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Bioorganic & Medicinal Chemistry Letters 17 (2007) 4495–4499
Bioorganic & Medicinal Chemistry Letters

Synthesis and matrix metalloproteinase (MMP)-12 inhibitory activity of ageladine A and its analogs

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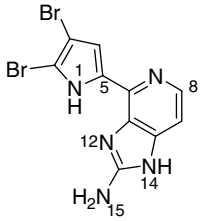
Received 22 March 2007; revised 25 May 2007; accepted 1 June 2007
Available online 6 June 2007

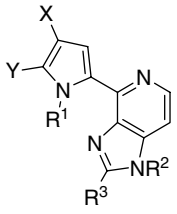
Abstract—Ageladine A (**1**) and its analogs **2**–**10** were expeditiously synthesized by featuring the biosynthetic route proposed for **1** (for **1**–**10**) and by employing 2-(*N*-*t*-butoxycarbonylamino)imidazol-4-carbaldehyde as the starting material (for **1**–**8**). From MMP-12 inhibitory activity assay, it appeared evident that the two bromine atoms and the three NH groups (1-NH, 14-NH, and 15-NH_2) were indispensable for **1** to exhibit excellent activity.

Ageladine A (**1**) is a pyrrol-2-aminoimidazole alkaloid isolated from the marine sponge *Agelas nakamurai* by Fusetani et al. (Fig. 1).[1] It has been reported that **1** inhibits various subtypes of matrix metalloproteinases (MMPs) such as MMP-1, 2, 8, 9, 12, and 13. Among these MMPs, MMP-12 has attracted our particular attention because it has been considered to be associated with inflammatory diseases caused by macrophages infiltration such as skin diseases,[2] atherosclerosis,[3] aneurysms,[4] and cancers.[5] Accordingly, we embarked on evaluating **1** as a new lead compound for novel MMP-12 inhibitors. Quite recently, two total syntheses of **1** have been reported by Weinreb et al.[6] and Karuso et al.[7] In this communication, we wish to describe an efficient convenient synthesis of **1** and its analogs and their MMP-12 inhibitory activity. The analogs **2**–**10** we planned to synthesize are shown in Figure 2. Compounds **2**–**4** are the debrominated analogs for evaluating the two bromine atoms of **1**. Compounds **5**–**9** are the N-methylated analogs and **10** is the deaminated analog for clarifying the effects of the three NH groups involved in **1**. We expected that these analogs might disclose the structural features of **1** required for its MMP-12 inhibitory activity, affording a novel analog that can show more excellent activity than **1**.



ageladine A (**1**)

Figure 1. Structure of ageladine A (**1**).



2: X = Br, Y = R^1 = R^2 = H, R^3 = NH_2
3: X = H, Y = Br, R^1 = R^2 = H, R^3 = NH_2
4: X = Y = R^1 = R^2 = H, R^3 = NH_2
5: X = Y = Br, R^1 = Me, R^2 = H, R^3 = NH_2
6: X = Y = Br, R^1 = R^2 = H, R^3 = NHMe
7: X = Y = Br, R^1 = H, R^2 = Me, R^3 = NH_2
8: X = Y = Br, R^1 = H, R^2 = Me, R^3 = NHMe
9: X = Y = Br, R^1 = R^2 = H, R^3 = NMe_2
10: X = Y = Br, R^1 = R^2 = R^3 = H

Figure 2. Structures of ageladine A analogs **2**–**10**.

Recently, we succeeded in developing a novel synthetic route to 2-aminoimidazol-4-carbaldehyde derivatives, the versatile synthetic intermediates for various structural types of 2-aminoimidazole alkaloids.[8] So, we planned to synthesize **1** and its analogs mainly employing 2-(*N*-*t*-butoxycarbonylamino)imidazol-4-carbaldehyde

Keywords: Ageladine A; Matrix metalloproteinase-12.
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doi:10.1016/j.bmcl.2007.06.005

(**11**) as the starting material. We first tried the total synthesis of **1** based on the biosynthetic route proposed by Fusetani et al. in which **1** may be biosynthesized from 4,5-dibromopyrrol-2-carbaldehyde and histamine by sequential imino formation, cyclization, and dehydrogenation (Fig. 3).[1] Just after we completed the total synthesis of **1** delineated below, we became aware that Karuso et al. succeeded in synthesizing **1** based on the proposed biosynthetic route similarly to us.[7]

As outlined in Scheme 1, nitroaldol condensation of **11** followed by reduction using $LiAlH_4$ gave 2-(*N*-*t*-butoxycarbonylamino)histamine (**12**). This was readily converted to the tetrahydroageladine A derivative **14a** by the Pictet–Spengler reaction[9] with 4,5-dibromo-1-(2-trimethylsilylethoxymethyl)pyrrol-2-carbaldehyde (**13a**) in good yield. Nicolaou et al. reported that dehydrogenation of tetrahydroisoquinoline to isoquinoline is cleanly effected using 2.5 equiv iodoxybenzoic acid (IBX) at 45 °C, and the use of 1.5 equiv IBX at 25 °C gave dihydroisoquinoline.[10] Therefore, we thought that the protected ageladine A **15a** can be readily produced by this method. Thus, the 8,9-dihydroageladin A derivative[11] was produced in good yield by treating **14a** with 1.5 equiv IBX. However, complete dehydrogenation of **14a** turned out to be fruitless by using 2.5 equiv IBX, resulting in the formation of a mixture of **15a** and the 8,9-dihydroageladine A derivative. Further addition of IBX was not effective for complete dehydrogenation and afforded the decreased yield. Therefore, we next examined a two-step dehydrogenation protocol, in which the additional dehydrogenation was attempted using another oxidant after obtaining the 8,9-dihydroageladine A derivative from **14a** using 1.5 equiv IBX. After experimentation, it was found that activated MnO_2 smoothly led the 8,9-dihydroageladine A derivative to **15a** in good yield. The yield of **15a** from **14a** for this reaction was 89% over two steps. On the other hand, when **14a** was directly oxidized using activated MnO_2, the yield of **15a** was 37%. This result obviously shows the efficacy of this two-step dehydrogenation. With **15a** in hand, we deprotected both the 2-trimethylsilylethoxymethyl and the *t*-butoxycarbonyl groups by using excess trifluoroboran–diethylether complex, giving rise to **1**. Ageladine A (**1**) thus obtained was isolated as its bistrifluoroacetate.[12,13]

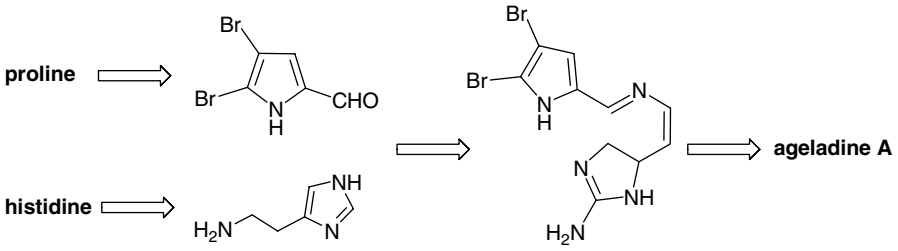
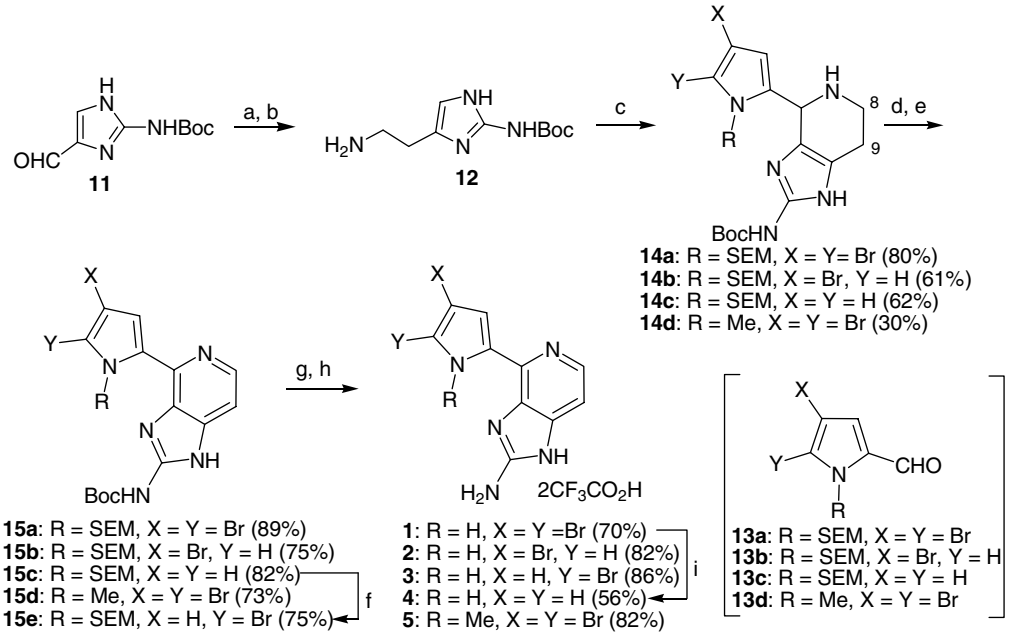


Figure 3. The biosynthetic route of ageladine A (**1**) proposed by Fusetani et al.

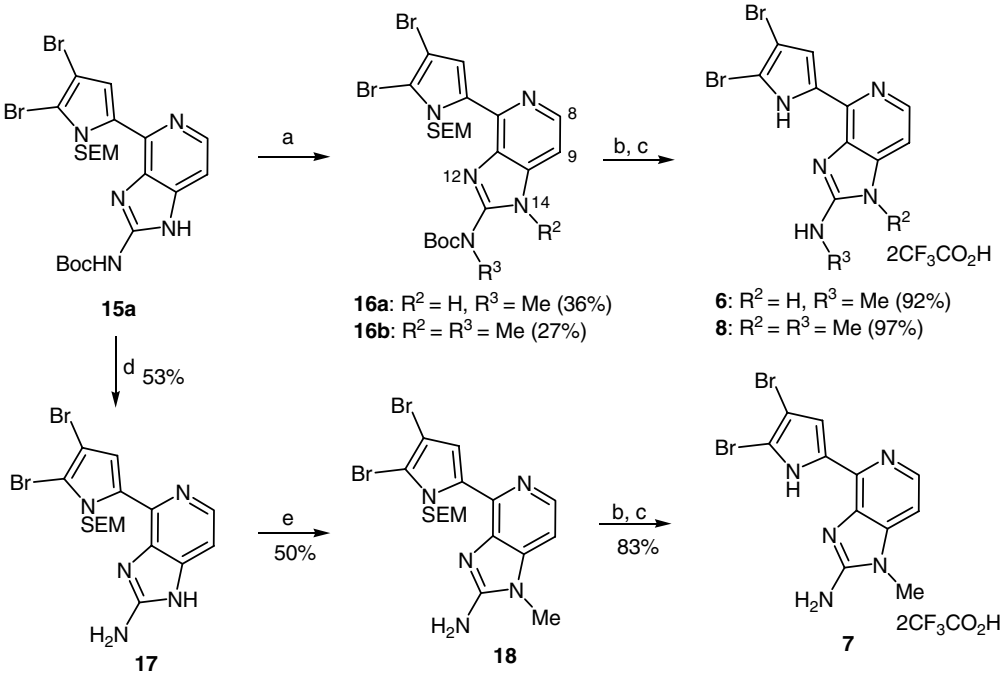


Scheme 1. Reagents and conditions: (a) $AcONH_4/MeNO_2$, reflux, 20 min, 94%; (b) $LiAlH_4$ (3 equiv)/THF, 50 °C, 1 h, 67%; (c) Compounds **13a–d**/EtOH, 50 °C, 4–6 h; (d) IBX (1.5 equiv)/DMSO, rt, 1.5–3 h; (e) MnO_2/CH_2CI_2, rt, 1–2.5 h; (f) tetra-*n*-butylammonium tribromide/MeOH, rt, 2 h; (g) BF_3–OEt_2 (10 equiv)/CH_2CI_2, rt, overnight; (h) TFA/MeOH, rt, 5 min, 70% (two steps); (i) 10% Pd–C, H_2(1 atm)/MeOH, rt, 18 h.

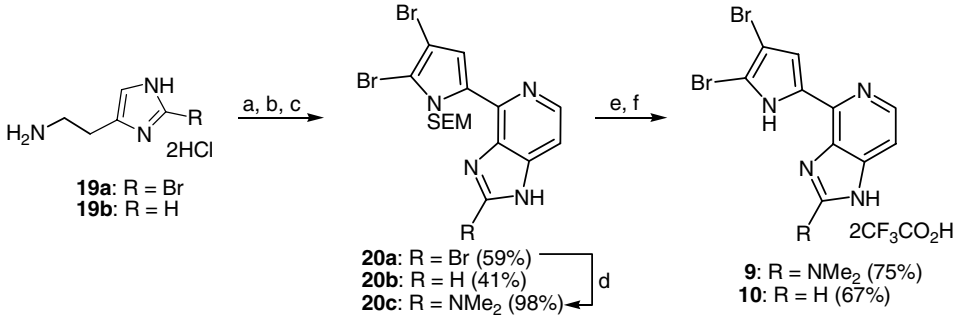
Next, we examined the synthesis of various analogs **2**–**5** of **1** by applying this synthetic route. Thus, **1** was debrominated by catalytic hydrogenation to yield **4**. Employing **13b** and **13d** in place of **13a**, **2** and **5** were prepared by the same method as that for **1**. Selective monobromination of intermediate **15c** synthesized from **12** and **13c** by way of **14c**, followed by removal of the protective groups, afforded **3** (Scheme 1).

The synthetic routes of analogs **6**–**8** are shown in Scheme 2. All these analogs were derived from the common intermediate **15a**. Methylation of **15a** using NaH and iodomethane produced **16a** and **16b** in 36% and 27% yield, respectively, after separation by column chromatography.[14] On the other hand, methylation after removal of the *t*-butoxycarbonyl group provided **18**. **16a**, **16b**, and **18** were converted to the corresponding target analogs **6**–**8** by the same deprotection method as that for the synthesis of **1**.

As depicted in Scheme 3, the synthesis of analogs **9** and **10** was completed in good yields by a similar method to that for **1**. Reaction of 2-bromohistamine (**19a**)[15] or histamine (**19b**) with aldehyde **13a** was carried out at higher temperature than that for the case of **1**. In addition, the second dehydrogenation using activated MnO_2 required longer reaction time. Transformation of the 2-bromine atom of **20a** into a dimethylamino group was readily performed by using excess dimethylamine at 100 °C in a sealed tube, giving rise to **20c**. Deprotection of **20a** and **20c** in the same manner as that for the preparation of **1** furnished **9** and **10**. Similarly to **1**, all the analogs **2**–**10** thus produced were isolated as their bistrifluoroacetates.



Scheme 2. Reagents and conditions: (a) Mel, NaH/DMF, rt, overnight; (b) BF_3–Et_2O (10 equiv)/CH_2CI_2, rt, overnight; (c) TFA/MeOH, rt, 5 min; (d) HCI-MeOH, rt, overnight; (e) Mel, NaH/DMF, rt, 2 h.



Scheme 3. Reagents and conditions: (a) Compound **13a**/EtOH, reflux, 5 h (for **20a**) or **13a**/$MeO(CH_2)_2OH$, reflux, 18 h (for **20b**); (b) IBX (1.5 equiv)/DMSO, rt, 2 h; (c) MnO_2/CH_2Cl_2, rt, 18 h; (d) 2 M Me_2NH in MeOH, 100 °C (sealed tube), 10 h; (e) BF_3–Et_2O (10 equiv)/CH_2Cl_2, rt, overnight; (f) TFA/MeOH, rt, 5 min.

Table 1. MMP-12 inhibitory activity of ageladine A (**1**) and its analogs **2**–**10**

Compound	MMP-12 inhibition IC_{50} (μM)
1	3.66
2	>100
3	>100
4	>100
5	>100
6	10.4
7	56.9
8	>100
9	>100
10	43.6

Ageladine A (**1**) and its analogs **2**–**10** were then subjected to MMP-12 inhibition assay.[16] The results are summarized in Table 1. It appeared that the debrominated analogs **2**–**4** did not inhibit MMP-12 even at a concentration of 100 μM. These results clearly disclosed that two bromine atoms in the pyrrole ring are indispensable for **1** to exhibit inhibitory activity. The lack of inhibitory activity of **5** bearing 1-*N*-methyl group might be explained by deformation of the conjugate system for **1**, caused by the bond rotation between the pyrrole and the 5-azabenzimidazole rings and/or by inhibition of the intramolecular hydrogen bond between the 1-NH and 12-N group. While 14- and 15-N-monomethylated analogs **6**, **7** were found to show inhibitory activity against MMP-12, which is obviously inferior to that of **1**, the 14,15- and 15,15-N,N-dimethylated analogs **8**, **9** exhibited no inhibitory activity even at a concentration of 100 μM. These results and the weak inhibitory activity observed for **10** clearly suggest that 14-NH and 15-NH_2 groups might play some role in the inhibitory activity of **1**, through intermolecular hydrogen and/or coordination bond in the catalytic domain of MMP-12 (ageladine A numbering). In summary, these results suggest that the two bromine atoms and the three NH groups (1-NH, 14-NH, and 15-NH_2) of **1** play important roles in its MMP-12 inhibitory activity.

In conclusion, we have succeeded in synthesizing ageladine A (**1**) and its analogs **2**–**10** by featuring the biosynthetic route proposed for **1** (for **1**–**10**) and by employing 2-(*N*-*t*-butoxycarbonylamino)imidazol-4-carbaldehyde as the starting material (for **1**–**8**). Contrary to our expectation, it appeared that, among these analogs, 14- or 15-N-monomethylated analogs **6**, **7** and 13-deaminated analog **10** (ageladine A numbering) only exhibited very weak MMP-12 inhibitory activity. However, these results clearly disclosed that the two bromine atoms and the three NH groups were indispensable for **1** to exhibit strong activity. Aiming at exploring novel congeners of **1** that can show more prominent inhibitory activity against MMP-12 is *in progress*.

Acknowledgments

We are grateful to Drs. T. Ishizaki and Y. Fukuda, Kyorin Pharmaceutical Co. Ltd, for their many valuable suggestions and encouragement. We would also like to thank Dr. Y. Kohno, Kyorin Pharmaceutical Co. Ltd, for his helpful suggestions and discussions. The MMP-12 inhibition assay was performed by Dr. Emme C. K. Lin, ActivX Biosciences, to whom our thanks are also due.

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11. This structure was assigned by its ^{1}H NMR spectra (^{1}H NMR (CD_3OD, 400 MHz) δ 0.02 (9H, s), 0.91–0.96 (2H, m), 1.51 (9H, s), 2.55–2.63 (2H, m), 2.94 (1H, dt, J = 5.0, 12.6 Hz), 3.00–3.07 (1H, m), 3.61–3.67 (2H, m), 5.12 (1H, s), 5.46 (2H, s), 5.89 (1H, s)) as well as by taking into account the result reported by Nicolaou et al.[10]
12. Yellow powder (lit.[1], yellow powder); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.17 (1H, s), 7.42 (1H, d, J = 6.4 Hz), 8.05 (1H, d, J = 6.4 Hz); ^{13}C NMR (CD_3OD, 400 MHz) δ 102.4, 105.5, 107.8, 115.2, 125.7, 128.6, 133.0, 136.7, 147.2, 160.9; LRMS (ESI^+): 356 $[M+H]^+$; HRMS (ESI^+): Calcd for $C_{10}H_8Br_2N_5$: 355.91465, found: 355.91340. These spectral properties were identical to those reported.
13. Although the total synthesis of **1** reported by Karuso et al. is almost the same as that independently developed by us, their reaction conditions for the Pictet–Spengler reaction using $Sc(OTf)_3$ (44% yield) and for the sequential dehydrogenation and deprotection using chloranil (65% yield) were completely different from those explored by us. Taking into account the chemical yield for each step, our synthetic route (the Pictet–Spengler reaction: 80% yield; the dehydrogenation: 89% yield for two steps; deprotection: 70% yield) to **1** is anticipated to be more efficient than that of Karuso et al.[7]
14. Specific N-methylations at the N-14 not at the N-12 position were verified by the ^{1}H NMR spectra of **16a**, **16b**, and **18** (**16a**: ^{1}H NMR (CD_3OD, 400 MHz) δ −0.25 (9H, s), 0.59 (2H, t, J = 8.1 Hz), 1.69 (9H, s), 3.17 (2H, t, J = 8.1 Hz), 3.57 (3H, s), 5.92 (2H, s), 7.07 (1H, s), 7.40 (1H, d, J = 5.2 Hz), 8.23 (1H, d, J = 5.2 Hz); **16b**: ^{1}H

NMR (CD_3OD, 400 MHz) δ −0.25 (9H, s), 0.61 (2H, t, J = 8.1 Hz), 1.47 (9H, s), 3.23 (2H, t, J = 8.1 Hz), 3.35 (3H, s), 3.71 (3H, s), 5.96 (2H, s), 7.11 (1H, s), 7.50 (1H, d, J = 5.8 Hz), 8.39 (1H, d, J = 5.8 Hz); **18**: ^{1}H NMR (CD_3OD, 400 MHz) δ −0.23 (9H, s), 0.64–0.68 (2H, m), 3.20–3.25 (2H, m), 3.59 (3H, s), 5.98 (2H, s), 6.06 (2H, br s), 6.99 (1H, d, J = 5.5 Hz), 7.03 (1H, s), 8.26 (1H, d, J = 5.5 Hz).). In **16b** and **18**, NOE was observed between the proton on the C-9 position and those of the N^{14}-methyl group.

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16. The MMP-12 inhibition assay was performed as per manufacturer's (BioMol) protocol.