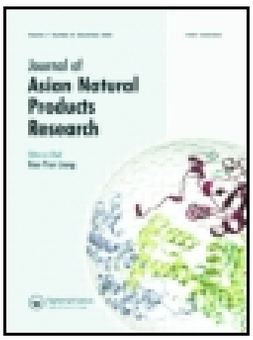

Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

A new selaginellin derivative and a new triarylbenzophenone analog from the whole plant of Selaginella pulvinata

Xin Liu, Gui-Hua Tang, Han-Zhuang Weng, Jun-Sheng Zhang, You-Kai Xu & Sheng Yin

To cite this article: Xin Liu, Gui-Hua Tang, Han-Zhuang Weng, Jun-Sheng Zhang, You-Kai Xu & Sheng Yin (2017): A new selaginellin derivative and a new triarylbenzophenone analog from the whole plant of Selaginella pulvinata, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2017.1378646

To link to this article: http://dx.doi.org/10.1080/10286020.2017.1378646

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 2017
https://doi.org/10.1080/10286020.2017.1378646

Taylor & Francis
Taylor & Francis Group

A new selaginellin derivative and a new triarylbenzophenone analog from the whole plant of *Selaginella pulvinata*

Xin Liu[a#], Gui-Hua Tang[a#], Han-Zhuang Weng[a], Jun-Sheng Zhang[a], You-Kai Xu[b] and Sheng Yin[a]

[a]School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China; [b]Key Laboratory of Tropical Plant Resource and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun 666303, China

ABSTRACT

Five selaginellin derivatives (**1** and **3–6**) including a new one, selaginellin T (**1**), and a new triarylbenzophenone analog, selagibenzophenone A (**2**), were isolated from the whole plants of *Selaginella pulvinata*. Their structures were determined by 1D- and 2D-NMR and HR-ESI-MS data. Selagibenzophenone A (**2**) is the first example of naturally occurring triarylbenzophenone. The results of the phosphodiesterase-4 (PDE4) inhibitory screening assays showed that compounds **1**–**6** exhibited potent activities with the IC_{50} values in the range of 1.04–9.35 μM.

ARTICLE HISTORY
Received 12 May 2017
Accepted 5 September 2017

KEYWORDS
Selaginellaceae; *Selaginella pulvinata*; selaginellin derivative; triarylbenzophenone; phosphodiesterase-4

1. Introduction

Selaginellins, a group of novel natural pigments characterized by a *p*-hydroxyphenylethynyl moiety and multiple phenolic groups, were currently found only in the genus *Selaginella* (Selaginellaceae) [1–14]. So far approximately 30 selaginellins have been reported, and these structures possessed important biological activities such as antimicrobial, cytotoxic, antioxidant, and phosphodiesterase-4 (PDE4) inhibitory properties [4,10,12,15]. *Selaginella pulvinata* (Hook. et Grev.) Maxim., a Traditional Chinese Medicine (TCM) widely distributed

CONTACT Sheng Yin yinsh2@mail.sysu.edu.cn
[#]X. Liu and G.-H. Tang contributed equally to this work.
Supplemental data for this article can be accessed at https://doi.org/10.1080/10286020.2017.1378646.

in China, was used for the treatment of asthma, dysmenorrhea, and traumatic injury [16]. Recently, our group have found a novel type of compounds featuring a fluorene core with remarkable PDE4 inhibitory activities from the plants of *S. pulvinata* [15,17]. Continued investigation on the plants of *S. pulvinata* let to the isolation of a new selaginellin derivative, selaginellin T (**1**), a new triarylbenzophenone analog, selagibenzophenone A (**2**) (Figure 1), and four known selaginellins (**3–6**) (Figure S1 in Supplemental Material). Bioassay verified that these isolates have potent PDE4 inhibitory activities. Herein, details of the isolation, structural elucidation, and PDE4 inhibitory activities of these metabolites are described.

2. Results and discussion

Compound **1**, a red powder, had a molecular formula of $C_{26}H_{18}O_5$ as determined by the HRESIMS data (*m*/*z* 433.1030 $[M + Na]^+$; calcd 433.1046) and 1D NMR data. The IR spectrum displayed the presence of hydroxyl (3402 cm^{-1}), carbonyl (1725 cm^{-1}), and phenyl (1611 and 1514 cm^{-1}) functionalities. The 1D NMR spectra of **1** showed signals for three 1,4-disubstituted benzene ring (two were overlapped) [δ_H 6.88 (4H, d, J = 8.5 Hz, H-3/5/8/12), 6.71 (4H, d, J = 8.5 Hz, H-2/6/9/11), 6.56 (2H, d, J = 8.6 Hz, H-21/23), and 6.54 (2H, d, J = 8.6 Hz, H-20/24); δ_C 158.4 (C × 2, C-1/10), 157.8 (C, C-22), 131.7 (CH × 2, C-20/24), 131.5 (C × 2, C-4/13), 131.0 (CH × 4, C-3/5/8/12), 130.6 (C, C-25), 115.4 (CH × 4, C-2/6/9/11), and 115.2 (CH × 2, C-21/23)], a 1,2,3-trisubstituted benzene ring [δ_H 7.91 (1H, d, J = 7.5 Hz, H-15), 7.70 (1H, t, J = 7.5 Hz, H-16), and 7.52 (1H, d, J = 7.5 Hz, H-17); δ_C 151.2 (C, C-19), 140.5 (C, C-18), 138.3 (CH, C-17), 130.7 (CH, C-16), 127.6 (C, C-14), and 125.1 (CH, C-15)], an ester carbonyl [δ_C 170.0], and an oxygenated quaternary carbon [δ_C 93.9], which highly resembled those of selaginellin H [5], a selaginellin derivative previously reported from the same plant, except that the signals for a hydroxymethyl group in selaginellin H was not observed in **1**. This information suggested that **1** was a dehydroxymethyl derivative of selaginellin H, which was supported by its MS data (30 mass units less than that of selaginellin H) and the presence of the characteristic 1,2,3-trisubstited pattern of ring A [δ_H 7.52 (d, J = 7.5 Hz, H-17), 7.70 (t, J = 7.5 Hz, H-16), and 7.91 (d, J = 7.5 Hz,

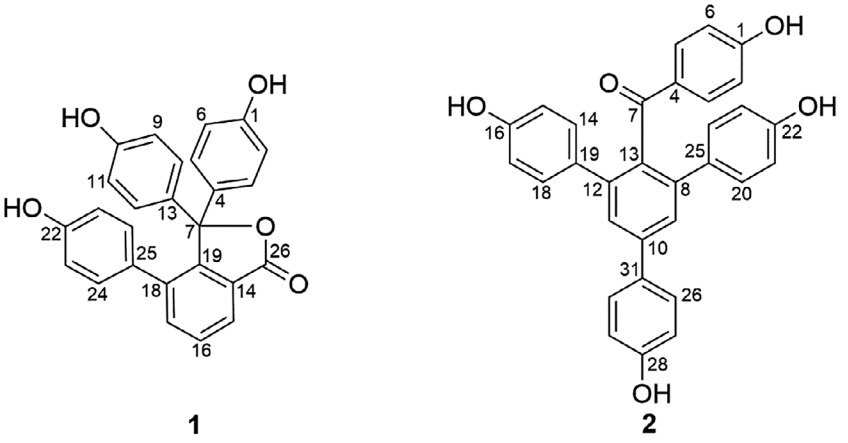

Figure 1. Structures of new compounds **1** and **2**.

H-15)]. Analysis of the HMBC correlations of compound **1** (Figure 2) further confirmed its structure. Thus, the structure of **1** was determined as shown and named as selaginellin T.

Compound **2** was obtained as a red powder. It possessed a molecular formula of $C_{31}H_{22}O_5$ as established by HRESIMS at *m*/*z* 473.1387 $[M–H]^-$ and 1D NMR data. The ^{1}H NMR spectrum showed signals for four *p*-hydroxylphenyl groups (two were overlapped) [δ_H 6.55 (2H, d, *J* = 8.7 Hz, H-2/6) and 7.38 (2H, d, *J* = 8.7 Hz, H-3/5), 6.63 (4H, d, *J* = 8.6 Hz, H-15/17/21/23) and 7.10 (4H, d, *J* = 8.6 Hz, H-14/18/20/24), and 6.89 (2H, d, *J* = 8.6 Hz, H-27/29) and 7.56 (2H, d, *J* = 8.6 Hz, H-26/30)] and a symmetric 1,2,3,5-tetrasubstituted benzene ring [δ_H 7.49 (2H, s)]. The ^{13}C NMR spectrum of **2** exhibited 31 carbon resonances which were classified by HSQC and HMBC experiments as two superimposed 4-hydroxylphenyl groups (rings C and D), two well distinguished 4-hydroxylphenyl groups (rings A and E), a symmetric tetrasubstituted benzene ring (ring B), and a ketone carbonyl (C-7). The connectivity of rings A–D and the ketone carbonyl was accomplished by the HMBC correlations (Figure 2) and consideration of the symmetrical characteristic of the molecule. In particularly, strong HMBC correlations from four symmetrical aromatic protons [δ_H 7.10 (H-14/18/20/24)] to the quaternary carbons at δ_C 142.4 suggested that the superimposed rings C and D were linked at the two symmetrical carbons (C-12 and C-8) of ring A. The HMBC correlations from a pair of symmetrical aromatic protons at δ_H 7.56 (H-26/30) of ring E to the quaternary carbon at δ_C 142.9 (C-10) of ring B and the HMBC correlations from H-4/6 (δ_H 7.49) to the C-31 (δ_C 132.8) revealed that ring E was located at C-10 of ring A. The HMBC correlation of H-3/5 of ring A to the ketone carbonyl (C-7) deduced the presence of the 4-hydroxylbenzoyl group, which must be placed at the only "loose end" of C-13 in ring B. Thus, a triarylbenzophenone scaffold of **2** was established as depicted, which represented the first example of naturally occurring triarylbenzophenone and named as selagibenzophenone A.

The known compounds were identified as selaginellin S (**3**) [14], selaginellin L (**4**) [6], selaginellin B (**5**) [2], and selaginellin G (**6**) [5] by comparison of their NMR and MS data with those in the literature.

All compounds were tested for their PDE4 inhibitory activities by using tritium-labeled adenosine 3′,5′-cyclic monophosphate ([^{3}H]–cAMP) as substrate with rolipram as the

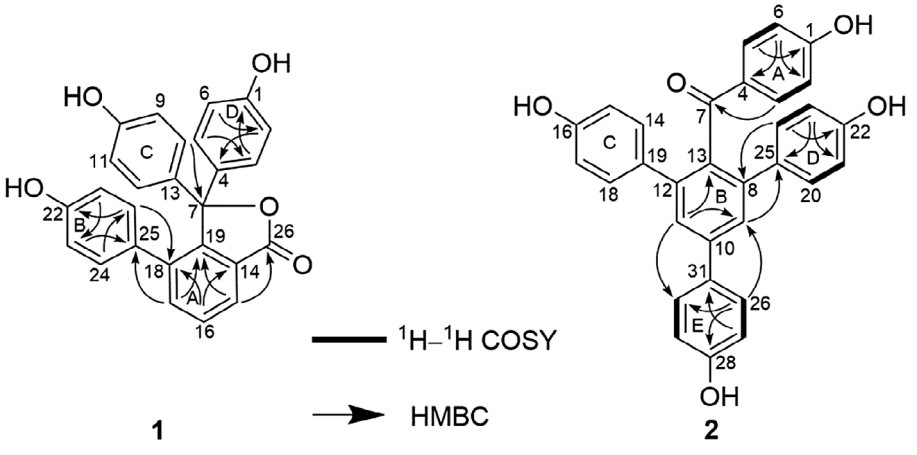

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1** and **2**.

positive control (IC_{50} = 0.62 μM). Compounds **2** and **5** showed remarkable inhibitory activity with the IC_{50} values of 1.04 and 1.25 μM, respectively, and **1**, **3**, **4**, and **6** had moderated activities with IC_{50} values in range of 2.67–9.34 μM (Table 1).

3. Experimental

3.1. General experimental procedures

UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) and IR spectra on a Bruker Tensor 37 infrared spectrophotometer (Bruker, Madison, USA) with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer (Bruker, Zurich, Switzerland) at 25 °C. ESIMS and HRESIMS were carried out on a Thermo Finnigan LCQ^{DECA} instrument (Thermo Finnigan, San Jose, USA). A Shimadzu LC-20AT equipped with a SPD-M20A PDA detector was used for HPLC, and a YMC-pack ODS-A column (250 × 10 mm, S-5 μM, 12 nm) was used for semipreparative HPLC separation. The column chromatography (CC) was performed on silica gel (300–400 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), MCI gel (CHP20P, 75–150 μM, Mitsubishi Chemical Industries Ltd.), Sephadex LH-20, and reversed-phase C_{18} (RP-C_{18}) silica gel (12 nm, S-50 μM, YMC Co., Ltd., Tokyo, Japan). Analytical grade solvents (Guangzhou Chemical Reagents Company, Ltd., Guangzhou, China) were used as eluents. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

3.2. Plant material

The whole plants of *Selaginella pulvinata* were collected in March 2015 from Yulin city, Guangxi Zhuang Autonomous Region. The plants were identified by one of the author (G.-H. Tang) and a voucher specimen (DZJB201503) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

3.3. Extraction and isolation

The air-dried powder of the aerial parts of *S. pulvinata* (10 kg) was extracted with 95% EtOH (3 × 5 L) at room temperature to give 700 g of crude extract. The extract was suspended in H_2O (3 L) and successively partitioned with petroleum ether (PE, 3 × 3 L) and EtOAc (3 × 3 L). The EtOAc extract (206 g) was subjected to MCI gel column chromatography (CC) eluted with a MeOH/H_2O gradient (3:7→10:0) to afford five fractions (Fr. I–V). Fr. III (12.6 g) was chromatographed over a silica gel CC eluted with PE/acetone (10:1→1:1) to afford five fractions (Fr. IIIa–IIId). Fr. IIIc (45 mg) was further purified on HPLC using MeCN/H_2O (45:55, 3 ml/min) as mobile phase to give **6** (8 mg, t_R 14 min) and **2** (6 mg, t_R

Table 1. IC_{50} values of compounds **1–6** against PDE4D2.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	9.35 ± 0.60	**5**	1.25 ± 0.04
2	1.04 ± 0.07	**6**	3.38 ± 0.09
3	2.67 ± 0.07	**Rolipram**[a]	0.62 ± 0.03
4	7.42 ± 0.31		

[a]Positive control.

16.5 min). Fr. IIIe (1.9 g) was chromatographed over a Sephadex LH-20 eluted with MeOH to give three fractions (Fr. IIIe1–IIIe3). Fr. IIIe2 (900 mg) was chromatographed by a RP-C_{18} column eluted with MeOH/H_2O (4:6→0:0) to afford **3** (6.2 mg), **5** (5 mg), and **1** (8.8 mg). Fr. IIIe3 (120 mg) was chromatographed by a Sephadex LH-20 (EtOH) to give **4** (11 mg). The purity of the compounds was tested by ^{1}H NMR spectra.

3.3.1. Selaginellin T (1)

Red powder; UV (CH_3OH) λ_{max} (log ε) 204.0 (4.55), 230.4 (4.13) nm; IR (KBr) ν_{max} 3402, 1725, 1611, 1514, 1240 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ 7.91 (1H, d, J = 7.5 Hz, H-15), 7.70 (1H, t, J = 7.5 Hz, H-16), 7.52 (1H, d, J = 7.5 Hz, H-17), 6.88 (4H, d, J = 8.5 Hz, H-3/5/8/12), 6.71 (4H, d, J = 8.5 Hz, H-2/6/9/11), 6.56 (2H, d, J = 8.6 Hz, H-21/23), 6.54 (2H, d, J = 8.6 Hz, H-20/24); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 170.0 (C, C-26), 158.4 (C × 2, C-1/10), 157.8 (C, C-22), 151.2 (C, C-19), 140.5 (C, C-18), 138.3 (CH, C-17), 131.7 (CH × 2, C-20/24), 131.5 (C × 2, C-4/13), 131.0 (CH × 4, C-3/5/8/12), 130.7 (CH, C-16), 130.6 (C, C-25), 127.6 (C, C-14), 125.1 (CH, C-15), 115.4 (CH × 4, C-2/6/9/11), 115.2 (CH × 2, C-21/23), 93.9 (C, C-7); HRESIMS m/z 433.1030 $[M + Na]^+$ (calcd for $C_{26}H_{18}O_5Na$, 433.1046).

3.3.2. Selagibenzophenone A (2)

Red powder; UV (CH_3OH) λ_{max} (log ε) 207.5 (4.41), 261.2 (4.07), 290.5 (3.98) nm; IR (KBr) ν_{max} 3253, 1592, 1512, 1262 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.56 (2H, d, J = 8.6 Hz, H-26/30), 7.49 (2H, s H-9/11), 7.38 (2H, d, J = 8.7 Hz, H-3/5), 7.10 (4H, d, J = 8.6 Hz, H-14/18/20/24), 6.89 (2H, d, J = 8.6 Hz, H-27/29), 6.63 (4H, d, J = 8.6 Hz, H-15/17/21/23), 6.55 (2H, d, J = 8.7 Hz, H-2/6); ^{13}C NMR (CD_3OD, 100 MHz) δ 200.5 (C, C-7), 165.9 (C, C-1), 158.7 (C, C-28), 157.8 × 2 (C, C-16/22), 142.9 (C, C-10), 142.4 × 2 (C, C-8/12), 137.8 (C, C-13), 133.6 × 2 (CH, C-3/5), 133.3 × 2 (C, C-19/25), 132.8 (C, C-31), 131.4 × 4 (CH, C-14/118/20/24), 130.6 (C, C-4), 129.3 × (CH, C-26/30), 127.6 × 2 (CH, C-9/11), 116.8 × 2 (CH, C-27/29), 116.6 × 2 (CH, C-2/6), 115.8 × 4 (CH, C-15/17/21/23); HRESIMS m/z 473.1387 $[M–H]^-$ (calcd for $C_{31}H_{21}O_5$, 473.1394).

3.4. PDE4D inhibitory screening assays

The PDE4D inhibitory screening assays for the active compounds were performed as described previously [18].

Acknowledgments

The authors acknowledge the financial support from the National High Technology Research and Development Program of China, the Guangdong Natural Science Funds for Distinguished Young Scholar, the National Natural Science Foundation of China, and the Fundamental Research Funds for the Central Universities.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the National High Technology Research and Development Program of China [863 project number 2015AA020928]; the Guangdong Natural Science Funds for Distinguished Young Scholar [grant number 2014A030306047]; the National Natural Science Foundation of China [grant number 81402813]; and the Fundamental Research Funds for the Central Universities [grant number 14YKPY10].

ORCID

Gui-Hua Tang http://orcid.org/0000-0002-8831-7154

References

[1] L.P. Zhang, Y.M. Liang, X.C. Wei, and D.L. Cheng, *J. Org. Chem*. **72**, 3921 (2007).
[2] X.L. Cheng, S.C. Ma, J.D. Yu, S.Y. Yang, X.Y. Xiao, J.Y. Hu, Y. Lu, P.C. Shaw, P.P.H. But, and R.C. Lin, *Chem. Pharm. Bull*. **56**, 982 (2008).
[3] G.S. Tan, K.P. Xu, F.S. Li, C.J. Wang, T.Y. Li, C.P. Hu, J. Shen, Y.J. Zhou, and Y.J. Li, *J. Asian Nat. Prod. Res*. **11**, 1001 (2009).
[4] Y. Cao, J.J. Chen, N.H. Tan, L. Oberer, T. Wagner, Y.P. Wu, G.Z. Zeng, H. Yan, and Q. Wang, *Bioorg. Med. Chem. Lett*. **20**, 2456 (2010).
[5] Y.A. Cao, J.J. Chen, N.H. Tan, Y.P. Wu, J. Yang, and Q.A. Wang, *Magn. Reson. Chem*. **48**, 656 (2010).
[6] K.P. Xu, H. Zou, F.S. Li, H.L. Xiang, Z.X. Zou, H.P. Long, J. Li, Y.J. Luo, Y.J. Li, and G.S. Tan, *J. Asian Nat. Prod. Res*. **13**, 356 (2011).
[7] K.P. Xu, H. Zou, G.R. Liu, H.P. Long, J. Li, F.S. Li, Z.X. Zou, J.W. Kuang, X. Xie, and G.S. Tan, *J. Asian Nat. Prod. Res*. **13**, 1051 (2011).
[8] K.P. Xu, H. Zou, Q.A. Tan, F.S. Li, J.F. Liu, H.L. Xiang, Z.X. Zou, H.P. Long, Y.J. Li, and G.S. Tan, *J. Asian Nat. Prod. Res*. **13**, 93 (2011).
[9] C. Yang, Y.T. Shao, K. Li, and W.J. Xia, *Beilstein J. Org. Chem*. **8**, 1884 (2012).
[10] G.G. Zhang, Y. Jing, H.M. Zhang, E.L. Ma, J. Guan, F.N. Xue, H.X. Liu, and X.Y. Sun, *Planta Med*. **78**, 390 (2012).
[11] Y. Cao, Y. Yao, X.J. Huang, L. Oberer, T. Wagner, J.M. Guo, W. Gu, W.D. Liu, G.X. Lv, Y.N. Shen, and J.A. Duan, *Tetrahedron* **71**, 1581 (2015).
[12] P.H. Nguyen, B.T. Zhao, M.Y. Ali, J.S. Choi, D.Y. Rhyu, B.S. Min, and M.H. Woo, *J. Nat. Prod*. **78**, 34 (2015).
[13] K.P. Xu, J. Li, G.Z. Zhu, X.A. He, F.S. Li, Z.X. Zou, L.H. Tan, F. Cheng, and G.S. Tan, *J. Asian Nat. Prod. Res*. **17**, 819 (2015).
[14] B. Zhu, T.B. Wang, L.J. Hou, H.X. Lv, A.M. Liu, P. Zeng, and A.H. Li, *Chem. Nat. Compd*. **52**, 624 (2016).
[15] X. Liu, H.B. Luo, Y.Y. Huang, J.M. Bao, G.H. Tang, Y.Y. Chen, J. Wang, and S. Yin, *Org. Lett*. **16**, 282 (2014).
[16] Editorial Board of "Zhong Hua Ben Cao", State Administration of Traditional Chinese Medicine of the People's Republic of China; Chinese Materia Medica (Zhong Hua Ben Cao), (Shanghai: Shanghai Scientific and Technical, 1999), Vol. 4, pp 387–389.
[17] J.S. Zhang, X. Liu, J. Weng, Y.Q. Guo, Q.J. Li, A. Ahmed, G.H. Tang, and S. Yin, *Org. Chem. Front*. **4**, 170 (2017).
[18] T.T. Lin, Y.Y. Huang, G.H. Tang, Z.B. Cheng, X. Liu, H.B. Luo, and S. Yin, *J. Nat. Prod*. **77**, 955 (2014).