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Synthesis, Antimicrobial Evaluation and Anti-MRSA Studies: 1-Aryl/Alkyl-4-Aminopyridinium Bromides

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A. YASODHA[*1], A. SIVAKUMAR[2], A. PURATCHIKODY[3]

[1]*Department of Pharmaceutical Chemistry, Dhanvanthri college of Pharmaceutical Sciences, Mahabubnagar - 509002,Telugana, India.*

[2]*Production department, AurobindoPharma Limited, Unit –VII, Jadcherla, Hyderabad.*

[3]*Department of Pharmaceutical Technology, Anna University Chennai, BIT Campus, Tiruchirappalli-620024, Tamilnadu, India.*

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ABSTRACT

A series of bromides (RCH_2Br) containing aryl/alkyl group readily reacted with 4-aminopyridine to give stable pyridinium bromide **(1-15)**. The physical and spectral (IR, ^1H &^{13}C NMR and MS) data for **1-15** were collected to confirm the structure (s) assigned. *In vitro* antibacterial and antifungal activities of **1-15** against Gram positive/Gram negative and fungal species were evaluated. Compound **13** was emerged as promising antimicrobial agent (MIC = 0.39 µg/ml).The amplification of genes *mecA* and *tsst* by PCR method showed that *mecA* gene present in 46 and *tsst* gene in 15 out of 100 *S. aureus* isolates. The Anti-MRSA activity of compound **13** was determined by the agar-well diffusion method against 11 selected isolates of MRSA which possess both *mecA* and *tsst* genes. The data were analyzed using one way analysis of variance (ANOVA) and showed significant difference at $P<0.1$.

1. INTRODUCTION

Pyridinium salts are unsaturated heterocyclic compounds having different functional groups present either on pyridine ring or nitrogen atom[1]. Due to their easier preparation and rich biological activity, pyridinium framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry[2,3]. Quarternized amine derivatives were reported to possess antimicrobial properties[4,5]. Several quaternary ammonium compounds including pyridinium salts with various alkyl/aryl chain lengths exert antimicrobial activity against both Gram positive and Gram negative bacteria and some pathogenic species of fungi and protozoa[4].

The continued emergence of multi drug resistance (MDR) to clinically available drugs has lent additional urgency to develop new antimicrobial agents likely to be unaffected by existing resistance mechanisms. As a result of widespread methicillin use, methicillin-resistant *S.aureus* (MRSA) has become a major problem globally. MRSA infections are difficult to treat because of its multidrug-resistance properties to existing β-lactams as well as several other classes of antibiotics[6,7]. In addition, the accurate and rapid diagnosis of antibiotic resistance genes in the treatment of staphylococcal infections is extremely important in preventing the spread of infections. PCR-based molecular methods are often preferred for determination of antibiotic resistance genes[8]. Keeping in view of this and in continuation of our search on biologically potent molecules[9-11], we hereby report the synthesis, antimicrobial evaluation and Anti-MRSA studies of 1-aryl/alkyl 4-substituted aminopyridinium bromides.

2. MATERIALS AND METHODS

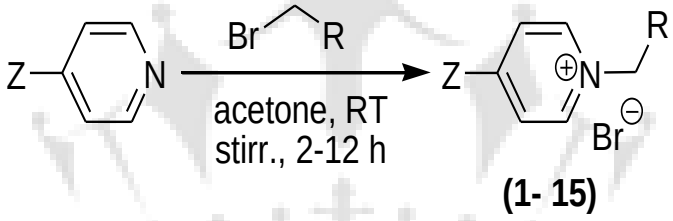
2.1 Synthesis

Melting points determined are uncorrected. IR (KBr) spectra of the compounds **1-30** were recorded on Perkin Elmer 1600 FT spectrophotometer. ^{1}H, ^{13}CNMR spectra were recorded in DMSO – d_6 on a Bruker AC, 300MHz spectrometer using TMS as standard and Mass Spectra on API 3000 Centroid Turbo Spray Analyzer. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer.

2.1.1 General procedure for synthesis of 1-aryl/alkyl- 4-aminopyridinium bromides (1-15)

A mixture of aryl/alkyl bromide (0.1 mol) and *4-aminopyridine* (0.1 mol) in dry acetone (50ml) was stirred for about 55– 60°C for 2-12 h. Colorless/colored solid that separated was filtered, washed with toluene, dried in vacuum and recrystallized from chloroform-acetone (1:1, w/v) to give **1-15**.

Compd. No	1	2	3	4	5	6	7	8
Z	$-NH_2$	$-NH_2$	$-NH_2$	$-NH_2$	$-NH_2$	$-NH_2$	$-NH_2$	$-NH_2$
R	$-C_6H_5$	$-C_6H_4$-Br(*4*)	$-C_6H_4-NO_2$(*4*)	$-C_6H_4-CH_3$(*4*)	$-COC_6H_5$	$-CH_2CH_3$	$-(CH_2)_2-CH_3$	$-(CH_2)_3-CH_3$
Compd. No	9	10	11	12	13	14	15	



SCHEME 1

4-amino-1-(phenylmethyl)pyridinium bromide (1)

IR (KBr, cm^{-1}): 3337 (NH str., primary amine), 3171 (CH str., aromatic), 2649 (CH str., aliphatic), 2077 (CH_2 str.), 1653 (C=C str.), 1542 (C-N str.), 1449 (N^+-C str.), 1381 (N-C str.); ^{1}H NMR, ppm: 8.37-8.36 (d, 2H, C_2- and C_6-H) , 8.28 (s, 2H, NH_2), 7.45-7.35 (m, 5H, -CH_2Ph), 7.07-7.06 (d, 2H, C_3-and C_5-H), 5.40 (s, 2H, N^+CH_2-); ^{13}C NMR, ppm: 158.70, 155.85, 142.95, 142.00, 135.80, 135.65, 129.00, 128.63, 128.01 (aromatic carbons), 109.56, 108.00 (N^+CH_2-), 59.47, 59.13; MS calculated mass for $C_{12}H_{13}BrN_2$: 185.03, Observed mass ESI-MS (m/z): 185 (M^+, 100%), 186 (M^++1, 13%), 184 (M^+-1, 17%), 165 (83%), 137 (53%), 120 (90%), 108 (70%); Anal cal%: C, 77.83; H, 7.02; N, 15.13; Found%: C, 77.81; H, 7.03; N, 15.12.

4-amino-1-[(4-bromophenyl)methyl]pyridinium bromide (2)

IR (KBr, cm^{-1}): 3280 (NH str., primary amine), 3128 (CH str., aromatic), 2690 (CH str., aliphatic), 2064 (CH_2 str.), 1668 (C=C str.), 1544 (C-N str.), 1490 (N^+-C str.), 1174 (N-C str.),

818 (C-Br str.); ^{1}H NMR, ppm: 8.32-8.29 (d, 2H, C_2- and C_6-H), 8.23 (s, 2H, NH_2), 7.63-7.61(d, 2H, -C'$_3$- and C'$_5$-H), 7.38-7.35 (d, 2H, C'$_2$ and C'$_6$-H), 6.89-6.87 (d, 2H, C_3-and C_5-H), 5.38 (s, 2H, N^+CH_2-); ^{13}C NMR, ppm: 158.74, 142.94, 134.97, 131.22, 130.30 (-C_4-Br), 122.01 (aromatic carbons), 109.64 (N^+CH_2-), 58.74; MS calculated mass for $C_{12}H_{12}Br_2N_2$: 262.94, Observed mass ESI-MS (m/z): 263 (M^+, 100%), 264 (M^++1, 5%), 262 (M^+-1, 4%), 205 (40%), 171 (86%), 123 (58%), 107 (95%); Anal cal%: C, 54.56; H, 4.58; N, 10.60; Found%: C, 54.54; H, 4.56; N, 10.59.

4-amino-1-[(4-nitrophenyl)methyl]pyridinium bromide (3)

IR (KBr, Cm^{-1}): 3399 (NH str., primary amine), 3035 (CH str., aromatic), 2717 (CH str., aliphatic), 2064 (CH_2 str.), 1648 (C=C str.), 1565 (C-N str.), 1344 (N^+-C str.), 1168 (N-C str.), 803 (C-NO_2 str.); ^{1}H NMR, ppm: 8.33-8.30 (d, 2H, C_2- and C_6-H), 8.25 (s, 2H, NH_2), 7.62-7.61 (d, 2H, -C'$_2$- and C'$_6$-H), 7.35-7.34 (d, 2H, -C'$_3$- and C'$_5$-H), 7.06-7.05 (d, 2H, C_3-and C_5-H), 5.30 (s, 2H, N^+CH_2-); ^{13}C NMR, ppm: 155.96, 147.52, 143.01, 142.18, 124.06 (aromatic carbons), 108.14 (N^+CH_2-), 58.30, 40.97 (-C_4-NO_2); MS calculated mass for $C_{12}H_{12}BrN_3O_2$: 230.01, Observed mass ESI-MS (m/z): 230 (M^+, 100%), 231 (M^++1, 10%), 229 (M^+-1, 4%), 212 (32%), 179 (82%), 163 (34%), 157 (9%); Anal cal%: C, 62.60; H, 5.21; N, 18.26; Found%: C, 62.58; H, 5.20; N, 18.27.

4-amino-1-[(4-methylphenyl)methyl]pyridinium bromide (4)

IR (KBr, cm^{-1}): 3289 (NH str., primary amine), 3122 (CH str., aromatic), 2699 (CH str., aliphatic), 2150 (CH_2 str.), 1652 (C=C str.), 1530 (C-N str.), 1368 (N^+-C str.), 1167 (N-C str.); ^{1}H NMR, ppm: 8.31-8.30 (d, 2H, C_2- and C_6-H), 8.21 (s, 2H, NH_2), 7.30-7.29 (d, 2H, C'$_3$- and C'$_5$-H), 7.22-7.21 (d, 2H, -C'$_2$- and C'$_6$-H), 6.88-6.86 (d, 2H, C_3- and C_5-H), 5.33 (s, 2H, N^+CH_2-), 2.29 (s, 3H, -C_4-CH_3); ^{13}C NMR, ppm: 158.86, 142.86, 138.12, 132.61, 129.54, 128.08 (aromatic carbons), 109.55 (N^+CH_2-), 59.37, 20.68 (-C_4-CH_3); MS calculated mass for $C_{13}H_{15}BrN_2$: 199.04, Observed mass ESI-MS (m/z): 199 (M^+, 100%), 200 (M^++1, 31%), 198 (M^+-1, 6%), 163 (22%), 149 (40%), 136 (44%), 123 (96%), 107 (84%); Anal cal%: C, 78.39; H, 7.53; N, 14.07; Found%: C, 78.38; H, 7.51; N, 14.10.

4-amino-1-(2-oxo-2-phenylethyl)pyridinium bromide (5)

IR (KBr, cm^{-1}): 3437 (NH str., primary amine), 3096 (CH str., aromatic), 2764 (CH str., aliphatic), 2052(CH_2 str.), 1649 (C=O str.), 1506 (C=Cstr.), 1443 (C-N str.), 1332 (N^+-C str.), 1215 (N-C str.); ^{1}H NMR, ppm: 8.37-8.36 (d, 2H, C_2- and C_6-H), 8.28 (s, 2H, NH_2), 7.45-7.35 (m, 5H, -COPh), 7.07-7.06 (d, 2H, C_3-and C_5-H), 5.41 (s, 2H, N^+CH_2-); ^{13}C NMR, ppm: 193.12(C=O), 154.17, 149.41, 137.21, 131.62, 129.02, 128.99, 127.00 (aromatic carbons), 108.82 (N^+CH_2-), 58.36; MS calculated mass for $C_{13}H_{13}BrN_2O$: 213.02, Observed mass ESI-MS (m/z): 213 (M^+, 100%), 214 (M^++1, 18%), 212 (M^+-1, 2%), 201 (36%), 200 (55%);Anal cal%: C, 73.23; H, 6.10; N, 13.14; Found%: C, 73.21; H, 6.09; N, 13.12.

4-amino-1-propylpyridinium bromide (6)

IR (KBr, cm^{-1}): 3506 (NH str., primary amine), 3331 (CH str., aromatic), 2334 (CH str., aliphatic), 2054 (CH_2 str.), 1646 (C=Cstr.), 1422 (C-N str.), 1324 (N^+-C str.), 1203 (N-C str.); ^{1}H NMR, ppm: 7.98-7.97 (d, 2H, C_2- and C_6-H), 6.47-6.46 (d, 2H, C_3-and C_5-H), 6.01 (s, 2H, NH_2), 3.72-3.70 (t, 2H, N^+CH_2-), 2.57(m, 5H, -CH_2-CH_2-CH_3); ^{13}C NMR, ppm: 154.24, 149.33, 139.50, 108.82 (N^+CH_2-), 46.10, 20.10, 18.03 (-CH_2-CH_3); MS calculated mass for $C_8H_{13}BrN_2$: 137.03, Observed mass ESI-MS (m/z): 137 (M^+, 100%), 138 (M^++1, 38%), 136 (M^+-1, 36%), 123 (42%), 115 (10%), 105 (78%); Anal cal%: C, 70.07; H, 9.48; N, 20.43; Found%: C, 70.05; H, 9.47; N, 20.42.

4-amino-1-butylpyridinium bromide (7)

IR (KBr, cm^{-1}): 3434 (NH str., primary amine), 3300 (CH str., aromatic), 3100 (CH str., aliphatic), 2538 (CH_2 str.), 1646 (C=C str.), 1531 (C-N str.), 1435 (N^+-C str.), 1332 (N-C str.); ^{1}H NMR, ppm: 8.20-8.18 (d, 2H, C_2- and C_6-H), 7.96 (s, 2H, NH_2), 6.85-6.82 (d, 2H, C_3-and C_5-H), 3.51-3.46 (m, 9H, N^+CH_2-CH_2-CH_2-CH_3); ^{13}C NMR, ppm: 158.52, 154.20, 149.33, 142.80, 109.33, 108.81 (N^+CH_2), 65.68,56.70, 32.17, 18.66, 13.30 (-CH_2-CH_2-CH_3); MS calculated mass for $C_9H_{15}BrN_2$: 151.04, Observed mass ESI-MS (m/z): 151 (M^+, 100%), 152 (M^++1, 8%), 150 (M^+-1, 23%), 145 (17%), 135 (54%), 121 (40%), 106 (52%); Anal cal%: C, 71.52; H, 9.93; N, 9.27; Found%: C, 71.51; H, 9.91; N, 9.26.

4-amino-1-pentylpyridinium bromide (8)

IR (KBr, cm^{-1}): 3428 (NH str., primary amine), 3209 (CH str., aromatic), 2938 (CH str., aliphatic), 2055 (CH_2 str.), 1651 (C=C str.), 1542 (C-N str.), 1437 (N^+-C str.), 1381 (N-C str.); ^1H NMR, ppm: 8.36-8.25 (d, 2H, C_2- and C_6-H), 8.19 (s, 2H, NH_2), 6.88-6.87 (d, 2H, C_3-and C_5-H), 4.15-4.12 (t, 2H, N^+CH_2),3.42-2.50 (m, 6H, N^+-CH_2-CH_2-CH_2-CH_2-CH_3), 1.19-1.15 (t, 3H, N^+-CH_2-CH_2-CH_2-CH_2-CH_3); ^{13}C NMR, ppm: 158.59, 154.28, 149.19, 142.81, 109.26, 108.79 (N^+CH_2), 56.41, 29.49, 21.70 (-CH_2-CH_2-CH_2-CH_3); MS calculated mass for $C_{10}H_{17}BrN_2$: 165.06, Observed mass ESI-MS (m/z): 165 (M^+, 100%), 166 (M^++1, 12%), 164 (M^+-1, 3%), 107 (3%), 106 (37%), 105 (82%); Anal cal%: C, 72.72; H, 10.30; N, 16.96; Found%: C, 72.70; H, 10.28; N, 16.96.

4-amino-1-hexylpyridinium bromide (9)

IR (KBr, cm^{-1}): 3306 (NH str., primary amine), 3095 (CH str., aromatic), 2698 (CH str., aliphatic), 2052 (CH_2 str.), 1649 (C=C str.), 1506 (C-N str.), 1435 (N^+-C str.), 1334 (N-C str.); ^1H NMR, ppm: 8.10 (s, 2H, NH_2), 7.98-7.96 (d, 2H, C_2- and C_6-H), 6.47-6.45 (d, 2H, C_3-and C_5-H), 4.16-4.15 (t, 2H, N^+-CH_2-), 1.75-1.23 (m, 11H, N^+-CH_2-CH_2-CH_2-CH_2-CH_3); ^{13}C NMR, ppm: 154.82, 141.95, 108.19 (N^+CH_2), 60.43, 56.50, 48.26, 34.99, 32.20, 31.93, 30.07, 24.65 (-CH_2-CH_2-CH_2-CH_3); MS calculated mass for $C_{11}H_{19}BrN_2$: 179.07, Observed mass ESI-MS (m/z): 179 (M^+, 100%), 180 (M^++1, 28%), 178 (M^+-1, 2%), 165 (79%), 135 (16%), 107 (75%); Anal cal%: C, 73.74; H, 10.61; N, 15.64; Found%: C, 73.72; H, 10.60; N, 15.63.

4-amino-1-(3-bromopropyl)pyridinium bromide (10)

IR (KBr, cm^{-1}): 3437 (NH str., primary amine), 3308 (CH str., aromatic), 2916 (CH str., aliphatic), 2540 (CH_2 str.), 1669 (C=C str.), 1508 (C-N str.), 1334 (N^+-C str.), 1269(N-C str.), 823 (C-Br str.); ^1H NMR, ppm: 8.21 (s, 2H, NH_2), 7.96-7.95 (d, 2H, C_2- and C_6-H), 6.46-6.44 (d, 2H, C_3-and C_5-H), 4.20-4.17 (t, 2H, N^+-CH_2), 2.50 (m, 4H, N^+-CH_2-CH_2-CH_2-Br); ^{13}C NMR, ppm: 158.66, 154.19, 142.80, 109.44 (N^+CH_2-), 66.18 (-CH_2-Br), 53.92, 46.10; MS calculated mass for $C_8H_{12}Br_2N_2$: 214.14, Observed mass ESI-MS (m/z): 214 (M^+, 100%), 215 (M^++1, 2%), 213 (M^+-1, 2%), 212 (30%), 207 (6%);Anal cal%: C, 44.85; H, 5.60; N, 13.08; Found%: C, 44.84; H, 5.61; N, 13.07.

4-amino-1-(4-bromobutyl)pyridinium bromide (11)

IR (KBr, cm^{-1}): 3437 (NH str., primary amine), 3304 (CH str., aromatic), 2698 (CH str., aliphatic), 2540 (CH_2 str.), 1600 (C=C str.), 1508 (C-N str.), 1435 (N^+-C str.), 1217 (N-C str.), 823 (C-Br str.); ^1H NMR, ppm: 8.18 (s, 2H, NH_2), 7.96-7.94 (d, 2H, C_2- and C_6-H), 6.86-6.85 (d, 2H, C_3- and C_5-H), 4.17 (t, 2H, N^+-CH_2), 2.50-1.71 (m, 6H, N^+-CH_2-CH_2-CH_2-CH_2-Br); ^{13}C NMR, ppm: 158.57, 154.22, 149.30, 142.79, 109.38, 108.79 (N^+CH_2-), 56.16 (-CH_2-Br), 26.79; MS calculated mass for $C_9H_{14}Br_2N_2$: 228.95, Observed mass ESI-MS (m/z): 229 (M^+, 100%), 230 (M^++1, 17%), 228 (M^+-1, 3%), 215 (21%), 210 (28%), 207 (27%); Anal cal%: C, 47.36; H, 6.14; N, 12.28; Found%: C, 47.35; H, 6.12; N, 12.27.

4-amino-1-(5-bromopentyl)pyridinium bromide (12)

IR (KBr, cm^{-1}): 3397 (NH str., primary amine), 3143 (CH str., aromatic), 2930 (CH str., aliphatic), 2076 (CH_2 str.), 1655 (C=C str.), 1540 (C-N str.), 1368 (N^+-C str.), 1186 (N-C str.), 834 (C-Br str.); ^1H NMR, ppm: 8.27-8.25 (d, 2H, C_2- and C_6-H), 8.19 (s, 2H, NH_2), 6.88-6.87 (d, 2H, C_3-and C_5-H), 4.15–4.12 (t, 2H, N^+-CH_2), 2.50-1.15 (m, 8H, N^+-CH_2-CH_2-CH_2-CH_2-CH_2-Br); ^{13}C NMR, ppm: 158.5, 154.28, 149.19, 142.84, 109.26, 108.79 (N^+CH_2-), 56.41 (-CH_2-Br), 29.49,21.70; MS calculated mass for $C_{10}H_{16}Br_2N_2$: 242.97, Observed mass ESI-MS (m/z): 242 (M^+, 100%), 243 (M^++1, 22%), 241 (M^+-1, 6%), 234 (34%), 222 (51%), 215 (78%); Anal cal%: C, 49.58; H, 6.61; N, 11.57; Found%: C, 49.58; H, 6.61; N, 11.57.

4-amino-1-(6-bromohexyl)pyridinium bromide (13)

IR (KBr, cm^{-1}): 3311 (NH str., primary amine), 3136 (CH str., aromatic), 2935 (CH str., aliphatic), 2065 (CH_2 str.), 1602 (C=C str.), 1539 (C-N str.), 1371 (N^+-C str.), 1188(N-C str.), 839 (C-Br str.); ^1H NMR, ppm: 8.25-8.24 (d, 2H, C_2- and C_6-H), 8.17 (s, 2H, NH_2), 7.95-7.94 (d, 2H, C_3- and C_5-H), 4.14– 4.10 (t, 2H, N^+-CH_2), 2.50-1.23 (m, 10H, N^+-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-Br); ^{13}C NMR, ppm: 158.50, 154.29, 149.17, 142.81, 109.26, 108.75 (N^+CH_2-), 56.70 (-CH_2-Br), 29.91, 24.74; MS calculated mass for $C_{11}H_{18}Br_2N_2$: 256.98, Observed mass ESI-MS (m/z): 256 (M^+, 100%), 257 (M^++1, 25%), 255 (M^+-1, 52%), 253 (23%), 251 (7%); Anal cal%: C, 51.56; H, 7.03; N, 10.90; Found%: C, 51.54; H, 7.02; N, 10.90.

4-amino-1-(2-ethoxy-2oxoethyl)pyridinium bromide (14)

IR (KBr, cm^{-1}): 3427 (NH str., primary amine), 2988 (CH str., aromatic), 2708 (CH str., aliphatic), 2080 (CH_2 str.), 1744 and 1654 (C=O str.), 1557 (C=C str.), 1382 (C-N str.), 1210 (N^+-C str.), 1019 (N-C str.); ^{1}H NMR, ppm: 8.16-8.14 (d, 2H, C_2- and C_6-H), 6.91-6.90 (d, 2H, C_3-and C_5-H), 5.05 (s, 2H, N^+CH_2-), 4.21-4.16 (quadret, 2H,-CH_2- CH_3), 2.51-2.50 (t, 3H,-CH_2-CH_3); ^{13}C NMR, ppm: 168.86 (carbon of ester group), 167.58, 159.79, 158.93, 158.81, 143.98, 139.77, 108.96, 108.68 (N^+CH_2-), 61.74 (-CH_2-CH_3), 57.12, 56.75 (-CH_2-CH_3); MS calculated mass for $C_9H_{13}BrN_2O_2$: 181.02, Observed mass ESI-MS (m/z): 181 (M^+, 100%), 182 (M^++1, 21%), 180 (M^+-1, 8%), 165 (98%), 147 (13%), 136 (98%), 108 (96%); Anal cal%: C, 59.66; H, 7.18; N, 15.46; Found%: C, 59.64; H, 7.17; N, 15.45.

4-amino-1-allylpyridinium bromide (15)

IR (KBr, cm^{-1}): 3259 (NH str., primary amine), 3091 (CH str., aromatic), 2710 (CH str., aliphatic), 2154 (CH_2 str.), 1658 (C=C str.), 1535 (C-N str.), 1506 (C=C str., olefinic), 1369 (N^+-C str.), 1170 (N-C str.); ^{1}H NMR, ppm: 8.31-8.21 (d, 2H, C_2- and C_6-H), 8.0 (s, 2H, NH_2), 6.94-6.92 (d, 2H, C_3-and C_5-H), 6.07-6.05 (d, 2H, N^+CH_2-), 5.30-5.27 (d, 1H,- CH_2-CH=CH_2), 4.84-4.83 (d, 2H,-CH_2-CH=CH_2); ^{13}C NMR, ppm: 158.60, 142.80, 138.25, 132.80 (-CH=CH_2), 119.68 (-CH=CH_2), 109.30 (N^+CH_2-), 58.51; MS calculated mass for $C_8H_{11}BrN_2$: 135.01, Observed mass ESI-MS (m/z): 135 (M^+, 100%), 136 (M^++1, 6%), 134 (M^+-1, 9%), 120 (40%), 106 (82%); Anal cal%: C, 71.11; H, 8.14; N, 20.74; Found%: C, 71.10; H, 8.12; N, 20.73.

2.2 Antimicrobial Testing:

The antibacterial activity was performed against Gram positive (*Staphylococcus aureus* and *Streptococcus mutants*) and Gram negative (*Escherichia coli* and *Klebesillapneumoniae*) bacteria and antifungal activity was performed against *Rhizopus arrhizus* and *Aspergillus niger* by tube dilution method[12]**.** Dilutions of test and standard compounds [ciprofloxacin (antibacterial) and fluconazole (antifungal)] were prepared in double strength nutrient broth – I.P. (bacteria) and Sabouraud dextrose broth I.P. (fungi). The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 72 h (*Aspergillus niger*) and at 37°C for 48 h (*Rhizopus arrhizus*), respectively, and the results were recorded in terms of minimum inhibitory concentration (MIC) (the lowest concentration of test substance which inhibited the growth of microorganisms).

2.3 Anti-MRSA activity

2.3.1 Sample collection and isolation

In the present study, a total of 205 clinical samples were obtained from Government Hospitals in Perambalur and Namakkal districts, Tamilnadu, India. They were collected from the following clinical specimens: sputum (28), wound discharge (70), burn discharge (33), urine (50) and blood (24). These specimens were inoculated into peptone water and incubated at 37°C for 24 h. A loopful of culture from peptone water was streaked to the mannitol salt agar plate. The plates were incubated at 37°C for 24-48 h. All isolates were identified according to colonial and microscopical morphology and standard tests like catalase, tube coagulase, motility, and oxidase and biochemical tests[13].

2.3.2 DNA isolation for PCR

For nucleic acid isolation from staphylococcal isolates, the frozen samples were thawed rapidly, and were cultivated in brain-heart infusion broth (Merck, Germany) at 37°C with continuous shaking overnight. Total DNA was isolated from 5 ml of a broth culture grown overnight. After incubation, bacterial cells were harvested by centrifugation at 3000 × *g* for 10 min; the cell pellet was re-suspended in phosphate-buffered saline with 100 µg of lysostaphin (Sigma, USA) per ml, and incubated at 37°C for 30 min. The phenol/chloroform extraction method was used for nucleic acid extraction and DNA was precipitated in 1 ml of 70 % ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer [10 mMTris chloride-1 mM EDTA (pH 8.0)], and stored at -20°C until processing[14].

2.3.3 PCR for *mecA* gene detection

The *mecA* gene was amplified with the following two oligonucleotides: forward primer (5-CTCAGGTACTGCTATCCACC-3') and backward primer (5'- CACTTGGTATATCTTCACC -3') which gave a PCR product of 449 bp[15]. The PCR was performed with an initial denaturation step of 5 min 94°C, followed by 25 cycles of 20s at 94°C, annealing at 55°C for 20s and the extension step of 50s at 72°C. Agarose gels were prepared with TBE buffer (Tris, Boric acid, EDTA, pH 8) and stained with ethidium bromide (1 µg /15 ml gel). PCR product (5 µl) of each sample was mixed with 5 µl of sample buffer (6X: 0.25% bromophenol, 0.25% xylene cyanol,

15% ficoll 400) and loaded on 1.5% agarose and electrophoresed in 75 volt for 60 min. The band of fragment was observed by ultraviolet (UV) transilluminator and was documented by gel analyzer machine[16].

2.3.4 PCR for *tsst* gene detection

The *tsst* gene was amplified with the following two oligonucleotides: forward primer (5AAGCCCTTTGTTGCTTGCGAC-3' and backward primer (5'AGCAGGGCTATAATAAGG -ACTC-'3) which gave a PCR product of 250 bp[15]. The same procedure was followed for detection of *tsst* gene as worked -up in PCR for *mecA* gene detection.

2.3.5 Antibacterial activity against MRSA

The antibacterial activity of the compounds **13** was tested against selected isolates of MRSA by the agar-well diffusion method[17]. A standard inoculum size of the *S.aureus* strains were evenly distributed and streaked on the surface of a sterile Muller Hinton Agar plate using sterile cotton swab. The density of the suspension was adjusted to approximately 10^8 CFU/ml by comparing its turbidity to a McFarland 0.5 $BaSO_4$ standard[18]. Five wells of 6 mm in diameter were punched in the plates using a sterile cork borer. Out of five wells, three wells were filled with 100 µl of 100, 50, 25 µg/ml concentration of the test compound (**13**) and another with ampicillin 5 µg/ml concentration as the positive control, whereas, the centre well was filled with 100 µg/ml of DMSO and was kept as negative control. Plates were then incubated at 37°C for 24 h. All tests were performed in triplicate and the antibacterial activity produced by the test compounds was expressed as the mean diameter of the inhibition zones (mm).

2.3.6 Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data was performed by analysis of variance (ANOVA), using the statistical package for social sciences (SPSS) program, version 17.0. A probability value of difference $p \leq 0.01$ was considered to be statistically significant. All data were presented as mean values ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Chemistry:

The reaction between aryl/alkyl bromide(s) (RCH_2Br) and 4-aminopyridine reacts to give 4-amino-1-aryl/alkyl pyridinium bromide **(1-15)** in dry acetone at room temperature under stirring for 2-12 hours **(Scheme 1)**. The physical data of **1-15** were collected and are presented in **Table 1**. The physical (m.p., yield, etc.) data of some of the 4-amino-1-arylpyridinium bromides and 4-amino-1-alkylpyridinium bromides are found comparable with their reported values elsewhere[19]. Each of 4-aminopyridineis small organic molecule but is a potential nucleophile[20]. The presence of $-NH_2$ group at 4-position of pyridine has electron releasing property, whereas, the nitrogen at 1-position has electron accepting tendency. As a result, the charge density on the endocyclic nitrogen is more than the exocyclic nitrogen[21]. Therefore, electrophile preferably attacks the endocyclic nitrogen. In the case of aryl bromide, electron withdrawing group (EWG) at 4-position of benzene ring causes for C-Br bond to be more facile to cleave which makes the reaction faster and form more yield more (>80%) **(1-5)**. Whereas, C-Br cleavage is less facile in the case of alkyl bromide(s) **(6-9)** which results the less yield (70-80%).

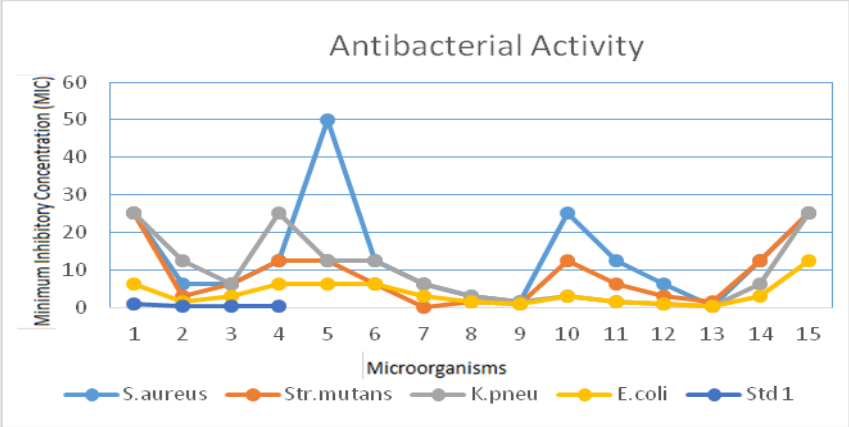
The presence of other bromine atom in the alkyl chain may cause for increasing the electrophilicity of the alkyl group. The reaction between 4-aminopyridine and dibromo alkane under 1:1 mole ratio prefers to attack either one of the C-Br bonds[22]. Increasing the alkyl chain length is also favored for the approach of 4-aminopyridine nucleophile which in turn increases the yield. The reaction between 4-aminopyridine and carboethoxymethyl/allyl bromide gave the corresponding pyridinium salt (**14 & 15**) with poor yield. This may be due to the formation of carbocation which is to be stabilized by inductive and conjugative effect.

Table1 Physicochemical characteristics of the synthesized compounds 1-15

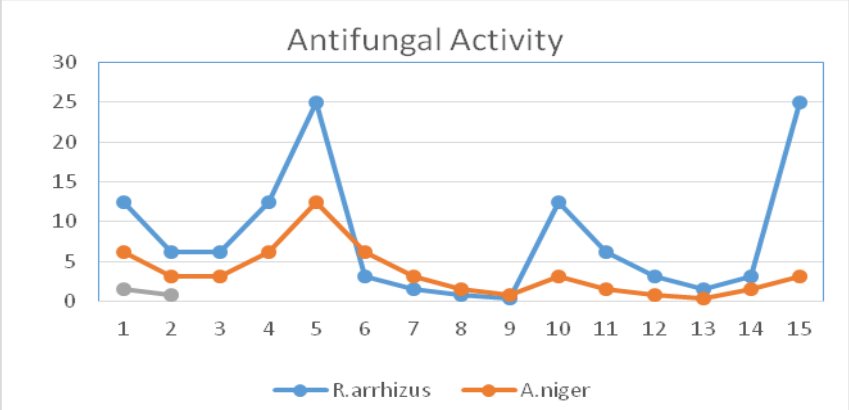
Compd. No	MF	Colour	Yield (%)	mp(°C)
1	$C_{12}H_{13}BrN_2$	Colourless	81	186-188
2	$C_{12}H_{12}Br_2N_2$	Pale yellow	85	230-232
3	$C_{12}H_{12}BrN_3O_2$	Yellow	83	242-244
4	$C_{13}H_{15}BrN_2$	Pale Yellow	80	222-224
5	$C_{13}H_{13}BrN_2O$	Pale yellow	92	122-124
6	$C_8H_{13}BrN_2$	Pale Yellow	78	102-104
7	$C_9H_{15}BrN_2$	Colourless	80	112-114
8	$C_{10}H_{17}BrN_2$	Pale Yellow	76	104-106
9	$C_{11}H_{19}BrN_2$	Yellow	74	118-120
10	$C_8H_{12}Br_2N_2$	Colourless	87	128-130
11	$C_9H_{14}Br_2N_2$	Colourless	91	99-100
12	$C_{10}H_{16}Br_2N_2$	Colourless	92	100-102
13	$C_{11}H_{18}Br_2N_2$	Colourless	93	108-110
14	$C_9H_{13}BrN_2O_2$	Yellow	66	220-222
15	$C_8H_{11}BrN_2$	Pale yellow	55	180-182

3.2 Antimicrobial activity:

The minimum inhibitory concentration (MIC) assay of the 4-amino-1-aryl/alkyl pyridinium bromides showed a significant activity against all the mentioned pathogenic organisms (**Figure 1 a & b**). The compounds **13** and **9** (MIC=0.39 and 1.5 µg/ml) showed good potency in inhibiting the growth of *S.aureus*. The compound **9** (MIC=1.5 µg/ml) has shown comparable antibacterial potential with standard ciprofloxacin against *S. mutans* (MIC=0.39 µg/ml). Compound **13** (MIC=0.39 µg/ml) was emerged as most effective antibacterial agent against gram negative bacteria *K. pneumoniae* and *E. coli*. The results of antifungal activity indicated that the compounds **9** and **13** have shown most potent antifungal potential (MIC = 0.39 µg/ml) than the standard ketoconazole against *R. arrhizus* and *A. niger* respectively. The most active compound **15** has a broad antimicrobial spectrum against all the tested organisms.



(a)



(b)

Figure 1. Graph showing relation between the mean value of MIC of compounds 1-30 against bacterial (a) & fungal (b) microorganisms.

SA: *Staphylococcus aureus* EC: *Escherichia coli*

SM: *Streptococcus mutans* RH: *Rhizopusarrhizus*

KP: *Klebsiella pneumonia* AN: aspergillus niger

STD 1- Ciprofloxacin STD- Fluconazole

3.3 Anti-MRSA activity

3.3.1 Sample collection and Isolation

A total of 100 clinical isolates of staphylococci were recovered and detected from the different types of 205 samples. This was identified on the basis of morphological characters. The selected

isolates were found to be gram positive, non motile grape like coccal clusters. Biochemical tests confirmed that all the isolates were *Staphylococcus aureus.*

In our study MRSA isolates were in majority from wound discharges (53.4%), followed by burn discharges (46%), blood samples (42.8%), urine (40%) and sputum (30%). The results are shown in **Table 2 and Figure 2.**

Table 2. Frequency of *S. aureus* and MRSA in specimens Samples

Samples	No. of Strains Tested	No. of Strains Resistant	Percentage
Pus from postoperative wound infections	43	23	53.4
Urine from urinary tract infections in hospitalized patients	25	10	40
Pus from burns	15	7	46
Sputum from Respiratory tract infections in hospitalized patients	10	3	30
Blood cultures from septicaemias	7	3	42.8
Total	100	46	46

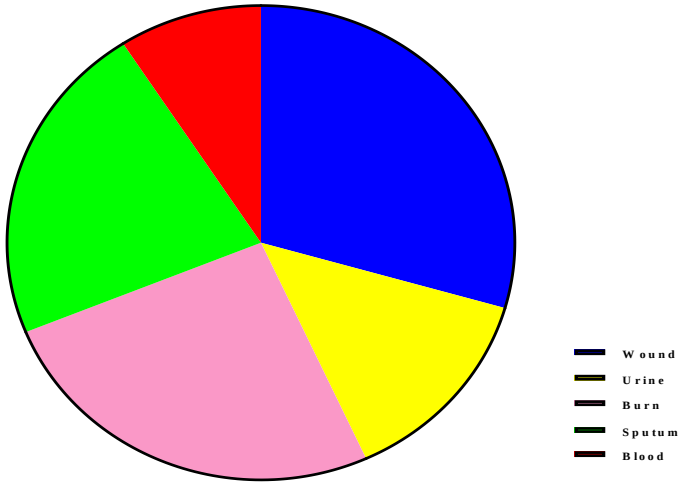


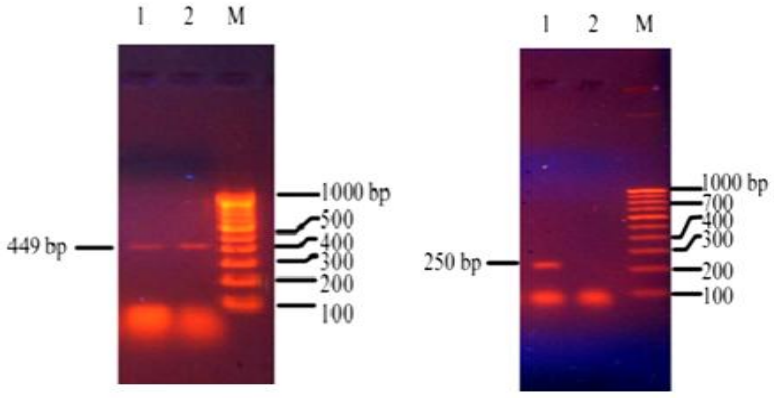
Figure 2. Percentage prevalence of MRSA by sample origin

3.3.2 PCR Assay

The results of PCR showed that the protection of enterotoxin genes, which play a major role in the pathogenicity of *S. aureus*. In the current study, the *mec*A and *tsst* genes were detected in all 100 isolates of *S.aureus*. Of 100 *S. aureus* isolates, 46 isolates (46%) were found to be *mec*A positive and 15 isolates (15%) were positive for the *tsst* gene. All primers produced amplicons of predicted sizes is shown in **Table 3.** The PCR products of *mecA* and tsst genes are shown in **Fig 3 a & b.** Detection of *mecA* gene by PCR has been found as potentially sensitive method and confirms the MRSA status of the isolate[23].The presence of gene *tsst* is also responsible for resistance against *S. aureus*[24]. Totally, 100 *S. aureus* isolates were examined by PCR in which 11 isolates carried both *mec*A and *tsst* genes which is responsible for the resistance against MRSA. From the experimental results and related literature[25], it was concluded that the presence of two virulence genes *mec*A and *tsst* confers the competitive advantage of resistance upon *S. aureus*. Hence, the 11 isolates were selected for anti-MRSA activity.

Table 3. The primer sequences and predicted sizes used in the PCR

Gene	Oligonucleotide sequences (5' - 3')	Size of amplified product (bp)
mecA	5' - CACTTGGTATATCTTCACC - 3'	449 bp
	5 - CTCAGGTACTGCTATCCACC - 3'	
tsst	5'-AGCAGGGCTATAATAAGGACTC-'3	250 bp
	5AAGCCCTTTGTTGCTTGCGAC-3'	



(a) **(b)**

Figure 3. PCR amplification of *mec*A (a) and *tsst* (b) genes

3.3.3 Antibacterial activity against MRSA (Agar-well diffusion method)

The compound **13** was screened against 11 MRSA isolates by agar-well diffusion assay at three different concentrations (25, 50 and 100 µg/ml). The highest level of inhibition of zone was found to be 15.2 mm (100 µg/ml) and the lowest level of inhibition was found to be 7.8 mm (25 µg/ml). The zone of inhibition of the compounds is listed in **Table 4**. The zone of inhibition was maximum against urine isolates of *S. aureus* than the others such as sputum, blood, burn and wound isolates **(Figure 4)**. Compound **13** (15.2 mm) showed comparable activity against MRSA isolates with standard drug ampicillin that is a 6-bromohexyl derivative containing amino group as well as pyridinium nuclei.

It is worth noting that the presence of a polar aminopyridinium head group and the long lipophilic carbon chain could give rise to surfactant like activity. This surfactant like character may be helpful in achieving successful penetration into the lipid cell membrane and better biological activity for the compounds[1]. Pyridinium salts have previously been reported to have anti-MRSA activity[26].

Table 4. Anti-MRSA activity of 4-amino 1-(6-bromohexyl)pyridinium bromide (13) expressed as inhibition zones (mm)

Isolate	Compound 13			Standard
	25 µg	50 µg	100 µg	30 µg
W Sa5	7.8±0.03*	10.5±0.03	13.3±0.06	20.2±0.05
WSa8	8.5±0.03*	11.5±0.03	14.7±0.05	21.5±0.03
WSa12	8.2±0.03*	12.2±0.03	13.4±0.03	20.3±0.05
WSa27	9.2±0.03	11.1±0.03	12.7±0.06	20.9±0.03
WSa41	7.8±0.03*	10.7±0.03	15.2±0.06	21.2±0.05
WSa46	8.1±0.03*	11.3±0.03	14.4±0.03	21.5±0.03
USa64	8.7±0.03*	12.4±0.03	12.5±0.06	19.8±0.05
BSa67	9.5±0.03	12.7±0.03	12.4±0.03	20.5±0.03
bSa85	9.2±0.03*	12.6±0.05	14.2±0.03	19.3±0.03
SSa92	8.7±0.03*	11.8±0.03	12.0±0.03	18.8±0.03
SSa100	9.7±0.03*	12.6±0.08	13.8±0.03	19.5±0.03

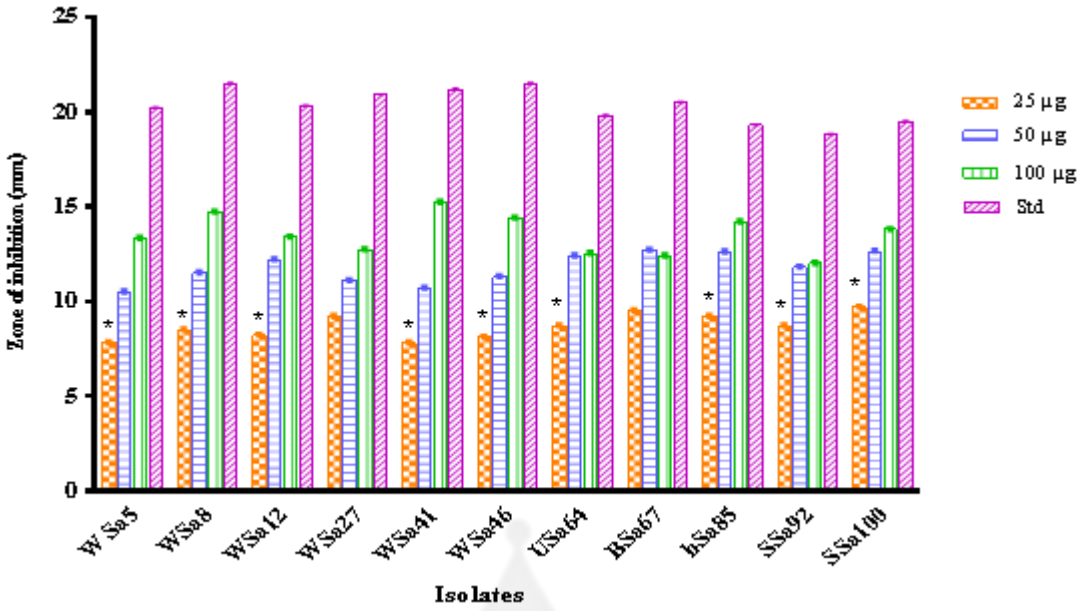


Figure 4. Anti-MRSA activity of Compound 15 expressed as inhibition zones (mm)

4. CONCLUSION

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. A series of 1-aryl/alkyl 4-substituted aminopyridinium bromides were synthesized were synthesized in good yield and evaluated for *in vitro* antimicrobial activity. Results of antimicrobial study revealed that all the synthesized compounds showed significant antimicrobial activity against tested microbial strains. Among the synthesized pyridinium bromides, compound **13** possessed highest antimicrobial activity and evaluated for anti-MRSA activity. The emergence of MRSA organisms with reduced susceptibility to many antibiotics is a serious and ongoing concern.

The compounds **13** has shown significant antibacterial activity against methicillin resistant *Staphylococcus aureus*, which suggests that they may be members of a promising new class of pyridinium anti-MRSA agents. These compounds present a good starting point for modelling antimicrobial activity.

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