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Synoxazolidinones A and B: Novel Bioactive Alkaloids from the Ascidian *Synoicum pulmonaria*

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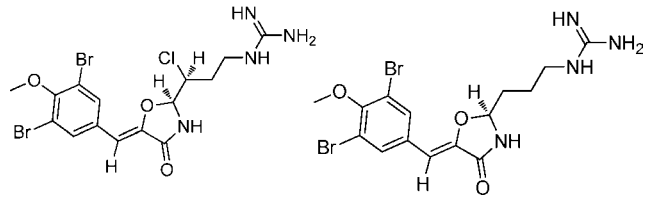
ABSTRACT



Synoxazolidinone A (1) **Synoxazolidinone B (2)**

Bioassay-guided fractionation of the sub-Arctic ascidian *Synoicum pulmonaria* collected off the Norwegian coast led to the isolation of a novel family of brominated guanidinium oxazolidinones named synoxazolidinones A and B (1 and 2). The backbone of the compounds contains a 4-oxazolidinone ring rarely seen in natural products. The structure of the compounds was determined by spectroscopic methods. The synoxazolidinones exhibited antibacterial and antifungal activities.

Synoicum sp. are colonial ascidians that have afforded a wide range of compounds with diverse biological activities. Among these are the cytotoxic palmerolide A,[1] ecdysteroids,[2] a tetrahydrocannabinol derivative,[3] prunolides A, B, and C,[4] a number of rubrolides,[5] the antidiabetic tiruchanduramine,[6] and *E*/*Z*-rubrolide O, which is an anti-inflammatory halogenated furanone.[7] *S. pulmonaria* (Ellis and Solander, 1786) is commonly found in the Arctic-boreal waters of the North

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Sea and the deep cold waters of West Scotland and Northern Ireland.[8] In a recent study, we have shown that extracts from this species contain antimicrobial compounds.[9] In the present study, we have isolated two novel brominated guanidines from *S. pulmonaria* that we have named synoxazolidinones A and B (**1** and **2**) due to their unique 4-oxazolidinone core structure (Figure 1). The *S. pulmonaria* species was collected off the coast of Troms in Northern Norway.

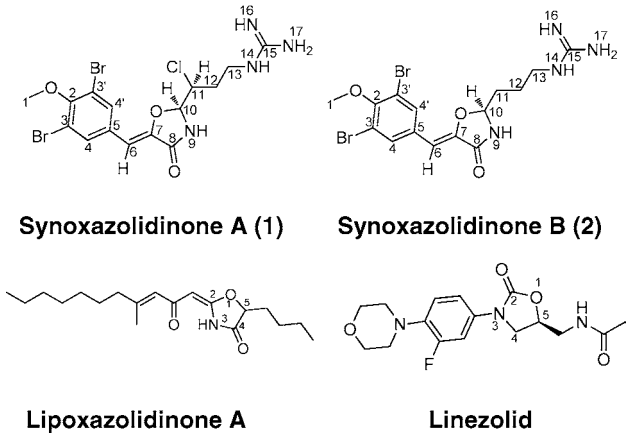

Figure 1. Structures of synoxazolidinones A and B (**1** and **2**), lipoxazolidinone A, and the synthetic linezolid.

To our knowledge, only one other report describes naturally occurring 4-oxazolidinones, namely, the antibacterial lipoxazolidinones which were isolated from marine actinomycetes from a Guam marine sediment (Figure 1).[10] In that study, the intact oxazolidinone ring was found to be essential for antibacterial activity. While very rare in nature, the oxazolidinones are important in medicinal chemistry, and a new class of totally synthetic antibiotic drugs is based on this unusual core. A recent successful drug from this class to reach the market is linezolid (a 2-oxazolidinone) (Figure 1).[11] Both the lipoxazolidinones and linezolid are highly potent antimicrobial agents, particularly against Gram-positive microorganisms. Linezolid possesses a novel mode of action, whereby it inhibits initiation of bacterial protein synthesis by binding to the 50S ribosomal subunit.[11] We also found that **1** and **2** isolated in the present study exhibited antibacterial and antifungal activity, as described more in detail below.

Synoxazolidinones A and B (**1** and **2**) were isolated from a crude acetonitrile extract of the lyophilized *S. pulmonaria* specimen after separation on a preparative RP-HPLC C_{18}-column using a gradient of acetonitrile/water. HPLC analysis revealed two major components with almost similar retention.

Synoxazolidinone A (**1**) was isolated as a colorless semi-crystalline oil with the molecular formula $C_{15}H_{17}Br_2ClN_4O_3$ (HRESIMS *m/z* 494.9437, Δ +0.3 mmu for $[M + H]^+$).[12] Neither UV or IR analysis revealed any unambiguous functionalities, and the structure was solved solely on the basis of a number of key 1D and 2D NMR experiments.

gHMBC correlations from the H-1 methyl protons to C-2 (Table 1) positioned the methoxy group at C-2 on the dibrominated aromatic ring of **1**. The bromine atoms were placed at C-3/3′ on the aromatic ring based on the C-3/3′ shift values (δ_C 119.0). The olefinic proton H-6 afforded gHMBC correlations to C-4/4′ and to the amide carbonyl C-8. Those signals, combined with the cross peaks seen in DMSO-d_6 for the amide proton H-9 to C-7, C-8, and C-10, were essential for proposing the 4-oxazolidinone ring.

Table 1. ^{1}H and ^{13}C NMR Assignments and gHMBC Correlations of **1**[a]

no.	δ_H (mult, *J* in Hz)	δ_C (mult)	gHMBC (^{1}H−^{13}C)
1	3.87 (s)	61.2, CH_3	2
2		154.3, C	
3/3′		119.0, C	
4/4′	7.85 (s)	134.0, CH	2, 3/3′, 5, 6
5		133.9, C	
6	6.06 (s)	100.6, CH	4/4′, 5, 7, 8
7		146.2, C	
8		164.7, C	
9	10.2[b] (s)		7[b], 8[b], 10[b]
10	5.90 (d, 2.2)	90.5, CH	11
11	4.38 (d, 10.7)	62.3, CH	10, 12, 13
12	2.30 (m)	32.3, CH_2	11
	1.98 (m)		
13	3.58 (m)	39.3, CH_2	11, 12, 15
14	8.0[b] (m)		
15		158.8, C	
16/17	7.4[b] (br m)		

[a] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C) in CD_3OD and DMSO-d_6. [b] Peaks/correlations only seen in DMSO-d_6.

gCOSY correlations revealed a carbon chain from H-10 extending all the way to the methylene protons H-13. The gHMBC correlation from H-13 to C-15 (only seen in DMSO-d_6) supported the terminal guanidine group. The shift value of C-15 (δ_C 158.8) also supported a terminal guanidine group. The guanidine protons H-16 and H-17/17′ gave overlapping signals at δ_H 7.4 in the ^{1}H NMR spectrum obtained in DMSO-d_6. The key gHMBC correlations of **1** are shown in Figure 2. The structure of **1** was further supported by the fragmentation pattern observed by tandem mass spectrometric analysis (see the Supporting Information). Synoxazolidinone A (**1**) contains stereochemical features which were not readily determined using standard techniques. The

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(12) Synoxazolidinone A (**1**) ((*Z*)-1-(3-chloro-3-(5-(3,5-dibromo-4-methoxybenzylidene)-4-oxooxazolidin-2-yl)propyl)guanidine): colorless semi-crystalline oil, $[\alpha]_D^{24}$ + 81.5° (*c* 0.184, MeOH); CD ($\Delta\varepsilon_{208}$ −3, $\Delta\varepsilon_{295}$ 12); UV (MeOH) λ_{max} (log ε) 292 nm (4.2); IR υ_{max} 3169, 1674; HRESIMS $[M + H]^+$ *m/z* 494.9438 (calcd for $C_{15}H_{18}Br_2ClN_4O_3$, 494.9443). NMR data can be found in Table 1.

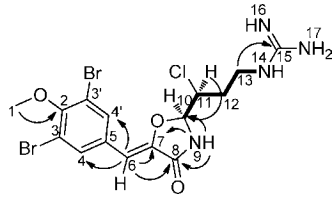

Figure 2. Key gHMBC (H→C) and gCOSY (—) correlations of **1**.

trisubstituted double bond between C-6 and C-7 was problematic and is proposed to be *Z* using the ^3J(C,H) coupling constant between H-6 and C-8 which was measured to be smaller than 3 Hz.[13] The assignment was based on the literature value for the ^3J(C,H) for a *cis* double bond (7.6 Hz), which is significantly lower than that for a *trans* double bond (14.1 Hz).[14] Calculations based on CD experiments also supported a *Z*-configuration at this position (see below).

The stereocenters at C-10 and C-11 were not amenable to *J*-based configurational analysis, and therefore the CD spectra of all eight possible stereoisomers (shown in the Supporting Information) were simulated using time-dependent density functional theory at the LDA/SVP level of theory.[15−17] Contributions to the CD spectra arising from different thermally accessible conformations were accounted for by summing Boltzmann weighted spectra of structures sampled from classical MD simulations. The best fit was obtained between the experimental spectrum and the CD spectrum simulated for the 6*Z*, 10*S*, 11*S* configuration (Figure 3). The 3D structure of **1** is shown in Figure 4.

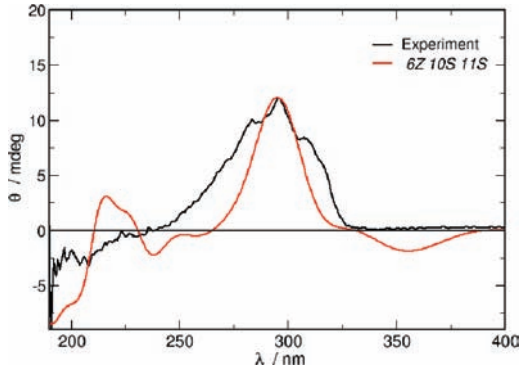

Figure 3. Overlay of the experimental CD spectrum with the TD-LDA/SVP calculated spectrum for **1** (6*Z*, 10*S*, 11*S* stereoisomer).

Accurate mass measurement of **2** indicated a molecular formula of $C_{15}H_{18}Br_2N_4O_3$ (HRESIMS *m/z* 460.9833, Δ +0.9 mmu for $[M + H]^+$).[18] Unlike **1**, compound **2** did not contain a chlorine atom. The NMR data for **2** again indicated an overall structure similar to **1** (Table 2). However, the absence of a chlorine atom caused a marked upfield shift for the methylene protons H-11 and for C-11. The shift value of H-6 in **2** was similar to that seen in **1**. Pending synthesis, the same absolute configuration as **1** is proposed for **2** (6*Z*, 10*S*) based on the similarities of their CD spectra (see the Supporting Information).

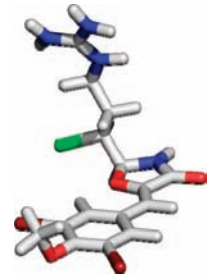

Figure 4. DFT-PBE/SVP optimized structure of **1** (6*Z*, 10*S*, 11*S* stereoisomer). CPK colors used: Br is brown, and Cl is green. Figure prepared with Pymol v1.2r1 (http://www.pymol.org/).

Table 2. ^{1}H and ^{13}C NMR Assignments and gHMBC Correlations of **2**[a]

no.	δ_H (mult, *J* in Hz)	δ_C (mult)	gHMBC (^{1}H−^{13}C)
1	3.87 (s)	61.2, CH_3	2
2		154.3, C	
3/3′		119.0, C	
4/4′	7.89 (s)	133.9, CH	2, 3/3′, 5, 6
5		134.4, CH	
6	6.05 (s)	100.1, CH	4/4′, 7, 8
7		146.9, C	
8		165.3, C	
9			
10	5.78 (1H, t, 5.0)	90.1, CH	11,12
11	1.97 (1H, m)	34.3, CH_2	10, 12, 13
	1.86 (1H, m)		
12	1.76 (2H, m)	23.4, CH_2	10, 11, 13
13	3.28 (2H, t, 7.0)	41.9, CH_2	11, 12, 15
14			
15		158.7, C	
16/17			

[a] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C) in CD_3OD.

A biogenesis for **1** and **2** can be postulated involving the initial formation of a dipeptide from a brominated tyrosine-derived metabolite[19,20] and an arginine/agmatine derivative[21] followed by the formation of an oxazolidinone ring (Figure 5). It is not clear at which point of the biosynthesis the chlorine atom in **1** is introduced. Experiments have not been carried out to determine whether *S. pulmonaria* or potentially symbiotic microorganisms are responsible for the biosynthesis.

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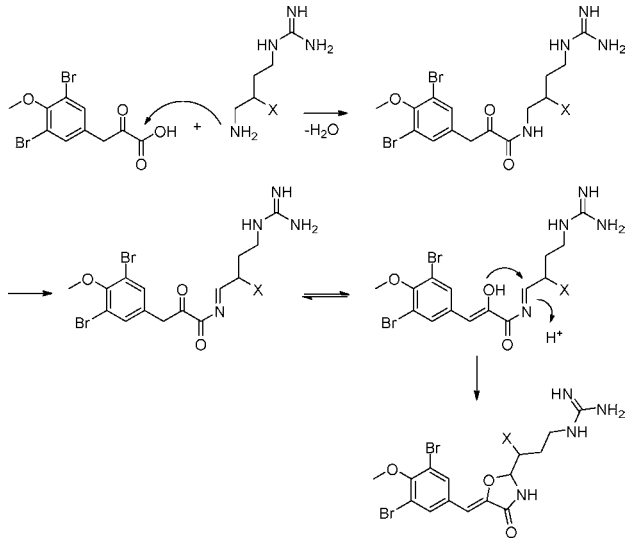

Figure 5. Suggested biosynthetic route of **1** and **2** (X = H, Cl).

Synoxazolidinone A (**1**) displayed MIC values against the Gram-positive bacteria *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) at a concentration of 10 μg/mL. Synoxazolidinone A (**1**) showed an MIC value of 6.25 μg/mL against the Gram-positive bacterium *Corynebacterium glutamicum* and an MIC of 12.5 μg/mL against the fungi *Saccharomyces cerevisiae*. Synoxazolidinone B (**2**) generally displayed lower activities against MRSA (MIC of 30 μg/mL). This lowered activity suggests that the chlorine atom is important for biological activity. The synoxazolidinones have provided a novel scaffold for synthetic structure−activity relationship studies, which are currently being carried out with the aim of improving the activities and reducing the toxicity of synoxazolidinones A and B (**1** and **2**). The significance of cold-water marine organisms for the discovery of new structural types is demonstrated.[22]

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Supporting Information Available: Experimental and computational procedures, bioactivity assays, ^{1}H, ^{13}C, gHSQC, gCOSY, and gHMBC data, and CD spectra of synoxazolidinones A and B (**1** and **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Synoxazolidinone B (**2**) ((*Z*)-1-(3-(5-(3,5-dibromo-4-methoxybenzylidene)-4-oxooxazolidin-2-yl)propyl)guanidine): colorless semi-crystalline oil, $[\alpha]_D^{24}$ + 57.8° (*c* 0.087, MeOH); UV (MeOH) λ_{max} (log ε) 292 nm (4.1); IR υ_{max} 3346, 1674; HRESIMS [M + H]$^+$ *m*/*z* 460.9835 (calcd for $C_{15}H_{19}Br_2N_4O_3$, 460.9846). NMR data can be found in Table 2.

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