Bromopyrrole Alkaloids from Okinawan Marine Sponges Agelas spp.

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In our continuing study for structurally and biogenetically interesting natural products from marine organisms, Okinawan marine sponges Agelas spp. were investigated, resulting in the isolation of 18 unique alkaloids including five dimeric bromopyrrole alkaloids (1–5), ten monomeric bromopyrrole alkaloids (6–15), and three conjugates of monomeric bromopyrrole alkaloid and hydroxykynurenine (16–18). In this mini-review, the isolation, structure elucidation, and antimicrobial activities of these alkaloids are summarized.

Key words: bromopyrrole alkaloid; Okinawan marine sponge; Agelas spp.

1. Introduction

Bromopyrrole alkaloids are one of the most common secondary metabolites found in marine sponges. These alkaloids are argued to be taxon-specific of at least the Agelasida order and can be used as chemical markers of these phylogenetically related sponges.1–3 It has been shown that oroidin and scep-trin, which are bromopyrrole alkaloids contained in sponges Agelas spp. (Agelasidae), are not associated with the symbiotic bacteria but with the sponge cells,4 and that sponges Agelas spp. share bromopyrrole alkaloids as a common chemical defense against fish predators.5,6 In addition to these chemo-taxonomic and ecological roles, bromopyrrole alkaloids are interesting for their diverse biological activities7 including antifeedent, antibiofilm, anticancer, antiinflammatory, antimicrobial, immunomodulatory, analgesic, antiserotonic, and antihistaminic activities.

Representative bromopyrrole alkaloids, oroidin8,9 and hymeninid,10 are composed of an aminoimidazole moiety and a brominated pyrrole-amide moiety linked through a C3 unit (Fig. 1). Proline and lysine have been suggested to be biogenetic precursors of oroidin.11 Cyclization and/or dimerization of the monomeric bromopyrrole alkaloids yielded polycyclic and dimeric bromopyrrole alkaloids, which have attracted widespread interest as challenging targets for total synthesis by virtue of their fascinating functionalized structures with a high N:C ratio (1:2).12,13 Though a number of bromopyrrole alkaloids have been isolated from marine sponges to date, stylissadines A and B14 from Stylissa flabellata, are the only known examples of tetrameric bromopyrrole alkaloids.

In our continuing search for structurally and biogenetically unique natural products from marine organisms, we have reported the isolation of some bromopyrrole alkaloids from Okinawan marine sponges.15 As part of that research project, bromopyrrole alkaloids in sponges Agelas spp. (SS-162, SS-307) were investigated, resulting in the isolation of 18 unique alkaloids including five dimeric bromopyrrole alkaloids (1–5), ten monomeric bromopyrrole alkaloids (6–15), and three conjugates of monomeric bromopyrrole alkaloid and hydroxykynurenine (16–18). In this mini-review, we describe the isolation, structure elucidation, and antimicrobial activities of these alkaloids.

2. Extraction and Isolation of New Bromopyrrole Alkaloids

The sponges Agelas spp. (SS-162, SS-307) were collected off Kerama Islands, Okinawa, and were individually extracted with MeOH. Repeated chromatographic separations of the extract from SS-162 using a silica gel column, an octadecylsilyl (ODS) column, a Sephadex LH-20 column, and a Toyopearl HW-40 column gave fractions containing bromopyrrole alkaloids. The fractions were purified by reversed-phase HPLC and/or hydrophilic interaction liquid chromatography (HILIC) HPLC to isolate new bromopyrrole alkaloids; agelamadins A (1, B (2), C (16), D (17), and E (18); nagelamides U (7), V (8), W (9), X (3), Y (4), and Z (5); tauroucidins C (10) and D (11); mukanadin G (13); 2-bromokeramadine (14); and 2-bromo-9,10-dihydrokeramadine (15). Similarly, the MeOH extract from SS-307 was separated to give agelamadin F (6) and tauroacidin E (12).

3. Dimeric Bromopyrrole Alkaloids, Agelamadins A and B and Nagelamides X–Z

Agelastatins, which are monomeric bromopyrrole alkaloids, possess a unique 5/5/6/5 tetracyclic ring system. Among these, agelastatin A16,17 (Fig. 2) is interesting for its potent cytotox-
icity against various cancer cell lines and for its inhibitory activity against glycogen synthase kinase-3β (GSK-3β). Agelamadins A (1) and B (2) 18) have a common structure consisting of an agelastatin-like tetracyclic moiety and an oroidin-like linear moiety (Fig. 2). The high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) and the one dimensional (1D) NMR spectra suggested that 1 and 2 were dimeric bro-mopyrrole alkaloids, and their gross structures were elucidated by analyses of the 1H–1H correlation spectroscopy (COSY) and heteronuclear multiple bond coherence (HMBC) spectra. Rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) analysis indicated that the relative configurations of the agelastatin-like tetracyclic moieties for 1 and 2 were coincident with that of agelastatin A. The optical resolutions on chiral HPLC revealed both 1 and 2 to be racemates.

The 1D NMR spectra implied that nagelamides X (3) and Y (4) 19) are dimeric bromopyrrole alkaloids with an N-ethanesulfonic acid moiety (Fig. 3). The novel tricyclic skeleton shared by 3 and 4, comprising spiro-bonded tetrahydrobenzaminoimidazole and aminomidazolodine moieties, was elucidated by 2D-NMR analyses. Nagelamides X (3) and Y (4) seemed to have been derived from oroidin (Fig. 1) and taurodispacamide A20) (Fig. 3) by [4+2] cycloaddition. Nagelamide Z (5) 19) was the first example of a dimer of oroidin-like linear bromopyrrole alkaloid involving dimerization at C-8 (Fig. 3). The absolute configuration of C-8 for 5 remained unsolved.

4. Monomeric Bromopyrrole Alkaloids, Agelamadine F and Nagelamides U–W

The 1D NMR spectra suggested that agelamadine F (6) 21) had an oroidin-like linear moiety and a 3-hydroxyxpyridinium moiety (Fig. 4). The connectivity between C-15 of the linear moiety and N-1' of the 3-hydroxyxpyridinium moiety was revealed by HMBC and ROESY analyses. Agelamadine F (6) is the first example of a bromopyrrole alkaloid with a 3-hydroxyxpyridinium moiety. Nagelamide U (7) 22) was a monomeric bromopyrrole alkaloid having a γ-lactam ring with an N-ethanesulfonic acid moiety and a guanidino moiety, while nagelamide V (8) 22) was assigned as a stereoisomer of 7. The relationships between the substituents at C-9 and C-11 for 7 and 8 were assigned as anti and syn, respectively, by ROESY analysis. Though nagelamides U (7) and V (8) were optically active, their absolute configurations were not assigned. Interpretation of the 2D-NMR spectra revealed nagelamide W (9) 22) to be a monomeric bromopyrrole alkaloid with two aminimidazole moieties in the molecule (Fig. 4). Nagelamide W (9)
of maleimide and oroidin has been reported. 25) Chiral HPLC
source, whereas the synthesis of
as a Diels–Alder adduct
13
was concluded as shown in Fig. 5
by ROESY analysis and a comparison of the experimental 1H
coupling constants with those calculated for the most stable
analyses disclosed tauroacidins C–E (15)
and mukanadin G (13) to be racemates. The structures of
14
and 13
were elucidated by
analyses of the NMR spectra implied that tauroacidins
12
18
possessed a unique hybrid
structure of oroidin and 3-hydroxykynurenine connected
through a dihydro-1,4-oxazine moiety (Fig. 5). The relative
stereochemistry of 13
was assigned as 2-bromo and 2-bromo-9,10-dihydro analogues 23)
of keramadine, 26) respectively (Fig. 5).

5. Conjugates of Monomeric Bromopyrrole Alkaloid
and Hydroxykynurenine, Agelamadins C–E
Agelamadins C–E (16–18)25) possessed a unique hybrid
structure of oroidin and 3-hydroxykynurenine connected
through a dihydro-1,4-oxazine moiety (Fig. 6). Interpretations
of the 1D and 2D-NMR spectra indicated that
16–18
had the same planar structure. To assign the absolute
configurations for the α-carbons (C-9) of 16–18, a phenylglycine
methyl ester (PGME) method was applied,27,28) suggesting
the configuration to be S in each case. ROESY analysis indicated
the H-9/H-10 anti relationship for 16 and 17, whereas the
H-9/H-10 syn relationship was assigned for 18. The absolute
configurations at C-9 and C-10 of 16–18 were elucidated by
comparison of the electronic circular dichroism (ECD) spectra
with the time-dependent density functional theory (TDDFT)
calculated spectra as shown in Fig. 6.

6. Antimicrobial Activities of Isolated Bromopyrrole
Alkaloids
In the course of our search for antimicrobial marine natural
products,29–34) we evaluated the antimicrobial activities
of isolated bromopyrrole alkaloids against Escherichia coli,
Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus,
Aspergillus niger (IFM62678), Trichophyton mentagrophytes
(IFM62679), Candida albicans (IFM62680), and Cryptococcus
neoformans (IFM62681) (Table 1). Among tested alkaloids,
nagelamide Z (5) exhibited significant antimicrobial activity
against C. albicans (MIC 0.25 μg/mL) and moderate activities

Table 1. Antimicrobial Activities of 1–5, 7, 9, 10, and 13–18

<table>
<thead>
<tr>
<th>Strain</th>
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<th>7</th>
<th>9</th>
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<tr>
<td><em>Escherichia coli</em>&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><em>Staphylococcus aureus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32</td>
<td>32</td>
<td>8.0</td>
<td>&gt;32</td>
<td>16</td>
<td>&gt;32</td>
<td>&gt;32</td>
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<tr>
<td><em>Bacillus subtilis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>16</td>
<td>&gt;32</td>
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<tr>
<td><em>Micrococcus luteus</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0</td>
<td>8.0</td>
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<td><em>Aspergillus niger</em>&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><em>Trichophyton mentagrophytes</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;32</td>
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<td><em>Candida albicans</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2.0</td>
<td>2.0</td>
<td>0.25</td>
<td>4.0</td>
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<tr>
<td><em>Cryptococcus neoformans</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0</td>
<td>4.0</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2.0</td>
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<sup>a</sup> MIC value (μg/mL). IC₅₀ value (µg/mL).
against several microorganisms, while agelamides A (1) and B (2), nagelamides X (3), Y (4), U (7), and W (9) also showed moderate antimicrobial activities.

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Conflict of Interest The authors declare no conflict of interest.

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