New Bromotyrosine Alkaloids from the Marine Sponge Psammaplysilla purpurea

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New bromotyrosine alkaloids from the marine sponge *Psammaplysilla purpurea*

Supriya Tilvi,* Celina Rodrigues, C. G. Naik, P. S. Parameswaran and Solimabi Wahidhulla

National Institute of Oceanography, Dona Paula-Goa, India

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Abstract—Seven new bromotyrosine alkaloids Purpurealidin A, B, C, D, F, G, H and the known compounds Purealidin Q, Purpurealidin E, 16-Debromoaplysamine-4 and Purpuramine I have been isolated from the marine sponge *Psammaplysilla purpurea.* Their structure was elucidated on the basis of detailed 1D, 2D NMR and MS spectroscopic data. Purpurealidin B, 16-Debromoaplysamine-4 and Purpuramine I exhibited in vitro antimicrobial activities against *E. coli*, *S. aureus*, and *V. cholerae*. In addition, Purpurealidin B and 16-Debromoaplysamine-4 were also active against *Shigella flexineri* and *Salmonella typhi* while Purealidin Q was bactericidal only against *Salmonella typhi*.

1. Introduction

Marine sponges of the order Verongida are characterized by their ability to synthesize brominated tyrosine derivatives, many of which possess potent antimicrobial and cytotoxic activities. Chemical modification occurs both in the side chain and the aromatic ring of the brominated tyrosine precursors, giving rise to a broad range of biosynthetically related compounds.[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] Macrocyclic peptides Bastadins[15] etc. have been previously reported from the sponge *Psammaplysilla* sp. In our earlier communication we reported the isolation of known compounds 16-Debromoaplysamine-4[9,16] and Purpuramine I.[13,16] The present paper deals with the isolation, structures and in vitro bioactivity of bromotyrosine metabolites Purpurealidin A–D and F–H along with Purealidin Q[10] and Purpurealidin E.[17]

2. Results and discussions

The animals were collected by scuba diving at a depth of 8–10 m from Mandapam, Tamil Nadu, India. A voucher specimen is deposited at the National Institute of Oceanography, Dona Paula Goa, India. The frozen sponge (250 g, dry weight) was extracted with Methanol (1 L×3) and concentrated under vacuo to obtain 10 g of crude extract. Successive chromatography of the crude MeOH extract on Silica gel, Sephadex LH-20 and a reverse phase column yielded 11 compounds (see Fig. 1). The structures and complete assignment of the ^{1}H and ^{13}C NMR spectra for the new compounds was determined based on extensive 1D and 2D NMR spectroscopic studies.

Compound **1**, was obtained as colourless oil. HRMS showed pseudomolecular ion peak at *m/z* 741.8, 743.8, 745.8, 747.8, 749.8 in the ratio 1.07:4.23:6.2:4.0:1.0, which indicated the presence of four bromine atoms in the molecule and established the molecular formula as $C_{23}H_{27}N_3O_4Br_4$. It was identified as Purealidin Q previously described from the Okinawan marine sponge *Psammaplysilla purea*, by comparison with the spectral data (UV, IR, 1D and 2D NMR) reported in the literature (see Table 1).[10] The stereochemistry at C1 and C6 of the spiroisoxazole ring in **1** was deduced to be *trans* from the proton chemical shift (ca. δ 4.05) of H-1 in CD_3OD.[18] The absolute configuration was not assigned. The HRMS of **1** also showed pseudomolecular peaks at *m/z* 755.8, 757.8, 759.8, 761.8, 763.8 for the minor compound Purpurealidin A (**2**) (see Table 1), which is 14 amu higher than Purealidin Q. This can be accounted from the presence of an additional methyl group either as –OMe at C-1 or –NMe at N-9. The position was established as –NMe at N-9 based on the fragmentation ion peaks. The MS/MS at *m/z* 755.8, 759.8 and 763.8 gave the product ions at *m/z* 418.9, 420.9, 422.9 for fragmentation at C8–C9 (Scheme 1) and the absence of mass peaks at *m/z* 404.9, 406.9, 408.9 (Scheme 2) as found in Purealidin Q.

Keywords: Antimicrobial activity; Bromotyrosine alkaloids; Marine sponge; *Psammaplysilla purpurea*; Purpurealidin.
* Corresponding author. Tel.: +91 832 2450394/2450392; fax: +91 832 2450603/2450604; e-mail: supriyat@darya.nio.org

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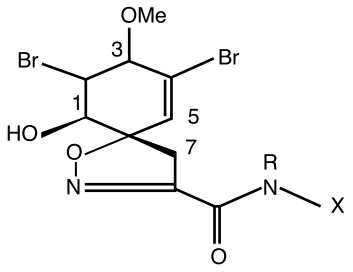
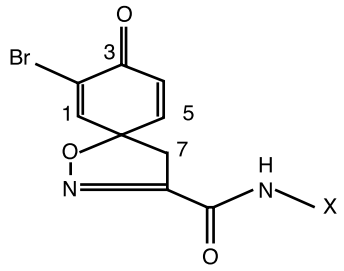
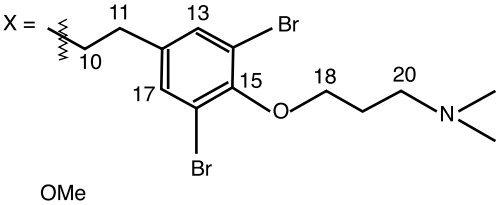
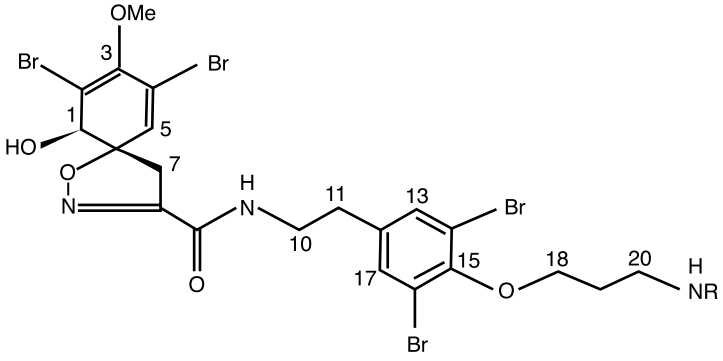




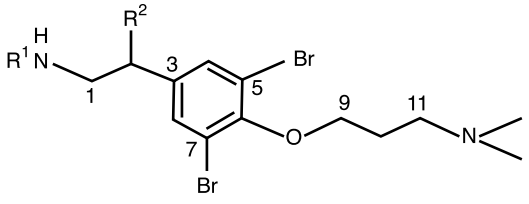
Purealidin Q (1) R=H
Purpurealidin A (2) R=Me

Purpurealidin B (3)

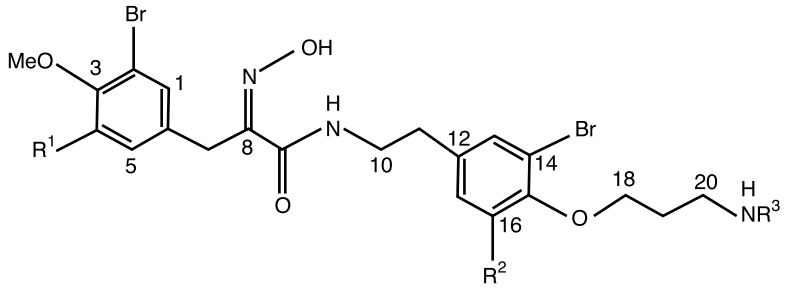




Purpurealidin C (4) R=-$CO(CH_2)_{11}CH(CH_3)_2$
Purpurealidin C (5) R=-$CO(CH_2)_{12}CH_2CH_2CH_3$



Purpurealidin E (6) R^1=H, R^2=H
Purpurealidin F (7) R^1=H, R^2=OH
Purpurealidin G (8) R^1=-$COCH_2CH_3$, R^2=OH

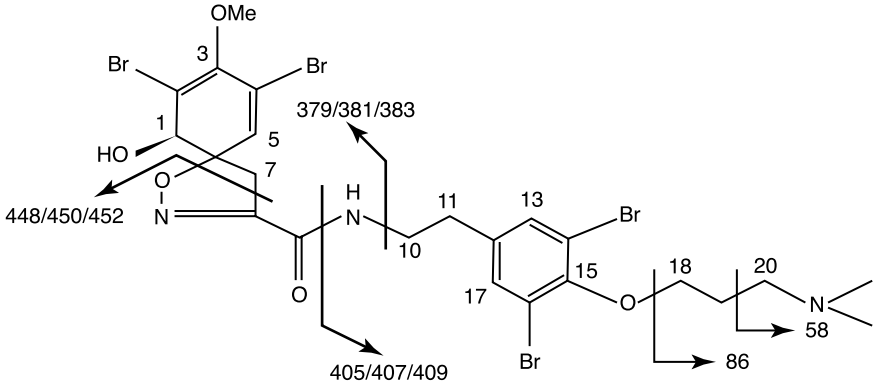


16-Debromoaplysamine-4 (9) R^1=Br, R^2=R^3=H
Purpurealidin H (10) R^1=Br, R^2=H, R^3=Me
Purpurealidin I (11) R^1=H, R^2=Br, R^3=Me

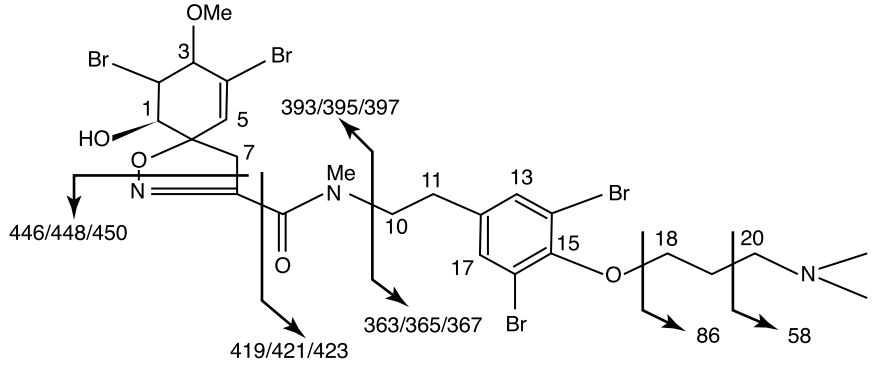
Figure 1. Structures of compounds **1–11** from the sponge *Psammaplysilla purpurea*.

Table 1. ^{1}H, ^{13}C NMR of Purealidin Q (**1**) and Purpurealidin A (**2**), in CD_3OD

Carbon Nos.	**1**		**2**	
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	130.9, d	6.29 (1H, s)	130.9, d	6.29 (1H, s)
2	121.4, s		121.4, s	
3	147.5, s		147.5, s	
4	113.3, s		113.3, s	
5	73.8, d	4.33 (1H, s)	73.8, d	4.33 (1H, s)
6	91.9, s		91.9, s	
7	38.8, t	2.98 (1H, d, J=18.3 Hz)	38.8, t	2.98 (1H, d, J=18.3 Hz)
		3.92 (1H, d, J=18.6 Hz)		3.92 (1H, d, J=18.6 Hz)
8	153.9, s		153.9, s	
9	159.2, s		159.2, s	
10	40.1, t	3.54 (2H, t, J=12.2, 6.6 Hz)	40.1, t	3.54 (2H, t, J=12.2, 6.6 Hz)
11	34.2, t	2.77 (2H, t, J=12.6, 6.8 Hz)	34.2, t	2.77 (2H, t, J=12.6, 6.8 Hz)
12	137.2, s		137.2, s	
13,17	132.9, d	7.35 (2H, s)	132.9, d	7.35 (2H, s)
14,16	118.1, s		118.1, s	
15	151.5, s		151.5, s	
18	71.0, t	4.05 (2H, t, J=12.0, 5.6 Hz)	71.0, t	4.05 (2H, t, J=12.0, 5.6 Hz)
19	27.0, t	2.19 (2H, m)	27.0, t	2.19 (2H, m)
20	56.0, t	2.92 (2H, t, J=5.6 Hz)	56.0, t	2.92 (2H, t, J=5.6 Hz)
$-OCH_3$	60.0, q	3.74 (3H,s)	60.0, q	3.74 (3H, s)
$-N(CH_3)_2$	44.5, q	2.89 (6H, s)	44.5, q	2.89 (6H, s)
–NH		7.40 (1H, s)		
$-N-CH_3$			39.3, t	3.4 (3H, s)



Scheme 1. Fragmentation patterns of Purealidin Q (**1**).



Scheme 2. Fragmentation patterns of Purpurealidin A (**2**).

The monoisotopic peaks at *m/z* 58, 86 also help in confirming the side chain to be dimethylpropylamine.

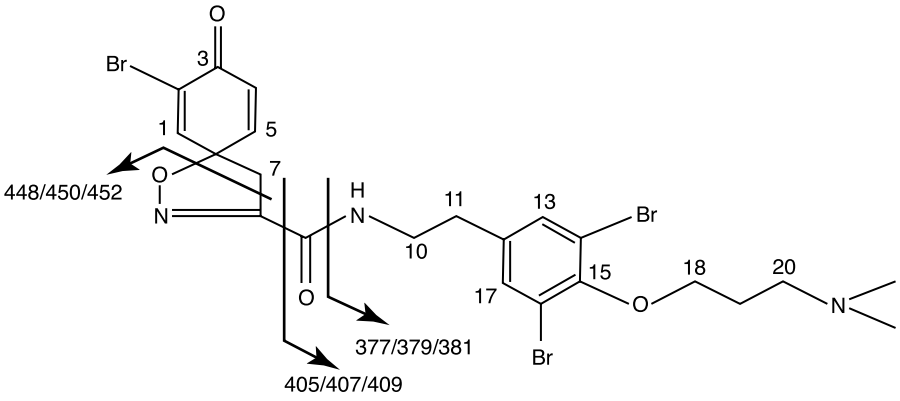
The Purpurealidin B (**3**) contains a dibromospirocyclohexadienonyldihydroisoxazole moiety of the type found in Verongida metabolites but differing in having one bromine atom and dienone ring system.[19,20] The mass spectrum of Purpurealidin B showed a 1.06:3.13:3.06:1.0 quartet for the pseudomolecular ion peak $[M+H]^+$ at *m/z* 631.8, 633.8, 635.8, 637.8, indicative of the presence of three bromine

Table 2. ^{1}H, ^{13}C NMR and COSY of Purpurealidin B (**3**), in $CDCl_3$

Carbon Nos.	^{13}C NMR	^{1}H NMR	COSY	HMBC
1	144.2, d	7.27 (1H, d, J=2.2 Hz)	H5	C2, C3, C5
2	125.8, s			
3	177.9, s			
4	127.7, d	6.34 (1H, d, J=9.8 Hz)	H5	C2, C6
5	144.2, d	6.87 (1H, dd, J=2.2, 9.8 Hz)	H1, H4	C3
6	84.5, s			
7	43.1, t	3.48 (1H, d, J=18.0 Hz)		
		3.90 (1H, d, J=18.0 Hz)		C5, C8
8	153.4, s			
9	158.4, s			
10	40.4, t	3.57 (2H, t, J=7.0 Hz)	H11	C9, C11, C12, C13, 17
11	34.1, t	2.81 (2H, t, J=7.2 Hz)	H10	C10
12	137.3, s			
13,17	132.9, d	7.34 (2H, s)		C11, C13,17, C15, C14,16
14,16	117.9, s			
15	150.8, s			
18	69.5, t	4.04 (2H, t, J=5.6 Hz)	H19	C19, C20
19	25.2, t	2.38 (2H, m)	H20, H18	C18, C20
20	55.7, t	3.42 (2H, t, J=5.6 Hz)	H19	C18, C19
–NH	7.4		C8	
–N $(CH_3)_2$	43.1, q	2.89(6H, s)		

atoms in the molecule, which is appropriate for the molecular formula $C_{22}H_{24}N_3O_4Br_3$. The ^{13}C NMR spectrum had 22 carbon signals, the multiplicities of which were assigned from a DEPT 135 experiment as two methyls, six methylenes, five methines, and nine quarternary carbons. The coupling pattern in proton signals at δ_H 7.27 (1H, d, J= 2.2 Hz), 6.87 (1H, dd, J=2.2, 9.8 Hz) and 6.34 (1H, d, J= 9.8 Hz) indicated the presence of a 2,3,6-trisubstituted aromatic moiety (see Table 2). Analysis of the proton COSY spectrum showed connectivities for H1–H5, H4–H5 and H5–H1–H4 for the 2, 3, 6-trisubstituted aromatic moieties. The HMBC experiment showed that the proton signal at δ_H 7.27 is connected to C2, C3, C5 and δ_H 6.34 to C2, C6, C5 and δ_H 6.87 to C3. The presence of signal at δ_C 177.18 in the ^{13}C NMR spectrum shows presence of a ketone in the ring system. Thus, the partial structure was confirmed to be monobromospirocyclohexadienoneisoxazole. The structure of the remaining part of the molecule, which is linked to the nitrogen atom of the carboxamide group at C-8, was similar to that of Purealidin Q, which was established by inspection of 1H–1H connectivities. This clearly indicates presence of H10–H11 and also the H18–H19–H20 methylene chain. The HMBC showed a proton signal at δ_H 7.34 (2H, s) connected to C11, C13, 17, C15, C14, 16 for the tetrasubstituted aromatic ring. A 6H singlet at δ_H 2.89 was assigned to be a dimethylamino group. This is also confirmed by pseudomolecular peaks at *m/z* 405, 407, 409, and 448, 450, 452 (Scheme 3).

Purpurealidin C (**4**) and D (**5**) exhibited the same characteristic features as Purealidin Q (**2**) except for one additional amide proton at δ_H 5.3, the carbonyl signal at δ_C 173.6, and methylene signals at δ_C 27.0–32.7 (δ_H 1.19) indicative of the presence of an additional amide carbonyl group and long straight fatty chain. A doublet at δ_H 0.80 (6H, J=6.8 Hz) was assigned to the isopropyl group. The structure is also confirmed by ^{1}H, ^{13}C, COSY and HMBC spectral data (see Table 3). The molecular weight of Purpurealidin C (**4**) was higher than that of Purealidin Q (**2**). The low resolution mass spectrum showed pseudomolecular ion peaks at *m/z* 938.0, 940.0, 942.0, 944.0, 946.0. The mass spectrum showed additional pseudomolecular ion peaks at *m/z* 952.0, 954.0, 956.0, 958.0, 960.0, which are 14 units higher than (**4**) indicative of an extra methylene group. The presence of a signal at δ_H 0.70 (t) and ^{13}C signal at δ_C 14.0 suggested for terminal methyl group in **5** (see Table 4). The



Scheme 3. Fragmentation patterns of Purpurealidin B (**3**).

Table 3. ^{1}H, ^{13}C NMR, COSY and HMBC of Purpurealidin C (**4**), in $CDCl_3$

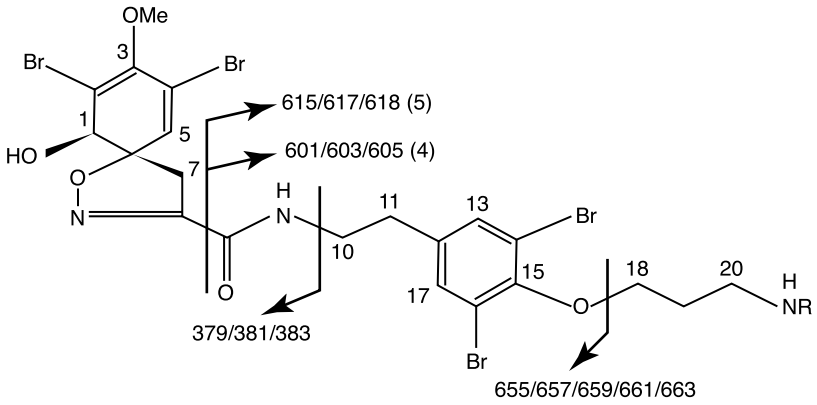
Carbon Nos.	^{13}C NMR	^{1}H NMR	COSY	HMBC
1	74.0, d	4.28 (1H, s)		C3, C2, C5
2	112.7, s			
3	148.0, s			
4	121.4, s			
5	130.0, s	6.24 (1H, s)		C4, C3
6	91.5, s			
7	38.8, t	Ha=2.93 (1H, d, J=18.6 Hz)	Hb	C5, C1, C8
		Hb=3.88 (1H, d, J=18.3 Hz)	Ha	
8	154.9, s			
9	159.1, s			
10	40.3, t	3.54 (2H, t, J=13.2, 6.6 Hz)	H11	C11
11	34.4, t	2.67 (2H, t, J=12.6, 7.8 Hz)	H10	C10, C12, C13
17				
12	138.0, s			
13,17	132.9, d	7.34 (2H, s)		C11, C13,17, C15, C14,16
14,16	118.2, s			
15	151.2, s			
18	71.0, t	4.01 (2H, t, J=12.0, 6.0 Hz)	H19	C15, C19, C20
19	29.2, t	2.06 (2H, m)	H18, H20	C18, C20
20	37.0, t	3.63 (2H, t)	H19	C18, C19
21	173.6, s			
22	34.4, t	2.67(2H, m)		
23–32	27.0–32.	1.19 (24H, s)		
	7, t			
33	29.0, d	1.53 (2H, m)		C34,35
34,35	22.6, q	0.80 (6H, d, J=6.8 Hz)	H33	
–N-9		7.43 (1H, d)		
–N-20		5.30 (1H, s)		
–OCH3	60.0, q	3.67 (3H, s)		C3

fragmentation pattern of **4** and **5** (Scheme 4) is different from the Arapplysillin II isolated from the *Psammaplysilla purpurea*[21] and agrees well with the structure assigned.

The mass spectrum of Purpurealidin E (**6**) showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 378.9768, 380.9757, 382.97 in the ratio 1.05:2.05:1.0, characteristic for the presence of two bromine atoms. Examination of the ^{1}H and ^{13}C and COSY showed that the structure is similar to the part structure of Purealidin Q. In addition, the mass spectrum of (**6**) showed minor pseudomolecular ion peaks at *m/z* 394.9, 396.9, 398.9 and 451.0, 453.0, 455.0 compounds Purpuealidin F (**7**) and G (**8**). The ^{1}H NMR signal at 3.77 (1H, m) and δ_C 59.6 is accounted for the hydroxy methine at

Table 4. ^{1}H, ^{13}C NMR, COSY and HMBC of Purpurealidin D (**5**), in $CDCl_3$

Carbon Nos.	^{13}C NMR	^{1}H NMR	COSY	HMBC
1	74.0, d	4.28 (1H, s)		C3, C2, C5
2	112.7, s			
3	148.0, s			
4	121.4, s			
5	130.0, s	6.24 (1H, s)		C4, C3
6	91.5, s			
7	38.8, t	Ha=2.93 (1H, d, J=18.6 Hz)	Hb	C5, C1, C8
		Hb=3.88 (1H, d, J=18.3 Hz)	Ha	
8	154.9, s			
9	159.1, s			
10	40.3, t	3.54 (2H, t, J=13.2, 6.6 Hz)	H11	C11
11	34.4, t	2.67 (2H, t, J=12.6, 7.8 Hz)	H10	C10, C12, C13, 17
12	138.0, s			
13,17	132.9, d	7.34(2H, s)		C11, C13,17, C15, C14,16
14,16	118.2, s			
15	151.2, s			
18	71.0, t	4.01 (2H, t, J=12.0, 6.0 Hz)	H19	C15, C19, C20
19	29.2, t	2.06 (2H, m)	H18, H20	C18, C20
20	37.0, t	3.63 (2H, t)	H19	C18, C19
21	173.6, s			
22	34.4, t	2.67 (2H, m)		
23–34	27.0–32.	1.19 (24H, s)		
	7, t)			
35	29.0, d	1.53 (2H, m)		C35
36	14.0, q	0.70 (3H, t)	H35	C34
–N-9		7.43 (1H, s)		
–N-20		5.40 (1H, s)		
–OCH3	60.0, q	3.67 (3H, s)		C3



Scheme 4. Fragmentation patterns of Purpurealidin C (**4**) and D (**5**).

C2 in 7 and 8 (see Table 5). The carbonyl signal at δ_C 173.0, methylene signal at δ_C 29.2 (δ_H 1.20, s) and methyl signal at δ_C 14.0 (δ_H 0.81, t, $J=7.0$ Hz) were indicative of the presence of an additional amide carbonyl group and ethyl groups.

The mass spectrum of the known compound that we have reported earlier, 16-Debromo aplysamine-4 (**9**), revealed characteristic isotope peaks for $[M+H]^+$ pseudo molecular ion at 619.8, 621.8, 623.8 and 625.8 in the ratio 1.05:3.1:3.06:1.0, indicating the presence of three bromine atoms in the molecule. Its ^{13}C NMR spectrum had 21 carbon signals, which were designated as one methyl, six methylenes, five methines, and nine quarternary carbons from a DEPT135 experiment. The signals at 7.52 (1H, s) and 7.40 (1H,s) and 7.33 (1H, d, $J=2.0$ Hz), 6.86 (1H, d, $J=8.4$ Hz) and 7.02 (1H, dd, $J=8.4$, 2.0 Hz) in the ^1H NMR spectrum indicated the presence of tetra and 1,2,4-trisubstituted aromatic moieties. The IR absorptions at 3350, 1655, and 1624 cm^{-1} and ^{13}C NMR signals at 163.8 and 152.3 ppm were indicative of amide and oxime groups. The exchangeable proton signals at δ_H 11.40 (2H, br m), 8.70 (1H, br s,) and 7.90 (1H, br s,) in the ^1H NMR spectrum indicated the presence of NH_2, NH and OH groups. The presence of a primary amine in the molecule is also confirmed by the positive ninhydrin test. The above results, as well as the assumption that this compound is a derivative of aplysamine/purpuramine, indicated its molecular formula to be $C_{21}H_{24}Br_3N_3O_4$. The upfield ^{13}C NMR chemical shift of C-7 (27.3 ppm) suggested *E* configuration of the oxime as the corresponding value for (*Z*)-oxime is >35 ppm.[1] It also shows additional singly charged $[M+H]^+$ at *m/z* at 633.8, 635.8, 637.8, 639.8 for (**10**), 14 units higher than that of the compound **9**. This is accounted for the methyl group at the terminal *N*-methyl.

Compounds **1**, **3**, **9** and **11** were evaluated for their antimicrobial activity (see Table 6) against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexineri*, *Klebsiella* sp and *V. cholerae* bacterial strains and fungal strains of *Aspergillus fumigatus*, *Fusarium* sp, *Cryptococcus neoformans*, *Aspergillus niger*, *Rhodotorula* sp, *Norcardia* sp, and *Candida albicans*. The compounds did not show any activity against bacterial strains *Klebsiella* sp, *Pseudomonas aeruginosa* and all fungal strains. Purealidin Q (**1**) showed good activity against *Salmonella typhi*. It was previously reported to show cytotoxic activity against tumor cell lines and moderate inhibitory against epidermal growth factor (EGF) kinase.[10] Purpurealidin B (**3**) showed good activity against *E. coli*, *S. aureus*, *V. cholerae* and weak activity against *Shigella flexineri*. 16-Debromo aplysamine-4 (**9**) showed moderate activity against *Salmonella typhi* and very weak acivity against *E. coli*, *Staphylococcus aureus* and *V. cholerae*.

Table 5. ^1H, ^{13}C NMR and COSY of Purpurealidin E (**6**), F (**7**), G (**8**) in CD_3OD

Carbon Nos.	**6**		**7**		**8**	
	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^1H NMR
1	40.0, t	2.73 (2H, t, $J=13.2$, 6.6 Hz)	40.0, t	2.73 (2H, t, $J=13.2$, 6.6 Hz)	40.0, t	2.73 (2H, t, $J=13.2$, 6.6 Hz)
2	33.6, t	3.24 (2H, t)	59.6, t	3.77 (1H, m)	59.6, t	3.77 (1H, m)
3	130.3, s		130.3, s		130.3, s	
4,8	133.0, t	7.43 (2H,s)	133.0, t	7.43 (2H, s)	133.0, t	7.43 (2H, s)
5,7	117.3, s		117.3, s		117.32, s	
6	150.7, s		150.7, s		150.72, s	
9	69.8, t	4.05 (2H, t, $J=5.6$ Hz)	69.8, t	4.05 (2H, t, $J=5.6$ Hz)	69.8, t	4.05 (2H, t, $J=5.6$ Hz)
10	25.0, t	2.23 (2H, m)	25.0, t	2.23 (2H, m)	25.0, t	2.23 (2H, m)
11	55.8, t	3.44 (2H, t)	55.8, t	3.44 (2H, t)	55.8, t	3.44 (2H, t, $J=5.6$ Hz)
		$J=5.6$ Hz)		$J=5.6$ Hz)		$J=5.6$ Hz)
12,13	42.7, q	2.90 (6H, s)	42.7, q	2.90 (6H, s)	42.7, q	2.90 (6H, s)
NH_2		7.63 (br, s)				
NH				8.10 (br, s)		8.10 (br, s)
CO					173.0	
CH_2					29.2	1.20 (2H, s)
CH_3					14.0	0.81 (3H, t, $J=7.0$ Hz)

Table 6. Effect of compounds **1**, **3**, **9** and **11** on growth of microbial strains (MIC in μg/ml)

Compounds	*E. Coli*	*S. aureus*	*Salmonella typhi*	*Shigella flexineri*	*Vibrio cholarae*
Purealidin Q (**1**)	—	—	>25	—	—
Purpurealidin B (**3**)	>12	10	—	100	25
16-Debromo aplysamine 4 (**9**)	250	200	>50	—	100
Purpuramine I (**11**)	100	50	—	—	100
Streptomycin	10	10	10	10	10

Good activity: 10–25 μg/ml. Moderate activity: 26–100 μg/ml. Weak activity: >100 μg/ml.

Moderate acivity against *S. aureus* was confirmed for Purpuramine I (**11**) according to the previous studies. It also showed moderate activity against *E. coli* and *V. cholerae*.

3. Experimental

3.1. General experimental procedures

Column chromatographies were carried out using Silica gel (60–120 mesh, Qualigens), gel filtrations were carried out using Sephadex LH20 17-0090-01 Pharmacia Biotech). Fractions were monitored on TLC using alumina-backed sheets (Si gel 60 F254, 0.25 mm thick) with visualization under UV (254 nm) and Ninhydrin spray reagent. All analytical reverse phase HPLC (Chromspher 5 C18 column 250×10 mm, MeOH/H2O 85/15) were performed with a P4000 pump (Spectra system) equipped with a UV2000 detector (spectra system).

UV spectra were recorded in MeOH, using a Shimadzu UV–Vis 2401PC Spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR 8201PC Spectrophotometer. Optical rotations were recorded in MeOH using Optical Polarimeter ADP220 (Bellingham Stanley Ltd).

Mass spectra were recorded on a PE Sciex-QSTAR and QSTAR-TOF MS/MS of Applied Biosystems.

NMR (^{1}H, ^{13}C, COSY, HMQC and HMBC) experiments were obtained on a Bruker (Avance 300) spectrometer with TMS as internal standard.

3.2. Animal material

The animals were collected by scuba diving at a depth of 8–10 m from Mandapam, Tamil Nadu, India, and identified by Dr. P. A. Thomas of Vizhingam Research Center of Central Marine Fisheries Research Institute, Kerala, India. A voucher specimen is deposited at the National Institute of Oceanography, Dona Paula Goa, India.

3.3. Extraction and isolation

The frozen sponge (250 g, dry weight) was extracted with methanol (1 L×3) and concentrated under vacuo to obtain 10 g of crude extract. The extract showed antimicrobial activity against pathological strains, which was chromatographed on silica gel (Qualigens silica gel 60–120 mesh) column using dichloromethane with increasing amounts of methanol as eluent. The fractions eluted with 8, 10 and 20% were purified separately. The fraction eluted with 8% MeOH (1.5 g) was further purified by repeated[1] gel chromatography (Sephadex LH20) columns using chloroform/methanol (1:1) to get Purealidin Q (200 mg), Purpurealidin B (800 mg) and Purpurealidin C and D (400 mg). The fractions eluted with 10% MeOH were purified on reverse phase HPLC using Chromspher 5 C18 column 250×10 mm^2, MeOH/H_2O 85/15, flow rate 2 mL/min and UV detection at λ_{max} 254 nm) which afforded 16-Debromo aplysamine-4, Purpurealidin-H (R_t 18.4 min) (20 mg) and Purpuramine I (R_t 27.5 min) (25 mg). The fractions eluted with 20% were subjected to silica gel column eluted with increasing amounts of methanol in dichloromethane to yield mixture of Purpurealidin E, F, G (300 mg).

3.3.1. Purealidin Q (1). Colorless oil, UV (MeOH) λ_{max} 277 nm (ε 1700), 284 nm (ε 1400); $[\alpha]_D^{28}=+9.5$ (c 0.2, MeOH); IR (neat) ν_{max} 3418, 2922, 1668, 1537, 1458.1, 1254, 1051, 920, 737 cm^{-1}; ^{1}H and ^{13}C recorded in CD_3OD see Table 1; HRMS: m/z (relative heights) 741.8691(450), 743.8871(1780), 745.8710(2600), 747.8762(1700), 749.7914(420) [1.07:4.23:6.2:4.0:1.0],† $[M+H]^+$, found 741.8691 $C_{23}H_{27}N_3O_5Br_4$ requires 741.8764; $[M+H-Br]^+$ 662.9, 664.9, 666.9, 668.9; $[M+H+Br+CH_3]^+$ 647.8, 649.8, 651.8, 653.8; 404.8, 406.8, 408.8; 378.9, 380.9, 382.9; 348.9, 350.8, 352.8; 58; 86.

3.3.2. Purpurealidin A (2). Colorless oil, UV (MeOH) λ_{max} 277 nm (ε 1700), 284 nm (ε 1400); $[\alpha]_D^{28}=+9.5$ (c 0.2, MeOH); IR (neat) ν_{max} 3418, 2922, 1668, 1537, 1458.1, 1254, 1051, 920, 737 cm^{-1}; ^{1}H and ^{13}C recorded in CD_3OD see Table 1; HRMS: m/z (relative heights) 755.8819(55), 757.8799(210), 759.8810(310), 761.8820(200), 763.8(50) [1.1:4.2:6.2:4.0:1.0],† $[M+H]^+$, found 755.8819 $C_{24}H_{29}N_3O_5Br_4$ requires 755.8920.

3.3.3. Purpurealidin B (3). White amorphous solid, mp 175.8 °C; UV (MeOH) λ_{max} 283 (1320); IR (KBr pellet) ν_{max} 3302, 2932, 2689, 1678, 1605, 1541, 1460, 1383, 1259, 910 and 739 cm^{-1}. ^{1}H and ^{13}C recorded in $CDCl_3$ see Table 2; HRMS: m/z (relative heights) 631.8403(1600), 633.8185(4700), 635.8118(4600), 637.8226(1500) [1.06:3.13:3.03:1.0],† $[M+H]^+$, found 631.8403 $C_{22}H_{24}N_3O_4Br_3$ requires 631.8396; 404.9, 406.9, 408.9; 376.9, 378.9, 380.9; 224.9, 226.9.

3.3.4. Purpurealidin C (4). Colorless oil, UV (MeOH) λ_{max} 282(10,000), 218(2500), $[\alpha]_D^{28}=+158.5$ (c 0.2, $CHCl_3$); IR (KBr pellet) ν_{max} 3319, 2925, 2854, 1660, 1605, 1456, 1257, 739 cm^{-1}, ^{1}H and ^{13}C recorded in $CDCl_3$ see Table 3 ESI-MS: m/z (relative heights) 938.0(22), 940.0(85), 942.0(125), 944.0(80), 946.0(20) [1.1:4.2:6.2:4.0:1.0],† $[M+H]^+$,

† Real ratios of the pseudomolecular ion peaks.

found 938.05 $C_{36}H_{51}N_3O_6Br_4$ requires 938.0591; 615, 617, 619; 379, 381, 383.

3.3.5. Purpurealidin D (5).[2] Colorless oil, UV (MeOH) λ_{max} 282(10,000), 218(2500); IR (KBr pellet) ν_{max} 3319, 2925, 2854, 1660, 1605, 1456, 1257, 739 cm^{-1}, ^1H ($CDCl_3$, 300 MHz) see Table 4 ESI-MS: *m/z* (relative heights) 952.0(16), 954.0(65), 956.0(95), 958.0(60), 960.0(15) [1.06:4.3:6.3:4.0:1.0],† $[M+H]^+$, found 952.07 $C_{37}H_{53}N_3O_6Br_4$ requires 952.0747; 655, 657, 659, 661, 662; 601, 603, 605; 379, 381, 383.

3.3.6. Purpurealidin E (6). Colourless oil, UV (MeOH) λ_{max} 282 (ε 950), 277 (ε 925); IR (neat) ν_{max} 3302, 2933, 1666, 1545, 1458, 1259, 1039, 739 cm^{-1}, ^1H and ^{13}C recorded in $CDCl_3$ see Table 5; HRMS: *m/z* (relative heights) 378.9768(37), 380.9757(72), 382.97(35) [1.05:2.05:1.0],† $[M+H]^+$, found 378.9768 $C_{13}H_{20}N_2OBr_2$ requires 378.9943.

3.3.7. Purpurealidin F (7). Colourless oil, UV (MeOH) λ_{max} 282 (ε 950), 277 (ε 925); IR (neat) ν_{max} 3302, 2933, 1666, 1545, 1458, 1259, 1039, 739 cm^{-1}, ^1H and ^{13}C recorded in $CDCl_3$ see Table 5; HRMS: *m/z* (relative heights) 394.9667(16), 396.9661(32), 398.9618(15) [1.06:2.1:1.0],† $[M+H]^+$, found 394.9667 $C_{13}H_{20}N_2O_2Br_2$ requires 394.9970.

3.3.8. Purpurealidin G (8). Colorless oil, UV (MeOH) λ_{max} 282 (ε 950), 277 (ε 925); IR (neat) ν_{max} 3302, 2933, 1666, 1545, 1458, 1259, 1039, 739 cm^{-1}, ^1H and ^{13}C recorded in $CDCl_3$ see Table 5; HRMS: *m/z* (relative heights) 451.0220(10), 453.0210(20), 455.0301(10) [1:2:1], $[M+H]^+$, found 451.0220 $C_{16}H_{24}N_2O_3Br_2$ requires 452.0232.

3.3.9. 16-Debromo aplysamine-4 (9). Colorless amorphous solid (MeOH): mp 124–126 °C; UV (MeOH) λ_{max} 218 nm (ε 12675), 280 nm (ε 2675); IR (KBr pellet) ν_{max} 3350, 3205, 2958, 1655, 1624, 1541, 1497, 1472, 1421, 1256, 1203, 1049, 993 and 739 cm^{-1}; ^1H (CD_3OD, 300 MHz) δ_H 11.40 (2H, br m, $-NH_2$), 8.70 (1H, brs, –NH), 7.90 (1H, brs, –OH), 7.4 (2H, s, H-1, 5), 7.33 (1H, d, $J=2.0$ Hz, H-13), 7.02 (1H, dd, $J=2.0$, 8.4 Hz, H-17), 6.86 (1H, d, $J=8.4$ Hz, H-16), 4.06 (2H, t, $J=6.5$ Hz, H-18), 3.75 (3H, s, $-OCH_3$), 3.74 (1H, s, H-7), 3.34 (2H, t, $J=7.0$ Hz, H-10), 3.15 (2H, t, $J=6.8$ Hz, H-20), 2.65 (2H, s, $J=7.0$ Hz, H-11), 2.09 (2H, m, H-19); ^{13}C NMR(CD_3OD, 300 MHz) (165.2 (s, C-9), 154.6 (s, C-3), 153.7 (s, C-8), 151.9 (s, C-15), 137.2 (s, C-12), 134.5 (s, C-6), 134.4 (d, C-1, 5), 134.4 (d, C-13), 130.2 (d, C-17), 118.5 (s, C-2, 4), 114.4 (d, C-16), 112.6 (s, C-14), 67.6 (t, C-18), 61.0 (q, -OCH_3), 41.7 (t, C-10), 38.8 (t, C-20), 35.1 (t, C-11), 28.7 (t, C-7), 28.1 (t, C-19); HRMS: *m/z* (relative heights) 619.8797 (525), 621.8535 (1550), 623.8444 (1530), 625.8845 (500) [1.05:3.1:3.06:1.0],† $[M+H]^+$, found 619.8797 $C_{21}H_{24}N_3O_4Br_3$ requires 619.9396.

3.3.10. Purpurealidin H (10). Colorless amorphous solid (MeOH); UV (MeOH) λ_{max} 218 nm (ε 12675), 280 nm (ε 2675); IR (KBr pellet) ν_{max} 3350, 3205, 2958, 1655, 1624, 1541, 1497, 1472, 1421, 1256, 1203, 1049, 993 and 739 cm^{-1}; ^1H (CD_3OD, 300 MHz) δ_H 11.40 (2H, br m, $-NH_2$), 8.70 (1H, brs, –NH), 7.90 (1H, brs, –OH), 7.4 (2H, s, H-1, 5), 7.33 (1H, d, $J=2.0$ Hz, H-13), 7.02 (1H, dd, $J=2.0$, 8.4 Hz, H-17), 6.86 (1H, d, $J=8.4$ Hz, H-16), 4.06 (2H, t, $J=6.5$ Hz, H-18), 3.75 (3H, s, $-OCH_3$), 3.74 (1H, s, H-7), 3.34 (2H, t, $J=7.0$ Hz, H-10), 3.15 (2H, t, $J=6.8$ Hz, H-20), 2.764 (3H, s, $-NCH_3$), 2.65 (2H, s, $J=7.0$ Hz, H-11), 2.09 (2H, m, H-19); ^{13}C NMR (CD_3OD, 300 MHz) δ 165.2 (s, C-9), 154.6 (s, C-3), 153.7 (s, C-8), 151.9 (s, C-15), 137.2 (s, C-12), 134.5 (s, C-6), 134.4 (d, C-1, 5), 134.4 (d, C-13), 130.2 (d, C-17), 118.5 (s, C-2, 4), 114.4 (d, C-16), 112.6 (s, C-14), 67.6 (t, C-18), 61.0 (q, $-OCH_3$), 41.7 (t, C-10), 38.8 (t, C-20), 35.1 (t, C-11), 28.7 (t, C-7), 28.1 (t, C-19), 27.615 (q, $-NCH_3$); HRMS: *m/z* (relative heights) 633.9220(420), 635.9091(1250), 637.9021(1220), 639.9104(400) [1.05:3.12:3.05:1.0],† $[M+H]^+$, found 633.9220 $C_{22}H_{26}N_3O_4Br_3$ requires 633.9550.

3.4. Antibacterial assays

Antibacterial activity was determined against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexineri*, *Klebsiella* sp. and *V. cholerae* using the paper disk assay method. The paper disk impregnated with the sample was placed on agar plate containing bacterium and the plates were incubated for 24 h at 37 °C, and observed for zone of inhibition halos. Streptomycin was used as a positive control.

3.5. Antifungal assays

Antifungal activity was determined against strains of *Aspergillus fumigatus*, *Fusarium* sps, *Cryptococcus neoformans*, *Aspergillus niger*, *Rhodotorula* sp., *Norcardia* sp., and *Candida albicans*. The paper disk impregnated with the sample was placed on agar plate containing fungus and plates were incubated for 18 h at 24 °C. *Nystatin* was used as a positive control.

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