RSC Advances

PAPER

View Article Online
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Cite this: *RSC Adv.*, 2017, **7**, 49910

Antiviral and anti-inflammatory meroterpenoids: stachybonoids A–F from the crinoid-derived fungus *Stachybotrys chartarum* 952†

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Received 5th September 2017
Accepted 9th October 2017

DOI: 10.1039/c7ra09859f

rsc.li/rsc-advances

Six new meroterpenoids, which have been named as stachybonoids A–F (**1–3** and **5–7**), and three known phenylspirodrimanes (**4**, **8** and **9**) were isolated from the crinoid-derived fungus *Stachybotrys chartarum* 952. Their structures were established on the basis of extensive spectroscopic data (1D and 2D NMR, MS, and ECD), as well as the modified Mosher's method and single-crystal X-ray structural analysis. Compound **1** exhibited inhibitory activity against the replication of dengue virus. Compounds **4**, **7**, and **8** exhibited moderate anti-inflammatory activity by inhibiting the production of nitric oxide (NO) in RAW264.7 cells activated by lipopolysaccharide, with IC_{50} values of 27.2, 52.5, and 17.9 μM, respectively.

Introduction

Stachybotrys chartarum is a species of filamentous fungus that is widespread in soil and plants, as well as marine sponges.[1–3] The fungus *S. chartarum* is known to produce a variety of secondary metabolites with diverse novel structures and interesting biological activities,[1] such as toxic macrocyclic trichothecenes,[4] anti-inflammatory atranones,[5] and antiviral phenylspirodrimanes.[3,6] Phenylspirodrimanes belong to the class of polyketide–terpenoid hybrid meroterpenoids, possess a common sesquiterpene unit containing a drimane skeleton to which a benzene ring is attached through a spirofuran ring, and have only previously been isolated from the genus *Stachybotrys*.[1,7] They display a wide range of bioactivities including antiviral activity (HIV-1 and IAV),[3,6] antihyperlipidemic activity,[2] inhibition of tyrosine kinase,[8] and cytotoxicity.[9]

Stachybotrys chartarum 952 was isolated from a crinoid (*Himerometra magnipinna*) from Xuwen Coral Reef Nature Reserve, Zhanjiang city, Guangdong Province, China. An EtOAc extract of a fermentation broth of the fungus *S. chartarum* 952 displayed moderate anti-inflammatory activity by inhibiting the production of nitric oxide (NO) in RAW264.7 cells activated by lipopolysaccharide, with IC_{50} values of 36 ± 2.1 μM. Subsequent chemical investigation led to the isolation of three new meroterpenoids, namely, stachybonoids A–C (**1–3**) and one new phenylspirodrimane, namely, stachybonoid D (**5**) from a rice medium and two new phenylspirodrimanes, namely, stachybonoids E and F (**6** and **7**), as well as three known phenylspirodrimanes (**4**, **8**, and **9**) from PDB medium. In this paper, we describe the isolation, structural elucidation, plausible biosynthetic pathways, and bioactivities of the isolates from the fungus.

Results and discussion

The crinoid-derived fungus *Stachybotrys chartarum* 952 was cultured on solid rice and PDB medium for four weeks, respectively. The fermentation extract was fractionated by repeated silica gel chromatography, Sephadex LH-20 column chromatography, and HPLC to afford compounds **1–9** (Fig. 1).

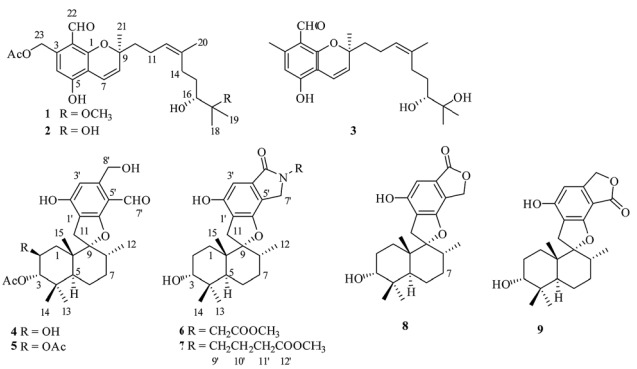

Fig. 1 Structures of compounds **1–9**.

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† Electronic supplementary information (ESI) available: Spectra of all new compounds (^{1}H NMR, ^{13}C NMR, 2D NMR, HRESIMS). CCDC 1563788. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ra09859f

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Stachybonoid A (**1**) was obtained as a yellow gum and possessed a molecular formula of $C_{26}H_{36}O_7$ according to HRESIMS data in combination with ^{1}H and ^{13}C NMR data (Table 1). The ^{1}H NMR data showed the presence of an aldehyde proton [δ_H 10.34 (1H, s)], an aromatic proton [δ_H 6.69 (1H, s)], a pair of *cis*-coupled olefinic protons [δ_H 5.63 (1H, d, J = 10.3 Hz) and 6.91 (1H, d, J = 10.3 Hz)], and five methyl groups [δ_H 2.03 (3H, s), 1.66 (3H, s), 1.42 (3H, s), 1.34 (3H, s), and 1.27 (3H, s)]. The ^{13}C and DEPT NMR spectra of **1** exhibited 26 carbon signals, namely, one aldehyde carbon (δ_C 194.3), one ester carbonyl carbon (δ_C 170.6), six aromatic carbons (δ_C 161.1, 161.1, 141.8, 113.3, 111.0, and 109.6), four olefinic carbons, two oxygen-linked quaternary carbons, two oxygen-linked methine groups, five methylene groups, and five methyl groups. All the above information suggested that compound **1** has a chromene skeleton similar to anthopogochromene A,[10] in addition to the different substituents on the aromatic ring and alkyl chain. In the HMBC spectrum (Fig. 2), the correlations of the aldehyde proton H-22 (δ_H 10.34) with C-1 and C-2 and the oxygen-linked methylene proton H-23 (δ_H 5.54) with C-2, C-3, and C-4 indicated that the aldehyde group and oxygen-linked methylene group were located at C-2 and C-3 of the chromene unit, respectively. The acetyl group was substituted on the C-23 methylene group, as supported by the HMBC correlation of H-23 with the ester carbonyl carbon (δ_C 170.6). The key COSY correlations of H-9/H-10/H-11 and H-14/H-15/H-16, as well as the HMBC correlations of H-20 with C-12, C-13 and C-14, H-18 with C-16 and C-17, H-19 with C-17 and C-18, and 17-OCH_3 with C-17, indicated a 2-methoxy-2,6-dimethylnon-6-en-3-ol unit. This unit and the remaining methyl group were both linked to C-9 of the chromene unit on the basis of the HMBC correlations from H-10 to C-8 and C-9 and H-21 to C-8 and C-10.

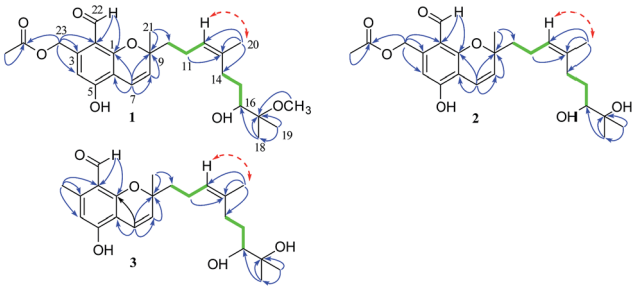

Fig. 2 Key HMBC (blue arrows), ^{1}H–^{1}H COSY (bold green lines), and NOESY (red arrows) correlations in **1–3**.

The NOESY correlation of H-12 (δ_H 5.32) with H-20 (δ_H 1.64) established that the C-12/C-13 double bond had the *Z* configuration. The absolute configuration of C-9 was established as *S* by a comparison of its CD spectrum (a positive cotton effect: λ = 274 nm, $\Delta\varepsilon$ = 10.20) with analogues in the literature (Fig. 3).[10,11] The assignment of the absolute configuration of C-16 was performed by the modified Mosher ester method.[12] The differences in the ^{1}H NMR chemical shifts between (*S*)- and (*R*)-MTPA esters ($\Delta\delta = \delta_S - \delta_R$) around the C-16 position were analyzed to

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **1–3** (in pyridine-d_5, J in Hz)

	1		**2**		**3**	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		161.1, C		161.0, C		161.5, C
2		113.3, C		113.3, C		114.2, C
3		141.8, C		141.8, C		145.1, C
4	6.69, s	111.0, CH	6.70, s	111.0, CH	6.32, s	111.7, CH
5		161.1, C		161.0, C		161.2, C
6		109.6, C		109.5, C		107.7, C
7	6.91, d (10.3)	116.3, CH	6.91, d (10.3)	116.2, CH	6.91, d (10.3)	116.6, CH
8	5.63, d (10.3)	128.4, CH	5.63, d (10.3)	128.4, CH	5.58, d (10.3)	127.4, CH
9		81.6, C		81.6, C		81.4, C
10	1.81, m; 1.68, m	42.3, CH_2	1.82, m; 1.71, m	42.3, CH_2	1.81, m; 1.70, m	42.4, CH_2
11	2.24, m	23.4, CH_2	2.24, m	23.3, CH_2	2.24, m	23.5, CH_2
12	5.31, t (7.6)	124.1, CH	5.32, t (7.6)	124.0, CH	5.34, t (7.6)	124.1, CH
13		136.7, C		136.7, C		136.8, C
14	2.30, m; 2.62, m	37.8, CH_2	2.33, m; 2.66, m	37.9, CH_2	2.66, m; 2.33, m	38.0, CH_2
15	1.97, m; 1.68, m	30.7, CH_2	2.08, m; 1.81, m	31.0, CH_2	2.09, m; 1.81, m	31.2, CH_2
16	3.70, d (10.2)	76.6, CH	3.77, d (10.2)	78.7, CH	3.77, d (10.2)	78.8, CH
17		78.2, C		73.0, C		73.1, C
18	1.34, s	21.8, CH_3	1.54, s	26.4, CH_3	1.54, s	26.5, CH_3
19	1.27, s	20.4, CH_3	1.50, s	26.3, CH_3	1.50, s	26.4, CH_3
20	1.65, s	16.6, CH_3	1.66, s	16.6, CH_3	1.67, s	16.6, CH_3
21	1.42, s	27.6, CH_3	1.42, s	27.6, CH_3	1.42, s	27.7, CH_3
22	10.36, s	194.3, CH	10.36, s	194.3, CH	10.13, s	194.5, CH
23	5.54, s	63.2, CH_2	5.54, s	63.2, CH_2	2.38, s	18.5, CH_3
23-OAc		170.6, C		170.6, C		
23-OAc	2.03, s	21.0, CH_3	2.03, s	21.0, CH_3		
17-OCH_3	3.24, s	49.6, CH_3				
5-OH	13.19, br s		13.16, br s		13.26, br s	

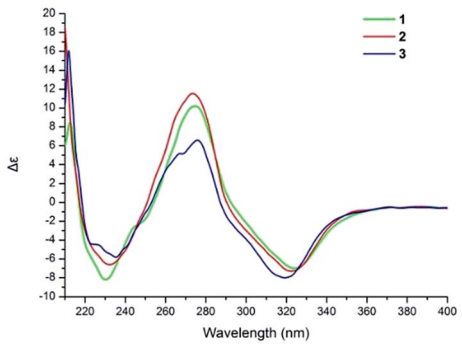

Fig. 3 ECD spectra of compounds **1–3**.

determine the absolute configuration of this position, which was found to be *R* (Fig. 4). Thus, compound **1** was revealed to be ((*S*)-8-formyl-5-hydroxy-2-((*S*,*Z*)-7-hydroxy-8-methoxy-4,8-dimethylnon-3-en-1-yl)-2-methyl-2*H*-chromen-7-yl)methyl acetate and was named as stachybonoid A.

Compound **2** was obtained as a yellow oil. The molecular formula of $C_{25}H_{34}O_7$ was confirmed from the molecular ion peak at *m*/*z* 445.2229 $[M - H]^-$ observed in the HRESIMS spectrum. The ^1H and ^{13}C NMR data (Table 1) showed that compound **2** lacked a carbon signal ($\delta_{H/C}$ 3.24/49.6) in comparison with **1**. It was deduced that the 17-OCH_3 group in **1** was replaced by a hydroxyl group in compound **2**. The positive cotton effect (λ = 273 nm, $\Delta\varepsilon$ = 11.53) observed in the ECD spectrum enabled the definition of the absolute configuration at C-9 (*S*) in **2** (Fig. 3). Therefore, the structure of **2** was determined to be demethoxystachybonoid A, and **2** was named as stachybonoid B.

Compound **3** gave a molecular formula of $C_{23}H_{32}O_5$, which was confirmed by the HRESIMS peak at *m*/*z* 387.2168 $[M - H]^-$. The NMR spectroscopic data of **3** (Table 1) were very similar to those of compound **2** but lacked two carbon signals (δ_C 170.6 and 63.2). This suggested that in compound **3** the ester group on the side chain at C-23 (δ_C 18.5) was absent, which was confirmed by the HMBC correlation from H-23 (δ_H 2.37) to C-1 (δ_C 161.1) and C-2 (δ_C 113.3). The absolute configuration of C-9 was also assigned as *S* by a comparison of its CD spectrum with analogues in the literature (Fig. 3).[10,11] Consequently, the structure of **3** was determined to be (*E*)-2-(7,8-dihydroxy-4,8-dimethylnon-3-en-1-yl)-6-hydroxy-2,7-dimethyl-2*H*-chromene-8-carbaldehyde, and **3** was named as stachybonoid C.

Compound **5** was obtained as a yellow oil, and its molecular formula was determined to be $C_{27}H_{36}O_8$ from the HRESIMS negative ion at *m*/*z* 487.2339 $[M - H]^-$ (calcd for $C_{27}H_{35}O_8$: 487.2339), which indicated nine degrees of unsaturation. The IR spectrum displayed absorption bands for phenolic hydroxyl (3408 cm^{-1}), carbonyl (1740 cm^{-1}), and aromatic groups (1650 and 1455 cm^{-1}). The ^1H NMR spectrum (Table 2) revealed the presence of one aldehyde proton [δ_H 10.85 (1H, s)], one penta-substituted aromatic proton [δ_H 7.53 (1H, s)], and six methyl groups [δ_H 2.05 (3H, s), 1.92 (3H, s), 1.05 (3H, s), 0.94 (3H, s), 0.91 (3H, s), and 0.87 (3H, d, J = 6.4 Hz)]. The ^{13}C NMR and DEPT spectra exhibited 27 carbon signals, which comprised six methyl groups, five methylene groups, five methine groups, eight quaternary carbons, and three carbonyl groups. The ^1H and ^{13}C NMR (Table 2) data of compound **5** were similar to those of stachybotrysin C (**4**) with its phenylspirodrimane skeleton,[6] except for the presence of an additional acetyl group and a variation in the chemical shift of C-2 ($\delta_{H/C}$ 5.46/68.9) in **5**. It was shown that the 2-OH group in **4** was replaced by an acetoxy group in compound **5**, which was further supported by the HMBC correlation from H-17 to C-16 and C-2 in **5** (Fig. 5). The NOE correlations from H-15 to H-14 and H-11b, H-8 to H-11a, and H-3 to H-14 indicated that H-3, H-8, H-11, H-14, and H-15 were *syn*-oriented, whereas H-5 and H-12 had the opposite orientation. The NOE correlation between H-2 and H-3 suggested that the corresponding bonds were both equatorial in association with the small coupling constant of $J_{2,3}$. The ECD spectrum of **5** exhibited a strong positive cotton effect (CE) at 210 nm and two weak negative CEs at 286 and 327 nm, which were similar to those of **4** (Fig. 7). This indicated that they have the same absolute configurations, namely, 2*S*, 3*S*, 8*S*, and 10*S*. Therefore, the structure of 5 was identified as shown in Fig. 1, and **5** was named as stachybonoid D.

Compound **6** was obtained as a yellow powder. The molecular formula of **6** was established as $C_{26}H_{35}O_6N$ on the basis of the $[M + H]^+$ ion signal at *m*/*z* 458.2537. The ^1H and ^{13}C NMR data for the phenylspirodrimane framework of **6** were quite similar to those of stachybotrylactam.[4] The HMBC correlations of H-9′ and H-11′ with C-10 and H-7′ with C-9′ revealed that a methyl acetate moiety was connected to the nitrogen atom. The relative configuration of the phenylspirodrimane moiety in **6** was further established by its NOESY correlations (Fig. 6). The absolute configuration of **6** was assigned to be 2*S*, 3*S*, 8*S*, 10*S* on the basis of its ECD spectrum, which exhibited a strong cotton effect (CE) at 218 nm and two weak negative CEs at 222 and 266 nm, which were similar to those of **8**. Hence, compound **6** was identified as shown in Fig. 1 and was named as stachybonoid E.

Compound **7** was obtained as a faint yellow powder. The HRESIMS data indicated a molecular formula of $C_{28}H_{39}O_6N$ on the basis of the $[M + H]^+$ ion signal at *m*/*z* 486.2850. The ^1H and ^{13}C NMR data showed that **7** had two additional methylene signals (δ_C 49.6) in comparison with **6**. The ^1H–^1H COSY correlation of H-10′ with H-9′ and H-11′, as well as the HMBC correlations from H-11′ and H-13′ to C-12′ and H-7′ to C-9′ indicated that a methyl butyrate moiety was linked to the nitrogen atom. Its relative configuration was identified to be the same as that of **6** from NOESY data (Fig. 6). At the same time, compounds **6** and **7** shared the same absolute configuration of 2*S*, 3*S*, 8*S*, 10*S* according to their similar ECD spectra (Fig. 8). Thus, compound **7** was identified and named as stachybonoid F.

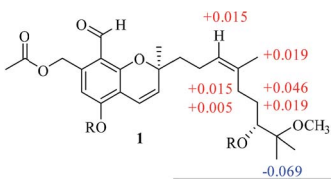

Fig. 4 Values of $\Delta\delta = \delta_S - \delta_R$ in ppm obtained from the MTPA esters of **1**.

Table 2 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **5–7** (in pyridine-d_5, J in Hz)

	5		**6**		**7**	
No.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.29, m; 1.63, dd (3.5, 11.9)	31.7, CH_2	1.98, t (14.0); 1.75, m	26.6, CH_2	1.73, m; 1.99, m	26.6, CH_2
2	5.45, m	68.9, CH	2.33, td (12.8, 3.5); 1.14, dt (12.8, 6.1)	25.2, CH_2	2.38, m; 1.20, m	25.1, CH_2
3	5.30, br s	77.5, CH	3.63, m	75.3, CH	3.62, m	75.3, CH
4		38.7, C		38.7, C		38.7, C
5	2.29, m	41.5, CH	2.62, dd (12.8, 2.5)	40.9, CH	2.65, dd (12.8, 2.5)	40.9, CH
6	1.48, m; 1.33, m	21.1, CH_2	1.45, dd (12.8, 3.5); 1.63, m	21.8, CH_2	1.66, m; 1.47, m	21.8, CH_2
7	1.58, m	31.2, CH_2	1.75, m; 1.60, m	32.1, CH_2	1.78, m; 1.62, m	32.1, CH_2
8	1.74, m	37.2, CH	1.75, m	37.8, CH	1.78, m	37.8, CH
9		99.5, C		99.4, C		99.3, C
10		44.4, C		43.2, C		43.3, C
11	3.46, d (16.7); 3.08, d (16.7)	32.0, CH_2	3.13, d (16.9); 3.54, d (16.9)	33.3, CH_2	3.16, d (16.9); 3.62, m	33.4, CH_2
12	0.87, d (6.4)	16.2, CH_3	0.83, d (5.8)	16.4, CH_3	0.89, s	16.4, CH_3
13	0.94, s	21.9, CH_3	1.25, s	29.7, CH_3	1.26, s	29.7, CH_3
14	0.91, s	28.4, CH_3	0.92, s	23.2, CH_3	0.93, s	23.2, CH_3
15	1.05, s	17.3, CH_3	0.99, s	16.7, CH_3	1.01, s	16.7, CH_3
16		170.9, C				
17	2.05, s	21.1, CH_3				
18		170.8, C				
19	1.92, s	21.5, CH_3				
1′		112.2, C		118.7, C		118.2, C
2′		169.1, C		156.1, C		156.0, C
3′	7.53, s	108.2, CH	7.34, s	102.5, CH	7.36, s	102.3, CH
4′		148.9, C		113.7, C		113.4, C
5′		110.0, C		134.9, C		135.8, C
6′		161.6, C		157.5, C		157.5, C
7′	10.86, s	188.1, CH		169.6, C		169.2, C
8′	5.56, m	63.7, CH_2	4.04, d (16.0); 4.32, d (16.0)	48.4, CH_2	3.83, d (16.5); 4.07, d (16.5)	47.8, CH_2
9′			4.57, d (17.7); 4.32, d (17.7)	44.6, CH_2	3.62, m; 3.40, m	42.3, CH_2
10′				170.5, C	1.83, m	24.5, CH_2
11′			3.54, s	52.4, CH_3	2.32, t (7.4)	31.8, CH_2
12′						173.7, C
13′					3.55, s	51.8, CH_3
2-OH			5.73, d (3.5)		5.76, d (3.7)	
2′-OH			12.23, s		12.20, s	

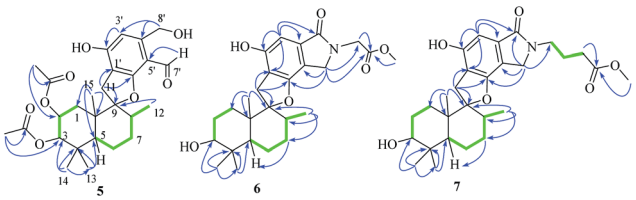

Fig. 5 Key HMBC (blue arrows) and $^{1}H-^{1}H$ COSY (bold green lines) correlations in **5–7**.

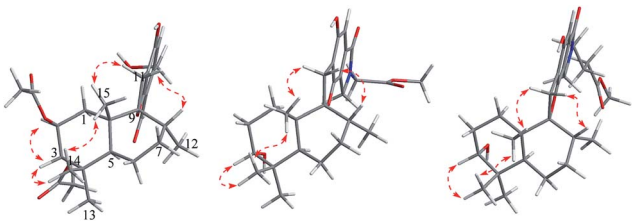

Fig. 6 Key NOESY correlations (red arrows) in **5–7**.

The known compounds were identified as stachybotrylactone (**8**)[13] and stachartin B (**9**)[13,14] on the basis of NMR data and mass spectrometric analysis and a comparison of their spectroscopic data with published values. The absolute configuration of compound **8** was further established by a single-crystal X-ray diffraction experiment using Cu Kα radiation (ESI†).

Compounds **1–9** belong to the class of polyketide–terpenoid hybrid meroterpenoids, which are derived from orsellinic acid and farnesyl diphosphate.[7] A plausible biosynthetic pathway for **1–9** was proposed, as shown in Fig. 9. Initially, orsellinic acid and farnesyl diphosphate underwent addition to form the intermediate ilicicolin B. Then, the terminal olefin bond in the prenyl group of ilicicolin B was epoxidized to yield another intermediate. When an aromatic hydroxyl group reacted with C-2 of the prenyl group, the series of phenylspirodrimane derivates **4–9** were generated by cyclization, oxidation, addition, and acetylation. When the aromatic hydroxyl group was linked to C-3 of the prenyl group by electrophilic addition, a series of chromene derivatives (**1–3**) were yielded.

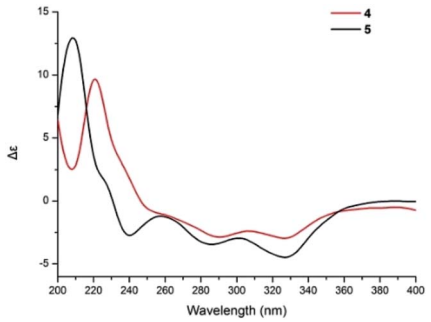

Fig. 7 ECD spectra of compounds 4 and 5.

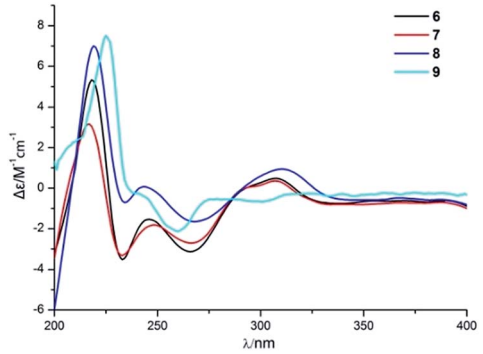

Fig. 8 ECD spectra of compounds 6–9.

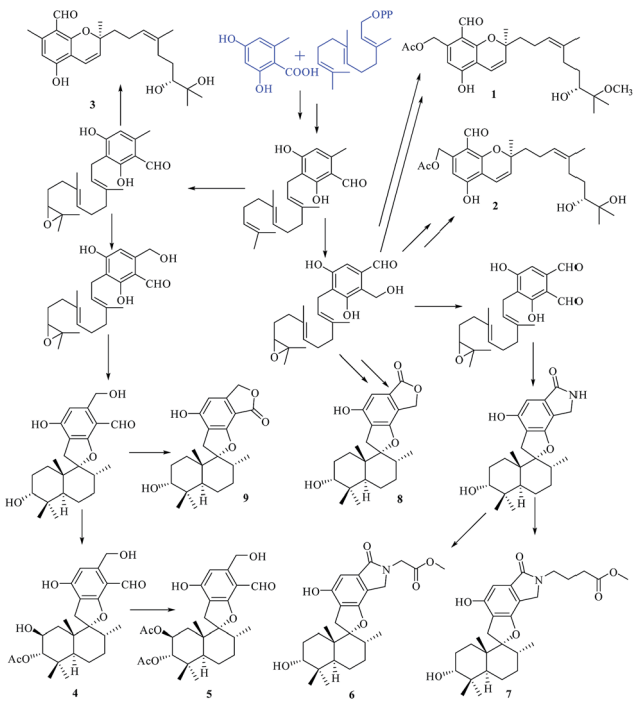

Fig. 9 Plausible biosynthetic pathway of compounds 1–9.

Compounds **1–3** were tested for their antiviral activities against dengue virus proteins using α-tubulin as a standard. The results of the anti-dengue virus assay indicated that compound **1** displayed

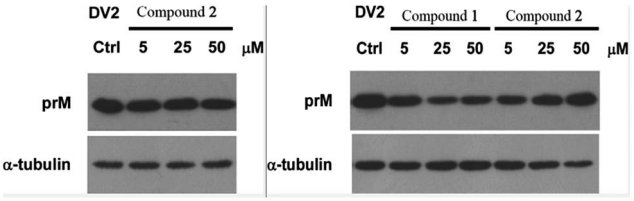

Fig. 10 Inhibitory activity of compounds 1–3 against dengue virus protein prM. Compound 1 displayed inhibitory activity against the replication of dengue virus, compound 2 increased the formation of dengue protein, but compound 3 exhibited no obvious activity in this assay.

inhibitory activity against the replication of dengue virus. A western blot showed that compound **1** reduced the expression of the dengue virus protein prM in a dose-dependent manner (Fig. 10). It is noteworthy that compound **1** inhibited the replication of dengue virus whereas compound **2** increased the formation of dengue protein, when the only difference between their structures was whether or not 17-OH was methylated. Compounds **4–9** were investigated for their inhibitory activities against the LPS-activated production of NO in RAW264.7 cells using the Griess assay with indomethacin as a positive control ($IC_{50} = 37.5 \pm 1.6$ μM). Compounds **4**, **7**, and **8** displayed moderate inhibitory effects on the production of NO, with IC_{50} values of 27.2 ± 1.2, 52.5 ± 1.8, and 17.9 ± 0.9 μM, respectively (ESI†). However, compounds **5**, **6** and **9** were inactive (<50% inhibition at 100 μM, which was the highest concentration tested). In order to investigate whether the inhibitory activities of the active compounds were due to their cytotoxicity, the effects of compounds **4**, **7** and **8** on cell proliferation/viability were determined using the MTT method. After treatment with LPS for 24 h, compounds **4** and **7** (up to 50 μM) did not exhibit any significant cytotoxicity. However, **8** displayed cytotoxicity at 50 μM, and the cell survival rate was 9.50%.

Experimental

General experimental procedures

Optical rotations were measured using an Abbemat 300 reflectometer with a 100 mm cell. IR spectra were recorded with an FT-Raman spectrometer (Nicolet NXR 9650). NMR spectra were recorded using a Bruker Avance 400 MHz spectrometer with tetramethylsilane as an internal standard. The semipreparative HPLC apparatus was an Essentia LC-16. ESIMS spectra were acquired using an LCQ Deca XP LC-MS spectrometer, and HRESIMS spectra were recorded with an LTQ Orbitrap LC-MS spectrometer (Thermo Corporation, USA). ECD and UV spectra were recorded with an Applied Photophysics Ltd spectrometer using MeOH as a solvent. Sephadex LH-20 and silica gel (100–200 mesh and 200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China) were used for column chromatography. Analytical thin-layer chromatography (TLC) was performed with GF254 plates (Qingdao Marine Chemical Factory). The reagents used in the research process were of analytical grade (Guangzhou Chemical Reagent Factory).

Fungal material

Crinoids (*Himerometra magnipinna*) were collected in Zhanjiang Mangrove National Nature Reserve in Guangdong Province, China, in August 2014. A strain, which was designated as 952, was isolated from the crinoids. On the basis of its morphological characteristics and the ITS region, the strain was identified to be *Stachybotrys chartarum*. The sequence data of the fungal strain have been deposited in the GenBank database under the accession number KC427079.1. The voucher specimen was preserved on potato dextrose agar slants at 4 °C at the School of Marine Science, Sun Yat-Sen University (HM001).

Fermentation, extraction and isolation

The fungus *S. chartarum* 952 was cultivated on rice culture medium (70 mL rice and 90 mL 3% artificial sea salt water per flask) and PDB medium (300 g potato and 20 g dextrose in 1 L 3% artificial sea salt water), respectively, in 1 L Erlenmeyer flasks for 35 days at 25 °C. After fermentation, the fungal culture in the rice medium was extracted exhaustively with MeOH three times to obtain crude extracts. The extracts were suspended in MeOH/H_2O (8 : 2) and extracted three times with *n*-hexane, $CHCl_3$, and EtOAc successively. The chloroform extract (98 g) was subjected to CC on silica gel (200–300 mesh) and was eluted with PE–EtOAc of increasing polarity (from 1 : 0 to 0 : 1) to afford five fractions (A–E). Fr. C was subjected to chromatography on Sephadex LH-20 (MeOH : CH_2Cl_2 = 1 : 1) to afford two fractions, which were denoted as Fr. C1 and Fr. C2. Fr. C1 was repurified by HPLC with isocratic elution with MeOH–H_2O (80 : 20) to obtain compound **2** (6 mg). Fr. C2 was subjected to chromatography on Sephadex LH-20 (MeOH : CH_2Cl_2 = 1 : 1) and was further purified by HPLC elution with MeOH–H_2O (80 : 20) to obtain compounds **1** (4 mg), **3** (7 mg), and **5** (7 mg).

The cultures in the PDB medium were filtered to remove mycelia. The filtrate was concentrated under reduced pressure and then extracted three times with *n*-hexane, $CHCl_3$, and EtOAc successively. The EtOAc extract (16 g) was subjected to CC on silica gel (200–300 mesh) and eluted with PE–EtOAc of increasing polarity (from 1 : 0 to 0 : 1) to afford five fractions (A–E). Fr. B was separated on Sephadex LH-20 (MeOH) and was then purified by HPLC (80% MeOH; flow rate 1.0 mL min^{-1}; C18 10 × 250 mm, 5 μm) to give **8** (8.0 mg). Fr. C was subjected to RP-18 gel CC (MeOH/H_2O, from 6 : 4 to 1 : 0) and was further purified by HPLC (80% MeOH; flow rate 1.0 mL min^{-1}; C18 10 × 250 mm, 5 μm) to yield **9** (8.9 mg). The $CHCl_3$ extract (80 g) was subjected to CC on silica gel (200–300 mesh) and eluted with PE–EtOAc of increasing polarity (from 1 : 0 to 0 : 1) to afford five fractions (A–E). Fr. D was separated on Sephadex LH-20 (MeOH) and was then purified by HPLC (80% MeOH; flow rate 1.0 mL min^{-1}; C18 10 × 250 mm, 5 μm) to give **4** (4.3 mg) and **6** (1.7 mg). Fr. E was subjected to CC on silica gel (200–300 mesh), eluted with PE–EtOAc and then purified by HPLC (80% MeOH; flow rate 1.0 mL min^{-1}; C18 10 × 250 mm, 5 μm) to give **7** (2.4 mg).

Stachybonoid A (1). Yellow oil; $[\alpha]_{20}^{D}$ −11.1 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 277 (3.28), 320 (3.18) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 230 (−8.17), 274 (10.20), 325 (−7.01) nm; IR (neat) ν_{max} 3467, 2935, 1747, 1619, 1376, 1225 cm^{-1}; HRESIMS *m*/*z* 459.2386 $[M - H]^-$ (calcd for $C_{26}H_{35}O_7$, 459.2388); ^1H and ^{13}C NMR, see Table 1.

Stachybonoid B (2). Yellow oil; $[\alpha]_{20}^{D}$ −1.4 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) 277 (3.26), 324 (3.16) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 232 (−6.60), 273 (11.53), 321 (−7.29) nm; IR (neat) ν_{max} 3471, 2926, 1741, 1622, 1382, 1232 cm^{-1}; HRESIMS *m*/*z* 445.2229 $[M - H]^-$ (calcd for $C_{25}H_{33}O_7$, 445.2232); ^1H and ^{13}C NMR, see Table 1.

Stachybonoid C (3). Yellow oil; $[\alpha]_{20}^{D}$ −2.6 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 276 (3.30), 319 (3.20) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 234 (−5.65), 276 (6.57), 319 (−8.00) nm; IR (neat) ν_{max} 3456, 2917, 1660, 1206 cm^{-1}; HRESIMS *m*/*z* 387.2168 $[M - H]^-$ (calcd for $C_{23}H_{31}O_5$, 387.2174); ^1H and ^{13}C NMR, see Table 1.

Stachybotrysin C (4). White powder; $[\alpha]_{20}^{D}$ −45.2 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.43), 284 (3.84), 329 (3.52) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 218 (12.24), 271 (−5.23), 324 (−2.80) nm; IR (neat) ν_{max} 3394, 2941, 2833, 1645, 1460, 1024 cm^{-1}; ESIMS *m*/*z* 444.9 $[M - H]^-$.

Stachybonoid D (5). Yellow oil; $[\alpha]_{20}^{D}$ −73 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.37), 285 (3.81), 330 (3.67) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (13.82), 286 (−3.51), 327 (−4.50) nm; IR (neat) ν_{max} 3408, 2964, 2938, 1740, 1609 cm^{-1}; HRESIMS *m*/*z* 487.2339 $[M - H]^-$ (calcd for $C_{27}H_{35}O_8$, 487.2337); ^1H and ^{13}C NMR, see Table 2.

Stachybonoid E (6). Yellow powder; $[\alpha]_{20}^{D}$ −17.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.28), 266 (3.52), 308 (3.06) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 218 (5.33), 233 (−3.52), 266 (−3.14) nm; IR (neat) ν_{max} 3362, 2943, 2839, 1649, 1456, 1032, 663 cm^{-1}; HRESIMS *m*/*z* 458.2531 $[M + H]^+$ (calcd for $C_{26}H_{36}O_6N$, 458.2537); ^1H and ^{13}C NMR, see Table 2.

Stachybonoid F (7). Yellow powder; $[\alpha]_{20}^{D}$ −21.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.36), 266 (3.65), 306 (3.23) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 217 (3.16), 233 (−3.33), 268 (−2.69) nm; IR (neat) ν_{max} 3364, 2943, 2839, 1649, 1456, 1032, 663 cm^{-1}; HRESIMS *m*/*z* 486.2842 $[M + H]^+$ (calcd for $C_{28}H_{40}O_6N$, 486.2850); ^1H and ^{13}C NMR, see Table 2.

Stachybotrylactone (8). White powder; $[\alpha]_{20}^{D}$ −9.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.58), 269 (3.74), 310 (3.44) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 219 (7.00), 234 (−0.70), 268 (−1.65) nm; IR (neat) ν_{max} 3394, 2952, 2839, 1645, 1448, 1022 cm^{-1}; ESIMS *m*/*z* 387 $[M + H]^+$.

Stachartin B (9). White powder; $[\alpha]_{20}^{D}$ −17.7 (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.43), 259 (3.92), 300 (3.59) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 224 (13.87), 240 (−0.95), 259 (−4.19) nm; IR (neat) ν_{max} 3378, 2943, 2837, 1660, 1410, 1448, 1119, 1024, 658 cm^{-1}; ESIMS *m*/*z* 387 $[M + H]^+$.

Preparation of the (*R*)- and (*S*)-MTPA esters of 1

A sample of **1** (1.0 mg), (*R*)-MTPACl (10.0 μL), and pyridine (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 30 min to yield the (*S*)-MTPA ester **1a** (0.8 mg). By the same procedure, the (*R*)-MTPA ester **1b** (0.7 mg) was obtained from the reaction of **1** (1 mg) with (*R*)-MTPACl (10.0 mL).

(*S*)-MTPA ester derivative (1a). ^1H NMR (selected signals, pyridine-d_5, 400 MHz) δ_H: 10.261 (1H, s, H-22), 5.291 (1H, t, H-12), 3.796 (3H, s, OCH_3–MTPA), 2.621 (1H, m, H-14a), 2.226

(1H, m, H-14b), 1.970 (1H, m, H-15a), 1.660 (1H, m, H-15b), 1.660 (3H, s, H-20), 1.263 (3H, s, H-18).

(*R*)-MTPA ester derivative (1b). ^{1}H NMR (selected signals, pyridine-d_5, 400 MHz) δ_H: 10.263 (1H, s, H-22), 5.276 (1H, t, H-12), 3.796 (3H, s, OCH_3–MTPA), 2.616 (1H, m, H-14a), 2.193 (1H, m, H-14b), 1.924 (1H, m, H-15a), 1.640 (1H, m, H-15b), 1.641 (3H, s, H-20), 1.334 (3H, s, H-18).

Bioactivity assay

The effectiveness against dengue virus of compounds **1–3** was determined by detecting the expression of the dengue virus protein prM using a western blot *via* a previously described method.[15] RAW264.7 cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, People's Republic of China). Cell maintenance, experimental procedures, the determination of data for the inhibition of the production of NO, and the viability assay were performed according to a modified literature procedure.[16,17] RAW 264.7 cells were seeded in 96-well plates (Nunc) at a density of 1×10^5 cells per well and incubated overnight. Then the cells were treated with LPS (1 μg mL^{-1}) and various concentrations of the compounds for 24 h. Subsequently, 50 μL cell culture supernatant solution was removed to a new 96-well plate (Nunc), and 50 μL nitric oxide detection reagent **I** and 50 μL nitric oxide detection reagent **II** were added to each well, respectively. The absorbance was measured at 540 nm with an Infinite M200 PRO microplate reader (TECAN). The IC_{50} values were determined using Origin 8Pro software from experiments performed in triplicate. Indomethacin (IC_{50} value of 37.5 ± 1.6 μM) was used as a positive control. All the tested compounds were prepared as stock solutions in DMSO, and the final solvent concentration was less than 0.2% in all assays.

Conclusions

In summary, nine meroterpenoids, including six new compounds, were isolated from the crinoid-derived fungus *Stachybotrys chartarum* 952. To the best of our knowledge, the nine meroterpenoids **1–9** were the first secondary metabolites to be reported from this crinoid-derived fungus. Chromenes **1–3** and phenylspirodrimanes **4–9** were isolated from the fungus *S. chartarum* 952. In addition, it was suggested that both kinds of compounds were derived from orsellinic acid and farnesyl diphosphate. Compound **1** exhibited inhibitory activity against the replication of dengue virus and would be a promising compound for the development of agents for the inhibition of dengue virus.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China under Grant No. 21272286 and 41606167 and China's Marine Commonwealth Research Project under Grant No. 201305017.

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