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PTP1B inhibitors from the seeds of *Iris sanguinea* and their insulin mimetic activities via AMPK and ACC phosphorylation

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ABSTRACT

To find PTP1B inhibitors from natural products, two new compounds (**1** and **2**), along with nine known compounds (**3**–**11**), were isolated from a methanol-soluble extract of *Iris sanguinea* seeds. The structures of compounds **1** and **2** were determined based on extensive spectroscopic data analysis including UV, IR, NMR, and MS. The IC_{50} value of compound **5** on protein tyrosine phosphatase 1B (PTP1B) inhibitory activity is 7.30 ± 0.88 μM with a little activity compared to the IC_{50} values of the tested positive compound. Compound **5** significantly enhanced glucose uptake and activation of pACC, pAMPK and partially Erk1/2 signaling. These results suggest that compound **5** from *Iris sanguinea* seeds are utilized as both PTP1B inhibitors and regulators of glucose uptake. These beneficial effects could be applied to treat metabolic diseases such as diabetes and obesity.

Diabetes mellitus (DM) has emerged as a major threats to human health, and the expected number of diabetic patients will exceed 300 million by 2025 globally.[1,2] DM is characterized by consistently high sugar levels in the bloodstream due to the insulin deficiency, insulin resistance or both.[3] Excessive glucose in the blood damages blood vessels and nerves, leading to various diseases such as hypertension, cardiovascular disease, blindness, stroke, amputations, kidney and dental diseases.[4–6] Aside from insulin, several classes of oral medications are being used clinically to treat DM, including sulfonylureas, meglitinides, alpha-glucosidase inhibitors, DPP-4 inhibitors, biguanides, incretin mimetics and thiazolidinediones.[7–9] However it is still difficult for many patients to reduce their levels of glycosylated hemoglobin (HbA1C) to target values.[10]

Protein tyrosine phosphatases (PTPs) play a pivotal role in cellular signaling through the regulation of tyrosine phosphorylation of target proteins and are considered as promising next-generation drug targets to treat type 2 diabetes and obesity.[11] Among the PTP family of enzymes, PTP1B, an intracellular PTP, has been shown to negatively regulate insulin and leptin receptor signaling through dephosphorylating IR (insulin receptor) kinase and JAK2 (Janus kinase 2), respectively.[12–14] Furthermore, PTP1B increases insulin sensitivity and regulates body weight in mice according to PTP1B knockout studies.[15] These observations suggest the potential of PTP1B inhibitors as regulators of glucose metabolism involved in insulin resistance.

Iris is a genus comprising approximately 300 different species of flowering plants worldwide. Many plants of this genus are widely used as landscape plants due to their showy flowers.[16] Among the *Iris* plants, *Iris sanguinea* (*I. sanguinea*) is native to Korea, China, Japan, Russia and Mongolia, and it has been widely cultivated as an ornamental plant in these countries. Although a series of chemical constituents including monoterpenoids, triterpenoids, steroids and flavonoids have been isolated from *Iris* species,[17–20] the chemical profiles of *I. sanguinea* have not been studied, aside from the compound swertiajaponin, which has hepatoprotective effects.[18]

In our efforts to search for novel phytochemicals with anti-diabetic potential from natural plants, we describe here, for the first time, a chemical investigation of *I. sanguinea* seeds, their effects on the regulation and mechanism of glucose uptake, and their PTP1B inhibitory activity.

Successive chromatographic procedures (silica gel, RP-C_{18}, LH-20 and HPLC) afforded two new triterpenoids (**1** and **2**) and nine known compounds (**3**–**11**) (Fig. 1) from a methanol extract of the seeds of *I. sanguinea*.[21]

Compound **1** was obtained as a white, amorphous powder with $[\alpha]_D^{20}$ +37.6 (*c* 0.5, $CHCl_3$).[22] The HRESIMS ion peak at *m*/*z* 661.5909 $[M-H_2O+H]^+$ suggested a molecular formula of $C_{46}H_{78}O_3$ with eight double-bond equivalents, which were ascribed to a pentacyclic ring system, two olefinic bonds and one ester carbonyl

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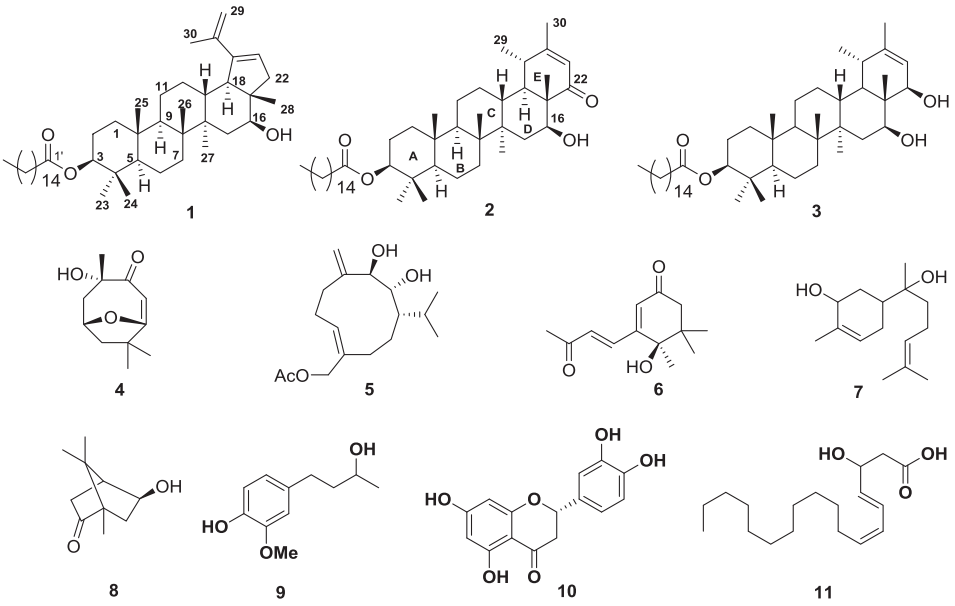

Fig. 1. Chemical structures of compounds **1**–**11** isolated from *Iris sanguinea* seeds.

group. The UV spectrum around λ_{max} 280–285 nm suggested the presence of a conjugated olefin system.[23] The IR spectrum showed absorptions of hydroxyl (3401 cm^{-1}), carbonyl ester (1741 cm^{-1}) and olefin (1465 cm^{-1}) functionalities. The ^1H NMR spectrum of compound **1** showed signals for seven methyl singlets [δ_H 0.65, 0.84, 0.85, 0.89, 1.03, 1.05 and 1.81], two oxygenated methine protons at δ_H 3.67 (1H, dd, J = 3.7, 11.8 Hz) and δ_H 4.48 (1H, dd, J = 5.2, 10.9 Hz), and three olefinic protons at δ_H 4.78 (1H, br s), 5.12 (1H, br s) and 5.40 (1H, br s). The ^{13}C NMR spectrum revealed the presence of two oxygenated carbons at δ_C 76.3 and 80.7, four olefinic carbons at δ_C 108.9, 122.8, 135.0 and 146.0, and one ester carbonyl carbon at δ_C 173.8. Its ^1H and ^{13}C NMR data showed the presence of a fatty acid ester in the structure: δ_H 2.29 (2H, t, J = 7.4 Hz), 1.24–1.29 (overlap), 0.86 (3H, t, J = 7.0 Hz); δ_C 173.8 and 29.6–30.2 (overlap). These observations indicated that compound **1** is a lupane-type triterpenoid possessing a conjugated olefin system, a hydroxy group, and a fatty acid ester (Table 1).[23] Detailed analysis of HSQC and HMBC spectra of compound **1** confirmed the location of the $\Delta^{19(21),20(29)}$ olefin system, analyzed as follows: H-29 (δ_H 4.78 and 5.12, each one proton) to C-20 (δ_C 135.0) and C-30 (δ_C 26.1), H-30 (δ_H 1.81) to C-19 (δ_C 146.0) and C-20 (δ_C 135.0), H-21 (δ_H 5.40) to C-18 (δ_C 46.8), C-19 (δ_C 146.0) and C-20 (δ_C 135.0). A hydroxy group was determined at position C-16 (δ_C 76.3) based on HMBC correlations from H-16 (δ_H 3.67) to C-15 (δ_C 36.2), C-17 (δ_C 41.3) and C-28 (δ_C 11.2). The relative configuration of OH-16 was elucidated as β from the coupling pattern of H-16 (dd, J = 3.7, 11.8 Hz). A fatty acid ester was located at C-3 (δ_C 80.7), as analyzed by HMBC cross-peaks from H-3 (δ_H 4.48) to C-1 (δ_C 38.0), C-2 (δ_C 23.9), C-4 (δ_C 37.3), C-23 (δ_C 28.1) and C-24 (δ_C 16.7), as well as from H-3 (δ_H 4.48) to the ester carbonyl carbon at δ_C 173.8, which also had HMBC cross-peak from H-2′ at δ_H 2.29 (Fig. 2). Besides all of the above NMR signals, there remain one methyl at δ_H 0.86 (t, J = 7.0 Hz) and resonances from δ_C 29.6 to δ_C 30.2. All of the above NMR and HRESIMS information were used to determine the structure of the fatty acid chain as shown. The proton H-3 was determined as α-oriented based on its coupling pattern (dd, J = 5.2, 10.9 Hz).[24] Finally the structure of compound **1** was determined as shown in Fig. 1, and named irisin A. Irisin A represents the first example of a lupane-type triterpenoid possessing the $\Delta^{19(21),20(29)}$ olefin moiety.

Compound **2** was obtained as a white and amorphous powder with $[\alpha]_D^{20}$ −57.4 (c 0.1, $CHCl_3$).[25] The HRESIMS ion at m/z 699.5667 $[M+Na-H_2O]^+$ (calcd 699.5687) indicated a molecular formula of $C_{46}H_{78}O_4$ with eight degrees of unsaturation. The UV absorption at λ_{max} 230 nm and IR absorption peaks of hydroxyl (3410 cm^{-1}), carbonyl ester (1731 cm^{-1}) and olefin (1460 cm^{-1}) strongly suggested the presence of an aliphatic chain and conjugated carbonyl functionalities. The ^1H NMR spectrum of compound **2** showed one olefin-connected methyl broad singlet at δ_H 1.93, one methyl doublet at δ_H 1.14 (J = 6.5 Hz), seven other methyl singlets at δ_H 0.84, 0.85, 0.89, 1.00, 1.02 and 1.07, two oxygenated methine protons at δ_H 4.05 (1H, dd, J = 4.8, 11.5 Hz) and δ_H 4.47 (1H, dd, J = 5.0, 11.5 Hz), and one olefin proton at δ_H 5.72 (1H, br s). The ^{13}C NMR spectrum revealed the presence of two olefin carbons at δ_C 122.9 and 165.2, two oxygenated carbons at δ_C 70.2 and 80.6, and one ester carbonyl carbon at δ_C 173.9. These spectroscopic data indicated that compound **2** possessed a very similar molecular structure to the known compound **3**.[24] The major difference is the replacement of OH-22 in **3** by a ketone group in compound **2** (Table 2). This conclusion was further confirmed by HMBC as shown Fig. 2. The relative configuration of OH-16 was elucidated as β based on the coupling pattern of H-16 (dd, J = 4.8, 11.5 Hz).[24] A fatty acid ester was located at position C-3 (δ_C 80.6), as analyzed by HMBC cross-peaks from H-3 (δ_H 4.47) to C-1 (δ_C 38.6), C-2 (δ_C 23.8),), C-4 (δ_C 37.9), C-23 (δ_C 28.1) and C-24 (δ_C 16.7), and from H-3 (δ_H 4.47) to the ester carbonyl carbon at δ_C 173.9, which also had HMBC cross-peak to H-2′ at δ_H 2.28 (Fig. 2). Besides the all above NMR signals, there remain one methyl at δ_H 0.88 (t, J = 7.0 Hz) and resonances from δ_C 29.6 to δ_C 30.2. All NMR and HRESIMS information determined the structure of the fatty acid chain as shown (Fig. 2). The coupling pattern of H-3 (dd, J = 5.0, 11.5 Hz) supported the α-orientation of H-3. The structure of compound **2** was determined and named irisin B.

Based on spectroscopic data and optical rotation values, the known compounds (Supporting Information) were identified as heliantriol C-3-pentadecylic acid ester (**3**),[24] pubinernoid A (**4**),[26] kikkanol F monoacetate (**5**),[27] (*E*,4*R*)-4-hydroxy-4,5,5-trimethyl-3-(3-oxobut-1-enyl)cyclohex-2-enone (**6**),[27] 3,6(11)-dien-disabolane-2,9-diol (**7**),[28] (1*S*,5*S*)-(−)-5-*exo*-hydroxycamphor (**8**),[29]