New alkaloids and isocoumarins from the marine gorgonian-derived fungus *Aspergillus sp.* SCSIO 41501

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ABSTRACT

Two new \(\beta\)-carboline alkaloids, aspergillspins A-B (1–2), three new quinolone alkaloids, aspergillspins C-E (3–5), and two new isocoumarins, aspergillspins F-G (6–7), together with four known alkaloids were isolated from the marine gorgonian-derived fungus *Aspergillus* sp. SCSIO 41501. Their structures were identified by spectroscopic analysis, and the absolute configurations of several chiral carbons in 2 and 3 were further established by quantum chemical calculations of the electronic circular dichroism (ECD) spectra. Their cytotoxic and antibacterial activities were also evaluated.

1. Introduction

\(\beta\)-Carboline and quinolone alkaloids are widely spread in plants, animals, and microorganisms, and are considered as important groups of natural products exhibiting diverse biological activities (Cao et al. 2007; Michael 2007). Many novel natural products with promising biological and pharmacological properties have been obtained from marine-derived fungi. In our previous study, we had obtained some cyclic and linear peptides with cytotoxic, antiviral, and antifouling activities from the marine gorgonian-derived fungus *Aspergillus* sp SCSIO 41501 (previouslyumbered as *Aspergillus* sp. SCSGAF 0076) (Bao et al. 2013; Ma et al. 2017). Recently, we continued to obtain two new \(\beta\)-carboline alkaloids, aspergillspins A-B (1–2), three new quinolone alkaloids,
aspergillspins C-E (3–5), and two new isocoumarins, aspergillspins F-G (6–7) (Figure 1), together with four known alkaloids (3S)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (8) (Wang et al. 2012), transtorine (9) (Al-Khalil et al. 1998), marinamide (10) (Zhu et al. 2013), and methyl marinamide (11) (Zhu et al. 2013) from the SCSIO 41501 strain. Herein, we describe the isolation and structure elucidation of 1–7 as well as their cytotoxic and antibacterial activities.

2. Results and discussion

Aspergillspin A (1) was obtained as deep yellow oil. Its molecular formula was determined to be C_{16}H_{10}N_{2}O_{3} on the basis of its HRESIMS signal at m/z 279.0771 [M + H]^+. The $^1$H NMR spectrum showed eight aromatic protons ($\delta_H$ 7.35, 7.56, 7.60, 7.66, 7.80, 8.34, 8.36, 8.50) and one exchangeable proton ($\delta_H$ 11.62). The $^{13}$C NMR spectrum showed 16 low-field carbon signals, including seven methines, eight quaternary carbons and one carbonyl group. These data indicated that 1 had a β-carboline alkaloid skeleton (Wang et al. 2012; Xie et al. 2013; Rao et al. 2006), which was supported by the $^1$H–$^1$H COSY and HMBC spectral data (Figure S55). In addition, the HMBC spectrum showed correlations of H-4 with C-2/C-3/C-5/C-6, and H-2 with C-1/C-3/C-4, which indicated a six-membered lactone attached on C-6 of the β-carboline skeleton. So, the structure of 1 was determined as shown.

Aspergillspin B (2) was isolated as yellow oil. Its molecular formula was determined as C_{16}H_{18}N_{2}O_{4} by HRESIMS (m/z 303.1348 [M + H]^+). The $^1$H and $^{13}$C NMR spectral data of 2 were similar to that of 1, and the obvious difference between them was the substituent at C-6 of the β-carboline skeleton. The COSY spectrum (Figure S55) showing as sequential correlation of H-1/H-2/H-3/H-4/H-5, and the HMBC spectrum (Figure S55) exhibiting correlations of H-4 and H-5 with C-6, which indicated a 1,2,3,5-tetrahydroxypentyl group attached on C-6 as shown. The chemical shifts for the geminal protons of H$_2$-4 were differentiated at $\delta_H$ 2.24 and 2.50, which indicated a syn 1,3-diol unit between C-3 and C-5 in 2 (Wu et al. 2014; Kikuzaki et al. 1992; Huang et al. 2018).

![Figure 1. Structures of compounds 1–11.](image-url)
Usually, the chemical shifts for the geminal protons of anti 1,3-diol unit overlap (Wu et al. 2014; Kikuzaki et al. 1992; Huang et al. 2018). The coupling constant between H-2 and H-3 ($J_{H-2,H-3} = 5.5$ Hz) suggested an anti relative configuration at C-2/C-3 (Chlipala et al. 2010; Hwang et al. 2014). The syn/anti relative configuration for C-3/C-5 and C-2/C-3 was also supported by the $^{13}$C chemical shift of C-3 ($\delta_{C} 68.9$) that was consistent to the reported universal NMR databases (Kobayashi et al. 2001; Benowitz et al. 2001; Kobayashi et al. 2000). The planar structure of 2 was the same as that of tangutorids E-F (Zhao et al. 2017) and 1-(1,3,4,5-tetrahydroxypent-1-yl)-β-carbolines (Diem and Herderich 2001), however, the relative structures of the three known compounds were not identified, and according to the coupling constants of the hydrogens of their side chains, we inferred that 2 was a diastereomer of the three known compounds.

The absolute configuration of C-5 was determined by ECD calculation. In order to save computation time, we simplified the structure as model in Figure S55. Three conformers were obtained from Spartan software with low-energy conformers within a 10 kcal/mol window, which were further geometry-optimized at the B3LYP/6-31G level single point energies were calculated at M062X/dex2TZVP using the Gaussian 16 program. The ECD spectra of three conformers were calculated using the TD-DFT method at the B3LYP/6-311G level. The experimental ECD spectra of 2 did not match well with the calculated spectrum (Figure S57). However, the final Boltzmann factor-weighted theoretical ECD spectrum in the range of 240–300 nm showed acceptable similarity to the experimental ECD spectrum (Figure S57). Thus, the absolute configuration of C-5 was tentatively established as $R$. Correspondingly, the absolute configurations of C-3 and C-2 were tentatively assigned as $S$ and $R$, respectively.

Aspergillspin C (3) was obtained as a light yellow solid, and its molecular formula C$_{16}$H$_{16}$N$_{2}$O$_{5}$ was determined by HRESIMS ($m/z$ 339.0949 [M + Na]$^+$). The $^1$H and $^{13}$C NMR spectral data showed the presence of one oxygenated methyl, three methylenes, one oxygenated methine, one 1,2-di-substituted benzene ring, two carbonyl groups, and one α, β-unsaturated ketone group. These data suggested that 3 had a 4-quinolone skeleton (Takayama et al. 1994; Jadulco et al. 2014). The suggestion was supported by the COSY spectrum (Figure S56) showing correlations of H-11 with H-10/H-12, and H-10 with H-9/H-11, and the HMBC spectrum (Figure S56) showing correlations of NH with C-7/C-9a, H-9 with C-9a/C-12a/C-13. In addition, the $^1$H-$^1$H COSY spectrum showing a sequential correlation of H-2/H-3/H-4/H-5, and HMBC spectrum showing correlations of H-3 with C-1/C-2/C-4/C-5, H-4 with C-3/C-5, H-5 to C-1/C-3/C-4/C-7, indicted a six-membered ring lactam attached to C-7. Thus, the planar structure of 3 was determined as shown.

The absolute configuration of C-2 in 3 was determined by ECD calculations. Nine conformers were obtained from the Spartan software, and among then, four conformers with low energy were further calculated by using DFT at the B3LYP/6-31G(d) level. The final Boltzmann factor-weighted theoretical ECD spectrum was reasonably similar to the experimental ECD spectrum (Figure S57). Thus, the absolute configuration of C-2 in 3 was established as $R$.

Aspergillspin D (4) was isolated as a yellow solid and had the molecular formula C$_{18}$H$_{22}$N$_{2}$O$_{3}$ as determined by HRESIMS. The $^1$H and $^{13}$C NMR spectral data of 4 were
very similar to those of quinolactacin C1 (Clark et al. 2006), and the only obvious difference between them was one additional oxygenated ethyl group instead of one hydroxy group attached to C-3 in 4, which was supported by the HMBC spectrum (Figure S56) showing a correlation of H-1" (δ_H 3.12, 3.49) with C-3 and the 1H-1H COSY spectrum (Figure S56) showing correlation of H-1" with H-2". The trans configuration between-OCH2CH3 and CH3-1’ was determined by the high field-shifted chemical shift of CH3-1’ (δ_H 0.55) that was induced by the anisotropic effect of the pyrrolo-quinolone ring (Kim et al. 2001).

Aspergillspin E (5) had the same molecular formula C_{18}H_{22}N_{2}O_{3} as 4. The 1H and 13C NMR spectral data of 5 were greatly similar to those of 4, and the only obvious difference between them was the chemical shifts of CH3-1’, H-2’, and H-3’. The 1D and 2D NMR spectral data revealed that 5 had the same planar structure as 4. The ECD spectra of 4 and 5 are almost mirror images (Figure S58), which indicated that the configuration of C-3 in 4 and 5 was opposite. The cis configuration between-OCH2CH3 and CH3-1’ was determined by the relatively low-field chemical shift of CH3-1’ (δ_H 1.12) that wasn’t induced by anisotropic effect of the pyrrolo-quinolone ring (Kim et al. 2001). These above data indicated that 4 and 5 were epimers at C-3.

Aspergillspin F (6) was obtained as a brown solid and had the molecular formula C_{11}H_{10}O_{6} as established by HRESIMS. The 1H and 13C NMR spectral data showed the presence of two acyl groups, one oxygenated methine, one methylene, one methyl, and one penta-substituted benzene ring. These data were very similar to those of 7-hydroxymellein (Pontius et al. 2008). Further analysis of its 2D NMR data revealed the planar structure of 6 as shown, and displayed that the only obvious difference between 6 and 7-hydroxymellein was the presence of an additional carboxyl group at C-6 in 6. The absolute configuration of C-3 in 6 was proposed to be R by comparing the optical rotations of 6 ([α]_{25}^{25}D -57° (c 0.20, MeOH)), (–)-(3R)-mellein ([α]_{25}^{25}D -102.5° (c 1.0, CHCl3)) (Zhu et al. 2013), (–)-(3R)-7-hydroxymellein (Devys et al. 1980), and (–)-(3R)-5-hydroxymellein (Devys et al. 1994).

Aspergillspin G (7) was obtained as a light yellow solid. Its molecular formula C_{11}H_{9}O_{6} was established by HRESIMS (m/z 221.0439 [M + H]^+). The 1H and 13C NMR spectral data of 7 showed similarity to that of 6. The COSY spectrum showed correlation of H-7 with H-8, and the HMBC spectrum displayed correlations of CH3-11 with C-5/C-6/C-7, H-8 with C-1/C-6/C-7/C-9/C-10, H-7 with C-8/C-9/C-10, and H-4 with C-3/C-5/C-10/C-12, which established the structure of 7 as shown.

Compounds 1–3 and 6–7 were also evaluated for their cytotoxicity against human carcinoma HL60, HepG2, and MCF-7 cell lines by MTT methods and for their antibacterial activity against Bacillus subtilis and Escherichia coli by standard disc diffusion assay. Unfortunately, none of them showed activity in these assays.

3. Experimental section
3.1. General experimental procedures
The procedures were the same as previous reported.4
3.2. Fungal material
The fungal strain SCSIO 41501 (previously numbered as SCSGAF 0076) was isolated from gorgonian Melitodes squamata collected from the South China Sea, Sanya (18°11’N, 109°25’E), Hainan Province, China (Bao et al. 2013).

3.3. Fermentation and extraction
The procedures were the same as previous reported (Ma et al. 2017).

3.4. Isolation and purification
The crude extract (85 g) was subjected to silica gel column chromatography eluting with CH₂Cl₂/MeOH (v/v 100:0–50:50) to give eight fractions (Fr.1–Fr.8). Fr.3 was eluted with CH₂Cl₂/MeOH (v/v 100:0–50:50) to give seven subfractions. Fr.3-4 was further purified by Sephadex LH-20, then by preparative HPLC with CH₃OH/H₂O-0.03% TFA (v/v 45:55, 5 ml/min) to yield 10 (19.4 mg, tᵣ = 47 min). Fr.5 was subjected to MPLC with an ODS column, eluting with CH₃OH/H₂O-0.03% TFA (v/v 60:40, 20 ml/min) to give four subfractions. Fr.5-3-2 was further purified by preparative HPLC, eluting with CH₃OH/H₂O-0.03% TFA (v/v 60:40) to yield 4 (11.8 mg, tᵣ = 15.5 min). Fr.5-3-3 was further purified by preparative HPLC, eluting with CH₃CN/H₂O-0.03% TFA (v/v 37:63) to give 11 (3.5 mg, tᵣ = 21.5 min). Fr.5-3-4 was further purified by preparative HPLC, eluting with CH₃CN/H₂O-0.03% TFA (v/v 68:32) to give 3 (15.5 mg, tᵣ = 30.8 min). Fr.6 was subjected to MPLC with an ODS column, eluting with CH₃OH/H₂O-0.03% TFA (v/v 50:40) to obtain five subfractions. Fr.6-5-3 was further purified by Pre-HPLC, eluting with CH₃CN/H₂O-0.03% TFA (v/v 22:78) to give 5 (13.4 mg, tᵣ = 28 min). Fr.6-5-5 was further purified by preparative HPLC, eluting with CH₃CN/H₂O-0.03% TFA (v/v 18:82) to give 2 (8.5 mg, tᵣ = 22 min). Fr.7 was subjected to silica gel using step gradient elution with CH₃Cl₂/CH₃OH (v/v 100:0–50:50) to give six subfractions. Fr.7-4 was further purified by Sephadex LH-20, then by preparative HPLC with CH₃OH/H₂O-0.03% TFA (v/v 45:56, 5 ml/min) to yield 9 (2.1 mg, tᵣ = 31 min). Fr.7-5 was further purified by MPLC with an ODS column, eluting with CH₃OH/H₂O-0.03% TFA (v/v 30:70) to give six subfractions. Fr.7-5-4 was further purified by preparative HPLC, eluting with CH₃CN/H₂O-0.03% TFA (v/v 31:69) to afford 1 (6.5 mg, tᵣ = 46 min). Fr.7-6 was further purified by preparative HPLC, eluting with CH₃CN/H₂O (v/v 20:80) to afford 8 (5.6 mg, tᵣ = 30 min).

3.5. Characteristics of compounds

3.5.1. Aspergillispin A (1)
Deep yellow oil; UV (MeOH) λₑ max (log ε) 216 (3.96), 306 (3.61), 354 (3.26), 389 (3.44) nm; IR (film) νₑ max 3379, 3352, 3335, 3232, 3287, 2951, 2926, 2849, 2845, 1672, 1661, 1633, 1456, 1204, 1144, 1016, 800, 721, 667, 599, 557 cm⁻¹; ¹³C NMR spectral data (175 MHz) δₑ: 159.7 (C-1), 153.6 (C-3), 146.1 (C-5), 142.3 (C-12a), 137.1 (C-7), 131.9 (C-6a), 131.6 (C-8a), 130.7 (C-6), 130.0 (C-11), 122.6 (C-9), 120.9 (C-10), 120.8 (C-9a), 120.2 (C-2), 116.0 (C-8a), 113.3 (C-12), 113.0 (C-11); ¹H NMR spectral data (700 MHz) δₜ: 11.62
(s, H-NH), 8.58 (d, J = 4.2 Hz, 1H, H-7), 8.36 (d, J = 7.7 Hz, 1H, H-9), 8.34 (d, J = 4.9 Hz, 1H, H-8), 7.80 (d, J = 8.4 Hz, 1H, H-12), 7.66 (t, J = 7.0 Hz, 1H, H-11), 7.60 (t, J = 2.8 Hz, 1H, H-4), 7.56 (d, J = 3 Hz, 1H, H-2), 7.35 (t, J = 7 Hz, 1H, H-10); (+)-HRESIMS m/z 279.0771 [M + H]+ (calcd for C_{16}H_{10}N_{2}O_{3}, 279.0764).

3.5.2. Aspergill_spin B (2)
Yellow oil; [α]^{25}_{D} +12° (c 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.13), 243 (4.15), 250 (4.14), 290 (3.80), 302 (3.80), 354 (3.82) nm; ν_{max} 2922, 2849, 1674, 1637, 1462, 1261, 1202, 1200, 1136, 1018, 950, 800,721, 598 cm^{-1}; ^{13}C NMR spectral data (125 MHz) δ_C: 143.9 (C-6), 143.5 (C-12a), 133.8 (C-8a), 131.5 (C-11), 131.6 (C-6a), 128.3 (C-7), 121.5 (C-10), 122.5 (C-9), 119.9 (C-9a), 115.5 (C-8), 112.5 (C-12), 74.8 (C-2), 68.9 (C-3), 67.0 (C-5), 62.9 (C-1), 38.8 (C-4); ^{1}H NMR spectral data (500 MHz) δ_H: 8.55 (d, J = 6.0 Hz, 1H, H-8), 8.40 (d, J = 7.5 Hz, 1H, H-9), 8.35 (d, J = 7.5 Hz, 1H, H-7), 7.80 (d, J = 8.5 Hz, 1H, H-12), 7.47 (m, 1H, H-10), 7.47 (m, 1H, H-11), 5.81 (t, J = 5.5 Hz, 1H, H-5), 3.88 (t, J = 5.5 Hz, 1H, H-3), 3.70 (dd, J = 11.5, 4.0 Hz, 1H, H-1), 3.58 (dd, J = 11.5, 5.5 Hz, 1H, H-1), 3.54 (br dd, J = 4.5, 6.0 Hz, 1H, H-2), 2.24 (ddd, J = 2.0, 4.5, 15.0 Hz, 1H, H-4), 2.50 (ddd, J = 6.5, 9.0, 15.0 Hz, 1H, H-4); (+)-HRESIMS m/z 303.1348 [M + H]+ (calcd for C_{16}H_{18}N_{2}O_{4}, 303.1339).

3.5.3. Aspergill_spin C (3)
Light yellow solid; [α]^{25}_{D} -6° (c 0.192, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.99), 251 (3.61), 307 (3.25) nm; IR (film) ν_{max} 3366, 2922, 2849, 1682, 1645, 1607, 1585, 1450, 1296, 1269, 1192, 1142, 1090, 1018, 756, 698, 667, 602, 554 cm^{-1}; ^{13}C NMR spectral data (125 MHz) δ_C: 174.2 (C-1), 166.3 (C-13), 166.3 (C-14), 162.1 (C-7), 138.6 (C-12a), 133.4 (C-11), 130.8 (C-12), 123.1 (C-10), 122.0 (C-9), 118.1 (C-9a), 112.0 (C-8), 63.5 (C-2), 52.3 (C-15), 42.8 (C-8), 27.7 (C-4), 27.3 (C-3); ^{1}H NMR spectral data (500 MHz) δ_H: 11.45 (s, NH), 8.49 (d, J = 8.0 Hz, 1H, H-9), 7.18 (t, J = 7.5 Hz, 1H, H-11), 7.58 (t, J = 7.5 Hz, 1H, H-10), 4.27 (t, J = 6.5 Hz, 1H, H-2), 3.89 (s, 3H, H-15), 3.09 (m, 1H, H-5), 3.43 (dd, J = 18.0, 8.0 Hz, 1H, H-5), 2.10 (m, 3H, H-3, H-4), 1.43 (m, 1H, H-3); (+)-HRESIMS m/z 339.0949 [M + Na]+ (calcd for C_{18}H_{16}N_{2}O_{5}, 339.0951).

3.5.4. Aspergill_spin D (4)
White solid; [α]^{25}_{D} -9.4° (c 0.10, CH_{3}OH); UV (MeOH) λ_{max} (log ε) 216 (3.93), 250 (3.67), 259 (3.67), 316 (3.44), 329 (3.43) nm; IR (film) ν_{max} 3368, 3348, 2922, 2849, 1682, 1611, 1530, 1462, 1385, 1261, 1202, 1140, 1018, 800, 662, 600, 554 cm^{-1}; ^{13}C NMR spectral data (125 MHz) δ_C: 171.4 (C-1), 171.4 (C-9), 160.6 (C-3a), 141.4 (C-4a), 132.9 (C-6), 128.4 (C-8a), 125.7 (C-8), 124.9 (C-7), 117.5 (C-5), 110.1 (C-9a), 93.4 (C-3), 58.3 (C-1’), 40.9 (C-1’), 34.1 (C-4-CH_{3}), 22.8 (C-2’), 15.0 (C-2’), 13.1 (C-1’-CH_{3}), 12.2 (C-3’); ^{1}H NMR spectral data (500 MHz) δ_H: 8.29 (br s, 1H, 2-NH), 8.26 (d, J = 7.5 Hz, 1H, H-8), 7.91 (d, J = 8.0 Hz, 1H, H-5), 7.86 (t, J = 6.5 Hz, 1H, H-6), 7.53 (t, J = 7.0 Hz, 1H, H-7), 4.04 (s, 3H, 4-CH_{3}), 3.49 (m, 1H, H-1’), 3.12 (m, 1H, H-1’), 2.25 (m, 1H, H-1’), 1.96 (m, 1H, H-2’), 1.16 (m, 1H, H-2’), 1.12 (t, J = 7.0 Hz, 3H, H-2’), 0.96 (t, J = 7.5 Hz, 3H, H-3’), 0.55 (d, J = 6.5 Hz, 3H, 1’-CH_{3}); (+)-HRESIMS m/z 315.1704 [M + H]+ (calcd for C_{18}H_{22}N_{2}O_{3}, 315.1703).
3.5.5. AspergillspinE (5)
White solid; [α]$_D^{25}$ +23.5° (c 0.10, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 216 (3.93), 250 (3.67), 259 (3.67), 316 (3.44), 329 (3.43) nm; IR (film) $\nu_{max}$ 3368, 3348, 2922, 2849, 1682, 1611, 1530, 1462, 1385, 1261, 1202, 1140, 1018, 800, 662, 600, 554 cm$^{-1}$; $^{13}$C NMR spectral data (125 MHz) $\delta_C$: 171.2 (C-9), 166.5 (C-1), 160.5 (C-3a), 141.4 (C-4a), 132.8 (C-6), 128.6 (C-8a), 125.7 (C-8), 124.9 (C-7), 117.4 (C-5), 110.4 (C-9a), 93.1 (C-3), 58.0 (C-1"), 40.7 (C-1'), 34.2 (C-4-CH$_3$), 23.2 (C-2"), 15.0 (C-2"'), 12.8 (C-1'-CH$_3$), 11.2 (C-3'); $^1$H NMR spectral data (500 MHz) $\delta_H$: 8.32 (br s, 1H, 2-NH), 8.26 (d, $J = 7.5$ Hz, 1H, H-8), 7.90 (d, $J = 8.5$ Hz, 1H, H-5), 7.85 (t, $J = 8.0$ Hz, 1H, H-6), 7.53 (t, $J = 7.0$ Hz, 1H, H-7), 4.04 (s, 3H, 4-CH$_3$), 3.46 (m, 1H, H-1"), 3.11 (m, 1H, H-1"'), 2.31 (s, 1H, H-1'), 1.12 (t, $J = 7.5$ Hz, 3H, H-2"), 1.11 (d, $J = 7.5$ Hz, 3H, H-2"'), 0.88 (m, 2H, H-2"'), 0.72 (t, $J = 7.0$ Hz, 3H, H-3'); (+)-HRESIMS m/z 315.1704 [M + H]$^+$ (calcd for C$_{18}$H$_{22}$N$_2$O$_3$, 315.1703).

3.5.6. Aspergillspin F (6)
Brown solid; [α]$_D^{25}$ –57° (c 0.20, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 202 (4.10), 232 (4.46), 264 (3.99), 314 (3.83) nm; IR (film) $\nu_{max}$ 3333, 3291, 2947, 2924, 2835, 2359, 2342, 1661, 1616, 1449, 1410, 1252, 1115, 1018, 658, 633, 600, 556 cm$^{-1}$; $^{13}$C NMR spectral data (125 MHz) $\delta_C$: 171.9 (C-12), 166.6 (C-1), 165.7 (C-7), 164.5 (C-8), 154.2 (C-10), 106.1 (C-5), 105.3 (C-6), 101.3 (C-9), 74.5 (C-3), 35.1 (C-4), 20.8 (C-11); $^1$H NMR spectral data (500 MHz) $\delta_H$: 6.21 (s, 1H, H-5), 4.57 (m, 1H, H-3), 2.88 (dd, $J = 16.5$, 2.5 Hz, 1H, H-4), 2.76 (dd, $J = 16.0$, 11.5 Hz, 1H, H-4), 1.36 (d, $J = 6.0$ Hz, 3H, H-11); (+)-HRESIMS m/z 261.0373 [M + Na]$^+$ (calcd for C$_{11}$H$_{10}$O$_6$, 261.0369).

3.5.7. Aspergillspin G (7)
Light yellow solid; UV (MeOH) $\lambda_{max}$ (log ε) 212 (3.97), 232 (4.05), 286 (3.70), 295 (3.73), 344 (3.72), 355 (3.68) nm; IR (film) $\nu_{max}$ 3333, 3316, 3300, 3285, 2953, 2845, 1661, 1636, 1616, 1449, 1410, 1204, 1117, 1015, 667, 600, 557 cm$^{-1}$; $^{13}$C NMR spectral data (125 MHz) $\delta_C$: 171.9 (C-12), 166.6 (C-1), 165.7 (C-7), 158.9 (C-5), 143.2 (C-3), 139.1 (C-7), 133.8 (C-9), 127.2 (C-6), 118.7 (C-8), 113.3 (C-4), 106.9 (C-10), 16.0 (C-11); $^1$H NMR spectral data (500 MHz) $\delta_H$: 11.1 (s, 2H, H-OH), 7.71 (d, $J = 5.5$ Hz, 1H, H-7), 7.64 (s, 1H, H-4), 7.27 (d, $J = 5.5$ Hz, 1H, H-8), 2.27 (s, 3H, H-11); (+)-HRESIMS m/z 221.0439 [M + H]$^+$ (calcd for C$_{11}$H$_{10}$O$_6$, 221.0444).

3.6. Computational methods
Molecular Merck force field (MMFF) and DFT/TDDFT calculations were carried out with Spartan’14 software package (Wave function Inc., Irvine, CA, 2013) and the Gaussian 09 program package (Gaussian, Inc., Pittsburgh PA, 2011), respectively, using default grids and convergence criteria. The MMFF conformational search afforded low-energy conformers with in a 10 kcal/mol energy window, which were subjected to geometry optimization using density functional theory (DFT) method at the B3LYP/6-31G(d) level. Vibrational frequency calculations were run at the same level to estimate their relative thermal free energies ($\Delta G$) at 298.15K. A series of single-point energy calculations for the conformers above were performed at the M06-2X/def2-TZVP level. Solvent effects were taken into account by using the polarizable continuum model (PCM). The DFT
optimized conformers with the Boltzmann distribution over 1% was then subjected to TDDFT calculations using the functional PBE1PBE and basis set 6-311G(d). The CD spectra were generated by the program SpecDis using a Gaussian band shape with 0.25–0.35 eV exponential half-width from dipole-length dipolar and rotational strengths. The equilibrium population of each conformer at 298.15 K was calculated from its relative free energies using Boltzmann statistics. The calculated spectra of compounds 2 and 3 were generated from the low-energy conformers according to the Boltzmann distribution of each conformer in MeOH solution.

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Disclosure statement

No conflict of interest was reported by the authors.

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