



COMPOUNDS FROM *PESTALOTIOPSIS NEGLECTA* WITH PTP1B AND SHP2 INHIBITORY EFFECTS

Hua-Guang LIU,^a Si-Qian QI,^a Run-Yan WANG,^a Yue CUI,^a Zhen-Sheng LI,^c Hong-Liang TANG,^{*b} Xiao-Long ZHAO^{*a} and Du-Qiang LUO^{*b}

^a College of Chemistry and Materials Science, Hebei University, Baoding 071002, P. R. China

^b College of life science, Hebei University, Baoding 071002, P. R. China

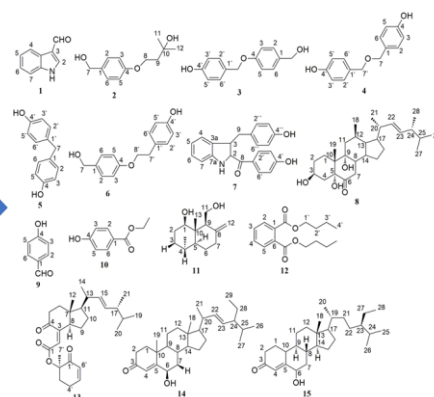
^c College of Chemical and Materials Engineering, Xuchang University, Xuchang 461000, P. R. China

Received January 14, 2025

A new naturally occurring compound (**2**) and an undescribed compound (**6**) along with 13 known compounds including alkaloids, phenols, sterols, esters, sesquiterpenes and norsteroid were isolated from an endophytic fungus *Pestalotiopsis neglecta*, which was arised from the leaves of plant Ceylon olive. According to the HRESI-MS and NMR data and comparison with literature, the structures of the compounds were defined. Among these compounds, compound **1** and **13** showed promising inhibitory effects on PTP1B and SHP2 enzymes with the inhibition rates of 71.33% and 50.59%, respectively.



leaves of plant Ceylon olive



INTRODUCTION

Elaeocarpus serratus L., commonly called Ceylon olive, is an evergreen tree widely planted in the subtropical and tropical Asia, including Hainan province, People's Republic of China. The fruit of Ceylon olive contains carbohydrates, protein, polyphenolic compounds such as flavonoids and condensed tannins, carotenoids, and vitamins with high nutritional value.^{1,2} Simultaneously, the leaves of Ceylon olive have

significant quantities of polyphenols and flavonoids with bioactive and pharmacological properties.^{3–5} It has been reported that the Ceylon olive leaf extract possessed antibacterial activity against *Plesiomonas*, *Salmonella typhi* and *Proteus* spp., and had the potential of being an antitumor, antimicrobial, and pesticidal reagent in the cytotoxicity investigation of brine shrimp lethality assay.⁶ Furthermore, antioxidant flavonol glycosides, including myricitrin, mearnsitin 3-*O*- β -D-glucopyranoside, mearnsitrin, and tamarixetin

* Corresponding authors: Hong-Liang Tang: thl_1980@163.com; Xiao-Long Zhao: longlong_666@sina.com; Du-Qiang Luo: duqiangluo999@126.com.

3-*O*- α -L-rhamnopyranoside, were isolated and identified from the Ceylon olive leaf extract.⁴ Therefore, Ceylon olive is appropriate to be an sustainable plant.

Diabetes mellitus is a metabolic disease characterized by hyperglycemia and the main types are type I diabetes, type II diabetes, gestational diabetes and diabetes of other etiologies.⁷ In recent years, the prevalence of diabetes and obesity has been increasing year by year, which brings about adverse effects on human health.⁸ Protein tyrosine phosphatase (PTP1B) is the main enzyme involved in insulin receptor desensitization and is responsible for regulating the insulin pathway, especially in adipose tissue and muscle, and has been shown to be closely associated with the development of type 2 diabetes mellitus (T2DM).^{9,10} PTP1B inhibitors improve insulin receptor sensitivity and have the ability to cure insulin resistance-related diseases.¹¹ Studies have shown that protein tyrosine phosphatase 1B (PTP1B) has emerged as a promising therapeutic target for the treatment of type 2 diabetes.¹² Due to the variety of mechanisms by which PTP1B can be inhibited, many synthetic compounds and natural products have been identified for the development of therapeutic agents.¹³⁻¹⁶ Therefore, the development of various PTP1B inhibitors is of increasing importance and has received increasing attention in recent years.

On the other hand, SHP2, also an important member of the PTP superfamily, is a non-receptor protein tyrosine phosphatase that removes tyrosine phosphorylation.¹⁷ Functionally, SHP2 is an important hub connecting multiple oncogenic signaling pathways in multiple cells,¹⁸ and several studies have shown that SHP2 plays an important role in regulating immune cell function in the tumor microenvironment and is a promising target for cancer therapy.¹⁹

The objectives of this study are to extract and separate compounds from the endophytic fungus *Pestalotiopsis neglecta* which was isolated from the leaves of Ceylon olive, and evaluate the inhibition activities against PTP1B and SHP2 of the compounds. In all, a new naturally occurring compound (**2**) and an undescribed compound (**6**) along with 13 known compounds including alkaloids, phenols, sterols, esters, sesquiterpenes and norsteroid were obtained. Among these compounds, compound **1** and **13** showed promising inhibitory effects on PTP1B and SHP2 enzymes, providing new ideas for the development of novel drugs or pioneer drugs.

RESULTS AND DISCUSSION

Characterization of the prepared compounds

Compound **1** was obtained as a brown solid. Analysis of the HRESI-MS ion at m/z 144.0442 [M-H]⁻ and 146.0660 [M-Na]⁺ and the NMR data revealed its molecular formula as C₉H₇NO with 7 degrees of unsaturation. The ¹H NMR (600 MHz, C₅D₅N) spectrum indicated the δ : 10.31 (1H, s, 3-CHO), 8.76 (1H, d, J = 7.8 Hz, H-4), 8.25 (1H, s, H-2), 7.62 (1H, d, J = 7.8 Hz, H-7), 7.40 (1H, t, J = 7.8 Hz, H-5), 7.36 (1H, t, J = 7.8 Hz, H-6). The ¹³C NMR (150 MHz, C₅D₅N) spectrum indicated the δ : 185.48 (3-CHO), 138.81 (C-7a), 138.32 (C-2), 125.98 (C-3a), 124.56 (C-6), 124.50 (C-5), 123.20 (C-4), 120.29 (C-3), 113.32 (C-7). On the basis of the spectra data and the reported reference [20], compound **1** was identified as 1H-indole-3-carbaldehyde.

Compound **2** was obtained as a colorless oil. The HRESI-MS ion at m/z 209.1178 [M-H]⁻ and 233.1146 [M+Na]⁺ and the NMR data established its molecular formula as C₁₂H₁₈O₃ with 4 degrees of unsaturation. The ¹H NMR (600 MHz, CD₃OD) spectrum indicated the δ : 7.16 (1H, d, J = 8.4 Hz, H-2), 7.16 (1H, d, J = 8.4 Hz, H-6), 6.75 (1H, d, J = 8.4 Hz, H-3), 6.75 (1H, d, J = 8.4 Hz, H-5), 4.39 (2H, s, H-7), 3.61 (2H, t, J = 6.6 Hz, H-8), 1.77 (2H, t, J = 6.6 Hz, H-9), 1.19 (3H, s, H-11), 1.19 (3H, s, H-11). The ¹³C NMR (150 MHz, CD₃OD) spectrum indicated the δ : 158.3 (C-4), 130.7 (C-2), 130.7 (C-6), 130.4 (C-1), 116.1 (C-3), 116.1 (C-5), 74.0 (C-10), 71.0 (C-7), 67.8 (C-8), 43.5 (C-9), 29.6 (C-11), 29.6 (C-12). Compound **2**, identified as 4-(3-hydroxy-3-methylbutoxy)-benzyl alcohol, was isolated for the first time in nature and is a new natural product with CAS No. 1503781-91-6.

Compound **3** was obtained as a white powder. The HRESI-MS ion at m/z 253.0835 [M+Na]⁺ and 229.0868 [M-H]⁻ and the NMR data deduced its molecular formula as C₁₄H₁₄O₃ with 8 degrees of unsaturation. The ¹H NMR (600 MHz, CD₃OD) spectrum indicated the δ : 7.28 (2H, d, J = 6.6 Hz, H-2,6), 7.26 (2H, d, J = 8.4 Hz, H-2',6'), 6.96 (2H, d, J = 6.6 Hz, H-3,5), 6.79 (2H, d, J = 8.4 Hz, H-3',5'), 4.96 (2H, s, ArCH₂OAr), 4.53 (2H, s, CH₂OH). The ¹³C NMR (150 MHz, CD₃OD) spectrum indicated the δ : 159.75 (C-4'), 158.4 (C-4), 134.94 (C-1), 130.46 (C-2,6), 129.63 (C-1'), 129.48 (C-2',6'), 116.20 (C-3',5'), 115.92 (C-3, 5), 71.11 (ArCHOAr), 64.96 (ArCHOH). Compound

3 was identified as 4-(4-hydroxyphenylmethoxy)-benzyl alcohol by comparing its physicochemical constants with those in the literature.²¹

Compound **4** was obtained as a colorless oil. Based on the HRESI-MS ion at m/z 229.0868 $[M-H]^-$ and the NMR data, its molecular formula was determined to be $C_{14}H_{14}O_3$ with 8 degrees of unsaturation. The 1H NMR (600 MHz, CD_3OD) spectrum indicated the δ : 7.15 (4H, d, $J = 8.4$ Hz,

H-2,2', H-6,6'), 6.76 (4H, d, $J = 8.4$ Hz, H-3,3', H-5,5'), 4.38 (4H, s, 7,7'-2CH). The ^{13}C NMR spectrum indicated the δ : (150 MHz, CD_3OD) δ : 158.26 (2C, C-4,4'), 130.88 (2C, C-1,1'), 130.38 (4C, C-2,2', C-6,6'), 116.19 (4C, C-3,3', C-5,5'), 72.71 (2C, C-7,7'). The physicochemical constants mentioned above were compared with those in the literature,²² leading to the identification of compounds **4** as 4,4-dihydroxydibenzyl ethers.

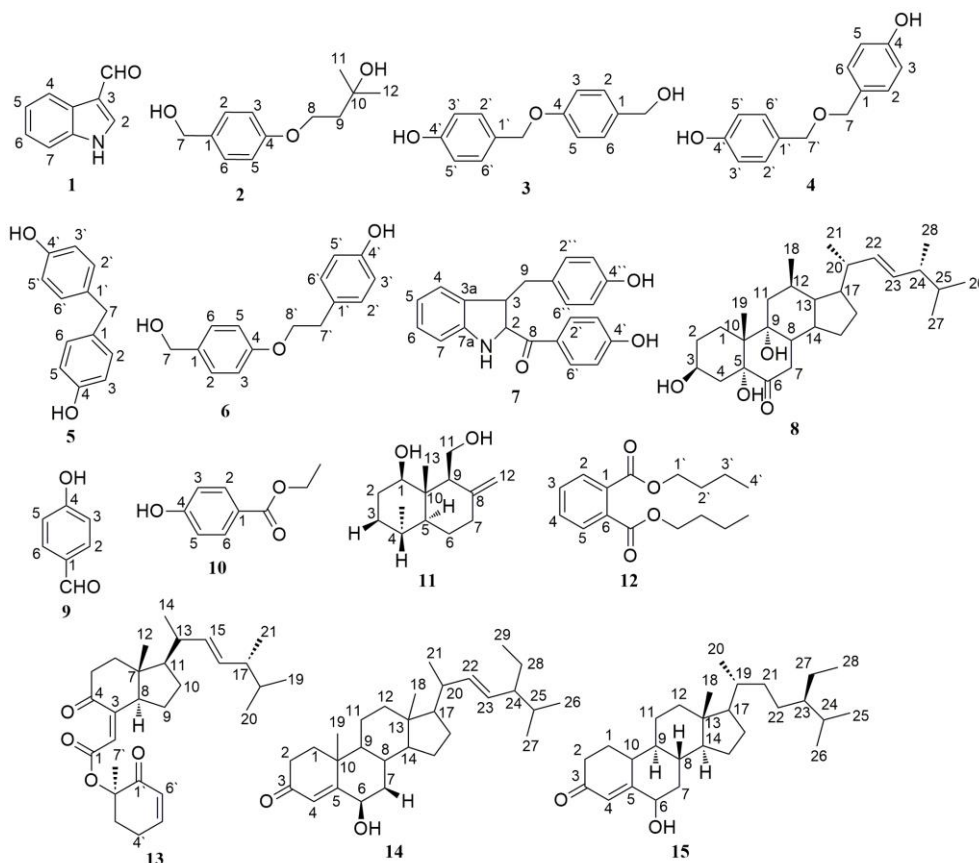


Fig. 1 – Structure of compounds **1-15**.

Compound **5** was obtained as a white crystal. The HRESI-MS ion at m/z 199.0758 $[M-H]^-$ and the NMR data deduced its molecular formula as $C_{13}H_{12}O_2$ with 8 degrees of unsaturation. The 1H NMR (600 MHz, CD_3OD) spectrum indicated the δ : 6.96 (4H, d, $J = 8.4$ Hz, H-2,2', H-6,6'), 6.67 (4H, d, $J = 8.4$ Hz, H-3,3', H-5,5'), 3.75 (2H, s, 7-CH₂). The ^{13}C NMR (150 MHz, CD_3OD) spectrum indicated the δ : 156.5 (C-4,4'), 134.25 (C-1,1'), 130.69 (C-2,2', C-6,6'), 116.10 (C-3,3', C-5,5'), 41.12 (C-7). The above physicochemical constants were compared with literature,²³ and compounds **5** was identified as 4,4'-dihydroxydiphenylmethane.

Compound **7** was obtained as a colorless oil. The compound had the molecular formula $C_{22}H_{17}O_3N$ with 15 degrees of unsaturation

according to the HRESI-MS ion at m/z 342.1136 $[M+H]^+$ and the NMR data. The 1H NMR (600 MHz, CD_3OD) spectrum indicated the δ : 7.70 (1H, d, $J = 8.4$ Hz, H-2', 6'), 7.51 (1H, d, $J = 8.4$ Hz, H-4), 7.42 (1H, d, $J = 8.4$ Hz, H-7), 7.25 (1H, t, $J = 7.2$ Hz, H-6), 7.01 (1H, t, $J = 7.2$ Hz, H-5), 6.91 (2H, d, $J = 6.6$ Hz, H-2'', 6''), 6.85 (2H, d, $J = 6.6$ Hz, H-3', 5'), 6.59 (2H, d, $J = 6.6$ Hz, H-3'', 5''), 4.16 (2H, s, H-9). The ^{13}C NMR (150 MHz, CD_3OD) spectrum indicated the δ : 190.6 (C-8), 163.44 (C-4'), 156.31 (C-4''), 138.5 (C-7a), 133.59 (C-2), 133.3 (C-1''), 133.14 (C-2',6'), 131.86 (C-1'), 130.28 (C-2'',6''), 129.31 (C-3a), 126.31 (C-5), 123.56 (C-3), 122.3 (C-6), 120.88 (C-4), 116.26 (C-3',5'), 115.92 (C-3'',5''), 113.24 (C-7), 30.98 (C-9). Compound **7** was identified as 2-(4'-

hydroxybenzoyl)-3-(4''-hydroxybenzyl) indole by comparing its physicochemical constants with those in literature.²⁴

Compound **8** was obtained as a white powder. The HRESI-MS ion at m/z 443.32 [M-H]⁻ and the NMR data deduced its molecular formula as C₂₈H₄₄O₄ with 8 degrees of unsaturation. The ¹H (600 MHz, CDCl₃) NMR spectrum indicated the δ : 5.61 (1H, s, H-7), 5.24 (1H, dd, $J = 15.0, 7.8$ Hz, H-23), 5.17 (1H, dd, $J = 15.0, 7.8$ Hz, H-22), 4.05 (1H, m, H-3), 1.03 (3H, d, $J = 6.6$ Hz, H-21), 0.98 (3H, s, H-19), 0.92 (3H, d, $J = 6.6$ Hz, H-28), 0.84 (3H, d, $J = 6.7$ Hz, H-27), 0.82 (3H, d, $J = 6.7$ Hz, H-26), 0.61 (3H, s, H-18). The ¹³C NMR (150 MHz, CDCl₃) spectrum indicated the δ : 198.41 (C-6), 164.97 (C-8), 135.03 (C-22), 132.44 (C-23), 119.66 (C-7), 79.47 (C-5), 74.65 (C-9), 67.17 (C-3), 56.01 (C-17), 51.76 (C-14), 45.28 (C-13), 42.79 (C-10), 41.7 (C-24), 40.18 (C-20), 36.76 (C-4), 34.98 (C-12), 33.12 (C-25), 29.90 (C-2), 28.65 (C-11), 27.8 (C-16), 25.49 (C-1), 22.38 (C-15), 21.04 (C-19), 20.27 (C-21), 19.89 (C-27), 19.59 (C-26), 17.67 (C-28), 12.23 (C-18). Compound **8** was identified as (22E,24R)-3 β ,5 α ,9 α -trihydroxyergosta-7,22-dien-6-one by comparing its physicochemical constants with those in literature.²⁵

Compound **9** was obtained as a white powder. The HRESI-MS ion was at m/z 121.03 [M-H]⁻. The molecular formula of the compound was confirmed as C₇H₆O₂ with 5 degrees of unsaturation according to the NMR data. The ¹H NMR (600 MHz, CD₃OD) spectrum indicated the δ : 9.76 (1H, s, CHO-1), 7.78 (2H, d, $J = 8.4$ Hz, H-2, 6), 6.92 (2H, d, $J = 8.4$ Hz, H-3, 5). The ¹³C NMR (150 MHz, CD₃OD) spectrum indicated the δ : 192.85 (C-7), 165.2 (C-1), 133.45 (C-3, 5), 130.33 (C-4), 116.89 (C-2, 6). Compound **9** was identified as p-hydroxybenzaldehyde by comparing their physicochemical constants with those in the literature.²⁶

Compound **10** was obtained as a colorless oil. It possesses the molecular formula C₉H₁₀O₃ with 5 degrees of unsaturation, which was deduced from the HRESI-MS ion at m/z 165.0549[M-H]⁻ and the NMR data. The ¹H NMR (600 MHz, CD₃OD) spectrum indicated the δ : 7.86 (2H, d, $J = 8.4$ Hz, H-3,5), 6.82 (2H, d, $J = 8.4$ Hz, H-2,6), 4.30 (2H, q, $J = 7.2$ Hz, -OCH₂), 1.36 (3H, t, $J = 7.2$ Hz, -CH₃). The ¹³C NMR (150 MHz, CD₃OD) spectrum indicated the δ : 168.31 (-C=O), 163.45 (C-4), 132.69 (C-2,6), 122.59 (C-1), 116.14 (C-3,5), 61.67 (-OCH₂), 14.65 (-CH₃). Compound **10** was established as ethyl p-hydroxybenzoate by comparing its physicochemical constants with those in the literature.²⁷

Compound **11** was obtained as a white crystal. Using the HRESI-MS ion at m/z 237.1860 [M-H]⁻ and the NMR data, its molecular formula was determined to be C₁₅H₂₆O₂ with 3 degrees of unsaturation. The ¹H NMR (600 MHz, CDCl₃) spectrum indicated the δ : 4.75 (1H, s, H-12a), 4.32 (1H, s, H-12b), 3.89 (1H, m, H-11a), 3.82 (1H, m, H-11b), 3.63 (1H, dd, $J = 10.2, 5.4$ Hz, H-1a), 2.32 (1H, m, H-7a), 2.06 (1H, m, H-7b), 2.01 (1H, m, H-9), 1.76 (1H, m, H-6a), 1.67 (2H, m, H-2a), 1.46 (1H, m, H-6b), 1.43 (1H, m, H-3a), 1.35 (2H, m, H-3b), 1.11 (1H, dd, $J = 12.6, 3.0$ Hz, H-5), 0.88 (3H, s, H-15), 0.82 (3H, s, H-14), 0.80 (3H, s, H-13). The ¹³C NMR (150MHz, CDCl₃) spectrum indicated the δ : 149.17 (C-8), 106.88 (C-12), 77.23 (C-1), 61.53 (C-11), 58.04 (C-9), 53.84 (C-5), 44.98 (C-10), 40.11 (C-3), 38.00 (C-7), 33.17 (C-4), 33.11 (C-15), 28.79 (C-2), 24.31 (C-6), 21.39 (C-14), 9.36 (C-3). Compound **11** was identified as funatrol D by comparing its physicochemical constants with those in the literature.²⁸

Compound **12** was obtained as a colorless oil. The HRESI-MS ion at m/z 277.15 [M-H]⁻ and the NMR data assigned the molecular formula of the compound as C₁₆H₂₂O₄ with 6 degrees of unsaturation. The ¹H NMR (600 MHz, CD₃OD) spectrum indicated the δ : 7.71 (2H, dd, $J = 6.0, 3.6$ Hz, H-3,6), 7.60 (2H, dd, $J = 6.0, 3.6$ Hz, H-4,5), 4.29 (4H, t, $J = 7.2$ Hz, H-1), 1.71 (2H, m, H-2'), 1.42 (4H, m, H-3'), 0.97 (6H, t, $J = 7.2$ Hz, H-4). The ¹³C NMR (150 MHz, CD₃OD) spectrum indicated the δ : 169.31 (COO-), 133.61 (C-1,2), 132.35 (C-4,5), 129.89 (C-3,6), 66.67 (C-1'), 31.74 (C-2'), 20.27 (C-3'), 14.06 (C-4'). Compound **12** was established as dibutyl phthalate by comparing its physicochemical constants with those in the literature.²⁹

Compound **13** was obtained as a white powder. It had a molecular formula C₂₈H₄₀O₄ with 9 degrees of unsaturation based on the [M-Na]⁺ ion at m/z 463.2819 in the HRESI-MS spectrum and the NMR data. The ¹H NMR (600 MHz, CDCl₃) spectrum indicated the δ : 6.89 (1H, m, H-5'), 6.04 (1H, dd, $J = 9.6$ Hz, H-6'), 5.64 (1H, d, $J = 2.4$ Hz, H-2), 5.26 (1H, ddd, $J = 15.0, 7.8, 2.4$ Hz, H-16), 5.15 (1H, m, H-15), 2.91 (1H, m, H-3'b), 2.72 (1H, m, H-5b), 2.50 (1H, m, H-3'a), 2.47 (1H, m, H-3'b), 2.44 (1H, m, H-8), 2.33 (1H, m, H-5a), 2.20 (1H, m, H-6b), 2.09 (1H, m, H-13), 2.03 (1H, m, H-3'), 1.88 (1H, m, H-10b), 1.84 (1H, m, H-17), 1.75 (1H, m, H-6a), 1.69 (1H, m, H-18), 1.55 (1H, m, H-9b), 1.52 (1H, m, H-9a), 1.48 (1H, m, H-10a), 1.43 (1H, s, H-7'), 1.38 (1H, m, H-11), 1.03 (3H, t, $J = 6.3$ Hz, H-14), 0.92 (3H, d, $J = 6.6$ Hz,

H-21), 0.88 (3H, s, H-12), 0.84 (3H, d, $J = 6.6$ Hz, H-19), 0.82 (3H, d, $J = 6.6$ Hz, H-20). The ^{13}C NMR (150MHz, CDCl_3) spectrum indicated the δ : 204.52 (C-4), 195.76 (C-1'), 164.26 (C-1), 156.16 (C-3), 148.75 (C-5'), 134.56 (C-15), 132.90 (C-16), 128.12 (C-6'), 117.51 (C-2), 81.19 (C-2'), 57.82 (C-8), 55.38 (C-11), 46.46 (C-7), 42.83 (C-17), 40.09 (C-13), 38.97 (C-5), 37.97 (C-6), 33.04 (C-18), 32.11 (C-3'), 29.01 (C-10), 24.76 (C-4'), 21.87 (C-9), 21.64 (C-7'), 20.97 (C-14), 19.93 (C-19), 19.64 (C-20), 17.55 (C-21), 12.07 (C-12). Compound **13** was identified as chaxine C by comparing its physicochemical constants with those in literature.³⁰

Compound **14** was obtained as a white crystal. Its molecular formula was deduced as $\text{C}_{29}\text{H}_{46}\text{O}_2$ with 7 degrees of unsaturation according to the $[\text{M}-\text{H}]^-$ and $[\text{M}-\text{Na}]^+$ ion at m/z 425.3433 and 449.3387 in the HRESI-MS spectrum and the NMR data. The ^1H NMR (600 MHz, CDCl_3) spectrum indicated the δ : 5.82 (1H, s, H-4), 5.15 (1H, dd, $J = 15.0, 8.4$ Hz, H-22), 5.00 (1H, dd, $J = 15.0, 8.4$ Hz, H-23), 4.34 (1H, s, H-6), 1.38 (3H, s, H-19), 1.02 (3H, d, $J = 6.6$ Hz, H-21), 0.85 (3H, m, H-26), 0.80 (3H, t, $J = 7.2$ Hz, H-29), 0.78 (3H, d, $J = 7.2$ Hz, H-27), 0.6 (3H, s, H-18). The ^{13}C NMR (150MHz, CDCl_3) spectrum indicated the δ : 200.29 (C-3), 168.34 (C-5), 138.08 (C-22), 129.53 (C-23), 126.35 (C-4), 73.32 (C-6), 56.02 (C-14), 55.99 (C-17), 53.68 (C-9), 51.25 (C-24), 42.41 (C-13), 40.43 (C-20), 39.53 (C-12), 38.58 (C-7), 38.00 (C-10), 37.13 (C-1), 34.26 (C-2), 31.86 (C-25), 29.74 (C-8), 28.83 (C-16), 25.43 (C-28), 24.21 (C-15), 21.17 (C-27), 21.05 (C-21), 20.97 (C-11), 19.50 (C-19), 18.99 (C-26), 12.01 (C-18), 11.97 (C-29). Compound **14** was established as (20R)-6 β -hydroxystigmasta-4,22-dien-3-one by comparing its physicochemical constants with those in literature.³¹

Compound **15** was obtained as a white crystal. The HRESI-MS ion at m/z 427.3581 $[\text{M}-\text{H}]^-$ and 451.3544 $[\text{M}-\text{Na}]^+$ and the NMR data assigned its molecular formula as $\text{C}_{29}\text{H}_{46}\text{O}_2$ with 7 degrees of unsaturation. The ^1H NMR (600 MHz, CDCl_3) spectrum indicated the δ : 5.81 (1H, s, H-4), 4.35 (1H, s, H-6), 1.69 (1H, m, H-25), 1.51 (1H, m, H-24), 1.38 (1H, s, H-20), 1.33 (1H, m, H-28b), 1.26 (1H, m, H-28a), 1.03 (3H, s, H-19), 0.93 (3H, d, $J = 6.6$ Hz, H-21), 0.86 (3H, s, H-29), 0.84 (3H, d, $J = 6.6$ Hz, H-27), 0.82 (3H, d, $J = 6.6$ Hz, H-26), 0.74 (3H, s, H-18). The ^{13}C NMR (150 MHz, CDCl_3) spectrum indicated the δ : 200.34 (C-3), 168.43 (C-5), 126.32 (C-4), 73.29 (C-6), 56.10 (C-14), 55.91 (C-17), 53.66 (C-9), 45.87 (C-24), 42.53 (C-13), 39.63 (C-12), 38.6 (C-7), 38.01 (C-10),

37.12 (C-1), 36.12 (C-20), 34.26 (C-2), 33.93 (C-22), 29.74 (C-8), 29.21 (C-25), 28.17 (C-16), 26.16 (C-23), 24.16 (C-15), 23.10 (C-28), 20.99 (C-11), 19.50 (C-19), 19.04 (C-27), 18.73 (C-21), 12.01 (C-18), 11.97 (C-29), 9.80 (C-26). Compound **15** was identified as 6-hydroxystigmast-4-en-3-one by comparing its physicochemical constants with those in the literature.³²

Representative compound analysis

Compound **6** was obtained as a white crystal. The IR spectrum of the compound showed absorption peaks at 3408, 2871, 1607, 1512, 1481, 1322 and 833 cm^{-1} . The UV spectrum of the compound in methanol (0.05 M) displayed two absorption bands at 226 nm and 278 nm, respectively. The methanol solution of the compound (0.1 M) exhibited a rotundity of -13.6° at 25°C . The HRESI-MS ion at m/z 243.1027 $[\text{M}-\text{H}]^-$ and the NMR data deduced its molecular formula as $\text{C}_{15}\text{H}_{16}\text{O}_3$ with 8 degrees of unsaturation. The ^1H -NMR (600 MHz, CD_3OD) and the ^{13}C -NMR (150 MHz, CD_3OD) spectra data were illustrated in Table 1, which indicated the presence of three methylene groups [δ_{H} : 4.38, (s, 2H), 3.60 (t, $J = 7.2$ Hz, 2H), 2.76 (t, $J = 7.2$ Hz, 2H)]; eight methine [δ_{H} : 7.11 (d, $J = 8.4$ Hz, H-3,5), 7.01 (d, $J = 8.4$ Hz, H-3',5'), 6.73 (d, $J = 8.4$ Hz, H-2,6), 6.68 (d, $J = 8.4$ Hz, H-2',6')]; four quaternary carbon signals [δ_{C} : 158.31 (C-4), 156.81 (C-4'), 131.05 (C-1'), 130.06 (C-1)]. It can be known from the one-dimensional spectra data that there were four groups of symmetrical carbons {116.11 (C-3',5'), 116.12 (C-3,5), 130.68 (C-2,6), 130.87 (C-2', C-6')}, which inferred there were two benzene rings present in the structure of the compound. Further deduction from the HMBC spectrum suggested that C-4 and C-4' were the carbon atoms that attached to the oxygen atoms in benzene rings. According to the HMBC signals (Fig. 2), H-7' correlated with C-1' and C-8'; H-7 correlated with C-1, H-2 correlated with C-3,5, C-1, C-7; H-3 correlated with C-2,6, C-1, C-4; H-2' correlated with C-3',5', C-1', C-4', C-7'; and H-3' correlated with C-2',6', C-1', C-4'. Based on the H-H COSY signals (Fig. 2), H-2 was correlated to H-3; H-5 was correlated to H-6; H-2' was correlated to H-3'; H-5' was correlated to H-6'; and H-7' was correlated to H-8'. By Summing up the above signals it can be proved that two benzene rings in the compound were connected by O-C8'-C7'. Furthermore, the presence of two OH in the structure of the compound was deduced from the

molecular formula. By combining HMBC, H-H COSY spectra and the literature,³³ the compound

was established and named as 4-(4-hydroxyphenethoxy)-benzyl alcohol.

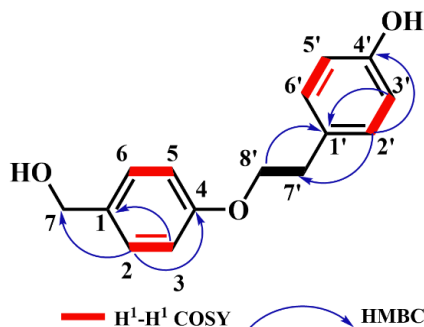


Fig. 2 – ¹H-¹H COSY correlations and the selected HMBC correlations of compound **6**.

Table 1
¹H NMR (600 MHz) and ¹³C NMR (150 MHz) data for compound **6**

Positions	δ_C	δ_H (mult., <i>J</i> in Hz)
1	130.36, C	
2	130.68, CH	7.11 (d, 8.4)
3	116.12, CH	6.73 (d, 8.5)
4	158.31, C	
5	116.12, CH	6.73 (d, 8.5)
6	130.68, CH	7.11 (d, 8.4)
7	73.76, CH ₂	4.38 (s)
1'	131.05, C	
2'	130.87, CH	7.01 (d, 8.3)
3'	116.11, CH	6.68 (d, 8.3)
4'	156.81, C	
5'	116.11, CH	6.68 (d, 8.3)
6'	130.87, CH	7.01 (d, 8.3)
7'	36.34, CH ₂	2.76 (t, 7.1)
8'	72.42, CH ₂	3.60 (t, 7.1)

Recorded in CD₃OD.

Activity test of PTP1B and SHP2

Several studies have shown that SHP2 plays an important role in regulating immune cell function in the tumor microenvironment and is a promising target for cancer therapy, and protein tyrosine phosphatase 1B (PTP1B) has emerged as an important therapeutic target in the treatment of type 2 diabetes and obesity. In our

continuing search for anticancer reagents and PTP1B inhibitors, most of the compounds with sufficient amount were screened at 50 μ g/mL for their SHP2 and PTP1B inhibition activities. As summarized in Table 2, compound **13** was found to be the most potent agent toward SHP2 and compound **1** could serve as the most effective inhibitor toward PTP1B with the inhibition rates being 50.59% and 71.33%, respectively.

Table 2
Inhibitory activities of the compounds against SHP2 and PTP1B

Compound (50 μ g/mL)	SHP2 Inhibition (%)	PTP1B Inhibition (%)
1	22.58	71.33
2	0.02	30.03
3	11.90	49.64
4	23.51	29.44
5	49.90	62.09
6	31.68	59.21
7	46.90	56.89
11	31.31	40.28
13	50.59	11.46
14	27.47	20.52
15	26.68	24.05

EXPERIMENTAL SECTION

Materials and Measurements

Bruker AM-600 spectrometer (Bruker, Bremen, Germany), Apex-Ultra 7.0 T (Bruker, Bremen, Germany), LC2000 semi-preparative liquid chromatography system (auno-tech, Beijing, China), Thermo Scientific UltiMate 3000 HPLC (Thermo, Shanghai, China), Rapid preparation of liquid chromatography (Biotage, Sweden), Hypersil GOLD Column (Thermo, Shanghai, China), Bruker VERTEX 70 Infrared spectro-photometer (Bruker, Bremen, Germany), Rotary evaporator (BUCHI, Switzerland), Portable UV Analyser (Shanghai Jihui scientific analysis instrument Co., Ltd.), Electronic balance (Sartorius, Gottingen, Germany), Double thermostatic shaker (Zhicheng, Shanghai, China), benchtop (Zhicheng, Shanghai, China), biochemical incubator (Yuejin, Shanghai, China), steam sterilizer (Shenan, Shanghai, China).

Silica gel (HSGF-254, Yantai Institute of Chemical Industry, Yantai, China) was employed for thin-layer chromatography (TLC), while Silica gel (60-80 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China) and silica gel (200-300 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China) and Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography. Chromatographic-grade reagents (methanol, acetonitrile) were procured from OCEANPAK. Analytical Reagent-grade reagents were procured from Tianjin jindong tianzheng chemical reagent factory.

The fungus was separated from the leaves of Ceylon olive which was collected from Xinglong County, Hainan Province, People's Republic of China. It was stored in glycerol tubes at -80°C in the strain bank of Hebei University, Baoding City, Hebei province, People's Republic of China. The strain was identified as *Pestalotiopsis neglecta* by genome sequencing and morphological analysis. It is registered in the NCBI Gene Bank with the tag number of AY687881.

Synthesis

Fermentation and Isolation

The strain *Pestalotiopsis neglecta* stored in glycerol tubes was removed to fresh PDA plates for activation at 28°C for 4 days. When the strains grew to about 2/3 of the PDA plate, the activated strains were divided into 3 mm × 3 mm squares, and

cultured in SDA medium and incubated in a shaker at 130 r/min, 28 °C for 4 days to give a seed liquid. Finally the seed liquid was transferred to rice culture medium and incubated in darkness at 28 °C for 30 days to obtain the fermentation product.

The fermentation product was stepped in equal volume of ethyl acetate and sonicated four times. The solvents were evaporated to give a crude product. Then the crude product was extracted with water/ethyl acetate (1:1) three times and the ethyl-acetate phase was collected. After the organic layer was concentrated in vacuo, the extraction (180g) was obtained.

The extraction (180 g) was fractionated on a positive silica column (petroleum-ether/ethyl-acetate 19:1, 17:3, 2:3, 3:2, 3:17, 0:1) to afford six primary fractions (Fr.1–Fr.6).

Fr. 3 was subjected to a normal-phase silica gel column chromatography (petroleum-ether/ethyl-acetate) and the 30% ethyl acetate fraction was subsequently fractionated on an open reversed-phase silica gel column eluting with a gradient solvent system of methanol/water (25:75, 35:65, 45:55, 50:50, 60:40, 70:30, 90:10, 100:0) to give five subfractions (Fr. 3.1–3.5). Purification of Fr. 3.1 by HPLC (flow rate: 2.5 mL/min, 28% methanol/72% acidic water) yielded compound **1** (4.0 mg). Separation of Fr.3.2 over column chromatography on Sephadex LH-20 (MeOH) and then recrystallisation obtained compound **2** (6.5 mg). Subfraction Fr.3.3 was purified by semipreparative HPLC (flow rate: 2.5 mL/min, 33% acetonitrile/67% water) to give compound **3** (31.0 mg), **4** (4.0 mg) and a mixture (55.0 mg). The mixture was further separated by semipreparative HPLC (flow rate: 2.5 mL/min, 22% acetonitrile/78% water) to afford compound **5** (3.5 mg) and **6** (7.5 mg). The Fr.3.4 was subjected to semipreparative HPLC (flow rate: 2.5 mL/min, 96% methanol/4% water) to give compound **7** (4.8 mg). The Fr.3.5 was fractionated by semipreparative HPLC (flow rate: 2.5 mL/min, 92% methanol/8% water) to yield compound **8** (11.5 mg).

Fr. 2 was applied to a normal-phase silica column (petroleum-ether/ethyl-acetate) and the 30% ethyl acetate fraction was then chromatographed over open reversed-phase silica gel by elution with stepwise methanol/water (35:65, 45:55, 55:45, 65:35, 70:30, 90:10, 100:0) to give seven subfractions (Fr. 2.1–2.7). Purification of Fr. 2.1 by semipreparative HPLC (flow rate: 2.5 mL/min, 45% methanol/55% water) yielded compound **9** (6.0 mg). Separation of Fr. 2.2 by semipreparative HPLC (flow rate: 2.5 mL/min, 52% methanol/48% acidic water) afforded compound **10** (3.0 mg). Subfraction Fr. 2.3 was

recrystallized twice to give compound **11** (6.5 mg). The Fr. 2.4 was purified over semipreparative liquid phase (flow rate: 2.5 mL/min, 79% methanol/21% water) to provide compound **12** (7.2 mg). The Fr. 2.5 was separated sequentially by column chromatography on Sephadex LH-20 (dichloromethane-methanol) and semipreparative HPLC (flow rate: 2.5 mL/min, 88% methanol/12% water) to obtain compound **13** (3.5 mg). The Fr. 2.6 was fractionated by column chromatography on Sephadex LH-20 (methanol) and subsequently semipreparative liquid phase (flow rate: 3 mL/min, 97% methanol/3% water) to give compound **14** (3.5 mg) and compound **15** (12 mg).

CONCLUSION

In conclusion, a total of fifteen compounds, including a new natural product, a new compound and thirteen known compounds, have been isolated from the fermentation product of *Pestalotiopsis neglecta*. The biological activity tests showed that compound **1** exhibited excellent inhibitory activity against PTP1B with the inhibition rate of 71.33% and compound **13** displayed promising inhibitory effect on SHP2 with the inhibition rate of 50.59%. However, persistent studies are needed to explore more effective lead compounds and investigate the therapeutic window, bioavailability and metabolism *in vivo* of the lead compounds.

Acknowledgements. This work was funded by the Natural Science Foundation-Biomedicine Joint Foundation of Hebei Province (B2020201090), Students Innovation Training Program of Hebei University (DC2005311) and Natural Science Foundation of Henan Province (212300410068).

REFERENCES

- C.Y. Tsui and C.Y. Yang, *Foods*, **2021**, *10*, 704–721.
- F.F. De Lima, C.A. Breda, C.A.L. Cardoso, M.C.T. Duarte and E.J. Sanjinez-Argandoña, *Afr. J. Food Sci.*, **2019**, *13*, 30–37.
- Y.H. Chen and C.Y. Yang, *Processes*, **2020**, *8*, 1218.
- L. Jayasinghe, N.R. Amarasinghe, B.G. Arundathie, G.K. Rupasinghe, N.H. Jayatilake and Y. Fujimoto, *Nat. Prod. Res.*, **2012**, *26*, 717–721.
- C.Y. Huang, I. Liu, X.Z. Huang, H.J. Chen, S.T. Chang, M.L. Chang, Y.T. Ho and H.T. Chang, *Pharmaceutics*, **2021**, *13*, 1059–1070.
- S.M.D. Sharker and I.J. Shahid, *J. Pharm. Pharmacol.*, **2010**, *4*, 66–69.
- B. Draznin, V.R. Aroda, G. Bakris, G. Benson, F.M. Brown, R. Freeman, J.B. Green, E.S. Huang, D.M. Isaacs, S. Kahan, J. Leon, S.K. Lyons, A.L. Peters, P. Prahalad, J.E. Reusch, D. Young-Hyman, S. Das and M.N. Kosiborod, *Diabetes care*, **2021**, *45* Supplement_1, 17–38.
- J.B. Xiao and P. Högger, *Curr. Med. Chem.*, **2015**, *22*, 23–38.
- H. Minoura, S. Takeshita, T. Yamamoto, M. Mabuchi, J. Hirosumi, S. Takakura, I. Kawamura, J. Seki, T. Manda, M. Ita and S. Mutoh, *Eur. J. Pharmacol.*, **2005**, *519*, 182–190.
- J.K. Kim, M.D. Michael, S.F. Previs, O.D. Peroni, F. Mauvais-Jarvis, S. Neschen, B.B. Kahn, C.R. Kahn and G. Shulman, *J. Clin. Investig.*, **2000**, *105*, 1791–1797.
- H. Hussain, I.R. Green, G. Abbas, S.M. Adekenov and W. Hussain, *Expert Opin. Ther. Pat.*, **2019**, *29*, 689–702.
- T.O. Johnson, J. Ermolieff and M.R. Jirousek, *Nat. Rev. Drug Discov.*, **2002**, *1*, 696–709.
- W.H. Jiao, J. Li, D. Wang, M.M. Zhang, L.Y. Liu, F. Sun, J.Y. Li, R.J. Capon and H.W. Lin, *J. Nat. Prod.*, **2019**, *82*, 2586–2593.
- L. Pompermaier, E.H. Heiss, M. Alilou, F. Mayr, M. Monizi, T. Lautenschlaeger, D. Schuster, S. Schwaiger and H. Stuppner, *J. Nat. Prod.*, **2018**, *81*, 2091–2100.
- S. G. Wubshet, Y. Tahtah, A. M. Heskes, K. T. Kongstad, I. Pateraki, B. Hamberger, B. L. Møller and D. Staerk, *J. Nat. Prod.*, **2016**, *79*, 1063–1072.
- J.F. Zong, Z. Hu, Y.Y. Shao, Q. Shi, M.M. Zhang, Y.B. Zhou, J. Li and A.J. Hou, *Org. Lett.*, **2020**, *22*, 2797–2800.
- X. Yuan, H. Bu, J. Zhou, C.Y. Yang and H. Zhang, *J. Med. Chem.*, **2020**, *63*, 11368–11396.
- S.G. Julien, N. Dubé, S. Hardy and M.L. Tremblay, *Nat. Rev. Cancer.*, **2011**, *11*, 35–49.
- Q. Liu, J. Qu, M. Zhao, Q. Xu and Y. Sun, *Pharmacol. Res.*, **2020**, *152*, 104595.
- M.T. Gutierrez-Lugo, G.M. Woldemichael, M.P. Singh, P.A. Suarez, W.M. Maiese, G. Montenegro and B.N. Timmermann, *Nat. Prod. Res.*, **2005**, *19*, 645–652.
- J. Hayashi, T. Sekine, S. Deguchi, Q. Lin, S. Horie, S. Tsuchiya, S. Yano, K. Watanabe and F. Ikegami, *Phytochemistry*, **2002**, *59*, 513–519.
- W. Zhang and Q. Song, *Chinese Herb. Med.*, **2010**, *41*, 1782–1785.
- J. Hayashi, T. Sekine, S. Deguchi, Q. Lin, S. Horie, S. Tsuchiya, S. Yano, K. Watanabe and F. Ikegami, *Phytochemistry*, **2002**, *59*, 513–519.
- L. Yang, R. Jiang, H.H. Li, Y.P. Pan, J.J. Lu, H. Zhang, S.J. Liu, J.L. Shen and J.M. Hu, *Rsc. Adv.*, **2020**, *10*, 14644–14649.
- X.L. Han, Z.J. Lin, H.W. Tao, P.P. Liu, Y. Wang and W.M. Zhu, *Chin. J. Mar. Drugs*, **2009**, *28*, 11–16.
- D. Shataer, R. Abdulla, Ma, Q.L. G.Y. Liu and H.A. Aisa, *Chem. Nat. Compd.*, **2020**, *56*, 338–340.
- L.M. Yang and H.Z. Fu, *J. Chin. Pharm. Sci.*, **2011**, *20*, 154–158.
- J.H. Ding, T. Feng, Z.H. Li, J. Si, H.Y. Yu, H.B. Zhang and J.K. Liu, *J. Asian Nat. Prod. Res.*, **2013**, *15*, 828–832.
- J.T. Li, B.L. Yin, Y. Liu, L.Q. Wang and Y.G. Chen, *Chem. Nat. Compd.*, **2009**, *45*, 234–236.
- J.H. Choi, A. Ogawa, N. Abe, K. Masuda, T. Koyama, K. Yazawa and H. Kawagishi, *Tetrahedron*, **2009**, *65*, 9850–9853.
- R.J. Li, D.X. Guo and H.X. Lou, *Chin. J. Nat. Med.*, **2013**, *11*, 74–76.
- P. Georges, M. Sylvestre, H. Ruegger and P. Bourgeois, *Steroids*, **2006**, *71*, 647–652.
- J. Hayashi, T. Sekine, S. Deguchi, Q. Lin, S. Horie, S. Tsuchiya, S. Yano, K. Watanabe & F. Ikegami, *Phytochemistry*, **2002**, *59*, 513–5