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Cytotoxic Aaptamine Derivatives from the South China Sea Sponge *Aaptos aaptos*

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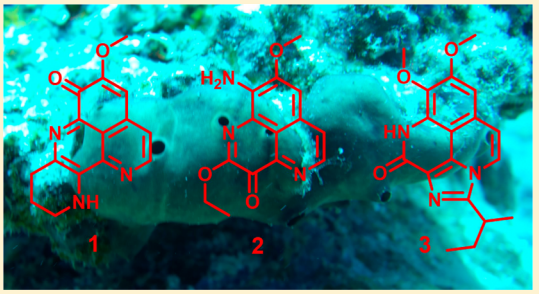
Supporting Information

ABSTRACT: Nine new aaptamine derivatives (**1**–**9**), together with three known related compounds (**10**–**12**), have been isolated from the South China Sea sponge *Aaptos aaptos*. The structures of all compounds were unambiguously elucidated on the basis of spectroscopic analyses. Structurally, compound **1** possesses a piperidinyl group fused to a demethyl(oxy)aaptamine moiety, whereas compounds **3**–**6** share an imidazole-fused 1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one skeleton. The cytotoxic activities of the compounds were evaluated against various human cancer cell lines, and compounds **2**, **8**, **11**, and **12** showed potent cytotoxicities against HL60, K562, MCF-7, KB, HepG2, and HT-29 cells, with IC_{50} values in the range of 0.03 to 8.5 μM.



Aaptamines, now collectively used to represent aaptamine and its natural derivatives, represent a structurally diverse family of typical marine alkaloids, which contain a benzo[*de*]-[1,6]naphthyridine framework.[1] The first and representative example of the heteroaromatic alkaloid belonging to this family is aaptamine, which was first reported by Nakamura et al. following its isolation from the Okinawa marine sponge *Aaptos aaptos* in 1982.[2] Only 46 natural alkaloids belonging to this structural class have been isolated to date.[1−10] With the exception of four congeners isolated from toadstools of the genus *Lactarius*,[11,12] the rest of these compounds have been isolated from members of the Demospongiae class of sponges, including the genera *Xestospongia* (Haplosclerida, Petrosiidae), *Suberites* (Hadromerida, Suberitidae), *Luffariella* (Dictyoceratida, Thorectidae), *Hymeniacidon* (Halichondrida, Halichondriidae), and *Aaptos* (Hadromerida, Suberitidae).[1−10] From a biosynthetic perspective, these metabolites can be derived from the original aaptamine core by diversified substitution,[13] dimerization,[7] rearrangement,[14] and ring expansion reactions.[14] As a unique type of marine alkaloids, aaptamines have attracted considerable interest from scientists working in a variety of different fields because of their unique structural diversity and varied biological activities, including their antioxidant, enzymatic inhibition, antiviral, antimicrobial, antifungal, antiparasitic, cytotoxic, α-adrenergic antagonistic, antifouling, and antidepressant activities.[1−9]

As part of our ongoing research on the isolation of new bioactive metabolites from marine sponges collected off the Xisha Islands in the South China Sea,[15,16] chemical investigation of the marine sponge *A. aaptos* led to the isolation of nine new aaptamine-type alkaloids (**1**–**9**), together with three known aaptamine derivatives (**10**–**12**). Herein, we report the details of the purification and structure elucidation of these compounds, as well as the evaluation of cytotoxic activity against a variety of different human cancer cell lines.

RESULTS AND DISCUSSION

Compound **1** was isolated as a light purple solid. The molecular formula was determined as $C_{15}H_{13}N_3O_2$ by an HRESIMS ion peak at m/z 290.0907 $[M + Na]^+$, implying the presence of nine degrees of unsaturation. The ^1H NMR spectrum of **1** displayed signals for a methoxy group at δ_H 3.95 (3H, s) and three olefinic protons at δ_H 6.58 (1H, s), 7.40 (1H, d, J = 4.2 Hz), and 8.70 (1H, d, J = 4.2 Hz). These signals are characteristic for the 8-OCH_3, the isolated H-7, and the coupled H-6 and H-5 protons, respectively, found in most of the aaptamine structures.[13] Unlike the other aaptamine analogues reported in the literature, compound **1** lacked a second pair of coupled protons at H-2 and H-3, indicating the

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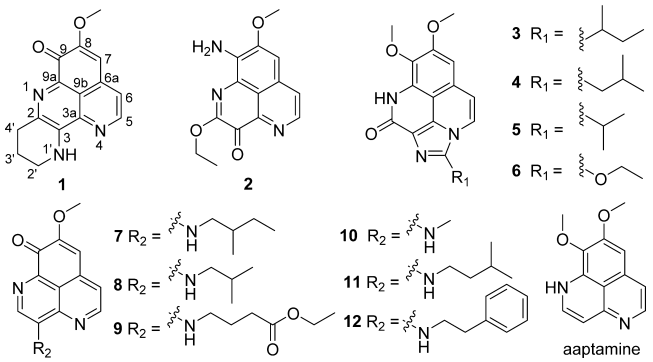

substitutions of both C-2 and C-3. Taken together with key HMBC correlations from H-5 to C-3a (δ_C 136.1), C-6 (δ_C 121.3), and C-6a (δ_C 134.6), from H-6 to C-5 (δ_C 150.2), C-7 (δ_C 105.6), and C-9b (δ_C 117.7), from H-7 to C-6, C-6a, C-8 (δ_C 158.0), C-9 (δ_C 175.6), and C-9b, and from 8-OCH$_3$ to C-8 (Figure 1) and a comparison of the NMR data for **1** with those of demethyl(oxy)aaptamine,[13] these data indicated that **1** was a 2,3-disubstituted-demethyl(oxy)aaptamine. The ^{1}H NMR spectrum of compound **1** also contained a broad singlet at δ_H 7.08 (1H, br s), two multiplets at δ_H 3.65 (2H, m) and 2.18 (2H, m), and a triplet at δ_H 3.24 (2H, t, J = 6.0 Hz). The latter three signals were attributed to three methylene groups, which were determined to be directly attached to the carbons at δ_C 41.5, 20.6, and 30.4, respectively, based on the HSQC analysis. However, the broad singlet at δ 7.08 was not directly attached to any carbon. Taken together with the IR absorption band at 3301 cm^{-1} and the molecular formula of **1**, this proton was deduced to be an NH proton, leading to the spin system 1′-NH/C-2′/C-3′/C-4′, supported by COSY correlations between 1′-NH and H$_2$-2′ (δ_H 3.65), H$_2$-2′ and H$_2$-3′ (δ_H 2.18), and H$_2$-3′ and H$_2$-4′ (δ_H 3.24). On the basis of the HMBC correlations from H$_2$-2′ to C-3 (δ_C 142.0) and from H$_2$-4′ to C-2 (δ_C 140.5) and C-3, and the one remaining unassigned degree of unsaturation for **1**, C-3 appeared to be connected to C-2′ (δ_C 41.5) via N-1′, whereas C-2 was connected to C-4′ (δ_C 30.4) (Figure 1). Finally, the structure of compound **1** was unambiguously assigned as piperidine[3,2-*b*]demethyl(oxy)-aaptamine.

Compound **2** was a dark red solid. Its molecular formula $C_{14}H_{13}N_3O_3$, which indicated 10 degrees of unsaturation, was obtained from the HRESIMS ion peak at m/z 294.0853 [M + Na]$^+$ in combination with the NMR spectroscopic data. The ^{1}H NMR spectrum of **2** exhibited a set of coupled doublet protons for a 2,3,4-trisubstituted pyridine group at δ_H 8.70 (1H, d, J = 4.8 Hz) and δ_H 7.88 (1H, d, J = 4.8 Hz), an isolated aromatic proton at δ_H 7.24 (1H, s), a methoxy group at δ_H 4.03 (3H, s), and an ethoxy group at δ_H 4.50 (2H, q, J = 7.2 Hz) and δ_H 1.39 (3H, t, J = 7.2 Hz). A broad singlet was also observed at δ_H 7.19 (2H, br s), which was attributed to two protons connected to a nitrogen atom. The ^{13}C NMR and DEPT spectra of **2** revealed the presence of 14 carbons, including one carbonyl carbon (δ_C 170.8), seven olefinic quaternary carbons (δ_C 155.1, 152.2, 131.9, 141.6, 141.8, 118.2, and 113.8), three olefinic methines (δ_C 144.4, 124.1, and 103.4), one oxygenated methylene (δ_C 62.0), one methoxy (δ_C 56.4), and one methyl (δ_C 14.2). These signals indicated that the coupled doublet protons at C-2 and C-3 were substituted. HMBC correlations from the methoxy (δ_H 4.03) to C-8 (δ_C 152.2) and from NH$_2$-9 (δ_H 7.19) to C-8 and C-9a (δ_C 113.8) indicated that the methoxy and amino groups were tethered to C-8 and C-9 (δ_C 141.6), respectively. In contrast to the other aaptamine analogues, the ethoxy group was determined to be attached to an olefinic quaternary carbon based on an HMBC correlation from H$_2$-1′ (δ_H 4.50) to the carbon resonance at δ_C 155.1, rather than C-8 or C-9 (Figure 1). Unfortunately, HMBC analysis did not allow for the unambiguous assignment of the remaining two carbon atoms at C-2 and C-3. A comparison of the ^{13}C NMR data for the C-2 to C-3 fragment in **2** with similar systems in the literature revealed that similar chemical shifts were reported by Kitahara et al. for the synthetic alkaloid 4*H*-naphtho[1,2,3-*ij*][2,7]naphthyridin-4-one.[17] Kobayashi et al. also reported similar chemical shifts for 2-methoxy-3-oxoaaptamine.[10] On the basis of these comparisons, the chemical shifts of C-2 and C-3 can be assigned at δ_C 155.1 and 170.8, respectively. Finally, the structure of **2** was established as 9-amino-2-ethoxy-8-methoxy-3*H*-benzo[*de*]-[1,6]naphthyridin-3-one. Compound **2**, however, could possibly be an artifact from the extraction process with EtOH as the solvent.

Compound **3** was isolated as a light brown solid and assigned the molecular formula of $C_{18}H_{19}N_3O_3$, based on HRESIMS data for the [M + Na]$^+$ ion at m/z 348.1323, which is consistent with 11 degrees of unsaturation. The IR spectrum showed bands due to NH (3408 cm^{-1}) and conjugated lactam carbonyl (1654 cm^{-1}) functions.[18] All 18 carbons were well resolved in the ^{13}C NMR spectrum and were classified by chemical shifts and DEPT and HSQC spectra as one lactam carbonyl (δ_C 158.4), three olefinic methines (δ_C 101.8, 113.2, and 121.8), eight quaternary carbons (δ_C 105.1, 120.5, 121.6, 129.3, 133.6, 133.9, 148.9, and 154.4), two methoxy groups (δ_C 56.4 and 60.8), one methine (δ_C 32.5), one methylene (δ_C 28.4), and two methyl groups (δ_C 18.7 and 11.5). The ^{1}H NMR spectrum displayed the characteristic signals of the methoxy protons 8-OCH$_3$ (δ_H 3.95), and 9-OCH$_3$ (δ_H 3.84),

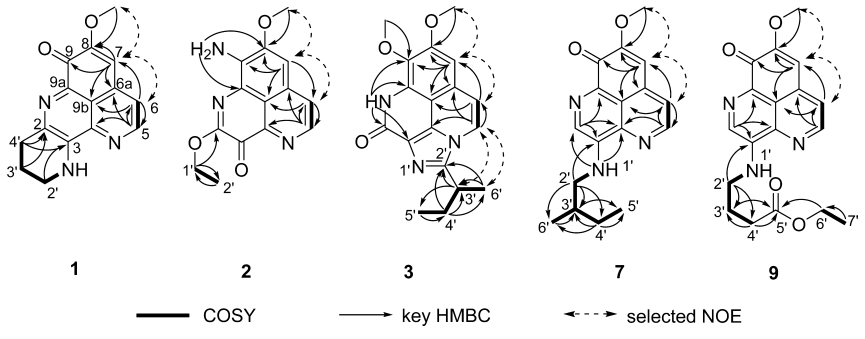

Figure 1. COSY, key HMBC, and selected NOE correlations of **1**−**3**, **7**, and **9**.

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Table 1. ^{1}H NMR Data for Compounds 1−9 (J in Hz)

position	1[a]	2[b]	3[b]	4[b]	5[c]	6[b]	7[a]	8[a]	9[a]
1			10.81 s	10.82 s	10.85, s	10.76, s			
2							8.37, s	8.38, s	8.37, s
5	8.70, d (4.2)	8.70, d (4.8)	8.29, d (7.2)	8.27, d (7.8)	8.30, d (7.2)	7.86, d (7.2)	8.74, d (4.8)	8.76, d (4.2)	8.74, d (4.8)
6	7.40, d (4.2)	7.88, d (4.8)	7.15, d (7.2)	7.16, d (7.8)	7.18, d (7.2)	7.03, d (7.2)	7.45, d (4.2)	7.46, d (4.2)	7.43, d (4.8)
7	6.58, s	7.24, s	7.28, s	7.28, s	7.31, s	7.25,s	6.60, s	6.61, s	6.59, s
8-OMe	3.95, s	4.03, s	3.95, s	3.95, s	3.96, s	3.95, s	3.96, s	3.97, s	3.96, s
9-OMe			3.84, s	3.83, s	3.84, s	3.83, s			
9-NH_2		7.19, br s							
1′	7.08, br s	4.50, q (7.2)					7.03, br s	7.01, br s	7.02, br s
2′a	3.65, m	1.39, t (7.2)					3.33, m	3.36, t (6.6)	3.60, q (6.6)
2′b							3.47, m		
3′a	2.18, m		3.43, m	3.01, d (7.2)	3.62, m	4.64, q (7.2)	1.89, m	2.12, m	2.14, m
3′b							1.34, m		
4′a	3.24, t (6.0)		1.73, m	2.20, m	1.41, d (6.8)	1.48, t (7.2)	1.58, m	1.10, d (6.6)	2.51,t (7.2)
4′b			1.88, m						
5′			0.84, t (7.2)	0.96, d (6.6)	1.41, d (6.8)		0.99, t (7.2)	1.10, d (6.6)	
6′			1.38, d (7.2)	0.96, d (6.6)			1.08, t (6.6)		4.15, q (7.2)
7′									1.25, t (7.2)

[a]Measured at 600 MHz in $CDCl_3$. [b]Measured at 600 MHz in DMSO-d_6. [c]Measured at 400 MHz in DMSO-d_6.

Table 2. ^{13}C NMR Data for Compounds 1−9

carbon	1[a]	2[b]	3[b]	4[b]	5[c]	6[b]	7[a]	8[a]	9[a]
2	140.5, C	155.1, C	158.4, C	158.4, C	158.4, C	157.9, C	129.7, CH	129.7 CH	129.5 CH
3	142.0, C	170.8, C	129.3, C	129.3, C	129.3, C	129.5, C	144.5, C	144.5 CH	144.0 CH
3a	136.1, C	141.8, C	133.9, C	133.9, C	134.1, C	132.3, C	136.3, C	136.4 C	136.3 C
5	150.2, CH	144.4, CH	121.8, CH	121.9, CH	121.8, CH	120.1, CH	150.5, CH	150.5 CH	150.7 CH
6	121.3, CH	124.1, CH	113.2, CH	113.3, CH	113.2, CH	115.7, CH	121.6, CH	121.6 CH	121.6 CH
6a	134.6, C	131.9, C	121.6, C	121.6, C	121.7, C	121.5, C	136.2, C	136.2 C	136.2 C
7	105.6, CH	103.4, CH	101.8, CH	101.9, CH	101.8, CH	102.3, CH	106.1, CH	106.4 CH	106.2 CH
8	158.0, C	152.2, C	154.4, C	154.4, C	154.4, C	154.2, C	157.9, C	158.0 C	157.8 C
9	175.6, C	141.6, C	133.6, C	133.7, C	133.6, C	133.8, C	175.9, C	175.9 C	175.9 C
9a	132.5, C	113.8, C	120.5, C	120.7, C	120.2, C	115.7, C	134.3, C	134.3 C	134.7 C
9b	117.7, C	118.2, C	105.1, C	105.1, C	105.1, C	104.9, C	117.9, C	118.0 C	117.9 C
8-OMe	56.0, CH_3	56.4, CH_3	56.4, CH_3	56.5, CH_3	56.4, CH_3	56.5, CH_3	56.0, CH_3	56.1, CH_3	56.0, CH_3
9-OMe			60.8, CH_3	60.8, CH_3	60.8, CH_3	60.8, CH_3			
1′		62.0, CH_2							
2′	41.5, CH_2	14.2, CH_3	148.9, C	144.4, C	149.6, C	148.5, C	48.7, CH_2	50.4, CH_2	42.2, CH_2
3′	20.6, CH_2		32.5, CH	34.7, CH_2	25.9, CH	67.1, CH_2	34.9, CH	28.5, CH	24.3, CH_2
4′	30.4, CH_2		28.4, CH_2	27.7, CH	20.9, CH_3	14.5, CH_3	27.2, CH_2	20.3, CH_3	31.6, CH_2
5′			11.5, CH_3	22.2, CH_3	20.9, CH_3		11.3, CH_3	20.3, CH_3	172.7, C
6′			18.7, CH_3	22.2, CH_3			17.5, CH_3		60.8, CH_2
7′									14.2, CH_3

[a]Measured at 150 MHz in $CDCl_3$. [b]Measured at 150 MHz in DMSO-d_6. [c]Measured at 100 MHz in DMSO-d_6.

the isolated proton H-7 (δ_H 7.28), the coupled protons H-5 (δ_H 8.29, d, J = 7.2 Hz) and H-6 (δ_H 7.15, d, J = 7.2 Hz), and the proton 1-NH (δ_H 10.81) of aaptamine, respectively. Furthermore, the HMBC spectrum of **3** revealed correlations from H-5 to C-3a (δ_C 133.9), C-6 (δ_C 113.2), and C-6a (δ_C 121.6), from H-6 to C-5 (δ_C 121.8), C-7 (δ_C 101.8), and C-9b (δ_C 105.1), from H-7 to C-6, C-6a, C-8 (δ_C 154.4), C-9 (δ_C 133.6), and C-9b, from 8-OCH_3 (δ_H 3.95) to C-8, from 9-OCH_3 (δ_H 3.84) to C-9, and from 1-NH to C-2 (δ_C 158.4), C-3 (δ_C 129.3), C-9a (δ_C 120.5), and C-9b, which indicated the 3,4-disubstituted 8,9-dimethoxy-1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one subunit in **3**. At this stage, one sp^2 quaternary carbon (δ_C 148.9, C-2′), one methine (δ_H/δ_C 3.43/32.5, CH-3′), one methylene (δ_H/δ_C 1.73, 1.88/28.4, CH_2-4′), and two methyls (δ_H/δ_C 0.84/11.5, CH_3-5′, and 1.38/18.7, CH_3-6′) remained unassigned. The continuous spin system CH_3(5′)−CH_2(4′)−CH(3′)−CH_3(6′), as indicated by the COSY correlations of H_3-6′/H-3′, H_2-4′/H-3′, and H_2-4′/H_2-5′, was determined to be attached to C-2′ based on the HMBC correlation from H-3′, H_2-4′, and H_3-6′ to C-2′. The deshielded resonance of C-2′ (δ_C 148.9) suggested its attachment to two nitrogen atoms,[19] which was confirmed by the key NOESY correlations of H-3′/H-5 and H_3-6′/H-5. Taking all of the information provided above into consideration, the unassigned nitrogen at N-1′ was assigned to a nonprotonated nitrogen based on the absence of an exchangeable proton in the ^{1}H NMR spectrum of **3** in DMSO-d_6. Moreover, the downfield chemical shift of C-3 (δ_C 129.3) suggested that C-3 must be attached to C-2′ via N-1′ to consume the remaining one degree of unsaturation. Hence, the structure of compound **3** can be

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Table 3. Evaluation of the Cytotoxicities for Compounds 2, 8, 11, and 12

	IC_{50} (μM)					
compound	HL60	K562	MCF-7	KB	HepG2	HT-29
2	0.11 ± 0.04	0.22 ± 0.04	1.2 ± 0.1	0.66 ± 0.04	0.6 ± 0.1	6.6 ± 1.1
8	0.28 ± 0.04	0.46 ± 0.04	2.3 ± 0.04	1.1 ± 0.04	1.0 ± 1.2	8.5 ± 2.1
11	0.03 ± 0.03	0.09 ± 0.01	0.44 ± 0.03	0.11 ± 0.03	0.17 ± 0.03	1.8 ± 0.4
12	0.03 ± 0.03	0.09 ± 0.03	0.4 ± 0.1	0.12 ± 0.03	0.2 ± 0.1	2.2 ± 0.03
paclitaxel (nM)	1.5 ± 0.1	2.4 ± 0.2	3.2 ± 0.3	3.8 ± 0.5	2.7 ± 0.1	3.5 ± 0.2

unambiguously assigned as a new compound with an imidazole-fused 1*H*-benzo[*de*][1,6]naphthyridin-2(4*H*)-one skeleton as shown. Unfortunately, the spectroscopic data alone were insufficient to define the absolute configuration of C-3′ in the side chain.

Compound **4** was isolated as a light brown solid. The molecular formula of $C_{18}H_{19}N_3O_3$ was deduced from its HRESIMS data in combination with extensive NMR analyses. Comparison of the NMR data for **4** with those of **3** indicated that **4** was an isomer of compound **3**, with the only difference at the C-2′-substituted side chain (Tables 1 and 2). The *sec*-butyl group in the structure of **3** was replaced by an isobutyl group in **4**, which was supported by the COSY correlations of H_2-3′ (δ_H 3.01)/H-4′ (δ_H 2.20), H-4′/H_3-5′ (δ_H 0.96), and H-4′/H_3-6′ (δ_H 0.96), as well as the HMBC correlations from H_2-3′ and H-4′ to C-2′ (δ_C 144.4).

Compound **5** was also isolated as a light brown solid, with a molecular formula of $C_{17}H_{17}N_3O_3$ as determined by HRESIMS. Comparison of the ^{1}H NMR data of **5** with those of **4** revealed that these two compounds were very similar, except for the absence of a methylene doublet (δ_H 3.01, d, J = 7.2 Hz). Therefore, compound **5** had an isopropyl group attached to C-2′ instead of the isobutyl group in **4**, which was also supported by the COSY correlations of H-3′ (δ_H 3.62, m)/H_3-4′ (δ_H 1.41, d, J = 6.8 Hz) and H-3′/H_3-5′ (δ_H 1.41, d, J = 6.8 Hz), as well as HMBC correlations from H-3′, H-4′, and H-5′ to C-2′ (δ_C 149.6).

Compound **6** was isolated as a light brown solid, with an adduct ion at m/z 336.0959 $[M + Na]^+$ in its HRESIMS spectrum, appropriate for a molecular formula of $C_{16}H_{15}N_3O_4$. The NMR data of **6** were almost identical to those for **3** with the same imidazole-fused skeleton except for the presence of a different substituent at C-2′. Furthermore, one oxymethylene (δ_H/δ_C 4.64/67.1) and one methyl (δ_H/δ_C 1.48/14.5) were determined as an ethoxy group attached to C-2′ based on the HMBC correlation from H-3′ (δ_H 4.64) to C-2′ (δ_C 148.5) and consideration of its molecular formula. Because EtOH was used for the extractions, it is probable that compound **6** is an artifact.

Compound **7** was obtained as an orange, amorphous solid. HRESIMS revealed an ion peak at m/z 320.1377 $[M + Na]^+$, indicating a molecular formula of $C_{17}H_{19}N_3O_2$. The ^{1}H NMR spectrum revealed resonances at δ_H 3.96 (3H, s), 6.60 (1H, s), 7.45 (1H, d, J = 4.8 Hz), 8.37 (1H, s), and 8.74 (1H, d, J = 4.8 Hz). The ^{13}C NMR spectrum analysis indicated the presence of one carbonyl (δ_C 175.9), six olefinic quaternary carbons (δ_C 117.9, 134.3, 136.2, 136.3, 144.5, and 157.9), four olefinic methines (δ_C 106.1, 121.6, 129.7, and 150.5), and one methoxy (δ_C 56.0). These characteristic signals indicated that compound **7** was a demethyl(oxy)aaptamine derivative.[13] Furthermore, H-2 appeared as a singlet, indicating that C-3 (δ_C 144.5) was substituted. Compared with demethyl(oxy)aaptamine, the downfield chemical shift for C-3 suggested that C-3 was connected to a nitrogen atom.[13] In the HSQC spectrum, the broad singlet resonance at δ_H 7.03 was not correlated to any carbon resonance, and based on the IR absorption band at 3374 cm^{-1}, the proton was deduced to be an NH proton. The COSY correlations of H-1′ (δ_H 7.03)/H_2-2′ (δ_H 3.33, 3.47), H_2-2′/H-3′ (δ_H 1.89), H-3′/H_2-4′ (δ_H 1.34, 1.58), and H_2-4′/H_3-5′ (δ_H 0.99), as well as the HMBC correlation from H_3-6′ (δ_H 1.08, d, J = 6.6 Hz) to C-3′ (δ_C 34.9), indicated the presence of a 2-methylbutylamino fragment, which was unambiguously determined to be tethered to C-3, based on the key HMBC correlations from H-1′ to C-2 (δ_C 129.7) and C-3a (δ_C 136.3) and from H_2-2′ to C-3 (Figure 1). Therefore, on the basis of these data, the structure of compound **7** was assigned as 3-(2-methylbutylamino)demethyl(oxy)aaptamine. However, the absolute configuration at C-3′ could not been definitively determined on the basis of spectroscopic data.

Compound **8** was also isolated as an orange, amorphous solid, assigned a molecular formula of $C_{16}H_{17}N_3O_2$ deduced from the HRESIMS and NMR data, implying 10 degrees of unsaturation. The ^{13}C NMR and DEPT spectra exhibited 16 carbon resonances corresponding to three methyls, one methylene, five methines, and seven quaternary carbons. The overall appearance of the NMR spectra showed close structural similarity between **7** and **8**, with the same C-3-substituted benzo[*de*][1,6]naphthyridine moiety, except for the absence of a methylene resonance in **8**, indicating C-3 was substituted by an isobutylamino subunit. This was also confirmed by the COSY correlations of H-1′ (δ_H 7.01)/H_2-2′ (δ_H 3.36)/H-3′ (δ_H 2.12)/H_3-4′ (δ_H 1.10) and H-3′/H_3-5′ (δ_H 1.10), as well as the HMBC correlations from H_2-2′ to C-3 (δ_C 144.5). Thus, compound **8** was identified as 3-isobutylaminodemethyl(oxy)-aaptamine.

Compound **9** was also obtained as an orange, amorphous solid, and its molecular formula was determined to be $C_{18}H_{19}N_3O_4$ by HRESIMS analysis, requiring 11 degrees of unsaturation. Meanwhile, the ^{1}H NMR spectrum of compound **9** closely resembled those of **7** and **8**, indicating the same N-1′-substituted 3-amino demethyl(oxy)aaptamine moiety. Interpretation of the COSY and HSQC spectra revealed the presence of two spin systems in the structure: NH-1′/H_2-2′/H_2-3′/H_2-4′ and H_2-6′/H_3-7′ (Figure 1). The latter spin system was determined as an ethoxy group attached to C-5′ on the basis of the HMBC correlation from H_2-6′ (δ_H 4.15) to C-5′ (δ_C 172.7). Additionally, C-5′ must be attached to C-3′ (δ_C 24.3) via C-4′ (δ_C 31.6) on the basis of HMBC correlations of H_2-3′ (δ_H 2.14)/C-5′ and H-4′ (δ_H 2.51)/C-5′. Therefore, compound **9** was identified as 3-(*N*-4-ethylbutanoate)amino-demethyl(oxy)aaptamine. However, it is possible that compound **9** is an artifact formed during the extraction and isolation procedure with the solvent containing EtOH.

Besides the nine new compounds **1**−**9**, the known compounds 3-(methylamino)demethyl(oxy)aaptamine (**10**),[5] 3-(isopentylamino)demethyl(oxy)aaptamine (**11**),[13] and 3-(phenethylamino)demethyl(oxy)aaptamine (**12**)[13] were also

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obtained and elucidated by comparison of their physical and spectroscopic features with the data reported in the literature.

The cytotoxicities of compounds **1**–**12** were evaluated against six human cancer cell lines, including HL60 (human acute leukemia), K562 (human chronic leukemia), MCF-7 (human breast cancer), KB (human nasopharyngeal cancer), HepG2 (human liver carcinoma), and HT-29 (human colon adenocarcinoma) cells, using paclitaxel as a positive control (Table 3). Compounds **2**, **8**, **11**, and **12** showed potent cytotoxicities against all the human cancer cell lines tested, with IC_{50} values in the range of 0.03 to 8.5 μM. The remaining compounds tested were found to be inactive against all of the six human cancer cell lines (IC_{50} value >10 μg/mL). In terms of the sensitivities of the different cell lines to compounds **2**, **8**, **11**, and **12**, the HL-60 cells were found to be the most sensitive, followed sequentially by the K562, HepG2, KB, MCF-7, and HT-29 cells. The biological evaluation indicated that substitution at C-3 does not result in a significant decrease of general cytotoxicity, as a series of similar compounds showed significant activity against various human cancer cell lines.[9,13] However, substitutions at both C-2 and C-3, as in the case of compound **1**, correlated with a loss of inhibitory activity. In addition, oxidation of C-2 to a carbonyl group and modification of the aaptamine structure with an imidazole-fused system, represented by compounds **3**–**6**, also negatively affected the activity.[13,19] On the basis of their significant cytotoxic activities, compounds **2**, **8**, **11**, and **12** should be investigated in greater detail to develop a deeper understanding of their cell growth inhibition characteristics and possible mechanisms of action.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation data were recorded on a PerkinElmer model 341 polarimeter with a 1 dm cell. UV and IR (KBr) spectra were obtained on a Hitachi U-3010 spectrophotometer and Jasco FTIR-400 spectrometer, respectively. ^{1}H, ^{13}C, DEPT135, COSY, HSQC, HMBC, and NOESY NMR spectra were acquired at room temperature (rt) on Bruker AVANCE-600, Bruker AMX-500, and Bruker AMX-400 instruments. HRESIMS and ESIMS data were obtained on a Waters Q-Tof micro YA019 mass spectrometer. Reversed-phase HPLC was performed on YMC-Pack Pro C18 RS (5 μm) columns with a Waters 1525 separation module equipped with a Waters 2998 photodiode array detector. Purifications by column chromatography were performed on silica gel 60 (200–300 mesh; Yantai, China), Sephadex LH-20 (18–110 μm, Pharmacia Co.), and ODS (50 μm, YMC Co.). Analytical thin-layer chromatography was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde-H_2SO_4 reagent.

Animal Material. The sponge samples were collected off Woody (Yongxing) Island and Seven Connected Islets in the South China Sea in June 2007 and identified as *Aaptos aaptos* by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China), A voucher sample (No. OLS) was deposited in the Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, China.

Extraction and Isolation. The air-dried sponge (2.3 kg, dry weight) was powdered and extracted with 95% aqueous EtOH at rt. The combined extracts were concentrated under reduced pressure to yield the crude extract (109.7 g), which was suspended in H_2O and extracted sequentially with CH_2Cl_2 and *n*-BuOH to afford CH_2Cl_2- and *n*-BuOH-soluble extracts. The CH_2Cl_2-soluble extract (84.4 g) was partitioned between 90% aqueous MeOH and *n*-hexane, and the *n*-hexane layer was collected and concentrated under reduced pressure to afford the *n*-hexane-soluble extract (52.0 g). The 90% aqueous MeOH phase was diluted to 60% aqueous MeOH with H_2O, which was extracted with CH_2Cl_2 to afford the CH_2Cl_2-soluble extract (14.2 g). The CH_2Cl_2-soluble extract was subjected to vacuum liquid chromatography on silica gel by gradient elution using CH_2Cl_2/MeOH (100:1, 80:1, 60:1, 50:1, 30:1, 20:1, 10:1, 8:1, 5:1, 1:1, 0:1, v/v) as solvents to give seven fractions (A–G). Fraction B (1.8 g) was subjected to column chromatography (CC) on Sephadex LH-20 with CH_2Cl_2/MeOH (1:1) as the eluting solvent to afford six subfractions (B1–B6). Subfraction B3 was separated by CC on ODS (50 μm) eluting with MeOH/H_2O (2:3–1:0) to give six fractions (Fr.B31–B36). Fr.B34 was also separated by reversed-phase HPLC eluting with 40% MeOH to afford compound **2** (2.0 mL/min, 288 nm, t_R = 62.0 min, 9.0 mg) and compound **1** (2.0 mL/min, 254 nm, t_R = 64.3 min, 4.8 mg). Fraction B4 was further separated on an ODS (50 μm) column followed by stepwise gradient elution with MeOH/H_2O (2:3, 3:2, 4:1, 1:0) to afford four fractions (B41–B44), and then fraction B42 was purified by reversed-phase HPLC with an elution of 30% MeCN detected at the wavelength of 256 nm to give compounds **5** (2.0 mL/min, t_R = 37.0 min, 2.9 mg), **6** (2.0 mL/min, t_R = 43.0 min, 3.1 mg), **3** (2.0 mL/min, t_R = 65.0 min, 13.5 mg), and **4** (2.0 mL/min, t_R = 69.0 min, 1.7 mg). Fraction D (1.8 g) was separated by CC on silica gel eluting in a stepwise manner with CH_2Cl_2/MeOH (60:1, 50:1, 30:1, 15:1, 10:1, 5:1, 0:1, v/v) to afford six fractions (D1–D6). Fraction D2 was purified by reversed-phase HPLC, eluting with 50% MeCN detected at 249 nm, to give **9** (2.0 mL/min, t_R = 13.3 min, 1.6 mg), whereas the purification of D3 by reversed-phase HPLC eluting with 50% MeCN detected at 244 nm resulted in the isolation of **7** (2.0 mL/min, t_R = 15.0 min, 3.3 mg), **8** (2.0 mL/min, t_R = 16.3 min, 5.2 mg), and **10** (2.0 mL/min, t_R = 17.0 min, 2.0 mg). The other two fractions, D4 and D5, were both isolated by reversed-phase HPLC eluting with 50% MeCN at 248 nm to afford **11** (2.0 mL/min, t_R = 11.5 min, 18.6 mg), and **12** (2.0 mL/min, t_R = 12.8 min, 5.8 mg).

*Piperidine[3,2-b]demethyl(oxy)aaptamine (**1**):* light purple solid; UV (MeOH) (log ε) λ_{max} 207 (3.68), 253 (3.34), 287 (3.31), 398 (2.81) nm; IR (KBr) ν_{max} 3301, 2926, 2854, 1720, 1642, 1615, 1563, 1458, 1397, 1367, 1324, 1264, 1199, 1122, 1016, 927, 937, 862, 801 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 290.0907 [M + Na]$^+$ (calcd for $C_{15}H_{13}N_3O_2Na$, 290.0905).

*9-Amino-2-ethoxy-8-methoxy-3H-benzo[de][1,6]naphthyridin-3-one (**2**):* dark red solid; UV (MeOH) (log ε) λ_{max} 211 (4.07), 224 (4.06), 290 (4.09), 402 (3.22) nm; IR (KBr) ν_{max} 3323, 2957, 2927, 2855, 1644, 1614, 1584, 1572, 1510, 1487, 1435, 1371, 1327, 1313, 1264, 1206, 1089, 1028, 1002, 958, 862, 811 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 294.0853 [M + Na]$^+$ (calcd for $C_{14}H_{13}N_3O_3Na$, 294.0855).

*2-(sec-Butyl)-7,8-dimethoxybenzo[de]imidazo[4,5,1-ij][1,6]-naphthyridin-10(9H)-one (**3**):* light brown solid; $[\alpha]^{25}_D$ +7.41 (*c* 0.10, MeOH); UV (MeOH) (log ε) λ_{max} 219 (4.40), 253 (4.56), 319 (3.96), 350 (3.55) nm; IR (KBr) ν_{max} 3408, 3175, 3087, 2966, 2932, 2873, 1693, 1654, 1579, 1503, 1459, 1417, 1393, 1353, 1316, 1221, 1157, 1108, 1058, 1026, 997, 964, 913, 827, 732 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 348.1323 [M + Na]$^+$ (calcd for $C_{18}H_{19}N_3O_3Na$, 348.1324).

*2-Isobutyl-7,8-dimethoxybenzo[de]imidazo[4,5,1-ij][1,6]-naphthyridin-10(9H)-one (**4**):* light brown solid; UV (MeOH) (log ε) λ_{max} 219 (4.38), 257 (4.55), 319 (3.89), 350 (3.57) nm; IR (KBr) ν_{max} 3183, 3095, 2956, 2929, 2865, 1693, 1652, 1580, 1504, 1491, 1461, 1416, 1391, 1347, 1321, 1261, 1233, 1157, 1122, 1093, 1059, 1028, 999, 823, 799 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 348.1323 [M + Na]$^+$ (calcd for $C_{18}H_{19}N_3O_3Na$, 348.1324).

*2-Isopropyl-7,8-dimethoxybenzo[de]imidazo[4,5,1-ij][1,6]-naphthyridin-10(9H)-one (**5**):* light brown solid; UV (MeOH) (log ε) λ_{max} 219 (4.38), 252 (4.54), 275 (4.18), 319 (3.93), 350 (3.57) nm; IR (KBr) ν_{max} 3177, 3063, 2965, 2929, 2854, 1693, 1652, 1578, 1504, 1460, 1418, 1395, 1355, 1314, 1257, 1225, 1157, 1102, 1057, 1026, 1000, 968, 923, 825, 759, 732 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 334.1169 [M + Na]$^+$ (calcd for $C_{17}H_{17}N_3O_3Na$, 334.1168).

*2-Ethoxy-7,8-dimethoxybenzo[de]imidazo[4,5,1-ij][1,6]-naphthyridin-10(9H)-one (**6**):* light brown solid; UV (MeOH) (log ε) λ_{max} 221 (4.43), 233 (4.42), 260 (4.51), 350 (3.64) nm; IR (KBr) ν_{max} 3217, 2928, 2856, 1698, 1648, 1580, 1535, 1494, 1460, 1415, 1384,

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1338, 1309, 1286, 1260, 1226, 1153, 1133, 1092, 1025, 970, 806 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 336.0959 $[M + Na]^+$ (calcd for $C_{16}H_{15}N_3O_4Na$, 336.0960).

*3-(2-Methylbutylamino)demethyl(oxy)aaptamine (**7**):* orange, amorphous solid; $[\alpha]^{25}_D$ +2.75 (*c* 0.15, MeOH); UV (MeOH) (log ε) λ_{max} 208 (3.84), 250 (3.56), 267 (3.40), 402 (3.57), 498 (3.56) nm; IR (KBr) ν_{max} 3374, 3051, 2960, 2927, 2855, 1719, 1644, 1615, 1565, 1461, 1378, 1340, 1313, 1273, 1202, 1141, 1105, 1065, 1003, 968, 923, 872, 828, 690, 658 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 320.1377 $[M + Na]^+$ (calcd for $C_{17}H_{19}N_3O_2Na$, 320.1375).

*3-Isobutylaminodemethyl(oxy)aaptamine (**8**):* orange, amorphous solid; UV (MeOH) (log ε) λ_{max} 203 (3.47), 247 (3.10), 277 (2.94), 398 (2.49), 498 (2.95) nm; IR (KBr) ν_{max} 3367, 3053, 2959, 2928, 2870, 1725, 1645, 1615, 1566, 1545, 1462, 1388, 1340, 1314, 1274, 1203, 1176, 1145, 1105, 1061, 1004, 967, 923, 872, 826, 733, 693 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 284.1397 $[M + H]^+$ (calcd for $C_{16}H_{18}N_3O_2$, 284.1397).

*3-(N-4-Ethylbutanoate)aminodemethyl(oxy)aaptamine (**9**):* orange, amorphous solid; UV (MeOH) (log ε) λ_{max} 209 (3.96), 245 (3.81), 277 (3.62), 383 (3.24), 498 (3.50) nm; IR (KBr) ν_{max} 3358, 3054, 2927, 2854, 1729, 1642, 1611, 1565, 1511, 1462, 1419, 1374, 1343, 1315, 1269, 1201, 1169, 1113, 1026, 923, 870, 825, 803, 658 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 364.1271 $[M + Na]^+$ (calcd for $C_{18}H_{19}N_3O_4Na$, 364.1273).

Cytotoxicity Assay. The HL60 and K562 cell lines were grown in suspension culture at 37 °C in RPMI-1640 medium supplemented with 10% nondialyzed fetal bovine serum (FBS), 2 mM/L glutamine, 100 units/mL of penicillin, and 10 μg/mL of streptomycin. The cells were allowed to grow undisturbed for 24 h before addition of the test compounds. After 48 h of incubation with the test compounds at 37 °C, the viable cells were counted with the trypan blue exclusion method to assess cell viability.[20] The MCF-7, KB, HepG2, and HT-29 cell lines were cultured at 37 °C in DMEM medium supplemented with 10% nondialyzed FBS, 2 mM/L glutamine, 100 units/mL of penicillin, and 10 μg/mL of streptomycin. After 72 h of incubation with the test compounds at 37 °C, the methylene blue assay was performed as described by Finlay et al.[21] The UV absorbance was read on a microplate reader at a wavelength of 595 nm. The results were expressed as the relative percentage of absorbance detected in the treated cells compared with untreated control cells.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of 1D and 2D NMR, HRESIMS, UV, and IR spectra for compounds **1**−**9** are 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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