Bioorganic & Medicinal Chemistry Letters 26 (2016) 4785–4789

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Bioactive diterpenoids from *Trigonostemon chinensis*: Structures, NO inhibitory activities, and interactions with iNOS

Jing Xu [a,*], Maoqin Peng [a], Xiaocong Sun [a], Xingyu Liu [a], Ling Tong [b], Guochen Su [a], Yasushi Ohizumi [c], Dongho Lee [d], Yuanqiang Guo [a,*]

[a] State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300350, People's Republic of China
[b] State Key Laboratory of Core Technology in Innovative Chinese Medicine, Pharmaceutical Analysis Institute, Tasly Academy, Tianjin 300410, People's Republic of China
[c] Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan
[d] Department of Biosystems and Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

ARTICLE INFO

Article history:
Received 5 July 2016
Revised 23 July 2016
Accepted 10 August 2016
Available online 10 August 2016

Keywords:
Trigonostemon chinensis
Diterpenoids
NO inhibitory activities
iNOS
Molecular docking

ABSTRACT

A phytochemical investigation to obtain new NO inhibitors led to the isolation of two new (**1** and **2**) and four known (**3**–**6**) diterpenoids from *Trigonostemon chinensis*. Their structures were elucidated on the basis of extensive 1D and 2D NMR spectroscopic data analyses, and the absolute configurations of new compounds were established by experimental and calculated ECD spectra. The inhibitory activities on lipopolysaccharide-induced NO production in murine microglial BV-2 cells of these diterpenoids were evaluated, and all of the compounds showed inhibitory effects. The interactions of bioactive compounds with iNOS protein were also studied by molecular docking.

Nitric oxide (NO) is a signaling molecule and has been well-known to regulate various physiological functions in many tissues of human body.[1,2] In the central nervous system (CNS), NO has been demonstrated to be involved in brain development, synaptic plasticity, and neuron growth.[3] While, NO is also considered as an inflammatory mediator, and excessive NO in the CNS is a signal of inflammatory response, which has been demonstrated to be neurotoxic and can cause neuron degeneration and following neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD).[4] Therefore, NO inhibitors may be considered as potential anti-inflammatory agents for prevention and/or treatment of inflammatory diseases and related disorders.[5]

The genus *Trigonostemon*, a member of the family Euphorbiaceae, comprises about 50 species that grows mainly in tropical and subtropical regions of Asia.[6] Some *Trigonostemon* species have been used traditionally as folk medicines for the treatment of various medical indications.[7] Compounds reported from this genus include mainly terpenoids, especially diterpenoids, alkaloids, steroids, flavonoids, ligans, coumarins, and phenolics,[7–18] displaying diverse biological effects, such as cytotoxic, antimicrobial, and antivirus activities.[7,8] In the course of an ongoing search for bioactive substances from plants,[19–21] attention has been given to the occurrence of bioactive compounds with NO inhibitory effects, since active compounds of this type are expected to be potentially useful for the treatment of inflammation and related neurodegenerative diseases.[4,5] In a preliminary screening procedure, an ethyl acetate-soluble extract of the twigs of *Trigonostemon chinensis* showed moderate NO inhibitory effects. The following investigation to obtain bioactive compounds led to the isolation of two new diterpenoids, chinensipenes A and B (**1** and **2**), and four known analogues. Their structures, including their absolute configurations, were established by analyses of their NMR spectroscopic data and their experimental and calculated ECD spectra. Herein, the isolation, structural elucidation, and NO inhibitory effects of these compounds as well as their interactions with iNOS protein are described.

The ethyl acetate-soluble part of the methanol extract of the twigs of *T. chinensis* was fractionated by silica gel column chromatography and MPLC and further purified by HPLC to afford two new (**1** and **2**) and four known (**3**–**6**) diterpenoids (Fig. 1) (detailed experimental procedures see Supplementary data).

Compound **1** was isolated as a white powder.[22] Its molecular formula was determined as $C_{36}H_{42}O_{11}$ on the basis of HR-ESIMS spectroscopic data (*m*/*z* 651.2802 $[M+H]^+$, calcd for $C_{36}H_{43}O_{11}$,

* Corresponding authors. Tel./fax: +86 22 23502595.
E-mail addresses: xujing611@nankai.edu.cn (J. Xu), victgyq@nankai.edu.cn (Y. Guo).

http://dx.doi.org/10.1016/j.bmcl.2016.08.026
0960-894X/

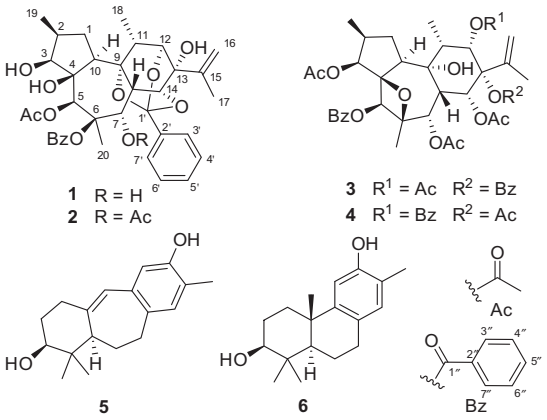

Figure 1. Structures of compounds **1**–**6** from *T. chinensis*.

651.2805), which was compatible with the NMR data. The ^{1}H and ^{13}C NMR spectra of **1** revealed the occurrence of one acetoxy group [δ_H 2.17 s; δ_C 170.7 (CO) and 21.0 (CH_3)], one benzoyloxy group (δ_H 8.00 d, 7.47 t, and 7.61 t; δ_C 165.2, 131.2, 129.7 × 2, 128.5 × 2, and 133.3), and one monosubstituted phenyl group (δ_H 7.67 d, 7.41 t, and 7.41 t; δ_C 138.0, 125.0 × 2, 128.3 × 2, and 129.8) (Table 1).

Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2** (δ in ppm and *J* in Hz)[a]

Position	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
1	34.2	2.02 m	33.9	2.11 m
		1.88 m		1.93 m
2	36.9	1.69 m	37.0	1.71 m
3	77.9	3.56 s	78.0	3.56 s
4	83.0		82.5	
5	78.4	5.12 s	78.7	4.93 s
6	87.4		85.6	
7	79.3	5.26 s	75.8	6.63 s
8	36.0	2.88 s	35.7	2.83 s
9	78.5		76.9	
10	52.0	3.01 dd (13.2, 5.4)	52.1	2.79 overlapped
11	37.6	2.49 dd (13.2, 6.5)	38.0	2.56 dd (12.6, 6.2)
12	83.5	4.00 s	83.5	3.94 s
13	70.0		70.3	
14	82.0	4.40 s	80.1	4.43 s
15	141.8		142.1	
16	115.4	4.73 s	115.3	4.74 s
		4.59 s		4.55 s
17	17.8	1.46 s	18.0	1.45 s
18	17.9	1.54 d (6.5)	17.9	1.55 d (6.0)
19	13.1	1.04 d (6.5)	13.1	1.06 d (6.0)
20	22.4	1.82 s	22.0	1.66 s
1′	108.1		107.5	
2′	138.0		138.5	
3′/7′	125.0	7.67 d (7.3)	125.1	7.64 d (7.5)
4′/6′	128.3	7.41 t (7.3)	128.0	7.38 t (7.5)
5′	129.8	7.41 t (7.3)	129.4	7.38 t (7.5)
1″	165.2		165.0	
2″	131.2		130.9	
3″/7″	129.7	8.00 d (7.6)	129.8	8.04 d (7.3)
4″/6″	128.5	7.47 t (7.6)	128.5	7.47 t (7.3)
5″	133.3	7.61 t (7.6)	133.4	7.64 t (7.3)
5-OAc	170.7		170.9	
	21.0	2.17 s	21.0	2.16 s
7-OAc			169.6	
			21.1	1.83 s
3-OH		2.57 s		2.73 s
4-OH		3.40 s		3.48 s
7-OH		3.15 s		
13-OH		3.74 s		3.75 s

[a] Assignments of NMR data are based on ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC NMR experiments.

Additionally, the ^{1}H NMR spectrum exhibited signals attributed to four methyl groups (1.46 s, 1.54 d, 1.04 d, and 1.82 s) and two terminal olefinic protons [4.73 and 4.59 (each 1H, s)]. Apart from these carbons for the substituent groups, the ^{13}C NMR spectrum, together with DEPT and HMQC spectra, showed an additional typical quaternary carbon signal at δ_C 108.1 (C-1′) and 20 skeletal carbons including four methyls, two methylenes, nine methines, and five quaternary carbons (Table 1). These spectroscopic features suggested compound **1** may be a diterpenoid derivative. Upon comparison of its NMR data with those of diterpenoids reported from the genus *Trigonostemon*, a daphnane-type diterpenoid orthoester with one acetyloxy and one benzoyloxy group for **1** was established.[23–25] This skeletal type of diterpenoid was substantiated by the following HMBC and ^{1}H–^{1}H COSY experiments, and the oxygenated and olefinic skeletal carbons at δ_C 77.9 (C-3), 83.0 (C-4), 78.4 (C-5), 87.4 (C-6), 79.3 (C-7), 78.5 (C-9), 83.5 (C-12), 70.0 (C-13), 82.0 (C-14), 141.8 (C-15), and 115.4 (C-16) were assigned. Further analyses of 1D and 2D NMR spectra resulted in the assignments of the remaining skeletal protons and carbons. The positions of the substituent groups were deduced from the HMBC spectrum. The HMBC correlation of H-5 (δ_H 5.12) with the carbonyl signal at δ_C 170.7 (CO of the acetoxy moiety), indicated the presence of the acetoxy group at C-5. Similarly, the long-range couplings of the obvious hydroxy signals at δ_H 2.57, 3.40, 3.15, and 3.74 with the corresponding carbons revealed that four hydroxy groups were attached at C-3, C-4, C-7, and C-13, respectively. Additionally, the HMBC correlations of the protons H-12 (δ_H 4.00) and H-14 (δ_H 4.40) with the typical quaternary carbon C-1′ (δ_C 108.1) were indicative of the presence of a 9,12,14-orthobenzoate. The remaining benzoyloxy could only be located at C-6 according to the chemical shift of C-6, which was supported by the NOESY correlations of H-3″ (H-7″) of the benzoyloxy group to H-8 and 4-OH. Thus, the planar structure of **1** was established.[26,27]

The configuration of **1** was elucidated as follows. NOESY correlations observed for H-8/4-OH, H-8/H-7, H-8/H-11, H-8/H-14, and H-12/H-11 (Fig. 2) indicated that 4-OH, H-7, H-8, H-11, H-12, and H-14 were on the same side and randomly assigned in β-positions. In turn, the NOESY cross-peaks of H-3/H-2, H-3/H-5, 4-OH/5-OAc, H-5/H-10, and H_3-20/H-5 revealed that H-3, H-5, H-10, and H_3-20 were α-oriented. NOESY correlations of H-12/H_2-16 and H-12/H_3-17 suggested that the 9,12,14-orthobenzoate group occupied the α-face.

The absolute configuration of **1** was established by the time-dependent density functional theory (TDDFT) ECD calculations. Starting from the conformation of **1** deduced from the NOESY correlations and Chem3D modeling, conformational searches with the MMFF94S force field by MOE software[28] and geometry optimizations by the Gaussian 09 package[29] were performed. Then, the ECD spectra were calculated at the CAM-B3LYP/SVP level with the CPCM model in acetonitrile. The obtained ECD spectrum of **1** (Fig. 3) matched the experimental results closely, which suggested an absolute configuration of 2*S*, 3*S*, 4*R*, 5*S*, 6*R*, 7*S*, 8*S*, 9*R*, 10*R*, 11*R*, 12*S*, 13*S*, and 14*R* for compound **1**. On the basis of the above evidence, the structure of **1** was established as shown in Figure 1 and named chinensipene A.

Compound **2** possessed a molecular formula $C_{38}H_{44}O_{12}$ based on the HR-ESIMS and ^{13}C NMR data.[22] The ^{1}H and ^{13}C NMR spectra of compound **2** were very similar to those of **1**. Compound **2** differs from compound **1** only by the presence of an additional acetyl group, indicating one hydroxy group in **1** was acetylated. This acetyloxy group was inferred to be located at C-7 from the ^{1}H NMR spectrum of **2**, in which the H-7 signal [δ_H 6.63 (1H, s)] was downfield shifted by about 1.4 ppm compared to that of **1** [δ_H 5.26 (H-7, 1H, s)]. The following interpretation of 2D NMR spectrum confirmed the above deduction. Further analyses of 1D and 2D NMR data led to the assignments of all proton and carbon