Structure related α-glucosidase inhibitory activity and molecular docking analyses of phenolic compounds from *Paeonia suffruticosa*

Po-Chun Chen¹,² · Bongani Sicelo Dlamini³ · Chiy-Rong Chen⁴ · Yueh-Hsiung Kuo⁵,⁶,⁷ · Wen-Ling Shih⁸ · Yun-Sheng Lin⁹ · Chien-Hsing Lee¹⁰,¹¹,¹² · Chi-I Chang⁰

Received: 1 October 2021 / Accepted: 23 November 2021 / Published online: 7 January 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

High postprandial hyperglycaemia is an important determinant of the development and progression of type 2 diabetes. Thus, inhibition of key digestive enzymes such as α-amylase and α-glucosidase is considered an efficient approach to control glycaemic levels in diabetics. In search of α-amylase and α-glucosidase inhibitors, the root bark of *Paeonia suffruticosa* was screened for inhibitors, resulting in the isolation of eleven phenolic compounds (1–11). Their enzymes inhibitory activities and inhibition mechanism were investigated using an in vitro inhibition assay and molecular docking studies. Compounds 2, 5, 6, and 8–11 (IC₅₀ between 290 and 431 µM) inhibited α-glucosidase more effectively than the reference compound acarbose (IC₅₀ = 1463.0 ± 29.5 µM). However, the compounds (IC₅₀ > 800 µM) were less effective against α-amylase than acarbose (IC₅₀ = 16.6 ± 0.9 µM). Among them, compound 10 exhibited the highest α-glucosidase inhibitory effect with an IC₅₀ value of 290.4 ± 9.6 µM. Compounds 2, 5, 9, 10 and 11 were found to be competitive inhibitors, while compounds 6 and 8 were noncompetitive inhibitors of α-glucosidase. Computational analyses showed that the main binding forces between the compounds and the main residues were hydrogen bonds. The results suggest that these compounds have the potential to be developed as α-glucosidase inhibitors.

Graphical Abstract

Keywords Enzymatic activity · Enzyme inhibition mechanism · α-glucosidase inhibitor · *Paeonia suffruticosa*

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00044-021-02830-6.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterised by excessive increases in plasma glucose levels and abnormalities in lipid and protein metabolism caused by deficient insulin secretion, insulin resistance, or both in
combination over time [1]. Changes in human behaviour and lifestyle have led to a substantial increase in the prevalence of diabetes worldwide over the past century. In 2014, approximately 422 million individuals were reported by the World Health Organization to have diabetes worldwide, with this figure projected to increase to over 650 million by 2040 [2]. One of the most effective approaches to treat DM is to suppress postprandial hyperglycaemia by inhibiting key digestive enzymes such as α-amylase and α-glucosidase. Therefore, commercial inhibitors such as voglibose, miglitol, and acarbose were developed for the treatment of hyperglycaemia and DM [3]. These oral drugs were efficient in inhibiting both α-amylase and α-glucosidase, which are responsible for the hydrolysis of polysaccharides to oligosaccharides and disaccharides and then to monosaccharides, subsequently lowering plasma glucose levels [4]. However, the use of these drugs (especially acarbose) is not without drawbacks, the most notable of which are diarrhoea and flatulence as a result of the prolonged suppression of starch hydrolysis [5]. The adverse effects (gastrointestinal complications) of the drugs are associated with excessive α-amylase inhibition leading to accumulation of undigested polysaccharides in the colon [6]. These limitations have highlighted the need for novel drug discovery techniques aimed at improving affinity and specificity while reducing existing adverse effects. Therefore, the focus in treating diabetes and managing its associated problems is shifting to widely available drugs with few side effects [7]. Medicinal plant extracts and their chemical constituents are gaining importance as potential therapies for diabetes and its sequelae because of their different modes of action and safety. Secondary metabolites of medicinal plants with pharmacological activity, including phenolic chemicals and flavonoids, are considered as potential sources of efficient and safe hypoglycaemic agents [8].

*Paeonia suffruticosa* (Paeoniaceae) is a medicinal plant indigenous to China with a long history of use in Traditional Chinese Medicine and has become an important ornamental plant worldwide [9]. Traditionally, the root of *P. suffruticosa* has been utilised as a crude medicine for the treatment of extravagant blood, elimination of stagnant blood, and cardiovascular complications [10]. The biological activities of the plant are mainly attributed to monoterpenoid glycosides, such as paeoniflorin, benzoylpaeoniflorin, albiflorin, and paeoniflorigenone, and the plant is also rich in galloylglucoses, gallic acid derivatives, flavonoids, triterpenoids, and acetophonones [11]. Although the crude extract of the plant is frequently employed in antidiabetic Chinese herbal formulations, scientific studies on its antidiabetic effects are limited [12]. A comprehensive study of its bioactive constituents against key digestive enzymes responsible for the hydrolysis of carbohydrates is still lacking. Hence, the root bark of *P. suffruticosa* was investigated for α-glucosidase inhibitors, resulting in the isolation of eleven compounds (1–11) (Fig. 1). The structurally related compounds were screened for their structure-activity association and interaction with α-glucosidase. Although the phenolic compounds have been studied for their α-amylase and α-glucosidase activities [13–15], their mode of interaction with α-glucosidase has rarely been fully explored, with the exception of gallic acid (1) [16]. Therefore, in the present study, kinetic analysis and molecular docking were conducted to understand the mechanism of interactions between phenolic compounds and α-glucosidase. This article presents the separation, characterisation, enzyme inhibitory effect and inhibition mechanisms of phenolic compounds from the root bark of *P. suffruticosa*.

**Materials and methods**

**General experimental procedures**

High performance liquid chromatography (HPLC) was conducted on a Hitachi L-7100 system coupled with Waters R410 differential refractometer using a Thermo Hypersil-Keystone BETASIL Silica-100 column (5 μm, 250 × 10 mm). Silica gel (63–200 mesh, Merck) was used for column chromatography. 1H and 13C NMR spectra were recorded on a Varian-Unity-Plus-400 spectrometer in DMSO-d6 or CDCl3 using residual solvent signals as reference. TLC was conducted on a silica gel 60 F254 (0.2 mm, Merck), illuminated under UV light (254 and 365 nm) and developed with 10% H2SO4 in ethanol (v/v). The absorbance was recorded in a Thermo Fisher Scientific (Rastatie 2, FI-01620 Vantaa, Finland) spectrophotometer. *Saccharomyces cerevisiae* α-glucosidase and porcine pancreas α-amylase were purchased from Sigma Aldrich (St. Louis, MO, USA). Acarbose, and 4-p-nitrophenyl-α-D-glucopyranoside (pNPG) were obtained from Acros Organics Company. 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) was obtained from Carbosynth Limited.
Plant material
The root bark of *P. suffruticosa* was collected from China’s Anhui province and was purchased from traders. Samples were authenticated by Prof. Sheng-Zehn Yang, Herbarium Curator, Department of Forestry, National Pingtung University of Science and Technology. A voucher specimen (No. BT360) was deposited at the herbarium of the Department of Biological Science and Technology.

Extraction and isolation
The root bark (3.6 kg) of *P. suffruticosa* was pulverised and extracted with methanol (3 × 20 L) at room temperature. The methanol was removed from the extract using a vacuum rotary evaporator to give crude extract (720 g), which was then suspended in water and separated sequentially with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) to obtain EtOAc (105 g), *n*-BuOH (320 g), and water (260 g) soluble fractions. The EtOAc fraction was further fractionated on a column chromatography (7 × 90 cm) using a gradient solvent mixture (100:0 to 0:100, v/v) to give twenty-three fractions (Fr. 1–23). Fr. 4 (1400 mg) was further purified by washing and recrystallisation with *n*-hexane/dichloromethane to obtain compound 5 (610 mg). Fr. 10 (302 mg) was separated on a semi-preparative normal phase HPLC eluted with dichloromethane/EtOAc (30:1, v/v) to obtain thirteen subfractions (Fr.10–1–Fr.10-13). Fr. 10-7 (34 mg) was further purified on a HPLC eluted with dichloromethane/isopropanol (100:1, v/v) to obtain twelve fractions (Fr.16–1–Fr.16-12). Fr. 16-1 (35 mg) was further purified on a HPLC using *n*-hexane/dichloromethane/acetone (7/7/1, v/v) to afford compound 3 (2 mg). Fr.17 (380 mg) was purified on a HPLC eluting with *n*-hexane/dichloromethane/acetone (7/7/1, v/v) to give four subfractions (Fr.17-1–Fr.17-4). Fr. 17-1 (35 mg) was further purified on HPLC using dichloromethane/isopropanol (100/1, v/v) to obtain compounds 2 (100 mg) and 9 (3 mg). Fr.19 (800 mg) was washed and recrystallised with dichloromethane and methanol to give compound 11 (75 mg). Fr. 20 (3200 mg) was subjected to silica gel column chromatography (2 × 50 cm) eluted with dichloromethane/EtOAc (10/1, v/v) to obtain compound 10 (810 mg).

Benzoic acid (1)
Colourless crystal; m.p. 121–122 °C; 1H-NMR (CDCl3, 400 MHz) δ 8.12 (2H, d, J = 8.4 Hz, H-2, 6), 7.60 (1H, d, J = 7.6 Hz, H-4), 7.47 (2H, d, J = 8.4 Hz, H-3,5); 13CN M R (CDCl3, 100 MHz) δ 172.3, 133.8, 130.2, 129.3, 128.5; EI-MS m/z (%): 122 [M]+ (100), 105 (95); Anal. Calcd. for C7H6O2: C, 68.84; H, 4.95. Found: C, 68.91, H, 4.96 [17].

4-Hydroxybenzoic acid (2)
White crystal; m.p. 214–217 °C; 1H-NMR (DMSO-d6, 400 MHz) δ (ppm): 10.23 (1H, s, 4-OH), 7.78 (2H, d, J = 8.8 Hz, H-3,5), 6.81 (1H, d, J = 8.8 Hz, H-2,6); EI-MS: m/z (%): 138 [M]+ (70), 121 (100); Anal. Calcd. for C7H6O2: C, 60.87; H, 4.38. Found: C, 60.92; H, 4.38 [18].

4-Methoxybenzoic acid (3)
White crystal; m.p. 184–185 °C; 1H-NMR (CDCl3, 400 MHz) δ 8.05 (2H, d, J = 8.4 Hz, H-2, 6), 6.92 (2H, d, J = 8.8 Hz, H-3,5), 3.86 (3H, s, 4-OCH3); 13CN M R (CDCl3, 100 MHz) δ 170.3, 163.9, 132.3, 121.7, 133.7, 55.5; EI-MS m/z (%): 152 [M]+ (65), 151 (100); Anal. Calcd. for C7H6O2: C, 63.15; H, 5.30. Found: C, 63.21; H, 5.31 [19].

1-(2,4-Dihydroxyphenyl)ethanone (4)
Colourless needless; m.p. 143–145 °C; 1H-NMR (CDCl3, 400 MHz) δ 12.68 (1H, s, 2-OH), 7.62 (1H, d, J = 8.4 Hz, H-6), 6.38 (1H, d, J = 8.4, 2.4 Hz, H-5), 6.35 (1H, d, J = 2.4 Hz, H-3), 2.54 (3H, s, 1-COCH3); 13CN M R (CDCl3, 100 MHz) δ 202.7, 165.1, 162.7, 150.4, 133.0, 114.2, 109.7, 103.4, 26.2; EI-MS m/z (%): 152 [M]+ (43), 137 (100); Anal. Calcd. for C7H6O2: C, 63.15; H, 5.30. Found: C, 63.21; H, 5.31 [20].

(5 mg). Fr.15 (1600 mg) was purified on a HPLC using a mobile phase of dichloromethane/EtOAc (30:1, v/v) to isolate compound 1 (70 mg). Fr.16 (1260 mg) was separated on a HPLC eluted with dichloromethane/EtOAc (100:1, v/v) to obtain twelve fractions (Fr.16-1–Fr.16-12). Fr.16-1 (35 mg) was further purified on a HPLC using *n*-hexane/dichloromethane/acetone (7/7/1, v/v) to afford compound 3 (2 mg). Fr.17 (380 mg) was purified on a HPLC eluting with *n*-hexane/dichloromethane/acetone (7/7/1, v/v) to give four subfractions (Fr.17-1–Fr.17-4). Fr.17-1 (35 mg) was further purified on HPLC using dichloromethane/isopropanol (100/1, v/v) to obtain compounds 2 (100 mg) and 9 (3 mg). Fr.19 (800 mg) was washed and recrystallised with dichloromethane and methanol to give compound 11 (75 mg). Fr.20 (3200 mg) was subjected to silica gel column chromatography (2 × 50 cm) eluted with dichloromethane/EtOAc (10/1, v/v) to obtain compound 10 (810 mg).
1-(2-Hydroxy-4-methoxyphenyl)ethanone (Paeonol) (5)

White powder; m.p. 47–48 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 12.72 (1H, s, 2-OH), 7.60 (1H, d, J = 8.8 Hz, H-6), 6.41 (1H, dd, J = 8.8, 2.4 Hz, H-5), 6.38 (1H, d, J = 2.4 Hz, H-3), 3.80 (3H, s, 4-OCH₃). ¹³C NMR (CDCl₃, 100 MHz) δ 202.5, 166.0, 165.2, 132.2, 113.8, 107.6, 100.8, 55.5, 26.2; EI-MS m/z (%): 166 [M]+ (24), 49 (100); Anal. Calcd. for C₉H₁₀O₃: C, 52.18; H, 5.54. Found: C, 52.22; H, 5.54 [23].

1-(3-Hydroxy-4-methoxyphenyl)ethanone (Isoacetovalonil) (6)

White solids; m.p. 85–87 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 7.51 (1H, dd, J = 8.8, 2.0 Hz, H-6), 7.49 (1H, d, J = 2.0 Hz, H-2), 6.85 (1H, d, J = 8.8 Hz, H-5), 6.04 (1H, s, 3-OH), 3.91 (3H, s, 4-OCH₃), 2.50 (3H, s, 1-COCH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 197.2, 150.7, 145.3, 130.8, 121.8, 114.4, 109.8, 56.0, 26.3; EI-MS m/z (%): 166 [M]+ (46), 151 (100); Anal. Calcd. for C₉H₁₀O₃: C, 65.22; H, 6.04. Found: C, 65.60; H, 6.07 [22].

1-(2,5-Dihydroxy-4-methylphenyl)ethanone (7)

Yellow solids; m.p. 139–141 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 11.85 (1H, s, 2-OH), 7.08 (1H, s, H-6), 6.75 (1H, s, H-2), 4.69 (1H, brs, 5-OH), 2.54 (3H, s, 1-COCH₃), 2.25 (3H, s, 4-CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 203.4, 156.6, 146.0, 135.7, 120.0, 117.5, 114.8, 26.6, 16.7; EI-MS m/z (%): 144 [M]+ (50), 151 (100); Anal. Calcd. for C₉H₁₀O₃: C, 52.18; H, 5.54. Found: C, 52.22; H, 5.54 [24].

1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8)

Yellow crystal; m.p. 165–166 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 12.47 (1H, s, 2-OH), 7.17 (1H, s, H-6), 6.41 (1H, s, H-2), 3.89 (3H, s, 4-OCH₃), 2.50 (3H, s, 1-COCH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 202.7, 158.8, 153.7, 137.9, 113.9, 112.4, 99.7, 56.1, 26.4; EI-MS m/z (%): 182 [M]+ (56), 167 (100); Anal. Calcd. for C₉H₁₀O₄: C, 59.51; H, 5.51. Found: C, 59.84; H, 5.54 [24].

1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9)

Light yellow powder; m.p. 131–132 °C; ¹H-NMR (CDCl₃, 400 MHz) δ 12.48 (1H, s, 2-OH), 7.31 (1H, d, J = 8.8 Hz, H-6), 6.49 (1H, d, J = 8.8 Hz, H-5), 5.54 (1H, brs, 3-OH), 3.94 (3H, s, 4-OCH₃), 2.56 (3H, s, 1-COCH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 203.5, 152.0, 150.2, 133.3, 122.7, 114.7, 102.7, 56.2, 26.3; EI-MS m/z (%): 182 [M]+ (47), 167 (100); Anal. Calcd. for C₉H₁₀O₄: C, 59.51; H, 5.51. Found: C, 59.84; H, 5.54 [25].

Gallic acid (10)

White crystals; m.p. 236–238 °C; ¹H-NMR (DMSO-d₆, 400 MHz) δ 12.17 (1H, brs, 1-COOH), 9.19 (2H, brs, 3, 5-OH), 8.23 (1H, brs, 4-OH), 6.90 (2H, s, H-2, 6); ¹³C NMR (DMSO-d₆, 100 MHz) δ 167.6, 145.5, 138.1, 120.5, 108.8; EI-MS m/z (%): 170 [M]+ (100), 153 (89); Anal. Calcd. for C₉H₈O₅: C, 49.13; H, 4.12. Found: C, 49.45; H, 4.15 [26].

Methyl gallate (11)

Pale yellowish crystal; m.p. 200–202 °C; ¹H-NMR (DMSO-d₆, 400 MHz) δ 6.92 (2H, s, H-2, 6), 3.72 (3H, s, 1-COOCH₃); ¹³C NMR (DMSO-d₆, 100 MHz) δ 166.4, 145.6, 138.5, 119.3, 108.5, 51.7; EI-MS m/z (%): 184 [M]+ (44), 153 (100); Anal. Calcd. for C₉H₁₀O₅: C, 52.18; H, 4.38. Found: C, 52.22; H, 4.38 [27].

α-Amylase inhibition assay

The inhibition of α-amylase by the tested phenolic compounds was carried out using the method of Okutan et al. [28] with minor changes. In a 96-well plate, 5 µL of the compounds solution (0–1000 µM, dissolved in DMSO), 160 µL of phosphate buffer (0.1 M, pH 6.0) with 0.02% sodium azide, and 10 µL of α-amylase (1 U/mL, final concentration) were mixed and incubated for 5 min at 37 °C. Then 10 µL of 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) solution (25 mM, in phosphate buffer) was introduced into the reaction and incubated at 37 °C for 30 min. Thereafter, 5 µL NaOH (5 M in distilled water) was added to the reaction mixture and the absorbance was recorded at 405 nm using a microplate reader. Acarbose (0–50 µM in DMSO) was used as reference compound. The amount of DMSO (2.5%) did not affect the enzyme activity [28]. The inhibition effect of the compounds was calculated using the following formula.

Inhibition effect (%) = \[\frac{[A_{\text{control}} - A_{\text{sample}}]}{A_{\text{control}}} \times 100\]  

where \[A_{\text{sample}}\] and \[A_{\text{control}}\] represent the absorbance of enzyme activity with and without the samples or standard, respectively.

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity of the tested compounds was measured following the methods of [29] and [30] with slight modifications. Briefly, 10 µL of α-glucosidase, 5 µL of the compounds solution and 170 µL of phosphate buffer (0.2 M, pH 6.8) were mixed and incubated
at 37°C for 5 min. After incubation, the reaction was initiated with the addition of 10 µL of pNPG solution into the reaction mixture and incubated for 60 min at 37°C. After incubation, the reaction was stopped by adding 5 µL of NaOH and the absorbance was measured at 405 nm using a microplate reader. The enzyme (1 U/mL) and substrate (25 mM) stock solutions were prepared in phosphate buffer and NaOH was dissolved in distilled water, while the compounds (0–500 µM) and acarbose (0–1500 µM) were dissolved in DMSO. The amount of DMSO (2.5%) did not interfere with the experiment [26]. The percentage of inhibition was calculated using equation 1.

Mode of inhibition against α-glucosidase

The same procedure as the enzyme inhibition assay was used to analyse the inhibition mechanisms and the reaction mixture contained 5 µL of the compounds at different concentrations [2 (0, 220, 440, 880 µM), 5 (0, 205, 410, 820 µM), 6 (0, 220, 240, 880 µM), 8 (0, 190, 380, 760 µM), 9 (0, 185, 370, 740 µM), 10 (0, 145, 290, 580 µM), 11 (0, 183, 365, 730 µM) and acarbose (0, 375, 750, 1500 µM), 10 µL of α-glucosidase (0.05 U/mL), 170 µL of phosphate buffer (0.2 M, pH 6.8), 10 µL of pNPG at four different concentrations (0, 0.31, 0.63, 1.25 mM) and 5 µL of NaOH. Kinetic parameters were determined using Lineweaver–Burk plots and described as follows [31, 32].

Competitive type:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

(2)

Non-competitive and mixed type:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{[I]}{K_{is}}\right)$$

(3)

Secondary plots were determined as follows

Slope = $\frac{K_m}{V_{max}} + \frac{K_{m}[I]}{V_{max}K_i}$

(4)

Y-intercept = $\frac{1}{V_{app}} = \frac{1}{V_{max}} + \frac{1}{K_{is}V_{max}}[I]$

(5)

Here $K_i$ and $K_m$ indicate the equilibrium constant of the inhibitor to the enzyme and the enzyme-substrate composite, respectively. $K_m$ represent the Michaelis-Menten constant, $v$ denotes enzyme velocity, $V_{max}$ represents maximal velocity, [I] and [S] represent the concentration of the compounds and pNPG, respectively.

$K_m$ was the negative reciprocal of the x-axis intercept while $V_{max}$ was the reciprocal of the y-axis intercept obtained from the double reciprocal plots of enzyme velocity versus pNPG with increasing concentrations of the compounds. $K_i$ was the additive inverse of the x-axis intercept derived from the secondary plots of the slope of the double reciprocal plots against the concentration of the compounds, whereas $K_{is}$ was the additive inverse of the x-axis intercept obtained from the y-axis intercept of the double reciprocal plots versus the concentrations of the compounds.

Molecular docking

The interactions between α-glucosidase and phenolic compounds were studied by computer simulations. A SWISS-MODEL was used to identify the protein for homology modelling from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) with high sequence identity to the target (α-glucosidase from Saccharomyces cerevisiae used in the experimental procedures). The crystallographic structure of the S. cerevisiae glucosidase enzyme has not yet been published. Therefore, S. cerevisiae isomaltase (PDB code: 3AA4; resolution: 1.60 Å) was selected as the receptor model for simulation, which was 72% identical and had 85% similarity to the target [33]. Ligands and water were removed from the enzyme to create a stable receptor for the phenolic compounds and docking calculations (binding energies) were carried out using the default setting for Discovery Studio 3.0 and the docking protocol was validated through redocking of co-crystallised ligand in protein. The angle of the grid box was 90 points (x, y, and z) with a spacing of 0.5 Å, and the grid box location was set at 11.9, −16.3, and 15.5 Å (x, y, and z), with 10 runs per molecule and up to 10 conformers per ligand. Binding events were visually analysed using Discovery Studio 3.0 software and geometry minimisation was performed using a CDOCKER (CHARMm-based DOCKER). ChemDraw Pro 5.0 software was used to create the three-dimensional structures of the compounds and standard acarbose. Blind docking was conducted on all the compounds and the conformation with the highest docking number and lowest binding energy was selected as the best results for analysis. The hydrogen bonding, pi-pi stacking and hydrophobic interactions generated between the phenolic compounds and the major residues in the active site of α-glucosidase were obtained from the docking results.

Druglikeness/ADME/toxicity analysis

Druglikeness, ADME properties (absorption, distribution, metabolism, and elimination), and toxicity of the compounds were analysed via the preADMET online server (http://preadmet.bmdrc.org) [34]. Lipinski’s rule of five [35], leadlike rule [36], Comprehensive Medicinal Chemistry (CMC) like rule [37] and MACCS-II drug data report (MDDR) like rule [38] were used for the prediction of drug likeness properties of phenolic compounds while for
ADME prediction, Blood brain barrier (BBB) penetration, Caco2 cell permeability, human intestinal absorption (HIA), plasma protein binding (PPB) and skin permeability were used and for toxicity analysis, Ames test [39] and rodent carcinogenicity were used.

Statistical analysis

SPSS version 25 was used for all statistical analyses. Compounds were statistically compared using one-way analysis of variance (ANOVA), and differences between means were assessed using Tukey’s HSD test, with p values < 0.05 considered significant. Each figure reflects three separate experiments, and results are reported as mean ± standard error of the mean (SEM). The variable slope non-linear regression method was used to determine IC50 values (GraphPad Prism 5.0.1, GraphPad Software, San Diego, California USA).

Results and discussion

Alpha-Glucosidase and alpha-amylase inhibitory activity

All the isolated compounds were investigated for their in vitro α-glucosidase and α-amylase inhibition activities. As shown in Table 1, all tested phenolic compounds (IC50 values between 290 and 431 µM) were more efficient in inhibiting α-glucosidase compared to the reference compound acarbose (IC50 value 1463.0 ± 29.5 µM), except for benzoic acid (1) and 4-methoxybenzoic acid (3), which were not active at a concentration of 500 µM. Among the tested compounds, gallic acid (10) (IC50 value 290.4 ± 9.6 µM) was the most potent α-glucosidase inhibitor, while isoacetovanillon (6) (IC50 value 431.3 ± 11.7 µM) showed the least inhibitory effect. Gallic acid (10) contained three hydroxyl groups positioned at C-3, C-4 and C-5 and one carboxylic acid group connected to C-1. Substitution of the carboxylic acid group with an ester group resulted in a decline in the inhibitory effect of methyl gallate (11) compared to that of gallic acid (10) (Fig. 2). When the data of gallic acid (10) was compared with that of 4-hydroxybenzoic acid (2), it was observed that hydroxylation of the compound contributes to the effectiveness of the compound in suppressing α-glucosidase. Moreover, a comparison of the inhibition data of paeonol (5) and isoacetovanillon (6) with those of 1-(2,5-dihydroxy-4-methoxyphenyl)ethanone (8) and 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (9) substantiated that the greater number of hydroxyl groups on the aromatic ring was favourable for their inhibitory activity. 1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8) and 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (9) had an additional hydroxyl group and showed lower IC50 values than paeonol (5) and isoacetovanillon (6). A similar phenomenon has been observed from the literature [40], suggesting that hydroxylation may increase the inhibitory activity of flavonoid compounds. Moreover, methoxylation at C-4 and substitution of the carboxylic acid group with an acetyl group further decreased the IC50 value of the compounds. The position of the hydroxyl group on the benzene ring had a minor effect on the potency of the compounds, which was observed when 1-(2,5-dihydroxy-4-methoxyphenyl)ethanone (8) was compared with 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (9), as well as paeonol (5) with isoacetovanillon (6).

Moreover, all tested compounds (1-11) were evaluated for their α-amylase inhibitory activity and the results showed that the compounds (IC50 values >800 µM) were significantly less active compared to acarbose (IC50 = 16.6 ± 0.85 µM) (Table 1). These compounds showed 50-fold lower α-amylase inhibition activities than acarbose while their α-glucosidase inhibition activities were threefold greater than acarbose. Therefore, α-glucosidase was selected for further kinetic analysis of tested compounds. 4-Hydroxybenzoic acid (2) and gallic acid (10) were previously reported for their α-amylase inhibitory activity with

| Compounds | IC50 (µM) | α-amylase | α-glucosidase |
|-----------|----------|-----------|---------------|
| Benzoic acid (1) | NA | NA | |
| 4-Hydroxybenzoic acid (2) | >1000 | | 398.6 ± 14.9<sup>ec</sup> |
| 4-Methoxybenzoic acid (3) | NA | NA | |
| 1-(2,4-Dihydroxyphenyl)ethanone (4) | – | – | |
| Paeonol (5) | >1000 | | 404.8 ± 12.3<sup>f</sup> |
| Isoacetovanillon (6) | >1000 | | 431.3 ± 11.7<sup>f</sup> |
| 1-(2,5-Hydroxy-4-methylphenyl)ethanone (7) | – | – | |
| 1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8) | 997.2 ± 11.5<sup>d</sup> | | 375.5 ± 12.8<sup>cd</sup> |
| 1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9) | 957.2 ± 14.8<sup>e</sup> | | 369.2 ± 10.2<sup>b</sup> |
| Gallic acid (10) | 835.4 ± 13.2<sup>a</sup> | | 290.4 ± 9.6<sup>a</sup> |
| Methyl gallate (11) | 898.6 ± 11.4<sup>b</sup> | | 365.5 ± 11.9<sup>b</sup> |
| Acarbose (positive control) | 16.6 ± 0.85 | | 1463.0 ± 29.5<sup>f</sup> |

Values are expressed as mean ± standard error of the mean (n = 3). Values with different superscripted letters (a–g) in the same column were significantly different at p < 0.05 (–): Not tested

NA: Not active at 1000 µM
IC₅₀ values >1000 µM and α-glucosidase inhibitory activity with comparable IC₅₀ values of 424.8 ± 30.40 µM and 296.2 ± 17.63 µM, respectively [41], which were similar to our result.

The compounds showed selectivity in inhibition of α-amylase and α-glucosidase. Compared with α-amylase, these compounds have stronger selective inhibition of α-glucosidase (Table 1). In contrast, the standard compound acarbose showed greater ability to selectively inhibit α-amylase over α-glucosidase. This result demonstrated that the structural composition of a compound can lead to large differences in its ability to selectively inhibit one enzyme over others. The reason for the selectivity of phenolic compounds in inhibiting α-glucosidase over α-amylase may be that α-amylase must incorporate larger oligosaccharide units into its active site than the small molecule inhibitors [42]. Therefore, acarbose inhibited α-amylase more effectively than α-glucosidase compared to the other compounds, which may be due to the trisaccharide moiety of acarbose that favours additional interactions. Acarbose was found to have a stronger affinity for the C-terminal than for the N-terminal subunits of the enzymes, which is due to its extended substrate binding site that enhances the interaction of C-terminal enzymes with longer oligomers [43, 44].

**Inhibition mechanisms of α-glucosidase**

The nature of inhibition on α-glucosidase of phenolic compounds was investigated using Lineweaver–Burk plots [45]. Figure 3 shows the reciprocal plots of the maximal velocity (Vₘₐₓ) of α-glucosidase reaction against pNPG concentration with increasing concentrations of the compounds. The apparent values of Kₘ (Michaelis-Menten constant) were calculated from linear curve fitting equations, while the Kᵢ (equilibrium constant for enzyme-inhibitor association) and Kᵢₛ (equilibrium constant for enzyme-substrate-inhibitor association) were derived from secondary plots of the slopes of the primary plots (Lineweaver–Burk plots) versus the concentration of the compounds. All the double reciprocal plots of the compounds (2, 5, 9, 10, and 11) intersected on the y-axis, indicating competitive inhibition of α-glucosidase. The values of Kᵢ increased and the values of Vₘₐₓ remained constant as shown by the increasing slope and constant y-intercept of the curves as the concentration of the compounds increased, indicating that the compounds formed the α-glucosidase-compound composite to slow down the catalytic efficiency of the enzyme, which showed that these compounds induce competitive inhibition.

In Fig. 3, the data lines of isoacetovanillon (6) and 1-(2,5-dihydroxy-4-methoxyphenyl)ethanone (8) crossed on the horizontal axis with a constant x-intercept. Additionally, both the y-intercept and gradient of the graphs increased with the increase in the concentration of the compounds, indicating that the Vₘₐₓ values decreased and the Kₘ values were constant. As shown in Table 2, the equilibrium constants (Kᵢ and Kᵢₛ) were the same, suggesting that the affinity of the α-glucosidase-compound complex is similar to the affinity of the α-glucosidase-pNPG-compound complex. These results indicated that isoacetovanillon (6) and 1-(2,5-dihydroxy-4-
Fig. 3 The Lineweaver–Burk plots of the active constituents of *Paeonia suffruticosa* root bark against α-glucosidase with pNPG as substrate. Insert represent the secondary plots of slope versus compounds. A 4-Hydroxybenzoic acid (2), (B) Paeonol (5), (C) Isoacetovanillon (6), (D) 1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8), (E) 1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9), (F) Gallic acid (10), (G) Methyl gallate (11) and (H), Acarbose
methoxyphenyl)ethanone (8) were noncompetitive inhibitors of α-glucosidase. Moreover, the secondary plots (insert of Fig. 3) of the slope against the concentration of the compounds fitted linearly, suggesting that the compounds bind to a single inhibition site on the enzyme [46].

Table 2 Kinetic analysis parameters of the compounds against α-glucosidase

| Compounds                                      | $K_i$ (µM)  | $K_m$ (µM) | Inhibition type |
|------------------------------------------------|-------------|-------------|-----------------|
| 4-Hydroxybenzoic acid (2)                      | 147.5 ± 8.6$^a$ | NA          | Competitive     |
| Paenol (5)                                      | 120.1 ± 3.5$^b$ | NA          | Competitive     |
| Isoacetovanillon (6)                           | 161.8 ± 9.2$^c$ | ND          | Noncompetitive  |
| 1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8)  | 136.9 ± 4.6$^d$ | ND          | Noncompetitive  |
| 1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9)  | 140.4 ± 3.8$^e$ | NA          | Competitive     |
| Gallic acid (10)                               | 107.5 ± 2.7$^f$ | NA          | Competitive     |
| Methyl gallate (11)                            | 135.9 ± 5.6$^g$ | NA          | Competitive     |
| Acarbose (positive control)                    | 527.4 ± 11.7$^h$ | NA          | Competitive     |

Values are expressed as mean ± standard error of the mean ($n = 3$). Values with different superscripted letters ($a$–$f$) in the same column were significantly different at $p < 0.05$

NA Not available
ND Not different from $K_i$

Fig. 4 Predominant interactions observed between the compounds and main residues of α-glucosidase. A The green region indicate the catalytic active site of α-glucosidase. B–C The compounds docked to enzyme on the molecular surface. D 4-Hydroxybenzoic acid (2), (E) Paenol (5), (F) Isoacetovanillon (6), (G) 1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (8), (H) 1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9), (I) Gallic acid (10), (J) Methyl gallate (11) and (K) Acarbose

Molecular docking analysis

Computer-assisted docking was conducted to analyse the interaction mechanisms of the compounds with α-glucosidase by visualising binding in the receptor-ligand composite
| Compounds | Binding energy (Kcal/mol) | Bonds between atoms of compounds and residues of active site |
|-----------|--------------------------|----------------------------------------------------------|
|           |                          | Compounds atom    | Receptor residues | Type of interaction bond | Distance (Å) |
| 2         | −0.32                    | -COOH             | Asp233            | Hydrogen bond            | 1.98         |
|           |                           | -OH               | Ser311, Asn317    | Hydrogen bond            | 2.07, 1.99   |
|           |                           | Ring              | Leu313            | Hydrophobic              | 4.59         |
| 5         | −0.38                    | -OH               | Asp233            | Hydrogen bond            | 2.00         |
|           |                           | -COCH₃            | Asn235            | Hydrogen bond            | 2.91         |
|           |                           | -OCH₃             | Asn317            | Hydrogen bond            | 2.04         |
|           |                           | Ring              | Leu313            | Hydrophobic              | 5.43         |
| 6         | −0.28                    | -OH               | Asp233            | Hydrogen bond            | 2.08         |
|           |                           | -COCH₃            | Asn317            | Hydrogen bond            | 1.96         |
|           |                           | -COCH₃            | Ser311            | Non-classic hydrogen bond| 2.62         |
|           |                           | Ring              | Leu313            | Hydrophobic              | 4.62         |
| 8         | −0.87                    | -OH               | Ser311            | Hydrogen bond            | 2.03         |
|           |                           | -COCH₃            | Lys432            | Hydrogen bond            | 1.81         |
|           |                           | -OCH₃             | Asp233            | Non-classic hydrogen bond| 2.56, 2.90   |
|           |                           | Ring              | Leu313            | Hydrophobic              | 4.92         |
| 9         | −0.92                    | -OH               | Asp233            | Hydrogen bond            | 2.16         |
|           |                           | -OH               | Asn317            | Hydrogen bond            | 2.15         |
|           |                           | -COCH₃            | Asn317            | Hydrogen bond            | 2.07         |
|           |                           | -COCH₃            | Ser311            | Non-classic hydrogen bond| 2.48         |
|           |                           | Ring              | Leu313            | Hydrophobic              | 4.72         |
| 10        | −1.34                    | -COOH             | Asn235            | Hydrogen bond            | 2.25         |
|           |                           | -OH               | Ser311, Asn317    | Hydrogen bond            | 1.92, 2.03   |
|           |                           | -OH               | Asn317            | Hydrogen bond            | 2.06         |
|           |                           | Ring              | Leu313            | Hydrophobic              | 5.03         |
| 11        | −1.17                    | -OH, -OH          | Asp233            | Hydrogen bond            | 1.94, 2.09   |
|           |                           | -COOCH₃           | Asn317            | Hydrogen bond            | 2.23         |
|           |                           | -COOCH₃           | Ser311            | Non-classic hydrogen bond| 2.42         |
|           |                           | -COOCH₃           | Val319            | Hydrophobic              | 4.81         |
|           |                           | Ring              | Leu313            | Hydrophobic              | 4.96         |
| Acarbose  | −0.24                    | -OH               | Lys156, Leu313    | Hydrogen bond            | 1.80, 1.83   |
|           |                           | -NH               | Tyr158            | Hydrogen bond            | 2.47         |
|           |                           | -OH               | Asp233            | Hydrogen bond            | 2.15         |
|           |                           | -OH, -OH          | Trp238, Glu422    | Hydrogen bond            | 2.54, 2.32   |
|           |                           | -OH               | Asp242            | Hydrogen bond            | 2.24         |
|           |                           | -OH               | Asn415            | Hydrogen bond            | 1.81, 3.30   |
|           |                           | -OH               | Glu422, His423    | Hydrogen bond            | 2.07, 2.27   |
|           |                           | -OH, -OCH₂OH      | Asn235            | Non-classic hydrogen bond| 2.64, 2.18   |
|           |                           | -OH               | Phe314            | Non-classic hydrogen bond| 2.06         |
Table 4 Druglikeness/ADME/Toxicity properties for the active α-glucosidase inhibitors (compound 2, 5, 6, 8, 9, 10, 11, and acarbose)

| Druglikeness/ADME/Toxicity | Compounds | 2 | 5 | 6 | 8 | 9 | 10 | 11 | Acarbose |
|----------------------------|------------|---|---|---|---|---|----|----|-----------|
| CMC-like rule              | Not qualified | Qualified | Qualified | Qualified | Qualified | Not qualified | Qualified | Not qualified |
| Lead-like rule             | Suitable | Suitable | Suitable | Suitable | Suitable | Not qualified | Not qualified | Suitable |
| MDDR-like rule             | Nondrug-like | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Violated | Violated | Violated |
| Rule of five               | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Drug-like |
| BBB                        | 0.6436 | 0.5604 | 0.5603 | 0.4673 | 0.4675 | 0.3481 | 0.3794 | 0.0271 |
| Caco2                      | 20.3138 | 18.0580 | 18.0563 | 14.8821 | 14.8762 | 13.8492 | 18.3188 | 9.4445 |
| HIA                        | 88.1391 | 93.5439 | 93.5427 | 86.1882 | 86.1817 | 53.6969 | 69.7464 | 0.0000 |
| PPB                        | 8.0405 | 54.9533 | 68.3810 | 84.8822 | 74.4363 | 65.3847 | 88.4397 | 0.0000 |
| Skin permeability          | −2.2282 | −2.0242 | −2.0422 | −3.0089 | −3.0161 | −3.6269 | −3.4013 | −5.1762 |
| Ames test                  | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Non-mutagen |
| Carcinco mouse             | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Positive |
| Carcinco rat               | Negative | Positive | Positive | Positive | Positive | Positive | Positive | Negative |
| hERG inhibition            | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Low risk | Ambiguous |

Ranges for BBB, blood-brain barrier permeability (low < −1, middle −1 ≤ −0.3, high > 0.3); Caco2, Caco2 cell permeability (low < 4, middle 4–70, high > 70 nm/s); HIA, human intestinal absorption (poor < 20%, moderate 20–70%, well 70–100%); PPB, plasma protein binding (week bound < 90%, strong bound > 90%); skin permeability (low < −1, optimal −1 ≤ −0.3, high > −1 cm/hr); carcino, carcinogenicity; hERG, human ether-a-go-go-related gene.

[47] As shown in Fig. 4A and B, the compounds were located at the active binding site of α-glucosidase. The major amino acid residues involved in the interaction of the compounds and α-glucosidase were Asp233, Asn235, Ser311, Leu313, Asn317, Val319, and Lys342, and these residues were found to be crucial for the catalytic mechanism (Table 3) [46]. All compounds formed π-interactions with the amino acid residue Leu313, and paeonol (5) and methyl gallate (11) also formed π-interactions with Val319. In Fig. 4C, 4-hydroxybenzoic acid (2) was stabilised by forming hydrogen bonds at the C4-OH and carboxyl group with amino acid residues Ser311 (2.07 Å), Asn317 (1.99 Å), and Asp233 (1.98 Å). The calculated binding energy was −0.32 Kcal/mol. In Fig. 4D, paeonol (5) interacted with Asp233 (2.00 Å), Asn235 (2.91 Å) and Asn317 (2.04 Å) via hydrogen bonding. The paeonol (5) showed higher binding affinity than 4-hydroxybenzoic acid (2) and isoacetovanillon (6) with the binding energy of −0.38 Kcal/mol. In Fig. 4E, isoacetovanillon (6) produced two hydrogen bonds at C3-OH and the carbonyl group with Asp233 (1.99 Å) and Asn317 (1.98 Å) with a calculated binding energy, −0.28 Kcal/mol. The binding energy was in agreement with the experimental results (Table 2), showing that isoacetovanillon (6) is the compound with lower affinity. The change in the position of the hydroxyl group from C2 to C3 has significant implications for the interactions of the compounds with the enzyme, as we observed that isoacetovanillon (6) forms fewer hydrogen bonds with the amino residues compared to paeonol (5), which weakens the interaction of the compound with the residues in the deep pocket of the active site [48]. This is consistent with the noncompetitive inhibition mode of isoacetovanillon (6) (Fig. 3). In addition, the C2 and C3 hydroxyl group substitution in the structure of the compound appears to have a significant impact explaining the observed difference in α-glucosidase inhibition between compounds 5 and 6 (Table 1). Similar to compounds 5 and 6, we observed that the C3 and C5 substitution of the hydroxyl group affected the manner in which compounds 8 and 9 inhibited the enzyme (Table 2). The change in position of the hydroxyl group altered the selectivity of the compounds on the amino acid residues with which they interact (Fig. 4).

In the case of 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (8), two hydrogen bonds were generated at C2-OH and the carbonyl group with Ser311 and Lys342, their distances were 2.03 and 1.81 Å, respectively (Fig. 4F). 1-(2,3-Dihydroxy-4-methoxyphenyl)ethanone (9) was stabilised by the interactions of Asn317 with C2-OH and the carbonyl group with distances of 2.13 and 2.00 Å, and C3-OH interacted with Asp233 (2.15 Å) through hydrogen bonds (Fig. 3G). Gallic acid (10) formed hydrogen bonds with Asn235, Ser311, and Asn317, while methyl gallate (11) interacted with Asp233 and Asn317 through hydrogen bonds (Fig. 4H and I). The more hydrogen bonds formed between the compounds and the amino acids, the higher the affinity of the compounds. The binding energies of 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (8) and 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (9) were −0.87 and −0.92 Kcal/mol, while the values of gallic acid (10) and methyl gallate (11) were −1.34 and −1.17 Kcal/mol,
respectively. The results were in agreement with the kinetic analysis (Table 2), which showed that gallic acid (10) had the highest affinity, while 1-(2,5-dihydroxy-4-methoxyphenyl) ethanone (8) and 1-(2,3-dihydroxy-4-methoxyphenyl)ethanone (9) had a similar effect. All tested compounds showed high affinity for α-glucosidase compared to acarbose, as indicated by the lower binding energies of the compounds (ranging from $-1.34$ to $-0.28$) compared to the binding energy of acarbose ($-0.24$) (Table 4).

**Druglikeness/ADME/toxicity parameters**

Many potential therapeutics do not make it into clinical trials due to poor ADME properties and toxicity and untested tolerability. The failure of compounds in vivo or in clinical trials results in monetary and time losses. Therefore, screening compounds for drug-like, ADME and toxicity properties before further studies in vivo or clinical trials can minimise losses. In this work, the phenolic compounds were analysed for drug likeness, pharmacokinetic and toxicity properties (Table 4). The results showed that all the evaluated compounds complied with drug likeness rules except for compounds (2, 10 and 11) while the reference compound acarbose only complied with the MDDR-like rule. These results suggest that compounds (5, 6, 8 and 9) are less likely to cause problems related to oral bioavailability, showing the potential value of the compounds in developing a compound with good drug-like properties. The projections for ADME showed that the compounds had high blood-brain barrier (BBB) penetration and moderate Caco2 cell permeability, while acarbose showed moderate permeability for both. Compounds with high penetration of the BBB may be a dynamic regulatory interface for the brain and have the potential to develop effective central nervous system drugs [49]. Moreover, the projections for the phenolic compounds showed effective intestinal absorption and low plasma protein affinity along with optimal skin permeability. Overall, the projections showed an optimal pharmacokinetic profile of the compounds, as they met all the requirements for intestinal absorption as well as BBB penetration. Toxicity analysis predicted that the compounds are mutagenic, while acarbose is not. In addition, the compounds were predicted to be carcinogenic in mice, whereas acarbose is carcinogenic in rats. In addition, the predictions showed that the compounds have a low risk of cardiotoxicity, while the cardiotoxicity risk of acarbose was not clear.

**Conclusions**

The results of enzymatic activities and molecular docking suggested that hydroxylation of the aromatic ring was favourable for the inhibitory effect of phenolic compounds compared to methoxylation or hydrogenation. In addition, the position of the hydroxyl group and the substitution of the carboxyl group were important in improving the inhibitory activity of the compounds. The most effective phe

**References**

1. Tang X, Olatunji OJ, Zhou Y, Hou X. Allium tuberosum: Antidiabetic and hepatoprotective activities. Food Res Int 2017;102:681–9. https://doi.org/10.1016/j.foodres.2017.08.034
2. Organization WH Global report on diabetes: executive summary. World Health Organization; 2016. http://www.who.int/publications/i/item/9789241565257
3. Chamberlain JJ, Kalyani RR, Leal S, Rhinehart AS, Shubrook JH, Skolnik N, et al. Treatment of type 1 diabetes: synopsis of the 2017 American diabetes association standards of medical care in diabetes. Ann Intern Med 2017;167:493–8. https://doi.org/10.7326/M17-1259
4. Li S. Pharmacodynamic bioequivalence testing. J Clin Pharm Ther 2012;37:497–8. https://doi.org/10.1111/j.1365-2710.2012.01338.x
5. Kim JG, Jo SH, Ha KS, Kim SC, Kim YC, Apostolidis E, et al. Effect of long-term supplementation of low molecular weight chitosan oligosaccharide (GO2K A1) on fasting blood glucose and HbA1c in db/db mice model and elucidation of mechanism of action. BMC Complement Alter Med 2014;14:272 https://doi.org/10.1186/1472-6882-14-272
6. Lee KH, Ha KS, Jo SH, Lee CM, Kim YC, Chung KH, et al. Effect of long-term dietary arginyl-fructose (AF) on hyperglycemia and HbA1c in diabetic db/db mice. Int J Mol Sci 2014;15:8352–9. https://doi.org/10.3390/ijms1508352
7. Ye JP. Challenges in drug discovery for thiazolidinedione substituent. Acta Pharm Sin B 2011;1:137–42. https://doi.org/10.1016/j.apsb.2011.06.011
8. Tang D, Chen QB, Xin XL, Aisa HA. Anti-diabetic effect of three new norditerpenoid alkaloids in vitro and potential mechanism via P3K/AKT signaling pathway. Biomed Pharmacother 2017;87:145–52. https://doi.org/10.1016/j.biopha.2016.12.058
9. De-Yuan H, Kai-Yu P. A revision of the Paonia suffruticosa complex (Paeoniaceae). Nord J Bot 1999;19:289–300. https://doi.org/10.1111/j.1756-1051.1999.tb01115.x
10. Huang Q, Chen JJ, Pan Y, He XF, Wang Y, Zhang XM, et al. Pharma.
11. Anh HLT, Cuc NT, Tai BH, Yen PH, Nhiem NX, Thao DT, et al. Mangrove fungus Xylaria sp. From the South China sea coast. J Org Chem 2001;66:2522–5. https://doi.org/10.1021/jo015522r
12. Lau C, Chan C, Chan Y, Lau K, Lau T, Lam F, et al. Phenolic compounds: Xyloketals from mangrove fungus Xylaria sp. From the South China sea coast. J Org Chem 2001;66:6252–5. https://doi.org/10.1021/jo015522r
13. Ryu SH, Kim SB, Yeon SW, Turk A, Jo YH, Hwang BY, et al. Moutan and its active component paeonol. Phytomedicine 2000;7:91–7. https://doi.org/10.1078/0944-7113-999
14. Abdelli I, Benariba N, Adjdir S, Fekhikher Z, Daoud I, Terki M, et al. New diterpene polyester and phenolic compounds from Hylotelephium erythrostictum (Crassulaceae). Acta Pharm Sin B 2011;1:137–44. https://www.koreascience.or.kr/article/JAKO2019059600629.pdf
15. Pan Y, Gao Z, Huang XY, Chen JJ, Geng CA. Chemical and biological comparison of different parts of Maconia suffruticosa (Mudan) based on LCMS-IT-TOF and multi-evaluation in vitro. Ind Crops Prod 2020;144:112028 https://doi.org/10.1016/j.indcrop.2019.112028
16. Lau C, Chan C, Chan Y, Lau K, Lau T, Lam F, et al. Pharmacological investigations of the anti-diabetic effect of Cortex Moutan and its active component paenol. Phytomedicine 2007;14:778–84. https://doi.org/10.1016/j.phymed.2007.01.007
17. Nandi J, Hutcheson EL, Leadbeater NE. Combining photoredox catalysis and oxoammonium cations for the oxidation of aromatic α-glucosidase and α-amylase inhibitors – proof of concept and α-amylase inhibitor in cinnamon. J Agric Food Chem 2014;62:11465–71. https://doi.org/10.1021/jf5047283
18. Sang D, Yue H, Fu Y, Tian J. Cleavage of carboxylic esters by α-glucosidase and anti-dipeptidyl peptidase-IV activities of extracts and purified compounds from Vitis thunbergii var. taiwaniana. J Agric Food Chem 2015;63:6393–401. https://doi.org/10.1021/jacsaf.5b02069
19. Nandi J, Hutcheson EL, Leadbeater NE. Combining photo
docking studies of new thiazole derivatives. Bioorg Chem 2016;71:444–50. https://doi.org/10.1016/j.bioorg.2016.08.010
20. Meng Y, Su A, Yuan S, Zhao H, Tan S, Hu C, et al. Evaluation of total flavonoids, myricetin, and quercetin from Hovenia dulcis Thunb. as inhibitors of α-amylase and α-glucosidase. Plant Foods Hum Nutr 2016;71:444–9. https://doi.org/10.1007/s11130-016-0581-2
21. Khan KM, Qurban S, Salar U, Taha M, Hussain S, Perveen S, et al. Synthesis, in vitro α-glucosidase inhibitory activity and molecular docking studies of new thiazole derivatives. Bioorg Chem 2016;68:245–58. https://doi.org/10.1016/j.biochim.2016.08.010
22. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharm Toxicol Methods 2000;44:235–49. https://doi.org/10.1016/S1056-8719(00)00017-6
23. Seah S, Chan AM, Oprea T. The design of leadlike combinatorial libraries. Angew Chem Int Ed 1999;38:3743–8. https://doi.org/10.1002/(SICI)1521-3773(19991216)38:24<3743::aid-ANIE3743>3.0.CO;2-U
24. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharm Toxicol Methods 2000;44:235–49. https://doi.org/10.1016/S1056-8719(00)00017-6
25. Teague SJ, Davis AM, Oprea T. The design of leadlike combinatorial libraries. Angew Chem Int Ed 1999;38:3743–8. https://doi.org/10.1002/(SICI)1521-3773(19991216)38:24<3743::aid-ANIE3743>3.0.CO;2-U
26. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J Comb Chem 1999;1:55–68. https://doi.org/10.1021/cc980007i
38. Oprea TI. Property distribution of drug-related chemical databases. J Comput Aided Mol Des 2000;14:251–64. https://doi.org/10.1023/A:1008130001697

39. Ames BN, Gurney EG, Miller JA, Bartsch H. Carcinogens as frame-shift mutagens: metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc Natl Acad Sci 1972;69:3128–32. https://doi.org/10.1073/pnas.69.11.3128

40. Yang J, Wang X, Zhang C, Ma L, Wei T, Zhao Y, et al. Comparative study of inhibition mechanisms of structurally different flavonoid compounds on α-glucosidase and synergistic effect with acarbose. Food Chem 2021;347:129056 https://doi.org/10.1016/j.foodchem.2021.129056

41. Tan Y, Chang SK, Zhang Y. Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. Food Chem 2017;214:259–68. https://doi.org/10.1016/j.foodchem.2016.06.100

42. Mohan S, Eskandari R, Pinto BM. Naturally occurring sulfonium glucosidase inhibitors and their derivatives: a promising class of potential antidiabetic agents. Acc Chem Res 2014;47:211–25. https://doi.org/10.1021/ar400132g

43. Sim L, Quezada-Calvillo R, Sterchi EE, Nichols BL, Rose DR. Human intestinal maltase-glucanamylase: Crystal structure of the N-terminal catalytic subunit and basis of inhibition and substrate specificity. J Mol Biol 2008;375:782–92. https://doi.org/10.1016/j.jmb.2007.10.069

44. Jones K, Sim L, Mohan S, Kumarasamy J, Liu H, Avery S, et al. Mapping the intestinal alpha-glucogenic enzyme specificities of starch digesting maltase-glucanamylase and sucrase-isomaltase. Bioorg Med Chem 2011;19:3929–34. https://doi.org/10.1016/j.bmc.2011.05.033

45. Zhang CC, Geng CA, Huang XY, Zhang XM, Chen JJ. Anti-diabetic stilbenes from peony seeds with PTP1B, α-glucosidase, and DPPIV inhibitory activities. J Agric Food Chem 2019;67:6765–72. https://doi.org/10.1021/acs.jafc.9b01193

46. Zeng L, Ding H, Hu X, Zhang G, Gong D. Galangin inhibits α-glucosidase activity and formation of non-enzymatic glycation products. Food Chem 2019;271:70–9. https://doi.org/10.1016/j.foodchem.2018.07.148

47. Zeng L, Zhang G, Lin S, Gong D. Inhibitory mechanism of apigenin on α-glucosidase and synergy analysis of flavonoids. J Agric Food Chem 2016;64:6939–49. https://doi.org/10.1021/acs.jafc.6b02314

48. Proença C, Freitas M, Ribeiro D, Oliveira EF, Sousa JL, Tomé SM, et al. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. J Enzym Inhib Med Chem 2017;32:1216–28. https://doi.org/10.1080/14756366.2017.1368503

49. Ma XL, Chen C, Yang J. Predictive model of blood-brain barrier penetration of organic compounds. Acta Pharm Sin 2005;26:500–12. https://doi.org/10.1111/j.1745-7254.2005.00068.x

**Affiliations**

**Po-Chun Chen**1,2 · **Bongani Sicelo Dlamini**3 · **Chiy-Rong Chen**4 · **Yueh-Hsiung Kuo**5,6,7 · **Wen-Ling Shih**8 · **Yun-Sheng Lin**9 · **Chien-Hsing Lee**10,11,12 · **Chi-I Chang**8

1 Department of Radiation oncology, Pingtung Christian Hospital, Pingtung 90054, Taiwan
2 Graduate Institute of Bioresources, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
3 Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
4 Department of Life Science, National Taitung University, Taitung 95002, Taiwan
5 Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 40402, Taiwan
6 Department of Biotechnology, Asia University, Taichung 41354, Taiwan
7 Chinese Medicine Research Center, China Medical University, Taichung 40402, Taiwan
8 Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
9 Department of Biological Science and Technology, Meiho University, Pingtung 91201, Taiwan
10 Department of Pharmacology, Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
11 Department of Pharmacology, School of Medicine; School of Post-Baccalaureate Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
12 Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung 80708, Taiwan