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Note

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# Ianthelliformisamines A–C, Antibacterial Bromotyrosine-Derived Metabolites from the Marine Sponge *Suberea ianthelliformis*

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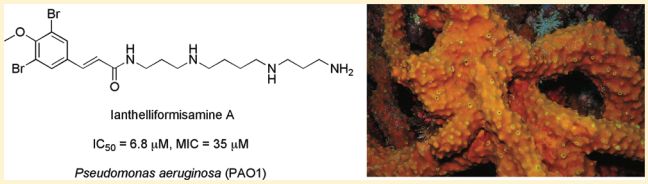
Supporting Information

**ABSTRACT:** A high-throughput screening campaign using a prefractionated natural product library and an in vitro *Pseudomonas aeruginosa* (PAO200 strain) assay identified two antibacterial fractions derived from the marine sponge *Suberea ianthelliformis*. Mass-directed isolation of the $CH_2Cl_2/CH_3OH$ extract from *S. ianthelliformis* resulted in the purification of three new bromotyrosine-derived metabolites, ianthelliformisamines A–C (**1**–**3**), together with the known natural products aplysamine 1 (**4**) and araplysillin I (**5**). The structures of **1**–**3** were determined following analysis of 1D and 2D NMR and MS spectroscopic data. This is the first report of chemistry from the marine sponge *S. ianthelliformis*. Ianthelliformisamine A (**1**) showed inhibitory activity against the Gram-negative bacterium *P. aeruginosa* with an $IC_{50}$ value of 6.8 $\mu$M (MIC = 35 $\mu$M).




Bacterial infections are associated with significant mortality and morbidity among immunocompromised and hospitalized patients, particularly those with pre-existing chronic ailments such as heart disease, diabetes, AIDS, and cancer.[1,2] Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus maltophilia*, and *Acinetobacter* spp. pose a particular threat to these patients, as they are all opportunistic pathogens that possess multidrug resistance.[2] Multidrug resistance is genetically acquired by these pathogens and is primarily due to the active transport of drugs out of the cell by efflux pump systems.[3,4] In addition, intrinsic resistance further decreases the efficacy of clinically used treatments, such as ampicillin, cephalosporins, and macrolide antibiotics, mainly due to impermeability.[1,5] Unfortunately, the increasing prevalence of resistance in bacteria has meant that most known antibacterial drugs now lack efficacy, and this highlights the need for the discovery and development of new anti-infective drugs.[6]

In order to discover new antibacterial compounds from nature, a high-throughput screening (HTS) campaign was undertaken using a prefractionated natural product library[7] and an in vitro assay using a *P. aeruginosa* efflux pump knockout strain (PAO200, MexAB-OprM-deficient mutant).[8] The PAO200 strain was used in an effort to increase initial hits by decreasing efflux clearance. HTS hits were retested against the wild-type strain PAO1 and a Gram-positive methicillin-resistant bacterium, *Staph. aureus* (MRSA strain 01A1095),[9] for estimation of Gram selectivity. Two fractions derived from the Australian marine sponge *Suberea ianthelliformis* showed activity against *P. aeruginosa* PAO1. (+)-LRESIMS of the active fractions identified ions at *m/z* 519/521/523 and 462/464/466, which were predicted to correspond to the bioactive natural products. Mass-directed isolation of the $CH_2Cl_2/CH_3OH$ extract of *S. ianthelliformis* resulted in the purification of three new bromotyrosine-derived metabolites, ianthelliformisamines A–C (**1**–**3**), together with two known compounds, aplysamine 1 (**4**)[10] and araplysillin I (**5**).[11] Herein we report the isolation and structure elucidation of ianthelliformisamines A–C (**1**–**3**) as well as the antibacterial activitities for compounds **1**–**5** against *P. aeruginosa* PAO1 and *Staph. aureus* 01A1095.

The freeze-dried and ground marine sponge *S. ianthelliformis* was sequentially extracted with *n*-hexane, $CH_2Cl_2$, and $CH_3OH$. The $CH_2Cl_2/CH_3OH$ extracts were combined and chromatographed using $C_{18}$-bonded silica HPLC ($CH_3OH/H_2O$/0.1% TFA) to yield 60 fractions. Fractions 34, 36–38, and 45 afforded ianthelliformisamines A (**1**, 7.9 mg, 0.100% dry wt), B (**2**, 8.4 mg, 0.110% dry wt), and C (**3**, 3.1 mg, 0.040% dry wt), respectively. Further purification of fractions 23–25 and 39 using $C_{18}$-bonded silica HPLC ($CH_3OH/H_2O$/0.1% TFA) resulted in the isolation of two known bromotyrosine derivatives, aplysamine 1 (**4**, 0.9 mg, 0.010% dry wt)[10] and araplysillin I (**5**, 0.6 mg, 0.008% dry wt).[11]

Ianthelliformisamine A (**1**) exhibited an ion cluster at *m/z* 519/521/523 (1:2:1) in the (+)-LRESIMS spectrum, indicating the presence of two bromine atoms. The molecular formula of $C_{20}H_{32}Br_2N_4O_2$ was determined on the basis of the (+)-HRESIMS and NMR data for **1** (Table 1). $^1$H and gHSQC NMR data analysis suggested that **1** contained one methoxy group ($\delta_H$ 3.82, s, 3H), one isolated *trans* olefin ($\delta_H$ 7.36, d, $J$ = 16.0 Hz, 1H and 6.66 d, $J$ = 16.0 Hz, 1H), one isolated aromatic signal ($\delta_H$ 7.88, s, 2H), one amide proton ($\delta_H$ 8.31, t, $J$ = 6.1 Hz, 1H), three exchangeable protons ($\delta_H$ 8.80,

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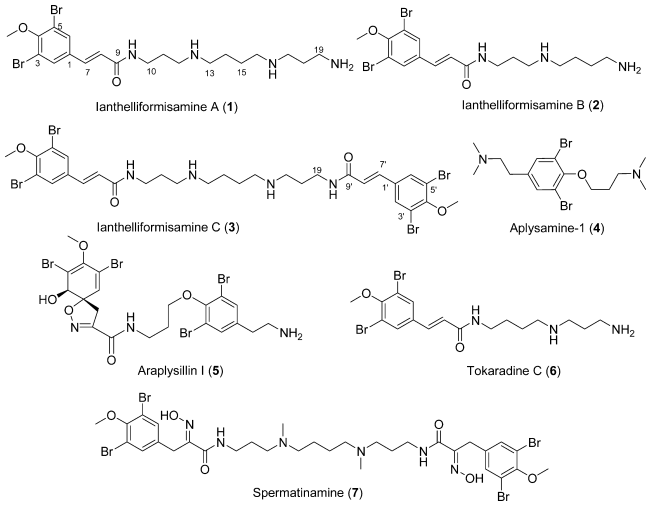


brs, 1H; 8.64, brs, 1H; and 7.94, brs, 1H), and several mutually coupled methylene signals. The $^{13}C$ NMR data of **1** displayed 10 aliphatic carbons, one methoxy carbon ($\delta_C$ 60.5), one carbonyl ($\delta_C$ 164.8), and six aromatic carbons. The COSY correlation data (Figure 1) provided unambiguous connectivities for the polyamine chain: -NH-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-$NH_2$. This unit is the known natural product spermine, which is a polyamine involved in eukaryotic cellular metabolism.[12] A symmetrical 1,3,4,5-tetrasubstituted benzene moiety was established by the gHMBC correlations from the aromatic methine at $\delta_H$ 7.88 (s, 2H) to the carbon to which it was directly attached at $\delta_C$ 131.2 (Figure 1). The position of the methoxy on the benzene ring was determined on the basis of the $^3J_{CH}$ correlations from the aromatic proton at $\delta_H$ 7.88 and methoxy protons at $\delta_H$ 3.82 to the oxygenated aromatic carbon at $\delta_C$ 153.9 (C-4). HMBC correlations from the two olefinic protons to the quaternary aromatic carbon at $\delta_C$ 134.5 and the carbonyl carbon at $\delta_C$ 164.8 indicated that an $\alpha,\beta$-unsaturated carbonyl group was linked to the 1,3,4,5-tetrasubstituted aromatic ring, thus forming a cinnamyl-derived moiety. The gHMBC correlations from the methylene protons at $\delta_H$ 3.26 and the exchangeable proton at $\delta_H$ 8.31 to the

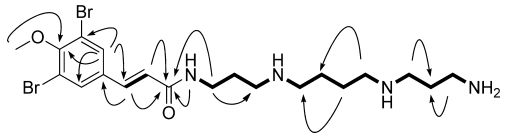

**Figure 1.** Selected HMBC (→) and COSY (—) correlations of **1**.

**Table 1. $^1H$ and $^{13}C$ NMR Data of Ianthelliformisamines A–C (1–3)[a]**

| | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| position | $\delta_C$ mult | $\delta_H$ mult ($J$ in Hz) | $\delta_C$ mult[b] | $\delta_H$ mult ($J$ in Hz) | $\delta_C$ mult | $\delta_H$ mult ($J$ in Hz) |
| 1 | 134.5, C | | 134.0, C | | 134.2, C | |
| 2,6 | 131.2, CH | 7.88, s | 131.2, CH | 7.89, s | 131.5, CH | 7.87, s |
| 3,5 | 117.9, C | | 117.1, C | | 118.0, C | |
| 4 | 153.9, C | | 153.7, C | | 153.9, C | |
| 4-$OCH_3$ | 60.5, $CH_3$ | 3.82, s | 60.0, $CH_3$ | 3.82, s | 60.5, $CH_3$ | 3.81, s |
| 7 | 135.4, CH | 7.36, d (16.0) | 135.0, CH | 7.35, d (16.0) | 135.4, CH | 7.35, d (16.0) |
| 8 | 124.1, CH | 6.66, d (16.0) | 123.6, CH | 6.66, d (16.0) | 124.1, CH | 6.66, d (16.0) |
| 9 | 164.8, C | | 165.0, C | | 164.9, C | |
| 10 | 35.9, CH | 3.26, dt (6.1, 6.0) | 35.6, CH | 3.25, dt (6.1, 6.0) | 35.9, CH | 3.25, dt (6.1, 6.0) |
| 11 | 26.0, CH | 1.79, tt (6.1, 6.5) | 25.8, CH | 1.79, tt (6.1, 6.5) | 26.0, CH | 1.80, tt (6.1, 6.5) |
| 12 | 44.6, $CH_2$ | 2.90, m | 44.5, $CH_2$ | 2.92, m | 44.6, $CH_2$ | 2.92, m |
| 13 | 46.3, $CH_2$ | 2.90, m | 44.5, $CH_2$ | 2.92, m | 46.0, $CH_2$ | 2.92, m |
| 14 | 22.6, $CH_2$ | 1.63, m | 22.7, $CH_2$ | 1.59, m | 22.6, $CH_2$ | 1.61, m |
| 15 | 22.6, $CH_2$ | 1.63, m | 22.7, $CH_2$ | 1.59, m | 22.6, $CH_2$ | 1.61, m |
| 16 | 46.3, $CH_2$ | 2.90, m | 37.3, $CH_2$ | 2.82, tq (6.1, 6.2) | 46.0, $CH_2$ | 2.92, m |
| 17 | 43.8, $CH_2$ | 2.90, m | | | 44.6, $CH_2$ | 2.92, m |
| 18 | 23.7, $CH_2$ | 1.86, m | | | 26.0, $CH_2$ | 1.80 tt (6.1, 6.5) |
| 19 | 36.2, $CH_2$ | 2.90, dd (6.1, 6.5) | | | 35.9, $CH_2$ | 3.25, m |
| 9-NH | | 8.31, t (6.1) | | 8.29, t (6.1) | | 8.25, t (6.1) |
| 12-NH | | 8.80, brs | | 8.57, brs | | 8.48, brs |
| 16-NH | | 8.64, brs | | 7.84, brs | | 8.48, brs |
| 19-$NH_2$ | | 7.94, brs | | | | |
| 1′ | | | | | 134.2, C | |
| 2′,6′ | | | | | 131.5, CH | 7.88, s |
| 3′,5′ | | | | | 118.0, CH | |
| 4′ | | | | | 153.9, C | |
| 4′-$OCH_3$ | | | | | 60.5, $CH_3$ | 3.81, s |
| 7′ | | | | | 135.4, CH | 7.35, d (16.0) |
| 8′ | | | | | 124.1, CH | 6.66, d (16.0) |
| 9′ | | | | | 164.9, C | |
| 9′-NH | | | | | | 8.25 t (6.1) |

[a]Recorded in DMSO-$d_6$ at 30 °C, 500 MHz for $^1H$ NMR of **1**–**3** and 125 MHz for $^{13}C$ NMR of **1** and **3**. [b]$^{13}C$ chemical shifts obtained from 2D NMR experiments.


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carbonyl group at $\delta_C$ 164.8 allowed the linkage of the spermine unit to the substituted cinnamyl moiety. Thus, the structure for ianthelliformisamine A (**1**) was established.

The major metabolite ianthelliformisamine B (**2**) exhibited a cluster of ions at *m/z* 462/464/466 (1:2:1) in the (+)-LRESIMS spectrum, indicating the presence of two bromine atoms. The molecular formula of $C_{17}H_{25}Br_2N_3O_2$ was assigned to **2** on the basis of the (+)-HRESIMS spectrum and NMR data (Table 1). In a similar manner to **1**, the $^1$H and gHSQC data of **2** (Table 1) displayed seven mutually coupled methylenes, one methoxy group, a *trans* olefin, a symmetrical benzene moiety, an amide proton, and two more downfield exchangeable protons. These data identified that **2** had the same cinnamyl derivative as **1**, but contained a different polyamine chain. Compound **2** lacked three methylenes and one nitrogen atom that were present in **1**; this was inferred from the $^1$H NMR and HRESIMS data. The polyamine spin system in **2** was elucidated as -NH-$(CH_2)_3$-NH-$(CH_2)_4$-$NH_2$ by gCOSY correlation data. This unit is the known natural product spermidine, which is a precursor to spermine.[12] The gHMBC correlations from the methylene protons at $\delta_H$ 3.25 and the amide proton at $\delta_H$ 8.29 to the carbonyl group at $\delta_C$ 165.0 and from the methylene protons at $\delta_H$ 1.79 to the methylene carbon at $\delta_C$ 35.6 allowed the linkage of the spermidine unit to the cinnamyl-substituted moiety. Thus, the structure of ianthelliformisamine B was assigned to **2**.

The minor compound ianthelliformisamine C (**3**) showed an ion cluster at *m/z* 835/837/839/841/843 (1:4:6:4:1) in the (+)-LRESIMS spectrum, indicating the presence of four bromine atoms. The molecular formula of **3** was determined to be $C_{30}H_{38}Br_4N_4O_4$ on the basis of the (+)-HRESIMS and NMR data (Table 1). The $^{13}$C NMR spectrum of **3** displayed only 15 carbon resonances, indicating this molecule was symmetrical. The $^1$H and gHSQC data of **3** (Table 1) were very similar to those of compound **1**. The differences included the replacement of a terminal $NH_2$ moiety in **1** with an amide proton ($\delta_H$ 8.25) in **3**, a downfield chemical shift of the methylene protons from $\delta_H$ 2.90 in **1** to $\delta_H$ 3.25 in **3**, and the doubling of the integration values for the aromatic singlets and the *trans* olefinic protons. These data established the existence of an additional cinnamyl moiety in **3**. The gHMBC correlations from the methylene protons at $\delta_H$ 3.25 and the amide proton at $\delta_H$ 8.25 to the carbonyl carbon at $\delta_C$ 164.9 and from the methylene protons at $\delta_H$ 1.80 to the methylene carbon at $\delta_C$ 35.9 (C-10/C-18) confirmed the linkage of the polyamine chain. Hence, ianthelliformisamine C was assigned to structure **3**.

Numerous bromotyrosine-derived secondary metabolites have been isolated from marine sponges of the order Verongida and related organisms.[13−16] Much of the variation of this structure class is associated with the amine component, which is typically derived from ornithine, lysine, tyrosine, cystamine, and histamine.[13] Very few bromotyrosine-derived marine natural products contain spermine or spermidine. Examples include tokaradine C (**6**),[17] spermatinamine (**7**),[18] and pseudoceramines A−D,[19] which have all been isolated from marine sponges belonging to the genus *Pseudoceratina*.

Compounds **1**−**5** were tested for their antibacterial activity against Gram-negative *P. aeruginosa* PAO1, and compounds **1**, **3**, **4** and **5** were tested against the Gram-positive bacterium *Staph. aureus* 01A1095. Compound **1** displayed selective activity against *P. aeruginosa*, with an $IC_{50}$ of 6.8 $\mu$M (MIC = 35 $\mu$M), and 77% inhibition against *Staph. aureus* at 175 $\mu$M. Compound **2** showed only minor inhibition (80% at 87.5 $\mu$M) against *P. aeruginosa* and was not tested against *Staph. aureus*, while **3** showed similar activity against both *P. aeruginosa* and *Staph. aureus*, with $IC_{50}$ values of 8.9 (MIC = 17.5 $\mu$M) and 4.1 $\mu$M (MIC = 8.75 $\mu$M), respectively. Aplysamine 1 (**4**) showed no activity against either bacterial strain at 350 $\mu$M, while araplysillin I (**5**) showed minor growth inhibition toward *P. aeruginosa* (65% at 175 $\mu$M) and *Staph. aureus* (60% at 87.5 $\mu$M). Although limited numbers of compounds were tested, some structure activity relationships were observed. The spermine moiety associated with **1** and **3** appeared to be important for *P. aeruginosa* activity, since replacement of spermine by spermidine as in **2** reduced the activity significantly. Furthermore the addition of an extra cinnamyl derivative in **3** to the terminal amine of the spermine chain decreased the antibacterial selectivity between *P. aeruginosa* against *Staph. aureus*; however the observed selectivity may be due to differential cell permeability between the Gram-negative and the Gram-positive bacteria.

In conclusion, this paper reports the isolation and structure elucidation of three new marine natural products, ianthelliformisamines A−C (**1**−**3**), and two known metabolites, aplysamine 1 (**4**) and araplysillin I (**5**). All the compounds were evaluated for their ability to inhibit the growth of the Gram-negative bacterium *P. aeruginosa*, and ianthelliformisamine A (**1**) was found to be the most active compound, with an $IC_{50}$ value of 6.8 $\mu$M (MIC = 35 $\mu$M). Ianthelliformisamine A also displayed some selectivity toward *P. aeruginosa* compared to *Staph. aureus*.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** UV and IR spectra were recorded on a Jasco V650 UV/vis spectrophotometer and a Bruker Tensor 27 spectrometer, respectively. NMR spectra were recorded at 30 °C on either a Varian 500 or 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple resonance cold probe. The $^1$H and $^{13}$C NMR chemical shifts were referenced to the solvent peak for DMSO-$d_6$ at $\delta_H$ 2.50 and $\delta_C$ 39.5. LRESIMS spectra were recorded on a Waters ZQ mass spectrometer. HRESIMS spectra were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. Alltech Davisil 40−60 $\mu$m 60 Å $C_{18}$ bonded silica was used for preadsorption work. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler was used for HPLC. A Thermo Scientific $C_{18}$ Betasil 5 $\mu$m 143 Å column (21.2 mm × 150 mm) and a Phenomenex Luna $C_{18}$ 5 $\mu$m 143 Å column (21.2 mm × 250 mm) were used for semipreparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade, and the $H_2O$ was Millipore Milli-Q PF filtered. A BIOLINE orbital shaker was used for the large-scale extraction of the sponge material.

**Sponge Material.** The sponge *Suberea ianthelliformis* (order, Verongida; family, Aplysinidae) was collected by scuba diving (−5 m) at Manta Ray Bommie, North Stradbroke Island, Australia, during February 2005 and kept frozen prior to freeze-drying and extraction. A voucher sample (G322245) has been lodged at the Queensland Museum, Brisbane, Australia.

**Extraction and Isolation.** The freeze-dried and ground sponge (10 g) was poured into a conical flask (1 L), *n*-hexane (250 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity, then discarded. $CH_2Cl_2$/$CH_3OH$ (4:1, 250 mL) was added to the defatted marine sample in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity and set aside. $CH_3OH$ (250 mL) was added, and the $CH_3OH$/sponge mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the sponge sample was extracted with another volume of $CH_3OH$ (250 mL), while being shaken at 200 rpm


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for 16 h. All $CH_2Cl_2/CH_3OH$ extractions were combined and dried down under reduced pressure to yield a dark brown solid (1.01 g). Some of this material (0.8 g) was preadsorbed to $C_{18}$-bonded silica (1 g), then packed into a HPLC stainless steel guard cartridge (10 × 30 mm) that was subsequently attached to a $C_{18}$ semipreparative HPLC column. Isocratic HPLC conditions of 90% $H_2O$ (0.1% TFA)/10% $CH_3OH$ (0.1% TFA) were initially employed for the first 10 min; then a linear gradient to $CH_3OH$ (0.1% TFA) was run over 40 min, followed by isocratic conditions of $CH_3OH$ (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected and then analyzed by (+)-LRESIMS. Fractions 34 (+MS, *m/z* 519/521/523), 36−38 (+MS, *m/z* 462/464/466), and 45 (+MS, *m/z* 417/418/419/420/421, 835/837/839/841/843) contained the ions of interest and following lyophilization yielded ianthelliformisamines A (**1**, 7.9 mg, 0.10% dry wt), B (**2**, 8.4 mg, 0.11% dry wt), and C (**3**, 3.1 mg, 0.04% dry wt), respectively. $^1$H NMR analysis of the remaining fractions from the first step of $C_{18}$ semipreparative HPLC identified that fractions 23−25 and 39 contained semipure bromotyrosine derivatives. We isolated these compounds in order to obtain potential structure activity relationships. Fractions 23−25 (6.0 mg) were combined and further purified on a $C_{18}$ HPLC column using a 50 min linear gradient from 55% $H_2O$ (0.1% TFA)/45% $CH_3OH$ (0.1% TFA) to $CH_3OH$ (0.1% TFA), followed by a linear gradient to $CH_3OH$ (0.1% TFA) in 10 min, at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected, and fraction 16 yielded pure aplysamine 1 (**4**, 0.9 mg, 0.01% dry wt). Fraction 39 (3.0 mg) was also further purified using $C_{18}$ HPLC. A linear gradient from 55% $H_2O$ (0.1% TFA)/45% $CH_3OH$ (0.1% TFA) was initially employed for 50 min and finally a linear gradient to $CH_3OH$ (0.1% TFA) in 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected, and fraction 26 yielded araplysillin I (**5**, 0.6 mg, 0.008% dry wt).

*Tris-TFA salt of ianthelliformisamine A (**1**):* brown, amorphous powder; UV $\lambda_{max}$ ($CH_3OH$) (log $\varepsilon$) 282 (3.11), 239 (3.19) nm; IR $\nu_{max}$ (KBr) 3300, 3023, 2956, 1675, 1629, 1546, 1468, 1262, 1198, 1133, 886 cm$^{-1}$; $^1$H and $^{13}$C NMR data (DMSO-$d_6$) see Table 1; (+)-LRESIMS *m/z* 519 (50%), 521 (100%), 523 (50%); (+)-HRESIMS *m/z* 519.0967 $[M + H]^+$ (calcd for $C_{20}H_{33}{}^{79}Br_2N_4O_2$, 519.0965).

*Bis-TFA salt of ianthelliformisamine B (**2**):* brown, amorphous powder; UV $\lambda_{max}$ ($CH_3OH$) (log $\varepsilon$) 283 (3.40), 225 (4.10) nm; IR $\nu_{max}$ (KBr) 3281, 1613, 1513, 1345, 1262, 1012, 839 cm$^{-1}$; $^1$H and $^{13}$C NMR data (DMSO-$d_6$) see Table 1; (+)-LRESIMS *m/z* 462 (50%), 464 (100%), 466 (50%); (+)-HRESIMS *m/z* 462.0340 $[M + H]^+$ (calcd for $C_{17}H_{26}{}^{79}Br_2N_3O_2$, 462.0386).

*Bis-TFA salt of ianthelliformisamine C (**3**):* brown, amorphous powder; UV $\lambda_{max}$ ($CH_3OH$) (log $\varepsilon$) 282 (5.07), 239 (4.66) nm; IR $\nu_{max}$ (KBr) 3276, 3019, 2585, 2358, 1673, 1623, 1543, 1467, 1260, 1198, 1133, 892 cm$^{-1}$; $^1$H and $^{13}$C NMR data (DMSO-$d_6$) see Table 1; (+)-LRESIMS *m/z* 418 $[M + 2H]^{2+}$ (15%), 419 (60%), 420 (100%), 421 (60%), 422 (15%), 835 $[M + H]^+$ (15%), 837 (60%), 839 (100%), 841 (60%), 843 (15%); (+)-HRESIMS *m/z* 834.9692 $[M + H]^+$ (calcd for $C_{30}H_{39}{}^{79}Br_4N_4O_4$, 834.9699).

*Pseudomonas aeruginosa Optical Density ($OD_{620}$) Viability Assay.* *P. aeruginosa* PAO1 or PAO200 strain (supplied by Pfizer Global Research and Development) cultures were prepared at 3.5 × $10^4$ cfu/mL in cation-adjusted Mueller Hinton broth (Difco), from concentrated frozen stocks of culture. The final bacterial concentration in the assay was 1500 cfu/well. Diluted bacteria (45 μL) were added to a 384-well, lidded, sterile, clear plate (Becton Dickinson) containing controls/fractions by a Multidrop liquid handler (Thermo Scientific). Plates were incubated at 37 °C in a humidified incubator for 18 h or until the wells reached an optical density ($OD_{620}$) of between 0.7 and 0.8, then allowed to cool for 30 min. Clear plate seals (Perkin-Elmer) were placed over the plate surface before reading on a Multiskan Ascent reader (Thermo Scientific) at 620 nm. Test fractions, compounds, or control samples (5 μL) were added to the assay plate prior to the addition of bacteria. Samples were prepared by dilution of stock fractions/compounds/controls in DMSO into the assay plate with addition of 0.875 μL of stock and 4.125 μL of autoclaved Milli-Q filtered $H_2O$ with a Minitrak (Perkin-Elmer) liquid handler. The final concentration of DMSO in the assay was 1.75%. Each assay plate contained both positive and negative controls in columns 23 and 24, respectively. The positive control, for uninhibited growth, consisted of 5 μL of DMSO/Milli-Q $H_2O$ to a final concentration of 1.75%, and the negative control, or 100% cell death, comprised 5 μL of the broad spectrum antibiotic ciprofloxacin at a final concentration of 5 μg/mL. Whole control plates were included for each assay run, which consisted of duplicate dose−response curves of ciprofloxacin, each in triplicate. Ciprofloxacin was shown to have an $IC_{50}$ value of 0.038 μM against *P. aeruginosa* PAO1.

*Staph. aureus Optical Density Viability Assay.* The *Staph. aureus* assay was carried out as per the *P. aeruginosa* assay, with the following modifications: the final bacterial concentration used was 1980 cfu/well and ciprofloxacin for the internal assay control wells was at 500 μg/mL. Incubation was for 19 h, or until the $OD_{620}$ reached 0.45. Ciprofloxacin was shown to have an $IC_{50}$ value of 125 μM against methicillin-resistant *Staph. aureus* 01A1095.

## ■ ASSOCIATED CONTENT

### Supporting Information

NMR and MS spectra for ianthelliformisamines A−C (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

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