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Synthesis and Antibacterial Study of Aaptamine Derivatives

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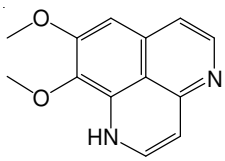
Aaptamine is a bioactive marine alkaloid containing a unique of 1*H*-benzo[de][1,6]naphthyridine skeleton. Aaptamine was isolated from marine sponge, *Aaptos aaptos* and was proposed as starting material for semi-synthetic modifications. A series of aaptamine derivatives were synthesized which consisted of 1,4-dialkyl (**2-7**), 4-alkyl (**8-10**) and 9-*O*-butyryl-1,4-dialkyl (**11-16**) aaptamine derivatives. Each derivative was characterized by FT-IR, UV-visible, NMR and MS. The synthesized compounds were evaluated for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus* sp. bacterial strains using the disc-diffusion method. Some of the derivatives showed potent antibacterial activity against certain bacterial strains.

Keywords: Aaptamine, Antibacterial, Disc-diffusion method.

INTRODUCTION

Marine sponges are one of the richest sources of potential secondary metabolites in the marine environment. Aaptamine (**1**) with a 1*H*-benzo[de][1,6]naphthyridine skeleton[1] is one of the potential secondary metabolites marine alkaloid which have remarkable biological properties.



Structure of aaptamine

Aaptamine (**1**) was initially isolated by Nakamura *et al.*[2] from the marine sponge *Aaptos aaptos*, collected off the shores of Okinawa. There are few of aaptamine-type alkaloids that have been isolated from *Aaptos aaptos* included isoaaptamine[3], 4-methylaaptamine[4], bisdimethylaaptamine[5], bisdimethylaaptamine-9-*O*-sulfate[5], aaptosine[6], 3-(phenethylamino) demethyl(oxy)aaptamine[7] and 3-(isopentylamino)demethyl (oxy)aaptamine[7]. Aaptamine and its congeners exhibit a broad spectrum of biological activities such as α-adrenoceptor inhibitor[2], anti-HSV-1[4], antineoplastic[8,9], antimicrobial[3,10] and cytotoxicity against cancer cell lines[11]. Aaptamine itself has good potential as a lead compound from which various derivatives can be synthesized. This prompted us to further explore the chemistry of this group of compound. Consequently, some new 1,4-dialkyl (**2-7**), 4-alkyl (**8-10**) and 9-*O*-butyryl-1,4-dialkyl (**11-16**) aaptamine derivatives have been synthesized in our laboratory and their antibacterial activity are reported herein.

EXPERIMENTAL

All reagents were obtained from Sigma-Aldrich Co., Merck Chemical Co. as well as Acros Organics Co. and were used without additional purification. The reactions were monitored by thin layer chromatography (TLC) using plastic precoated sheets (Silica gel 60 F254, 0.25 mm thick). Plates were visualized under UV 365 nm and UV 254 nm without treatment. Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). Melting points were determined using Stuart-Scientific SMP3 apparatus and are uncorrected. Infrared spectra were recorded on Perkin Elmer 100 FT-IR Spectrometer. UV-visible spectra were recorded on Shimadzu UV-1601PC Spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on either Bruker FT-400 (400 MHz) or Jeol (500 MHz) Spectrometer, using CD_3OD as the solvent.

Isolation of aaptamine: The marine sponges, *Aaptos aaptos* (934 g) were collected *via* scuba diving at a depth of 5 to 10 meters from the islands in Terengganu. The sponges were freeze-dried and later ground into powder. The powdered samples (245.20 g) were extracted with methanol followed by solvent evaporation by rotary evaporator. Aaptamine (**1**)

was isolated from methanolic extract (20.56 g) by column chromatography (silica gel, $CHCl_3$:CH_3OH 8:2)[2] to yield large amounts of aaptamine (2.18 g, 10.62 %).

Preparation of compounds 2-10[8,9]: Aaptamine (1.09 mmol) was dissolved in anhydrous *N,N*-dimethylformamide (25 mL). To the solution, potassium carbonate (5.47 mmol) and alkyl halide (5.47 mmol) were added at room temperature. The reaction was allowed to stir for 48 h at the same temperature. The solution was filtered and the solvent was evaporated under reduced pressure to give various colour of residue. The residue was checked by TLC and purified by column chromatography (silica gel, $CHCl_3$:CH_3OH) to afford 1,4-dialkylaaptamine (**2-7**) together with by-products 4-alkylaaptamine (**8-10**).

Preparation of compounds 11-16[12,13]: 1,4-Dialkylaaptamine (0.20 mmol) was dissolved in 48 % HBr (2-3 mL) and heated at 115-120 °C (preheated oil bath) for approximately 3 h with stirring. The reaction was quenched with 1-2 mL water and extracted with $CHCl_3$. The organic layer was washed with brine solution and dried over Na_2SO_4. The residue after evaporation was purified by column chromatography (silica gel, $CHCl_3$:CH_3OH) to afford 9-*O*-demethyl-1,4-dialkylaaptamines.

9-*O*-Demethyl-1,4-dialkylaaptamines (0.18 mmol) was dissolved in dry dichloromethane (0.5 mL) at 0 °C. Triethylamine (0.1 mL) was added and the mixture was stirred for 0.5 min. An excess of butyryl chloride was added dropwise to the mixture over a period of 15 min. The reaction was stirred for an additional 0.5 min at 0 °C and slowly warmed to room temperature. Then, the reaction was continued to stir for 24 h. The residue was checked by TLC and fractionated by column chromatography (silica gel, $CHCl_3$:CH_3OH) to give 9-*O*-demethyl-1,4-dialkylaaptamine (**11-16**).

1,4-Diethylaaptamine (2): Yield: 68 %, bright yellow solid. m.p. 208-213 °C (dec); IR (KBr, ν_{max}, cm^{-1}) 2981, 2931, 1645, 1568, 1371, 1307, 1092; UV (MeOH) λ_{max} (log ε) 221 (3.98), 243 (3.85), 262 (3.86), 272 (3.84), 281 (3.82), 306 (3.08), 317 (3.08), 411 (3.34) nm; ^{1}H NMR (CD_3OD): 7.98 (1H, d, *J* 7.6 Hz, H-2), 7.45 (1H, d, *J* 7.2 Hz, H-5), 7.25 (1H, s, H-7), 7.04 (1H, d, *J* 7.2 Hz, H-6), 6.60 (1H, d, *J* 7.6 Hz, H-3), 4.55 (2H, q, *J* 7.2 Hz, H-1'), 4.16 (2H, q, *J* 7.2 Hz, H-1"), 4.09 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 1.44 (6H, t, *J* 7.2 Hz, H-2', 2"); ^{13}C NMR (CD_3OD): 58.8, 148.8, 148.2, 134.4, 133.3, 133.1, 133, 119, 114.5, 102.4, 97.0, 61.2, 55.9, 53.2, 48.6, 14.6, 12; EIMS *m/z* $[M - HI]^+$ 284.20 ($C_{17}H_{21}N_2O_2$, 285.16).

1,4-Dipropylaaptamine (3): Yield: 61 %, yello wish green solid. m.p. 216-220 °C (dec); IR (KBr, ν_{max}, cm^{-1}) 2965, 2929, 1648, 1567, 1366, 1301, 1080; UV (MeOH) λ_{max} (log ε) 222 (4.27), 243 (4.13), 263 (4.12), 272 (4.10), 282 (4.08), 318 (3.37), 413 (3.67) nm; ^{1}H NMR (CD_3OD): 7.92 (1H, d, *J* 7.5 Hz, H-2), 7.40 (1H, d, *J* 7.5 Hz, H-5), 7.21 (1H, s, H-7), 6.98 (1H, d, *J* 6.9 Hz, H-6), 6.52 (1H, d, *J* 8.1 Hz, H-3), 4.40 (2H, t, *J* 7.5 Hz, H-1'), 4.03 (5H, t, *J* 7.5 Hz, H-1", OCH_3), 3.86 (3H, s, OCH_3), 1.82-1.80 (4H, m, H-2', 2"), 1.02 (3H, t, *J* 7.5 Hz, H-3"), 0.95 (3H, t, *J* 7.5 Hz, H-3'); ^{13}C NMR (CD_3OD): 158.9, 149.1, 148.6, 134.5, 134, 133.2, 133.1, 119.1, 114.2, 102.5, 96.7, 61.2, 59.5, 55.8, 54.8, 23, 20.7, 9.6, 9.5; EIMS *m/z* $[M - HI]^+$ 312.25 ($C_{19}H_{25}N_2O_2$, 313.19).

1,4-Dihexylaaptamine (4): Yield: 36 %, bright yellow solid. m.p. 236-238 °C (dec); IR (KBr, ν_{max}, cm^{-1}) 2919, 2850, 1649, 1467, 1369, 1308, 1078; UV (MeOH) λ_{max} (log ε) 225 (4.49), 242 (4.44), 262 (4.42), 271 (4.40), 281 (4.39), 309 (3.70), 318 (3.72), 360 (3.80), 412 (3.98) nm; ^{1}H NMR (CD_3OD): 7.93 (1H, d, *J* 7.5 Hz, H-2), 7.40 (1H, d, *J* 6.9 Hz, H-5), 7.21 (1H, s, H-7), 6.99 (1H, d, *J* 7.5 Hz, H-6), 6.51 (1H, d, *J* 7.5 Hz, H-3), 4.43 (2H, t, *J* 7.5 Hz, H-1'), 4.06 (5H, t, *J* 7.5 Hz, H-1", OCH_3), 3.85 (3H, s, OCH_3), 1.77 (4H, quint, *J* 7.5 Hz, H-2', 2"), 1.43-1.26 (12H, m, H-3'-5', 3"-5"), 0.89-0.87 (6H, m, H-6', 6"); ^{13}C NMR (CD_3OD): 158.9, 149, 148.6, 134.6, 134, 133.2, 133.1, 119, 114.3, 102.5, 96.7, 61.2, 58.1, 55.9, 53.5, 31.2, 31.2, 29.7, 27.4, 25.7, 22.3, 13; EIMS *m/z* $[M - HI]^+$ 396 ($C_{25}H_{37}N_2O_2$, 397.28).

1,4-Dinonylaaptamine (5): Yield: 33 %, yellowish green solid. *m.p*. 246-248°C (dec); IR (KBr, ν_{max}, cm^{-1}) 2928, 2855, 1631, 1588, 1351, 1308, 1098; UV (MeOH) λ_{max} (log ε) 208 (4.04), 221 (4.05), 242 (3.91), 262 (3.89), 271 (3.86), 281 (3.83), 308 (3.26), 318 (3.27), 411 (3.38) nm; ^{1}H NMR (CD_3OD): 7.96 (1H, d, *J* 7.6 Hz, H-2), 7.44 (1H, d, *J* 7.2 Hz, H-5), 7.25 (1H, s, H-7), 7.02 (1H, d, *J* 7.2 Hz, H-6), 6.55 (1H, d, *J* 8 Hz, H-3), 4.48 (2H, t, *J* 7.2 Hz, H-1'), 4.09 (5H, t, *J* 6 Hz, H-1", OCH_3), 3.89 (3H, s, OCH_3), 1.82 (4H, quint, *J* 9.2 Hz, H-2', 2"), 1.49-1.28 (24H, m, H-3'-8', 3"-8"), 0.91 (6H, t, *J* 6.4 Hz, H-9', 9"); ^{13}C NMR (CD_3OD): 158.9, 149, 148.5, 135, 133.9, 133.2, 133.1, 119, 114.2, 102.5, 96.7, 61.1, 58, 55.8, 53.4, 31.6, 29.6, 29.1, 29.1, 28.9, 28.9, 28.8, 28.8, 27.3, 25.9, 25.9, 22.3, 13; EIMS *m/z* $[M - HI]^+$ 480.15 ($C_{31}H_{49}N_2O_2$, 481.38).

1,4-Didodecylaaptamine (6)[12]: Yield: 21 %, bright yellow solid *m.p*. 260-262 °C (dec); IR (KBr, ν_{max}, cm^{-1}) 2923, 2852, 1589, 1463, 1352, 1306, 1160; UV (MeOH) λ_{max} (log ε) 222 (4.42), 243 (4.31), 262 (4.30), 271 (4.28), 281 (4.26), 317 (3.57), 413 (3.85) nm; ^{1}H NMR (CD_3OD): 7.99 (1H, d, *J* 7.6 Hz, H-2), 7.45 (1H, d, *J* 7.2 Hz, H-5), 7.26 (1H, s, H-7), 7.03 (1H, d, *J* 7.2 Hz, H-6), 6.57 (1H, d, *J* 7.6 Hz, H-3), 4.48 (2H, t, *J* 7.2 Hz, H-1'), 4.11 (5H, t, *J* 7.2 Hz, H-1", OCH_3), 3.89 (3H, s, OCH_3), 1.82-1.80 (4H, m, H-2', 2"), 1.35-1.29 (36H, m, H-3'-11', 3"-11"), 0.91 (4H, t, *J* 6 Hz, H-12', 12"); ^{13}C NMR (CD_3OD): 158.9, 148.9, 148.6, 134.6, 134, 133.2, 133.1, 119, 114.2, 102.5, 96.8, 61.2, 58, 55.9, 53.4, 31.7, 29.6, 29.3, 29.2, 29.2, 29.1, 29.1, 28.9, 28.8, 27.3, 25.9, 25.9, 22.3, 13.1; EIMS *m/z* $[M - I]^+$ 565.80 ($C_{37}H_{61}N_2O_2$, 565.47).

1,4-Dihexadecylaaptamine (7): Yield: 19 %, bright yellow solid. m.p. 288-290 °C (dec); IR (KBr, ν_{max}, cm^{-1}) 2919, 2851, 1651, 1466, 1382, 1323, 1094; UV (MeOH) λ_{max} (log ε) 211 (4.14), 222 (4.19), 243 (4.05), 262 (4.04), 271 (4.02), 281 (4.01), 308 (3.28), 317 (3.30), 412 (3.59) nm; ^{1}H NMR (CD_3OD): 7.96 (1H, d, *J* 7.5 Hz, H-2), 7.44 (1H, d, *J* 6.9 Hz, H-5), 7.25 (1H, s, H-7), 7.02 (1H, d, *J* 7.5 Hz, H-6), 6.55 (1H, d, *J* 7.5 Hz, H-3), 4.48 (2H, t, *J* 7.2 Hz, H-1'), 4.11 (5H, t, *J* 7.2 Hz, H-1", OCH_3), 3.89 (3H, s, OCH_3), 1.82 (4H, m, H-2', 2"), 1.36-1.29 (52H, m, H-3'-15', 3"-15"), 0.91 (6H, m, H-16', 16"); ^{13}C NMR (CD_3OD): 158.9, 148.9, 148.6, 134.6, 134, 133.1, 129.5, 119.1, 114.2, 102.5, 96.7, 61.1, 58.0, 55.8, 53.5, 31.7, 29.5, 29.4, 29.3, 29.2, 29.2, 29.1, 29.1, 29.1, 28.9, 28.8, 27.3, 25.9, 25.8, 22.3, 13; EIMS *m/z* $[M - HI]^+$ 676.95 ($C_{45}H_{77}N_2O_2$, 677.60).

4-Propylaaptamine (8): Yield: 8 %, dark yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2973, 2932, 1655, 1603, 1324, 1248, 1091;

^{1}H NMR (CD_3OD): 7.91 (1H, d, *J* 7.6 Hz, H-2), 7.36 (1H, d, *J* 7.6 Hz, H-5), 7.09 (1H, s, H-7), 6.93 (1H, d, *J* 7.2 Hz, H-6), 6.54 (1H, d, *J* 7.2 Hz, H-3), 4.05 (5H, q, *J* 7.6 Hz, OCH_3, H-1'), 3.95 (3H, s, OCH_3), 1.90-1.81 (2H, m, H-2'), 1.07 (3H, t, J 7.6 Hz, H-3'); ^{13}C NMR (CD_3OD): 156.8, 149.8, 141.8, 134.1, 133.4, 132.2, 131.5, 117.2, 113.8, 101.2, 96.3, 59.9, 55.8, 54.5, 20.7, 9.6.

4-Hexylaaptamine (9): Yield: 9 %, dark yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2930, 2857, 1650, 1602, 1320, 1249, 1091; ^{1}H NMR (CD_3OD): 7.92 (1H, d, *J* 7.2 Hz, H-2), 7.36 (1H, d, *J* 7.2 Hz, H-5), 7.13 (1H, s, H-7), 6.96 (1H, d, *J* 7.6 Hz, H-6), 6.53 (1H, d, *J* 7.2 Hz, H-3), 4.07 (5H, q, *J* 8 Hz, OCH_3, H-1'), 3.97 (3H, s, OCH_3), 1.82 (2H, quint, *J* 8 Hz, H-2'), 1.49-1.35 (6H, m, H-3'-5'), 0.94 (3H, t, *J* 7.2 Hz, H-6'); ^{13}C NMR (CD3OD): 157.0, 150, 141.8, 134, 133.5, 132.4, 131.6, 117.4, 113.8, 101.2, 96.2, 59.9, 55.7, 53.1, 31.1, 27.3, 25.7, 22.2, 12.9.

4-Nonylaaptamine (10): Yield: 10 %, dark green solid: IR (KBr, ν_{max}, cm^{-1}) 2919, 2851, 1651, 1599, 1323, 1228, 1096; ^{1}H NMR (CD_3OD): 7.92 (1H, d, *J* 7.2 Hz, H-2), 7.36 (1H, d, *J* 7.6 Hz, H-5), 7.13 (1H, s, H-7), 6.96 (1H, d, *J* 7.6 Hz, H-6), 6.53 (1H, d, *J* 7.2 Hz, H-3), 4.07 (5H, q, *J* 8 Hz, OCH_3, H-1'), 3.97 (3H, s, OCH_3), 1.82 (2H, quint, *J* 7.6 Hz, H-2'), 1.48-1.30 (12H, m, H-3'-8'), 0.90 (3H, t, *J* 7.2 Hz, H-9'); ^{13}C NMR (CD_3OD): 157, 149.9, 141.8, 134.0, 133.5, 132.4, 131.6, 117.4, 113.8, 101.2, 96.2, 59.9, 55.7, 53.1, 31.6, 29.1, 28.9, 28.9, 27.3, 25.9, 22.3, 13.

9-*O*-butyryl-1,4-diethylaaptamine (11): Yield: 88 %, bright yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2968, 2875, 1771, 1594, 1351, 1125, 1073; UV (MeOH) λ_{max} (log ε) 205 (4.23), 223 (4.22), 240 (4.20), 268 (4.26), 278 (4.23), 305 (3.48), 315 (3.51), 403 (3.86) nm; ^{1}H NMR (CD_3OD): 8.01 (1H, d, *J* 7.6 Hz, H-2), 7.56 (1H, d, *J* 7.2 Hz, H-5), 7.27 (1H, s, H-7), 7.12 (1H, d, *J* 7.2 Hz, H-6), 6.69 (1H, d, *J* 8 Hz, H-3), 4.43 (2H, q, *J* 6.8 Hz, H-1'), 4.20 (2H, q, *J* 7.2 Hz, H-1"), 4.02 (3H, s, OCH_3), 2.73 (2H, t, *J* 7.2 Hz, CH_2), 1.88-1.79 (2H, m, CH_2), 1.50-1.43 (4H, m, H-2', 2"), 1.11 (3H, t, *J* 7.2 Hz, CH_3); ^{13}C NMR (CD_3OD): 171.3, 157.1, 148.6, 147.8, 135.2, 134.2, 133.2, 124.5, 118.7, 114.6, 101.7, 97.4, 56.0, 52.6, 48.8, 35.2, 17.8, 14.5, 12.5, 11.9; EIMS *m/z* $[M - HI]^+$ 340.25 ($C_{20}H_{25}N_2O_3$, 341.19).

9-*O*-butyryl-1,4-dipropylaaptamine (12): Yield: 60 %, bright yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2877, 2812, 1772, 1595, 1351, 1124, 1086; UV (MeOH) λ_{max} (log ε) 208 (3.71), 223 (3.74), 239 (3.60), 263 (3.63), 269 (3.64), 279 (3.60), 316 (2.83), 390 (3.26), 404 (3.26) nm; ^{1}H NMR (CD_3OD): 7.99 (1H, d, *J* 7.6 Hz, H-2), 7.56 (1H, d, *J* 7.2 Hz, H-5), 7.28 (1H, s, H-7), 7.11 (1H, d, *J* 7.6 Hz, H-6), 6.67 (1H, d, *J* 8 Hz, H-3), 4.33 (2H, t, *J* 7.6 Hz, H-1'), 4.13 (2H, t, *J* 7.6 Hz, H-1"), 4.01 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.6 Hz, CH_2), 1.92-1.78 (6H, m, H-2', 2", CH_2), 1.12-1.02 (9H, m, H-3', 3", CH_3); ^{13}C NMR (CD_3OD): 171.3, 157.1, 148.8, 148.2, 135.2, 134.8, 133.2, 124.6, 118.7, 114.2, 101.8, 97.2, 58.9, 56, 54.9, 35.2, 22.9, 20.6, 17.8, 12.5, 9.5, 9.5; EIMS *m/z* $[M - HI]^+$ 368.10 ($C_{22}H_{29}N_2O_3$, 369.27).

9-*O*-butyryl-1,4-dihexylaaptamine (13): Yield: 55 %, bright yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2814, 2727, 1751, 1594, 1351, 1160, 1072; UV (MeOH) λ_{max} (log ε) 205 (4.21), 223 (4.20), 269 (4.06), 279 (4.04), 315 (3.29), 402 (3.73) nm; ^{1}H NMR (CD_3OD): 7.99 (1H, d, *J* 7.2 Hz, H-2), 7.55 (1H, d, *J* 7.2 Hz, H-5), 7.27 (1H, s, H-7), 7.11 (1H, d, *J* 7.2 Hz, H-6), 6.64 (1H, d, *J* 7.2 Hz, H-3), 4.36 (2H, t, *J* 7.6 Hz, H-1'), 4.15 (2H, t, *J* 7.2 Hz, H-1"), 4.01 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.6 Hz, CH_2), 1.87-1.78 (6H, m, H-2', 2", CH_2), 1.48-1.28 (12H, m, H-3'- 5', 3"- 5"), 1.11 (3H, t, *J* 7.2 Hz, CH_3), 0.95-0.92 (6H, m, H-6', 6"); ^{13}C NMR (CD_3OD): 171.3, 157.1, 148.8, 148.2, 135.2, 134.7, 133.2, 124.7, 118.8, 114.3, 101.8, 97.2, 57.6, 56.0, 53.6, 35.2, 31.1, 31.1, 29.6, 27.3, 25.7, 25.6, 22.1, 17.8, 12.8, 12.5; EIMS *m/z* $[M - HI]^+$ 452.55 ($C_{28}H_{41}N_2O_3$, 453.31).

9-*O*-butyryl-1,4-dinonylaaptamine (14): Yield: 82 %, bright yellow solid. IR (KBr, ν_{max}, cm^{-1}) 2976, 2811, 1751, 1594, 1351, 1170, 1036 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (4.35), 223 (4.23), 240 (4.16), 269 (4.19), 279 (4.16), 305 (3.46), 316 (3.48), 404 (3.77) nm; ^{1}H NMR (CD_3OD): 7.98 (1H, d, *J* 7.6 Hz, H-2), 7.55 (1H, d, *J* 7.2 Hz, H-5), 7.28 (1H, s, H-7), 7.11 (1H, d, *J* 7.6 Hz, H-6), 6.65 (1H, d, *J* 7.6 Hz, H-3), 4.37 (2H, t, *J* 7.6 Hz, H-1'), 4.15 (2H, t, *J* 7.6 Hz, H-1"), 4.01 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.2 Hz, CH_2), 1.87-1.78 (6H, m, H-2', 2", CH_2), 1.42-1.28 (24H, m, H-3'- 8', 3"- 8"), 1.11 (3H, t, *J* 7.2 Hz, CH_3), 0.92-0.89 (6H, m, H-9', 9"); ^{13}C NMR (CD_3OD): 171.3, 157.1, 148.7, 148.2, 135.2, 134.8, 133.2, 124.7, 118.7, 114.3, 101.8, 97.2, 57.6, 56, 53.6, 35.2, 31.5, 31.5, 29.6, 29.1, 29, 28.9, 28.8, 28.8, 27.3, 26, 25.9, 22.2, 17.8, 13, 12.5, 7.8; EIMS *m/z* $[M - I]^+$ 537.10 ($C_{34}H_{53}N_2O_3$, 537.41).

9-*O*-butyryl-1,4-didodecylaaptamine (15): Yield: 33 %, bright yellow solid: IR (KBr, ν_{max}, cm^{-1}) 2925, 2852, 1761, 1594, 1351, 1130, 1092; UV (MeOH) λ_{max} (log ε) 206 (4.26), 224 (4.20), 240 (4.18), 269 (4.22), 279 (4.19), 315 (3.47), 404 (3.82) nm; ^{1}H NMR (CD_3OD): 7.98 (1H, d, *J* 8 Hz, H-2), 7.55 (1H, d, *J* 7.2 Hz, H-5), 7.28 (1H, s, H-7), 7.11 (1H, d, *J* 7.2 Hz, H-6), 6.64 (1H, d, *J* 7.6 Hz, H-3), 4.37 (2H, t, *J* 7.2 Hz, H-1'), 4.15 (2H, t, *J* 7.2 Hz, H-1"), 4.01 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.2 Hz, CH_2), 1.87-1.78 (6H, m, H-2', 2", CH_2), 1.44-1.29 (36H, m, H-3'- 11', 3"- 11"), 1.11 (3H, t, *J* 7.2 Hz, CH_3), 0.91 (6H, t, *J* 6.8 Hz, H-12', 12"); ^{13}C NMR (CD_3OD): 171.3, 157.1, 148.7, 148.2, 135.2, 134.8, 133.2, 124.8, 118.7, 114.3, 101.8, 97.2, 57.6, 56.0, 53.6, 35.2, 31.6, 29.6, 29.3, 29.3, 29.1, 29.1, 29.1, 29.0, 29.0, 28.8, 27.3, 25.9, 25.8, 22.3, 17.8, 13, 12.5; EIMS *m/z* $[M - HI]^+$ 620.80 ($C_{40}H_{65}N_2O_3$, 621.50).

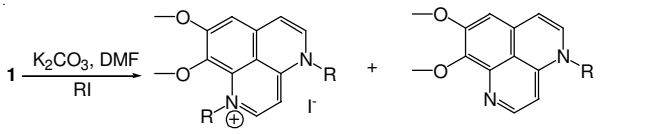
9-*O*-Butyryl-1,4-dihexadecylaaptamine (16): Yield: 28 %, bright yellow solid. IR (KBr, ν_{max}, cm^{-1}) 2921, 2851, 1763, 1591, 1370, 1162, 1097; UV (MeOH) λ_{max} (log ε) 205 (4.31), 224 (4.23), 240 (4.22), 269 (4.29), 279 (4.27), 305 (3.53), 316 (3.54), 404 (3.89) nm; ^{1}H NMR (CD_3OD): 7.99 (1H, d, *J* 7.6 Hz, H-2), 7.56 (1H, d, *J* 7.2 Hz, H-5), 7.29 (1H, s, H-7), 7.11 (1H, d, *J* 7.6 Hz, H-6), 6.65 (1H, d, *J* 7.6 Hz, H-3), 4.37 (2H, t, *J* 7.6 Hz, H-1'), 4.16 (2H, t, *J* 7.6 Hz, H-1"), 4.01 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.2 Hz, CH_2), 1.87-1.78 (6H, m, H-2', 2", CH_2), 1.45-1.29 (52H, m, H-3'- 15', 3"- 15"), 1.11 (3H, t, *J* 7.2 Hz, CH_3), 0.92 (6H, t, *J* 6.8 Hz, H-16', 16"); ^{13}C NMR (CD_3OD): 171.2, 157.2, 148.7, 148.2, 135.2, 134.8, 133.2, 124.8, 118.8, 114.3, 101.9, 97.2, 57.6, 56.1, 53.6, 35.2, 31.6, 29.5, 29.3, 29.2, 29.2, 29.1, 29.1, 29.0, 28.8, 28.8, 27.2, 25.9, 25.8, 22.3, 17.8, 13, 12.5; EIMS *m/z* $[M - HI]^+$ 731.95 ($C_{48}H_{81}N_2O_3$, 733.62).

Antibacterial assay (disc-diffusion method): Antibacterial activity was determined against six bacteria, *i.e.*,

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative), *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus sp*. (Gram-positive). The target bacteria were cultured as described by Mackeen *et al.*[14]. 1 mg of the aaptamine derivatives was diluted in 1 mL of methanol. 20 µL of diluted compounds were loaded onto sterile paper discs (Whatman; 6 mm diameter) and placed on inoculated agar surface. The plates were done in triplicates and were incubated for ± 24 h at 30 °C. Antibacterial activity was indicated by the occurrence of clear inhibition zones around the discs. The diameters of the inhibition zones were measured in millimeters. Gentamicin and streptomycin were served as controls.

RESULTS AND DISCUSSION

Alkylation reactions: 1,4-Dialkylaaptamine (**2-7**) were obtained by alkylation of aaptamine with various alkyl iodide in the presence of K_2CO_3 (**Scheme-I**)[8,9]. K_2CO_3 acted as a base and deprotonated the hydrogen atom of aaptamine. It was believed that the steric hindrance around the alkyl group of alkyl iodide influenced the yield of 1,4-dialkylaaptamine (19-61 %). Interestingly, the minor amounts of by-products; 4-alkylaaptamine (**8-10**) were also isolated from this reaction when propyl iodide, hexyl iodide and nonyl iodide were used as reagents. 4-Alkylaaptamine (**8-10**) were partially alkylated due to incomplete conversion to 1,4-dialkylaaptamine[8,9]. The ^{1}H NMR data of by-products proved to be 4-alkylaaptamines (**8-10**) where the alkyl group was bonded at N-4 position. It was anticipated that the alkylation reaction would first occur at the less hindered N-4 ratherthan N-1.



2-7 **8-10**

2: R = CH_2CH_3, (68%)
3: R = $CH_2CH_2CH_3$, (61%)
4: R = $CH_2(CH_2)_4CH_3$, (36%)
5: R = $CH_2(CH_2)_7CH_3$, (33%)
6: R = $CH_2(CH_2)_{10}CH_3$, (21%)
7: R = $CH_2(CH_2)_{14}CH_3$, (19%)

8: R = $CH_2CH_2CH_3$, (8%)
9: R = $CH_2(CH_2)_4CH_3$, (9%)
10: R = $CH_2(CH_2)_7CH_3$, (10%)

Scheme-I: Semi-synthetic route for preparation of compounds **2-10**

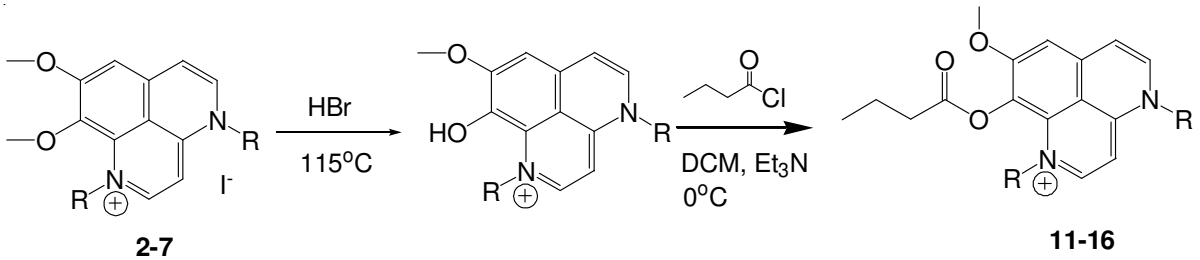
Demethylation and acylation reactions: With a successful route to 1,4-dialkylaaptamine (**2-7**), the selective *O*-demethylation and acylation reactions could then be examined. Compounds **2-7** were treated with 48 % HBr at 115-120 °C[12], yielding unstable 9-*O*-demethylated derivatives due to the free base[15]. 9-*O*-demethylated derivatives were further treated with butyryl chloride in the presence of Et_3N to give derivatives **11-16** (28-88 %) (**Scheme-II**)[13].

The antibacterial activity of **2-16** was examined towards selected bacterial strains. The results are summarized in Table-1. In general, the carbon number of side chain at N-1 and N-4 positions had no relationship with antibacterial activity. It seems that, introducing the alkyl substituent to N-1 and N-4 positions of aaptamine (**1**) had increased and decreased the antibacterial activity. Compound **4** with a side chain of six carbons, showed the most potent antibacterial activity against *Micrococcus* sp., *S. aureus* and *B. cereus* compare to other

TABLE-1
ANTIBACTERIAL ACTIVITY OF AAPTAMINE DERIVATIVES **2-16**

Compounds	*Inhibition Zone (mm)*					
	E. c	*P. a*	*K. p*	*B. c*	*S. a*	*M. sp*
1	-	35	7	-	-	-
2	-	-	-	-	-	7
3	-	-	-	-	-	11
4	-	-	-	17	15	23
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	8	7	12
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	11
13	-	-	-	13	11	16
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	-	-	-	-	-	-
G	20	23	19	22	-	26
S	13	12	16	22	-	24

Control: *G. gentamycin*, *S. streptomycin*; Bacteria: *E. c: Escherichia coli*; *P. e: Pseudomonas aeruginosa*; *K. e*: *Klebsiella pneumonia*, *B. c*: *Bacillus cereus*, *S. a*: *Staphylococcus aureus*, *M. sp.*: *Micrococcus sp.*



2-7 **11-16**

2, R = CH_2CH_3
3, R = $CH_2CH_2CH_3$
4, R = $CH_2(CH_2)_4CH_3$
5, R = $CH_2(CH_2)_7CH_3$
6, R = $CH_2(CH_2)_{10}CH_3$
7, R = $CH_2(CH_2)_{14}CH_3$

11, R = CH_2CH_3, (88%)
12, R = $CH_2CH_2CH_3$, (60%)
13, R = $CH_2(CH_2)_4CH_3$, (55%)
14, R = $CH_2(CH_2)_7CH_3$, (82%)
15, R = $CH_2(CH_2)_{10}CH_3$, (33%)
16, R = $CH_2(CH_2)_{14}CH_3$, (28%)

Scheme-II: Semi-synthetic route for preparation of compounds **11-16**

compounds. Meanwhile, compounds **2** and **3** with side chains two and three carbons were inactive towards all bacteria except *Micrococcus* sp. This result is consistence with previous study done by Bowling *et al*.[12]. They found that compounds with side chains five or less carbon units were inactive. Compounds **5-7** with side chains of nine, twelve and sixteen carbons were also inactive towards all the bacteria. The acylation at C-9 position of compounds **2-7** led to a decreasing in potency of the first generation 1,4-dialkylated derivatives. For example, 9-*O*-butyryl-1,4-dihexylaaptamine (**10**), a compound obtained from acylation of **4** had shown decreasing in activity. The decreasing in activity of acylated compounds might be explained by low water solubility and low bioavailability[11].

Conclusion

In conclusion, the convenient synthesis of 1,4-dialkylated, 4-alkylated and 9-*O*-butyryl-1,4-dialkylated aaptamine derivatives has been developed. Several derivatives had potential as antibacterial agents.

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REFERENCES

1. B. Fugmann, B. Steffan and W. Steglich, *Tetrahedron Lett*., **25**, 3575 (1984).
2. H. Nakamura, J. Kobayashi, Y. Ohizumi and Y. Hirata, *Tetrahedron Lett*., **23**, 5555 (1982).
3. L. Calcul, A. Longeon, A.A. Mourabit, M. Guyot and M.L. Bourguet-Kondracki, *Tetrahedron*, **59**, 6539 (2003).
4. R. de A. Epifanio, A.F. Coutinho, B. Chanas, T.M.L. e Souza and I.C.P.P. Frugrulhetti, *Heterocycles*, **57**, 1265 (2002).
5. A. Herlt, L. Mander, W. Rombang, R. Rumampuk, S. Soemitro, W. Steglich, P. Tarigan and F. von Nussbaum, *Tetrahedron*, **60**, 6101 (2004).
6. A. Rudi and Y. Kashman, *Tetrahedron Lett*., **34**, 4683 (1993).
7. K. Shaari, K.C. Ling, Z. Mat Rashid, T.P. Jean, F. Abas, S.M. Raof, Z. Zainal, N.H. Lajis, H. Mohamad and A.M. Ali, *Mar. Drugs*, **7**, 1 (2009).
8. G.R. Pettit, H. Hoffmann, D.L. Herald, P.M. Blumberg, E. Hamel, J.M. Schmidt, Y. Chang, R.K. Pettit, N.E. Lewin and L.V. Pearce, *J. Med. Chem*., **47**, 1775 (2004).
9. G.R. Pettit, H. Hoffmann, D.L. Herald, J. Mcnulty, A. Murphy, K.C. Higgs, E. Hamel, N.E. Lewin, L.V. Pearce, P.M. Blumberg, R.K. Pettit and J.C. Knight, *J. Org. Chem*., **69**, 2251 (2004).
10. Y. Takahashi, N. Tanaka, T. Kubota, H. Ishiyama, A. Shibazaki, T. Gonoi, J. Fromont and J. Kobayashi, *Tetrahedron*, **68**, 8545 (2012).
11. Y.C. Shen, T.T. Lin, J.H. Sheu and C.Y. Duh, *J. Nat. Prod*., **62**, 1264 (1999).
12. J.J. Bowling, H.K. Pennaka, K. Ivey, S. Wahyuono, M. Kelly, R.F. Schinazi, F.A. Valeriote, D.E. Graves and M.T. Hamann, *Chem. Biol. Drug Des*., **71**, 205 (2008).
13. W. Gul, N.L. Hammond, M. Yousaf, J.J. Bowling, R.F. Schinazi, S.S. Wirtz, G.C. Andrews, C. Cuevas and M.T. Hamann, *Bioorg. Med. Chem*., **14**, 8495 (2006).
14. M.M. Mackeen, A.M. Ali, N.H. Lajis, K. Kawazu, Z. Hassan, M. Amran, M. Habsah, L.Y. Mooi and S.M. Mohamed, *J. Ethnopharmacol*., **72**, 395 (2000).
15. E.L. Larghi, M.L. Bohn and T.S. Kaufman, *Tetrahedron*, **65**, 4257 (2009).