Three New Manzamine Alkaloids from a Common Indonesian Sponge and Their Activity against Infectious and Tropical Parasitic Diseases[⊥]

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Three new manzamine-type alkaloids, 12,34-oxamanzamine E (**3**), 8-hydroxymanzamine J (**4**), and 6-hydroxymanzamine E (**8**), as well as 12 previously characterized manzamine alkaloids have been isolated from a common Indonesian sponge of the genus *Acanthostrongylophora*. The structures of the new compounds have been established on the basis of 1D and 2D NMR spectroscopic analysis and comparison of the data to literature values of related compounds. The biological activities and structure−activity relationship of the manzamines against malaria, *Mycobacterium tuberculosis*, Leishmania, HIV-1, and AIDS opportunistic infections are discussed. A plausible pathway for the formation of the 12,34-oxaether bridge in compound **3** is also provided.

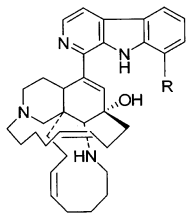
As part of our ongoing investigations[1−4] to identify new leads from marine invertebrate and microbial communities, an extract of the Indonesian sponge *Acanthostrongylophora* sp. was subjected to a detailed investigation, as it showed promising antimalarial activity against *Plasmodium falciparum* and also significant antimicrobial activity against a number of microbial strains. Fractionation of this extract led to the isolation of the manzamines, a well-established class of marine antimalarial agents, and in this paper we describe their isolation, structural characterization, and biological activities.

The manzamines have previously been reported to show a number of significant biological activities including cytotoxicity,[5] insecticidal,[6] antibacterial,[7] antiinflammatory,[8] antiinfective,[9] and antiparasitic,[10] activities with some of the greatest potential for possible clinical applications existing for malaria and *Mycobacterium tuberculosis*.[11]

Manzamines are complex β-carboline alkaloids isolated from Indo-Pacific sponges and characterized as having an intricate and novel nitrogen-containing polycyclic system. In 1986, Higa and co-workers first reported manzamine A (**2**) from the Okinawan sponge of the genus *Haliclona*.[5] Following manzamine A, a series of β-carboline-containing manzamine-type alkaloids have been isolated from marine sponges over the past two decades, which include the fascinating unsymmetrical manzamine dimer from the Scheuer group, kauluamine,[12] and a nearly symmetrical dimer called *neo*-kauluamine.[1] To date, there are 16 species belonging to eight families of marine sponges that have been confirmed to yield β-carboline manzamine and manzamine-related alkaloids.[13] The occurrence of manzamine alkaloids in a diversity of unrelated sponges has led to speculation of a possible microbial origin for the biosynthesis of these compounds.

Results and Discussion

The sponge *Acanthostrongylophora* sp. was collected from Manado, Indonesia, and exhaustively extracted with acetone. The extract as concentrated under reduced pressure, and the aqueous acetone concentrate was treated with chloroform. The chloroform portion was purified by Si gel



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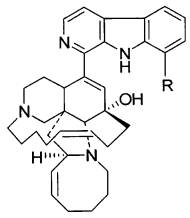

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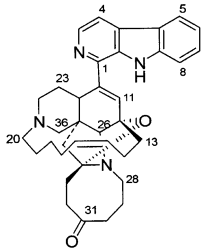

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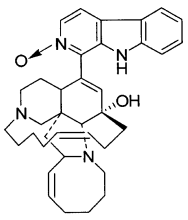

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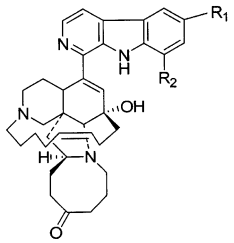
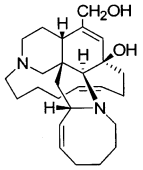


7 R_1 = H, R_2 = H
8 R_1 = OH, R_2 = H
9 R_1 = H, R_2 = OH



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[⊥] Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

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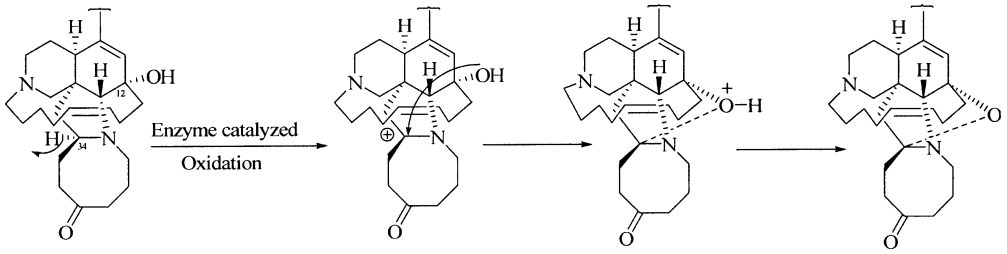

Figure 1. Plausible mechanism of formation of 12,34-oxaether bridge in **3**.

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **3**, **4**, and **8** (δ in ppm, J in Hz)[a]

	12,34-oxamanzamine E (**3**)[b]		8-hydroxymanzamine J (**4**)[b]		6-hydroxymanzamine E (**8**)[c]	
position	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	143.9 s		144.5 s		143.6 s	
3	138.8 d	8.41 d, 5.2	138.7 d	8.43 d, 5.3	136.5 d	8.21 d, 5.3
4	114.2 d	7.84 d, 5.2	112.8 d	8.08 d, 5.3	113.4 d	7.82 d, 5.3
4a	129.9 s		130.5 s		130.2 s	
4b	122.0 s		122.2 s		122.4 s	
5	121.8 d	8.08 d, 7.8	109.7 d	7.82 d, 7.8	105.5 d	7.50 d, 2.3
6	120.4 d	7.26 t, 8.0	120.2 s	7.24 t, 7.9	151.6 s	
7	128.8 d	7.51 t, 7.4	113.9 d	7.49 d, 7.8	118.7 d	7.09 dd, 2.3, 8.8
8	112.3 d	7.55 d, 8.0	131.5 s		113.3 d	7.53 d, 8.8
8a	140.8 s		136.4 s		134.8 s	
9a	133.8 s		128.4 s		136.3 s	
10	142.8 s		140.9 s		139.7 s	
11	132.7 d	6.24 s	132.2 d	6.28 s	137.1 d	6.28 s
12	80.5 s		70.9 s		70.1 s	
13	40.3 t	1.66 m, 2.35 m	40.8 t	1.46 m, 1.96 m	41.2 t	1.80 m, 1.89 m
14	23.1 t	2.45 m, 2.85 m	22.5 t	2.01 m, 2.24 m	30.1 t	2.11 m, 2.21 m
15	129.9 d	5.34 br s	129.8 d	5.34 m	128.9 d	5.65 dt, 4.7, 11.4
				7.2, 8.2, 10.8		
16	129.8 d	5.29 br s	132.2 d	5.46 dt, 7.8, 10.8	132.1 d	5.52 q, 9.1
17	25.4 t	1.73 m, 1.86 m	28.6 t	2.89 m, 2.92 m	26.5 t	1.67 m, 2.48 m
18	30.0 t	1.24 m, 1.52 m	28.4 t	1.54 m, 1.91 m	27.4 t	1.36 m, 1.41 m
19	30.1 t	1.38 m, 1.46 m	28.8 t	1.54 m, 1.92 m	23.1 t	1.34 m, 1.72 m
20	59.3 t	2.28 m, 2.71 m	53.9 t	2.81 m, 2.92 m	53.6 t	2.37 m, 2.63 m
22	50.1 t	2.04 m, 3.02 m	50.1 t	1.94 m, 2.92 m	50.2 t	1.83 m, 2.72 m
23	32.1 t	2.59 m, 2.67 m	33.1 t	1.75 m, 1.98 m	32.4 t	1.45 m, 1.84 m
24	46.3 d	2.52 dd, 5.5, 11.8	44.8 d	2.45 m	40.3 d	3.15 dd, 7.3, 11.2
25	38.6 s		43.8 s		45.7 s	
26	67.2 d	4.36 s	59.7 d	3.88 s	75.8 d	3.65 s
28	54.1 t	2.84 m, 3.38 m	58.6 t	2.36 m, 3.55 m	53.7 t	2.66 m, 3.56 m
29	23.3 t	1.72 m, 1.76 m	29.4 t	1.69 m, 1.79 m	32.5 t	1.83 m, 1.90 m
30	33.1 t	1.64 m, 1.78 m	29.6 t	1.25 m, 1.78 m	48.4 t	2.65 m, 2.98 m
31	206.2 s		22.5 t	1.73 m, 1.48 m	216.6 s	
32	30.9 t	2.75 m, 3.20 m	128.8 d	5.62 br t, 11.0	37.4 t	1.80 m, 2.21 m
33	30.5 t	2.15 m, 2.25 m	131.4 d	5.36 br t, 8.8	26.5 t	1.60 m, 1.79 m
34	101.8 s		26.6 t	2.19 m, 2.74 m	65.5 d	3.20 m
35	47.4 t	2.27 m, 2.34 m	37.8 t	1.44 m, 1.98 m	47.4 t	1.34 m, 1.42 m
36	66.3 t	2.24 d, 3.15 m	66.1 t	2.04 m, 2.35 m	67.1 t	2.31 m, 2.65 m

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT experiments. s = C, d = CH, t = CH_2. Coupling constants (J) are in Hz. [b] NMR obtained in $CDCl_3$. [c] NMR obtained in d_4-methanol.

vacuum liquid chromatography to afford 10 major fractions, and further workup resulted in the isolation and purification of 12 known and three new manzamine alkaloids.

The previously reported compounds were identified as ircinal A,[14] manzamine J (**1**),[14] manzamine A (**2**),[5,15] manzamine A *N*-oxide (**5**),[6] 8-hydroxymanzamine A (**6**),[16,17] 6-deoxymanzamine X,[6] manzamine E (**7**),[18] manzamine X,[16] manzamine F (**9**),[18] *neo*-kauluamine,[1] ircinol A (**10**),[19] and 3,4-dihydromanzamine A *N*-oxide,[6] by comparison of spectral data with the values reported in the literature.

12,34-Oxamanzamine E (**3**) showed a molecular ion peak at *m*/*z* 563.3904 (M + H^+) (calc 563.3386) in high-resolution ESIMS, which suggests a molecular composition of $C_{36}H_{42}N_4O_2$. The UV absorption maxima at 239, 252, 275, and 354 nm suggested the presence of a β-carboline chromophore.[20] The IR exhibited an absorption band at 1716 cm^{-1}, indicating the presence of a carbonyl functionality in **3**. Comparison of the ^{1}H and ^{13}C NMR, optical rotation, and melting point data for compound **3** and *ent*-12,34-oxamanzamine E revealed similar physical properties with the exception of opposite optical rotation values.[2] Compound **3** has $[\alpha]_D^{25}$ +44.32 (*c* 0.6, $CHCl_3$), while *ent*-12,34-oxamanzamine E has $[\alpha]_D^{25}$ −54.6 (*c* 0.3), in the same solvent. These data support that *ent*-12,34-oxamanzamine E is an enantiomer rather than a diastereomer of compound **3**, and thus compound **3** was characterized as 12,34-oxamanzamine E. The proposed mechanism of formation of the 12,34-oxaether bridge in **3** is illustrated in Figure 1. In an enzyme-catalyzed reaction, hydrogen would be oxidatively cleaved as a hydride ion with the formation of a carbocation stabilized by the tertiary nitrogen (enamine). Subsequent attack of OH in an S_N1 fashion and loss of the proton would result in the formation of the 12,34-oxaether bridge.

Compound **4** was obtained as a pale yellow powder from $CHCl_3$, $[\alpha]_D^{25}$ +23.4 (*c* 0.2, $CHCl_3$), and it was revealed to have a molecular formula of $C_{36}H_{46}N_4O_2$ by HRESIMS (*m*/*z* 567.3960 [M + $H]^+$). The ^{1}H and ^{13}C NMR spectra of **4** (Table 1) gave signals including nine quaternary carbons (7 × sp^2, 2 × sp^3), 12 methines (10 × sp^2, 2 × sp^3), and 15

Table 2. In Vitro Activity of the Manzamine Alkaloids against *Mycobacterium tuberculosis*, Malaria, and Leishmania[a]

	activity in vitro					
				L. donovani		
compound	*M. tuberculosis* (H37Rv) MIC, μg/mL	*P. falciparum* (D6 clone) IC_{50}, ng/mL	*P. falciparum* (chlorine-resistant W2 clone) IC_{50}, ng/mL	IC_{50}, μg/mL	IC_{90}, μg/mL	cytotoxicity (Vero), μg/mL
manzamine A (**2**)	1.5	4.5	8.0	0.9	1.8	1.2
(+)-8-hydroxymanzamine A (**6**)	0.9	6.0	8.0	6.2	11	1.1
manzamine A *N*-oxide (**5**)	3.9	11	13	1.1	3.8	4.2
3,4-dihydromanzamine A-*N*-oxide	NT	1600	3700	NT	NT	NC
12,34-oxamanamine E (**3**)	NT	NA	NA	NA	NA	NC
manzamine E (**7**)	3.8	3400	4760	3.8	6.8	NC
6-hydroxymanzamine E (**8**)	0.4	780	870	2.5	4.3	4.3
manzamine F (**9**)	2.6	780	1700	4.2	7.0	NC
manzamine J (**1**)	1.7	1300	750	25	45	NC
6-deoxymanzamine X	1.8	1300	1400	3.2	7.5	4.7
manzamine X	NT	950	2000	5.7	11	NC
neo-kauluamine	2.0	1700	2800	4.2	8.2	NC
ircinal A	30.2	NA	NA	4.6	8.5	NC
ircinol A (**10**)	1.9	2400	3100	0.9	0.7	NC
rifampin	0.5	NT	NT	NT	NT	NT
chloroquine	NT	15.5	170	NT	NT	NT
artemisinin	NT	10	6.3	NT	NT	NT
pentamidine	NT	NT	NT	2.1	10	NT
amphotercin B	NT	NT	NT	0.06	0.15	NT

[a] NA = not active (concentration 5.0 μg/mL); NT = not tested; NC = no cytotoxicity (concentration: 4.7 μg/mL).

methylenes, suggesting that compound **4** has a skeleton similar to that of the common manzamine alkaloids. The significant upfield shifts of C-26 (59.7 ppm), C-34 (26.6 ppm), and C-35 (37.8 ppm), the latter two appearing as CH_2 signals in the DEPT, indicated that compound **4** was an analogue of manzamine J (**1**).[14] Comparison of the ^{1}H and ^{13}C NMR data of **4** with those of manzamine J revealed that the 8-position of the β-carboline moiety of **4** was substituted by a hydroxyl group, and thus compound **4** was shown to be 8-hydroxymanzamine J.

Compound **8** was obtained as a pale yellow powder, and the high-resolution ESIMS established the molecular formula as $C_{36}H_{44}N_4O_3$ (*m*/*z* 581.3477 $[M + H]^+$ Δ 1.5 mmu of calcd). The UV absorption maxima at 218, 239, 280, 288, and 346 nm suggested the presence of a β-carboline chromophore.[20] The ^{1}H NMR spectrum of **8** showed five characteristic signals in the aromatic region, indicating the presence of a disubstituted β-carboline moiety. The olefinic signals at δ 6.28 (s), 5.65 (dt, J = 4.7, 11.4 Hz), and 5.52 (q, J = 9.1 Hz) revealed the presence of one tri- and one disubstituted double bond. The presence of a ketonic carbonyl was observed by IR (1725 cm^{-1}) and ^{13}C NMR (δ 216.6, s). These features together with ^{13}C NMR data (Table 1) suggested that **8** has the same structural features as manzamine E (**7**). The hydroxyl functionality in **8** was placed at C-6 due to the ^{1}H NMR signal for the C-7 proton at δ 7.09 (dd, 2.3 and 8.8 Hz) and was confirmed by 2D NMR (^{1}H$-^1$H-COSY, HMQC, and HMBC) data. Thus compound **8** was characterized as 6-hydroxymanzamine E.

The in vitro activity of manzamines against *Mycobacterium tuberculosis* (H37Rv), the malaria parasite *Plasmodium falciparum*, and *Leishmania donovani*, the causative agent for visceral leishmaniasis, is reported in Table 2. Most manzamines were active against *M. tuberculosis* with MICs < 12.5 μg/mL. (+)-8-Hydroxymanzamine A (**6**) had an MIC of 0.91 μg/mL, indicating improved activity for the (+) over the (−) enantiomer. The difference in the antimalarial and antileishmanial activities of manzamines A (**2**) and J (**1**) indicates that the bond between N-27 and C-34 may be important for the antimalarial and leishmania activity and provides valuable insight into the structural moieties required for activity against malaria and leishmania parasites. The minimal difference in the *Mycobacterium tuberculosis* activity of manzamines A (**2**) and J (**1**) may indicate that the structure−activity relationship and targets for *M. tuberculosis* and malaria are significantly different. Comparison of the *M. tuberculosis* and antimalarial activities of manzamine E (**7**) and its hydroxy derivatives (**8** and **9**) indicates that the hydroxyl functionality and its position on the β-carboline moiety may play a role in biological activity. Although there is no significant difference in the leishmanicidal activities of manzamine E (**7**), 6-hydroxymanzamine E (**8**), and manzamine F (**9**), these results may provide valuable information regarding the structural moieties required for activity against malaria. The significant leishmanicidal activity of ircinol A (**10**, IC_{50} 0.9 μg/mL and IC_{90} 1.7 μg/mL) indicates that the β-carboline moiety is not essential for activity against the leishmania parasite in vitro, which is significantly different from the malaria structure−activity relationship.

The antimicrobial and HIV-1 activities of the manzamines are given in Table 3. From Table 3, it is evident that manzamine A (**2**), manzamine A *N*-oxide (**5**), and 8-hydroxymanzamine A (**6**) are more active than manzamines E (**7**) and F (**9**), and these results provide valuable data to show that the eight-membered ring is essential for antimicrobial and anti-HIV-1 activity. This observation suggests that reduction of the C32−C33 olefin and oxidation of C-31 to the ketone significantly reduces the antimicrobial activity for the manzamine alkaloids.

The diversity of manzamines isolated from different species of sponge is unusual and clearly raises the question of the origin of these metabolites. The microbial fauna/flora associated with these sponges may ultimately be responsible for the production of the manzamines, and the observed chemical variation may be due to differences in the sponge-associated microbial community. The sponge or sponge-associated microbes could easily be responsible for the oxidation of the manzamine A skeleton.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-310 digital polarimeter. UV and IR spectra were respectively obtained using a Perkin-Elmer Lambda 3B UV/vis spectrophotometer and an AATI Mattson, Genesis Series FTIR. The ^{1}H and ^{13}C NMR spectra

Table 3. HIV-1 and AIDS Opportunistic Infections Data for Manzamines[a]

compound	activity IC_{50} (μg/mL)				
	S. aureus	MRS	*C. neoformans*	*M. intracellulare*	HIV-1 EC_{50} (μM)
manzamine A (**2**)	0.5	0.7	3.0	0.35	4.2
8-hydroxymanzamine A (**6**)	0.9	4.0	3.0	1.0	0.59
manzamine A *N*-oxide (**5**)	0.5	0.4	1.0	0.2	NT
3,4-dihydromanzamine A *N*-oxide	NA	NA	NA	NA	NA
12,34-oxamanzamine E (**3**)	NA	NA	NA	NA	NT
manzamine E (**7**)	NA	NA	NA	12.5	13.1
6-hydroxymanzamine E (**8**)	NT	NT	5.5	3.5	NT
manzamine F (**9**)	NA	NA	6.5	6.25	7.3
manzamine J (**1**)	NA	NA	NA	15	NT
8-hydroxymanzamine J (**4**)	3.0	5.0	3.5	0.45	NT
6-deoxymanzamine X	1.5	1.5	2.0	6.25	1.6
manzamime X	1.0	0.75	3.0	1.5	2.3
neo-kauluamine	NA	NA	3.0	3.0	2.3
ircinal A	NA	NA	NA	50	6.8
ircinol A (**10**)	NA	30	NA	50	4.3
amphotericin B	NT	NT	0.15	NT	NT
ciprofloxacin	0.10	0.10	NT	0.25	NT

[a] NA = not active. NT = not tested.

were recorded in $CDCl_3$ and methanol-d_4 using NMR spectrometers operating at 400 or 500 MHz for ^1H and 100 or 125 MHz for ^{13}C NMR. The HRMS spectra were measured using a Bioapex FTESI-MS with electrospray ionization. TLC analysis was carried out on precoated silica gel G_{254} or aluminum oxide ALOX-100 UV_{254} 500 μm.

Biological Material. The sample *Acanthostrongylophora* sp. was collected in March 2002 from Manado, Indonesia, and identified by Michelle Kelly of National Institute of Water and Atmospheric Research Ltd., Auckland, New Zealand. A voucher specimen is deposited in National Institute of Water and Atmospheric Research Ltd., Auckland, New Zealand, and the Department of Pharmacognosy, The University of Mississippi.

Extraction and Isolation. The sponge *Acanthostrongylophora* sp. was stored frozen until extracted. The lyophilized sponge (4.2 kg, dry weight) was crushed, homogenized, and then extracted with acetone at room temperature. The extract was concentrated under reduced pressure, and the resultant aqueous acetone extract was treated with chloroform. TLC analysis indicated that the extracts contained manzamine A, together with various related minor alkaloids as detected by Dragendorff reagent. The chloroform extract (120 g) was subjected to Si gel vacuum liquid chromatography and eluted beginning with hexane (100%), hexane−acetone (9:1, 3:1, 1:1), acetone (100%), chloroform−methanol (1:1), and finally methanol (100%). A total of 10 major fractions were collected, and the elution of the metabolites was monitored by TLC.

Fraction 2 (14 g) was rechromatographed on Si gel and eluted with hexane−acetone to obtain ircinal A (120 mg, 0.0029% dry wt), manzamine J (**1**, 120 mg, 0.0029% dry wt), manzamine A (**2**, 50 mg, 0.0012% dry wt), and 8-hydroxymanzamine J (**4**, 6 mg, 0.00014% dry wt).

Fraction 3 (35 g) was rechromatographed over alumina and eluted with a gradient system of hexane−acetone and then with methanol to obtain 12,34-oxamanzamine E (**3**, 5 mg, 0.00012% dry wt), manzamine A (**2**, 4.0 g, 0.095% dry wt), manzamine A *N*-oxide (**5**, 6 mg, 0.00014% dry wt), 8-hydroxymanzamine A (**6**, 3.0 g, 0.071% dry wt), and 3 g of 6-deoxymanzamine X (0.071% dry wt).

Purification of fraction 4 (15 g) over a Si column (gradient elution with chloroform−methanol) gave manzamine E (**7**, 3.0 g, 0.071% dry wt).

Flash column chromatography of fraction 5 (26 g) over Si gel by eluting with a hexane−acetone gradient, acetone, and ethyl acetate gave 20 fractions. 6-Hydroxymanzamine E (**8**, 4.2 mg, 0.0001% dry wt) was obtained from fraction 8. Twelve grams of manzamine F (**9**, 0.29% dry wt) was obtained from fractions 10−14. Further workup of fractions 15−19 (VLC, Si gel, hexane−acetone gradient) gave 400 mg of *neo*-kauluamine (0.0095% dry wt).

Purification of fraction 6 (22 g), over Si gel, using a chloroform−methanol gradient, gave 1.2 g of ircinol A (**10**, 0.03% dry wt) and 5 mg of 3,4-dihydromanzamine A *N*-oxide (0.00012% dry wt).

12,34-Oxamanzamine E (3): pale yellow powder (MeOH); mp 152 °C (dec); $[\alpha]_D^{25}$ +44.32 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 239 (3.38), 252 (3.82), 275 (3.65), 354 (3.41) nm; IR ($CHCl_3$) ν_{max} 3650, 3001−2818, 1716, 1620, 1592, 1533, 1452, 1267, 1144, 1052 cm^{-1}; NMR data, see Table 1; HRESIMS *m*/*z* 563.3904 (calcd for $C_{36}H_{43}N_4O_2$, $[M + H]^+$, 563.3386).

8-Hydroxymanzamine J (4): pale yellow powder ($CHCl_3$); $[\alpha]_D^{25}$ +23.4 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) (MeOH) 251 (3.62), 274 (3.68), 358 (3.39) nm; NMR data, see Table 1; HRESIMS *m*/*z* 567.3960 (calcd for $C_{36}H_{47}N_4O_2$, $[M + H]^+$, 567.3699).

6-Hydroxymanzamine E (8): yellow powder ($CHCl_3$); mp >198 °C dec; $[\alpha]_D^{25}$ +34.4 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (3.64), 239 (3.63), 280 (3.25), 288 (3.09), 346 (3.42) nm; IR ($CHCl_3$) ν_{max} 3629, 3388, 3001−2815, 1725, 1690, 1630, 1548, 1443, 1145, 1048 cm^{-1}; NMR data, see Table 1; HRESIMS *m*/*z* 581.3477 (calcd for $C_{36}H_{45}N_4O_3$, $[M + H]^+$, 581.3492).

In Vitro Antileishmanial Assay. Compounds were screened for antileishmanial activity in vitro on *Lesishmania donovani* promastigotes. A transgenic cell line of *L. donovani* promastigotes showing stable expression of luciferase was used as the test organism. Pentamidine and amphotericine B were tested as standard antileishmanials. Antimalarial,[21] antituberculosis,[22] and HIV[23] assays were performed by previously published procedures.

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