

# The isolation and synthesis of polyandrocarpamines A and B. Two new 2-aminoimidazolone compounds from the Fijian ascidian, *Polyandrocarpa* sp.

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**Abstract**—Chemical investigation of a Fijian ascidian, *Polyandrocarpa* sp., has resulted in the isolation of two new 2-aminoimidazolone-derived compounds, polyandrocarpamines A (**1**) and B (**2**). The structures of these unique metabolites were determined by the interpretation of spectroscopic data and confirmed by total synthesis. The stereospecific synthesis of **1** was accomplished using aldol condensation chemistry to generate an arylidene thiohydantoin that was subsequently transaminated to yield polyandrocarpamine A. Demethylation of synthetic **1** afforded polyandrocarpamine B. Both the natural product and synthetic polyandrocarpamines were assigned *Z* geometries about the exocyclic double bond (C-5/C-7) on the basis of <sup>13</sup>C/<sup>1</sup>H long-range coupling constants, which were measured using a gHSQMBC experiment. © 2002 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Marine organisms have proven to be a rich source of novel and structurally diverse metabolites that display a wide variety of biologically significant activity. Examples include the anti-fouling oroidin dimer, mauritiamine,<sup>1</sup> the MEK-1 inhibitor, hymenialdisine,<sup>2</sup> and the histaminergic antagonist, dispacamide.<sup>3,4</sup> The marine metabolites listed above all belong to the 2-aminoimidazolone class of compounds that have been predominantly isolated from marine sponges. Interestingly, ascidians, which are known for their production of nitrogenous metabolites,<sup>5</sup> have only contributed two compounds to this structure class thus far. These include the *N,N*-dimethylaminoimidazolone compounds, **3** and **4** (Fig. 1), both of which were isolated

from the ascidian *Dendrodoa grossularia*.<sup>6,7</sup> This paper reports the isolation, structural elucidation and total synthesis of two new members of this structure class, which we have named polyandrocarpamines A (**1**) and B (**2**). These novel alkaloids were purified as part of our continuing investigations of Fijian ascidians for new structural and/or potentially therapeutic chemistries.

## 2. Results and discussion

A freeze-dried sample of an undescribed *Polyandrocarpa* sp.<sup>8</sup> (Styelidae) was exhaustively extracted with MeOH. This extract was evaporated to dryness then chromatographed on a C<sub>18</sub> bonded silica flash column using a

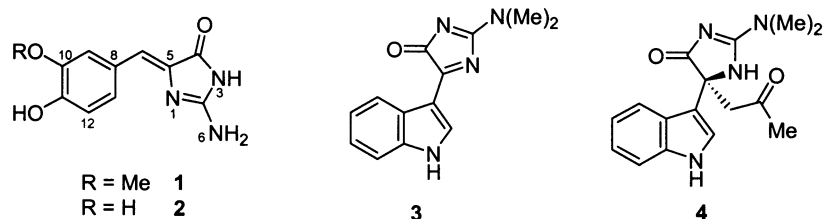


Figure 1. Ascidian isolated 2-aminoimidazolone compounds.

**Keywords:** *polyandrocarpa*; 2-aminoimidazolone; polyandrocarpamines A and B; synthesis; aldol condensation; arylidene thiohydantoin; transamination; gHSQMBC.

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**Table 1.** NMR data for polyandrocarpamine A (**1**)

Position	<sup>13</sup> C (δ)	<sup>1</sup> H (δ, mult., <i>J</i> in Hz)	COSY	HMBC	ROESY
2	166.3				
4	178.2				
5	132.0				
7	114.8	6.54 (s)		4, 9, 13	9, 13
8	127.3				
9	114.1	7.24 (d, 2.0)	13	7, 11, 13	7, 10-OMe
10	149.3				
10-OMe	56.6	3.91 (s)		10	9
11	149.0				
12	116.7	6.84 (d, 8.0)	13	8, 10	13
13	124.5	7.09 (dd, 8.0, 2.0)	9, 12	7, 9, 11	7, 12

Spectra were recorded in CD<sub>3</sub>OD at 25°C.

MeOH/H<sub>2</sub>O gradient. One of the earlier eluting fractions was subjected to Sephadex LH-20 (MeOH) chromatography and yielded pure polyandrocarpamines A (**1**, 0.04% dry wt) and B (**2**, 0.23% dry wt).

Polyandrocarpamine A (**1**) was isolated as a stable yellow solid. An [M+H]<sup>+</sup> ion in the positive high-resolution fast atom bombardment mass spectrum [(+)-HRFABMS] at *m/z* 234.08816 (Δ+1.2 ppm) allowed a molecular formula of C<sub>11</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub> to be assigned to **1**. Eight degrees of unsaturation were calculated for compound **1**. Strong absorption bands at 1666 and 3600–3000 cm<sup>-1</sup> in the IR spectrum suggested that **1** contained a carboxyl moiety and an amine and/or hydroxyl groups, respectively. The <sup>13</sup>C NMR spectrum (Table 1) displayed signals for all eleven carbons and the DEPT experiment indicated that **1** contained one methyl and four methine carbons. The <sup>1</sup>H NMR spectrum of **1** showed one methoxyl signal at δ 3.91 (3H, s), an isolated olefinic resonance at δ 6.54 (1H, s) and three aromatic signals at δ 7.09 (1H, dd, *J*=8.0, 2.0 Hz), 6.84 (1H, d, *J*=8.0 Hz) and 7.24 (1H, d, *J*=2.0 Hz) that were assigned to a 1,3,4-trisubstituted benzene ring. The HMQC spectra enabled all the proton signals to be assigned to their directly attached carbons. Three bond HMBC correlations for the three aromatic proton signals at δ 7.09 (H-13), 6.84 (H-12) and 7.24 (H-9) revealed that the benzenoid ring was substituted by two *ortho*-orientated oxygenated substituents at C-10 (149.3 ppm) and C-11 (149.0 ppm) and an isolated olefinic moiety at C-8 (127.3 ppm). Confirmation of the C-8 substitution was provided by <sup>3</sup>J<sub>CH</sub> correlations for the proton singlet at δ 6.54 (H-7) to both benzenoid carbons C-9 (114.1 ppm) and C-13 (124.5 ppm). The methoxyl group

at δ 3.91 was attached to C-10 due to a strong <sup>3</sup>J<sub>CH</sub> correlation to 149.3 ppm and a ROESY cross-peak with H-9 (δ 7.24). A hydroxyl group was attached to C-11 based on the characteristic downfield <sup>13</sup>C chemical shift (149.0 ppm) of this carbon.<sup>9,10</sup> The presence of a phenol was supported by the UV spectrum, which underwent a bathochromic shift from 352 to 404 nm on addition of base. While the majority of the structure for polyandrocarpamine A had been assigned, a C<sub>3</sub>H<sub>3</sub>N<sub>3</sub>O subunit still remained to be elucidated. This unidentified substructure contained three low-field quaternary <sup>13</sup>C chemical shifts that resonated at 178.2, 166.3 and 132.0 ppm. These data indicated that the remaining three hydrogens were attached to heteroatoms. The carbon at 178.2 ppm, and the strong IR absorption band at 1666 cm<sup>-1</sup> suggested the presence of a 5-membered lactam ring.<sup>11,12</sup> Incorporation of the remaining atoms (H<sub>3</sub>N<sub>3</sub>) into compound **1** required a 2-aminoimidazolone system. A <sup>3</sup>J<sub>CH</sub> correlation from the isolated olefinic proton at δ 6.54 (H-7) to the imidazolone carbonyl (178.2 ppm) provided the only linkage between this C<sub>3</sub> subunit and the rest of the molecule. Comparison of the 2-aminoimidazolone <sup>13</sup>C chemical shifts of polyandrocarpamine A (**1**) [(C-2), 166.3; (C-4), 178.2; (C-5) 132.0] with dispacamide (**5**) [(C-2), 168.3; (C-4), 179.1; (C-5) 136.9] showed only minor discrepancies.<sup>3,4</sup> The geometry of the exocyclic double bond in **1** was determined on the basis of <sup>13</sup>C/<sup>1</sup>H long range coupling constants, which were measured using a gHSQMBC experiment.<sup>13</sup> Analysis of this 2D NMR experiment revealed that the coupling constant between the exocyclic proton at δ 6.54 (H-7) and the imidazolone carbon at 178.2 ppm (C-4) was 5.5 Hz, consistent for *Z* geometry.<sup>14,15</sup> With the stereochemistry defined, structure **1** was assigned to polyandrocarpamine A.

**Table 2.** NMR data for polyandrocarpamine B (**2**)

Position	<sup>13</sup> C (δ)	<sup>1</sup> H (δ, mult., <i>J</i> in Hz)	COSY	HMBC	ROESY
2	165.5				
4	176.8				
5	130.2				
7	115.3	6.52 (s)		4, 9, 13	9, 13
8	126.9				
9	117.6	7.04 (d, 2.0)	13	7, 10, 11, 13	7
10	146.8				
11	148.1				
12	116.8	6.82 (d, 8.4)	13	8, 10, 11	13
13	123.3	6.97 (dd, 8.4, 2.0)	9, 12	7, 9, 11	7, 12

Spectra were recorded in CD<sub>3</sub>OD at 25°C.

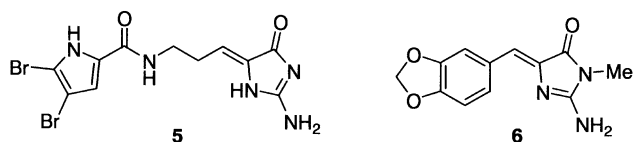


Figure 2. Sponge metabolites, dispacamide (**5**) and leucettamine B (**6**).

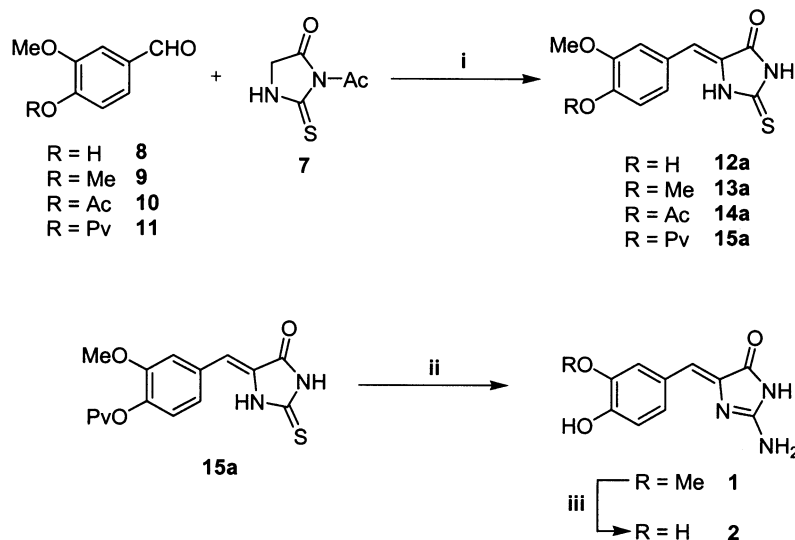
The major metabolite, polyandrocarpamine B (**2**) was isolated as a stable yellow solid. The molecular formula  $C_{10}H_9N_3O_3$  was determined by interpretation of the  $[M+H]^+$  ion at  $m/z$  220.07225 ( $\Delta +0.1$  ppm) in the (+)-HRFABMS. Comparison of the  $^{13}C$  and  $^1H$  NMR data of **2** with **1** (Tables 1 and 2) showed the only major difference to be the absence of a methoxyl substituent in polyandrocarpamine B. A gHSQMBC experiment was again used to establish the exocyclic double bond geometry of **2** as *Z*. With the stereochemistry defined, structure **2**, the *O*-demethyl analog of **1**, was assigned to polyandrocarpamine B.

Although polyandrocarpamines A and B were not isolated using bioassay guided fractionation, we wished to obtain more material for future biological evaluations, since a variety of bioactivities have been reported for members of this structure class. The recent syntheses of dispacamide (**5**) and leucettamine B (**6**), both of which have a high degree of structural similarity to the polyandrocarpamines, presented us with already developed chemistry for the synthesis of 2-aminoimidazolones (Fig. 2).<sup>16,17</sup> The key step in both these syntheses involved a facile one-pot conversion of an alkyl- or aryl-idene thiohydantoin to the corresponding 2-aminoimidazolones by employing *tert*-butylhydroperoxide (TBHP) in the presence of aqueous  $NH_4OH$ . We decided to use this relatively new chemistry for the production of both polyandrocarpamines A and B.

We initially synthesized a small series of vanillin-derived thiohydantoin analogs **12a–15a**, all of which had the potential to be converted into either polyandrocarpamine A or B following a TBHP promoted transamination reaction. The readily available 3-acetyl-2-thioxoimidazolidin-4-one (**7**)<sup>18</sup>

was separately condensed with each of the vanillin derivatives **8–11** in a NaOAc/AcOH slurry under refluxing conditions (Scheme 1),<sup>16</sup> and yielded the pure 5-arylidene-2-thiohydantoin systems, **13a** (82% yield), **14a** (87% yield) and **15a** (65% yield). Interestingly, the condensation reaction that used unprotected vanillin **8** yielded an 8:1 mixture of the *Z* and *E* thiohydantoin isomers, **12a** and **12b**, respectively. Although the *Z* isomer **12a** (63% yield) was finally purified by recrystallization (MeOH/ $H_2O$ ), all previously published syntheses of 5-arylidene-2-thiohydantoin systems had reported the production of only the *Z* isomer, with no fractional crystallization or chromatography required.<sup>16,18</sup> Closer inspection of the literature revealed that all the aromatic aldehydes that had previously been used for these aldol condensation reactions were devoid of phenol functionality. Hence, our results suggested that the free phenol interfered with the stereochemical integrity of the aldol reaction.

The development of a synthesis containing a phenol-based product often requires mandatory protection in order to prevent side reactions with oxidizing agents, electrophiles or the reaction of the nucleophilic phenoxide ion with appropriate receptors/reagents.<sup>19</sup> Since the required transamination reaction included the use of an oxidizing agent (TBHP), and basic conditions (aq.  $NH_4OH$ ) that would generate the phenoxide ion, we decided to pursue the remaining synthetic step with one of the phenol-protected thiohydantoin. Ester hydrolysis studies were performed on both the acetate and pivaloate thiohydantoin analogs, **14a** and **15a**, using the proposed literature transamination conditions (MeOH, aqueous  $NH_4OH$ , rt, 72 h) but without the reagent, TBHP.<sup>16</sup> These hydrolysis reactions were monitored using analytical  $C_{18}$  HPLC with samples taken at 1, 2, 4, 8, 24, 48 and 72 h intervals. The acetate analog **14a** was shown to be fully hydrolyzed within the first 2 h, whereas the pivaloate derivative **15a** did not show significant signs of ester hydrolysis until 24 h. Full ester cleavage of **15a** was achieved after 72 h. No side products were detected by HPLC during these hydrolysis studies. The hydrolysis data were consistent with the literature, which had previously reported that the pivaloate group was more resilient than



Scheme 1. Reagents and conditions: (i) NaOAc, AcOH, reflux, 2 h; (ii) TBHP, aq.  $NH_4OH$ , MeOH, rt, 72 h; (iii)  $BBr_3 \cdot S(Me)_2$ , DCE, reflux, 15 min.

the acetate moiety under basic conditions, due to steric hindrance considerations.<sup>19,20</sup> As a result of these studies, the more robust pivaloate thiohydantoin **15a** was chosen for the final transamination step. Literature procedures were initially employed using 3 mol equiv. of TBHP, in aqueous  $\text{NH}_4\text{OH}$  and MeOH.<sup>16,17</sup> After 72 h the reaction mixture was subjected to  $\text{C}_{18}$  flash chromatography followed by  $\text{C}_{18}$  semipreparative HPLC to yield pure synthetic polyandrocarpamine A (**1**, 16% yield). An appreciable amount of pivaloate cleaved starting material **12a** was also recovered from this initial reaction, suggesting the need for increased amounts of TBHP to convert all the thiohydantoin **15a** to either the sulfinic or sulfonic acid reactive intermediate, which undergoes the nucleophilic displacement with  $\text{NH}_4\text{OH}$ .<sup>16,17</sup> Adding 15 mol equiv. of TBHP with the same quantities of aqueous  $\text{NH}_4\text{OH}$  and MeOH afforded, after chromatography, polyandrocarpamine A (**1**) in the more respectable yield of 68%. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data along with the MS, IR and UV spectra of this synthetic 2-aminoimidazolone compound were shown to be identical to that of the natural product, polyandrocarpamine A (**1**).

Synthetic polyandrocarpamine A (**1**) was demethylated using  $\text{BBr}_3 \cdot \text{S}(\text{Me})_2$  in refluxing DCE<sup>21</sup> to yield compound **2** in a yield of 20%. Spectroscopic data for this demethylated synthetic compound was identical to that of the natural product, polyandrocarpamine B (**2**).

Marine metabolites containing either alkyl- or aryl-lidene aminoimidazolone systems have been reported to undergo double bond isomerization under a variety of conditions. The aplysinopsin class of metabolites have been shown to photoisomerize in solution under either UV irradiation or daylight exposure in the laboratory,<sup>14,22</sup> while 10*E*-debrohmohymenialdisine was observed to partially convert to its 10*Z* isomer upon attempted recrystallization using hot MeOH.<sup>23</sup> Due to these literature reports we decided to perform stability studies on both the natural products and the synthetic compounds from this project. All the thiohydantoin analogs **12a–15a** were shown to undergo double bond isomerization to varying degrees when stored in the laboratory at rt in  $\text{DMSO}-d_6$ . The samples **12–15** were analyzed weekly by  $^1\text{H}$  NMR spectroscopy and the relative ratios of the *E* to *Z* isomers (Fig. 3) were calculated by integration of the exocyclic proton singlet, H-6 (Table 3). Apart from the *E* isomers, no other decomposition material was identified during these NMR studies. Interestingly, neither the natural products or synthetic compounds of the polyandrocarpamines showed any degradation or isomerization during the 1D and 2D NMR studies in  $\text{CD}_3\text{OD}$ . Synthetic polyandrocarpamines A and B were also stored

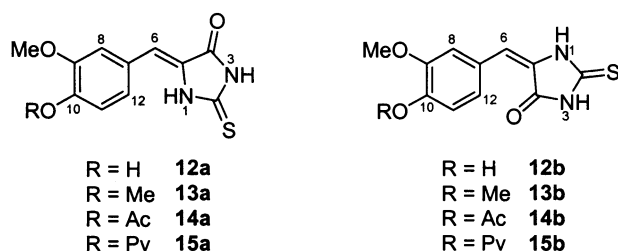


Figure 3. Thiohydantoin isomers **12a/b–15a/b**.

Table 3. Isomerization studies for synthesized thiohydantoin **12a–15a**

Isomers	<i>Z/E</i> ratios			
	Day 7	Day 14	Day 21	Day 28
<b>12a/12b</b>	4:1	4:1	4:1	4:1
<b>13a/13b</b>	10:1	9:1	8:1	6:1
<b>14a/14b</b>	12:1	12:1	11:1	11:1
<b>15a/15b</b>	19:1	16:1	16:1	14:1

Compounds stored on the laboratory bench in  $\text{DMSO}-d_6$  at rt.

in  $\text{DMSO}-d_6$  at rt in the laboratory without degradation or isomerization even after two weeks.

It should also be noted that both polyandrocarpamines A and B were only sparingly soluble in  $\text{CD}_3\text{OD}$ , but that this deuterated solvent afforded the best NMR signal dispersion and linewidths. In analogy to the hymenialdisine class of compounds,<sup>23</sup> the  $^1\text{H}$  NMR spectra of compounds **1** and **2** were found to be concentration dependent. While no doubling of proton signals was detected, the chemical shifts and linewidths did vary depending on the amount of material present in the NMR tube.

### 3. Conclusion

To the best of our knowledge this study represents only the third report on new chemistry from the ascidian genus, *Polyandrocarpa*. Previous investigations have reported the isolation of the antimicrobial guanidine-derived metabolites, the polyandrocarpidines,<sup>24–26</sup> and the  $\alpha$ -carbonyl indole-based alkaloids, polyandrocarpamides A–D.<sup>27</sup> This is the first report of 2-aminoimidazolone-derived compounds from this genus. The synthesis of both polyandrocarpamines A (**1**) and B (**2**) confirmed the spectroscopically assigned structures. The synthetic approach provided larger quantities of these novel secondary metabolites for future biological evaluation, and is also readily amenable to analog production.

### 4. Experimental

#### 4.1. General procedures

NMR spectra were recorded on either a Varian Mercury 400 spectrometer ( $^1\text{H}$ : 399.880 MHz  $^{13}\text{C}$ : 100.559 MHz) at 25°C, or a Varian Unity 500 spectrometer ( $^1\text{H}$ : 500.620 MHz  $^{13}\text{C}$ : 125.893 MHz) at 26°C. The  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts are reported in parts per million relative to the reference solvent peaks at  $\delta$  3.30 and 49.00 ppm for  $\text{CD}_3\text{OD}$ ,  $\delta$  2.49 and 39.51 ppm for  $\text{DMSO}-d_6$ , and  $\delta$  7.26 and 77.00 ppm for  $\text{CDCl}_3$ . FTIR and UV spectra were recorded on a Jasco FT/IR-420 spectrophotometer and a Hewlett Packard 8452A diode array spectrophotometer, respectively. High- and low-resolution mass spectral measurements were performed on a Finnegan MAT 95 high-resolution spectrometer. Melting points were determined using a Laboratory Devices, MEL-TEMP II apparatus and are uncorrected. A glass column (85×45 mm<sup>2</sup>) or SPE cartridge (10×45 mm<sup>2</sup>) packed with J.T. Baker Bakerbond  $\text{C}_{18}$  bonded silica 40  $\mu\text{m}$  60 Å was

used for reversed-phase flash chromatography. A column (25×120 mm<sup>2</sup>) packed with Merck silica 40–63 μm 60 Å was used for normal-phase flash chromatography. Size-exclusion chromatography was performed on Sigma Lipophilic Sephadex LH-20 (45×420 mm<sup>2</sup>) connected to a Spectra/Chrom CF-1 fraction collector. A Beckman Gold solvent module equipped with a 7725i Rheodyne injector and a Beckman 168 PDA detector was used for HPLC separations. Phenomenex Luna C<sub>18</sub>(2) 5 μm 100 Å (analytical: 4.6×250 mm<sup>2</sup>, semipreparative: 10×250 mm<sup>2</sup>) HPLC columns were used. All solvents used for HPLC, UV and MS were Fisher HPLC grade, and the water used was Barnstead E-pure 0.2 μm filtered. All synthetic reagents used were purchased from Sigma-Aldrich.

#### 4.2. Animal material

A specimen of the *Polyandrocarpa* sp. was collected during January of 2001 by SCUBA diving (–10 m) at Great Astrolabe Reef (S 18° 42.569', E 178° 29.889'), Fiji Islands, and kept frozen prior to freeze-drying and extraction. Voucher specimen MZUSP13992 has been deposited at the Museu de Zoologia, Universidade Federal de São Paulo, São Paulo, Brazil. The colonial ascidian consisted of a long stalk and a globular head that was compressed on two sides; the zooids lay in each of these sides and there is a layer of tunic without zooids between them. The high quantity of round orange vesicles in the body wall and in the branchial sac is also striking. Further diagnostic characteristics are: peripharyngeal ring forming a deep 'v' around the dorsal tubercle, which opens in a vertical slit; a small digestive tract, which contains a stomach that has six or seven folds and a small curved caecum, two or three endocarps in the intestinal loop and no other body wall; very large longitudinal vessels on the branchial sac.

#### 4.3. Extraction and isolation

The freeze-dried *Polyandrocarpa* sp. (13.1 g dry wt) was exhaustively extracted with MeOH (3×200 mL) then concentrated under vacuum to yield a dark brown solid (3.38 g). This material was redissolved in 20% MeOH/80% H<sub>2</sub>O (100 mL) and chromatographed on a C<sub>18</sub> bonded silica flash column using a 20% stepwise gradient from 20% MeOH/80% H<sub>2</sub>O to 100% MeOH. The 60% MeOH/40% H<sub>2</sub>O fraction was evaporated to dryness under reduced pressure, dissolved in 100% MeOH (10 mL) and chromatographed on a Sephadex LH-20 column using 100% MeOH as the eluant at a flowrate of 5.0 mL/min. This afforded pure polyandrocarpamines A (**1**, 5.6 mg, 0.04% dry wt) and B (**2**, 30 mg, 0.23% dry wt).

**4.3.1. Polyandrocarpamine A (1).** Stable yellow solid; UV (MeOH) λ<sub>max</sub> 246 (ε 2000), 352 nm (ε 2000); UV (MeOH+NaOH) λ<sub>max</sub> 250 (ε 3000), 404 nm (ε 2000); IR ν<sub>max</sub> (NaCl) 3600–3000, 1666, 1626, 1594, 1566, 1519, 1484, 1364, 1285, 1246, 1166, 1129, 1031, 779 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; (+)-LRFABMS *m/z* (rel. int.) 234 (100); (+)-HRFABMS *m/z* 234.08816 (C<sub>11</sub>H<sub>12</sub>N<sub>3</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 234.08787).

**4.3.2. Polyandrocarpamine B (2).** Stable yellow solid; UV (MeOH) λ<sub>max</sub> 248 (ε 2000), 352 nm (ε 2000); UV

(MeOH+NaOH) λ<sub>max</sub> 248 (ε 2000), 396 nm (ε 2000); IR ν<sub>max</sub> (NaCl) 3600–3000, 1696, 1666, 1633, 1599, 1528, 1486, 1455, 1377, 1335, 1242, 1089, 1047, 970, 863, 839, 760 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 2; (+)-LRFABMS *m/z* (rel. int.) 220 (60); (+)-HRFABMS *m/z* 220.07225 (C<sub>10</sub>H<sub>10</sub>N<sub>3</sub>O<sub>3</sub> [M+H]<sup>+</sup> requires 220.07222).

**4.3.3. Synthesis of 3-acetyl-2-thioxoimidazolidin-4-one (7).** Compound **7** was synthesized using a modified method from Villemin et al.<sup>18</sup> Ac<sub>2</sub>O (10 mL) was added to glycine (750 mg, 10 mmol) and ammonium thiocyanate (760 mg, 10 mmol) and the mixture was heated at reflux for 2 h. After cooling the solution was added to chilled H<sub>2</sub>O (50 mL) and left overnight for crystal formation. The precipitate was then collected, washed with chilled H<sub>2</sub>O (3×15 mL) then dried under vacuum overnight. 3-Acetyl-2-thioxoimidazolidin-4-one was obtained as fine brown needles (905 mg, 57% yield); mp 171–173°C (lit.<sup>18</sup> 175°C); UV (MeOH) λ<sub>max</sub> 234 (ε 16,000), 276 nm (ε 18,000); IR ν<sub>max</sub> (NaCl) 3300–3000, 1813, 1769, 1757, 1674, 1539, 1476, 1408, 1368, 1346, 1330, 1306, 1228, 1200, 1088, 1039, 973, 904, 818, 747, 660, 631, 606, 588, 515 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 2.67 (3H, s, 3-Ac), 4.39 (2H, s, H-5), 12.56 (1H, br s, 1-NH); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 26.6 (3-Ac), 52.2 (C-5), 169.3 (3-Ac), 170.3 (C-4), 182.5 (C-2); LREIMS (70 eV) *m/z* (rel. int.) 43 (100), 116 (100) 158 (100); HREIMS (70 eV) *m/z* 158.01515 (C<sub>5</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>S requires 158.01500).

**4.3.4. Synthesis of 3-methoxy-4-pivaloyloxybenzaldehyde (11).** Pivaloyl chloride (3025 μL, 25 mmol) was added dropwise over 10 min to a stirred and cooled (5°C) solution of anhydrous pyridine (10 mL) and vanillin **8** (760 mg, 5 mmol) over Ar. The resulting mixture was allowed to warm to rt over the next 7 h with constant stirring. The solvents were evaporated to dryness. The remaining residue was resuspended in a 1:5 mix of CHCl<sub>3</sub> and hexanes (10 mL total) and chromatographed on a silica flash column using a 10% stepwise gradient from 100% hexanes to 50% EtOAc/50% hexanes. The 20% EtOAc/80% hexanes wash yielded pure pivaloylated vanillin **11** as a white amorphous solid (824 mg, 70% yield); mp 49–51°C; UV (MeOH) λ<sub>max</sub> 220 (ε 20,000), 258 (ε 11,000), 306 nm (ε 4000); IR ν<sub>max</sub> (NaCl) 1759, 1703, 1600, 1504, 1480, 1464, 1422, 1394, 1368, 1323, 1268, 1200, 1148, 1108, 1031, 958, 943, 889, 866, 841, 805, 780, 755, 733, 644, 598, 580, 549 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 1.38 (9H, s, 4-OPv), 3.88 (3H, s, 3-OMe), 7.18 (1H, d, *J*=8.0 Hz, H-5), 7.47 (1H, d, *J*=8.0 Hz, H-6), 7.48 (1H, s, H-2), 9.94 (1H, s, 1-CHO); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 27.1 (4-OPv), 39.2 (4-OPv), 56.0 (3-OMe), 110.7 (C-2), 123.4 (C-5), 124.8 (C-6), 135.0 (C-1), 145.6 (C-4), 152.1 (C-3), 176.1 (4-OPv), 191.1 (1-CHO); LREIMS (70 eV) *m/z* (rel. int.) 57 (100), 85 (20), 151 (30), 152 (60), 168 (20), 236 (10); HREIMS (70 eV) *m/z* 236.10366 (C<sub>13</sub>H<sub>16</sub>O<sub>4</sub> requires 236.10486).

#### 4.4. General synthetic procedure for thiohydantoin analogs 12a–15a

The appropriate benzaldehyde derivative **8–11** (2.32 mmol) was added to a solution of 3-acetyl-2-thioxoimidazolidin-4-one (**7**, 368 mg, 2.32 mmol) in AcOH (880 μL) and NaOAc

(190 mg, 2.32 mmol). The mixture was heated at reflux for 2 h then allowed to cool to rt. The precipitate was collected, washed with chilled H<sub>2</sub>O (3×5 mL) and dried under vacuum overnight to yield the corresponding thiohydantoin analog.

**4.4.1. Thiohydantoin 12a [(5Z)-5-(3-methoxy-4-hydroxybenzylidene)-2-thioxoimidazolidin-4-one].** Compound **12a** was purified by fractional recrystallization using MeOH/H<sub>2</sub>O (3:1). After filtration and drying under vacuum the thiohydantoin **12a** was obtained as brown needles (367 mg, 63% yield); mp 234–236°C; UV (MeOH)  $\lambda_{\max}$  258 ( $\epsilon$  11,000), 300 ( $\epsilon$  7000), 392 nm ( $\epsilon$  31,000); IR  $\nu_{\max}$  (NaCl) 3600–3000, 1710, 1638, 1588, 1506, 1374, 1349, 1290, 1268, 1213, 1183, 1128, 1097, 1029, 971, 946, 894, 865, 840, 800, 753, 725, 672, 633, 613 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.84 (3H, s, 9-OMe), 6.42 (1H, s, H-6), 6.80 (1H, d, *J*=8.8 Hz, H-11), 7.23 (1H, d, *J*=2.0 Hz, H-8), 7.24 (1H, dd, *J*=8.8, 2.0 Hz, H-12), 9.63 (1H, br s, 10-OH), 12.05<sup>†</sup> (1H, br s, 1-NH), 12.25<sup>†</sup> (1H, br s, 3-NH); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  55.9 (9-OMe), 113.3 (C-6), 114.0 (C-8), 115.8 (C-11), 123.8 (C-7), 124.8 (C-12), 125.3 (C-5), 147.8 (C-9), 148.6 (C-10), 165.9 (C-4), 178.3 (C-2); LREIMS (70 eV) *m/z* (rel. int.) 163 (100), 250 (100); HREIMS (70 eV) *m/z* 250.04013 (C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>S requires 250.04121).

**4.4.2. Thiohydantoin 12b [(5E)-5-(3-methoxy-4-hydroxybenzylidene)-2-thioxoimidazolidin-4-one].** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.79 (3H, s, 9-OMe), 6.51 (1H, s, H-6), 6.77 (1H, d, *J*=8.4 Hz, H-11), 7.35 (1H, dd, *J*=8.4, 2.0 Hz, H-12), 8.16 (1H, d, *J*=2.0 Hz, H-8), 9.70 (1H, br s, 10-OH), 11.92<sup>†</sup> (1H, br s, 1-NH), 12.13<sup>†</sup> (1H, br s, 3-NH).

**4.4.3. Thiohydantoin 13a [(5Z)-5-(3,4-dimethoxybenzylidene)-2-thioxoimidazolidin-4-one].** Yellow solid (504 mg, 82% yield); mp 231–233°C; UV (MeOH)  $\lambda_{\max}$  256 ( $\epsilon$  12,000), 300 ( $\epsilon$  7000), 388 nm ( $\epsilon$  32,000); IR  $\nu_{\max}$  (NaCl) 1718, 1647, 1593, 1512, 1490, 1362, 1331, 1273, 1253, 1229, 1175, 1143, 1084, 1019, 946, 874, 807, 759, 668, 548 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.80 (3H, s, 10-OMe), 3.83 (3H, s, 9-OMe), 6.45 (1H, s, H-6), 6.98 (1H, d, *J*=8.0 Hz, H-11), 7.24 (1H, d, *J*=2.0 Hz, H-8), 7.35 (1H, dd, *J*=8.0, 2.0 Hz, H-12), 12.12 (1H, br s, 1-NH), 12.29 (1H, br s, 3-NH); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  55.6 (10-OMe), 55.8 (9-OMe), 111.7 (C-11), 112.7 (C-6), 113.3 (C-8), 124.4 (C-12), 125.0 (C-7), 125.8 (C-5), 148.8 (C-9), 150.2 (C-10), 165.8 (C-4), 178.6 (C-2); LREIMS (70 eV) *m/z* (rel. int.) 69 (100), 131 (30), 162 (20), 264 (95); HREIMS (70 eV) *m/z* 264.05535 (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S requires 264.05686).

**4.4.4. Thiohydantoin 13b [(5E)-5-(3,4-dimethoxybenzylidene)-2-thioxoimidazolidin-4-one].** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.78<sup>†</sup> (3H, s, 10-OMe), 3.79<sup>†</sup> (3H, s, 9-OMe), 6.54 (1H, s, H-6), 6.97 (1H, d, *J*=8.8 Hz, H-11), 7.46 (1H, dd, *J*=8.8, 2.0 Hz, H-12), 8.14 (1H, d, *J*=2.0 Hz, H-8), 11.97<sup>†</sup> (1H, br s, 1-NH), 12.17<sup>†</sup> (1H, br s, 3-NH).

**4.4.5. Thiohydantoin 14a [(5Z)-5-(3-methoxy-4-acetoxybenzylidene)-2-thioxoimidazolidin-4-one].** Yellow solid (587 mg, 87% yield); mp 252–253°C; UV (MeOH)  $\lambda_{\max}$

248 ( $\epsilon$  10,000), 298 ( $\epsilon$  6000), 370 nm ( $\epsilon$  26,000); IR  $\nu_{\max}$  (NaCl) 1747, 1726, 1711, 1657, 1586, 1511, 1493, 1452, 1427, 1366, 1293, 1270, 1219, 1193, 1165, 1124, 1025, 1011, 948, 918, 897, 831, 682, 616, 540 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.26 (3H, s, 10-OAc), 3.85 (3H, s, 9-OMe), 6.49 (1H, s, H-6), 7.12 (1H, d, *J*=8.4 Hz, H-11), 7.34 (1H, dd, *J*=8.4, 2.0 Hz, H-12), 7.36 (1H, d, *J*=2.0 Hz, H-8), 12.21<sup>†</sup> (1H, br s, 1-NH), 12.39<sup>†</sup> (1H, br s, 3-NH); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  20.4 (10-OAc), 56.1 (9-OMe), 111.1 (C-6), 114.3 (C-8), 122.9 (C-12), 123.2 (C-11), 127.7 (C-5), 131.2 (C-7), 140.0 (C-10), 151.0 (C-9), 165.8 (C-4), 168.4 (10-OAc), 179.3 (C-2); LREIMS (70 eV) *m/z* (rel. int.) 43 (10), 163 (40), 250 (100), 292 (15); HREIMS (70 eV) *m/z* 292.05075 (C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>S requires 292.05178).

**4.4.6. Thiohydantoin 14b [(5E)-5-(3-methoxy-4-acetoxybenzylidene)-2-thioxoimidazolidin-4-one].** <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  2.25 (3H, s, 10-OAc), 3.79 (3H, s, 9-OMe), 6.56 (1H, s, H-6), 7.08 (1H, d, *J*=8.4 Hz, H-11), 7.48 (1H, dd, *J*=8.4, 2.0 Hz, H-12), 8.10 (1H, d, *J*=2.0 Hz, H-8), 12.08<sup>†</sup> (1H, br s, 1-NH), 12.27<sup>†</sup> (1H, br s, 3-NH).

**4.4.7. Thiohydantoin 15a [(5Z)-5-(3-methoxy-4-pivaloyloxybenzylidene)-2-thioxoimidazolidin-4-one].** Pale yellow solid (504 mg, 65% yield); mp 215–217°C; UV (MeOH)  $\lambda_{\max}$  248 ( $\epsilon$  12,000), 296 ( $\epsilon$  8000), 370 nm ( $\epsilon$  32,000); IR  $\nu_{\max}$  (NaCl) 1732, 1653, 1592, 1508, 1489, 1452, 1362, 1318, 1271, 1237, 1180, 1126, 1030, 963, 946, 894, 835, 754, 675, 633, 620, 590 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.29 (9H, s, 10-OPv), 3.83 (3H, s, 9-OMe), 6.48 (1H, s, H-6), 7.09 (1H, d, *J*=9.0 Hz, H-11), 7.36 (1H, dd, *J*=9.0, 2.0 Hz, H-12), 7.37 (1H, d, *J*=2.0 Hz, H-8), 12.22<sup>†</sup> (1H, br s, 1-NH), 12.38<sup>†</sup> (1H, br s, 3-NH); <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  26.8 (10-OPv), 38.6 (10-OPv), 56.2 (9-OMe), 111.2 (C-6), 114.4 (C-8), 123.0 (2C, C-11, C-12), 127.6 (C-5), 131.1 (C-7), 140.5 (C-10), 151.0 (C-9), 165.8 (C-4), 175.7 (10-OPv), 179.3 (C-2); LREIMS (70 eV) *m/z* (rel. int.) 57 (100), 91 (25), 163 (30), 218 (30), 250 (50), 334 (30); HREIMS (70 eV) *m/z* 334.09707 (C<sub>16</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S requires 334.09873).

**4.4.8. Thiohydantoin 15b [(5E)-5-(3-methoxy-4-pivaloyloxybenzylidene)-2-thioxoimidazolidin-4-one].** <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.29 (9H, s, 10-OPv), 3.78 (3H, s, 9-OMe), 6.56 (1H, s, H-6), 7.06 (1H, d, *J*=8.0 Hz, H-11), 7.48 (1H, dd, *J*=8.0, 2.0 Hz, H-12), 8.11 (1H, d, *J*=2.0 Hz, H-8), 12.08<sup>†</sup> (1H, br s, 1-NH), 12.27<sup>†</sup> (1H, br s, 3-NH).

## 4.5. Synthesis of polyandrocaramine A (1)

70% TBHP (385  $\mu$ L, 3.0 mmol) and 30% aqueous NH<sub>4</sub>OH (1 mL) were added to a solution of the pivaloylated thiohydantoin **15a** (66.8 mg, 0.2 mmol) in MeOH (3 mL) and the mixture was stirred for 72 h at rt. The solvents were removed under reduced pressure and the resulting residue chromatographed on a C<sub>18</sub> packed SPE cartridge using 20% stepwise elutions from 20% MeOH/80% H<sub>2</sub>O to 100% MeOH. The 40% MeOH/60% H<sub>2</sub>O fraction was further purified by HPLC using a Phenomenex Luna C<sub>18</sub>(2) semi-preparative column with a linear gradient from 100% H<sub>2</sub>O to 100% MeOH in 20 min at a flowrate of 4 mL/min. This yielded a pure 2-aminoimidazolone compound (32 mg, 68%

<sup>†</sup> Interchangeable signals.

yield), which was spectroscopically identical to the natural product, polyandrocarpamine A (1).

#### 4.6. Synthesis of polyandrocarpamine B (1)

Synthetic polyandrocarpamine A (1, 12.2 mg, 0.052 mmol) was added to a solution of the  $\text{BBr}_3 \cdot \text{S}(\text{Me})_2$  (65 mg, 0.0208 mmol) in anhydrous DCE (3 mL) and the mixture heated at 83°C for 15 min. Upon cooling,  $\text{H}_2\text{O}$  (1 mL) was added and the solvents evaporated to dryness under reduced pressure. The resulting residue was purified by HPLC using a Phenomenex Luna  $\text{C}_{18}(2)$  semipreparative column with a linear gradient from 100%  $\text{H}_2\text{O}$  to 100% MeOH in 20 min at a flowrate of 4 mL/min. This yielded a pure 2-aminoimidazolone compound (2.3 mg, 20% yield), which was spectroscopically identical to the natural product, polyandrocarpamine B (2). A small amount (2.2 mg) of unreacted polyandrocarpamine A was recovered after HPLC.

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