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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 *Ircinia 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 libraries[1-3] among them; sponges of the genus *Iricina* have been proven to be a rich source of diverse biologically active secondary metabolites with novel chemical structures[4-7]. Several molecules isolated form *Iricina* species exhibited antineoplastic, antifouling, anti-inflammatory, cytotoxicity[8-10] and antimicrobial properties[11].

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 ^{1}H and ^{13}C, DEPT-135, COSY, TOCSY, HSQC, HMBC at Bruker 400 MHz (or 100 MHz for ^{13}C). 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 *Ircinia fusca* (Carter, 1880) was collected from Bagwatibandhan (N 18º19.092,' E 072º57.343' 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 ºC 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*

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Supplementary data available in the online version only.

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 diameters of the halos of inhibition can be rationalized on a qualitative basis as follows: Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active. 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[12].

Results and Discussion

During the course of our search for bioactive substances from marine sponges, we collected *Iricnia 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)[7] (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 ºC and $[\alpha]^{25}_{D}$= 13.0 (c 1.0, CH_3OH). 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 $C_{17}H_{30}N_4O_6K$, indicating four degrees of unsaturation and four nitrogen atoms in the molecule. The UV absorption at λ_{max} 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 ^{1}H NMR chemical shifts at δ_H 8.85 (s, H-2), δ_H 7.90 (s, H-5) and ^{13}C NMR signals at δ_C 140.0 (C-2), 131.8 (C), and 128.4 (C-5) were a characteristic feature of imidazole ring with C-4 substitution[13]. The ^{1}H NMR spectrum of compound 1 (Fig.S3) showed three singlet's at δ_H 3.05, 3.91, 4.09 indicating three N-CH_3 groups and one singlet at δ_H 3.41 (6H, s) for two methoxy groups and five methine signals at 3.73 (1H, m, H-2'), δ_H 3.27 (1H, d, *J* = 10 Hz, H-3'), 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 δ_H 3.05 (2H, d, *J* = 10Hz, H-4'), 3.60 (2H, d, *J* = 10Hz, H-1'), 3.53 (2H, m, H-5').The ^{13}C, DEPT 135, ^{13}C HSQC NMR spectrum of 1 (Fig. S4, S5, S6) exhibited total of 17 carbons (Table 1) including a carbonyl signal at δ_C 159.0 (C-2"), four olefinic methines at δ_C 140.0 (C-2), 128.4(C-5), 75.1 (C-5"), 100.8 (C-3"), one quaternary carbon δ_C 131.4(C-4), two methylenes δ_C 50.4 (C-5'), 39.8(C-4'), three oxygenated methines at δ_C 71.8

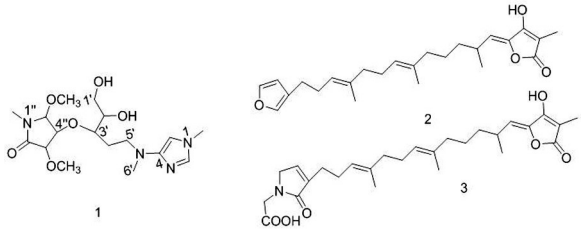

Fig. 1 — Chemical structure of isolated compounds from *I. fusca*

Table 1 — NMR data of compounds 1 (400 MHz, CD_3OD)

Position	Compound 1	
	δ_H/ppm, multi(J/Hz)	δ_C/ppm
1	N	-
2	8.85, s	140.0, CH
3	N	-
4	-	131.4, C
5	7.90, s	128.4, CH
1'	3.60, d, *J* = 10 Hz	62.7, CH_2
2'	3.73, m	73.5, CH
3'	3.27, br d, *J* = 10 Hz	71.8, CH
4'	3.05, d, *J* =10 Hz	39.8, CH_2
5'	3.53, m	50.4, CH_2
1"	N	-
2"	-	159.0, CO
3"	4.67, d, *J* =3.7 Hz	100.8, CH
4"	3.38, brd, *J* = 10, 3.7 Hz	73.5, CH
5"	3.60, d, *J* =10 Hz	75.1, CH
1' N	4.09,s	36.6
6'N	3.91, s	36.5
1"N	3.05,s	38.4
-	3.41, s	55.5, OCH_3
-	3.41, s	55.5, OCH_3

(C-3'), 73.5 (C-2'), 73.5 (C-4"), an oxygenated methylene at δ_C 62.7 (C-1'), two methoxy carbons at δ_C 55.5, three N-methyl carbons at δ_C 36.5, 36.6, 38.4 respectively.

The COSY (Fig.S7) correlations between H-4"/H-3", H-4"/H-5', as well as the HMBC (Fig.S8) correlations from H-3" to C-2" revealed the presence of pyrrolidin-2-one ring moiety. Further HMBC correlations of N-methyl proton δ_H 3.05 to C-2", δ_H 3.41 (Methoxy) with C-3", C-5" asserted the location of the methoxy groups at H-3" and H-5" positions respectively as shown in Fig. 2, which confirmed 3, 5 dimethoxy -1-methyl pyrrolidin-2-one moiety in compound 1. The ^{1}H signals at δ_H 3.54 and 3.68 and correlations of H-5'-H-4'-H-3'-H-2'-H-1' observed in the COSY spectrum, in combination with HMBC correlations of H-5' to N-methyl carbon δ_C 36.6 (C-6') revealed the presence of amino pentyl moiety in compound 1. Furthermore, HMBC correlations of 6'N to C-4 and H-3' to C-4" revealed 3, 5 dimethoxy -1-methyl pyrrolidin-2-one was linked to 1-methyl-1H-imidazol-4-yl-amino-pentan-3-yl-oxy through ether linkage showed in Fig. 2. Thus, the structure compound **1** 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 as shown in Fig. 1

Compound 1 to 3 were evaluated for their antifungal activity against *A. niger* (NCIM 1207), *P. chrysogenum* (NCIM 1315), *Alternaria sp* (NCIM 900), *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[14]. 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.

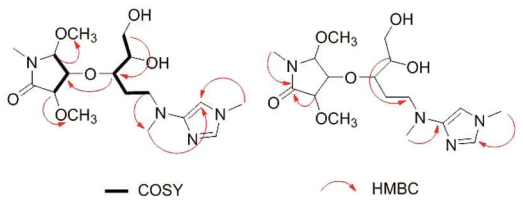

Fig. 2 — Key COSY and HMBC correlations of compound 1

Conclusion

Until now, sponge species like *Leucetta*[15] and *Leucosolenia*[16] 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, ircinialactams, 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: ^{1}H 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, 3.7 Hz, 1H), 3.41 (s, 6H), 3.27 (d, *J* = 10 Hz, 1H), 3.05 (d, *J* = 10.0 Hz, 5H).

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Conflict of Interest

The authors declare no competing financial interests.

References

1 Faulkner D J, Marine natural products, *Nat Prod Rep*, 2002, **19**, 1-49.

2 Blunt J W, Copp B R, Ping Hu W, Munro M H G, Northcote P T, *et al.*, Marine natural products, *Nat Prod Rep*, 2009, **26**, 170-244.

3 Blunt J W, Copp B R, Keyzers R A, Munro M H G and Prinsep M R, Marine natural products, *Nat Prod Rep*, 2015, **32**, 116-211.

4 Malcolm S B, Annette E, Gordon K, Jacqueline W and Ronald J Q, Cheilanthane sesterterpenes, protein kinase inhibitors, from a marine Sponge of the genus *Ircinia*, *J Nat Prod*, 2001, **64**, 300-303.

5 George R P, Jun-Ping X, Jean-Charles C, Robin K P, Larry P T, *et al.*, Antineoplastic agents. 520. Isolation and structure of Irciniastatins A and B from the Indo-pacific marine sponge *Ircinia ramosa*, *J Med Chem,* 2004, **47**, 1149–1152.

6 Maria T, Claire H, Constantinos V, Catherine H and Vassilios R, Chemical defense and antifouling activity of three Mediterranean sponges of the genus *Ircinia*, *Z Naturforsch*, 2002, **57c**, 161-171.

7 Walter B, Robiul I, Frank F, Andrew M P, Hua Z, *et al.*, Ircinialactams: Subunit-selective glycine receptor modulators from Australian sponges of the family Irciniidae, *Bioorganic Med Chem*, 2010, **18**, 2912–2919.

8 Rashid M A, Gustafson K R and Boyd M R, New chondropsin macrolide lactams from marine sponges in the genus *Ircinia*, *Tetrahedron Lett*, 2001, **42**, 1623-1626.

9 Yan S, Zhang G, Su J and Zeng L, A rare long conjugated diterpene ketene from the marine sponge *Ircinia selaginea* (Lamark), *Gaodeng Xuexiao Huaxue Xuebao*, 2001, **22**, 949-951.

10 Kokubo S, Yogi K, Udin M J, Inuzuka T, Suenaga K, *et al.*, Kohamaic acids A and B, novel cytotoxic sesterterpenic acids, from the marine sponge *Ircinia sp.*, *Chem Lett*, 2001, **30**, 176-177.

11 Sharad S, Satish M, Gupta S G, Vijay L and Dinesh K, Antimicrobial potential of the marine intertidal sponge, *Ircinia fusca* from West coast of India, *Indian J Pharm Biol Res*, 2015, **3**, 12-14.

12 Andrews J M, Determination of minimum inhibitory concentrations, *J Antimicrob Chemother*, 2001, **48**, 5-16.

13 Shin J, Rho J R, Seo Y, Lee H S, Cho K W, *et al.*, Wondonins A and B, new bis(dihydroxystyryl)imidazoles from a two-sponge association, *Tetrahedron Lett*, 2001, **42**, 1965–1968.

14 Lippert H, Brinkmeye R, Mulhaupt T and Iken K, Antimicrobial activity in sub-Arctic marine invertebrates, *Polar Biol*, 2003, **26**, 591-600.

15 Tsukamoto S, Kawabata T, Kato H, Ohta T, Rotinsulu H, *et al.*, Naamidines H, Cytotoxic Imidazole alkaloids from the Indonesian Marine sponge *Leucetta chagosensis, J Nat Prod*, 2007, **70**, 1658–1660

16 Ralifo P, Tenney K, Valeriote F A and Crews P, A distinctive structural twist in the aminoimidazole alkaloids from a calcareous Marine sponge: Isolation and characterization of Leucosolenamines A and B, *J Nat Prod*, 2007, **70**, 33-38.