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NOTE

Sch 1385568, a new azaphilone from *Aspergillus* sp.

Shu-Wei Yang, Tze-Ming Chan, Joseph Terracciano[1], David Loebenberg, Mahesh Patel[2], Vincent Gullo[3] and Min Chu[1]

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In the course of our continuing search for novel antimicrobial agents,[1,2] we have identified a novel azaphilone Sch 1385568 (**1**) (Scheme 1) from an *Aspergillus* sp. culture (SPRI-0814). Various azaphilones and hydrogenated azaphilones have been isolated mainly from fungal species, such as *Emericella* sp.,[3–5] *Penicillium* sp.,[6–8] *Phomopsis* sp.,[9] *Chaetomium* sp.,[10] *Pseudohalonectria* sp.[11] and *Anuulohypoxylon* sp.[12] Some of them have been described to show biological activities against various targets related to the different therapeutic areas, including cardiovascular, lipid metabolism, inflammatory, anti-infectious and antitumor areas. More specifically, azaphilones have been reported to display inhibitory activity against the following targets: acyl-CoA: cholesterol acyltransferase,[6] endothelin receptor,[8] cholesteryl ester transfer protein,[13] platelet-derived growth factor,[14] gp120-CD4,[15] monoamine oxidase,[16] phospholipase A_2[17] and nitric oxide production.[18] Some azaphilones have also been reported to display antitumor[10,19] and antimicrobial activities.[10–12] In this communication, we describe the fermentation, isolation, structure elucidation and antimicrobial activity of **1**.

Fermentation of *Aspergillus* sp. culture SPRI-0814 was conducted in shake flasks. Stock cultures were maintained as frozen whole broths at −80 °C in a final concentration of 10% glycerol. The germination medium contained proteus peptone 5.0 g, NaCl 5.0 g, KH_2PO_4 5.0 g, yeast extract 3.0 g, cerelose 20 g and soybean grits 5.0 g in 1.0 l tap water with pH 7.0 before autoclaving. Each 250 ml flask containing 70 ml of this medium was inoculated with 2 ml of the stock culture. The flasks were incubated at 24 °C on a rotary shaker at 250 rpm for 4 days to obtain the first stage seed. The above procedure was repeated using the first stage seed to obtain the second stage seed. This second stage seed was then used to inoculate the fermentation medium at 5% v/v. The fermentation was carried out in 500 ml flasks, each containing 100 ml of the fermentation medium, which consisted of neopeptone 10 g and cerelose 40 g in 1.0 l tap water. The pH was adjusted to 7.4, and $CaCO_3$ (4 g l^{-1}) was added. The flasks were incubated at 24 °C in a rotary shaker at 250 rpm for 7 days.

The harvested fermentation broth (10 l) was mixed with NaCl (2 kg) and acetonitrile (MeCN, 20 l) for 15 min. The organic layer was separated and concentrated to a slurry, and then the slurry material was absorbed onto the polymeric resin, CG161 (~200 ml, Tosoh Biosep LLC, Montgomeryville, PA, USA). The salts and hydrophilic substances were removed by washing with water (20 l). Then, the absorbed organic material was eluted with 85% aq. MeOH (4 l) to yield ~2.4 g of dried material after concentration *in vacuo*. Part of this organic material was purified on a semi-preparative ODS-A HPLC column (YMC, 120 Å, S-7, 20×250 mm; Waters HPLC, Millennium System (Milford, MA, USA), equipped with a photodiode array detector). The column was eluted with a gradient of MeCN-H_2O: 5–100% MeCN in 50 min, and then held isocratically with 100% MeCN for an additional 15 min with a flow rate of 15 ml min^{-1}. All fractions were collected and analyzed on the basis of a UV chromatogram. Pure **1** (~5 mg) was obtained from three injections of the enriched material (40 mg each).

The structure of **1** was mainly elucidated by extensive one- and two-dimensional NMR analyses. In the ^{1}H-NMR spectrum, a total of 17 carbon-attached protons were detected. Three methyl and one methine signals were observed in the aliphatic region, and eight resonances were observed in the low-field region. In the ^{13}C NMR spectrum, 21 carbon signals were detected, in which a conjugated ketone functionality (C-6, δ 200.0) was identified. The molecular ion *m/z* 385, $[M+H]^+$ was observed on an electrospray ionisation-MS instrument (Applied Biosystem, Foster City, CA, USA, API-150Ex spectrometer), and therefore the molecular formula of **1** was calculated as $C_{21}H_{20}O_7$. From the analyses of NMR and MS data, three hydroxyl groups were proposed to be present in the molecule based on only 17 protons observed in the ^{1}H-NMR spectrum. The azaphilone skeleton was mainly determined by ^{1}H–^{13}C long-range correlations measured in a heteronuclear multiple bond correlation (HMBC) experiment. The methyl group showing a doublet–doublet resonance (H_3-3′, δ 1.88, dd, J=7.0, 1.7 Hz) in ^{1}H-NMR was determined to be

Schering-Plough Research Institute, Kenilworth, NJ, USA
[1]Current address: Cubist Pharmaceuticals, Inc. 65 Hayden Ave., Lexington, MA 02421, USA.
[2]Current address: SMP International LLC, 42 Brentwood Drive, Verona, NJ 07044, USA.
[3]Current address: Drew University, Charles A. Dana Research Institute, 36 Madison Ave., Madison, NJ 07940, USA.
Correspondence: Dr S-W Yang, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-1300, USA.
E-mail: shu-wei.yang@spcorp.com
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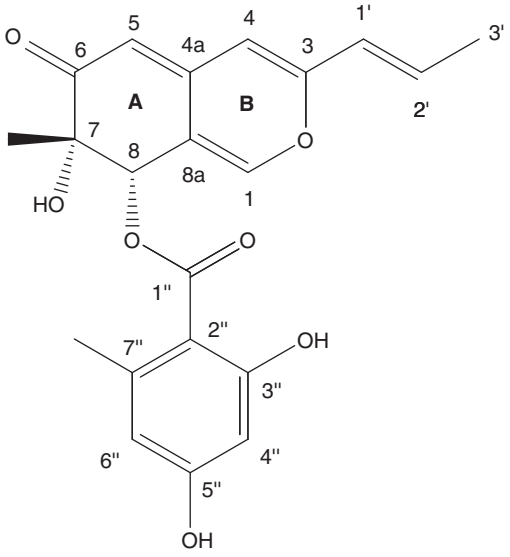

Sch1385568 (**1**)

Scheme 1 Structure of **1**.

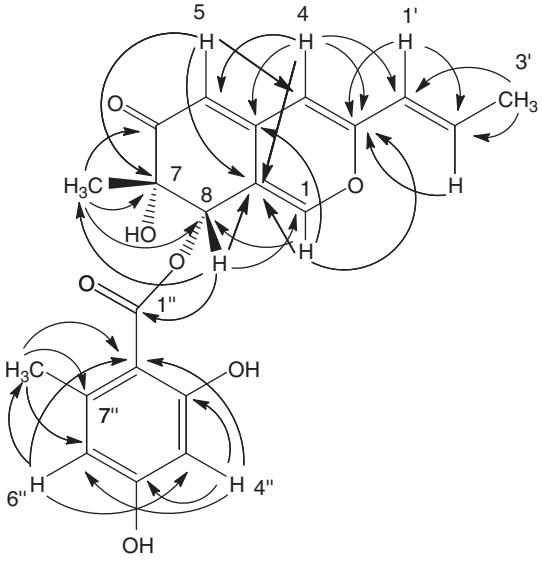

Figure 1 HMBC correlations of **1**.

adjacent to a double bond consisting of two olefinic methine carbons (C-1′ and C-2′) because of the observation of the correlations between H_3-3′ and C-1′ (δ 124.4) and C-2′ (δ 136.0). This double bond was conjugated to the additional two double bonds and further extended to the ketone (C-6) at the other end on the basis of the following correlations: H-1′ to C-3; H-2′ to C-3; H-4 to C-3, C-4a, C-5, and C-1′; H-5 to C-4 and C-7; CH_3-7 to C-6 and C-7 (Figure 1). The NOE correlations observed between H-1′ and H_3-3′, H-1′ and H-4′, and H-4 and H-5 established linear conjugation and regiochemistry of the ketone-tri-ene moiety. The oxygenated olefin proton signal (H-1, δ 7.75) showed correlations to the oxygenated olefinic C-3 (δ 157.5), C-4a (δ 146.7) and C-8a (δ 117.3) assembling a pyran moiety (ring B). The second methyl group (7-CH_3, δ 1.36, s) substituted on an oxygenated quaternary carbon (C-7, δ 75.3) was located adjacent to

Table 1 NMR spectral data for compound 1 in CD_3OD[a]

C/H no.	*^1H (δ)*	*^{13}C (δ)*[b]	*^1H-^1H COSY*
1	7.75, 1H, d, *J*=1.2	150.9 d	H-5
3		157.5 s	
4	6.25, 1H, s	103.9 d	
4a		146.7 s	
5	5.44, 1H, d, *J*=1.2	105.9 d	
6		200.0 s	
7		75.3 s	
7-Me	1.36, 3H, s	24.6 q	
8	5.88, 1H, s	76.2 d	
8a		117.3 s	
1′	6.11, 1H, dq, *J*=15.8, 1.7	124.4 d	H-2′
2′	6.57, 1H, dq, *J*=15.8, 7.0	136.0 d	H-1′, H-3′
3′	1.88, 3H, dd, *J*=7.0, 1.7	18.7 q	H-2′
1″		172.3 s	
2″		105.7 s	
3″		166.5 s	
4″	6.11, 1H, d, *J*=2.5	101.8 d	H-6″
5″		164.3 s	
6″	6.13, 1H, d, *J*=2.5	112.8 d	H-4″
7″		145.2 s	
7″-Me	2.25, 3H, s	24.7 q	

[a]Recorded on a Varian Unity 500 NMR instrument (Varian, Palo Alto, CA, USA) at 500 MHz for ^1H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). δ in ppm; *J* in Hz.
[b]The proton-attached carbon signals showed cross peaks with its corresponding proton signals in the HSQC-COSY spectrum.

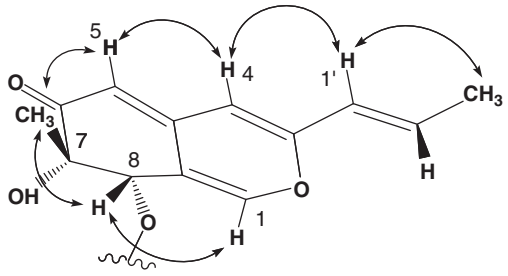

Figure 2 The relative configuration of **1** and NOE correlations observed in NOESY spectrum (represented by double arrows).

the conjugated ketone (C-6) and an aliphatic oxygenated methine carbon (C-8, δ 76.2) because of the significant coupling of CH_3-7 and C-7, C-6 and C-8. C-8 was further identified to connect to C-8a based on the following long-range correlations: H-8 to C-1 and C-8a; H-1 to C-8. Therefore, the six-member ring A was constructed on the basis of these evidences. Thus, the 7,8-dihydro-6H-isochromen-6-one ring skeleton was determined.

The remaining seven carbons were constructed to represent a 2,4-dihydroxy-6-methyl-benzoyl moiety based on the following observation: correlations H-4″ to C-2″, C-3″, C-5″ and C-6″; H-6″ to C-2″, C-4″, C-5″ and CH_3-7″; CH_3-7″ to C-2″, C-6″ and C-7″. The ^{13}C chemical shifts of the highly substituted benzoyl moiety were identical to the data of the same benzoyl moiety reported in the literature.[20] The benzoyl group was unambiguously assigned to the 8-*O* position based on the correlation of H-8 and C-1″ (δ 172.3). Thus, the two-dimensional structure of **1** was determined, and the unambiguous assignment of the ^1H and ^{13}C chemical shifts was accomplished on the basis of the two-dimensional NMR data analyses including ^1H–^1H COSY, HSQC and HMBC as detailed in Table 1.

The stereochemistry of **1** was established by the analyses of ^1H–^1H coupling constants and NOESY. The double bond Δ1′,2′ was determined as *trans* because of the typical large coupling constant (J=15.8 Hz). The relative stereochemistry on C-7 and C-8 was assigned as *cis* configuration on the basis of the NOE correlations between H-8 and CH_3-7 as shown in Figure 2. The highly conjugated system and the double bond Δ1,8a led to the flat bicyclic ring system, causing pseudoaxial orientation of the 7-methyl group, sterically close to H-8. The absolute configuration of **1** was not studied because of the limited amount of the sample.

Most of the previously reported azaphilones possess benzoyl substitution on the C-7 position.[3–5] Benzoyl substitution on C-8 for azaphilone is rare.[6,7,21] To the best of our knowledge, Sch1385568 (**1**) represents the fourth example of a C-8 benzoyl-substituted azaphilone. It is a close analog of Sch 725680 and mitorubrinic acid B. Sch 725680 is a 1,8a-dihydroazaphilone derivative of **1**[21] and mitorubrinic acid B is an oxidative acidic analog of **1** at the 3′ position.[7]

Sch1385568 (**1**) was evaluated for its antimicrobial activity. It displayed antifungal activity against *Saccharomyces cerevisiae* (PM503)[22] with an MIC of 32 μg ml^{-1}, and it was inactive against *Candida albicans* (C43) with an MIC of 256 μg ml^{-1}. In addition, **1** did not show antibacterial activity against *Staphylococcus aureus* at 256 μg ml^{-1}.

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