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Synthesis and anticancer activities of ageladine A and structural analogs

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ABSTRACT

A series of ageladine A analogs that include 2-aminoimidazo[4,5-*c*]azepines (seven-membered rings) and 2-amino-3*H*-imidazo[4,5-*c*]pyridine (six-membered rings) derivatives were synthesized and evaluated for their anticancer effects against several human cancer cell lines and MMP-2 inhibition in vitro. Only compounds possessing the aromatic azepine (seven-membered ring) core showed anticancer activity with IC_{50} values in the low micromolar range.

Ageladine A (**1a**) is a unique imidazole–pyrrole-based marine natural product which was isolated in 2003 from the sponge *Agelas nakamurai* (Fig. 1).[1] Bioactivity investigations determined that **1a** is an inhibitor of matrix metalloproteinases (MMPs). Its use as a potential pH sensitive membrane permeable dye has also been described.[2] Several syntheses of ageladine A and related analogs have been published in recent years.[3–8] In an effort to discover new anticancer lead structures for drug development, a collection of agelidine A-related compounds was synthesized and tested against different human cancer cell lines. Herein, we report the results of this study and a novel class of anticancer fused seven-membered ring 2-aminoimidazole azepine heterocycles **2**.

In 1994, we disclosed that 2-aminoimidazoles such as **3** undergo a facile Pictet–Spengler reaction with aldehydes to yield 2-aminoimidazoazepines **4** in excellent yields (Scheme 1).[9] This key reaction enables the construction of the bicyclic core of ageladine A and was independently adapted by Karuso in 2004 for the synthesis of the natural product.[8] Due to the important biological activities reported for ageladine A, we recently decided to pursue this reaction in greater depth and extend the methodology for the preparation and biological evaluation of ageladine A analogs.

Of the 12 initial analogs prepared, eight of them are 3*H*-imidazo[4,5-*c*]pyridin-2-amine (six-membered ring) analogs that include the natural product, ageladine A (**1a**) and four are imidazo[4,5-*c*]azepin-2-amine (seven-membered ring) derivatives comprising **2** (Fig. 2).

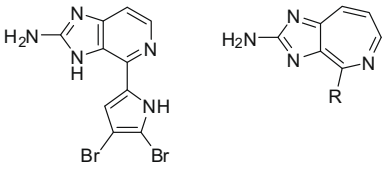
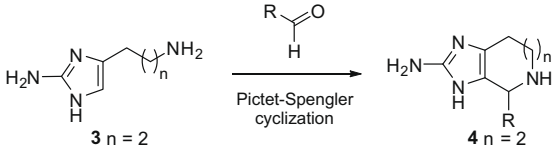


Figure 1. Structure of ageladine A and compound **2**.



Scheme 1. Pictet–Spengler cyclization of 2-aminoimidazoles.

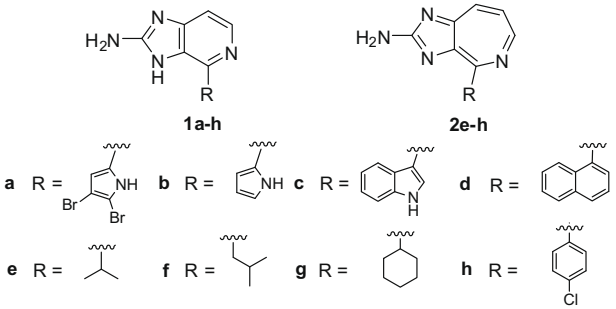


Figure 2. Ageladine A and analogs.

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These analogs were prepared using Pictet–Spengler methodology followed by oxidation to the requisite aromatic system.

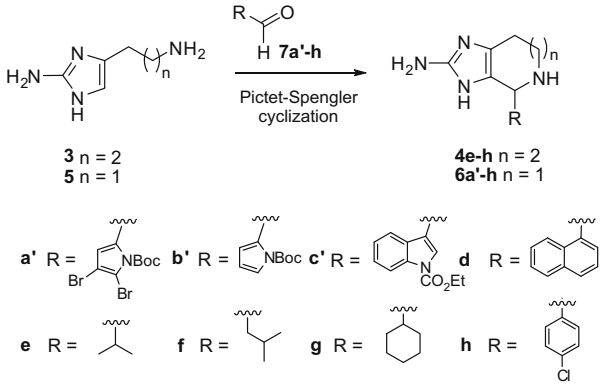
Starting from readily available 2-amino-4-(3-aminopropyl)-1*H*-imidazole (**3**) and 2-aminohistamine (**5**),[10,11] the corresponding Pictet–Spengler cyclization products **4**[12] and **6** were produced in good yields from reaction with aldehyde substrates **7** (Scheme 2).

With tetrahydro ring products **4** and **6** in hand, oxidation to the corresponding aromatic system was investigated. This was not as straightforward as anticipated. While Karuso describes the use of chloranil for the synthesis of ageladine A, the use of this oxidant could not be generally applied to the azepine series. For instance, attempts to use chloranil in oxidizing pyrrole–azepines **4**, no aromatic products corresponding to **2** could be isolated. After experimenting with several different reagents and reaction conditions, moderate success was achieved from a two-step procedure utilizing elemental bromine in methanesulfonic acid followed by treatment with potassium *tert*-butoxide in air (Scheme 3).

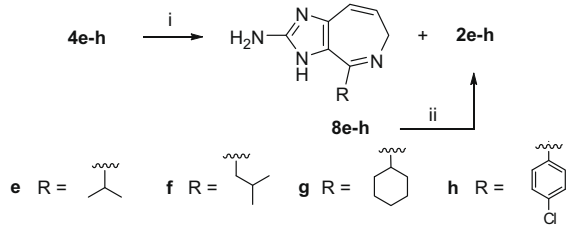
Initial oxidation with bromine at elevated temperatures (110 °C, sealed tube) produced a mixture of products which primarily consisted of dehydro intermediate **8**.[13] This intermediate can be isolated and characterized. Only small amounts of the desired aromatic system **2**[14] were obtained under these conditions. Upon exposure of **8** to potassium *tert*-butoxide in air, modest but sufficient amounts of **2** were produced and used for bioactivity studies.

The preparation of ageladine A (**1a**) and analogs **1b–d** was achieved by treatment of precursor **6** with chloranil which afforded the desired aromatic derivatives after deprotection with either trifluoroacetic acid or potassium carbonate (Scheme 4).

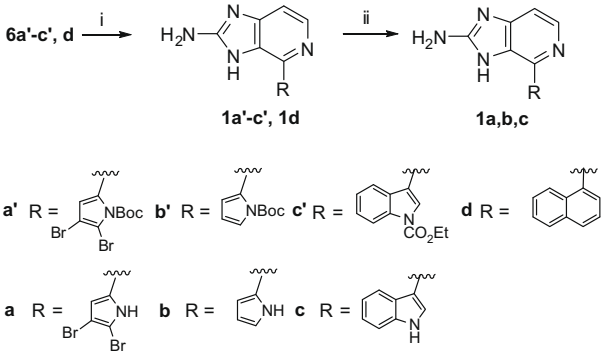
Finally, oxidation of **6e–f** was best achieved using bromine in methanesulfonic acid to afford the desired ageladine A analogs **1e–f** (Scheme 5).



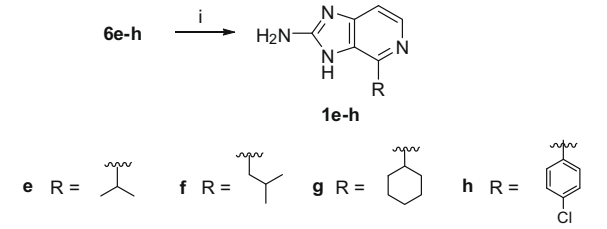
Scheme 2. Reagents and conditions: **3**·2HCl, **7e–h**, H_2O/EtOH, Na_2CO_3, 1 d, 23 °C: products **4e** (86%), **4f** (70%), **4g** (85%), and **4h** (60%). Compounds **5**, **7a′** or **7b′**, $Sc(OTf)_3$, EtOH, 23 °C, 1 d: products **6a′** (21%) or **6b′** (22%); **5**, **7c′–h**, EtOH, 23 °C, 1 d: products **6c′** (32%), **6d** (40%), **6e** (63%), **6f** (51%), **6g** (36%), **6h** (38%).



Scheme 3. Reagents and conditions: (i) Br_2 (2 equiv), $MeSO_3H$, 110 °C, 16 h: products **8e** (25%) and **2e** (11%), **8f** (30%) and **2f** (8%), **8g** (33%) and **2g** (6%), **8h** (41%) and **2h** (5%); (ii) *t*-BuOK (1 equiv), THF, 16 h: products **2e** (26%), **2f** (47%), **2g** (37%), and **2h** (25%).



Scheme 4. Reagents and conditions: (i) chloranil (3 equiv), $CHCl_3$, 80 °C, 16 h, **1′** (50%), **1b′** (10%), **1c′** (15%), **1d** (13%); (ii) TFA/CH_2Cl_2, **1a** (95%), **1b** (95%) or K_2CO_3, MeOH, **1c** (80%).



Scheme 5. Reagents and conditions: (i) Br_2 (2 equiv), $MeSO_3H$, 110 °C, 16 h, **1e** (32%), **1f** (30%), **1g** (31%), **1h** (30%).

Next, biological assays were performed to determine the effects of these analogs on cancer cell viability and MMP-2 activity. All derivatives were screened for anticancer activities against human

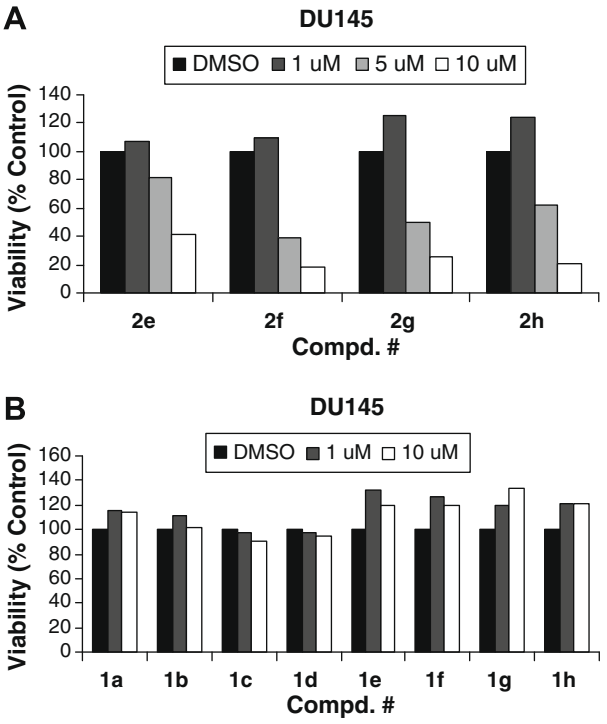


Figure 3. Seven-membered ring aromatic analogs inhibit viabilities of DU145 cells (A) but not six-membered analogs (B). Cells were treated with compounds at various concentrations for 48 h. Cell viability was determined by an MTS assay using manufacturer's (Promega, Madison, WI) protocol. Values are mean values of three determinations with <10% deviation from the mean value.

DU145 prostate cancer cells (Fig. 3). Aromatic azepines **2e–h** dispalyed anticancer activities in a dose-dependent manner. Moreover, the aromatic 2-aminoimidazo[4,5-*c*]azepine core plays an important role in the anticancer activity since neither the non-aromatic seven-membered azepine derivatives (data not shown) nor the six-membered pyridine (ageladine A) series showed activities.

Analogs of **1** and **2** were further tested with A2058 melanoma and MDA-MB-435 breast cancer cell lines. Similarly, the seven-membered ring aromatic compounds inhibited cell viabilities as shown in Table 1.

Table 1
Inhibition of cell viability by compounds **2e–h**[a]

Compds	IC_{50} (μM) DU145	IC_{50} (μM) A2058	IC_{50} (μM) MDA-MB-435
2e	7.5	9.5	6.7
2f	4.3	4.1	2.9
2g	5	4.5	3.4
2h	6.2	5.1	3.6

[a] IC_{50} values were determined with an MTS assay after 48 h treatment. Values are mean values of three determinations with <10% deviation from the mean value.

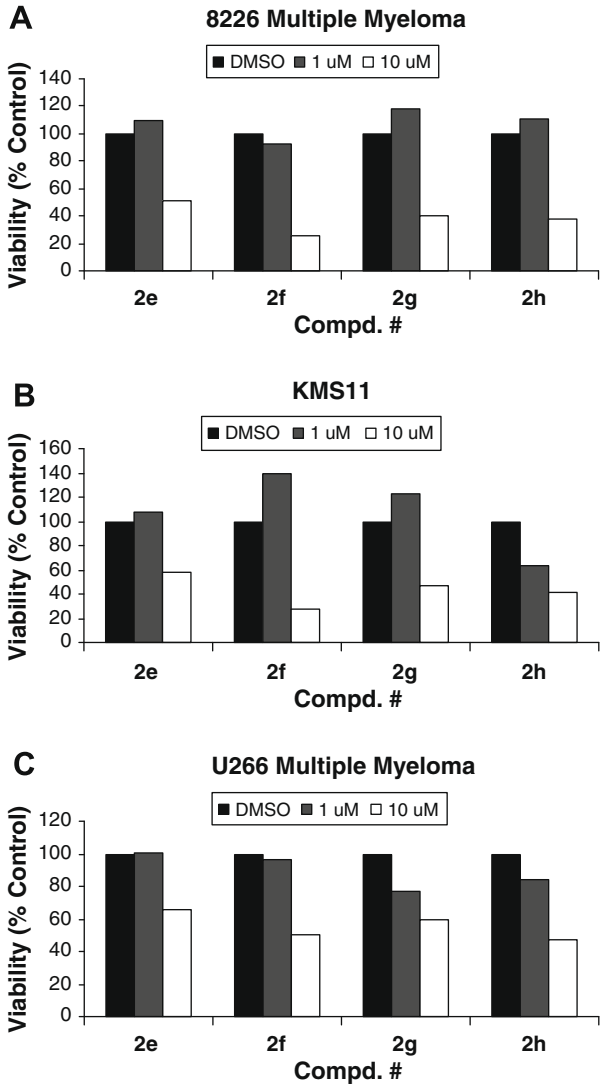


Figure 4. Compounds **2e–h** inhibit viabilities of multiple myeloma cells. Cells were treated with compounds at 1 μM or 10 μM for 48 h. Cell viability was determined by an MTS assay. Values are mean values of three determinations with <10% deviation from the mean value.

Table 2
MMP-2 inhibitory activity of compounds **1a–h** and **2e–f**[a]

Compds	% Inhibition @ 5 μM	% Inhibition @ 50 μM
1b	N/A[b]	N/A[b]
1c	25	26
1d	20	N/A[b]
1e	25	17
1f	31	22
1g	9	40
1h	N/A[b]	N/A[b]
2e	28	52
2f	27	60
2g	23	53
2h	27	54

[a] MMP-2 inhibition assay was performed using manufacturer's (Enzo Life Sciences AK-409) protocol. Values are mean values of two determinations with <10% deviation from the mean value.
[b] Strong fluorescence of the compound interfered with the assay measurement.

In addition, compounds **2e–h** substantially reduced viabilities of human multiple myeloma cells, including 8226, KMS11 and U266 (Fig. 4).

Previous studies have reported that ageladine A (**1a**) inhibits MMP-2 activities, which are involved in tumor angiogenesis. Consistently, we observed potent inhibition of MMP-2 activity by ageladine A (**1a**) (IC_{50} = 1.7 ± 0.2 μM) among all derivatives (**1a–h** and **2e–h**). All other compounds showed only weak inhibition of MMP-2 activities at higher concentrations (Table 2).

In conclusion, six- and seven-membered ring ageladine A analogs have been synthesized via a two-step process that centers on a Pictet–Spengler cyclocondensation followed by oxidative aromatization. The choice of oxidant and reaction conditions is highly dependent on the desired ring system. While these two classes of heterocycles have structural similarities, the bioactivities of the two systems are dramatically different. The seven-membered analogs **2e–h** represent a new pharmacophore that possesses in vitro anticancer activity. This is contrast to the six-membered pyridine series found in ageladine A. Further studies expanding the medicinal chemistry of these derivatives along with investigations directed at the biological mechanism of action are under investigation.

Acknowledgments

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12. *Compound* **4e**·2HCl: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12 .31 (d, J = 17.0 Hz, 2H), 9.92 (br, 1H), 9.33 (br, 1H), 7.49 (s, 2H), 4.04 (d, J = 9.4 Hz, 1H), 3.38 (br, 2H), 2.67 (br, 2H), 2.26 (m, 1H), 1.92 (br, 2H), 1.02 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 146.8, 126.1, 124.6, 60.8, 44.4, 29.4, 26.3, 23.3, 19.1, 18.1.
13. *Compound* **8e**: ^{1}H NMR (CD_3OD, 400 MHz) δ 6.73 (d, J = 9.4 Hz, 1H), 5.66 (m, 1H), 3.62 (d, J = 6.7 Hz, 2H), 3.21 (m, 1H), 1.12 (d, J = 6.8 Hz, 6H), ^{13}C NMR (CD_3OD, 100 MHz) δ 167.2, 155.7, 143.6, 130.3, 124.3, 119.4, 47.5, 33.3, 19.8.
14. *Compound* **2e**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.79 (d, J = 6.8 Hz, 1H), 8.02 (d, J = 10.0 Hz, 1H), 7.39 (dd, J = 10.0 Hz, J = 6.8 Hz, 1H), 7.29 (s, 2H), 4.24 (m, 1H), 1.34 (d, J = 6.8 Hz, 6H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0, 164.0, 163.8, 156.9, 149.8, 130.3, 127.3, 34.4, 22.2, HRMS(ESI) m/z calcd for $C_{10}H_{13}N_4$ (M+H): 189.1140; found: 189.1141.