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

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One new phenoxazin analogue, strepoxazine A (1), along with two known phenazine compounds 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 compound 1, 2, and 3 were determined by spectroscopic analysis including 1D and 2D NMR, HR-ESI-MS experiments as well as comparison to literatures. We investigated the apoptotic effect of the three compounds on the human promyelocytic leukemia cells HL-60 and human breast adenocarcinoma cells MCF-7. Only strepoxazine A (1) showed cytotoxicity against HL-60 cells with IC50 at 16 µg/ml. These results demonstrate that sponge-associated actinomycetes are rich sources for natural products with new pharmacological activities and relevance to drug discovery.

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six sp² aromatic proton signals at δH 7.38 (1H, brs, 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, brs, 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 δC 121.5 (1C, C-10a) ppm, H-3 to δC 144.2 (1C, C-4a) ppm, H-7 to δC 142.5 (1C, C-5a) ppm, and H-6 to δC 125.0 (1C, C-9a) ppm led to the assignment of a phenoxazin nucleus [16] 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 Venezuelan C and Venezuelan D [17]. Additional NMR data conducted to assign one carboxylic acid group to C-4 by correlation observed between the methoxy protons at δH 3.88 (3H, s, H-15) ppm to the other carbonyl at δC 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 10eV) by the loss mass of 43.9898 Da (cald. for C₈H₁₂NO₃) to fragment ion mass m/z 297.0867 (cald. for C₂₀H₂₄N₂O₅) (Figure 2). The presence of the methoxyl ester was also verified by the loss mass of 59.0234 Da (cald. for C₈H₁₂O₃) in both negative and positive ionisation modes from the molecular ion mass m/z 341.0766 (cald. for C₂₀H₂₄N₂O₅) to fragment ion mass m/z 282.0539 (cald. for C₁₇H₁₅N₂O₄) (Figure 2), as well as the molecular ion mass m/z 365.0757 (cald. for C₂₃H₂₃N₃O₃Na) to fragment ion mass m/z 306.0619 (cald. for C₁₇H₁₅N₂O₄Na) (Figure 3) respectively. The fragment ion mass m/z 265.0611 (cald. for C₁₇H₁₅N₂O₄) in negative ionisation mode (Figure 2) and m/z 288.0515 (cald. for C₁₇H₁₅N₂O₄Na) in the positive ionisation mode (Figure 3) were deduced by losing the carboxylic acid and methoxyl groups from the molecular ion. Furthermore, m/z 237.0662 (cald. for C₁₄H₁₃N₂O₃) and m/z 260.0564 (cald. for C₁₇H₁₅N₂O₄Na) were the products of losing both carboxylic acid group and methoxyl ester side chain (Figure 2 and 3). The high-resolution fragment ion masses and the NMR data assigned the phenoxazine based structure, one carboxylic acid, and one methoxyl ester side chain which established a partial elementary composition as C₁₇H₁₅N₂O₄Na. The remaining elements C₂H₁NO were deduced as an olefinic structure that substituted by a hydroxyl and an amine group, and connects between the methoxyl ester and the aromatic carbon C-9. The enol group was deduced to connect to C-13 due to the broad proton resonance at H-8 which could be affected by the intramolecular hydrogen bond with enol proton. The enamin proton was also speculated to form the other intramolecular hydrogen bond with the ketone oxygen at C-14 to form a keto-enamine structure, which makes the whole structure more stable than the presence of single enol or enamin group [18]. Additionally, the fragment ion mass m/z 209.0710 (cald. for C₁₃H₁₆N₂O₃) presented in negative mass spectrum was interpreted with the structure shown in Figure 2 by the web server named as CFM-ID designed for annotation, spectrum prediction and metabolite identification from tandem mass spectra using the data from HMDB, MassBank, and Metlin databases [20]. The deprotonated imine at C-12 (Figure 2) further demonstrated the elucidation of enamin part.

Compound 2 was isolated as yellow solid from fraction 3 and the molecular formula was established as C₁₇H₁₆N₂O₃ with 10 degrees of unsaturation by ESI-HRMS (found at m/z 225.0655 [M + H]+, cald. 225.0664). The ¹H NMR and COSY spectra revealed six sp² aromatic protons at δH 7.67 (1H, t, J = 8.7 Hz, H-2), 7.88 (1H, t, J = 8.7, 15.4 Hz, H-3), 8.02 (1H, d, J = 8.7 Hz, H-4), 8.19 (1H, d, J = 8.7 Hz, H-7), 7.97 (1H, t, J = 8.7, 15.6 Hz, H-8), and 8.38 (1H, d, J = 8.7 Hz, H-9) ppm in two different aromatic rings and 1 methoxyl proton at δH 4.01 (3H, s, H-13) ppm. The ¹H-¹C HMBC spectrum assigned the nine aromatic carbons δC 129.5 (1C, C-2), 127.3 (1C, C-4), 142.0 (1C, C-4a), 139.1 (1C, C-5a), 132.1 (1C, C-6), 131.1 (1C, C-7), 132.0 (1C, C-9), 142.1 (1C, C-9a), 140.4 (1C, C-10a) ppm, and two carboxylic carbonyls δC 170.5 (1C, C-11), and 167.8 (1C, C-12) ppm. The carbon resonances of C-4a, C-5a, C-9a, and C-10a indicated a phenazine based structure. An exact mass search in the Database of Natural Products and comparison of the spectral data with literature [21] determined compound 2 as a known phenazine compound phencomycin which was previously isolated from *Streptomyces* sp. derived from terrestrial [21, 22] and marine sources [23].

![Figure 2. ESI-HRMS² fragmentation of strepoxazine A in negative ionization mode](image2)

![Figure 3. ESI-HRMS² fragmentation of strepoxazine A in positive ionization mode](image3)

**Figure 2.** ESI-HRMS² fragmentation of strepoxazine A in negative ionization mode

**Figure 3.** ESI-HRMS² fragmentation of strepoxazine A in positive ionization mode

Compound 3 was isolated as yellow solid from fraction 3 and the molecular formula was established as C₁₉H₁₆N₂O₄ with 10 degrees of unsaturation by ESI-HRMS (found at m/z 225.0655 [M + H]+), cald. 225.0664). The ¹H NMR revealed seven sp² aromatic protons at δH 8.72 (1H, d, J = 8.8 Hz, H-2), 8.12 (1H, m, H-3), 8.53 (1H, d, J = 8.8 Hz, H-4), 8.37 (1H, d, J = 8.3 Hz, H-6), 8.08 (1H, m, H-7), 8.10 (1H, m, H-8), 8.34 (1H, d, J = 8.3 Hz) ppm in two different aromatic rings. The ¹H-¹C HMBC spectrum assigned eight aromatic carbons δC 133.3 (1C, C-2), 143.2 (1C, C-5a), 129.7 (1C, C-6), 132.2 (1C, C-7), 131.6 (1C, C-8), 129.7 (1C, C-9), 143.2 (1C, C-9a), and 140.1 (1C, C-10a) ppm. A search in Database of Natural Products and comparison of spectral data with phencomycin and literatures [24, 25] resulted in a known phenazine compound tubermycin B which was previously isolated from *Pseudomonas* sp. [26] and *Streptomyces* sp. [25].

Various phenoxazines and phenazines have been reported for their anticancer activities against a panel of tumor cell lines, including intestinal adenocarcinoma cell lines [17, 27], gastric...
cancer cell lines, pancreatic cancer cell lines, lung tumor cell lines, breast cancer cell lines, human hepatoma cell lines, multiple myeloma cell lines, and human promyelocytic leukemia cells. The antiproliferative activity of compounds 1–3 was evaluated against human promyelocytic leukemia cells HL-60 and human breast adenocarcinoma cells MCF-7 using Vitality Test and MTT assay. The new phenoxazine strepoxazine A (1) exhibited significant cytotoxic properties against HL-60 with IC₅₀ at 16 µg/ml. However, the other two phenazines 2 and 3 did not display any activity.

In conclusion, strepoxazine I is a new phenoxazine analogue isolated from the solid culture of sponge-associated Streptomyces sp. SBT345. and exhibited potent apoptotic effect against human promyelocytic leukemia cells HL-60. The results presented in this paper highlight sponge-associated actinomycetes as a rich source for novel biologically active natural products.

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References and notes


**Supplementary Material**

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.