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Published

2011

Journal Title

Bioorganic & Medicinal Chemistry Letters

DOI

[10.1016/j.bmcl.2010.11.081](https://doi.org/10.1016/j.bmcl.2010.11.081)

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Psammaplysin H, a new antimalarial bromotyrosine alkaloid from a marine sponge of the genus *Pseudoceratina*

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This is where the receipt/accepted dates will go; Received Month XX, 2000; Accepted Month XX, 2000 [BMCL RECEIPT]

Abstract— Mass-directed isolation of the CH₂Cl₂/CH₃OH extract from a marine sponge of the genus *Pseudoceratina* resulted in the purification of a new antimalarial bromotyrosine alkaloid, psammaplysin H (**1**), along with the previously isolated analogues psammaplysin G (**2**) and F (**3**). The structure of **1** was elucidated following 1D and 2D NMR, and MS data analysis. All compounds were tested *in vitro* against the 3D7 isolate of *Plasmodium falciparum* and mammalian cell lines (HEK293 and HepG2), with **1** having the most potent (IC₅₀ 0.41 μM) and selective (>97 fold) antimalarial activity.

Malaria is an infectious disease caused by the protozoan parasite *Plasmodium falciparum*. Each year around 500 million clinical cases of malaria occur with approximately one million of these cases resulting in death.¹ Natural products have played a key role in antimalarial drug discovery and therapy.²⁻⁵ Well known antimalarial natural products include quinine and artemisinin, which were first isolated from the South-American “quinine bark” (*Cinchona succiruba*) and the Chinese “sweet wormwood” (*Artemisia annua*), respectively.^{6,7} Numerous antimalarial drugs have subsequently been developed from these two natural products.^{7,8} Unfortunately, most antimalarial drugs have lost effectiveness due to the emergence of drug-resistant *Plasmodium* strains.⁹⁻¹¹ Thus there is an urgent need for the discovery and development of new antimalarial drugs.

Recently we have reported the isolation, structure elucidation and antimalarial activity of a number of natural products from a variety of Australian biota,¹²⁻¹⁵ including the bromotyrosine derivatives psammaplysin G (**2**) and F (**3**) from a marine sponge belonging to the genus *Hyattella*.¹⁵ Psammaplysin F (**3**)

displayed some activity towards chloroquine-resistant (Dd2) and chloroquine-sensitive (3D7) *P. falciparum* lines (IC₅₀ 1.38 μM and 0.867 μM, respectively). Psammaplysin G (**2**) showed only minimal inhibition at 40 μM against the Dd2 isolate. In order to obtain further analogues of this particular chemical class for structure activity relationship studies, and to further evaluate these molecules as potential antimalarial leads, we performed analytical HPLC and off-line MS data analysis on 50 marine sponges belonging to the order Verongida. This taxonomic order has been the source of numerous bromotyrosine-derived secondary metabolites, many of which contain a distinctive spiroisoxazoline system.¹⁵⁻²⁵ HPLC/MS data of the CH₂Cl₂/CH₃OH extract from one *Pseudoceratina* sp. identified UV-active peaks that contained ion clusters in the (+)-LRESIMS at *m/z* 787/789/791/793/795, 744/746/748/750/752, and 772/774/776/778/780. The first and second ion clusters were predicted to correspond to psammaplysin G (**2**) and F (**3**), respectively. The third ion cluster could not be identified following literature searching, and was thought to correspond to a new brominated natural

product. Subsequent mass-directed isolation of the large-scale $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ extract from marine sponge *Pseudoceratina* sp. afforded the new bromotyrosine alkaloid, psammaplysin H (**1**) along with the previously reported analogues psammaplysin G (**2**) and F (**3**).^{15,25} This paper reports the isolation, structure elucidation of psammaplysin H (**1**), as well as comparative antimalarial activity and selectivity studies of this new compound with psammaplysin G (**2**) and F (**3**).

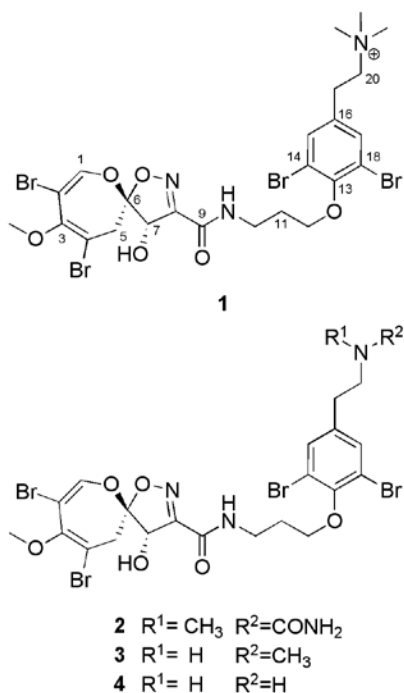


Figure 1. Chemical structures of **1-4**.

The freeze-dried and ground marine sponge *Pseudoceratina* sp. (G319257) was sequentially extracted with *n*-hexane, CH_2Cl_2 , and CH_3OH . The $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ extracts were combined and chromatographed using C_{18} HPLC ($\text{CH}_3\text{OH}/\text{H}_2\text{O}/0.1\%$ TFA) to yield 60 fractions. Fraction 39 contained the ion cluster of interest (+MS, m/z 772/774/776/778/780) and was further purified using C_{18} HPLC ($\text{CH}_3\text{OH}/\text{H}_2\text{O}/0.1\%$ TFA) to yield the new natural product psammaplysin H (**1**, 26.5 mg, 0.53 % dry wt). Fractions 43-50 from the first HPLC separation contained the other ion clusters of interest [+MS: m/z 744/746/748/750/752 and 787/789/791/793/795] and were combined and subjected to C_{18} HPLC ($\text{CH}_3\text{OH}/\text{H}_2\text{O}/0.1\%$ TFA) fractionation to yield the known alkaloids psammaplysin F (**3**, 10.6 mg, 0.22 % dry wt) and G (**2**, 14.2 mg, 0.23% dry wt). Compounds **2** and **3** were identified following spectroscopic data comparison with literature values.^{15,25} Compounds **1-3** were isolated as their TFA salts.

Compound **1** was obtained as an optically active gum.²⁶ The (+)-LRESIMS displayed a 1:4:6:4:1 ion cluster at m/z 772/774/776/778/780 [$\text{M} + \text{H}$]⁺, indicated the presence of four bromine atoms. The

molecular formula of **1** was determined to be $\text{C}_{24}\text{H}_{30}\text{Br}_4\text{N}_3\text{O}_6$ by (+)-HRESIMS. Analysis of the ^1H NMR data (Table 1) and the correlations observed in the HSQC experiment showed that the molecule contained three isolated aromatic protons at δ_{H} 7.68 (s, 2H) and 7.28 (s, 1H), two geminal protons at δ_{H} 3.34 (d, $J = 15.0$ Hz, 1H), 3.03 (d, $J = 15.0$ Hz, 1H), three methylenes at δ_{H} 3.44 (dt, $J = 6.1, 6.3$ Hz, 2H), 2.02 (tt, $J = 6.3, 6.6$ Hz, 2H) and 3.97 (t, $J = 6.6$ Hz, 2H), two mutually coupled methylenes at δ_{H} 3.03 (t, $J = 6.8$ Hz, 2H) and 3.52 (t, $J = 6.8$ Hz, 2H), an isolated methylene (δ_{H} 4.91, s, 2H), a methoxyl (δ_{H} 3.58, s, 3H), as well as three equivalent *N*-methyl groups (δ_{H} 3.11, s, 9H).

Table 1. NMR data for psammaplysin H (**1**).^a

Position	^{13}C	^1H (mult., J in Hz, int.)
1	145.4	7.28 (s, 1H)
2	101.4	
3	147.7	
3-OCH ₃	58.5	3.58 (s, 3H)
4	103.1	
5	36.9	3.34 (d, 15.0, 1H), 3.03 (d, 15.0, 1H)
6	117.4	
7	79.1	4.91 (s, 1H)
8	157.9	
9	158.2	
9-NH	-	8.77 (t, 6.1, 1H)
10	36.2	3.44 (dt, 6.1 6.3, 2H)
11	29.3	2.02 (tt, 6.3, 6.6, 2H)
12	71.1	3.97 (t, 6.6, 2H)
13	151.1	
14	117.6	
15	133.4	7.68 (s, 1H)
16	135.8	
17	133.4	7.68 (s, 1H)
18	117.6	
19	27.0	3.03 (t, 6.8, 2H)
20	65.3	3.52 (t, 6.8, 2H)
20-NCH ₃	52.3	3.11 (s, 9H)

^aRecorded in $\text{DMSO}-d_6$ at 30 °C.

The ^{13}C NMR spectrum displayed 20 signals, nine of which resonated above δ_{C} 100. The above NMR data was very similar to that of the known bromotyrosine psammaplysin F (**3**).²⁵ The main differences between **1** and **3** included the MW and the chemical shifts of the protons and carbons associated with the terminal methylated amine moiety. The MW of **1** was 28 Da greater than that of psammaplysin F (**3**). In the ^1H and ^{13}C NMR spectra of **1** the terminal *N*-CH₃ signal resonated further downfield (δ_{H} 3.11; δ_{C} 52.3), compared to psammaplysin F (δ_{H} 2.56; δ_{C} 32.3). In addition, the methylene linked to the terminal *N*-CH₃ moiety in **1** was also further downfield (δ_{H} 3.52; δ_{C} 65.3), compared to psammaplysin F (δ_{H} 3.17; δ_{C} 48.9). These data indicated the presence of two additional *N*-methyl groups attached to the terminal amine of **1**. This was confirmed by HMBC correlations of the *N*-CH₃

protons at δ_H 3.11 to carbons at δ_C 65.3 (C-20) and 27.0 (C-19) (Fig. 2).

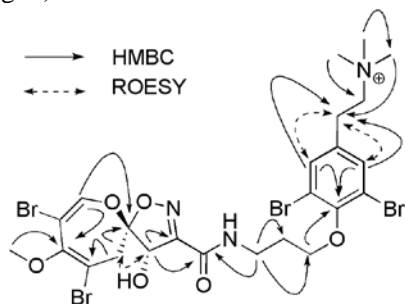


Figure 2. Key HMBC and ROESY correlations for compound **1**

The above data was essentially identical to the trimethylated amine moiety in purealidin U.²⁰ The ^1H - ^1H COSY and HMBC data analysis further supported the psammaplysin skeleton of **1** as shown in Fig. 2. The relative configuration of compound **1** was determined on the basis of the optical rotation data comparison and key ROESY correlations (Fig. 2). The relative configuration of psammaplysin A (**4**) was previously assigned following X-ray data analysis.²² This same relative configuration was assumed for psammaplysin H since both compounds had similar optical rotations [psammaplysin A $[\alpha]_D^{22} - 65.2$ (0.5, CH_3OH)²¹; psammaplysin H $[\alpha]_D^{25} - 63.8$ (0.1, CH_3OH)]. With the relative stereochemistry determined, structure **1** was assigned to psammaplysin H.

Compounds **1-3** were all tested *in vitro* for their ability to inhibit the growth of the 3D7 isolate of *P. falciparum* (Table 2). The new compound psammaplysin H (**1**) displayed the most potent *in vitro* antimalarial activity, with an IC_{50} of 0.41 μM . This activity is at least four fold better than psammaplysin G (**2**) or F (**3**). Importantly, when the activity of these three analogues was compared against the two mammalian cell lines HEK293 and HepG2, psammaplysin H (**1**) showed only minimal toxicity at the highest concentration tested (40 μM), giving this compound a parasite-specific selectivity index (SI) of >97. In contrast, psammaplysin G (**2**) and F (**3**) were more toxic to these cell lines with IC_{50} values between 3.71 and 18.96 μM , respectively (SI 2-5; Table 2). This preliminary structure activity data suggests that the substitution of the terminal nitrogen is important for the

antimalarial activity and selectivity. In addition, the *in silico* physicochemical profiling for compounds **1-3** is summarized in Table 2. The compounds are too large to be considered leads²⁷ and fail the MW criterion for drug-likeness as defined by Lipinski.²⁸ However, Leeson²⁹ has reasoned that the most important oral drug-like properties are actually those that have remained most constant over time. In light of this, compounds **1-3** are well positioned in terms of log P, the so-called "Lord of the Rules",³⁰ percent polar surface area (%PSA) and hydrogen bond donor count (HBD). Interestingly, Leeson's analysis²⁹ also found that anti-infectives had the most extreme property profiles of any therapy area.

In conclusion we have isolated a new antimalarial bromotyrosine alkaloid, psammaplysin H, that displays promising activity (IC_{50} 0.41 μM) towards the 3D7 strain of *P. falciparum*, and promising selectivity (SI >97) when compared to the human cell lines HEK293 and HepG2. Further chemical investigations around the spiroisoxazoline scaffold are currently underway, which will provide additional structure activity data and assist target identification studies.

Acknowledgements

We acknowledge the Australian Red Cross Blood Service for the provision of human blood and sera. KTA acknowledges funding from the Australian Research Council. We also thank H. T. Vu from Griffith University for acquiring the HRESIMS measurement. The opinions expressed herein are those of the author's and do not necessarily reflect those of the Australian Defence Force, Joint Health Command or any extant policy.

Supplementary data

Supplemental material available: 1D (^1H , ^{13}C) and 2D (gCOSY, HSQC, gHMBC and ROESY) NMR spectra for psammaplysin H (**1**), general experimental procedures, sponge collection and identification details, extraction and isolation procedures, biological assay details. Supplemental data associated with this article can be found in the online version at doi:

Table 2. Physicochemical and biological activity for compounds **1-3**.

Compound	Physicochemical Parameters ^a					$\text{IC}_{50} \pm \text{SD}$ (μM)			SI
	MW	ALog P	HBA	HBD	%PSA	3D7 ^b	HEK293	HepG2	
1	776	2.17	7	2	16.36	0.41 \pm 0.1	>40 ^c	>40 ^d	>97
2	790	2.58	8	3	24.14	5.22 \pm 1.6	18.7 \pm 2.7	17.4 \pm 1.8	3
3	747	2.99	8	3	19.94	1.92 \pm 1.1	10.9 \pm 1.7	3.7 \pm 1.1	2-5
Chloroquine	319	4.35	3	1	8.47	0.02 \pm 0.006	48.0 \pm 7.9	12.9 \pm 4.4	514-1921

^a*In silico* calculations performed using Pipeline Pilot version 6.1.5 software. ^bChloroquine sensitive *P. falciparum* line. ^cThe highest concentration tested was 40 μM which caused 35% \pm 10% growth inhibition from 3 assays, thus insufficiently toxic to provide IC_{50} value. ^dThe highest concentration tested was 40 μM which caused 33% \pm 3% growth inhibition from 3 assays, thus insufficiently toxic to provide IC_{50} value. MW = molecular weight (Da), HBA = H-bond acceptors, HBD = H-bond donors, %PSA = % polar surface area.

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26. Brown gum; $[\alpha]_D^{26} - 63.8$ (*c* 0.1, CH₃OH); UV (CH₃OH) λ_{\max} (log) 207 (4.76), 232 sh (4.23), 263 sh (3.91) nm; IR ν_{\max} (film) 3212, 2362, 1676, 1540, 1455, 1259, 1121 cm⁻¹; (+)-LRESIMS (rel. int.) *m/z* 772 [C₂₄H₃₀⁷⁹Br₄N₃O₆ + H]⁺ (15), 774 [C₂₄H₃₀⁷⁹Br₃⁸¹BrN₃O₆ + H]⁺ (60), 776 [C₂₄H₃₀⁷⁹Br₂⁸¹Br₂N₃O₆ + H]⁺ (100), 778 [C₂₄H₃₀⁷⁹Br⁸¹Br₃N₃O₆ + H]⁺ (60), 780 [C₂₄H₃₀Br₃⁸¹N₃O₆ + H]⁺ (15); (+)-HRESIMS *m/z* 771.8843 (C₂₄H₃₀⁷⁹Br₂⁸¹Br₂N₃O₆ [M + H]⁺ requires 771.8863).
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