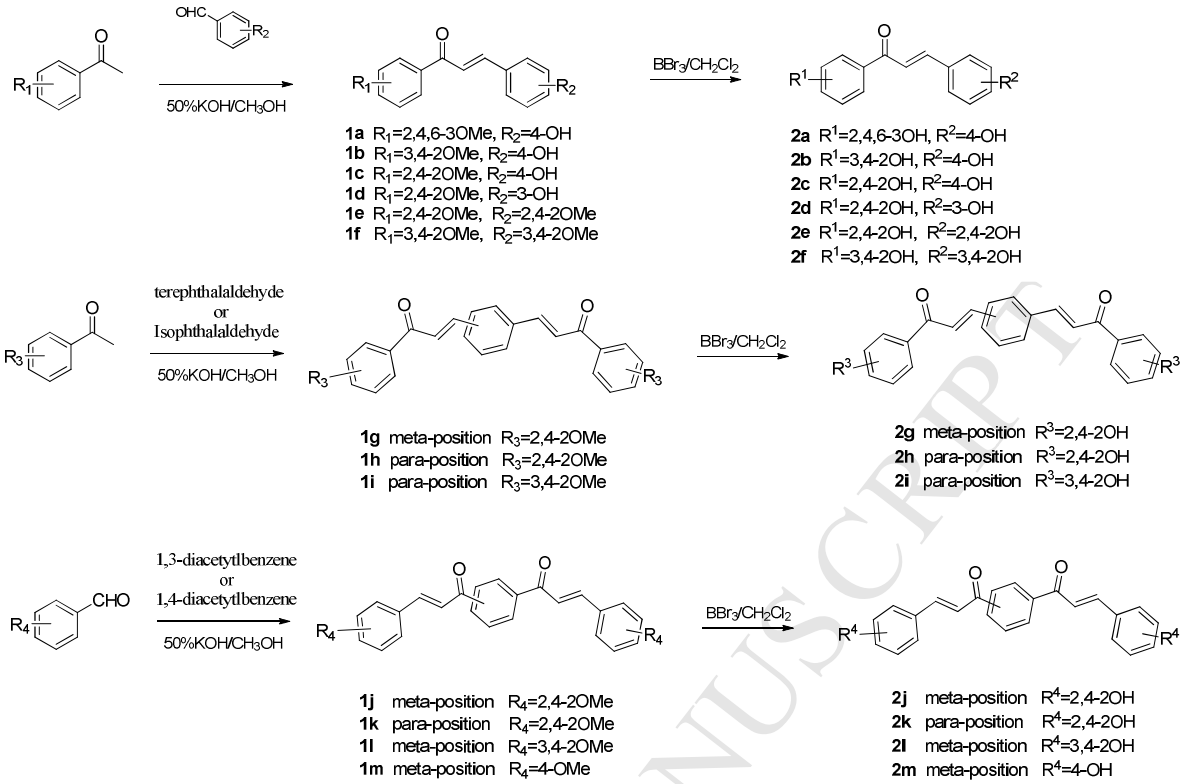
2.1 Chemistry

2.1.1 Synthesis of chalcones and bis-chalcones

Two series of compounds (chalcones and bis-chalcones), including four new compounds (**2g**, **2i**, **2j** and **2k**), were synthesized respectively, the structures of all compounds were confirmed with IR, ^{1}H NMR, ^{13}C NMR, and HRMS techniques.

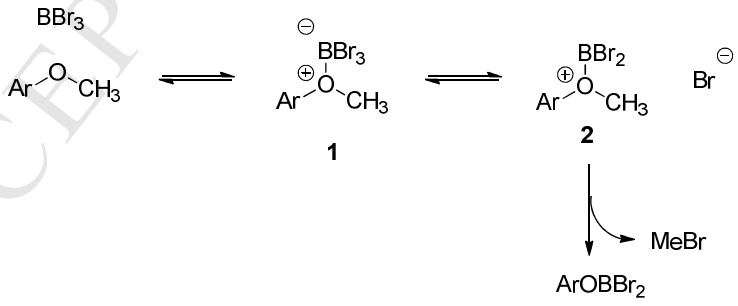
Initially, we tried to synthesize compounds **2a**-**2d** using $SOCl_2$/EtOH as catalytic system according to literature [15]. Unfortunately, the yields were lower than 10% because substrates contain multi-hydroxyl groups. Therefore, we switched to two steps methods, that is, synthesizing compounds **1** and then demethylating to generate target compounds **2**. As expected, **1a**-**1d** (with only one hydroxyl group) were provided through the reaction catalyzed by $SOCl_2$ with moderate yields (30%~57%). **1e** and **1f**, without hydroxyl groups, were easily synthesized via Aldol condensation reactions in the presence of aqueous KOH with good yields (71%~78%). Then compounds **2a-2m** were obtained by the demethylation of **1a**-**1m** in the presence of BBr_3/CH_2Cl_2, respectively.

It should be noted that the demethylation conditions would be substrate-dependent. For example, when we synthesized **2d**, **2f**, **2g**, **2h**, **2j** and **2k** from **1d**, **1f**, **1g**, **1h**, **1j** and **1k**, some by-products with one or two methoxy groups retaining were obviously observed, which led to the low yields (15%~31%) of desired **2d**, **2f**, **2g**, **2h**, **2j** and **2k**.



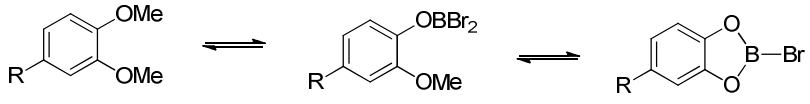
Scheme 1 Synthesis of the target compounds **1a-1m** and **2a-2m**

Conceptually, the demethylation proceeds via an adduct **1** formed between BBr_3 and methoxyl chalcone followed by the loss of bromide. Then the methoxyl group of the cationic intermediate **2** is attacked by the free bromide cleaving the C-O bond (Scheme 2) [16]. Then $ArOBBr_2$ would be hydrolyzed to generate ArOH. Interestingly, for chalcones with 3, 4-methoxyl groups, a cyclic borate can be formed when the demethylation is proceeded (Scheme 3) [17].



$$ArOBBr_2 + 3H_2O \longrightarrow ArOH + H_3BO_3 + 2HBr$$

Scheme 2 Conceptual reaction mechanism for demethylation



Scheme 3 Conceptual reaction mechanism for demethylation of dimethoxyl groups

2.1.2 Stereochemistry of chalcones and bis-chalcones

Generally, an AB two-spin system overlapping with a typical *trans* coupling of $^3J_{AB}$=12~18 Hz reveals a CC double bond with protons in *trans* configuration (Figure 2) [18]. Take **1a** as example, the AB two-spin system (δ_A=7.20 and δ_B=6.75) with a typical *trans* coupling of $^3J_{AB}$=16.0 Hz reveals the CC double bond of chalcone **1a** in *trans* configuration. From the NMR spectroscopy of chalcones and bis-chalcones, the methoxyl chalcones and bis-chalcones **1a**-**1m** are all in *trans* configuration. The configuration of hydroxyl chalcones and bis-chalcones **2b**, **2f**, **2i**, **2l** and **2m** could not be confirmed by ^{1}H NMR because the spectral peaks are overlapped. Since these five compounds were synthesized from methoxyl chalcones and bis-chalcones **1b**, **1f**, **1i**, **1l** and **1m** which are in *trans* configuration and the demethylation would not change the configuration of these compounds. It can be inferred that **2b**, **2f**, **2i**, **2l** and **2m** are in *trans* configuration.

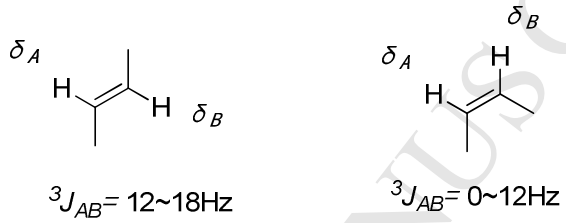
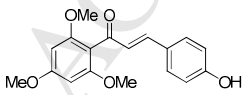
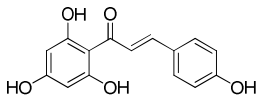
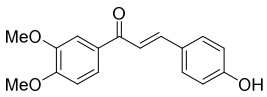
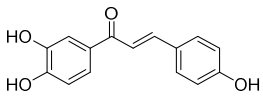
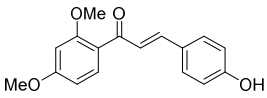
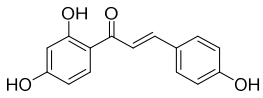


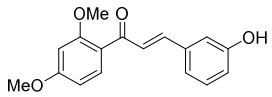
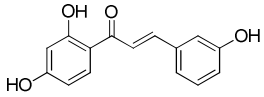
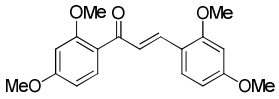
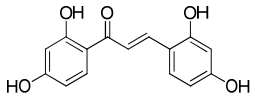
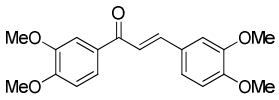
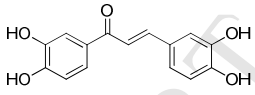
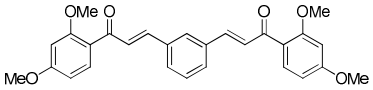
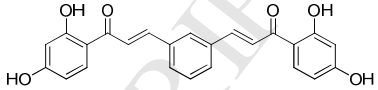
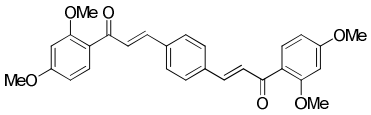
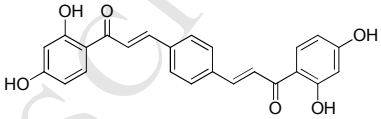
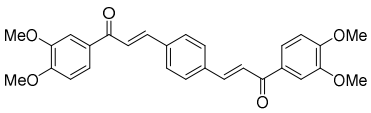
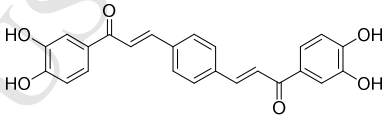
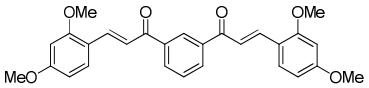
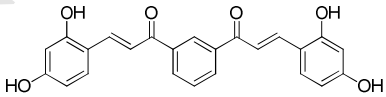
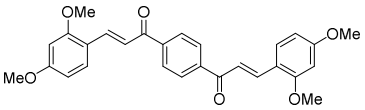
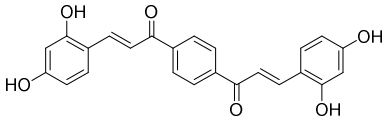
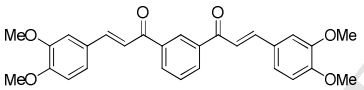
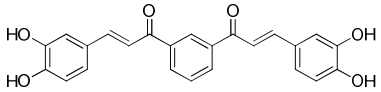
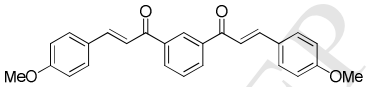
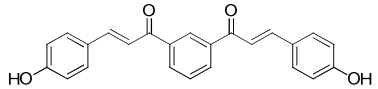
Figure 2 The stereochemistry of chalcones

2.2 α-Glucosidase inhibitory activity

As shown in Table 1, nearly all the hydroxyl chalcones and bis-chalcones (**21**-**2m**) showed higher activities than 1-deoxynojirimycin, one of the well-known AGIs. Compound **2k** showed the highest inhibitory activity. The IC_{50} value was only 1.0 μM. On the contrary, the chalcones and bis-chalcones containing methoxyl groups (**1a**-**1m**) showed much lower inhibitory activities, and due to the poor solubility, only the inhibition ratio at 40 μM level were obtained for some compounds. Therefore, it is reasonable to consider that the H-bond donor effect of the hydroxyl group is the key effect during the interaction of these compounds with the enzyme.

Table 1 The IC_{50} value or inhibition of the compounds

Compounds	Structure	Inhibition or IC_{50}/μM	Compounds	Structure	IC_{50}/μM
1a		>200	**2a**		71.1±2.6
1b		>200	**2b**		35.2±0.2
1c		58.7±3.0	**2c**		13.2±2.7

1d		>200	**2d**		42.0±6.0
1e		83.2±1.0	**2e**		12.5±2.1
1f		123.1±9.9	**2f**		15.6±2.8
1g		27.2% [a]	**2g**		23.7±0.3
1h		27% [a]	**2h**		22.5±3.2
1i		32.0% [a]	**2i**		10.1±1.7
1j		55.2% [a]	**2j**		5.5±1.2
1k		66.9±2.5	**2k**		1.0±0.1
1l		16.0% [a]	**2l**		6.5±0.2
1m		29% [a]	**2m**		18.3±0.7

[a] : the inhibition of the compounds at the concentration of 40 μM (The solubility of these methoxyl bis-chalcones in PBS are not good). The IC_{50} value of positive control (1-deoxynojirimycin, PG) is 21.3±8.7μM.

The position of the hydroxyl groups in chalcones is another key factor for their AG inhibitory activities. As shown in Figure 3, in ring A, the 4-hydroxyl group was stronger than the 3-hydroxyl group in enhancing the inhibitory effect, with the IC_{50} values of **2c** (13.4±2.7 μM) vs. **2d** (42.0±6.0 μM). However, introducing one more hydroxyl groups to the ring A at the C3 position of **2b** (35.2±0.2 μM) led to stronger AGIs **2f** (15.6±2.8 μM). In addition, the 2'-hydroxyl group is more effective than the 3'-hydroxyl group in ring B to improve the inhibitory activity towards chalcones (**2b** and **2c**).

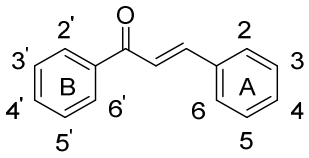


Figure 3 The structure of chalcone

The effect of the number of hydroxyl groups on the inhibitory activity of chalcones was much intriguing. Compound **2a** with 2', 4', 6'-trihydroxyl groups in ring B, showed much weaker activity than **2c** does, which has 2', 4'-dihydroxyl groups in ring B; while **2c** with 4-hydroxyl group in ring A, displayed almost the same activity to **2e** with 2,4-hydroxyl groups in ring A. On the other hand, bis-chalcones **2j** (5.5±1.2 μM) and **2l** (6.5±0.4 μM) showed a much strong inhibitory activity than **2m** (18.3±0.7 μM), which indicated that introducing more hydroxyl groups in bis-chalcones were very important in discovering stronger AGIs.

Surprisingly, compound **2g** and **2h** had lower inhibitory activities than compound **2k**, probably due to the formation of intramolecular hydrogen bonds in **2g** and **2h** (Figure 4), which devastates their binding to the enzyme as hydrogen bond donors. Similarly, exemplified by the fact that, methoxy groups are hydrogen bond acceptors, chalcones and bis-chalcones with methoxy groups all provided lower inhibitory activities than hydroxyl groups containing counterparts.

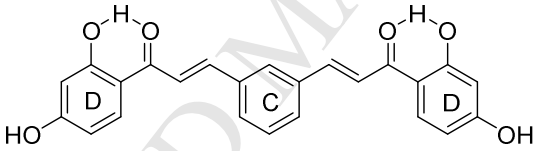
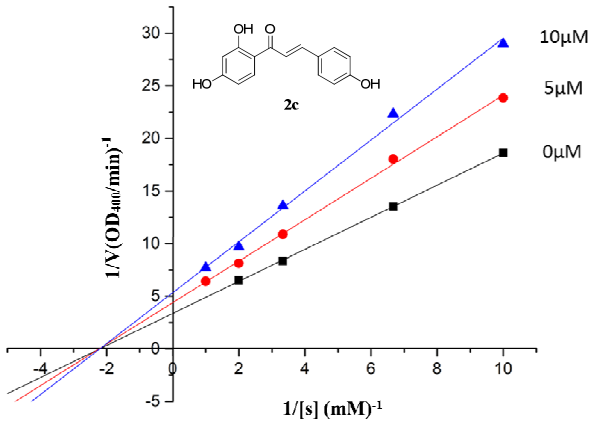


Figure 4 The structure of **2g**

2.3 The inhibition kinetics of a-glucosidase



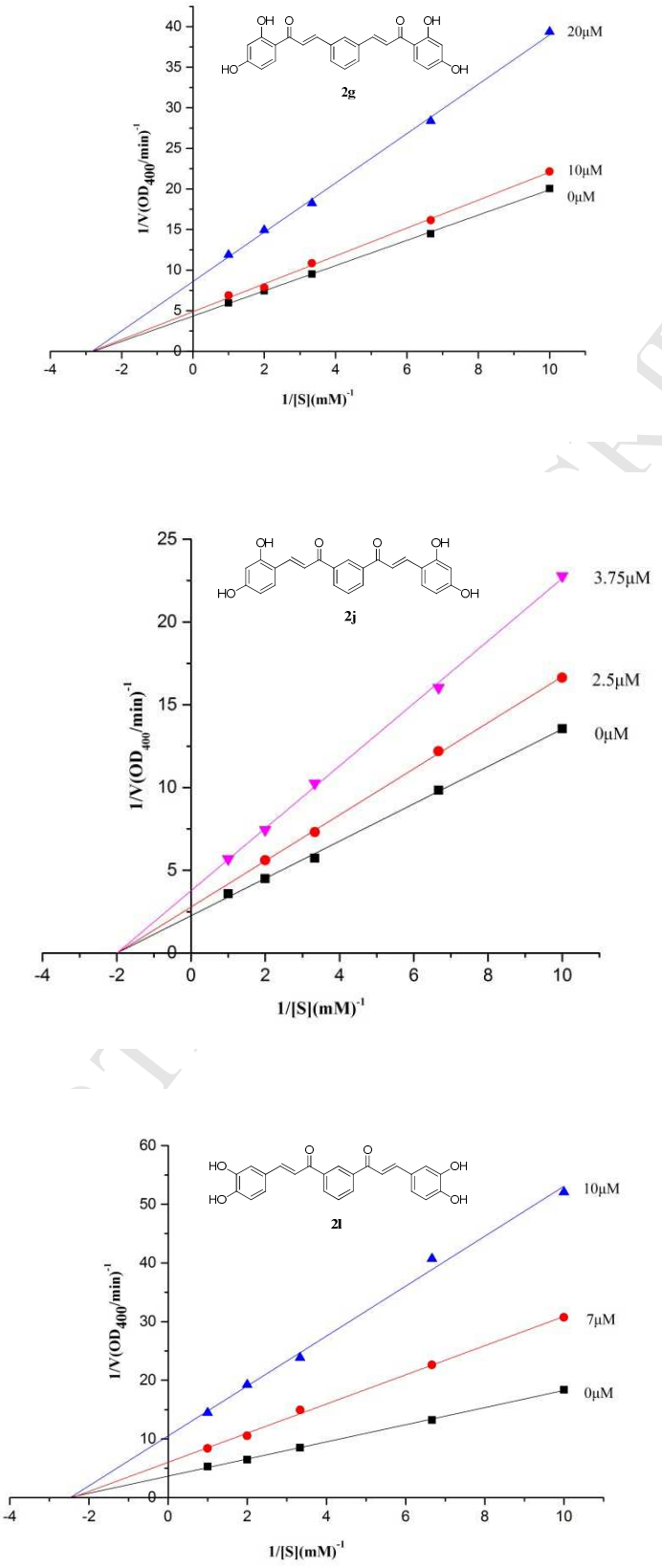


Figure 5 The inhibition types of compounds **2c**, **2g**, **2j** and **2l**

To further explore interaction mechanism of chalcones and bis-chalcones with a-glucosidase, the inhibition types of typical compounds **2c**, **2g**, **2j** and **2l** were

determined from Lineweavere Burk plots according to the methods reported in literatures [19-20]. As shown in Figure 5, the straight lines with the same Michaelis-Menten constant Km suggested that these compounds were non-competitive a-glucosidase inhibitors by binding to the non-competitive sites of the enzyme.

2.4 The analysis of glucose level and glucose up-take in cell

To further investigate the α-glucosidase inhibitory activities of chalcones and bis-chalcones, the glucose level in HepG-2 cells were evaluated.

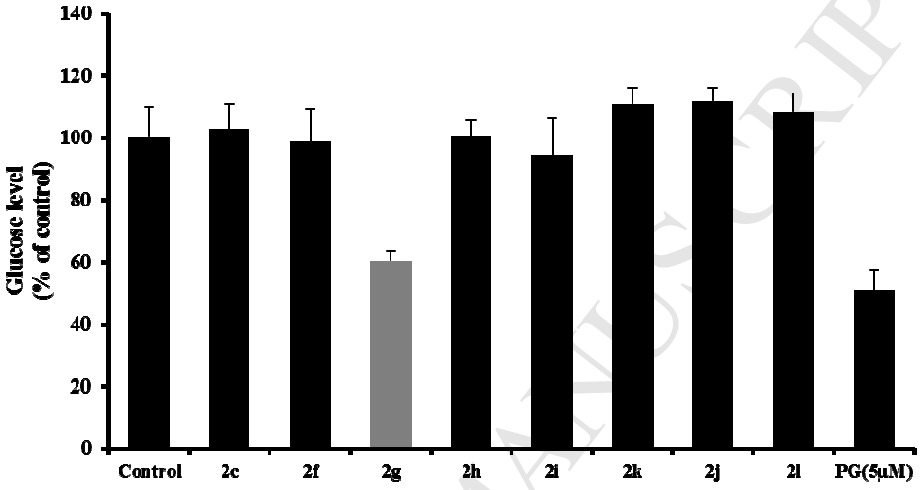


Figure 6 The effect of the compounds (1μM) on the glucose level in HepG-2 cells

As shown in Figure 6, 1-deoxynojirimycin (**PG**) served as a positive control. PG exerted subtle effect on reducing glucose level in HepG-2 cells at 5 μM, with the evidence of an approximately 50% decrease in glucose level in HepG-2 after the PG administration. Compared with PG at 5μM, compound **2g** exerted a similar effect with the PG treatment at 1 μM for 24 h, suggesting that compound **2g** had a better effect than 1-deoxynojirimycin on reducing the glucose level in cell.

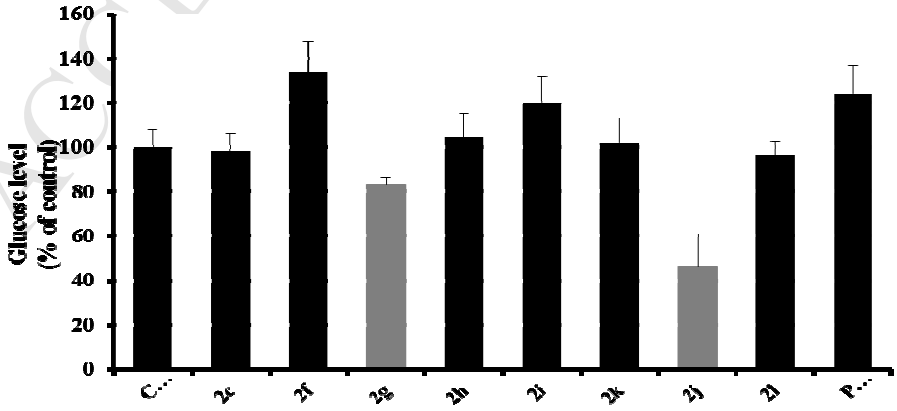


Figure 7 The effect of the compounds (1 μM) on glucose uptake activity in HepG-2 cells

Furthermore, the effect of the compounds on glucose uptake activity was

evaluated. As shown in Figure 7, PG showed no major effect on the glucose uptake activity in HepG-2 cells. Similar with positive control **PG**, most compounds except **2j** showed no major effect on glucose uptake activity, demonstrating that compound **2g** reducing glucose level in HepG-2 cells via inhibiting a-glucosidase activity.

Interestingly, compound **2k** exhibited the best inhibitory activities (1.0±0.1μM) in the enzyme assays *in vitro*, compound **2g** (at 1 μM concentration) has stronger effect on reducing the glucose level in HepG-2 cells. To elucidate this bias, the Log P value of compound **2g** and **2k** was tested. The results were shown in Table 2. Compound **2g** showed a higher log P value than **2k**, which means compound **2g** could be easier to cross cell membranes. This may reveal the reason of ambivalent resulted in the enzyme assay and glucose levels in cell.

Table 2 The log P values for selected compounds

compounds	Log P
2g	1.60
2k	1.10

3. Conclusion

As the analogues of xanthones, twenty-six chalcones and bis-chalcones were designed, synthesized, and evaluated as AGIs. Most of the hydroxyl chalcones and bis-chalcones showed better inhibitory activities than 1-deoxynojirimycin, and bis-chalcone **2k** showed the highest inhibitory activity, the IC_{50} value was only 1.0μM. The structure and activity relationship revealed that, the number and the position of the hydroxyl group(s) are the key factors to determining the a-glucosidase inhibitory activity of chalcones probably because that the hydroxyl groups of the compounds bound to a-glucosidase as hydrogen-bond donors. Moreover, kinetic analysis revealed that compounds **2c**, **2g**, **2j** and **2l** are noncompetitive inhibitors. Inspiringly, compound **2g** displayed stronger activity than 1-deoxynojirimycin in reducing the glucose level in HepG-2 cells, which means that compound **2g** could be a potential drug candidates for treating diabetes.

4. Experimental and methods

4.1 General

Melting points were determined using a SGW X-4 digital melting point apparatus, and the temperatures are not corrected. IR spectra were determined on an EQUINOX 55 Fourier transformation infrared spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 500, Bruker AVANCE AV400 or Mercury-Plus 300 NMR spectrometer in $CDCl_3$, DMSO-d_6 or Acetone-d_6 and TMS as an internal standard. High resolution (HR) mass spectra were measured on Thermo MAT95XP mass spectrometer. UV spectra were recorded on a Shimadzu UV-2450 scanning. 1-Deoxynojirimycin, *p*-nitrophenyl-a-D-glucopyranoside (PNP) and a-glucosidase (from Saccharomyces cerevisiae) were purchased from Sigma (St. Louis, MO, USA). All the other reagents

were used as purchased without further purification.

4.1.1 General methods for the synthesis of 1a-1d

Acetyl benzenes (1.0 mmol) were reacted with substituted benzaldehydes (1.2 mmol) at 80℃ for 36 hours in the presence of $SOCl_2$ (1.42 mmol) using ethanol as a solvent. Then the solution was poured into water, the precipitate after filtered and dried was purified by flash chromatography.

4.1.1.1. (*E*)*-4-hydroxy-2',4',6'-trimethoxychalcone (**1a**)*
Yellow solid, 30% yield, m.p. 208-211℃. ^{1}H NMR (300 MHz, Acetone-d_6) δ =8.85 (s, 1H, ArOH), 7.50 (d, *J* = 8.7Hz, 2H), 7.20 (d, *J* = 16.0Hz, 1H), 6.87 (d, *J* = 8.7Hz, 2H), 6.75 (d, *J* = 16.0Hz, 1H), 6.30 (s, 2H), 3.87 (s, 3H), 3.75 (s, 6H); LRMS (EI) m/z 314 M^+

*4.1.1.2. (E)-4-hydroxy-3',4'-dimethoxychalcone (**1b**)*
Yellow solid, 57% yield, m.p. 165-168℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.84 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.71 (m, 3H), 7.62 (d, *J* = 15.6, 1H), 7.59 (d, *J* = 2.1 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 3.85 (d, *J* = 3.3 Hz, 6H); LRMS (EI) m/z 284 M^+

*4.1.1.3. (E)-4-hydroxy-2',4'-dimethoxychalcone (**1c**)*
Yellow solid, 50% yield, mp 153~155℃. ^{1}H NMR (300 MHz, Acetone-d_6) δ 8.88 (s, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.57 (m, 3H), 7.45 (d, *J* = 15.0 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 2.1 Hz, 1H), 6.62 (dd, *J* = 8.1, 2.7 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H) ; LRMS (EI) m/z 284 M^+

*4.1.1.4. (E)-3-hydroxy-2',4'-dimethoxychalcone (**1d**)*
Orange solid, 40% yield, m.p. 159-160℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.8 Hz, 1H), 7.67 (d, *J* = 15.6 Hz, 1H), 7.52 (d, *J* = 15.6 Hz, 1H), 7.31, 7.27, 7.20 (d, *J* = 7.6 Hz, 1H), 7.14 (s, 1H), 6.90 (dd, *J* = 8.0, 2.4 Hz, 1H), 6.59 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 3.93 (s, 3H), 3.90 (s, 3H); LRMS (EI) m/z 284 M^+

4.1.2 General methods for the synthesis of 1e-1f

To a stirred solution of benzaldehydes (0.2 mmol) and acetyl benzenes (0.25 mmol) in methanol was added aqueous KOH (50%). The mixture was stirred at 70℃ for 3 hours, then the solution was poured into water and methanol was removed under reduced pressure. The solution was extracted with two portions of CH_2Cl_2 and the organic layer was separated and evaporated under reduced pressure. The residue after evaporation was purified by flash chromatography.

*4.1.2.1. (E)-2,4,2',4'-tetramethoxychalcone (**1e**)*
Pale yellow solid, 78% yield, mp 134~136℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (d, *J* = 15.9 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 15.9 Hz, 1H), 6.65 (d, *J* = 2.1 Hz, 1H), 6.63 – 6.54 (m, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.6, 153.1, 151.3, 149.3, 144.1, 131.6, 128.1, 122.9, 119.7, 111.2, 110.9, 110.3, 110.0, 56.1, 56.0; LRMS (EI) m/z 328 M^+

*4.1.2.2. (E)-3,4,3',4'-tetramethoxychalcone (**1f**)*

Yellow solid, 71% yield, mp 114~116℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (dd, J = 8.4, 2.0 Hz, 1H), 7.84 (d, J = 15.2 Hz, 1H), 7.68 (d, J = 15.6 Hz, 1H), 7.61 (d, J = 2.0 Hz, 1H), , 7.54 (d, J = 2.0 Hz, 1H) 7.41 (dd, J = 8.4, 2.0 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 3.89-3.87 (m, 9H), 3.83(s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.6, 153.1, 151.3, 149.3, 144.1, 131.6, 128.1, 122.9, 119.7, 111.2, 110.9, 110.3, 110.0, 56.1, 56.0; LRMS (EI) m/z 328 M^+

4.1.3 General methods for the synthesis of 1g-1i

Phthalaldehyde (0.1 mmol) reacted with acetyl benzenes (0.21 mmol) in methanol at 70℃ for 3 hours after adding aqueous KOH (50%). The post treatment is the same with the synthesis of **1e-1f**.

*4.1.3.1. (2E,2'E)-3,3'-(1,3-phenylene)bis(1-(2,4-dimethoxyphenyl)prop-1-en-2-one) (**1g**)*

Yellow solid, 56% yield, mp 169~170℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.8 Hz, 3H), 7.72 (d, J =15.6 Hz, 2H), 7.63 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 15.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 1H), 6.60 (dd, J = 8.4, 2.0 Hz, 2H), 6.53 (d, J = 2.0 Hz, 2H), 3.95 (s, 6H), 3.90 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.2, 164.4, 160.5, 141.1, 136.2, 133.0, 129.3, 129.3, 128.3, 127.9, 122.1, 105.3, 98.6, 55.8, 55.6; LRMS (EI) m/z 458 M^+

*4.1.3.2. (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2,4-dimethoxyphenyl)prop-1-en-2-one) (**1h**)*

Yellow solid, 60% yield, mp 215~217℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 15.6 Hz, 2H), 7.64 (s, 4H), 7.59 (d, J = 15.6 Hz, 2H), 6.60 (dd, J = 8.8, 2.4 Hz, 2H), 6.53 (d, J = 2.4 Hz, 2H), 3.95 (s, 6H), 3.91 (s, 6H) ; LRMS (EI) m/z 458 M^+

*4.1.3.3. (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(3,4-dimethoxyphenyl)prop-2-en-1-one) (**1i**)*

Yellow solid, 33% yield, mp 231~232℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 15.6 Hz, 2H), 7.73 – 7.71 (m, 6H), 7.66-7.61 (m, 4H), 6.96 (d, J = 8.4 Hz, 2H), 4.00 (d, J = 1.6 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.3, 153.5, 149.4, 142.7, 136.9, 131.2, 128.9, 123.1, 122.6, 110.8, 110.0, 56.1, 56.1; LRMS (EI) m/z 458 M^+

4.1.4 General methods for the synthesis of 1j-1m

Diacetylbenzene (0.1 mmol) reacted with substituted benzaldehydes (0.21 mmol) in methanol at 70℃ for 3 hours after adding aqueous KOH (50%). The post treatment is the same with the synthesis of **1e-1f**.

*4.1.4.1. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(2,4-dimethoxyphenyl)prop-2-en-1-one) (**1j**)*

Yellow solid, 43% yield, mp 160~162℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.21 (dd, J = 7.6, 1.6 Hz, 2H), 8.12 (d, J = 15.6 Hz, 2H), 7.63 (m, 5H), 6.56 (dd, J = 8.8, 2.4 Hz, 2H), 6.50 (d, J = 2.4 Hz, 2H), 3.92 (s, 6H), 3.88 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.5, 163.3, 160.5, 141.2, 139.1, 132.0, 131.1, 128.8, 128.3, 120.1, 117.0, 105.5, 98.5, 55.6, 55.5; LRMS (EI) m/z 458 M^+

*4.1.4.2. (2E,2'E)-1,1'-(1,4-phenylene)bis(3-(2,4-dimethoxyphenyl)prop-2-en-1-one) (**1k**)*
Yellow solid, 37% yield, mp 220~221℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 4H), 8.05 (d, J = 15.6 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 15.6 Hz, 2H), 6.68-6.65 (m, 4H), 3.93 (s, 6H), 3.87 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 163.3, 160.6, 141.7, 141.4, 131.1, 128.5, 120.4, 117.0, 108.4, 105.5, 98.5, 55.6, 55.5; LRMS (EI) m/z 458 M^+

*4.1.4.3. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(3,4-dimethoxyphenyl)prop-2-en-1-one) (**1l**)*
Yellow solid, 33% yield, mp 120~121℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.39 (dd, J = 7.5, 1.5 Hz, 2H), 7.89 (d, J = 15.6 Hz, 2H), 7.78 – 7.72 (m, 3H), 7.56 (d, J = 1.8 Hz, 2H), 7.43 (dd, J = 8.1, 1.8 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 3.86 (s, 6H), 3.82 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.9, 151.7, 149.3, 145.9, 138.9, 132.2, 129.0, 128.2, 127.7, 123.5, 119.6, 111.2, 110.2, 56.1; LRMS (EI) m/z 458 M^+

*4.1.4.4. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(4-methoxyphenyl)prop-2-en-1-one) (**1m**)*
Yellow solid, 59% yield, mp 192~194℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (t, J = 1.6 Hz, 1H), 8.24 (dd, J = 7.6, 1.6 Hz, 2H), 7.87 (d, J = 15.6 Hz, 2H), 7.69-7.66 (m, 5H), 7.50 (d, J = 15.6 Hz, 2H), 7.00 – 6.97 (m, 4H), 3.90 (s, 6H); LRMS (EI) m/z 398 M^+

4.1.5 General methods for the synthesis of 2a-2m

The solution of methoxychalcone (0.4 mmol) in dried dichloromethane was added to the stirred boron tribromide BBr_3 (1 mol/L, 5 mL) at 0℃. The mixture was stirred for 24 or 48 hours at room temperature, after that the solution was poured into ice water. The precipitate after filtered and dried was purified by flash chromatography.

*4.1.5.1. (E)-4-hydroxy-2',4',6'-trihydroxychalcone (**2a**)*
Yellow solid, 11% yield, m.p. 235-236℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.50 (s, 2H, ArOH), 10.38 (s, 1H, ArOH), 10.04 (s, 1H, ArOH), 7.94 (d, J = 15.3 Hz, 1H), 7.62 (d, J = 15.6 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.81 (s, 2H); LRMS (ESI) m/z 271 $[M-H]^-$

*4.1.5.2. (E)-4-hydroxy-3',4'-dihydroxychalcone (**2b**)*
Brown solid, 86% yield, m.p. 199-201℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 9.98 (s, 1H, ArOH), 9.84 (s, 1H, ArOH), 9.29 (s, 1H, ArOH), 7.66 (d, J = 8.7 Hz, 2H), 7.53 ~ 7.68 (m, 3H), 7.46 (d, J = 2.1 Hz, 1H), 6.76 ~ 6.85 (m, 3H); LRMS (ESI) m/z 255 $[M-H]^-$

*4.1.5.3. (E)-4-hydroxy-2',4'-dihydroxychalcone (**2c**)*
Yellow solid, 48% yield, m.p. 198~200℃. ^{1}H NMR (500 MHz, Acetone-d_6) δ 13.65 (s, 1H), 9.49 (s, 1H), 9.03 (s, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 15.0 Hz, 1H), 7.81 – 7.75 (m, 3H), 6.95 (d, J = 8.5 Hz, 2H), 6.49 (dd, J = 9.0, 2.5 Hz, 1H), 6.39 (d, J = 2.5 Hz, 1H); LRMS (ESI) m/z 255 $[M-H]^-$

*4.1.5.4. (E)-3-hydroxy-2',4'-dihydroxychalcone (**2d**)*
Yellow solid, 21% yield, m.p. 222-223℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 13.34 (s, 1H, ArOH), 10.71 (s, 1H, ArOH), 9.60 (s, 1H, ArOH), 8.16 (d, J = 9.0 Hz, 1H), 7.85(d, J = 15.3Hz, 1H), 7.67 (d, J = 15.6 Hz, 1H), 7.18 ~ 7.33 (m, 3H), 6.85 (d, J = 7.5 Hz, 1H), 6.39 (dd, J = 9.0, 2.4 Hz, 1H), 6.27 (d, J = 2.4 Hz, 1H); LRMS (ESI) m/z 255 [M-H]$^-$

*4.1.5.5. (E)-2,4,2',4'-tetrahydroxychalcone (**2e**)*
Orange solid, 30% yield, Td 151~152℃. ^{1}H NMR (400 MHz, Acetone-d_6) δ 13.80(s,1H), 9.40(s,1H), 9.22(s,1H), 8.91(s,1H), 8.24(dd, J=15.6 Hz,1.6 Hz, 1H), 8.05(d, J=9.2Hz, 1H), 7.82(d, J=15.2Hz 1H), 7.73(d, J=8.4Hz 1H), 6.53 (d, J=1.6Hz, 1H), 6.49(d, J=2.0Hz, 1H), 6.47(d, J=2.4Hz, 1H), 6.38(s, 1H); LRMS (ESI) m/z 271 [M-H]$^-$

*4.1.5.6. (E)-3,4,3',4'-tetrahydroxychalcone (**2f**)*
Yellow solid, 26 % yield, Td 199~200℃. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.66 (s, 1H), 8.49 (s, 1H), 8.31 (s, 1H), 8.12 (s, 1H), 7.66 – 7.55 (m, 4H), 7.32 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 8.0, 2.0 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H); LRMS (ESI) m/z 271 [M-H]$^-$

*4.1.5.7. (2E,2'E)-3,3'-(1,3-phenylene)bis(3-(2,4-dihydroxyphenyl)prop-1-en-2-one) (**2g**)*
Yellow solid, 25% yield, mp 213~215℃. IR (KBr) 3163, 2922, 2851, 1637, 1594, 1577, 1520, 1435, 1361, 1310, 1288, 1227, 1173, 1129, 1029, 996, 975, 838, 781, 750, 663, 631, 583, 560, 526, 501, 484, 452 cm^{-1}. ^{1}H NMR (400 MHz, Acetone-d_6) δ 13.48 (s, 2H), 9.59 (s, 2H), 8.43 (s, 1H), 8.21 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 15.5 Hz, 2H), 8.02 – 7.90 (m, 4H), 7.61 (t, J = 7.8 Hz, 1H), 6.53 (dd, J = 8.9, 2.4 Hz, 2H), 6.42 (d, J = 2.4 Hz, 2H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 191.8, 166.9, 165.4, 143.1, 135.9, 132.8, 130.7, 129.6, 128.8, 121.8, 113.5, 108.2, 102.9; HRMS (ESI) m/z 401.10296 (calcd for $C_{24}H_{17}O_6$, 401.10306)

*4.1.5.8. (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(2,4-dihydroxyphenyl)prop-2-en-1-one) (**2h**)*
Yellow solid, 15% yield, mp 252~254℃. ^{1}H NMR (400 MHz, Acetone-d_6) δ 13.48 (s, 2H), 9.66 (s, 2H), 8.21 (d, J = 9.2 Hz, 2H), 8.08 (d, J = 15.6 Hz, 2H), 8.00 (s, 4H), 7.93 (d, J = 15.2 Hz, 2H), 6.52 (dd, J = 8.8, 2.4 Hz, 2H), 6.42 (d, J = 2.0 Hz, 2H); LRMS (ESI) m/z 401 [M-H]$^-$

*4.1.5.9. (2E,2'E)-3,3'-(1,4-phenylene)bis(1-(3,4-dihydroxyphenyl)prop-2-en-1-one) (**2i**)*
Brown solid, 33% yield, IR (KBr) 3477, 3244, 3086, 2749, 1653, 1608, 1571, 1528, 1512, 1437, 1377, 1327, 1283, 1246, 1184, 1113, 1050, 977, 936, 898, 865, 830, 799, 785, 740, 646, 622, 573, 548, 508 cm^{-1}.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 2H), 9.40 (s, 2H), 7.97 – 7.93 (m, 6H), 7.70 – 7.67 (m, 4H), 7.54 (d, J = 2.0 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ187.6, 151.5, 146.0, 142.0, 137.1, 130.0, 129.6, 123.5, 122.8, 115.9, 115.6; HRMS (ESI) m/z 401.10303 (calcd for $C_{24}H_{17}O_6$, 401.10306)

*4.1.5.10. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(2,4-dihydroxyphenyl)prop-2-en-1-one) (**2j**)*
Reddish brown solid, 31% yield, mp 138~140℃. IR (KBr) 3185, 2973, 2926, 2601, 1643,

1597, 1550, 1514, 1449, 1383, 1316, 1253, 1176, 1099, 1064, 1038, 979, 847, 796, 771, 737, 682, 693, 602, 570, 534, 499, 450 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.26 (s, 2H), 10.01 (s, 2H), 8.56 (s, 1H), 8.32 – 8.27 (m, 2H), 8.03 (d, J = 15.5 Hz, 2H), 7.79 – 7.65 (m, 5H), 6.40 (d, J = 2.0 Hz, 2H), 6.34 (dd, J = 8.5, 2.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 188.9, 161.7, 159.3, 140.9, 138.8, 131.8, 130.7, 129.2, 127.3, 117.0, 113.3, 108.1, 102.5; HRMS (ESI) m/z 401.10300 (calcd for $C_{24}H_{17}O_6$, 401.10306)

*4.1.5.11. (2E,2'E)-1,1'-(1,4-phenylene)bis(3-(2,4-dihydroxyphenyl)prop-2-en-1-one) (**2k**)* Black solid, 17% yield, IR (KBr) 3150, 2608, 1643, 1576, 1539, 1501, 1444, 1390, 1341, 1318, 1258, 1214, 1134, 1035, 1010, 981, 846, 801, 771, 701, 500, 444 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 2H), 10.05 (s, 2H), 8.18 (s, 4H), 8.03 (d, J = 15.6 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 7.69(d, J = 15.2, 2H), 6.41 (d, J = 2.0 Hz, 2H), 6.34 (dd, J = 8.4, 2.4 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 189.76, 162.20, 159.84, 141.65, 141.58, 131.11, 128.88, 117.62, 113.76, 108.67, 102.94. HRMS (ESI) m/z 401.10301 (calcd for $C_{24}H_{17}O_6$, 401.10306)

*4.1.5.12. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(3,4-dihydroxyphenyl)prop-2-en-1-one) (**2l**)* Dark yellow solid, 61% yield, mp 236~237℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.74 (s, 2H), 9.13 (s, 2H), 8.61 (s, 1H), 8.32 (dd, J = 7.8, 1.5 Hz, 2H), 7.71 (t, J = 7.8 Hz, 1H), 7.65 (s, 4H), 7.28 (d, J = 1.8 Hz, 2H), 7.19 (dd, J =8.4, 2.1 Hz, 2H), 6.80 (d, J = 8.1 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 189.2, 149.5, 146.1, 139.0, 132.8, 129.8, 128.1, 126.7, 123.1, 118.8, 116.3, 116.1; LRMS (ESI) m/z 401 $[M-H]^-$

*4.1.5.13. (2E,2'E)-1,1'-(1,3-phenylene)bis(3-(4-hydroxyphenyl)prop-2-en-1-one) (**2m**)* Yellow solid, 57% yield, mp 271~273℃. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.00 (s, 2H), 8.76 (t, J = 1.6 Hz, 1H), 8.37 (dd, J = 7.6, 1.6 Hz, 2H), 7.86 – 7.74 (m, 9H), 6.96 (d, 4H); LRMS (ESI) m/z 369 $[M-H]^-$

4.2. Biological study

4.2.1 Enzyme assays

The inhibitory activities of all the chalcones and bis-chalcones were measured by using the methods similar to those articles previously. Typically, a-glucosidase activity was assayed in 50 mM phosphate buffer (pH 6.8) containing 5% v/v dimethylsulfoxide. The inhibitors were pre-incubated with the enzyme in phosphate buffer at 37℃ for 30 minutes. PNP glycoside, the substrate, was then added and the enzymatic reaction was carried out at 37℃ for 60 seconds. The reaction was monitored spectrophotometrically by measuring the absorbance at 400 nm. The assay was performed in triplicate with five different concentrations around the IC_{50} values, and the mean values were calculated [19-21].

4.2.2 Kinetics of enzyme inhibition

The inhibition types of compounds **2c**, **2h**, **2j** and **2l** were determined from Lineweavere Burk plots, using the methods similar to those reported in literatures [18-19]. Typically, two different concentrations of each compound lower than the IC_{50} values were

chosen. Under each concentration, a-glucosidase activity was assayed by varying the concentration of PNP glycoside. The enzyme reaction was performed under the above-mentioned reaction condition. The mixtures of the enzyme and the inhibitor were dissolved in 50mM phosphate buffer (pH 6.8) containing 5% v/v DMSO, and pre-incubated at 37℃ for 30 min, and then the substrate was added. The enzymatic reaction was carried out at 37℃ for 60 seconds, and monitored spectrophotometrically by measuring the absorbance at 400 nm. Inhibition types and Ki values of the inhibitors were determined by Double-reciprocal plots.

4.2.3 Cell cultures

HepG-2 cells (ATCC, USA) were purchased from the American Type Culture Collection. Cells were maintained in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin and streptomycin (BI, USA) in a humidified atmosphere containing with 5% CO_2 in air at 37℃.

4.2.4 Glucose level Analysis

Cells were seeded and cultured in serum-free medium for indicated time, and then treated with compounds at 1 μM. After stimulation with compounds for 24 h. Cells were collected and lysed in protein extraction buffer. Glucose content and protein level were determined by Glucose analysis Kit (JianCheng, China) and BCA determine Kit (Thermo, USA). The glucose level in cells was expressed as “mmol glucose / g protein”.

4.2.5 Glucose Up-take analysis

Glucose-uptake analysis was measured as follow. Cells were seeded and cultured in serum-free medium for 2 h, and treated with compounds at 1 μM. After stimulation with compounds for 24 h. Culture medium and cells were collected and centrifuged at 1500 rpm for 5 min, respectively. Supernatant was used to determine the glucose level. Protein level was also determined by BCA determine Kit (Thermo, USA). The glucose level in culture medium was expressed as “mmol glucose /g protein”.

4.2.6 The determination of the lipid-water distribution coefficient.

The distribution coefficients were determined by using the shake–flask method in 1-octanol/water system [22]. After shaking the tested compounds in 1-octanol/water (1:1) solution for 24h, the distribution of the compounds in 1-octanol phase and water phase was determined by HPLC analysis. The lipid–water distribution coefficient was calculated according the following equation:

$$\log P = \log \frac{C_o}{C_w}$$

where C_o is the concentration of the test compound in water and C_w is its concentration in 1-octanol. Log P is a mean value from at least three independent tests.

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Table

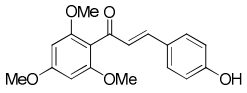
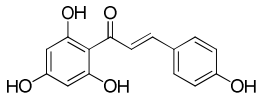
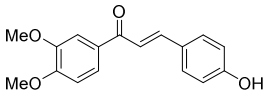
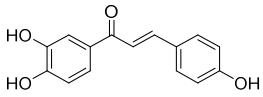
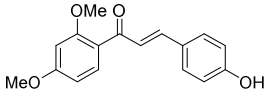
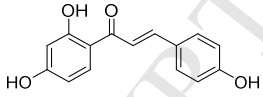
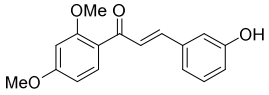
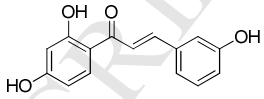
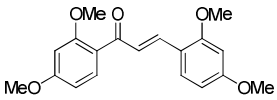
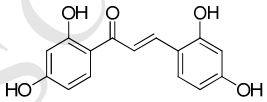
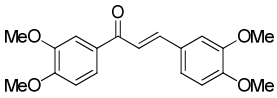
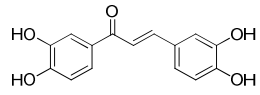
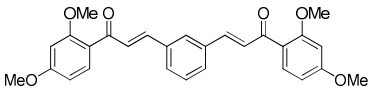
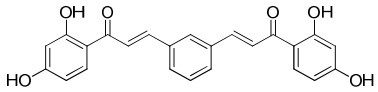
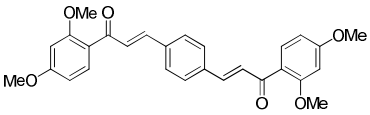
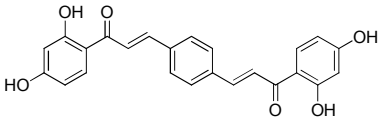
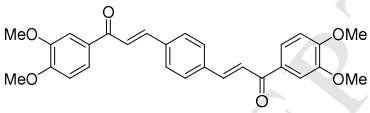
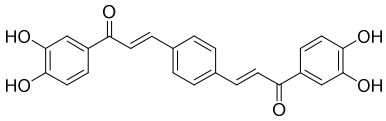
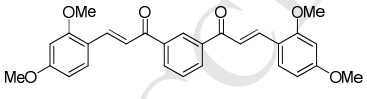
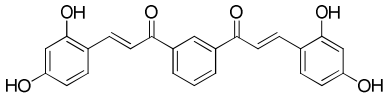
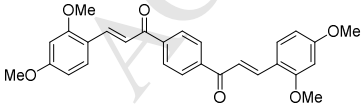
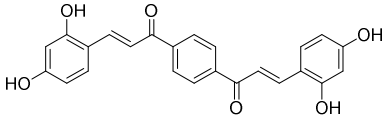
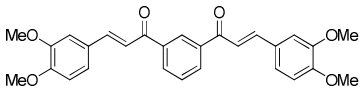
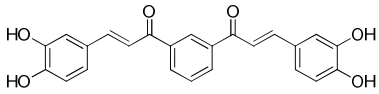
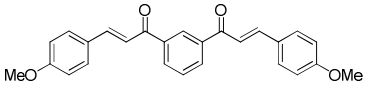
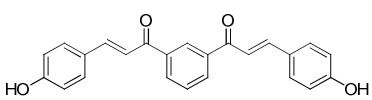
Figure Captions

Schemes

Tables

Table 1 The IC_{50} value or inhibition of the compounds

Compounds	Structure	Inhibition or	Compounds	Structure	$IC_{50}/\mu M$

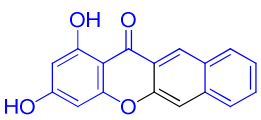
		$IC_{50}/\mu M$			
1a		>200	**2a**		71.1±2.6
1b		>200	**2b**		35.2±0.2
1c		58.7±3.0	**2c**		13.2±2.7
1d		>200	**2d**		42.0±6.0
1e		83.2±1.0	**2e**		12.5±2.1
1f		123.1±9.9	**2f**		15.6±2.8
1g		27.2% [a]	**2g**		23.7±0.3
1h		27% [a]	**2h**		22.5±3.2
1i		32.0% [a]	**2i**		10.1±1.7
1j		55.2% [a]	**2j**		5.5±1.2
1k		66.9±2.5	**2k**		1.0±0.1
1l		16.0% [a]	**2l**		6.5±0.2
1m		29% [a]	**2m**		18.3±0.7

[a] : the inhibition of the compounds at the concentration of 40 μM (The solubility of these methoxyl bis-chalcones in PBS are low). The IC_{50} value of positive control (1-deoxynojirimycin, PG) is 21.3±8.7 μM.

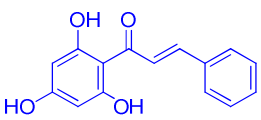
Table 2 The logP values for selected compounds

compounds	logP
2g	1.60
2k	1.10

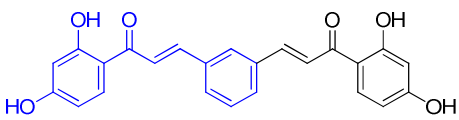
Figures



benzoxanthone



chalcone



bis-chalcone

Figure 1 The structures of benzoxanthone, chalcone and bis-chalcone

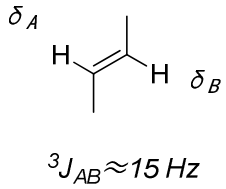


Figure 2 The stereochemistry of chalcones

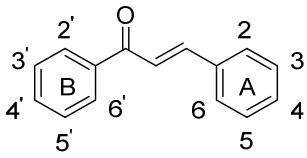


Figure 3 The structure of chalcone

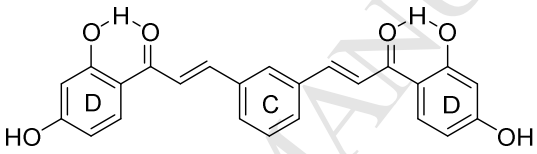
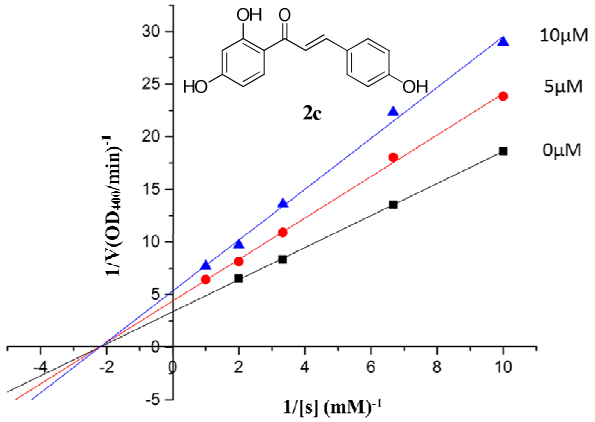


Figure 4 The structure of **2g**



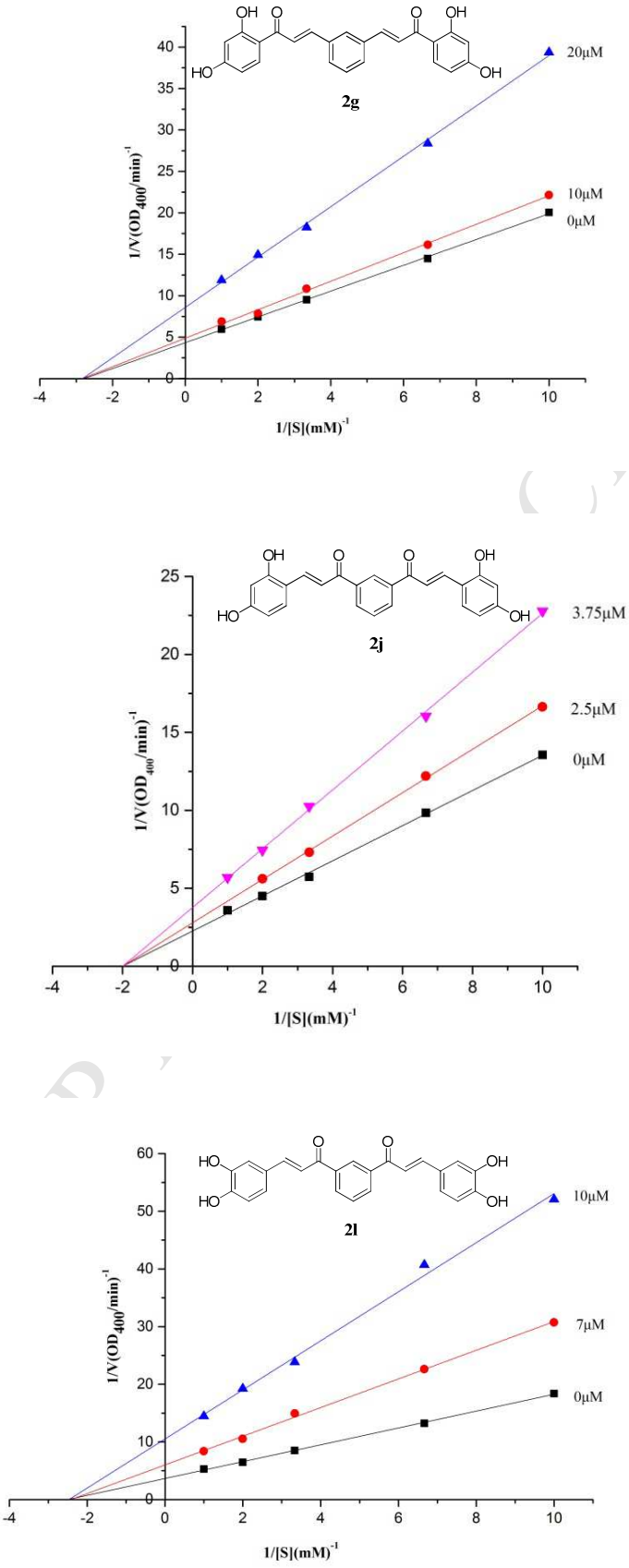


Figure 5 The inhibition types of compounds **2c**, **2g**, **2j** and **2l**

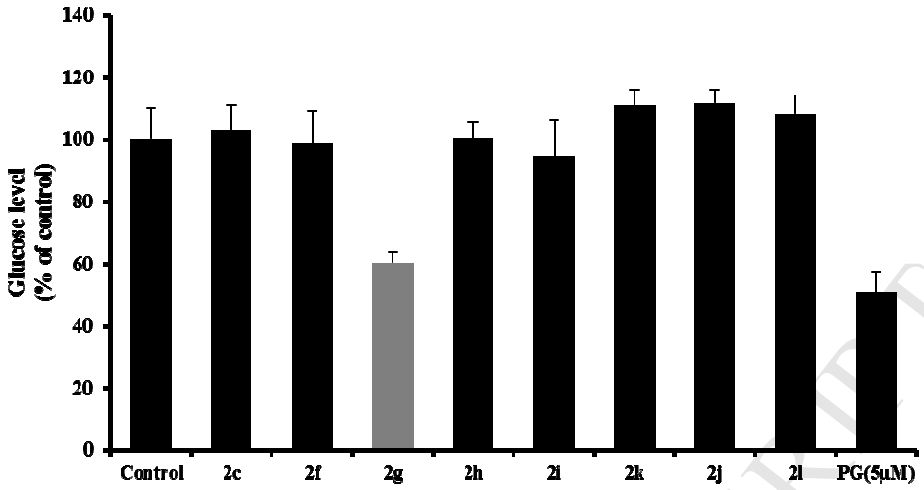


Figure 6 The effect of the compounds (1μM) on the glucose level in HepG-2 cells

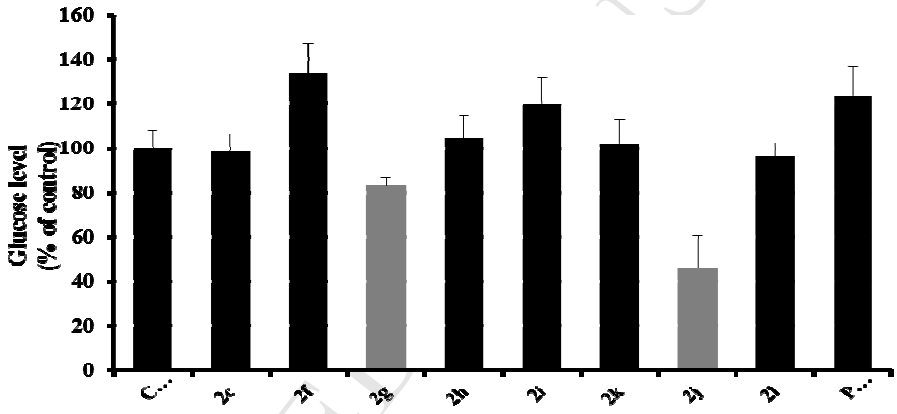
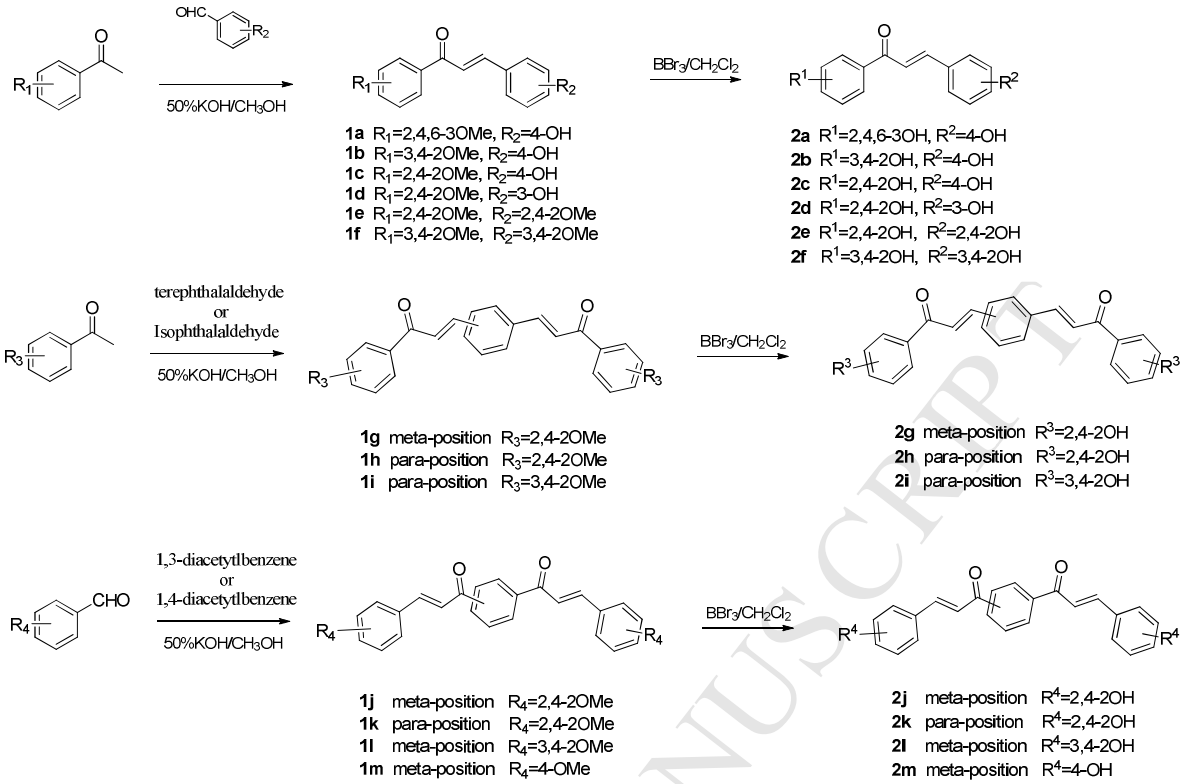
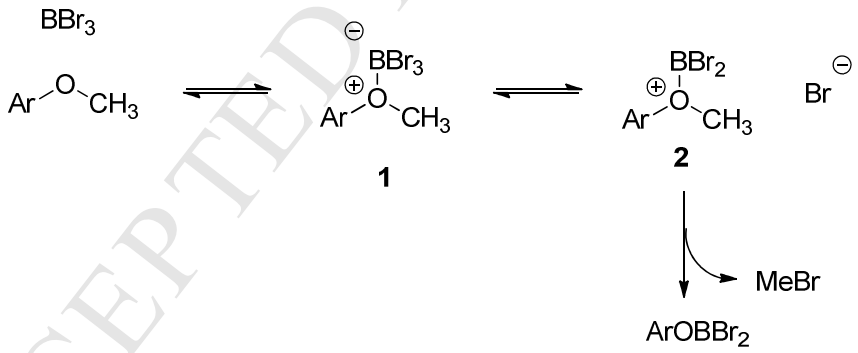


Figure 7 The effect of the compounds (1 μM) on glucose uptake activity in HepG-2 cells

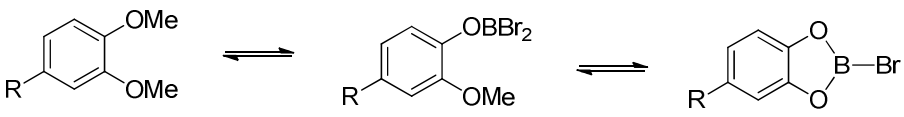


Scheme 1 Synthesis of the target compounds **1a-1m** and **2a-2m**



$$ArOBBr_2 + 3H_2O \longrightarrow ArOH + H_3BO_3 + 2HBr$$

Scheme 2 Conceptual reaction mechanism for demethylation



Scheme 3 Conceptual reaction mechanism for demethylation of dimethoxyl groups

Research Highlights

- Twenty-six chalcone and bis-chalcone derivatives were synthesized and four of them are first reported.
- Some compounds exhibited excellent inhibitory activities against α-glucosidase, the best IC_{50} value reaches to 1.0 μM.
- Valuable structure-activity relationship was obtained.
- The selected compounds (**2c**, **2g**, **2j**, **2l**) were showed as non-competitive inhibitors.
- The compound **2g** had a better effect than the positive control 1-deoxynojirimycin on reducing the glucose level in HepG-2 cells and could be a potential drug candidates for treating diabetes.