

Synthesis and antimicrobial activity of N-alkyl substituted *p*-methyl (*E*)-3- and 4-azachalconium bromides

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Twenty new N-alkyl substituted *p*-methyl (*E*)-3- and 4-azachalcones (**1a-j**, **2a-j**) {3-[(1*E*)-3-(4-methyl-phenyl)-3-oxoprop-1-en-1-yl]-1-alkyl (C_{5-12, 14-15}) pyridinium bromides (**1a-j**) and 4-[(1*E*)-3-(4-methyl-phenyl)-3-oxoprop-1-en-1-yl]-1-alkyl (C_{5-12, 14-15}) pyridinium bromides (**2a-j**)} were synthesized and tested for antimicrobial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Proteus vulgaris*, and *Escherichia coli*. They showed good antimicrobial activity especially against the gram-positive bacteria tested with minimal inhibitory concentration (MIC) values less than 4.7 μg/mL in most cases. The optimum length of the alkyl chain for better and broader activity is situated in the range of 8-12 carbon atoms in the series of compounds **1a-j**, **2a-j**. The non-alkylated compounds **1-2** were not effective as were the ones alkylated with 14 or 15 C alkyl groups (**1i**, **1j**, **2i**, **2j**). N-Alkyl derivatives of *p*-methyl (*E*)-3-azachalcone (**1a-h**) showed better activity in comparison to those of *p*-methyl (*E*)-4-azachalcone (**2a-h**). The antimicrobial activity increased as the length of the alkyl substitution increased from 5 to 12 carbons.

Key Words: N-Alkyl *p*-Methyl-(*E*)-3-, 4-azachalconium bromides; antimicrobial activity.

Introduction

Chalcones are a group of natural compounds possessing a broad spectrum of biological activity.^{1,2} Chalcones are reported to display antimicrobial, anticancer, antichinivirus, antipicornavirus, insecticidal, and bacteriostatic

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activities,³⁻⁹ and also act as an antibiotic.¹⁰ The analogues of chalcones with an annular nitrogen atom in the A or B phenyl rings are called azachalcones.^{3,4,11-18} Over the last 25 years, azachalcones and their N-alkyl derivatives have been synthetically prepared by several investigators^{3,4,7-9} and shown to possess a wide variety of biological activities, such as antituberculostatic, antimicrobial, anti-inflammatory, and antibacterial potentials.^{3,4,13-15,19-24}

In our previous work, nitro substituted (*E*)-3-, and 4-azachalcones, and their N-alkyl derivatives were synthesized and showed very good antimicrobial activities especially against gram-positive bacteria.¹³⁻¹⁶ In view of the continuing interest in new antimicrobial agents, we synthesized other N-alkyl(C_{5-12,14,15}) substituted *p*-methyl-(*E*)-3- and 4-azachalconium bromides in this respect. We aimed to determine the influence of the length of the carbon chain in the N-alkyl substituent and position of N in the pyridyl ring. The attempts to N-alkylate the *p*-methyl (*E*)-2-azachalcone was unsuccessful probably because of the steric effects.

The present work deals with the synthesis, spectral characterization, and antimicrobial activity of 20 new N-alkyl substituted *p*-methyl (*E*)-3- and 4-azachalcones (**1a-j**, **2a-j**) (Scheme).

Experimental

General

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in CDCl₃. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrometer. The elemental analyses were performed on a Leco CHNS 932 instrument. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-400 cm⁻¹) spectrophotometer. Melting points were determined using a Thermo-var apparatus fitted with a microscope and are uncorrected. UV-Vis spectral analyses were carried out on a Unicam UV2-100 spectrophotometer at 25 °C. Thin-layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F₂₅₄ analytical aluminum plates. Column chromatography studies were carried out on silica gel.

Materials and methods

p-Methylacetophenone and 3-/4-pyridine carboxaldehydes were purchased from Aldrich/Merck and used without further purification. The solvents used (chloroform, *n*-hexane, ethanol, methanol, acetonitrile, and diethyl ether) were either of analytical grade or bulk solvents distilled before use. The known compounds **1-2** were prepared according to the literature.^{3,4,13-16}

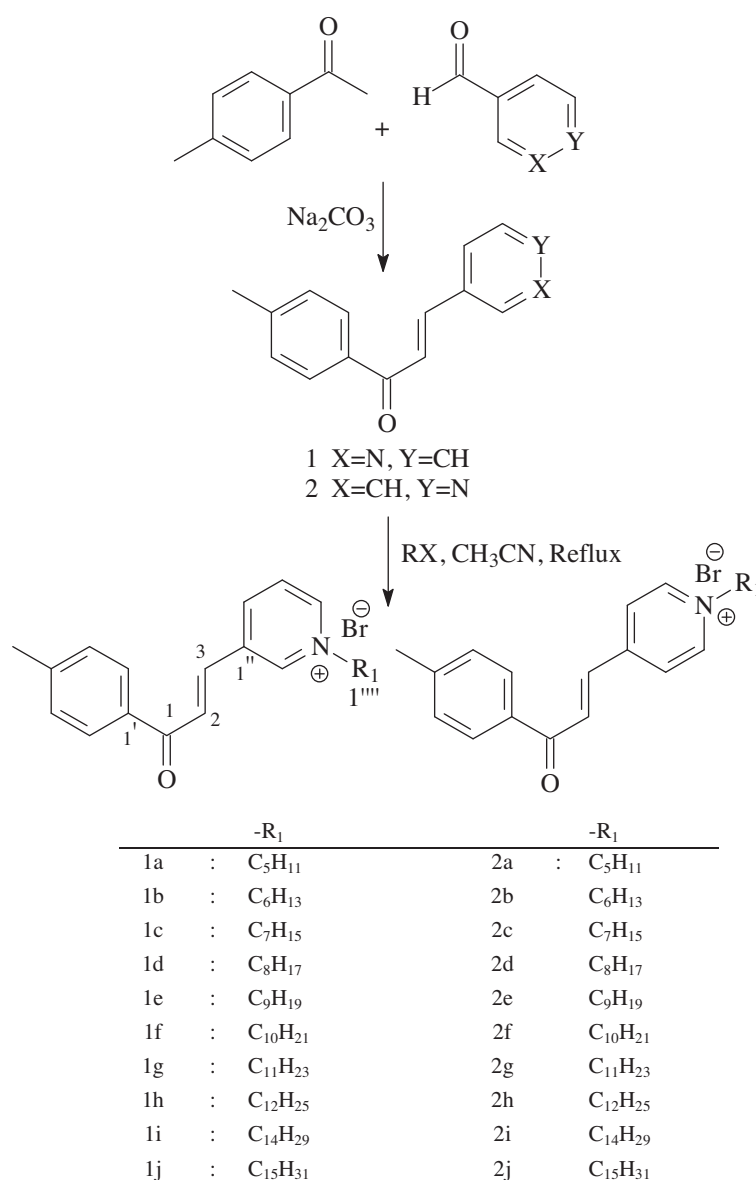
General procedure for synthesis of compounds **1a-j**, **2a-j**

p-Methyl (*E*)-3- or 4-azachalcones (0.02 mol each) and *n*-bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, 1-bromotetradecane, and 1-bromopentadecane (0.05 mol each) in acetonitrile (30 mL) were refluxed separately for 6-16 h.^{4,16,17} The reactions were followed and monitored by TLC. After the reactions were completed, the acetonitrile was removed using a rotary evaporator and the residues were separated by column chromatography on silica gel (30 × 2 cm, ~25 g each, Merck, 230-400 mesh) first with ethyl acetate (30 mL) and then with ethyl

acetate-methanol (3:1, 20 mL and 3:2, 20 mL), methanol (30 mL), and finally methanol-water (4:1, 30 mL). Fractions (5-10 mL each) were collected and monitored by analytical TLC. The desired dark red amorphous solids (**1a-j**, **2a-j**) were obtained from fractions 10-18 (yields are in Table 1).

3-[(1*E*)-3-(4-methylphenyl)-3-oxoprop-1-en-1-yl]-1-alkyl(C_{5-12,14-15})pyridinium bromides, 1a-j

See the physico-chemical, and ¹H- and ¹³C-NMR data of compounds **1a-j** in Tables 1-3.



Scheme

Table 1. Physico-chemical data of compounds **1a-j**, **2a-j**.

| Comp. | IR, cm ⁻¹ C=O | Formula | LC-MS/MS (%) | | Yield (%) | mp (°C) | UV-Vis λ _{nm} (log ε) | TLC ^a (R _f) | Elemental analyses (%) ^b | | |
|-------|-----------------------------|--------------------------------------|-------------------------------------|-------------------------------------|-----------|---------|--------------------------------|---------------------------------------|-------------------------------------|-----------|-----------|
| | | | [M(⁷⁹ Br)] ⁺ | [M(⁸¹ Br)] ⁺ | | | | | C | H | N |
| 1a | 1663 | C ₂₀ H ₂₄ BrNO | 374(32) | 376(45) | 68 | 105-107 | 312(3.7) | 0.40 | 64.17/64.20 | 6.46/6.46 | 3.74/3.73 |
| 1b | 1666 | C ₂₁ H ₂₆ BrNO | 388(62) | 390(44) | 60 | 112-114 | 311(4.2) | 0.37 | 64.95/64.96 | 6.75/6.74 | 3.61/3.62 |
| 1c | 1665 | C ₂₂ H ₂₈ BrNO | 402(28) | 404(26) | 85 | 110-112 | 313(4.1) | 0.41 | 65.67/65.67 | 7.01/7.04 | 3.48/3.56 |
| 1d | 1666 | C ₂₃ H ₃₀ BrNO | 416(32) | 418(10) | 77 | 71-73 | 312(4.1) | 0.46 | 66.34/66.34 | 7.26/7.25 | 3.36/3.36 |
| 1e | 1664 | C ₂₄ H ₃₂ BrNO | 430(10) | 432(08) | 69 | 109-111 | 313(3.7) | 0.42 | 66.97/66.97 | 7.49/7.48 | 3.25/3.26 |
| 1f | 1666 | C ₂₅ H ₃₄ BrNO | 444(06) | 446(07) | 75 | 79-81 | 312(4.1) | 0.50 | 67.56/67.57 | 7.71/7.72 | 3.15/3.16 |
| 1g | 1666 | C ₂₆ H ₃₆ BrNO | 458(32) | 460(10) | 59 | 115-117 | 312(4.2) | 0.51 | 68.11/68.20 | 7.91/7.92 | 3.06/3.15 |
| 1h | 1637 | C ₂₇ H ₃₈ BrNO | 472(24) | 474(15) | 65 | 114-116 | 307(4.1) | 0.51 | 68.63/68.62 | 8.11/8.23 | 2.96/2.99 |
| 1i | 1667 | C ₂₉ H ₄₂ BrNO | 500(42) | 502(25) | 62 | 118-120 | 311(4.1) | 0.54 | 69.59/69.59 | 8.46/8.49 | 2.80/2.80 |
| 1j | 1666 | C ₃₀ H ₄₄ BrNO | 514(28) | 516(66) | 70 | 89-91 | 311(4.4) | 0.58 | 70.02/70.03 | 8.62/8.62 | 2.72/2.72 |
| 2a | 1663 | C ₂₀ H ₂₄ BrNO | 374(42) | 376(18) | 63 | 169-171 | 377(3.6) | 0.45 | 64.17/64.44 | 6.46/6.46 | 3.74/3.74 |
| 2b | 1663 | C ₂₁ H ₂₆ BrNO | 388(26) | 390(32) | 53 | 181-183 | 333(3.8) | 0.45 | 64.95/64.97 | 6.75/6.75 | 3.61/3.62 |
| 2c | 1662 | C ₂₂ H ₂₈ BrNO | 402(68) | 404(18) | 62 | 173-174 | 325(4.1) | 0.40 | 65.67/65.67 | 7.01/7.02 | 3.48/3.48 |
| 2d | 1663 | C ₂₃ H ₃₀ BrNO | 416(24) | 418(28) | 71 | 170-172 | 332(3.9) | 0.45 | 66.34/66.34 | 7.26/7.27 | 3.36/3.38 |
| 2e | 1663 | C ₂₄ H ₃₂ BrNO | 430(18) | 432(22) | 64 | 160-162 | 326(3.9) | 0.45 | 66.97/66.96 | 7.49/7.48 | 3.25/3.27 |
| 2f | 1660 | C ₂₅ H ₃₄ BrNO | 444(32) | 446(08) | 73 | 146-148 | 328(3.6) | 0.46 | 67.56/67.56 | 7.71/7.72 | 3.15/3.05 |
| 2g | 1663 | C ₂₆ H ₃₆ BrNO | 458(16) | 460(48) | 58 | 152-154 | 328(4.1) | 0.44 | 68.11/68.42 | 7.91/7.93 | 3.06/3.06 |
| 2h | 1662 | C ₂₇ H ₃₈ BrNO | 472(12) | 474(08) | 72 | 141-143 | 326(3.7) | 0.50 | 68.63/68.64 | 8.11/8.13 | 2.96/2.97 |
| 2i | 1663 | C ₂₉ H ₄₂ BrNO | 500(15) | 502(34) | 58 | 156-158 | 324(3.9) | 0.50 | 69.59/69.35 | 8.46/8.47 | 2.80/2.88 |
| 2j | 1663 | C ₃₀ H ₄₄ BrNO | 514(18) | 516(42) | 63 | 144-146 | 326(4.0) | 0.50 | 70.02/70.12 | 8.62/8.83 | 2.72/2.76 |

^a Ethyl acetate-methanol (5:1)

^b First number calculated and second number found values for C, H, and N.

Table 2. ¹H-NMR data of compounds **1a-j**, **2a-j** (δppm, CDCl₃)^{a,b}.

| Comp. | H ₂ | H ₃ | H ₂ , H ₆ | H ₃ , H ₅ | H ₂ ^u , H ₄ ^r or H ₂ ^v , H ₆ | H ₅ ^v , H ₆ ^r or H ₃ ^v , H ₅ ^v | N-CH ₂ - | -(CH ₂) _n | Ph-CH ₂ -CH ₃ |
|-------|----------------|----------------|---------------------------------|---------------------------------|---|--|---------------------|----------------------------------|-------------------------------------|
| 1a | 7.7 | 8.5 | 8.2 | 7.2 | 10.5, s, 9.4, d, 6.0 | 8.2, dd, 8.8, d, 8.0 | 5.1, t, 7.4 | 2.1, m, 2H, 1.4, m, 4H | 2.4, s, 0.8, t, 6.2 |
| 1b | 7.7 | 8.5 | 8.2 | 7.3 | 10.5, s, 9.3, d, 5.8 | 8.2, dd, 8.8, d, 8.2 | 5.1, t, 7.4 | 2.1, m, 2H, 1.3, m, 6H | 2.4, s, 0.8, t, 6.8 |
| 1c | 7.7 | 8.5 | 8.2 | 7.3 | 10.6, s, 9.3, d, 5.6 | 8.2, dd, 8.8, d, 8.0 | 5.1, t, 7.4 | 2.1, m, 2H, 1.3, m, 8H | 2.4, s, 0.8, t, 6.8 |
| 1d | 7.7 | 8.6 | 8.2 | 7.3 | 10.5, s, 9.2, d, 6.0 | 8.1, dd, 8.7, d, 7.8 | 5.1, t, 7.4 | 2.2, m, 2H, 1.3, m, 10H | 2.4, s, 0.8, t, 6.4 |
| 1e | 7.7 | 8.5 | 8.2 | 7.3 | 10.6, s, 9.3, d, 5.6 | 8.2, dd, 8.7, d, 8.0 | 5.1, t, 7.0 | 2.1, m, 2H, 1.3, m, 12H | 2.4, s, 0.8, t, 6.0 |
| 1f | 7.7 | 8.5 | 8.2 | 7.3 | 10.5, s, 9.3, d, 6.0 | 8.1, dd, 8.7, d, 8.0 | 5.1, t, 7.0 | 2.1, m, 2H, 1.2, m, 14H | 2.4, s, 0.9, t, 6.4 |
| 1g | 7.7 | 8.5 | 8.2 | 7.3 | 10.4, s, 9.2, d, 5.6 | 8.1, dd, 8.7, d, 7.8 | 5.1, t, 7.4 | 2.1, m, 2H, 1.2, m, 16H | 2.4, s, 0.9, t, 6.8 |
| 1h | 7.7 | 8.5 | 8.2 | 7.3 | 10.5, s, 9.2, d, 5.8 | 8.1, dd, 8.7, d, 8.1 | 5.1, t, 7.0 | 2.1, m, 2H, 1.2, m, 18H | 2.4, s, 0.9, t, 6.8 |
| 1i | 7.7 | 8.6 | 8.3 | 7.3 | 10.5, s, 9.0, d, 6.0 | 8.0, dd, 8.6, d, 8.0 | 5.1, t, 7.4 | 2.1, m, 2H, 1.2, m, 22H | 2.4, s, 0.9, t, 6.4 |
| 1j | 7.7 | 8.6 | 8.3 | 7.3 | 10.5, s, 9.1, d, 6.0 | 8.0, dd, 8.6, d, 8.0 | 5.1, t, 7.0 | 2.1, m, 2H, 1.2, m, 24H | 2.4, s, 0.9, t, 6.4 |
| 2a | 7.7 | 8.3 | 7.9 | 7.3 | 8.5, d, 6.4 | 9.6, d, 6.4 | 4.9, t, 7.4 | 2.1, m, 2H, 1.4, m, 4H | 2.4, s, 0.9, t, 6.4 |
| 2b | 7.7 | 8.3 | 8.8 | 7.3 | 8.6, d, 6.4 | 9.5, d, 6.4 | 4.9, t, 7.4 | 2.1, m, 2H, 1.3, m, 6H | 2.4, s, 0.8, t, 6.8 |
| 2c | 7.7 | 8.3 | 8.1 | 7.3 | 8.5, d, 6.8 | 9.5, d, 6.8 | 4.9, t, 7.2 | 2.1, m, 2H, 1.3, m, 8H | 2.4, s, 0.8, t, 6.4 |
| 2d | 7.7 | 8.3 | 8.1 | 7.3 | 8.5, d, 6.4 | 9.5, d, 6.4 | 4.9, t, 7.0 | 2.0, m, 2H, 1.2, m, 10H | 2.4, s, 0.8, t, 6.0 |
| 2e | 7.7 | 8.3 | 8.1 | 7.3 | 8.6, d, 6.8 | 9.5, d, 6.8 | 4.9, t, 7.0 | 2.1, m, 2H, 1.2, m, 12H | 2.4, s, 0.8, t, 6.2 |
| 2f | 7.7 | 8.2 | 8.1 | 7.3 | 8.4, d, 6.4 | 9.4, d, 6.4 | 5.0, t, 7.4 | 2.0, m, 2H, 1.3, m, 14H | 2.4, s, 0.9, t, 6.0 |
| 2g | 7.8 | 8.3 | 8.1 | 7.3 | 8.6, d, 6.2 | 9.6, d, 6.2 | 5.0, t, 6.2 | 2.1, m, 2H, 1.3, m, 16H | 2.4, s, 0.9, t, 6.0 |
| 2h | 7.7 | 8.3 | 8.1 | 7.3 | 8.5, d, 6.8 | 9.5, d, 6.8 | 4.9, t, 7.2 | 2.1, m, 2H, 1.2, m, 18H | 2.4, s, 0.9, t, 6.0 |
| 2i | 7.7 | 8.3 | 8.1 | 7.3 | 8.6, d, 6.4 | 9.6, d, 6.4 | 5.0, t, 6.8 | 2.1, m, 2H, 1.2, m, 22H | 2.4, s, 0.9, t, 6.0 |
| 2j | 7.7 | 8.3 | 8.1 | 7.3 | 8.6, d, 6.4 | 9.6, d, 6.4 | 5.0, t, 7.2 | 2.1, m, 2H, 1.2, m, 24H | 2.4, s, 0.9, t, 6.0 |

^a Assignment based on ¹H, ¹H-¹H COSY, and comparison with ACD NMR program.

^b J (Hz): H₂:d (~15.4-15.8), H₃:d (~15.4-15.8), H₂:d (~8.0), H₃:d (~8.0), n: 3-13.

4-[(1E)-3-(4-methylphenyl)-3-oxoprop-1-en-1-yl]-1-alkyl(C_{5-12,14-15})pyridinium bromides, 2a-jSee the physico-chemical, and ¹H- and ¹³C-NMR data of compounds **2a-j** in Tables 1, 2, and 4.**Antimicrobial activity and microbial strains**

The compounds were tested individually against 6 microbial species, 4 gram-positive, and 2 gram-negative bacteria. The bacterial strains, obtained from the American Type Culture Collection (ATCC), were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Proteus vulgaris* (ATCC 13315), and *Escherichia coli* (ATCC 25922). All the synthesized compounds were weighed and dissolved in methanol to prepare sample stock solutions of 300 µg/mL.

Table 3. ¹³C-NMR (δ_C ppm) data of compounds **1a-j** in CDCl₃.

| CNo. | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1h | 1i | 1j |
|-------------------------------------|----------------|-------------------------|----------------------------------|----------------------------------|---|---|---|--|---|---|
| 1 | 187.88 | 188.40 | 188.04 | 188.24 | 188.0 | 188.07 | 188.22 | 188.18 | 187.96 | 187.90 |
| 2 | 135.74 | 136.15 | 135.87 | 136.16 | 135.81 | 136.04 | 136.13 | 136.08 | 135.91 | 135.87 |
| 3 | 133.78 | 134.89 | 134.42 | 134.41 | 134.39 | 134.32 | 134.43 | 134.36 | 133.90 | 133.86 |
| 1' | 132.90 | 134.23 | 134.78 | 134.10 | 133.86 | 133.98 | 134.10 | 134.03 | 132.44 | 133.01 |
| 2' / 6' | 129.12 | 129.57 | 129.32 | 129.43 | 129.40 | 129.58 | 129.45 | 129.47 | 129.77 | 129.27 |
| 3' / 5' | 129.54 | 129.75 | 129.69 | 129.67 | 129.60 | 129.96 | 129.69 | 129.63 | 132.44 | 129.61 |
| 4' | 145.50 | 145.01 | 144.70 | 144.91 | 144.66 | 144.84 | 144.93 | 144.87 | 145.33 | 145.50 |
| 1'' | 132.93 | 130.14 | 130.27 | 130.10 | 130.09 | 130.38 | 130.05 | 130.05 | 129.38 | 130.08 |
| 2'' | 150.82 | 144.32 | 144.53 | 144.33 | 144.45 | 144.58 | 144.32 | 144.53 | 145.33 | 145.11 |
| 4'' | 145.50 | 143.92 | 144.17 | 144.06 | 144.07 | 144.00 | 144.80 | 143.97 | 144.50 | 144.18 |
| 5'' | 126.60 | 128.74 | 128.30 | 128.35 | 128.31 | 128.25 | 128.37 | 128.28 | 127.24 | 126.71 |
| 6'' | 145.50 | 143.79 | 143.86 | 143.88 | 143.85 | 143.75 | 143.88 | 143.80 | 145.33 | 143.97 |
| 1''' | 61.37 | 61.98 | 61.95 | 61.95 | 61.97 | 61.98 | 61.99 | 61.97 | 61.79 | 61.51 |
| 2''' | 31.41 | 32.18 | 31.91 | 32.02 | 31.91 | 32.75 | 32.13 | 32.06 | 31.90 | 31.75 |
| (CH ₂) _n ''' | 28.49 21.61 | 22.50 31.27 31.27 | 22.29 25.81 28.54 31.29 | 22.43 28.93 28.93 31.54 | 22.37 25.87 28.89 29.11 31.53 | 22.57 26.03 29.04 29.15 29.39 | 22.66 26.11 29.13 29.29 29.38 29.54 31.86 | 22.62 26.06 29.08 29.27 29.54 31.83 | 22.68 26.12 29.09 29.36 29.67 | 22.53 25.99 28.98 29.20 29.50 |
| -CH ₃ ''' | 13.83 | 14.11 | 13.82 | 14.07 | 13.87 | 14.03 | 14.13 | 14.08 | 14.13 | 13.99 |
| Ph-CH ₃ | 22.08 | 21.93 | 21.60 | 21.83 | 21.57 | 21.72 | 21.80 | 21.75 | 21.83 | 21.67 |

^a Assignment based on APT and comparison with ACD NMR program.

Table 4. ^{13}C -NMR (δ_{C} ppm) data of compounds **2a-j** in CDCl_3 .

| C No. | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 2h | 2i | 2j |
|-------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1 | 187.99 | 187.80 | 187.84 | 187.81 | 187.79 | 187.88 | 187.74 | 187.81 | 188.10 | 187.88 |
| 2 | 135.91 | 135.96 | 135.78 | 137.76 | 135.57 | 135.79 | 135.71 | 135.76 | 136.08 | 135.86 |
| 3 | 133.10 | 132.93 | 132.99 | 132.90 | 133.22 | 133.24 | 132.82 | 132.91 | 133.19 | 133.00 |
| 1' | 133.94 | 133.74 | 133.79 | 133.73 | 133.74 | 133.95 | 133.67 | 133.76 | 134.05 | 133.84 |
| 2' / 6' | 129.32 | 129.31 | 129.18 | 129.19 | 129.31 | 129.36 | 129.08 | 129.18 | 129.45 | 129.24 |
| 3' / 5' | 129.70 | 129.66 | 129.54 | 129.49 | 129.66 | 129.80 | 129.42 | 129.52 | 129.78 | 129.58 |
| 4' | 145.23 | 145.01 | 145.03 | 145.01 | 145.01 | 145.40 | 144.91 | 145.03 | 145.27 | 145.08 |
| 1'' | 150.97 | 150.76 | 150.83 | 150.75 | 150.76 | 151.11 | 150.67 | 150.78 | 151.04 | 150.83 |
| 2'' / 6'' | 126.72 | 126.70 | 126.64 | 126.61 | 126.74 | 126.59 | 126.59 | 126.62 | 126.93 | 126.69 |
| 3'' / 5'' | 145.23 | 144.87 | 144.89 | 144.96 | 144.84 | 145.16 | 144.87 | 144.93 | 145.09 | 144.77 |
| 1''' | 61.54 | 61.30 | 61.40 | 61.37 | 61.36 | 61.79 | 61.74 | 61.40 | 61.67 | 61.50 |
| 2''' | 31.46 | 31.56 | 31.67 | 31.63 | 31.64 | 31.88 | 31.53 | 31.64 | 31.93 | 31.73 |
| (CH ₂) _n ''' | 22.07 | 22.08 | 22.27 | 22.31 | 22.33 | 22.65 | 22.32 | 22.43 | 22.69 | 22.51 |
| | 28.01 | 25.47 | 25.83 | 25.86 | 25.86 | 26.12 | 25.81 | 25.91 | 26.17 | 25.98 |
| | | 30.85 | 28.49 | 28.77 | 28.89 | 29.06 | 28.80 | 28.88 | 29.17 | 28.97 |
| | | | 31.26 | 31.40 | 29.05 | 29.21 | 28.95 | 29.09 | 29.37 | 29.18 |
| | | | | | 31.48 | 29.33 | 29.06 | 29.15 | 29.66 | 29.48 |
| | | | | | 31.54 | 29.42 | 29.20 | 29.29 | 29.66 | 29.48 |
| | | | | | 31.80 | | 29.34 | | | |
| -CH ₃ ''' | 13.76 | 13.68 | 13.80 | 13.82 | 13.84 | 14.12 | 13.81 | 13.90 | 14.16 | 13.96 |
| Ph-CH ₃ | 21.76 | 21.55 | 21.61 | 21.58 | 21.57 | 21.85 | 21.50 | 21.59 | 21.85 | 21.65 |

^aAssignment based on APT and comparison with ACD NMR program.

Determination of the in vitro antimicrobial activity by the microwell dilution assay

The minimum inhibitory concentration (MIC) of all the test compounds was determined by microwell dilution assay. The inocula of the bacterial strains were prepared from 12-h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The compounds dissolved in methanol were first diluted to the highest concentration (300 $\mu\text{g}/\text{mL}$) to be tested, and then serial 2-fold dilutions were made to obtain a concentration range from 2.3 to 300 $\mu\text{g}/\text{mL}$ in 1-mL sterile test tubes containing Mueller-Hinton broth (MHB). The MIC values of the synthetic compounds against bacterial strains were determined on the basis of a microwell dilution method.²⁵⁻²⁷ The 96-well plates were prepared by dispensing 100 μL of MHB containing the inoculum into each well. One hundred microliters from the stock solutions of synthetic compounds prepared at the 300 $\mu\text{g}/\text{mL}$ concentration was added to the first wells. Then 100 μL from the serial dilutions was transferred into the 7 consecutive wells. The last well, containing 200 μL of MHB without the test compound and with the inoculum on each strip, was used as a negative control. The final volume in each well was 200 μL . Ampicillin at a concentration range of 300-0.12 $\mu\text{g}/\text{mL}$ was prepared in MHB and used as a standard drug for positive control.

The plate was covered with a sterile plate sealer. The contents of each well were incubated at 37 °C for 24 h. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm using the spectrophotometer (Molecular Devices, SpectraMax M2) and confirmed by plating 10- μ L samples from each well on Mueller-Hinton agar (MHA) medium. The compounds tested in this study were screened twice against each organism. The results are shown in Table 5.

Results and discussion

The Scheme illustrates the synthetic approach chosen for the preparation of *p*-methyl (*E*)-3- and 4-azachalcones (**1a-j**, **2a-j**) [5-6, 12-23] by Claisen-Schmidt condensation¹³⁻¹⁶ of an appropriate *p*-methylacetophenone and

Table 5. Minimum inhibitory concentrations (MIC, μ g/mL) of the compounds **1a-j**, **2a-j** based on dilution assay.

| Comp. No. | Stock conc. (μ g/mL) | Minimum inhibitory concentrations (MIC, μ g/mL) | | | | | |
|-----------|---------------------------|---|----------------|----------------|----------------|----------------|----------------|
| | | <i>Sa</i> (G+) | <i>Se</i> (G+) | <i>Bs</i> (G+) | <i>Ef</i> (G+) | <i>Pv</i> (G-) | <i>Ec</i> (G-) |
| 1 | 300 | - | - | - | - | - | - |
| 2 | 300 | - | - | - | - | - | - |
| 1a | 300 | 9.4 | < 2.3 | - | - | - | - |
| 1b | 300 | 9.4 | < 4.7 | 37.5 | 9.4 | 75 | - |
| 1c | 300 | < 4.7 | < 4.7 | 9.4 | < 4.7 | 37.5 | 37.5 |
| 1d | 300 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | 37.5 | 37.5 |
| 1e | 300 | 9.4 | 9.4 | 9.4 | 9.4 | 37.5 | 37.5 |
| 1f | 300 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | 9.4 | 18.75 |
| 1g | 300 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | 9.4 | - |
| 1h | 300 | < 4.7 | < 4.7 | < 4.7 | - | - | - |
| 1i | 300 | - | - | - | - | - | - |
| 1j | 300 | - | - | - | - | - | - |
| 2a | 300 | 75 | 75 | 300 | 150 | 75 | - |
| 2b | 300 | 18.75 | 37.5 | 37.5 | 37.5 | 18.75 | - |
| 2c | 300 | 18.75 | 18.75 | 37.5 | 18.75 | 18.75 | - |
| 2d | 300 | < 4.7 | < 4.7 | 9.4 | < 4.7 | 9.4 | 18.75 |
| 2e | 300 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | 9.4 |
| 2f | 300 | < 4.7 | < 4.7 | 9.4 | 9.4 | 9.4 | 18.75 |
| 2g | 300 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | < 4.7 | - |
| 2h | 300 | - | < 4.7 | < 4.7 | < 4.7 | - | - |
| 2i | 300 | - | - | - | - | - | - |
| 2j | 300 | - | - | - | - | - | - |
| Amp. | 300 | 0.12 | 0.5 | 0.5 | 0.5 | 0.5 | 2.0 |

Sa: Staphylococcus aureus, Se: Staphylococcus epidermidis, Bs: Bacillus subtilis, Ef: Enterococcus faecalis, Pv: Proteus vulgaris, Ec: Escherichia coli, Amp.: Ampicillin, (-): no activity.

3- or 4-pyridinecarboxaldehydes with 2 equivalents of Na₂CO₃ solution (EtOH, 95%), which yields the *trans*-isomer of the corresponding α,β -unsaturated ($J=15.5/15.6$ Hz, respectively) compounds (**1-2**).^{3,4,11-18}

N-Alkyl derivatives of *p*-methyl (*E*)-3- or 4-azachalcones were synthesized from the corresponding azachalcones with *n*-bromoalkanes (1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane, 1-bromoundecane, 1-bromododecane, 1-bromotetradecane, and 1-bromopentadecane) in acetonitrile solution by reflux.^{3,4,13-15} In the ¹H-NMR spectra of **1a-j**, **2a-j**, the characteristic -CH₂- signal of N-alkyl groups was exhibited at δ 4.9-5.1 ppm (2H, t, $J=6.2-7.4$ Hz) (Table 2) due to pyridinium salt.⁴

N-Alkyl derivatives of azachalcones attract widespread interest because many of them have exhibited a wide variety of biological activities.^{3,4,13-15,20-24} All the synthesized compounds (**1a-j**, **2a-j**) were characterized on the basis of spectral data analyses (¹H, ¹³C, APT, ¹H-¹H COSY NMR, ACD-NMR, FT-IR, UV, LC-MS/MS, and elemental analysis), whose results were in agreement with the proposed structures (Tables 1-4). The LC mass spectra of **1a-j**, **2a-j** exhibited molecular ion peaks for [M(⁷⁹Br)]⁺ and [M(⁸¹Br)]⁺ (Table 1) and the base ion peaks were [M(⁷⁹Br)-79]⁺ and [M(⁸¹Br)-81]⁺ with other fragment ions such as [M(⁷⁹Br)-R]⁺ and [M(⁸¹Br)-R]⁺. Based upon the above observations, the complete chemical shift assignments for **1a-j**, **2a-j** were deduced to be 3-[(1*E*)-3-(4-methylphenyl)-3-oxoprop-1-en-1-yl]-1-alkyl (C_{5-12,14,15}) pyridinium bromides (**1a-j**) and 4-[(1*E*)-3-(4-methylphenyl)-3-oxoprop-1-en-1-yl]-1-alkyl (C_{5-12,14,15}) pyridinium bromides (**2a-j**).

The antimicrobial activity of compounds **1**, **2**, **1a-j**, **2a-j** was determined against *S. aureus*, *S. epidermidis*, *B. subtilis*, *E. faecalis*, *P. vulgaris*, and *E. coli* (Table 5). The activities of the synthesized compounds were investigated by the dilution method.²⁵⁻²⁷ Some compounds showed slight to pronounced antimicrobial activity against gram-positive and gram-negative bacteria. The non-alkylated compounds **1-2** were not effective as were the ones alkylated with 14 or 15 C alkyl groups (**1i**, **1j**, **2i**, **2j**). The derivatives with 8-12 alkyl groups were active against the bacteria, and their activity was more pronounced against the gram-positive bacteria compared to the gram-negative ones. Nowakowska et al. have previously shown a similar trend in activity with respect to the bacterial type with 3'-hydroxy-substituted and unsubstituted N-alkyl derivatives of 4-azachalcones.⁴ N-Alkyl derivatives of 3-azachalcone (**1a-h**) showed better activity in comparison to those of 4-azachalcone (**2a-h**). Compound **1h**, C₁₂ alkylated 3-azachalcone, was interestingly inactive against *E. faecalis*, while its 4-azachalcone analogue (**2h**) showed inhibitory activity at the lowest test concentration. Similarly, compound **2h** was inactive against *S. aureus*, while its 3-azachalcone analogue (**1h**) showed inhibitory activity at the lowest test concentration. The solvent control methanol showed no inhibition effect on all test microorganisms. The antimicrobial activity increased as the length of the alkyl substitution increased from 5 to 10, and similar findings have been observed by Nowakowska et al. for N-bromoalkyl derivatives of 4-azachalcones.^{3,4}

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References

1. Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*, edited by Agrawal, P. K., Elsevier, New York, 1989, pp. 365-431.
2. Harborne, J. B. *The Flavonoids. Advances in Research*, Chapman & Hall, London, 1988, pp. 329-388.
3. Nowakowska, Z.; Wyrzykiewicz, E.; Kedzia, B. *Il Farmaco* **2001**, *56*, 325-329.
4. Nowakowska, Z.; Wyrzykiewicz, E.; Kedzia, B. *Il Farmaco* **2002**, *57*, 657-661.
5. Nissan Chemical Industries Ltd, Japan Kokai, Tokkyo Koho J P 58,08,035; Chem. Abstr. **1983**, *98*, 178947a.
6. Swallow, D.L. (Ed.) *Progress in Drug Research*, Birkhouser Verlag, Basel, **1984**, p. 140.
7. Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; Vaya, J. *Phytochemistry* **2004**, *65*, 1389-1395.
8. Ishitsuka, H.; Ohasawa, C.; Ohiwa, T.; Umeda, T.; Suhara, Y. *Antimicrob. Agents Chemother.* **1982**, *22*, 611-616.
9. Schraufstatter, E. *Experientia* **1948**, *4*, 484-485.
10. Dhar, D.N. (Ed.) *The Chemistry of Chalcones and Related Compounds*, Wiley, New York, 1981, p. 214.
11. Nowakowska, Z. *Magn. Reson. Chem.* **2000**, *38*, 382-383.
12. Jovanović, B. Z.; Vuković, M. M.; Marinković, A. D.; Csanádi, J. *J. Molec. Struc.* **1999**, *482-483*, 371-374.
13. Yaylı, N.; Üçüncü, O.; Yaşar, A.; Küçük, M.; Yaylı, N.; Akyüz, E.; Karaoğlu, Ş. A. *Turk. J. Chem.*, **2006**, *30*, 505-514.
14. Yaylı, N.; Küçük, M.; Üçüncü, O.; Yaşar, A.; Yaylı, N.; Karaoğlu, Ş. A. *J. Photochem. Photobiol. A: Chem.* **2007**, *188*, 161-168.
15. Usta, A.; Yaşar, A.; Yılmaz, N.; Güleç, C.; Yaylı, N.; Karaoğlu, Ş. A.; N. Yaylı, *Helv. Chim. Acta* **2007**, *90*, 1482-1490.
16. Yaylı, N.; Yaşar, A.; Üçüncü, O.; Sivrikaya, S. Ö.; Güleç, C.; Küçük, M.; Abbasov, R. *J. Photochem. Photobiol. A: Chem.* **2005**, *171*, 291-298.
17. Yaylı, N.; Üçüncü, O.; Yaşar, A.; Gök, Y.; Küçük, M.; Kolaylı, S. *Turk. J. Chem.* **2004**, *28*, 515-521.
18. Yaylı, N.; Üçüncü, O.; Yaşar, A.; Küçük, M.; Yaylı, N. Burnaz, N. A. Karaoğlu, Ş. A.; Küçük, M. *J. Photochem. Photobiol. A: Chem.*, **2009**, *203*, 85-91.
19. Edwards, M. L.; Stemerick, D. M.; Sabol, J. S.; Diekema, K.A.; Dinerstein, R. J. *J. Med. Chem.* **1994**, *37*, 4357-4362.
20. Zamocka, J. *Pharmazie* **1993**, *48*, 857-859.
21. Szajda, M.; Kedzia, B. *Pharmazie* **1991**, *46*, 745-746.
22. Wyrzykiewicz, E.; Bartkowiak, G.; Nowakowska, Z.; Kedzia, B. *Farmaco* **1993**, *48*, 979-988.
23. Tomcufcik, A.; Wilkinson, R.; Child, G. Ger. Offen Patent **1974**, *2*, 502, 490. Chem. Abstr. **1975**, *83*, P. 179067r.
24. Rao, M. N.; Naidoo, L.; Ramanan, P. N. *Pharmazie* **1991**, *46*, 542-543.
25. Amelia, A.; Almeida, P.; Farah, A.; Silva, D. A. M.; Nunan, E. A.; Gloria, B. A. *J. Agric. Food Chem.* **2006**, *54*, 8738-8743.
26. Demirbağ, Z.; Beldüz, A. O.; Sezen, K.; Nalçacıoğlu, R. *Kükem Dergisi* **1997**, *20*, 47-53.
27. Willanova, P. A. *NCCLS Document M7-A313 (25)*, National Committee for Clinical Laboratory Standards, USA, **1993**.