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Imidazole and Dihydrofuran Derivative from a Sponge *Iricinia Fusca*

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Abstract

Chemical investigation of the sponge *Ircinia fusca* collected from the Arabian Sea, afforded two new metabolites (1-2). The compounds 1-2 showed neither antifungal nor antibacterial activity. The compounds 1-2 showed weak cytotoxic activity against HeLa, SiHa, and MDA-MB-231 cell lines. The structures of the new compounds were unambiguously established by 1D and 2D NMR and MS data.

Keywords

Sponge; Imidazole;NMR & Mass spectrometry; Natural Products; Antimicrobial and anticancer activity

Abbreviations

Methanol (MeOH).

Introduction

Marine invertebrates are a rich source of new metabolites as reported in marine libraries[1,2]. Till date majority of these compounds have been identified from marine invertebrates sources predominantly sponges[3]. Marine sponges are widely distributed from intertidal zones to thousands of meters deep in the ocean[4]. The demosponge, *Ircinia fusca* (Carter 1880) is a commonly found on the intertidal rocky shores of the Arabian Sea, India. Sponges of the genus Iricina have been proven to be a rich source of diverse secondary metabolites like Cheilanthane sesterterpenoids[5], Irciniastatins[6], quinones [7] and Ircinialactams [8]. Some of the metabolites from *Iricinia sps* exhibited antifouling, anti-inflammatory and antimicrobial activities[9]. In addition to this, a number of cytotoxic compounds have also been reported from *Iricinia* genus[10,11,12].

Material and Methods

General 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-decimeter cell with a total volume of 1.0mL. The UV spectra were measured on 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, ROESY and 400 MHz (or 100 MHz for ^{13}C) at Bruker 400 MHz (Internal standard: TMS). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The positive ion HR-ESI-MS spectrum was recorded on Mass Q-TOF-LC-MS spectrometer (Bruker Daltonics).

Collection of sponge

The sponge *Ircinia fusca* (Carter, 1880) was collected from Vayngani (N 16^{0}55.827, E 073^{0}16.973),

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West coast of Maharashtra, INDIA, in Feb 2016. The sponge was identified by Dr. Satish S.Mokashe, Associate Professor, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, 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) reduced to small pieces and extracted with methanol. Desalting of sponge methanolic extract with acetone. The methanolic extracts were concentrated under vacuum using a rotavapor at 40^0 C followed by a partition with hexane. All the partition layers were subjected to preliminary bioactivity studies (antibacterial & antifungal) by disc diffusion method [13].

Antimicrobial activity

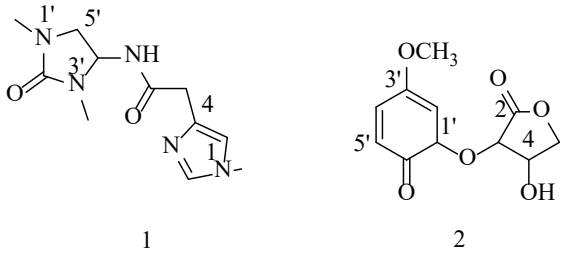
The isolated compounds 1–2 were tested against antibacterial i.e., *Escherichia coli* (NCIM 2065), *Salmonella typhimurium* (NCIM 2501), *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Mycobacterium smegmatis* (NCIM 5138) and antifungal strains *Aspergillus niger* (NCIM 1207), *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 concentration's (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). The tests were performed in triplicate and the mean values are given in Table 1. 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.

Cytotoxic assay

Cytotoxicity was evaluated against HeLa, SiHa (cervical cancer cells) and MDA-MB-231 (Breast cancer cell line) lines. Cell proliferation was followed by the colorimetric MTT test [14].

Results

During the course of our search for bioactive substances from marine sponges, we collected *Iricnia fusca* from the Arabian Sea, West coast of Maharashtra, India. The crude methanolic extract of the specimen exhibited cytotoxicity in preliminary studies was selected for further purification. Chromatographic separation of the MeOH extracts using C18 semi-preparative reverse phase HPLC yielded lead two new compounds i.e.: compound 1 (10 mg), 2 (13 mg), as showed in Fig. S1. Herein we describe the structure elucidation and bioactivity metabolites.



1 2

Figure 1. Chemical structures of isolated compounds 1-2 from *Ircinia fusca*

Compound 1

Compound 1 was obtained as white color. The specific rotation was $[\alpha]^{25}_{D}$ = 0.1 (c 1.0, CH_3OH). The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 251.18 $[M+H]^+$ (Fig. S2), corresponding to the molecular formula of $C_{11}H_{17}N_5O_2$, indicating six degrees of unsaturation. The chemical shifts of three carbons at δ_C 138.7 (CH, H-2), 131.8 (C), and 123.4 (CH, H-5) was a characteristic feature of imidazole ring with C-4 sum tuition [15]. The presence of methylene at δ_H 3.94 (2H, s, H-6) and HMBC correlations H-6 to δ_C 171.1 (amide carbon), C-2 (δ_C 138.7), C-5 (δ_C 123.4) revealed N-methyl-Imidazole-4-yl- acetamide moiety in compound 1. Further ^{1}H NMR data showed two singlets at δ_H 3.08 & 3.87 as N-methyl protons, one methine proton at δ_H 3.68 (1H, t, J = 6.5 Hz, 4'), single methylene at δ_H 3.10 (2H, d, J = 6.5 Hz, 5') as showed in Table 1. The COSY correlations between H-4'/H-3', as well as the HMBC correlations δ_H 3.08 (N- methyl proton) and H-4' to a quaternary carbon δ_C 157.8, (C-2') was a characteristic feature of the 1,3 dimethyl -2-Oxo-imidazole as showed in Fig. 2.

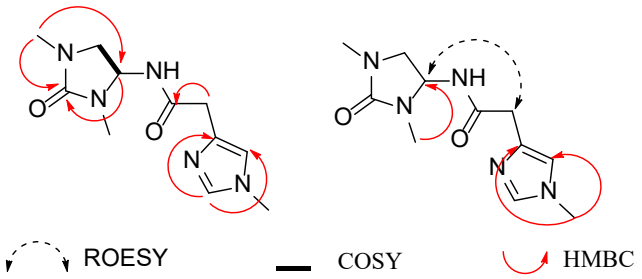


Figure 2. Key COSY, HMBC, NOE correlations of compound 1

The long range HMBC correlations of H-4' with C-6 (δ_C 29.6) confirmed that compound1 has N-(1,3-dimethyl-2-oxoimidazolidin-4-yl)-2-(1-methyl-1H-imidazol-4-yl) acetamide, this was supported by the NOE cross peaks H-4' to H-6 as showed in Fig. 2. Thus, the planar structure of 1 was elucidated as shown in Fig. 1 named as Iricimidazole.

Compound 2

Compound 2 was obtained as pale yellow viscous, and its molecular formula was calculated as $C_{11}H_{12}O_6Na$ from HR-ESI-MS at m/z 263.21 $[M+Na]^+$ (Fig. S 11) indicating six degrees of unsaturation. The specific rotation was $[\alpha]^{25}_D$ = -1.6 (c 1.0, CH_3OH).The IR spectrum showed absorption bands for 1675, 1195, 1136 cm^{-1} suggested the presence of ester and ether groups in the molecule, respectively. The ^{13}C NMR spectrum of 2 exhibited total of 11 carbons (Table 2) including two carbonyl carbons δ_C 169.2(C-2), δ_C 164.4(C-6'), one quaternary carbon C-3' (δ_C 152.5), six methine sp^2 carbons δ_C 86.5 (C-4), 71.1 (C-3), 75.1(C-1'), 90.4 (C-2'), 142.7 (C-4'), 102.3 (C-5'), oxymethylene sp^2 carbons at δ_C 62.1 (C-5), methoxy sp^2 carbon at δ_C 53.3.

The COSY correlations of H-3/H-4/H-5 indicate an isolated spin system, as well as HMBC correlations of H-5 to C-2 Fig. 3 confirmed the dihydrofuran 2-one framework in compound 2. The presence of COSY correlations H-4'/H-5' and H1'/H-2' exhibited AB spin system as showed in Fig. 3, and HMBC correlations of H-4' to C-3', C-6' revealed the presence of cyclohexene ring. The long range HMBC correlations of methoxy proton at δ_H 3.35 to C-3' asserted the location of a methoxy group at C-3' position and H-2'/H-5' to C-4', C-3' confirmed the 4 hydroxy - 3-methoxy-6-oxocyclohexa- 2,4-dien-1-yl moiety. Further, HMBC cross peaks of H-1' and to C-3 (δ_C 71.1) and H-4 to C-1' establishes that compound 1 has 4-hydroxy-3-((3-methoxy-6-oxocyclohexa-2,4-dien-1-yl)oxy) dihydrofuran-2(3H)-one. Thus, the planar structure of 1 was elucidated as shown in Fig.1, named as 'Dihydrofuranol' The NOE cross peaks of δ_H 3.35 to H-5' and H-1' supports the above confirmation as showed in Fig. 3

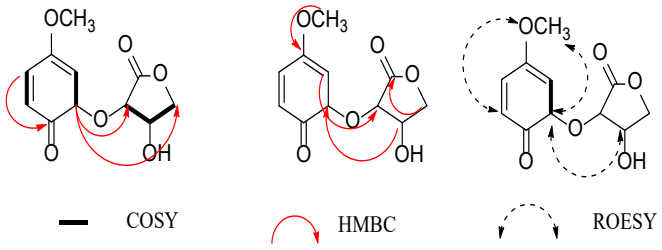


Figure 3. Key COSY, HMBC, NOE correlations of compound 2

Discussion

Compounds 1 & 2 have been reported as new metabolites from I.fusca and its structure was elucidated by NMR and mass spectroscopic analysis. Isolated Compound 1 and 2 were also evaluated for their antifungal and antibacterial activity, but none of them showed significant inhibitory activity. Compounds 1 & 2 exhibited cytotoxic activity against HeLa, SiHa (cervical cancer cells), and MDA-MB-231(Breast cancer cell line) lines with IC_{50} values 200 µg/mL.

Compound 1: ^1H NMR (400 MHz, MeOD) δ 8.87 (s, 1H), 7.53 (s, 1H), 3.93 (s, 2H), 3.87 (s, 3H), 3.68 (t, J = 6.5 Hz, 1H), 3.10 (d, J = 6.5 Hz, 2H), 3.08 (s, 3H).

Compound 2:^1H NMR (500 MHz, MeOD) δ 8.01 (d, J = 8.3 Hz, 1H), 5.90 (d, J = 4.8 Hz, 1H), 5.70 (d, J = 8.3 Hz, 1H), 4.18 (d, J = 4.8 Hz, 1H), 4.15 (d, J = 4.7 Hz, 1H), 4.01 (dd, J = 7.2, 4.7 Hz, 1H), 3.80 (m, 2H), 3.35 (s, 3H).

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