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Strepoxazine A, a new cytotoxic phenoxazin from the marine sponge-derived bacterium *Streptomyces* sp. SBT345

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ABSTRACT

One new phenoxazin analogue, strepoxazine A (**1**), along with two known antibiotic phenazines phencomycin (**2**) and tubermycin B (**3**) were isolated from the solid culture of *Streptomyces* sp. SBT345 which had previously been recovered from the Mediterranean sponge *Agelas oroides*. The structures of compounds **1**, **2** and **3** were determined by spectroscopic analyses including 1D and 2D NMR, and HR-ESI-MS experiments as well as comparison to the literature. We further investigated the apoptotic effect of the three compounds on the human promyelocytic leukaemia cells HL-60 and human breast adenocarcinoma cells MCF-7. Only strepoxazine A (**1**) showed cytotoxicity against leukaemia cells HL-60 cells. These results demonstrate that sponge-associated actinomycetes are rich sources for natural products with new pharmacological activities and relevance to drug discovery.

Introduction

The quest for novel biologically active structures is a challenge in natural product-directed drug discovery. The biological richness and diversity of marine source attracted scientists to seek for unique and novel molecules from the previously underexplored area, such as the deep sea sediments, marine algae, marine invertebrates, and their associated microbes. Marine sponge-associated microorganisms have exhibited good potential in producing natural products with diverse medicinal and pharmaceutical properties.[1–5] In particular, sponge-associated actinomycetes provide novel secondary metabolites[6,7] with various biological activities, such as antimicrobial,[8,9] anti-parasitic,[10,11] immunomodulatory,[12] antichlamydial,[13] antioxidant[7] and cytotoxic[14,15] activities. Our efforts on searching for novel biologically active compounds from marine sponge-associated actinomycetes led to the isolation of one new phenoxazin analogue strepoxazine A (**1**), and two known phenazine analogues phencomycin (**2**) and tubermycin B (**3**) from a sponge derived *Streptomyces* sp. SBT345. Phenoxazines and phenazines are known as antibiotics against a broad spectrum of pathogen microorganisms from plant[16,17] and human.[18,19] Herein, we reported on the structure of the new phenoxazin analogue strepoxazine A and the cytotoxic properties of the three Streptomycete's secondary metabolites.

Results and discussion

On our continuous effort to search for new cytotoxic natural products from sponge-associated actinomycetes, *Streptomyces* sp. SBT345 was selected due to its activity against the human promyelocytic leukaemia cells HL-60. *Streptomyces* sp. SBT345 (GenBank accession Nr. KP238414) was cultivated from the Mediterranean sponge *Agelas oroides* that was collected offshore Pollonia, Milos, Greece (N36.76612°; E24.51530°) in May 2013.[20] 10^5 cells of *Streptomyces* sp. SBT345 were inoculated on ISP2 medium agar plates[21] (a total of 300 plates) and incubated at 30 °C for 5 days. Colonies and agar were extracted with ethyl acetate. Colonies and agar were cut into small pieces and were macerated twice with 500 mL ethyl

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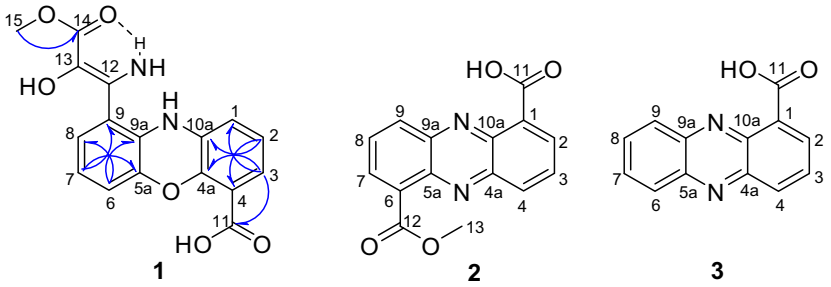

Figure 1. Structures of strepoxazine A (**1**) with HMBC correlations (arrows from H to C), phencomycin (**2**), and tubermycin B (**3**).

Table 1
NMR-spectroscopic data of strepoxazine A (**1**) in MeOD-d_4 (^{1}H: 600 MHz; ^{13}C: 150 MHz)

Position C/H No.	δ_H, Mult	COSY	δ_C, Mult	HMBC (J = 8.3 Hz)
1	7.38 (1H, s, br)	7.01	127.8 (1C)	
2	7.01 (1H, t)	7.93, 7.38	120.6 (1C)	121.5, 126.1, 127.8, 131.3, 144.3
3	7.93 (1H, d)	7.38, 7.01	131.3 (1C)	127.8, 144.2, 173.3
4			126.1 (1C)	
4a			144.2 (1C)	
5a			142.5 (1C)	
6	7.18 (1H, d)	7.24	119.6 (1C)	125.0, 122.9
7	7.24 (1H, t)	7.18	127.9 (1C)	129.4, 142.5
8	7.43 (1H, s, br)	7.24	122.9 (1C)	
9			129.4 (1C)	
9a			125.0 (1C)	
10a			121.5 (1C)	
11			173.3 (1C)	
12			133.7 (1C)	
13			134.1 (1C)	
14			168.5 (1C)	
15	3.88 (3H, s)		52.8 (1C)	168.5

acetate which was followed by 500 mL acetone under continuous shaking at 150 rpm overnight. The ethyl acetate extract (630 mg) was fractionated on a Sephadex LH20 column (32–64 μm, 100 × 10 mm, Merck) eluting with H_2O/MeOH (90:10%) to MeOH (100%), to yield 6 fractions. Fraction Nr. 5 was subjected to C18 reversed-phase chromatography and led to the isolation of compounds **1–3**[22] (Fig. 1).

Compound **1** was obtained as a yellowish powder and the molecular formula was established as $C_{17}H_{14}N_2O_6$ by ESI-HR-MS (found at m/z 341.0766 $[M-H]^-$, calcd 341.0774; and m/z 365.0757 $[M+Na]^+$, calcd 365.0750) requiring 12° of unsaturation. The following NMR spectral data were acquired using a 600 MHz instrument: ^{1}H, ^{13}C, ^{13}C-DEPT135, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC (optimized to J = 8.3 Hz) in MeOD-d_4[23] and were tabulated in Table 1. The ^{1}H NMR spectrum exhibited the resonances for six sp^2 aromatic proton signals at δ_H 7.38 (1H, br s, H-1), 7.01 (1H, t, J = 7.9, 15.7 Hz, H-2), 7.93 (1H, d, J = 7.9 Hz, H-3), 7.19 (1H, d, J = 7.7 Hz, H-6), 7.24 (1H, t, J = 7.7, 15.4 Hz, H-7), 7.43 (1H, br s, H-8) ppm, of which two independent aromatic systems were observed based on the analysis of COSY spectrum. The HMBC cross-peaks from the aromatic protons of H-2 to δ_C 121.5 (1C, C-10a) ppm, H-3 to δ_C 144.2 (1C, C-4a) ppm, H-7 to δ_C 142.5 (1C, C-5a) ppm and H-6 to δ_C 125.0 (1C, C-9a) ppm led to the assignment of a phenoxazin nucleus, in which the carbon resonance of C-4a, C-5a, C-9a and C-10a were consistent with the other analogues in the literature exemplified by Venezueline C and Venezueline D.[24] Additional NMR data conducted to assign one carboxylic acid group to C-4 by correlation observed between H-3 and a carboxylic carbon at δ_C 173.3 (1C, C-11) ppm, and one methyl ester by correlation observed between the methoxy protons at δ_H 3.88 (3H, s, H-15) ppm to the other carbonyl at δ_C 168.5 (1C, C-14) ppm in the HMBC spectrum. The presence of the carboxylic acid group was further verified in ESI-HRMS/MS spectra (collision voltage of 10 eV) by the loss mass of 43.9898 Da (calcd for COO) from the molecular ion mass m/z 341.0766 (calcd for $C_{17}H_{13}N_2O_6^-$) to fragment ion mass m/z 297.0867 (calcd for $C_{16}H_{13}N_2O_4^-$) (Fig. 2). The presence of the methyl ester was also verified by the loss mass of 59. 0234 Da (calcd for $C_2H_3O_2$) in both negative and positive ionization modes from the molecular ion mass m/z 341.0766 (calcd for $C_{17}H_{13}N_2O_6^-$) to fragment ion mass m/z 282.0639 (calcd for $C_{15}H_{10}N_2O_4^-$) (Fig. 2), as well as the molecular ion mass m/z 365.0757 (calcd for $C_{17}H_{14}N_2O_6Na^+$) to fragment ion mass m/z 306.0619 (calcd for $C_{15}H_{11}N_2O_4Na^+$) (Fig. 3) respectively. The fragment ion mass m/z 265.0611 (calcd for $C_{15}H_9N_2O_3^-$) in negative ionization mode (Fig. 2), and m/z 288.0515 (calcd for $C_{15}H_9N_2O_3Na^+$) in the positive ionization mode (Fig. 3) were deduced by losing the carboxylic acid and methoxy groups from the molecular ion.

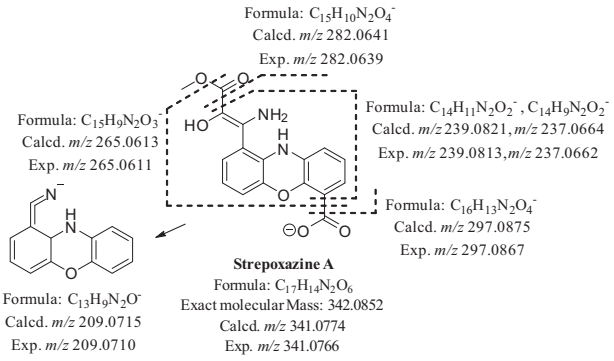

Figure 2. ESI-HRMS/MS fragmentation of strepoxazine A in negative ionization mode.