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Pseudoceratinazole A: a novel bromotyrosine alkaloid from the Australian sponge *Pseudoceratina* sp.

Yunjiang Feng, Rohan A. Davis, Melissa L. Sykes, Vicky M. Avery, David Camp, Ronald J. Quinn *

Eskitis Institute, Griffith University, Brisbane QLD 4111, Australia

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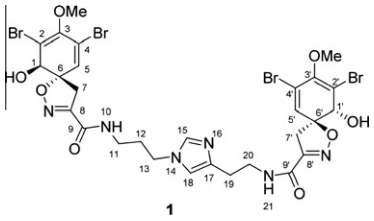
ABSTRACT

Mass-directed fractionation based on a *Trypanosoma brucei brucei* active fraction from the Australian sponge *Pseudoceratina* sp. led to the isolation of a novel bromotyrosine alkaloid, pseudoceratinazole A (**1**). Compound **1** is the first dimeric bromotyrosine alkaloid containing an imidazole-bridging moiety.

Marine sponges belonging to the order Verongida have proven to be a remarkable source of chemically diverse bromotyrosine derivatives.[1] The diversity of this structural class arises from the degree of bromination of the tyrosine moiety, as well as the subsequent oxidation, reduction, decyclization and rearrangement.[1] Many of the metabolites display a variety of activities, including cytotoxicity,[2,3] antimicrobial activity[4] and epidermal growth factor (EGF) receptor kinase inhibition.[5] Spirocyclohexadiene isoxazoline-based natural products have been frequently isolated from *Pseudoceratina* species of the Pseudoceratiniidae family,[6,5] and this unique structural feature could be considered as a marker for chemotaxonomic identification.[7]

In our ongoing search for new lead compounds for neglected diseases,[8–12] a drug discovery programme was initiated to identify the novel trypanocidal compounds. A 384-well fluorescence-based trypanosomal high throughput screening (HTS) assay[13] was developed against *Trypanosoma brucei brucei*, and used to screen our prefractionated natural product library of 202,983 fractions. *T. b. brucei* has been routinely used in screening for initial identification of antitrypanosomal leads.[14] The library was constructed by fractionation of over 18,000 marine and terrestrial extracts enhanced for lead- and drug-likeness with 11 fractions collected per sample. From the eleven fractions derived from an extract of the Australian sponge *Pseudoceratina* sp., a single active fraction was identified with activity against *T. b. brucei*. (+)-LRESIMS of the active fraction showed a cluster of five isotopic ions at *m/z* 894, 896, 898, 900 and 902 (1:4:6:4:1) that were predicted to correspond to the bioactive natural product(s). Mass-directed fractionation of the crude extract led to the isolation of a novel bromotyrosine alkaloid, pseudoceratinazole A (**1**).



1

The Australian sponge *Pseudoceratina* sp. was collected from Georges Rock Main, Tasmania, at a depth of 8 m by SCUBA diving in 2004. A voucher sample (G321486) is stored at the Queensland Museum, Brisbane, Australia. A freeze-dried and ground sample of the sponge (5 g) was sequentially extracted with *n*-hexane, CH_2Cl_2/MeOH (4:1) and MeOH. The CH_2Cl_2/MeOH extracts (0.9 g) were combined and chromatographed using C_{18}-bonded silica HPLC (MeOH/H_2O/0.1% TFA). Mass analysis of all the HPLC fractions indicated that the three fractions contained the desired cluster of five isotopic ions. These fractions were combined and further purified by C_{18} HPLC (MeOH/H_2O/0.1% TFA) to give the novel bromotyrosine alkaloid, pseudoceratinazole A (**1**, 4.5 mg, 0.18% dry weight). Compound **1** had moderate antitrypanosomal activity with 80% inhibition of *T. b. brucei* at 83 μM.

Compound **1** was obtained as an optically active solid, $[\alpha]_D^{24}$ +81 (*c* 0.1, MeOH).[15] The cluster of five isotopic ions at *m/z* 894, 896, 898, 900 and 902 (1:4:6:4:1) in the (+)-LRESIMS indicated the presence of 4 bromine atoms in the molecule. HRESIMS measurement on the $[M+H]^+$ ion (*m/z* 894.8958), in combination with ^1H and ^{13}C NMR spectroscopic data (Table 1), supported the molecular formula of $C_{28}H_{30}Br_4N_6O_8$. The ^1H and ^{13}C NMR signals, in particular, the C=N signal (δ_C 154.3) and the characteristic AB quartet for H_2-7 (δ_H 3.18, d, *J* = 15.6 Hz; 3.62, d, *J* = 15.6 Hz) were consistent with a spirocyclohexadiene isoxazoline moiety. The duplication of the ^1H NMR signals at H-1/H-1′, H-5/H-5′ and H-7/H-7′ suggested that **1** was a dimeric spirocyclohexadiene isoxazoline analogue.

* Corresponding author. Tel.: +61 7 3735 6000; fax: +61 7 3735 6001.
E-mail address: r.quinn@griffith.edu.au (R.J. Quinn).

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Table 1
NMR data for pseudoceratinazole A (**1**)[a]

Position	^{13}C	^{1}H mult. (*J* in Hz)	gHMBC
1	73.5	3.93 s	2, 3, 6
1′	73.5	3.94 s	2′, 3′, 6′
2	113.0	—	—
2′	113.0	—	—
3	147.1	—	—
3′	147.1	—	—
4	120.9	—	—
4′	120.9	—	—
5	131.1	6.57 s	1, 2, 3, 4, 6, 7
5′	131.1	6.58 s	1′, 2′, 3′, 4′, 6′, 7′
6	90.3	—	—
6′	90.3	—	—
7	39.2	3.62 d (15.6) 3.18 d (15.6)	1, 5, 6, 8, 9
7′	39.2	3.65 d (15.6) 3.21 d (15.6)	1′, 5′, 6′, 8′, 9′
8	154.3	—	—
8′	154.3	—	—
9	159.1	—	—
9′	159.0	—	—
10	—	8.62 t (5.5)	8, 9, 11, 12
11	35.5	3.17 m	9, 12, 13
12	29.2	2.01 q (7.0)	11, 13
13	46.3	4.16 t (7.0)	11, 12, 15, 18
15	134.9	9.01 s	13, 17, 18
17	131.3	—	—
18	118.8	7.58 s	13, 15, 17, 19
19	24.3	2.85 t (7.0)	17, 18, 20
20	37.6	3.45 m	17, 19, 9′
21	—	8.66 t (5.5)	19, 20, 8′, 9′
3/3′-OCH$_3$	59.6	3.66 s	3′/3

[a] Spectra were recorded at 500 MHz for ^{1}H and at 125 MHz for ^{13}C in DMSO-d_6 at 30 °C.

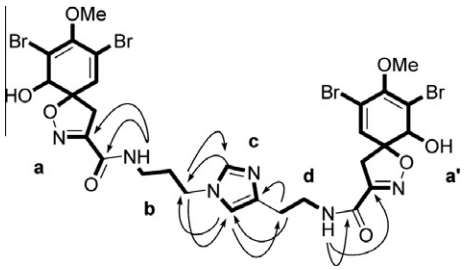


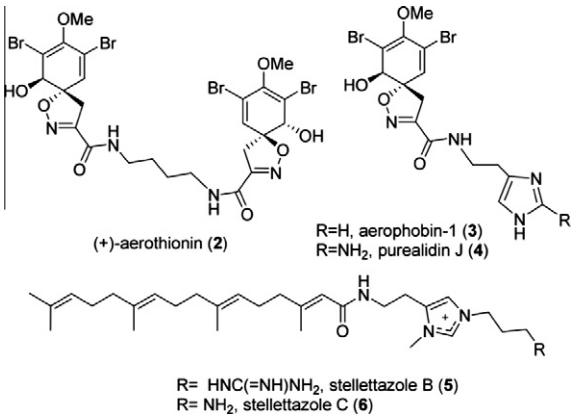
Figure 1. Partial structures **a**, **b**, **c**, **d** and **a**′ (in bold) and key HMBC correlations for **1**.

A series of COSY, HSQC and HMBC experiments further confirmed the presence of two spirocyclohexadiene isoxazoline moieties **a** and **a**′, and established the additional partial structures including an aminopropyl group **b** and an aminoethyl group **d** (Fig. 1). The structure of an imidazole moiety **c** was proposed based on the ^{1}H and ^{13}C NMR chemical shifts at the C-15 (δ_H 9.01, 1H, s; δ_C 134.9), C-17 (δ_C 131.3) and C-18 (δ_H 7.58, 1H, s; δ_C 118.8) positions, and was confirmed by the HMBC correlations from H-15 to C-17 and C-18, and from H-18 to C-15 and C-17. This imidazole unit also accounted for the remaining elements, $C_3H_2N_2$, of **1**.

The connectivities between substructures **a**, **b**, **c**, **d** and **a**′ were established following the detailed analysis of the HMBC correlations (Table 1 and Fig. 1). The HMBC correlations from the two exchangeable NH protons (δ_H 8.62 and 8.66) to the two carbonyls (δ_C 159.0 and 159.1) and C-8/C-8′ (δ_C 154.3) established the formation of an amide bond between **a** and **b**, and **d** and **a**′, respectively. Correlations from the H-13 methylene (δ_H 4.16) to C-15 and C-18 (δ_C 134.9 and 118.8) established the connectivity of **b** and **c**, which was confirmed by the correlations from H-15 and H-18 (δ_H 9.01 and 7.58) to C-13 (δ_C 46.3). Further correlations from H-18 (δ_H 7.58) to C-19 (δ_C 24.3) afforded the connectivity of **c** and **d**, which was confirmed by the HMBC correlations from H-19 (δ_H 2.85) to C-17 and C-18 (δ_C 131.3 and 118.8). The final structure of pseudoceratinazole A was therefore elucidated as **1**.

The relative stereochemistry of the chiral centres in the spirocyclohexadiene isoxazoline moieties in **1** was deduced by 2D ROESY data. The absence of a ROESY correlation between H-1 and H-7 (H-1′ and H-7′) suggested a trans-configuration between H-1 and C6–C7 (H-1′ and C6′-C7′), as is known in other spirocyclohexadiene isoxazoline compounds.[6,16] It has been well established that the absolute configuration at C-1/C-1′ and C-6/C-6′ can be determined from the signs of optical rotation and the Cotton effects at 248 and 284 nm in the CD spectra, as reported for (+)-aerothionin.[17,18] It is also relevant to note that the absolute configuration of (+)- and (−)-aerothionin has recently been confirmed by the total synthesis of optically pure aerothionin.[19] Pseudoceratinazole A (**1**) has a positive optical rotation ($[\alpha]_D^{24}$ +81, *c* 0.1, MeOH), as does (+)-aerothionin ($[\alpha]_D^{22}$ +210, *c* 1.7, MeOH).[18] More significantly, compound **1** has two prominent positive Cotton effects at 249 and 284 nm (Fig. 2), which were of the same sign and similar magnitudes to those of (+)-(1*R*,6*S*)-aerothionin,[18] suggesting that **1** has an (1/1′-*R*) and (6/6′-*S*) absolute configuration.

Compound **1** contains two spirocyclohexadienyl isoxazoline units and a unique 1,4-disubstituted imidazole-bridging unit. It is the first example of an imidazole-bridging bromotyrosine derivative, and represents a departure from the common dimeric spiroisoxazoline natural products where a 1,4-diaminoalkane acts as the bridging moiety such as in (+)-aerothionin (**2**). Several spiroisoxazoline cyclohexadienes including aerophobin-1 (**3**) and purealidin J (**4**)[5] also contain an imidazole moiety; although they are monomeric and contain only a 4-substituted imidazole. A literature search revealed that natural products rarely contain a 1,4-disubstituted imidazole moiety.[20–22] The only class of compounds which possesses a similar 1,4-imidazole unit as that of pseudoceratinazole A are the antibacterial stellettazoles B (**5**) and C (**6**) from the marine sponge *Stelletta* sp.[23]



A plausible biosynthetic pathway for compound **1** is proposed in Scheme 1. The spirocyclohexadienyl isoxazolines in **1** are likely derived from a bromotyrosine via an arene oxide intermediate, as proposed by Andersen and Faulkner.[24] This unit might participate in amide bond formation with a histamine to give the known natural product aerophobin-1 (**3**). Aerophobin-1 (**3**) could undergo alkylation at the N-1 position of the imidazole unit to form the intermediate *N*-(2-aminobutanoic acid-4-yl) aerophobin-1 (**7**). The N-alkylation is likely achieved from *S*-adenosylmethionine (SAM) as postulated by Rogers and Molinski for the biosynthesis of araplysillin-1 and its analogues.[25] The proposed mechanism involves SAM participating in an aberrant S_N2-type alkylation of

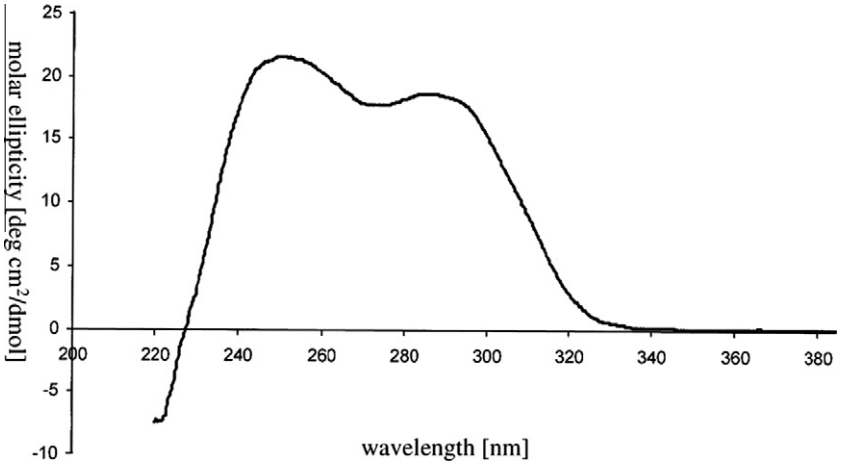
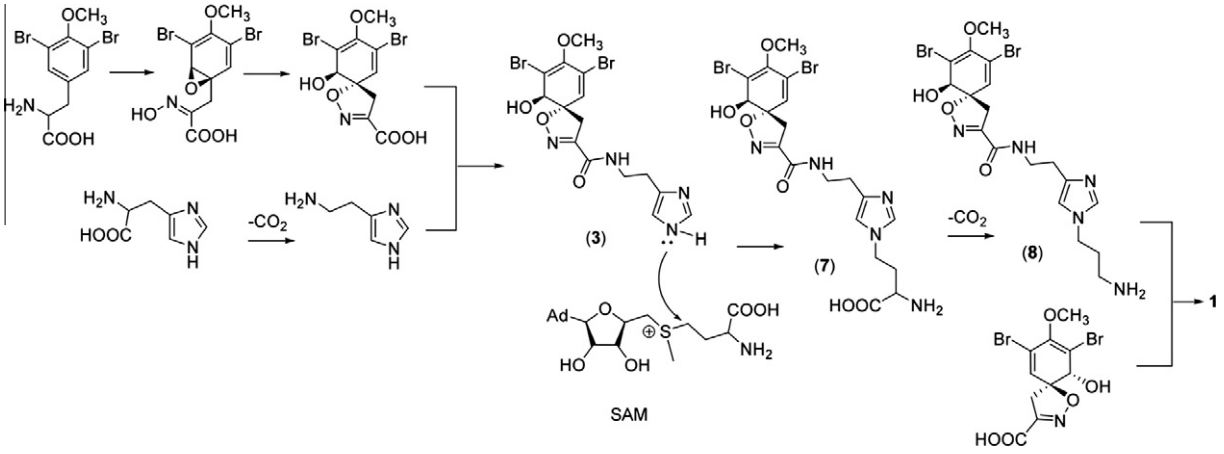


Figure 2. CD spectrum of pseudoceratinazole A (**1**).



Scheme 1. Proposed biosynthesis of pseudoceratinazole A (**1**).

the imidazole N-1 nitrogen at the more substituted S–CH_2 carbon of the sulfonium ion instead of the S–Me carbon.[24] The intermediate (**7**) could then be decarboxylated to give *N*-(3-aminopropyl) aerophobin-1 (**8**), which participate in another amide bond formation with a spirocyclohexadienyl isoxazoline unit to give the final product, pseudoceratinazole A (**1**).

In conclusion, we have isolated a novel spirocyclohexadienyl isoxazoline analogue, pseudoceratinazole A (**1**). Compound **1** is the first dimeric bromotyrosine alkaloid containing an imidazole-bridging moiety.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.052.

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15. Compound **1** was obtained as an amphorous solid: $[\alpha]_D^{24}$ +81 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε 235 (3.41) and 284 (3.38 nm; IR (KBr) ν_{max} 3400, 2930, 2850, 1675, 1520, 1135 and 1120 cm^{-1}; CD (*c* 0.001, MeOH) $\Delta\varepsilon^{25}$ (degrees cm^2/dmol) (nm) +22.9 (249) and +19.4 (284); ^1H and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* 894.8958 ($C_{28}H_{31}Br_4N_6O_8$ $[M+H]^+$ requires 894.8931).

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