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Pseurotin A: An Antibacterial Secondary Metabolite from *Aspergillus fumigatus*

M.A.U. Mehedi†, Abul Hossain Molla†, Proma Khondkar‡, Sherin Sultana¶, M. Anwarul Islam‡, Mohammad A. Rashid* and Rasheduzzaman Chowdhury
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh
E-mail: rashidma@univdhaka.edu; rashid_phdu@yahoo.com

Bioassay guided fractionation of a chloroform extract of *Aspergillus fumigatus* culture afforded pseurotin A (**1**). The compound was identified by a series of spectral data including 1D/2D NMR and MS analyses. The extract and pseurotin A (**1**) showed mild to moderate antimicrobial activity against a panel of gram +ve and gram -ve bacteria and a fungus. The minimum inhibitory concentration of **1** was found to be 64 µg/mL against the most susceptible, *Bacilus cereus* and *Shigella shiga*.

Key Words: *Aspergillus fumigatus*, Pseurotin A, Antibacterial.

INTRODUCTION

Over the past few years, a significant number of secondary metabolites have been isolated from fungal sources that exhibited bioactivity[1]. Indeed, the structural diversity of these metabolites makes the filamentous fungi a potential source of new leads for drug discovery and development[2]. *Penicilium* and *Aspergillus* are amongst the richest sources of fungal antibiotics from the family Aspergillaceae[3].

As a part of our ongoing research for antimicrobial principles[4], we isolated a fungus, *A. fumigatus* that was previously reported to produce a wide range of bioactive compounds, including pyripyropenes, fumagillin, fumiquinones A and B, spinulosin, synerazol and many others[5-8]. Herein, the antibacterial activity and the revised assignment of ^{13}C NMR data of pseurotin A (**1**) from *A. fumigatus* are reported. Although the compound was previously isolated from a marine-derived *A. fumigatus*, its antibacterial activity has not been tested hitherto.

EXPERIMENTAL

General procedures: NMR spectra (1D and 2D) were obtained on a Varian VXR 500S (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer, using the residual non-deuterated solvent ($CDCl_3$) as internal standard. The number of attached protons

†Department of Applied Chemistry and Chemical Technology, University of Rajshahi, Rajshahi, Bangladesh.
‡Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh.
¶Khademul Islam Girls College, Rajshahi, Bangladesh.

for ^{13}C signals was determined using the DEPT pulse sequence. HSQC and HMBC spectra were optimized for $^{1}J_{C\text{-}H}$ of 140 Hz and $^{n}J_{C\text{-}H}$ of 8.3 Hz, respectively. COSY-45 spectra were used to determine the proton-proton connectivities. Accurate mass measurements were determined on a JEOL SX 102 mass spectrometer using *m*-nitro benzyl alcohol (NBA) as matrix. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Column chromatography was carried out using Merck Si gel 60 H (70-230 mesh). TLC was carried out using Merck Kieselgel 60 PF_{254} plates.

Microorganisms: An antagonist organism was isolated from a soil sample of Rajshahi University graveyard by crowded plate technique[9] and identified as *A. fumigatus* on the basis of morphological characteristics and biochemical studies.

Extraction and isolation of compound 1: The culture filtrate (15 L) was extracted with chloroform (5 L) at room temperature followed by evaporation of solvent by rotary evaporator yielding chocolate colored amorphous powder (0.125 g). Compound **1** (0.004 g) was purified by column chromatography followed by preparative TLC using a mixture of chloroform and methanol (40:3) as mobile phase.

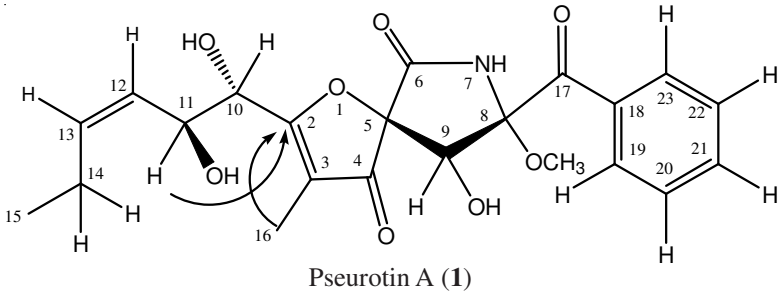
Pseurotin A (1): White crystalline solid; $[\alpha]_D$: -40° (c 0.5, MeOH) (Lit.[10] -5°); ^{1}H NMR (500 MHz, $CDCl_3$): 0.98 (3H, d, J = 7.5 Hz, H-15), 1.68 (3H, s, H-16), 2.10, 2.16 (2H, sext, J = 7.5 Hz, H-14), 3.44 (3H, s, OMe), 4.60 (1H, d, J = 4.5 Hz, H-10), 4.70 (1H, s, H-9), 4.76 (1H, dd, J = 9.0, 4.5 Hz, H-11), 5.30 (1H, t, J = 9.0 Hz, H-12), 5.60 (1H, br dd, J = 9.0, 7.5 Hz, H-13), 7.50 (2H, t, J = 7.0 Hz, H-20/H-22), 7.65 (1H, t, J = 7.0 Hz, H-21), 8.30 (2H, d, J = 7.0 Hz, H-19, H-23), 8.37 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$): 6.0 (C-16), 14.1 (C-15), 21.4 (C-14), 51.8 (OMe), 70.6 (C-10), 70.7 (C-11), 73.1 (C-9), 90.5 (C-8), 92.7 (C-5), 113.3 (C-3), 126.4 (C-12), 128.6 (C-20), 128.6 (C-22), 130.7 (C-19), 130.7 (C-23), 132.4 (C-18), 134.7 (C-21), 136.6 (C-13), 166.8 (C-6), 186.0 (C-2), 195.2 (C-17), 196.5 (C-4); FABMS: m/z $[M + H]^+$ 432.

Bioassays: The antimicrobial assay was performed by measuring zones of inhibition (mm) using standard disc diffusion technique[11]. A positive control, amoxicillin (10 μg/disc) was used for comparison purpose, whilst a blank disc impregnated with appropriate solvent was used as a negative control. In addition, the minimum inhibitory concentrations (MICs) of pseurotin A (**1**) against *Bacillus cereus* and *Shigella shiga* were determined by serial dilution technique[12]. DMSO solution of compound **1** was assayed for cytotoxic properties in a 2-day *in vitro* assay[13] and for anti-HIV activities in an *in vitro* XTT-based assay[14].

RESULTS AND DISCUSSION

Repetitive chromatographic purification of a chloroform extract of *A. fumigatus* culture yielded compound **1**. The molecular formula of **1** was determined by FABMS (m/z, 432 $[M + H]^+$) as $C_{22}H_{25}NO_8$ that suggested 11 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectral data of **1** revealed the presence of a monosubstituted benzene ring (2H at δ 7.50, t, J = 7.0 Hz; 2H at d 8.30, t, J = 7.0 Hz and 1H at δ 7.65,

t, J = 7.0 Hz), a disubstituted *cis* double bond (δ 5.30, t, J = 9.0 Hz and 5.60, br dd, J = 9.0, 7.5 Hz), two oxymethine protons (δ 4.60, d, J = 4.5 Hz and 4.76, dd, J = 9.0 and 4.5 Hz) and three methyl groups: O-(δ 3.44, s), allylic (δ 1.68, s) and aliphatic (δ 0.98, s) methyls. Based on these characteristic structural features and a comprehensive database search for possible compounds in the literature, the compound was identified as pseurotin A, previously isolated from microbial sources including *Pseudewotaum ovalis*[10] and a marine-derived *A. fumigatus*[6].



Pseurotin A (**1**)

In order to unambiguously determine the structure we performed 2D NMR including ^{1}H-^{1}H COSY, HSQC and HMBC experiments. Detailed analyses of spectral data revealed that most of the ^{1}H and ^{13}C NMR data were in line with the previously published values[6,10] except two inconsistencies. In the literature, two quaternary carbons C-2 and C-6 were assigned to δ_C of *ca.* 167 and *ca.* 187 ppm, respectively. In contrast in the current study, we observed clear ^{3}J correlations of H-11 (4.76, dd, J = 9.0, 4.5 Hz) and H-16 (1.68, s) to δ_C 186.0. Therefore, we propose that the C-6 ketone and the quaternary-olefinic C-2 should be reassigned to δ_C 166.8 and 186.0, respectively. The assignment of C-6 to δ_C 166.8 is consistent with the previously reported values for amide carbons (δ_C *ca.* 160-178) in peptides[15] and macrolides[16].

The chloroform extract of *A. fumigatus* culture and pseurotin A (**1**) were tested for antimicrobial, anticancer and HIV inhibitory activities. Although the extract was mildly growth inhibitory against the tested organisms at 200 μg, the compound (**1**) showed relatively higher activities at the same dose. Notably, the compound was found inactive against the tested fungus, *Saccharomyces cerevisiae*. The result is consistent with the previous yeast assay data using wild-type as well as cell cycle-related mutant strains of *S. cerevisiae*[6]. The minimum inhibitory concentration (MIC) of **1** was measured to be 64 μg/mL against both *Bacillus cereus* and *Shigella shiga*. The data (Table-1) indicate that pseurotin A (**1**) is selectively antibacterial. The activity (at higher dose) is comparable in some cases with the standard antibiotic such as amoxicillin. Although speculative, the compound may bind to a general macromolecular target that is present in bacteria but not in fungi.

The purified compound exhibited no anti-HIV or anticancer activity at a test concentration of 50 μg/mL (data not shown).

TABLE-1
ANTIMICROBIAL ACTIVITIES OF CHLOROFORM EXTRACT (CH) AND PSEUROTIN A (**1**)

Microorganisms	Diameter of zone of inhibition (mm)		
	CH (200 µg/disc)	Pseurotin A (**1**) (200 µg /disc)	Amoxicillin (10 µg/disc)
Gram positive			
Bacillus cereus	10	21	12
B. megaterium	11	17	14
Sarcina lutea	15	15	18
Staphylococcus aureus	07	13	31
Staphylococcus β-*haemolyticus*	–	14	27
Gram negative			
Escherichia coli	08	14	15
Shigella boydii	10	17	13
Shigella shiga	10	16	08
Fungus			
Saccharomyces cerevisiae	15	–	31

'–' indicates no zone of inhibition.

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