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[c002]

Synthesis of 5-BromoVerongamine, an Antibacterial Dibromotyrosine Metabolite from Pseudoceratina sponge

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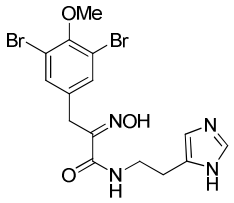
Abstract- 5-BromoVerongamine has been successfully synthesized in 2 steps in good yield from readily available starting materials. Structural elucidation has been confirmed through direct comparison with spectroscopic data of isolated natural product. 5-BromoVerongamine has been shown to have moderate bacteriacidal activity against MRSA.

Keywords: marine sponge metabolites, bromotyramine, bromotyrosine, anti-bacterials



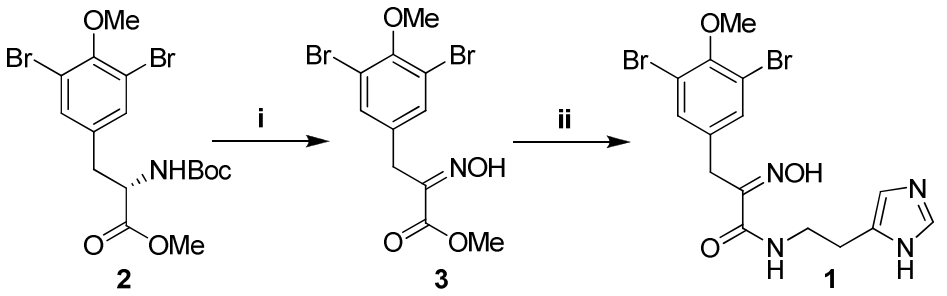
Introduction

A remarkable feature of sponges within the order Verongida are their activated chemical defense mechanisms based upon bromotyrosine derived secondary metabolites.[1] Up to 10% of the total dry biomass of the sponge consists of these metabolites stored in spherolous cells, which upon rupture act as a deterrent toward other organisms through a cascade of reactions, yet show low toxicity to the host.[2] Due to the immediate and considerable dilution effects inherent in inhabiting an aquatic environment the compounds must display high potency towards their intended targets. This makes these compounds attractive targets for systematic biological evaluation and total synthesis. 5-BromoVerongamine (**1**) was originally isolated from a Caribbean sponge of Pseudoceratina (40.3 mg as a pale yellow oil) and only tested for its anti-fouling properties, inhibiting the settlement of barnacle larvae at 10 mg mL^{-1} (EC_{50}=1.03 mg mL^{-1}) without toxic effects at that concentration.[3] The natural product was identified through ^{1}H, ^{13}C NMR and Low Resolution Mass Spectra Data.

Following on from our previous synthetic endeavors,[4] we have investigated the synthesis and biological evaluation of 5-BromVerongamine (**1**) against 40 clinical isolates of Methicillin Resistant *Staphylococcus Aureus* (MRSA).

Results and Discussion

The starting material for the synthesis of 5-Bromoverongamine was the known compound (**2**)[5] Boc-deprotection of **2**, followed by oxidation and finally amination utilising known methodology[4b] provided 5-Bromoverongamine (**1**) in good yield (59 % over 2 steps).



Reagents and Conditions:

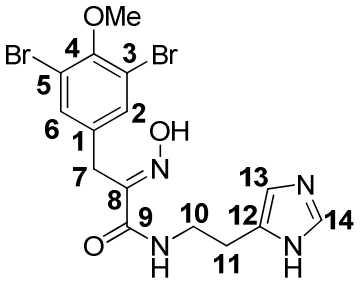
(i) TFA, CH_2Cl_2, then Na_2WO_4, 30% H_2O_2, EtOH/H_2O **69%**; (ii) Histamine, MeOH, 60°C, 72 hr **85%**.

Structure elucidation was achieved by direct comparison of natural versus synthetic product (**Table 1**). Excellent spectral correlation was achieved. Notably the ^{13}C chemical shift of C-7 at 28.8 ppm suggests an *E*-geometry.[6]

5-Bromoverongamine was tested against 40 clinical isolates of MRSA composed of community acquired (16) and hospital acquired (24) samples. 5-BromoVerongamine showed bacteriacidal activity against MRSA species minimum inhibitory concentration (MIC) range was 0.0625-0.5 mg L^{-1}. Ongoing work has shown synergistic activity when combined with Gentamycin against MRSA.

We are currently exploiting the versatility of the synthetic strategy towards other marine sponge metabolites and unnatural analogues. Further investigations will be published in due course.

Table 1. NMR and MS Data for 1



Position	^{1}H NMR		^{13}C NMR	
	A	B	A	B
1			137.7	137.2
2	7.46 (s, 1H)	7.48 (s, 1H)	134.8	135.8
3			118.8	118.5
4			154.2	153.6
5			118.8	118.5
6	7.46 (s, 1H)	7.48 (s, 1H)	134.8	135.8
7	3.84 (s, 2H)	3.84 (s, 2H)	29.1	28.8
8			152.4	153.6
9			165.6	165.2
10	3.49 (t, *J* = 7.0 Hz, 2H)	3.54 (t, *J* = 7.2 Hz, 2H)	40.6	40.2
11	2.79 (t, *J* = 7.0 Hz, 2H)	2.83 (t, *J* = 7.2 Hz, 2H)	27.9	27.6
12			134.0	134.3
13	6.84 (brs, 1H)	6.84 (brs, 1H)	118.0	117.7
14	7.62 (brs, 1H),	7.60 (brs, 1H)	136.2	136.0
OCH_3	3.80 (s, 3H)	3.78 (s, 3H)	61.3	61.0
MS	HR-EI $[M]^+$ ($C_{15}H_{16}{}^{79}Br_2N_4O_3$) Calculated 457.959 Observed 457.962	HR-EI $[MH]^+$ ($C_{15}H_{17}{}^{79}Br_2N_4O_3$) Calculated 458.967 Observed 458.967		

A – Thirionet *et al*. J. C.; *Nat.Prod.Lett.* **1998**, 12, 209. (^{1}H 400 MHz, ^{13}C 100 MHz, CD_3OD)
B – The results we obtained. (^{1}H 500 MHz, ^{13}C 125 MHz, CD_3OD)

General Experimental

Methyl 3-(1,5-Dibromo-4-methoxyphenyl)-2(*E*)-(hydroxylamino)propanoate (**3**)

TFA (0.19 mL, 2.57 mmol) was added dropwise to the Boc-protected tyrosine derivative (**2**, 1.00 g, 2.14 mmol) in CH_2Cl_2 (10 mL) and stirred for 5 h. The solvent was removed *in vacuo*, and the residue was dissolved in EtOAc (75 mL). The EtOAc solution was washed with $NaHCO_3$ (2 x 75 mL), saturated NaCl (75 mL), dried over $MgSO_4$ and solvent removed *in vacuo* to give a colourless gum (0.761 g). The crude amine was dissolved in absolute EtOH (5 mL). $Na_2WO_4.2H_2O$ (0.709 g, 2.15 mmol) and then 30% H_2O_2 (21.42 mL, 0.214 mol) were added and the mixture was stirred for 2 h at which point a white precipitate formed. The solid was filtered and washed with H_2O (50 mL) then $CHCl_3$ (100 mL) and dried *in vacuo* to give a colourless microcrystalline solid (**3**, 0.566 g, 69 %); mpt 199-201^oC; IR (Diamond) υ_{max} 3236, 2924, 1731, 1472 cm^{-1}; ^1H NMR (300 MHz, d_6-DMSO) δ 12.59 (s, 1H), 7.43 (s, 2H), 3.80 (s, 2H), 3.78 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (75 MHz, d_6-DMSO) δ 164.35, 152.43, 148.87, 136.23, 133.23, 117.65, 112.07, 60.69, 52.64, 29.31; Anal. Calcd for $C_{11}H_{11}Br_2NO_4$: C, 34.67; H, 2.91; N, 3.68. Found: C, 35.00; H, 2.97; N, 3.44.

Bromoverongamine (**1**)

A solution of oxime (**3**, 0.20 g, 0.525 mmol) and histamine (0.175 g, 1.57 mmol) in MeOH (2 mL) was heated for 72 h at 60 ^oC. The solvent was removed *in vacuo* and the orange gum obtained purified by column chromatography (SiO_2, EtOAc /MeOH, 5:2) to give a pale yellow gum (**1**, 0.206 g, 85 %). IR (Diamond) υ_{max} 3197, 2925, 1654, 1527, 1470 cm^{-1}. ^1H, ^{13}C NMR and HR-MS see Table 1

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