PTP1B INHIBITORS FROM ISODON TERNIFOLIUS COLLECTED IN VIET NAM

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Abstract. From the whole plant of Isodon ternifolius collected in Viet Nam, four triterpenes including ursaldehyde (1), ursolic acid (2), β-sitosterol (3), and β-sitosteryl ferulate (4) were isolated. Their chemical structures were determined using NMR and MS spectroscopy methods in combination with literature comparison. Inhibitory activity against PTP1B enzyme activity of isolated compounds also evaluated using in vitro assay. Compounds 1 and 2 displayed potential activities with IC50 values of 16.92 ± 0.12 and 3.42 ± 0.45 μM, respectively. For the first time, compounds 1 and 4 were isolated from the Isodon genus and I. ternifolius, as well as evaluated for the PTP1B inhibitory activity.

Keywords: Isodon ternifolius, Lamiaceae, whole plant, ursane-type triterpene, PTP1B.

Classification numbers: 1.1.1, 1.2.1.

1. INTRODUCTION

Isodon ternifolius is a member of the Isodon genus, which belongs to the Lamiaceae family. This genus contained approximately 150 species worldwide which distributed mainly in tropical and subtropical countries [1]. There have been several records of the use of Isodon genus in traditional medicine to cure several diseases such as esophageal cancer, rheumatism, and chronic pharyngitis [2, 3]. Isodon species have been reported in previous studies to exhibit various pharmacological activities such as cytotoxic, anti-tumor, anti-inflammatory, antivirus, antibiosis, and anti-proliferative activities [1]. Previous data on chemical constituents of the Isodon genus indicated the presence of phenolics, diterpenoids and triterpenoids [1, 4-10]. I. ternifolius is rich in diterpenoids [4-7, 9, 10], lignans, and phenylethanoid [8]. This species was commonly
used in folk medicine to treat icterohepatitis, enteritis [11], inflammation [8 - 11], and cancer [5, 6]. Type 2 diabetes (T2D) used to be known as noninsulin-dependent diabetes mellitus, is the most common type among three types of diabetes which accounting for about 90% of the total cases. Protein tyrosine phosphatase 1B (PTP1B) is described role as a negative regulator of leptin and insulin signaling. The overexpression of PTP1B was proved to inhibit the expression of insulin in insulin-resistant states; in addition, recent genetic evidence demonstrated the correlation between PTP1B gene variants and changes in insulin sensitivity. Thus, PTP1B is considered as a promising drug target for at-risk obese patients and T2D treatment [12 - 14].

Up to date, there has been no investigation on PTP1B inhibitory activity of I. ternifolius chemical constituents. Thus, this study presented the isolation, structural elucidation of four compounds from I. ternifolius and their activity against PTP1B enzyme.

2. MATERIALS AND METHODS

2.1. Plant material

The whole plant of I. ternifolius was collected in July, 2017 at Thai Nguyen province, Viet Nam (Longitude 105° 50’ 53.41” E; Latitude 21° 35’ 39.19” N) and authenticated by Assoc. Prof. Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST. A voucher specimen (IS20171001.HN) was deposited at the Institute of Natural Products Chemistry, VAST.

2.2. General experimental procedures

1H-NMR (500 MHz) and 13C-NMR (125 MHz) spectra were measured on Bruker Avance 500 MHz spectrometer. Varian FT-MS spectrometer and MicroQ-TOF III (Bruker Daltonics, Ettlingen, Germany) were utilized to obtain ESI-MS spectral data. Column chromatography was conducted using silica gel (Si 60 F254, 40–63 mesh, Merck, St. Louis, MO, USA). Pre-coated thin layer chromatography (TLC) plates (Si 60 F254) were used for analytical purposes. Compounds were visualized using UV radiation (254, 365 nm) and by spraying plates with 10% H2SO4 followed by heating. All solvents were redistilled before use.

2.3. Extraction and isolation

The dried powder of the whole plant of I. ternifolius (4.0 kg) was extracted with MeOH (3 × 8 L) accompanied by refluxing at 60 °C, to give 380 g of crude extract. The crude extract was suspended in water (800 mL) and successively partitioned with n-hexane (4 × 1.5 L), EtOAc (4 × 1.5 L), and BuOH (4 × 1.5 L). The n-hexane extract was chosen for further activity-guided fractionation study.

The n-hexane extract (48.84 g) was subjected to chromatography on a silica gel column (CC) using n-hexane-acetone as eluent (20:1 to 0:1) to yield ten fractions (CMH.1–CMH.10) based on their TLC profiles. The combined fractions (CMH.6 and CMH.7, 4.44 g) were further subjected to silica gel CC, eluting with n-hexane-EtOAc (8:1 to 0:1) to give nine small fractions (CMH.6-1 to CMH.6-9). Fraction CMH.6-2 was chromatographed on RP-C18 CC, using a stepwise gradient of acetone:water (1:1 to 1:0) to obtain twenty-five sub-fractions (CMH.6-2-1 to CMH.6-2-25). Further purification of sub-fraction CMH.6-2-12 (450 mg) by an RP-C18 column, eluting with acetone:water (3:1) yielded compounds 1 (152.0 mg) and 2 (19 mg). Fraction CMH.6-2-25 (650 mg) was further subjected to silica gel CC, using a gradient of n-hexane:acetone (10:1 to 5:1) yielded compounds 3 (443 mg) and 4 (153 mg).
**Ursaldehyde (1):** White powder; ESI-MS m/z 441.4 [M + H]+ (C20H48O2); 1H-NMR (500 MHz, CDCl3) δH (ppm): 0.72 (1H, dd, J = 13.0, 1.5 Hz, H-5), 0.77 (3H, s, H-24), 0.78 (3H, s, H-25), 0.89 (3H, d, J = 7.0 Hz, H-29), 0.92 (3H, s, H-26), 0.97 (3H, d, J = 7.0 Hz, H-30), 0.98 (3H, s, H-23), 1.08 (3H, s, H-27), 3.21 (1H, dd, J = 11.0, 5.0 Hz, H-3), 5.31 (1H, t, J = 4.0 Hz, H-12), 9.32 (1H, d, J = 1.0 Hz, H-28); 13C-NMR (125 MHz, CDCl3) δC (ppm): 207.4 (C-28), 137.8 (C-13), 126.2 (C-12), 79.0 (C-3), 55.2 (C-5), 52.6 (C-18), 50.1 (C-17), 47.6 (C-9), 42.2 (C-14), 39.8 (C-19), 39.0 (C-20), 38.8 (C-8), 38.7 (C-4), 38.7 (C-1), 37.0 (C-10), 33.1 (C-7), 31.7 (C-22), 30.2 (C-21), 29.7 (C-23), 28.1 (C-2), 27.2 (C-15), 26.9 (C-16), 23.3 (C-11), 23.2 (C-27), 21.0 (C-30), 18.3 (C-6), 17.2 (C-26), 16.6 (C-24), 15.6 (C-29), 15.5 (C-25).

**Ursolic acid (2):** White powder; ESI-MS m/z 457 [M + H]+ (C25H40O3); 1H-NMR (500 MHz, CDCl3+t-Methanol-d4) δH (ppm): 0.72 (1H, br d, J = 11.5 Hz, H-5), 0.76 (3H, s, H-24), 0.87 (3H, d, J = 6.0 Hz, H-29), 0.93 (3H, s, H-25), 0.94 (3H, d, J = 6.0 Hz, H-30), 0.96 (3H, s, H-26), 0.96 (3H, s, H-23), 1.09 (3H, s, H-27), 3.15 (1H, dd, J = 11.5, 7.5 Hz, H-3), 5.31 (1H, br s, H-12); 13C-NMR (125 MHz, CDCl3+t-Methanol-d4) δC (ppm): 181.0 (C-28), 139.3 (C-13), 126.6 (C-12), 79.5 (C-3), 56.5 (C-5), 54.1 (C-18), 49.1 (C-17), 48.7 (C-9), 43.1 (C-14), 40.5 (C-19), 40.2 (C-20), 40.1 (C-8), 39.8 (C-4), 39.7 (C-1), 37.9 (C-10), 34.1 (C-7), 31.7 (C-22), 29.0 (C-21), 28.8 (C-23), 27.7 (C-2), 25.2 (C-15), 25.2 (C-16), 24.2 (C-11), 24.2 (C-27), 21.7 (C-30), 19.3 (C-6), 17.7 (C-26), 17.7 (C-24), 16.4 (C-29), 16.1 (C-25).

**β-sitosterol (3):** Colorless crystals; ESI-MS m/z: 415 [M + H]+ (C29H44O); 1H-NMR (500 MHz, CDCl3) δH (ppm): 5.35 (1H, t, J = 3.0 Hz, H-6), 3.53 (1H, m, H-3), 1.00 (3H, s, H-19), 0.92 (3H, d, J = 6.5 Hz, H-29), 0.85 (3H, d, J = 7.5 Hz, H-27), 0.83 (3H, d, J = 6.5 Hz, H-26), 0.81 (3H, t, J = 6.5 Hz, H-24), 0.68 (3H, s, H-28); 13C-NMR (125 MHz, CDCl3) δC (ppm): 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 29.7 (C-7), 31.9 (C-8), 50.1 (C-9), 37.2 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.0 (C-14), 24.3 (C-15), 28.2 (C-16), 56.7 (C-17), 11.9 (C-18), 19.3 (C-19), 36.5 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.2 (C-25), 19.8 (C-26), 19.0 (C-27), 24.3 (C-28), 11.8 (C-29).

**β-sitosteryl furlulate (4):** White solid; ESI-MS m/z: 591 [M + H]+ (C39H58O6); 1H-NMR (500 MHz, CDCl3) δH (ppm): 7.60 (1H, d, J = 16.0 Hz, H-3'), 7.07 (1H, br d, J = 8.5 Hz, H-9'), 7.03 (1H, br s, H-5'), 6.91 (1H, d, J = 8.5 Hz, H-8'), 6.29 (1H, d, J = 16.0 Hz, H-2'), 5.34 (1H, t, J = 2.5 Hz, H-6), 3.92 (3H, s, 6'-OCH3), 3.51 (1H, m, H-3), 1.00 (3H, s, H-19), 0.92 (3H, d, J = 6.5 Hz, H-29), 0.88 (3H, t, J = 7.0 Hz, H-24), 0.83 (3H, d, J = 7.0 Hz, H-26), 0.82 (3H, d, J = 7.0 Hz, H-27), 0.68 (3H, s, H-28); 13C-NMR (125 MHz, CDCl3) δC (ppm): 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 29.7 (C-7), 31.9 (C-8), 50.1 (C-9), 37.3 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 55.9 (C-14), 24.3 (C-15), 28.2 (C-16), 56.8 (C-17), 12.0 (C-18), 19.4 (C-19), 36.5 (C-20, 18.8 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.0 (C-27), 24.3 (C-28), 11.8 (C-29), 167.3 (C-1'), 115.7 (C-2'), 144.6 (C-3'), 127.1 (C-4'), 109.3 (C-5'), 147.3 (C-6'), 146.8 (C-7'), 114.7 (C-8'), 123.0 (C-9'), 56.1 (6'-OCH3).

2.4. PTP1B inhibition assay

PTP1B (human recombinant) was obtained from Biomol International LP, Plymouth Meeting, PA, USA. All isolated compounds were tested for inhibitory activities using the method as described [15]. Briefly, 0.05–0.1 μg of PTP1B and 4 mM p-NPP in a buffer containing 1 mM EDTA, 1 mM dithiothreitol, 0.1 M NaCl and 50 mM citrate (pH 6.0), with or
without test compounds, were added as 100 μL of a final volume to each of the 96 well plate. After incubation for 30 mins at 37 °C, the reaction mixture was quenched by adding 10 M NaOH. PTP1B enzyme activity was determined by measuring the amount of p-nitrophenol produced at 405 nm. The non-enzymatic hydrolysis of the substrate means the control sample without PTP1B enzyme.

3. RESULTS AND DISCUSSION

The methanol extract of the whole plant of *I. ternifolius* was partitioned into *n*-hexane, ethyl acetate, *n*-butanol, and water-soluble fractions. The inhibitory effect of these fractions were tested in vitro using PTP1B enzyme assay. Obtained results showed that the *n*-hexane fraction exhibited the most inhibitory effect against enzyme activity (Table 1). Therefore, this fraction was selected for further studies. Repeated column chromatography of the *n*-hexane fraction resulted in the isolation of four known compounds (1–4) (Figure 1).

Compound 1 was isolated as a white powder. The electrospray impact ionization mass spectrometry (ESI-MS) showed the pseudo-molecular ion [M + H]+ at m/z 441.4 suggesting the molecular formula C$_{30}$H$_{48}$O$_2$. The $^1$H-NMR spectrum of 1 revealed seven methyl groups [$\delta_H$ 0.98 (3H, s, H-23), 0.77 (3H, s, H-24), 0.78 (3H, s, H-25), 0.92 (3H, s, H-26), 1.08 (3H, s, H-27), 0.89 (3H, d, H-29) and 0.97 (3H, d, H-30)], an axial oxymethine proton at $\delta_H$ 3.21 (1H, dd, H-3), an olefinic proton [$\delta_H$ 5.31 (1H, t, H-12), and an aldehyde proton at $\delta_H$ 9.32 (1H, d, H-28). Furthermore, the presence of five methines and nine methylene groups were displayed in the $^1$H-NMR spectrum (Figure 1).

The $^{13}$C NMR spectrum of 1 exhibited thirty carbon signals, including one aldehyde carbon at $\delta_C$ 207.4 (C-28), two olefinic carbons at $\delta_C$ 137.8 (C-13) and 126.2 (C-12), an oxymethine carbon at $\delta_C$ 79.0, and seven methyl carbons at $\delta_C$ 29.7 (C-23), 16.6 (C-24), 15.5 (C-25), 17.2 (C-26), 23.2 (C-27), 15.6 (C-29) and 21.0 (C-30). The other carbon signals were observed and assigned to five methines and nine methylene carbons (Figure 1). The obtained $^1$H- and $^{13}$C-NMR spectral data suggested compound 1 to be an ursane-type triterpene [14, 15]. In the HMBC spectrum, the correlations from H-23 and H-24 to C-3 and C-5, as well as from H-9 to C-2 and H-27 to C-13 confirmed the positions of the oxymethine and olefinic groups (Figure 2). In addition, the HMBC correlations from H-18 to C-28, as well as the HMBC correlation from aldehyde proton H-28 to C-16 and C-22 confirmed the position of the aldehyde group at C-28.
(Figure 2). Based on the above evidences in comparison with the published data, compound 1 was identified as ursaldehyde [16].

Compound 2 was obtained as a white powder. The molecular formula of 2 was clarified as C_{39}H_{56}O_{2} based on the ion at m/z 457 [M + H]^+ in the ESI-MS spectrum. The ^1H- and ^13C-NMR spectra of 2 were similar to those of 1 except for the replacement of an aldehyde group [δ_H 9.32 (1H, d, H-28) and δ_C 207.4 (C-28)] in 1 by a carboxyl group [δ_H 181.0 (C-28)] in 2 (Figure 1). It was confirmed by HMBC correlations from H-16, H-18, and H-22 to C-28 (Figure 2). Compared with literature data, compound 2 was determined as ursolic acid [17].

Compound 3 was identified as β-Sitosterol by comparison of co-TLC and data from the literature [18].

Compound 4 was obtained as a white solid. Its molecular formula was determined as C_{39}H_{58}O_{3} using ESI-MS measurement with [M + H]^+ peak at m/z 591. The ^1H-NMR spectrum of 4 indicated the presence of six methyls [δ_H 1.00 (3H, s, H-19), 0.92 (3H, d, H-29), 0.88 (3H, t, H-24), 0.83 (3H, d, H-26), 0.82 (3H, d, H-27) and 0.68 (3H, s, H-28)], an oxymethylene proton at δ_H 5.34 (1H, t, J = 8.5 Hz, H-3’), 5.07 (1H, br d, J = 8.5 Hz, H-9’), 7.03 (1H, br s, H-5’), 6.91 (1H, d, J = 8.5 Hz, H-8’), 6.29 (1H, d, J = 16.0 Hz, H-2’) and 3.92 (3H, s, 6’-OCH_3)] were also presented in the ^1H-NMR spectrum (Figure 1). The ^13C-NMR spectrum of 3 exhibited 39 carbon signals, consisting of six methyl carbons, an oxymethylene carbon at δ_C 71.8 (C-3), two olefinic carbons [δ_C 140.8 (C-5) and 121.7 (C-6)] and eleven methylene carbon signals (Figure 1). Furthermore, the carbon signals of feruloyl moiety [δ_C 167.3 (C-1’), 115.7 (C-2’), 144.6 (C-3’), 127.1 (C-4’), 109.3 (C-5’), 147.3 (C-6’), 146.8 (C-7’), 114.7 (C-8’), 123.0 (C-9’), 56.1 (6’-OCH_3)] were also indicated in the ^13C-NMR spectrum (Figure 1). These above ^1H- and ^13C-NMR spectral data suggested compound 4 to be a sterol containing a feruloyl moiety in the side chain [19, 20]. In the HMBC spectral data, the correlations from the H-1 to C-3, H-3 and H-28 to C-5, H-4 and H-8 to C-6, as well as from H-6 to C-10 confirmed the positions of the oxymethylene and olefinic groups (Figure 2). The correlations from H-19 to C-17 and C-20, as well as the correlation from H-24, H-26, and H-27 to C-22, were observed in the HMBC further confirming compound 4 was to be a sterol (Figure 2). In the feruloyl moiety, the HMBC correlations from H-2’ to C-4’, H-3’ to C-1’ and C-9’, H-5’ to C-3’, as well as from H-8’ and OCH_3 to C-6’ were also observed (Figure 2). Especially, the HMBC correlations from H-3 to C-1’ confirmed the feruloyl moiety located at C-3 (Figure 2).

Base on above information in comparison with literature data [17, 18], compound 4 was determined as β-sitosterol ferulate.

![Figure 2. HMBC correlations (H→C) of compounds 1, 2 and 4.](image-url)
PTP1B was confirmed to play an important role in obesity and diabetes diseases through inhibiting the expression of insulin in insulin-resistant states [13, 14]. Therefore, all the isolated compounds (1–4) were evaluated for inhibitory effect against PTP1B enzyme activity, the results are presented as IC₅₀ values (Table 1). In this study, RK-682 was selected as positive control which displayed an IC₅₀ value of 4.52 ± 0.25 μM. Among the tested samples, compounds 1 and 2 displayed potential inhibitory activities with IC₅₀ values of 16.92 ± 0.12 and 3.42 ± 0.45 μM, respectively. Compounds 3 and 4 showed no activity (IC₅₀ value > 50 μM). Ursolic acid (2) was previously identified from persimmon as the major constituent and showed potential PTP1B inhibitory activity (IC₅₀ value was 3.1 ± 0.2 μM) [21]. In our assay, compound 2 also indicated as the major compound of I. ternifolius and showed the similar results of its activity with the value in the published article indicating the reliability of the data obtained. In addition, compound 1 has been identified from this plant for the first time together with its PTP1B inhibitory activity.

**Table 1. Results of PTP1B inhibition effect of studied compounds.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀, µMᵃ</th>
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<tr>
<td>1</td>
<td>16.92 ± 0.12</td>
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<tr>
<td>2</td>
<td>3.42 ± 0.45</td>
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<tr>
<td>3</td>
<td>&gt; 50</td>
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<tr>
<td>4</td>
<td>&gt; 50</td>
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<tr>
<td>n-Hexane fraction</td>
<td>63.37 ± 1.42'</td>
</tr>
<tr>
<td>RK-682ᵇ</td>
<td>4.52 ± 0.25</td>
</tr>
</tbody>
</table>

ᵃResults are expressed as IC₅₀ values (µM), determined by regression analysis and expressed as the means ± SD of three replicates;ᵇPositive control;’% inhibition at a final concentration of 30 µg/mL.

4. CONCLUSIONS

The methanol extract of the whole plant of *I. ternifolius* was partitioned into n-hexane-, ethyl acetate-, n-butanol soluble fractions, and a water-soluble fractions. Four compounds, ursaldehyde (1), ursolic acid (2), β-sitosterol (3), and β-sitosteryl ferulate (4) were isolated for the first time from the whole plant of *Isodon ternifolius*. The chemical structures of these compounds were clarified using NMR spectroscopy, mass spectra and compare with data from literatures. For the first time, ursaldehyde (1) and sitosteryl ferulate (4) were isolated from the *Isodon* genus. In addition, ursaldehyde (1) and ursolic acid (2) showed potent inhibition against PTP1B with IC₅₀ values of 16.92 ± 0.12 and 3.42 ± 0.45 μM, respectively. Obtained results suggested that *I. ternifolius* and its major constituents might exert the hypoglycemic effect via the insulin signaling pathway targeting inhibition of PTP1B enzyme activity.

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