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Inhibition of α-Glucosidase by Abietane-Type Diterpenoids Isolated from Roots of *Salvia miltiorrhiza*

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Abstract – *Salvia miltiorrhiza* is a traditional medicinal plant used in Asian medicine for various therapeutic purposes. This plant contains numerous bioactive secondary metabolites, particularly abietane-type diterpenoids. In this study, 16 abietane-type diterpenoids were isolated from *S. miltiorrhiza* root extracts and structurally identified through advanced spectroscopic techniques. Among them, tanshinone IIA (**6**) and 15,16-dihydrotanshinone I (**11**) exhibited potent α-glucosidase inhibition, with IC_{50} values of 48.38 ± 0.57 and 48.02 ± 0.47 μM, respectively. Enzyme kinetic studies revealed that these compounds served as non-competitive inhibitors of α-glucosidase. Our findings indicate that natural compounds from *S. miltiorrhiza* show promise as safe and effective α-glucosidase inhibitors, providing an alternative approach to diabetes treatment. This study contributes to the growing interest in utilizing natural sources for α-glucosidase inhibition and their potential application in healthcare and disease management.
Keywords – *Salvia miltiorrhiza*, diterpenoid, α-glucosidase inhibition, enzyme kinetics

Introduction

α-Glucosidase, a crucial enzyme in carbohydrate digestion, is primarily located at the border of the small intestine, where it acts on glycosidic bonds.[1] This enzyme plays a key role in converting carbohydrates into absorbable monosaccharides.[2,3] α-Glucosidase inhibitors are oral antidiabetic medications used to manage type 2 diabetes by preventing carbohydrate digestion.[4] Given its important role in metabolizing polysaccharides, particularly in regulating plasma blood levels, the investigation of α-glucosidase activity and its inhibition holds significant interest. Several α-glucosidase inhibitors, such as miglitol, voglibose, and acarbose, have been employed as antidiabetic drugs.[5,6] These drugs function by inhibiting α-glucosidase activity, preventing the release of digested single sugars during carbohydrate digestion.[7] However, these medications have been associated with side effects, such as stomach discomfort and diarrhea.[8] Consequently, the discovery of potential α-glucosidase inhibitors with no side effects and safety for health, especially from natural sources, is highly necessary. Furthermore, α-glucosidase also participates in immune responses related to various diseases, including Pompe disease,[9] P-glycoprotein trafficking,[10] and cancer.[11]

Salvia miltiorrhiza Bunge, a perennial plant belonging to the Salvia genus in the Lamiaceae family, has a long history of use.[12] Its roots and rhizomes have been employed in ethnopharmacology for over a thousand years and have been officially documented in the Chinese pharmacopeia since 1963.[13] Traditional Asian medicine has reported various therapeutic properties of this plant, including enhancing blood circulation, reducing inner stress, and treating cardiovascular and cerebrovascular diseases as well as chronic renal failure.[14-16] In addition, *S. miltiorrhiza* is frequently used with other herbs to treat various diseases,

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including diabetes,[17] coronary heart disease,[18] osteoporosis,[19] Alzheimer's,[20] and cancer.[21] Phytochemical analysis has revealed that the major components contained in this plant are lipophilic diterpenoids, including various tanshinone analogues, as well as hydrophilic phenolic compounds like salvianolic acids and their derivatives.[12,22,23] Tanshinones isolated from *S. miltiorrhiza*, including cryptotanshinone, tanshinol B, and dehydrodanshenol A, were previously reported to exhibit significant protein tyrosine phosphatase 1B (PTP1B) inhibition, with IC_{50} values ranging from 4.7 to 8.5 μM.[24] Furthermore, these active compounds could tightly bind to the allosteric site of the target enzyme through *in silico* studies.

In our ongoing efforts to identify bioactive secondary metabolites from Korean medicinal plants, we successfully isolated 16 compounds from the roots of *S. miltiorrhiza*. The structures of all isolated compounds were determined using state-of-the-art spectroscopic techniques. Moreover, the *in vitro* α-glucosidase inhibitory activity of all isolated compounds was evaluated. To gain a more comprehensive understanding of the inhibition mechanism and the inhibition constant, kinetic studies involving the active compounds and the α-glucosidase enzyme were conducted.

Experimental

General experimental procedures – Experiments in this study were conducted similarly to our previously described procedures.[25-27] Compound isolation processes were carried out through column chromatography (CC) using silica gel 60 (0.063-0.200 mm) and RP-18 (40-63 μM) (Merck, Darmstadt, Germany). HPLC procedures were performed on a Waters Alliance HPLC system (MA, USA) featuring a 1525 Binary pump, a Waters 2998 PDA detector, and a C18 column (21.2 × 250 mm, 10 μM). NMR data were obtained on a Bruker Avance Digital 500 MHz spectrometer (Bruker, Karlsruhe, Germany), *J* in Hz. TLC was conducted using glass plates precoated with silica gel 60 F_{254} and RP-18 F_{254s} (Merck). The TLC plates were examined under UV light at 254 nm and 356 nm to visualize the isolated compounds. Additionally, the plates were visualized by spraying them with a 10% sulfuric acid solution, followed by heating at 110°C for 1 min.

Plant material – *S. miltiorrhiza* roots were obtained from the Kyungdong market (Dongdaemun-gu, Seoul) in February 2015. The plant was authenticated by Professor Byung Sun Min, Daegu Catholic University, and deposited at the Pharmacognosy laboratory, College of Pharmacy, Kyungpook National University (voucher code: 18ASM6).

Extraction and isolation – The dried roots of *S. miltiorrhiza* (10.0 kg) were sliced into small pieces and extracted three times using MeOH (15 L × 3 h) under reflux conditions. After concentration, the MeOH residue (1.0 kg) was dissolved with 2 L of water and subsequently partitioned with $CHCl_3$ and EtOAc to obtain $CHCl_3$ (74.3 g) and EtOAc (55.4 g) extracts, respectively. The detailed isolation procedure was described in our previous study.[12]

Ferruginol (1) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 2.17 (1H, m, H-1a), 1.39 (1H, dd, *J* = 13.1, 3.6 Hz, H-1b), 1.85 (1H, m, H-2a), 1.68 (1H, m, H-2b), 1.47 (1H, m, H-3a), 1.20 (1H, m, H-3b), 1.32 (1H, m, H-5), 1.73 (1H, m, H-6a), 1.63 (1H, m, H-6b), 2.82 (2H, m, H-7), 6.63 (1H, br s, H-11), 6.83 (1H, s, H-14), 3.11 (1H, m, H-15), 1.23 (each 3H, dd, *J* = 8.2, 6.9 Hz, H-16, H-17), 0.91 (3H, s, H-18), 0.94 (3H, s, H-19), 1.17 (3H, d, *J* = 0.4 Hz, H-20); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 39.0 (C-1), 19.4 (C-2), 41.8 (C-3), 33.6 (C-4), 50.5 (C-5), 19.5 (C-6), 29.9 (C-7), 127.5 (C-8), 148.8 (C-9), 37.7 (C-10), 111.1 (C-11), 150.8 (C-12), 131.5 (C-13), 126.8 (C-14), 27.0 (C-15), 22.9 (C-16), 22.7 (C-17), 21.8 (C-18), 33.5 (C-19), 24.9 (C-20).[28]

Deoxyneocryptotanshinone (2) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.24 (2H, t, *J* = 6.4 Hz, H-1), 1.83 (2H, m, H-2), 1.69 (2H, m, H-3), 7.88 (1H, d, *J* = 8.2 Hz, H-6), 7.94 (1H, d, *J* = 8.2 Hz, H-7), 3.37 (1H, m, H-15), 1.25 (3H, s, H-16), 1.27 (3H, s, H-17), 1.33 (each 3H, s, H-18, H-19); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.7 (C-1), 19.0 (C-2), 37.7 (C-3), 34.6 (C-4), 152.2 (C-5), 133.1 (C-6), 125.4 (C-7), 132.6 (C-8), 126.9 (C-9), 139.9 (C-10), 183.2 (C-11), 154.4 (C-12), 124.3 (C-13), 184.1 (C-14), 24.2 (C-15), 19.3 (C-16), 19.3 (C-17), 31.1 (C-18), 31.1 (C-19).[29]

Normiltirone (3) – Amorphous powder; ^{1}H and ^{13}C NMR data, see reference.[12]

Salviprzol A (4) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.23 (1H, m, H-1a), 2.97 (1H, m, H-1b), 1.90 (1H, m, H-2a), 1.73 (1H, m, H-2b), 1.74 (2H, m, H-3), 7.80 (1H, d, *J* = 8.3 Hz, H-6), 7.77 (1H, d, *J* = 8.3 Hz, H-7), 2.06 (3H, s, H-17), 3.28 (1H, d, *J* = 13.6 Hz, H-18a), 2.92 (1H, d, *J* = 13.6 Hz, H-18b), 2.40 (3H, s, H-20), 1.33 (each 3H, s, H-21, H-22); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.9 (C-1), 19.4 (C-2), 38.0 (C-3), 34.6 (C-4), 149.7 (C-5), 134.6 (C-6), 124.2 (C-7), 138.3 (C-8), 125.1 (C-9), 140.7 (C-10), 195.9 (C-11), 80.6 (C-12), 152.9 (C-13), 99.8 (C-14), 128.1 (C-15), 170.5 (C-16), 9.3 (C-17), 49.5 (C-18), 209.7 (C-19), 33.5 (C-20), 32.0 (C-21), 32.0 (C-22).[30]

Cryptotanshinone (5) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.22 (2H, t, *J* = 6.4 Hz, H-1), 1.79 (2H, m, H-2), 1.65 (2H, m, H-3), 7.63 (1H, d, *J* = 8.2 Hz,

H-6), 7.49 (1H, d, J = 8.2 Hz, H-7), 4.88 (1H, t, J = 9.5 Hz, H-15a), 4.36 (1H, dd, J = 9.5, 6.0 Hz, H-15b), 3.59 (1H, m, H-16), 1.35 (3H, d, J = 6.8 Hz, H-17), 1.30 (each 3H, s, H-18, H-19); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.8 (C-1), 19.2 (C-2), 38.0 (C-3), 34.9 (C-4), 143.9 (C-5), 132.7 (C-6), 122.7 (C-7), 128.5 (C-8), 126.5 (C-9), 152.4 (C-10), 184.4 (C-11), 175.9 (C-12), 118.4 (C-13), 171.1 (C-14), 81.6 (C-15), 34.7 (C-16), 19.0 (C-17), 32.1 (C-18), 32.2 (C-19).[31]

Tanshinone IIA (6) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.18 (2H, t, J = 6.4 Hz, H-1), 1.81 (2H, m, H-2), 1.63 (2H, m, H-3), 7.63 (1H, d, J = 8.2 Hz, H-6), 7.55 (1H, d, J = 8.2 Hz, H-7), 7.22 (1H, q, J = 1.2 Hz, H-15), 2.26 (3H, d, J = 1.3 Hz, H-17), 1.31 (each 3H, s, H-18, H-19); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.9 (C-1), 19.2 (C-2), 38.0 (C-3), 34.9 (C-4), 144.4 (C-5), 133.5 (C-6), 120.4 (C-7), 127.7 (C-8), 126.5 (C-9), 150.4 (C-10), 183.9 (C-11), 175.9 (C-12), 120.0 (C-13), 161.9 (C-14), 141.4 (C-15), 121.3 (C-16), 8.9 (C-17), 32.1 (C-18), 32.1 (C-19).[31]

Tanshinol B (7) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.10 (2H, m, H-1), 1.67 (1H, m, H-2a), 1.87 (1H, m, H-2b), 1.80 (2H, m, H-3), 7.95 (1H, d, J = 7.9 Hz, H-6), 7.60 (1H, d, J = 7.9 Hz, H-7), 7.71 (1H, br s, H-15), 2.16 (3H, s, H-17), 1.40 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 28.7 (C-1), 19.5 (C-2), 37.6 (C-3), 69.0 (C-4), 148.3 (C-5), 133.5 (C-6), 119.9 (C-7), 127.6 (C-8), 125.8 (C-9), 142.4 (C-10), 182.5 (C-11), 175.0 (C-12), 120.1 (C-13), 160.4 (C-14), 142.3 (C-15), 119.6 (C-16), 8.5 (C-17), 31.3 (C-18).[32]

Tanshinone IIB (8) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.17 (2H, dt, J = 13.5, 6.5 Hz, H-1), 7.65 (1H, d, J = 8.1 Hz, H-6), 7.52 (1H, d, J = 8.1 Hz, H-7), 7.21 (1H, br s, H-15), 2.26 (3H, s, H-17), 1.27 (3H, s, H-18), 3.79 (1H, d, J = 11.1 Hz, H-19a), 3.61 (1H, d, J = 11.1 Hz, H-19b); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.8 (C-1), 19.0 (C-2), 32.4 (C-3), 40.3 (C-4), 146.3 (C-5), 133.8 (C-6), 119.9 (C-7), 127.8 (C-8), 126.7 (C-9), 146.1 (C-10), 183.4 (C-11), 175.6 (C-12), 121.3 (C-13), 161.6 (C-14), 120.4 (C-15), 141.4 (C-16), 8.9 (C-17), 26.9 (C-18), 71.6 (C-19).[33]

Tanshinonal (9) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.24 (2H, m, H-1), 1.85 (2H, dd, J = 12.3, 6.3 Hz, H-2), 2.07 (1H, m, H-3a), 1.66 (1H, m, H-3b), 7.33 (1H, d, J = 8.1 Hz, H-6), 7.60 (1H, d, J = 8.1 Hz, H-7), 7.25 (1H, d, J = 1.1 Hz, H-15), 2.27 (3H, d, J = 0.9 Hz, H-17), 1.46 (3H, s, H-18), 9.49 (1H, br s, H-19); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.2 (C-1), 18.7 (C-2), 30.1 (C-3), 51.3 (C-4), 139.9 (C-5), 135.5 (C-6), 120.7 (C-7), 129.0 (C-8), 127.0 (C-9), 145.6 (C-10), 183.4 (C-11), 175.5 (C-12), 121.5 (C-13), 161.1 (C-14), 120.7 (C-15), 141.9 (C-16), 8.9 (C-17), 24.1 (C-18), 201.8 (C-19).[34]

Methyl tanshinonate (10) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.23 (2H, m, H-1), 2.29 (1H, m, H-2a), 1.74 (1H, m, H-2b), 1.82 (2H, td, J = 13.7, 6.8 Hz, H-3), 7.56 (1H, d, J = 8.1 Hz, H-6), 7.48 (1H, d, J = 8.1 Hz, H-7), 7.23 (1H, br s, H-15), 2.26 (3H, s, H-17), 1.58 (3H, s, H-18), 3.67 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 29.2 (C-1), 19.3 (C-2), 34.2 (C-3), 47.3 (C-4), 143.2 (C-5), 135.1 (C-6), 120.4 (C-7), 126.7 (C-8), 128.7 (C-9), 141.7 (C-10), 183.5 (C-11), 175.6 (C-12), 121.4 (C-13), 161.5 (C-14), 120.4 (C-15), 144.5 (C-16), 8.9 (C-17), 27.7 (C-18), 177.1 (C-19), 52.6 (C-20).[34]

15,16-Dihydrotanshinone I (11) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 9.28 (1H, d, J = 8.9 Hz, H-1), 7.56 (1H, dd, J = 8.9, 6.9 Hz, H-2), 7.39 (1H, d, J = 6.9 Hz, H-3), 8.29 (1H, dd, J = 8.7, 0.9 Hz, H-6), 7.74 (1H, d, J = 8.7 Hz, H-7), 4.97 (1H, t, J = 9.5 Hz, H-15a), 4.43 (1H, dd, J = 9.5, 6.3 Hz, H-15b), 3.65 (1H, m, H-16), 1.41 (3H, d, J = 6.8 Hz, H-17), 2.69 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 126.3 (C-1), 130.6 (C-2), 128.4 (C-3), 135.1 (C-4), 134.8 (C-5), 132.2 (C-6), 118.8 (C-7), 129.0 (C-8), 125.5 (C-9), 132.1 (C-10), 184.4 (C-11), 175.6 (C-12), 120.4 (C-13), 170.7 (C-14), 81.8 (C-15), 34.9 (C-16), 18.9 (C-17), 20.0 (C-18).[35]

Tanshinone I (12) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 9.24 (1H, d, J = 8.9 Hz, H-1), 7.54 (1H, dd, J = 8.9, 6.9 Hz, H-2), 7.29 (1H, d, J = 6.9 Hz, H-3), 8.28 (1H, d, J = 8.7 Hz, H-6), 7.79 (1H, d, J = 8.7 Hz, H-7), 7.34 (1H, d, J = 6.9 Hz, H-15), 2.28 (3H, d, J = 1.3 Hz, H-17), 2.68 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 124.9 (C-1), 130.8 (C-2), 128.5 (C-3), 135.1 (C-4), 134.1 (C-5), 132.9 (C-6), 118.8 (C-7), 129.8 (C-8), 123.5 (C-9), 132.9 (C-10), 183.6 (C-11), 175.8 (C-12), 121.9 (C-13), 161.3 (C-14), 142.2 (C-15), 120.6 (C-16), 8.9 (C-17), 20.0 (C-18).[35]

Dehydrodanshenol A (13) – Amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 9.02 (1H, d, J = 8.8 Hz, H-1), 7.50 (1H, m, H-2), 7.40 (1H, d, J = 6.9 Hz, H-3), 8.12 (1H, d, J = 8.8 Hz, H-6), 7.93 (1H, d, J = 8.8 Hz, H-7), 7.34 (1H, br s, H-15), 2.33 (3H, s, H-17), 2.73 (3H, s, H-18), 3.36 (1H, d, J = 13.5 Hz, H-19a), 3.05 (1H, d, J = 13.5 Hz, H-19b), 2.03 (3H, s, H-21); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C 125.4 (C-1), 127.0 (C-2), 128.0 (C-3), 135.4 (C-4), 134.1 (C-5), 126.1 (C-6), 118.8 (C-7), 120.8 (C-8), 137.1 (C-9), 131.5 (C-10), 80.7 (C-11), 197.4 (C-12), 120.3 (C-13), 162.4 (C-14), 116.7 (C-15), 141.6 (C-16), 8.9 (C-17), 20.4 (C-18), 56.3 (C-19), 205.3 (C-20), 32.2 (C-21).[36]

Isosalviamide F (14) – Amorphous powder; ^{1}H and

^{13}C NMR data, see reference.[12]

Isosalviamide G (15) – Amorphous powder; ^{1}H and ^{13}C NMR data, see reference.[12]

Isosalviamide H (16) – Amorphous powder; ^{1}H and ^{13}C NMR data, see reference.[12]

Inhibitory activity on α-glucosidase – The assay for inhibiting α-glucosidase was carried out following established procedures described in previous studies.[5,37,38] To initiate the enzymatic reaction, 20 μL of α-glucosidase enzyme from *Saccharomyces cerevisiae* (EC 3.2.1.20, G5003; Sigma-Aldrich, MO, USA) at a concentration of 0.1 unit/mL was added into a 96-well plate, which contained 20 μL of test compounds and 20 μL of phosphate buffer (100 mM, pH = 6.8). Afterward, the enzyme reaction was started by adding 20 μL substrate *p*-NPG (2.5 mM) and incubated at 37°C. After 10 min, 80 μL Na_2CO_3 (0.2 M) was used for each well to finalize the reaction. The absorbance was measured at 405 nm using a PHOmo microplate reader (Autobio Labtec Instruments, Zhengzhou, China). Acarbose (A8980; Sigma-Aldrich) was used as a positive control. The percentage of α-glucosidase inhibitory activity was calculated using the following equation:

Inhibitory activity (%) = [(⊿Control − ⊿Inhibitor)/⊿Control] × 100, where ⊿Control and ⊿Inhibitor represent the signal intensities of the control and inhibitor solutions after a 10 min incubation period, respectively.

The determination and calculation of IC_{50} values were performed using Microsoft Excel (Microsoft 365, WA, USA). The results were expressed as the means ± standard error based on data from at least three experiments. Statistical significance ($p < 0.05$) was calculated through the application of Duncan's tests and ANOVA.

Enzyme kinetics – To understand the inhibition mechanism of α-glucosidase by active compounds, two graphical kinetic methods, including Lineweaver–Burk and Dixon plots, were carried out.[39] The Dixon plot was em-

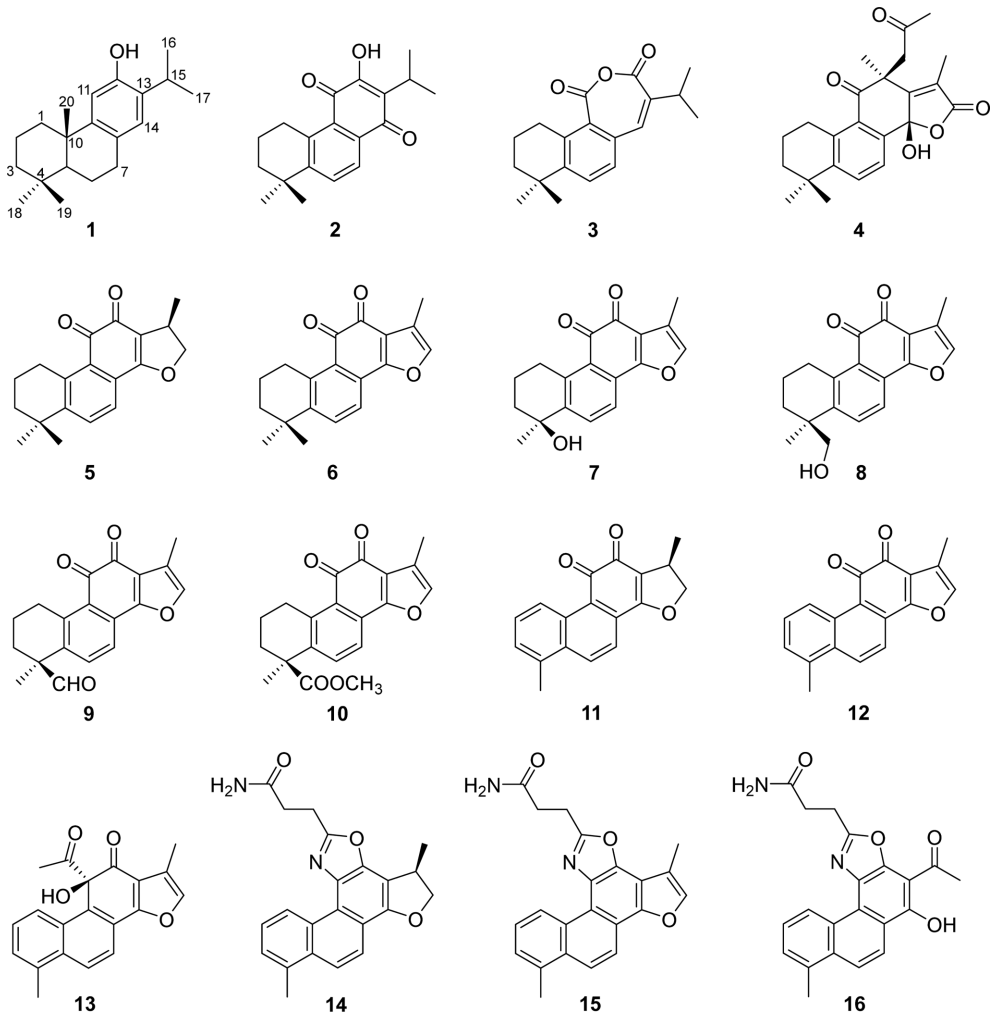

Fig. 1. Abietane-type diterpenoids (**1**–**16**) isolated from the roots of *S. miltiorrhiza*.

ployed to assess enzymatic reactions across different concentrations of substrate, specifically 0.625, 1.25, and 2.5 mM. The inhibition constant (K_i) was determined *via* the Dixon plot, where the x-axis value was taken as -K_i.

In addition, the Lineweaver–Burk plot was employed to identify the α-glucosidase inhibition mode of test compounds through various concentrations (0, 19.75, 48, and 83.75 μM for compound **6**; and 0, 25.25, 55.25, and 100 μM for compound **11**).

Results and Discussion

S. miltiorrhiza roots were extracted with methanol. After removing the solvent, the residue was mixed with water and partitioned into $CHCl_3$ and EtOAc extracts. These fractions were separated using chromatography techniques, leading to the isolation of 16 compounds (Fig. 1). Their chemical structures were elucidated through NMR spectroscopic analysis, including ^{1}H and ^{13}C-NMR, as well as literature comparisons: ferruginol (**1**),[28] deoxyneocryptotanshinone (**2**),[29] normiltirone (**3**),[12] salviprzol A (**4**),[30] cryptotanshinone (**5**),[31] tanshinone IIA (**6**),[31] tanshinol B (**7**),[32] tanshinone IIB (**8**),[33] tanshinonal (**9**),[34] methyl tanshinonate (**10**),[34] 15,16-dihydrotanshinone I (**11**),[35] tanshinone I (**12**),[35] dehydrodanshenol A (**13**),[36] and isosalviamides F–H (**14**–**16**).[12]

Compound **6** was yielded as an amorphous powder. The ^{1}H-NMR spectrum of **6** displayed signals characteristic of an abietane-type diterpenoid, including three methyl groups at δ_H 2.26 (3H, d, J = 1.3 Hz, H-17) and 1.31 (each 3H, s, H-18, H-19), three methylene groups at δ_H 3.18 (2H, t, J = 6.4 Hz, H-1), 1.81 (2H, m, H-2), and 1.63 (2H, m, H-3), a methine proton at δ_H 7.22 (1H, q, J = 1.2 Hz, H-15), and two aromatic protons at δ_H 7.63 (1H, d, J = 8.2 Hz, H-6) and 7.55 (1H, d, J = 8.2 Hz, H-7). In addition, the ^{13}C-NMR and DEPT spectra of compound **6** exhibited signals of two carbonyl groups at δ_C 183.9 (C-11) and 175.9 (C-12), nine olefinic carbons at δ_C 161.9, 150.4, 144.4, 141.4, 133.5, 127.7, 126.5, 121.3, 120.4, and 120.0, and three methyl groups at δ_C 32.1, 32.1 and 8.9. The position of the methyl group at C-17 was determined based on the HMBC correlations of H-17 with C-13, C-15, and C-16, whereas the positions of the two remaining methyl groups were determined based on the HMBC correlations of H-18 and H-19 with C-3, C-4, and C-5. This analysis, along with published data from the literature, led to the elucidation of compound **6** as tanshinone IIA.[31]

Compound **11** was obtained as an amorphous powder. The ^{1}H-NMR spectrum of this compound showed the

Table 1. α-Glucosidase inhibtion of abietane-type diterpenoids from *S. miltiorrhiza*

Compounds	α-Glucosidase inhibition		
	IC_{50} (μM)	Inhibition type[a]	K_i (μM)[b]
1	> 100	–	–
2	58.06 ± 0.97	–	–
3	67.06 ± 0.97	–	–
4	> 100	–	–
5	> 100	–	–
6	48.38 ± 0.57	Non-competitive	47.17 ± 0.41
7	> 100	–	–
8	55.13 ± 1.03	–	–
9	89.33 ± 0.72	–	–
10	> 100	–	–
11	48.02 ± 0.47	Non-competitive	45.41 ± 0.40
12	51.69 ± 0.86	–	–
13	> 100	–	–
14	> 100	–	–
15	> 100	–	–
16	> 100	–	–
Acarbose[c]	222.62 ± 1.98	–	–

[a]Determined by Lineweaver–Burk plots.
[b]Determined by Dixon plots.
[c]Positive control.
(–) No test.

presence of two methyl groups at δ_H 1.41 (3H, d, J = 6.8 Hz, H-17) and 2.69 (3H, s, H-18), a methylene group at δ_H 4.97 (1H, t, J = 9.5 Hz, H-15a) and 4.43 (1H, dd, J = 9.5, 6.3 Hz, H-15b), and five aromatic protons at δ_H 9.28 (1H, d, J = 8.9 Hz, H-1), 7.56 (1H, dd, J = 8.9, 6.9 Hz, H-2), 7.39 (1H, d, J = 6.9 Hz, H-3), 8.29 (1H, dd, J = 8.7, 0.9 Hz, H-6), and 7.74 (1H, d, J = 8.7 Hz, H-7). They correlated with the carbons resonating at δ_H 18.9, 20.0, 81.8, 126.3, 130.6, 128.4, 132.2, and 118.8 in the HMQC spectrum, respectively. Furthermore, the ^{13}C-NMR spectrum of compound **11** showed signals of two carbonyl groups at δ_C 184.4 (C-11) and 175.6 (C-12), and seven quaternary carbons at δ_C 135.1 (C-4), 134.8 (C-5), 129.0 (C-8), 125.5 (C-9), 132.1 (C-10), 120.4 (C-13), and 170.7 (C-14). This evidence, similar to that reported for compound **6**, indicates that compound **11** is an abietane-type diterpenoid. Compared with the literature, compound **11** was clearly identified as 15,16-dihydrotanshinone I.[35]

The examination of their ability to inhibit α-glucosidase activity was evaluated on all isolated compounds (**1**–**16**). For reference, acarbose, a well-known α-glucosidase inhibitor, served as positive control and exhibited an IC_{50} value of 222.62 ± 1.98 μM (Table 1).

Compounds **2** and **3** demonstrated moderate α-glucosidase inhibition, with IC_{50} values of 58.06 ± 0.97 and 67.06 ± 0.97 μM, respectively. Compound **6**, which had an additional double bond at C-15 and C-16 in comparison to compound **5**, displayed α-glucosidase inhibitory activity with an IC_{50} value of 48.38 ± 0.57 μM, while compound **5** showed no α-glucosidase inhibition. This suggests that the double bond at C-15 and C-16 may contribute to the inhibitory activity of abietane diterpenoids against α-glucosidase.

Compounds **7**–**10** shared a similar structure with compound **6**, except for the differences in the functional groups at C-19. They exhibited weaker α-glucosidase inhibitory activity compared to compound **6**, with IC_{50} values ranging from 55.13 to more than 100 μM. This

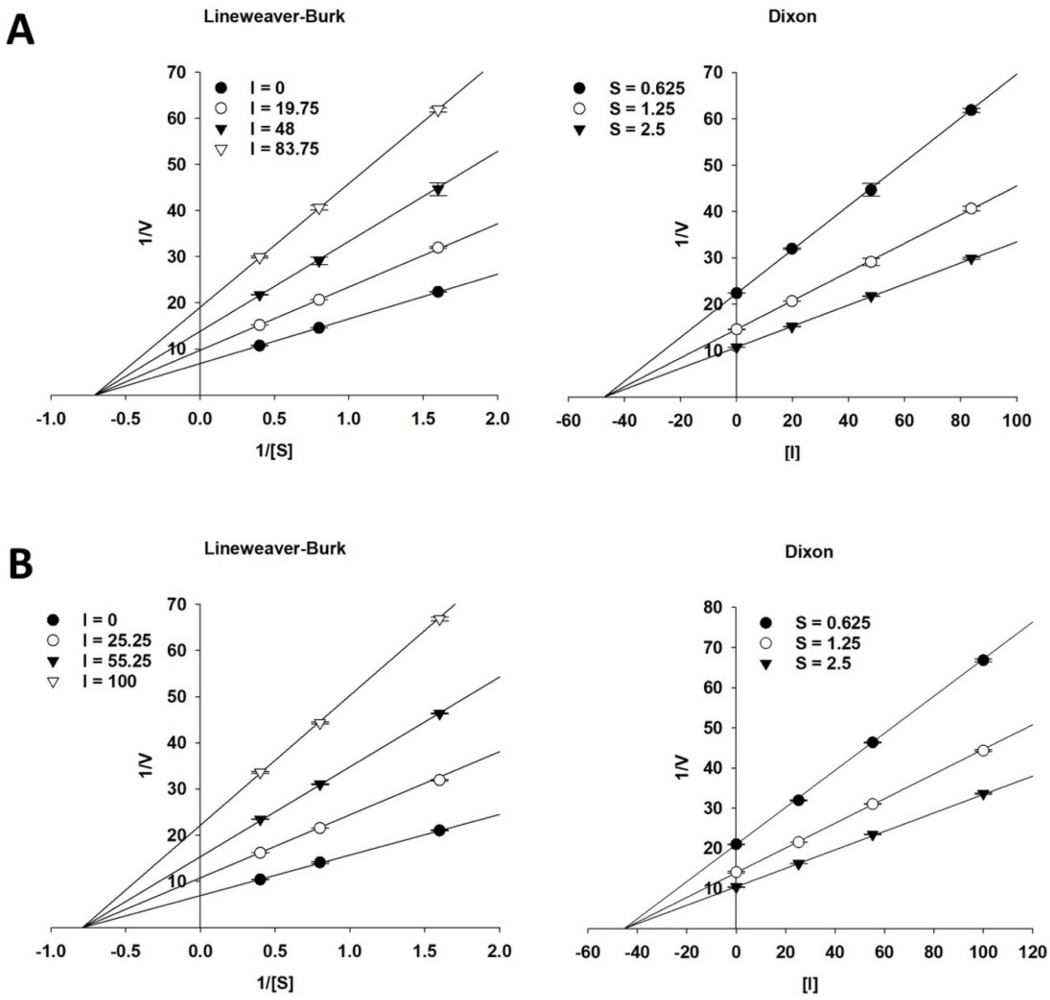

Fig. 2. Lineweaver−Burk and Dixon plots for the inhibition of α-glucosidase by active compounds **6** (**A**) and **11** (**B**).

evidence considers the idea that less polar substituents in the structure of abietane diterpenoids may influence α-glucosidase inhibition.

Compounds **11** and **12**, which had an aromatic ring in ring A and lacked a methyl group linked to C-4, exhibited α-glucosidase inhibition with IC_{50} values of 48.02 ± 0.47 and 51.69 ± 0.86 μM, respectively. The remaining compounds (**13**–**16**) showed no α-glucosidase inhibitory effect.

In our research framework, we have chosen the two most potent active compounds (**6** and **11**) to continue investigating the inhibition mechanism and determining the inhibition constant (K_i) through enzyme kinetics studies. Lineweaver–Burk and Dixon plots were employed. The Lineweaver–Burk graph showed that the spot where the lines of the inhibitors crossed on the x-axis or y-axis indicated whether the inhibition was non-competitive or competitive respectively.[39,40]

As presented in Fig. 2, compounds **6** and **11** exhibited a family of intersecting straight lines on the x-axis (1/[S]-axis), indicating that these active compounds served as a non-competitive inhibitor of α-glucosidase. The K_i values for compounds **6** and **11** were determined through Dixon plots and calculated to be 47.17 ± 0.41 and 45.41 ± 0.40 μM, respectively.

In conclusion, 16 abietane-type diterpenoids (**1**–**16**) were isolated from extracts of *S. miltiorrhiza* roots. The structures of all compounds were determined through extensive spectroscopic analysis, including ^{1}H- and ^{13}C-NMR, in conjunction with data from existing literature. Our investigation into α-glucosidase inhibitory activity revealed that tanshinone IIA (**6**) and 15,16-dihydrotanshinone I (**11**) exhibited the most potent α-glucosidase inhibition, with IC_{50} values of 48.38 ± 0.57 and 48.02 ± 0.47 μM, respectively, surpassing the positive control acarbose (IC_{50} = 222.62 ± 1.98 μM). Our kinetic studies demonstrated that these active compounds acted as non-competitive inhibitors of α-glucosidase. These promising results establish that natural compounds from *S. miltiorrhiza* with α-glucosidase inhibition potential could serve as a safe and primary complement to treating diabetes and related diseases. These leading secondary metabolites may warrant further exploration through *in silico* approaches or *in vivo* investigations of their α-glucosidase inhibitory activities using animal models.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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