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Synthesis and inhibition of α-glucosidase of methyl glycyrrhetinate glycosides

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ABSTRACT

The synthesis of the methyl glycyrrhetinate glycosides and inhibition of α-glucosidase were studied. The carboxyl group of glycyrrhetinic acid was methylated, and glucose and galactose were introduced into the hydroxyl group to obtain compounds **7** and **12**. Compound **1**, **2**, **7**, **12** and glycyrrhizic acid (GL) were evaluated for their inhibitory activities against α-glucosidase. As a result, Compound **1**, **2**, **7**, **12** and GL all showed significant α-glucosidase inhibitory activity and IC_{50} values were 0.465, 1.352, 0.759, 0.687 and 2.085 mM, respectively, and acted as non-competitive inhibitors. The activity of the compound **2**, **7**, **12** was lower than compound **1**, but significantly higher than GL. Therefore, it was concluded that the change of structure in glycyrrhetinic acid by chemical modification had certain effect on bioactivity, and the change of carboxyl group, hydroxyl group and the type of monosaccharide introduced were the influencing factors.

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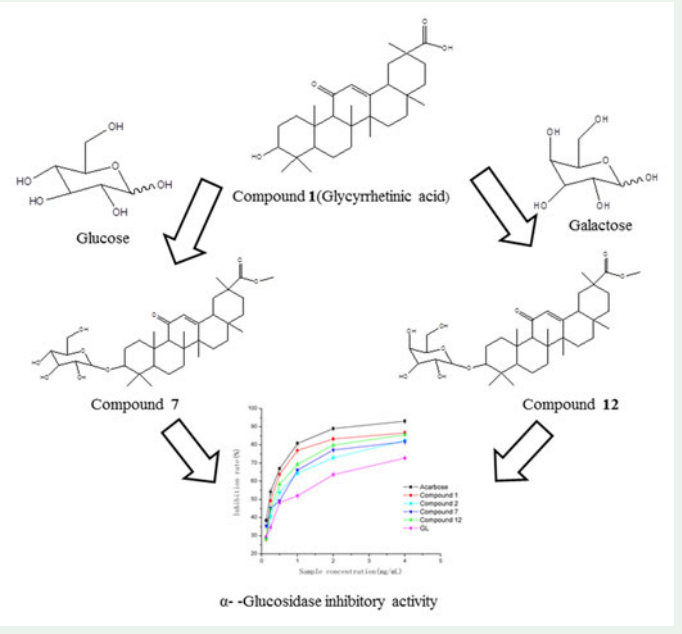

1. Introduction

Diabetes mellitus (DM) is a common chronic metabolic disorder characterised by hyperglycemia. It is now widely recognised that its main hazard is due to complications caused by long-term hyperglycemia, including hyperglycemia, diabetic ketoacidosis and non-ketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure and retinal damage. Therefore, it has always been considered as the main health killer. DM is mainly divided into insulin-dependent I (Type I Diabetes mellitus, T1DM) and non-insulin-dependent II (Type II Diabetes mellitus, T2DM). T1DM accounts for 10% of all DM cases. It occurs mostly in children and adolescents, and it is caused by the destruction of insulin-producing pancreatic islet β cells by the autoimmune system, which leads to decreased insulin production. T2DM accounts for 90% of DM cases, and is more common in adults and the elderly. Current studies suggest that its occurrence and development are closely related to islet cell damage and insulin resistance, and familial genetic factors also are significant. With the improvement of people's living standards and lifestyle changes, the incidence of T2DM continues to grow and develops toward youthfulness. According to predictions, the prevalence of T2DM will increase from the current 350 million to 592 million by 2035. Therefore, the prevention and treatment of diabetes has become an urgent problem to be solved (Wu et al. 2011; Zhang et al. 2006).

One of the drugs for the treatment of T2DM is α-glucosidase inhibitor. These drugs act on the α-glucosidase in the small intestine mucosa, competitively inhibiting the activity of this enzyme in the small intestine. The absorption of glucose and the hydrolysis rate of starch are reduced, and even achieving to inhibit completely,

thereby achieving the purpose of reducing postprandial blood glucose (Zhen et al. 2017). α-Glucosidase inhibitors which have been used in clinical treatment include *acarbose*, *voglibose*, *miglitol* and so on (Picazo et al. 2017).

Glycyrrhetinic acid (GA, compound **1**) belongs to pentacyclic triterpenoids, which is naturally present in various licorices (such as *Glycyrrhiza glabra*, *Ural licorice*, *Yunnan licorice*, etc.) and is one of the main active constituents of licorice. It has a variety of pharmacological effects, such as anti-cancer (Shen et al. 2017), anti-ulcer (Radwan et al. 2016), anti-oxidation (Li et al. 2012), anti-inflammatory (Li et al. 2017), and reducing blood lipids function (Kalaiarasi et al. 2009), etc. Consequently, GA is a leading compound of Chinese medicine with great research and development potential, and is widely used in medicines and health foods. Not only that, GA is also added to all kind of cosmetics, in addition to better whitening effect, but also anti-inflammatory, emollient, scavenging oxygen free radicals and UV protection (Ljiljana et al. 2016; Kong et al. 2015).

Since there are two configurations of hydrogen on C-18 in the GA structure, GA is further divided into 18α-GA and 18β-GA. But the naturally occurring 18β-GA is much more than 18α-GA, so the GA mentioned in the paper is 18β-GA. The previous researchers firstly protected the C-3 hydroxyl group of GA with acetyl group, and the C-30 carboxyl group was activated by $SOCl_2$ and then reacted with some aromatic groups, alicyclic groups, heterocyclic groups and aromatic groups to obtain amides and esters. Comparing the anti-inflammatory activities of these derivatives, it was found that these derivatives basically exhibited excellent anti-inflammatory activity, and even some derivatives showed better anti-inflammatory effects than prednisolone and indomethacin (Radwan et al. 2016). Furthermore, other researchers took derivatives of GA amino phosphonates as inhibitors of NF-κB pathway and cell proliferation to achieve the anti-tumor effect (Jin et al. 2018). A large number of literatures have reported the anti-inflammatory, anti-tumor and anti-cancer activities of GA and its derivatives, but the hypoglycemic activity of GA and its derivatives have yet to be deeply investigated.

The previous research found that GA and *glibenclamide* have comparable hypoglycemic effects in a rat model of hyperglycemia established by streptozotocin (Kalaiarasi & Pugalendi. 2009).

What's more, glycyrrhizic acid (GL), its aglycone is GA and its structure also includes glucuronic acid, reduced blood glucose and HOMA-IR values in type 2 diabetic rats according to the previous report. It also reduced the level of hyperglycemia, hyperglycosylated hemoglobin and serum cholesterol in streptozotocin-induced diabetic rats, and improved the level of serum insulin, glucose tolerance and the number of islet cells (Eu et al. 2010).

In the light of above considerations, in this article, some GA derivatives containing methyl glycyrrhetinate glycosides were carefully designed and obtained. These GA derivatives were expected to have better α-glucosidase inhibitory activity than GA.

2. Results and discussion

2.1. Chemistry

The general procedures for the synthesis of methyl glycyrrhetinate glycosides are shown in Scheme 1. In order to prevent the GA C-30 carboxyl group from reacting, it

was first protected by ester group (compound **2**). The hydroxyl groups of glucose and galactose were protected by benzoyl groups (compounds **4** and **9**) and then treated with 33% HBr to give the corresponding bromides to react with GA (compounds **5** and **10**) (Ruiz et al. 2009). Compounds **5** and **10** were reacted with compound **2** under catalysis by silver trifluoromethanesulfonate, respectively, to obtain compounds **6** and **11**, and finally the benzoyl groups were removed using a sodium methoxide–methanol system to obtain the final product compounds **7** and **12** (Schwarz et al. 2014; Fan et al. 2016). All compounds were identified by nuclear magnetic resonance spectroscopy and high resolution mass spectrometry.

The signal peaks of –OMe in ^{1}H NMR (δ 3.69) and ^{13}C NMR (δ 55.07) of compound **2** were substantially consistent with the previous literature (Zou et al. 2016), indicating that –COOH in compound **1** had been methylated (Supplementary information Figure S1). In the ^{1}H NMR of compound **6**, δ 8.02–7.33 (m, 20, ArH), δ 5.67(d, $J = 7.8$ Hz, 1H, H-1′) were the signal peaks of benzoyl hydrogen and anomeric hydrogen in glucose, respectively. In ^{13}C NMR, δ 133.56–128.42, δ 103.27 were the signal peaks of benzoyl carbon and anomeric carbon, respectively (Supplementary information Figure S2); δ 8.05–7.13 (m, 20, ArH), δ 5.89 (d, $J = 7.24$ Hz, 1H, H-1′) in the ^{1}H NMR of compound **11** were the signal peaks of benzoyl hydrogen and anomeric hydrogen in galactose. And δ 136.73–124.75, δ 102.70 in ^{13}C NMR were the signal peaks of benzoyl carbon and anomeric carbon in galactose (Supplementary information Figure S4), which fully demonstrated that benzoylated glucose and galactose had reacted with GA successfully. What's more, the coupling constants of compounds **6** and **11** anomeric hydrogen were7.8 Hz, 7.24 Hz, respectively, which proved that the bonds formed were β-type. In ^{1}H NMR and ^{13}C NMR of compounds **7** and **12**, the signal peaks of benzoyl group disappeared, indicating that the hydroxy group on glucose and galactose had been deprotected (Supplementary information Figures S3, S5). The mass of the molecular peaks of compounds **6**, **7**, **11**, and **12** were 1085.4971, 669.3984, 1085.4996, 669.3979 in the mass spectrums, respectively, and the error did not exceed 5 ppm (Supplementary information Figure S6).

2.2. α-Glucosidase inhibition

2.2.1. α-Glucosidase inhibitory activity

As described in Supplementary information Figure S7, compound **1**, **2**, **7**, **12** and GL had certain inhibition activity on α-glucosidase at different concentrations and it was in a dose-dependent manner (Juncheng et al. 2018). The α-glucosidase inhibitory activity of these five compounds increased with the increasing sample concentrations from 0.125 to 4 mg/mL and the inhibition rate tended to be gentle at the concentration of 2 mg/mL. The half-maximal inhibitory efficiency of inhibitor (IC_{50}) value could be used to assess inhibitory efficiency of the inhibitor (Hashim et al. 2015). The data in Supplementary information Table S1 showed that the IC_{50} value of the acarbose (positive control) was 0.035 mM and the IC_{50} values of compounds **1**, **2**, **7**, **12** and GL were 0.465, 1.352, 0.759, 0.687 and 2.085, respectively.

Referring to Supplementary information Figure S7 and Table S1, when the carboxyl group of GA was esterified (compound **2**), the α-glucosidase inhibitory activity was

obviously decreased, indicating that the carboxyl group of GA had an important influence on the α-glucosidase inhibition activity. When the hydroxyl group of GA was introduced glucose and galactose (compound **7** and **12**), the α-glucosidase inhibition activity decreased, but compared with GL, their inhibition activity was greatly enhanced. This indicated that the sugar introduced at the C-3 position was different and the activity was different. The type of monosaccharide introduced was also very an important factor.

2.2.2. Type of α-glucosidase inhibitory

To clarify the α-glucosidase inhibition mode of compounds **1**, **2**, **7** and **12**, Lineweaver-Burk plots were generated. As shown in Supplementary information Figure S8, the rate of enzymatic reaction varies with substrate concentration and was linear. When the concentration of these four compounds increased from 0.5 to 2 mg/mL, the slope of the straight line decreased, the *y*-intercept ($1/V_{max}$) increased, and the *x*-axis intercept ($1/K_m$) remained unchanged, indicating that the initial velocity of the maximum reaction began to decrease and K_m did not change. The reaction process conformed to the non-competitive reversible inhibition type, that is, the reversible inhibition types of α-glucosidase of these four samples were non-competitive.

3. Experimental section

All experimental procedures are described in the supplementary materials.

4. Conclusions

In conclusion, the final products compounds **7** and **12** were obtained by a stepwise reaction of compound **1** with glucose and galactose. Analysis of ^{1}H NMR and ^{13}C NMR of compounds **7** and **12**, δ 4.12 ppm and δ 4.19 ppm in ^{1}H NMR were the signals of glucose and galactose anomeric hydrogen, respectively, δ 105.85 ppm and δ 105.91 ppm in ^{13}C NMR were the signals of glucose and galactose anomeric carbon, respectively. The coupling constants of compounds **6** and **11** anomeric hydrogen were 7.8 and 7.24 Hz, respectively. This indicated that glucose and galactose had successfully reacted with the hydroxyl group of compound **1** and were linked in β configuration. The α-glucosidase inhibition activity assay illustrated the significant change of bioactivity in compound **1**, **2**, **7**, **12** and GL. Thus, the conclusion that the change of structure in GA by chemical modification had certain effect on bioactivity could be drawn, and the change of carboxyl group, hydroxyl group and the type of monosaccharide introduced were the influencing factors.

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