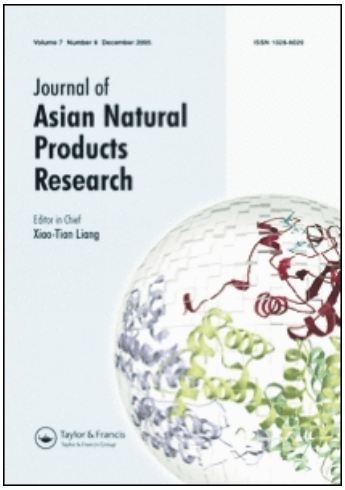

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PTP1B inhibitors from *Ardisia japonica*

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In bioassay-directed isolation from the whole plant of *Ardisia japonica*, sixteen known compounds: chrysophanol (**1**), physcion (**2**), oleanolic acid (**3**), euscaphic acid (**4**), tormentic acid (**5**), quercetin (**6**), quercitrin (**7**), myricitrin (**8**), kaempferol 3-*O*-α-L-rhamnopyranoside (**9**), cyclamiretin A 3-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→4)]-α-L-arabinopyranoside (**10**), (7*E*)-9-hydroxymegastigma-4, 7-dien-3-on-9-*O*-β-D-glucopyranoside (**11**), bergenin (**12**), norbergenin (**13**), rutin (**14**), kaempferol 3,7-*O*-α-L-dirhamnopyranoside (**15**), (−)-epigallacatechin 3-*O*-gallate (**16**) were obtained. Compounds **1**–**5**, **9**, **11** and **14**–**16** have not been reported previously from this plant. Among these isolates, **2**, **3**, **6** and **12** showed moderate bioactivity against PTP1B *in vitro* with IC_{50} values of 121.50, 23.90, 28.12 and 157 μM, respectively.

Keywords: Myrsinaceae; *Ardisia japonica*; Chemical constituents; PTP1B inhibitor

1. Introduction

Type 2 diabetes mellitus (T2DM), also known as non-insulin dependent diabetes mellitus (NIDDM), develops in middle or late life and affects 2–6% of adults in most Western societies. Insulin resistance in the liver and peripheral tissues, together with a pancreatic cell defect, is the common causes of Type 2 diabetes [1]. However, this is difficult since the current therapies for type II diabetes have inherent problems, including compliance, ineffectiveness and hypoglycemic episodes with insulin and the sulfonylureas [2]. Glitazone-type therapeutic agents are not effective in all type II patients [3]; therefore, there still remains a great need for more effective, orally administered agents, particularly ones that normalize both glucose and insulin levels [4].

It is now appreciated that insulin resistance is the result of a defect in the receptor signaling system, at a site post binding of insulin to its receptor. The interaction of insulin with its receptors leads to the phosphorylation of certain tyrosine molecules with the receptor protein, thus activating the receptor kinase. But PTPase dephosphorylate the activated insulin receptor, and attenuate the tyrosine kinase activity. Therefore, it can be concluded that

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dephosphorylation of PTPases is one of the reasons for insulin resistance. The PTPases that appear most likely to be closely associated with insulin receptors kinase activity include PTP1B, LAR, PTPα and SH-PTP2 [5].

PTP1B plays a major role in the dephosphorylation of the insulin receptor in many cellular and biochemical studies. Therefore, orally active PTP1B inhibitors could be potential pharmacological agents for the treatment of Type-II diabetes and obesity [6].

As part of our research work on natural products with anti-diabetes activity [7], we have intensively screened our plant extract bank for inhibitors of PTP1B enzyme to find that three active fractions (chloroform fractions and 30%, 50% ethanol-eluted fractions) from ethanol extracts of the whole plant of *Ardisia japonica* (Thunb.) Bl. (Myrsinaceae), used to treat tuberculosis and chronic bronchitis as a folk medicine of China [8], showed strong inhibitory bioactivity against PTP1B with $IC_{50} = 1.57$, 1.73 and 1.37 $\mu g\,ml^{-1}$, respectively. Using the PTP1B bioassay as a guide, the chromatography of the chloroform and the 30, 50% ethanol-eluted fractions afford sixteen compounds: chrysophanol (**1**), physcion (**2**), oleanolic acid (**3**), euscaphic acid (**4**), tormentic acid (**5**), quercetin (**6**), quercitrin (**7**), myricitrin (**8**), kaempferol 3-*O*-α-L-rhamnopyranoside (**9**), cyclamiretin A 3-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→4)]-α-L-arabinopyranoside (**10**), (7*E*)-9-hydroxymegastigma-4,7-dien-3-on-9-*O*-β-D-glucopyranoside (**11**), bergenin

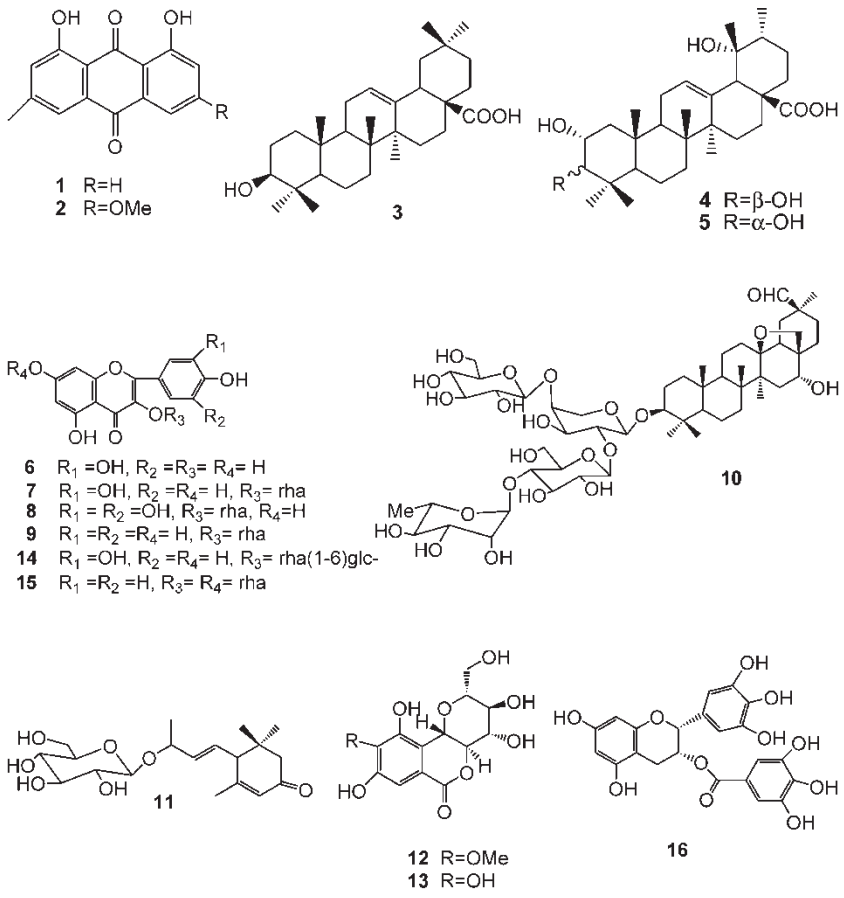

Figure 1. Structures of **1**–**16**.

(**12**), norbergenin (**13**), rutin (**14**), kaempferol 3,7-*O*-α-L-dirhamnopyranoside (**15**) and (−)-epigallacatechin 3-*O*-gallate (**16**) (figure 1).

2. Results and discussion

Sixteen known compounds, as given above, were isolated from the three active fractions of the whole plant of *Ardisia japonica*, as described in the Experimental Section, and were identified by comparison of their physical and spectral data with those of reported values. Compounds **1**–**5**, **9**, **11** and **14**–**16** have not been reported previously from this plant.

All of the isolates obtained from the whole plant of *Ardisia japonica* were evaluated for their potential to inhibit human protein tyrosine phosphatase 1B (hPTP1B) activity. The results showed that **2**, **3**, **6**, and **12** inhibited hPTP1B activity with IC_{50} values of 121.50, 23.90, 28.12 and 157 μM, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined using an XT-4 point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer Model 341 polarimeter. IR spectra were measured with a Perkin-Elmer 559B apparatus. NMR spectra were recorded with Varian Mercury 300 and Bruker AMX 400 NMR spectrometer in pyridine-d_5 and acetone-d_6 using TMS as internal standard. MS were determined on a MAT 711 mass spectrometer. D-101 macroporous resin (Nankai University, China); MCI gel CHP 20P (Mistubishi Kasei Industry Co. Ltd, Japan) and Sephadex LH-20 (Merck, Germany) were used.

3.2 Plant material

The whole plant of *Ardisia japonica* (Thunb.) Bl. was collected at Nanchuan, Chongqing, China, in February 2001. A voucher sample (NPLE00157) of the plant has been deposited at the Chinese National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

3.3 Biological assays for the inhibition of human Protein Tyrosine Phosphatase 1B (hPTP1B)

hPTP1B phosphatase activity was assayed at room temperature using appropriate concentrations of *p*-nitrophenylphosphate (PNPP) as substrate. The buffer used was pH 7.6, 10 mM Tris.Cl, 2%DMSO. The reaction was initiated by addition of enzyme. The non-enzymatic hydrolysis of the substrate was corrected by measuring the control without the addition of enzyme. The amount of product *p*-nitrophenol was determined from the absorbance at 410 nm. The data were exported from Softmax software as a text file and transferred to Excel (STR·xls) for percent inhibition calculation. IC_{50} s were determined in re-screen with an inhibition bigger than 50%.

3.4 Extraction and isolation

The whole plant of *Ardisia japonica* (2.0 kg) was powdered and extracted three times at room temperature with 95% EtOH (each time for 3 days). After concentration *in vacuo*, the extract was diluted with H_2O and extracted with $CHCl_3$, and evaporated to dryness *in vacuo* to provide the $CHCl_3$ part (23.5 g), The remaining aqueous solutions were chromatographed over a D-101 macroporous resin column and gradiently eluted H_2O–EtOH, to obtain water, 15, 30, 50, 75 and 95% EtOH-eluted fractions.

The $CHCl_3$ part (23.5 g, $IC_{50} = 1.57\ \mu g\ ml^{-1}$) was chromatographed over silica gel (200–300 mesh) using petroleum–EtOAc mixtures of increasing polarity. Repeated chromatography with the same eluent over silica gel ($>$ 400 mesh) afforded compounds **1** (8 mg), **2** (5 mg), **3** (14 mg), **4** (10 mg), **5** (8 mg) and **6** (115 mg); the 50% EtOH part (6.0 g, $IC_{50} = 1.73\ \mu g\ ml^{-1}$) was chromatographed over MCI gel using acetone–water (1:1 to 7:3) mixtures of decreasing polarity. Repeated chromatography with the same eluent over MCI gel and Sephadex LH-20 afforded compounds **7** (10 mg), **8** (125 mg) and **9** (10 mg).

The 30% EtOH fraction (21.0 g, $IC_{50} = 1.37\ \mu g\ ml^{-1}$) was chromatographed over MCI gel using acetone–water (1:1 to 7:3) mixtures of decreasing polarity. Repeated chromatography with the same eluent over MCI gel and Sephadex LH-20 afforded compounds **10** (54 mg), **11** (13 mg), **12** (450 mg), **13** (32 mg), **14** (11 mg), **15** (8 mg) and **16** (43 mg).

Compounds **1**–**8** were identified by comparing their physical and spectra data with the literature values: chrysophanol (**1**), physcion (**2**), oleanolic acid (**3**), tormentic acid (**4**), euscaphic acid (**5**), quercetin (**6**), quercitrin (**7**), myricitrin (**8**) [8–12].

3.4.1 Kaempferol 3-*O*-α-L-rhamnofuranoside (9). Yellow amorphous powder, mp 165–168°C (acetone–H_2O); ^{1}H NMR (DMSO-d_6) δ (ppm): 6.37 (1H, d, $J = 1.8$ Hz, H-8), 6.20 (1H, d, $J = 1.8$ Hz, H-6), 7.24 (2H, d, $J = 8.7$ Hz, H-2′,6′), 6.90 (2H, d, $J = 8.4$ Hz, H-3′,5′), 5.29 (1H, br s, H-1 of rha), 12.62 (1H, s, OH-5), 0.78 (3H, d, $J = 6.0$ Hz, H-6 of rha), ^{13}C NMR (DMSO-d_6) δ (ppm): C-2 → C-10: 151.7, 134.8, 178.4, 161.9, 99.4, 165.0, 94.4, 157.8, 104.7, C-1′ → C-6′: 121.2, 131.3, 116.0, 160.5, 116.0, 131.3, 3-*O*-rha C-1″ → C-6″: 102.4, 70.9, 71.2, 71.7, 70.7 and 18.1. ^{1}H and ^{13}C NMR data were consistent with literature values [9].

3.4.2 Cyclamiretin A 3-*O*-α-L-rhamnopyranosyl(1→4)β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→4)] α-L-arabinopyranoside (10). White amorphous powder, mp 218–220 °C (MeOH), ESI-MS (*m/z*): 1097.5 $[M + Na]^+$, 1114.4 $[M + K]^+$; Acid hydrolysis of **10** gave the aglycone cyclamiretin A, identified by co-TLC with authentic sample and comparison of the ^{13}C NMR chemical shift from the literature. ^{1}H NMR (pyridine-d_5, δ (ppm): 6.38 (1H, s, H-1 of rha), 5.25 (1H, d, $J = 7.4$ Hz, H-1 of glc), 5.41 (1H, d, $J = 7.7$ Hz, H-1 of glc), 5.07 (1H, d, $J = 4.2$ Hz, H-1 of glc), 1.88 (3H, d, $J = 6$ Hz, H-6 of rha); ^{13}C NMR (pyridine-d_5, δ (ppm): C-1 → C-30: 40.7 (t), 27.9 (t), 90.6 (d), 41.7 (s), 57.2 (d), 19.4 (t), 34.2 (t), 45.5 (s), 54.7 (d), 44.0 (s), 20.6 (t), 32.0 (d), 87.8 (s), 46.1 (s), 34.7 (t), 77.1 (t), 49.7 (s), 51.9 (d), 35.9 (t), 38.4 (s), 33.8 (t), 30.5 (t), 29.5 (q), 17.8 (q), 17.9 (q), 21.2 (q), 19.9 (q), 79.0 (t), 25.6 (q), 208.9 (q), 3-*O*-Ara: C-1 → C-6: 105.8 (d), 76.2 (d), 74.1 (d), 81.9 (d), 64.4 (t), 2′-*O*-Glc: C-1 → C-6: 106.7 (d), 80.9 (d), 79.4 (d), 78.9 (d), 78.4 (d), 64.1 (t), 4′-*O*-Glc′: C-1 → C-6: 104.5 (d), 77.3 (d), 79.6 (d), 73.7 (d), 79.5 (d), 64.4 (t),

4″-*O*-Rha: C-1 → C-6: 102.9 (d), 73.3 (d), 73.4 (d), 76.2 (d), 70.9 (d), 20.2 (q). ^{1}H and ^{13}C NMR data were consistent with literature values [13].

3.4.3 (7*E*)-9-Hydroxymegastigma-4,7-dien-3-on-9-*O*-β-D-glucoside (11). Colorless oil, ^{1}H NMR (DMSO-d_6, δ (ppm): 2.34, 1.95 (2H, d, $J = 6.5$ Hz, H-2), 5.78 (1H, s, H-4), 2.58 (1H, d, $J = 9.0$ Hz, H-6), 5.55 (1H, dd, $J = 6.4$, 16.4 Hz, H-7), 5.68 (1H, dd, $J = 6.4$, 16.4 Hz, H-8), 4.28 (1H, m, H-9), 1.18 (3H, d, $J = 6.4$ Hz, H-10), 0.89 (3H, s, H-11), 0.93 (3H, s, H-12), 1.83 (3H, s, H-13), 4.18 (2H, d, $J = 8.0$ Hz, H-1 of glc); ^{13}C NMR (DMSO-d_6, δ (ppm): C-1 → C-13: 35.6 (s), 47.1 (t), 197.9 (s), 124.9 (d), 162.0 (s), 54.6 (d), 127.2 (d), 136.6 (d), 73.7 (d), 20.7 (q), 26.6 (q), 27.4 (q), 22.9 (q), 9-*O*-Glc: C-1 → C-6: 100.8 (d), 73.7 (d), 76.7 (d), 69.9 (d), 76.7 (d), 60.9 (t). ^{1}H and ^{13}C NMR data were consistent with literature values [14].

3.4.4 Bergenin (12). White crystals (acetone-H_2O), ^{1}H NMR (MeOH-d_4, δ (ppm): 3.3-4.1 (6H, m), 4.99 (1H, d, $J = 10.5$ Hz, H-10b), 7.06 (1H, s, H-7), 3.88 (3H, s, OMe); ^{13}C NMR (MeOH-d_4, δ (ppm): 61.5 (C-CH_2), 70.6 (C-2), 73.0 (C-3), 74.4 (C-4), 80.2 (C-4a), 164.6 (C-6), 118.2 (C-6a), 109.8 (C-7), 151.2 (C-8), 141.0 (C-10), 116.1 (C-10a), 81.8 (C-10b), 59.5 (OMe). ^{1}H and ^{13}C NMR data were consistent with literature values [15].

Comparing their physical and spectral data with the literature values [15–17]. Compounds **13**–**15** were identified as norbergenin (**13**), rutin (**14**), and kaempferol 3,7-*O*-α-L-dirhamnopyranoside (**15**).

3.4.5 (−)-Epigallacatechin 3-*O*-gallate (16). Brown powder, mp 160–162°C (MeOH); ^{1}H NMR (DMSO-d_6, δ (ppm): 6.40 (2H, s, H-2′, 6′), 6.80 (2H, s, H-2″,6″), 5.92 (1H, d, $J = 2.2$ Hz, H-6), 5.83 (1H, d, $J = 2.2$ Hz, H-8), 4.84 (1H, s, H-2), 5.38 (1H, br s, H-3), 2.93, 2.68 (2H, dd, $J = 4.4$, 16.2 Hz, H-4); BB + DEPT (DMSO-d_6, δ (ppm): C-2 → C-10: 76.6 (d), 68.1 (d), 25.8 (t), 156.6 (s), 95.7 (d), 155.6 (s), 94.6 (d), 156.5 (s), 97.5 (s), C-1′ → C-6′: 128.8 (s), 105.6 (d), 145.7 (s), 132.5 (s), 145.7 (d), 105.6 (d), C-1″ → C-6″: 119.5 (s), 108.8 (d), 145.4 (s), 138.0 (s), 145.4 (d), 108.8 (d), 165.4 (s, C═O). ^{1}H and ^{13}C NMR data were consistent with literature values [18].

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