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Butenolide derivatives from the plant endophytic fungus *Aspergillus terreus*

Feng Guo [a,b], Zhanlin Li [a,b], Xiangwei Xu [a,b], Kaibo Wang [a,b], Meili Shao [a,b], Feng Zhao [c,*], Haifeng Wang [a,b], Huiming Hua [a,b], Yuehu Pei [a,b], Jiao Bai [a,b,*]

[a] *Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, PR China*
[b] *School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, PR China*
[c] *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, PR China*

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ABSTRACT

Three new butenolides containing 5-hydroxyfuran-2(5*H*)-one core, asperteretal A (**1**), asperteretal B (**2**), and asperteretal C (**3**), together with seven known butenolides (**4**–**10**), were obtained from an endophytic fungus *Aspergillus terreus* PR-P-2 isolated from the plant *Camellia sinensis* var. *assamica*. The structures of compounds **1**–**3** were elucidated on the basis of detailed spectroscopic analysis including UV, IR, HRESIMS, 1D and 2D NMR, and ECD spectra. Compounds **1, 3, 5** and **6**–**8** showed potent inhibitory effects on NO production in RAW 264.7 lipopolysaccharide-induced macrophages, and compounds **5** and **8** also exhibited moderate cytotoxicity against HL-60 cell line.

1. Introduction

Endophytic fungi have proved to be a rich source of bioactive secondary metabolites [1,2]. *Aspergillus terreus*, a common fungus used in chemical and pharmaceutical industry, is well-known for the production of itaconic acid and lovastatin [3]. In the past decades, various kinds of secondary metabolites, such as butenolides, meroterpenoid [4], sesquiterpenes [5], sestertepenoids [6], alkaloids [7], and polyketides [8,9] have been subsequently isolated from terrestrial or marine strains of *A. terreus*. Particularly, butenolides are considered as typical metabolites in this strain, which exert anticancer [10,11], α-glucosidase inhibitory and antioxidant activity [12,13], antimalarial activity [14], and anti-phytopathogen activity [15]. With the aim of searching for novel bioactive compounds from endophytic fungi, we investigated the chemical components of the fermentation broth of the strain *A. terreus* PR-P-2 which was isolated from *Camellia sinensis* var. *assamica* (Mast.) *Kitam* in Pu'er City, Yunnan, China. As a result, three new butenolides, asperteretal A (**1**), asperteretal B (**2**) and asperteretal C (**3**), together with seven known butenolides, 3-hydroxy-5-[[4-hydroxy-3-(3-methyl-2-buten-1-yl)phenyl]methyl]-4-(4-hydroxyphenyl)-2(5*H*)-furanone (**4**) [16,17], butyrolactone I (**5**) [11,18], butyrolactone II (**6**) [11,19], butyrolactone III (**7**) [11,19], aspernolide A (**8**) [10], aspernolide B (**9**) [10], aspernolide D (**10**) [20] were obtained (Fig. 1). Compounds **1**–**10** were tested for their inhibitory effects on nitric oxide production in lipopolysaccharide (LPS)-induced macrophages. Herein, we report the isolation, structure elucidation, and plausible biosynthetic pathway of the new compounds, as well as anti-inflammatory activity and cytotoxicity of these butenolide derivatives.

2. Experimental

2.1. General

UV spectrum was recorded on a Shimadzu UV-1601 (Kyoto, Japan). IR spectrum was obtained on a Bruker IFS-55 infrared spectrophotometer with KBr pellets. The HR-ESI-MS data were detected on a Bruker microTOF-Q mass spectrometer. ECD spectrum was measured on a JASCO CD-2095 Chiral Detector. The NMR spectral data were recorded on Bruker AVIII-400 and AV-600 (400 or 600 MHz for 1H, 100 or 150 MHz for ^{13}C, and 600 MHz for HSQC and HMBC) with TMS as the internal standard. TLC analyses were carried out using precoated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, China). Column chromatography was performed on silica gel (200–300mesh; Qingdao Marine Chemical Plant, Qingdao, China), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA). Semi-preparative HPLC was performed on a

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* Corresponding authors.
E-mail addresses: ytuzhaofeng@163.com (F. Zhao), baijiao@hotmail.com (J. Bai).

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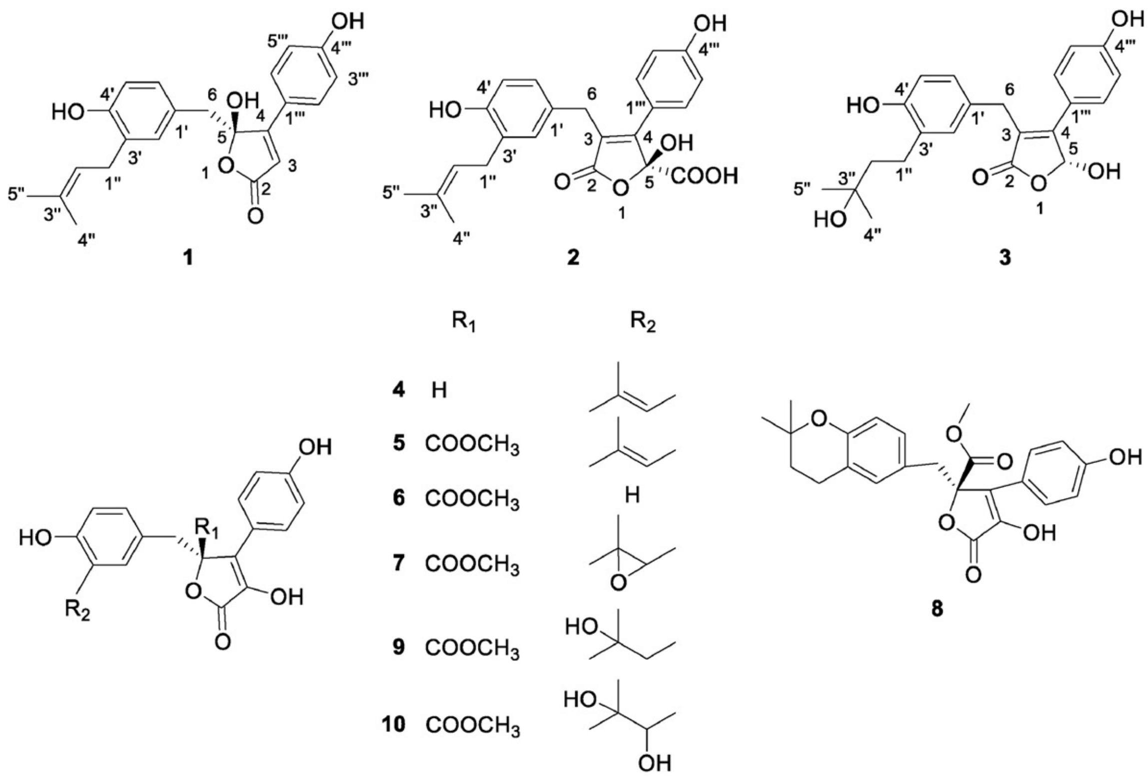


Fig. 1. Chemical structures of compounds **1**–**10**.

Shimadzu LC-6AB preparative liquid chromatograph (Shimadzu, SPD-20AB·UV.detector).

2.2. Fungal material

The endophytic fungus PR-P-2 was originally isolated from fresh plant of *Camellia sinensis* var. *assamica* (Mast.) Kitam, which was collected in September 2012 in Yunnan, China. This strain was identified as *A. terreus* on the basis of *in vitro* colony growth and micromorphology. The strain was also identified using DNA amplification and sequencing of the ITS. The sequence data has been deposited at GenBank (Accession no. KP260560). A voucher strain has been deposited in School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

2.3. Fermentation, extraction and isolation

The fungus PR-P-2 was cultured on a rotary shaker (180 rpm) at 28 °C for 7 days in liquid medium (containing mannitol 2%, D-glucose 2%, yeast extract 0.5%, peptone 1%, KH_2PO_4 0.05%, $MgSO_4$ 0.03%, corn syrup 0.1%). After 7 days, the fermented broth (65 L) was centrifuged to be separated into the supernatant and the mycelia. Finally, the supernatant was concentrated to 15 L and successively extracted with ethyl acetate, and then the crude extract (62.4 g) was obtained. The EtOAc crude extract was subjected to silica gel column chromatography (CC) (50 g), eluted with CH_2Cl_2 with increasing amounts of CH_3OH to give 6 fractions (Fr. 1–6). Fraction 2 was subjected to ODS CC eluted by CH_3OH-H_2O (5%–100%) to give 4 fractions (subfr. 2.1–2.4). Subfraction 2.1 (357.2 mg) was subjected to Sephadex LH-20 CC to yield compound **4** (3.2 mg). Fraction 3 (2.8 g) was subjected to ODS CC eluted by CH_3OH-H_2O (5%–100%) to give 4 fractions (subfr. 3.1–3.4). The subfraction 3.2 (314 mg) was purified with reverse phase semi-preparative HPLC (75% MeOH) to give compound **5** (19.4 mg), compound **6** (13.3 mg). Subfraction 4 (478.8 mg) was subjected to Sephadex LH-20 CC (40 g) eluted by methanol to give 2 subfractions (subfr. 4.1–4.2). The subfraction 4.2 (57 mg) was purified with reverse phase semi-preparative HPLC (70% MeOH) to give compound **1** (5.0 mg). Fraction 6 (3 g) was subjected to silica gel column eluted by petroleum ether-EtOAc (100:0–0:100) to give 5 subfractions (subfr. 6.1–6.5). The subfraction 6.2 (132 mg) was purified with reverse phase semi-preparative HPLC (65% MeOH) to give compound **7** (5.2 mg), compound **8** (4.6 mg), and compound **9** (8.8 mg). Subfraction 6.4 (185 mg) was purified with reverse phase semi-preparative HPLC (50% MeOH) to give compound **2** (6.1 mg), compound **3** (19.2 mg), compound **10** (22.2 mg).

2.3.1. Asperteretal A (*1*)

Colorless oil (CH_3OH); $[\alpha]^{20}_{D}$ $-6.80°$ (*c* 0.26, CH_3OH); UV (CH_3OH) λ_{max}: 225, 308 nm; IR (KBr) ν_{max} 3424, 1731, 1649, 1605 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$): 198 (-17.7), 208 (18.6), 213 (-2.82), 217 (7.15), 222 (-2.92), 253 (0.18), 305 (-3.56) nm; HR-ESI-MS *m/z*: 389.1354 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_5Na$, 389.1359). 1H NMR (600 MHz, DMSO-d_6) and 13C NMR (150 MHz, DMSO-d_6) spectral data see Table 1.

2.3.2. Asperteretal B (*2*)

Colorless oil (CH_3OH); $[\alpha]^{20}_{D}$ $-18.6°$ (*c* 0.12, CH_3OH); UV (CH_3OH) λ_{max}: 224, 283 nm; IR (KBr) ν_{max} 3431, 1735, 1670, 1607 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$): 194 (-8.83), 200 (15.9), 204 (-0.69), 206 (4.26), 210 (-0.43), 212 (3.11), 224 (-0.40), 250 (0.93), 305 (1.19) nm; HR-ESI-MS *m/z*: 411.1435 $[M + H]^+$ (calcd for $C_{23}H_{23}O_7$, 411.1438). 1H NMR (600 MHz, DMSO-d_6) and 13C NMR (100 MHz, DMSO-d_6) spectral data see Table 1.

2.3.3. Asperteretal C (*3*)

Colorless oil (CH_3OH); $[\alpha]^{20}_{D}$ $-9.00°$ (*c* 0.20, CH_3OH); UV (CH_3OH) λ_{max}: 225, 283, 305 nm; IR (KBr) ν_{max} 3427, 1738, 1607 cm^{-1}; CD (CH_3OH) λ_{max} ($\Delta\varepsilon$): 192 (-8.66), 197 (12.2), 205 (-4.25), 209 (4.67), 212.5 (-1.29), 217 (1.95), 227 (-1.16), 249 (0.30), 303 (1.19) nm; HRESIMS *m/z*: 407.1480 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_6Na$,

Table 1
1H and 13C NMR data for compounds **1**–**3** in DMSO-d_6.

	1		2		3	
Position	δ_H (mult. J in Hz)[a]	δ_C[b]	δ_H (mult. J in Hz)[a]	δ_C[c]	δ_H (mult. J in Hz)[d]	δ_C[b]
2		169.8		173.1		172.4
3	6.15 (1H, s)	112.5		124.4		123.7
4		163.6		157.5		156.3
5		107.7		103.2	6.51 (1H, s)	97.3
6	3.19 (1H, d, 13.4) 3.08 (1H, d, 13.4)	43.2	3.62 (1H, d, 15.5) 3.58 (1H, d, 15.5)	28.8	3.62 (2H, br s)	28.6
1′		124.4		128.0		127.7
2′	6.36 (1H, br s)	130.7	6.91 (1H, s)	129.2	6.89 (1H, d, 1.9)	129.2
3′		126.4		127.4		129.1
4′		153.5		153.2		153.5
5′	6.50 (1H, br s)	114.1	6.67 (1H, d, 8.0)	114.8	6.65 (1H, d, 8.2)	114.8
6′	6.50 (1H, br s)	128.1	6.83 (1H, d, 8.0)	125.8	6.76 (1H, dd, 8.2.1.9)	125.6
1″	2.89 (2H, d, 7.0)	27.5	3.15 (2H, d, 7.0)	28.0	2.49 (2H, m)	24.7
2″	4.99 (1H, t, 7.0)	122.4	5.23 (1H, t, 7.0)	122.8	1.53 (2H, m)	43.7
3″		131.3		131.2		68.8
4″	1.61 (3H, s)	25.5	1.66 (3H, s)	25.5	1.11 (3H, s)	29.3
5″	1.52 (3H, s)	17.5	1.63 (3H, s)	17.6	1.11 (3H, s)	29.3
1‴		120.9		122.0		121.5
2‴(6‴)	7.70 (2H, d, 8.5)	130.3	7.36 (2H, d, 7.5)	130.1	7.42 (2H, d, 8.6)	130.3
3‴(5‴)	6.88 (2H, d, 8.5)	115.7	6.74 (2H, d, 7.5)	115.1	6.82 (2H, d, 8.6)	115.6
4‴		160.0		158.7		159.3
5-OH	7.86 (1H, br s)					
4′–OH	9.07 (1H, s)		9.22 (1H, br s)			
4‴–OH	10.17 (1H, br s)		10.10 (1H, br s)			
5-COOH				167.8		

[a] ^{1}H NMR data were measured at 600 MHz.
[b] ^{13}C NMR data were measured at 150 MHz.
[c] ^{13}C NMR data were measured at 100 MHz.
[d] ^{1}H NMR data were measured at 400 MHz.

407.1471). 1H NMR (400 MHz, DMSO-d_6) and 13C NMR (150 MHz, DMSO-d_6) spectral data see Table 1.

2.3.4. 3-Hydroxy-5-[[4-hydroxy-3-(3-methyl-2-buten-1-yl)phenyl]methyl]-4-(4-hydroxyphenyl)-2(5H)-furanone (4)

Colorless oil (CH$_3$OH); $[\alpha]^{20}_{D}$ −14.3° (c 0.29, CH$_3$OH); 1H NMR (600 MHz,DMSO-d_6): δ_H 10.00 (1H, s, –OH), 9.82 (1H, s, –OH), 9.08 (1H, s, –OH), 7.55 (2H, d, J = 8.7 Hz, H-2‴/6‴), 6.87 (2H, d, J = 8.7 Hz, H-3‴/5‴), 6.62 (1H, dd, J = 8.1, 1.4 Hz, H-6′), 6.59 (1H, d, J = 8.1 Hz, H-5′), 6.55 (1H, d, J = 1.4 Hz, H-2′), 5.63 (1H, dd, J = 5.4, 3.6 Hz, H-5), 5.12 (1H, t, J = 7.3 Hz, H-2″), 3.10 (1H, dd, J = 14.9, 3.6 Hz, H-6a), 3.07 (2H, d, J = 7.3 Hz, H-1″), 2.71 (1H, dd, J = 14.9, 5.4 Hz, H-6b), 1.65 (3H, s, H-4″), 1.60 (3H, s, H-5″). 13C NMR (150 MHz, DMSO-d_6): δ_C 169.1 (C-2), 157.7 (C-4‴), 153.4 (C-4′), 136.3 (C-3), 131.0 (C-3″), 130.5 (C-2′), 129.6 (C-6′), 128.9 (C-2‴/6‴), 127.8 (C-4), 126.6 (C-3′), 125.1 (C-1′), 122.7 (C-2″), 121.8 (C-1‴), 115.5 (C-3‴/5‴), 114.2 (C-5′), 77.8 (C-5), 38.1 (C-6), 27.8 (C-1″), 25.5 (C-5″), 17.6 (C-4″).

2.4. Computational methods for ECD

The CONFLEX [21,22] searches based on molecular mechanics with MMFF94S force fields were performed for compounds **1**–**3**, which gave several stable conformers for each compound. Selected conformers of each compound with the lowest energy were further optimized by the density functional theory method at the B3LYP/6-31G* (d, p) level in Gaussian 09 program package [23], which was further checked by frequency calculation and resulted in no imaginary frequencies. The ECD of the conformer of each compound was then calculated by the TDDFT method at the B3LYP/6-31G (d) level with the CPCM model in methanol solution. The calculated ECD curve was generated using SpecDis 1.51 [24] with $\sigma = 0.10$ ev.

2.5. Anti-inflammatory assay

According to the Griess reaction, the concentration of nitrite in the medium was tested as a production index of NO. Briefly, RAW 264.7 cells were inoculated into 96-well plates at a density of 1×10^5 cells/well and treated with LPS (1 μg/mL) in the presence or absence of test compounds. After incubation at 37 °C for 24 h, 100 μL of cell-free supernatant was mixed with 100 μL of Griess reagent containing equal volumes of 2% (w/v) sulfanilamide in 5% (w/v) phosphoric acid and 0.2% (w/v) N-(1-naphthyl)-ethylenediamine solution to determine nitrite production. The absorbance was detected in a microplate reader at 540 nm and compared with a calibration curve prepared using NaNO$_2$ standards. The experiments were performed in triplicate, and the data are expressed as the means ± SD of three independent experiments [25,26].

2.6. Cytotoxicity assay

RPMI-1640 medium (Gibco, New York, NY, USA) contained 100 U/mL penicillin, 100 mg/mL streptomycin, 1 mmol glutamine, and 10% heat-inactivated fetal bovine serum (Gibco). Human leukemia HL-60 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in the above medium at a density of 5×10^4 cells/mL at 37 under an atmosphere of 5% CO$_2$. Cell growth inhibition assay was performed as reported previously. The compounds were dissolved in DMSO, and the amount of DMSO was controlled lower than 0.1% in the final concentration. Cells were incubated with various drug concentrations for 3 days. The number of cells was determined by hemocytometer, and its viability was determined using trypan blue staining. The growth inhibitory ability of the compound was calculated and expressed using the IC$_{50}$ value (half-inhibitory concentration). 5-Fluorouracil (5-FU) and 0.1% DMSO were used as a positive control and a negative control, respectively.

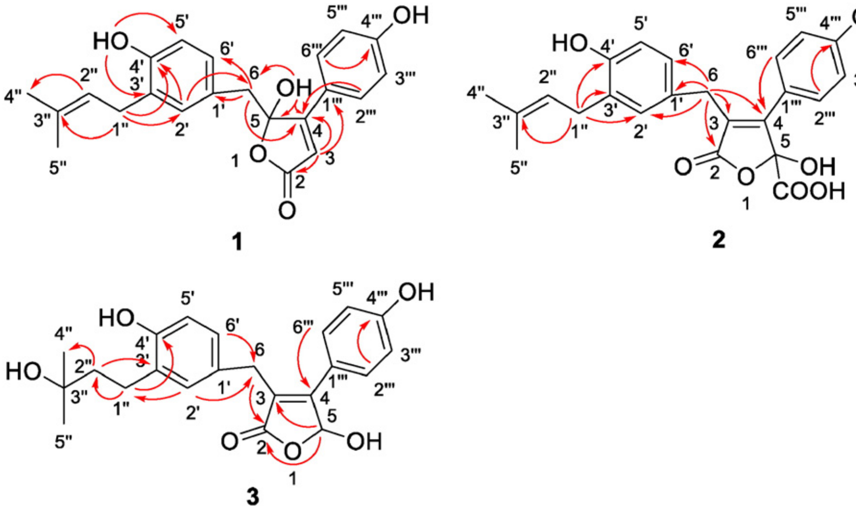


Fig. 2. Key HMBC correlations (H → C) of compounds **1**–**3**.

3. Results and discussion

Compound **1** was obtained as colorless oil. Its molecular formula was determined as $C_{22}H_{22}O_5$ by HR-ESI-MS at *m/z* 389.1354 $[M + Na]^+$ (calcd. 389.1359) indicating 12 degrees of unsaturation. The IR spectrum showed absorptions at 3424, 1731, 1649, 1604 cm^{-1}, indicating the presence of hydroxyl, carbonyl and phenyl. The 1H NMR (600 MHz, DMSO-d_6) spectrum (Table 1) showed the signals of two phenolic hydroxyls at δ_H 10.17 (1H, br s) and 9.07 (1H, br s); an alcoholic hydroxyl at δ_H 7.86 (1H, br s); a para-disubstituted benzene ring at δ_H 7.70 (2H, d, 8.5 Hz) and 6.88 (2H, d, 8.5 Hz); an olefinic proton at δ_H 6.15 (1H, s); a 1,3,4-trisubstituted benzene ring at δ_H 6.50 (2H, br s) and 6.36 (1H, s) ; an isolated methylene at δ_H 3.19 (1H, d, 13.4 Hz) and 3.08 (1H, d, 13.4 Hz); and a prenyl group at δ_H 4.99 (1H, t, 7.0 Hz), 2.89 (2H, d, 7.0 Hz), 1.61(3H, s) and 1.52 (3H, s). The 13C NMR (150 MHz, DMSO-d_6) spectrum (Table 1) exhibited 22 carbon signals, including one conjugated ester carbonyl at δ_C 169.8, sixteen sp^2 carbons (δ_C 163.6, 160.0, 153.5, 131.3, 130.7, 130.3, 130.3, 128.1, 126.4, 124.4, 122.4, 120.9, 114.1, 115.7, 115.7, 112.5), and five sp^3 carbons (δ_C 107.7, 43.2, 27.5, 25.5, 17.5). The spectroscopic data similar to those of the known compound **4** suggested that **1** should be a butenolide derivative. Furthermore, the protons and protonated carbon resonances in the NMR spectra were unambiguously assigned by the HSQC experiment. The HMBC spectrum (Fig. 2) revealed the correlations from H-2″ to C-4″ and C-5″; from H-1″ to C-3″, C-2′, C-3′, and C-4′; from H-2′ to C-1″, C-4′, and C-6; from H-6 to C-1′, C-2′, and C-6′; from 4′–OH to C-3′ and C-5′; from H-2‴(6‴) to C-4‴; from H-3‴(5‴) to C-1‴, indicating the presences of 4′-hydroxy-3′-isopentenyl benzyl moiety and 4‴-hydroxyl phenyl group. According to the HMBC correlations from H-3 to C-2 and C-4 combining with the chemical shifts of C-2 to C-4, an α,β-unsaturated γ-lactone was established. In addition, the HMBC correlations from H-6 to C-4 and C-5, from H-2‴(6‴) to C-4, and from H-3 to C-1‴, implied that the 4′-hydroxy-3′-isopentenyl benzyl moiety and the 4‴-hydroxyl phenyl group located at C-6 and C-4 of the γ-lactone, respectively. The HMBC correlations from 5-OH to C-4, C-5, and C-6 revealed that the hydroxyl connected to C-5 in compound **1** instead of C-3 in compound **4**. The absolute configuration of **1** was determined by comparison of the calculated ECD spectrum with the experimental one (Fig. 3). There are two possible isomers of **1** (*R*-**1** and *S*-**1**) and for each isomer an optimized conformation was calculated using DFT at the B3LYP/6-31G* (d, p) in the GAUSSIAN 09 program to generate its ECD property. The ECD spectrum of **1** showed the Cotton effects at 197 nm ($\Delta\varepsilon - 17.70$), 208 nm ($\Delta\varepsilon + 18.60$) and 217 nm ($\Delta\varepsilon + 7.15$) which matched well with those of the isomer *R*-**1**, it allowed us to unambiguously determine the stereo-chemistry of **1** with the absolute configuration of 5*R*. Consequently, the structure of compound **1** was established as (*R*)-5-hydroxy-5-[4-hydroxy-3-(3-methylbut-2-en-1-yl)benzyl]-4-(4-hydroxyphenyl) furan-2(5*H*)-one, named asperteretal A (**1**).

Although the structure of compound **1** has been reported as butryrolactone IX previously in the literature [27], the 1H and 13C NMR data of butryrolactone IX were not consistent with those of compound **1**, especially those of the lactone ring. Through careful analysis on the chemical shifts and the HMBC correlations of these two compounds, compound **1** was considered more convictive for the structure shown in Fig. 1.

Compound **2** was obtained as colorless oil. The molecular formula $C_{23}H_{22}O_7$ was established on the basis of $[M + H]^+$ peak at *m/z* 411.1435 (calcd. 411.1438) in the HR-ESI-MS, implying 13 degrees of unsaturation. The IR spectrum showed absorptions at 3431, 1735, 1670, 1607 cm^{-1}, indicating the presence of hydroxyl, carbonyl and phenyl functionalities. The 1H NMR spectrum of **2** (Table 1) showed a similar signal pattern to that of compound **1** for a *para*-disubstituted benzene ring [δ_H 7.36 (2H, d, 7.5 Hz, H-2‴ and 6‴) and 6.67 (2H, d, 7.5 Hz, H-3‴ and 5‴)] and a 1,3,4-trisubstituted benzene ring [δ_H 6.91 (1H, s, H-2′), 6.67 (1H, d, 8.0 Hz, H-5′), and 6.83 (1H, d, 8.0 Hz, H-6′)] with a prenyl unit [δ_H 5.23 (1H, t, 7.0 Hz, H-2″), 3.15 (2H, d, 7.0 Hz, H-1″), 1.66 (3H, s, H-4″) and 1.63 (3H, s, H-5″)], except for the absence of the proton signal of H-3. The 13C NMR spectrum (Table 1) showed an additional carbonyl signal at δ_C 167.8 and the chemical shifts for C-

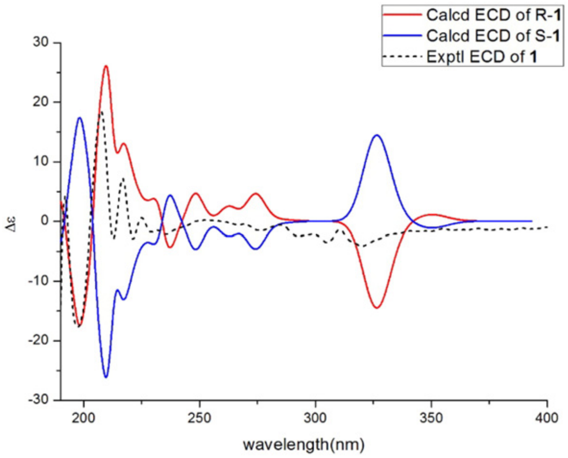


Fig. 3. The calculated ECD spectra of the two stereo-isomers and experimental ECD spectrum of compound **1**.

2 to C-5 different from those of **1**. It suggested that the pattern of substitution in the γ-lactone core of **2** had changed compared to that of **1**. The HMBC correlations (Fig. 2) from H-6 to C-2, C-3 and C-4, and from H-2‴(6‴) to C-4 confirmed the connections of C-6 to C-3 and C-1‴ to C-4. Furthermore, according to the chemical shift of C-5 (δ_C 103.2) and the carbonyl signal at δ_C 167.8 , as well as the molecular formula deduced from HR-ESI-MS, both a carboxyl group and a hydroxyl group were allotted to C-5. The absolute configuration of C-5 of **2** was determined by using the same method as **1**. The experimental ECD spectrum of **2** showing the Cotton effects at 194 nm ($\Delta\varepsilon - 8.83$), 200 nm ($\Delta\varepsilon + 15.9$), 204 nm ($\Delta\varepsilon - 0.69$) and 206 nm ($\Delta\varepsilon + 4.26$) was in accordance with the calculated one of the stereoisomer *R*-**2** (Fig. 4). Therefore, the structure of **2** was identified to be (*R*)-5-hydroxy-3-(4-hydroxy-3-(3-methylbut-2-en-1-yl)benzyl)-4-(4-hydroxyphenyl)-2-oxo-2,5-dihydrofuran-5-carboxylic acid, named asperteretal B.

Compound **3** was also obtained as colorless oil. The molecular formula was determined to be $C_{22}H_{24}O_6$ by HR-ESI-MS at *m/z* 407.1480 $[M + Na]^+$ (calcd. 407.1471). The IR spectrum showed absorptions at 3427, 1738, and 1607 cm^{-1}, indicating the presence of hydroxyl, carbonyl and phenyl groups. The 1H and 13C NMR spectral data (Table 1) were similar to those of **2**, except for the absence of the carboxyl carbon signal and the presence of an extra downfield proton signal at δ_H 6.51 (1H, s, H-5), which correlated with C-2 and C-3 in the HMBC spectrum (Fig. 2). It suggested that the proton was connected to C-5 in compound **3** instead of the carboxyl group for compound **2**. In addition, the significant upfield shifts of H-2“ [δ_H 1.53 (2H, m)], C-2″ (δ_C 43.7) and C-3″ (δ_C 68.8) combined with the HMBC correlations (Fig. 2) from H-4″(5″) to C-2″ and C-3″, and from H-1″ to C-2″ were indicative of the presence of a hydroxyl group at C-3″ instead of the $\Delta^{2'',3''}$ double bond for **2**. The absolute configuration of C-5 in **3** was also determined by using the same method as **1** and **2**. The ECD spectrum of **3** showed the Cotton effects at 192 nm ($\Delta\varepsilon - 8.66$), 197 nm ($\Delta\varepsilon + 12.20$) and 205 nm ($\Delta\varepsilon - 4.25$) which matched well with those of isomer *R*-**3** (Fig. 5). Thus, the structure of **3** was assigned as (*R*)-5-hydroxy-3-(4-hydroxy-3-(3-hydroxy-3-methylbutyl)benzyl)-4-(4-hydroxyphenyl)furan-2(5*H*)-one, named asperteretal C.

In addition, the known compounds **5**–**10** were identified as butyrolactone I (**5**) [11,18], butyrolactone II (**6**) [11,19], butyrolactone III (**7**) [11,19], aspernolide A (**8**) [10], aspernolide B (**9**) [10], and aspernolide D (**10**) [20] by comparing their measured spectroscopic data with those reported in the literatures. Although the structure of compound **4** has been reported in the literatures [16,17], the 1H and 13C NMR data were not given in these literatures. Therefore, we assigned the 1H and 13C NMR signals in this paper (see Experimental section) by comparing to those of the similar compound **5** [11,18] and 3-hydroxy-5-(4-hydroxybenzyl)-4-(4-hydroxyphenyl)-2(5*H*)-furanone [16].

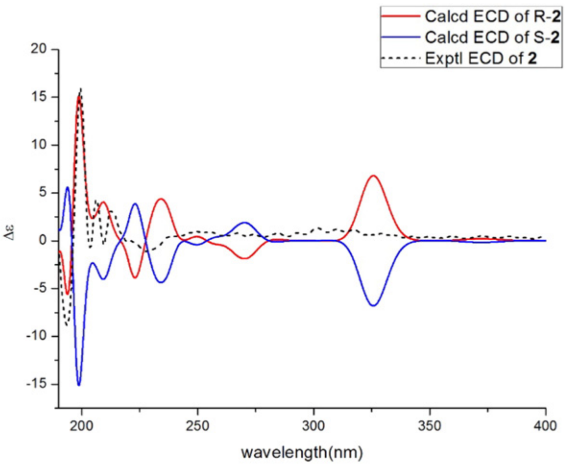


Fig. 4. The calculated ECD spectra of the two stereo-isomers and experimental ECD spectrum of compound **2**.

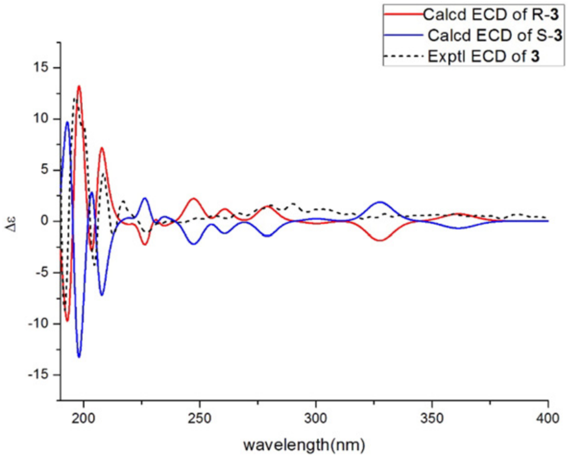


Fig. 5. The calculated ECD spectra of the two stereo-isomers and experimental ECD spectrum of compound **3**.

According to the structure and substituted pattern of the central lactone core, butenolide derivatives isolated form *A. terreus* can be classified mainly into four types, some butyrolactones and aspernolides (*e.g.* compounds **4**–**10**) [5,6,20,28] with the lactone core of 4.5-diarylsubstituted 3-hydroxyfuran-2(5*H*)-one, aspulvinones (*e.g.* aspulvinone E and isoaspulvinone E) [13] with the lactone core of 3.5-diarylsubstituted 4-hydroxyfuran-2(5*H*)-one, furandiones (*e.g.* asperjinone, asperterones A-C) [2,5,8] with the lactone core of 3.4-diarylsubstituted furan-2.5-dione, and butyrolactone VIII [29] with 3.4-diarylsubstituted 5-hydroxyfuran-2(5*H*)-one. Compound **1** was the first natural butenolide with the lactone core of 4.5-diarylsubstituted 5-hydroxyfuran-2(5*H*)-one from *A. terreus*.

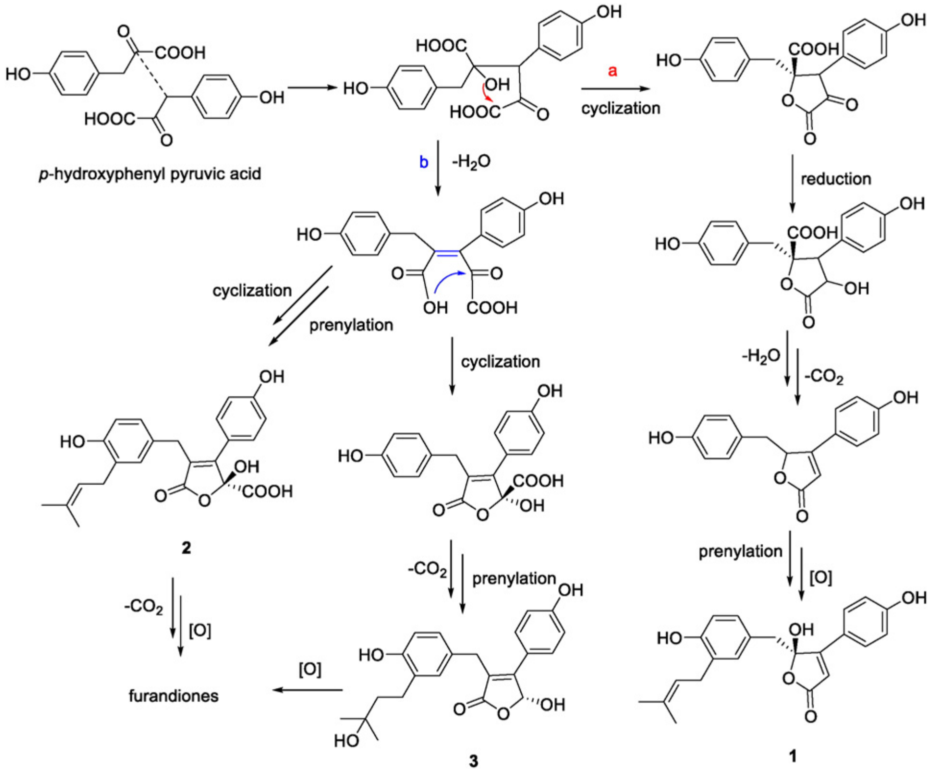
The biosynthetic research of some butenolide derivatives has revealed that they were formed by aldol condensation of two phenyl pyruvic acids or *p*-hydroxyphenyl pyruvic acids, followed by cyclization and prenylation [10,19,30,31]. Based on these findings, the plausible biosynthesis pathway of the new butenolides **1**–**3** was proposed as shown in Scheme 1. Two *p*-hydroxyphenyl pyruvic acids occurred condensation, then cyclization *via* path a, followed by reduction, decarboxylation, dehydration, oxidation at C-5, and prenylation to give compound **1**. While compounds **2** and **3** were biogenerated *via* path b, which undergoing dehydration, then intramolecular cyclization and prenylation. Since compounds **2** and **3** seem to be easily changed into furandiones through decarboxylation and/or oxidation, they also could be considered as the biosynthetic intermediates of the furandions.

The anti-inflammatory activities of compounds **1**–**10** were evaluated by their ability to inhibit NO production *in vitro* in LPS-induced macrophages. Hydrocortisone was used as a positive control. Cell viability was also examined by the MTT assay, and none of the test compounds exhibited cytotoxicity at their effective concentrations. As shown in Table 2, compounds **1**, **3**, **5** and **6**–**8** showed potent NO inhibitory activity with IC_{50} values of 26.64, 16.80, 17.21, 44.37, 20.60 and 45.37 μM (while that of hydrocortisone was 48.66 μM), respectively.

The primary cytotoxic activities of compounds **1**–**10** against HL-60 (human promyelocytic leukemia cells) cell line were tested by the method of trypan blue staining. Compounds **5** and **8** exhibited moderated growth inhibitory with IC_{50} values of 18.85 and 39.36 μM (IC_{50} value of 5-FU was 2.80 μM), respectively, and the other compounds were inactive toward HL-60 cells ($IC_{50} > 80$ μM).

Conflict of interest

The authors declare no conflict of interest.



Scheme 1. The proposed biosynthetic pathway of compounds **1**–**3**.

Table 2
Inhibitory effect of compounds **1**–**10** on LPS-induced NO production in macrophages.

Compounds	IC_{50}(μM)	Compounds	IC_{50}(μM)
1	26.64 ± 1.81	**6**	44.37 ± 3.02
2	61.36 ± 3.37	**7**	20.60 ± 1.53
3	16.80 ± 1.15	**8**	45.37 ± 3.31
4	61.43 ± 4.05	**9**	67.13 ± 4.88
5	17.21 ± 1.27	**10**	79.76 ± 5.42
Hydrocortisone	48.66 ± 3.26		

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Appendix A. Supplementary data

The UV, IR, HR-ESI-MS, 1D NMR, 2D NMR and ECD spectra for compounds **1**–**3**, and 1H and 13C NMR spectra for compound **4** were available in the Supplementary data. Supplementary data for this article can be found online http://dx.doi.org/10.1016/j.fitote.2016.06.014.

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