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New Manzamine Alkaloids from an Indo-Pacific Sponge. Pharmacokinetics, Oral Availability, and the Significant Activity of Several Manzamines against HIV-I, AIDS Opportunistic Infections, and Inflammatory Diseases

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Abstract

12,28-Oxamanzamine A (**1**), 12,28-oxa-8-hydroxymanzamine A (**2**), and 31-keto-12,34-oxa-32,33-dihydroircinal A (**3**) were isolated from two collections of an Indo-Pacific sponge, and their structures were assigned on the basis of 1D and 2D NMR spectroscopic data. These compounds possess a novel manzamine-type ring system generated through a new ether bridge formed between carbons 12 and 28 or between carbons 12 and 34 of the typical manzamine structure and add to our growing understanding of manzamine SAR and metabolism. Based on molecular modeling studies, the formation of these oxidation products is highly sterically favored. The potent antiinflammatory, antifungal, and anti-HIV-1 activity for a number of previously reported manzamines is also presented in addition to the pharmacokinetic studies of manzamine A (**5**). Oral and intravenous pharmacokinetic studies of manzamine A in rats indicated the compound to have low metabolic clearance, a reasonably long pharmacokinetic half-life, and good absolute oral bioavailability of 20.6%, which supports the value of these compounds as potential leads for further preclinical assessment and possible development.

Introduction

As part of a continuing investigation of new man-zamine-related alkaloids with activity against infectious and tropical disease,^{1–3} three naturally occurring oxidation products including 12,28-oxamanzamine A (**1**), 12,28-oxa-8-hydroxymanzamine A (**2**), and 31-keto-12,34-oxa-32,33-dihydroircinal A (**3**) were isolated and characterized from an

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undescribed species of the genus *Acanthostrongylophora*. Each new manzamine contains an ether linkage unique to the structural class. Here, we describe the isolation, structure elucidation, and bioactivity of **1-3**. In addition, key oral and intravenous pharmacokinetic studies of manzamine A (**5**) have been completed and are reported here. Following exploratory dosing studies conducted in rats, the intravenous (iv) pharmacokinetic parameters of half-life, volume of distribution, and clearance could not be accurately determined from data obtained over an 8 h sampling period. To better define the iv and oral pharmacokinetic parameters of this promising drug lead, further dosing studies were conducted in rats employing an extended sampling period of 96 h.

Results and Discussion

The lipophilic extract of the freeze-dried sponge 01IND35 (4.5 kg) afforded, after repeated chromatography on silica gel, alumina and RP-HPLC, the new (+)-12,28-oxamanzamine A (**1**) (10.5 mg, 2.3×10^{-4} % dry wt), (+)-12,28-oxa-8-hydroxymanzamine A (**2**) (1.7 mg, 3.7×10^{-5} % dry wt), and a significant quantity of the antimalarial drug lead neokauluamine (3 g, 6.6×10^{-2} % dry wt). The lipophilic extract of a second sample of the freeze-dried sponge 01IND35 collected on May 18, 2002, afforded the known (+)-manzamine A⁶ (**5**) (10.5 g, 2.3×10^{-1} % dry wt), (+) manzamine F⁷ (9.6 g, 2.1×10^{-1} % dry wt), and the new (+)-31-keto-12,34-oxa-32,-33-dihydroircinal A (**3**) (16.8 mg, 3.7×10^{-4} % dry wt).

The high-resolution Fourier transform mass spectrum (HRFTMS) of **1** displayed a molecular ion peak ($M + 1$) at m/z 547.3414, which combined with ^1H and ^{13}C NMR data (Table 1) suggested a molecular formula of $\text{C}_{36}\text{H}_{42}\text{N}_4\text{O}$ and 18 degrees of unsaturation. A comparison of the data for this new compound with the literature data for (+)-12,34-oxamanzamine E (**4**)¹ indicated that compounds **1** and **4** had similar structures. The ^{13}C NMR signals in both compounds matched closely, while only C-28 and C-31 to C-34 differed substantially, indicating that these compounds have the same skeleton but have some differences in functionalities and oxygen substitution. The heteronuclear multiple-bond correlation

(HMBC) spectra of both compounds showed similar correlations except for C-28 and C-34, confirming the same skeleton. The proton singlet resonating at δ 4.35 correlated to the nitrogenated methine carbon at δ 76.0 (C-26) in the heteronuclear multiple-quantum coherence (HMQC) spectrum and was assigned H-26. This proton showed HMBC correlations to a quaternary carbon (δ 77.7) and a methine carbon (δ 94.7), which were assigned as C-12 and C-28, respectively. The downfield shift of C-12 (δ 77.7) and C-28 (δ 94.7) in **1** compared with that of **4** suggested that the new ether bridge is between C-12 (C) and C-28 (CH) in **1** instead of between C-12 (C) and C-34 (C) as in **4**.¹ The lack of a keto group at C-31 and the presence of a double bond between C-32 and C-33 in **1** are clearly indicated by the compounds ¹H and ¹³C NMR data.

¹H and ¹³C NMR data of compound **2** revealed that it differed from **1** only in the carbocyclic ring of the β -carboline moiety (C-5 to C-8a, Table 1). An additional oxygen atom in the molecular formula of **2** (C₃₆H₄₂N₄O₂) suggested a phenolic hydroxyl, which is also shown by the ¹H NMR spectrum exhibiting five aromatic proton signals instead of six, combined with a new downfield oxygenated aromatic quaternary carbon at δ 143.4 (C-8) in **2**. The relative stereochemistry of **1** and **2** was deduced from the nuclear Overhauser effect spectrometry (NOESY), which showed NOE correlations between H-26 and H-28 as well as between H-24 and H-35. The 12,28-oxaether bridge was assigned α -orientation based on retention of stereochemistry of the C-12 oxygen in the parent compounds manzamine A⁶ (**5**) and 8-hydroxymanzamine A⁸ (**6**) during the formation of the ether bridge through a proposed enzymatic oxidation followed by intramolecular quenching of the cation by the 12-OH group (Figure 1). The absolute and relative stereochemistry of **1** and **2** would then be analogous to that of **5** and **6** and is further supported by NOE data (NOE correlations between H-26 and H-28 as well as between H-24 and H-35), optical rotation values, and molecular modeling [α]_D²⁵ +38 for **1** and +8 for **2** in CHCl₃).

The lipophilic extract (3.5 kg) of the sponge 01IND35 (second collection) afforded compound **3**. The ¹H and ¹³C NMR data of compound **3** exhibited a close resemblance to that of ircinal A (**7**) (Table 1). The HRFTMS of **3** suggested the molecular formula C₂₆H₃₆N₂O₃ and indicated the presence of one more double bond equivalent compared with that of ircinal A (**7**). This suggested the presence of a new ether bridge between C-12 and C-34, similar to that of **4**, which was confirmed by ¹³C NMR data. The downfield quaternary carbon signal at δ 201.2 was assigned as the C-31 ketone group, based on its HMBC correlations with H2-29 and H2-33. Other key HMBC correlations were observed between H-1 and C-10, between C-11 and C-24, and between H-26 and C-12 and C-34 supporting the structure of compound **3**. The relative stereochemistry was determined to be analogous to that of ircinal A (**7**), based on NOESY, which showed NOE correlations between H-24 and H-35. In addition, the retention of stereochemistry of the C-12 oxygen in the parent compound **7** during the formation of the ether bridge is supported by the optical rotation value of [α]_D²⁵ +44 in CHCl₃ combined with molecular modeling.

The highly promising antituberculosis and antimalarial activities of the manzamines have been reported previously. The common manzamines were assayed for antiinflammatory, antibacterial, antifungal, and antihuman immunodeficiency virus (HIV-1) activity, and these

additional data against infectious diseases are reported here (Table 2). Manzamine A, 8-hydroxyman-zamine A, 6-deoxymanzamine X, and neokaulumine were active against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Cryptococcus neoformans*, and *Mycobacterium intracellulare*, having MIC values in the range 0.5-6.25 $\mu\text{g/mL}$. Manzamine A, 8-hydroxyman-zamine A, 6-deoxymanzamine X, and neokaulumine showed significant anti-HIV-1 activity with EC_{50} of 4.2, 0.59, 1.6, and 2.3 μM , respectively. The absence of activity for **1-3**, 12,34-oxamanzamine E and F, and manzamine E and F is clearly associated with the presence of a C-31 ketone group, reduction of the double bond, or the formation of the ether bridge between carbons 12 and 28 or between carbons 12 and 34. The antiinflammatory activity of ent-8-hydroxymanzamine A and neokaulumine was determined as previously described by investigating the effect of these compounds on the release of rat neonatal brain microglia superoxide anion and thromboxane B_2 , mediators thought to be involved in neuroinflammatory conditions and lactate dehydrogenase (LDH), a marker for cell toxicity. Both ent-8-hydroxymanzamine A and neokaulumine were relatively ineffective inhibitors of both phorbol ester stimulated thromboxane B_2 ($\text{IC}_{50} > 30 \mu\text{M}$) and superoxide anion ($\text{IC}_{50} > 30 \mu\text{M}$) generation as well as rather nontoxic to the microglia cells in vitro ($\text{LDH}_{50} > 30 \mu\text{M}$).

The plasma concentration versus time profiles for manzamine A are presented in Figure 2, with concentrations remaining well above the assay limit of quantitation (LOQ) (10 ng/mL) throughout the entire 96 h sampling period following both iv and oral administration. After iv administration of manzamine A to rats at a dose of 10 mg/kg, the half-life, apparent volume of distribution, and plasma clearance were estimated from the mean plasma concentration data (from $n = 3$) to be 53.7 h, 23.7 L/kg, and 5.1 (mL/min)/kg, respectively. As noted in the earlier dosing studies, urine collected from these animals was red for approximately 2 h following the iv administration of manzamine A. After this time, however, it was observed that the color of the urine returned to a normal clear-yellow color. Collected plasma samples also showed evidence of hemolysis, and no evidence of discoloration in urine or plasma samples was noted in a control animal dosed intravenously with a drug-free iv formulation, indicating that these effects were unlikely due primarily to the vehicle.

After oral administration to rats at a dose of 50 mg/kg, the absolute oral bioavailability of manzamine A was approximated using truncated area under the curve (AUC) values for the oral and iv profiles (AUC_{0-96}), resulting in an absolute oral bioavailability of $20.6 \pm 1.3\%$ (mean $\pm$ SEM for four rats). It is probable that while this estimate is still somewhat lower than the “actual” value because of the need to use truncated AUC values, it nevertheless indicates reasonable oral bio-availability in rats after administration in an aqueous suspension. The C_{max} and T_{max} values were $1066 \pm 177 \text{ ng/mL}$ and $10 \pm 5 \text{ h}$, respectively. This report describes the first published pharmacokinetic studies of man-zamine A. Most importantly, the oral bioavailability of manzamine A in rats was estimated to be at least 20.6%. Furthermore, plasma concentrations were observed to remain well above the assay LOQ in all animals for the duration of the 96 h study.

The sampling period employed in the current study enabled the estimation of the iv pharmacokinetic parameters of manzamine A, which demonstrated a very long half-life and

low plasma clearance. The in vitro metabolism studies of manzamine A conducted previously in human liver microsomes support the likelihood of slow hepatic metabolism, predicting a low hepatic extraction ratio of 0.4.

The basis for the good oral bioavailability of man-zamine A is likely to result from reasonable metabolic stability and good absorption. The acid solubility of manzamine A suggests that the compound should readily dissolve in the stomach, while the compound is expected to be reasonably permeable in light of its high log *P* (8.34) that was determined using a chromatographic method.

This study has provided the first published information regarding the pharmacokinetic properties of man-zamine A in rats. It is likely that the very long half-life and low plasma clearance of this interesting compound may have significant pharmacodynamic implications with respect to its reported excellent antimalarial activity.

Molecular Modeling

Manzamine A exhibited significant antibacterial, antifungal, and anti-HIV activity, while 12,28-oxaman-zamine A, the oxidative cyclization product of man-zamine A, did not show notable biological activity. To better understand the structure-activity relationship and possible steric effects contributing to this oxidation, a molecular modeling study was performed using a Sybyl 6.8 software package. The minimized energy conformations of manzamine A and 12,28-oxaman-zamine A are shown in Figures 3 and 4, respectively. The minimized energy conformation of manzamine A is highly comparable to the conformation of crystalline manzamine A hydrochloride determined by X-ray diffraction with the exception of the C₂₅-C₂₆-N₂₇-C₃₄-C₃₅ five-membered ring and the N₂₇-C₃₄ eight-membered ring. On the basis of the crystal structure of manzamine A hydrochloride, the difference between the solution-phase structure and X-ray structure clearly arose from the ionic bond between N₂₇ and the chloride ion. The minimized energy conformation of 12,28-oxamanzamine A is very close to that of manzamine A with minor differences appearing at the N₂₇-C₃₄ eight membered ring. The N₂₇-C₃₄ eight-membered ring is folded upward in 12,28-oxamanzamine A and the conformation of the N-27 is enantiomeric, suggesting that the antimicrobial and anti-HIV activity may be closely related to N-27 and the conformation of the N₂₇-C₃₄ eight-membered ring.

Experimental Section

General Experimental Procedures

The ¹H and ¹³C NMR spectra were recorded in CDCl₃ on a Bruker DRX NMR spectrometer operating at 400 MHz for ¹H and at 100 MHz for ¹³C NMR. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals of CDCl₃ at $\delta_{\text{H}}/\delta_{\text{C}}$ 7.26/77.0. The HRMS data were measured on a Bioapex FTMS with electrospray ionization. The IR spectra were recorded on an ATI Mattson Genesis series FTIR spectrophotometer. UV spectra were scanned on a Perkin-Elmer Lambda 3B UV/vis spectrometer. 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 was performed on aluminum sheets (silica gel 60 F254, Merck KGaA, Germany).

Sponge Collection, Identification, and Taxonomy

Sponge collection, identification, taxonomy, and extraction for 01IND35 have been described elsewhere. The sponge was collected for the second time from reef slopes and vertical surfaces between 6 and 33 m from Black Reef Point, Manado Bay, Indonesia, on May 18, 2002, by Professor Subagus Wahyuono and his research group.

(+)-**12,28-Oxamanzamine A (1)**: amorphous solid (CHCl_3); mp 148 °C dec, $[\alpha]_{\text{D}}^{25} +38$ (c 0.1, CHCl_3); UV λ_{max} (log ϵ) (MeOH) 253 (3.81), 274 (3.66), 352 (3.41) nm; IR ν_{max} (CHCl_3) 3650 (NH), 3001-2818, 1620, 1592, 1533, 1452, 1267, 1144, 1052 cm^{-1} ; HRFABMS m/z calcd for $\text{C}_{36}\text{H}_{43}\text{N}_4\text{O}$ ($\text{M} + \text{H}$)⁺ 547.3386, found 547.3414. For ^1H and ^{13}C NMR, see Table 1.

(+)-**12,28-Oxa-8-hydroxymanzamine A (2)**: amorphous powder (EtOH); mp 160 °C dec; $[\alpha]_{\text{D}}^{25} +8$ (c 0.10, CHCl_3); UV λ_{max} (log ϵ) (MeOH) 251 (3.85), 273 (3.78), 356 (3.38) nm; IR ν_{max} (CHCl_3) 3658 (NH), 3377 (OH), 3002-2822, 1620, 1592, 1533, 1452, 1267, 1144, 1052 cm^{-1} ; HRFABMS m/z calcd for $\text{C}_{36}\text{H}_{43}\text{N}_4\text{O}_2$ ($\text{M} + \text{H}$)⁺ 563.3335, found 563.3313. For ^1H and ^{13}C NMR, see Table 1.

(+)-**31-Keto-12,34-oxa-32,33-dihydroircinal A (3)**: white powder (MeOH); mp 164 °C (dec); $[\alpha]_{\text{D}}^{25} +44$ (c 0.1, CHCl_3); UV λ_{max} (log ϵ) (MeOH) 235 (3.929) nm; IR ν_{max} (CHCl_3) 3368 (OH), 3001-2815, 1695 (C=O), 1625, 1590, 1535, 1451, 1265, 1145, 1050 cm^{-1} ; HRFABMS m/z calcd for $\text{C}_{26}\text{H}_{37}\text{N}_2\text{O}_3$ ($\text{M} + \text{H}$)⁺ 425.3408, found 425.3458. For ^1H and ^{13}C NMR, see Table 1.

Pharmacokinetic Studies

Pharmacokinetic studies were conducted in male Sprague-Dawley rats and included individual iv and oral administration of manzamine A. Briefly, on the day prior to dosing, rats (280-320 g) had polyethylene cannulae surgically implanted in the right jugular vein and/ or left carotid artery. Animals were fasted but had free access to water until 12 h following dosing, after which free access to food was also resumed.

Intravenous formulations were prepared at an approximate concentration of 3 mg/mL manzamine A in 10% v/v ethanol in 50 mM tartrate buffer, pH 3.0, to enable a nominal iv dose of 10 mg/kg with a 1 mL dosing volume. The oral formulation was prepared using a standard aqueous suspending vehicle (SSV, 0.5% CMC, 0.5% benzyl alcohol, 0.4% Tween-80 in 0.9% NaCl) to provide a nominal dose of 50 mg/kg. Intravenous doses were administered as a 5 min infusion via the jugular vein cannula, and oral doses were administered by gavage.

Blood samples were withdrawn from an in-dwelling carotid cannula over a 96 h period. Samples were stored on ice until centrifugation to separate plasma from red blood cells, and a 100 μL aliquot of plasma was transferred to a fresh Eppendorf tube and stored at -20 °C

until analysis. A validated HPLC/MS method was developed and employed for determination of manzamine A plasma concentrations, and Winnonlin software (version 1.5, Scientific Consulting, Inc.) was utilized for pharmacokinetic parameter estimation.

Molecular Modeling

The molecular modeling studies were performed on a Silicon Graphics Octane2 workstation. The molecular structure was constructed using standard geometries and standard bond lengths in the Sybyl 6.8 software package and was manipulated using the standard Tripos force. The initial conformations of the molecule were obtained from 10 rounds of simulated annealing experiments. In each round of simulation, the molecule was heated to 500 K within 500 fs and then allowed to cool to 200 K within 5000 fs. Ten energy optimal conformations were selected and refined by minimization using Powell's method, MMFF94 force field, and partial charges until a root-mean-square deviation of 0.001 kcal/(mol·Å) was achieved. A distance-dependent dielectric of 1.00 was used throughout the calculation. A strategy of simplex algorithm followed by conjugate gradient algorithm was used in the minimization. Finally, from these refined conformations, the conformer with the lowest energy was selected as the final molecular conformation.

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Figure 1.
Plausible formation of 12,28-oxaether bridge via an oxidation mechanism.

Figure 2.

Average plasma concentration versus time profiles for TDR 19620 after iv (top) and oral (bottom) administration to rats at a dose of approximately 10 and 50 mg/kg, respectively ($\pm$ SEM for $n = 3-4$). The LOQ of the HPLC/MS assay for TDR 19620 was 10 ng/mL.

Figure 3.
Minimized energy conformation of manzamine A

Figure 4.
Minimized energy conformation of 12,28-oxaman-zamine A (1).

Table 1

 ^{13}C and ^1H NMR Data of **1–3**^a

position	12,28-oxamanzamine A (1), δ		12,28-oxa-8-hydroxymanzamine A (2), δ		31-keto-12,34-oxa-32,33-dihydroircinal A (3), δ		ircinal A (7), ⁹ δ
	^{13}C or ^{15}N	^1H	^{13}C	^1H	^{13}C or ^{15}N	^1H	^{13}C
1	142.9, s		143.7, s		193.9, d	9.47, s	193.3, d
N2	ND		ND				
3	138.8, d	8.46, d (5.1)	138.9, d	8.45, d (5.0)			
4	113.7, d	7.83, d (5.1)	114.3, d	7.83, d (5.0)			
4a	129.6, s		129.8, s				
4b	121.9, s		122.2, s				
5	121.6, d	8.10, d (7.8)	121.2, d	7.66, d (7.6)			
6	120.2, d	7.27, t (7.4)	120.5, d	7.14, t (7.4)			
7	128.4, d	7.53, t (7.1)	113.0, d	7.01, d (7.7)			
8	111.7, d	7.55, d (7.8)	143.4, s				
8a	141.4, s		136.3, s				
N9	ND	8.64, s	ND	9.18, s			
9a	133.6, s		133.7, s				
10	140.2, s		142.3, s		144.5, s		142.6, s
11	135.1, d	6.58, s	136.1, d	6.70, s	151.5, d	6.53, s	157.6, d
12	77.7, s		77.8, s		79.3, s		70.2, s
13	41.0, t	2.34, m	41.0, t	2.23, m	39.6, t	2.65, m	38.9, t
		1.63, m		2.01, m		2.32, m	
14	22.2, t	2.95, m	22.4, t	2.35, m	22.6, t	2.31, m	21.0, t
		2.35, m		1.95, m		2.41, m	
15	128.2, d	5.64, br s	138.3, d	5.60, br s	129.7, d	5.28, m	127.9, d
16	133.0, d	5.53, br s	133.7, d	5.53, br s	129.3, d	5.26, m	132.5, d
17	26.1, t	1.85, m	23.3, t	1.60, m	30.0, t	2.15, m	25.6, t
		1.78, m		1.54, m		2.23, m	
18	25.7, t	1.54, m	25.9, t	1.89, m	25.3, t	1.86, m	26.7, t
		1.34, m		1.55, m		1.73, m	
19	24.3, t	1.48, m	24.5, t	1.82, m	23.3, t	1.84, m	25.3, t
		1.40, m		1.72, m		1.69, m	
20	52.5, t	2.82, m	52.7, t	2.75, m	59.3, t	2.75, m	53.5, t
		2.37, m		2.32, m		2.44, m	
N21	ND		ND		38.3, S		
22	49.1, t	3.22, m	49.3, t	3.23, br d (9.3)	54.4, t	3.05, m	49.4, t
		2.14, m		2.25, m		2.15, m	
23	33.7, t	2.39, m	34.2, t	2.69, m	32.1, t	2.46, m	31.6, t
		2.64, m		2.15, m		2.31, m	
24	43.0, d	2.49, dd (11.8, 5.5)	43.4, d	2.63, dd (12.1, 5.2)	39.5, d	2.45, dd (12.0, 5.4)	34.0, d
25	42.5, s		42.3, s		37.7, s		46.4, s

position	<u>12,28-oxamanzamine A (1), δ</u>		<u>12,28-oxa-8-hydroxymanzamine A (2), δ</u>		<u>31-keto-12,34-oxa-32,33-dihydroircinal A (3), δ</u>		<u>ircinal A (7),⁹ δ</u>
	¹³ C or ¹⁵ N	¹ H	¹³ C	¹ H	¹³ C or ¹⁵ N	¹ H	¹³ C
26	76.0, d	4.35, s	76.0, d	4.37, s	68.0, d	4.24, s	76.3, d
N27	ND		ND		74.2, S		
28	94.7, d	4.69, t (8.3)	95.4, d	4.72, t (8.2)	49.8, t	3.37, dd (12.7, 11.6) 2.83, dd (12.8, 4.6)	51.4, t
29	26.7, t	1.75, m 1.78, m	26.4, t	1.54, m 1.46, m	30.0, t	1.83, m 1.56, m	29.8, t
30	26.9, t	1.62, m 1.48, m	26.9, t	1.95, 2H, m	31.4, t	1.69, m 1.94, m	25.3, t
31	23.3, t	3.25, m 2.70, m	27.0, s	3.32, m 2.82, m	201.2, s		28.2, t
32	132.8, d	5.37, m	133.1, t	5.37, m	30.8, t	2.85, m 1.86, m	137.1, d
33	123.9, d	5.35, m	124.1, t	5.31, m	29.9, t	2.85, m 1.86, m	127.7, d
34	60.9, d	3.75, d (7.2, 7.0)	61.3, s	3.79, dd (7.2, 7.1)	102.2, s		55.4, d
35	48.8, t	2.24, d (12.4) 2.44, d (12.4)	49.0, t	2.32, d (12.4) 2.26, d (12.4)	45.7, t	2.35, d (12.5) 2.21, d (12.3)	44.6, t
36	68.6, t	3.14, d (11.1) 2.26, d (11.1)	68.8, t	3.18, d (11.1) 2.32, d (11.1)	66.1, t	3.12, d (11.2) 3.22, d (11.1)	69.2, t

^aIn CDCl₃, 400 MHz for ¹H and 100 MHz for ¹³C NMR and 50 MHz for ¹⁵N NMR. Nitromethane was used as the external standard for ¹⁵N NMR. Carbon multiplicities were determined by distortionless enhancement by polarization transfer (DEPT) experiments. s = quaternary, d = methine, t = methylene carbons. Coupling constants (*J*) are in Hz. NO = not observed. ND = not determined.

Table 2

Bioactivity Data for Manzamines^a

compd	assay				
	antibacterial IC ₅₀ (μg/mL)		antibacterial IC ₅₀ (μg/mL)		
	<i>Staphylococcus aureus</i>	methicillin-resistant <i>Staphylococcus aureus</i>	<i>Cryptococcus neoformans</i>	<i>Mycobacterium intracellulare</i>	HIV EC ₅₀ (μM)
12,28-oxamanamine A (1)	NA	NA	NA	NA	22.2
12,28-oxa-8-hydroxymanzamine A (2)	NA	NA	NA	NA	NT
31-keto-12,34-oxa-32,33-dihydroircinal A (3)	NA	NA	NA	NA	NT
12,34-oxamanzamine E	NA	NA	NA	NA	17.5
12,34-oxamanzamine F	NA	NA	NA	NA	14.9
neokauluamine	NA	NA	3.0	3.0	2.3
manzamine A (5)	0.5	0.7	3.0	0.35	4.2
8-hydroxymanzamine A (6)	0.9	4.0	3.0	1.0	0.59
6-deoxymanzamine X	1.5	1.5	2.0	6.25	1.6
manzamine E	NA	NA	NA	12.5	13.1
manzamine F	NA	NA	6.5	6.25	7.3
ircinal A	NA	NA	NA	NA	6.8
ircinol A	NA	30	NA	50	4.3
amphotericin B	NT	NT	0.15	NT	NT
ciprofloxacin	0.10	0.10	NT	0.25	NT
AZT ¹³	NT	NT	NT	NT	0.004

^aNA = not active. NT = not tested.