SHORT COMMUNICATION

A new antibacterial imidazole from the marine sponge *Iricinia fusca*

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A new imidazole alkaloid (1) along with two known compounds, variabilin (2) and iricinialactam A (3) have been isolated from the Arabian marine sponge *Iricinia fusca*. The structure of the new compound was established as 4-((1, 2-dihydroxy-5-((methyl (1-methyl-1H-imidazol-4-yl) amino) pentan-3-yl) oxy)-3, 5-dimethoxy-1-methylpyrrolidin-2-one (1) by 1D and 2D NMR, and high-resolution electrospray ionization mass spectrometry (HRESIMS). Compound 1 exhibited selective growth inhibitory activity against gram-positive bacteria *S. aureus* at 100 µg/mL.

Keywords: Anticancer activity, Antimicrobial, Imidazole alkaloids, Mass spectrometry, NMR, Sponge.

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Introduction

Marine sponges are a rich source of metabolites reported in marine libraries1-3 among them; sponges of the genus *Iricina* have been proven to be a rich source of diverse biologically active secondary metabolites with novel chemical structures4-7. Several molecules isolated form *Iricina* species exhibited antineoplastic, antifouling, anti-inflammatory, cytotoxicity8-10 and antimicrobial properties11.

Materials and Methods

Experimental details

Optical rotations were determined on a Rudolph Research Analytical (AUTOPOL V) polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0 dm cell with a total volume of 1.0 mL. The UV spectra were measured on an Agilent technologies carry series UV-VIS spectrophotometer and Infrared spectra on Bruker ALPHA. All solvents were of analytical grade. Column chromatography was performed on Merck silica gel (120-200 mesh) and Sephadex LH-20 (Sigma-Aldrich Chemie GmbH). Thin layer chromatography was carried out with silica gel GF254 plates, Merck, USA. The 1H and 13C, DEPT-135, COSY, TOCSY, HSQC, HMBC at Bruker 400 MHz (or 100 MHz for 13C). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The positive ion HR-ESI-MS spectra were recorded on a Mass Q-TOF-LC-MS spectrometer (Bruker Daltonics).

Collection of sponge

The sponge *Iricinia fusca* (Carter, 1880) was collected from Bagwatibandhan (N 18°19.092’, E 072°57.343’ West coast of Maharashtra, India in February 2016. The sponge was identified by Dr. Satish S.Mokashe, Associate Professor, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, India. A voucher specimen (No. 55-25P) was deposited in the College of Fisheries, Maharashtra, India.

Extraction and isolation

In the laboratory, the sponge was washed with distilled water to remove surface salts, sand, and epiphytes. The sponge was dabbed with tissue paper to remove excess water, cut into small pieces and placed in a lyophilizer to completely dry. The dried material was (2 g dried weight) reduced to small pieces and extracted with MeOH (0.86 g). Desalting of sponge methanolic extract was done with acetone. The methanolic extract was concentrated under vacuum using a rotary evaporator at 40 °C followed by partition between hexane, DCM, water. All the partition layers were subjected to preliminary bioactivity studies (antibacterial and antifungal) by disc diffusion method.

Antimicrobial activity

The isolated compounds 1-3 were tested against antibacterial i.e., *Escherichia coli* (NCIM 2065), *Salmonella typhimurium* (NCIM 2501), *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Mycobacterium smegmatis* (NCIM 5138) and antifungal strains *Aspergillus niger* (NCIM 1207), *Penicillium chrysogenum* (NCIM 1315), *Alternaria*
sp. (NCIM 900), and Fusarium sp. (NCIM 1372). The crude extracts were dissolved in DMSO at a concentration of 1 mg/mL. The discs were loaded with different concentrations (10-500 μg/disk) of the pure compound, to find out the inhibitory potential. The diameters of the inhibition zones generated around the discs were measured (Ø in mm). Ampicillin was taken as positive control. The tests were performed in triplicates. DMSO used to dissolve the extracts and the compounds were checked for the absence of antimicrobial activity. The crude organic methanolic extract of the specimen exhibited antimicrobial activity in preliminary studies. Chromatographic separation of the MeOH extract using C18 semi-preparative reverse phase HPLC led to the isolation of two known compounds variabilin (2) and new imidazole derivative (1) as showed in Fig. S1. Preparative reverse phase HPLC led to the isolation of iricinialactam A (3) from the elucidation and bioactivity of the new compound. The compound which showed ≥ 9 mm was selected for MIC studies.

Minimum inhibitory concentration (MIC)

Purified compound was evaluated for MIC values against various Gram-positive and Gram-negative bacteria test cultures using nutrient broth described by Andrews.

Results and Discussion

During the course of our search for bioactive substances from marine sponges, we collected Iricinia fusca from Bagwatibandhan (N 18° 19.092'E 072°57.343'), Arabian Sea, West coast of Maharashtra, India. The crude organic methanolic extract of the specimen exhibited antimicrobial activity in preliminary studies. Chromatographic separation of the MeOH extract using C18 semi-preparative reverse phase HPLC led to the isolation of new imidazole derivative (1) as showed in Fig. S1 and two known compounds variabilin (2) and iricinialactam A (3) (Fig. 1). Herein, the structure elucidation and bioactivity of the new compound 1 from the I. fusca is described.

Compound 1 was obtained as a white crystalline solid, melting point 110 °C and [α]D 25 = 13.0 (c 1.0, CH3OH). The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 425.2155 [M+K] + (Fig. S2), corresponding to the molecular formula of C17H30N4O2K, indicating four degrees of unsaturation and four nitrogen atoms in the molecule. The UV absorption at λmax 214 nm indicating the absence of chromophore in the compound 1. The IR spectra showed bands at 3339, 2946, 2034, 1450, 1120, 1027 suggested the presence of hydroxyl, lactam, and aromatic moieties. Its 1H NMR chemical shifts at δH 8.85 (s, H-2), δH 7.90 (s, H-5) and 13C NMR signals at δC 140.0 (C-2), 131.8 (C), and 128.4 (C-5) were a characteristic feature of imidazole ring with C-4 substitution.

The 1H NMR spectrum of compound 1 (Fig. S3) showed three singlet's at δH 3.05, 3.91, 4.09 indicating three N-CH3 groups and one singlet at δH 3.41 (6H, s) for two methoxy groups and five methine signals at 3.73 (1H, m, H-2'), 3.60 (1H, d, J = 10 Hz, H-5'), 4.67 (1H, d, J = 3.7 Hz. H-3'), 3.60 (1H, d, J = 10Hz, H-5''), 3.38 (1H, dd, J = 3.7, 10 Hz, H-4''), and three methylene at δH 3.05 (2H, d, J = 10Hz, H-4'), 3.60 (2H, d, J = 10Hz, H-1'), 3.53 (2H, m, H-5'). The 13C, DEPT 135, 13C HMQC NMR spectrum of 1 (Fig. S4, S5, S6) exhibited total of 17 carbons (Table 1) including a carbonyl signal at δC 159.0, one olefinic methines at δC 140.0 (C-2'), four olefinic methines at δC 128.4(C-5), 75.1 (C-5''), 100.8 (C-3''), one quaternary carbon δC 131.4(C-4), two methylenes δC 50.4 (C-4'), 39.8(C-4'), three oxygenated methines at δC 71.8 (OCH3).

<table>
<thead>
<tr>
<th>Position</th>
<th>Compound 1</th>
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<th>δC/ppm</th>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
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<td>131.4, C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.90, s</td>
<td>128.4, CH</td>
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<td>50.4, CH2</td>
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</table>

Table 1 — NMR data of compounds 1 (400 MHz, CD3OD)
Staphylococcus aureus activity of this drug against methicillin-resistant hospitals, especially positive bacteria which causes nosocomial infections in This indicates its use as a potential drug against gram-

The MIC of compound 1 was found to be 280 µM. when compared with ampicillin disc at 100 µg/mL.

selective inhibitory growth activity against the compounds (2 and 3), but compound 1 exhibited antifungal, nor antibacterial activity was recorded for was also tested using the disc diffusion assay B. subtilis (NCIM 1372) as well as antibacterial activity against (NCIM 2063), and S. aureus (NCIM 2065), S. typhimurium (NCIM 2501), and P. chrysogenum (NCIM 1207), Fusarium sp (NCIM 1372) as well as antibacterial activity against E. coli (NCIM 2065), S. typhimurium (NCIM 2501), B. subtilis (NCIM 2063) and S. aureus (NCIM 2079) was also tested using the disc diffusion assay. Neither antifungal, nor antibacterial activity was recorded for the compounds (2 and 3), but compound 1 exhibited selective inhibitory growth activity against S. aureus when compared with ampicillin disc at 100 µg/mL.

The MIC of compound 1 was found to be 280 µM. This indicates its use as a potential drug against gram-positive bacteria which causes nosocomial infections in hospitals, especially S. aureus. We are pursuing the activity of this drug against methicillin-resistant Staphylococcus aureus strains.

Conclusion

Until now, sponge species like Leucetta and Leucosolenia have been reported as rich sources of imidazole alkaloids. This is the first report that the sponge Iricinia fusca, taken from the West coast of Maharashtra, produces a new imidazole alkaloid as a natural product. The isolated compound 1 was similar to 2,5-Pyrrolidinedione, 1,1′-(1,4-butanediyl)bis[3-ethoxy-] with CAS Registry Number 1403656-78-9, which was found in SciFinder to be synthesized via a chemical reaction.

In summary, three compounds were isolated from I. fusca. Compound 1 has been reported as a new alkaloid metabolite isolated from I. fusca and the structure was elucidated by NMR and mass spectroscopic analysis. The genus Iricina has been known to produce diverse metabolites like sesterterpenoids, irciniastatins, quinones, ircinalactams, and pyrroles, but the current study also showed the presence of new imidazole derivative from I. fusca. These reports indicate that a large resource of metabolites with biological significance may yet be disclosed in I. fusca.

Compound 1: 1H NMR (400 MHz, MeOD) δ 8.85 (s, 1H), 7.90 (s, 1H), 4.67 (d, J = 3.7 Hz, 1H), 4.09 (s, 3H), 3.91 (s, 3H), 3.73 (m, 1H), 3.60 (d, J = 10H, 3Hz, 3H), 3.53 (m, 2H), 3.38 (br d, J = 10 Hz, 5H), 3.14 (s, 3H), 2.66 (s, 3H), 1.48 (t, J = 7 Hz, 3H), 0.90 (t, J = 7 Hz, 3H).

Acknowledgement

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Conflict of Interest

The authors declare no competing financial interests.

References


Fig. 2 — Key COSY and HMBC correlations of compound 1


