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Antimalarial β-Carbolines from the New Zealand Ascidian *Pseudodistoma opacum*

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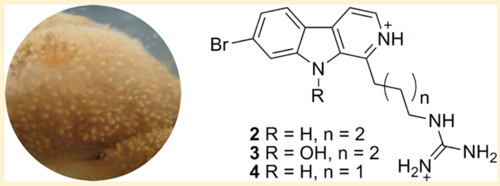
S *Supporting Information*

ABSTRACT: One tetrahydro-β-carboline, (−)-7-bromohomotrypargine (**1**), and three alkylguanidine-substituted β-carbolines, opacalines A, B, and C (**2**−**4**), have been isolated from the New Zealand ascidian *Pseudodistoma opacum*. The structures of the metabolites were determined by analysis of mass spectrometric and 2D NMR spectroscopic data. Natural products **2** and **3**, synthetic debromo analogues **8** and **9**, and intermediate **16** exhibited moderate antimalarial activity toward a chloroquine-resistant strain of *Plasmodium falciparum*, with an IC_{50} range of 2.5−14 μM. The biosynthesis of **1**−**4** is proposed to proceed via a Pictet−Spengler condensation of 6-bromotryptamine and the α-keto acid transamination product of either arginine or homoarginine. Cell separation and ^{1}H NMR analysis of *P. opacum* identified tetrahydro-β-carboline **1** to be principally located in the zooids, while fully aromatized analogues **2**−**4** were localized to the test.



Habitats of ascidians of the genus *Pseudodistoma* (family Pseudodistomidae) are globally well dispersed, with species reported from most oceans and seas of the world.[1] Previous studies of specimens of *Pseudodistoma* ascidians have led to the discovery of a diverse array of nitrogenous secondary metabolites encompassing piperidine, alkyl amine, and amino alcohol, β-carboline, and quinoline alkaloids.[2,3] In particular, New Zealand specimens of *Pseudodistoma* species ascidians have afforded cytotoxic and antifungal alkyl amines from *P. novazelandiae*,[4] purines from *P. cereum*,[5−7] and 6-hydroxyquinoline alkaloids from *P. aureum*.[8] In the context of our continuing study of the chemical diversity of natural products isolated from New Zealand and Antarctic ascidians,[9,10] we have investigated an extract prepared from a Maori Bay, Auckland, New Zealand, collection of *Pseudodistoma opacum* (Brewin, 1950). Herein we report the isolation, structure elucidation, and biological evaluation against a panel of tropical parasitic diseases of four new β-carboline alkaloids: (−)-7-bromohomotrypargine (**1**) and opacalines A−C (**2**−**4**). To aid in establishing a structure−activity relationship for the natural products, debromo analogues of opacalines A and C, **8** and **9**, were synthesized and biologically evaluated, details of which are also presented.

RESULTS AND DISCUSSION

Specimens of *P. opacum*, collected from intertidal rocks at Maori Bay, Auckland, were extracted with MeOH and fractionated repeatedly by reversed-phase C_{18} flash and Sephadex LH-20 column chromatography eluting with solvents acidified with TFA, yielding (−)-7-bromohomotrypargine (**1**) and opacalines A−C (**2**−**4**) as brown or yellow oils.

The positive ion ESI mass spectrum for **1** contained a pseudomolecular ion cluster at m/z 364 and 366, with ions in a 1:1 ratio, indicating the presence of bromine in the structure. High-resolution ESIMS data were consistent with a free base molecular formula for **1** of $C_{16}H_{22}BrN_5$ requiring eight degrees of unsaturation. All 16 carbons required by the molecular formula were accounted for in the ^{13}C NMR spectrum (Table 1). Combined analysis of ^{13}C chemical shifts and results of a multiplicity-edited HSQC NMR experiment established the presence of three olefinic methine, six olefinic quaternary, one alkyl methine, and six alkyl methylene carbon environments in the molecule. The ^{1}H NMR spectrum (CD_3OD) contained resonances attributable to a 1,2,4-trisubstituted benzene ring (δ_H 7.52−7.18), a highly deshielded aliphatic methine (δ_H 4.71), three deshielded methylenes (δ_H 3.72−3.06, 6H), and three alkyl-substituted methylenes (δ_H 2.26−1.65, 6H). COSY NMR data identified the presence of three separate proton spin systems: a 1,2,4-trisubstituted benzene ring [δ_H 7.52 (d, J = 1.5 Hz), 7.41 (d, J = 8.4 Hz), 7.18 (dd, J = 8.4, 1.5 Hz)], an alkyl spin system comprised of a pair of diastereotopic protons at δ_H 3.72 and 3.47 and a methylene resonance at δ_H 3.06, and a third spin system extended from the highly deshielded aliphatic methine resonance at δ_H 4.71 to a pair of diastereotopic protons at δ_H 2.26, 2.00, through two methylene proton resonances at δ_H 1.65 and 1.71, before terminating at a fourth methylene proton resonance at δ_H 3.24. HMBC correlations observed from the aromatic proton resonance at δ_H 7.52 (H-8) to δ_C 126.5 (C-4b) and from δ_H 7.41

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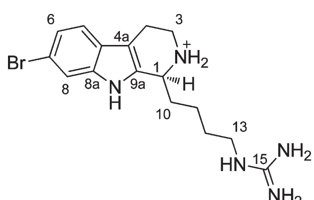
Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz), COSY, and HMBC NMR Data for 7-Bromohomotrypargine (1)[a]

position	δ_C	δ_H (J in Hz)	COSY	HMBC[b]
1	54.6	4.71, br m	10	9a, 11
3	42.8	3.72, dt (12.5, 4.7)	4	1, 4, 4a
		3.47, m		
4	19.4	3.06, m	3	3, 4a, 9a
4a	107.8			
4b	126.5			
5	120.7	7.41, d (8.4)	6	4a, 4b, 7, 8a
6	124.0	7.18, dd (8.4, 1.5)	5, 8	4b, 7, 8
7	117.0			
8	115.3	7.52, d (1.5)	6	4b, 6, 7
8a	139.1			
9a	131.0			
10	32.9	2.26, m	11	1, 9a, 11, 12
		2.00, m		
11	23.4	1.65, m	10, 12	1, 10, 12, 13
12	29.8	1.71, m	11, 13	10, 11, 13
13	42.2	3.24, t (7.0)	12	11, 12, 15
15	158.7			

[a] Spectra recorded in CD_3OD at 27 °C. [b] HMBC correlations, optimized for 8.3 Hz, are reported from the proton resonance to the indicated carbon resonance(s).

(H-5) to carbons at δ_C 139.1 (C-8a) and 107.8 (C-4a) allowed the construction of an indole substructure. The proton spin system, comprised of two contiguous methylene groups, was connected to the indole ring substructure at C-4a by virtue of HMBC correlations being observed between δ_H 3.06 (H_2-4) and δ_H 3.72/3.47 (H-3a and H-3b) and C-4a (δ_C 107.8). An additional HMBC correlation observed between H_2-4 and a carbon resonance at δ_C 131.0 was useful in establishing connectivity to the remaining aliphatic spin system. This spin system, comprised of a deshielded aliphatic methine and four contiguous methylene resonances, was placed at C-1 of a 1,2,3,4-tetrahydro-β-carboline ring system, on the basis of the observed HMBC correlation from the methine proton resonance (δ_H 4.71) to δ_C 131.0, assigned as C-9a. Finally, a HMBC NMR correlation observed between the terminating spin system methylene proton resonance (δ_H 3.24, H_2-13) and a carbon signal at δ_C 158.7 (C-15) indicated the presence of a guanidine group at the end of the side chain.[11,12] The presence of the guanidine group was confirmed by a positive Sakaguchi test. The planar structure of **1** was completed by placement of the bromine atom at C-7 (δ_C 117.0).[11] This structure is a homologue of the recently reported sponge metabolite (+)-1*R*-7-bromotrypargine (**5**), with **1** containing one additional methylene residue in the guanidinylated side chain.[11] There was excellent agreement between the ^{1}H and ^{13}C NMR data reported for **5** and those determined for **1** in the current study, providing further evidence for the structure of the new alkaloid. Structurally related trypargine metabolites 1-carboxytrypargine (**6**) and trypargimine (**7**) have been reported from the ascidian *Eudistoma* sp.[12] We observed **1** to be levorotatory with $[\alpha]_D$ −35.7 (*c* 0.056, MeOH), which compares well with the reported value of $[\alpha]_D$ −37.3 (*c* 1.00, MeOH) for African frog skin-sourced (−)-1*S*-trypargine, the configuration of which has been defined by analysis of electronic circular dichroism spectra[13] and asymmetric synthesis.[14] On this basis, we conclude that (−)-**1** most likely also has the 1*S* absolute configuration.

Opacaline A (**2**) was isolated as a yellow, oily ditrifluoroacetate salt. Positive ion FAB mass spectrometric analysis of **2** determined a free base molecular formula of $C_{16}H_{18}BrN_5$ (m/z 360.0828 and 362.0809 $[M + H]^+$), four mass units less than **1**. The ^{1}H NMR spectrum (CD_3OD) of opacaline A was superficially similar to that observed for tetrahydro-β-carboline **1**, with the noticeable absence of H-1, H_2-3, and H_2-4 resonances and the presence of two mutually coupled pyridine protons, H-3 (δ_H 8.38, d, J = 6.2 Hz) and H-4 (δ_H 8.55, d, J = 6.2 Hz) (Table 2). ^{1}H−^{13}C HMBC NMR correlations from the proton at δ_H 8.32 (H-5) to the quaternary carbon resonances at δ_C 145.9 (C-8a), 134.8 (C-4a), and 127.3 (C-7) and from the proton resonance at δ_H 7.96 (H-8) to both a quaternary carbon at δ_C 120.6 (C-4b) and the protonated carbon resonance at δ_C 126.7 (C-6) defined the chemical shifts of the 1,2,4-trisubstituted benzene ring of **2**. In addition, this latter proton resonance showed a long-range ^{1}H−^{15}N NMR heteronuclear correlation to a ^{15}N resonance at δ_N 120.6 (N-9), confirming the presence of the indole ring moiety.[15] The fully aromatized β-carboline nature of opacaline A was established on the basis of the observation of ^{1}H−^{13}C HMBC NMR correlations from H-4 (δ_H 8.55) to the quaternary sp^2 carbons at δ_C 135.5 (C-9a), 130.6 (C-3), and 120.6 (C-4b) and to a ^{15}N resonance at δ_N 190.0 (N-2) in the ^{1}H−^{15}N HMBC NMR spectrum. The upfield shifts of N-2 (δ_N 190.0) and H-3 (δ_H 8.38) are consistent with the pyridine ring of **2** being a pyridinium salt.[16] Combined analysis of COSY, HSQC, and HMBC spectroscopic data established the presence of a 4-substituted 1-butylguanidine side chain located at C-1. COSY and HSQC NMR data established a contiguous chain of four methylenes, starting at δ_H 3.45 (δ_C 30.9), through to a second methylene at δ_H 1.98 (δ_C 27.0), which in turn correlated to a third methylene signal at δ_H 1.75 (δ_C 29.6), and finally terminating at the methylene resonance at δ_H 3.25 (δ_C 42.0). The presence of long-range heteronuclear correlations from δ_H 1.98 (H_2-11) to C-1 (δ_C 143.1) and δ_H 3.45 (H_2-10) to C-1, C-9a (δ_C 135.5) and N-2 (δ_N 190.0) confirmed the connection of the butyl aliphatic chain at C-1 of the β-carboline ring, while an HMBC NMR correlation from H_2-13 (δ_H 3.25) to a carbon resonance at δ_C 158.7 supported the



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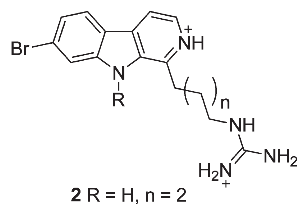
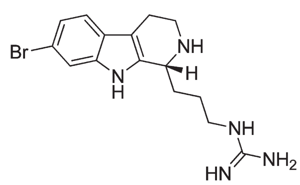


2 R = H, n = 2
3 R = OH, n = 2
4 R = H, n = 1



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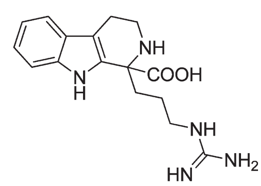

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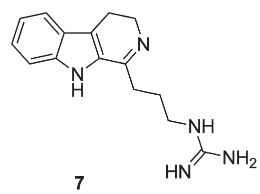

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Table 2. ^{1}H (600 MHz), ^{13}C (150 MHz), ^{15}N (40.6 MHz), and HMBC NMR Data for Opacalines A (**2**), B (**3**), and C (**4**)[a]

	opacaline A (**2**)			opacaline B (**3**)			opacaline C (**4**)		
position	δ_C/δ_N[b]	δ_H (*J*, Hz)	HMBC[c]	δ_C/δ_N[b]	δ_H (*J*, Hz)	HMBC[c]	δ_C[d]	δ_H (*J*, Hz)	HMBC[c]
1	143.1			142.9			142.6		
2	190.0		3, 4, 10	191.9		3, 4, 10	nd[e]		
3	130.6	8.38, d (6.2)	1, 2, 4, 4a	131.1	8.39, d (6.0)	1, 2, 4, 4a	131.2	8.37, d (6.2)	1, 4, 4a
4	117.0	8.55, d (6.2)	2, 3, 4b, 9a	117.1	8.57, d (6.0)	1, 2, 3, 4b, 9, 9a	116.6	8.47, d (6.2)	9a
4a	134.8			130.9			134.3		
4b	120.6			116.5			120.5		
5	125.8	8.32, d (8.6)	4a, 7, 8a	125.7	8.33, d (8.4)	4a, 4b, 7, 8a	125.2	8.29, d (8.5)	4a, 7, 8a
6	126.7	7.59, dd (8.6, 1.5)	4b, 8	127.1	7.62, dd (8.4, 1.5)	4b, 7, 8	126.3	7.57, dd (8.5, 1.1)	4b, 8
7	127.3			127.8			126.7		
8	116.9	7.96, br s	4b, 6, 9	114.0	7.99, d (1.5)	4b, 6, 7, 8a, 9	116.6	7.94, br s	4b, 6
8a	145.9			144.5			145.6		
9	120.6		8	164.6		4, 5, 8	nd		
9a	135.5			132.8			135.5		
10	30.9	3.45, t (7.8)	1, 2, 9a, 11, 12	30.9	3.63, t (7.7)	1, 2, 9a, 11, 12	28.6	3.45, t (7.4)	1, 9a, 11, 12
11	27.0	1.98, m	1, 10, 13	29.6	2.03, m	1, 10, 12, 13	28.3	2.20, tt (7.4, 7.4)	1, 10, 12
12	29.6	1.75, tt (7.4, 7.4)	10, 11, 13, 14	28.4	1.79, tt (7.4, 7.4)	10, 11, 13, 14	41.6	3.37, t (7.4)	10, 11, 14
13	42.0	3.25, t (7.4)	11, 12, 14, 15	42.0	3.26, t (7.4)	11, 12, 14, 15	nd		
14	84.0		12, 13	82.3		12, 13	158.8		
15	158.7			158.7					

[a] Spectra recorded in CD_3OD at 27 °C. [b] ^{15}N chemical shifts determined indirectly from $^{1}H-^{15}N$ HMBC NMR experiments optimized for $^{x}J_{NH}$ = 6.0 Hz and referenced externally to liquid NH_3 using urea as an external standard. [c] HMBC correlations, optimized for 8.3 Hz ($^{1}H-^{13}C$) or 6.0 Hz ($^{1}H-^{15}N$), are reported from the proton resonance to the indicated carbon/nitrogen resonance(s). [d] ^{13}C NMR chemical shifts determined indirectly from $^{1}H-^{13}C$ HMBC NMR data. [e] nd: not determined.

presence of a guanidinium group (Sakaguchi positive) at the terminus of the aliphatic chain. As with **1**, the planar structure of **2** was completed by placement of the bromine atom at C-7 (δ_C 127.3).[17] Thus the structure of opacaline A (**2**) represents the fully aromatized analogue of 7-bromohomotrypargine (**1**).

The pseudomolecular ion cluster observed in the HRFAB mass spectrum of opacaline B (**3**) (*m*/*z* 376.0770 and 378.0748 $[M + H]^+$) established a free base molecular formula of $C_{16}H_{18}BrN_5O$, differing from **2** by the inclusion of an oxygen atom. Comparative analysis of ^{13}C chemical shifts (CD_3OD) of **3** with those observed for opacaline A (**2**) highlighted differences centered upon the pyrrole ring of the β-carboline skeleton, while the only discernible difference in ^{1}H chemical shifts was a 0.18 ppm downfield shift observed for the H_2-10 resonance of **3** (Table 2). Comparison of ^{15}N NMR chemical shifts observed for **3**, again indirectly acquired from a $^{1}H-^{15}N$ HMBC NMR experiment, with those observed for opacaline A identified a noticeable difference for the N-9 resonance (δ_N 120.6 (**2**), 164.6 (**3**)), establishing opacaline B to be the N-9 hydroxy analogue of opacaline A. A similar magnitude change in the chemical shift of N-9 versus N-9 hydroxyl β-carboline alkaloids has been previously reported.[15,18]

Mass spectrometric analysis of opacaline C (**4**) (HRFABMS *m*/*z* 346.0669 and 348.0649 $[M + H]^+$) determined a free base molecular formula for the alkaloid of $C_{15}H_{16}BrN_5$, being 14 mass units (one methylene) less than that observed for opacaline A. Comparative analysis of ^{1}H and ^{13}C NMR chemical shifts, $^{1}H-^{1}H$ spin systems determined by COSY, and $^{1}H-^{13}C$ connectivities determined by multiplicity edited HSQC and HMBC NMR experiments (Table 2) rapidly established that opacaline C (**4**) was one methylene unit shorter in the alkyl guanidinium side chain than opacaline A (**2**), making the new metabolite the fully oxidized β-carboline analogue of 7-bromotrypargine (**5**).[11]

Further confirmation of the structures of opacalines A and C was achieved by the synthesis of debromo analogues **8** and **9** (Scheme 1). The same reaction sequence was used for the synthesis of both model compounds. In the case of **8**, the sequence commenced with the Pictet−Spengler reaction of phthalimide-protected aldehyde **10** with tryptamine to yield 1,2,3,4-tetrahydro-β-carboline **11**. Oxidation with DDQ afforded β-carboline **12** in low yield. Removal of the phthalimide protecting group, to yield amine **13**, was achieved by reaction with hydrazine in EtOH, with subsequent installation of the terminal guanidine group being undertaken using a two-step sequence via the di-*tert*-butoxycarbonyl-protected intermediate **14**. Removal of protecting groups afforded debromo opacaline A (**8**), the spectroscopic data for which were in complete agreement with the expected structure. The debromo analogue of opacaline C, **9**, was prepared in a similar fashion (see Experimental Section for details).

Biological evaluation of **2**, **3**, **8**, **9**, and **16** against the neglected disease parasite targets *Trypanosoma brucei rhodesiense*, *T. cruzi*, *Leishmania donovani*, and *Plasmodium falciparum* (K1 chloroquine-resistant strain) established that opacalines A (**2**) and B (**3**) exhibited moderate activity toward *P. falciparum* (IC_{50} 2.5 and 4.5 μM, respectively), while being only poorly cytotoxic toward the nonmalignant L6 cell line (Table 3). Similar levels of antimalarial activity, and selectivity versus the L6 cell line, were observed for the debromo analogues **8** and **9** and also for the phthalimide-protected 1,2,3,4-tetrahydro-β-carboline intermediate **16**. These results, when combined with the previously reported antimalarial activity of (−)-7-bromotrypargine (**5**) (IC_{50} 5.4 μM (Dd2 chloroquine-resistant), 3.5 μM (3D7 chloroquine-sensitive))[11]

Scheme 1. Synthesis of β-Carboline Model Compounds 8 and 9

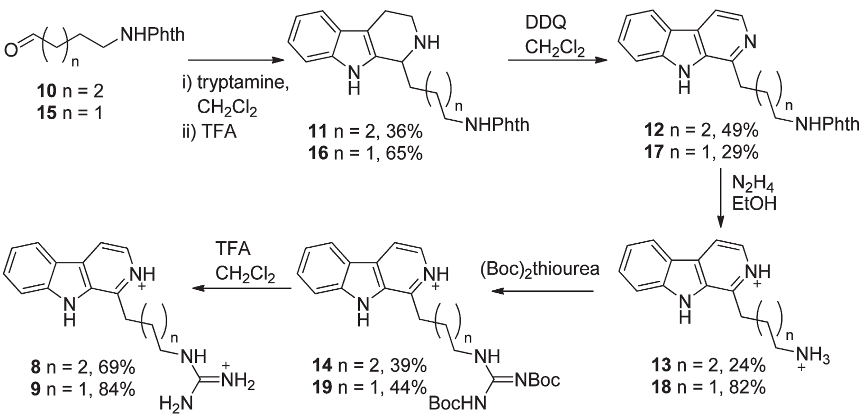


Table 3. Antiparasitic and Cytotoxic Activities of 2, 3, 8, 9, and 16

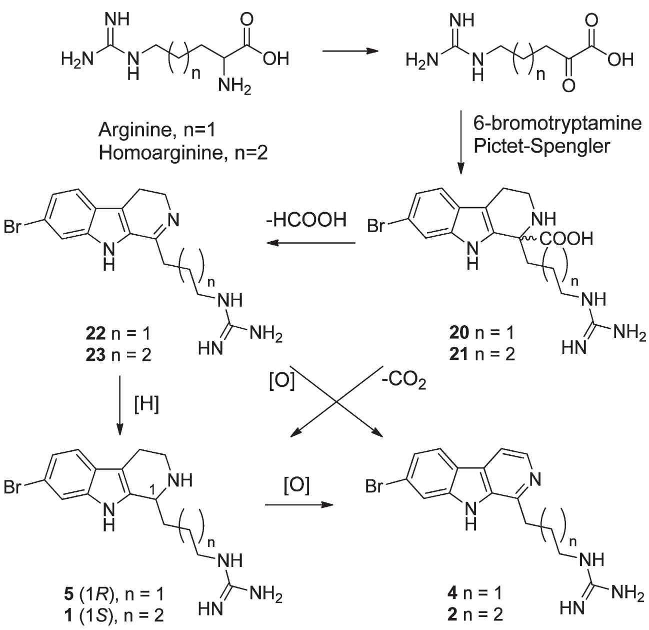
	IC$_{50}$ (μM)				
	T.b.rhod.[a]	*T. cruzi*[b]	*L.don.*[c]	*P. falc.* K1[d]	L6[e]
2	30	86	130	2.5	79
3	27	107	101	4.5	120
8	12	110	>200	6.4	84
9	7.7	130	>200	7.8	101
16	17	51	150	14	110
melarsoprol[f]	0.005				
benznidazole[f]		1.8			
miltefosine[f]			0.53		
chloroquine[f]				0.28	
podophyllotoxin[f]					0.019

[a] *Trypanosoma brucei rhodesiense*, STIB 900 strain, trypomastigotes stage. [b] *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage. [c] *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage. [d] *Plasmodium falciparum*, K1 strain, IEF stage. [e] L6 rat skeletal myoblast cell line. [f] Melarsoprol, benznidazole, miltefosine, chloroquine, and podophyllotoxin were used as positive controls. IC$_{50}$ values reported are the average of two independent assays.

suggest little influence of β-carboline oxidation state, N-hydroxylation, presence or absence of bromine, length of side chain (C$_3$/C$_4$), or presence or absence of a guanidine group on the observed antimalarial activity of this small set of compounds. Of the compounds tested, **9** was the most potent inhibitor of *Trypanosoma brucei rhodesiense*, a causative agent of human Africa trypanosomiasis. None of the compounds exhibited activity toward the other two parasite species, *T. cruzi* and *Leishmania donovani*.

There are two accepted biosynthetic routes to β-carboline alkaloids. The first of these is more completely characterized, whereby enzyme-mediated Pictet−Spengler condensation between aldehyde and tryptamine reactants followed by rounds of oxidation yield the β-carboline skeleton.[19] An alternative route for the biogenesis of the simple 1-methyl-β-carboline alkaloid harman or the 1-methyl-1-carboxy-1,2,3,4-tetrahydro-β-carboline eleagnine has been found to proceed in the plants *Passiflora edulis* and *Eleagnus angustifolia* via a condensation of tryptamine with an α-keto acid to yield eleagnine, with subsequent decarboxylation and oxidation yielding harman.[20] The isolation of **1**, **2**, and **4** in the current study adds to the body of evidence for the operation of the latter pathway in ascidians of the genera *Pseudodistoma*, *Eudistoma*,[12] and *Lissoclinum*.[21] A plausible biosynthesis of **1**, **2**, **4**, and **5** (Scheme 2) proceeds via the transamination of arginine or homoarginine (or their biosynthetic precursors) to the respective α-keto acids, which by condensation with 6-bromotryptamine yields 1-carboxy analogues **20** and **21**. Loss of formic acid from **20** and **21** would afford 3,4-dihydro-β-carbolines **22** and **23**,[12] while the action of decarboxylase(s) would yield 1,2,3,4-tetrahydro-β-carbolines **5** and **1**. Oxidation of imines **22** and **23** would directly afford opacalines A (**2**) and C (**4**), with an alternative route of stereoselective reduction (**22**, **23** to **5**, **1**) and oxidation (**5**, **1** to **4**, **2**) also being plausible. Of the natural products, and anticipated natural products, shown in Scheme 2, debromo analogues of **20** and **22** are metabolites of a *Eudistoma* sp. ascidian,[12] and the 1*R* stereoisomer of **5** is a sponge metabolite,[11] as noted earlier. MS analysis of fractions isolated during the purification of **1**−**4** failed to identify the presence of the putative

Scheme 2. Proposed Biosynthetic Pathway to Natural Products 1, 2, 4, and 5



1-carboxy (**20**, **21**) or imine-containing (**22**, **23**) precursors. It is relevant to note that an L-amino acid oxidase (transaminase) enzyme exhibiting substrate selectivity for L-arginine and L-lysine, as required in the first step of this proposed biosynthetic scheme, has been identified in a marine organism.[22] Escapin, isolated and sequenced from the purple ink secretion of the sea hare *Aplysia californica*, was predominantly selective for L-lysine and L-arginine (precursors of **1**, **2**, **4**, and **5**). L-Tyrosine, and L-histidine, the likely biosynthetic precursors of eudistomins Y_1-Y_7 (*Eudistoma* sp.),[23] and lissoclin C (*Lissoclinum* sp.),[21] respectively, were considered low-quality substrates, and the remaining 17 amino acids tested were poor substrates.

The pale yellow colored zooids of *P. opacum* are relatively pronounced and easily removed from the opaque test by gentle squeezing of fresh ascidian. Extracts of separated zooids and test identified **1** to be the dominant metabolite present in the zooids, while fully oxidized β-carbolines **2**−**4** were concentrated in the test. The ecological consequences of this translocation of specific metabolites warrant further study.

In conclusion, β-carbolines (−)-(1*S*)-**1** and **2**−**4** were isolated from the New Zealand ascidian *P. opacum* and shown to exhibit modest activity toward a chloroquine-resistant strain of *Plasmodium falciparum*. The relatively straightforward synthesis of the compound class, as exemplified by debromo analogues **8** and **9**, provides the foundation for further exploration of the structure−activity relationship of these bioactive natural products.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter using a 0.1 dm cell. Ultraviolet−visible spectra were run as MeOH solutions on a UV-2102 PC Shimadzu UV−vis scanning spectrophotometer. Infrared spectra were recorded using a Perkin-Elmer spectrum One Fourier-transform IR spectrometer as a dry film. NMR spectra were recorded on either a Bruker Avance DRX-600 spectrometer operating at 600 MHz for ^{1}H nuclei and 150 MHz for ^{13}C nuclei, a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei, or a Bruker Avance DRX-300 spectrometer operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.43; CD_3OD: δ_H 3.30, δ_C 49.05; $CDCl_3$: δ_H 7.25, δ_C 77.0). Standard Bruker pulse sequences were utilized. ^{15}N NMR chemical shifts were obtained indirectly by interpretation of ^{1}H−^{15}N HMBC NMR data that had been acquired with $^xJ_{NH}$ = 6.0 Hz and referenced externally to liquid NH_3 using urea as an external standard. HRMS data were acquired on either a VG-7070 or a Bruker micrOTOF Q II mass spectrometer. Flash column chromatography was performed using reversed-phase Merck Lichroprep RP-18 (40−63 μm), Davisil silica gel (40−63 μm), and size exclusion chromatography on Pharmacia Biotech Sephadex LH-20. Analytical reversed-phase HPLC was run on either a Waters 600 HPLC photodiode array system using an Alltech C_8 column (3 μm Econosphere Rocket, 33 × 7 mm) (system A) or a Dionex UltiMate 3000RS using a Grace C_8 column (3 μm Platinum, 33 × 7 mm) (system B) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min and monitoring at 330 nm.

Animal Material. Specimens of the ascidian *Pseudodistoma opacum* were collected by hand at low tide from intertidal rocks at Maori Bay, Auckland, New Zealand (36°50′9.57″ S, 174°25′34.48″ E) on March 10, 2008, and kept frozen until used. A voucher specimen of the organism, taxonomically identified by one of us (M.P.), is held at the School of Chemical Sciences, University of Auckland as 2008MB1-1.

Isolation and Purification. Frozen specimens (wet weight 69.0 g) were immersed repeatedly in MeOH (5 × 200 mL) overnight, filtered, and concentrated in vacuo until the extract was colorless. The combined green extract (2.85 g) was subjected to reversed-phase C_{18} flash column chromatography eluting with a step gradient from H_2O to MeOH. Three fractions (25%, 50%, and 75% MeOH in H_2O) were combined and subjected to repeated C_{18} flash column chromatography with a step gradient from H_2O (0.05% TFA) to MeOH (0.05% TFA) until the fractions eluted were exclusively mixtures of related compounds, as judged by HPLC retention times and HPLC-PDA UV−visible spectrum traces. The first mixture (eluting with 10−20% MeOH in H_2O (0.05% TFA)), with t_R around 5.5 min and no UV absorbances greater than 300 nm, was subjected to another round of purification by C_{18} flash column chromatography, by first eluting with H_2O (0.05% TFA) before being flushed from the column with MeOH (0.05% TFA). This MeOH fraction was subjected to further purification using Sephadex LH20 eluting with MeOH (0.05% TFA) to yield (−)-7-bromohomotrypargine (**1**) (0.56 mg, 0.0008% wet weight). The second mixture (20−40% MeOH in H_2O (0.05% TFA)), t_R around 6.0 min and distinctive HPLC-PDA UV−visible spectrum absorbance maxima at 350−400 nm, was subjected to column chromatography using Sephadex LH20 eluting with MeOH (0.05% TFA) to obtain a yellow band, which was further purified by reversed-phase C_{18} flash column chromatography eluting with a constant solvent mixture of 20% MeOH/H_2O to afford, in order of elution, opacaline A (**2**) (1.94 mg, 0.0028%), opacaline B (**3**) (6.43 mg, 0.0093%), and opacaline C (**4**) (~0.5 mg, ~0.0007%).

*(−)-7-Bromohomotrypargine (**1**):* brown oil; t_R 5.18 min (system B); $[\alpha]_D$ −35.7, $[\alpha]_{365}$ −75, $[\alpha]_{436}$ −22 (*c* 0.056, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.37), 284 (3.74), 294 sh (3.69) nm; ^{1}H and ^{13}C NMR data, see Table 1; (+)-ESIMS *m/z* 364 (57%), 366 (43%) $[M + H]^+$, (+)-HRESIMS *m/z* 364.1110 $[M + H]^+$ (calcd for $C_{16}H_{23}{}^{79}BrN_5$, 364.1131), 366.1144 (calcd for $C_{16}H_{23}{}^{81}BrN_5$, 366.1111).

*Opacaline A (**2**):* yellow oil; t_R 6.08 min (system A); UV (MeOH) λ_{max} (log ε) 207 (3.83), 248 (4.14), 311 (3.93), 367 (3.33) nm; IR ν_{max} (ATR) 3449, 3366, 3189, 2925, 2861, 1668, 1634, 1435 cm^{-1}; ^{1}H, ^{13}C, and ^{15}N NMR data, see Table 2; (+)-FABMS *m/z* 360 (48%), 362 (52%) $[M + H]^+$; (+)-HRFABMS *m/z* 360.0828 $[M + H]^+$ (calcd for $C_{16}H_{19}{}^{79}BrN_5$, 360.0824), 362.0809 (calcd for $C_{16}H_{19}{}^{81}BrN_5$, 362.0803).

*Opacaline B (**3**):* yellow oil; t_R 5.68 min (system A); UV (MeOH) λ_{max} (log ε) 223 (4.49), 257 (4.70), 264 (4.68), 271 sh (4.57), 314 (4.45), 381 (3.83) nm; (MeOH/KOH) λ_{max} (log ε) 219 (4.32), 248 sh (4.44), 259 (4.47), 282 (4.53), 307 sh (3.90), 328 sh (3.58), 422 (3.36) nm; IR ν_{max} (ATR) 3369, 3196, 2915, 2860, 1693, 1618, 1436 cm^{-1}; ^{1}H, ^{13}C, and ^{15}N NMR data, see Table 2; (+)-FABMS *m/z* 376 (51%), 378 (49%) $[M + H]^+$; (+)-HRFABMS *m/z* 376.0770 $[M + H]^+$ (calcd for $C_{16}H_{19}{}^{79}BrN_5O$, 376.0773), 378.0748 (calcd for $C_{16}H_{19}{}^{81}BrN_5O$, 378.0753).

*Opacaline C (**4**):* yellow oil; t_R 6.07 min (system A); ^{1}H and ^{13}C NMR data, see Table 2; (+)-FABMS *m/z* 346 (47%), 348 (53%) $[M + H]^+$; (+)-HRFABMS *m/z* 346.0669 $[M + H]^+$ (calcd for $C_{15}H_{17}{}^{79}BrN_5$, 346.0667), 348.0649 (calcd for $C_{15}H_{17}{}^{81}BrN_5$, 348.0647).

*2-(4-(2,3,4-Tetrahydro-1H-pyrido[3,4-b]indol-1-yl)butyl)isoindoline 1,3-dione (**11**).*[24,25] 5-(1,3-Dioxoisoindolin-2-yl)pentanal (**10**)[25] (0.16 g, 0.70 mmol) and tryptamine (0.17 g, 1.06 mmol) were dissolved in CH_2Cl_2 (7 mL) and cooled to −78 °C while stirring under a nitrogen atmosphere. TFA (0.11 mL, 0.16 g, 1.41 mmol) was added to the cooled, stirring mixture, and it was allowed to warm to room temperature overnight. Triethylamine (0.29 mL, 0.21 g, 2.08 mmol) was added and stirred for 15 min. The resulting mixture was washed with H_2O (10 mL). The organic layer was dried with anhydrous $MgSO_4$ and the solvent evaporated in vacuo. Purification by silica gel column chromatography eluting with MeOH (0−6%) in CH_2Cl_2 gave the product as a yellow glass (0.094 g, 36%): mp 55−57 °C (dec); R_f (5% MeOH/CH_2Cl_2) 0.42; IR ν_{max} (ATR) 3385, 2936, 2858, 1768, 1646, 1616, 1465, 1366 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.23 (1H, br s, NH-9), 7.83 (2H, m, H-17, H-20), 7.70 (2H, m, H-18, H-19), 7.45 (1H, d,

J = 7.7 Hz, H-5), 7.35 (1H, d, J = 7.9 Hz, H-8), 7.14 (1H, td, J = 7.7, 1.1 Hz, H-7), 7.07 (1H, td, J = 7.9, 1.1 Hz, H-6), 4.14 (1H, m, H-1), 3.74 (2H, m, H_2-13), 3.34 (1H, m, H-3a), 3.05 (1H, m, H-3b), 2.75 (2H, m, H_2-4), 1.92 (2H, m, H_2-10), 1.77 (2H, m, H_2-12), 1.49 (2H, m, H_2-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.6 (C-15, C-22), 135.8 (C-8a), 135.2 (C-9a), 134.0 (C-18, C-19), 132.0 (C-16, C-21), 127.3 (C-4b), 123.2 (C-17, C-20), 121.5 (C-7), 119.2 (C-6), 118.0 (C-5), 110.8 (C-8), 108.7 (C-4a), 52.5 (C-1), 42.4 (C-3), 37.1 (C-13), 33.4 (C-10), 28.3 (C-12), 22.2 (C-4), 22.2 (C-11); (+)-FABMS m/z 374 $[M + H]^+$; (+)-HRFABMS m/z 374.1865 $[M + H]^+$ (calcd for $C_{23}H_{24}N_3O_2$, 374.1868).

*2-(4-(9H-Pyrido[3,4-b]indol-1-yl)butyl)isoindoline-1,3-dione (**12**).* 2-(4-(2,3,4-Tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)butyl)isoindoline-1,3-dione (**11**) (0.10 g, 0.27 mmol) was dissolved in CH_2Cl_2 (20 mL), and DDQ (1.22 g, 5.37 mmol) was added. The reaction suspension was stirred at 40 °C for 7 min. The suspension was then washed with 1 M KOH until the aqueous layer was slightly yellow. The organic layer was dried with anhydrous $MgSO_4$, and the solvent removed in vacuo. Purification by silica gel column chromatography eluting with MeOH (0−1%) in CH_2Cl_2 gave 2-(4-(9*H*-pyrido[3,4-*b*]indol-1-yl)butyl)isoindoline-1,3-dione (**12**) as a yellow oil (0.048 g, 49%): R_f (50% EtOAc/hexanes) 0.18, (1% MeOH/CH_2Cl_2) 0.24; IR ν_{max} (ATR) 3330, 3059, 2947, 1768, 1700, 1624, 1455, 1362 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.48 (1H, br s, NH-9), 8.34 (1H, d, J = 5.3 Hz, H-3), 8.10 (1H, d, J = 7.8 Hz, H-5), 7.87 (2H, m, H-17, H-20), 7.80 (1H, d, J = 5.3 Hz, H-4), 7.72 (2H, m, H-18, H-19), 7.68 (1H, d, J = 8.3 Hz, H-8), 7.58 (1H, td, J = 7.8, 1.1 Hz, H-7), 7.27 (1H, m, H-6), 3.94 (2H, t, J = 6.1 Hz, H_2-13), 3.28 (2H, m, H_2-10), 1.92 (2H, m, H_2-12), 1.83 (2H, m, H_2-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2 (C-15, C-22), 145.4 (C-1), 140.4 (C-8a), 138.5 (C-3), 134.2 (C-18, C-19), 133.8 (C-9a), 131.9 (C-16, C-21), 128.5 (C-4a), 128.1 (C-7), 123.3 (C-17, C-20), 121.8 (C-4b), 121.7 (C-5), 119.7 (C-6), 112.9 (C-4), 111.6 (C-8), 36.5 (C-13), 33.4 (C-10), 28.3 (C-12), 25.3 (C-11); (+)-FABMS m/z 370 $[M + H]^+$; (+)-HRFABMS m/z 370.1546 $[M + H]^+$ (calcd for $C_{23}H_{20}N_3O_2$, 370.1555).

*1-(4-Ammoniobutyl)-9H-pyrido[3,4-b]indol-2-ium Ditrifluoroacetic Acid Salt (**13**).*[26] 2-(4-(9*H*-Pyrido[3,4-*b*]indol-1-yl)butyl)isoindoline-1,3-dione (**12**) (0.04 g, 0.01 mmol) was dissolved in absolute EtOH (10 mL), and hydrazine monohydrate (0.08 mL, 0.08 g, 1.65 mmol) was added. The reaction was stirred at room temperature for 1−2 days until all starting material was consumed, as judged by C_{18} analytical HPLC retention times and HPLC-PDA UV spectrum trace. The reaction mixture was dried in vacuo. Purification by Sephadex LH-20 column chromatography eluting with MeOH (0.05% TFA) gave the product as a yellow oil (0.012 g, 24%): R_f (10% MeOH/CH_2Cl_2/0.1% triethylamine) 0.13; IR ν_{max} (ATR) 3059, 2948, 2861, 1694, 1625, 1504, 1482, 1355 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.24 (1H, d, J = 5.6 Hz, H-3), 8.19 (1H, d, J = 8.0 Hz, H-5), 8.08 (1H, d, J = 5.6 Hz, H-4), 7.60 (2H, m, H-7, H-8), 7.29 (1H, m, H-6), 3.27 (2H, t, J = 7.6 Hz, H_2-10), 2.98 (2H, t, J = 7.5 Hz, H_2-13), 1.97 (2H, tt, J = 7.6, 7.5 Hz, H_2-11), 1.79 (2H, tt, J = 7.6, 7.5 Hz, H_2-12); ^{13}C NMR (CD_3OD, 100 MHz) δ 145.1 (C-1), 143.4 (C-8a), 136.0 (C-3), 135.7 (C-9a), 131.6 (C-4a), 130.6 (C-7), 123.1 (C-5), 122.4 (C-4b), 121.5 (C-6), 115.0 (C-4), 113.2 (C-8), 40.4 (C-13), 32.9 (C-10), 28.2 (C-12), 26.7 (C-11); (+)-FABMS m/z 240 $[M + H]^+$; (+)-HRFABMS m/z 240.1504 $[M + H]^+$ (calcd for $C_{15}H_{18}N_3$, 240.1501).

*tert-Butyl (4-(9H-pyrido[3,4-b]indol-1-yl)butylamino)(tert-butoxycarbonylamino) methylenecarbamate (**14**).*[27] To a solution of 1-(4-ammoniobutyl)-9*H*-pyrido[3,4-*b*]indol-2-ium ditrifluoroacetate salt (**13**) (0.03 g, 0.07 mmol) in anhydrous DMF (0.05 mL) were added *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[28] (0.04 g, 0.14 mmol) and triethylamine (0.29 mL, 2.07 mmol). A suspension of Mukaiyama's reagent[27] (0.04 g, 0.14 mmol) in anhydrous DMF (0.1 mL) was added dropwise to the reaction mixture, which turned from yellow to brown-red. The reaction mixture was stirred at room temperature for 2 days until completion was judged by TLC. The reaction mixture was diluted with H_2O (3 mL) and extracted with CH_2Cl_2 (5 × 3 mL). The combined organic layer was dried over anhydrous $MgSO_4$, and the solvent removed in vacuo. Purification by silica gel column chromatography eluting with EtOAc (0−30%) in hexanes yielded the product as a yellow oil (0.013 g, 39%): R_f (50% EtOAc/hexanes) 0.31; IR ν_{max} (ATR) 3326, 3159, 2978, 2934, 1717, 1640, 1619, 1326 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.55 (1H, br s, NH), 9.82 (1H, br s, NH), 8.39 (2H, d, J = 5.2 Hz, H-3), 8.39 (1H, br s, NH), 8.11 (1H, d, J = 7.8 Hz, H-5), 7.81 (1H, d, J = 5.3 Hz, H-4), 7.58 (1H, m, H-8), 7.53 (1H, m, H-7), 7.27 (1H, m, H-6), 3.47 (2H, dt, J = 6.5, 6.5 Hz, H_2-13), 3.24 (2H, t, J = 7.3 Hz, H_2-10), 2.01 (2H, m, H_2-11), 1.55 (2H, m, H_2-12), 1.50 (18H, s, 2 × Boc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5 (C-17), 156.6 (C-15), 153.2 (C-24), 145.1 (C-1), 140.4 (C-8a), 138.6 (C-3), 134.8 (C-9a), 128.4 (C-4a), 127.9 (C-7), 121.8 (C-4b), 121.6 (C-5), 119.6 (C-6), 112.6 (C-4), 112.0 (C-8), 83.3/79.6 (C-19, C-25), 39.4 (C-13), 32.0 (C-10), 28.3/28.1 (C-20, C-21, C-22, C-26, C-27, C-28), 27.6 (C-12), 24.6 (C-11); (+)-FABMS m/z 482 $[M + H]^+$; (+)-HRFABMS m/z 482.2765 $[M + H]^+$ (calcd for $C_{26}H_{36}N_5O_4$, 482.2767).

*1-(4-(9H-Pyrido[3,4-b]indol-1-yl)butyl)guanidine Trifluoroacetic Acid Salt (**8**).* *tert*-Butyl (4-(9*H*-pyrido[3,4-*b*]indol-1-yl)butylamino)-(*tert*-butoxycarbonyl-amino)methylenecarbamate (**14**) (0.01 g, 0.023 mmol) was stirred in a 45% solution of TFA in CH_2Cl_2 for 30 min and dried in vacuo. Purification by Sephadex LH-20 column chromatography eluting with MeOH (0.05% TFA) gave the product as a yellow oil (0.008 g, 69%): IR ν_{max} (ATR) 3366, 3171, 2963, 2885, 1669, 1635, 1432 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.53 (1H, d, J = 6.4 Hz, H-4), 8.38 (1H, d, J = 8.0 Hz, H-5), 8.34 (1H, d, J = 6.4 Hz, H-3), 7.78 (1H, m, H-7), 7.77 (1H, m, H-8), 7.45 (1H, m, H-6), 3.46 (2H, t, J = 7.7 Hz, H_2-10), 3.25 (2H, t, J = 7.0 Hz, H_2-13), 1.99 (2H, tt, J = 7.7, 7.0 Hz, H_2-11), 1.76 (2H, tt, J = 7.7, 7.0 Hz, H_2-12); ^{13}C NMR (CD_3OD, 100 MHz) δ 158.7 (C-15), 145.5 (C-8a), 142.6 (C-1), 135.1 (C-4a), 135.1 (C-9a), 133.2 (C-7), 129.8 (C-3), 124.2 (C-5), 123.1 (C-6), 121.6 (C-4b), 116.7 (C-4), 113.9 (C-8), 42.0 (C-13), 30.8 (C-10), 29.6 (C-12), 27.0 (C-11); ^{15}N NMR (CD_3OD, 60.8 MHz, obtained from ^{1}H−^{15}N 2D data) δ 187.3 (N-2), 118.0 (N-9), 82.4 (N-14); (+)-FABMS m/z 282 $[M + H]^+$; (+)-HRFABMS m/z 282.1719 $[M + H]^+$ (calcd for $C_{16}H_{19}N_5$, 282.1719).

*2-(3-(2,3,4-Tetrahydro-1H-pyrido[3,4-b]indol-1-yl)propyl)isoindoline-1,3-dione (**16**).*[29] 4-(1,3-Dioxoisoindolin-2-yl)butanal (**15**)[25] (0.93 g, 4.28 mmol) and tryptamine (1.03 g, 6.43 mmol) were dissolved in CH_2Cl_2 (80 mL), and the mixture was cooled to −78 °C while stirring under nitrogen. TFA (1.60 mL, 21.5 mmol) was added to the cooled, stirring mixture, which was then allowed to warm to room temperature overnight. Triethylamine (3.10 mL, 22.4 mmol) was added, and the reaction stirred for another 15 min. The resulting mixture was washed with H_2O (50 mL). The organic layer was dried with anhydrous $MgSO_4$, and the solvent evaporated in vacuo. Purification by silica gel column chromatography eluting with MeOH (0−3%) in CH_2Cl_2 gave the product as a yellow glass (0.99 g, 65%): Mp 63−65 °C (dec); R_f (4% MeOH/CH_2Cl_2) 0.25; IR ν_{max} (ATR) 3381, 2932, 2842, 1767, 1615, 1466, 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.45 (1H, br s, NH), 7.82 (2H, m, H-16, H-19), 7.68 (2H, m, H-17, H-18), 7.45 (1H, d, J = 7.5 Hz, H-5), 7.32 (1H, d, J = 7.5 Hz, H-8), 7.12 (1H, td, J = 7.5, 1.0 Hz, H-7), 7.07 (1H, td, J = 7.5, 1.0 Hz, H-6), 4.10 (1H, m, H-1), 3.76 (2H, t, J = 6.8 Hz, H_2-12), 3.26 (1H, m, H-3a), 3.01 (1H, m, H-3b), 2.70 (2H, m, H_2-4), 1.88 (3H, m, H_2-10a, H-11), 1.73 (1H, m, H-10b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.6 (C-14, C-21), 135.8* (C-8a), 135.6* (C-9a), 133.9 (C-17, C-18), 131.8 (C-15, C-20), 127.2 (C-4b), 123.2 (C-16, C-19), 121.3 (C-7), 119.1 (C-6), 117.9 (C-5), 110.8 (C-8), 108.8 (C-4a), 51.3 (C-1), 41.7 (C-3), 37.4 (C-12), 31.7 (C-10), 25.0 (C-11), 22.6 (C-4); (+)-ESIMS m/z 360 $[M + H]^+$; (+)-HRESIMS m/z 360.1720 $[M + H]^+$ (calcd for $C_{22}H_{22}N_3O_2$, 360.1707).

*2-(3-(9H-Pyrido[3,4-b]indol-1-yl)propyl)isoindoline-1,3-dione (**17**).* 2-(3-(2,3,4-Tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)propyl)isoindoline-1,3-dione (**16**) (0.2 g, 0.56 mmol) was dissolved in CH_2Cl_2 (10 mL), and DDQ (2.53 g, 11.1 mmol) was added. The reaction suspension was stirred at 40 °C for 5 min. The suspension was then washed with 1 M KOH until the aqueous layer was slightly yellow. The organic layer was dried with anhydrous $MgSO_4$, and the solvent removed in vacuo. Purification by silica gel column chromatography eluting with EtOAc (0−50%) in hexanes gave the product as a yellow oil (0.058 g, 29%): R_f 0.60 (100% EtOAc); IR ν_{max} (ATR) 3404, 3059, 2947, 1765, 1697, 1623, 1505, 1354 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.79 (1H, br s, NH), 8.36 (1H, d, J = 5.4 Hz, H-3), 8.07 (1H, d, J = 7.5 Hz, H-5), 7.77 (3H, m, H-4, H-16, H-19), 7.66 (2H, m, H-17, H-18), 7.57 (1H, m, H-8), 7.53 (1H, m, H-7), 7.26 (1H, td, J = 7.5, 1.2 Hz, H-6), 3.81 (2H, t, J = 6.0 Hz, H_2-12), 3.18 (2H, t, J = 7.1 Hz, H_2-10), 2.35 (2H, tt, J = 7.1, 6.0 Hz, H_2-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.9 (C-14, C-21), 144.5 (C-1), 140.4 (C-8a), 138.6 (C-3), 134.6 (C-9a), 133.9 (C-17, C-18), 131.8 (C-15, C-20), 128.7 (C-4a), 128.1 (C-7), 123.2 (C-16, C-19), 121.8 (C-4b), 121.6 (C-5), 119.8 (C-6), 112.9 (C-4), 111.7 (C-8), 38.0 (C-12), 31.4 (C-10), 28.3 (C-11); (+)-ESIMS m/z 356 $[M + H]^+$; (+)-HRESIMS m/z 356.1400 $[M + H]^+$ (calcd for $C_{22}H_{18}N_3O_2$, 356.1394).

*1-(3-Ammoniopropyl)-9H-pyrido[3,4-b]indol-2-ium Ditrifluoroacetic Acid Salt (**18**).* 2-(3-(9*H*-Pyrido[3,4-*b*]indol-1-yl)propyl)isoindoline-1,3-dione (**17**) (0.073 g, 0.21 mmol) was dissolved in absolute EtOH (10 mL), and hydrazine monohydrate (0.20 mL, 4.10 mmol) was added. The reaction was stirred for 2 days and then dried in vacuo. The residue was subjected to purification by Sephadex LH-20 column chromatography eluting with MeOH (0.05% TFA) to give the product as a brown oil (0.077 g, 82%): IR ν_{max} (ATR) 3164, 3010, 2890, 1666, 1634, 1490, 1327 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.50 (1H, d, J = 6.2 Hz, H-4), 8.38 (1H, m, H-3), 8.36 (1H, m, H-5), 7.77 (2H, m, H-7, H-8), 7.44 (1H, m, H-6), 3.50 (2H, t, J = 7.8 Hz, H_2-10), 3.12 (2H, t, J = 7.8 Hz, H_2-12), 2.27 (2H, tt, J = 7.8, 7.8 Hz, H-11); ^{13}C NMR (CD_3OD, 100 MHz) δ 145.5 (C-8a), 141.0 (C-1), 135.3* (C-4a), 135.2* (C-9a), 133.2 (C-7), 130.3 (C-3), 124.2 (C-5) 123.1 (C-6), 121.6 (C-4b), 117.0 (C-4), 113.9 (C-8), 40.0 (C-12), 28.4 (C-10), 27.6 (C-11); (+)-ESIMS m/z 226 $[M + H]^+$; (+)-HRESIMS m/z 226.1336 $[M + H]^+$ (calcd for $C_{14}H_{16}N_3$, 226.1339).

*tert-Butyl (3-(9H-pyrido[3,4-b]indol-1-yl)propylamino)(tert-butoxycarbonylamino) Methylenecarbamate (**19**).* To a solution of 1-(3-ammoniopropyl)-9*H*-pyrido[3,4-*b*]indol-2-ium ditrifluoroacetic acid salt (**18**) (0.02 g, 0.04 mmol) in CH_2Cl_2 (2 mL) were added triethylamine (0.25 mL, 1.80 mmol), *N,N*′-bis(*tert*-butoxycarbonyl)thiourea[28] (0.061 g, 0.22 mmol), and Mukaiyama's reagent[27] (0.056 g, 0.22 mmol), which turned the color from yellow to brown-red. The reaction mixture was stirred at room temperature for 2 days until completion, as judged by TLC. The reaction mixture was washed with H_2O (5 mL), the organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo. Purification by silica gel column chromatography eluting with EtOAc (0−20%) in hexanes gave the product as a yellow oil (0.009 g, 44%): R_f (50% EtOAc/hexanes) 0.46; IR ν_{max} (ATR) 3326, 3100, 2978, 2931, 1721, 1645, 1615, 1412 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.62 (1H, br s, NH), 10.17 (1H, br s, NH), 8.64 (1H, br t, NH-13), 8.37 (1H, d, J = 5.3 Hz, H-3), 8.11 (1H, d, J = 7.8 Hz, H-5), 7.82 (1H, d, J = 5.3 Hz, H-4), 7.58 (1H, m, H-8), 7.53 (1H, m, H-7), 7.26 (1H, m, H-6), 3.52 (2H, dt, J = 6.3, 6.3 Hz, H_2-12), 3.29 (2H, t, J = 6.6 Hz, H_2-10), 2.16 (2H, tt, J = 6.6, 6.3 Hz, H_2-11), 1.52/1.48 (18H, s, 2 × Boc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5 (C-16), 156.8 (C-14), 153.4 (C-23), 145.3 (C-1), 140.4 (C-8a), 138.6 (C-3), 134.7 (C-9a), 128.5 (C-4a), 127.8 (C-7), 121.7 (C-4b), 121.6 (C-5), 119.6 (C-6), 112.9 (C-4), 112.1 (C-8), 83.4 (C-25), 79.7 (C-18), 40.4 (C-12), 31.9 (C-10), 30.2 (C-11), 28.1/28.2/28.3 (Boc); (+)-ESIMS m/z 468 $[M + H]^+$; (+)-HRESIMS m/z 468.2610 $[M + H]^+$ (calcd for $C_{25}H_{34}N_5O_4$, 468.2605).

*1-(3-(9H-Pyrido[3,4-b]indol-1-yl)propyl)guanidine Ditrifluoroacetate Salt (**9**).* *tert*-Butyl (3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propylamino)-(*tert*-butoxycarbonylamino)methylenecarbamate (**19**) (0.009 g, 0.019 mmol) was stirred in a 45% solution of TFA in CH_2Cl_2 for 30 min and dried in vacuo. Purification by Sephadex LH-20 column chromatography eluting with MeOH (0.05% TFA) gave the product as a yellow oil (0.008 g, 84%): IR ν_{max} (ATR) 3362, 2981, 2883, 1674, 1634, 1437 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.53 (1H, d, J = 6.1 Hz, H-4), 8.39 (1H, d, J = 8.0 Hz, H-5), 8.36 (1H, d, J = 6.1 Hz, H-3), 7.80 (1H, m, H-7), 7.77 (1H, m, H-8), 7.46 (1H, m, H-6), 3.50 (2H, t, J = 7.9 Hz, H_2-10), 3.39 (2H, t, J = 6.9 Hz, H_2-12), 2.22 (2H, tt, J = 7.9, 6.9 Hz, H_2-11); ^{13}C NMR (CD_3OD, 100 MHz) δ 158.9 (C-14), 145.4 (C-8a), 141.9 (C-1), 135.2 (C-4a), 135.2 (C-9a), 133.2 (C-7), 130.3 (C-3), 124.2 (C-5), 123.1 (C-6), 121.6 (C-4b), 116.8 (C-4), 113.9 (C-8), 41.9 (C-12), 28.8* (C-10), 28.7* (C-11); (+)-ESIMS m/z 268 $[M + H]^+$; (+)-HRESIMS m/z 268.1561 $[M + H]^+$ (calcd for $C_{15}H_{18}N_5$, 268.1557).

Biological Assays. Details of the whole organism parasite assay protocols have been reported elsewhere.[30]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Color in situ photo of the *Pseudodistoma opacum* ascidian, ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectra (CD_3OD) for **1**−**4**, and ^{1}H and ^{13}C NMR spectra of synthetic compounds **8**, **9**, **11**−**14**, and **16**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=103475 on 03-07-2011

(2) Wang, W.; Namikoshi, M. *Heterocycles* **2007**, *74*, 53–88.

(3) Ciavatta, M. L.; Manzo, E.; Nuzzo, G.; Villani, G.; Varcamonti, M.; Gavagnin, M. *Tetrahedron* **2010**, *66*, 7533–7538.

(4) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1991**, *44*, 627–633.

(5) Copp, B. R.; Wassvik, C. M.; Lambert, G.; Page, M. J. *J. Nat. Prod.* **2000**, *63*, 1168–1169.

(6) Appleton, D. R.; Page, M. J.; Lambert, G.; Copp, B. R. *Nat. Prod. Res.* **2004**, *18*, 39–42.

(7) Sakurada, T.; Gill, M. B.; Frausto, S.; Copits, B.; Noguchi, K.; Shimamoto, K.; Swanson, G. T.; Sakai, R. *J. Med. Chem.* **2010**, *53*, 6089–6099.

(8) Pearce, A. N.; Appleton, D. R.; Babcock, R. C.; Copp, B. R. *Tetrahedron Lett.* **2003**, *44*, 3897–3899.

(9) Finlayson, R.; Pearce, A. N.; Page, M. J.; Kaiser, M.; Bourguet-Kondracki, M.-L.; Harper, J. L.; Webb, V. L.; Copp, B. R. *J. Nat. Prod.* **2011**, *74*, 888–892.

(10) Appleton, D. R.; Chuen, C. S.; Berridge, M. V.; Webb, V. L.; Copp, B. R. *J. Org. Chem.* **2009**, *74*, 9195–9198.

(11) Davis, R. A.; Duffy, S.; Avery, V. M.; Camp, D.; Hooper, J. N. A.; Quinn, R. J. *Tetrahedron Lett.* **2010**, *51*, 583–585.

(12) Van Wagoner, R. M.; Jompa, J.; Tahir, A.; Ireland, C. M. *J. Nat. Prod.* **1999**, *62*, 794–797.

(13) Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 909–914.

(14) Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T.; Yamaguchi, K.; Sakai, S.-I. *Chem. Pharm. Bull.* **1984**, *32*, 1313–1325.
(15) Costa, E. V.; Pinheiro, M. L. B.; De Souza, A. D. L.; Dos Santos, A. G.; Campos, F. R.; Ferreira, A. G.; Barison, A. *Magn. Reson. Chem.* **2008**, *46*, 69–74.
(16) Appleton, D. R.; Pearce, A. N.; Lambert, G.; Babcock, R. C.; Copp, B. R. *Tetrahedron* **2002**, *58*, 9779–9783.
(17) Schumacher, R. W.; Davidson, B. S. *Tetrahedron* **1995**, *51*, 10125–10130.
(18) Costa, E. V.; Pinheiro, M. L. B.; Xavier, C. M.; Silva, J. R. A.; Amaral, A. C. F.; Souza, A. D. L.; Barison, A.; Campos, F. R.; Ferreira, A. G.; Machado, G. M. C.; Leon, L. L. P. *J. Nat. Prod.* **2006**, *69*, 292–294.
(19) Maresh, J. J; Giddings, L.; Friedrich, A.; Loris, E. A.; Panjikar, S.; Trout, B. L.; Stoeckigt, J.; Peters, B.; O'Connor, S. E. *J. Am. Chem. Soc.* **2008**, *130*, 710–723.
(20) Herbert, R. B.; Mann, J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1523–1525.
(21) Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1994**, *59*, 6600–6605.
(22) Yang, H.; Johnson, P. M.; Ko, K.; Kamio, M.; Germann, M. W.; Derby, C. D.; Tai, P. C. *J. Exp. Bio.* **2005**, *208*, 3609–3622.
(23) Wang, W.; Nam, S.-J.; Lee, B.-C.; Kang, H. *J. Nat. Prod.* **2008**, *71*, 163–166.
(24) McNulty, J.; Still, I. W. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1329–1337.
(25) Jackson, D. S.; Fraser, S. A.; Ni, L.; Kam, C.; Winkler, U.; Johnson, D. A.; Froelich, C. J.; Hudig, D.; Powers, J. C. *J. Med. Chem.* **1998**, *41*, 2289–2301.
(26) (a) Sasaki, T.; Minamoto, K.; Itoh, H. *J. Org. Chem.* **1978**, *43*, 2320–2325. (b) Cheng, E.; Dekker, H. L.; van Gelder, B. F.; Koomen, G.-J. *Biochim. Biophys. Acta* **1995**, *1253*, 189–192.
(27) Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540–1542.
(28) Iwanowicz, E. J.; Poss, M. A.; Lin, J. *Synth. Commun.* **1993**, *23*, 1443–1445.
(29) Mahboobi, S.; Wagner, W.; Burgemeister, T. *Arch. Pharm.* **1994**, *328*, 371–376.
(30) Orhan, I.; Şener, B.; Kaiser, M.; Brun, R.; Tasdemir, D. *Mar. Drugs* **2010**, *8*, 47–58.