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Synthesis and anti-hyperglycemic activity of hesperidin derivatives

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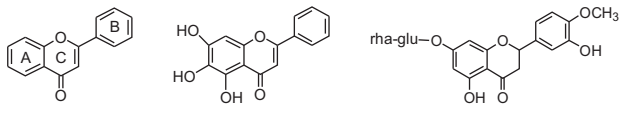
ABSTRACT

A series of hesperidin derivatives were prepared and identified by IR, ^{1}H NMR, and MS spectra. These compounds were evaluated in vitro and in vivo based on α-glucosidase inhibition, glucose consumption of HepG2 cells, and blood glucose level in streptozotocin-induced diabetic mice. The results revealed that all the compounds exhibited anti-hyperglycemic activities. The inhibition at 10^{-3} M of compounds **3** and **7a** on α-glucosidase were 55.02% and 53.34%, respectively, as compared to 54.80% by acarbose. Treated by compound **3** and the reference drug metformin, glucose consumption of HepG2 cell were 1.78 and 2.11 mM, respectively. After the streptozotocin-induced diabetic mice were oral administrated with compound **3** at 100 mg kg^{-1} d^{-1} for 10 days, the blood glucose level of **3** treated mice (13.23 mM, P <0.05) showed significant difference when compared to model control (23.03 mM). Thus, compound **3** exhibited promising anti-hyperglycemic activity.

Diabetes mellitus is a common chronic disease characterized by high level of blood glucose, insulin resistance and abnormal insulin secretion.[1] It is associated with the complications such as obesity, hypertension, and hyperlipidemia leading to cardiovascular risks.[2] According to the World Health Organization, the incidence of diabetes mellitus was 210 million people worldwide in 2010 and the number is expected to increase to 330 million by 2025.[3] Treatment of hyperglycemia in diabetes involves diet control, exercise, and the use of hypoglycemic drugs.[4] Caloric restriction and exercise were adopted by only a small part of patients. Current therapy is mostly dependent on the hypoglycemic agents. For example, acarbose,[5] an alpha-glucosidase inhibitor, can delay the digestion and absorption of dietary carbohydrates. Metformin acts by decreasing hepatic glucose output and increasing peripheral glucose utilization.[6] Sulfonylureas lower the blood level by blocking ATP-dependent potassium channels in pancreatic beta cells to stimulate insulin secretion.[7] Thiazolidinedione acts by activating peroxisome proliferator-activated receptors (PPARs) that combat insulin resistance and increase insulin sensitivity.[8] Although these oral therapeutic agents have already been used in clinical situations, it's difficult to inhibit their adverse effects, such as hepatotoxicity, weight gain, and edema.[9] Therefore, it's crucial to search for new compounds of different chemical classes with better treatment outcomes and less side effects.

Flavonoids, which are based on 2-phenylchromone or 2-phenyl benzopyrone (Fig. 1), have a C_6–C_3–C_6 carbon skeleton where two benzene rings (A- and B-ring) are linked through a heterocyclic pyran or pyrone ring (C-ring) in the middle. The B-ring is located at the 2-position and the C-ring contains a C2–C3 double bond. Flavonoids have shown beneficial effects in the treatment of hyperglycemic disease probably resulting from changes in the activity of intracellular enzymes, such as glucosidase enzyme.[10] The glucosidase enzymes are located in the brush border of the small intestine and are required for the breakdown of carbohydrates before monosaccharide absorption. Baicalein (5,6,7-trihydroxyflavone) and its 4′-hydroxyl substituted derivative (Fig. 1) exhibit promising inhibitory activity against the α-glucosidase enzyme and apparently delay the absorption of ingested carbohydrates, reducing the postprandial glycemia and insulin peaks. The inhibition on α-glucosedase by this kind of compounds maybe due to the 6-hydroxyl group at A-ring and 4′-hydroxyl substitution at B-ring.[11] Nomura et al[12] assessed the effects of different classes of flavonoids on insulin-stimulated 2-deoxy-D-[1-3*H*] glucose uptake by mouse MC3T3-G2/PA6 cells and found that the key structural features of flavonoids for inhibition of insulin-stimulated glucose uptake were



2-Phenylchromone Baicalein Hesperidin

Figure 1. Structures of some flavonoids.

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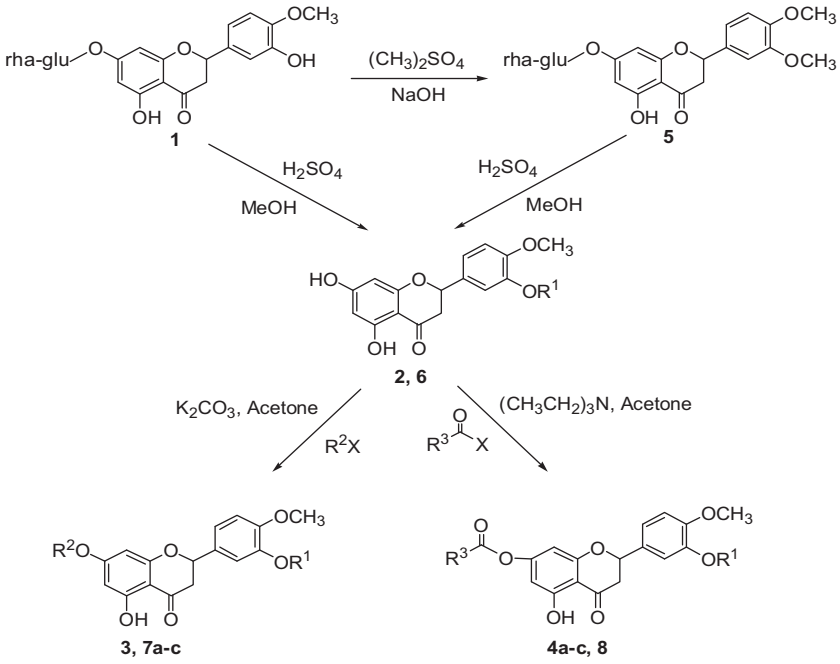
the B-ring 4′- or 3′, 4′-OH group, the C-ring C2–C3 double bond of the flavones, and the A-ring 5-OH of isoflavones. Rawat et al [13] evaluated the anti-hyperglycemic activities of various phenolic C-glycosides on glucose uptake by rat muscle cell lines (L-6) and low dosed-streptozotocin-induced diabetic rats, the structure–activity profile revealed that flavonoids with hydroxyl group at position 3 and 4′, and the absence of double bond in C-ring showed an appreciable increase in activities.

Hesperidin is a flavanone glycoside, consisting of an aglycone and an attached disaccharide (Fig. 1). It is present in a large number of fruits and vegetables. Until now, no signs of toxicity have been observed with normal intake. Hesperidin and its derivatives have been found to possess a wide range of pharmacological properties, including anti-hyperglycemic,[14] antihypercholesterolaemic,[15] anti-inflammatory[16] and antihypertensive[17] activities. Jeong et al[15] have synthesized some hesperidin derivatives with a long alkoxy chain at the 7 position and examined their hypocholesterolemic activities in high cholesterol-fed mice. The 7-*O*-lauryl and 7-*O*-oleyl substitution exhibited strong cholesterol-lowering effects. DuBois et al[18] have prepared sulfonated hesperetin dihydrochalcone (DHC) derivatives that had high water solubility throughout the useful pH range and provided DHC sweeteners with improved taste-timing characteristics.

Based on the above findings, many new hesperidin derivatives and their bioactivities were investigated. However, no previous studies have been reported on the synthesis and pharmacological evaluation of hesperetin ethers and esters with anti- hyperglycemic activities. The aim of the present study is to synthesize these compounds and examine their effects on α-glucosidase inhibition, glucose consumption of HepG2 cells, and blood glucose level in streptozotocin-induced diabetic mice.

The synthetic pathways adopted for the preparation of novel hesperidin derivatives are illustrated in Scheme 1. Hydrolysis of compound **1** and **5** was done in the presence of H_2SO_4/CH_3OH to give the corresponding **2** and **6**. The ether derivatives of the title compounds were prepared by further reacting with different substituted aryl and alkyl halides to obtain **3**, **7a–c**. The ester derivatives **4a–c** and **8** were afforded by treatment with acyl halides in refluxing acetone with triethylamine as base.

All the synthetic compounds were screened in vitro for inhibiting α-glucosidase activities by a modified method of Chapdelaine and Rao.[19] Acarbose was used as a positive control. The inhibition



X = Cl, Br

Entry	R^1	R^2	R^3
2	H	-	-
3	H	$C_6H_5CH_2$	-
4a	H	-	CH_3
4b	H	-	CH_3CH_2
4c	H	-	C_6H_5
6	CH_3	-	-
7a	CH_3	$C_6H_5CH_2$	-
7b	CH_3	$CH_3(CH_2)_3$	-
7c	CH_3	$BrCH_2(CH_2)_3$	-
8	CH_3	-	CH_3CH_2

Scheme 1. Synthesis of hesperidin derivatives.

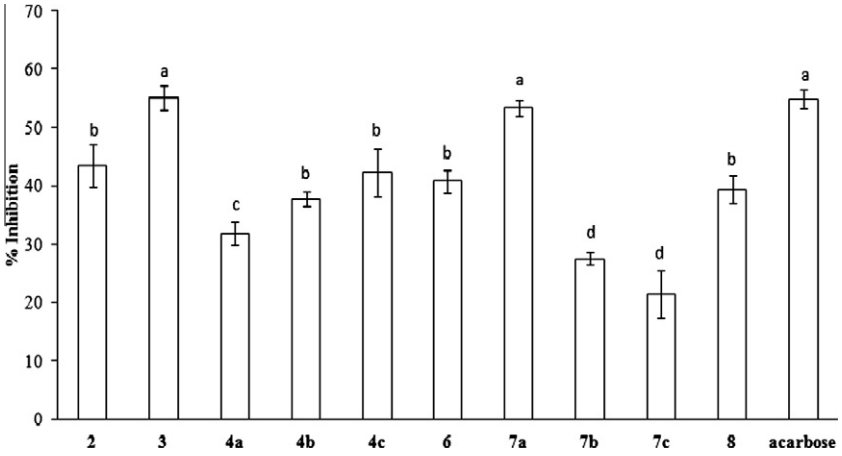


Figure 2. Inhibitory effects of compounds (at 10^{-3} M) on α-glucosidase activities.[a-d] Values are the means ± SD of α-glucosidase inhibitory activities ($n = 3$). Bars with different letters indicate statistically significant differences among groups at $P < 0.05$.

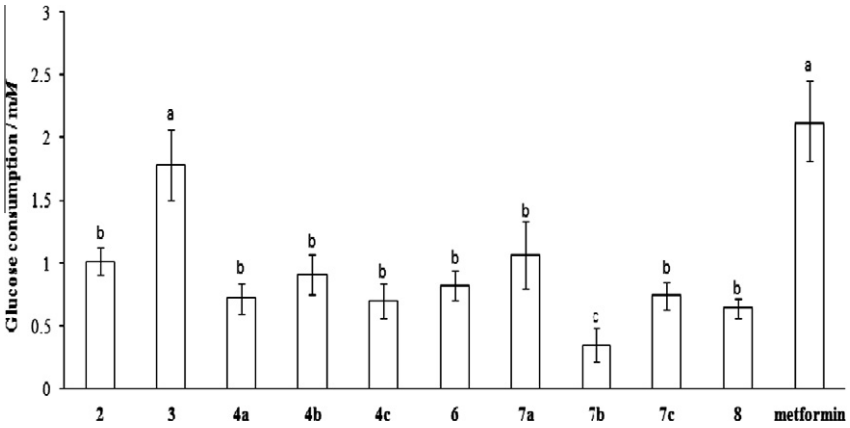


Figure 3. Influence of compounds (at 10^{-4} M) on glucose consumption of HepG2 cells.[a-c] Values are the means ± SD of glucose consumption (mM, $n = 3$). Bars with different letters indicate statistically significant differences among groups at $P < 0.05$.

Table 1
Effect of compound **3** and metformin on the blood glucose levels (mM) of the streptozotocin-induced diabetic mice at various doses and time intervals ($\bar{x} \pm s$, $n = 6$)

Group	Dose/mg·kg^{-1}	1 d	3 d	7 d	10 d
Control	0	6.12 ± 0.68	6.56 ± 0.31	6.40 ± 0.94	7.02 ± 0.57
Model	0	19.93 ± 6.36	23.97 ± 3.79	23.60 ± 4.25	23.03 ± 3.94
Metformin	100	16.98 ± 6.87	20.72 ± 8.23	14.47 ± 3.23*	10.00 ± 3.09**
3	50	17.50 ± 5.30	21.25 ± 4.78	19.35 ± 4.80	20.10 ± 9.59
	100	16.78 ± 6.24	20.12 ± 8.74	19.18 ± 7.38	13.23 ± 3.88*

* $P < 0.05$ vs model group.
** $P < 0.01$ vs model group.

against α-glucosidase was calculated following the formula of the inhibitory ratio. The results are shown in Figure 2.

Comparing the inhibitory ratio of compounds **2**–**6**, as well as compounds **4b**–**8**, it indicated that when the B ring 3′-OH was replaced by methoxy group, the inhibition on α-glucosidase became lower; when the hydroxyl group at C-7 position in A ring was substituted by short alkyl ether or ester, the inhibitory activities were also lower. These results are in good agreement with the previous report by Nomura.[12] **7b** and **7c** showed no difference in inhibiting α-glucosidase activity, this may result from the fact that the inductive effect of bromo atom decreases as its distance increasing from the oxygen atom at C-7 position.[20] The α-glucosidase inhibition of **3** and **7a** were 55.0% and 53.3%, respectively, as compared to acarbose (54.8%) at the concentration of 10^{-3} M. They had the highest inhibition among the ten compounds. The reason was most likely that the introduction of a benzyl group at the hydroxyl oxygen in C-7 position could increase the selectivity of the biotransformation. The benzyl group may act as an excellent affinity group, leading to the enhancement of selective interaction of the substrate with the active site of the enzyme.[21]

These compounds were tested for their effects on glucose consumption in HepG2 cell by the method of He.[22] Metformin was taken as the reference drug. The data are depicted in Figure 3. Treated by compound **3** and metformin at 10^{-4} M, glucose consumption of HepG2 cell were 1.78 and 2.11 mM, respectively. Compound **3** had the highest glucose consumption among these

synthetic compounds. However, **7a** was less effective than **3**, which may be related to the absence of hydroxyl group in B ring.[11,12]

All the compounds **2–8** were evaluated for their in vivo anti-hyperglycemic activity on blood glucose level in streptozotocin-induced diabetic mice.[23] Metformin was also chosen as the positive control. The results are shown in Table 1.

The results indicated that compound **3** has apparent effects on blood glucose levels in streptozotocin-induced diabetic mice just as the metformin. After respectively, oral administration of compound **3**, metformin, and saline water at 100 mg kg^{-1} d^{-1} for 10 days, the compound **3** group (13.23 mM, P <0.05) and the metformin group (10.00 mM, P <0.01) showed significant difference when compared to the model group (23.03 mM) on blood glucose levels. Compound **3** exhibited promising anti-hyperglycemic activity.

At this stage, the structure–activity relationship (SAR) could not be established because of the limited number of compounds. In order to further study the relationship between the structures and the anti-hyperglycemic activity, more hesperidin derivatives would need to be designed and synthesized.

In conclusion, the present studies showed that some novel hesperidin derivatives exhibited anti-hyperglycemic activities. Substitution of benzyloxy group on compounds **3** and **7a** might be the main factor contributing to their pharmacological properties. Further studies in developing them as lead compounds and exploring their mechanisms of action are in progress.

Acknowledgments

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Supplementary data

Supplementary data (General procedures and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.049.

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