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Xiamycin, a pentacyclic indolosesquiterpene with selective anti-HIV activity from a bacterial mangrove endophyte

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ABSTRACT

A novel pentacyclic indolosesquiterpene, named xiamycin (**1**), and its methyl ester (**2**) have been obtained from *Streptomyces* sp. GT2002/1503, an endophyte from the mangrove plant *Bruguiera gymnorrhiza*. The structures were established by 1D and 2D NMR, MS, and X-ray crystallography, and the absolute configuration of **1** was elucidated by the modified Mosher method. Compound **1** exhibits selective anti-HIV activity; it specifically blocks R5 but has no effects on X4 tropic HIV-1 infection. In a panel of cytotoxicity assays, compound **2** showed to be more potent (geometric mean IC_{50} = 10.13 μM) compared to compound **1** (geometric mean IC_{50} >30 μM), with antitumor potency being generally less pronounced. Xiamycin represents one of the first examples of indolosesquiterpenes isolated from prokaryotes.

Endophytes are a prolific source of pharmacologically active natural products with potential medicinal or agrochemical applications.[21,19,18,28,16] In recent years, it has been shown that the mangrove plants harbor various fungal[25,14,23,11,24,13,26] and bacterial[2,1,12,7] endophytes, a largely unexplored source for novel natural products. The large-leafed mangrove (*Bruguiera gymnorrhiza*) is one of the most important and widespread mangrove species in the Pacific. While several compounds have been isolated from this plant,[3–5,8] to our knowledge, no investigations on metabolites from its endophytes have been reported. Here we describe the isolation, structure elucidation, and biological activity of two novel indolosesquiterpenes from an endophytic actinomycete from *B. gymnorrhiza*.

Streptomyces sp. GT20021503 was isolated from the stem of *B. gymnorrhiza*. HPLC-UV/MS analysis of the crude extract from the bacterial broth indicated the presence of diverse secondary metabolites. To obtain sufficient material for their isolation and full characterization, the fermentation was scaled up. Broth and mycelium from a 300 L fermentation were separated and individually extracted using an XAD-161M resin column and ethyl acetate, respectively. The combined extracts were subjected to flash chromatography on silica, followed by a Sephadex LH-20 column. Final purification was achieved by preparative reversed phase HPLC to yield compounds **1** (50 mg) and **2** (1.5 mg). Compound **1** was obtained as a pale-yellow powder, which gives a strong violet color reaction with anisaldehyde–sulfuric acid. The HRESIMS established the molecular formula of $C_{23}H_{25}NO_3$ (*m*/*z* 362.177 $[M-H]^-$). In the aromatic region of the ^1H NMR spectrum four proton signals (δ 7.96, 7.34, 7.27, 7.08) were observed that are diagnostic for a 1,2-disubstituted benzene ring. Furthermore, two singlets (δ 7.92, 7.05) suggested the presence of a carbazole ring.[22] The aliphatic region of the ^1H NMR spectrum revealed signals for one oxygenated methine (δ 4.09, H-15) and for two methyl groups (appearing as singlets, δ 1.29, Me-22, and δ 1.23, Me-23), as well as various complicated coupling systems. The ^{13}C NMR spectrum showed 23 carbon signals including one indicating a carbonyl group (δ 181.3), 12 aromatic carbon signals, one oxygenated carbon signal (δ 76.3) and another nine aliphatic carbons. The 1,2-disubstituted benzene ring was readily confirmed by H,H COSY correlations. In the HMBC spectrum, correlations between H-5, H-21 and C-3; H-10 and C-2, and H-21 and C-10, 11 substantiated the structure of the carbazole ring (Fig. 1). The H,H COSY spectrum revealed the connectivities for H-13, H-14, and H-15, as well as for H-17, H-18, and H-19. Further HMBC correlations observed for H-17 and C-17, Me-22, 23 and C-24, H-14 and C-12, 16 corroborated the partial structure for the aliphatic part as a decaline-like substructure. Based on the HMBC correlations between H-17, H-19, Me-22 and

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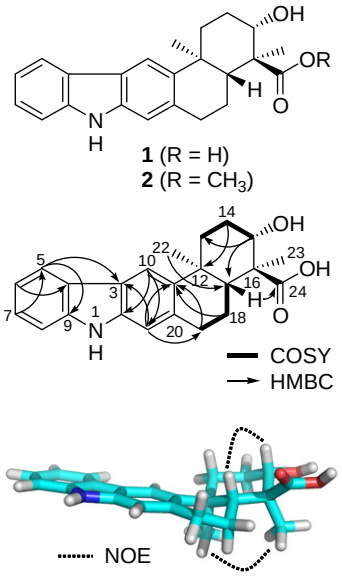

Figure 1. Structure of 1. Selected COSY, HMBC, and NOE correlations for compound **1**. (Arrows point from proton to carbon.)

C-11, and H-21 and C-19, we could connect the aliphatic part to the carbazole ring system. Compound **1**, named xiamycin, was thus established as a novel pentacyclic carbazole derivative (Fig. 1).

For compound **2**, the congener of **1** that is produced only in minute amounts, the molecular formula of $C_{24}H_{27}NO_3$ was established through HRESIMS (m/z [M+Na]$^+$ 400.1846). The NMR spectra were similar to that of **1** except for one additional methyl signal from one methoxy group (δ 3.70, δ 52.6). Consequently, compound **2** represents the methyl ester of **1**.

The relative configuration of **1** was elucidated by NOESY experiments. The NOE effect was observed between H-15 and H-17, as well as between Me-22 and Me-23, thus providing strong evidence for the substitution pattern shown in Figure 1. To unequivocally prove the proposed configuration, we crystallized compound **1** from a mixture of $CHCl_2$/MeOH and subjected a single crystal to X-ray crystallography.

The crystal structure (CCDC-780372) fully confirmed the constitution of the pentacyclic ring system and the relative configuration of **1** (Fig. 2). To establish the absolute configuration of **1**, the modified Mosher method was applied.[15] Through analysis of the specific ^{1}H NMR shifts (Fig. 3 and *Table S2*) we were able to determine the absolute configuration of **1** as 12*S*, 15*S*, 16*S*, 17*R*.

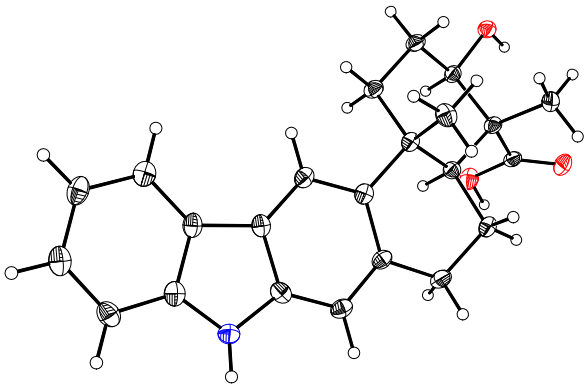

Figure 2. Molecular structure of **1**. The ellipsoids represent a probability of 40%, H atoms are drawn with arbitrary radii.

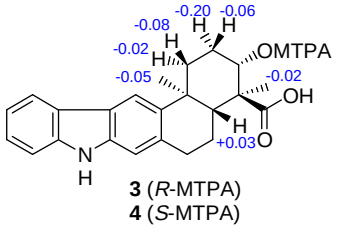

Figure 3. Elucidation of the absolute configuration using the advanced Mosher method. $\Delta\delta$ values ($\Delta S - \Delta R$) obtained for (*R*)- and (*S*)-MTPA esters of **1**.

Compounds **1** and **2** represent unusual pentacyclic indolosesquiterpenes. Indole terpene alkaloids are typical plant and fungal metabolites, such as the well-known ergot alkaloids from *Claviceps purpurea* and the anticancer agents vincristine and vinblastine from the tropical plant *Catharanthus roseus*.[17] Indolosesquiterpenes constitute a rather small group, with only few examples reported with plants origin, such as polyalthenol from *Polyalthia oliveri*,[10] isopolyalthenol and neopolyalthenol from *Polyalthia suaveolens*,[9] polyveoline from *P. suaveolens* and suaveolindole[6] isolated from *Greenwayodendron suaveolens*.[27] Tubingensin A, an indoloditerpene from the fungus *Aspergillus tubingensis* has been the only known natural product sharing the pentacyclic carbazole skeleton of **1**.[22] Surprisingly, there have been no reports of indolosesquiterpenes from bacteria. However, during the preparation of this Letter, Imamura and co-workers published the isolation and structure elucidation of the structurally related oridamycin A from a *Streptomyces* sp.[20]

To evaluate its bioactivity profile, **1** was subjected to a panel of biological assays involving antimicrobial, cytotoxic (compounds **1** and **2**) and antiviral test systems. In the cytotoxicity assays (modified propidium iodide assay), **1** exhibited only weak to moderate activities, while compound **2** showed to be more potent (geometric mean IC_{50} = 10.13 μM) (see Supplementary data). Compound **1** was also tested for its antiviral activity against entry by CXCR4 (X4) and CCR5 (R5) tropic HIV-1. Interestingly, **1** specifically blocks R5 but has no effects on X4 tropic HIV-1 infection (Fig. 4).

Albeit only being moderate, the antiviral activity of **1** is intriguing in light of the reported activity of the related fungal metabolite tubingensin against herpes simplex virus type 1.[22] Thus, it seems that the pentacyclic carbazole system could be a suitable scaffold for antiviral agents.

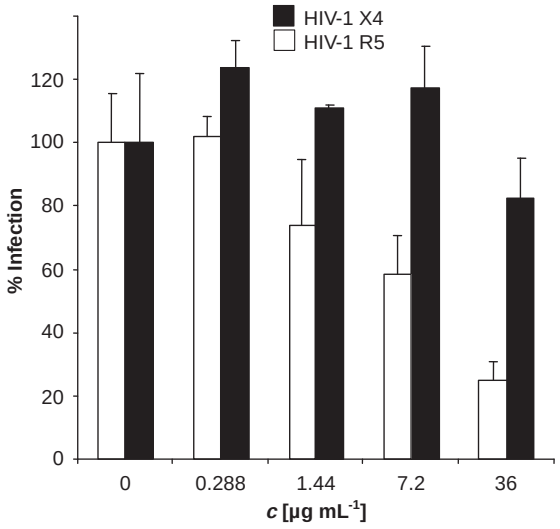

Figure 4. Anti-HIV activity of **1** against X4 and R5 tropic HIV.

In summary, we have isolated an endophytic *Streptomyces* sp. from the large-leafed mangrove, *B. gymnorrhiza*, and identified a novel indolosesquiterpene, xiamycin (**1**), along with its methyl ester (**2**). The structure of **1** was fully elucidated by HR-MS, various NMR techniques, and X-ray crystallography. The absolute configuration of **1** was established by the advanced Mosher method using MTPA. A broad bioactivity screen revealed that **1** is moderately active against HIV. Along with the recently reported oridamycins, xiamycin represents one of the first examples of bacterial indolosesquiterpenes.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.010.

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