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A New Antimicrobial Dibenzofuran Sch 725421 from an Unidentified Fungus

Shu-Wei Yang,* Tze-Ming Chan, Reena Patel, Joseph Terracciano, David Loebenberg, Mahesh Patel and Min Chu†

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

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In the course of our continuing search for novel antimicrobial agents,[1~4] we have isolated a novel dibenzofuran, Sch 725421 (**1**), from an unidentified culture. In this paper, we describe the isolation and structure elucidation of **1** using extensive NMR spectroscopic analysis. The antimicrobial activity of **1** was also reported.

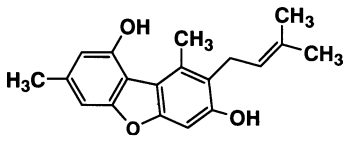
In the preliminary screening, the unidentified fungal culture, ILF016055, showed antimicrobial activity. Thus, further scale-up fermentation and bioassay-guided fractionation were carried out. Fermentation studies of the unidentified fungus were carried out in shake flasks. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The germination medium contained Proteus Peptone (5 g/liter), NaCl (5 g/liter), KH_2PO_4 (5 g/liter), yeast extract (3 g/liter), cerelose (20 g/liter) and soybean grits (5 g/liter). The pH was adjusted to 7.2 prior to autoclaving. A 250 ml Erlenmeyer flask containing 70 ml of this medium was inoculated with 2.0 ml of the stock culture. The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 96 hours. The 2.5 ml of this seed culture was used to inoculate another 250 ml Erlenmeyer flask containing 70 ml of the same seed medium and the flask was incubated under the same conditions as above for 96 hours.

Five percent of the second germination was used to inoculate the fermentation medium. The fermentation was carried out in a 500 ml Erlenmeyer flask containing 100 ml of the fermentation medium. The fermentation media used contained sucrose (30 g/liter), glucose (10 g/liter), Instant Ocean (1 g/liter), K_2HPO_4 (3 g/liter), yeast extract (3 g/liter) and corn steep powder (3 g/liter). The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 120 hours.

The harvested fermentation broth (10 liters) was stirred with 2 kg of NaCl and 20 liters of acetonitrile (MeCN) for 15 minutes. The organic layer was separated and dried to slurry in vacuum. The salt in the extract was further removed by a solid phase extraction method. Extract was absorbed onto the polymeric resin, CG161 (~200 ml) and the NaCl salt was washed out with water (20 liters). The absorbed organic material was then eluted with 200 ml 85% aq. MeOH (4 liters) to yield ~2 g of dried material, after removing solvent *in vacuo*. This organic material was fractionated on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 20×250 mm). The column was eluted with a gradient of MeCN - H_2O: 5~100% MeCN in 35 minutes, and then 100% MeCN isocratic for another 15 minutes, with a flow rate of 15 ml/minute. Fractions were collected (13 ml/fraction) by a fraction collector. Crude compound **1** (~9 mg) was obtained with five injections of 40 mg each of the crude material at retention time ~34 minutes. Crude **1** was further purified thru another HPLC column ODS-H80, (YMC J'sphere, 4 μm, 15×100 mm). The column was eluted with a two-step gradient of MeCN - H_2O: 5~50% MeCN (0.1% TFA) in 30 minutes, and then 50~100% MeCN (0.1% TFA) for another 20 minutes, with a flow rate of 3 ml/minute. Fractions were collected (~2 ml/fraction) by a fraction collector. Pure **1** (~1.6 mg) was obtained with three injections of 3 mg each of the crude **1** at retention time ~42 minutes.

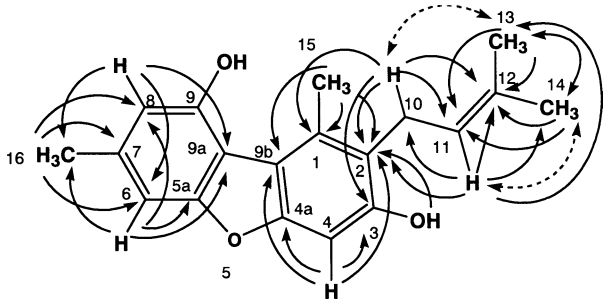
The structure of **1** was mainly elucidated by extensive 1D and 2D NMR data analysis. In the ^{13}C- and ^{1}H-NMR spectra, 19 carbons and 20 protons were observed, respectively. The molecular formula of **1** was therefore established as $C_{19}H_{20}O_3$ ($(M+H)^+$: m/z 297) from positive ESI-MS measurement (performed on a Waters MicromassZQ mass spectrometer). Multiplicity of the carbons was determined by APT experiment, and the proton-attached carbon resonances were assigned to the corresponding proton signals by analysis of HSQC spectrum. The pattern of the proton signals from H-10 to H-14 (Table 1) represented a typical prenyl moiety. This was confirmed by the HMBC correlations. For instance, olefin H-11 (δ 5.04, t, J=7.0) had long range correlations with C-10 (δ 5.0), C-12 (δ 129.8), C-13 (δ 17.8), and C-14 (δ 25.5), and the detail correlations were shown in Figure 1. Thus the prenyl group was established and assigned. Excluded this five-carbon unit, 12 aromatic and two aryl-substituted methyl carbon signals were observed in the ^{13}C

* Corresponding author: shu-wei.yang@spcorp.com
† Present address: Cubist Pharmaceuticals, Inc. 65 Hayden Ave. Lexington, MA 02421, USA.



Sch 725421 (**1**)

Fig. 1. 2D NMR correlations of **1** (solid arrows: HMBC correlations; dashed arrows: NOE correlations).



NMR spectrum. The analysis of HMBC correlations further confirmed the presence of bi-phenyl moiety. Both H-6 (δ 6.79) and H-8 (δ 6.54) adjacent to the methyl group (C-16, δ 21.1) were determined by the observation of the long-range correlations between H_3-16 (δ 2.31) and C-6 (δ 102.6), C-7 (δ 135.8), and C-8 (δ 110.3). Non-protonated sp^2 carbon C-9a (δ 110.1) was determined at the *meta* position to both C-6 and C-8 based on the observation of the correlations of both H-6 and H-8 to C-9a. The observation of the cross peaks of H-6 to C-5a (δ 157.3) and H-8 to C-9 (δ 150.9) located the two oxygen-bearing sp^2 carbons C-5a and C-9, and completed the connectivity of the first phenyl ring. The substitution of the second aromatic ring was determined by the similar method. The last unassigned methyl group (H_3-15) neighboring to two substituted aromatic carbons (C-2 and C-9b) was determined by the correlations between H_3-15 (δ 2.84) and both C-2 (δ 122.3) and C-9b (δ 115.2). Observation of the correlations between both H_2-10 (δ 3.37) and H-11 and C-2 in the HMBC spectrum defined the attachment of the prenyl group at C-2 through C–C linkage. Hydroxyl-substituted sp^2 carbon (C-3) was *ortho* to the prenyl group due to the correlations between H_2-10 and C-3 (δ 154.1) and between 3-O*H* (δ 9.59) and C-2. Proton H-4 (δ 6.81) was settled on the *meta* position of C-9b (δ 115.2) and C-2 based on the long-range correlations between H-4 and both C-9b and C-2. Eventually, the connectivity of C-3 (δ 154.1), C-4 (δ 94.9), and C-4a (δ 153.8) was established from the evidence of the cross peaks observed between H-4 (δ 6.81) and two oxygen-bearing carbons, C-3 and C-4a. Thus, the second phenyl ring was constructed.

At this point all the functional groups had been assigned to the two phenyl rings except for the substitutions on quaternary carbons C-9a and C-9b. The only possibility is the direct connection of these two carbons since no other functional groups were available. From the molecular weight information, only three oxygen atoms allowed in the molecule, therefore the bi-phenyl system had to be cyclized through an oxygen linkage, and thus formed a dibenzofuran system. This was also consistent with the only two phenol protons observed in the ^{1}H NMR and the degree of unsaturation. Thus, the full structure was concluded. The observation of NOE correlations between H_3-14 and H-11, and between H_3-13 and H_2-10 allowed us to unambiguously assign all the proton and carbon resonances, as shown in Table 1.

Table 1. NMR spectral data for Sch 725421 (**1**) in DMSO-d_6[a].

C/H no.	^{1}H (δ)	^{13}C (δ)
1		131.4 s
2		122.3 s
3		154.1 s
4	6.81, s	94.9 d
4a		153.8 s
5a		157.3 s
6	6.79, s	102.6 d
7		135.8 s
8	6.54, s	110.3 d
9		150.9 s
9a		110.1 s
9b		115.2 s
10	3.37, d, *J* = 7.0	25.0 t
11	5.04, t, *J* = 7.0	123.4 d
12		129.8 s
13	1.75, s	17.8, q
14	1.63, s	25.5, q
15	2.84, s	17.9, q
16	2.31, s	21.1, q
3-O*H*	9.59, s	
9-O*H*	10.08, brs	

[a] Recorded on a Varian Unity 500 NMR instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). δ in ppm; *J* in Hz.

The dibenzofuran natural products have been reported in lichens,[5~9] cellular slime molds,[10] the fruits of *Rhodomyrtus macrocarpa*,[11] the bulbs of *Allium porrum* L.[12] *etc.* However, dibenzofuran substituted with a prenyl unit through C–C bond is rare.[11,13] The carbon skeleton

Table 2. Antimicrobial activity of Sch 725421 (**1**), MIC-48 hours (μg/ml).

Strain[a]	MIC (μg/mL)	
	1	Gentamicin
S. aureus supersensitive (HS999)	2	8
S. aureus (ATCC 29213)	4	0.06
E. faecalis (ATCC 27270)	2	4
S. pneumoniae (ATCC 49619)	>32	0.5
E. coli supersensitive (HS294)	32	2
E. coli (ATCC 10536)	>32	0.125
S. cerevisiae supersensitive (PM503)	1	>64
C. albicans (C43)	8	>64
A. fumigatus (ND158)	>32	>64

[a] Incubation for 24 hours for bacteria in the solution; 48 hours for fungi in the solution

of **1** is unique, comparing to the identified natural dibenzofurans.[5~13] Several dibenzofurans have been reported to possess antimicrobial activity.[10,12]

Compound **1** exhibited antibacterial and antifungal activity against various strains. The MIC values of **1** and the control were shown in Table 2. Sch 725421 (**1**) showed potent inhibitory activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Saccharomyces cerevisiae*, and *Candida albican* strains with MIC values of 4, 2, 1 and 8 μg/ml, respectively.

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