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Four New Bioactive Manzamine-Type Alkaloids from the Philippine Marine Sponge *Xestospongia ashmorica*

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Analysis of the Philippine marine sponge *Xestospongia ashmorica* afforded four new manzamine congeners **1**−**4** and four known compounds **5** and **7**−**9**. Compound **1** is the 6-deoxy derivative of manzamine X, while **2**−**4** are the *N*-oxides of manzamine J (**5**), 3,4-dihydromanzamine A (**6**), and manzamine A (**7**), respectively. The structures of the new compounds were unambiguously established on the basis of NMR spectroscopic (^{1}H, ^{13}C, COSY, ^{1}H-detected direct, and long-range ^{13}C−^{1}H correlations) and mass spectrometric (EI, FAB-MS, and electrospray ionization) data. Alkaloid *N*-oxide structures were confirmed by conversion to the corresponding tertiary bases by reduction with Zn/HCl. This is the first report of the occurrence of bioactive manzamine *N*-oxides in marine sponges. Compound **7** exhibited insecticidal activity toward neonate larvae of the polyphagous pest insect *Spodoptera littoralis* (with an ED50 of 35 ppm) when incorporated in artificial diet and offered to larvae in a chronic feeding bioassay. Compound **7** was also active against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Cytotoxicity was studied *in vitro* using L1578y mouse lymphoma cells. From the alkaloids studied, the *N*-oxides **3** and **4** were the most active (ED50 = 1.6 μg/mL) followed by compound **7** (ED50 = 1.8 μg/mL).

Marine invertebrates are known as rich sources of alkaloids with unique chemical features and pronounced chemical activities, which suggests potential value as lead structures for the development of new pharmaceuticals.[1−3] The manzamine alkaloids are characterized by a complex pentacyclic diamine linked to C-1 of a β-carboline moiety. Manzamines have been isolated from six different genera of marine sponges: *Haliclona*,[4,5] *Pellina*,[6] *Xestospongia*,[7,8] *Ircinia*,[9] *Pachypellina*,[10] and *Amphimedon*.[11] In this paper, we describe the isolation and structure elucidation of new manzamine derivatives obtained from the Philippine marine sponge *Xestospongia ashmorica* n. comb. (order Haplosclerida, family Petrosiidae) and report on their insecticidal, antibacterial, and cytotoxic properties. We also present evidence of the occurrence of manzamine *N*-oxides using spectroscopic data and compare the biological activity with their parent compounds.

Results and Discussion

The marine sponge *Xestospongia ashmorica* was collected off the shores of Mindoro Island, Philippines. The samples were freeze-dried prior to transport and extraction. The *n*-BuOH-soluble material was subjected to Si gel column chromatography, and seven major fractions were obtained. The first fraction yielded **1** together with the known compound **5** (Chart 1). Compound **1** was obtained from the methanolic supernatant upon precipitation of **5** at 5 °C for 24 h. The second fraction afforded the known compounds manzamine A (**7**), manzamine E (**8**), and manzamine F (**9**). The last three polar fractions 5, 6, and 7 yielded the manzamine *N*-oxides **2**, **3**, and **4**, respectively.

A considerable amount of NMR data exist in the literature for manzamine derivatives. Since solvent and protonation effects can cause significant variations in chemical shifts, we used dichloromethane as the solvent for recording the NMR data, as all compounds were soluble in this solvent and it avoided the protonation problems encountered with chloroform solutions. Undue emphasis has not been placed on the interpretation of either ^{13}C or ^{1}H chemical shifts, but through-bond homonuclear (^{1}H COSY) and heteronuclear (^{1}H-detected one-bond and multiple-bond ^{13}C multiple-coherence) correlations have been used to establish assignments and atom connectivities in **1**−**4**. Shift data were then compared with literature data for compounds containing similar subunits in the structure. The identities of **5**−**9** were established by comparison with published data.

Compound **1** showed the molecular peak $(M + H)^+$ at *m/z* 565 in FAB-MS and electrospray ionization, which is compatible with the molecular composition of $C_{36}H_{44}N_4O_2$. The ^{1}H and ^{13}C-NMR data are similar to those of **8** (Tables 1 and 2) and manzamine X (**10**).[7,8] It has the same molecular composition as **8**, but its ^{13}C-NMR spectrum lacks the carbonyl signal at 216 ppm, which was attributed to C-31 of **8**, and instead this portion of the lower pentacyclic moiety has a signal at 79.8 ppm that correlates with a broad doublet at 4.54 ppm (J = 7.9 Hz) in the HMQC spectrum that is

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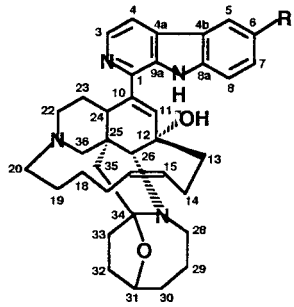
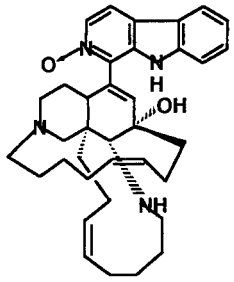
Chart 1



R = H: 1
R = OH: 10



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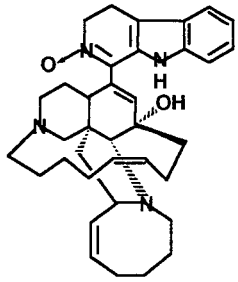

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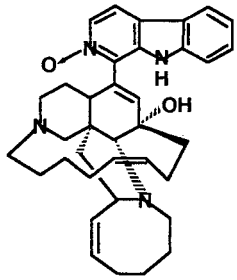

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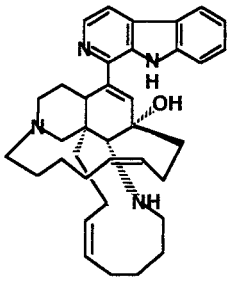

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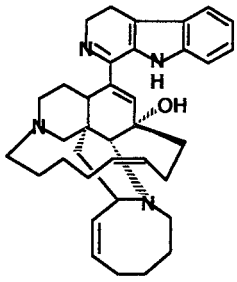
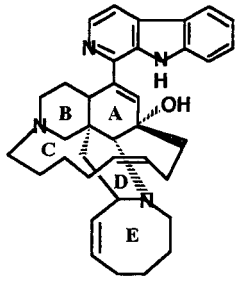
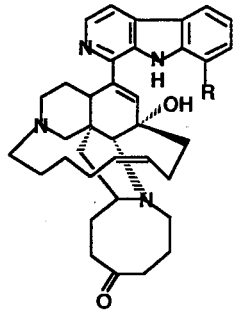


6



7



R = H: 8
R = OH: 9

Table 1. ^{13}C-NMR Data of Compounds **1**−**4** in CD_2Cl_2[a]

C no.	1	2	3	4
1	143.8 s	135.0 s	136.6 s	135.0 s
3	137.8 d	133.1 d	61.9 t	132.9 d
4	113.5 d	115.3 d	20.3 t	115.2 d
4a	129.4 s	120.3 s	108.3 s	120.5 s
4b	122.1 s	122.5 s	126.5 s	122.5 s
5	121.8 d	121.2 d	119.1 d	121.2 d
6	120.3 d	121.4 d	121.0 d	121.3 d
7	129.0 d	127.8 d	123.5 d	127.8 d
8	112.0 d	111.9 d	112.0 d	112.0 d
8a	140.5 s	141.1 s	137.8 s	141.3 s
9a	133.9 s	136.9 s	129.9 s	136.8 s
10	140.0 s	137.8 s	134.5 s	141.3 s
11	138.8 d	134.6 d	141.1 d	140.8 d
12	69.7 s	70.5 s	69.9 s	70.0 s
13	41.9 t	41.0 t	41.2 t	41.3 t
14	22.2 t	26.5 t	21.9 t	22.0 t
15	128.6 d	131.1 d	128.8 d	128.8 d
16	132.6 d	132.4 d	132.9 d	132.9 d
26	78.8 d	59.4 d	75.1 d	75.3 d
28	55.8 t	54.8 t	51.3 t	51.4 t
29	23.2 t	29.8 t	33.9 t	33.7 t
30	37.4 t	29.0 t	26.0 t	26.0 t
31	79.8 d	32.9 t	28.4 t	28.4 t
32	28.4 t	130.0 d	134.8 d	134.7 d
33	41.0 t	129.5 d	130.3 d	130.4 d
34	104.2 s	25.4 t	55.0 d	55.7 d
35	51.9 t	22.8 t	44.1 t	43.9 t
36	67.1 t	65.8 t	69.3 t	69.3 t

[a] s, singlet; d, doublet; t, triplet.

attributable to the insertion of the tetrahydrofuran ring system. This was confirmed by a number of long-range ^{13}C−^{1}H correlations, in particular the correlation between H-31 and C-34. The structure of **1**, compared with that of **10**, lacks the ^{13}C signal at 150 ppm, indicating the absence of a hydroxyl group at C-6 and the signals of the β-carboline moiety correspond closely with those of **8**.

The presence of manzamine *N*-oxides was evident in an HPLC chromatogram of the crude extract, indicating that these compounds are present as natural products and not as oxidation artifacts formed during isolation. The *N*-oxides **2**−**4** are all found to be more polar and lack the characteristic flourescence on Si 60 TLC plates when compared with their parent alkaloids (365 nm). In all cases the mass spectral data of the *N*-oxides indicate that the molecular weight is 16 mass units higher than that expected after analysis of the NMR spectra. For each of the *N*-oxides, the 1D and 2D NMR spectra allowed signal assignments that readily confirm the chemical shift changes found in the upper aromatic system. These differences between the shifts of the *N*-oxides compared with those of their parent compounds are pronounced (Table 1) but appear to be characteristic, with large upfield shifts for aromatic carbons in *ortho* and *para* positions to the substituent, caused by mesomeric redistribution of electron density (C-1, C-3, and C-4a in **2** and **4** and C1 and C-4a in **3**), and downfield shifts for directly bound sp^3 carbon atoms.[15] For *N*-oxides **2** and **4**, the carbons that are directly attached to the N-atom, C-1, C-3, and C4a, are shifted upfield by 5−9 ppm compared to the parent compounds. In the ^{1}H-NMR spectrum, H-3 and H-4 still appear as doublets but with coupling constants that are larger by 2 Hz than those of the parent compunds. For compound **3**, an *N*-oxide of 3,4-dihydromanzamine A,[11] there was an upfield shift for C-1 and C-4a (−22 and −8.8 ppm, respectively), and a downfield shift of 13.1 ppm for C-3.

The IR spectra of the *N*-oxides were identical to the parent compounds, and the expected *N*-oxide signals are too weak to be noticed from the fingerprint region. The decisive experiment for ascertaining the *N*-oxide char-

Table 2. ^{1}H-NMR Data of Compounds **1**−**4** in CD_2Cl_2

H no.	1	2	3	4
3	8.42 d, J = 5.1 Hz	8.08 d, J = 6.6 Hz	4.36 m	8.06 d, J = 6.7 Hz
			4.21 m	
4	7.87 d, J =5.2 Hz	7.79 d, J = 6.2 Hz	3.22 m	7.77 d, J = 6.7 Hz
5	8.16 d, J = 7.8 Hz	8.04 d, J = 7.5 Hz	7.51 d, J = 7.8 Hz	8.04 d, J = 8.0 Hz
6	7.33 m	7.32 m	7.15 d, J = 8.0 Hz	7.32 t, J = 8.0 Hz
7	7.57 m	7.51 m	7.21 dt, J = 7.6, 1.1 Hz	7.51 m
8	7.57 m	7.51 m	7.41 d, J = 8.0 Hz	7.54 m
11	6.41 s	6.06 s	5.97 s	6.07 s
13	1.69 m	1.59 br	1.75 m	1.85 m
	2.10 m	2.08 m	2.02 m	2.15 m
14	2.40 m	2.18 br	2.41 m	2.40 m
	2.14 m		2.14 m	2.10 m
15	5.68 q, J = 10.0	5.66 dt, J = 4.4, 10.8 Hz	5.69 m	5.72 m
16	5.58 dt, J = 5.0, 10.8 Hz	5.47 d, J = 10.9 Hz	5.59 dt, J = 10.8, 4.7 Hz	5.60 dt, J = 7.9, 4.7 Hz
17	1.86−1.76 m		1.85 m	1.75 m
	2.64 m		2.52 m	2.55 m
18	1.51 m		1.34 m	1.30 m
			1.70 m	1.41 m
19	1.47 m	1.20 br d, J = 10.1 Hz	1.34 m	1.41 m
			1.70 m	1.81 m
20	2.54 m	2.38 br	2.42 m	2.38 m
	2.78 m	2.59 br d, J = 11.6 Hz	2.63 dt, J = 11.7, 4.8 Hz	2.80 m
22	2.01 m	1.81 br	1.95 m	2.50 m
	2.80 m	2.88 br	2.79 m	2.98 m
23	1.58 m		1.60 m	1.75 m
			1.97 m	3.15 m
24	3.01 dt, J = 6.4, 9.3 Hz	2.90 br s	2.98 m	3.00 dd, J = 7.3, 11.5 Hz
26	3.62 s	3.77 br s	3.45 s	3.72 s
28	2.89 m	2.88 br	3.19 m	3.20 m
	3.36 m	2.98 br		3.96 m
29	1.86−1.76 m		1.55 m	1.98 m
			1.70 m	2.84 m
30	1.66 m	1.50 m	1.34 m	1.38 m
	1.86−1.76 m		1.85 m	1.91 m
31	4.54 brd, J = 7.9 Hz	1.75 m	2.35 m	2.30 m
		1.59 br	2.15 m	
32	3.20 d, J = 11.9	5.35 m	5.94 m	5.95 m
	2.05 m			
	1.63 m			
33	2.25−2.16 m	5.50 m	5.30 t, J = 9.5 Hz	5.35 m
34		1.48 m	4.21 m	4.29 m
35	1.94 d, J = 13.9 Hz	2.19 br	1.65 m	1.68 m
	2.33 d, J = 13.9 Hz	2.47 br	2.95 m	2.43 m
36	2.27 d, J = 11.9 Hz		2.30 d, J = 2.30 Hz	2.33 d, J = 11.3 Hz
			2.80 d, J = 11.6 Hz	2.80 d, J = 11.6 Hz
NH-9	8.80 br s			
OH-12	3.40 br s			

acter of the β-carboline moiety was its reduction with zinc dust and 1-N HCl, which is a specific reducing agent for the conversion of an *N*-oxide to its corresponding tertiary base.[16] In the EIMS, the fragmentation pattern of the resulting reduced product was identical in each case with that of the parent compound and showed conclusive evidence of the loss of 16 mass units, corresponding to the O-atom of the *N*-oxide. The UV spectra of the reduction products were also identical with those of the parent compounds. Compound **3** was unstable under the reduction conditions and gave two products, compound **6** (the parent compound) and its corresponding aromatized derivative, **7**, as confirmed by the HPLC/electrospray ionization−MS experiment. The parent compound **6** also gave the same UV pattern as previously reported.[11]

The relative and absolute configurations of the various asymmetric centers in the molecule are assumed to be the same as those established by X-ray analysis for similar related compounds. The optical rotation values obtained for the known compounds were similar to those previously reported,[4,5,8] while the optical rotation values of the *N*-oxides were less than those of the parent compounds by a mean amount of 35%.

All compounds isolated from *X. ashmorica* were analyzed for insecticidal activity, antibacterial activity, as well as cytotoxicity using mouse lymphoma cells grown *in vitro.* Insecticidal activity was studied by incorporating each compound into an artificial diet at an arbitrarily chosen concentration (132 ppm) and offering the spiked diet to neonate larvae of the vigorous pest insect *S. littoralis* in a chronic feeding experiment. After 6 days of exposure, larval survival and larval weight were monitored and compared to controls. All compounds except **7** were inactive or only weakly active in inhibiting the growth (40−100% larval weight compared to controls) of the larvae of *S. littoralis.* Compound **7** caused 80% inhibition of larval growth at a concentration at 132 ppm. In a subsequent experiment the active compound **7** was analyzed for activity at a range of doses (26−132 ppm). From the dose response curve obtained, the ED50 of **7** was calculated by probit analysis as 35 ppm.

All isolated compounds were also tested for antibacterial activity. Only compound **7** was found to be active against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and caused an inhibition zone of **7** mm diameter after 24 h of incu-

Table 3. Bioactivities of the Compounds Isolated from *X. ashmorica*

compd no.	growth inhibition of *S. littoralis* larvae (dose = 132 ppm) (%)	cytotoxicity assay with L5178 mouse lymphoma cells (ED50) (μg/mL)
1	18.5	1.8
2	16.2	3.2
3	26.4	1.6
4	9.5	1.6
5	2.0	25.0
7	80.0	1.8
8	0.0	6.6
9	50.6	2.3

bation at 37 °C. No inhibition of *E. coli* was observed.

Cytotoxicity of the manzamine alkaloids isolated from *X. ashmorica* was assessed *in vitro* with a mouse lymphoma cell line using the microculture tetrazolium (MTT) assay. Each alkaloid was tested for its cytotoxic activity at a range of concentrations (0.3−20 μg/mL). All compounds, except for **5**, were found to be active against the cell line chosen (Table 3). From the results of this assay, we were able to establish a structure−activity relationship between the different manzamine derivatives. Taking compound **7** (ED50 1.8 μg/mL) as the reference compound, the formation of an oxide bridge in ring E does not have a major effect, but addition of a carbonyl group at C-31 (**8**) or opening of ring E (**5**) decreases the activity. When changes in the upper β-carboline moiety are taken into consideration, the *N*-oxides of compounds **5** and **7**, and the 8-hydroxy derivative of compound **8** are more active than their parent compounds. The *N*-oxide compounds **3** and **4** are the most active compounds found in this study with ED50's of 1.6 μg/mL. These cytotoxicity data are not paralleled by the antibacterial activity or insecticidal activity toward neonate larvae of *S. littoralis*, which was also observed previously.[12] Thus, it is possible that the observed antibacterial activity and insecticidal activity of **7** is not caused by a general cytotoxicity but may be due to a different mode of action.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded on Bruker WM 400 NMR and AVANCE DMX 600 NMR spectrometers, respectively. Mass spectra (FAB, NBA as matrix) were measured on a Finnigan MAT 8430 mass spectrometer. Optical rotations were determined on a Perkin-Elmer-241 MC polarimeter. UV spectra were recorded in MeOH. Percent purity of isolated compounds was analyzed by HPLC. For HPLC analysis, samples were injected into a HPLC system (Pharmacia, LKB, Sweden) coupled to a photodiode-array detector (Waters Millipore GmbH, Eschborn, Germany). Routine detection was at 254 nm. The separation column (125 × 4 mm i.d.) was prefilled with Nucleosil C-18 (Knauer GmbH, Germany).

Solvents were distilled prior to use, and spectral-grade solvents were used for spectroscopic measurements. TLC were performed on precoated TLC plates with Si gel 60 F254 (Merck, Darmstadt, Germany). The compounds were detected from their UV absorbance at 254 and 366 nm.

Animal Material. Specimens of *X. ashmorica* were collected from a near vertical wall at a depth of 25−30 meters at the Manila Channel off Mindoro Island, Philippines, in April 1994. They form irregular thick columns with a coarse surface. The oscules were several mm in diameter, on low hummocks or flush. Consistency is firm, incompressible, and crumbly. The color is brown. The skeleton consists of a reticulation of thick spicule tracts averaging 75 μm in diameter, forming rather squarishly rounded meshes of 200−280 μm in diameter. No special ectosomal skeleton exists. Spicules are exclusively strongyles of 125−154 × 7−9.5 μm, with rare oxeote growth stages. The specimens were compared with a fragment of the type specimen of *Acanthostronglyophora ashmorica* described by Hooper[17] from North West Australia, and they were found to be closely similar in all respects. The genus *Acanthostronglyophora* is considered a junior synonym of the genus *Xestospongia,* and accordingly, we use here the combination *X. ashmorica* n. comb. The samples were frozen immediately after collection and then freeze-dried prior to transport to the University of Würzburg, Germany. A voucher fragment is kept in 70% ethanol under the registration number ZMA POR.10909 in the Zoölogisch Museum, Amsterdam.

Extraction and Isolation. The freeze-dried samples of *X. ashmorica* (45 g) were extracted successively with acetone and MeOH (300 mL × 2 for each). The total extract was evaporated under reduced pressure to give a residue of 5.0 g. This was partitioned between *n*-BuOH (50 mL × 5) and H_2O (50 mL). The organic fraction was taken to dryness (5 g) and chromatographed over a Si gel column (mobile phase CH_2Cl_2−MeOH 90:10), and seven major fractions were obtained. The nonpolar fractions (fractions 1 and 2) were separated from chlorophyll on a Sephadex column (LH-20) using acetone. After evaporation of all fractions under reduced pressure, each of the residues was taken up in a minimum amount of MeOH and stored at 5 °C for 24 h. Fractions 1 and 2 afforded a precipitate of colorless crystals, which were compounds **5** (39.9 mg, 0.10%) and **7** (107.1 mg, 0.33%), respectively. The supernatants were taken to dryness and further purified by column chromatography on Si 60 Lobar (Merck, Darmstadt, Germany) with mixtures of CH_2Cl_2, *i*-PrOH, and 0.5% NH_4OH as eluents. Compound **1** (17.5 mg, 0.04%) was obtained from the methanolic supernatant of fraction 1 and was further purified on Si 60 Lobar (CH_2Cl_2−*i*-PrOH 97:3 + 0.5% NH_4OH). The supernatant of fraction 2 afforded **8** (27.2 mg, 0.08%) and **9** (49.9 mg, 0.11%), which were separated on Si 60 Lobar (CH_2Cl_2-*i*-PrOH 97:3 + 0.5% NH_4OH). The last three polar fractions (5−7) yielded the *N*-oxides compounds **2** (40.1 mg, 0.09%), **3** (20.0 mg, 0.04%), and **4** (13.3 mg, 0.03%), respectively. The fractions containing the *N*-oxide congeners were further purified on Si 60 Lobar (CH_2Cl_2-*i*-PrOH 90:10 + 0.5% NH_4OH). The identity of the fractions was confirmed by HPLC and UV spectra recorded online.

Reduction of the *N*-Oxides. A 3.0 mg sample of the *N*-oxide was dissolved in 2 mL of 1 N HCl, and a small amount of Zn dust was added to produce H_2 gas that was indicated by a bubbling reaction. The reaction mixture was continuously stirred for 5 h (Zn dust was added when necessary). After 5 h, the reaction mixture was filtered through glass wool. The filtrate was made alkaline with 25% NH_4OH, and the free alkaloid was extracted with EtOAc (2 mL × 4). The EtOAc-soluble

portion was taken to dryness and further purified by column chromatography on Si gel in CH_2Cl_2-*i*-PrOH 95:5 + 0.5% NH_4OH.

HPLC/Electrospray Ionization−MS Method. HPLC-ESIMS was carried out using a Finnigan MAT TSQ-7000 mass spectrometry connected to a UV detector. HPLC was performed on a Nucleosil C-18 (60 × 2 mm i.d.) reversed-phase column. The mobile phase was H_2O (0.05% TFA, A), to which MeOH (B) was added by a linear gradient: initial, 0% of B; 45 min, 80% of B; 55 min, 80% of B. The flow rate was 3 μL/min. UV at 254 nm, capillary temperature at 200 °C, and drift voltage at 20 eV.

6-Deoxymanzamine X (**1**) was obtained as a pale yellow amorphous powder: percent purity 98%; UV λ_{max} (MeOH) 210 (ϵ 26 000), 260 (ϵ 11 800), 312 sh (ϵ 10 000), 378 (ϵ 3000); $[\alpha]_D$ +30.1° (*c* 0.35, $CHCl_3$); ($C_{36}H_{44}N_4O_2$) ESI (35 eV) *m*/*z* $[M + H]^+$ 565 (39), 547 (100), 529 (10), 406 (25), 382 (25), 195 (12), 178 (40).

Manzamine J *N*-oxide (**2**) was obtained as a yellow crystalline powder: percent purity 94%; UV λ_{max} (MeOH) 261 (ϵ 25 000), 325 (ϵ 19 000); $[\alpha]_D$ +15.0° (*c* 0.40, $CHCl_3$); ($C_{36}H_{46}N_4O_2$) ESI (25 eV) *m*/*z* $[M + H]^+$ 567 (58), 549 (100), 532 (10), 377 (17), 365 (36), 188 (5), 175 (10); FAB-MS *m*/*z* $[M + H]^+$ 567.

3,4-Dihydromanzamine A *N*-oxide (3) was obtained as a yellow crystalline powder: percent purity 95%; UV λ_{max} (MeOH) 201 (ϵ 25 000), 355 (ϵ 11 000); $[\alpha]_D$ +34.1° (*c* 0.59, $CHCl_3$); ($C_{36}H_{46}N_4O_2$) ESI (25 eV) *m*/*z* $[M + H]^+$ 567 (78), 549 (24), 531 (13), 426 (100); FAB-MS *m*/*z* $[M + H]^+$ 567.

Manzamine A *N*-oxide (4) was obtained as yellow crystalline powder: percent purity 95%; UV λ_{max} (MeOH) 201 (ϵ 26 000), 241 (ϵ 23 000), 261 (ϵ 23 000), 310 (ϵ 21 000); $[\alpha]_D$ +18.6° (*c* 0.35, $CHCl_3$); ($C_{36}H_{44}N_4O_2$) ESI (25 eV) *m*/*z* $[M + H]^+$ 565 (100), 547 (34), 529 (27), 424 (34), 407 (21), 311 (24); FAB-MS *m*/*z* $[M + H]^+$ 565.

Experiments with Insects. Larvae of *S. littoralis* were from a laboratory colony reared an artificial diet under controlled conditions as described previously.[13] Feeding studies were conducted with neonate larvae (*n* = 20) that were kept on an artificial diet that had been treated with various concentrations of the compounds under study. After 6 days, survival of the larvae and weight of the surviving larvae were protocolled and compared to controls. ED50s were calculated from the dose−response curves by probit analysis.

Agar Plate Diffusion Assay. Susceptibility disks (5 mm diameter) were impregnated with 100 μg of the isolated compound and then placed on agar plates inoculated with the test bacterium: *B. subtilis* 168 and *S. aureus* ATCC 25923 (for the Gram-positive bacteria) and *E. coli* ATCC 25922 (for Gram-negative bacteria). The plates were observed for zones of inhibition, after 24 h of incubation at 37 °C.

Cytotoxicity Studies. L5178y mouse lymphoma cells were grown in Eagle's minimal essential medium supplemented with 10% horse serum in roller tube culture as described previously.[14] For the dose−response experiments, 5 mL cultures were initiated by inoculation of 5 × 1000 cells/mL and were incubated at 37 °C for 72 h. Controls showed a population doubling time of 10.5 h. Cell growth was determined by a cell count with a Cytocomp counter (128-channel counter, system Michaelis, Mainz, Germany) incorporating a 32-channel size-distribution plotter.

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