SHORT REPORT

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A New Benzofuran from the Roots of *Eupatorium chinense* L. and Its *α*-Glucosidase and PTP1B Inhibitory Activities

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Abstract: A new benzofuran, named eupbenzofuran A (**1**), along with four known compounds, 10,11-dihydroxy-10,11-dihydroeuparin (**2**), 5-[1'-hydroxyethyl]-2-1'-hydroxyisoprolyl]-benzofuran (**3**), 3α,6-dihydroxytremetone (**4**), odoratin (**5**) were isolated from the roots of *Eupatorium chinense* L. The structure of compounds were identified by NMR, MS, CD and other spectroscopic methods and comparisons with relevant literature data. Compound **1** had favorable dual inhibitory activities against *α*-glucosidase and protein tyrosine phosphatase 1B (PTP1B). However, compounds **2**-**5** had no significant inhibitory activities effects on *α*-glucosidase and PTP1B (IC_{50}＞50 μg/mL). Molecular docking technique was used to calculate the interaction of compound **1** with *α*-glucosidase and PTP1B, respectively, and the calculated results showed that compound **1** had both strong binding to *α*-glucosidase and PTP1B.

Keywords: *Eupatorium chinense* L.; chemical constituents; antidiabetic activity; *α*-glucosidase; protein tyrosine phosphatase 1B.

1. Plant Source

The *Eupatorium chinense* L. (*E. chinense*) were collected from Changyang Tujia autonomous county, Hubei province in China, and identified as *Eupatorium chinense* L. by Prof. Yu-Bin Wang of China Three Gorges University. A voucher specimen (No. TRCW20210910) has been deposited at the Hubei Key Laboratory of Natural Products Research and Development, China Three Gorges University, China.

2. Previous Studies

E. chinense, primarily distributed in the western and southern regions of China. It plays an important role in Chinese herbal medicine because of the wide usage in Tujia and Miao minorities in some provinces of China. Traditionally, it has been used in folk medicine as a tea substitute for disease prevention. *E. chinense* was utilized for the treatment of throat diseases such as pharyngitis, diphtheria, and tonsillitis in Guangdong province in China, the roots of *E. chinense* were sliced and brewed into tea for their hypoglycemic properties in Jiangsu and Zhejiang provinces in China [1]. In our previous research, more than 100 compounds were isolated from *E. chinense*, including sesquiterpenes,

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diterpenes, flavonoids, benzofuran and other types [2-5]. Modern pharmacological research had shown that *E. chinense* exhibited significant anti-inflammatory [2], anti-tumor [6], and anti-diabetes [7].

3. Present Study

Dried roots of *E. chinense* (10.0 kg) was pulverized to a coarse powder and extracted with 95% ethanol by heating to 80℃ and refluxing three times for 6 hours each time. 297.6 g of crude extract was obtained under reduced pressure, The crude extract was suspended in water and partitioned successively with different polarity solvents; PE, EtOAc, and *n*-BuOH respectively. Then, PE (petroleum ether, 83.6 g), EtOAc (ethyl acetate, 48.4 g) and *n*-BuOH (*n*-butyl alcohol, 58.2 g) fractions were obtained. The *n*-butanol extract (58.2 g) was dissolved in 300 g of deionized water using ultrasonic dissolution and subjected to macroporous adsorption resin chromatography (D101). A 1000 g resin column was prepared and washed with deionized water. The sample was applied and adsorbed overnight. Elution was carried out with ethanol:deionized water (50:50, v/v) until colorless. 34.5 g of the extract was obtained under reduced pressure.

The fraction (34.5 g) was chromatographed by C_{18} reversed-phase silica gel with a gradient of MeOH:H_2O (10:90 to 100:0, v/v) to give 8 fractions, A-H. Fr.F was separated on C_{18} reversed-phase silica gel with MeOH:H_2O (10:90 to 100:0, v/v) as eluent to obtain 10 subfractions Fr.F (1-10). Subfraction F9 was separated to C_{18} reversed-phase silica gel eluted with MeOH:H_2O (75:25, v/v), 4 fractions (F9-1-F9-4) were obtained. Fr.F9-3 was purified by semi-preparative HPLC to give compound **1** (5.9 mg, ACN:H_2O=60:40, v/v, 2 mL/min, 15 min). Fr.F9-4 was purified by semi-preparative HPLC to give compound **4** (21.8 mg, ACN:H_2O=47:53, v/v, 2 mL/min, 33 min). Fr.E was isolated by HW-40F with MeOH:H_2O (2:8, v/v) as eluent to obtain 17 subfractions Fr.E (1-17). Subfraction Fr.E11 was subjected to C_{18} reversed-phase silica gel with MeOH:H_2O (63:36, v/v) elution to afford 3 fractions Fr.E-11(1-3), Fr.E11-2 was purified by semi-preparative HPLC to give compound **5** (8.8 mg, ACN:H_2O=35:65, v/v, 2 mL/min, 25 min), Fr.E11-3 was purified by semi-preparative HPLC to give compound **3** (22.8 mg, ACN:H_2O=45:55, v/v, 2 mL/min, 18 min). Fr.E12 was separated by C_{18} reversed-phase silica gel with MeOH:H_2O (63:36, v/v) elution to afford 3 fractions Fr.E-12(1-3). Fr.E12-3 was purified by semi-preparative HPLC to give compound **2** (8.9 mg, ACN:H_2O=37:63, v/v, 2 mL/min, 14 min).

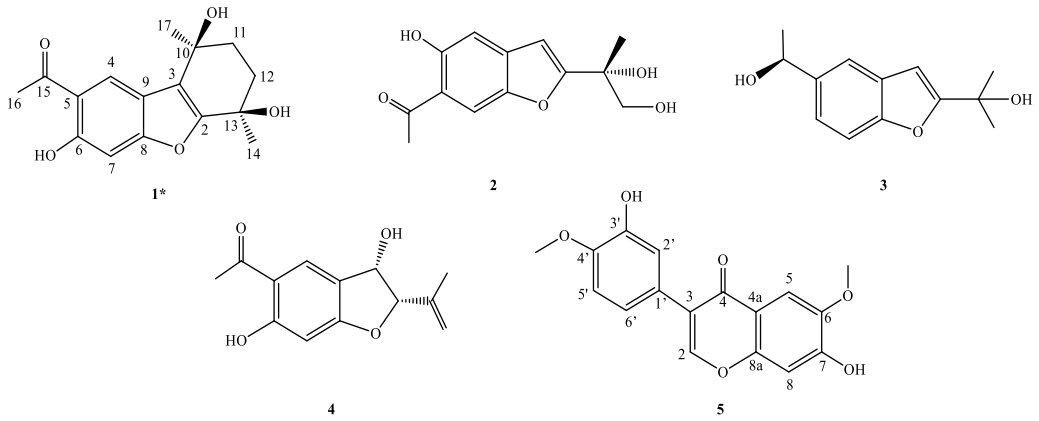

Figure 1. Chemical structures of compounds **1**-**5.**

Compound **1**: colorless oil, UV (MeOH) λ_{max} = 239, 350 nm; ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz) data, see Table **1**; HRESIMS *m/z* measured 313.1049 $[M+Na]^+$ ($C_{16}H_{18}NaO_5$, calcd. 313.1052).

In this study, we isolated and characterized the chemical constituents of the *n*-butanol portion of the *E. chinense*. We obtained a new benzofuran compound and four known compounds (Figure 1). The known compounds were identified as 10,11-dihydroxy-10,11-dihydroeuparin (**2**), 5-[1'-hydroxyethyl]-2-1'-hydroxyisoprolyl]-benzofuran (**3**), 3α,6-dihydroxytremetone (**4**), odoratin (**5**)

Table. 1 The NMR data of compound **1** in DMSO-d_6 (δ in ppm, J in Hz).

Position	**1**	
	δ_C	δ_H J (Hz)
2	156.86	-
3	117.22	-
4	124.92	8.31 (s)
5	120.73	-
6	160.04	-
7	99.52	7.07 (s)
8	158.92	-
9	119.66	-
10	68.74	-
11	36.77	2.13 (m), 1.73 (m)
12	37.71	1.95 (ddd, 13.8, 5.8, 3.0), 1.81 (m)
13	65.69	-
14	27.18	1.45 (s)
15	205.05	-
16	27.99	2.72 (s)
17	27.99	1.50 (s)
6-OH	-	12.33 (s)
10-OH	-	5.20 (s)
13-OH	-	5.28 (s)

Compound **1** was a colorless oil; $[\alpha]^{25}_D$ + 8.6° (c=0.1, CH_3OH); its molecular formula was established as $C_{16}H_{18}NaO_5$ (8 degrees of unsaturation) by HRESIMS data (*m/z* 313.1049 $[M+Na]^+$, calcd. for 313.1052). The ^{1}H-NMR spectral data (Table 1) of **1** displayed two olefinic proton signals at δ_H 8.31 (H-4) and 7.07 (H-7) and three methyl signals at δ_H 2.72 (H-16), 1.50 (H-17) and 1.45 (H-14). Analysis of the ^{13}C-NMR spectrum (Table 1) and DEPT135 spectrum of compound **1** revealed 16 carbons signals, including three methyl carbons δ_C 27.9 (C-16), 27.9 (C-17) and 27.2 (C-14), two methylene carbon δ_C 37.69 (C-12) and 36.77 (C-11), two oxygenated carbons δ_C 68.74 (C-10) and 65.69 (C-13), one carbonyl carbon signal δ_C 205.05 (C-15), eight olefinic carbon signals δ_C 160.04 (C-6), 158.92 (C-8), 156.9 (C-2), 124.9 (C-4), 120.7 (C-5), 119.7 (C-9), 117.22 (C-3), 99.52 (C-7). The carbon skeleton of the compound was similar to (-)-eupachinin A reported in the literature (TableS1, see supporting information) [8]. Compound **1** showed great similarity to (-)-eupachinin A reported in the literature (TableS1, see supporting information), differing in the additional of methyl carbon signal at δ_C 27.99 (C-17) and an oxygenated quaternary carbon signal of δ_C 65.69 (C-10), while the absence of a carbonyl carbon signal. After comprehensive analysis, it was preliminarily inferred that the compound was with one methyl, and one hydroxyl substituents at C-10. In the HMBC (Figure 2) profile of compound **1**, the correlations between H-17 (δ_H 1.50), H-11 (δ_H 1.73) and C-10 (δ_C 65.69) indicated the positions of the hydroxyl and methyl was at the C-10 position. In the HMBC, H-14 (δH 6.59) correlated with C-13, C-12, C-2, respectively. Meanwhile the ^{1}H-^{1}H COSY spectrum (Figure 2) showed H-11/H-12 correlations. Thus, the planar structure of **1** was thereby established as depicted.

In the NOESY spectrum, correlations such as H-17/H-11β, H-14/H-11β; H-17/H-12β, H-14/H-12β were observed, indicated H-14 and H-17 at the same side of C ring. In order to assign the absolute configuration of compound **1**, the theoretical ECD data were calculated using the TDDFT method. The calculated ECD spectrum showed ECD curve with positive Cotton effect at 240 nm, comparable to the Cotton effect observed at 242 nm in the experimental spectrum of compound **1** (Figure 3). Thus, the absolute configuration was determined to be 10*S*, 13*R*, respectively. Compound **1** was named eupbenzofuran A.

A new benzofuran from *Eupatorium chinense*

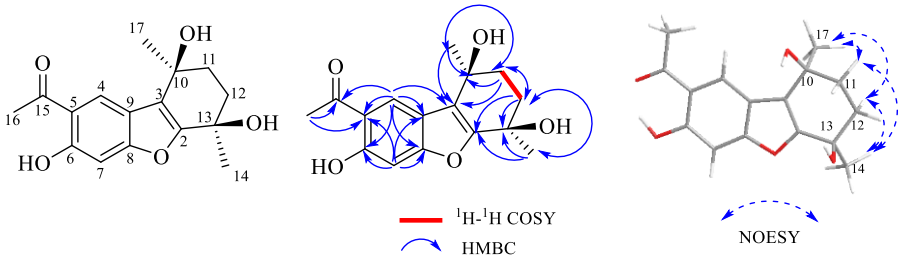

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of **1**

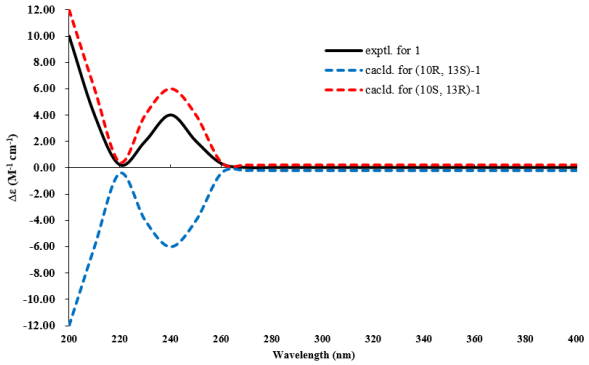

Figure 3. Experimental ECD spectrum and the calculated ECD spectrum of **1** in MeOH.

Compounds **2-5** was identified to be 10,11-dihydroxy-10,11-dihydroeuparin (**2**) [8], 5-[1'-hydroxyethyl]-2-1'-hydroxyisoprolyl]-benzofuran (**3**) [9], 3α,6-dihydroxytremetone (**4**) [8], odoratin (**5**) [11], by comparing their NMR data and optical rotations with those reported in the literature, respectively.

All compounds were tested for *α*-glucosidase and PTP1B inhibitory activities (Table 2.) according to the literature [12-15] Compound **1** showed potent dual inhibitory activities against *α*-glucosidase and PTP1B with the IC_{50} (μg/mL) values of 19.07±0.21, 36.82±0.54, respectively (positive control acarbose, IC_{50}=4.61±0.09, and positive control oleanolic acid , IC_{50}=9.82±0.19, respectively). But compounds **2-5** had no significant inhibitory effects on the activities of *α*-glucosidase and PTP1B (IC_{50}＞50 μg/mL).

Table 2. The inhibitory activities against *α*-glucosidase and PTP1B for **1-5**

Compounds	***α*-glucosidase (IC_{50}, μg/mL)**	**PTP1B (IC_{50}, μg/mL)**
1	19.07±0.21	36.82±0.54
2-5	＞50	＞50
acarbose	4.61±0.09	-
oleanolic acid	-	9.82±0.19

Molecular docking technique was used to calculate the interaction of compound **1** with *α*-glucosidase and PTP1B [16], respectively, and the calculated results showed that compound **1** have both strong binding to *α*-glucosidase and PTP1B.

Compound **1** exhibited multiple hydrophobic interactions with the amino acids PHE-163 and ALA-200 at the active site of the *α*-glucosidase protein and formed multiple hydrogen bonds with the amino acids GLN-256, ASP-327, GLN-328, and ARG-411 (Figure 4). The binding energy was calculated to be -7.9 kcal/mol.

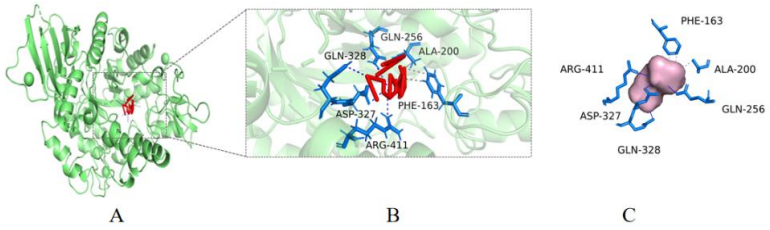

Figure 4. The binding mode of *α*-glucosidase protein with compound **1**
(**A**: 3D binding mode of compound **1** with *α*-glucosidase, **B**: Amino acid residue binding interactions of compound **1** with *α*-glucosidase, **C**: 2D binding mode of compound **1** with α-glucosidase)

Compound **1** exhibited multiple hydrophobic interactions with the amino acids GLU-75, GLU-76, LEU-234, LYS-248, VAL-249, and GLU-252 at the active site of the PTP1B, and formed multiple hydrogen bonds with the amino acids GLU-76, ALA-77, ARG-238, and VAL-249 (Figure 5). The binding energy was calculated to be -20.8 kcal/mol.

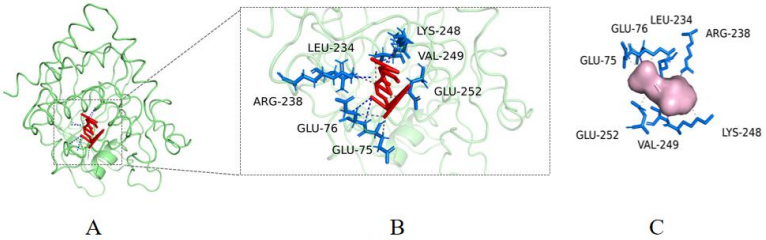

Figure 5. The binding mode of PTP1B with compound **1**
(**A**: 3D binding mode of compound **1** with PTP1B, **B**: Amino acid residue binding interactions of compound **1** with PTP1B, **C**: 2D binding mode of compound **1** with PTP1B).

In conclusion, our investigation towards the roots of *E. chinense* lead to the isolation of a new benzofuran compound and four known constituents. Compound **1** represents a new compound named eupbenzofuran A while compounds (**2-5**) were known compounds. All isolated compounds were evaluated for *α*-glucosidase and PTP1B inhibitory activities. This article enriched the chemical compound library of *E. chinense* and provided experimental evidence for elucidating the basis of its antidiabetic activity. It offered potential lead compounds derived from natural products for antidiabetic therapy, provided reliable activity data and s tructural informations for the further development and application of *E. chinense* resources and its benzofuran derivatives.

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Supporting Information

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