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Halogenated Tyrosine Derivatives from the Tropical Eastern Pacific Zoantharians *Antipathozoanthus hickmani* and *Parazoanthus darwini*

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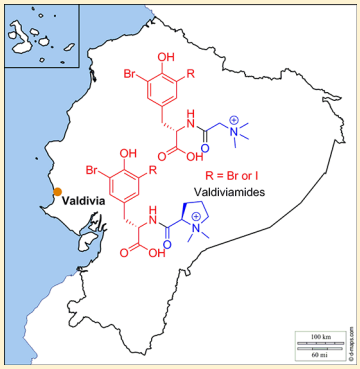
Supporting Information

ABSTRACT: In the search for bioactive marine natural products from zoantharians of the Tropical Eastern Pacific, four new tyrosine dipeptides, named valdiviamides A−D (**1**−**4**), were isolated from *Antipathozoanthus hickmani*, and two new tyramine derivatives, **5** and **6**, from *Parazoanthus darwini*. The phenols of all six tyrosine derivatives are substituted by bromine and/or iodine atoms at the *ortho* positions of the hydroxyl. The planar structures of these aromatic alkaloids were elucidated from 1D and 2D NMR experiments in combination with HRESIMS data, and the absolute configurations of **1**−**4** were deduced from comparison between experimental and calculated electronic circular dichroism spectra. As halogenated tyrosine derivatives could represent chemotaxonomic markers of these genera, we decided to undertake the first chemical investigation of another species, *Terrazoanthus* cf. *patagonichus*. As expected, no halogenated metabolite was evidenced in the species, but we report herein the identification of two new zoanthoxanthin derivatives, named zoamides E (**7**) and F (**8**), from this species. Antimicrobial and cytotoxicity bioassays revealed that valdiviamide B (**2**) displayed moderate cytotoxicity against the HepG2 cell line with an IC_{50} value of 7.8 μM.



The structures of marine natural products are usually characterized by a higher occurrence of halogen atoms than their terrestrial counterparts. Within the marine environment, most of the marine invertebrates but especially algae and sponges are sources of halogenated secondary metabolites.[1−5] By far, the most common halogen in marine natural products is bromine.[6,7] The incorporation of halogens into natural products is catalyzed by enzymes such as vanadium-dependent haloperoxidases, which have been reported mainly from algae, sponges, or bacteria.[2,6−11] Among the different halogenated structural units reported from marine organisms, bromotyrosine derivatives are the most common and mainly found in marine sponges of the order Verongida,[12−14] but also some species of ascidians.[15−18] Interestingly, a wide range of biological activities including antibacterial, anticancer, antiparasitic, antiplasmodial, anti-inflammatory, and antifouling have been reported for halogenated tyrosine derivatives.[18−22]

Zoantharians are sessile invertebrates inhabiting diverse marine environments such as coral reefs, shallow waters, and deep seas around the world. They are known to produce a number of interesting metabolites including zoanthamine alkaloids,[23,24] fluorescent pigments such as zoanthoxanthins,[25,26] ecdysteroids,[27,28] and some halogenated metabolites. The first halogenated natural product from zoantharians was the fatty acid 6-bromo-5,9-eicosadienoic acid isolated from *Palythoa caribaeorum* in 1995.[29] Later, two monobrominated tyrosine derivatives named parazoanthines D and E were

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reported in 2009 from the Mediterranean zoantharian *Parazoanthus axinellae*.[30] Additionally, two mono- and two dibrominated parazoanthines G–J were reported in 2014 from the same species.[31] In another context, two halogenated zoanthamine alkaloids, 5α-iodozoanthenamine and 11β-chloro-11-deoxykuroshine A, were isolated from *Zoanthus kuroshio* collected off the coast of Taiwan.[32] Despite the great diversity of natural products isolated from zoantharians, most of the chemical studies are reported from species collected in the Central Indo-Pacific, while only a few studies have been undertaken from species inhabiting the Tropical Eastern Pacific.[33−35]

During a dereplication process on zoantharians from the Ecuadorian coast,[36] the UPLC-HRMS analyses of both species *Antipathozoanthus hickmani* and *Parazoanthus darwini* revealed the presence of some metabolites with unreported masses and isotopic patterns characteristic of mono- and dibrominated compounds. The first chemical study of *A. hickmani* revealed ecdysteroid derivatives named ecdysonelactones A–D as the major compounds of the methanolic fraction.[34] As no chemical studies from *P. darwini* have been reported so far, we decided to undertake a deeper chemical study of *A. hickmani* and the first one on *P. darwini* collected off the Marine Protected Area El Pelado, Santa Elena, Ecuador.

In this study, we describe the isolation and structure elucidation of four new halogenated tyrosine dipeptides named valdiviamides A–D (**1**–**4**) from *A. hickmani* and two halogenated tyramine derivatives, **5** and **6**, from *P. darwini* (Figure 1). These compounds were isolated as trifluoroacetic

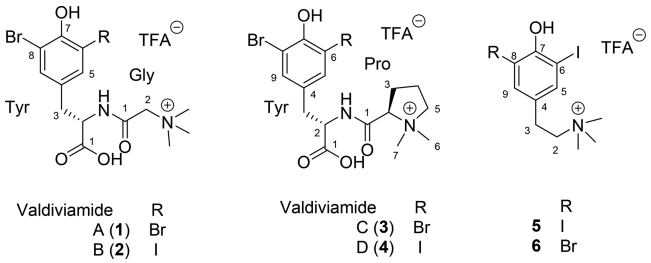

Figure 1. Structures of valdiviamides A–D (**1**–**4**) from *Antipathozoanthus hickmani* and tyramine derivatives **5** and **6** from *Parazoanthus darwini*.

acid (TFA) salts, as they contain an ammonium ion, and they are characterized by the presence of bromine and iodine atoms around the phenol. Because halogenated tyrosine derivatives could serve as chemotaxonomic markers for these two genera of the family Parazoanthidae, we decided to perform the first chemical study of a nonstudied species of this area, *Terrazoanthus* cf. *patagonichus*, belonging to the family Hydrozoanthidae. In agreement with this hypothesis, no halogenated derivative was obtained from this species of the genus *Terrazoanthus*, but two new zoanthoxanthin derivatives named zoamides E and F (**7** and **8**) were isolated as neutral molecules.

RESULTS AND DISCUSSION

The freeze-dried samples of *A. hickmani* (68 g) and *P. darwini* (54 g) were extracted with a mixture of CH_2Cl_2/MeOH (1:1) under ultrasonication. The extracts (2.60 and 2.10 g, respectively) were fractionated by RP-C18 vacuum liquid chromatography with mixtures of solvents of decreasing polarity from H_2O to MeOH to CH_2Cl_2. The hydromethanolic fractions containing the halogenated derivatives were purified by semipreparative RP-C18 HPLC, yielding pure compounds **1**–**6** as their TFA salts.

From *A. hickmani*, compound **1** was purified as a yellow, amorphous solid. The (+)-HRESIMS analysis revealed an isotopic cluster of ions $[M]^+$ at m/z 436, 438, and 440 in the ratio 1:2:1, consistent with the presence of two bromine atoms and the molecular formula $C_{14}H_{19}Br_2N_2O_4$. A first inspection of the ^{1}H NMR data revealed a singlet at δ_H 7.39 (s, H_2-5/9), suggesting the presence of a symmetric *ortho*/*ortho*/*para* tetrasubstituted aromatic ring. Additionally, the ^{1}H NMR, COSY, and HSQC spectra evidenced an ABX spin system with three signals at δ_H 4.72 (dd, J = 9.5, 5.0 Hz, Tyr-H-2), δ_H 3.21 (dd, J = 14.5, 5.0 Hz, Tyr-H-3a), and δ_H 2.88 (dd, J = 14.5, 9.5 Hz, Tyr-H-3b) and another AB system at δ_H 4.09 (d, J = 15.0 Hz, Gly-H-2a) and δ_H 4.00 (d, J = 15.0 Hz, Gly-H-2b). The deshielded singlet integrating for nine protons at δ_H 3.25 was assigned to the three methyls of a quaternary ammonium ion. The ^{13}C NMR spectrum disclosed signals corresponding to six nonprotonated carbons: one carboxylic acid carbon at δ_C 173.4 (Tyr-C-1), one amide carbonyl group at δ_C 164.5 (Gly-C-1), one aromatic signal at δ_C 132.3 (Tyr-C-4), an oxygenated aromatic carbon signal at δ_C 151.3 (Tyr-C-7), and the brominated aromatic signal at δ_C 112.1 (C-6/8). The *ortho* position of the two bromine atoms and the *meta* position of the aromatic protons with respect to the hydroxy group were supported by key Tyr-H_2-3/C-5 and Tyr-H-5/C-6 and C-7 HMBC correlations (Figure 2). The Tyr-H_2-3/Tyr-H-2

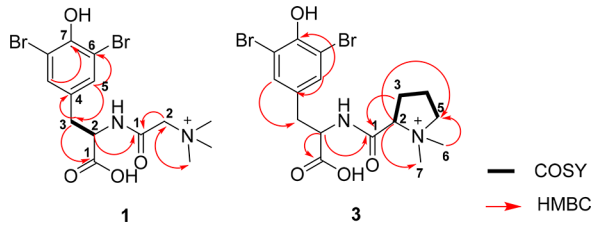

Figure 2. Key COSY and HMBC (H to C) correlations of **1** and **3**.

coupled system together with the key Tyr-H-2/C-1, C-4 HMBC correlations secured the structure of the tyrosine moiety. For the other part of the molecule, key Gly-H_2-2/C-1 and $(CH_3)_3N$ HMBC correlations confirmed the trimethylated glycine subunit. Finally, the key Tyr-H-2/Gly-C-1 HMBC correlation allowed the connection between both amino acids.

With the planar structure of the dipeptide in hand, the absolute configuration of the tyrosine stereogenic center was assigned by comparison of experimental and predicted electronic circular dichroism (ECD) spectra. After conformational analysis and geometry optimization, the ECD spectra of the two enantiomers of **1** were calculated using time-dependent density functional theory (TDDFT) with the B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) level of theory. The calculated ECD spectrum of the 2*S* enantiomer matched the experimental spectrum of **1**, confirming the presence of a L-tyrosine-derived residue (Figure 3).

Valdiviamide B (**2**) was isolated as an amorphous, white solid. Its HRESIMS spectrum revealed an isotopic cluster of ions at m/z 484 and 486 $[M]^+$ in a ratio of 1:1, consistent with the presence of one bromine atom and the molecular formula $C_{14}H_{19}BrIN_2O_4$. The analysis of the ^{1}H NMR spectrum revealed similar signals to those of compound **1** except for the

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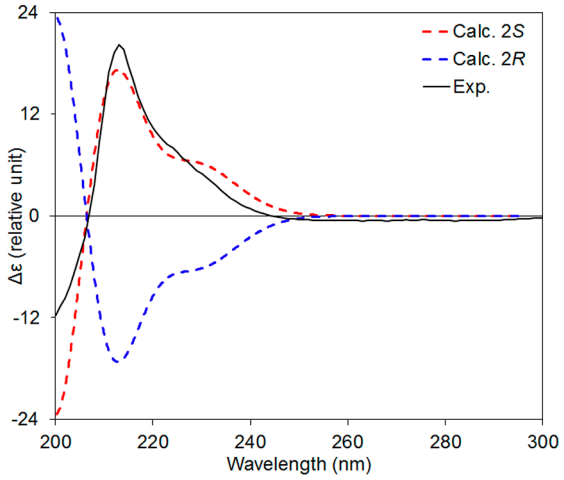

Figure 3. Comparison of the calculated ECD spectra of the two enantiomers of **1** at Tyr-C-2, with the experimental ECD spectrum.

presence of two doublets in the aromatic regions at δ_H 7.60 (d, J = 1.5 Hz, H-5) and δ_H 7.41 (d, J = 1.5 Hz, H-9), indicative of a loss of symmetry around the aromatic ring. The replacement of one bromine in **1** by one iodine atom in **2** in the molecular formula suggested a similar change on the phenol ring. Despite a small amount of pure compound **2** preventing the acquisition of interpretable ^{13}C and 2D NMR spectra, and based on the MS data and the significantly increased chemical shift of the aromatic proton at δ_H 7.60 in comparison to those of **1**, we are confident in assigning **2** as the iodo analogue of **1**. The absolute configuration at C-2 was assigned as *S* due to a similar positive Cotton effect at 219 nm in the ECD spectrum of **2** compared to that of **1**.

Valdiviamide C (**3**) was isolated as a colorless, amorphous solid, and the molecular formula $C_{16}H_{21}Br_2N_2O_4$ was determined by HRESIMS with an isotopic cluster of ions at m/z 462, 464, and 466 $[M]^+$ in the ratio 1:2:1, indicative of the presence of two bromine atoms. Analysis of the ^1H NMR spectrum of **3** revealed similar tyrosine signals to those of **1** with a singlet at δ_H 7.41 (s, Tyr-H-5/9) and a deshielded methine at δ_H 4.72 (dd, J = 10.5, 5.0 Hz, Tyr-H-2), indicating the presence of the same dibromotyrosine moiety in **1**. The ^1H NMR and COSY spectra of **3** differed from those of **1** with the presence of an additional spin-coupled system replacing the AB system of the glycine moiety in **1**. This spin-coupled system includes resonances associated with a methine at δ_H 4.10 (H-2) and three methylene groups at δ_H 2.51 (H-3a), δ_H 2.38 (H-3b), δ_H 2.27 (H_2-4), and δ_H 3.78 (H-5a), δ_H 3.57 (H-5b). The methyl singlet integrating for nine in **1** was also replaced by two singlets at δ_H 3.11 (s, H_3-7) and δ_H 2.95 (s, H_3-6), corresponding to nonequivalent *N*-methyl groups. These data combined with the key Pro-H_3-6/C-5, Pro-H-2/C-7, and Pro-H_2-3/C-1 HMBC correlations suggest the presence of an *N*,*N*-dimethyl proline subunit (Figure 2). Finally, the connection between the two subunits was confirmed by the key Tyr-H-2/Pro-C-1 HMBC correlation.

First, we assumed an *S* absolute configuration for the tyrosine residue due to a positive Cotton effect at 220 nm

Table 1. ^1H and ^{13}C NMR Data for Valdiviamides A–D (1–4) in CD_3OD (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR data)

	1		2	3		4
position	δ_C, type	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_C type	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)
Tyrosine						
1	173.4, C			173.7, C		
2	54.7, CH	4.72, dd (9.5, 5.0)	4.72, dd (9.5, 5.0)	54.8, CH	4.72, dd (10.5, 5.0)	4.72, dd (10.5, 5.0)
3a	36.6, CH_2	3.21, dd (14.5, 5.0)	3.20, dd (14.5, 5.0)	36.5, CH_2	3.27, dd (14.0, 5.0)	3.19, dd (14.0, 5.0)
3b		2.88, dd (14.5, 9.5)	2.86, dd (14.5, 9.5)		2.91, dd (14.0, 10.5)	2.89, dd (14.0, 10.5)
4	132.3, C			132.9, C		
5	134.0, CH	7.39, s	7.61, d (2.0)	134.1, CH	7.41, s	7.62, s
6	112.1, C			112.1, C		
7	151.3, C			151.3, C		
8	112.1, C			112.1, C		
9	134.0, C	7.39, s	7.41, d (2.0)	134.1, CH	7.41, s	7.43, s
Glycine						
1	164.5, C					
2a	65.6, CH_2	4.09, d (15.0)	4.09, d (15.0)			
2b		4.00, d (15.0)	4.01, d (15.0)			
$N(Me)_3$	54.8, CH_3	3.25, s	3.25, s			
Proline						
1				166.4, C		
2				75.7, CH	4.10, t (8.0)	4.08, t (8.0)
3a				26.6, CH_2	2.51, dddd (14.5, 9.0, 8.0, 5.0)	2.55, m
3b					2.38, ddt (14.5, 8.0, 6.5)	2.38, m
4a				21.3, CH_2	2.28, m	2.28, m
4b					2.24, m	2.24, m
5a				67.8, CH_2	3.78, ddd (11.5, 8.5, 6.0)	3.77, ddd (11.5, 8.5, 6.0)
5b					3.57, ddd (11.5, 8.5, 7.5)	3.61, ddd (11.5, 8.5, 7.5)
6				48.2, CH_3	2.95, s	2.94, s
7				53.1, CH_3	3.11, s	3.10, s

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similar to those found for **1** and **2**. For the relative configuration between the two residues, comparisons between the experimental and theoretical ECD spectra of *S*, *S* and *S*, *R* diastereoisomers were not conclusive, and therefore we decided to proceed with a DP4 probability calculation.[37] Even though the purification was performed with TFA in the eluent, the conformational analyses were undertaken on both possible ionic forms: first the zwitterion and then the ammonium ion with TFA^- as a counteranion. After a conformational analysis using a 1.5 kcal/mol energy threshold, four conformers were obtained for both the *S*, *S* and the *S*, *R* diastereoisomers. ^{13}C NMR chemical shifts were calculated using the B3LYP/6-31G(d)//MPW1PW91/6-311+G(2d) level. For both ionic forms, the *S*, *R* diastereoisomer was predicted as the most probable structure with 100% DP4 probability (Supporting Information). Despite possible influences of the solvent and concentration on the chemical shifts of ionic molecules, we were quite confident in this assignment, as the prediction was completely in favor of one of the two diasteroisomers. However, this unexpected result led us to inspect the NOESY spectrum of **3** and the distances between the atoms of the lowest energy conformers. On the most stable conformer of the diastereoisomers (2*S*)-Pro and (2*R*)-Pro, the distances between Tyr-H-5/9 and the methyls on the proline nitrogen were calculated as 2.9 and 3.8 Å, respectively. These observations came as a confirmation of the *S*, *R* diastereoisomer, as no NOE was observed between these two signals in the experimental spectrum of **3**.

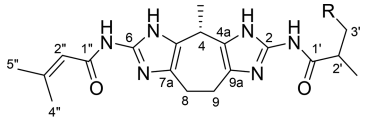
Valdiviamide D (**4**) was isolated as a colorless, amorphous powder. The HRESIMS data exhibited an isotopic cluster of ions at *m*/*z* 510 and 512 $[M]^+$ in a 1:1 ratio, consistent with the presence of one bromine atom and a molecular formula of $C_{16}H_{21}BrIN_2O_4$. Analysis of the ^1H NMR spectrum of **4** revealed similar signals to those of compound **3** (Table 1), except for the replacement of the aromatic singlet in **3** by two doublets at δ_H 7.61 (1H, d, *J* = 2.0 Hz) and δ_H 7.43 (1H, d, *J* = 2.0 Hz). This was indicative of the substitution of one bromine for an iodine around the phenol ring in **4**, similar to that observed for **2**.

From *P. darwini*, compound **5** was isolated as a colorless powder, and its HRESIMS spectrum revealed a major molecular ion peak at *m*/*z* 431.9349 $[M]^+$, consistent with the molecular formula $C_{11}H_{16}I_2NO$. A first inspection of the ^1H NMR spectrum revealed a deshielded aromatic singlet integrated for two protons at δ_H 7.74 (2H, s, H-5/9), and these signals were HMBC correlated to C-6/8 at δ_C 85.5, revealing an unusual shielding for the aromatic nonprotonated carbon (Table 1). These chemical shifts were in accordance with the data obtained for the iodinated compounds **2** and **4**, therefore suggesting two iodine atoms on the aromatic ring. Furthermore, the deshielded signal at δ_C 156.3 (C-7) confirmed the occurrence of a hydroxyl group at C-7 and was confirmed by a key H-5/C-7 HMBC correlation. The ^1H NMR and HSQC spectra also evidenced an AA′BB′ system with signals at δ_H 3.50 (H_2-2) and δ_H 3.00 (H_2-3). The remaining methyl singlet integrating for nine protons at δ_H 3.18 (9H, s) was assigned to a $N(CH_3)_3$ group. Altogether, these data suggest that compound **5** is a halogenated tyramine derivative. Key H_2-3/C-2, C-4, C-5, $N(CH_3)_3$/C-2, and H_2-2/C-3, C-4 HMBC correlations allowed the establishment of the structure of **5** as 2-(4-hydroxy-3,5-diiodophenyl)-*N*,*N*,*N*-trimethylethanaminium. This new natural product has a similar structure to that of iodocionin.[20]

Compound **6** was isolated as a pale yellow solid. The HRESIMS data revealed an isotopic cluster of ions at *m*/*z* 383 and 385 $[M]^+$ in a ratio of 1:1, suggesting the presence of one bromine atom, and it was consistent with the molecular formula $C_{11}H_{16}BrINO$, differing from **5** by the replacement of one iodine with one bromine atom. This was confirmed by the loss of symmetry of the phenol with two aromatic doublets at δ_H 7.71 (d, *J* = 2.0 Hz) and 7.51 (d, *J* = 2.0 Hz) and the shift of the C-8 carbon signal at δ_C 110.6. Therefore, the structure of **6** was assigned as 2-(4-hydroxy-3-bromo-5-iodophenyl)-*N*,*N*,*N*-trimethylethanaminium.

Valdiviamides A–D (**1**–**4**) isolated from the zoantharian *A. hickmani* are bromotyrosine derivatives obtained by condensation from the amine group of the tyrosine with the carboxylic acid group of the glycine for valdiviamides A and B (**1** and **2**) and with the carboxylic acid group of proline for valdiviamides C and D (**3** and **4**). The name valdiviamide is given as a tribute to the Valdivia culture, one of the oldest settled cultures in Ecuador and South America and still present in the area of the sample collection. Interestingly, the absolute configuration for compound **3** is proposed to be L-Tyr,D-Pro. Even though the presence of the D-Pro was unexpected, there are several reports of this enantiomer in natural products and especially in the presence of a betaine proline.[38] We anticipate that the epimerization from *S* to *R* configuration at the α position of the proline would be favored by the presence of the positive charge on the nitrogen when doubly substituted. Indeed, the strong withdrawing effect of the ammonium group would increase the acidity of the proton α.

All six metabolites are characterized by the presence of halogens such as bromine and/or the less common iodine in the *ortho* positions of the phenol. These results raise the question of the use of simple bromotyrosine derivatives as chemical markers of zoantharians from the family Parazoanthidae, containing the genera *Parazoanthus* and *Antipathozoanthus*. This assumption has to be confirmed, as for instance, only one morphotype of the Mediterranean *P. axinellae* contains the bromotyrosine parazoanthines.[39] However, this proposition is consistent with all the chemical studies we undertook on tropical eastern Pacific zoantharians. As we recently collected an undescribed species of zoantharian named *T.* cf. *patagonichus* from this area, we wanted to verify if it contained any halogenated tyrosine derivatives. Even though the dereplication process did not suggest the presence of such halogenated natural products, we decided to identify its major metabolites due to the presence of unidentified masses. We were finally able to characterize two new acylated zoanthoxanthin derivatives named zoamides E (**7**) and F (**8**).



Zoamide	R
E (**7**)	Me
F (**8**)	H

Zoamide E (**7**) was isolated as a colorless powder, and the (+)- HRESIMS analysis revealed a major peak at *m*/*z* 385.2369 $[M + H]^+$, consistent with the molecular formula $C_{20}H_{29}N_6O_2$. A first inspection of the ^1H NMR spectrum revealed an olefinic proton signal at δ_H 5.94 (septet, *J* = 1.0 Hz, H-2″), two methines at δ_H 4.30 (q, *J* = 7.0 Hz, H-4) and δ_H 2.56 (sextet, *J* = 7.0 Hz, H-2′), three methylenes at δ_H 3.05 (m,

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H_2-8/H_2-9), δ_H 1.78 (dquint, J = 15.0, 7.0 Hz, H-3′a), and δ_H 1.55 (dquint, J = 15.0, 7.0 Hz, H-3′b), and five methyls at δ_H 2.30 (d, J = 1.0 Hz, H_3-5″), δ_H 2.01 (d, J = 1.0 Hz, H_3-4″), δ_H 1.59 (d, J = 7.0 Hz, CH_3-C-4), δ_H 1.23 (d, J = 7.0 Hz, H-5′), and δ_H 0.97 (t, J = 7.5 Hz, H-4′). The 1D NMR data were in good agreement with the data for zoanthoxanthin derivatives already reported.[39] However, additional signals were evident in the 1D NMR spectra for saturated protons/carbons. Comparing with data reported for terrazoanthines isolated recently from *T. patagonichus* (ex *T. onoi*) we could quickly assign a 2-methylbutanoyl substituent on the primary amine of a terminal guanidine.[33] A second acyl substituent was identified as 3-methyl-2-butenoyl and placed on the second primary amine. Similar acylated zoanthoxanthin derivatives were already reported from a *Parazoanthus* sp. and named zoamides A−D.[40] The only difference between our compound and those reported previously is the nature of one acyl substituent. For the absolute configuration we decided to work on compound **8**, as it contains only one stereogenic center.

Zoamide F (**8**) was isolated as a white, colorless powder, and the (+)-HRESIMS analysis revealed a major peak at m/z 371.2214 $[M + H]^+$, consistent with the molecular formula $C_{19}H_{26}N_6O_2$ and therefore **8** is a nor derivative of **7**. Analysis of the ^{1}H NMR spectrum revealed similar signals to those of **7** with the difference of the loss of one methylene signal in **8**. The COSY spectrum allowed us to locate this loss on one acyl group replacing the 2-methylbutanoyl in **7** by an isobutanoyl in **8**. To assign the absolute configuration at the unique C-4 stereogenic center of the two zoamides, we used ECD analysis of **8**. The calculation of the ECD spectrum was not straightforward because of the structural difference on both sides of the stereogenic center were very minor. The ECD and UV spectra calculated at the commonly used B3LYP/6-31G(d) level did not match with the experimental UV spectrum and did not allow a definitive assignment of the chirality. Therefore, to confidently assign the absolute configuration of **8**, the ECD spectrum was predicted with different combinations of reported functions and basis sets with a higher computational power.[41] Spectra calculated using ωB97X-D/6-311+G(d,p)//B3LYP/6-31G(d,p) and cam-B3LYP/def2-TZVP//B3P86/aug-cc-pVDZ had successful matches with the experimental UV and ECD spectra, allowing us to confidently assign the C-4 stereogenic center in an *R* configuration (Figure S60). This highlights the need for TDDFT calculations to be performed with multiple functional/basis set combinations to conclusively identify the chirality of complex molecules. The structures of zoamide E and F were finally confirmed by a direct comparison of their ^{13}C NMR data to those of zoamides A−D isolated from a black unidentified species of *Parazoanthus*.[40] The MS and NMR data supported unambiguously the structures of these new zoanthoxanthin derivatives (see Supporting Information).

This species of *T.* cf. *patagonichus* is morphologically related to the previously studied *T. patagonichus* from which some other acylated zoanthoxanthin analogues named terrazoanthines were isolated.[33] Based on the similarity of these bisguanidinylated alkaloids, acylation seems restricted to species of the genus *Terrazoanthus*. Therefore, as there was no clear taxonomic description of the zoantharian from which zoamides A−D were isolated, we propose that the organism reported as *Parazoanthus* sp. could be a species of *Terrazoanthus*. Furthermore, the absence of halogenated compounds in *Terrazoanthus* species strengthens the hypothesis of halogenated tyrosine derivatives as a common feature of the genera *Antipathozoanthus* and *Parazoanthus*.

Compounds **1**−**8** were subjected to antimicrobial activity testing against a panel of Gram-positive (methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). None of the compounds displayed antimicrobial activity against any of the pathogenic strains at the highest concentration tested (128 μg/mL). Compounds **1**−**3** and **5**−**8** were additionally evaluated as cytotoxic agents against three human tumor cell lines, namely, HepG2 (liver), A549 (colon), and MIA PaCa-2 (pancreas). Regarding their cytotoxic activity, only compound **2** displayed moderate activity against the HepG2 cell line, with an IC_{50} value of 7.8 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations and UV and ECD data were obtained on a Chirascan V100 (Applied Photophysics). IR data was obtained on a PerkinElmer FT-IR spectrometer, spectrum 100. NMR experiments were performed on a 500 MHz spectrometer (Varian) or a 600 MHz spectrometer (Agilent) for compounds **2** and **4**, and signals were referenced in ppm to the residual solvent signals (CD_3OD, at δ_H 3.31 and δ_C 49.0). HRESIMS data were obtained with an Agilent UHR-qTOF 6540 mass spectrometer. HPLC purification was carried out on a JASCO HPLC equipped with a PU4087 pump and a UV4070 UV/vis detector.

Animal Material. Samples of *Antipathozoanthus hickmani*, *Parazoanthus darwini*, and *Terrazoanthus* cf. *patagonichus* were collected by scuba at 24 m depth at the Marine Protected Area El Pelado (Santa Elena, Ecuador). Vouchers of the samples with codes 150924EP01-02 (*A. hickmani*), 150820EP01-05 (*P. darwini*), and 170416EP02-01 (*T.* cf. *patagonichus*) are held at the Centro Nacional de Acuicultura e Investigaciones Marinas CENAIM-ESPOL (San Pedro, Santa Elena, Ecuador) (Supporting Information).

Extraction and Isolation. The freeze-dried samples of *A. hickmani* (68 g), *P. darwini* (54 g), and *T.* cf. *patagonichus* (8 g) were extracted with a mixture of solvents, MeOH/CH_2Cl_2 (1:1), three times (500 mL) at room temperature. The solvents were removed under pressure to obtain an organic extract of *A. hickmani* (2.60 g), *P. darwini* (2.10 g), and *T.* cf. *patagonichus* (0.308 g). Each extract was then subjected to RP-C18 vacuum liquid chromatography (VLC) (LiChroprep RP-18, 40−63 μm) using a mixture of solvents of decreasing polarity: (1) H_2O; (2) H_2O/MeOH (1:1); (3) H_2O/MeOH (1:3); (4) MeOH; (5) MeOH/CH_2Cl_2 (3:1); (6) MeOH/CH_2Cl_2 (1:1); and (7) CH_2Cl_2 using 500 mL of each solvent. The mobile phases used for HPLC purification of the three samples were (A) H_2O/TFA 0.1%; (B) CH_3CN/TFA 0.1%. The aqueous methanolic fractions F2 and F3 of *A. hickmani* were combined and purified by semipreparative RP-HPLC with a C-18 column (SymmetryPrep, 7.8 × 300 mm, 7 μm), using an isocratic method for 0−5 min with 95% A, 5% B; then a linear gradient from 5 to 20 min until 65% A, 35% B; then isocratic for 10 min at a flow rate of 3 mL/min and UV detection at λ 210 nm for 30 min, leading to pure compounds valdiviamide A (**1**) (14.8 mg), valdiviamide B (**2**) (0.2 mg), valdiviamide C (**3**) (1.8 mg), and valdiviamide D (**4**) (0.2 mg).

The aqueous methanolic fraction F3 of *P. darwini* was purified by semipreparative RP-HPLC with a C-18 column (SymmetryPrep, 7.8 × 300 mm, 7 μm), using an isocratic method for 0−8 min with 92% A, 8% B; then a linear gradient from 8 to 25 min with 60% A, 40% B; then isocratic for 5 min at a flow rate of 3 mL/min and UV detection at λ 210 for 30 min to obtain compounds **5** (3.8 mg) and **6** (0.9 mg).

The aqueous methanolic fractions F3 and F4 of *T.* cf. *patagonichus* were combined and purified by semipreparative RP-HPLC using a C-18 column (SymmetryPrep, 7.8 × 300 mm, 7 μm), starting with an isocratic method for 0−15 min with 80% A, 20% B; linear gradient for

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16−30 min until 60% A, 40% B; then isocratic for 30−45 min at a flow rate of 3.5 mL/min with UV detection at λ 254 nm to yield zoamide E (**7**) (1.5 mg), zoamide F (**8**) (2.4 mg), and the known zoamide D (6.4 mg).

*Valdiviamide A (**1**):* amorphous, yellow solid; $[\alpha]^{20}_{D}$ +18 (*c* 1.0, CH_3CN); UV (CH_3CN) λ_{max} (log ε) 290 (3.72), 224 (4.34), 207 (4.92) nm; ECD (*c* 2.28 × 10^{-5} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 224 (+10.74), 212 (+26.7) nm; IR data (Figure S3b); ^{1}H NMR and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 436.9723 $[M]^+$ (calcd for $C_{14}H_{19}Br_2N_2O_4$, 436.9706, Δ +3.8 ppm).

*Valdiviamide B (**2**):* amorphous, white solid; UV (CH_3CN) λ_{max} (log ε) 273 (3.66), 230 (4.29) nm; ECD (*c* 2.06 × 10^{-5} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 295 (−0.46), 255 (−1.39), 219 (+4.78), 208 (−1.06) nm; ^{1}H NMR data, Table 1; HRESIMS *m*/*z* 484.9566 $[M]^+$ (calcd for $C_{14}H_{19}BrIN_2O_4$, 484.9567, Δ −2.0 ppm).

*Valdiviamide C (**3**):* amorphous, pale yellow solid; $[\alpha]^{20}_{D}$ +16 (*c* 0.25, CH_3CN); UV (CH_3CN) λ_{max} (log ε) 290 (3.56), 224 (4.14), 207 (4.62) nm; ECD (*c* 2.15 × 10^{-5} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 277 (−0.89), 251 (−2.61), 225 (+5.19), 214 (+10.5) nm; ^{1}H NMR and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 462.9879 $[M]^+$ (calcd for $C_{16}H_{21}Br_2N_2O_4$, 462.9863, Δ +3.4 ppm).

*Valdiviamide D (**4**):* amorphous, white solid; UV (CH_3CN) λ_{max} (log ε) 293 (2.94), 261 (3.23), 241 (3.45), 212 (3.86) nm; ECD (*c* 1.95 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 285 (−0.15), 251 (−0.86), 220 (+1.61), 212 (+0.59) nm; ^{1}H NMR data, Table 1; HRESIMS *m*/*z* 510.9749 $[M]^+$ (calcd for $C_{16}H_{21}BrIN_2O_4$, 510.9724, Δ +4.6 ppm).

*2-(4-Hydroxy-3,5-diiodophenyl)-N,N,N-trimethylethan-1-aminium (**5**):* amorphous, white solid; UV (DAD, H_2O/CH_3CN) λ_{max} 225, 207 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.74 (2H, s, H-5/H-9), 3.50 (2H, AA′BB′, *J* = 9.0, 5.0 Hz, H_2-2), 3.18 (9H, s, $N(CH_3)_3$), 3.00 (2H, AA′BB′, *J* = 9.0, 5.0 Hz, H_2-3); ^{13}C NMR (CD_3OD, 125 MHz) δ 156.3 (C-7), 141.1 (C-5/C-9), 132.4 (C-4), 85.5 (C-6/C-8), 68.0 (C-2), 53.6 ($N(CH_3)_3$), 28.1 (C-3); HRESIMS*m*/*z* 431.9334 $[M]^+$ (calcd for $C_{11}H_{16}I_2NO$, 431.9316, Δ +4.1 ppm).

*2-(4-Hydroxy-3-bromo-5-iodophenyl)-N,N,N-trimethylethan-1-aminium (**6**):* amorphous, white solid; UV (DAD, H_2O/CH_3CN) λ_{max} 223, 207 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.71 (1H, d, *J* = 2.0 Hz, H-5), 7.51 (1H, d, *J* = 2.0 Hz, H-9), 3.51 (2H, AA′BB′, *J* = 9.0, 5.0 Hz, H_2-2), 3.19 (9H, s, $N(CH_3)_3$), 3.02 (2H, AA′BB′, *J* = 9.0, 5.0 Hz, H_2-3); ^{13}C NMR (CD_3OD, 125 MHz) δ 153.9 (C-7), 140.3 (C-5), 134.7 (C-9), 131.6 (C-4), 110.6 (C-8), 86.7 (C-6), 68.0 (C-2), 53.6 ($N(CH_3)_3$), 28.3 (C-3); HRESIMS *m*/*z* 383.9467 $[M]^+$ (calcd for $C_{11}H_{16}BrINO$, 383.9454, Δ +3.3 ppm).

*Zoamide E (**7**):* amorphous, yellow powder; $[\alpha]^{20}_{D}$ −13 (*c* 0.15, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 294 (4.15), 265 (4.28), 236 (4.18), 213 (4.09) nm; ECD (*c* 2.15 × 10^{-5} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 318 (−1.55), 303 (−9.40), 258 (−58.99), 211 (−29.02) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.94 (1H, septet, *J* = 1.2 Hz, H-2″), 4.30 (1H, q, *J* = 7.0 Hz, H-4), 3.05 (4H, m, H_2-8−9), 2.56 (1H, sext, *J* = 7.0 Hz, H-2′), 2.30 (3H, d, *J* = 1.0 Hz, H_3-5″), 2.01 (3H, d, *J* = 1.1 Hz, H_3-4″), 1.78 (1H, dq, *J* = 15.0, 7.5 Hz, H-3′a), 1.59 (3H, d, *J* = 7.0 Hz, H-4(CH_3)), 1.55 (1H, dq, *J* = 15.0, 7.5 Hz, H-3′b), 1.23 (3H, d, *J* = 7.0 Hz, H_3-5′), 0.97 (3H, t, *J* = 7.5 Hz, H_3-4′); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.3 (C-1′), 165.6 (C-1″), 161.8 (C-3″), 139.4 (C-6), 138.7 (C-2), 125.5 (C-4a), 125.2 (C-3a), 124.7 (C-7a), 124.5 (C-9a), 116.7 (C-2″), 43.6 (C-2′), 29.3 (C-4), 27.9 (C-4″), 27.8 (C-3′), 23.4 (C-4(CH_3)), 22.7 (C-8−9), 20.7 (C-5″), 17.1 (C-5′), 11.8 (C-4′); HRESIMS *m*/*z* 385.2358 $[M + H]^+$ (calcd for $C_{20}H_{29}N_6O_2$,385.2347, Δ +2.8 ppm).

*Zoamide F (**8**):* amorphous, yellow powder; $[\alpha]^{20}_{D}$ −21 (*c* 0.10, CH_3OH); UV (CH_3CN) λ_{max} (log ε) 374 (1.25), 266 (2.49), 230 (2.36), 212 (2.28) nm; ECD (*c* 1.35 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 293 (−0.01), 268 (−0.05), 215 (+0.04) nm; ^{1}H NMR (CD_3OD, 125 MHz) δ 5.94 (1H, septet, *J* = 1.2 Hz, H-2″), 4.31 (1H, q, *J* = 7.0 Hz, H-4), 3.06 (4H, m, H_2-8−9), 2.75 (1H, septet, *J* = 7.0 Hz, H-2′), 2.30 (3H, d, *J* = 1.0 Hz, H_3-5″), 2.01 (3H, d, *J* = 1.0 Hz, H_3-4″), 1.59 (3H, d, *J* = 7.0 Hz, H-4(CH_3)), 1.25 (6H, d, *J* = 7.0 Hz, H_3-3′-4′); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.8 (C-1′), 165.6 (C-1″), 161.8 (C-3″), 139.4 (C-6), 138.9 (C-2), 124.7 (C-7a, C-9a), 125.2 (C-3a, C-4a), 116.7 (C-2″), 36.5 (C-2′), 29.3 (C-4), 27.8 (C-4″), 23.4 (C-4(CH_3)), 22.7 (C-8−9), 20.7 (C-5″), 19.2 (C-3′-4′); HRESIMS *m*/*z* 371.2202 $[M + H]^+$ (calcd for $C_{19}H_{26}N_6O_2$, 371.2190, Δ +3.2 ppm).

Computational Methods. The methods used for the calculation of ECD spectra are as described previously.[42] The conformational analysis of each compound was performed with Schrodinger MacroModel 2018 as described by Willoughby et al.[43] Using DFT, at the B3LYP/6-311+G(d,p) level, the conformers were optimized using Gaussian 16. At the same time, the zero-point energy, electronic transition, and rational strength of all conformers were calculated.[44] The ECD spectra were calculated using Gaussian 16 at the B3LYP/6-311+G(d) level, and spectra were produced and corrected with the UV spectra using the freely available software SpecDis 1.7.[45] NMR properties were predicted at the B3LYP/6-31G(d)//MPW1PW91/6-311+G(2d) level on the four most stable conformers (conformational analysis performed using the GMMX module implemented in Gaussian 16). DP4 probabilities were calculated using an in-house implementation of the Goodman original script.

Bioactivity Tests. The antimicrobial activity of compounds **1**−**8** was tested against a panel of human pathogens including Gram-positive (*S. aureus* ATCC29213 (MSSA) and *S. aureus* MB5393 (MRSA)) and Gram-negative bacteria (*E. coli* ATCC25922, *K. pneumoniae* ATCC700603, *P. aeruginosa* PAO1, and *A. baumannii* MB5973) and a yeast (*C. albicans* ATCC64124), following previously described methodologies.[46,47] Cytotoxic activities of compounds **1**−**3** and **5**−**8** against the human-derived cell lines A549 (lung carcinoma), HepG2 (hepatocellular carcinoma), and MIA PaCa-2 (pancreatic carcinoma) were determined as previously reported.[48]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00173.

Biological description of the species; UV, ECD, NMR, and MS data of compounds **1**−**8** together with the IR spectrum of **1**; and computational analyses for compounds **3** and **8** (PDF)

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Notes

The authors declare no competing financial interest.

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