New Crambescidin-Type Alkaloids from the Indonesian Marine Sponge *Clathria bulbotoxa*

Kasmiati Kasmiati 1,2, Yukio Yoshioka 3, Tetsuji Okamoto 3 and Makoto Ojika 1,*

1 Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan; kasmiati74@yahoo.com
2 Faculty of Marine Science and Fishery, Hasanuddin University, Jalan Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia
3 Graduate School of Biomedical and Health Sciences, Hiroshima University, Minami-ku, Hiroshima 734-8553, Japan; yyoshioka@hiroshima-u.ac.jp (Y.Y.); tetsuok@hiroshima-u.ac.jp (T.O.)

* Correspondence: ojika@agr.nagoya-u.ac.jp; Tel.: +81-52-789-4116

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Abstract: A crude methanolic extract of the Indonesian sponge *Clathria bulbotoxa* showed a potent cytotoxic activity against the human epidermoid carcinoma A431 cells. An investigation of the active components led to the isolation of three new compounds named crambescidins 345 (1), 361 (2), and 373 (3), together with the known related metabolites crambescidins 359 (4), 657 (5), and 800 (6). The structures of the compounds were determined by spectroscopic analysis. These compounds 1–4 that possess a simple pentacyclic guanidine core exhibited moderate cytotoxicity against the A431 cells with the IC50 values of 7.0, 2.5, 0.94, and 3.1 µM, respectively, while the known compounds 5 and 6 that possess a long aliphatic side chain were found to be significantly cytotoxic. On the other hand, in an anti-oomycete activity test against the fungus-like plant pathogen *Phytophthora capsici*, 1–4 showed a higher activity than that of 5 and 6, suggesting that the long aliphatic side chain plays a significant role for cytotoxicity, but is not effective or suppressive for anti-oomycete activity.

Keywords: Indonesian sponge; *Clathria bulbotoxa*; crambescidin; cytotoxicity; anti-oomycete activity

1. Introduction

Marine sponges (phyla Porifera) are one of the most prolific and the largest sources of novel bioactive compounds among marine organisms [1,2], with more than 200 new compounds reported each year [3]. During the last decade from 2001 to 2010, approximately 2400 new natural products had been discovered from 671 species of sponges, contributing 29% of the marine natural products reported within the period [4]. Ecological studies reported that sponges produce a wide array of secondary metabolites for defensive purposes to protect them from threats of competitors, predators, and pathogens [5,6]. Furthermore, sponges are frequently a host for microbial symbionts, which are regarded as one of the most important sources of bioactive molecules [7].

Studies on Indonesian marine sponges are interesting because Indonesia is the largest archipelagic country in the world, encompassing approximately 86,700 square kilometers of coral reef ecosystems [8], which are important for sponges as the most dominant benthic inhabiting coral reefs [9]. The Indonesian coral reefs are located in the coral triangle area, which is the global center of marine biodiversity and is recognized as the richest region on the earth [10]. Therefore, sponges from the coral ecosystems produce metabolites with various biological properties and become a target of continuing searching for new bioactive compounds [11,12].
We recently investigated the biological activity of extracts from Indonesian marine organisms, including six sponge species (Agelas conifera, Carteriospongia foliascens, Clathria bulbotoxa, Clathria reinwardti, Haliclona koromella, and Tedania ignis), and found that an extract of the sponge C. bulbotoxa was highly cytotoxic. The sponges of the genus Clathria are widely distributed in the tropical shallow waters and temperate regions, especially along the coast of the southern hemisphere [13,14]. This genus has been recognized as an excellent producer of novel secondary metabolites exhibiting diverse chemical structures including alkaloids [15–20], carotenoids [21,22], peptides [23], sugars [24], terpenoids [25,26], and sterols [27–29].

For the above reasons as well as the abundant population of the Clathria bulbotoxa in the Samalona Island, South Sulawesi Sea, we were interested in the investigation of bioactive compounds from this sponge, leading to the isolation of three new crambescidin-type guanidine alkaloids 1–3 as well as three known related products 4–6. Herein, we present the isolation, structure elucidation, and biological characterization of these guanidine alkaloids.

2. Results and Discussion

2.1. Isolation and Structural Elucidation

The MeOH extract of the freeze-dried sponge was found to be highly cytotoxic against the human epidermoid carcinoma cell line A431 at an IC$_{50}$ value of 0.046 µg/mL and to exhibit anti-oomycete activity against the fungus-like plant pathogen Phytophthora capsici at a dose of 50 µg/disk. The active extract was subjected to bioassay-guided fractionation, followed by the final reversed-phase high performance liquid chromatography (HPLC) to yield six compounds 1–6. The compounds 4–6 were identified as crambescidins 359 [30], 657 [31], and 800 [32] (Figure 1), respectively, by comparison with published spectroscopic data, whereas the compounds 1–3 were found to be new crambescidin analogs.

![Chemical structures of 1–6.](image)

Crambescidin 345 (1) possesses the molecular formula of C$_{20}$H$_{31}$N$_{3}$O$_{2}$ deduced from a high resolution electrospy ionization mass spectrum (HR-ESIMS) using the pseudo-molecular ion at m/z 346.2495 [M + H]$^+$ (calculated for C$_{20}$H$_{32}$N$_{3}$O$_{2}$ 346.2489). The infra red (IR) spectrum of 1 exhibited a characteristic absorption at 3109 cm$^{-1}$, which was also observed for 2 (3107 cm$^{-1}$), 3 (3111 cm$^{-1}$), and the other known crambescidin-type analogs [31,33–35]. It was reported that this absorption was due to the N-H stretching mode of the cyclic guanidine structure [36].
The $^1$H and $^{13}$C NMR spectra (Tables 1 and 2) showed that 1 consisted of 30 hydrogen and 20 carbon atoms. A hetero-nuclear single quantum coherence (HSQC) experiment indicated that all of the hydrogen atoms were attached to carbons, revealing the presence of five CH, eleven CH$_2$, one CH$_3$, and three C. Two additional protons were observed at $\delta_H$ 10.20 and 10.28 in CDCl$_3$ (Figure S2), supporting the presence of the above-mentioned guanidine moiety characteristic of the crambescidin alkaloids. The signals of the three quaternary carbons were found at $\delta_C$ 85.1 (C-8), 81.3 (C-15), and 149.4 (C-20). Other NMR signals were characterized as an olefinic bond [$\delta_C$ 134.2/$\delta_H$ 5.50 (C-4) and $\delta_C$ 131.4/$\delta_H$ 5.71 (C-5)], one methyl [$\delta_C$ 10.8/$\delta_H$ 0.84 (C-1)], one oxymethylene [$\delta_C$ 62.6/$\delta_H$ 3.69 (C-19)], one oxymethine [$\delta_C$ 72.1/$\delta_H$ 4.35 (C-3)], two N-substituted methines [$\delta_C$ 54.9/$\delta_H$ 4.03 (C-10) and $\delta_C$ 53.5/$\delta_H$ 3.96 (C-13)], and ten methylenes ($\delta_C$ 19.5–39.1/$\delta_H$ 1.45–2.59) based on their chemical shifts. These NMR data exhibited a close similarity to those for crambescidin 359 (4) [30], except for the CH$_2$-19 in 1, which is replaced with an ethylidene (CH$_3$-CH<) in 4.

### Table 1. $^1$H NMR data for 1-3 (CD$_3$OD).

| Position | 1  | 2  | 3  |
|----------|----|----|----|
|          | $^a$ | $^b$ | $^a$ |
| 1        | 0.84, t (7.2) | 0.87, t (6.8) | 0.84, t (7.2) |
| 2a       | 1.46, m      | 1.38, m      | 1.46, m      |
| 2b       | 1.54, m      | 1.54, m      | 1.54, m      |
| 3        | 4.35, brd (10.8) | 1.41, m | 1.47, m | 4.33, brd (10.2) |
| 4        | 5.50, dt (10.8, 2.1) | 3.63, brt (12.6) | 5.50, dt (11.2, 2.1) |
| 5a       | 5.71, m      | 1.29, m      | 5.71, m      |
| 5b       | 1.67, m      | 1.67, m      | 1.67, m      |
| 6a       | 2.15, dt (15.3, 7.2) | 1.74, m | 1.74, m | 2.15, dt (15.3, 7.2) |
| 6b       | 2.42, brt (15.3) | 1.85, m | 1.85, m | 2.42, brt (15.3) |
| 7a       | 1.97, dd (13.5, 6.0) | 1.74, m | 1.74, m | 1.97, dd (13.5, 6.0) |
| 7b       | 2.27, t (13.5) | 2.27, t (13.5) | 2.27, t (13.5) |
| 9a       | 1.45, t (12.7) | 1.57, t (12.8) | 1.45, t (12.9) |
| 9b       | 2.59, dd (12.7, 4.8) | 2.19, dd (12.8, 4.2) | 2.59, dd (12.9, 4.8) |
| 10       | 4.03, m      | 4.00, m      | 4.05, m      |
| 11a      | 1.75, m      | 1.73, m      | 1.75, m      |
| 11b      | 2.31, m      | 2.30, m      | 2.32, m      |
| 12a      | 1.75, m      | 1.73, m      | 1.75, m      |
| 12b      | 2.31, m      | 2.30, m      | 2.32, m      |
| 13       | 3.96, m      | 4.00, m      | 4.03, m      |
| 14a      | 1.53, t (13.0) | 1.59, t (12.8) | 1.59, t (13.0) |
| 14b      | 2.33, dd (13.0, 4.5) | 2.21, dd (12.8, 4.2) | 2.21, dd (13.0, 4.8) |
| 16a      | 1.77, m      | 1.74, m      | 1.73, m      |
| 16b      | 1.77, m      | 1.77, m      | 1.77, m      |
| 17a      | 1.80, m      | 1.74, m      | 1.77, m      |
| 17b      | 1.85, m      | 1.85, m      | 1.85, m      |
| 18a      | 1.61, m      | 1.26, m      | 1.26, m      |
| 18b      | 1.70, m      | 1.70, m      | 1.70, m      |
| 19       | 3.69, m      | 3.74, m      | 3.50, m      |
| 20       | 1.11, d (9.0) | 1.42, m      | 1.42, m      |
| 21       | 0.85, t (7.2) | 0.85, t (7.2) | 0.85, t (7.2) |

Data were observed at $^a$ 600 MHz or $^b$ 400 MHz; $^{cd}$ Interchangeable signal within the same marks.
Table 2. $^{13}$C NMR data for 1–3 (CD$_3$OD).

| Position | 1 $^a$ | 2 $^b$ | 3 $^a$ |
|----------|-------|-------|-------|
| 1        | 10.8, CH$_3$ | 13.8, CH$_3$ | 11.3, CH$_3$ |
| 2        | 30.3, CH$_2$ | 19.4, CH$_2$ | 30.3, CH$_2$ |
| 3        | 72.1, CH | 38.7, CH$_2$ | 72.1, CH |
| 4        | 134.2, CH | 71.1, CH | 134.3, CH |
| 5        | 131.4, CH$_2$ | 31.7, CH$_2$ | 131.4, CH |
| 6        | 24.4, CH$_2$ | 19.5, CH$_2$$^c$ | 24.5, CH$_2$ |
| 7        | 38.5, CH$_2$ | 34.7, CH$_2$$^d$ | 38.5, CH$_2$ |
| 8        | 85.1, C | 81.6, C | 85.1, C |
| 9        | 37.9, CH$_2$ | 40.4, CH$_2$ | 37.9, CH$_2$ |
| 10       | 54.9, CH | 53.7, CH$_2$$^e$ | 54.9, CH |
| 11       | 30.8, CH$_2$ | 30.7, CH$_2$ | 30.8, CH$_2$ |
| 12       | 30.8, CH$_2$ | 30.7, CH$_2$ | 30.8, CH$_2$ |
| 13       | 53.5, CH | 53.4, CH$_2$ | 53.5, CH |
| 14       | 39.1, CH$_2$ | 40.4, CH$_2$ | 40.4, CH$_2$ |
| 15       | 81.3, C | 81.6, C | 81.5, C |
| 16       | 35.1, CH$_2$ | 34.6, CH$_2$$^d$ | 34.7, CH$_2$ |
| 17       | 19.5, CH$_2$ | 19.7, CH$_2$$^c$ | 19.5, CH$_2$ |
| 18       | 25.9, CH$_2$ | 33.3, CH$_2$ | 31.3, CH$_2$ |
| 19       | 62.6, CH$_2$ | 68.2, CH | 73.3, CH |
| 20       | 149.4, C | 22.0, CH$_3$ | 30.0, CH$_2$ |
| 21       | 149.0, C | 149.0, C | 10.2, CH$_3$ |
| 22       | 149.4, C |

Data were obtained at $^a$ 150 MHz or $^b$ 100 MHz; The number of hydrogen on carbon was determined by a hetero-nuclear single quantum coherence (HSQC); $^{c-e}$ Interchangeable signals within the same marks.

A double quantum filtered correlation spectroscopy (DQF-COSY) experiment was performed to determine the connectivity of the proton-bearing carbons described above, suggesting the presence of four substructures, CH$_3$-1–CH$_2$-2–CH-3–CH$_2$-4=CH-5–CH$_2$-6–CH$_2$-7, CH$_2$-9–CH-10–CH$_2$-11, CH$_2$-12–CH-13–CH$_2$-14, and CH$_2$-18–CH$_2$-19 (Figure 2a). A hetero-nuclear multiple-bond connectivity (HMBC) experiment was conducted to connect these substructures and the quaternary carbons. The HMBC correlations from H$_2$-6, H$_2$-7, and H-9 to C-8, and from H-9 to C-7 revealed the position of the quaternary carbon C-8, and the correlations from H$_2$-14, H$_2$-16, and H$_2$-19 to C-15 confirmed the position of the quaternary carbon C-15. The other important HMBC signals were found from H$_2$-14, H$_2$-17, and H$_2$-18 to C-16, from H$_2$-16 to C-14, and from H$_2$-19, H$_2$-18, and H$_2$-16 to C-17, supporting the position of the methylene carbons C-16 and C-17. Although the HMBC signal from H-3 to C-8 was not found, the chemical shift of the C-3 ($\delta_C$ 72.1) indicated that this carbon had an oxygen functionality. Based on these analyses, crambescidin 345 (1) was deduced as a new crambescidin analog, lacking the alkyl group at C-19, which is the second example among the crambescidins that were reported previously [35].

The relative configuration for 1 was assigned by nuclear Overhauser effect spectroscopy NOESY experiments (Figure 2b). The NOESY correlations of $\delta_H$ 4.35 (H-3)/2.27 (H-7b), $\delta_H$ 1.97 (H-7a)/1.45 (H-9a), and $\delta_H$ 2.42 (H-6b)/2.59 (H-9b) indicated the relative configuration between C-3 and C-8. The relative stereochemistry between C-10 and C-13 was confirmed by the NOESY correlations of H-9a/$\delta_H$ 1.75 (H-11a), $\delta_H$ 1.75 (H-12a)/1.53 (H-14a), H-9b/$\delta_H$ 4.03 (H-10), H-10/$\delta_H$ 3.96 (H-13), and H-13/$\delta_H$ 2.33 (H-14b). An additional NOESY correlation of $\delta_H$ 1.77 (H-16)/H-14a determined the relative configuration between C-15 and other positions. Furthermore, the large coupling constants of H-9a/H-10 ($J = 12.7$ Hz) and H-14a/H-13 ($J = 13.0$ Hz) confirmed the 1,2-diaxial orientation of these hydrogen pairs, concluding the relative stereochemistry of 1, as described in Figure 2b. The specific rotation value of 1 (−7.1) indicated a close similarity to that of the structurally similar analog 4 (−8.9), suggesting that 1 possesses the same absolute configuration as that of 4 (Figure 1).
Crambescidin 361 (2) possesses the molecular formula of C_{21}H_{35}N_{3}O_{2} deduced from a HR-ESIMS using the pseudo-molecular ion at m/z 362.2765 [M + H]^{+} (calculated for C_{21}H_{36}N_{3}O_{2}, 362.2802). Detailed analysis of the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) indicated that 2 consisted of 34 hydrogen and 21 carbon atoms, and HSQC experiments implied that all the protons were connected to carbons (four CH, twelve CH_{2}, two CH_{3}, and three C). In addition, two exchangeable proton signals were observed at δ_{H} 10.13, and 10.16 in acetone-d_{6} (Figure S17 and Table S1). The signals of the three quaternary carbons were observed at δ_{C} 81.6 (C-8 and C-15) and 149.0 (C-21). Other important NMR signals were recognized as two methyls [δ_{C} 13.8/δ_{H} 0.87 (C-1) and δ_{C} 22.0/δ_{H} 1.11 (C-20)], two oxymethines [δ_{C} 71.1/δ_{H} 3.63 (C-4) and δ_{C} 68.2/δ_{H} 3.74 (C-19)], two N-substituted methines [δ_{C} 53.7/δ_{H} 4.00 (C-10) and δ_{C} 53.4/δ_{H} 4.00 (C-13)], and twelve methylenes [δ_{C} 19.4–40.4/δ_{H} 1.26–3.30]. Although these data were characteristic of the crambescidin alkaloids, they showed the absence of the olefinic function that was found in most of the reported crambescidin-type alkaloids. In addition, the pentacyclic guanidine core is symmetrical, as indicated by highly overlapping chemical shifts of the corresponding proton and carbon signals (Tables 1 and 2).

The DQF-COSY correlations for 2 indicated the five partial structures CH_{3}-1–CH_{2}-2, CH_{2}-3–CH_{2}-4–CH_{2}-5–CH_{2}-6, CH_{2}-9–CH_{2}-10–CH_{2}-11, CH_{2}-12–CH_{13}–CH_{2}-14, and CH_{2}-17–CH_{2}-18–CH_{19}–CH_{2}-20 (Figure 3a). The HMBC correlations from H_{2}-1 to C-3, and from H_{2}-3 to C-2 and C-1 confirmed the presence of a propyl group on C-4. Furthermore, the correlations from H_{2}-6 to C-8, from H_{2}-7 to C-6, from H_{2}-17 to C-15, and from H_{2}-16 to C-17 confirmed the connectivities of C-6–C-7 and C-16–C-17. An HMBC spectrum was obtained in acetone-d_{6} (Figure 3a, dotted arrows), indicating the following important correlations: from NHb to C-7, C-8, C-9, and C-21 and from NHa to C-14, C-15, and C-16, revealing not only the presence of guanidine moiety, but also the position of C-7, C-8, C-15, and C-16. Based on these analyses, crambescidin 361 (2) was deduced as a new crambescidin analog with two tetrahydropyran rings instead of the combination of the left-side unsaturated seven membered ring and the right-side tetrahydropyran ring as found in 1 and most of the crambescidin analogs. Another structural feature of 2 is the presence of a propyl group as an alkyl substituent, which is quite rare in the crambescidin-type alkaloids [37,38].

The relative configuration of 2 was examined by the interpretation of NOESY correlations (Figure 3b). The chair conformation of both the tetrahydropyran rings was determined by the 1,3-diaxial correlations of δ_{H} 3.63 (H-4)/1.85 (H-6b) and δ_{H} 3.74 (H-19)/1.85 (H-17b) (Figure 3b, dotted double arrows). The NOESY correlations of δ_{H} 10.16 (NHb)/H-4, NHb/H-6b, δ_{H} 10.13 (NHa)/H-19, and NHa/H-17b indicated that both the guanidine NHs were also in the axial orientation about the tetrahydropyran rings. The additional NOESY correlations of δ_{H} 1.74 (H-7)/1.57 (H-9a), H-7/H-2.19 (H-9b), H-9b/δ_{H} 4.00 (H-10), δ_{H} 4.00 (H-13)/2.21 (H14b), δ_{H} 1.59 (H14a)/1.74 (H-16), and H14b/H-16 suggested the relative configuration between C-8 and C-10 and between C-13 and C-15. Although the
NOESY correlation of H-10/H-13 was not obtained due to their identical chemical shifts, the large coupling constants of 12.8 Hz between H-9a and H-10 and between H-14a and H-13 described that both H-10 and H-13 were in the axial-like α orientation. These findings support the relative stereochemistry of 2, as shown in Figure 3b. Since it was difficult to determine the position of two alkyl groups due to the highly symmetrical nature of 2, we tentatively assigned the alkyl position as shown because most of the related guanidine alkaloids possess a methylated tetrahydropyrane ring at the right side of the molecule. The specific rotation of 2 (−7.9) similar to that of 1 suggests the identical absolute configuration of 1 and 2.

Crambescidin 373 (3) possesses the molecular formula C\(_{22}\)H\(_{35}\)N\(_{3}\)O\(_{2}\) as determined by the pseudo-molecular ion at m/z 374.2786 [M + H]\(^+\) (calculated for C\(_{22}\)H\(_{36}\)N\(_{3}\)O\(_{2}\), 374.2802) in HR-ESIMS. The NMR data (Tables 1 and 2) were found to be similar to those for 1 and 4, indicating that 3 was another crambescidin analog with an ethyl group as supported by the signals at \(\delta\_H\) 3.38 (H-3) and \(\delta\_H\) 1.75 (H-14b). An additional NOESY correlation of H-13/H-11a, H-9b/H-12a, and H-9a/H-10 indicated the five partial structures CH\(_3\)-1–CH\(_2\)-2, CH\(_2\)-3–CH\(_2\)-4, CH\(_2\)-5–CH\(_2\)-6, CH\(_2\)-9–CH-10–CH\(_2\)-11, CH\(_2\)-12–CH-13–CH\(_2\)-14, and CH\(_2\)-17–CH\(_2\)-18–CH-19–CH\(_2\)-20. The exchangeable protons of 3 were observed at \(\delta\_H\) 11.17, 10.49 and 10.06 in CDCl\(_3\), revealing that 3 is the 19-ethyl homolog of 1. Although satisfactory NOESY data for 3 was not obtained due to the lack of the sample, the close similarity of the NMR data and the specific rotation (−8.0 for 3 and −8.9 for 4) to those for 4 suggests that the absolute configuration of 3 is the same as that of 4.

Guanidine compounds were mostly reported from marine organisms [39]. They have intriguing structures and wide range of biological activities and have attracted much attention of chemists and pharmacologists for their potential as drug leads [40]. Due to the strongest organic bases, guanidines are fully protonated under physiological conditions to form guanidinium cation, which can interact with biopolymers, such as DNA and proteins through hydrogen bonds and/or electrostatic interactions [41,42]. Since the first pentacyclic guanidine alkaloid ptilomycin A was isolated from the Caribbean sponge *Ptilocaulis spiculifer* and a Red Sea sponge *Hemimycale* sp. in 1989 [43], an array of cyclic guanidine alkaloids has been reported to date, including ptilomycins [44,45], crambescidins [30,32,33,35,46,47], monanchocidins [48,49], and monanchomycals [37,38]. Particularly, a number of metabolites of these types have been isolated mainly from marine sponges of the genera *Monanchora* and *Crambe*. The crambescidins and related alkaloids are characterized by a pentacyclic guanidine skeleton (vessel part) with two alkyl groups (ethyl at C-3 and methyl at C-19 in most cases) and a long aliphatic chain with a terminal carboxylate or a terminal spermidine amide. Crambescidins 359 (4) and 431 were reported in 2000 as the first crambescidin analogs lacking the long aliphatic chain (at C-14 in 5 and 6), which is replaced by a
hydrogen atom and an ethyl ester group, respectively [30]. Our compounds 1–3 are additional analogs of this type and are structurally characteristic in the following points. Crambescidin 345 (1) is the first analog with a non-alkylated tetrahydropyrane ring. Crambescidin 361 (2) possesses a rare propyl substituent as well as two tetrahydropyrane rings instead of the combination of one unsaturated oxepane and one tetrahydropyrane rings, which are found in most of the crambescidin-type alkaloids. Crambescidin 373 (3) is the first analog with an ethyl group at the right-side tetrahydropyrane ring, which possesses a methyl group in most of the reported crambescidin-type alkaloids.

![Figure 4](image_url) Figure 4. Two dimensional NMR correlations of 3. DQF-COSY and HMBC correlations are indicated by bold bonds and arrows, respectively.

2.2. Biological Activity

Biological activities of the isolated crambescidins 1–6 were evaluated as cytotoxic and anti-oomycete agents against the human epidermoid carcinoma cell line A431 and the oomycete plant pathogen Phytophthora capsici, respectively. All of the compounds showed cytotoxicity with an IC\(_{50}\) value lower than 10 \(\mu\)M (Figure 5a). The strongest cytotoxicity was observed for the long side chain-bearing crambescidins 5 and 6 with IC\(_{50}\)’s of 12 and 48 nM, respectively. Meanwhile, other crambescidins (1–4) without the long side chain part indicated a moderate cytotoxicity with IC\(_{50}\)’s of 7.0, 2.5, 0.94, and 3.1 \(\mu\)M, respectively. On the contrary, all new crambescidins 1–3 and the known analog 4 showed a higher anti-oomycete activity [minimum inhibitory dose (MID) of 50 \(\mu\)g/disk] than that for 5 and 6 (MID of 100 \(\mu\)g/disk or higher) (Figure 5b). It is interesting to note that the highly cytotoxic crambescidins (5 and 6) with a long side chain showed a lower anti-oomycete activity than the others. Especially, the most cytotoxic compound 5 showed no anti-oomycete activity even at 500 \(\mu\)g/disk (not indicated in Figure 5b). Consequently, these biological activities are approximately in an inverse relationship (Figure 5c).

Previous structure-activity relationship (SAR) studies on the crambescidins and their analogs reported that the presence of the long aliphatic side chain enhanced the cytotoxic effect of the guanidine core [50–53]. Our cytotoxicity data indicating the significant effect of the long aliphatic side chain are in good agreement with the previous reports. The long aliphatic side chain could affect the permeability of the guanidine alkaloid into animal cells. In contrast, it did not affect or rather diminished the anti-oomycete activity, which might be attributable to a low permeability through the microbial cell wall (mainly \(\beta\)-glucan) or/and into the hydrophilic agar medium that is used in the test.
3. Materials and Methods

3.1. General Procedures

Thin layer chromatography (TLC) was conducted by using precoated silica gel 600 F254 plates (Art. 5715, Merck, Darmstadt, Germany) or reverse phase C18 F254 plates (Art. 15389, Merck, Darmstadt, Germany). Flash column chromatography was carried out on silica gel by a medium-pressure gradient system equipped with a Pump Module C-605 and a Pump Manager C-615 (BUCHI, Flawil, Switzerland). High resolution ESIMS were recorded on a Mariner Biospectrometry Workstation (Applied Biosystems of Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ion source in the positive mode. High performance liquid chromatography (HPLC) was performed on a high pressure gradient system that is composed of pumps PU-2087, a degasser DG-2080-53, a mixer MX-2080-32, and a detector UV-2075 (JASCO, Tokyo, Japan). FT-IR spectra were recorded on an FT/IR-400 spectrometer instrument (JASCO). Specific rotations were observed on a DIP-370 polarimeter (JASCO). NMR spectra were investigated on an Avance ARX400 (400 MHz for $^1$H) or Avance III HD 600 MHz Cryo-probe spectrometer (600 MHz for $^1$H) (Bruker Bio Spin, Yokohama, Japan). The chemical shifts (ppm) were referenced to the solvent residual peak at $^\delta_H$ 7.26 ppm (CDCl$_3$), $^\delta_H$ 3.30/$^\delta_C$ 49.0 ppm (CD$_3$OD), or $^\delta_H$ 2.06/$^\delta_C$ 29.9 ppm (acetone-$d_6$).

3.2. Isolation of Bioactive Compounds

A reddish Indonesian sponge was collected by hand using a snorkeling equipment at a depth between 0.5 and 3 m in Samalona Island (5°8’16.4” S–119°23’22.60” E), South Sulawesi Sea, in August 2015. The species was identified as Clathria bulbifera based on the observation of its morphology and spicule elements under a microscope. The sponge possesses bulbous toxa spicule bulging toward the center that distinguishes it from other species [54]. The organism (750 g, wet weight) was lyophilized, homogenized in MeOH (1.5 L) and stand at room temperature for 3 days. The mixture was filtrated and the filtrate was concentrated to give an aqueous residue, which was extracted three times with EtOAc (225 mL). The combined organic layers were concentrated to yield EtOAc extract (6.4 g). This extract was dissolved in 90% MeOH (75 mL) and extracted twice with hexane (150 mL). Both of the layers were concentrated to obtain 90% MeOH (3.1 g) and hexane (2.5 g) fractions.

The 90% MeOH fraction, which showed a cytotoxicity (IC$_{50} = 0.046$ μg/mL), was separated by open column chromatography (silica gel, 100 g) eluted with 2, 5, 10, 100% of MeOH in CHCl$_3$
(600 mL each). The fractions were collected by every 100 mL, and appropriately combined to give six fractions (fr.1–fr.6), where fr.3 (222 mg) eluted with 2–5% MeOH and fr.6 (2.1 g) eluted with 100% MeOH showed significant cytotoxic activity of IC_{50} = 0.013 and 0.092 µg/mL, respectively. Fr.3 was further chromatographed on silica gel (HI-FLASH Size L, 30 g, Yamazen Co., Osaka, Japan) with linear gradient of 10–80% CHCl_3-MeOH-H_2O (90:9:1) in EtOAc (40 min) at a flow rate of 10 mL/min to yield eight fractions (fr.3-1–fr.3-8). The cytotoxic fr.3-5 (33.1 mg, IC_{50} = 0.8 mg/mL) was purified by HPLC [Develosil ODS-HG-5 (20 × 250 mm, Nomura Chemical Co., Ltd., Seto, Aichi, Japan), 80–100% MeOH-20 mM NH_4H_2COO (40 min), 6 mL/min, detected at 215 nm] to give crambescidin 800 (R_t = 25.0 min), 2.4 mg, α_1O (90:10) in CHCl_3 (600 mL each). The three fractions, fr.3-6–fr.3-8 (110 mg in total, IC_{50} = 0.081 µg/mL) were combined and subjected to HPLC [Develosil ODS-UG-5 (10 × 3.2 mm), 250 mm, Nomura Chemical Co., Ltd., Seto, Aichi, Japan), 60% MeOH-0.1% TFA, 2 mL/min, detected at 215 nm] to give pure crambescidin 345 (1, 0.6 mg, t_R = 19.7 min). The fr.6 (2.1 g, IC_{50} = 0.092 µg/mL) was fractionated through a silica gel (50 g) open column, eluted with 10, 20, 40, 60, 100% MeOH-H_2O (90:10) in CHCl_3 to afford four fractions (fr.6-1–fr.6-4). The active fr.6-3 (500 mg, IC_{50} = 0.019 µg/mL) eluted with 40% MeOH-H_2O (90:10) in CHCl_3 was then chromatographed on silica gel (HI-FLASH Size L, 30 g) with gradient elution of 10–100% MeOH in CHCl_3 for 40 min at a flow rate of 10 mL/min to give four fractions (fr.6-3-1–fr.6-3-4). The active fr. 6-3-3 (180 mg, IC_{50} = 0.081 µg/mL) was subjected to HPLC [Develosil ODS-HG-5 (20 × 250 mm), 40–60% MeCN-0.1% TFA (60 min), 8 mL/min, detected at 230 nm] to obtain pure crambescidin 345 (1.5 mg, t_R = 40.8 min), crambescidin 359 (4, 2.60 mg, t_R = 45.6 min), crambescidin 361 (2, 2.4 mg, t_R = 55.4 min), and crambescidin 373 (3, 1.1 mg, t_R = 62.0 min). The I-containing fraction was further purified by HPLC [Develosil ODS-UG-5 (10 × 250 mm), 60% MeOH-0.1% TFA, 2 mL/min, detected at 215 nm] to obtain pure crambescidin 345 (1, 0.6 mg, t_R = 19.7 min). The fr.6 (2.1 g, IC_{50} = 0.092 µg/mL) was fractionated through a silica gel (50 g) open column, eluted with 10, 20, 40, 60, 100% MeOH-H_2O (90:10) in CHCl_3 to afford four fractions (fr.6-1–fr.6-4). The active fr.6-3 (500 mg, IC_{50} = 0.017 µg/mL) eluted with 40% MeOH-H_2O (90:10) in CHCl_3 was then chromatographed on silica gel (HI-FLASH Size L, 30 g) with gradient elution of 10–100% MeOH in CHCl_3 for 40 min at a flow rate of 10 mL/min to give four fractions (fr.6-3-1–fr.6-3-4). The active fr. 6-3-3 (180 mg, IC_{50} = 0.019 µg/mL) eluted with 54–86% MeOH was subjected to HPLC [Develosil ODS-HG-5 (20 × 250 mm), 40–60% MeCN-0.1% TFA (60 min), 8 mL/min, detected at 230 nm] to obtain crambescidin 800 (6, 21.7 mg, t_R = 36.3 min).

3.2.1. Crambescidin 345 (1)

Colorless powder; [α]_D^{26} = -7.1 (0.051, MeOH); IR (film) ν_max 3222, 3109, 3019, 1678, 1654, 1607, 1201, 1177, 1131, and 720 cm⁻¹; HR ESIMS m/z 346.2495 [M + H]^+; calcd. for C_{20}H_{32}N_{3}O_{2} 346.2489.

3.2.2. Crambescidin 361 (2)

Pale yellow solid; [α]_D^{26} = -7.9 (0.13, MeOH); IR (film) ν_max 3236, 3107, 1676, 1652, 1606, 1201, 1177, 1131, 1017, and 719 cm⁻¹; HR ESIMS m/z 362.2765 [M + H]^+; calcd. for C_{21}H_{30}N_{3}O_{2} 362.2802.

3.2.3. Crambescidin 373 (3)

Pale yellow solid; [α]_D^{26} = -8.8 (0.025, MeOH); IR (film) ν_max 3228, 3111, 3019, 1678, 1652, 1606, 1201, 1177, 1131, and 720 cm⁻¹; HR ESIMS m/z 374.2786 [M + H]^+; calcd. for C_{22}H_{36}N_{3}O_{2} 374.2802.

3.2.4. Crambescidin 359 (4)

Pale yellow solid; [α]_D^{26} = -8.9 (0.23, MeOH) (reference [45]: [α]_D^{20} = -12.7 (0.4, MeOH)).

3.2.5. Crambescidin 657 (5)

Yellowish solid; [α]_D^{25} = -11.0 (0.18, MeOH) (reference [55]: [α]_D^{25} = -12.1 (0.34, MeOH)).

3.2.6. Crambescidin 800 (6)

Yellowish solid; [α]_D^{25} = -8.7 (1.6, MeOH) (reference [45]: [α]_D^{20} = -7.8 (4.1, MeOH)).

3.3. Anti-oomycete Assay

The test was performed by the paper disk diffusion method [56]. Briefly, a piece of the mycelia of the plant pathogen Phytophthora capsici NBRC 30696 was pre-cultured on a potato-glucose-agar medium in a 9-cm dish at 25 °C and 60% humidity for seven days in the dark. A piece (5 × 5 mm) of the colony was then inoculated on the center of a 5% V8 juice-1.5%-agar medium in a 9-cm dish.
and incubated for 48 h at 25 °C and 60% humidity. A paper disk (8 mm in diameter) containing a sample at an appropriate dose (none, 25, 50, and 100 µg/disk) was placed at 1 cm away from the colony front. After incubation for another 22–24 h, the inhibition zone formed around the sample disk was measured. The activity was represented by the minimum dose that expressed an obvious inhibition zone (usually 0.5 mm or wider).

3.4. Cytotoxicity Assay

A431 human vulva-derived epidermoid carcinoma cells were used to evaluate the cytotoxicity of the compounds under the conditions reported previously [57]. Briefly, the cells at a density of 1.0 × 10^4 cells/well were cultured in a 24-well plate (BD Falco) in DF6F medium with various concentrations of compounds (none, 1, 3, 10 µM for 1–4; none, 0.001, 0.01, 0.1, and 1.0 µM for 5 and 6) at 37 °C in a humidified 95% air/5% CO₂ condition in a CO₂ incubator (Thermo Fisher Scientific, Waltham, MA, USA), followed by cell counting with a Coulter Counter (Coulter Electronics Inc., Hialeah, FL, USA) on day 5. The DF6F medium was composed of a 1:1 ratio of DMEM and Ham F-12 medium (DF), supplemented with six factors, i.e., insulin (10 µg/mL), transferrin (5 µg/mL), 2-aminoethanol (10 µM), sodium selenite (10 nM), 2-mercaptoethanol (10 µM), and oleic acid conjugated with fatty acid-free bovine serum albumin (9.4 µg/mL) (all of the chemicals were from Sigma-Aldrich, St. Louis, MO, USA). IC₅₀ values are shown by the means of two (for 1–4 and 6) or three (5) replicates.

4. Conclusions

In the present study, we discovered three new guanidine alkaloids, crambescidins 345 (1), 361 (2), and 373 (3), together with three known crambescidins 4–6 from the Indonesian sponge Clathria bulbotoxa. The structures of 1–3 with absolute stereochemistry were determined by spectroscopic analysis, including two-dimensional NMR and specific rotation. Although the pentacyclic guanidine core has been found in a number of the crambescidins and related natural compounds, a high diversity in the alkyl substituents (methyl, ethyl, propyl) on the cyclic ether rings of our compounds was observed for the first time, whereas most related products possess ethyl group at C-3 and methyl group at C-19. The biological assays revealed that the long aliphatic side chain in compounds 5 and 6 plays a quite important role for the cytotoxicity against cancer cells (possibly due to the increase of permeability through cell membrane), but conversely not for the inhibition of an oomycete plant pathogen.

Supplementary Materials: The following materials are available online at http://www.mdpi.com/1660-3397/16/5/384/s1 Table S1: ¹H and ¹³C NMR data of 2 (600 MHz, acetone-d₆), Figure S1: ¹H NMR spectrum of 1 (600 MHz, CD₂OD), Figure S2: ¹H NMR spectrum of 1 (400 MHz, CDCl₃), Figure S3: ¹³C NMR spectrum of 1 (150 MHz, CD₂OD), Figure S4: DQF-COSY spectrum of 1 (600 MHz, CD₂OD), Figure S5: HSQC spectrum of 1 (600 MHz, CD₂OD), Figure S6: HMBC spectrum of 1 (600 MHz, CD₂OD), Figure S7: NOESY spectrum of 1 (600 MHz, CD₂OD), Figure S8: IR spectrum of 1, Figure S9: ESI-TOF-MS(+) spectra of 1–3, Figure S10: ¹H NMR spectrum of 2 (400 MHz CD₂OD), Figure S11: ¹³C NMR spectrum of 2 (100 MHz, CD₂OD), Figure S12: DQF-COSY spectrum of 2 (400 MHz, CD₂OD), Figure S13: HSQC spectrum of 2 (400 MHz, CD₂OD), Figure S14: HMBC spectrum of 2 (400 MHz, CD₂OD), Figure S15: NOESY spectrum of 2 (400 MHz, CD₂OD), Figure S16: IR spectrum of 2, Figure S17: ¹H NMR spectrum of 2 (600 MHz acetone-d₆), Figure S18: ¹³C NMR spectrum of 2 (150 MHz, acetone-d₆), Figure S19: DQF-COSY spectrum of 2 (600 MHz, acetone-d₆), Figure S20: HSQC spectrum of 2 (600 MHz, acetone-d₆), Figure S21: HMBC spectrum of 2 (600 MHz, acetone-d₆), Figure S22: NOESY spectrum of 2 (600 MHz, acetone-d₆), Figure S23: ¹H NMR spectrum of 3 (600 MHz, CD₂OD), Figure S24: ¹³C NMR spectrum of 3 (150 MHz, CD₂OD), Figure S25: HSQC spectrum of 3 (600 MHz, CD₂OD), Figure S26: DQF-COSY spectrum of 3 (600 MHz, CD₂OD), Figure S27: HSQC spectrum of 3 (600 MHz, CD₂OD), Figure S28: HMBC spectrum of 3 (600 MHz, CD₂OD), Figure S29: IR spectrum of 3, Figure S30: ¹H NMR spectrum of 4 (400 MHz, CD₂OD), Figure S31: ¹H NMR spectrum of 5 (400 MHz, CD₂OD), Figure S32: ¹H NMR spectrum of 6 (400 MHz, CD₂OD), Figure S33: Anti-oomycete activity of compounds 1–6.

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Author Contributions: K.K. collected the organism, isolated and elucidated the compounds, conducted the anti-oomycete assay, analyzed the cytotoxic activity, and prepared the manuscript. Y.Y. and T.O. performed the cytotoxicity assay. M.O. designed the project and wrote the manuscript.

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References
1. Proksch, P. Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. *Toxicon* 1994, 32, 639–655. [CrossRef] [PubMed]
2. Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* 1998, 15, 113–158. [CrossRef] [PubMed]
3. Laport, M.; Santos, O.; Muricy, G. Marine sponges: Potential sources of new antimicrobial drugs. *Curr. Pharm. Biotechnol.* 2009, 10, 86–105. [CrossRef] [PubMed]
4. Mehbub, M.F.; Lei, J.; Franco, C.; Zhang, W. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs* 2014, 12, 4539–4577. [CrossRef] [PubMed]
5. Paul, V.J.; Puglisi, M.P. Chemical mediation of interactions among marine organisms. *Nat. Prod. Rep.* 2004, 21, 189–209. [CrossRef] [PubMed]
6. Kim, S.-K.; Dewapriya, P. Bioactive compounds from marine sponges and their symbiotic microbes: A potential source of nutraceuticals. *Adv. Food Nutr. Res.* 2012, 65, 137–151. [CrossRef] [PubMed]
7. Lopanik, N.B. Chemical defensive symbioses in the marine environment. *Funct. Ecol.* 2014, 28, 328–340. [CrossRef]
8. Huffard, C.L.; Erdmann, M.V.; Gunawan, T.R. Geographic Priorities for Marine Biodiversity Conservation in Indonesia; Ministry of Marine Affairs and Fisheries and Marine Protected Areas Governance Program: Jakarta, Indonesia, 2012; pp. 1–6. ISBN 978-602-98450-6-8.
9. Pawlik, J.R. The chemical ecology of sponges on Caribbean reefs: Natural products shape natural systems. *BioScience* 2011, 61, 888–898. [CrossRef] [PubMed]
10. Veron, J.E.N.; Devantier, L.M.; Turak, E.; Green, A.L.; Kininmonth, S.; Stafford-Smith, M.; Peterson, N. Delineating the coral triangle. *Galaxea J. Coral Reef Stud.* 2009, 11, 91–100. [CrossRef]
11. Sabdono, A.; Radjasa, O.K. Microbial symbionts in marine sponges: Marine natural product factory. *J. Coast. Dev.* 2008, 11, 57–61.
12. Perdicaris, S.; Vlachogianni, T.; Valavanidis, A. Bioactive natural substances from marine sponges: New developments and prospects for future pharmaceuticals. *Nat. Prod. Chem. Res.* 2013, 1. [CrossRef] [PubMed]
13. Gomez, P. The genus *Clathria* from the Gulf of Mexico and Mexican Caribbean, with description and resurrection of *Clathria carteri* (Poecilosclerida: Microcionidae). *Zootaxa* 2014, 3790, 51–85. [CrossRef] [PubMed] [PubMed]
14. Zuleta, I.A.; Vitelli, M.L.; Baggio, R.; Garland, M.T.; Seldes, A.M.; Palermo, J.A. Novel pteridine alkaloids from the sponge *Clathria sp.* *Tetrahedron* 2002, 58, 4481–4486. [CrossRef]
15. Laville, R.; Thomas, O.P.; Berrue, F.; Marquez, D.; Vacelet, J.; Amade, P. Bioactive guanidine alkaloids from two Caribbean marine sponges. *J. Nat. Prod.* 2009, 72, 1589–1594. [CrossRef] [PubMed]
16. El-Naggar, M.; Conte, M.; Capon, R.J. Mirabilins revisited: Polyketide alkaloids from a southern Australian marine sponge, *Clathria sp.* *Org. Biomol. Chem.* 2010, 8, 407–412. [CrossRef] [PubMed]
17. Wei, X.; Henriksen, N.M.; Skalicky, J.J.; Harper, M.K.; Cheatham, T.E.; Ireland, C.M.; Van Wagoner, R.M. Ariaosamines A-D: Tris-bromoindole cyclic guanidine alkaloids from the marine sponge *Clathria (Thalysias) araiesa.* *J. Org. Chem.* 2011, 76, 5515–5523. [CrossRef] [PubMed]
18. Sun, X.; Sun, S.; Ference, C.; Zhu, W.; Zhou, N.; Zhang, Y.; Zhou, K. A potent antimicrobial compound isolated from *Clathria cervicornis*. *Biol. Med. Chem.* 2015, 25, 67–69. [CrossRef] [PubMed]
19. Tanaka, Y.; Katayama, T. Biochemical studies on carotenoids in Porifera. The structure of cathriaxanthin in sea sponge, *Clathria frondifera* (Bowerbank). *Bull. Jpn. Soc. Sci. Fish.* 1976, 42, 801–805. [CrossRef]
22. Dattelbaum, J.D.; Sieg, D.; Minieri, C.M.; Thomson, G.; Hill, M. Plasticity of acquired secondary metabolites in *Clathria prolifera* (Demospongia: Poecilosclerida): Putative photoprotective role of carotenoids in a temperate intertidal sponge. *Open Mar. Biol. J.* 2010, 4, 87–95. [CrossRef]

23. Davis, R.A.; Mangalindan, G.C.; Bojo, Z.P.; Antemano, R.R.; Rodriguez, N.O.; Concepcion, G.P.; Samson, S.C.; de Guzman, D.; Cruz, L.J.; Tasdemir, D.; et al. Microcionamides A and B, bioactive peptides from the Philippine sponge *Clathria* (*Thalysias*) *abietina*. *J. Org. Chem.* 2004, 69, 4170–4176. [CrossRef] [PubMed]

24. Capon, R.J.; MacLeod, J.K. 5-Thio-d-mannose from the marine sponge *Clathria pyramida* (Lendenfeld). The first example of a naturally occurring 5-thiosugar. *J. Chem. Soc. Chem. Commun.* 1987, 1200–1201. [CrossRef]

25. Gupta, P.; Sharma, U.; Schulz, T.C.; McLean, A.B.; Robins, A.J.; West, L.M. Bicyclic C₂₁ terpenoids from the marine sponge *Clathria compressa*. *J. Nat. Prod.* 2012, 75, 1223–1227. [CrossRef] [PubMed]

26. Kim, S.-H.; Shin, J. Additional sesterterpenes and a nortriterpene saponin from the sponge *Clathria gombauwensis*. *J. Nat. Prod.* 2015, 78, 218–224. [CrossRef] [PubMed]

27. Rudi, A.; Yosief, T.; Loya, S.; Hizi, A.; Schleyer, M.; Kashman, Y. Clathsterol, a novel anti-HIV-1 RT sulfated sterol from the sponge *Clathria* species. *J. Nat. Prod.* 2001, 64, 1451–1453. [CrossRef] [PubMed]

28. Santalova, E.A.; Makarieva, T.N.; Gorshkova, I.A.; Dmitrenok, A.S.; Krasokhin, V.B.; Stonik, V.A. Sterols from six marine sponges. *Biochem. Syst. Ecol.* 2004, 32, 153–167. [CrossRef]

29. Keyzars, R.A.; Northcote, P.T.; Webb, V. Clathriol, a novel polyoxygenated 14β steroid isolated from the New Zealand marine sponge *Clathria lissosclera*. *J. Nat. Prod.* 2002, 65, 598–600. [CrossRef]

30. Braekman, J.C.; Daloze, D.; Tavares, R.; Hadju, E.; Van Soest, R.W.M. Novel polycyclic guanidine alkaloids from two marine sponges of the genus *Monanchora*. *J. Nat. Prod.* 2000, 63, 193–196. [CrossRef] [PubMed]

31. Coffey, D.S.; McDonald, A.I.; Overman, L.E.; Rabinowitz, M.H.; Renhowe, P.A. A practical entry to the crambescidin family of guanidine alkaloids. Enantioselective total syntheses of ptilomycalin A, crambescidin 657 and its methyl ester (neofolitispates 2), and crambescidin 800. *J. Am. Chem. Soc.* 2000, 122, 4893–4903. [CrossRef] [PubMed]

32. Jares-Erijman, E.A.; Sakai, R.; Rinehart, K.L. Crambescidins: New antiviral and cytotoxic compounds from the sponge *Crambe crambe* Polycyclic guanidine alkaloids from the marine sponge *Clathria pyramida*. *J. Nat. Prod.* 1993, 56, 1007–1015. [CrossRef] [PubMed]

33. Berlinck, R.G.S.; Braekman, J.C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. Polycyclic guanidine alkaloids from the marine sponge *Crambe crambe* and Ca²⁺ channel blocker activity of crambescidin 816. *J. Nat. Prod.* 1993, 56, 1490–1494. [CrossRef] [PubMed]

34. Chang, L.C.; Whittaker, N.F.; Bewley, C.A. Crambescidin 826 and dehydrocrambine A: New polycyclic guanidine alkaloids from the marine sponge *Monanchora* sp. that inhibit HIV-1 fusion. *J. Nat. Prod.* 2003, 66, 1490–1494. [CrossRef] [PubMed]

35. El-Demerdash, A.; Moriou, C.; Martin, M.T.; Rodrigues-Stien, A.S.; Petek, S.; Demoy-Schneider, M.; Hall, K.; Hooper, J.N.A.; Debitus, C.; Al-Mourabit, A. Cytotoxic guanidine alkaloids from a French Polynesian *Monanchora* n. sp. sponge. *J. Nat. Prod.* 2016, 79, 1929–1937. [CrossRef] [PubMed]

36. Jones, W.J. The infra-red spectrum and structure of guanidine. *Trans. Faraday Soc.* 1959, 55, 524–531. [CrossRef]

37. Makarieva, T.N.; Tabakmaher, K.M.; Guzii, A.G.; Denisenko, V.A.; Dmitrenok, P.S.; Kuzmich, A.S.; Lee, H.-S.; Stonik, V.A. Monanchomycalin A and B, unusual guanidine alkaloids from the sponge *Monanchora pulchra*. *Tetrahedron Lett.* 2012, 53, 4228–4231. [CrossRef]

38. Tabakmaker, K.M.; Denisenko, V.A.; Guzii, A.G.; Dmitrenok, P.S.; Dyshlovoy, S.A.; Lee, H.-S.; Makarieva, T.N. Monanchomycalin C, a new pentacyclic guanidine alkaloid from the far-eastern marine sponge *Monanchora pulchra*. *Nat. Prod. Commun.* 2013, 8, 1399–1402. [PubMed]

39. Cong, H.-J.; Zhang, S.-W.; Shen, Y.; Huang, Y.-J.; Wang, W.-Q.; Leng, Y.; Xuan, L.-J. Guanidine alkaloids from *Plumbago zeylanica*. *J. Nat. Prod.* 2013, 76, 1351–1357. [CrossRef] [PubMed]

40. Berlinck, R.G.S.; Trinidad-Silva, A.E.; Santos, M.F.C. The chemistry and biology of organic guanidine derivatives. *Nat. Prod. Rep.* 2012, 29, 1382–1406. [CrossRef] [PubMed]

41. Gobbi, A.; Frenking, G. Y-conjugated compounds: The equilibrium geometries and electronic structures of guanidine, guanidinium cation, urea, and 1,1-diaminoethylene. *J. Am. Chem. Soc.* 1993, 115, 2362–2372. [CrossRef]

42. Feichtinger, K.; Zapf, C.; Sings, H.L.; Goodman, M. Diprotected triflyguanidines: A new class of guanidinylation reagents. *J. Org. Chem.* 1998, 63, 3804–3805. [CrossRef]
43. Kashman, Y.; Hirsh, S.; McConnell, O.J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. Ptilomycalin A: A novel polycyclic guanidine alkaloid of marine origin. *J. Am. Chem. Soc.* 1989, 111, 8925–8926. [CrossRef]

44. Bensemhoun, J.; Bombarda, I.; Aknin, M.; Vacelet, J.; Gaydou, E.M. Ptilomycalin D, a polycyclic guanidine alkaloid from the marine sponge *Monanchora diamchora*. *J. Nat. Prod.* 2007, 70, 2033–2035. [CrossRef] [PubMed]

45. Campos, P.-E.; Wolfender, J.-L.; Queiroz, E.F.; Marcourt, L.; Al-Mourabit, A.; Frederich, M.; Bordignon, A.; De Voogd, N.; Illien, B.; Gauvin-Bialecki, A. Unguiculin A and ptilomycalinins E-H, antimalarial guanidine alkaloids from the marine sponge *Monanchora unguiculata*. *J. Nat. Prod.* 2017, 80, 1404–1410. [CrossRef] [PubMed]

46. Jares-Erijman, E.A.; Ingrum, A.L.; Carney, J.R.; Rinehart, K.L.; Sakai, R. Polycyclic guanidine-containing compounds from the Mediterranean sponge *Crambe crambe*: The structure of 13,14,15-isocrambescidin 800 and the absolute stereochemistry of the pentacyclic guanidine moieties of the crambescidins. *J. Org. Chem.* 1993, 58, 4805–4808. [CrossRef]

47. Bondu, S.; Genta-Jouve, G.; Leiros, M.; Vale, C.; Guigonis, J.-M.; Botana, L.M.; Thomas, O.P. Additional bioactive guanidine alkaloids from the Mediterranean sponge *Crambe crambe*. *RSC Adv.* 2012, 2, 2828–2835. [CrossRef]

48. Makarieva, T.N.; Tabakmaher, K.M.; Guzii, A.G.; Denisenko, V.A.; Dmitrenok, P.S.; Shubina, L.K.; Kuzmich, A.S.; Lee, H.-S.; Stonik, V.A. Monanchocidins B-E: Polycyclic guanidine alkaloids with potent antileukemic activities from the sponge *Monanchora pulchra*. *J. Nat. Prod.* 2011, 74, 1952–1958. [CrossRef] [PubMed]

49. Guzii, A.G.; Makarieva, T.N.; Denisenko, V.A.; Dmitrenok, P.S.; Kuzmich, A.S.; Dyshlovoy, S.A.; Krasokhin, V.B.; Stonik, V.A. Monanchocidin: A new apoptosis-inducing polycyclic guanidine alkaloid from the marine sponge *Monanchora pulchra*. *Org. Lett.* 2010, 12, 4292–4295. [CrossRef] [PubMed]

50. Aron, Z.D.; Pietraszkiewicz, H.; Overman, L.E.; Valeriote, F.; Cuevas, C. Synthesis and anticancer activity of side chain analogs of the crambescidin alkaloid. *Bioorg. Med. Chem. Lett.* 2004, 14, 3445–3449. [CrossRef] [PubMed]

51. Aron, Z.D.; Overman, L.E. Total synthesis and properties of the crambescidin core zwitterionic acid and crambescidin 359. *J. Am. Chem. Soc.* 2005, 127, 3380–3390. [CrossRef] [PubMed]

52. Lazaro, J.E.H.; Nitcheu, J.; Mahmoudi, N.; Ibana, J.A.; Mangalindan, G.C.; Black, G.P.; Howard-Jones, A.G.; Moore, C.G.; Thomas, D.A.; Mazier, D.; et al. Antimalarial activity of crambescidin 800 and synthetic analogues against liver and blood stage of *Plasmodium* sp. *J. Antibi.* 2006, 59, 583–590. [CrossRef] [PubMed]

53. Moore, C.G.; Murphy, P.J.; Williams, H.L.; McGown, A.T.; Smith, N.K. Synthetic studies towards ptilomycalin A: Total synthesis of crambescidin 359. *Tetrahedron* 2007, 63, 11771–11780. [CrossRef]

54. Van Soest, R.W.M. Marine sponges from Curacao and other Caribbean localities. Part III. Poecilosclerida. *Stud. Fauna Curacao Caribb. Isl.* 1984, 66, 1–167.

55. Rinehart, K.L.; Shi, J.-G.; Sun, F. Crambescidin Compounds. US Patent 6028077, 22 February 2000.

56. Sun, Y.; Tomura, T.; Sato, J.; Iizuka, T.; Fudou, R.; Ojika, M. Isolation and biosynthetic analysis of haliamide, a new PKS-NRPS hybrid metabolite from the marine myxobacterium *Haliangium ochraceum*. *Molecules* 2016, 21, 59. [CrossRef] [PubMed]

57. Nakamura, M.; Kakuda, T.; Qi, J.; Hirata, M.; Shintani, T.; Yoshioka, Y.; Okamoto, T.; Oba, Y.; Nakamura, H.; Ojika, M. Novel relationship between the antifungal activity and cytotoxicity of marine-derived metabolite xestoquinone and its family. *Biosci. Biotechnol. Biochem.* 2005, 69, 1749–1752. [CrossRef] [PubMed]

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