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NOTE

JBIR-44, a new bromotyrosine compound from a marine sponge *Psammaplysilla purpurea*

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Marine sponges of the order Verongida are characterized by their ability to synthesize brominated tyrosine derivatives, many of which possess potent anti-microbial and cytotoxic activities.[1] Purealin,[2–4] Lipopurealin A–E,[5,6] Purealidin A–S,[6–11] Psammaplysin A–B,[12] Purpuramine A–J,[11,13] Aplysamines 2–5[14] and Macrocyclic peptides Bastadins[15] isolated from Verongida have been previously reported as brominated tyrosine derivatives. The diverse modification of these biosynthetically related compounds occurs in both the side chain and aromatic ring of the brominated tyrosine precursors. It has been reported that many brominated tyrosine derivatives were isolated from Verongida, *Psammaplysilla purpurea*;[1,2,5–10,13–15] therefore, *P. purpurea* is a potential resource for the chemical screening of novel compounds. We thus attempted to isolate new compounds from *P. purpurea*, and resulted in the isolation of a new compound designated as JBIR-44 (**1**) (Figure 1). In this paper, we report the isolation, structure elucidation and brief biological activity of **1**.

P. purpurea (class, Demospongiae; order, Verongida; family, Aplysinellidae) was collected at a depth of −25 m from Kinwan bay, Okinawa prefecture, Japan, in February 2007. *P. purpurea* (300 g, wet) was extracted with MeOH and, after concentration in vacuo, MeOH–H_2O (3:7) was added to the aqueous concentrate. The MeOH–H_2O solution (500 ml) was partitioned with EtOAc (three times). After drying over Na_2SO_4, the EtOAc layer was evaporated to dryness. The dried residue was subjected to normal-phase MPLC (Purif-Pack SI-60, Moritex, Tokyo, Japan) eluted with a Hexane-EtOAc (0–30% EtOAc) linear gradient system, followed by elution with a $CHCl_3$–MeOH (0–90% MeOH) linear gradient system. The 10–30% MeOH elute fractions were further purified by reversed-phase HPLC using a PEGASIL ODS column (Senshu Pak, 20 i.d. ×150 mm, Senshu Scientific, Tokyo, Japan) with a H_2O–MeOH (0–100% MeOH) linear gradient system containing 0.1% formic acid to yield JBIR-44 (**1**, 4.4 mg), together with four known compounds, purealidin C (2.1 mg),[6] purpuramine F (1.8 mg),[13] araplysillin I (45.3 mg)[16] and aplysamine 4 (175 mg).[14]

Compound **1** was obtained as a colorless oil (UV (MeOH) λ_{max} (ε) 283 (1700), 208 (17 600)). The electrospray ionization-mass spectrum (ESI-MS) displayed an isotopic cluster (640.9, 642.9, 644.9, 646.9, 648.9) that was consistent with the presence of four bromine atoms. Its molecular formula was established as $C_{18}H_{16}Br_4N_2O_4$ (*m/z* $[M+H]^+$ 640.7918, −0.4 mmu) by high-resolution ESI-MS. Compound **1** also displayed the IR spectrum [(KBr) ν_{max}] at 3560, 3510, 3415, 1675 and 1630 cm^{-1}.

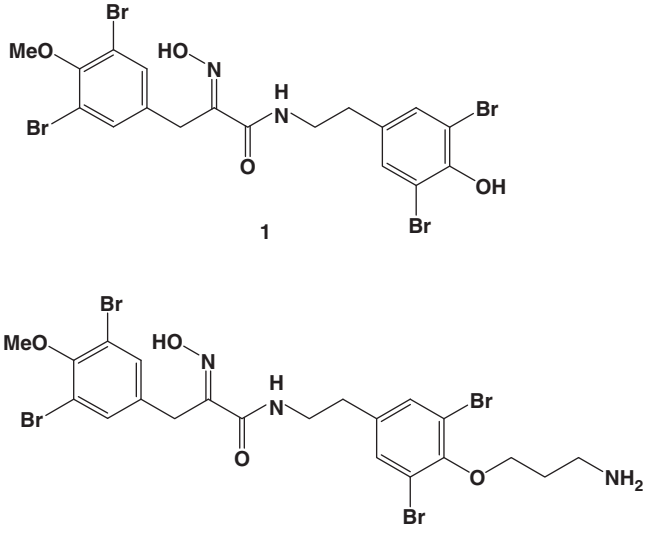

Figure 1 Structures of **1** and aplysamine 4.

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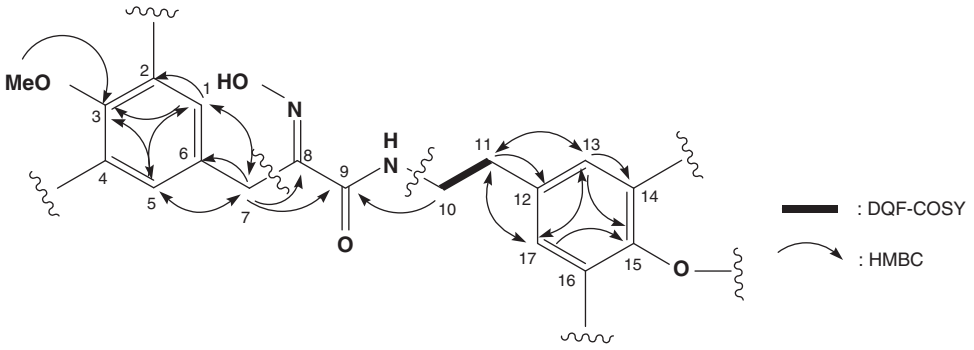

Figure 2 Key correlations in DQF-COSY (bold line) and HMBC (arrow) spectra of **1**.

The structure of **1** was elucidated as follows: The ^{1}H and ^{13}C NMR spectral data for **1** are shown in Table 1 and the structural information on **1** was further obtained by a series of 2D NMR analyses, such as heteronuclear single-quantum coherence, heteronuclear multiple-bond correlation (HMBC) and double-quantum-filtered correlation (DQF-COSY) spectra (Figure 2). Analyses of ^{1}H-^{13}C long-range couplings in the HMBC spectrum revealed two partial structures. In the HMBC spectrum, aromatic protons 1/5-H (δ_H 7.52) strongly *m*-coupled with each other and with an aromatic carbon C-3 (δ_C 152.0), which in turn long-range coupled to a methoxyl proton 3-OMe (δ_H 3.85, δ_C 61.0). A singlet methylene proton 7-H (δ_H 3.87) was long-range coupled to aromatic methine carbons C-1/5 (δ_C 134.4) and to an aromatic quaternary carbon C-6 (δ_C 137.3). Thus, the methylene carbon C-7 was deduced to be substituted at the position of C-6. The all assignment of this tetrasubstituted benzene ring moiety was established by ^{1}H-^{13}C long-range couplings as shown in Figure 2. These results revealed the presence of a 2,4-disubstituted-3-methoxy-phenylene moiety as shown in Figure 2. In the same manner, long-range couplings observed in the HMBC spectrum and the ^{13}C chemical shift at C-15 (δ_C 150.7) revealed a 4-oxygenated 1,3,4,5-tetrasubstituted benzene moiety. A methylene proton 11-H (δ_H 2.73), which was spin coupled to a methylene proton 10-H (δ_H 3.45), was ^{1}H-^{13}C long-range coupled to aromatic methine carbons C-13/17 (δ_C 133.6) and to an aromatic quaternary carbon C-12 (δ_C 134.6). ^{1}H-^{13}C long-range couplings from the methylene proton 10-H to an amide carbonyl carbon C-9 (δ_C 165.4), from the methylene proton 7-H to a hydroxyimino carbon C-8 (δ_C 153.8) and to the amide carbonyl carbon C-9, together with IR absorptions assignable to an amide and an imino functional group (1655 and 1620 cm^{-1}, respectively)[11] revealed that two tetrasubstituted benzene substructures were connected through a 2-(hydroxyimino) acetamide moiety[11] as shown in Figure 2. This structure was also supported by the ^{13}C chemical shifts of the amide and imino carbons and IR absorptions observed in aplysamine 4.[14] From the molecular formula of **1**, four bromine atoms were determined to be substituted at the position of C-2, C-4, C-14 and C-16, and a remaining hydroxyl group was assigned to an oxime functional group at the imino moiety. The geometry of C-8 at oxime moiety was elucidated as *E* from the up-field ^{13}C chemical shift of C-7 (δ_C 28.7) because of the γ-effect of the hydroxyl group of the oxime function. The different ^{13}C chemical shifts between *E* (δ_C 27.5) and *Z* (δ_C 35.7) observed in (*E,Z*)-*N,N′*-Bis [3-(3′-bromo-4′-hydroxyphenyl)-2-oximidopropionyl] cystamine,[17] of which positions corresponded to C-7 in **1**, supported the stereochemistry at C-8.

The cytotoxic effects of **1** and aplysamine 4 against human cervical carcinoma HeLa cells were determined by WST-8 colorimetric assay (Dojindo, Kumamoto, Japan). HeLa cells were treated for 48 h with various concentrations of **1** and aplysamine 4, which showed the cytotoxic effects in a dose-dependent manner with the IC_{50} values of 3.7 and 3.4 μM, respectively. These results indicate that a 3-aminopropanol chain structure at C-15 does not affect cytotoxic activity. As **1** is a structurally interesting compound, it is expected to be a potential candidate for a novel anti-cancer drug targeting new molecules. Studies on detailed biological activities are now underway.

Table 1 ^{1}H and ^{13}C NMR spectral data for 1 and aplysamine 4

	1		Aplysamine 4	
No.	*^{13}C*	*^{1}H (J/Hz)*	*^{13}C*	*^{1}H (J/Hz)*
1	134.4	7.52 (1H, s)	134.2	7.47 (1H, s)
2	118.6		118.5	
3	152.0		152.1	
4	118.6		118.6	
5	134.4	7.52 (1H, s)	134.4	7.47 (1H, s)
6	137.3		137.2	
7	28.7	3.87 (2H, s)	28.7	3.82 (1H, s)
8	153.8		153.6	
9	165.4		165.2	
10	41.7	3.45 (2H, t, 7.09, 7.33)	41.3	3.43 (2H, t, 7.09, 7.33)
11	34.9	2.73 (2H, t, 7.09, 7.33)	35.1	2.75 (2H, t, 7.09, 7.33)
12	134.6		139.9	
13	133.6	7.36 (1H, s)	134.4	7.38 (1H, s)
14	112.1		118.7	
15	150.7		152.0	
16	112.1		118.7	
17	133.6	7.36 (1H, s)	134.4	7.38 (1H, s)
18			71.6	4.06 (2H, t, 5.8)
19			28.9	2.18 (2H, tt, 5.8, 7.8)
20			38.7	3.29 (2H, t, 7.8)
MeO	61.0	3.85 (3H, s)	60.9	3.81 (3H, s)

1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were taken on an NMR System 500 NB CL (Varian, Palo Alto, CA, USA) in CD_3OD, and the solvent peak was used as an internal standard (δ_C 49.0, δ_H 3.30).

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