SHORT REPORT

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Eupbenzofuranside C: A New Benzofuranside with Dual Inhibitory Activities against α-Glucosidase and PTP1B from *Eupatorium chinense L.*

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Abstract: A new benzofuranside compound (eupbenzofuranside C) was isolated from the *n*-butanol fraction of ethanol extract of *Eupatorium chinense L.* along with three known compounds. The structures of the isolated compounds were elucidated by 1D and 2D NMR techniques, mass spectrometry and circular dichromism (CD). The isolated compounds were investigated for their α-glucosidase and PTP1B inhibitory activities by *p*-nitrophenyl-β-galactopyranoside method and *p*-nitrophenyl phosphate method, respectively. Although eupbenzofuranside C showed potential dual inhibitory activity against α-glucosidase and PTP1B with IC_{50} values of 40.07±0.38 μg/mL and 39.37±0.36 μg/mL, respectively, these values were determined to be far from the data of the positive control acarbose. In contrast, compound **2** showed inhibitory activities against both α-glucosidase and PTP1B with IC_{50} (μg/mL) values of 4.83±0.29 and 17.29±0.17, which were closer to acarbose. Compounds **3** and **4** showed no inhibition in both tests (IC_{50} > 50 μg/mL). In addition, the interactions of compound **1** with α-glucosidase and PTP1B were analyzed using the active site analysis for computer-aided drug design.

Keywords: *Eupatorium chinense L.*; bioactive components; inhibitory activity; *α*-glucosidase; PTP1B.

1. Plant Source

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

2. Previous Studies

Eupatorium chinense L, commonly known as Guangdong soil hyssop [1], is a plant in the family Asteraceae and primarily distributed in southern China. It is extensively utilized in the regions of Western Hubei and Western Hunan for treating throat disorders, as well as for its anti-inflammatory [2], analgesic, and trauma-healing properties [3]. *Eupatorium chinense L* contains diverse bioactive components, including benzofurans, flavonoids, terpenoids, alkaloids, and quinones [4]. Pharmacological studies have demonstrated multi-target activities such as anti-inflammatory, antiviral, anti-diabetes and antitumor effects [5-8].

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This study investigated the chemical constituents of the *n*-butanol fraction from *Eupatorium chinense L.* roots. Using in vitro enzyme assays, we systematically evaluated its inhibitory potency against α-glucosidase and protein tyrosine phosphatase 1B [11], ultimately identifying bioactive compounds with dual inhibitory effects on these enzymes. These findings provide a material basis for developing novel anti-diabetic therapeutics.

3. Present Study

Dried roots (10.0 kg) of *Eupatorium chinense* L were chopped to get tiny parts and extracted by refluxing with 95% ethanol three times for 6 hours each time. The ethanol extract was filtered through regular filter paper and concentrated under vacuum and 297 g of residue was obtained. The whole ethanol extract was suspended in water, then with *n*-butanol diethyl ether in a separation funnel five times and concentrated under vacuum to give a subextract (58.2 g). This extract (58.2 g) was suspended in 300 g deionized water and subjected to ultrasonication. It was then passed through a D101 macroporous resin column (1000 g, pre-soaked for 6 h) for adsorption and separation. The column was sequentially washed with deionized water (≥3 column volumes) and eluted with an ethanol:deionized water (50:50, v/v) until colorless. The eluate was concentrated to yield 34 g of extract. This extract was mixed with reversed-phase silica gel (90 g, 200–300 mesh) and loaded onto a reversed-phase silica gel column (1200 g, packed by the wet method). Gradient elution with MeOH:H_2O (10:90 to 100:0, v/v, 2 column volumes per gradient) was performed to obtain eight fractions (Fr. A–H).

Fr. F was initially separated using C_{18} reversed-phase silica gel, with a gradient elution system of MeOH:H_2O (10:90 to 100:0, v/v), yielding 10 subfractions F (1-10). Subsequently, subfraction F9 was further separated on C_{18} reversed-phase silica gel with an eluent composition of MeOH:H_2O (75:25, v/v), resulting in 4 fractions (F9 1-4). The specific fraction Fr. F9-4 was then purified via semi-preparative HPLC, ultimately yielding compound **2** (16.8 mg) under the conditions of ACN:H_2O=47:53 (v/v), 2 mL/min, 45 min. For Fr. E, isolation was achieved using HW-40F with an eluent mixture of MeOH:H_2O (2:8, v/v), affording 17 subfractions (E 1-17). Subfraction E8 was processed on C_{18} reversed-phase silica gel with an elution system of MeOH:H_2O (5:5, v/v), producing 4 fractions (E8 1-4). The Fr. E8-2 was subsequently purified by semi-preparative HPLC to obtain compound **1** (28.9 mg) under the conditions of ACN:H_2O=30:70 (v/v), 2 mL/min, 16 min. Additionally, subfraction E12 was separated on C_{18} reversed-phase silica gel with an eluent of MeOH:H_2O (63:36, v/v), resulting in 3 fractions (E12 1-3). Fr. E12-3 was purified by semi-preparative HPLC to yield compound **3** (4.3 mg) under the conditions of ACN:H_2O = 37:63 (v/v), 2 mL/min, 19 min. Fr. D was isolated using HW-40F with an eluent of MeOH:H_2O (1.5:8.5, v/v), generating 6 subfractions (D 1-6). The subfraction D6 was purified by semi-preparative HPLC to obtain compound **4** (4.8 mg) with the elution conditions of ACN:H_2O=25:75 (v/v), 2 mL/min, 32 min (Figure 1).

In this study, four compounds including a new benzofuranside (compound **1**) as well as three known compounds (**2**-**4**) (Figure 1) were isolated from the *n*-butanol portion of the *Eupatorium chinense L.* The three known compounds were verified to be 10-ethoxy-11-hydroxy-10,11-dihydroeuparin (**2**)[13], (-)-12β-hydroxygynunone (**3**)[7] and picein(**4**)[14].

Compound **1**, Yellow oil; $[\alpha]^{25}_D$ +36.7° (c = 0.1, MeOH); UV (MeOH) λ_{max} 247, 278 nm. Its molecular formula was identified as $C_{19}H_{26}O_8$ and eight degrees of unsaturation via the HR-ESI-MS spectrum data (*m/z* 405.1529 [M+ Na] +, calcd. 405.1525). The ^{1}H-NMR spectral data (Table 1) of **1** displayed one olefinic proton signal at δ_H 6.62 (d, *J*=0.8 Hz, H-3) and three methyl signals at δ_H 1.51 (s, H-13), 1.51 (s, H-14) and 1.41 (d, *J*=6.5 Hz, H-11). Analysis of the ^{13}C-NMR (Table 1) and DEPT135 spectra of **1** revealed 19 carbons signals, including three methyl carbons δ_C 29.4 (C-13), 29.4 (C-14) and 25.2 (C-11), two methylene carbon δ_C 61.7 (Glc-6') , two oxygenated carbons δ_C 68.1 (C-12) and 74.1 (C-10), eight olefinic carbon signals δ_C 164.8 (C-2), 154.9 (C-8), 137.9 (C-5), 128.2 (C-9), 122.9 (C-6), 119.4 (C-4), 111.0 (C-7), 100.1 (C-3).

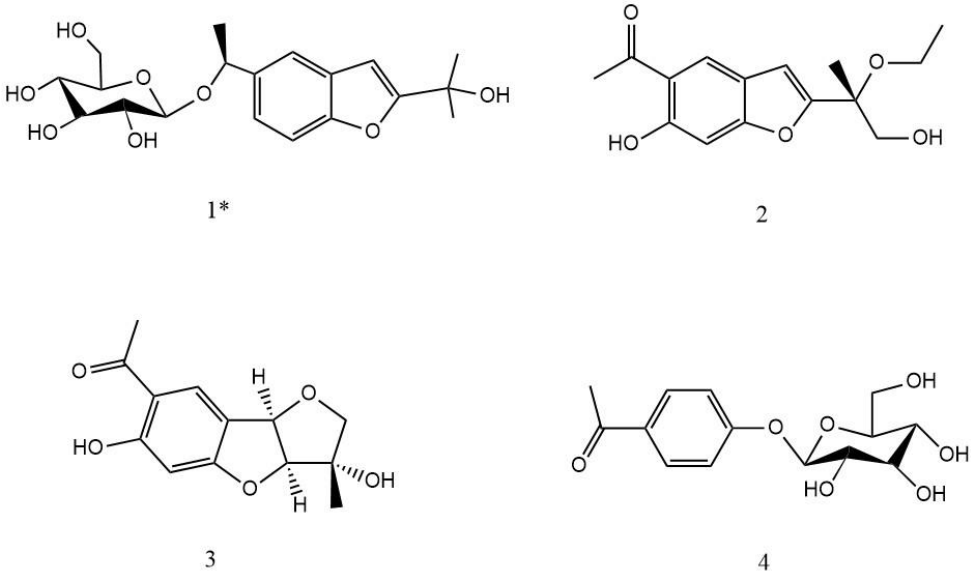

Figure 1. Chemical structures of compounds **1**-**4**

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

Position	**1**	
	δ_C	$\delta_H J$(Hz)
2	164.8	-
3	100.1	6.62 (d, 0.8)
4	119.4	7.60 (d, 1.5)
5	137.9	-
6	122.9	7.29 (dd, 8.6, 1.5)
7	111.0	7.48 (d, 8.6)
8	154.9	-
9	128.2	-
10	74.1	5.05 (q, 6.5)
11	25.2	1.41 (d, 6.5)
12	68.1	-
13	29.4	1.51 (s)
14	29.4	1.51 (s)
Glc-1'	100.5	3.89（d, 7.5）
Glc-2'	77.4	2.87(m)
Glc-3'	77.2	2.97(m)
Glc4'	74.1	2.89(m)
Glc-5'	70.8	3.02(m)
Glc-6'	61.7	3.67(m),3.44(m)
12-OH		5.41(s)

The ^{1}H-NMR spectrum of **1** exhibited a signal at δ_H 3.89 (d, J=7.5 Hz, H-1'), indicating the presence of an anomeric proton. The combined ^{1}H-NMR and ^{13}C-NMR spectral data (δ_C 100.5, 77.4, 77.2, 74.1, 70.8, 61.7) indicated characteristic signals of a glucosyl unit, confirming that **1** was a glycoside. Acid hydrolysis with 2.0 M HCl followed by HPLC analysis identified D-glucose as the sugar moiety. The coupling constant (J = 7.5 Hz) of the anomeric proton signal at δ_H 3.89 in the ^{1}H-NMR, combined with the absence of NOESY correlation between H-1' and H-6', established the β-configuration of the glucose unit. Comparative analysis of the aglycone moiety with the known compound (5-[1'hydroxyethyl]-2-1'-hydroxyisoprolyl]-benzofuran) [15] revealed a chemical shift change at C-10 from δ_C 68.7 in the known compound to δ_C 74.1 in **1**, speculating glycosylation at this position. This connectivity was further confirmed by the observed HMBC correlation between H-1' and C-10. Comprehensive analysis of the 2D NMR spectra demonstrated that the aglycone structure of

1 was identical to 5-[1'hydroxyethyl]-2-1'-hydroxyisoprolyl]-benzofuran. Based on these findings, the planar structure of compound **1** was elucidated.

The absolute configuration at the C-10 position of **1** was determined as *S*-configuration by comparing the theoretical ECD spectra calculated using TD-DFT with the experimental ECD spectra (which showed positive cotton effects at 250 nm and 280 nm). A literature search revealed that this compound is a new benzofuranside compound, and it was named eupbenzofuranside C.

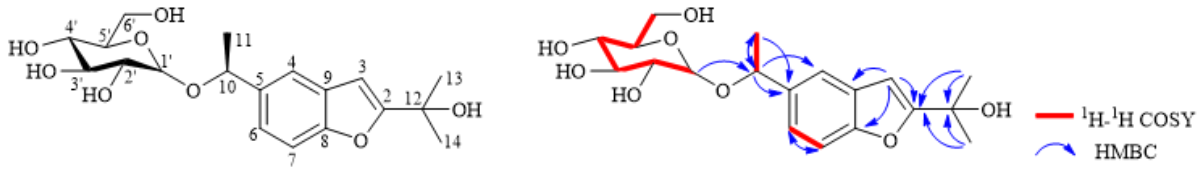

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations of **1**

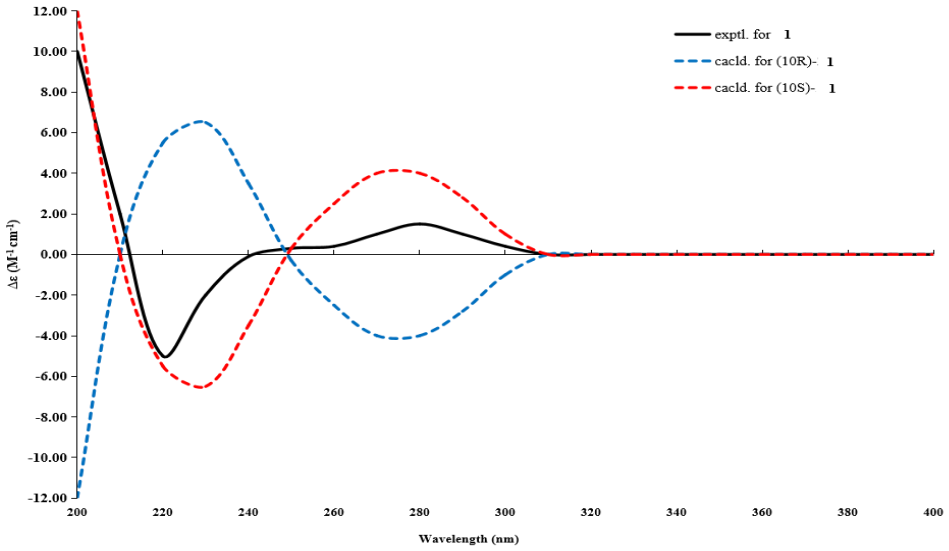

Figure 3. Experimental ECD spectrum and the calculated ECD spectrum of **1** in MeOH

The α-glucosidase and PTP1B inhibitory activities of four compounds were tested (Table 2). The results indicated that **1** had potential dual inhibitory activities against α-glucosidase and PTP1B with the IC_{50} (μg/mL) values of 40.07±0.38, 39.37±0.36. Compound **2**, a known compound, also showed dual inhibitory activities against α-glucosidase and PTP1B with the IC_{50} (μg/mL) values of 4.83±0.29, 17.29±0.17. However, **3** and **4** had no dual inhibitory activities effects on α-glucosidase and PTP1B (IC_{50} > 50 μg/mL).

Table 2. The inhibitory activities against α-glucosidase and PTP1B for **1-4**

Compounds	**α-glucosidase (IC_{50}, μg/mL)**	**PTP1B (IC_{50}, μg/mL)**
1*	40.07±0.38	39.37±0.36
2	4.83±0.29	17.29±0.17
3	>50	7.73±0.18
4	>50	>50
acarbose	4.61±0.09	-
Oleanolic acid	-	9.82±0.19

Calculations of **1** with α-glucosidase and PTP1B interactions were carried out using active site analysis for computer-aided drug design. The results indicated that compound **1** had an obvious combination for α-glucosidase and PTP1B.

Compound **1** forms multiple hydrogen bonds or hydrophobic interactions with PHE-144, ASP-60, GLN-256, ARG-411, THR-409 amino acids in the active site of α-glucosidase protein, thereby exhibiting strong binding affinity and effectively anchoring the small molecule within the protein pocket (Figure 4). The docking results were calculated to be -8.5 kcal/mol. (The positive drug acarbose -8.5 kcal/mol).

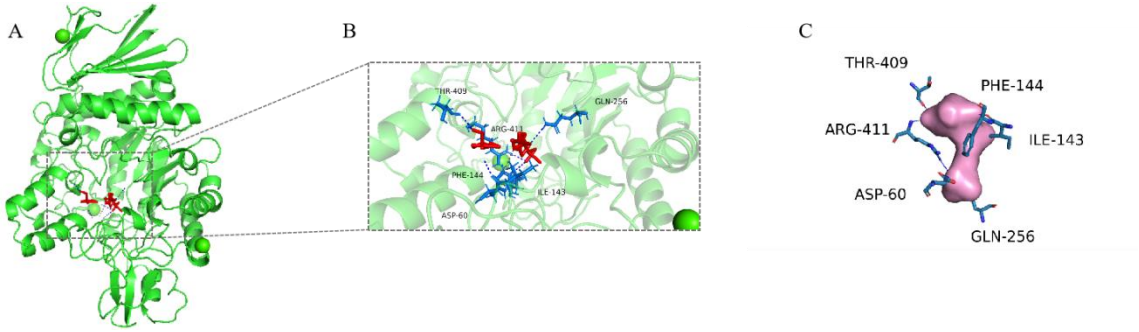

Figure 4. Interaction analysis of α-glucosidase protein with compound **1**
(A: 3D B: Amino acid residue C: 2D)

Compound **1** was found to form multiple hydrogen bonds or hydrophobic interactions with amino acids VAL-49, GLN-266, TYR-46, ASP-48 in the active site of PTP1B protein by theoretical calculations. Thus, the compound can effectively hold in the protein pocket by exhibiting strong binding affinity to the target site (Figure 5). The energy of the docking results was calculated to be -21.4 kcal/mol. (Oleanolic acid -24.3 kcal/mol).

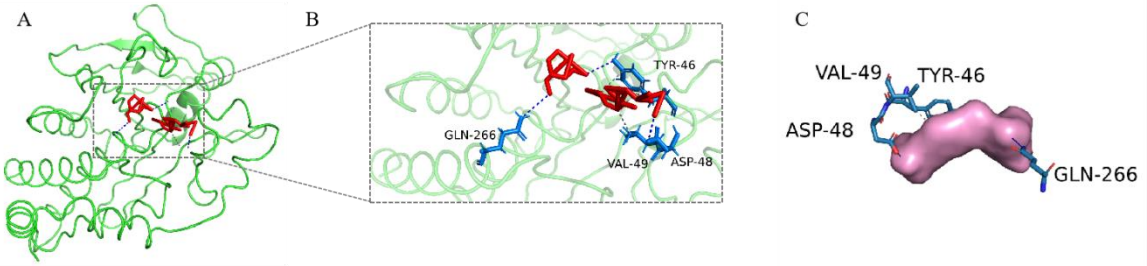

Figure 5. Interaction analysis of PTP1B protein with compound **1**
(A: 3D B: Amino acid residue C: 2D)

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Supporting Information

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