New Manzamine Alkaloids with Activity against Infectious and Tropical Parasitic Diseases from an Indonesian Sponge

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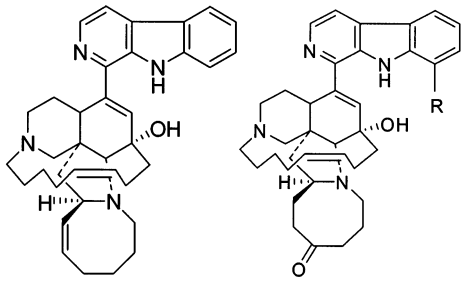
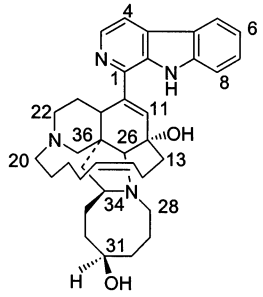
Eleven manzamine type alkaloids, two β-carbolines, and five nucleosides have been isolated from an Indonesian sponge. Among these are the previously characterized 12,34-oxamanzamine A, 12,34-oxamanzamine E, manzamine A (**1**), 8-hydroxymanzamine A, 6-deoxymanzamine X, manzamine E (**2**), manzamine X, manzamine F (**4**), norharman, thymine, 2′,3′-didehydro-2′,3′-dideoxyuridine, uracil, thymidine, and 2′-deoxyuridine. The structures for the five new compounds have been assigned as 32,33-dihydro-31-hydroxymanzamine A (**3**), 32,33-dihydro-6-hydroxymanzamine A-35-one (**5**), des-*N*-methylxestomanzamine A (**6**), 32,33-dihydro-6,31-dihydroxymanzamine A (**7**), and 1,2,3,4-tetrahydronorharman-1-one (**8**), on the basis of NMR and X-ray data. The bioactivity and SAR of the manzamines against malaria, TB, and leishmania are also presented. The structural revision of two previously reported pyrazoles as uracil and thymine is also discussed.

Manzamines are unique β-carboline alkaloids isolated from Indo-Pacific sponges and characterized by having an intricate nitrogen-containing polycyclic system. In 1986, Higa and co-workers first reported manzamine A from the Okinawan sponge of the genus *Haliclona*.[1] These compounds exhibit a diverse range of bioactivities including cytotoxicity,[1] insecticidal,[2] and antibacterial[3] as well as the exciting curative activity against malaria in animal models.[4,5] Since the first report of manzamine A, an additional 40 manzamine-type alkaloids have been reported from nine different sponge genera.[6,7] In our continuing search for manzamine-related alkaloids with significant activity against infectious diseases, we have identified several novel manzamine alkaloids from an Indonesian sponge, and herein we describe their structure determination and biological activity.



1 **2** R = H
4 R = OH



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Results and Discussion

The sponge was collected from Indonesia and successively extracted with hexane and acetone. Further workup on the combined extract led to the isolation of the known manzamines: (+)-12,34-oxamanzamine A,[8] (−)-12,34-oxamanzamine E,[8] (+)-manzamine A (**1**),[1,9] (+)-8-hydroxymanzamine A,[10,11] (+)-6-deoxymanzamine X,[2] (+)-manzamine E (**2**),[12] (+)-manzamine X,[9] (+)-manzamine F (**4**),[12] and norharman,[13] along with the new (+)-32,33-dihydro-31-hydroxymanzamine A (**3**), (+)-32,33-dihydro-6-hydroxymanzamine A-35-one (**5**), des-*N*-methylxestomanzamine A (**6**), (+)-32,33-dihydro-6,31-dihydroxymanzamine A (**7**), and 1,2,3,4-tetrahydronorharman-1-one (**8**).

Compound **3** was obtained as colorless crystals from MeOH, $[\alpha]_D$ +34.44 (*c* 0.9, $CHCl_3$). 32,33-Dihydro-31-hydroxymanzamine A (**3**) was positive to Dragondorff's reagent, and its IR spectrum suggested the presence of OH and NH functionalities. Compound **3** showed the molecular ion peak at *m/z* 567.4052 (M + H^+), in HRESIMS, which is compatible with the molecular composition of $C_{36}H_{46}N_4O_2$ (Δ 3.5 mmu of calcd). The ^{1}H NMR data (Table 1) includes aromatic proton resonances similar to those of manzamine E.[12] Spectral data of how **3** differs from manzamine E (**2**) includes the presence of 13 CH carbons (manzamine E has 12 CH carbons) and the absence of the low-field C-31 carbonyl carbon signal of manzamine E. In place of the carbonyl carbon compound **3** has a signal at 70.9 ppm, which correlates with a multiplet at 4.05 ppm in the HMQC

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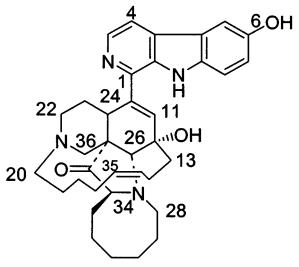
Table 1. ^{1}H and ^{13}C NMR Data for Compounds **3**, **4**, **5**, and **7** (MeOD)[a]

	32,33-dihydro-31-hydroxy-manzamine A (**3**)		manzamine F (**4**)		32,33-dihydro-6-hydroxy-manzamine A−35-one (**5**)[b]		32,33-dihydro-6,31-dihydroxy-manzamine A (**7**)	
C no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	143.1, s		142.3, 5		139.4, s		143.5, s	
3	137.7, d	8.30 d, 5.2	137.4, d	8.35 d, 5.3	136.9, d	8.44 d, 5.3	136.7, d	8.22 d, 5.3
4	113.7, d	7.93 d, 5.2	113.8, d	7.82 d, 5.3	114.2, d	8.35 d, 5.3	113.6, d	7.84 d, 5.3
4a	130.3, s		130.1, s		134.8, s		129.9, s	
4b	121.6, s		122.7, s		123.5, s		122.2, s	
5	121.3, d	8.14 d, 8.14	111.3, d	7. 61 d, 7.9	105.6, d	7.63 d, 2.3	105.3, d	7.49 d, 2.3
6	119.9, d	7.24 t, 8.0	120.8, d	7.04 t, 8.1	153.2, s		151.4, s	
7	128.5, d	7.53 t, 8.0	113.2, d	7.14 d, 8.0	123.1, d	7.33 dd, 2.3, 8.8	118.5, d	7.09 dd, 2.3, 8.8
8	112.3, d	7.66 d, 8.5	143.5, s		114.1, d	7.68 d, 8.8	113.0, d	7.52 d, 8.8
8a	141.1, s		129. 2, s		139.4, s		134.7, s	
9a	133.8, s		133.4, s		136.9, s		136.1, s	
10	141.7, s		141.2, s		143.7, s		139.5, s	
11	137.2, d	6.44 s	137.4, d	6.51 s	143.7, d	6.65 s	136.9, d	6.29 s
12	68.6, s		69.1, s		72.5, s		69.9, s	
13	40.5, t	1.83 m, 1.88 m	40.1, t	1.86 m, 2.02 m	41.2, t	1.85 m, 1.99 m	41.0, t	1.81 m, 1.89 m
14	21.7, t	2.15 m, 2.18 m	21.5, t	2.12 m, 2.21 m	21.6, t	2.11m, 2.20 m	21.9, t	2.11 m, 2.20 m
15	127.8, d	5.63 q, 12.08	127.9, d	5.64 m	134.8, d	5.62 q, 9.8	128.7, d	5.65 dt, 4.7, 11.38
16	132.7, d	5.52 dt, 4.4, 10.8	130.3, d	5.54 m	127.5, d	5.66 q, 10.1	132.0, d	5.52 q, 9.09
17	25.7, t	1.64 m, 2.49 m	25.2, t	1.63 m, 2.49 m	23.6, t	1.65 m, 2.51 m	26.3, t	1.66 m, 2.48 m
18	26.9, t	1.35 m, 1.42 m	26.8, t	1.32 m, 1.39 m	26.1, t	1.32 m, 1.40 m	27.1, t	1.35 m, 1.41 m
19	25.0, t	1.36 m, 1.69 m	24.8, t	1.40 m, 1.72 m	22.8, t	1.42 m, 1.73 m	22.8, t	1.34 m, 1.71 m
20	52.8, t	2.39 m, 2.60 m	53.1, t	2.37 m, 2.59 m	53.2, t	2.36 m, 2.59 m	53.5, t	2.37 m, 2.62 m
22	49.8, t	1.82 m, 2.70 m	49.6, t	1.90 m, 2.80 m	49.7, t	1.93 m, 2.81m	50.2, t	1.83 m, 2.71m
23	33.4, t	1.48 m, 1.95 m	33.6, t	1.56 m, 2.25 m	31.5, t	1.55 m, 2.75 m	32.5, t	1.46 m, 1.85 m
24	42.4, d	3.15 dt, 6.4, 9.3	42.1, d	3.19 m	39.5, d	3.05 dd, 7.1, 10.8	40.1, d	3.15 dd, 7.3, 11.2
25	45.9, s		46.5, s		42.7, s		45.5, s	
26	81.9, d	3.79 s	81.6, d	3.69 s	79.7, d	5.01 s	75.6, d	3.64 s
28	53.5, t	2.66 m, 3.56 m	53.3, t	2.73 m, 3.39 m	53.2, t,	2.69 m, 3.38 m	53.5, t	2.66 m, 3.56 m
29	31.7, t	1.83 m, 1.90 m	32.8, t	1.92 m, 2.02 m	38.1, t	1.83 m, 2.10m	32.4, t	1.83 m, 1.90 m
30	48.2, t	2.65 m, 2.98 m	45. 4, t	2.29 m, 2.55 m	41.2, t	2. 45 m, 2.87 m	48.2, t	2.65 m, 2.98 m
31	70.9, d	4.05 m	216.6, s		25.8, t	2.05 m	79.9, d	4.05 m
32	36.8, t	1.80 m, 2.21 m	38.9, t	2.34 m, 2.61 m	23.6, t	1.98 m, 2.31 m	37.2, t	1.80 m, 2.21 m
33	25.0, t	1.60 m, 1.79 m	24.9, t	1.69 m, 2.08 m	31.5, t	1.70 m, 2.10 m	26.3, t	1.60 m, 1.79 m
34	63.8, d	3.05 m	63.6, d	3.01 m	66.9, d	4.13 m	65.4, d	3.2 m
35	46.2, t	1.35 m, 1.42 m	46.4, t	1.52 m, 1.61 m	197.2, s		47.3, t	1.34 m, 1.42 m
36	68.9, t	2.30 m, 2.65 m	68.9, t	2.29 m, 2.48 m	63.5, t	2.33 m, 2.55 m	66.9, 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 = quaternary, d = methine, t = methylene carbons. Coupling constants (*J*) are in Hz. [b] NMR obtained at 300 MHz.



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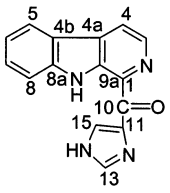

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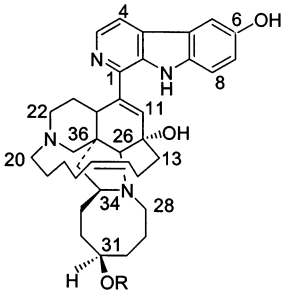
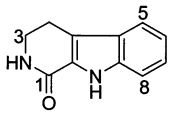


7 R = H
7a R = (*S*)-MTPA
7b R = (*R*)-MTPA



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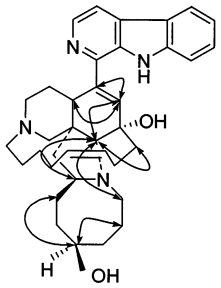


Figure 1. Selected HMBC correlations of **3**.

spectrum. The presence of this new OH− functionality is confirmed by a number of long-range ^{1}H− ^{13}C correlations H_2-29 and H_2-33 to C-31, Figure 1). Data from ^{1}H−^{1}H-COSY, HMQC, and HMBC provided a wealth of additional support to justify the gross structure shown for **3**. The relative configurations at C-12, C-24, C-25, C-26, and C-34 of **3** were elucidated to be the same as those of manzamine E by NOESY data as well as comparable ^{13}C chemical shifts. In addition both compounds were isolated from the same sponge and possessed dextrorotation. The relative configuration was confirmed by X-ray analysis (Figure 4) using a single crystal of **3** obtained from MeOH and unequivocally establishes the structure of **3** as 32,33-dihydro-31-hydroxymanzamine A.

Compound **5** was obtained as pale yellow powder. High-resolution ESIMS established the molecular formula as $C_{36}H_{44}N_4O_3$ (*m/z* 581.3467 $[M + H]^+$, Δ 2.5 mmu of calcd). 32,33-Dihydro-6-hydroxymanzamine A-35-one (**5**) tested positive to Dragendorff reagent, and the ^{1}H and ^{13}C NMR data for **5** were similar to those of the known manzamine F.[12] Differences between compound **5** and manzamine F (**4**) include the chemical shift of the carbonyl carbon and the position of the hydroxyl functionality on the *β*-carboline

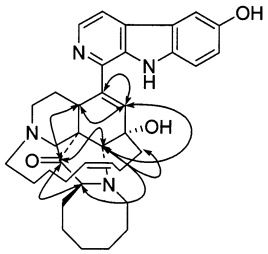


Figure 2. Selected HMBC correlations of **5**.

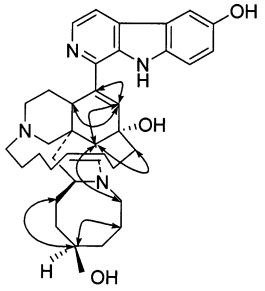


Figure 3. Selected HMBC correlations of **7**.

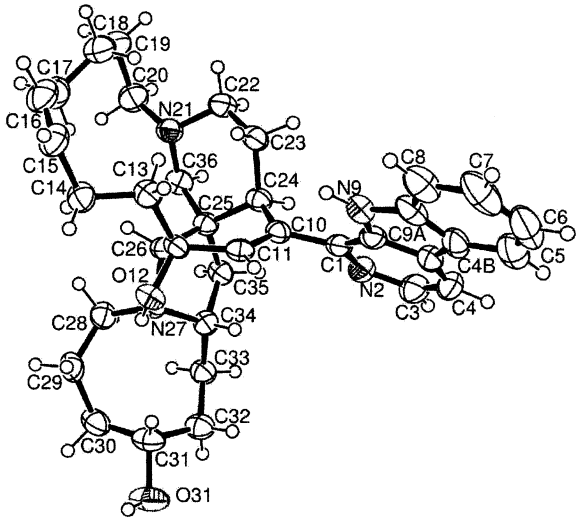


Figure 4. ORTEP[24] drawing of one independent molecule in compound **3**. For simplicity, the numbering scheme used for the X-ray structure depicts connectivity and does not follow the standard IUPAC convention.

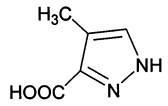
moiety. The carbonyl signal at 197.2 ppm indicated that it is not located at C-31 as in manzamine F. The signal at 197.2 ppm was assigned as C-35 on the basis of HMBC correlations for H_1-24, H_1-26, and H_1-34, all correlating to C-35 (Figure 2). The absence of the corresponding CH_2 signal in the DEPT confirmed this novel ketone among the manzamine alkaloids. The difference in the biological activities of manzamine E, manzamine F, and compound **5** (Table 2) further supports that the carbonyl carbon in **5** is not located at C-31. Thus compound **5** was characterized as 32,33-dihydro-6-hydroxymanzamine A-35-one.

Compound **6** was obtained as a yellow powder and showed a molecular $(M + H)^+$ ion peak at *m/z* 263.0915 in its HRESIMS, and the resulting molecular formula was defined as $C_{15}H_{10}N_4O$. The ^1H and ^{13}C NMR of **6** showed the signals assignable to a 1-substituted β-carboline, which were confirmed by COSY, HMQC, and HMBC analysis. The proton at δ 11.98 was assigned to the NH group and showed long-range correlations with C-4a, C-4b, C-8a, and C-9a carbons of the β-carboline moiety. Furthermore, the ^1H and ^{13}C NMR spectra showed signals ascribable to one carbonyl carbon (δ 184.5) and two singlet imidazole protons [δ 8.02 (1H, s), 8.59 (1H, s)]. From the spectral data it is evident that the β-carboline moiety was attached to the imidazole moiety through the carbonyl carbon. The absence of long-range correlations between the carbonyl carbon and the imidazole protons may be due to the tautomeric nature of imidazole moiety. The spectral data (^1H and ^{13}C NMR) of **6** match well with the data of xestomanzamine A,[9] which differs from **6** only by having an additional methyl group. On the basis of the spectral properties and biogenesis,[9] the structure for **6** was established as des-*N*-methylxestomanzamine A.

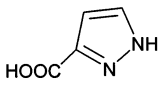
Compound **7** was obtained as a pale yellow powder. High-resolution ESIMS established the molecular formula as $C_{36}H_{46}N_4O_3$ (*m/z* 583.3477 $[M + H]^+$ (Δ 0.2 mmu of calcd). The spectral data of **7** were similar to those of **3**, with an additional oxygen functionality. The third oxygen atom is shown to be another hydroxy group on the aromatic ring by the ^1H NMR spectrum, which exhibits five aromatic proton signals instead of six as in compound **3**. By thorough analysis of 2D NMR data (HMBC, Figure 3), the hydroxyl group was placed at C-6, and the structure for **7** was assigned as 32,33-dihydro-6,31-dihydroxymanzamine A. The absolute configuration at the remaining five chiral centers of **7** also appears to be the same as those of **3** inasmuch as both compounds were isolated from the same sponge, and also are comparable to the NOE data and possessed dextrorotation. The absolute stereochemistry at C-31 was elucidated by applying Mosher's method.[14] The values of $\Delta\delta$ [($\delta_S - \delta_R$), H_2-29 $\Delta\delta$ -0.10 and -0.08, H_2-30 $\Delta\delta$ -0.58 and -0.56, H_2-32 $\Delta\delta$ $+0.59$ and $+0.52$, H_2-33 $\Delta\delta$ $+0.29$ and $+0.25$] obtained from the ^1H NMR spectra of MTPA esters indicated that the absolute configuration at C-31 was *R*.

Compound **8** was obtained as a pale yellow powder and was shown by HRESIMS to have the molecular formula $C_{11}H_{10}N_2O_3$ [*m/z* 187.0866, $(M + H)^+$, Δ 0.5 mmu of calcd]. The IR spectrum indicated the presence of NH and C=O groups, and the ^1H NMR spectrum exhibited the four aromatic protons at δ 7.59 (d, 8.3 Hz), 7.43 (d, 8.3 Hz), 7.26 (t, 8.5 Hz), and 7.23 (t, 8.5 Hz) and two vicinal aliphatic protons at δ 3.64 (t, 14.2) and 3.02 (t, 14.1), which suggested a 3,4-dihydro-β-carboline moiety. In the ^{13}C NMR spectrum, 11 carbon signals including five quaternary carbons, four CH carbons, and two CH_2 carbons were observed. On the basis of 2D NMR spectral data compound **8** was characterized as 1,2,3,4-tetrahydronorharman-1-one. Though this compound is commercially available, this is the first report in which the compound occurred from a natural source.

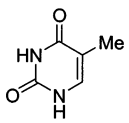
In the present study, the isolated nucleosides or nucleoside bases were identified on the basis of the spectral data (NMR and MS) as thymine,[15,16] 2′,3′-didehydro-2′,3′-dideoxyuridine,[17] uracil,[15,16] thymidine,[18] and 2′-deoxyuridine.[18]



9 (4-Methyl-pyrazole 3(5)-carboxylic acid)



10 (Pyrazole-3(5)-carboxylic acid)



9a

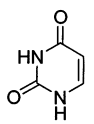


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Table 2. Bioactivity Data for Manzamines[a]

	activity in vitro					
	M. tuberculosis (H37Rv)	*P. falciparum* (D6 clone)	*P. falciparum* (chlorine-resistant W2 clone)	*L. donovani*		cytotoxicity (Vero)
compound	MIC μg/mL	IC 50 ng/mL	IC 50 ng/mL	IC 50 μg/mL	IC 90 μg/mL	ng/mL
12,34-oxamanzamine A	NT	4760	NA	14	40	NC
ent-12,34-oxamanamine E	128	NA	NA	NA	NA	NC
manzamine A (**1**)	1.53	4.5	8.0	0.9	1.8	1200
(+)-8-hydroxymanzamine A	0.91	6.0	8.0	6.2	11	1100
6-deoxymanzamine X	1.77	1300	1400	3.2	7.5	4760
manzamine E (**2**)	3.76	3400	4760	3.8	6.8	NC
manzamine X	NT	950	2000	5.7	11	NC
manzamine F (**4**)	2.56	780	1700	4.2	7.0	NC
32,33-dihydro-31-hydroxy-manzamine A (**3**)	NT	NA	NA	NA	NA	NC
32,33-dihydro-6-hydroxy-manzamine A-35-one (**5**)	NT	NA	NA	NA	NA	NC
des-*N*-methylxestomanzamine A (**6**)	NT	NA	NA	35	>50	NC
1,2,3,4-tetrahydronorharman-1-one (**8**)	NT	NA	NA	NA	NA	NC
norharman	NT	NA	NA	NA	NA	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; NT = not tested; NC = no cytotoxicity (concentration: 4760 ng/mL).

Careful examination of spectral data (NMR, MS) of thymine and uracil showed that they are similar to the data reported for **9** and **10**, isolated by Mishra et al.[19] (**9**) from the sponge *Suberites vestigium* and Parameswaran et al.[20] (**9** and **10**) from the sponge *Tedania anhelans*. The value of the vicinal proton coupling constant (J = 7.57 Hz) reported for **10** differs from pyrazoles (J = 2.1 Hz).[21] The IR absorption bands reported for **9** at 1724 and 1674 cm^{-1} and of **10** at 1730 and 1673 cm^{-1} support that they have two carbonyl functional groups. The IR recorded for pyrazole and 4-pyrazolecarboxylic acid (from Aldrich) did not show two carbonyl carbon bands, and the coupling constants in the ^{1}H NMR match with the literature values for pyrazoles.[21] On the basis of the reported data,[19,20] we believe that the structures of compounds **9** and **10** should be revised to the common primary metabolites thymine (**9a**) and uracil (**10a**). Without careful examination of coupling constants and IR data, these primary metabolites could easily be assigned as a pyrazole system.

In summary the compounds **3** and **7** are the first manzamine congeners with a hydroxyl group on the C-28−C-34 chain. Compound **5** is the first manzamine congener with a carbonyl group on C-35.

The enormous potential of the marine environment to provide new structural classes with activity against tuberculosis has been recently reported.[22] The in vitro activity of manzamines against *Mycobacterium tuberculosis* (H37Rv), 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 had an MIC of 0.91 μg/mL, indicating improved activity for the (+) over the (−) enantiomer.[8] Although compounds **3** and **5** were inactive against malaria and leishmania, these results provide valuable information on the structural moieties required for activity against malaria and leishmania. This observation further supports our previous report[4] which indicates that reduction of the C32−C33 olefin and oxidation of C-31 also significantly reduces the antimalarial activity for the manzamine alkaloids in vivo. These data combined strongly suggest that the ability of the C-34 allylic carbon to form a stabilized carbocation after oxidation both in cell culture and in animals followed by the inherent nucleophilic attack may play a critical role in the biological activity of the manzamine alkaloids against the malarial parasite. The significant differences in biological activities of manzamine A, manzamine E, and their corresponding 12,34-*oxa*-derivatives indicate that the C-12 hydroxy, C-34 methine, or the conformation of the lower aliphatic rings plays a key role in the antimalarial and leishmanicidal activity and provides valuable insight into the structural moieties required for activity against the malaria and leishmania parasites. The significant leishmaniacidal activity of ircinol A (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. The cytotoxicities of 6-deoxymanzamine X and manzamine X against A-548, HT-29, H-116, and MS-1 cell lines with IC_{50} (μg/mL), respectively, are as follows: 1.0, 5.1; 0.5, 0.5; 0.5, 5.1; 1.0, 5.1. The anti-HIV activity of manzamine A, 8-hydroxymanzamine A, and 6-deoxymanzamine X against human peripheral blood mononuclear (PBM) cells was determined, with median effective concentrations (EC_{50}) respectively of 0.59, 4.2, and 1.6 μM.

The diversity of manzamines that have been isolated from different species of sponges is unusual and raises the question of the origin of these metabolites. Microbial fauna/flora associated with these sponges may be responsible for the production of manzamines, and the observed chemical variation may be due to differences in the sponge-associated microbes in the host as well as the host's ability to transform these unusual alkaloids into secondary products.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-310 digital polarimeter. The IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrophotometer. UV spectra were recorded using a Perkin-Elmer Lambda 3B UV/vis spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, MeOD, and

DMSO-d_6 on NMR spectrometers operating at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C NMR. The HRESIMS spectra were measured using a Bioapex FTESI-MS with electrospray ionization. The X-ray diffraction data were collected on an Enraf-Nonius Kappa CCD area detector equipped with a rotating anode X-ray generator and Mo Kα radiation. The SIR-92 direct methods package[23] was used to locate the non-hydrogen atoms, and the WinGX package[24] was used for completing the structure determination. ORTEP[25] was used to generate Figure 4. Silica gel (200−400 mesh) and alumina (63−200 μm) were obtained from Natland International Corporation (www.natland.com) and Scientific Adsorbents Incorporated (www.saisorb.com), respectively. TLC analyses were carried out on precoated silica gel G_{254} or aluminum oxide ALOX-100 UV_{254} 500 μm. HPLC was performed on a Waters 510 model system.

Animal Material. The sponge was collected from vertical slopes between 33 and 40 m from Knife Cape, Manado Bay, Indonesia, on March 20, 2001, and is massively encrusting and extremely fragile. The details of taxonomy and morphology were described previously.[8]

Extraction and Isolation. The sponge was stored frozen until extracted. The lyophilized sponge (3.8 kg, dry weight) was crushed, homogenized, and then successively extracted with hexane and acetone at room temperature. TLC analysis indicated that both extracts contained manzamine A, together with various minor alkaloids as detected by Dragendorff reagent. The combined extract (110 g) was subjected to Si gel vacuum liquid chromatography and eluted in order, with dichloromethane (100%), dichloromethane−acetone (9:1, 3:1, 1:1), acetone (100%), chloroform−methanol (1:1), and methanol (100%). A total of nine major fractions were collected, and TLC was utilized to monitor the elution of metabolites.

Fraction 5 (34 g) was rechromatographed on Si gel and eluted with chloroform−acetone to obtain crude manzamine A (3.8 g), which was further purified over alumina (hexane−acetone, 95:5), HPLC (C-8, 100 mm, λ 410 nm, flow 19.8 mL/min), and acetonitrile−water (0.1% TFA), to obtain 8-hydroxymanzamine A (40 mg, 1.0 × 10^{-3}% dry wt), manzamine A (**1**, 3.2 g, 8.4 × 10^{-3}% dry wt), 12,34-oxamanzamine E (4 mg, 1.0 × 10^{-4}% dry wt), and 12,34-oxamanzamine A (2.2 mg, 5.8 × 10^{-5}% dry wt).

Purification of fraction 6 (22 g, CC, alumina, with hexane−acetone, HPLC using acetonitrile−water gradient (0.1% TFA with a 22 × 250 mm C8 column, 254 nm) gave 6-deoxymanzamine X (40 mg, 1.0 × 10^{-3}% dry wt), manzamine A (**1**, 20 mg), manzamine E (**2**, 4 mg, 1.0 × 10^{-4}% dry wt), 32,33-dihydro-31-hydroxymanzamine A (**3**, 4 mg, 1.0 × 10^{-4}% dry wt), manzamine X (40 mg, 1.0 × 10^{-3}% dry wt), manzamine F (**4**, 20 mg, 5.3 × 10^{-4}% dry wt), 32,33-dihydro-6-hydroxymanzamine A-35-one (**5**, 4 mg, 1.0 × 10^{-4}% dry wt), thymine (12 mg, 3.2 × 10^{-4}% dry wt), and des-*N*-methylxestomanzamine-A (**6**, 4 mg, 1.0 × 10^{-4}% dry wt).

Column chromatography of fraction 7 (20 g) over Si gel, by eluting with chloroform−methanol (gradient), gave five fractions. Further workup of fraction 2 (CC over alumina, hexane−acetone, followed by HPLC, gradient elution, acetonitrile−water (0.1% TFA, 22 × 250 mm, C8 column, at 380 nm) gave 32,33-dihydro-6,31-dihydroxymanzamine A (**7**, 4 mg, 1.0 × 10^{-4}% dry wt), 1,2,3,4-tetrahydronorharman-1-one (**8**, 5 mg, 1.3 × 10^{-4}% dry wt), and norharman (5 mg, 1.3 × 10^{-4}% dry wt). Purification of fraction 3 gave 10 mg of 2′,3′-didehydro-2′,3-dideoxyuridine (2.6 × 10^{-4}% dry wt), fraction 4 on further workup gave 20 mg of uracil (5.3 × 10^{-4}% dry wt) and deoxythymidine (25 mg, 6.6 × 10^{-4}% dry wt), and the fifth subfraction gave 2′-deoxyuridine (12 mg, 3.2 × 10^{-4}% dry wt).

32,33-Dihydro-31-hydroxymanzamine A (3): colorless crystals; $[\alpha]^{25}_D$ +34.44 (*c* 0.9, $CHCl_3$); UV (MeOH) λ_{max} 215, 248, 281, 291, 352, 359 nm; IR ($CHCl_3$) ν_{max} 3280, 2954, 2927, 1560, 1493, 1453, 1370, 1276, 1150, 748, 665 cm^{-1}; NMR data, see Table 1; HRESIMS *m*/*z* 567.4052 (calcd for $C_{36}H_{46}N_4O_2$, $[M + H]^+$, 567.4087).

X-ray Crystallographic Analysis of 3. A suitable needle, 0.06 × 0.09 × 0.45 mm, of **3** was obtained by slow crystallization from MeOH at room temperature for one week. Crystal data: $C_{36}H_{46}N_4O_2 \cdot CH_3OH$, orthorhombic, space group $P2_12_12_1$, $Z = 8$, unit cell parameters $a = 10.2728(1)$ Å, $b = 15.6215(2)$ Å, $c = 39.9081(6)$ Å, $V = 6404.3(1)$ Å^3, $T = 150(1)$ K, $F(000) = 2520$, $\lambda = 0.71073$ Å, μ(Mo Kα) = 0.076 mm^{-1}. There are two independent molecules (atoms numbered 1−40 and 51−90) in the asymmetric unit and a molecule of methanol. Final refinement with 8250 reflections ($\theta_{max} = 22.5°$) led to $R(F)$, $R(F>2\sigma)$, and GOF of 0.0577, 0.0400, and 1.023. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 192398. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

32,33-Dihydro-6-hydroxymanzamine A-35-one (5): pale yellow powder; mp >200 °C (dec); $[\alpha]^{25}_D$ +10.0 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 219, 248, 268, 356, 395; IR ($CHCl_3$) ν_{max} 3324, 2935, 1661, 1559, 1461, 1197, 664 cm^{-1}; NMR data, see Table 1; HRESIMS *m*/*z* 581.3467 (calcd for $C_{36}H_{44}N_4O_3$, $[M + H]^+$, 581.3492).

Des-*N*-methylxestomanzamine A (6): yellow powder; mp 192 °C (dec); UV (MeOH) λ_{max} 218, 258, 298, 356, 395; IR (KBr) ν_{max} 3425, 3075, 1620, 1210, 1208, 1130 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.98 (1H, s, NH-9), 8.59 (1H, s, H-15), 8.57 (1H, d, J = 5.1 Hz, H-3), 8.45 (1H, d, J = 8.2 Hz, H-5), 8.31 (1H, d, J = 5.1 Hz, H-4), 8.02 (1H, s, H-13), 7.82 (1H, d, J = 7.9 Hz, H-8), 7.59 (1H, t, J = 7.9 Hz, H-7), 7.30 (1H, t, J = 7.9 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ 184.5 (C-10), 142.6 (C-9a), 140.3 (C-15), 140.0 (C-13), 138.3 (C-3), 136.6 (C-1), 136.1 (C-8a), 131.9 (C-4b), 129.9 (C-11), 129.8 (C-7), 122.7 (C-5), 121.1 (C-6), 120.9 (C-4a), 119.8 (C-4), 113.9 (C-8); HRESIMS *m*/*z* 263.0915 (calcd for $C_{15}H_{10}N_4O$, $[M + H]^+$, 263.0933).

32,33-Dihydro-6,31-dihydroxymanzamine A (7): pale yellow powder; $[\alpha]^{25}_D$ +25.9 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 218, 240, 280, 291, 356, 359 nm; IR ($CHCl_3$) ν_{max} 3324, 2928, 1649, 1559, 1461, 1194, 675 cm^{-1}; NMR data, see Table 1; HRESIMS *m*/*z* 583.3641 (calcd for $C_{36}H_{46}N_4O_3$, $[M + H]^+$, 583.3643).

(*R*)- and (*S*)-MTPA Esters of 32,33-Dihydro-6,31-dihydroxymanzamine A (7). Compound **7** (0.5 mg) was dissolved in 500 μL of pyridine and treated with 5 μL of (*R*)-(−)-MTPA chloride at room temperature for 24 h. After addition of MeOH (800 μL), the solvent was removed in vacuo, and the residue was purified on SiO_2 TLC (hexane−acetone, 1:1) to afford the (*S*)-MTPA ester (0.1 mg) of **7a**. The (*R*)-MTPA ester (**7b**) of **7** was prepared with (*S*)-(−)-MTPA chloride according to the same procedure as described above.

(*S*)-MTPA ester of 7: ^{1}H NMR ($CDCl_3$) δ 8.36 (1H, d, J = 5.2 Hz, H-3), 8.08 (1H, d, J = 5.2 Hz, H-4), 7.84 (1H, d, J = 8.8 Hz, H-8), 7.25−7.54 (7H, m, Ph and H-5 and H-7), 6.42 (1H, s, H-11), 5.59 (1H, m, H-15), 5.53 (1H, m, H-16), 4.23 (1H, m, H-31), 2.81 (1H, m, H-30), 2.59 (1H, m, H-30), 2.36 (1H, m, H-32), 1.99 (1H, m, H-32), 1.81 (1H, m, H-33), 1.75 (1H, m, H-33), 1.72 (1H, m, H-29), 1.63 (1H, m, H-29); HRESIMS *m*/*z* 799.4894 (calcd for $C_{46}H_{53}F_3N_4O_5$, $[M + H]^+$, 799.4046).

(*R*)-MTPA ester of 7: ^{1}H NMR ($CDCl_3$) δ 8.35 (1H, d, J = 5.3 Hz, H-3), 8.09 (1H, d, J = 5.3 Hz, H-4), 7.85 (1H, d, J = 2.2 Hz, H-8), 7.25−7.54 (7H, m, Ph and H-7 and H-5), 6.59 (1H, s, H-11), 5.57 (2H, br m, H-15 and H-16), 4.21 (1H, m, H-31), 3.37 (1H, m, H-30), 3.17 (1H, m, H-30), 1.84 (1H, m, H-32), 1.80 (1H, m, H-29), 1.73 (1H, m, H-29), 1.52 (1H, m, H-33), 1.50 (1H, m, H-33), 1.40 (1H, m, H-32); HRESIMS *m*/*z* 799.4864 $[M + H]^+$ (calcd for $C_{46}H_{53}F_3N_4O_5$, $[M + H]^+$, 799.4046).

1,2,3,4-Tetrahydronorharman-1-one (8): pale yellow powder; UV (MeOH) λ_{max} 210, 240, 325; IR ($CHCl_3$) ν_{max} 3424, 2920, 1629, 1085 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.59 (1H, d, J = 8.3 Hz, H-5), 7.43 (1H, d, J = 8.3 Hz, H-8), 7.26 (1H, t, J = 8.5 Hz, H-7), 7.23 (1H, t, J = 8.5 Hz, H-6), 3.64 (1H, t, J = 14.2 Hz, H-3) 3.02 (1H, t, J = 14.1 Hz, H-4); ^{13}C NMR (CD_3OD) δ 164.5 (C-1), 138.2 (C-8a), 125.8 (C-9a), 125.0 (C-4b), 124.9 (C-7), 120.2 (C-6), 120.0 (C-4a), 112.4 (C-8), 111.9 (C-5), 41.8 (C-3), 20.6 (C-3); HRESIMS *m*/*z* 187.0866 (calcd for $C_{11}H_{10}N_2O_3$, $[M + H]^+$, 187.0871).

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,[26] antituberculosis,[22] and HIV[27] assays were performed using the referenced procedures.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for all new compounds, HMQC spectra for **3**, **5**−**7**, HMBC spectra for **5**−**8**, and X-ray data for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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