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Placovinane: 1″*β*-Ethoxy-6,4′-dimethoxy-3″,3″-dimethyl-1″,2″-dihydropyranoisoflavone, a New Isoflavone Derivative

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Abstract: Isoflavonoids possess a 3-phenylchroman skeleton and are the biologically active secondary metabolites of various plants that are used for different health promoting and restoring effects through a variety of mechanisms. Chromatographic separation of the *n*-hexane extract from the stems of *Placolobium vietnamense* led to the isolation of a new isoflavone derivative, placovinane (**1**), together with four known compounds (**2**–**5**). The structures of isolated compounds were identified from their spectroscopic data and by comparison with the literature. All isolated compounds were evaluated for their *α*-glucosidase inhibition. They all exhibited potent *α*-glucosidase inhibition with IC_{50} values ranging from 11.0 to 87.3 μM, which was significantly less than the positive control acarbose (IC_{50} 179 μM). The cytotoxicity of **1** was evaluated against KB, Hep G2, and MCF7 cell lines, and displayed weak cytotoxicity toward KB and Hep G2 cell lines, with the IC_{50} values of 89.6 and 93.8 μM, respectively.

Keywords: *Placolobium vietnamense*; placovinane; isoflavone derivative; *α*-glucosidase inhibition; cytotoxicity

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1. Introduction

Flavonoids are polyphenolic compounds that are ubiquitous in nature. Flavonoids have a variety of therapeutic properties, including anticancer, antioxidant, anti-inflammatory, and antiviral effects [1,2]. Isoflavonoids are a subclass of flavonoids derived from the phenylpropanoid and polyketonic chain pathway, which is established both enzymatically and genetically. There are many biological properties associated with isoflavones, including antioxidant, anticancer, antimicrobial, and anti-inflammatory properties [3]. *Placolobium vietnamense* N.D.Khoi & Yakovlev is distributed in primary forests in the Central Highlands of Vietnam. It is a perennial tree with brown bark, straight, and cylindrical trunk. Its fruit is a small pod with one seed. This plant is used in folk medicine to treat debility, snake bites, and to recover after childbirth [4]. As part of our research program focusing on new isoflavonoids from *P. vietnamense*, an indigenous species in Vietnam, we report herein the isolation and structural characterization of a new isoflavonoid derivative, placovinane (**1**), along with four known compounds, daidzein (**2**) [5], 8-*O*-methylretusin (**3**) [6], isorhamnetin (**4**) [7], and vanillic acid (**5**) [8] (Figure 1) from the stems of *P. vietnamense*. The structure of **1** was elucidated by the spectroscopic data interpretation and comparison with those reported in the literature. All isolated compounds were assayed for their *α*-glucosidase inhibition, and the new compound **1** was tested for its cytotoxicity against the KB, Hep G2, and MCF7 cell lines.

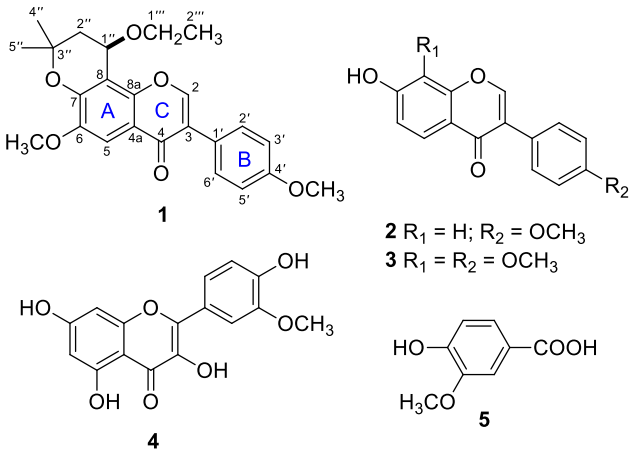

Figure 1. Chemical structures of **1**–**5**.

2. Results and Discussion

*2.1. Structural Elucidation of **1***

Compound **1** was isolated as a white amorphous powder. The HRESIMS revealed a protonated molecular ion peak at m/z 411.1831 $[M + H]^+$ (Supplementary Materials, Figure S6), corresponding to the formula $C_{24}H_{26}O_6$. The ^1H and ^{13}C NMR spectra of **1** (Table 1) exhibited low-field resonances at δ_H 8.45 (1H, s) and δ_C 152.8, which were indicative of H-2 and C-2, respectively, of an isoflavone skeleton [9]. A simple *para*-substituted B-ring was revealed by the presence of the AA′BB′ spin-system. [10]. The presence of a 4-ethoxy-2,2-dimethyldihydropyrano moiety [δ_H 4.80 (1H, dd, J = 4.5, 2.5 Hz), 3.77 (1H, dd, J = 9.0, 2.0 Hz), 3.68 (1H, dd, J = 9.0, 2.0 Hz), 2.35 (1H, dd, J = 15.0, 2.5 Hz), 1.90 (1H, dd, J = 15.0, 4.5 Hz), 1.18 (3H, dd, J = 3.0, 2.0 Hz), 1.46 (3H, s), and 1.43 (3H, s)] [11] and two methoxy substituents [δ_H 3.85 (3H, s) and 3.79 (3H, s)] were identified from both ^1H and ^{13}C NMR spectra (Table 1). Additionally, the singlet signal at δ_H 7.41 (1H, s) was assigned to H-5, as confirmed by the HMBC correlations from H-5 to C-4 (δ_C 174.2), C-6 (δ_C 147.1), and C-4a (δ_C 116.1). Furthermore, the HMBC correlations (Figure S5), particularly from the methoxy resonances at δ_H 3.85 (3H, s) to C-6 and 3.79 (3H, s) to C-4′ (δ_C 158.6), supported the attachment of the methoxy groups at C-6 and C-4′, respectively. The HMBC correlations from H-2″ [δ_H 2.35, (1H, dd, J = 15.0, 2.5 Hz) and 1.90 (1H, dd, J = 15.0, 4.5 Hz)] to C-8 (δ_C 110.8), C-1″ (δ_C 65.4), and C-3″ (δ_C 75.9) indicated that the 4-ethoxy-2,2-dimethyldihydropyrano group was located at the C-7 and C-8 positions of the A-ring (Figure 2), with the anticipated oxygenation at C-7 being supported by the HMBC correlation from H-5 to C-7. The relative configuration of the ethoxy group at C-1″ was deduced to be β by spin coupling analysis of **1** (J = 4.5, 2.5 Hz) with the known compound, horriperfin B (J = 4.9, 3.0 Hz) [11]. Based on the spectral data mentioned above, compound **1** was determined as 1″β-ethoxy-6,4′-dimethoxy-3″,3″-dimethyl-1″,2″-dihydropyranoisoflavone and was named placovinane.

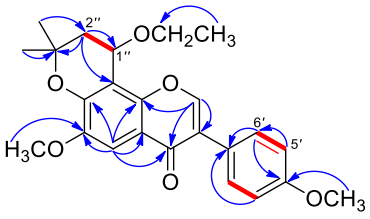

Figure 2. Key COSY (red bold line) and HMBC (blue arrow) correlations of **1**.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of **1** recorded in DMSO-d_6 (δ in ppm).

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
2	8.45, s	152.8	5′	6.99, dd (7.0, 2.0)	113.6
3		122.9	6′	7.55, dd (7.0, 2.0)	130.0
4		174.2	1″	4.80, dd (4.5, 2.5)	65.4
4a		116.1	2″	2.35, dd (15.0, 2.5)	30.6
5	7.41, s	103.5		1.90, dd (15.0, 4.5)	
6		147.1	3″		75.9
7		148.3	4″	1.46, s	26.3
8		110.8	5″	1.43, s	26.3
8a		150.2	1‴	3.77, dd (9.0, 2.0)	63.6
1′		124.4		3.68, dd (9.0, 2.0)	
2′	7.55, dd (7.0, 2.0)	130.0	2‴	1.18, dd (3.0, 2.0)	15.4
3′	6.99, dd (7.0, 2.0)	113.6	6-OCH_3	3.85, s	55.6
4′		158.9	4′-OCH_3	3.79, s	55.1

It should be noted that **1** has an ethoxy group at the C-1″. Since the ethoxy group is relatively rare in nature, it seems probable that this new compound is an artifact of the isolation process, which involves the use of EtOH as a solvent.

2.2. α-Glucosidase Inhibitory Activity

All isolated compounds were evaluated for their α-glucosidase inhibitory activity, with acarbose used as a reference compound. The resulting IC_{50} values, as listed in Table 2, indicated that all the compounds showed stronger α-glucosidase inhibitory activity than acarbose (IC_{50} 179 μM). Compound **2,** especially, displayed the most highly potent α-glucosidase inhibition with an IC_{50} value of 11.0 μM. The results suggested that compounds **1**–**5** from the stems of *P. vietnamense* were active α-glucosidase inhibitors that could be used as effective hypoglycemic drugs for diabetes therapy.

Table 2. α-Glucosidase inhibition of **1**–**5**.

Compound	IC_{50} (μM) [a]
1	18.7 ± 2.50
2	11.0 ± 1.50
3	18.9 ± 2.90
4	87.3 ± 3.13
5	24.5 ± 2.20
Acarbose [b]	179 ± 6.02

[a] Results were expressed as the means ± SD of three replicates. [b] Positive control.

2.3. Cytotoxicity against Human Cancer Cell Lines

Further evaluation of **1** for its in vitro cytotoxicity against three human cancer cell lines (KB, Hep G2, and MCF7) was performed using the MTT assay, which is detailed in the Materials and Methods. The resulting IC_{50} values displayed in Table 3 were compared to ellipticine as a positive control. Compound **1** was shown to have mild cytotoxicity against KB and Hep G2 cell lines, with IC_{50} values of 89.6 and 93.8 μM, respectively.

Table 3. Cytotoxic activity of **1** against three human cancer cell lines.

Compound	IC_{50} ± SD (μM) [a]		
	KB	Hep G2	MCF7
1	89.6 ± 4.78	93.8 ± 5.5	>100
Ellipticine [b]	0.31 ± 0.05	0.33 ± 0.05	0.40 ± 0.05

[a] Results were expressed as the means ± SD of three replicates. [b] Positive control.

3. Materials and Methods

3.1. General Experimental Procedures

The NMR spectra were recorded on Bruker AvanceNEO 600 MHz and Bruker Avance III™ HD 500 MHz NMR spectrometers in DMSO-d_6 (Merck, Darmstadt, Germany). Optical rotations were measured on a A.KRÜSS Optronic P8000 polarimeter (KRÜSS, Hamburg, Germany). The IR data were obtained with a Jasco 6600 FT-IR spectrometer using an ATR technique (Jasco, Japan). The HRESIMS spectral data were generated with a $X500_R$ QTOF model mass spectrometer (Sciex, Framingham, MA, USA) and Dionex Ultimate 3000 HPLC system hyphenated with a QExactive Hybrid Quadrupole Orbitrap MS (Thermo Fisher Scientific, Waltham, MA, USA). Silica gel 70–230 mesh (Merck) and Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography.

3.2. Plant Material

The stems of *P. vietnamense* were collected in Dak Nong province, Vietnam, in February 2017. This plant species was identified by botanist Vo Van Chi. A voucher specimen (No. SGU-A001) was deposited in the Herbarium of the Laboratory of Chemistry-Biology-Environment, Sai Gon University, Vietnam.

3.3. Extraction and Isolation

The air-dried *P. vietnamense* stems (23 kg) were powdered prior to being extracted with 95% EtOH (45 L × 5) at room temperature. The filtered solution was concentrated in vacuo to afford an EtOH crude extract (1200 g). This crude extract was suspended in water and partitioned with *n*-hexane and then EtOAc to yield *n*-hexane (271.2 g) and EtOAc (301.3 g) extracts, respectively. The *n*-hexane extract was subjected to silica gel column chromatography (CC) eluted with *n*-hexane–EtOAc (9:1–0:10, *v*/*v*) and then EtOAc–MeOH (10:0–0:10, *v*/*v*). Based on their TLC behavior, the eluted fractions were grouped into fractions HEX.1–HEX.7. Fraction HEX.2 (7.4 g) was subjected to further silica gel CC eluted with *n*-hexane–EtOAc (8:2, *v*/*v*) to give subfractions HEX.2.1–HEX.2.6. Subfraction HEX.2.4 (1.4 g) was subjected to silica gel CC eluted with *n*-hexane–EtOAc (8.5:1.5, *v*/*v*) to afford **1** (6.0 mg). Subfraction HEX.2.5 (1.1 g) was further purified using silica gel CC eluted with *n*-hexane–EtOAc (8:2, *v*/*v*), followed by CC on Sephadex LH-20 gel eluted with $CHCl_3$–MeOH (1:4 *v*/*v*) to yield **2** (8.5 mg), **3** (7.5 mg), and **4** (7.5 mg). Subfraction HEX.2.6 (0.3 g) was separated using silica gel CC eluted with *n*-hexane–EtOAc (7:3 *v*/*v*) to give **5** (8.8 mg).

Placovinane (**1**). White amorphous powder. $[\alpha]_D^{25}$ + 6.0 (c 0.01, acetone). IR (ATR) ν_{max} 3392, 2977, 1712, 1635, 1450, 1287, 1168 cm^{-1}. HRESIMS *m*/*z* 411.1831 $[M + H]^+$ (calcd. for $C_{24}H_{27}O_6$ 411.1802); ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1.

3.4. α-Glucosidase Inhibitory Assay

The in vitro α-glucosidase inhibitory evaluation of **1**–**5** was performed according to the previously described protocol [12]. Then, 100 mM pH 6.9 sodium phosphate buffer was used as a solvent to dissolve the substrate (5.0 mM *p*-nitrophenyl-D-glucopyranoside) and α-glucosidase (0.2 U/mL). The substrate (40 µL) was added to the reaction mixture after the inhibitor (50 µL) had previously been preincubated with α-glucosidase. The reaction was stopped after 20 min of enzymatic activity at 37 °C by adding 130 µL of 0.2 M Na_2CO_3. The amount of enzyme activity was determined by calculating absorbance at 405 nm. After each sample was tested in triplicate at five different concentrations around the IC_{50} values, the mean values were retained. The inhibition percentage (%) was determined as follows:

$$\text{Inhibition (\%)} = [1 - (A_{sample}/A_{control})] \times 100.$$

3.5. Cytotoxic Assay

Following a previous procedure [13], the cytotoxic assessment of **1** was performed against human epidermoid carcinoma (KB), hepatocellular carcinoma (Hep G2), and breast adenocarcinoma (MCF7) cell lines. The positive control was ellipticine, a powerful anti-cancer medication with various modes of action. The cancer cells were grown in Dulbecco's Modified Eagle's Medium at 37 °C in a 5 % CO_2 environment, with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin, and 1% L-glutamine. By dissolving the compounds under investigation in DMSO (20 mg/mL), the compounds were added at 0.5 to 128 µg/mL concentrations, and the incubation was carried out once more for 72 h under the same circumstances. Following the procedure, an MTT solution (10 µL, 5 mg/mL) was added to each well. The percentage of cell viability vs. sample concentration was plotted using SigmaPlot 10 (Systat Software Inc., San Jose, CA, USA) to calculate the IC_{50} values.

4. Conclusions

The stems of *P. vietnamense* were chemically investigated, leading to the isolation of a new isoflavonoid derivative, placovinane (**1**), together with four known compounds (**2**–**5**). Compounds **1**–**5** were isolated from *P. vietnamense* for the first time. Compounds **1**–**5** have much stronger α-glucosidase inhibition than the positive control acarbose. On the other hand, a new compound **1** displayed only weak cytotoxicity against KB and Hep G2 cell lines. The findings of this study indicate that *P. vietnamense* stems could become an alternative source of bioactive compounds that should be used as effective hypoglycemic agents for diabetes therapy.

Supplementary Materials: Figures S1–S6: HRESIMS, 1D, and 2D NMR spectra of **1**.

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