Structures and Cytotoxicity Relationship of Isoaaptamine and Aaptamine Derivatives

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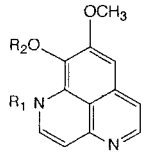
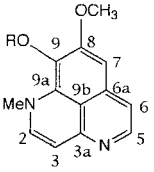
A series of 9-*O*-acylisoaaptamine (**3**−**14**) and 4-*N*-acyl-dihydroaaptamine (**16**−**19**) derivatives have been prepared and evaluated for antitumor activity against murine P-388 and human tumor cells including KB16, A549, and HT-29 cell lines. All of compounds showed significant cytotoxicity against P-388 cells. Among them, compounds **9**−**11** showed potent activity as isoaaptamine (**1**). There was an apparent lack of linear relationship between cytotoxicity and carbon number of the side chain. The structure and activity relationship for these particular compounds are discussed.

Marine sponges are a source of many unusual compounds including a series of 1*H*-benzo[*de*]1,6-naphthyridine that have not been reported from terrestrial sources.[1−3] Aaptamine (**2**) and isoaaptamine (**1**) were isolated from *Aaptos aaptos* as major components.[1,8] Interestingly, these compounds possess α-adrenoreceptor blocking activity on vascular smooth muscle and potent cytotoxicity against HeLa tumor cells.[2,4] We reported previously the isolation, structural elucidation, and cytotoxicity of four alkaloids, isoaaptamine (**1**), aaptamine (**2**), demethyl(oxy)aaptamine (**20**), and aaptosine (**21**), from the Taiwanese marine sponge *A. aaptos*.[5] Compounds **1**, **2**, and **20** showed significant cytotoxicity against human KB16, A549, and HT-29 tumor cells while **21** was inactive. To investigate the structure−activity relationship and the possible active center for antitumor activity of these novel compounds, **1** was acylated to give derivatives **3**−**14**. Compounds **16**−**19** were obtained from acylation of dihydroaaptamine (**15**) which was prepared from aaptamine via hydrogenation of aaptamine (**2**). Herein, we wish to report the preparation, structural elucidation and biological activity of 9-*O*-acyl-isoaaptamine and 4-*N*-acyldihydroaaptamine derivatives (Chart 1).

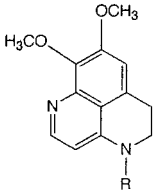
Chart 1



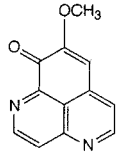
1 $R_1 = CH_3$, $R_2 = H$
2 $R_1 = H$, $R_2 = CH_3$



3 $R = COCH_3$
4 $R = COCH_2CH_3$
5 $R = CO(CH_2)_3CH_3$
6 $R = CO(CH_2)_4CH_3$
7 $R = CO(CH_2)_5CH_3$
8 $R = CO(CH_2)_6CH_3$
9 $R = CO(CH_2)_7CH_3$
10 $R = CO(CH_2)_8CH_3$
11 $R = CO(CH_2)_{10}CH_3$
12 $R = CO(CH_2)_{12}CH_3$
13 $R = CO(CH_2)_{14}CH_3$
14 $R = CO(CH_2)_{16}CH_3$



15 R = H
16 $R = COCH_3$
17 $R = CO(CH_2)_4CH_3$
18 $R = CO(CH_2)_{10}CH_3$
19 $R = CO(CH_2)_{16}CH_3$



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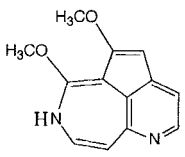

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Results and Discussion

Compounds **3**−**14** were prepared from **1** in pyridine with the following reagents, respectively: acetic anhydride, propionic anhydride, valeric anhydride, hexanoic anhydride, heptanoic anhydride, octanoyl chloride, nonanoyl chloride, decanoic anhydride, lauric anhydride, myristic anhydride, palmitic anhydride, and stearic anhydride under room temperature (acylation). Hydrogenation of **2** with PtO_2 in HOAc−HCl solution at 80 °C yielded dihydroaaptamine (**15**). Subsequent acylation of **15** with acetic anhydride, hexanoic anhydride, lauric anhydride, and stearic anhydride, respectively, provided 4-*N*-acetyldihydroaaptamine, 4-*N*-hexanoyldihydroaaptamine, 4-*N*-lauryldihydroaaptamine, and 4-*N*-stearyldihydroaaptamine (**16**−**19**). The structures of compounds **3**−**19** were determined by UV, IR, ^1H, ^{13}C NMR, and EIMS spectroscopic methods. Their molecular formulas were further confirmed by high-resolution EIMS spectral analysis. The assignment of each proton and carbon of compound **16** was facilitated by 2D-NMR experiments such as HMQC and HMBC. The spectral data for **3**−**19** are included in the Experimental Section.

Table 1 shows the biological results for compounds **1**−**21** as tested against murine P-388 (leukemia) and human tumor cells including KB16 (mouth epidermoid carcinoma), A549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma) in vitro. For P-388 inhibitory activity, compounds **9**−**11** and **18** were more potent than or comparable to **1** and **2**. The other derivatives were weaker than the starting materials. All the derivatives showed no or mild inhibitory activity against human KB16, A549, and HT-29 tumor cells.

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Table 1. Results of Cytotoxicities (IC_{50}, μg/mL)[a] of **1**−**21** in the *in Vitro* Primary Screen

compound	P-388	KB16	A549	HT-29
1 isoaaptamine	0.04	0.4	0.3	0.4
2 aaptamine	0.6	3.9	2.8	6.9
3	4.2	>50	>50	>50
4	1.4	28	18	13
5	1.8	35	13	9
6	0.7	33	13	12
7	0.7	38	11	13
8	1.5	15	14	12
9	0.04	33	12	3.0
10	0.03	20	6.1	2.5
11	0.03	20	3.7	2.5
12	0.3	3.3	5.6	2.7
13	0.1	8.0	7.3	9.0
14	1.1	>50	>50	21
15 dihydroaaptamine	1.8	22	23	47
16	2.6	32	20	17
17	3.7	>50	>50	>50
18	0.1	7.8	5.6	9.6
19	2.7	20	>50	>50
20 demethyl(oxy)aaptamine	0.01	0.1	0.3	NT[b]
21 aaptosine	NT[b]	NT[b]	>50	>50

[a] The concentration of compound which inhibited 50% (IC_{50}) of the growth tumor cell lines (P-388, murine lymphocytic leukemia; KB16, human mouth epidermoid carcinoma; A549, human lung adenocarcinoma; HT-29, human colon adenocarcinoma). All data estimated by interpolation method. [b] Not tested.

The cytotoxicity study revealed that the carbon number of the side chain at C-9 or N-4 was a nonlinear relationship with activity. General speaking, introducing an acyl side chain to C-9 position of **1** or to N-4 position of **15** gave rise to decrease in activity. However, compounds **9**−**11** contained a side chain of 9, 10, and 12 carbons, respectively, showing potent activity as **1** in P-388 assay. The result clearly indicated that the hydroxyl group at the C-9 was critical. Oxidation of the hydroxyl group to give a carbonyl function such as compound **20** resulted in an increase of activity. Compound **4** which contains a methoxyl group was also less active than **1**. Compound **15**, a product obtained from hydrogenation of **2** also showed less active than compound **2**, indicating that aromaticity in ring B was still important in activity. On the other hand, the decrease in activity of acylated products against human tumor cells may be explained by low water solubility and low bioavailability.

Experimental Section

General Experimental Procedures. UV and IR spectra were taken on a Hitachi U-3210 and JASCO A-100 IR spectrophotometers, respectively. EIMS spectra were obtained on a MAT 112S-JMS D300 and JMX-HX 110 mass spectrometer, using direct inlet systems. ^{1}H, ^{13}C NMR, HMQC, and HMBC spectra were recorded on a Varian FT-300 and a Bruker FT-400 spectrometers. Analytical TLC was carried out on the Kiesel gel GF_{254} plates and detection was made under UV light. EM Kieselgel 60 (230−400 mesh ASTM) was used for column chromatography.

Preparation of Compounds 3−14. To a solution of isoaaptamine (25 mg, **1**) in pyridine (2 mL) was added an appropriate anhydride or acyl chloride and kept at room temperature overnight. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The residue after evaporation was basified with 10% Na_2CO_3, added THF (15 mL) and stirred for 10 min. The solution was extracted with ether and the ether layer was concentrated to give a residue. The residue was chromatographed on a silica gel column and eluted with solvent mixture of EtOAc/MeOH by the following ratios and volumes (20:1, 10:1, 5:1, 4:1, 3:1, and 2:1; each 50 mL), to afford compounds **3**−**14** with a yield which varied in a range of 50−94%.

9-*O*-Acetylisoaaptamine (3): pale brown amorphous solid, 84%; IR (KBr) ν_{max} 2928, 1712, 1616 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 293 (3.15) nm; ^{1}H NMR (CD_3OD) δ 7.60 (1H, d, J = 7.2 Hz, H-2), 6.35 (1H, d, J = 7.2 Hz, H-3), 7.44 (1H, d, J = 7.1 Hz, H-5), 6.94 (1H, d, J = 7.1 Hz, H-6), 7.06 (1H, s, H-7), 3.96 (3H, s, NCH_3), 3.90 (3H, s, OCH_3), 2.37 (3H, s, H-2′); ^{13}C NMR (CD_3OD) δ 146.6 (C-2), 100.8 (C-3), 151.4 (C-3a), 135.7 (C-5), 114.7 (C-6), 135.9 (C-6a), 103.1 (C-7), 157.7 (C-8), 137.8 (C-9), 124.5 (C-9a), 119.6 (C-9b), 45.8 (NCH_3), 57.7 (OCH_3), 170.7 (C-1′), 21.6 (C-2′); EIMS *m*/*z* (rel int.) 270 (M^+, 16), 228 (90), 213 (39), 184 (33), 168 (14), 155 (14), 114 (14), 81 (41), 73 (26), 69 (100), 43 (73); HREIMS *m*/*z* 270.1004 (obsd), 270.1005 (calcd for $C_{15}H_{14}N_2O_3$); R_f value 0.20 (EtOAc/MeOH, 4:1).

9-*O*-Propionylisoaaptamine (4): pale brown amorphous solid, 80%; IR (KBr) ν_{max} 1644, 1608, 1352, 1112 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 237 (4.01) nm; ^{1}H NMR (CD_3OD) δ 7.53 (1H, d, J = 4 Hz, H-2), 6.28 (1H, d, J = 4 Hz, H-3), 7.40 (1H, d, J = 2.1 Hz, H-5), 6.88 (1H, d, J = 2.1 Hz, H-6), 6.98 (1H, s, H-7), 3.93 (3H, s, NCH_3), 3.83 (3H, s, OCH_3), 2.70 (2H, t, J = 7.5 Hz, H-2′), 1.27 (3H, m, H-3′); ^{13}C NMR (CD_3OD) δ 148.3 (C-2), 101.5 (C-3), 151.3 (C-3a), 134.0 (C-5), 114.8 (C-6), 136.1 (C-6a), 101.8 (C-7), 158.6 (C-8), 137.8 (C-9), 125.3 (C-9a), 119.3 (C-9b), 45.7 (NCH_3), 57.3 (OCH_3), 174.6 (C-1′), 28.2 (C-2′), 23.7 (C-3′); EIMS *m*/*z* (rel int.) 284 (M^+, 11), 228 (100), 213 (31), 184 (31), 168 (12), 155 (15), 81 (22), 69 (54), 57 (45), 41 (24); HREIMS *m*/*z* 284.1161 (obsd), 284.1162 (calcd for $C_{16}H_{16}N_2O_3$); R_f value 0.25 (EtOAc/MeOH, 4:1).

9-*O*-Valerylisoaaptamine (5): pale brown amorphous solid, 79%; IR (KBr) ν_{max} 2932, 1762, 1640, 1610, 1466, 1308, 1230, 1174, 1084, 848 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 242 (4.16) nm; ^{1}H NMR (CD_3OD) δ 7.49 (1H, d, J = 6.3 Hz, H-2), 6.25 (1H, d, J = 6.3 Hz, H-3), 7.39 (1H, d, J = 6.5 Hz, H-5), 6.86 (1H, d, J = 6.5 Hz, H-6), 6.96 (1H, s, H-7), 3.92 (3H, s, NCH_3), 3.82 (3H, s, OCH_3), 2.67 (2H, t, J = 7.1 Hz, H-2′), 1.74 (2H, m, H-3′), 1.48 (2H, H-4′), 1.00 (3H, t, J = 7.4 Hz, H-7′); ^{13}C NMR (CD_3OD) δ 148.1 (C-2), 101.6 (C-3), 151.4 (C-3a), 134.2 (C-5), 114.8 (C-6), 136.1 (C-6a), 101.7 (C-7), 158.5 (C-8), 137.8 (C-9), 125.2 (C-9a), 119.3 (C-9b), 45.6 (NCH_3), 57.4 (OCH_3), 173.8 (C-1′), 34.4 (C-2′), 27.8 (C-3′), 23.2 (C-4′), 0.14.0 (C-5′); EIMS *m*/*z* (rel int.) 312 (M^+, 8), 228 (100), 213 (20), 184 (18), 168 (7), 155 (8), 85 (13), 81 (14), 69 (40), 57 (27), 41 (24); HREIMS *m*/*z* 312.1471 (obsd), 312.1475 (calcd for $C_{18}H_{20}N_2O_3$); R_f value 0.30 (EtOAc/MeOH, 4:1).

9-*O*-Hexanoylisoaaptamine (6): pale brown amorphous solid, 76%; IR (KBr) ν_{max} 2924, 1768, 1644, 1614, 1464, 1274, 1118 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 242 (4.16) nm; ^{1}H NMR ($CDCl_3$) δ 7.57 (1H, d, J = 6.0 Hz, H-2), 6.63 (1H, d, J = 6.0 Hz, H-3), 7.17 (1H, d, J = 6.5 Hz, H-5), 6.72 (1H, d, J = 6.5 Hz, H-6), 7.26 (1H, s, H-7), 3.88 (3H, s, NCH_3), 3.76 (3H, s, OCH_3), 2.56 (2H, t, J = 7.7 Hz, H-2′), 1.75 (2H, m, H-3′), 1.30−1.41 (4H, overlap, H-4′-5′), 0.92 (3H, t, J = 7.2 Hz, H-6′); ^{13}C NMR ($CDCl_3$) δ 144.8 (C-2), 99.1 (C-3), 150.3 (C-3a), 134.8 (C-5), 113.4 (C-6), 135.4 (C-6a), 102.8 (C-7), 156.2 (C-8), 136.6 (C-9), 123.0 (C-9a), 118.7 (C-9b), 44.0 (NCH_3), 56.4 (OCH_3), 172.1 (C-1′), 34.0 (C-2′), 24.4 (C-3′), 31.2 (C-4′), 22.3 (C-5′), 14.0 (C-6′); EIMS *m*/*z* (rel int.) 326 (M^+, 7), 228 (100), 213 (18), 184 (16), 168 (6), 155 (6), 99 (6), 81 (2), 69 (4), 57 (8), 43 (24); HREIMS *m*/*z* 326.1630 (obsd), 326.1632 (calcd for $C_{19}H_{22}N_2O_3$); R_f value 0.35 (EtOAc/MeOH, 4:1).

9-*O*-Heptanoylisoaaptamine (7): pale brown amorphous solid, 94%; IR (KBr) ν_{max} 2936, 1760, 1636, 1612, 1460, 1304, 1284, 1110 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 242 (4.16) nm; ^{1}H NMR ($CDCl_3$) δ 7.57 (1H, d, J = 5.9 Hz, H-2), 6.54 (1H, d, J = 5.9 Hz, H-3), 7.13 (1H, d, J = 6.8 Hz, H-5), 6.72 (1H, d, J = 6.8 Hz, H-6), 6.62 (1H, s, H-7), 3.88 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 2.58 (2H, t, J = 7.4 Hz, H-2′), 1.74 (2H, m, H-3′), 1.32−1.43 (6H, overlap, H-4′-6′), 0.89 (3H, t, J = 6.9 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 144.6 (C-2), 98.9 (C-3), 150.5 (C-3a), 134.8 (C-5), 113.4 (C-6), 136.0 (C-6a), 103.0 (C-7), 156.1 (C-8), 136.6 (C-9), 123.0 (C-9a), 118.7 (C-9b), 44.5 (NCH_3), 56.3 (OCH_3), 172.1 (C-1′), 34.0 (C-2′), 24.6 (C-3′), 28.7 (C-4′), 31.3 (C-5′), 22.4 (C-6′), 14.0 (C-7′); EIMS *m*/*z* (rel int.) 340 (M^+, 6), 228 (100), 213 (17), 184 (15), 155 (6), 113 (5), 81 (1), 69 (8), 57 (6), 43 (22); HREIMS *m*/*z* 340.1789 (obsd), 340.1788 (calcd for $C_{20}H_{24}N_2O_3$); R_f value 0.20 (EtOAc/MeOH, 4:1).

9-*O*-Octanoylisoaaptamine (8): pale brown amorphous solid, 52%; IR (KBr) ν_{max} 2924, 1766, 1642, 1612 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 260 (4.21) nm; ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, d, J = 6.9 Hz, H-2), 6.32 (1H, d, J = 6.9 Hz, H-3), 6.78 (1H, d, J = 5.4 Hz, H-5), 6.76 (1H, d, J = 5.4 Hz, H-6), 6.53 (1H, s, H-7), 3.87 (3H, s, NCH_3), 3.65 (3H, s, OCH_3), 2.59 (2H, t, J = 7.4 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.42 (8H, overlap, H-4′-7′), 0.88 (3H, t, J = 7.5 Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ 142.4 (C-2), 97.4 (C-3), 152.2 (C-3a), 135.0 (C-5), 113.4 (C-6), 137.0 (C-6a), 105.9 (C-7), 155.3 (C-8), 141.0 (C-9), 122.1 (C-9a), 119.6 (C-9b), 44.0 (NCH_3), 56.1 (OCH_3), 172.3 (C-1′), 34.1 (C-2′), 24.8 (C-3′), 29.1 (C-4′), 29.0 (C-5′), 31.7 (C-6′), 22.6 (C-7′), 14.1 (C-8′); EIMS m/z (rel int.) 354 (M^+, 4), 228 (100), 213 (16), 184 (12), 155 (4), 125 (3), 81 (8), 55 (11); HREIMS m/z 354.1946 (obsd), 354.1945 (calcd for $C_{21}H_{26}N_2O_3$); R_f value 0.25 (EtOAc/MeOH, 4:1).

9-*O*-Nonanoylisoaaptamine (9): pale brown amorphous solid, 50%; IR (KBr) ν_{max} 1766, 1612, 1562, 1350, 1106, 784 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 260 (4.21) nm; ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, d, J = 7.1 Hz, H-2), 6.27 (1H, d, J = 7.1 Hz, H-3), 6.76 (1H, d, J = 6.5 Hz, H-5), 6.74 (1H, d, J = 6.5 Hz, H-6), 6.50 (1H, s, H-7), 3.86 (3H, s, NCH_3), 3.63 (3H, s, OCH_3), 2.58 (2H, t, J = 7.4 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.42 (12H, overlap, H-4′-9′), 0.90 (3H, t, J = 6.6 Hz, H-9′); ^{13}C NMR ($CDCl_3$) δ 142.1 (C-2), 97.1 (C-3), 152.6 (C-3a), 135.1 (C-5), 113.5 (C-6), 137.1 (C-6a), 106.4 (C-7), 155.2 (C-8), 142.0 (C-9), 122.0 (C-9a), 119.8 (C-9b), 43.9 (NCH_3), 56.0 (OCH_3), 172.5 (C-1′), 34.1 (C-2′), 24.7 (C-3′), 29.0−29.2 (overlap, C-4′-6′), 31.7 (C-7′), 22.6 (C-8′), 14.0 (C-9′); EIMS m/z (rel int.) 368 (M^+, 5), 228 (100), 213 (19), 170 (5), 141 (5), 71 (7), 57 (15), 43 (18) HREIMS m/z 368.2100 (obsd), 368.2101 (calcd for $C_{22}H_{28}N_2O_3$); R_f value 0.30 (EtOAc/MeOH, 4:1).

9-*O*-Decanoylisoaaptamine (10): pale brown amorphous solid, 84%; IR (KBr) ν_{max} 2928, 2856, 1764, 1640, 1610, 1594, 1466, 1310, 1176, 1104, 780, 760 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 261 (4.14) nm; ^{1}H NMR ($CDCl_3$) δ 7.62 (1H, d, J = 6.0 Hz, H-2), 6.52 (1H, d, J = 6.0 Hz, H-3), 7.10 (1H, d, J = 6.2 Hz, H-5), 6.74 (1H, d, J = 6.2 Hz, H-6), 6.61 (1H, s, H-7), 3.89 (3H, s, NCH_3), 3.74 (3H, s, OCH_3), 2.60 (2H, t, J = 6.8 Hz, H-2′), 1.76 (2H, m, H-3′), 1.28−1.43 (12H, overlap, H-4′-9′), 0.90 (3H, t, J = 5.7 Hz, H-10′); ^{13}C NMR ($CDCl_3$) δ 144.3 (C-2), 98.6 (C-3), 150.8 (C-3a), 134.8 (C-5), 113.4 (C-6), 136.7 (C-6a), 103.4 (C-7), 156.0 (C-8), 136.8 (C-9), 122.9 (C-9a), 118.8 (C-9b), 44.4 (NCH_3), 56.2 (OCH_3), 172.3 (C-1′), 34.0 (C-2′), 24.6 (C-3′), 29.0−29.4 (overlap, C-4′-7′), 31.7 (C-8′), 22.5 (C-9′), 14.0 (C-10′); EIMS m/z (rel int.) 382 (M^+, 6), 228 (100), 213 (14), 184 (11), 155 (5), 83 (3), 71 (5), 69 (5), 64 (6), 57 (11), 55 (12); HREIMS m/z 382.2255 (obsd), 382.2258 (calcd for $C_{23}H_{30}N_2O_3$); R_f value 0.35 (EtOAc/MeOH, 4:1).

9-*O*-laurylisoaaptamine (11): pale brown amorphous solid, 67%; IR (KBr) ν_{max} 2924, 2856, 1760, 1608, 1576, 1460, 1348, 1174, 1108, 836 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 260 (4.18) nm; ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, d, J = 6.5 Hz, H-2), 6.52 (1H, d, J = 6.5 Hz, H-3), 6.92 (1H, d, J = 6.6 Hz, H-5), 6.77 (1H, d, J = 6.6 Hz, H-6), 6.59 (1H, s, H-7), 3.89 (3H, s, NCH_3), 3.70 (3H, s, OCH_3), 2.59 (2H, t, J = 7.4 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.43 (16H, overlap, H-4′-15′), 0.88 (3H, t, J = 6.9 Hz, H-12′); ^{13}C NMR ($CDCl_3$) δ 143.5 (C-2), 98.2 (C-3), 151.5 (C-3a), 135.0 (C-5), 113.5 (C-6), 137.0 (C-6a), 104.6 (C-7), 155.8 (C-8), 138.6 (C-9), 122.7 (C-9a), 119.3 (C-9b), 44.3 (NCH_3), 56.2 (OCH_3), 172.3 (C-1′), 34.0 (C-2′), 24.7 (C-3′), 29.1−29.6 (overlap, C-4′-9′), 31.8 (C-10′), 22.6 (C-11′), 14.0 (C-12′); EIMS m/z (rel int.) 410 (M^+, 4), 228 (100), 213 (11), 184 (10), 155 (5), 125 (13), 111 (22), 97 (33), 95 (23), 83 (26), 57 (12); HREIMS m/z 410.2568 (obsd), 410.2571 (calcd for $C_{25}H_{34}N_2O_3$); R_f value 0.40 (EtOAc/MeOH, 4:1).

9-*O*-Myristylisoaaptamine (12): pale brown amorphous solid, 67%; IR (KBr) ν_{max} 2920, 2852, 1764, 1608, 1464, 1354, 1110, 834 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 261 (4.14) nm; ^{1}H NMR ($CDCl_3$) δ 7.62 (1H, d, J = 6 Hz, H-2), 6.70 (1H, d, J = 6 Hz, H-3), 7.20 (1H, d, J = 5 Hz, H-5), 6.78 (1H, d, J = 5 Hz, H-6), 6.70 (1H, s, H-7), 3.92 (3H, s, NCH_3), 3.81 (3H, s, OCH_3), 2.61 (2H, t, J = 7.5 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.43 (20H, overlap, H-4′-13′), 0.88 (3H, t, J = 6.3 Hz, H-14′); ^{13}C NMR ($CDCl_3$) δ 145.3 (C-2), 99.6 (C-3), 152.1 (C-3a), 134.8 (C-5), 113.5 (C-6), 136.8 (C-6a), 102.5 (C-7), 155.8 (C-8), 138.4 (C-9), 122.8 (C-9a), 118.2 (C-9b), 44.8 (NCH_3), 56.5 (OCH_3), 172.2 (C-1′), 34.0 (C-2′), 24.7 (C-3′), 29.1−29.6 (overlap, C-4′-11′), 31.9 (C-12′), 22.6 (C-13′), 14.0 (C-14′); EIMS m/z (rel int.) 438 (M^+, 3), 228 (100), 213 (9), 212 (5), 211 (5), 184 (7), 83 (3), 57 (13), 43 (20) HREIMS m/z 438.2882 (obsd), 438.2884 (calcd for $C_{27}H_{38}N_2O_3$); R_f value 0.30 (EtOAc/MeOH, 4:1).

9-*O*-Palmitylisoaaptamine (13): pale brown amorphous solid, 68%; IR (KBr) ν_{max} 2920, 2852, 1760, 1630, 1616, 1465, 1360, 1110, 840 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 261 (4.17) nm; ^{1}H NMR ($CDCl_3$) δ 7.72 (1H, d, J = 6.3 Hz, H-2), 6.69 (1H, d, J = 6.3 Hz, H-3), 6.97 (1H, d, J = 6.5 Hz, H-5), 6.77 (1H, d, J = 6.5 Hz, H-6), 6.62 (1H, s, H-7), 3.90 (3H, s, NCH_3), 3.73 (3H, s, OCH_3), 2.60 (2H, t, J = 7.1 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.43 (24H, overlap, H-4′-15′), 0.88 (3H, t, J = 6.3 Hz, H-16′); ^{13}C NMR ($CDCl_3$) δ 143.6 (C-2), 98.3 (C-3), 151.4 (C-3a), 135.0 (C-5), 113.6 (C-6), 137.0 (C-6a), 104.5 (C-7), 155.9 (C-8), 138.2 (C-9), 122.8 (C-9a), 119.2 (C-9b), 44.3 (NCH_3), 56.2 (OCH_3), 172.3 (C-1′), 34.1 (C-2′), 24.7 (C-3′), 29.1−29.6 (overlap, C-4′-13′), 31.9 (C-14′), 22.6 (C-15′), 14.0 (C-16′); EIMS m/z (rel int.) 466 (M^+, 2), 228 (100), 213 (9), 184 (7), 168 (3), 137 (4), 123 (3), 109 (5), 97 (11), 83 (12), 69 (16), 57 (26), 43 (40); HREIMS m/z 466.3195 (obsd), 466.3198 (calcd for $C_{29}H_{42}N_2O_3$); R_f value 0.35 (EtOAc/MeOH, 4:1).

9-*O*-Stearylisoaaptamine (14): pale brown amorphous solid, 65%; IR (KBr) ν_{max} 2920, 2852, 1760, 1636, 1614, 1468, 1384, 1174, 1136 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 260 (4.09) nm; ^{1}H NMR ($CDCl_3$) δ 7.68 (1H, d, J = 6.3 Hz, H-2), 6.69 (1H, d, J = 6.3 Hz, H-3), 7.04 (1H, d, J = 6.8 Hz, H-5), 6.77 (1H, d, J = 6.8 Hz, H-6), 6.64 (1H, s, H-7), 3.90 (3H, s, NCH_3), 3.75 (3H, s, OCH_3), 2.60 (2H, t, J = 7.4 Hz, H-2′), 1.77 (2H, m, H-3′), 1.25−1.43 (28H, overlap, H-4′-17′), 0.88 (3H, t, J = 6.6 Hz, H-18′); ^{13}C NMR ($CDCl_3$) δ 144.2 (C-2), 98.8 (C-3), 150.8 (C-3a), 135.0 (C-5), 113.5 (C-6), 136.9 (C-6a), 103.6 (C-7), 156.2 (C-8), 138.4 (C-9), 123.1 (C-9a), 119.3 (C-9b), 44.5 (NCH_3), 56.3 (OCH_3), 172.3 (C-1′), 34.0 (C-2′), 24.7 (C-3′), 29.1−29.6 (overlap, C-4′-15′), 31.9 (C-16′), 22.6 (C-17′), 14.0 (C-18′); EIMS m/z (rel int.) 494 (M^+, 2), 267 (1), 228 (100), 213 (8), 184 (6), 169 (4), 109 (5), 97 (7), 83 (8), 69 (11), 57 (22), 43 (34); HREIMS m/z 494.3505 (obsd), 494.3511 (calcd for $C_{31}H_{46}N_2O_3$); R_f value 0.40 (EtOAc/MeOH, 4:1).

Hydrogenation of Aaptamine (2). To a mixture of aaptamine (42 mg, **2**) and 22 mL of HOAc/35% HCl (10:1) in a hydrogenation apparatus was added PtO_2 (30 mg); the solution was reduced under hydrogen atmosphere, while the reaction mass was maintained at 80 °C for 24 h. The reaction mixture was filtrated and evaporated under vacuum to yield a residue. The residue was chromatographed on a silica gel column and eluted with solvent mixture of EtOAc/MeOH by the following ratios and volumes (30:1, 20:1, 10:1, 5:1, and 3:1; each 50 mL), to give dihydroaaptamine (25 mg, **15**).

Dihydroaaptamine (15): pale yellow solid, 59%; IR (KBr) ν_{max} 3420, 1620, 1464, 1388, 1252, 1120, 1070, 868 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 238 (3.86) nm; ^{1}H NMR (CD_3OD) δ 8.05 (1H, d, J = 6.0 Hz, H-2), 6.58 (1H, d, J = 6.0 Hz, H-3), 3.67 (1H, dt, J = 6.9, 3.9 Hz, H-5), 3.21 (1H, t, J = 6.9 Hz, H-6), 7.25 (1H, s, H-7), 4.06 (3H, s, OCH_3-8), 3.98 (3H, s, OCH_3-9), 2.37 (3H, s, H-2′); ^{13}C NMR (CD_3OD) δ 142.8 (C-2), 100.5 (C-3), 156.2 (C-3a), 41.1 (C-5), 27.6 (C-6), 134.0 (C-6a), 112.1 (C-7), 157.0 (C-8), 131.7 (C-9), 135.6 (C-9a), 110.2 (C-9b), 61.8 (OCH_3-8), 57.2 (OCH_3-9); EIMS m/z (rel int.) 230 (M^+, 28), 229 (58), 227 (4), 215 (75), 213 (23), 201 (34), 199 (37), 184 (21), 178 (18), 170 (29), 169 (26), 129 (21), 107 (26), 84 (30), 69 (45), 61 (56), 57 (38), 55 (35), 43 (100); HREIMS m/z 230.1066 (obsd), 230.1056 (calcd for $C_{15}H_{14}N_2O_3$); R_f value 0.40 (EtOAc/MeOH, 4:1).

Preparation of Compounds 16−19. To a solution of dihydroaaptamine (5 mg, **15**) in pyridine was added an appropriate anhydride at room-temperature overnight. The reaction mixture was treated as in the preparation of compounds **3**−**14** mentioned above. Further purification by preparative TLC plate (silica gel, 1 mm thickness) using EtOAc/MeOH (10:1) as developing solvent yielded compounds **16**−**19** with a yield which varied in a range of 31−59%.

4-*N*-Acetyldihydroaaptamine (16): pale yellow amorphous solid, 59%; IR (KBr) ν_{max} 2936, 1762, 1610, 1410, 1300, 1222, 1118, 1022, 862 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 244 (4.07) nm; ^{1}H NMR ($CDCl_3$) δ 8.70 (1H, d, J = 5.0 Hz, H-2), 7.63 (1H, d, J = 5.0 Hz, H-3), 4.13 (1H, d, J = 5.7 Hz, H-5), 3.24 (1H, d, J = 5.7 Hz, H-6), 7.34 (1H, s, H-7), 4.03 (3H, s, OCH_3-8), 3.96 (3H, s, OCH_3-9), 2.43 (3H, s, H-2′); ^{13}C NMR ($CDCl_3$) δ 150.6 (C-2), 111.2 (C-3), 144.3 (C-3a), 43.3 (C-5), 29.7 (C-6), 128.2 (C-6a), 113.1 (C-7), 151.6 (C-8), 141.7 (C-9), 115.4 (C-9b), 61.9 (OCH_3-8), 56.9 (OCH_3-9), 169.8 (C-1′), 23.9 (C-2′); EIMS *m*/*z* (rel int.) 272 (M^+, 9), 271 (9), 257 (15), 243 (6), 229 (15), 215 (15), 213 (8), 201 (8), 199 (11), 178 (10), 170 (13), 169 (10), 149 (13), 95 (12), 81 (35), 69 (97), 43 (100); HREIMS *m*/*z* 272.1141 (obsd), 272.1162 (calcd for $C_{15}H_{16}N_2O_3$); R_f value 0.50 (EtOAc/MeOH, 4:1).

4-*N*-Hexanoyldihydroaaptamine (17): pale yellow amorphous solid, 46%; IR (KBr) ν_{max} 2940, 2856, 1686, 1608, 1406, 1300, 1204, 1124, 816 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 245 (4.24) nm; ^{1}H NMR ($CDCl_3$) δ 8.68 (1H, d, J = 6 Hz, H-2), 8.09 (1H, d, J = 6 Hz, H-3), 4.21 (1H, d, J = 6 Hz, H-5), 3.30 (1H, d, J = 6 Hz, H-6), 7.49 (1H, s, H-7), 4.09 (3H, s, OCH_3-8), 4.04 (3H, s, OCH_3-9), 2.82 (2H, t, J = 7.4 Hz, H-2′), 1.76 (2H, m, H-3′), 1.29−1.40 (4H, overlap, H-4′-5′), 0.93 (3H, t, J = 7.1 Hz, H-6′); ^{13}C NMR ($CDCl_3$) δ 150.6 (C-2), 110.4 (C-3), 144.7 (C-3a), 43.6 (C-5), 29.7 (C-6), 128.5 (C-6a), 113.7 (C-7), 151.5 (C-8), 141.0 (C-9), 114.4 (C-9b), 62.0 (OCH_3-8), 56.9 (OCH_3-9), 173.5 (C-1′), 36.0 (C-2′), 25.1 (C-3′), 31.4 (C-4′), 22.4 (C-5′), 13.9 (C-6′); EIMS *m*/*z* (rel int.) 328 (M^+, 18), 231 (13), 230 (78), 216 (15), 215 (100), 200 (11), 169 (11), 71 (15), 55 (20), 43 (72); HREIMS *m*/*z* 328.1764 (obsd), 328.1788 (calcd for $C_{19}H_{24}N_2O_3$); R_f value 0.60 (EtOAc/MeOH, 4:1).

4-*N*-Lauryldihydroaaptamine (18): pale yellow amorphous solid, 42%; IR (KBr) ν_{max} 2920, 1632, 1410, 1304, 1188, 1094, 854 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 246 (4.18) nm; ^{1}H NMR ($CDCl_3$) δ 8.83 (1H, d, J = 5 Hz, H-2), 7.29 (1H, d, J = 5 Hz, H-3), 4.16 (1H, d, J = 5.7 Hz, H-5), 3.17 (1H, d, J = 5.7 Hz, H-6), 7.10 (1H, s, H-7), 4.09 (3H, s, OCH_3-8), 4.03 (3H, s, OCH_3-9), 2.66 (2H, t, J = 7.5 Hz, H-2′), 1.67−1.71 (2H, m, H-3′), 1.24−1.29 (16H, overlap, H-4′-15′), 0.88 (3H, t, J = 6.8 Hz, H-12′); ^{13}C NMR ($CDCl_3$) δ 150.5 (C-2), 113.3 (C-3), 144.3 (C-3a), 43.4 (C-5), 29.7 (C-6), 128.2 (C-6a), 113.1 (C-7), 151.5 (C-8), 141.1 (C-9), 115.4 (C-9b), 61.9 (OCH_3-8), 56.9 (OCH_3-9), 173.0 (C-1′), 35.3 (C-2′), 25.8 (C-3′), 29.3−30.3 (overlap, C-4′-9′), 31.9 (C-10′), 22.7 (C-11′), 14.1 (C-12′); EIMS *m*/*z* (rel int.) 412 (M^+, 13), 411 (14), 397 (18), 229 (28), 215 (51), 213 (13), 201 (18), 199 (18), 183 (12), 169 (10), 71 (12), 69 (23), 57 (46), 55 (45), 45 (12), 43 (100); HREIMS *m*/*z* 412.2705 (obsd), 412.2728 (calcd for $C_{25}H_{36}N_2O_3$); R_f value 0.40 (EtOAc/MeOH, 4:1).

4-*N*-Stearyldihydroaaptamine (19): pale yellow amorphous solid, 31%; IR (KBr) ν_{max} 2920, 2852, 1664, 1412, 1304, 1252, 1114, 864 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 245 (4.46) nm; ^{1}H NMR ($CDCl_3$) δ 8.84 (1H, d, J = 5.1 Hz, H-2), 7.26 (1H, d, J = 5.1 Hz, H-3), 4.16 (1H, d, J = 5.9 Hz, H-5), 3.17 (1H, d, J = 5.9 Hz, H-6), 7.10 (1H, s, H-7), 4.10 (3H, s, OCH_3-8), 4.03 (3H, s, OCH_3-9), 2.66 (2H, t, J = 7.7 Hz, H-2′), 1.50−1.80 (2H, m, H-3′), 1.25−1.34 (28H, overlap, H-4′-17′), 0.88 (3H, t, J = 6.6 Hz, H-18′); ^{13}C NMR ($CDCl_3$) δ 150.5 (C-2), 113.3 (C-3), 144.3 (C-3a), 43.4 (C-5), 29.7 (C-6), 128.3 (C-6a), 113.1 (C-7), 151.5 (C-8), 141.4 (C-9), 115.5 (C-9b), 61.9 (OCH_3-8), 56.9 (OCH_3-9), 173.0 (C-1′), 35.3 (C-2′), 25.7 (C-3′), 29.2−30.1 (overlap, C-4′-15′), 31.9 (C-16′), 22.6 (C-17′), 14.1 (C-18′); EIMS *m*/*z* (rel int.) 496 (M^+, 7), 495 (8), 481 (8), 469 (8), 229 (15), 215 (25), 199 (9), 149 (7), 97 (17), 95 (14), 71 (30), 69 (52), 57 (63), 55 (62), 43 (100); HREIMS *m*/*z* 496.3646 (obsd), 496.3667 (calcd for $C_{31}H_{48}O_3N_2$); R_f value 0.65 (EtOAc/MeOH, 4:1).

Biological Assay. The cytotoxic activities of compounds against P-388, KB16, A549, and HT-29 cells were assayed by the MTT{3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} colorimetric assay with some modifications. [6,7] Each cell line was plated at 1000 cells/well in 96-well microtiter plates. Two-fold serial dilutions of tested compounds were added to the cells and P-388, KB16, A549, and HT-29 cells were enumerated using MTT after 3, 3, 6, and 6 days, respectively. MTT (50 μL, 1 mg/mL) was added to each well, and the plates were incubated at 37° C for 5 h. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and plates were then immediately read on a microtiter plate reader (Dynatech) at 540 nm. The ID_{50} was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. Results are given in Table 1.

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