Structure Elucidation of a New Diketopiperazine Sch 725418 from *Micromonospora* sp.

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In the course of our continuing search for novel antimicrobial agents,[1~4] we have isolated a novel diketopiperazine, Sch 725418 (**1**), from culture *Micromonospora* sp. In this paper, we describe the isolation and structure elucidation of **1** using high resolution ESI-MS and extensive NMR spectroscopic analysis.

Fermentation studies of the SP culture 94-00889 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 (g/liter) glucose (10 g), trehalose (10 g), Difco Tryptone (5 g), soyflour (5 g) and yeast extract (5 g). The pH was adjusted to 7.2 and $CaCO_3$ (2.0 g/liter) was added. 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 28°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 (g/liter) PD-650 Dextrin (50 g), ProFlo Flour (35 g), cerelose (5 g), $CaCO_3$ (7 g), and $CoCl_2$ (0.24 mg). The flasks were incubated at 28°C on a rotary shaker at 250 rpm for 96 hours.

The fermentation culture broth (2 liters) was stirred with 100 g of NaCl and 4 liters of acetonitrile (ACN). The organic layer was separated and dried in vacuum. The salt in the extract was further removed by a solid phase extraction (SPE) method. Extract was absorbed onto the polymeric resin, CG161 (~100 ml) and the NaCl salt was washed out with water (200 ml). The absorbed organic material was then eluted with 200 ml 40% aq. ACN, and 80% aq. ACN to yield 432 and 73 mg of dried material, respectively, after removing solvent *in vacuo*. The organic material of 80% ACN fraction was fractionated on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 20×250 mm). The column was eluted with a three-step gradient of ACN - H_2O: 5~40% ACN in 60 minutes, 40~85% gradient in 35 minutes, and then 85%~100% in another 15 minutes, with a flow rate of 15 ml/minute. Fractions were collected (13 ml/fraction) by a fraction collector. Pure **1** (~1 mg), **2** (~2 mg), and **3** (~1 mg) were obtained with two injections of 40 mg of above 80% ACN fraction at retention time ~59, 49, and 46 minutes, respectively.

The molecular formula of **1** was established as $C_{24}H_{24}N_4O_2$ ($[M+H]^+$: found 401.1980, calcd. 401.1972) from high-resolution positive ESI-MS (performed on a PE Sciex QSTAR mass spectrometer, positive ion ESI-HR-MS measurements). The structure of **1** was further elucidated by extensive 1D and 2D NMR data analysis. Only 12 carbon signals and 10 proton signals (12 protons by integration calculation) observed in the 1D NMR spectra, indicating the possibility of symmetric dimmer skeleton. The pattern of the aromatic proton signals was the typical pattern of indole moiety: typical H-2 (δ 6.55, s), *N*–H (δ 10.85, s) signals, and four neighboring aromatic carbons (between δ 6.4~7.4). The observation of 8 aromatic carbon (C-2~C-9) resonances also supported this assumption (see Table 1). The analysis of HMBC correlations further confirmed the presence of indole moiety, and thus the proton and carbon signals in the indole moiety were assigned unambiguously as shown in Table 1. Determination of the sp^3 methylene (C-10) substitution on C-3 position was based on the observation of the long range correlation of H_2-10 (δ 2.10, 2.71) to C-2 (δ 124.2), C-3 (δ 109.5), and C-8 (δ 127.1). Proton H-11 (δ 4.07) was adjacent to H_2-10 based on ^{1}H-^{1}H COSY and HMBC correlations. The methine carbon (C-11) was determined to be next to an acyl carbonyl (C-12) functionality and a *N*–CH_3 group based on the correlations of H_2-10 to C-11 (δ 62.3) and C-12 (δ 165.5), *N*–CH_3 (δ 2.60) to C-11, and H-11 to C-12 and *N*–CH_3 (δ 32.3). At this point, all proton and carbon signals were connected and assigned to the formula $C_{12}H_{12}N_2O$, which is the half of the previously

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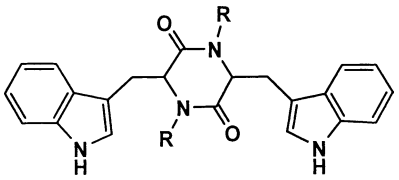
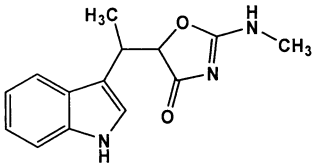
Table 1. NMR spectral data for Sch725418 (**1**) in DMSO-d_6[a].

C/H no.	^{1}H (δ)	^{13}C (δ)
2, 2'	6.55, s	124.2 d
3, 3'		109.5 s
4, 4'	7.31, d, *J* = 8.0	118.4 d
5, 5'	6.98, t, *J* = 8.0	118.5 d
6, 6'	7.06, t, *J* = 8.0	121.0 d
7, 7'	7.29, d, *J* = 8.0	111.4 d
8, 8'		127.1 t
9, 9'		136.1 s
10, 10'	2.10, dd, *J* = 15.0, 5.0	28.4 d
	2.71, dd, *J* = 15.0, 5.0	
11, 11'	4.07, t, *J* = 5.0	62.3 d
12, 12'		165.5 s
N-CH_3	2.60, s	32.3, q
N-H	10.85, s	

[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.

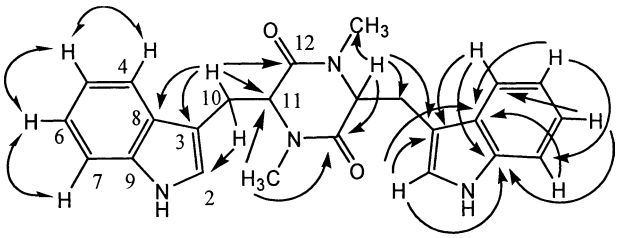


Sch 725418 (**1**), R = CH_3
Fellutanine A (**2**), R = H



Indolmycin (**3**)

Fig. 1. 2D NMR correlations of **1** (single arrows: HMBC correlations; double arrows: ^{1}H-^{1}H correlations).



determined molecular formula $C_{24}H_{24}N_4O_2$. In order to arrange the structure as a symmetric dimmer to be consistent with the molecular formula, the diketopiperazine moiety was proposed. The ^{1}H and ^{13}C chemical shifts of the diketopiperazine moiety in **1** matched the literature data.[5~8] This also explained the long range correlation of *N*–CH_3 to C-12′ (δ 165.5). The detailed 2D NMR correlations were shown in Figure 1, and the assignments were listed in Table 1. Thus, the full structure was concluded. The stereochemistry of **1** was not determined due to the insufficient amount of material for X-ray crystallographic analysis.

Fellutanine A (**2**)[9] and indolmycin (**3**)[10] were also isolated in this study. Fellutanine A (**2**) is a *N*,*N*-di-demethylated analog of **1**, according to its MS and NMR data by comparison to the literature data.[5] Indolmycin (**3**) has been reported as an antibacterial agent.[10]

Compound **1** exhibited inhibitory activity against a supersensitive strain of *Saccharomyces cerevisiae* (PM503).[1] The MIC values of **1** is 32 μg/ml. Sch 725418 (**1**) did not show significant antimicrobial effect against other tested strains, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *E. coli*, and *C. albicans*.

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