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NOTE

Aspergone, a new chromanone derivative from fungus *Aspergillus* sp. SCSIO41002 derived of mangrove soil sample

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The mangrove habitats in tropical regions represent the terrestrial fringe of the marine habitats. Mangrove forests represent an ecosystem of high biodiversity. The environment of the mangrove soil is changeable and complex, which causes the diversity of marine microorganism. Mangrove marine-derived fungus could produce numerous novel and bioactive compounds, so it has an expansive foreground to study.[1–5] Fungi of the family *Aspergillus* are of particular interest, as crude extracts from this regularly exhibit biological activity, particularly anticancer, antioxidant, antimicrobial and antiviral activities.[6–9] In our previous research toward the discovery of biologically active metabolites from mangrove soil-derived fungus, we obtained some active compounds.[10,11] During our ongoing research, a new chromanone derivative Aspergone (**1**) was isolated from mangrove soil-derived fungus *Aspergillus* sp. SCSIO41002, together with 10 known compounds (**2–11**): 8-ethyl-5,7-dihydroxy-2-metlylchroman-4-one (**2**),[12] dothiorelone C (**3**),[13,14] citreoisocoumarinol (**4**),[15–17] dothiorelone B (**5**),[18] 14,15-secocurvularin (**6**),[19,20] (1*R**,2*S**,3*R**,4*R**,6*S**)-*p*-menthane-1,2,3,6-tetrol (**7**),[21] 5,6-dihydroxy-2,3,6-trimethylcyclohex-2-enone (**8**),[13] 4-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one (**9**), tyrosol (**10**),[13] and *p*-hydroxybenzaldehyde (**11**)[22] (Figure 1). The cytotoxicity and cyclooxygenase-2 (COX-2) inhibitory activities of these compounds were individually evaluated. Herein we described the isolation, structure elucidation and bioactivities of these compounds.

Compound **1** was obtained as an amorphous powder. Its molecular formula was established as $C_{15}H_{18}O_5$ on the basis of the positive HR-ESI/MS at *m*/*z* 301.1051 $[M+Na]^+$, accounting for seven degrees of unsaturation. The ^1H NMR spectrum showed one olefinic proton (δ_H 6.31, 1H, s, H-4), one *O*-methyl singlet (δ_H 3.68, 3H, s, H-15), one methyl doublet (δ_H 1.50, 3H, d, $J=6.0$ Hz, H-12) and one methyl triplet (δ_H 1.12, 3H, t, $J=7.5$ Hz, H-14). The ^1H and ^{13}C NMR, DEPT and HSQC data revealed the presence of two carbonyl groups, including one ketone (δ_C 195.5), one ester carbonyl (δ_C 174.3), one benzoic moiety (δ_C 164.3, 159.3, 136.8, 119.0, 115.1 and 113.3), one oxymethine (δ_C 75.0), three methylenes and three methyls, including *O*-methyl (δ_C 52.2). As three of the seven degrees of unsaturation were attributed to one benzoic moiety, one carbonyl and one ketone, **1** was assumed to contain another ring. These spectroscopic features suggested that **1** belongs to the family of chromones and is very similar to 8-ethyl-5,7-dihydroxy-2-metlylchroman-4-one (**2**).[12] This was confirmed by HMBC correlations of H_3-14 to C-13 and C-6, H-13 to C-5 and C-6, H-10 to C-9 and H-12 to C-10 and C-11. The distinction was attributed to the presence of a methyl acetate at the C-3 of **1**. The assignments were supported by the HMBC correlations, H_3-15 to C-1, H-2 to C-1, C-3 and C-8 and H-4 to C-2, C-6 and C-8 (Figure 2). Accordingly, the planar structure of **1** was constructed as shown in Figure 1. In the CD spectrum of **1**, a positive cotton effect for the n–p^* transition was observed at around 270 nm, which determined the *S* configuration at C-11 on the basis of the octant rule (Supplementary Figure S6).[23] This was further confirmed by comparison of the ^1H NMR data and the optical rotation with those of the homologs described previously.[24]

Compounds **1–11** were assessed for its cytotoxicity against K562, MCF-7, HeLa, DU145, U937, H1975, SGC-7901, A549, MOLT-4 and HL60 cell lines by standard MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) screening method. Trichostatin A and taxol were used as positive controls. However, all compounds were inactive even at 50 μM. All the compounds were subjected for COX-2-inhibitory activity, where **3**, **5** and **6** exhibited moderate COX-2-inhibitory activity with the IC_{50} values of 58.6, 42.0 and 12.7 μM, respectively. Celecoxib (Sigma, St Louis, MO, USA) was used as the positive control with the IC_{50} value of 0.018 μM.

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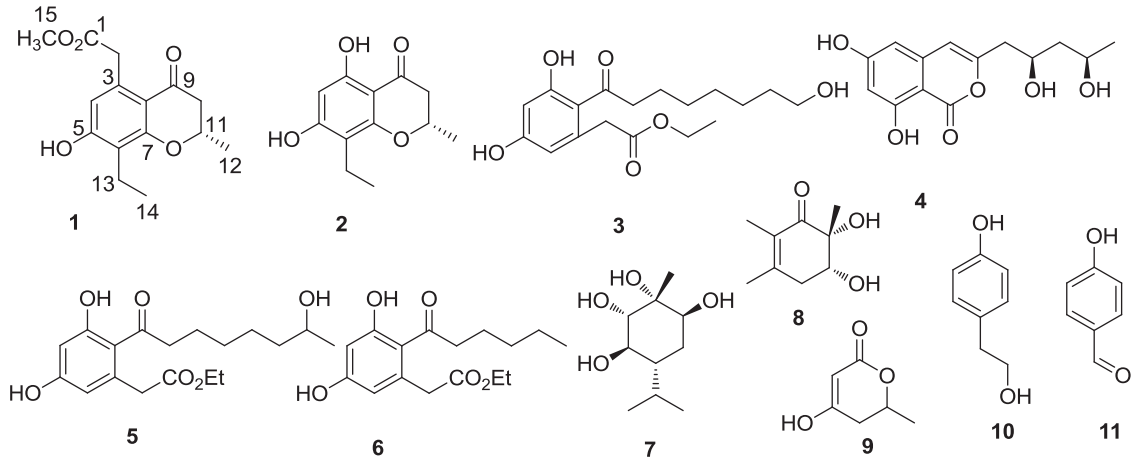

Figure 1 Structures of compounds **1**–**11**.

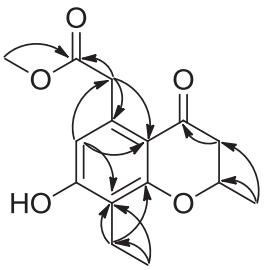

Figure 2 Key HMBC correlations of compound **1**.

MATERIALS AND METHODS

General experimental procedures

The NMR spectra were measured on a Bruker AC 500 MHz NMR spectrometer (Bruker, Fällanden, Switzerland) with tetramethylsilane as an internal standard. High-resolution mass spectra were recorded on a Bruker micro TOF-QII mass spectrometer (Bruker). CD spectra were measured with a Chirascan circular dichroism spectrometer (Applied Q3 Photophysics). Size exclusion chromatography was performed on Sephadex LH-20 gel (GE Healthcare, Uppsala, Sweden). Column chromatography was carried out on silica gel (200–300 μm, Qingdao Marine Chemical Factory, Qingdao, China).

Single-crystal X-ray diffraction data were measured on an Oxford Gemini S Ultra diffractometer. The TLC spots were detected under UV light or by heating after spraying with 5% H_2SO_4 in EtOH.

Fungal strain

The culture of *Aspergillus* sp. SCSIO41002 was isolated from a mangrove sediment sample collected in Sanya (18°13′50.2″N, 109°37′15.8″E) in August 2010. The strain was identified as *Aspergillus* sp. based on a molecular biological protocol calling for DNA amplification and ITS region sequence comparison with the GenBank database and shared a similarity of 99% with *Aspergillus versicolor* UOA/HCPF8640. The strain was deposited in the RNAM Center, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

Fermentation, extraction and isolation

Strain stored on potato dextrose agar slants at 4 °C was cultured on potato dextrose agar plates and incubated for 7 days. Seed medium (potato 200 g, dextrose 20 g, NaCl 2.5 g, distilled water 1000 ml) in 50 ml Erlenmeyer flasks (each containing 10 ml) was inoculated with strain SCSIO41002 and incubated at 25 °C for 48 h on a rotating shaker (180 r.p.m.). Production medium of solid rice in 1000 ml flasks (rice 200 g, NaCl 0.5 g, distilled water 200 ml) was inoculated with 10 ml seed solution. Flasks were incubated at 25 °C under static stations and daylight. After 60 days, cultures from 30 flasks were harvested for the isolation of substances. The total 6 kg of rice culture was crushed and extracted with acetone three times. The acetone extract was evaporated under reduced pressure to afford an aqueous solution, and then the aqueous solution was extracted with ethyl acetate (EtOAc) to yield 29 g of a crude gum. The EtOAc portion was subsequently separated by Si gel column chromatography using $CHCl_3$-MeOH gradient elution to give 17 fractions (*Frs. A–Q*). *Fr. G* was subjected to silica gel column chromatography, using a gradient of EtOAc in petroleum ether (PE), to give seven fractions (*Frs. G1–G7*). *Fr. G4* was purified by semipreparative RP HPLC (70% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **11** (6.8 mg). *Fr. H* was subjected to silica gel column chromatography, using a gradient of EtOAc in PE, to give seven fractions (*Frs. H1–H11*). *Fr. H3* was further separated by semipreparative reversed-phase HPLC (70% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **1** (2.7 mg), **2** (2.9 mg) and **9** (7.8 mg). *Fr. H4* was further separated by semipreparative reversed-phase HPLC (70% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **7** (5.7 mg). *Fr. H6* was further separated by semipreparative reversed-phase HPLC (60% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **6** (12.0 mg). *Fr. I* was further separated by semipreparative reversed-phase HPLC (60% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **8** (2.8 mg) and **10** (5.0 mg). *Fr. K* was subjected to silica gel column chromatography, using a gradient of EtOAc in PE, to give 12 fractions (*Frs. K1–K12*). *Fr. K8* was further separated by semipreparative reversed-phase HPLC (40% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **3** (8.5 mg) and **5** (3.6 mg). *Fr. O* was subjected to silica gel column chromatography, using a gradient of MeOH in $CHCl_3$, to give 15 fractions (*Frs. O1–O15*). *Fr. O8* was further separated by semipreparative reversed-phase HPLC (30% MeOH in H_2O) at a flow rate of 2 ml min^{-1} to afford **4** (5.7 mg).

Aspergone (**1**): $[\alpha]_D^{25} = -5.67$ (MeOH; $c = 0.12$); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon$ 336 −2.49, $\Delta\varepsilon$ 277 +3.32, $\Delta\varepsilon$ 221 −1.97 (MeOH; $c = 0.2$); ^1H NMR (500 MHz, CD_3OD): δ 6.31 (1H, s, H-4), 4.54 (1H, m, H-11), 3.85 (2H, s, H-2), 3.68 (3H, s, H-15), 2.66 (2H, m, H-13), 2.59 (2H, m, H-10), 1.50 (3H, d, $J = 6.0$ Hz, H-12), 1.12(3H, t, $J = 7.5$ Hz, H-14); ^{13}C NMR (125 MHz, CD_3OD): δ 195.5 (C-9, C), 174.3 (C-1, C), 164.3(C-5, CH), 159.3 (C-7, C), 136.8 (C-8, C), 119.0 (C-6, C), 115.1 (C-4, CH), 113.3 (C-3, C), 75.0 (C-11, CH), 52.2 (C-15, CH_3), 46.0 (C-10, CH_2), 42.1 (C-2, CH_2), 21.1 (C-12, CH_3), 17.2 (C-13, CH_2), 14.3 (C-14, CH_3). HRESIMS *m/z* 301.1051 $[M+Na]^+$ (calcd for $C_{15}H_{18}NaO_5$, 301.1046).

Biological activities

Cytotoxicity assay. The cytotoxic activity of compounds **1–11** was screened against the growth panel of 10 tumor cell lines (K562, MCF-7, HeLa,

DU145, U937, H1975, SGC-7901, A549, MOLT-4 and HL60) according to Bergeron *et al.*[25]

COX-2-inhibitory activity assay. COX-2, as a well-established target, is an inducible enzyme whose expression is activated by cytokines, mitogens, endotoxin and tumor promoters. The anti-inflammatory and analgesic properties of traditional non-steroidal anti-inflammatory drugs are primarily due to the inhibition of COX-2.[26] Hence, the compounds isolated were tested for COX-2-inhibitory activity using the COX (ovine) inhibitor screening kit, according to the manufacturer's instructions. The test compounds were dissolved in DMSO and the final concentration was set as 100 μM. The percentage inhibition has been calculated by comparison with control incubations. Celecoxib was used as the positive control.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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