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Stachybotrysams A–E, prenylated isoindolinone derivatives with anti-HIV activity from the fungus *Stachybotrys chartarum*

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ABSTRACT

Four new farnesylated isoindolinone derivatives, named stachybotrysams A–D (**2–5**), and one new farnesyl-cyclized analogue, named stachybotrysam E (**6**), as well as one known congener (**1**), were isolated from the filamentous fungus *Stachybotrys chartarum* CGMCC 3.5365. The structures of these compounds were elucidated on the basis of spectroscopic data analysis and by comparison with reported data. Compounds **2–4** exhibited significant HIV-inhibitory activity with IC_{50} values of 9.3, 1.0, and 9.6 μM, respectively.

1. Introduction

The fungal genus *Stachybotrys* comprises approximately 100 species (Wu et al., 2014). Members of *Stachybotrys* spp. are distributed worldwide and are commonly isolated from soil and various decaying plant substrates. Among these species, the strains of *S. chartarum* are reported to produce a variety of secondary metabolites, including trichothecene mycotoxins (Hinkley et al., 2000), diterpenes (Hinkley et al., 1999), and phenylspirodrimanes (Li et al., 2014a and Ma et al., 2013). These structurally unusual compounds exhibit a wide range of pharmacological activities, including inhibition of pancreatic cholesterol esterase (Sakai et al., 1995), anticomplement (Kaise et al., 1979) and antiviral effects (Li et al., 2014a and Ma et al., 2013). To search for diverse metabolites with structure novelty and pharmacological potency, we fermented 70 L of *S. chartarum* CGMCC 3.5365. The preliminary purification of its ethyl acetate extract led to the isolation of four new farnesylated isoindolinone derivatives stachybotrysams A−D (**2**−**5**) and one new farnesyl-cyclized analogue stachybotrysam E (**6**) along with a known compound, chartarutine B (**1**) (Fig. 1) (Li et al., 2014b). These compounds' structures were determined by spectroscopic analysis and comparison with reported data. In this study, we report the detailed isolation, structure elucidation and anti-HIV activity of these compounds.

2. Results and discussion

Stachybotrysam A (**2**) was obtained as white amorphous powder. This compound has a molecular formula $C_{25}H_{35}O_4N$, as determined by the HR-ESI-MS ion at *m/z* 414.2639 $[M + H]^+$ (calcd for $C_{25}H_{36}O_4N$, 414.2639), corresponding to nine degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3302 cm^{-1}) and carbonyl (1628 cm^{-1}) groups. The UV spectrum displayed absorptions at 215 and 261 nm. The ^1H NMR spectrum (Table 1) and HSQC data displayed three hydroxyls [δ_H 9.45 (s, OH-2), δ_H 9.20 (s, OH-4), and δ_H 4.81 (t, J = 5.4 Hz, OH-2″)], an aromatic methine (δ_H 6.62, s, H-1), a methylene (δ_H 4.31, s, H_2-8), and typical signals of a farnesyl unit [three olefinic protons at δ_H 5.17 (t, J = 7.1 Hz, H-2′), δ_H 5.05 (t, J = 7.0 Hz, H-10′), and δ_H 5.03 (t, J = 7.0 Hz, H-6′); five methylenes at δ_H 3.28 (d, J = 7.1 Hz, H_2-1′), δ_H 1.89 (m, H_2-4′/8′), and δ_H 1.96 (m, H_2-5′/9′); four methyls at δ_H 1.73 (s, H_3-15′), δ_H 1.62 (s, H_3-12′), and δ_H 1.52 (6H, s, H_3-13′/14′)]. The ^{13}C NMR and DEPT spectra (Table 1) assigned a total of 25 carbon resonances, including a carbonyl carbon (δ_C 167.8, C-7), six aromatic carbons, and those characteristic of the farnesyl group [six olefinic carbons (δ_C 134.3, 133.6, 130.6, 124.1, 123.9, and 122.6), five methylene carbons (δ_C 39.3, 39.3, 26.1, 26.1, and 22.4), and four methyl carbons (δ_C 25.5, 17.5, 16.0, and 15.8)]. The IR, UV, ^1H and ^{13}C NMR data strongly suggested that **2** possessed a prenylated isoindolinone skeleton similar to that of **1**, the known compound chartarutine B (Li et al., 2014b). The only difference was that an ethyl alcohol group was assigned as an N-linked side chain based on the ^1H-^1H COSY correlations of H_2-1″/H_2-2″/OH-2″, as well as the HMBC interactions of H_2-8/C-1″, H_2-1″/C-2″, C-7 and C-8, and H_2-2″/C-1″ (Fig. 2). Thus, the structure of stachybotrysam A was elucidated as shown in Fig. 1.

Stachybotrysam B (**3**) was obtained as white amorphous powder.

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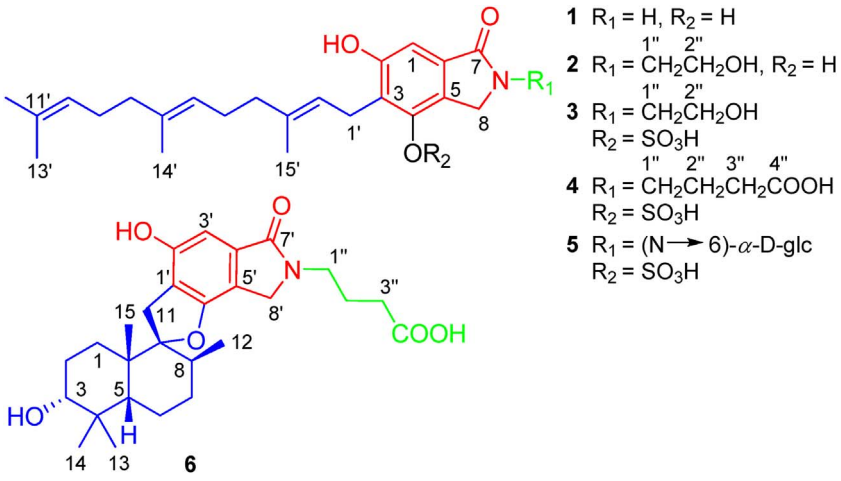

Fig. 1. Structures of compounds **1–6** from *Stachybotrys chartarum*.

This compound's molecular formula was deduced as $C_{25}H_{35}O_7NS$ by the HR-ESI-MS ion at *m/z* 494.2197 $[M + H]^+$ (calcd for $C_{25}H_{36}O_7NS$, 494.2207), which accounts for nine degrees of unsaturation. The molecular weight of **3** was 80 *amu* more than that of **2**, suggesting the existence of a sulphate moiety. This was supported by the fragment ion at *m/z* 414.2627 $[M - SO_3 + H]^+$ in its HR-ESI-MS spectrum. Careful analysis of its NMR data (Table 1) revealed that **3** virtually had an identical prenylated isoindolinone skeleton and N-linked side chain to that of **2**. The difference was that the hydroxyl in downfield (OH-4) was absent, and H-1 was shifted downfield to δ 6.86, indicating that the sulphate moiety might be attached at C-4 through an ester bond. The conclusion was further supported by the upfield shift of C-4 as well as downfield shifts of C-1, C-3, and C-5. Thus, the structure of stachybotrysam B was determined as shown in Fig. 1.

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **2**−**5** in DMSO-d_6.

position	2		3		4		5	
	δ_C[a], type	δ_H[b] (*J* in Hz)	$_{\delta C}$[c], type	δ_H[d] (*J* in Hz)	δ_C[a], type	δ_C[b], type	δ_C[c], type	$_{\delta C}$[d], type
1	100.3, CH	6.62, s	104.6, CH	6.86, s	104.6, CH	6.85, s	105.1, CH	6.88, s
2	156.1, C		156.2, C		156.2, C		156.6, C	
3	118.6, C		126.6, C		126.1, C		127.7, C	
4	150.1, C		146.6, C		146.6, C		146.9, C	
5	118.9, C		126.7, C		126.7, C		127.5, C	
6	131.2, C		131.2, C		131.2, C		131.1, C	
7	167.8, C		167.4, C		167.2, C		169.1, C	
8	48.7, CH_2	4.31, s	49.4, CH_2	4.48, s	48.4, CH_2	4.41, s	51.3, CH_2	4.55, d (11.7)
1′	22.4, CH_2	3.28, d (7.1)	23.3, CH_2	3.44, d (7.3)	23.3, CH_2	3.44, overlapped	23.8, CH_2	3.42, overlapped
2′	122.6, CH	5.17, t (7.1)	122.7, CH	5.15, t (7.3)	122.7, CH	5.15, t (7.4)	123.0, C	5.15, t (7.0)
3′	133.6, C		133.6, C		133.6, C		134.3, C	
4′	39.3, CH_2	1.89, m	39.2, CH_2	1.88, m	39.2, CH_2	1.88, m	39.8, CH_2	1.88, m
5′	26.1, CH_2	1.96, m	26.2, CH_2	1.97, m	26.1, CH_2	1.94, m	26.6, CH_2	1.97, m
6′	124.1, CH	5.03, t (7.0)	124.1, CH	5.04, t (7.5)	124.1, CH	5.03, overlapped	124.5, CH	5.05, overlapped
7′	134.3, C		134.3, C		134.2, C		134.7, C	
8′	39.3, CH_2	1.89, m	39.2, CH_2	1.88, m	39.2, CH_2	1.88, m	39.8, CH_2	1.88, m
9′	26.1, CH_2	1.96, m	26.2, CH_2	1.97, m	26.1, CH_2	1.98, m	26.7, CH_2	1.97, m
10′	123.9, CH	5.05, t (7.0)	124.0, CH	5.04, t (7.5)	123.9, CH	5.03, overlapped	124.6, CH	5.05, overlapped
11′	130.6, CH		130.6, CH		130.6, CH		130.0, C	
12′	25.5, CH_3	1.62, s	25.5, CH_3	1.61, s	25.5, CH_3	1.61, s	26.0, CH_3	1.62, s,
13′	17.5, CH_3	1.52, s	17.6, CH_3	1.53, s	17.5, CH_3	1.52, s	18.1, CH_3	1.53, s
14′	15.8, CH_3	1.52, s	15.8, CH_3	1.52, s	15.8, CH_3	1.52, s	16.6, CH_3	1.53, s
15′	16.0, CH_3	1.73, s	16.1, CH_3	1.72, s	16.1, CH_3	1.72, s	16.2, CH_3	1.72, s
1″	44.6, CH_2	3.50, t-like (5.7)	44.6, CH_2	3.49, t-like (5.5)	41.5, CH_2	3.44, overlapped	98.9, CH	4.39, d (3.0)
2″	59.4, CH_2	3.57, dd (5.7, 11.3)	59.4, CH_2	3.55, dd (5.3, 11.5)	24.3[e], CH_2	1.76, m	69.3, CH	3.59, overlapped
3″					32.3[e], CH_2	2.07, brs	69.8, CH	3.77, overlapped
4″					175.6[e], C		69.4, CH	3.33, overlapped
5″							77.8, CH	4.42, m
6″							48.7, CH_2	3.76/3.47, overlapped
OH-2		9.45, s		9.62, s		9.63, s		9.66, s
OH-4		9.20, s						
OH-2″		4.81, t (5.4)		4.80, t (5.4)				
OH-1″								5.74, s

[a] 150 MHz.
[b] 600 MHz.
[c] 125 MHz.
[d] 500 MHz.
[e] assigned with the aid of the HSQC and HMBC experiment.