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Note

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Neopetrocyclamines A and B, Polycyclic Diamine Alkaloids from the Sponge *Neopetrosia* cf *exigua*

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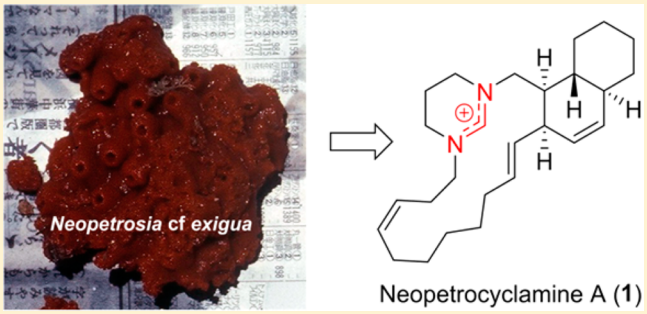
Supporting Information

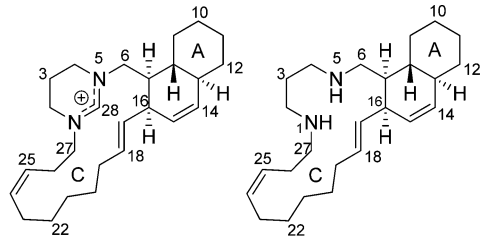
ABSTRACT: Two new polycyclic alkaloids, neopetrocyclamines A and B (**1** and **2**), along with the known metabolites papuamine (**3**) and haliclonadiamine (**4**), were isolated from the Indonesian sponge *Neopetrosia* cf *exigua*. Neopetrocyclamine A contains a formamidinium moiety, a rare functional group. While these compounds share the same basic biosynthetic building blocks, the size of the ring system differs in **1** and **2** because of the formamidinium moiety. Biological evaluations of **1**−**4** revealed that papuamine is cytotoxic against glioblastoma SF-295 cells (GI_{50} = 0.8 μM).

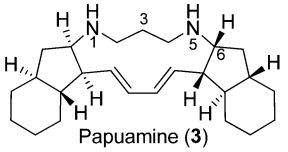


Polycyclic diamine alkaloids are characteristic of marine sponges in the order Haplosclerida. To date, over 120 such alkaloids have been reported, most isolated from four families of Haplosclerida (Callyspongiidae, Chalinidae, Niphatidae, and Petrosiidae).[1] This class of alkaloids is proposed to be derived from ammonia, propenal, and long-chain dialdehydes as the universal building blocks.[2] Due to the structural complexity and biological activity of polycyclic diamine alkaloids, these compounds have attracted a great deal of interest from natural product chemists.[1] Extensive studies on an extract derived from the Indonesian sponge *Neopetrosia* cf *exigua* (Kirkpatrick, 1900) (order Haplosclerida, family Petrosiidae) have led to the isolation of two new alkaloids, neopetrocyclamines A and B (**1** and **2**), along with two known alkaloids, papuamine (**3**) and haliclonadiamine (**4**).[3,4] Herein, we report the structure elucidation of **1** and **2**.

Neopetrocyclamine A (**1**) was isolated as an optically active yellow oil. The HR-ESITOFMS data for **1** provided a molecular ion of m/z 381.3269 $[M]^+$. This datum defined a molecular formula of $C_{26}H_{41}N_2^+$, which suggested **1** contained 7.5 double-bond equivalents and a positive charge. Seven sp^2 carbons ($\delta_{C\text{-}14}$ 132.4; $\delta_{C\text{-}15}$ 128.8; $\delta_{C\text{-}17}$ 129.6; $\delta_{C\text{-}18}$ 134.0; $\delta_{C\text{-}24}$ 133.5; $\delta_{C\text{-}25}$ 124.3; $\delta_{C\text{-}28}$ 155.0) were observed in the ^{13}C NMR spectrum (Table 1), which indicated one carbon−nitrogen and three carbon−carbon double bonds were present in **1**. The multiplicity-edited HSQC spectrum recorded in pyridine-d_5 showed that the 26 carbon resonances correlated to 41 protons, which were ascribed to 11 methines and 15 methylenes.

Extensive analyses of the NMR spectroscopic data (recorded in pyridine-d_5) established the four units that comprised the planar structure of **1** (Figure 1). While most of the backbone was readily deduced through this analysis, one piece of datum deserves comment. Examination of the NMR data of **1** revealed a 9.60 ppm proton singlet uncorrelated to any carbon signal in





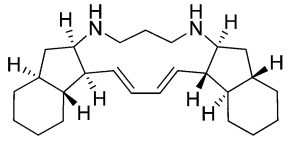


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Table 1. NMR Spectroscopic Data for 1 in Pyridine-d_5

pos.	δ_C, type	δ_H (J in Hz)	COSY	HMBC	ROESY
2	42.6, CH_2	3.47, m	3b	27,[a] 28[a]	
		3.37, m			
3	18.9, CH_2	2.02, m			
		1.96, m	2a, 4a		
4	42.1, CH_2	3.34, m	3b	6a, 28	
		3.27, m			
6	55.3, CH_2	3.96, t (13.2)	7	28[a]	
		3.56, d (13.2)			
7	38.3, CH	2.00, m	6a, 6b, 8, 16	13, 15, 16, 17	9b, 13, 16
8	38.2, CH	1.27, q (10.8)	7, 9b, 13	6a, 10a, 12b, 14, 16	12b, 17
9	28.7, CH_2	1.54, m		7	8
		0.74, dt (11.8, 10.8)	8, 10b		
10	26.5, CH_2	1.59, m		12b	
		1.05, m	9b		
11	26.5, CH_2	1.59, m		13	
		1.14, m	12b		
12	33.1, CH_2	1.61, m		10a, 14	
		0.99, m	11b, 13		8
13	43.7, CH	1.64, td (10.8, 2.7)	8, 12b, 14	11a, 12b, 14, 15	11b, 14
14	132.4, CH	5.50, brd (9.2)	13, 15	12a, 13, 16	12a, 12b, 15
15	128.8, CH	5.63, ddd (9.2, 4.7, 2.7)	14, 16	13, 14, 17	14, 16
16	40.8, CH	2.79, m	7, 15, 17	14, 15, 17, 18	7, 15, 18
17	129.6, CH	5.72, dd (15.0, 8.1)	16, 18	16, 18, 19	8, 19
18	134.0, CH	5.50, dt (15.0, 6.6)	17, 19a, 19b	16, 19a, 19b, 20	16, 19a, 19b, 20
19	30.8, CH_2	2.59, dt (13.7, 6.6)	18, 20	17, 18, 21a, 21b	17, 18, 20, 21a
		2.14, dt (13.7, 6.6)			17, 18
20	27.4, CH_2	1.49, m	19a, 19b, 21a	18, 19a, 19b, 21a, 21b	18, 19a, 19b
21	26.7, CH_2	1.38, m	20, 22a	19a, 19b, 23a, 23b	22a
		1.31, m			
22	27.8, CH_2	1.47, m	21a, 21a, 23b	21a, 21b, 23a, 23b, 24	21a, 23b, 24
		1.37, m	23b		23a, 24
23	26.2, CH_2	2.11, m	22a, 24	21a, 21b, 24, 25	22b, 24, 26b
		1.91, m	22a, 22b, 24		22b, 24
24	133.5, CH	5.61, dt (9.6, 7.5)	23a, 23b, 25	22a, 22b, 23a, 23b	22a, 22b, 23a, 23b, 25
25	124.3, CH	5.27, ddd (10.0, 9.6, 4.8)	24, 26a, 26b	23a, 23b, 26a	24, 26a, 26b
26	26.5, CH_2	2.50, m	25, 27a, 27b	24	25
		2.38, m	25, 27a, 27b		23a, 25
27	54.8, CH_2	4.10, m	26a, 26b	25, 28	
		3.49, m	26a, 26b		
28	155.0, CH	9.60, s		6a	

[a]HMBC correlations observed in $CDCl_3$.

the HSQC spectrum (optimized for 140 Hz), yet still present in MeOH-d_4, indicating $^1J_{C,H}$ outside of the typical range. A literature search focusing on uncommon functional groups

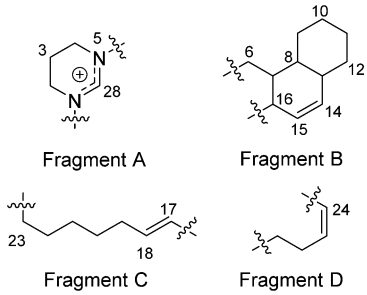

Figure 1. Initial fragments of **1**.

suggested that this downfield proton singlet could be ascribed to a formyl-like sp^2 carbon, with a $^1J_{C,H}$ coupling greater than 170 Hz. An HSQC experiment optimized for a $^1J_{C,H}$ of 200 Hz showed a correlation between δ_H 9.60 and a sp^2 carbon at 155.0 ppm, verifying this hypothesis. Therefore, in addition to the three olefins and the delocalized double bond of the formamidinium group that accounted for four degrees of unsaturation, **1** contained four rings.

The fragments were assembled via analyses of the HMBC and COSY spectra. The terminal methylene ($\delta_{C\text{-}6}$ 55.3) of fragment B was linked to the nitrogen atom (N-5) of fragment A based on HMBC correlations from H-6 to C-4 and H-28 to C-6. In addition, the methine proton signal ($\delta_{H\text{-}16}$ 2.79) at the other terminus displayed COSY cross-peaks to two olefinic protons ($\delta_{H\text{-}15}$ 5.63 and $\delta_{H\text{-}17}$ 5.72) that connected fragments B and C. Fragment D was located between the C-23 methylene of fragment C and the nitrogen atom (N-1) of fragment A based on a COSY correlation from H-24 to H-23 and HMBC correlations from H-27 to C-2 and H-28 to C-27. Taken as a whole, these data defined a 17-membered macrocycle (C ring) with an exocyclic tetrahydropyrimidinium ring.

Neopetrocyclamine B (**2**) was isolated as an optically active, colorless powder. A comparison between the HR-ESITOFMS data of **1** and **2** indicated that **2** ($C_{25}H_{42}N_2$) was 11 amu smaller than **1** and contained only six double-bond equivalents. Analysis of the multiplicity-edited HSQC spectrum indicated that **2** possessed the same basic carbon skeleton as that of **1** except that **2** did not contain the H-28/C-28 resonances corresponding to the formamidinium cation. Taken together, the missing signals and analysis of the 2D NMR spectroscopic data (Table 2) confirmed **2** contained the presence of two secondary amines rather than a formamidinium cation.

The relative configuration of the stereogenic centers in **1** was assigned by analyses of the $^3J_{H,H}$ coupling constants and corresponding 2D ROESY and 1D NOE correlations. For example, the proton signal for H-8 was an apparent quartet with a $^3J_{H,H}$ value of 10.8 Hz, which indicated that H-8 was *anti* to three axial protons (H-7, H-9b, and H-13) (Figure 2). On the basis of these data and the observation of ROESY cross-peaks between H-8/H-12b ($\delta_{H\text{-}12b}$ 0.99), H-8/H-9a ($\delta_{H\text{-}9a}$ 1.54), H-13/H-9b ($\delta_{H\text{-}9b}$ 0.74), and H-13/H-11b ($\delta_{H\text{-}11b}$ 1.14), the A ring was in a chair conformation. In addition, H-7 showed an NOE with H-16 and was thus assigned a *syn* position relative to H-16. Due to the presence of the double bond, the conformation of the B ring containing C-7 and C-16 was a half-chair conformation, a conclusion supported by stepwise molecular modeling calculations using the MMFF94 and AM1 force fields. A similar analysis of the NMR data of **2** established that it possessed the same relative configuration in those rings.

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Table 2. NMR Spectroscopic Data for 2 in MeOH-d_4

pos.	δ_C, type[a]	δ_H (J in Hz)	COSY	HMBC	1D-TOCSY
2	49.4, CH_2	3.08, m	3	4b, 27	
		2.96, ddd (12.2, 8.4, 5.1)			
3	26.3, CH_2	1.78, m	2a, 2b, 4a, 4b	4b	
4	49.0, CH_2	3.09, m	3	2a, 3, 6b	
		2.74, ddd (13.0, 8.7, 5.5)			2a, 2b, 3
6	49.5, CH_2	2.87, dd (12.6, 4.8)	7	4b	7, 8, 9a, 9b, 10b, 13, 15, 16, 17
		2.62, dd (12.6, 13.7)			
7	40.5, CH	1.85, ddd (13.7, 11.3, 4.8)	6a, 6b, 8, 16	9a, 9b	6a, 6b, 8, 9a, 9b, 13, 16
8	40.0, CH	1.17, q (11.3)	7, 9a, 9b, 13	6a, 6b, 7	7, 9a, 9b, 10a, 10b, 12a, 12b, 13
9	29.9, CH_2	1.78, m	8, 10b		
		0.99, qd (11.3, 4.0)			7, 8, 10a, 10b, 11a, 11b, 13
10	27.8, CH_2	1.83, m		12b	
		1.32, m	9b, 11b		
11	27.2, CH_2	1.76, m		9b	
		1.33, m	10b, 12b		
12	34.4, CH_2	1.75, m	11a, 11b, 13	14	
		1.06, ddd (12.8, 11.5, 3.8)			11a, 11b, 13
13	44.3, CH	1.72, t (11.5)	8, 12b, 14	7, 15	
14	133.0, CH	5.47, d (9.7)	13, 15		
15	129.4, CH	5.43, ddd (9.7, 4.0, 1.5)	14, 16		
16	42.2, CH	2.87, br s	7, 15, 17	6a, 7, 14, 18	
17	130.2, CH	5.36, dd (14.7, 8.3)	16, 18	7, 18, 19a, 19b	
18	134.3, CH	5.54, ddd (14.7, 8.6, 4.7)	17, 19a, 19b	17	16, 17, 19a, 19b
19	32.4, CH_2	2.13, m	18, 20a	17	17, 18, 20a, 20b, 21
		2.05, m			
20	29.3, CH_2	1.43, m	19a, 19b, 21		
		1.38, m			
21	27.7, CH_2	1.33, m	20b, 22b	19a, 19b, 22a	
22	29.0, CH_2	1.47, m	23, 23a, 23b		
		1.42, m			
23	27.8, CH_2	2.16, m	22a, 23b, 24	22a	
		2.05, m			
24	134.9, CH	5.58, dt (9.6, 8.3)	23a, 23b, 25	22a, 23a, 23b, 26a, 26b	
25	126.3, CH	5.37, dt (9.6, 7.8)	24, 26a, 26b	23a, 23b, 26a, 23b, 27	
26	26.6, CH_2	2.41, tt (14.7, 7.8)	25, 27		23a, 23b, 24, 25, 27
		2.31, tt (14.7, 7.8)			
27	48.7, CH_2	2.88, m	26a, 26b	2a, 2b, 26a, 26b	

[a]Determined from the meHSQC spectrum.

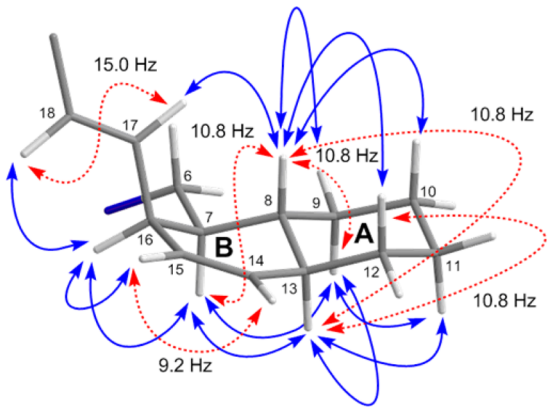

Figure 2. Conformational model of the AB ring system and selected experimental ROESY correlations (solid arrows) along with key $^3J_{H,H}$ values (dashed arrows) of **1**.

The configurations of the olefins in **1** and **2** were assigned by analysis of the $^3J_{H,H}$ coupling constants. Due to the strong second-order coupling for these olefinic protons (recorded in CD_3OD or $CDCl_3$), $^3J_{H,H}$ coupling constants were determined in pyridine-d_5 in order to allow potential $\pi-\pi$ interactions to disperse the proton chemical shifts. The resulting $^3J_{H,H}$ coupling constants for the olefins ($\Delta_{14,15}$ and $\Delta_{24,25}$) were 9.2 and 9.6 Hz, respectively, suggesting *Z*-configurations, while the other olefin ($\Delta_{17,18}$) displayed a vicinal coupling value of 15.0 Hz that indicated an *E*-configuration. Evaluation of the $^3J_{H,H}$ values for the olefinic methines in **2** established the same configurations.

Natural products containing a tetrahydropyrimidinium ring are uncommon. This motif is a component of haliclorensin B,[5] convolutamine J,[6] *N*-methylmanzacidin C,[7] efrapeptin F,[8] phloeodictines,[9] and incasines B and B′.[10,11] In our case, neopetrocyclamine B (**2**) appears to be a precursor to **1**, as a one-carbon transfer reaction would convert **2** to **1**. The mechanism by which formylation occurs may be similar to the biosynthesis of tetrahydrofolate cofactors.[12] Similar proposals have been put forth for the biosynthesis of other formamidinium-containing natural products. For example, the tetrahydropyrimidinium ring of incasine B has been proposed to be generated via a one-carbon enzymatic formylation and subsequent cyclization.[13]

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One final point of discussion is necessary concerning the relationship between **1** and **2** and whether **1** is an artifact of a reaction with our HPLC additive formic acid or its derivative, always a concern with natural products. Several lines of evidence suggest this is not the case here. First, direct coupling between **2** and formic acid is thermodynamically unfavorable, as it requires a nucleophilic acyl substitution reaction between an amine and a carboxylic acid when a thermodynamically more favorable reaction manifold exists, an acid−base reaction. The addition of simple acids or bases has little effect on the general feasibility of this nucleophilic acyl substitution reaction at room temperature, hence ruling out the formation of **1** via this route. A more likely scenario is the coupling of **2** with methyl formate, itself formed via a reaction between the chromatography solvent MeOH and the additive formic acid. Fortunately, this can be ruled out as well, as the m/z corresponding to **1** and **2** are clearly visible after the initial Kupchan partitioning in different fractions (CH_2Cl_2 and hexane, respectively), before formic acid was even introduced to the samples during the final HPLC purification step. Taken together, the above considerations indicate that **1** is not an isolation artifact.

Recently, LaBarbera et al.[14] reported that papuamine was an antimetastatic agent against MDA-MB-231 breast cancer cells, while Kanno et al.[15] reported it reduces cell survival through mitochondrial damage and JNK activation. On the basis of these reports, compounds **1**−**4** were screened *in vitro* against a human glioblastoma (SF-295) cancer cell line and two human renal cancer cell lines (UO-31 and A498). Neither **1** nor **2** showed significant cytotoxicity at 20 μM. However, both **3** and **4** inhibited the growth of these three carcinoma cell lines, with GI_{50} values ranging from 0.8 to 8.0 μM (Table 3). In particular, **3** was more potent than **4** against glioblastoma SF-295 cells, as its GI_{50} value was 8-fold lower. This suggested that the stereogenic center at C-6 of **3** was important for the cytotoxic effect. Compound **3** was nearly 4-fold more potent against glioblastoma cells when compared with renal cancer cells, whereas, in contrast, **4** was not. At this time, the specific molecular targets and mechanisms by which these compounds exert their antitumor effects are unknown.

Table 3. Cytotoxicity of 1−4[a]

	UO-31	A498	SF-295
3	3.0	2.9	0.8
4	8.0	5.9	6.3
1	>20	>20	>20
2	>20	>20	>20

[a]The numbers represent the GI_{50} values as determined from quadruplicate measurements and are expressed in μM.

This is the first report of the isolation of C_3-diamine polycyclic alkaloids from a sponge of the genus *Neopetrosia*, which has been a rich source of marine alkaloids.[16−19] While the neopetrocyclamines represent a new carbon skeleton of polycyclic diamine alkaloids, the cross-distribution of papuamine alkaloids in different sponge genera, as well as their structural homology to the neopetrocyclamines, supports the hypothesis of the monophyletic evolution for sponges in the order Haplosclerida.[1,20]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-370 digital polarimeter at the sodium D-line (589 nm). IR spectroscopy was measured as a thin film on a CaF_2 disk using a Shimadzu IRAffinity-1 FTIR. ^1H, ^{13}C, and 2D NMR experiments were performed on a Varian Unity Inova 500 MHz spectrometer. NMR spectra were referenced to the appropriate residual solvent signal (δ_H 3.30, δ_C 49.1 for MeOH-d_4; δ_H 7.58, δ_C 135.9 for pyridine-d_5). The HSQC experiments were optimized for $^1J_{C,H}$ = 140 Hz, and HMBC experiments for $^3J_{C,H}$ = 7 Hz. Mixing times for ROESY and NOESY experiments were 500 ms, and generally 80 ms for the 1D TOCSY experiments. High-resolution mass spectrometric data were obtained on an LC-MS-TOF spectrometer using ESI mode. HPLC separations were performed on a Shimadzu LC-20AP system.

Collection and Identification. The sponge sample designated 96-IND-70 was collected by scuba from a depth of 24 m at Old Derawan Pier, Indonesia (2.458° N, 118.237° E), on March 19, 1996. The sponge is most closely comparable to *Neopetrosia exigua* (Kirkpatrick, 1900) (order Haplosclerida, family Petrosiidae), characterized in life by a dark reddish-brown external coloration and cream interior, and has relatively small spicules of about 150 μm long, set in a dense round-meshed reticulation, which produces a velvety surface. The sponge is thickly encrusting, and the texture relatively crumbly and very sticky to the touch. The surface is irregular with small oscules on mounds on the surface. Voucher specimens have been deposited at the Natural History Museum, London (NMHUK2012.3.27.1), as well as at the UH Manoa, Department of Chemistry (96-IND-70).

Isolation. A portion of the freeze-dried sample was exhaustively extracted with MeOH to yield 3.6 g of crude extract. This extract was subjected to a successive partition using a modified Kupchan procedure with hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O to yield five fractions of 0.2, 0.4, 0.02, 0.7, and 2.2 g, respectively. LC-MS analyses of these fractions revealed that organic phases contained a series of compounds with molecular weights between 360 and 400 amu corresponding to the pure compounds **1**−**4**. TLC experiments revealed that the spots of the organic fractions remained at the origin on either normal (silica, EtOAc) or reversed-phase (RP-C18, MeOH/H_2O, 1:1) plates, but moved in both modes when either diethylamine (5%) or formic acid (0.1%) was added. The residue from the CH_2Cl_2 phase (0.4 g) was chromatographed on a silica gel flash column (75 g) eluting with a step gradient of EtOAc/Et_2NH (95:5), MeOH/EtOAc/Et_2NH (25:70:5) (×2), MeOH/EtOAc/Et_2NH (50:45:5) (×2), MeOH/EtOAc/Et_2NH (75:20:5), and MeOH/Et_2NH (95:5) to provide seven fractions (A−G). The known alkaloid haliclonadiamine (**4**) (122 mg) was thus isolated from fraction B. Separation of fraction C (130 mg) by reversed-phase HPLC [Luna C8, 250 × 10 mm, a linear gradient over 20 min from 20% to 100% MeOH in H_2O with 0.1% formic acid added to both solvents, flow rate 2.75 mL/min, PDA detection] afforded neopetrocyclamine A (**1**) (t_R 17.2 min, 3.8 mg, 0.106% yield). The organic residue from the hexane phase (0.2 g) was thus chromatographed on a silica gel flash column (75 g) using EtOAc/Et_2NH (95:5) to afford 25 fractions. These fractions were analyzed by LC-MS and combined with fraction 8 to 14 (total of 51 mg). This fraction was then washed with 10 mL of 1 N HCl and extracted with hexane (10 mL × 5) to remove fatty acids. The residue from the HCl aqueous phase (4 mg) was separated by reversed-phase HPLC [Kinetex C8, 100 × 4.6 mm, a linear gradient over 20 min from 10% to 50% MeOH in H_2O with 0.1% formic acid added to both solvents, flow rate 0.7 mL/min, PDA detection] to afford neopetrocyclamine B (**2**) (t_R 12.4 min, 0.85 mg, 0.024% yield). The residue from the *n*-BuOH phase (0.7 g) was also chromatographed on a silica gel flash column (125 g) using EtOAc/Et_2NH (95:5) to obtain known alkaloids papuamine (**3**) (220 mg, 6.1% yield) and haliclonadiamine (**4**) (108 mg, 6.4% total yield) as the free bases. Compounds **3** and **4** were identified by comparison with published spectroscopic data.[3,4]

*Neopetrocyclamine A (**1**):* light yellow oil; $[\alpha]^{22}_D$ −63 (c 0.20, $CHCl_3$); IR (CaF_2) ν_{max} 3009, 2924, 2855, 1682, 1667, 1651, 1605, 1450, 1396, 1327, 1211, 1119, 972 cm^{-1}; see Table 1 for NMR data; HR-ESITOFMS m/z 381.3269 $[M]^+$ (calcd for $C_{26}H_{41}N_2$, 381.3270, −0.2 ppm error).

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Neopetrocyclamine B (2): colorless powder; $[\alpha]^{22}_{D}$ −24 (*c* 0.20, $CHCl_3$); IR (CaF_2) ν_{max} 3416, 3009, 2924, 2847, 1589, 1450, 1411, 1381, 1350, 1204, 1126, 972 cm^{-1}; see Table 2 for NMR data; HR-ESITOFMS *m/z* 371.3434 $[M + H]^+$ [calcd for $C_{25}H_{43}N_2$, 371.3426, −2.2 ppm error], *m/z* 393.3236 $[M + Na]^+$ (calcd for $C_{25}H_{42}N_2Na$, 393.3245, 2.3 ppm error).

Molecular Modeling. Molecular modeling was constructed in the Chem3D interface. An MMFF94 force field was used to optimize the energy and geometry of **1** at a simulated temperature of 298 K (5000 iterations, rms convergence of 0.01 kcal/mol). The resulting structure was then subjected to the AM1 semiempirical method for a secondary minimization using a gradient algorithm at a simulated temperature of 298 K (1000 iterations, rms convergence of 0.001 kcal/mol). All bonds were treated as freely rotatable except for the alkenyl and formamidinium bonds.

Cell Viability Assay. Cells were plated at a density of 5000 cells per well in a 96-well plate in 90 μL of culture media without phenol red or antibiotics. Cells were allowed to attach for 60 min under regular culture conditions. After the attachment, 10 μL of diluted compound or the solvent DMSO as control was added to the wells. Cells were incubated for 48 h with the compound before subjecting them to a cell proliferation assay (XTT) according to the manufacturer's protocol (Roche Diagnostics).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H, ^{13}C, and 2D NMR spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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■ REFERENCES

(1) Andersen, R. J.; Van-Soest, R. W. M.; Kong, F. 3-Alkylpiperidine alkaloids isolated from marine sponges in the order haplosclerida. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: New York, 1996; Vol. *10*, pp 301−355.

(2) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughtflower, R. J.; Mutton, I. M.; Upton, R. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2661−2663.

(3) Baker, B. J.; Scheuer, P. J.; Shoolery, J. N. *J. Am. Chem. Soc.* **1988**, *110*, 965−966.

(4) Fahy, E.; Molinski, T. F.; Harper, M. K.; Sullivan, B. W.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. *Tetrahedron Lett.* **1988**, *29*, 3427−3428.

(5) Sorek, H.; Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *J. Nat. Prod.* **2010**, *73*, 456−458.

(6) Davis, R. A.; Sykes, M.; Avery, V. M.; Camp, D.; Quinn, R. J. *Biorg. Med. Chem.* **2011**, *19*, 6615−6619.

(7) Tsukamoto, S.; Tane, K.; Ohta, T.; Matsunaga, S.; Fusetani, N.; van Soest, R. W. M. *J. Nat. Prod.* **2001**, *64*, 1576−1578.

(8) Krasnoff, S.; Gupta, S. *J. Chem. Ecol.* **1991**, *17*, 1953−1962.

(9) Li, X.-C.; Babu, K. S.; Jacob, M. R.; Khan, S. I.; Agarwal, A. K.; Clark, A. M. *ACS Med. Chem. Lett.* **2011**, *2*, 391−395.

(10) Chi, Y.-M.; Hashimoto, F.; Yan, W.-M.; Nohara, T. *Tetrahedron Lett.* **1997**, *38*, 2713−2716.

(11) Drandarov, K.; Guggisberg, A.; Linden, A.; Hesse, M. *Helv. Chim. Acta* **1998**, *81*, 1773−1791.

(12) Rabinowitz, J. C.; Pricer, W. E. *J. Am. Chem. Soc.* **1956**, *78*, 5702−5704.

(13) Guggisberg, A.; Drandarov, K.; Hesse, M. *Helv. Chim. Acta* **2000**, *83*, 3035−3042.

(14) Li, Q.; Chen, C.; Kapadia, A.; Zhou, Q.; Harper, M. K.; Schaack, J.; Labarbera, D. V. *J. Biomol. Screening* **2011**, *16*, 141−154.

(15) Kanno, S.-i.; Yomogida, S.; Tomizawa, A.; Yamazaki, H.; Ukai, K.; Mangindaan, R. E. P.; Namikoshi, M.; Ishikawa, M. *Int. J. Oncol.* **2013**, *43*, 1413−1419.

(16) Williams, D. E.; Austin, P.; Diaz-Marrero, A. R.; Van Soest, R.; Matainaho, T.; Roskelley, C. D.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 4173−4176.

(17) Brastianos, H. C.; Vottero, E.; Patrick, B. O.; Van Soest, R.; Matainaho, T.; Mauk, A. G.; Andersen, R. J. *J. Am. Chem. Soc.* **2006**, *128*, 16046−16047.

(18) de Almeida Leone, P.; Carroll, A. R.; Towerzey, L.; King, G.; McArdle, B. M.; Kern, G.; Fisher, S.; Hooper, J. N. A.; Quinn, R. J. *Org. Lett.* **2008**, *10*, 2585−2588.

(19) Oku, N.; Matsunaga, S.; Van Soest, R. W. M.; Fusetani, N. *J. Nat. Prod.* **2003**, *66*, 1136−1139.

(20) Redmond, N. E.; Raleigh, J.; Van-Soest, R. W. M.; Kelly, M.; Travers, S. A. A.; Bradshaw, B.; Vartia, S.; Stephens, K. M.; McCormack, G. P. *PLoS One* **2011**, *6*, e24344.

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