



New furoisocoumarins and isocoumarins from the mangrove endophytic fungus *Aspergillus* sp. 085242

Ze'en Xiao^{‡1,2}, Senhua Chen^{‡1}, Runlin Cai¹, Shao'e Lin¹, Kui Hong^{*3} and Zhigang She^{*1}

Full Research Paper

Open Access

Address:

¹School of Chemistry and Chemical Engineering, Sun Yat-Sen University, No. 135 of Xingang West Road, Guangzhou, 510275, China, ²Shenzhen Academy of Metrology and Quality Inspection, No. 144 of Minkan Road, Minzhi Street, Longhua District, Shenzhen, 518102, China and ³Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education of China, School of Pharmaceutical Sciences, Wuhan University, Wuhan, 430071, China

Email:

Kui Hong* - kuihong31@gmail.com;
Zhigang She* - cesszhg@mail.sysu.edu.cn

* Corresponding author ‡ Equal contributors

Keywords:

α -glucosidase; *Aspergillus*; DPPH; furoisocoumarin; isocoumarin

Beilstein J. Org. Chem. **2016**, *12*, 2077–2085.

doi:10.3762/bjoc.12.196

Received: 17 July 2016

Accepted: 08 September 2016

Published: 23 September 2016

Associate Editor: J. S. Dickschat

© 2016 Xiao et al.; licensee Beilstein-Institut.

License and terms: see end of document.

Abstract

The chemical investigation of the mangrove endophytic fungus *Aspergillus* sp. 085242 afforded eight isocoumarin derivatives **1–8** and one isoquinoline **9**. Asperisocoumarins A and B (**1** and **2**) were new furoisocoumarins, and asperisocoumarins E and F (**5** and **6**) were new isocoumarins. Their structures were established by analysis of their spectroscopic data and the absolute configuration of compound **2** was unambiguously determined by X-ray structure analysis and ECD calculation. Moreover, the absolute configurations of compounds **3–5** were assigned by comparison of their ECD spectra with isocoumarins described in the literature. Asperisocoumarins C and D (**3** and **4**) were fully characterized spectroscopically and isolated from a natural source for the first time. Asperisocoumarins A–D (**1–4**) related to the class of furo[3,2-*h*]isocoumarins are rarely occurring in natural sources. Compounds **2**, **5**, and **6** showed moderate α -glucosidase inhibitory activity with IC₅₀ of 87.8, 52.3, and 95.6 μ M, respectively. In addition, compounds **1** and **3** exhibited weak radical scavenging activity with EC₅₀ values of 125 and 138 μ M, respectively.

Introduction

Isocoumarins are an important group of natural products with diverse structural features and interesting biological activities. They have been widely isolated from fungi, lichens, bacteria, plants, and insects [1,2]. Furoisocoumarins combining a furan ring and an isocoumarin moiety are divided into two subclasses

depending on their fusion type: linear furo[2,3-*g*]isocoumarins and angular type furo[3,2-*h*]isocoumarins. The linear furo[2,3-*g*]isocoumarins are relatively common in nature and coriandrin [3,4], dihydrocoriandrin [3,4], coriandrone C [5], and coriandrone E [5] are a few examples. However, an angular-type

furo[3,2-*h*]isocoumarin has up to date only been once reported from a natural source: Coriandrone A isolated from the aerial parts of *Coriandrum sativum* [6] and other furo[3,2-*h*]isocoumarins have been reported as synthetic products [7-9].

In the last decade, our research group has been devoted to finding novel bioactive compounds from mangrove endophytic fungi derived from the South China Sea [10-15]. In a previous study, a chemical investigation of the endophytic fungal strain *Aspergillus* sp. 085242 allowed us to identify two novel sesquiterpenoids, asperterpenols A and B with an unusual 5/8/6/6 tetracyclic ring skeleton [15]. The unique structures of these sesquiterpenoids encouraged us to further study this fungal strain and continuous chemical investigation of it led to the isolation of two new furo[3,2-*h*]isocoumarins, asperisocoumarins A and B (**1** and **2**) and two new isocoumarins, asperisocoumarins E and F (**5** and **6**), together with five known compounds (**3**, **4**, **7–9**) (Figure 1). Details of the isolation, structure elucidation, and biological activity of these compounds are reported herein.

Results and Discussion

The mangrove endophytic fungus *Aspergillus* sp. 085242 was cultured on solid rice medium with saline water for four weeks. The MeOH extract of the fermentation was fractionated by repeated silica gel chromatography and Sephadex LH-20 column chromatography to yield compounds **1–9**.

Compound **1** was obtained as pale yellow crystal. Its molecular formula was established as C₁₅H₁₂O₄ on the basis of HREIMS

(*m/z* 256.0729, calcd for C₁₅H₁₂O₄, 256.0730) and NMR data, implying ten degrees of unsaturation. The IR spectrum displayed intense absorption bands at 1737 and 1695 cm⁻¹ indicating the presence of two carbonyl functionalities. The ¹H NMR data (Table 1) showed two aromatic AB spin system protons δ_H 7.92 (1H, d, *J* = 8.0 Hz, H-4) and δ_H 7.01 (1H, d, *J* = 8.0 Hz, H-5), one olefinic proton δ_H 6.30 (1H, s, H-6), and three methyl groups δ_H 2.39 (3H, s, H-13), δ_H 2.32 (3H, s, H-10), and δ_H 2.24 (3H, s, H-12). ¹³C and DEPT NMR spectra of **1** revealed the resonance of two carbonyl, six aromatic, four olefinic, and three methyl carbons. Key HMBC correlations (Figure 2) from H-10 to C-6 and C-7, H-6 to C-5, C-5a, and C-9a, H-4 to C-3a, C-5a, and C-9b, and the upfield appearance of carbonyl group C-9 (δ_C 158.2) established a 3a,9b-disubstituted 7-methylisocoumarin unit. A 2-oxy-3-methyl-2-butenoyl moiety was assigned by the HMBC correlations of two methyl protons H-12 and H-13 to C-2, C-3, and C-11, as well as the chemical shifts of these carbons. This moiety connected to the aromatic ring at C-3a was supported by the HMBC correlations of H-4 with C-3. An ether linkage between C-2 and C-9b was fused as a 3-oxobenzofuran unit according to the chemical shifts of C-2 (δ_C 145.7) and C-9b (δ_C 165.1) as well as the required degrees of unsaturation. Thus, the structure of **1** was identified as 7-methyl-2-(propan-2-ylidene)-9*H*-furo[3,2-*h*]isochromene-3,9(2*H*)-dione, named asperisocoumarin A.

Compound **2** was obtained as colorless crystals, and analyzed for the molecular formula C₁₅H₁₈O₄ by interpretation of HREIMS (*m/z* 262.1201, calcd for C₁₅H₁₈O₄, 262.1200). The IR spectrum revealed the presence of an additional hydroxy

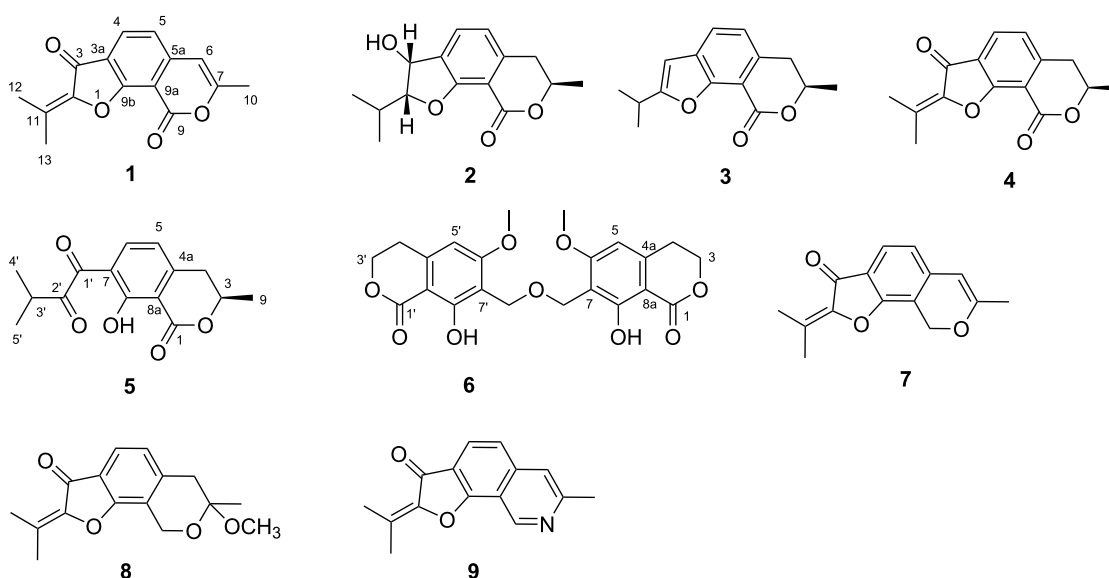
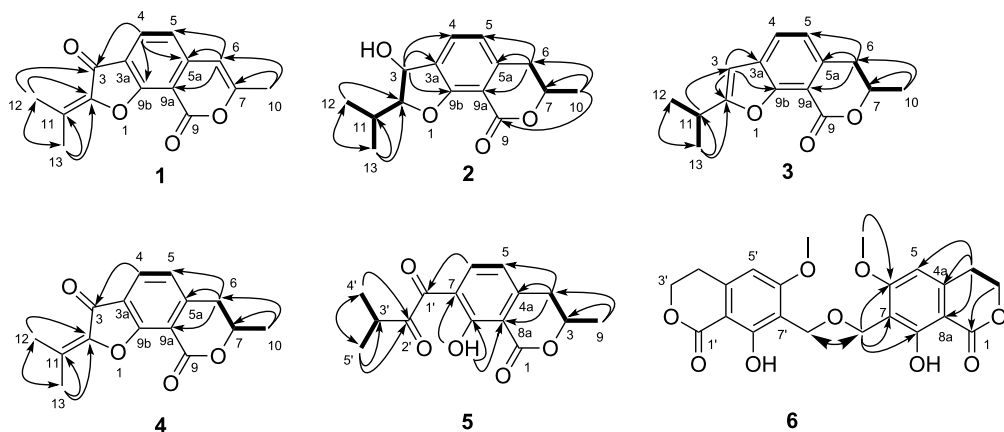


Figure 1: The structure of compounds **1–9**.

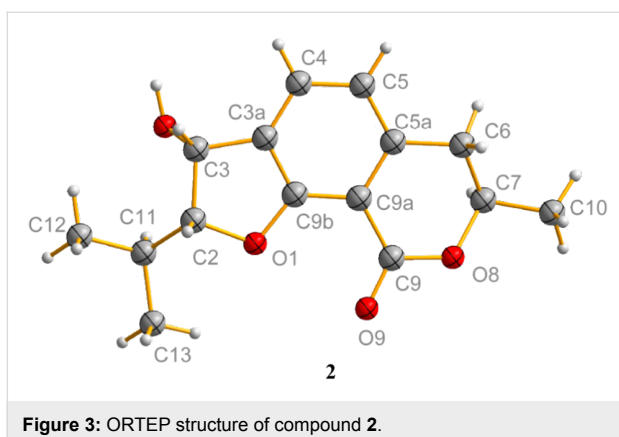
Table 1: NMR spectroscopic data of compounds 1–4.^a

No.	1		2		3		4	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	145.7, C		94.5, CH	4.17, dd (5.5, 10.2)	167.2, C		145.3, C	
3	181.6, C		71.0, CH	5.09, d (5.5)	99.6, CH	6.39, d (0.9)	182.3, C	
3a	123.1, C		131.2, C		130.1, C		124.6, C	
4	130.3, CH	7.92, d (8.0)	130.9, CH	7.53, d (7.5)	125.8, CH	7.61, d (6.6)	129.4, CH	7.88, d (7.7)
5	119.1, CH	7.01, d (8.0)	119.6, CH	6.76, d (7.5)	121.5, CH	7.03, d (6.6)	121.5, CH	7.01, d (7.7)
5a	145.7, C		142.3, C		135.3, C		148.4, C	
6	104.2, CH	6.30, s	35.3, CH ₂	2.90, d (8.4); 2.89, d (5.4)	35.3, CH ₂	3.01, m	36.0, CH ₂	3.02, m
7	159.0, C		74.6, CH	4.61, m	75.4, CH	4.70, m	74.7, CH	4.70, m
9	158.2, C		162.4, C		162.8, C		161.4, C	
9a	105.7, C		109.3, C		109.9, C		110.9, C	
9b	165.1, C		161.6, C		153.8, C		164.3, C	
10	20.1, CH ₃	2.32, s	20.8, CH ₃	1.43, d (6.3)	21.3, CH ₃	1.54, d (6.4)	20.8, CH ₃	1.55, d (6.3)
11	135.3, C		27.3, CH	2.31, m	28.3, CH	3.20, m	135.6, C	
12	20.8, CH ₃	2.24, s	20.0, CH ₃	1.29, d (6.6)	21.0, CH ₃	1.38, d (6.9)	20.9, CH ₃	2.22, s
13	17.7, CH ₃	2.39, s	19.4, CH ₃	1.14, d (6.6)	21.0, CH ₃	1.37, d (6.9)	17.8, CH ₃	2.39, s

^a¹H (400 MHz) and ¹³C (100 MHz) in CDCl₃.**Figure 2:** Key HMBC (arrows) and COSY (bold lines) correlations of compounds 1–6.

group at 3409 cm^{-1} and the absence of a carbonyl group at 1737 cm^{-1} in comparison with compound **1**. The ¹H NMR spectrum (Table 1) showed the signals corresponding to two ortho-coupled aromatic protons, four alkyl methine protons, one methylene, and three methyl groups. The ¹³C NMR spectrum of **2** displayed the resonance of one carbonyl, six aromatic, four methine centers including three oxygenated, one methylene, and three methyl carbons. The above spectroscopic data suggested that compound **2** was a hexahydro-analogue of **1**. This deduction was further evidenced by the HMBC correlations of H-10 to C-9, C-7 and C-6, H-6 to C-5, C-5a and C-9a, and H-3 to C-3a, C-4 and C-9b, as well as the COSY correlations of H-2 with H-3 and H-11, H-11 with H-12 and H-13, H-4 with H-5,

and H-7 with H-6 and H-10 (Figure 2). The relative configuration of compound **2** was determined by X-ray crystallographic analysis (Figure 3). The final refinement of the CuK α data resulted in a Flack parameter of 0.12(16) and the Hooft parameter of 0.06(8) [16,17], which allowed the assignment of the absolute configuration of **2** as (2*R*,3*R*,7*R*) (Figure 3). Moreover, the predicted ECD curves of **2** and its relevant enantiomer were computed at the [B3LYP/6-31 G(2d,p)] level, and the experimental ECD curve of **2** agreed well with the predicted one (Figure 4), in accordance with the deduction from the X-ray crystallography analysis. Therefore, the structure of compound **2** was established as depicted in Figure 3 and named asperisocoumarin B.

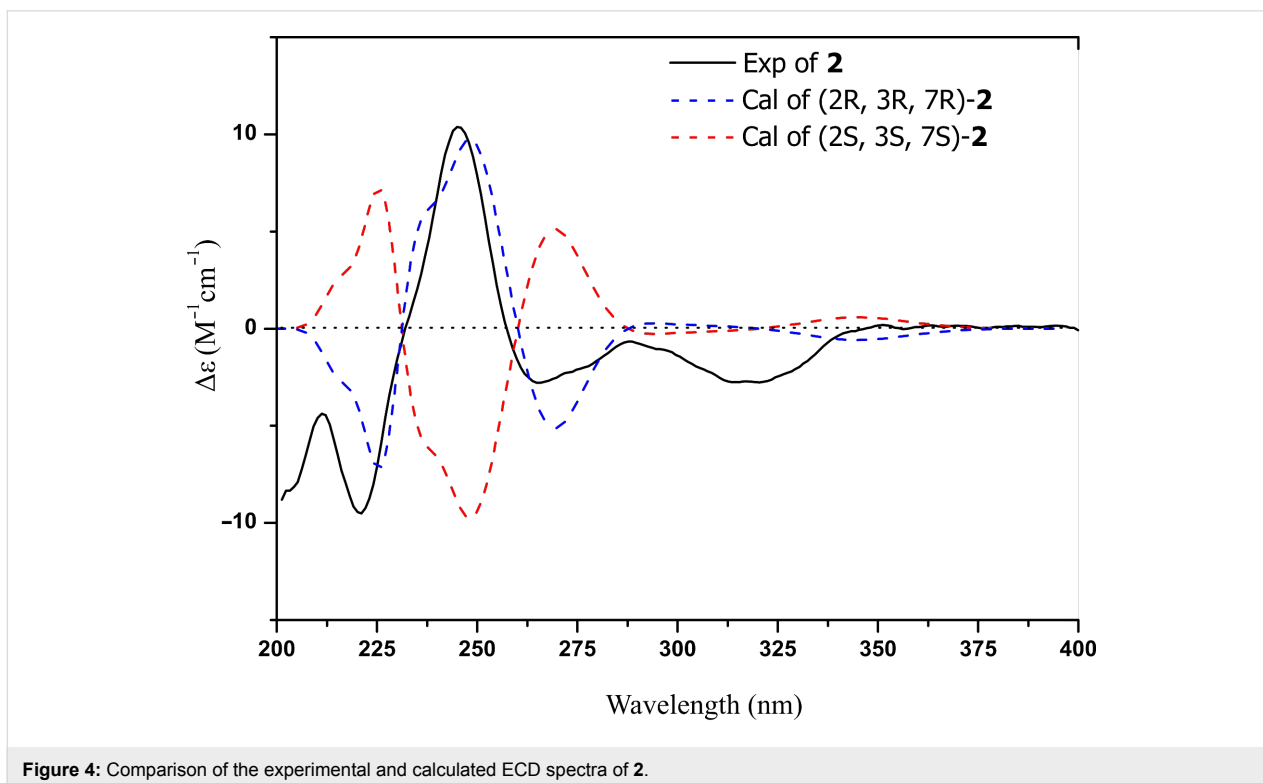


Asperisocoumarin C (**3**) was obtained as a pale yellow crystalline solid and displayed an $[M + H]^+$ ion in HRESIMS at m/z 245.1174, suggesting a molecular formula of $C_{15}H_{16}O_3$. A careful comparison of its 1H and ^{13}C NMR spectra (Table 1) with those of **2** indicated that compound **3** also shared the same isocoumarin skeleton as compound **2**. The main differences were that an additional olefinic methine carbon at δ_C 99.6 and a quaternary carbon at δ_C 167.2 were observed, whereas two oxygenated methine carbons at δ_C 94.5 and 71.0 were absent in the spectrum of compound **3**. These differences were further supported by the HMBC correlations of olefinic proton H-3 to C-2, C-3a and C-9b (Figure 2). By comparison of the ECD spectrum (Figure 5) and the optical rotation of **3** with data re-

ported for dihydrocoumarins [18], it was possible to assign the absolute configuration of C-7 as *R*. So, the structure of compound **3** was identified as (*R*)-2-isopropyl-7-methyl-6,7-dihydro-9*H*-furo[3,2-*h*]isochromen-9-one.

Asperisocoumarin D (**4**) was isolated as a white amorphous powder. Its molecular formula was determined as $C_{15}H_{14}O_4$ by HREIMS (m/z 258.0888, calcd for $C_{15}H_{14}O_4$, 258.0887). The 1H and ^{13}C NMR spectra of **4** were similar to those of asperisocoumarin A (**1**), except that the NMR resonances assigned to olefinic carbons C-6 and C-7 were replaced by sp^3 hybridized methylene (δ_C 36.0, δ_H 3.02) and methine (δ_C 74.7, δ_H 4.70) signals. HMBC correlations from H-10 (δ_H 2.12) to C-6 and C-7 and COSY correlations of H-7 with H-6 and H-10 further supported the above deduction (Figure 2). Finally, negative cotton effect ($\lambda = 265$ nm, $\Delta\epsilon = -15.10$) observed in the ECD spectrum (Figure 5), allowed the definition of the absolute configuration at C-7 (*R*) of compound **4** [18]. Thus, the structure of compound **4** was identified as (*R*)-2-isopropyl-7-methyl-6,7-dihydro-9*H*-furo[3,2-*h*]isochromen-9-one.

Asperisocoumarin E (**5**) was obtained as a pale yellow powder and the molecular formula was deduced by HREIMS analysis as $C_{15}H_{16}O_5$ (m/z 276.0092, calcd for $C_{15}H_{16}O_5$, 276.0092), indicating eight degrees of unsaturation. The 1H and ^{13}C NMR spectra of compound **5** were quite similar to those of **3** except for absence of an olefinic proton at H-3 and presence of two ad-



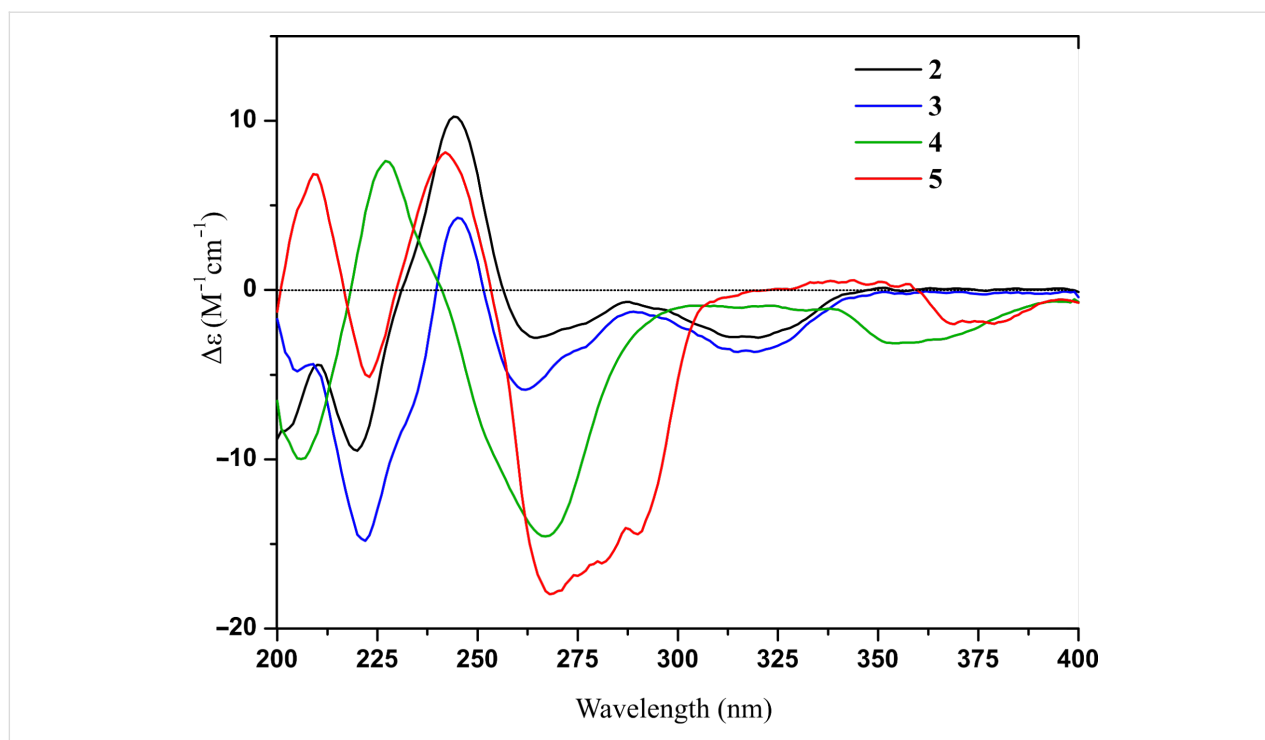


Figure 5: The experimental ECD spectra of 2–5.

ditional carbonyl carbons at C-1' (δ_C 206.2) and C-2' (δ_C 193.9), respectively (Table 2). This evidence suggested that compound **5** lacks a furan ring and is most likely derived from the furan ring-opening and oxidation of compound **3**, which was estab-

lished by HMBC correlations of the aromatic proton H-6 to C-1', two methyl protons H-4' and H-5' to C-2', and chelated hydroxy proton 8-OH to C-8, C-7, and C-8a (Figure 2). The negative circular dichroism at 265 nm (Figure 5) suggested *R*

Table 2: NMR Spectroscopic data for **5** and **6**.^a

No.	5		No.	6	
	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
1	169.4, C		1/1'	169.7, C	
3	76.0, CH	4.80, m	3/3'	67.7, CH ₂	4.51, t (6.1)
4	34.8, CH ₂	3.01, d (9.9), 3.03, d (5.0)	4/4'	28.3, CH ₂	3.01, t (6.1)
4a	146.4, C		4a/4a'	141.6, C	
5	118.8, CH	8.03, d (7.9)	5/5'	101.4, CH	6.27, s
6	137.0, CH	6.87, d (7.9)	6/6'	164.6, C	
7	121.9, C		7/7'	112.8, C	
8	162.4, C		8/8'	162.7, C	
8a	109.3, C		8a/8a'	102.4, C	
9	20.7, CH	1.58, d (6.3)	9/9'	60.1, CH ₂	4.67, s
1'	206.2, C		6/6'-OCH ₃	56.1, CH ₃	3.87, s
2'	193.9, C		8/8'-OH		11.31, s
3'	36.6, CH	3.18, m			
4'	17.2, CH ₃	1.29, d (7.0)			
5'	17.3, CH ₃	1.31, d (7.0)			
8-OH		11.94, s			

^a¹H (400 MHz) and ¹³C (100 MHz) in CDCl₃.

configuration at C-3, by comparison with data for isocoumarin derivatives described in the literature [13]. Thus, asperisocoumarin E (**5**) was elucidated as (*R*)-1-(8-hydroxy-3-methyl-1-oxoisochroman-7-yl)-3-methylbutane-1,2-dione.

Asperisocoumarin F (**6**) was obtained as a white powder. The molecular formula of **6** was deduced as C₂₂H₂₂O₉ from HRESIMS analysis (*m/z* 429.1186 [M – H][–]), implying 12 degrees of unsaturation. Its ¹H NMR spectrum resembled that of stellatin [13], except the absence of a hydroxy proton at δ_H 2.24. In the ¹³C NMR spectrum, the chemical shift value of the oxygenated methylene at C-9 was 3.9 ppm higher than that of stellatin [19]. At the same time, there was a strong HMBC correlation between H-9 and C-9' or H-9' and C-9. The above evidences allowed us to conclude that compound **6** was a polyether dimer of stellatin as shown in Figure 2.

In addition, the following known compounds were identified: ustusorane B (**7**) [20], penicisochroman A (**8**) [21], and TMC-120B (**9**) [22], on the basis of the spectroscopic comparison with those reported in the literature as well as to the specific rotation. The structures of ustusorane B (**7**) and penicisochroman A (**8**) were analyzed by X-ray crystallography analysis (Figure 6) for the first time.

Numerous isocoumarin and 3,4-dihydroisocoumarin derivatives have been isolated from various natural sources [1]. However, the furo[3,2-*h*]isocoumarin is a very uncommon class of isocoumarins and to date only a few members have been reported [1,6]. Asperisocoumarin A–D (**1–4**) were the second examples belonging to furo[3,2-*h*]isocoumarins from natural sources. Asperisocoumarin B (**2**) shared the same planar structure as (2*S*,3*S*,7*R*)-6,9-dihydro-3-hydroxy-7-methyl-2-(1-methylethyl)-7*H*-furo[3,2-*h*][2]benzopyran-9(2*H*)-one, which was the intermediate of the syntheses of (–)-ustusorane D and (+)-penicisochroman B [8]. Asperisocoumarin C (**3**) and D (**4**) had the same structure as (*R*)-2-isopropyl-7-methyl-6,7-

dihydro-9*H*-furo[3,2-*h*]isochromen-9-one and (*R*)-7-methyl-2-(propan-2-ylidene)-6,7-dihydro-9*H*-furo[3,2-*h*]isochromene-3,9-(2*H*)-dione, respectively. Both have been synthesized as intermediates during synthesis and structural characterization of natural benzofuranoids [9]. Asperisocoumarin E (**5**) containing an isopentenyl substituent with two adjacent carbonyl groups seems to be rare in natural isocoumarin derivatives and asperisocoumarin F (**6**) presents as a scaffold with an ether dimer of isocoumarin.

All isolates were evaluated for their α-glucosidase inhibitory activity using clinical acarbose (IC₅₀ of 628.3 μM) as a positive control. Compounds **2**, **5**, and **6** showed moderate α-glucosidase inhibitory activity with IC₅₀ of 87.8, 52.3, and 95.6 μM, respectively. The other compounds were inactive (> 300 μM). Compounds **1–9** were also evaluated for antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*. None of the compounds was active at a concentration of 50 μg/mL. In the free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH), only compounds **1** and **3** exhibited weak activity with EC₅₀ values of 125 and 130 μM, respectively (vitamin C was used as a positive control with EC₅₀ = 35 μM).

Experimental

General experimental procedures. Analogously as described in reference [12]. Melting points were determined with a Fisher-Johns hot-stage apparatus apparatus and are uncorrected. UV data were measured on a PERSEE TU-1900 spectrophotometer. Infrared spectra were recorded on a Nicolet Nexus 670 spectrophotometer using KBr discs. EIMS data were measured on a DSQ EI-mass spectrometer (Thermo, Shanghai, China) and HREIMS data were carried out on a DMAT95XP high-resolution mass spectrometer. ESIMS spectra were recorded on a Finnigan LCQ-DECA mass spectrometer and HRESIMS spectra were determined on a Shimadzu LCMS-IT-TOF mass spec-

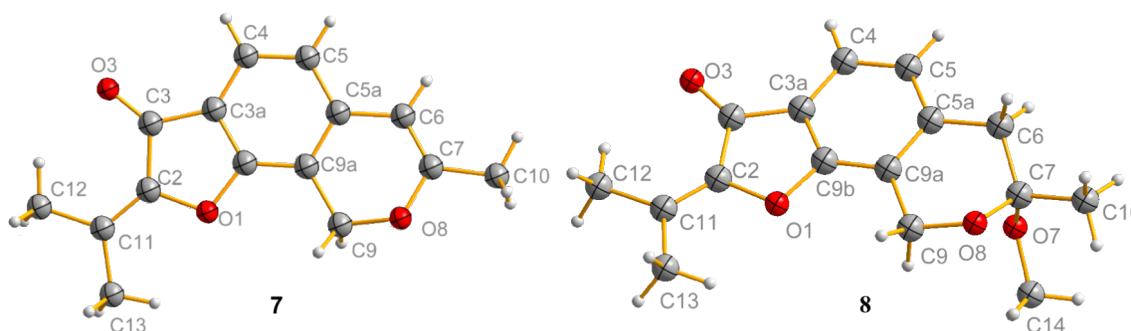


Figure 6: ORTEP structures of compound **7** and **8**.

trometer. 1D and 2D NMR spectra were carried out on Bruker Avance 400 spectrometer (^1H 400 MHz, ^{13}C 100 MHz). Chemical shifts (δ) are given in ppm with reference to the solvent signal (δ_{C} 77.1/ δ_{H} 7.26 for CDCl_3) and coupling constants (J) are given in Hz. ECD spectra were measured on a Chirascan CD spectrometer (Applied Photophysics, London, UK). Single-crystal data were collected on an Agilent Gemini Ultra diffractometer (CuK α radiation). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia, Piscataway) were used for column chromatography (CC). Thin layer chromatography was performed on precoated silica gel plates (Qingdao Huang Hai Chemical Group Co., G60, F-254).

Fungal material. The fungus *Aspergillus* sp. 085242 was isolated from healthy roots of *Acanthus ilicifolius*, which were collected from the Shankou Mangrove National Nature Reserve in Guangxi Province, China. Fungal identification was carried out using a molecular biological protocol by DNA amplification and sequencing of the ITS region and the sequence data have been submitted to GenBank with accession no. KC816018.1. A BLAST search result indicated that the sequence was the most similar (99%) to the sequence of *Aspergillus* sp. (compared to KP059102.1 and KJ567455.1). A voucher strain is deposited in the China Center for Type Culture Collection under patent depository number CCTCC M 2013081.

Fermentation, extraction, and isolation. Analogously as described in reference [12] the fungus was grown on autoclaved rice solid substrate medium (thirty 500 mL Erlenmeyer flasks, each containing 50 g rice and 50 mL 3‰ of saline water) at room temperature under static conditions and daylight for 28 days. Following incubation, the mycelia and solid rice medium were extracted with MeOH three times. The extract was evaporated under reduced pressure to yield 41 g of residue. The residue was then divided into 20 fractions (Fr. 1–Fr. 20) by column chromatography on silica gel eluted by a gradient of petroleum ether/EtOAc from 1:0 to 0:1. Fr. 4 (309 mg) was applied to the Sephadex LH-20 CC ($\text{CHCl}_3/\text{MeOH}$, v/v, 1:1) to give subfraction Fr. 4.9, which was purified on silica gel (petroleum ether/EtOAc, v/v, 8:2) to yield **1** (2.8 mg). Fr. 6 (105 mg) was rechromatographed on silica gel (petroleum ether/EtOAc, v/v, 8:2) to give subfraction Fr. 6.8, which was purified by Sephadex LH-20 CC ($\text{CHCl}_3/\text{MeOH}$, v/v, 1:1) to yield **7** (2.3 mg) and **8** (2.1 mg). Fr. 7 (264 mg) was subsequently separated by Sephadex LH-20 CC eluted with ($\text{CHCl}_3/\text{MeOH}$, v/v, 1:1) to give subfraction Fr. 7.9, which was purified on silica gel (petroleum ether/EtOAc, v/v, 7:3) to yield **2** (5.4 mg) and **3** (2.5 mg), respectively. Fr. 9 was chromatographed on Sephadex LH-20 CC ($\text{CHCl}_3/\text{MeOH}$, v/v, 1:1) to give subfraction Fr. 9.7,

which was purified using silica gel (petroleum ether/EtOAc, v/v, 7:3) to give **4** (4.1 mg) and **5** (2.6 mg). Fr. 11 (180 mg) was subsequently separated by Sephadex LH-20 CC eluted with MeOH to obtain **6** (3.1 mg). Fr. 12 was chromatographed on silica gel (petroleum ether/EtOAc, v/v, 6:4) to produce **9** (5.2 mg).

Asperisocoumarin A (1): pale yellow crystals; mp 189.5–192.0 °C; UV (MeOH) λ_{max} (log ϵ): 240 (4.25), 357 (3.56) nm; IR (KBr) ν_{max} : 3074, 2998, 2904, 1737, 1695, 1656, 1606, 1576, 1458, 1343, 1265, 1164, 1078 cm^{-1} ; EIMS (m/z): 256; HRMS–EI (m/z): $\text{C}_{15}\text{H}_{12}\text{O}_4$ calcd for 256.0730; found, 256.0729; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 1.

Asperisocoumarin B (2): colorless crystals; mp 179.4–181.4 °C; $[\alpha]_{\text{D}}^{20}$ +25.3 (c 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ): 220 (4.83), 248 (4.25), 317 (3.56) nm; IR (KBr) ν_{max} : 3391, 2973, 2935, 2868, 1691, 1610, 1451, 1383, 1175, 1054 cm^{-1} ; EIMS (m/z): 262; HRMS–EI (m/z): $\text{C}_{15}\text{H}_{18}\text{O}_4$ calcd for 262.1200; found, 262.1201; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 1.

Asperisocoumarin C (3): pale yellow amorphous powder; mp 122.4–124.8 °C; $[\alpha]_{\text{D}}^{20}$ –78.9 (c 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ): 221 (4.85), 252 (4.26), 324 (3.54) nm; IR (KBr) ν_{max} : 3391, 2973, 2935, 2868, 1691, 1610, 1451, 1383, 1175, 1054 cm^{-1} ; EIMS (m/z): 244; HRMS–ESI (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{16}\text{O}_3$, 245.1173; found, 245.1174; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 1.

Asperisocoumarin D (4): white amorphous powder; mp 182.2–184.4 °C; $[\alpha]_{\text{D}}^{20}$ –70 (c 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ): 222 (4.89), 249 (4.28), 319 (3.50) nm; IR (KBr) ν_{max} : 3390, 2976, 2934, 2866, 1692, 1612, 1453, 1381, 1176, 1056 cm^{-1} ; EIMS (m/z): 258; HRMS–EI (m/z): $\text{C}_{15}\text{H}_{14}\text{O}_4$ calcd for 258.0887; found, 258.0888; ^1H NMR (CDCl_3 , 400 MHz), see Table 1.

Asperisocoumarin E (5): pale yellow crystals; mp 136.1–138.0 °C; $[\alpha]_{\text{D}}^{20}$ –78.6 (c 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ): 220 (4.84), 335 (3.56) nm; IR (KBr) ν_{max} : 3376, 2979, 1707, 1607, 1431, 1389, 1273, 1135, 806 cm^{-1} ; EIMS (m/z): 276; HRMS–EI (m/z): $\text{C}_{15}\text{H}_{16}\text{O}_5$ calcd for 276.0092; found, 276.0092; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 2.

Asperisocoumarin F (6): white amorphous powder; mp 201.1–203.0 °C; UV (MeOH) λ_{max} (log ϵ): 268 (4.64), 299 (3.36) nm; IR (KBr) ν_{max} : 3476, 2998, 1670, 1581, 1369, 1283, 1125 cm^{-1} ; EIMS (m/z): 430; HRMS–ESI (m/z): $[\text{M} - \text{H}]^-$

calcd for $C_{15}H_{18}O_4$, 429.1186; found, 429.1186; 1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2.

X-ray crystallographic analysis. Single crystal X-ray diffraction data were collected at 123 K on an Agilent Gemini Ultra diffractometer with $CuK\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the “ride on” method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. Crystallographic data for **2**, **7** and **8** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44-(0)1223-336033, or email: deposit@ccdc.cam.ac.uk).

Crystal data of **2**: $C_{15}H_{18}O_4$, $M_r = 262.29$, monoclinic, $a = 16.4513(3) \text{ \AA}$, $b = 11.2236(2) \text{ \AA}$, $c = 8.27930(10) \text{ \AA}$, $\alpha = 90.00$, $\beta = 116.344$, $\gamma = 90.00$, $V = 1369.95(4) \text{ \AA}^3$, space group $C2$, $Z = 4$, $D_{\text{calcd}} = 1.272 \text{ mg/m}^3$, $\mu = 0.754 \text{ mm}^{-1}$, and $F(000) = 560$. Crystal dimensions: $0.44 \times 0.41 \times 0.40 \text{ mm}^3$. Independent reflections: 1987 ($R_{\text{int}} = 0.0180$). The final R_1 values were 0.0237, $\omega R_2 = 0.0614$ ($I > 2\sigma(I)$). The goodness of fit on F^2 was 1.078. Flack parameter = 0.12(16). CCDC number: 1458037.

Crystal data of **7**: $C_{15}H_{14}O_3$, $M_r = 242.26$, monoclinic, $a = 7.6380(3) \text{ \AA}$, $b = 13.8996(6) \text{ \AA}$, $c = 11.22829(4) \text{ \AA}$, $\alpha = 90.00$, $\beta = 95.368(4)$, $\gamma = 90.00$, $V = 1192.60(8) \text{ \AA}^3$, space group $P2_1/n$, $Z = 4$, $D_{\text{calcd}} = 1.349 \text{ mg/m}^3$, $\mu = 0.093 \text{ mm}^{-1}$, and $F(000) = 512$. Crystal dimensions: $0.42 \times 0.33 \times 0.23 \text{ mm}^3$. Independent reflections: 2569 ($R_{\text{int}} = 0.0228$). The final R_1 values were 0.0419, $\omega R_2 = 0.0961$ ($I > 2\sigma(I)$). The goodness of fit on F^2 was 1.037. Flack parameter = 0.09(10). CCDC number: 1458039.

Crystal data of **8**: $C_{32}H_{36}O_8$, $M_r = 548.60$, triclinic, $a = 6.5460(2) \text{ \AA}$, $b = 8.6973(3) \text{ \AA}$, $c = 12.0611(4) \text{ \AA}$, $\alpha = 88.934(3)$, $\beta = 85.192(3)$, $\gamma = 85.357(3)$, $V = 1369.95(4) \text{ \AA}^3$, space group $P-1$, $Z = 2$, $D_{\text{calcd}} = 1.336 \text{ mg/m}^3$, $\mu = 0.782 \text{ mm}^{-1}$, and $F(000) = 296$. Crystal dimensions: $0.42 \times 0.28 \times 0.23 \text{ mm}^3$. Independent reflections: 2423 ($R_{\text{int}} = 0.0263$). The final R_1 values were 0.0350, $\omega R_2 = 0.0899$ ($I > 2\sigma(I)$). The goodness of fit on F^2 was 1.067. CCDC number: 1458040.

Calculation of ECD spectra. Molecular Merck force field (MMFF) and DFT/TD-DFT calculations were carried out with Spartan[®] 14 software (Wavefunction Inc., Irvine, CA, USA) and

Gaussian 09 program, respectively. Conformers within 10 kcal/mol energy window were generated and optimized using DFT calculations at B3LYP/6-31G(d) level. Conformers with Boltzmann distribution over 1% were chosen for ECD calculations in methanol at B3LYP/6-311+g(2d,p) level. The IEF-PCM solvent model for MeOH was used. ECD spectra were generated using the program SpecDis 3.0 (University of Würzburg, Würzburg, Germany) and OriginPro 8.5 (OriginLab, Ltd., Northampton, MA, USA) from dipole-length rotational strengths by applying Gaussian band shapes with $\sigma = 0.30 \text{ eV}$ and UV shift = +21 nm. All calculations were performed with High-Performance Grid Computing Platform of Sun Yat-Sen University.

Biological assays. The assays for antibacterial [23] and α -glucosidase inhibitory [23] were carried out as described previously.

The assay for DPPH radical scavenging activity was measured by a reported method [24,25], with slight modifications. Firstly, 180 μL of DPPH[•] (150 μM in MeOH) and 20 μL of a series of test compound solutions (31.2, 62.5, 125, 250, 500 μM in MeOH) were mixed in each well of a 96-well microtiter plate. The reaction was measured by determination of the absorbance $A_{\text{sample+DPPH}^{\bullet}}$ using a microtiter plate reader at 490 nm after shaking for 30 min at room temperature in the dark. Twenty μL test samples of each concentration with 180 μL of MeOH were used as the blank measurement for each tested compound, and the absorbance was recorded as A_{sample} . The absorbance of the mixture of 20 μL of MeOH and 180 μL of DPPH[•] was recorded as $A_{\text{DPPH}^{\bullet}}$, and the absorbance of the 200 μL of MeOH was measured as A_{blank} . The natural antioxidant vitamin C was used as a positive control. Calculations of the DPPH[•] scavenging activity was performed according to the following equation: scavenging activity (%) = $[1 - (A_{\text{sample+DPPH}^{\bullet}} - A_{\text{sample}}) / (A_{\text{DPPH}^{\bullet}} - A_{\text{blank}})] \times 100\%$. All measurements were done in triplicate from two independent experiments. The reported EC_{50} was the average value of two independent experiments.

Supporting Information

Supporting Information File 1

1D and 2D NMR, HREIMS, and HRESIMS spectra of the new compounds.

[<http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-196-S1.pdf>]

Acknowledgements

We thank the National Natural Science Foundation of China (21472251, 41276146), the Science & Technology Plan Project

of Guangdong Province of China (2013B021100011), the Key project of Natural Science Foundation of Guangdong Province (2016A040403091), Special Financial Fund of Innovative Development of Marine Economic Demonstration Project (GD2012-D01-001), China's Marine Commonwealth Research Project (201305017) for generous support.

References

- Saeed, A. *Eur. J. Med. Chem.* **2016**, *30*, 290–317. doi:10.1016/j.ejmech.2016.03.025
- Hill, R. A. *Prog. Chem. Org. Nat. Prod.* **1986**, *49*, 1–78.
- Ceska, O.; Chaudhary, S. K.; Warrington, P.; Ashwood-Smith, M. J.; Bushnell, G. W.; Poulton, G. A. *Phytochemistry* **1988**, *27*, 2083–2087. doi:10.1016/0031-9422(88)80101-8
- Ashwood-Smith, M. J.; Warrington, P. J.; Jenkins, M.; Ceska, O.; Romaniuk, P. J. *Photochem. Photobiol.* **1989**, *50*, 745–751. doi:10.1111/j.1751-1097.1989.tb02905.x
- Taniguchi, M.; Yanai, M.; Xiao, Y. Q.; Kido, T.-i.; Baba, K. *Phytochemistry* **1996**, *42*, 843–846. doi:10.1016/0031-9422(95)00930-2
- Baba, K.; Xiao, Y.-Q.; Taniguchi, M.; Ohishi, H.; Kozawa, M. *Phytochemistry* **1991**, *30*, 4143–4146. doi:10.1016/0031-9422(91)83482-Z
- Saito, F.; Kuramochi, K.; Nakazaki, A.; Mizushina, Y.; Sugawara, F.; Kobayashi, S. *Eur. J. Org. Chem.* **2006**, *21*, 4796–4799. doi:10.1002/ejoc.200600702
- Kuramochi, K.; Tsubaki, K.; Kuriyama, I.; Mizushina, Y.; Yoshida, H.; Takeuchi, T.; Kamisuki, S.; Sugawara, F.; Kobayashi, S. *J. Nat. Prod.* **2013**, *76*, 1737–1745. doi:10.1021/np400460m
- Kuramochi, K.; Tsubaki, K. *J. Nat. Prod.* **2015**, *78*, 1056–1066. doi:10.1021/np5010483
- Liu, Z.; Chen, Y.; Chen, S.; Liu, Y.; Lu, Y.; Chen, D.; Lin, Y.; Huang, X.; She, Z. *Org. Lett.* **2016**, *18*, 1406–1409. doi:10.1021/acs.orglett.6b00336
- Liu, Y.; Yang, Q.; Xia, G.; Huang, H.; Li, H.; Ma, L.; Lu, Y.; He, L.; Xia, X.; She, Z. *J. Nat. Prod.* **2015**, *78*, 1816–1822. doi:10.1021/np500885f
- Chen, S.; Liu, Z.; Liu, Y.; Lu, Y.; He, L.; She, Z. *Beilstein J. Org. Chem.* **2015**, *11*, 1187–1193. doi:10.3762/bjoc.11.133
- Li, H.; Jiang, J.; Liu, Z.; Lin, S.; Xia, G.; Xia, X.; Ding, B.; He, L.; Lu, Y.; She, Z. *J. Nat. Prod.* **2014**, *77*, 800–806. doi:10.1021/np400880w
- Huang, X.; Huang, H.; Li, H.; Sun, X.; Huang, H.; Lu, Y.; Lin, Y.; Long, Y.; She, Z. *Org. Lett.* **2013**, *15*, 721–723. doi:10.1021/ol303549c
- Xiao, Z.; Huang, H.; Shao, C.; Xia, X.; Ma, L.; Huang, X.; Lu, Y.; Lin, Y.; Long, Y.; She, Z. *Org. Lett.* **2013**, *15*, 2522–2525. doi:10.1021/ol401005j
- Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A39*, 876–881. doi:10.1107/S0108767383001762
- Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681–690. doi:10.1002/chir.20473
- Choukchou-Braham, N.; Asakawa, Y.; Lepoittevin, J. P. *Tetrahedron Lett.* **1994**, *35*, 3949–3952. doi:10.1016/S0040-4039(00)76710-4
- Simpson, T. J. *J. Chem. Soc., Chem. Commun.* **1978**, 627–628. doi:10.1039/C39780000627
- Lu, Z.; Wang, Y.; Miao, C.; Liu, P.; Hong, K.; Zhu, W. *J. Nat. Prod.* **2009**, *72*, 1761–1767. doi:10.1021/np900268z
- Trisuwan, K.; Rukachaisirikul, V.; Sukpondma, Y.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J. *Tetrahedron* **2010**, *66*, 4484–4489. doi:10.1016/j.tet.2010.04.073
- Kohno, J.; Sakurai, M.; Kameda, N.; Nishio, M.; Kawano, K.; Kishi, N.; Okuda, T.; Komatsubara, S. *J. Antibiot.* **1999**, *52*, 913–916. doi:10.7164/antibiotics.52.913
- Chen, S.; Liu, Y.; Liu, Z.; Cai, R.; Lu, Y.; Huang, X.; She, Z. *RSC Adv.* **2016**, *6*, 26412–26420. doi:10.1039/C6RA02566H
- Wang, P.-P.; Zhao, G.-W.; Xia, W.; Han, E.-J.; Xiang, L. *Chin. J. Nat. Med.* **2014**, *12*, 377–381. doi:10.1016/S1875-5364(14)60047-9
- Jiao, Z.-Z.; Yue, S.; Sun, H.-X.; Jin, T.-Y.; Wang, H.-N.; Zhu, R.-X.; Xiang, L. *J. Nat. Prod.* **2015**, *78*, 2588–2597. doi:10.1021/acs.jnatprod.5b00524

License and Terms

This is an Open Access article under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The license is subject to the *Beilstein Journal of Organic Chemistry* terms and conditions: (<http://www.beilstein-journals.org/bjoc>)

The definitive version of this article is the electronic one which can be found at: doi:10.3762/bjoc.12.196