Axinellamines A−D, Novel Imidazo−Azolo−Imidazole Alkaloids from the Australian Marine Sponge *Axinella* sp.

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Four imidazo−azolo−imidazole alkaloids, axinellamines A−D, have been isolated from an Australian marine sponge, *Axinella* sp. (order: Halichondrida: family: Axinellidae). These compounds contain a unique perhydrocyclopenta−imidazo−azolo−imidazole carbon skeleton. Three of these compounds had bactericidal activity against *Helicobacter pylori* at 1000 μM.

Introduction

Bromopyrrole-containing alkaloids such as hymenidin and oroidin have been isolated from sponges of the genera *Axinella* and *Agelas*.[1−3] More complex bromopyrroles which also contain guanidine moieties such as 2,3-dibromostyloguanidine and dimers such as dibromoageliferin acetate have been isolated from *Stylotella aurantium*[4] and *Agelas conifera*,[5,6] respectively. In this report the isolation, characterization, and identification of four imidazo−azolo−imidazole bromopyrrole-containing metabolites, axinellamines A−D (**1**−**4**) from an Australian marine sponge, *Axinella* sp., is described. Axinellamines B−D (**2**−**4**) had bactericidal activity against *Helicobacter pylori*, a gram negative bacterium associated with pepticular and gastric cancer, at 1000 μM.

Results and Discussion

Bioassay guided fractionation of the crude methanol extract of *Axinella* sp. revealed that fractions inhibiting the growth of *H. pylori* consistently had ion peaks at *m*/*z* 846 and 860 in the electrospray mass spectrum (ESIMS). The application of LC/positive ESIMS, using reverse-phase C18 HPLC, allowed chromatographic conditions to be established. A larger scale purification of the crude methanol extract was successfully achieved by gel permeation chromatography (LH-20 Sephadex, using methanol as the eluant) followed by reverse-phase HPLC (using 1% trifluoroacetic acid (TFA) with acetonitrile/H_2O gradients), resulting in the isolation of axinellamines A−D (**1**−**4**).

The structures of axinellamines A−D (**1**−**4)** were deduced from 1D and 2D NMR data (Tables 1, 2, and 4) and MS analysis. The positive electrospray mass spectra of **1** and **2** displayed clusters of ion peaks [(M + H) − $2CF_3COOH]^+$ at *m*/*z* 842/844/846/848/850/852, while the negative electrospray mass spectra displayed clusters of molecular ion peaks $[M - H]^-$ at *m*/*z* 1069/1071/1073/1075/1077/1079, which was consistent with a $ClBr_4$ isotope pattern. The positive electrospray mass spectra of **3** and **4** displayed clusters of ion peaks [(M + H) − $2CF_3COOH]^+$ at *m*/*z* 856/858/860/862/864/866, while the negative electrospray mass spectra displayed clusters of molecular ion peaks $[M - H]^-$ at *m*/*z* 1083/1085/1087/1089/1091/1093, which were also consistent with a $ClBr_4$ isotope pattern with the incorporation of an additional 14 mass units compared with **1** and **2**. Axinellamines A (**1**) and B (**2**) were thus isomeric, as were axinellamines C (**3**) and D (**4**), and were deduced to have the molecular formulas $C_{22}H_{23}Br_4ClN_{10}O_4 \cdot 2CF_3COOH$ for **1** and **2** and

1 R=H
3 R=CH_3

2 R=H
4 R=CH_3

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Table 1. NMR Data for Axinellamine A (1) in DMSO-d_6[a]

position no.	^{13}C NMR,[b] δ	^{1}H NMR,[c] δ (mult, J in Hz)	HMBC (C no.)	g-COSY	ROESY[d]
1	78.4 (d)	5.27 (d, J = 7.2 Hz)	C3, C13	1-O*H*, 2-N*H*	H12, H13, H1$_b$″, 1-O*H*, 2-N*H*
2					
3	155.7 (s)				
4					
5	79.0 (d)	5.29 (s)	C7, C9, C14	6-NH	H13, 6-NH, 9-OH
6					
7	156.7 (s)				
8					
9	99.9 (s)				
10	50.9 (d)	2.84 (d, J = 4.2 Hz)	C1, C5, C9, C11, C13, C14, C1″	H11, 9-OH	H1$_a$″, H1$_b$″, 1-OH, 8-NH, 2″-NH
11	33.6 (d)	2.60 (ddd, J = 4.2, 5.4, 9.6 Hz)	C9, C10, C12, C1″	H10, H12, (H1″)$_2$	H13, 9-OH, 2′-NH, 2″-NH, H1″
12	48.7 (d)	1.96 (dddd, J = 4.2, 4.2, 9.6, 12.0 Hz)	C13	H11, H13, (H1″)$_2$	H1, H13, (H1′)$_2$, 2″-NH
13	65.7 (d)	4.28 (d, J = 12.0 Hz)	C1, C10, C14, C1′	H12	H1, H5, H11, H12, (H1′)$_2$, 9-OH, 2′-NH
14	83.2 (s)				
1′	36.8 (t)	3.74 (ddd, J = 4.2, 7.8, 15.0 Hz)	C11, C13, C3′	H12, 2′−NH	H12, H13, H1$_b$′, 2′-NH, 2″-NH, H8″
		3.24 (ddd,J= 4.2, 4.2, 15.0 Hz)			H12, H13, H1$_a$′, 2′-NH
2′					
3′	160.2 (s)				
4′	128.0 (s)				
5′					
6′	104.2 (s)				
7′	98.0 (s)				
8′	112.8 (d)	6.99 (s)	C3′, C4′, C6′	5′-NH	2′-NH
1″	41.9 (t)	3.47 (m)	C10, C11, C12, C3″	H11, 2″-NH	H1, H10, H11, H1$_b$″, 2″-NH
		3.34 (m)			H1, H10, H1$_a$″, 2″-NH, 5″-NH
2″					
3″	159.5 (s)				
4″	128.2 (s)				
5″					
6″	108.2 (s)				
7″	98.0 (s)				
8″	113.0 (d)	6.97 (s)	C4″, C6″	5″-NH	H10, 9-OH, 7-NH$_2$
1-OH		7.39 (d, J = 7.2 Hz)	C1, C14	H1	H1, H10
9-OH		7.76 (s)	C9, C10	H10	H5, H11, H13, 8-NH, 8″-NH
2-NH		9.88 (s)	C1, C3, C14	H1	H1
3-NH$_2$		8.99 (bs) and 8.68 (bs)			
6-NH		8.96 (s)	C5, C7, C9	H5, 8-NH	H5, 7-NH$_2$
7-NH$_2$		8.41 (bs)			6-NH, 8-NH
8-NH		9.67 (s)	C5, C7, C9	6-NH	H10, 9-OH, 7-NH$_2$
2′-NH		8.26 (t, J = 5.4 Hz)	C1′, C3′	(H1′)$_2$	H11, H13, H1$_a$′, H1$_b$′, H8′
5′-NH		12.69 (s)	C4′, C7′, C8′	H8′	
2″-NH		8.14 (t, J = 5.4 Hz)	C3″	(H1″)$_2$	H10, H11, H12, H1$_a$′, H1$_a$″, H1$_b$″, H8″
5″-NH		12.67 (s)	C4″, C7″, C8″	H8″	H1$_b$″

[a] Spectra were recorded in DMSO-d_6. [b] ^{13}C NMR at 150 MHz referenced to DMSO (δ 39.5). [c] ^{1}H NMR at 600 MHz referenced to residual solvent (δ 2.49) [d] ROESY experiment was acquired using a mixing time of 500 ms.

$C_{23}H_{25}Br_4ClN_{10}O_4$·2$CF_3COOH$ for **3** and **4**, respectively, on the basis of HRESIMS data.

Examination of the ^{1}H NMR and HMQC spectra of **1** (Table 1) revealed the presence of 11 exchangeable protons. On the basis of HMBC and COSY correlations, these could be identified as follows: 7 amine (δ 9.88, 9.67, 8.96, 8.26, 8.14, 12.67, and 12.69), 2 amide (δ 8.99, 8.68, and 8.41), and 2 hydroxyl moieties (δ 7.39 and 7.76). Analysis of the HMQC spectrum indicated the presence of 6 aliphatic methine resonances: 3 doublets at δ 5.27/78.4 ppm (C1), δ 4.28/65.7 ppm (C13), and 2.84/50.9 ppm (C10), 2 doublet of doublet of doublets at δ 2.60/33.6 ppm (C11) and 1.96/48.7 ppm (C12), and 1 singlet at 5.29/79.0 ppm (C5). The signal at δ 4.28/65.7 ppm (C13) was consistent with a chlorinated methine.[5] The presence of two methylenes C1′ and C1″ was indicated by HMQC correlations from proton resonances at δ 3.34 and 3.47 to 41.9 ppm and from the signals at δ 3.24 and 3.74 to 36.8 ppm. Two aromatic methines (C8′ 6.99/112.8 ppm and C8″ 6.97/113.0 ppm) were also assigned. ^{1}H−^{1}H COSY correlations showed that four of the methines (H10, H11, H12, H13) and the four methylene protons (H1′$_a$, H1′$_b$, H1″$_a$, H1″$_b$) formed one ^{1}H−^{1}H spin system, giving a RClCHCH(CH_2)CH(CH_2)CHR partial structure. HMBC correlations from H13 to C10, as well as to a quaternary carbon C14, and from H10 to C13 and C14 established the presence of a five-membered ring (C10−C11−C12−C13−C14−C10). HMBC correlations from the proton resonating at δ 6.99 (H8′) to carbons at 128.0 ppm (C4′) and 104.2 ppm (C6′), and from the proton resonating at δ 6.97 (H8″) to carbons at 128.2 ppm (C4″) and 108.2 ppm (C6″) suggested the presence of two dibromopyrrole carboxylates. This was supported by the fact that the ^{1}H and ^{13}C NMR chemical shifts associated with the dibromopyrroles were in agreement with those of a 2,3-

Table 2. NMR Data for Axinellamine B (2) in DMSO-d_6[a]

position no.	^{13}C NMR,[b] δ	^{1}H NMR,[c] δ (mult, J in Hz)	HMBC (C no.)	g-COSY	ROESY[d]
1	79.1 (d)	5.35 (d, J = 7.0 Hz)	C3, C13	1-O*H*	H12, 1-O*H*, −N*H*
2					
3	158.9 (s)				
4					
5	80.2 (d)	5.32 (s)	C7, C9, C14	6-N*H*	9-O*H*, 6-N*H*, 3-NH_2, 7-NH_2
6					
7	157.3 (s)				
8					
9	102.6 (s)				
10	51.7 (d)	3.05 (d, J = 3.6 Hz)	C1, C5, C9, C11, C13, C14, C1″	H11	1-O*H*, 9-O*H*, 2″-N*H*
11	37.5 (d)	2.24 (m)	C9, C10, C12	H10, H12, (H1″)$_2$	H12, H13, 8-N*H*, 2′-N*H*, 2″-N*H*
12	48.3 (d)	2.06 (m)	C11, C13, C1″	H11, H13, (H1")$_2$	H1, H11, H13, H1$_a$′, 2′−N*H*, 2″-N*H*
13	66.7 (d)	3.97 (d, J = 12.0 Hz)	C1, C11, C12, C14	H12	H11, H12, 2′-N*H*
14	83.3 (s)				
1′	37.7 (t)	3.60 (m) 3.33 (m)	C11, C12, C13, C3′	H12, 2′-N*H*	H12, H1$_b$′, 2′-N*H* H1$_a$′, 2′-N*H*
2′					
3′	159.4 (s)				
4′	128.0 (s)				
5′					
6′	104.9 (s)				
7′	97.7 (s)				
8′	112.9 (d)	6.93 (s)	C4′, C6′	5′-N*H*	2″-N*H*
1″	40.9 (t)	3.34 (m) 2.52 (m)	C10, C11, C12, C3″	H11, 2″-N*H*	2″-N*H*
2″					
3″	159.2 (s)				
4″	128.0 (s)				
5″					
6″	104.9 (s)				
7″	97.9 (s)				
8″	112.9 (d)	6.91 (s)	C4″, C6″	5″-N*H*	2′-N*H*
1-O*H*		7.37 (d, J = 7.0 Hz)	C1, C14	H1	H1, H10
9-O*H*		7.44 (s)	C9, C10		H5, H10
2-N*H*		10.06 (s)	C1, C3, C14	H1	H1
3-NH_2		9.10 (bs) and 9.85 (bs)			H5
6-N*H*		8.77 (s)		H5, 8-N*H*	H5
7-NH_2		9.10 (bs) and 9.85 (bs)			H5
8-N*H*		9.82 (s)	C5, C7	6-N*H*	H11
2′-N*H*		8.22 (t, J = 5.4 Hz)	C1′, C3′	(H1′)$_2$	H11, H12, H13, H1$_a$′, H1$_b$′, H8′
5′-N*H*		12.64 (s)	C4′, C7′, C8′	H8′	
2″-N*H*		7.93 (t, J = 5.4 Hz)	C1″, C3″	(H1″)$_2$	H10, H11, H12, H1$_a$″, H8″
5″-N*H*		12.61 (s)	C4″, C7″, C8″	H8″	

[a] Spectra were recorded in DMSO-d_6. [b] ^{13}C NMR at 150 MHz referenced to DMSO (δ 39.5). [c] ^{1}H NMR at 600 MHz referenced to residual solvent (δ 2.49). [d] ROESY experiment was acquired using a mixing time of 500 ms.

dibromopyrrole carbonyl moiety.[7] The two dibromopyrrole carboxylate moieties were attached through amide bonds to the methylene groups C1′ and C1″, since ^{1}H−^{1}H COSY and HMBC correlations were observed between the methylene protons H1′$_a$ and H1′$_b$, and H1″$_a$ and H1″$_b$, and the amide protons 2′-NH and 2″-NH and the amide carbonyl carbons C3′ and C3″ respectively.

Correlations in the HMQC spectrum between δ 5.29 and 79.0 ppm indicated that C5 was a deshielded methine occurring between two heteroatoms. HMQC correlations between δ 5.27 and 78.4 ppm indicated that C1 was also a methine attached to two heteroatoms. The presence of two guanidine moieties was supported by the presence of the two quaternary carbons at 155.7 ppm (C3) and 156.7 ppm (C7), indicative of guanidinium carbons. The observation of HMBC correlations from the methine protons at δ 5.27 (H1) and 5.29 (H5) to the carbons at 155.7 ppm (C3) and 156.7 ppm (C7), respectively, indicate their proximity to the guanidine moieties. HMBC correlations from H5 (δ 5.29) to C7 and C9 and from 9-OH (δ 7.76) to C9, together with a ^{1}H−^{1}H COSY correlation from H5 to 6-NH, indicated that a five-membered ring (C5−N6−C7−N8−C9−C5) could be delineated. HMBC correlations from H1 (δ 5.27) to C3 and from the hydroxyl proton 1-OH (δ 7.39) to C1 and C14, together with COSY correlations from H1 to 1-OH and 2-NH, indicated **1** contained another five-membered ring, delineated by C1−N2−C3−N4−C14−C1. A fourth five-membered-ring system (N4−C5−C9−C10−C14−N4) was established from HMBC correlations from H10 to C5, C9, and C14, from H5 to C9, and C14 and from 9-OH to C10. Hence, the presence of a novel perhydrocyclopenta−imidazo−azolo−imidazole ring system was established in the structure of axinellamine A (**1**).

Axinellamine B (**2**) differed from axinellamine A (**1**) only in stereochemistry at C5 and C9 (Table 2). This was supported by the observation that the only protons

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Table 3. Protons Affected (δ in ppm) by the Stereochemical Changes for 1 and 2

position no.	1	2	Δ
C1	5.27	5.35	−0.08
C10	2.84	3.05	−0.21
C11	2.60	2.24	+0.36
C12	1.96	2.06	−0.1
C13	4.28	3.97	+0.31

Table 4. NMR Data for Axinellamine C (3) and Axinellamine D (4) in DMSO-d_6[a]

	axinellamine C (**3**)		axinellamine D (**4**)	
position no.	^{13}C NMR,[b] δ	^{1}H NMR,[c] δ (mult, J in Hz)	^{13}C NMR,[b] δ	^{1}H NMR,[c] δ (mult, J in Hz)
1	84.2 (d)	4.92 (s)	86.0 (d)	5.07 (s)
2				
3	155.5 (s)		158.8 (s)	
4				
5	77.8 (d)	5.34 (s)	80.3 (d)	5.41 (s)
6				
7	155.8 (s)		157.0 (s)	
8				
9	99.1 (s)		102.3 (s)	
10	49.4 (d)	2.97 (d, J = 4.8 Hz)	48.5 (d)	3.13 (d, J = 4.8 Hz)
11	34.0 (d)	2.56 (m)	39.7 (d)	2.22 (m)
12	46.9 (d)	2.06 (m)	47.6 (d)	2.11 (m)
13	64.8 (d)	4.24 (d, J = 12.0 Hz)	66.3 (d)	3.96 (d, J = 12.0 Hz)
14	82.0 (s)		82.7 (s)	
1′	35.6 (t)	3.79 (m) 3.26 (m)	37.9 (t)	3.66 (m) 3.40 (m)
2′				
3′	159.6 (s)		159.1 (s)	
4′	127.3 (s)		127.9 (s)	
5′				
6′	104.2 (s)		104.5 (s)	
7′	97.1 (s)		97.5 (s)	
8′	112.2 (d)	6.99 (s)	113.1 (d)	6.95 (s)
1″	39.6 (t)	3.47 (m) 3.34 (m)	40.7 (t)	3.40 (m) 2.61 (m)
2″				
3″	161.6 (s)		159.3 (s)	
4″	127.3 (s)		127.9 (s)	
5″				
6″	104.2 (s)		104.5 (s)	
7″	97.1 (s)		97.6 (s)	
8″	112.2 (d)	7.00 (s)	113.1(d)	6.94 (s)
1-OCH_3	54.4 (q)	3.24 (s)	55.6 (q)	3.32 (s)
9-OH		7.86 (s)		7.40 (s)
2-NH		10.52 (s)		10.72 (s)
3-NH_2		9.31 (s) and 9.21 (s)[d]		9.40 (s)[d]
6-NH		9.13 (s)		8.81 (s)
7-NH_2		9.02 (bs)[d]		9.09 (bs)[d]
8-NH		9.90 (s)[d]		9.87 (s)
2′-NH		8.21 (t, J = 5.4 Hz)		8.24 (t, J = 6.0 Hz)
5′-NH		12.69 (s)		12.68 (s)
2″-NH		8.17 (t, J = 5.4 Hz)		8.05 (t, J = 6.0 Hz)
5″-NH		12.69 (s)		12.61 (s)

[a] Spectra were recorded in DMSO-d_6. [b] ^{13}C NMR at 150 MHz referenced to DMSO (δ 39.5). [c] ^{1}H NMR at 600 MHz referenced to residual solvent (δ 2.49). [d] These signals are interchangeable and could not be definitively assigned.

affected by the stereochemical change were those at positions C1, C10, C11, C12, and C13 (see Table 3).

Axinellamines C (**3**) and D (**4**) were the C1 methoxyl analogues of **1** and **2**, respectively. HMBC correlations from the methoxyl protons (δ 3.24; 54.4 and δ 3.32; 55.6) to C1 (84.2 and 86.0 ppm, respectively) were observed for **3** and **4** (Table 4).

The relative stereochemistries of **1**−**4** were established from interpretation of ^{1}H−^{1}H coupling constants and

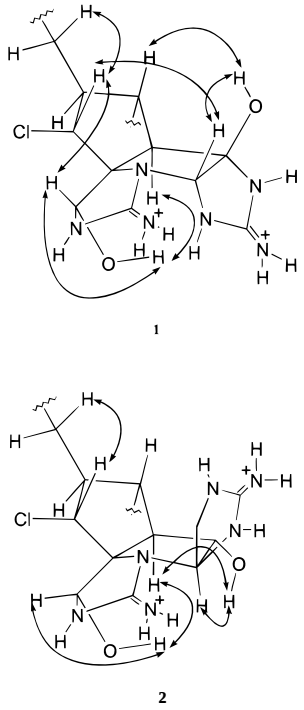

Figure 1. Selected ROESY correlations of axinellamine A (**1**) and axinellamine B (**2**).

ROESY correlations. A coupling constant of 12.0 Hz between H12 and H13 indicated that these protons were diaxial. The diagnostic ROESY correlations for **1** and **2** are shown in Figure 1. In the ROESY spectrum for **1**, the most informative correlations were from 9-OH (δ 7.76) to H11 (δ 2.60) and H5 (δ 5.29) and from H13 (δ 4.28) to H5 (δ 5.29) and H1 (δ 5.27), which placed H1, H5, 9-OH, H11, and H13 on the same face of the molecule. In the ROESY spectrum for **2** correlations from 9-OH (δ 7.44) to H5 (δ 5.32) and H10 (δ 3.05) and from H10 (δ 3.05) to 1-OH (δ 7.37) placed 1-OH, H10, 9-OH, and H5 on the same face of the molecule. These ROESY correlations indicated that the B/D ring geometry was *cis* in both **1** and **2** and that rings A and D were *anti* in **1** and *syn* in **2**. The absolute stereochemistries of **1**−**4** remain unassigned.

Axinellamines B−D (**2**−**4**) had minimum inhibitory concentration (MIC) for bactericidal action against *H. pylori* at 1000 μM. Axinellamine A (**1**) was not bactericidal at 1000 μM.

Experimental Section

General Procedures. For general experimental details see ref 8.

Animal Material. Eight *Axinella* sp. were collected via a remotely controlled submersible from depths of 50 m at either North Head or Long Reef, Sydney, NSW, Australia, and kept frozen prior to freeze-drying and extraction. Of these specimens one specimen was investigated in detail, and the voucher specimen QMG303751 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia. The remaining seven *Axinella* sp. were only briefly investigated (also found to contain 1−4), and the voucher specimens (QM303735, QM303747, QM303724, QM303767, QM303753, QM303721, and QM303727) have also been deposited at the Queensland Museum, South Brisbane, Queensland, Australia. These speci-

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mens of *Axinella* are believed to represent two new species of *Axinella* which are yet to be described.

Extraction and Isolation. The crude methanol extract of this sponge was found to inhibit the growth of *H. pylori* at a concentration equivalent of 1000 μg of sample per 200 μL. LC/MS was performed on a portion of the crude methanol extract using a Rainin C18 analytical HPLC column with a flow rate of 1.0 mL/min (with a gradient from 100% H_2O (containing 0.1% TFA) to 100% acetonitrile over 15 min) and monitored by UV at 280 nm and by positive electrospray ionization. It was found that **1**−**4** were well-resolved from all other compounds present in this extract eluting on LC/ESI MS with the following retention times: 14.51 min for **1**, 14.31 min for **2**, 14.79 min for **3**, and 14.51 min for **4**. A final concentration of 1% TFA in H_2O was used for the isolation and purification procedure. The remaining sample was purified by reverse-phase C18 HPLC with UV detection at 280 nm. In addition to the sponge described in this report, seven other *Axinella* sp. sponges collected from either Long Reef, Sydney, NSW, Australia, or North Head, Sydney, NSW, Australia, were also examined and found to contain the same compounds.

The freeze-dried sponge (9 g dry weight) was extracted sequentially with dichloromethane and methanol. The methanol extract was concentrated under vacuum to give an orange/brown gum (2.1 g). This gum was subjected to gel permeation chromatography on LH-20 Sephadex using 100% methanol as the eluant with detection at 300 nm. Fractions were subjected to analysis by positive electrospray mass spectrometry and combined on this basis. Fractions containing ions at *m/z* 846 and 860 were further purified by reverse-phase C18 HPLC using 30% acetonitrile/H_2O (1% TFA) with UV detection at 280 nm, yielding axinellamine A (**1**; 37.3 mg, 0.62%), axinellamine B (**2**; 32.3 mg, 0.54%), axinellamine C (**3**; 26 mg, 0.29%), and axinellamine D (**4**; 16.5 mg, 0.18%).

Biological Testing. Bacterial Preparation. *H. pylori* was added to sterile Brucella broth containing foetal calf serum (10%). Incubation was at 37 °C under microaerophilic (10% CO_2) conditions with shaking occurring for 24 h.

Addition of Bacteria to Extract Plate. The culture was observed under the microscope to check the purity of the *H. pylori*. The bacteria was then diluted 1:100 using Brucella broth containing foetal calf serum at pH 7.2. A 100 μL portion of the diluted bacteria was then added to the extract plate (each well containing 4 μL of extract that was filter-sterilized and 36 μL of sterile water). The negative control was uninoculated broth, the bacterial control was inoculated broth, and the positive control was the sulfide form of omeprazole. The plates were gently mixed before incubation and then incubated at 37 °C, under microaerophilic conditions for 3 days.

Plate Replication and Reading. After 3 days, 10 μL was transferred from each well onto a large predried Columbia blood agar plate (highly nutrious agar plate) and incubated at 37 °C for 3 days, under microaerophilic conditions.

Plate Reading. The Columbia blood agar plate was visualized for "growth"/"no growth" spots. Spots containing ≤10 colonies are considered no growth, indicating an inhibition.

Axinellamine A (1), 4-Chloro-5,6-bis(4,5-dibromo-1*H*-2-azolylcarboxamidomethyl)-3,6β-dihydroxy-1,8-diimino-perhydrocyclopenta[2,3]imidazo[4′,5′:4,5]azolo[1,2-*c*]imidazole:[9] isolated as a white powder; $[\alpha]_D^{20}{}_{589} = -18°$ (*c* 0.16, MeOH); UV (MeOH) λ_{max} (ϵ) 277 nm (11 400); IR ν_{max} (film) 3182, 1675, 1432, 1204 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; MS positive ESI *m/z* 846 [(M + H) − 2CF_3COOH]$^+$, negative ESI *m/z* 827 [(M − H) − 2CF_3COOH − H_2O]$^-$, 845 [(M − H) − 2CF_3COOH]$^-$, 959 [(M − H) − CF_3COOH]$^-$, 1073 [M − H]$^-$, 1187 [M + CF_3COO^-]$^-$; HRESIMS *m/z* 846.8383 ($C_{22}H_{24}{}^{79}Br_2{}^{81}Br_2ClN_{10}O_4$ requires 846.8364, Δ = 4.0 ppm).

Axinellamine B (2), 4-Chloro-5,6-bis(4,5-dibromo-1*H*-2-azolylcarboxamidomethyl)-3,6β-dihydroxy-1,8-diimino-perhydrocyclopenta[2,3]imidazo[4′,5′:4,5]azolo[1,2-*c*]imidazole: isolated as a pale yellow oil; $[\alpha]_D^{20}{}_{589} = -7°$ (*c* 0.21, MeOH); UV (MeOH) λ_{max} (ϵ) 277 nm (8000); IR ν_{max} (film) 3432, 1641, 1206 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; MS positive ESI *m/z* 846 [(M + H) − 2CF_3COOH]$^+$; negative ESI *m/z* 827 [(M − H) − 2CF_3COOH − H_2O]$^-$, 845 [(M − H) − 2CF_3COOH]$^-$, 959 [(M − H) − CF_3COOH]$^-$, 1073 [M − H]$^-$, 1187 [M + CF_3COO^-]$^-$; HRESIMS *m/z* 846.8360 ($C_{22}H_{24}{}^{79}Br_2{}^{81}Br_2ClN_{10}O_4$ requires 846.8364, Δ = −1.9 ppm).

Axinellamine C (3), 4-Chloro-5,6-bis(4,5-dibromo-1*H*-2-azolylcarboxamidomethyl)-6β-hydroxy-1,8-diimino-3-methoxyperhydrocyclopenta[2,3]imidazo[4′,5′:4,5]azolo-[1,2-*c*]imidazole: isolated as a pale yellow oil; $[\alpha]_D^{20}{}_{589} = -9°$ (*c* 1.1, MeOH); UV (MeOH) λ_{max} (ϵ) 277 nm (6500); IR ν_{max} (film) 3436, 1642, 1437, 1206 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; MS positive ESI *m/z* 860 [(M + H) − 2CF_3COOH]$^+$; negative ESI *m/z* 827 [(M − H) − 2CF_3COOH − OH − CH_3]$^-$, 842 [(M − H) − 2CF_3COOH − OH]$^-$, 859 [(M − H) − 2CF_3COOH]$^-$, 973 [(M − H) − CF_3COOH]$^-$, 1087 [M − H]$^-$, 1201 [M + CF_3COO^-]$^-$; HRESIMS *m/z* 860.8547 ($C_{23}H_{26}{}^{79}Br_2{}^{81}Br_2ClN_{10}O_4$ requires 860.8624, Δ = −2.3 ppm).

Axinellamine D (4), 4-Chloro-5,6-bis(4,5-dibromo-1*H*-2-azolylcarboxamidomethyl)-6β-hydroxy-1,8-diimino-3-methoxyperhydrocyclopenta[2,3]imidazo[4′,5′:4,5]azolo-[1,2-*c*]imidazole: isolated as a pale yellow oil; $[\alpha]_D^{20}{}_{589} = -6°$ (*c* 0.5, MeOH); UV (MeOH) λ_{max} (ϵ) 276 nm (8000); IR ν_{max} (film) 3393, 1679, 1440, 1205 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 4; MS positive ESI *m/z* 860 [(M + H) − 2CF_3COOH]$^+$; negative ESI *m/z* 827 [(M − H) − 2CF_3COOH − OH − CH_3]$^-$, 842 [(M − H) − 2CF_3COOH − OH]$^-$, 859 [(M − H) − 2CF_3COOH]$^-$, 973 [(M − H) − CF_3COOH]$^-$, 1087 [M − H]$^-$, 1201 [M + CF_3COO^-]$^-$; HRESIMS *m/z* 860.8517 ($C_{23}H_{26}{}^{79}Br_2{}^{81}Br_2ClN_{10}O_4$ requires 860.8624, Δ = −5.4 ppm).

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Supporting Information Available: Figures giving ^{1}H NMR spectra for axinellamines A−D (**1**−**4**) (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead for ordering information.

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(9) Please note that the IUPAC naming of axinellamines A−D (**1**−**4**) uses a numbering system different from that used in this report.