Bioorganic & Medicinal Chemistry Letters 19 (2009) 5461–5463

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel ageladine A analogs showing more potent matrix metalloproteinase (MMP)-12 inhibitory activity than the natural product

Naoki Ando [a,*], Shiro Terashima [b]

[a] Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd, 2399-1, Nogi, Nogi-machi, Shimotsuga-gun, Tochigi 329-0114, Japan
[b] Sagami Chemical Research Center, Hayakawa 2743-1, Ayase, Kanagawa 252-1193, Japan

ARTICLE INFO

Article history:
Received 25 June 2009
Revised 16 July 2009
Accepted 18 July 2009
Available online 23 July 2009

Keywords:
Ageladine A
Matrix metalloproteinase (MMP)-12

ABSTRACT

By employing a previously established synthetic scheme, the synthesis described in the title was carried out in order to explore the substituent effects in the pyrrole ring of ageladine A on MMP-12 inhibitory activity. It became evident that a halogen atom (Br or Cl) at the 2-position and an additional bromine atom at the 4-position are highly effective for improving the inhibitory activity. These studies led us to discover three novel ageladine A analogs (**4a**, **c**, **o**) showing more potent MMP-12 inhibitory activity than the natural product.

In 2003, Fusetani et al. isolated ageladine A (**1**), one of the pyrrol-2-aminoimidazole alkaloids, from the marine sponge *Agelas nakamurai*.[1] It was also reported that **1** exhibits inhibitory activity against various subtypes of matrix metalloproteinases (MMPs) such as MMP-1, 2, 8, 9, 12 and 13.[1] Accordingly, we envisioned that **1** has potential as the new lead compound for our ongoing project aimed at exploring novel MMP-12 inhibitors. It has been anticipated that MMP-12 closely associates with inflammatory diseases caused by macrophage infiltration such as skin diseases,[2] atherosclerosis,[3] aneurysms[4] and cancers.[5]

Recently, we completed the total synthesis of **1** and reported the MMP-12 inhibitory activity of **1** and its analogs.[6] The latter analogs were prepared by featuring the synthetic route established for **1**.[6] Quite interestingly, it was disclosed that, as shown in Figure 1, two debromo-ageladine A analogs, **2** and **3**, showed no inhibitory activity against MMP-12. This result strongly suggested that a slight change of the substituent on the pyrrole ring affords great influence on the inhibitory activity. Therefore, we continued synthetic studies on ageladine A analogs to reveal more detailed substituent effects for the pyrrole ring and to find analogs showing improved inhibitory activity. These studies revealed novel aspects of the structure–activity relationships for ageladine A analogs, especially concerning substituents on the pyrrole ring, and led us to discover three novel types of ageladine A analogs **4a**, **c**, **o** exhibiting more potent MMP-12 inhibitory activity than **1**.

As shown in Figure 2, the general structures of the analogs bearing various substituents on their pyrrole rings were designed as the target molecules we planned to synthesize. We achieved the synthesis of these analogs using the synthetic scheme previously established.[6] Thus, as outlined in Scheme 1, the Pictet–Spengler reaction of 2-(*N*-*t*-butoxycarbonylamino)histamine (**5**)[7] with the pyrrole-2-aldehydes **6a**–**p** (vide infra) gave the corresponding tetrahydro-ageladine A derivatives **7**. Sequential two-step dehydrogenations of **7** using IBX and activated MnO_2 afforded the protected ageladine A derivatives **8**. Depending upon the structures of the

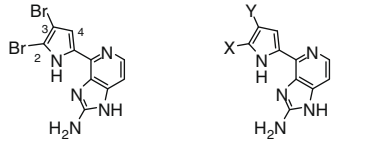


Figure 1. Structures of ageladine A (**1**) and its previously synthesized analogs **2**, **3**.

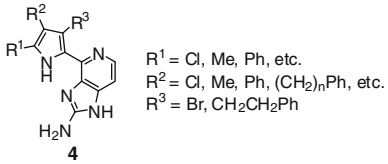
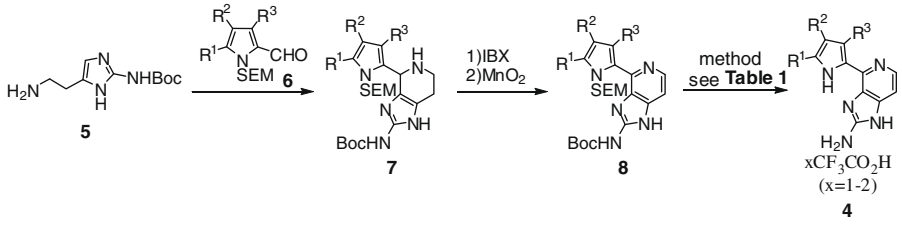


Figure 2. Structures of the novel ageladine A analogs **4**.

* Corresponding author.
E-mail address: naoki.andou@mb.kyorin-pharm.co.jp (N. Ando).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.099



Scheme 1.

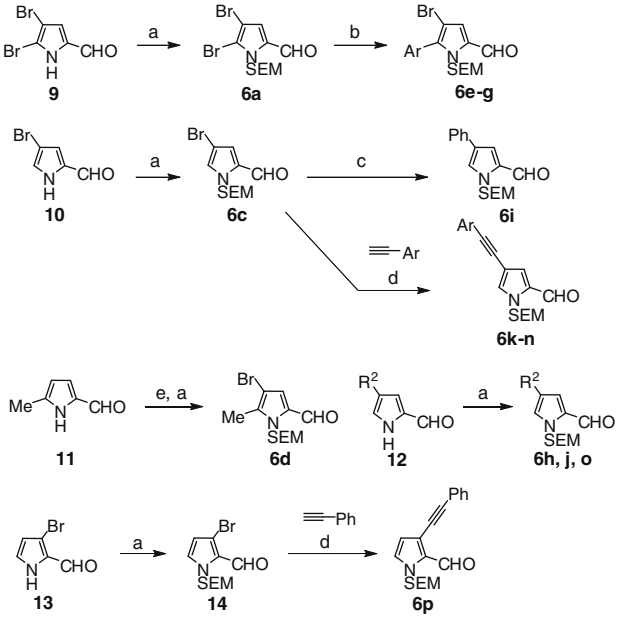
target molecules and the protective groups used, conversion of **8**–**4**[8] was attempted by employing various methods detailed in Table 1. Thus, analogs **4d**, **f**–**h** were prepared by deprotection of the corresponding derivatives **8d**, **f**–**h**. Analogs **4a**–**c**, **e**, **i**–**m**, **s**, **t**[8] were produced by chlorination or bromination of the corresponding derivatives **8a**–**c**, **e**, **i**–**m**, **s**, **t** followed by deprotection. Analogs **4n**–**r**, **u**[8] were prepared by hydrogenation of **8n**–**r**, **u** followed by sequential bromination and deprotection. All of the synthesized analogs **4a**–**u** were isolated as their trifluoroacetate(s).[6] On the other hand, aldehyde derivatives **6a**–**p** used for the Pictet–Spengler reaction (vide supra) were synthesized by the methods shown in Scheme 2. Thus, protection of **9**,[9] **10**,[10] **12**[11] and **13**[12] with a 2-(trimethylsilyl)ethoxymethyl group provided **6a**, **c**, **h**, **j**, **o** and **14**, respectively. Aldehyde **6a** was further converted to **6e**–**g** by a regioselective Suzuki–Miyaura cross-coupling reaction carried out following the procedure reported by Handy et al.[13] Aldehyde **6i** was prepared by Suzuki–Miyaura cross-coupling reaction of **6c**. The Sonogashira cross-coupling reaction of **6c** and **14** gave **6k**–**n**, **p**, respectively. The synthesis of **6d** was performed by bromination of **11**[14] followed by protection.

The 21 structurally discrete ageladine A analogs **4a**–**u** thus obtained were subjected to MMP-12 inhibition assay.[15] The results are summarized in Table 2. As for the 2-position of the pyrrole ring, replacement of the bromine atom with a chlorine atom obviously increased the inhibitory activity (see **4c**). However, introduction of other groups such as methyl, phenyl and biphenyl groups clearly decreased the activity (see **4d**, **f**–**h**). From these results, it appeared that the bromine or chlorine atom at the 2-position plays an important role for MMP-12 inhibitory activity. On the other hand, the 3-position of the pyrrole ring was found to show slightly

Table 1
Synthetic methods and results for the novel ageladine A analogs **4a**–**u**

Run	Aldehyde **6**				Method[a]	Compound **4**			
	No.	R^1	R^2	R^3		R^1	R^2	R^3	No. (yield)[d]
1	**6a**	Br	Br	H	A–D	Br	Br	Br	**4a** (43%)
2	**6b**	H	H	H	B[b]–D	Cl	Cl	H	**4b** (14%)
3	**6c**	H	Br	H	B–D	Cl	Br	H	**4c** (10%)
4	**6d**	Me	Br	H	D	Me	Br	H	**4d** (29%)
5	**6d**	Me	Br	H	A–E	Me	Br	Br	**4e** (27%)
6	**6e**	Ph	Br	H	D	Ph	Br	H	**4f** (34%)
7	**6f**	3-Ph–Ph	Br	H	D	3-Ph–Ph	Br	H	**4g** (39%)
8	**6g**	4-Ph–Ph	Br	H	D	4-Ph–Ph	Br	H	**4h** (37%)
9	**6h**	H	Me	H	A–E	Br	Me	H	**4i** (26%)
10	**6h**	H	Me	H	A–A–E	Br	Me	Br	**4j** (10%)
11	**6i**	H	Ph	H	A–D	Br	Ph	H	**4k** (13%)
12	**6j**	H	$PhCH_2$	H	A–D	Br	$PhCH_2$	H	**4l** (41%)
13	**6j**	H	$PhCH_2$	H	A–A–D	Br	$PhCH_2$	Br	**4m** (40%)
14	**6k**	H	Ph–≡	H	C–A–E	Br	$Ph(CH_2)_2$	H	**4n** (37%)
15	**6k**	H	Ph–≡	H	C–A–A–E	Br	$Ph(CH_2)_2$	Br	**4o** (25%)
16	**6l**	H	*o*-CF_3-Ph–≡	H	C–A[c]–D	Br	*o*-CF_3–$Ph(CH_2)_2$	Br	**4p** (47%)
17	**6m**	H	*m*-CF_3-Ph–≡	H	C–A[c]–D	Br	*m*-CF_3–$Ph(CH_2)_2$	Br	**4q** (29%)
18	**6n**	H	*p*-CF_3-Ph–≡	H	C–A[c]–D	Br	*p*-CF_3–$Ph(CH_2)_2$	Br	**4r** (12%)
19	**6o**	H	$Ph(CH_2)_3$	H	A–E	Br	$Ph(CH_2)_3$	H	**4s** (47%)
20	**6o**	H	$Ph(CH_2)_3$	H	A–A–E	Br	$Ph(CH_2)_3$	Br	**4t** (37%)
21	**6p**	H	H	Ph–≡	C–A[c]–F	Br	Br	$Ph(CH_2)_2$	**4u** (11%)

[a] Method A: tetra-*n*-butylammonium tribromide (1 equiv), method B: NCS (1 equiv), method C: H_2, Pd/C, method D: (1) BF_3OEt_2, (2) Na_2CO_3aq, (3) TFA, method E: (1) TFA, (2) Na_2CO_3aq, (3) TFA, method F: (1) NaOHaq, (2) TFA.
[b] NCS (2 equiv).
[c] Tetra-*n*-butylammonium tribromide (2 equiv).
[d] Isolated yield based on **5**.



Scheme 2. Reagents and conditions: (a) SEMCl, *t*BuOK/DMF, 0 °C, 1 h; (b) $ArB(OH)_2$, $Pd(OAc)_2$, K_2CO_3/DMF, 100 °C, 7–8 h; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3/DMF, 100 °C, 8 h; (d) $PdCl_2(PPh_3)_2$, CuI, TEA/DMF, 100 °C, 5 h; (e) NBS/THF.

Table 2
MMP-12 inhibitory activity of ageladine A (**1**) and its analogs **2**, **3** and **4a**–**u**

Compound	MMP-12 IC_{50} (μM)	Compound	MMP-12 IC_{50} (μM)
1	3.66	**4j**	5.02
2	>100	**4k**	18.7
3	>100	**4l**	>100
4a	1.24	**4m**	>100
4b	5.02	**4n**	10.2
4c	2.02	**4o**	2.99
4d	>100	**4p**	>100
4e	>100	**4q**	>100
4f	>100	**4r**	>100
4g	>100	**4s**	>100
4h	>100	**4t**	>100
4i	>100	**4u**	>100

different substituent effects from the 2-position. The bromine atom was most promising (see **4b**, **c**), but the phenyl and phenethyl analog also showed weak inhibitory activity (see **4k**, **n**). The most interesting effects were obtained by introducing a substituent into the 4-position of the pyrrole ring. It became evident that introducing a bromine atom into the 4-position significantly increased the inhibitory activity (see **4a**, **j**, **o**). Especially, 4-bromo-ageladine A, **4a**, showed the most potent activity among all the synthesized analogs, and its activity was ca. three times as much as that of natural ageladine A (**1**). On the other hand, the 4-phenetyl analog, **4u**, was found to show no inhibitory activity.

In conclusion, we have succeeded in synthesizing 21 structurally discrete ageladine A analogs in order to explore the substituent effects in the pyrrole ring on MMP-12 inhibitory activity. From the inhibitory activity assay, it became evident that three analogs, **4a**, **c**, **o**, show more potent activity than natural ageladine A (**1**) and that the activity of the most potent, **4a**, is ca. three times as great as that of **1**. Based on the studies of the structure–activity relationships, it was also disclosed that a halogen atom such as bromine or chlorine atom was indispensable at the 2-position and that introduction of a bromine atom into the 4-position was highly promising for improving MMP-12 inhibitory activity. Taking into account these results, further studies aimed at discovering even more potent ageladine A analogs are in progress.

Acknowledgements

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.

References and notes

1. Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; Yamashita, J.; van Soest, R. W. M.; Fusetani, N. *J. Am. Chem. Soc.* **2003**, *125*, 15700.
2. (a) Vaalamo, M.; Kariniemi, A. L.; Shapiro, S. D.; Saarialho-Kere, U. *J. Invest. Dermatol.* **1999**, *112*, 499; (b) Saarialho-Kere, U.; Kerkela, E.; Jeskanen, L.; Hasan, T.; Pierce, R.; Starcher, B. *J. Invest. Dermatol.* **1999**, *113*, 664; (c) Suomela, S.; Kariniemi, A. L.; Snellman, E.; Saarialho-Kere, U. *Exp. Dermatol.* **2001**, *10*, 175.
3. Matsumoto, S.; Kobayashi, T.; Katoh, M.; Saito, S.; Ikeda, Y.; Kobori, M. *Am. J. Pathol.* **1998**, *153*, 109.
4. Curci, J. A.; Liao, S.; Huffman, M. D.; Shapiro, S. D.; Thompson, R. W. *J. Clin. Invest.* **1998**, *102*, 1900.
5. (a) Cornelius, L. A.; Nehring, L. C.; Harding, E.; Bolanowski, M.; Welgus, H. G.; Kobayashi, D. *J. Immunol.* **1998**, *161*, 6845; (b) Kerkela, E.; Bohling, T.; Herva, R.; Uria, J. A.; Saarialho-Kere, U. *Bone* **2001**, *29*, 487; (c) Kerkela, E.; Ala-Aho, R.; Jeskanen, L.; Rechardt, O.; Grenman, R.; Shapiro, S. D. *J. Invest. Dermatol.* **2000**, *114*, 1113.
6. Ando, N.; Terashima, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4495.
7. Ando, N.; Terashima, S. *Synlett* **2006**, 2836.
8. **4a**/CF_3CO_2H: pale yellow powder; mp 185 °C (decomp.); IR (KBr) 3333, 3179, 1681, 1661, 1636, 1433, 1205, 1184, 1115, 720 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.53 (1H, d, J = 6.4 Hz), 8.24 (1H, d, J = 6.4 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 103.0, 104.3, 106.7, 108.5, 116.8, 119.7, 122.4, 126.4, 135.4, 162.3; LRMS (ESI^+) 434 $[M+H]^+$; HRMS (ESI^+) Calcd for $C_{10}H_7Br_3N_5$ 433.82516, found: 433.82799; Anal. Calcd for $C_{10}H_6Br_3N_5$, $C_2HF_3O_2$: C, 26.21; H, 1.28; N, 12.74. Found: C, 25.99; H, 1.24; N, 12.56. **4c**/$2CF_3CO_2H$: pale yellow powder; Mp 130 °C (decomp.); IR (KBr) 3147, 2923, 1719, 1664, 1474, 1438, 1202, 1135, 794, 724 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.16 (1H, s), 7.42 (1H, d, J = 6.4 Hz), 8.06 (1H, d, J = 6.4 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 98.3, 105.3, 114.8, 121.0, 123.5, 128.6, 133.0, 136.6, 146.9, 160.7; LRMS (ESI^+) 312 $[M+H]^+$; HRMS (ESI^+) Calcd for $C_{10}H_8BrClN_5$ 311.96516, found: 311.96500; Anal. Calcd for $C_{10}H_7BrClN_5$, $2C_2HF_3O_2$: C, 31.10; H, 1.68; N, 12.95. Found: C, 31.15; H, 1.65; N, 13.31. **4o**/$1.5CF_3CO_2H$: pale yellow powder; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.26–2.72 (2H, m), 2.76–2.81 (2H, m), 7.18–7.32 (5H, m), 7.50 (1H, d, J = 6.1 Hz), 7.75 (2H, br s), 8.26 (1H, d, J = 6.1 Hz), 12.73 (1H, br s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 27.7, 35.3, 100.0, 102.4, 112.6, 115.6, 118.6, 121.5, 121.9, 122.2, 126.1, 128.28, 128.34, 128.36, 128.41, 140.6; LRMS (ESI^+) 460 $[M+H]^+$; HRMS (ESI^+) Calcd for $C_{18}H_{16}Br_2N_5$ 459.97725, found: 459.97738; Anal. Calcd for $C_{18}H_{15}Br_2N_5$, $1.5C_2HF_3O_2$: C, 39.90; H, 2.63; N, 11.08. Found: C, 39.62; H, 2.65; N, 11.34.
9. Handy, S. T.; Sabatini, J. J.; Zhang, Y.; Vulfova, I. *Tetrahedron Lett.* **2004**, *45*, 5057.
10. Laha, J. K.; Muthiah, C.; Taguchi, M.; McDowell, B. E.; Ptaszek, M.; Lindsey, J. S. *J. Org. Chem.* **2006**, *71*, 4092.
11. Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 3215.
12. Ghosez, L.; Franc, C.; Denonne, F.; Cuisinier, C.; Touillaux, R. *Can. J. Chem.* **2001**, *79*, 1827.
13. Handy, S. T.; Sabatini, J. J. *Org. Lett.* **2006**, *8*, 1537.
14. Muchowski, J. M.; Hess, P. *Tetrahedron Lett.* **1988**, *29*, 777.
15. The MMP-12 inhibition assay was performed as per manufacturer's (BioMol) protocol. Human MMP-12 catalytic domain (residues 84-255) obtained from Biomol (Plymouth Meeting, PA) was diluted in Assay buffer (50 mM Tris pH7.5, 0.05% Brij-35, 10 mM $CaCl_2$, 1 mM DTNB) to a concentration of 0.007 U/μL. The MMP-12 solution (44 μL) was premixed with 1 μL of inhibitors dissolved in DMSO in a 384-well plate, and the mixture was incubated for 20 min at RT. Then, 5 μL of 1 mM MMP chromogenic substrate (thiopeptolide) obtained from Biomol was added to the mixture and the reaction mixture was incubated for 30–80 min at 37 °C. Reaction was terminated by adding 7 μL of 0.2 M EDTA (pH 8.0). The intensity of the color developed by the digested substrate was measured at 405 nm.