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Ageladine A: An Antiangiogenic Matrixmetalloproteinase Inhibitor from the Marine Sponge *Agelas nakamurai*[1]

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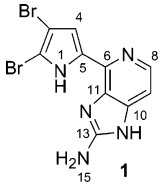
Matrix metalloproteinases (MMPs) regulate multiple steps of the angiogenic process which are essential for tumor growth and metastasis.[2] In the process of tumor metastasis, gelatinase A (MMP-2) is known to form a complex with MT1-MMP and TIMP2 on the migration front of the malignant tumor cells[3] and degrade type IV collagen which is a main component of the extracellular matrix (ECM). MMP-2 is also known to associate with integrin $\alpha_V\beta_3$ on the surface of endothelial cells in the process of angiogenesis.[4] Inhibitors against MMP-2 are therefore expected to be antimetastatic as well as antiangiogenic agents. In fact, a number of MMP-2 inhibitors are under clinical trials.[5]

In our search for MMPs inhibitors from Japanese marine invertebrates, we isolated tetramic acid glycosides,[6] phosphorylated steroids,[7] and sulfated alcohols.[8] Subsequently, we found that the hydrophilic extract of the marine sponge *Agelas nakamurai* inhibited MMP-2 significantly. Bioassay-guided isolation afforded a fluorescent alkaloid ageladine A (**1**) which was also found to be antiangiogenic. We report the isolation, structure elucidation, and antiangiogenic activity of the new alkaloid.



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The combined extracts of the frozen sponge was fractionated by solvent partitioning, ODS flash chromatography, gel filtration, and ODS HPLC. Repetitive reversed phase HPLC of active fractions afforded ageladine A (**1**) as yellowish powder (1.2×10^{-3} % yield, based on wet weight).

LR-FABMS of **1** showed the $(M + H)^+$ ion cluster at *m*/*z* 356/358/360 in a ratio of 1:2:1, indicating the presence of two bromine atoms. On the basis of HR-FABMS and ^{13}C NMR data of **1**, its molecular formula was established as $C_{10}H_7N_5Br_2$, indicating nine degrees of unsaturation.

The ^1H NMR spectrum of ageladine A (**1**) measured in CD_3OD exhibited only three signals at δ 7.17, 7.41, and 8.04, which were attached to the carbons at δ 115.1, 105.4, and 133.0, respectively. The molecular formula indicated that ageladine A bears four exchangeable protons, but the ^1H NMR spectra measured in CD_3OH, DMSO-d_6, C_5D_5N, and $CDCl_3$ with or without 1% TFA at −30, 27, and 50 °C revealed no exchangeable proton.

Interpretation of two-dimensional (2D) NMR data led to three partial units **a**−**c**. Unit **a** was assigned as 2-substituted 4,5-dibromopyrrole on the basis of the diagnostic NMR signals at δ_H 7.17; δ_C 125.7, 115.1, 107.7, and 102.3 (H-4, C-2, -4, -5, and -3, respectively) as well as HMBC cross-peaks δ_H 7.17/δ_C 125.7 and 107.7.[9] Unit **b** contained two aromatic protons at δ_H 8.04 and 7.41 (H-8 and H-9, respectively) which were mutually coupled by 6.3 Hz. The chemical shifts, coupling constant, and $^1J_{C-H}$ values of 192 Hz (H-8/C-8) and 176 Hz (H-9/C-9) were consistent with the 2,3,4-trisubstituted pyridine ring, which was supported by HMBC cross-peaks, δ_H 8.04/δ_C 147.1, 128.5, and 105.4 and δ_H 7.41/δ_C 136.7 and 133.0. The remaining unit **c** consisted of an isolated carbon at δ_C 160.8, three nitrogen atoms, and exchangeable protons. Considering the carbon chemical shift, they were presumed to be a guanidine (Figure 1).

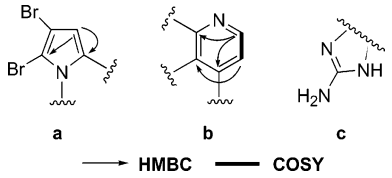

Figure 1. Partial structures of **1**.

Connection of these units could not be accomplished by further 2D experiments including INADEQUATE[10] and ^{15}N HMBC due to the low solubility. To circumvent this problem, *N*-methyl derivatives of **1** were prepared. Treatment of **1** with MeI in the presence of NaH[11] afforded three methylated derivatives **2**−**4** (Figure 2).

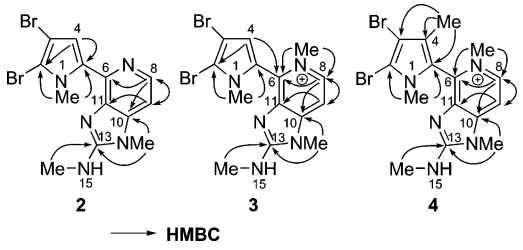

Figure 2. Key HMBC correlations of mathylated derivatives **2**−**4**.

Tri-*N*-methyl derivative **2** exhibited HMBC cross-peaks Me-1/C-2 and -5 and Me-14 and -15/C-13, indicating that 1-NH, 14-NH, and 15-NH_2 in **1** were methylated. An HMBC cross-peak Me-14/C-10 connected 14-N to C-10 and the remaining 12-N therefore must be attached to C-11 to form a 2-aminoimidazole ring.

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For tetra-*N*-methyl derivative **3**, an HMBC cross-peak was observed between H-4/C-6, which indicated connection between C-5 and C-6. Thus, the structure of ageladine A (**1**) was constructed as 4-(4,5-dibromo-1*H*-pyrrol-2-yl)]-1*H*-imidazo[4,5-*c*]pyridin-2-amine.

Although C-3 did not correlate with any protons in **1**−**3**, methyl protons at C-4 in penta-*N*-methyl derivative **4** showed HMBC correlation to C-3 and secured this assignment.

Ageladine A (**1**) inhibited not only MMP-2, but also MMPs-1, -8, -9, -12, and -13 with IC_{50} values of 2.0, 1.2, 0.39, 0.79, 0.33, and 0.47 μg/mL, respectively, while its *N*-methylated derivatives **2**−**4** did not inhibit MMP-2. Many potent MMP inhibitors are known to bind Zn^{2+} in the catalytic domain. Therefore, Zn^{2+} chelating ability of **1** was examined,[12] which disclosed that ageladine A was not capable to chelate Zn^{2+}.[13] Moreover, the kinetic analysis indicated that inhibition of MMP-2 by **1** was not competitive judging from the Lineweaver−Burk plot. Thus, the inhibition mechanism of **1** was presumed to be different from those of other MMP-2 inhibitors.

Ageladine A (**1**) showed 33.3% inhibition of cell migration using bovine aortic endothelial (BAE) cells at 5 μg/mL (IC_{10} for BAE cell proliferation) and 65.9% inhibition at 25 μg/mL (IC_{50}).

Furthermore, antiangiogenic effect of ageladine A was evaluated by the in vitro vascular organization model using mouse ES cells.[14] Administration of 10 μg/mL ageladine A (**1**) significantly inhibited vascular formation from aggregates of vascular progenitor cells in 3D culture using type-I collagen gel (Figure 3).

As BB-94, a potent MMPs inhibitor, showed similar effect at a concentration of 1 μg/mL (data not shown), **1** should exert antiangiogenic effect through the inhibition of both MMP activites and endothelial cell migration.

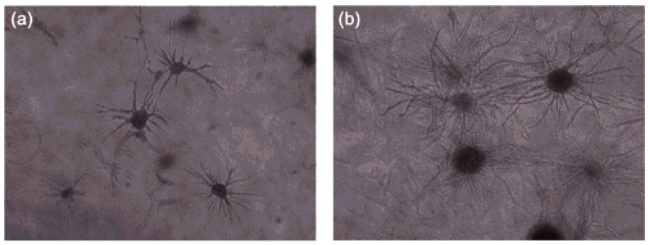

Figure 3. Antiangiogenic effect of ageladine A (**1**). Five-days culture of vascular progenitor cell aggregates in type-I collagen gel: (a) with 10 μg/mL of **1** and (b) without **1** (control).

On the basis of the finding that biosynthetic precursors of oroidin derivatives were revealed as proline (**6**), histidine (**7**), or ornithine,[15,16] biosynthesis of **1** may be proposed as follows; Schiff base **10** derived from aldehyde **8** and histamine **9** undergoes intramolecular cyclization and dehydrogenation to afford **1** (see Figure 4).

Marine sponges of the genus *Agelas* have been reported to contain more than 60 bioactive bromopyrroles.[17] Most of them are C_{11} or C_{22} oroidine derivatives with further modifications. Exceptions are latondulin A with C_{10} skeleton,[16] and 4,5-dibromopyrrole-2-carbonitrile which contains 2-carbonitrile.[18] Ageladine A (**1**) is the first example of this family to contain 2-aminoimidazolopyridine. With its fluorescent nature and interesting bioactivity, **1** is expected as a fluorescent bioprobe for studying angiogenesis.

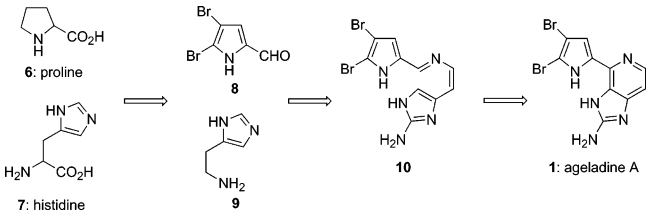

Figure 4. Proposed biogenesis of ageladine A (**1**).

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Supporting Information Available: Experimental details and spectral data for **1**, **2**, **3**, and **4**; spectral data of $ZnCl_2$ titration experiment for **1**; results of kinetics experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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