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Article

Marmaricines A-C: Antimicrobial Brominated Pyrrole Alkaloids from the Red Sea Marine Sponge *Agelas* sp. *aff. marmarica*

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Abstract: The Red Sea is the home of a rich diversity of sponge species, with unique ecological adaptations that thrive in its saline, warm, and nutrient-poor waters. Red Sea sponges offer potential as sources of novel drugs and bioactive compounds. The organic extract of the Red Sea sponge *Agelas sp. aff. marmarica* was investigated for its antimicrobial constituents. Through bioassay-guided fractionation of the antimicrobial fraction of the extract on SiO_2, Sephadex LH-20, and HPLC purification, three bioactive compounds, marmaricines A-C (**1-3**), were isolated. Structural elucidation of the compounds was performed using 1D (^1H and ^{13}C) and 2D (COSY, HSQC, HMBC and NOESY) NMR, as well as (+)-high-resolution electron spray ionization mass spectroscopy, leading to the identification of the compounds. Marmaricines A-C exhibited significant antimicrobial activity against *methicillin-resistant Staphylococcus aureus* (MRSA), with inhibition zones of 14, 15, and 12 mm, respectively. Further, marmaricines B and C showed activity against *Candida albicans*, with inhibition zones of 15 and 14 mm, while compound **1** displayed no activity. The results indicate that compounds **1-3** are selectively active against MRSA, and compounds **2** and **3** demonstrate potential against *C. albicans*, making them promising candidates for the development of novel antimicrobial agents targeting resistant pathogens.

Keywords: Res Sea sponges; *Agelas* sp. *aff. marmarica*; bioactive compounds; brominated pyrrole alkaloids; marmaricines A-C; antimicrobial activity

1. Introduction

Marine sponges (Porifera) have been a primary focus of research aimed at discovering biologically active secondary metabolites. The Red Sea sponges' biodiversity is characterized by a wide variety of morphologies and secondary metabolites, some of which play critical roles in marine ecosystems, including symbiosis with microorganisms and the production of bioactive compounds with potential pharmaceutical applications. Marine sponges of the genus *Agelas* (class Demospongiae, order Agelasida, family Agelasidae) are among the most common sponges found in tropical and subtropical regions worldwide, with 36 valid species currently recognized. The understanding of these species continues to grow. The secondary metabolites isolated from *Agelas* sponges, since their initial discovery, represent a fascinating area of research that has driven significant advancements in the field of marine natural products [1]. Over five decades (1971-2021),

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more than 355 compounds have been reported from several members of the genus *Agelas [1]*. The most producres of the compounds are *A. oroides* (15%), *A. nakamurai* (13%) and *A. mauritiana* (11%), while the rest was reported from unclassified *Agelas* species [1].

Members of the genus *Agelas* exhibit notable structural diversity in their pyrrole and terpenoidal alkaloids [1]. Following the isolation of specific bromopyrrole derivatives from *Agelas oroides* in 1971 [2] and the identification of agelasine, a quaternary 9-methyladenine derivative of an unidentified diterpene from *Agelas dispar* in 1975 [3], beside to pyrrole and terpenoidal alkaloid, s numerous bioactive metabolites of varying biogenetic origins such as glycosphingolipids, sterols, caretonoids and many others have been discovered within genus *Agelas* [1,4–8]. Pyrrole alkaloids of this genus typically possess a backbone consisting of a bromo- or debromo-pyrrole-2-carboxamide structure, which is associated with various side chains and cyclic formations [9–11]. In contrast, the less common diterpene alkaloids primarily include those containing a 9-N-methyladeninium group (such as agelines, agelasines, and nemoechines) [12–14], as well as diterpenes related to hypotaurocyamine (for example, agelasidines) [15]. Secondary metabolites of the genus *Agelas* show a wide range of biological activities, including antimicrobial [16,17], antihistaminic [18], antimalarial [19], antileukemic [20], cytotoxic [17,21], antifouling [21,22], Na^+,K^+-adenosine triphosphatase (ATPase) inhibitory effects [12,15] and antiangiogenic matrixmetalloproteinase inhibitory effect [23]. In our continuous effort to identify bioactive compounds from Red Sea marine sponges [24,25], we investigated the sponge *Agelas sp. aff. marmarica. Bioassay-guided partition of the antimicrobial fraction of the organic extract of the sponge and final HPLC purification afforded three new brominated pyrrole-derived alkaloids, marmaricines A-C.* The current study describes the isolation, structural elucidation, and antimicrobial activities of these compounds.

2. Results and Discussion

2.1. Purification of Compounds 1-3.

Fractionation of the antimicrobial fraction of the organic extract of the Red Sea sponge *Agelas sp. aff. Marmarica* on normal silica gel, Sephadex LH 20, and purification of active fraction on HPLC afforded compounds **1-3** (Figure 1).

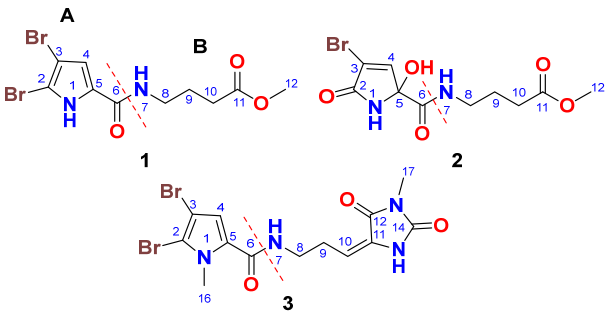

Figure 1. Chemical structures of compounds **1-3**.

2.2. Structure of Compound 1.

Compound **1** (Figure 1) was isolated as a yellowish powder with the molecular formula $C_{10}H_{12}Br_2N_2O_3$, determined from the positive HRESIMS pseudomolecular ion peak at *m/z* 388.9111 $[M + Na]^+$. The presence of two bromine atoms in compound **1** was confirmed by the observation of three pseudomolecular ion peaks at *m/z* 388.9, 390.9, and 392.9 in a 1:2:1 ratio. The structure of **1** was elucidated through the interpretation of its 1D and 2D NMR spectra. The ^{13}C NMR spectrum showed signals for 10 carbon atoms (Table 1). Analysis of the ^{13}C NMR spectrum, supported by the HSQC experiment, led to the assignment of five quaternary carbons, one methine, three methylene groups, and one methyl group. The ^{13}C NMR chemical shifts suggested the presence of two distinct parts (A

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and B) in compound **1** including a 4,5-dibromo-2-carboxylic acid moiety (A) linked to a 4-amino-1-methylbutanoate (B) unit via an amidic linkage (CO-NH) at C-6/NH-7. The ^{13}C NMR spectrum exhibited two carbonyl signals at δ_C 159.4 and 173.6, which were attributed to carboxamide (C-6) and carboxylic acid ester (C-11) groups, respectively. The $^{1}H/^{13}C$ signals at $\delta_{H/C}$ 12.65 (s) (N*H*-1), 104.9 (C, C-2), 98.2 (C, C-3), 6.90 (s)/112.9 (CH, C-4), and 128.6 (C, C-5) were consistent with the structure of an amidic derivative of 4,5-dibromo-pyrrole-2-carboxylic acid (substructure A).

Table 1. NMR data of compound **1** (DMSO-d_6).[1]

Position	δ_C, type	δ_H (mult., *J* in Hz)
1		12.65 (s)
2	104.9, C	
3	98.2, C	
4	112.9, CH	6.90 (s)
5	128.6, C	
6	159.4, C	
7		8.14 (t, 5.6)
8	38.3, CH_2	3.21 (q, 6.5)
9	25.0, CH_2	1.73 (quin., 7.1)
10	31.1, CH_2	2.34 (t, 7.4)
11	173.6, C	
12	51.7, CH_3	3.57 (s)

[1] Acquired at 800 MHz for ^{1}H and 200 MHz for ^{13}C NMR spectra.

The ^{1}H-^{1}H COSY spectrum revealed a single spin-coupling system from N*H*-7 through H_2-8 to H_2-10 (Figure 2). The signals at $\delta_{H/C}$ 8.14 (t, *J* = 5.6 Hz, N*H*-7), 3.21 (q, *J* = 6.5 Hz)/38.3 (CH_2, C-8), 1.73 (quin., *J* = 7.0 Hz)/25.0 (CH_2, C-9), and 2.34 (t, *J* = 7.4 Hz)/31.1 (CH_2, C-10) confirmed this coupling system and this portion of the molecule.

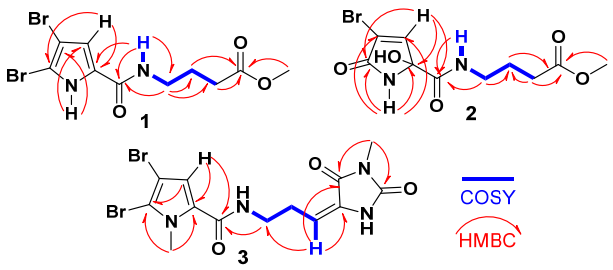

Figure 2. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC correlations of compounds **1-3**.

Further HMBC correlations from the three-proton singlet at δ_H 3.57 (s) to C-11 (δ_C 173.6) supported the presence of a methyl ester group. HMBC couplings from H_2-9 and H_2-10 to C-11 (δC 174.9) further confirmed this assignment. The connectivity between the two parts of the compound was established through HMBC correlations (Figure 2) from H-4 (δ_H 6.90) to C-6 (δ_C 159.4), from N*H*-7 (δ_H 12.65) to C-6 (δ_C 159.4), and from H_2-8 (δ_H 3.21) to C-6 (δ_C 159.4). Additional HMBC correlations from N*H*-1 (δ_H 12.65) to C-3 (δ_C 98.2) and C-4 (δ_C 112.9), and from H-4 (δ_H 6.90) to C-2 (δ_C 104.9) and C-5 (δ_C 128.6), confirmed the assignments of these carbons. Therefore, compound **1** was identified as 4-(4,5-dibromo-1H-pyrrole-2-carboxamido)-1-methylbutanoate. This is the first report of its isolation from a natural source, making it a newly identified natural product. The generic name marmaricine A was given to compound **1**.

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2.3. Structure of Compound 2.

Compound **2** (Figure 1) was isolated as an optically inactive yellowish powder ([α]$_D$ 0°, c 0.10, MeOH) with the molecular formula $C_{10}H_{13}BrN_2O_5$, as determined from the positive HRESIMS pseudomolecular ion peak at m/z 342.9906 $[M + Na]^+$. The observation of two pseudomolecular ion peaks at m/z 342.9 and 344.9 in a 1:1 ratio confirmed the presence of a single bromine atom in compound **2**. The structure of compound **2** was determined through the analysis of its 1D and 2D NMR spectra. The ^{13}C NMR spectrum showed signals for 10 carbon atoms (Table 2).

Table 2. NMR data of compound **2** (DMSO-d_6).[1]

Position	δ_C, type	δ_H (mult., *J* in Hz)
1		9.05 (brs)
2	167.5, C	
3	120.3, C	
4	147.0, CH	7.24 (t, 1.6)
5	87.8, C	
6	167.3, C	
7		8.21 (t, 5.9)
8	38.8, CH_2	3.07 (q, 7.8)
9	24.7, CH_2	1.65 (quin., 7.2)
10	31.0, CH_2	2.27 (t, 7.5)
11	173.6, C	
12	51.7, CH_3	3.55 (s)

[1] Acquired at 800 MHz for ^1H and 200 MHz for ^{13}C NMR spectra.

Interpretation of the ^{13}C NMR data, combined with the HSQC experiment, allowed the assignment of the carbons as five quaternary carbons, one methine, three methylene groups, and one methyl group. The COSY, ^{13}C NMR, HSQC, and HMBC data facilitated the identification of two main parts (A and B) in compound **2** including a 4-bromo-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-2-carboxamide moiety (A) linked to a 4-amino-1-methylbutanoate unit (B) via an amidic bond (C-6/NH-7). When compared to compound **1**, which contains a 4,5-dibromo-1H-pyrrole-2-carboxamide moiety as part A, compound **2** features a 4-bromo-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-2-carboxamide moiety. The assignment of this substructure in compound **2** was supported by the $^1H/^{13}C$ NMR signals at $\delta_{H/C}$ 9.05 (NH-1), 167.5 (C, C-2), 120.3 (C, C-3), 7.24 (d, J = 1.6 Hz)/147.0 (CH, C-4), 87.8 (C, C-5), and 167.3 (C, C-6). HMBC correlations from NH-1 to C-2, C-3, C-5, and C-6, as well as from H-4 to C-2 and C-5, supported this assignment. The chemical shifts of the ^1H and ^{13}C NMR signals for the second part of compound **2** (B) are similar to those in compound **1**, suggesting that the same substructure is present in both molecules. The connection between the two parts of compound **2** was further confirmed by HMBC correlations (Figure 2) from H-4 to C-6, from NH-7 to C-6, and from H_2-8 to C-6 (δ_C 167.3). The racemic nature of compound **2** was confirmed by the lack of any optical activity and the absence of any Cotton effects (CE) in the experimental ECD spectrum. Therefore, compounds **2** was determined to be a racemic mixture and assigned as (±)-4-(4-bromo-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-2-carboxamido)-1-methylbutanoate. Compound **2** is reported here as a new natural product and named marmaricine B.

2.4. Structure of Compound 3.

Compound **3** (Figure 1) was purified and obtained as a yellowish powder with the molecular formula $C_{13}H_{14}Br_2N_4O_3$, as indicated by the (+)-HRESIMS pseudomolecular ion peak at m/z 454.9327 $[M + Na]^+$, which suggests the presence of eight degrees of unsaturation. The detection of three ion peaks at m/z 454.9, 456.9, and 458.9 in a 1:2:1 ratio further corroborates the dibrominated nature of compound **3**. The structure for compound **3** was determined through analysis of its 1D (^1H and ^{13}C) and 2D (COSY, HSQC, HMBC, and NOESY) NMR spectra. The NMR data (Table 3) supports the

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presence of two substructures (A and B). With the exception of the absence of the NH-1 signal, compound **3** exhibited similar ^{1}H and ^{13}C NMR signals to the substructure A of compound **1**. Notably, the $^{1}H/^{13}C$ NMR signals at 3.90 (3H, s, H_3-16)/36.4 (CH_3, C-16) in the ^{1}H and ^{13}C NMR spectra of compound **3**, along with the HMBC correlations from H_3-16 (δ_H = 3.90) to C-2 (δ_C = 111.9), C-5 (δ_C = 129.4), and from H-4 (δ_H = 6.24) to C-2, C-5, and C-6 (δ_C = 161.4), support the assignment of substructure A as the 4,5-dibromo-1-methyl-1H-pyrrole-2-carboxamide moiety.

Table 3. NMR data of compound **3** (CD_3OD.[1].

Position	δ_C, type	δ_H (mult., *J* in Hz)
2	111.9, C	
3	99.2, C	
4	116.1, CH	6.82 (s)
5	129.4, C	
6	161.4, C	
8	39.2, CH_2	3.45 (t, 7.5)
9	28.8, CH_2	2.61 (q, 7.5)
10	120.5, CH	6.24 (t, 7.5)
11	130.0, C	
12	163.1, C	
14	154.0, C	
16	36.4, CH_3	3.90 (s)
17	26.5, CH_3	3.20 (s)

[1] Acquired at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR spectra.

The remaining signals for compound **3** were attributed to 5-(3-aminopropylidene)-3-methylimidazolidine-2,4-dione moiety, based on the ^{1}H and ^{13}C NMR signals at $\delta_{H/C}$ 3.45 (t, J = 7.5 Hz)/39.2 (CH_2, C-8), 2.61 (q, J = 7.5 Hz)/28.8 (CH_2, C-9), 6.24 (q, J = 7.5 Hz)/120.5 (CH, C-10), 130.0 (C, C-11), 163.1 (C, C-12), 154.0 (C, C-14), and 3.20 (3H, s, H_3-17)/26.5 (CH_3, C-17). This assignment is further validated by COSY correlations (Figure 2) from H_2-8 to H-10 and HMBC correlations (Figure 2) from H-10 to C-8 (δ_C = 39.2), C-11 (δ_C = 130.0), and C-12 (δ_C = 163.1) as well as from CH_3-17 to C-12 (δ_C = 163.1) and C-14 (δ_C = 154.0). The interconnection between the substructures in compound **3** is also supported by an HMBC correlation (Figure 2) from H_2-8 to C-6. Additionally, the assignment of the ^{1}H and ^{13}C NMR signals for substructure B was confirmed through COSY (Figure 2) and HMBC correlations. The E configuration at $\Delta^{10,11}$ is supported by NOESY correlations between H-10 and H_2-8, between H_3-17 and H-4 as well as between H_2-8 and H_3-16 (Figure 3). Therefore, compound **3** is assigned as (E)-4,5-dibromo-1-methyl-N-(3-(1-methyl-2,5-dioxoimidazolidin-4-ylidene)propyl)-1H-pyrrole-2-carboxamide and is being reported here as a new natural product and named marmaricine C.

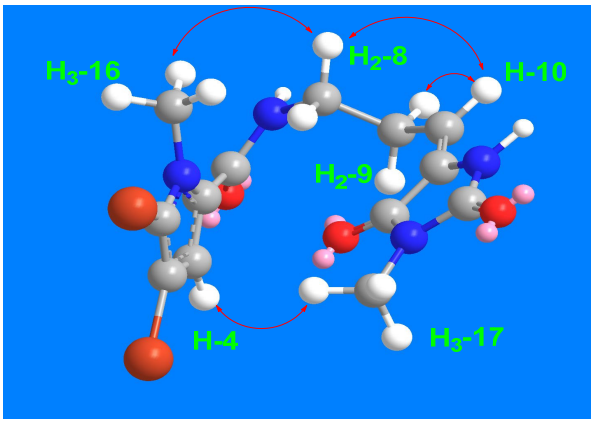

Figure 3. Significant ^{1}H-^{1}H NOESY correlations of compound **3**.

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2.5. Antimicrobial Activities of the Compounds

Compounds **1-3** were screened for their antimicrobial effects using a disc diffusion assay, testing against *methicillin-resistant Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candida albicans* at a concentration of 50 µg/disc. Compounds **1-3** displayed significant inhibition zones of 14, 15, and 12-15 mm, respectively, against MRSA (Table 3). In contrast, compounds **2** and **3** exhibited inhibition zones of 15 and 14 mm, respectively, against *C. albicans*, while compound **1** showed no activity (Table 3). None of the compounds exhibited any effect against *E. coli* (Table 3). These findings indicate a strong selectivity of compounds **1-3** against MRSA and of compounds **2** and **3** against *C. albicans.* These results suggest that compounds **1-3** represent promising candidates for the development of new antibiotics.

Table 4. Antimicrobial activities of compounds **1-3**.

Compound	Inhibition zone (mm, 50 µg/disc)		
	MRSA	*E. coli*	*C. albicans*
1	14	NI	NI
2	15	NI	15
3	12	NI	14
Ciprofloxacin[1]	3.5	19	NT
Clotrimazole[2]	NT	NT	18

[1] Positive antibacterial control (50 µg/disc); [2] Positive antifungal control (10 µg/disc).

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were recorded using a JASCO DIP-370 digital polarimeter (Jasco Co., Tokyo, Japan) at 25 °C, with measurements taken at the sodium D line (589 nm). One-dimensional and two-dimensional NMR spectra (chemical shifts in ppm and coupling constants in Hz) were acquired on Bruker Avance DRX 800 MHz (800 MHz for ^{1}H and 200 MHz for ^{13}C) or 500 MHz (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometers (Bruker, Rheinstetten, Germany), using DMSO-d_6 or CD_3OD as the solvent. HPLC separation was carried out on a C18 column (150 × 4.6 mm, 2.5 µm, Waters Atlantis®, Massachusetts, USA), with a CH_3CN:H_2O gradient as the mobile phase, monitored at 220 nm and a flow rate of 2.0 mL/min.

3.2. Biological Materials

The Red Sea sponge Agelas sp. aff. marmarica (Figure 4) was collected from the Saudi Red Sea coast (N021°39′17.5″,E038°52′26.3″). The sponge belongs to Kingdom: Animalia, Phylum: Porifera, Class: Demospongiae, Subclass: Heteroscleromorpha, Order: Agelasida, Family: Agelasidae, Genus: Agelas, Species: Agelas sp. aff. marmarica. The sponge was kindly identified by Rob van Soest. A specimen of the sponge was kept at the collection of the Naturalis Biodiversity Center at Leiden, The Netherlands under registeration number RMNHPOR 9165. Another specimen was stored at the Red Sea Invertebrates Collection at King Abdulaziz University under code No. DY-16.

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Figure 4. A photograph of the Red Sea sponge *Agelas* sp. *aff. marmarica*.

3.3. Purification of the Compounds

The freeze-dried sponge materials (0.35 Kg) were macerated in a mixture of CH_2Cl_2:CH_3OH (1:1) (3 × 2000 mL) at room temperature. The combined extracts were dried under reduced pressure to give a brown residue. The dried residue (17.5 g) was subjected to partition on VLC silica gel column using n-hexane-CH_2Cl_2-MeOH gradients affording 12 main fractions (Fr. 1-12). The antimicrobial fraction eluted with 100% CH_2Cl_2, Fr. 6 (0.41 g) (inhibition zone = 8 mm against *C. albicans*), was subjected to partition on Sephadex LH-10 using MeOH to afford five factions (Fr. A-E). The antimicrobial fraction (Fr. C) (126 mg) (inhibition zone = 10 mm against *C. albicans*) was purified on reversed-phase HPLC column (XDB-C18, 250 x 9.4 mm 5 µm, Agilent) using CH_3CN:H_2O gradients at 2 mL/min starting from 20% CH_3CN to 0% CH_3CN in 50 min to yield compounds **1** (2.5 mg, t_R = 11 min), **2** (3.9 mg, t_R = 20.5 min), and **3** (4.7 mg, t_R = 17.5 min).

3.4. Spectra Data of 1-3

3.4.1. Marmaricine A (1). Yellowish powder; NMR data: see Table 1; HRESIMS m/z 388.9111 (calcd for $C_{10}H_{12}Br_2N2O_3Na$ [M + Na]+, 388.9106).

3.4.2. Marmaricine B (2). Yellowish powder; NMR data: see Table 2; HRESIMS m/z 342.9906 (calcd for $C_{10}H_{13}BrN_2O_5Na$ [M + Na]+, 342.9900).

3.4.3. Marmaricine C (3): Yellowish powder; $[\alpha]_D$ 0° (c 0.1, MeOH); NMR data: see Table 3; HRESIMS m/z 454.9327 (calcd for $C_{13}H_{14}Br_2N_4O_3Na$ [M + Na]+, 454.9324).

3.5. Antimicrobial Activities of the Compounds

The antimicrobial effects of the compounds was performed against methicillin-resistant *Staphylococcus aureus* (ATCC 43300) , *Escherichia coli* (ATCC 35218), and *Candida albicans* (ATCC 76615) were performed at 50 µg/disc as previously reported in a disk diffusion assay [26–29].

4. Conclusions

In this study, the organic extract of the Red Sea sponge *Agelas sp. aff. marmarica* was studied. Bioassay-guided partition of the antimicrobial-active fraction on SiO_2, Sephadex LH-20, and HPLC purification, led to the isolation of three compounds, marmaricines A-C (**1-3**). The structures of these compounds were elucidated through spectroscopic analysis, including 1D and 2D NMR and (+)-HRESIMS measurements. The compounds were identified as 4-(4,5-dibromo-1H-pyrrole-2-carboxamido)-1-methylbutanoate (**1**), (±)-4-(4-bromo-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-2-carboxamido)-1-methylbutanoate (**2**), and (*E*)-4,5-dibromo-1-methyl-N-(3-(1-methyl-2,5-dioxoimidazolidin-4-ylidene)propyl)-1H-pyrrole-2-carboxamide (**3**). Compounds **1-3** exhibited notable antimicrobial activity with inhibition zones of 14, 15, and 12 mm, respectively, against *methicillin-resistant Staphylococcus aureus* (MRSA). Furthermore, compounds **2** and **3** demonstrated

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inhibition zones of 15 and 14 mm, respectively, against *Candida albicans*, while compound **1** showed no activity. These findings highlight the strong selectivity of compounds **1-3** against MRSA and compounds **2** and **3** against *C. albicans*. The results suggest that compounds **1-3** represent promising scaffolds for further development as therapeutic agents targeting antimicrobial resistance.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figures S1-S16: 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC, NOESY) NMR spectra of compounds **1-3**.

Author Contributions: Conceptualization, D.T.A.Y. and L.A.S.; methodology, D.T.A.Y, A.S.A., T.A. and L.A.S.; formal analysis, D.T.A.Y, A.S.A., T.A., A.M.A. and L.A.S.; investigation, D.T.A.Y, A.S.A., A.M.A., T.A. and L.A.S.; resources, D.T.A.Y.; data curation, D.T.A.Y. and L.A.S..; writing—D.T.A.Y. and L.A.S.; writing—review and editing, D.T.A.Y.; supervision, D.T.A.Y.; project administration, D.T.A.Y.; funding acquisition, D.T.A.Y. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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