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Synthesis of methyl (*E*)-2′,4″-thiazachalcones and their *N*-alkyl derivatives, photochemistry with theoretical calculations and antimicrobial activities

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A series of 9 new (*E*)-thiazachalcones **(1-3)**, and their *N*-alkyl substituted derivatives **(4-6)**, and stereoselective dimerization products **(7-9)** were synthesized, then tested for antimicrobial activity against all test microorganisms except *Pseudomonas aeruginosa*. The new compounds **(1-6)** without dimerization products **(7-9)** showed good antimicrobial property against *Staphylococcus aureus*, *Listeria monocitogenes*, and *Enterococcus faecalis*. The possible dimerization products of compounds **(1-3)** were calculated theoretically. Experimental and theoretical calculation showed that *δ-truxinic* type dimer is the most stable isomer.

Key Words: Thiazachalcones, *N*-decyl-4-thiazaclaconium bromide, photodimerization, antimicrobial activity.

Introduction

Chalcones are medicinally important α,β-unsaturated ketones and constitute a class of naturally occurring substances. These compounds are reported to show insecticidal, antimicrobial, cyctotoxic, anticancer, and

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antihinovirus activities.[1–4] They found numerous applications as pesticides, photoprotectors in plastics, solar creams, and food additives.[5–7] Azachalcones are the derivatives of chalcones with an annular nitrogen atom in the phenyl ring. In recent years, synthesis of azachalcones,[8–14] their *N*-alkyl substituted derivatives,[8–11,14] and their photochemistry[10,14–15] have been studied. Furthermore, the preparation of furan and thiophene analogues of azachalcones have been described,[14,16–21] and some of them possess a wide variety of biological activities, such as antituberculostatic, antimicrobial, antioxidant, and anti-inflammatory potential.[8–12]

In consideration of the antimicrobial properties of azachalcones and its derivatives, we previously reported the synthesis of a series of thiazachalcones, their *N*-alkyl derivatives, and their photodimerization products belonging to the azachalcone family by using Claisen-Schmidt reaction.[14] In this study, a series of new methyl substituted 2′,4″-thiazachalcones, their *N*-alkyl substituted derivatives, and stereoselective dimerization products were synthesized, and tested their antimicrobial activity against test microorganisms.

In our previous work, methyl (*E*)-2′,3″-thiazachalcones, their N-alkyl derivatives, and photochemical dimerization products have been synthesized and evaluated for their antimicrobial activities and they have shown very good antibacterial activities.[14] Thus, in the present work, we focused on the synthesis of analogous methyl (*E*)-2′,4″-thiazachalcones, and their dimerization products.

Experimental

Chemistry

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$. NMR data assignment was based on ^{1}H, ^{13}C, APT, ^{1}H-^{1}H COSY, and ACD NMR program. The mass spectral analyses were carried out on a Micromass Quattro LC-MS/MS spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer 1600 FT-IR (4000-100 cm^{-1}) spectrometer. The elemental analyses were performed on a Costech 4010 CHNS instrument. UV-VIS spectral analyses were carried out on a Unicam UV2-100 spectrophotometer at 25 °C. Melting points were determined using a Thermo-var apparatus fitted with a microscope and are uncorrected. Thin layer chromatography (TLC) was carried out on Merck precoated 60 Kieselgel F_{254} analytical aluminum plates. Preparative TLC was carried out on Merck precoated 60 Kieselgel F_{254} (20 × 20, 0.25 mm) silica gel plates.

2-Acetyl-5-methylthiophene, 2-acetyl-4-methylthiophene, 2-acetyl-3-methylthiophene, and 4-pyridinecarboxaldehyde were purchased from Fluka or Merck and used without further purification. The solvents (chloroform, *n*-hexane, ethanol, methanol, ethyl acetate, acetonitrile, and diethyl ether) used were either of analytical grade or bulk solvents distilled before use.

General procedure for synthesis of compounds (1-3): The heterocyclic thiazachalcones **1-3** were readily prepared by the condensation of the appropriate pyridinecarboxaldehyde and methyl thiophene ketones as described in the literature.[4,10–15] To a cooled solution (∼ 1-5 °C) of sodium hydroxide (0.8 g, 20 mmol) in 50 mL dist. water was added 2-acetyl-3-, 4-, or 5-methyl thiophene (1.40 g, 10 mmol for each) solution in ethanol drop by drop. The resulting mixture was stirred for 30 min, and then was added 4-pyridinecarboxaldehyde (1.07 g, 10 mmol) drop by drop. After addition was completed, the reaction mixture was stirred at the same temperature (TLC control). The aqueous phase was extracted with $CHCl_3$ (3 × 30 mL). The combined organic

phases were dried over Na_2SO_4 and the solvent was removed using a rotary evaporator. The residue was purified by column chromatography (column, 30 × 2 cm) on silica gel (35 g, Merck, 230-400 mesh). The column was eluted successively with the following solvent and solvent mixture *n*-hexane (50 mL), *n*-hexane-diethyl ether (8:1, 50 mL; 7:2, 50 mL; 6:3, 75 mL; 4:5, 75 mL; 2:7, 50 mL and 1:8, 50 mL), and diethyl ether (50 mL). Fractions (15-20 mL each) were collected and monitored by analytical TLC. The desired products **1-3** were obtained from fractions 4-15 (91%, 80%, and 90% yield), respectively.

(2*E*)-1-(3-methyl-2-thienyl)-3-pyridyl-2-propen-1-one (1): Light yellowish solid (yield: 91%). R_f = 0.40 (diethyl ether-ethyl acetate, 2:1); mp 93-95 °C. UV (λ_{max} nm, $CHCl_3$): 320 (ε, 23435), 282 (ε, 34924); Analysis (Calc/found %): for $C_{13}H_{11}NOS$ C: 68.10/68.20, H: 4.84/5.00, N: 6.11/6.10, S: 13.98/13.90, O: 6.98/6.80; FT-IR ($CHCl_3$) cm^{-1}: 3025 (ν_{sp^2CH}), 2917, 2852 (ν_{sp^3CH}), 1648 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1594, 1542, 1499, 1455, 1415; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.49 (1H, AB, H^2), 7.69 (1H, AB, H^3), 6.87 (1H, d, $H^{4'}$), 7.70 (1H, d, $H^{5'}$), 2.45 (3H, s, thiophene-CH_3), 7.45 (2H, d, $H^{2'',6''}$), 8.67 (2H, bd, $H^{3'',5''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 180.8 (C=O), olephinic CH: [125.5 (CH), 140.2 (CH)], thiophene-C: [151.3 (C), 131.0 (C), 133.1 (CH), 127.2 (CH)], 16.3 (thiophene-CH_3), pyridine-C: [142.0 (C), 122.0 (CH), 150.6 (CH)]. (+)LC-MS/MS: m/z 229,97 $[M+H]^+$.

(2*E*)-1-(4-methyl-2-thienyl)-3-pyridyl-2-propen-1-one (2): Yellowish solid (yield: 80%). R_f = 0.44 (diethyl ether-ethyl acetate, 2:1); mp 84-86 °C; UV (λ_{max} nm, $CHCl_3$): 336 (ε, 17176), 290 (ε, 28015); Analysis (Calc/found %): for $C_{13}H_{11}NOS$ C: 68.10/68.10, H: 4.84/5.30, N: 6.11/6.10, S: 13.98/13.90, O: 6.98 / 6.60; FT-IR ($CHCl_3$) cm^{-1}: 3028 (ν_{sp^2CH}), 2922, 2849 (ν_{sp^3CH}), 1650 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1602, 1594, 1546, 1501, 1421; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.49 (1H, AB, H^2), 7.69 (1H, AB, H^3), 7.31 (1H, bs, $H^{3'}$), 7.68 (1H, bs, $H^{5'}$), 2.35 (3H, s, thiophene-CH_3), 7.45 (2H, bd, $H^{2'',6''}$), 8.66 (2H, bd, $H^{3'',5''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 181.2 (C=O), olephinic CH: [130.7 (CH), 140.5 (CH)], thiophene-C: [144.3 (C), 125.6 (CH), 139.3 (C), 134.4 (CH)], 15.6 (thiophene-CH_3), pyridine-C: [141.9 (C), 122.0 (CH), 150.6 (CH)]. (+)LC-MS/MS: m/z 229.97 $[M+H]^+$.

(2*E*)-1-(5-methyl-2-thienyl)-3-pyridyl-2-propen-1-one (3): Light yellowish solid (yield: 90%). R_f = 0.50 (diethyl ether-ethyl acetate, 2:1); mp 76-78 °C; UV (λ_{max} nm, $CHCl_3$): 330 (ε, 22615), 286 (ε, 24036); Analysis (Calc/found %): for $C_{13}H_{11}NOS$ C: 68.10/68.10, H:4.84/5.30, N: 6.11/6.10, S: 13.98/14.00, O: 6.98/6.50; FT-IR ($CHCl_3$) cm^{-1}: 3025 (ν_{sp^2CH}), 2921, 2851 (ν_{sp^3CH}), 1653 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1604, 1541, 1518, 1400; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.46 (1H, AB, H^2), 7.67 (1H, AB, H^3), 7.52 (1H, d, $H^{3'}$), 7.03 (1H, d, $H^{4'}$), 2.62 (3H, s, thiophene-CH_3), 7.45 (2H, dd, $H^{2'',6''}$), 8.68 (2H, d, $H^{3'',5''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 182.1 (C=O), olephinic CH: [130.4 (CH), 140.2 (CH)], thiophene-C: [146.9 (C), 128.1 (CH), 133.1 (CH), 135.6 (C)], 17.1 (thiophene-CH_3), pyridine-C: [141.9 (C), 121.9 (CH), 150.5 (CH)]. (+)LC-MS/MS: m/z 229.85 $[M+H]^+$.

General procedure for synthesis of compounds (4-6): 3-, 4-, and 5-methyl substituted (*E*)-2′,4″-thiazachalcone (0.0005 mol) and 1-bromodecane (0.005 mol) in acetonitrile (30 mL) were refluxed respectively 35-40 h. Then the acetonitrile was removed using a rotary evaporator. The residue was purified by column chromatography (column, 30x2 cm) on silica gel (25 g, Merck, 230-400 mesh). The column was eluted successively with the following solvent and solvent mixture *n*-hexane (30 mL), *n*-hexane-ethyl acetate (3:1, 120 mL; 7:2, 45 mL), ethyl acetate-methanol (3:1, 120 mL). Fractions (10-15 mL each) were collected and

monitored by analytical TLC. The desired products **4-6** were obtained from fractions 10-20 (85%, 80%, and 80% yield), respectively.

1-Decyl-4-[(1*E*)-3-(3-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide (4): Dark yellow amorphous solid (85% yield). R_f = 0.82 (ethyl acetate-methanol, 3:2); mp 182-184 °C; UV (λ_{max} nm, $CHCl_3$): 364 (ε, 5820), 296 (ε, 14838); Analysis (Calc/found %): for $C_{23}H_{32}BrNOS$ C: 61.32/61.34, H: 7.16/7.16, N: 3.11/3.15, S: 7.12/7.10; FT-IR ($CHCl_3$) cm^{-1}: 3025 (ν_{sp^2CH}), 2925, 2854 (ν_{sp^3CH}), 1738 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1638, 1620, 1515, 1455; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.68 (1H, AB, H^2), 8.23 (1H, AB, H^3), 6.91 (1H, d, $H^{4'}$), 8.22 (1H, d, $H^{5'}$), 2.57 (3H, s, thiophene-CH_3), 8.53 (2H, d, $H^{2'',6''}$), 9.27 (2H, d, $H^{3'',5''}$), 4.89 (2H, t, $H^{1'''}$), 1.97 (2H, m, $H^{2'''}$), 1.23 – 1.32 (14H, m, $H^{3'''-9'''}$), 0.86 (3H, t, $H^{10'''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 179.9 (C=O), olephinic CH: [128.1 (CH), 136.2 (CH)], thiophene-C: [152.9 (C), 142.6 (C), 134.9 (CH), 133.3 (CH)], 16.4 (thiophene-CH_3), pyridine-C: [151.2 (C), 126.9 (CH), 144.9 (CH)], alkyl-C: [61.9 (CH_2), 31.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 26.2 (CH_2), 22.7 (CH_2), 14.1 (CH_3)]. (+)LC-MS/MS: m/z 452.32 $[M+2(^{81}Br)]^+$, 451.38 $[M+1(^{79}Br)]^+$.

1-Decyl-4-[(1*E*)-3-(4-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide (5): Dark yellow amorphous solid (80% yield). R_f = 0.79 (ethyl acetate-methanol, 3:2); mp 145-147 °C; UV (λ_{max} nm, $CHCl_3$): 356 (ε, 1915), 292 (ε, 5384); Analysis (Calc/found %): for $C_{23}H_{32}BrNOS$ C: 61.32/61.34, H: 7.16/7.18, N: 3.11/3.10, S: 7.12/7.14; FT-IR ($CHCl_3$) cm^{-1}: 3035 (ν_{sp^2CH}), 2924, 2854 (ν_{sp^3CH}), 1654 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1629, 1602, 1459, 1418; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.69 (1H, AB, H^2), 8.15 (1H, AB, H^3), 7.39 (1H, bs, $H^{3'}$), 8.15 (1H, bs, $H^{5'}$), 2.35 (3H, s, thiophene-CH_3), 8.45 (2H, d, $H^{2'',6''}$), 9.27 (2H, d, $H^{3'',5''}$), 4.88 (2H, t, $H^{1'''}$), 1.76 (2H, m, $H^{2'''}$), 1.18 – 1.26 (14H, m, $H^{3'''-9'''}$), 0.87 (3H, t, $H^{10'''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 180.2 (C=O), olephinic CH: [132.3 (CH), 136.9 (CH)], thiophene-C: [151.2 (C), 133.4 (CH), 140.1 (C), 135.0 (CH)], 15.6 (thiophene-CH_3), pyridine-C: [144.9 (C), 126.7 (CH), 143.7 (CH)], alkyl-C: [61.9 (CH_2), 31.9 (CH_2), 30.0 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 26.1 (CH_2), 22.7 (CH_2), 14.1 (CH_3)]. (+)LC-MS/MS: m/z 452.20 $[M+2(^{81}Br)]^+$, 451.26 $[M+1(^{79}Br)]^+$.

1-Decyl-4-[(1*E*)-3-(5-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide (6): Dark yellow amorphous solid (80% yield). R_f = 0.76 (ethyl acetate-methanol, 3:2); mp 138-140 °C; UV (λ_{max} nm, $CHCl_3$): 344 (ε, 7658), 292 (ε, 19009); Analysis (Calc/found %): for $C_{23}H_{32}BrNOS$ C: 61.32/61.33, H: 7.16/7.16, N: 3.11/3.16, S: 7.12/7.10; FT-IR ($CHCl_3$) cm^{-1}: 3010 (ν_{sp^2CH}), 2917, 2851 (ν_{sp^3CH}), 1650 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1598, 1513, 1459, 1400; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.68 (1H, AB, H^2), 7.76 (1H, AB, H^3), 7.05 (1H, d, $H^{3'}$), 7.61 (1H, d, $H^{4'}$), 2.64 (3H, s, thiophene-CH_3), 8.28 (2H, d, $H^{2'',6''}$), 9.56 (2H, d, $H^{3'',5''}$), 4.96 (2H, t, $H^{1'''}$), 2.04 (2H, m, $H^{2'''}$), 1.22 – 1.32 (14H, m, $H^{3'''-9'''}$), 0.85 (3H, t, $H^{10'''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 180.5 (C=O), olephinic CH: [132.0 (CH), 135.3 (CH)], thiophene-C: [150.6 (C), 133.4 (CH), 134.6 (CH), 135.0 (C)], 17.3 (thiophene-CH_3), pyridine-C: [148.2 (C), 126.2 (CH), 145.6 (CH)], alkyl-C: [61.7 (CH_2), 31.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 26.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3)]. (+)LC-MS/MS: m/z 452.32 $[M+2(^{81}Br)]^+$, 451.32

$[M+1(^{79}Br)]^+$.

General procedure for photodimerizations of 1-3 in solution: Solutions of compounds **1-3** (0.40 g, 0.41 g, and 0.47 g, respectively) in 20-25 mL of acetonitrile, kept in quartz tube, were exposed to UV light

(400 watt high-pressure Hg lamp). The progress of the reactions was followed by silica gel TLC (acetonitrile-ethyl acetate, 1:2). The reactions were stopped after ∼10-12 h. The solutions were evaporated and a portion of the residues (0.200 g, 0.213 g, and 0.258 g, respectively) purified by PTLC (20 × 20 cm, 0.5 mm, 2 plates, each) to give compounds **7-9** (24%, 18%, and 20% yield), respectively.

[2-(3-methylthiophene-2-carbonyl)-3,4-dipyridin-4-yl-cyclobutyl](3-methylthiophen-2-yl) methanone (7): Light brown amorphous solid (24% yield). R_f = 0.54 (chloroform-ethyl acetate-methanol, 1:1:2); mp 47-49 °C; UV (λ_{max} nm, $CHCl_3$): 306 (ε, 52366), 268 (ε, 39733); Analysis (Calc/found %): for $C_{26}H2_2N_2O_2S_2$ C: 68.10/68.05, H: 4.84/4.89, N: 6.11/6.14, S: 13.98/13.96, O: 6.98/6.96; FT-IR ($CHCl_3$) cm^{-1}: 3021 (ν_{sp^2CH}), 2972, 2884 (ν_{sp^3CH}), 1645 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1674, 1597, 1454, 1401; ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.36 (2H, AA′BB′, $H^{1,2}$), 4.00 (2H, AA′BB′, $H^{3,4}$), 6.68 (2H, dd, $H^{4'/4''}$), 7.29 (2H, d, $H^{5'/5''}$), 2.43 (6H, s, thiophene-CH_3), 7.23 (4H, d, $H^{2'''/2'''',6'''/6''''}$), 8.57 (4H, bd, $H^{3'''/3'''',5'''/5''''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): cyclobutane ring-C: [47.4 (CH), 45.0 (CH)], 189.6 (C=O), thiophene-C: [152.1 (C), 140.4 (C), 134.3 (CH), 127.4 (CH)], 16.2 (thiophene-CH_3), pyridine-C: [148.9 (C), 122.4 (CH), 149.9 (CH)]. LC-MS/MS: m/z 459.20 $[M+H]^+$.

[2-(4-methylthiophene-2-carbonyl)-3,4-dipyridin-4-yl-cyclobutyl](4-methyl-thiophen-2-yl) methanone (8): Light brown amorphous solid (18% yield). R_f = 0.44 (chloroform-ethyl acetate-methanol, 1:1:2); mp 78-80 °C; UV (λ_{max} nm, $CHCl_3$): 298 (ε, 11771), 266 (ε, 12257); Analysis (Calc/found %): for $C_{26}H2_2N_2O_2S_2$ C: 68.10/68.08, H: 4.84/4.80, N: 6.11/6.13, S: 13.98/13.94, O: 6.98/7.05; FT-IR ($CHCl_3$) cm^{-1}: 3084 (ν_{sp^2CH}), 2964, 2926 (ν_{sp^3CH}), 1660 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1595, 1559, 1418; ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.34 (2H, AA′BB′, $H^{1,2}$), 4.06 (2H, AA′BB′, $H^{3,4}$), 7.23 (2H, bs, $H^{3'/3''}$), 7.26 (2H, bs, $H^{5'/5''}$), 2.14 (6H, s, thiophene-CH_3), 7.28 (4H, d, $H^{2'''/2'''',6'''/6''''}$), 8.58 (4H, bd, $H^{3'''/3'''',5'''/5''''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): cyclobutane ring-C: [47.9 (CH), 44.6 (CH)], 189.9 (C=O), thiophene-C: [149.5 (C), 131.5 (CH), 139.3 (C), 135.4 (CH)], 15.4 (thiophene-CH_3), pyridine-C: [141.9 (C), 122.4 (CH), 149.9 (CH)]. LC-MS/MS: m/z 459.20 $[M+H]^+$.

[2-(5-methylthiophene-2-carbonyl)-3,4-dipyridin-4-yl-cyclobutyl](5-methyl-thiophen-2-yl) methanone (9): Light brown amorphous solid (20% yield). R_f = 0.49 (chloroform-ethyl acetate-methanol, 1:1:2); mp 75-77 °C; UV (λ_{max} nm, $CHCl_3$): 286 (ε, 20862); Analysis (Calc/found %): for $C_{26}H2_2N_2O_2S_2$ C: 68.10/68.06, H: 4.84/4.86, N: 6.11/6.14, S: 13.98/13.96, O: 6.98/6.98; FT-IR ($CHCl_3$) cm^{-1}: 3026 (ν_{sp^2CH}), 2925, 2854 (ν_{sp^3CH}), 1645 ($\nu_{\alpha,\beta-unsaturated-C=O}$), 1597, 1557, 1519, 1403; ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.34 (2H, AA′BB′, $H^{1,2}$), 3.95 (2H, AA′BB′, $H^{3,4}$), 7.35 (2H, d, $H^{3'/3''}$), 6.94 (2H, d, $H^{4'/4''}$), 2.62 (6H, s, thiophene-CH_3), 7.25 (4H, dd, $H^{2'''/2'''',6'''/6''''}$), 8.56 (4H, bd, $H^{3'''/3'''',5'''/5''''}$); ^{13}C NMR ($CDCl_3$) δ (ppm): cyclobutane ring-C: [49.7 (CH), 45.5 (CH)], 190.8 (C=O), thiophene-C: [149.5 (C), 131.3 (CH), 133.1 (CH), 133.7 (C)], 17.1 (thiophene-CH_3), pyridine-C: [147.6 (C), 122.4 (CH), 150.1 (CH)]. LC-MS/MS: m/z 459.20 $[M+H]^+$.

Theoretical calculations. Theoretical investigations were performed with HYPERCHEM 7.5 program on an IBM PC Pentium IV computer. The HOMO and LUMO energies in the ground state and the HSOMO and LSOMO energies in the exited state were calculated by using the PM3 and PM3-UHF semi-empirical methods.[22−24]

Antimicrobial activity assessment. All test microorganisms were obtained from the Hıfzıssıhha

Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 10145, *Bacillus cereus* 709 Roma, *Listeria monositogenes* ATCC 43251, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Candida tropicalis* ATCC 13803. All the newly synthesized compounds were dissolved in dimethylsulphoxide (DMSO) to prepare chemicals stock solution of 5000-5600 μg/mL.

Determination of minimal inhibitory concentration (MIC). The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (μg/mL) were determined.[25] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (YNB) (Difco, Detroit, MI) at pH 7.0, respectively. Dilution of each chemical substance to be tested was prepared in 0.1 mL volumes of sterile MH and YNB broth to give concentrations ranging from 300 μg/mL to 0.05 μg/mL. After preparation of suspensions of test microorganisms in MH and YNB broth (approximately 10^6 microorganisms per mL), one drop of suspension (0.02 mL) was added to the extract/broth dilutions. After 18 h at 35 °C incubation, the tubes were examined for growth. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and triflucan were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide was used as solvent control.

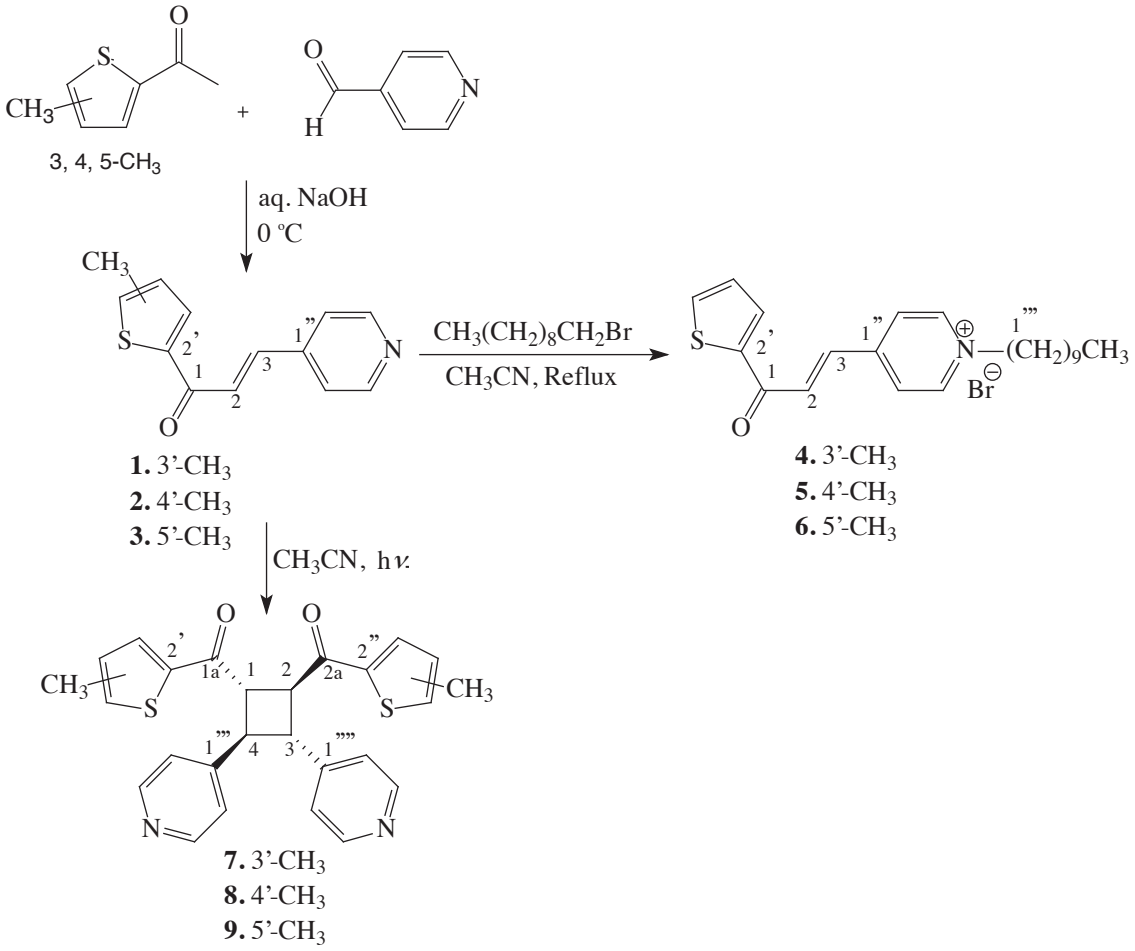
Results and discussion

The aim of this investigation is to synthesize 3-, 4-, and 5-methyl substituted (*E*)-1-decyl-prydinium bromide **(4-6),** and **[2-(3, 4, 5-methylthiophene-2-carbonyl)-3,4-dipyridin-4-yl-cyclobutyl](3, 4-, 5-methylthiophen-2-yl)methanone (7-9)** from 3-, 4-, and 5-methyl substituted (*E*)- 2′,4″-thiazachalcone **(1-3)**, which might show biological activity. The synthetic route is shown in Scheme 1.

The most noticeable feature of structural characterization of 3-, 4-, and 5-methyl substituted (*E*)-2′,4″-thiazachalcone **(1-3)** is the assignment of the ^{1}H resonances of H_α and H_β. The ^3J values of 15.4 / 15.8 / 15.4 Hz of H_α and H_β are consistent with a *trans* relationship at the C=C double bond of the α,β-unsaturated moiety of **1-3**, respectively.[8−15]

The synthesis of *N*-alkyl derivatives of (*E*)-4-azachalcones attracts widespread interest because many of them have exhibited antimicrobial activities.[8−9,13−14] A series of 3 new *N*-alkyl substituted derivatives of 3-, 4-, and 5-methyl substituted (*E*)-2′,4″-thiazachalcone **(4-6)** were synthesized by the reaction of compounds **1-3** with 1-bromodecane in boiling acetonitrile (Scheme 1). The spatial relation of the olefinic H-atoms of $(E)-N$-alkyl-4-azachalconium bromides **(4-6)** was also *E* based on their ^3J values ($^3J_{H\alpha-H\beta}$ = 15.4, 15.8, 15.4 Hz, respectively).[8−15]

3-, 4-, and 5-methyl substituted (*E*)- 2′,4″-thiazachalcone **(1-3)**, when exposed to UV light (400 Watt high-pressure Hg lamp) in acetonitrile, were converted to the respective cyclobutanes **(7-9)** with yields (chromatographed products, PTLC) of 24%, 18%, and 20%, respectively. The structures of the cyclobutyl rings of compounds **7-9** were elucidated from their ^{1}H-NMR spectra, which show 2 symmetrical multiplets, highly shielded, for the CH parts (AA′BB′ system) at δ_H 4.36 (δ_C 47.4) / δ_H 4.00 (δ_C 45.0) for compound **7,** at δ_H 4.34 (δ_C 47.9) / δ_H 4.06 (δ_C 44.6) for compound **8**, at δ_H 4.34 (δ_C 49.7) / δ_H 3.95 (δ_C 45.5) for compound **9**. Simulation of these NMR patterns allowed the calculation of the H,H-coupling constants of the cyclobutane



Scheme 1

ring (J_{AA} = 9.0/9.0/8.8, J_{AB} = 5.6/5.4/5.4, $J_{AB'}$ = 3.4/3.6/3.4, $J_{BB'}$ = 9.2/8.6/8.6, respectively). The size of these coupling constants and the ^{1}H- and ^{13}C-NMR signal patterns of the cyclobutane ring of **7-9** suggest that its formation occurs by *trans*-type 'head-to-head' junction to give the *δ-truxinic* type structure.[10−12,18−22] The residual ^{1}H- and ^{13}C-NMR data of compounds **7-9** are shown in Table 1.

For the photochemical reactions of compounds **1-3,** we have examined the possibility of frontier-orbital control on the stereochemical behavior, and some theoretical calculations were performed to derive their optimized structure. We calculated the ground-state HOMO and LUMO energies, as well as the singlet- and triplet-state HSOMO-LSOMO energies of **1-3** to compare the coefficients and superposition of frontier molecular orbitals by means of the PM3 and PM3-UHF semi-empirical methods. The results of these calculations are collected in Table 2. As can be seen, the frontier orbitals of **1-3** generally allow dimerization through orbital-symmetry and electron-density control. The results show that total superposition occurs between both ground state HOMO and LSOMO of triplet state of the compound **1**, ground state LUMO and HSOMO of singlet state of the compound **2**, and **3**. In excited triplet state and ground state of compound **2** and **3**, electron density of HOMO ($qi = 2c_i^2$) of unsaturated bonds of compounds are too low to have any dimerization reaction occur.

Table 1. NMR data of compounds **(7-9).**

Position	**7**[a,b]		**8**[a,b]		**9**[a,b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1, 2	4.36, AA'BB', 9.0, 5.6, 3.4, 2.2	47.4	4.34, AA'BB', 9.0, 5.4, 3.6, 1.8	47.9	4.34, AA'BB', 8.8, 5.4, 3.4, 2.0	49.7
3, 4	4.00, AA'BB', 9.2, 5.4, 3.4, 2.0	45.0	4.06, AA'BB', 8.6, 5.0, 3.4, 1.6	44.6	3.95, AA'BB', 8.6, 5.6, 3.2, 2.4	45.5
1a, 2a	-	189.6	-	189.9	-	190.8
1'/ 1"	-	152.1	-	149.5	-	149.5
3'/ 3"	-	140.4	7.23, bs	131.5	7.35, d, 4.8	131.3
4'/ 4"	6.68, dd, 3.8, 1.0	134.3	-	139.3	6.94, d, 5.0	133.1
5'/ 5"	7.29, d, 3.8	127.4	7.26, bs	135.4	-	133.7
$-CH_3$	2.43, s	16.2	2.14, s	15.4	2.62, s	17.1
1'''/ 1''''	-	148.9	-	141.9	-	147.6
2'''/ 2''''	7.23, d, 5.8	122.4	7.28, bd, 6.0	122.4	7.25, dd, 6.6, 1.2	122.4
3'''/ 3''''	8.57, bd, 4.6	149.9	8.58, bd, 5.4	149.9	8.56, bd, 5.4	150.1
5'''/ 5''''	8.57, bd, 4.6	149.9	8.58, bd, 5.4	149.9	8.56, bd, 5.4	150.1
6'''/ 6''''	7.23, d, 5.8	122.4	7.28, bd, 6.0	122.4	7.25, dd, 6.6, 1.2	122.4

[a]Assignment based on ^1H, ^{13}C, APT, ^1H-^1H-COSY, and ACD NMR program.

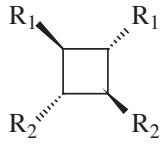
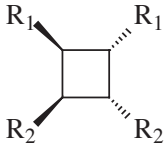
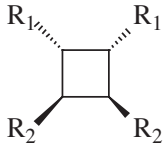
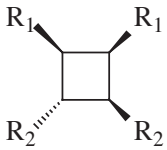
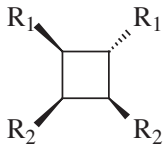
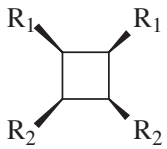
[b]In $CDCl_3$.

Table 2. Relative HOMO/LUMO and HSOMO/LSOMO energies (in eV) and electron coefficients for the α- and β-C-atoms of **(1-3).**

Electronic state	**1**			**2**			**3**		
	S_o	S_1	T_1	S_o	S_1	T_1	S_o	S_1	T_1
HOMO (eV)	-9.67			-9.56			-9.59		
C_α	-0.47			0.02			-0.02		
C_β	-0.31			0.02			-0.02		
LUMO (eV)	-1.20			-1.20			-1.20		
C_α	0.30			0.32			-0.31		
C_β	-0.35			0.36			0.36		
HSOMO (eV)		-4.03	-7.54		-4.47	-7.99		-4.47	-7.69
C_α		-0.01	-0.12		-0.39	0.03		-0.48	-0.18
C_β		0.11	-0.09		-0.46	0.06		0.44	0.04
LSOMO (eV)		-0.84	-0.63		-0.98	-1.13		-0.97	-0.95
C_α		0.43	-0.36		-0.40	-0.43		-0.39	-0.31
C_β		0.38	0.40		0.27	0.41		0.28	0.34

Furthermore, in excited singlet and triplet state of the compound **1,** electron density of HOMO ($qi = 2c_i^2$) of unsaturated bonds of compounds are too low to have any dimerization reaction occur.

Table 3. Calculated heats of formation (in kcal/mol) for different configurations of the "head-to-head" dimmers **(7-9).**

Isomers[a]	-E			$\Delta H^{\neq}$		
	7	**8**	**9**	**7**	**8**	**9**
 **a**	108375.48	108377.80	108376.34	92.02	89.71	91.17
 **b**	108372.62	108358.16	108371.18	94.89	109.34	96.33
 **c**	108358.14	108356.68	108372.75	109.36	110.83	94.75
 **d**	108353.44	108371.51	108371.48	114.06	95.99	96.02
 **e**	108353.22	108355.92	108369.16	114.29	111.58	98.35
 **f**	108342.07	108346.91	108359.49	125.25	120.60	108.02

[a]R_1 = 3-, 4-, or 5-methyl-2-thienoyl, R_2 = 4-pyridyl.

In the case of compound **2** and **3**, the energy of the HOMO-S_0 and LUMO-S_0 are -9.56/9.59 and -1.20/-1.20 eV, and in the first excited singlet state the HSOMO-S_1 and LSOMO-S_1 showed the energy of -4.47/-4.47 and -0.98/-0.97 eV, respectively.

Theoretical calculations were done in order to see compounds **7-9** to be kinetically the most stable isomers. In the photochemical reactions of compounds **1 - 3,** possible eleven different isomers are obtained according to the kinetic theory.[10,22–24] As a result of experimental irradiation of compound **1-3**, isomer **7a-9a** was obtained, respectively. We calculate all possible isomers to show how to closure dimerization of cyclobutane and the energy of the transition state of the ring-closure reactions from the biradical *syn* and *anti* forms, [10,22–24] and results are reported in Table 3. According to the results obtained with semi-empirical method, the most stable of the dimers possible to form, having the lowest strain energy and heat of formation, is head-to-head isomer that has R_1 and R_2 groups in cyclobutane ring at *trans–cis–trans–cis* configuration. The results showed that isomers **7a, 8a,** and **9a** were the most stable isomers of all in this method (Table 3).

The compounds **1-9** were characterized on the basis of spectral data evaluations (^{1}H, ^{13}C, ^{1}H-^{1}H COSY NMR, FT-IR, UV-VIS, EA, and LC-MS/MS), whose results were in agreement with the proposed structures.

The antimicrobial activities of all new compounds **(1-9)** were determined (Table 4). The activities of the synthesized compounds were investigated by broth micro dilution method.[25] The compounds **1-9** showed good antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and a yeast-like fungus.

Table 4. Minimum inhibitory concentrations (MIC, μg/mL) of the synthesized compounds.

Comp. No.	Stock Solution (μg/mL)	Microorganisms and Minimal Inhibition Concentration Value							
		Ec	Yp	Pa	Bc	Li	Sa	Ef	Ct
1	5100	-	-	-	127.5	31.87	7.96	15.9	7.96
2	5400	-	-	-	135	33.75	8.44	16.87	8.44
3	5600	-	-	-	140	17.5	8.75	35	8.75
4	5500	2.15	8.59	-	4.29	0.54	>0.27	1.07	1.07
5	5000	7.81	7.81	-	3.91	1.95	1.95	3.91	0.98
6	5000	1.95	15.63	-	3.91	0.98	0.49	0.98	>0.24
7	5100	-	-	-	127.5	127.5	255	127.5	127.5
8	5000	-	-	-	125	250	250	125	125
9	5000	-	-	-	125	125	250	125	125
Amp.	100	8	32	32	>128	2	2	<1	
Trif.	50								8
DMSO		-	-	-	-	-	-	-	-

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 10145, Bc: *Bacillus cereus* 702 Roma, Li: *Listeria monocitogenes* ATCC 43251, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Ct: *Candida. tropicalis* ATCC 13803. Amp.: Ampicillin, Trif.: Triflucan, (-): stock soln. not active.

Compounds **4-6** exhibited broad-spectrum antimicrobial activity. Compounds **4-6** had MIC values of 3.91-4.29 μg/mL against the food-bound bacterium *Bacillus cereus*. Compounds **4-6** were also highly active against the yeast-like fungus (*C. tropicalis*), and the bacteria with non-sporidical basillus (*L. monocitogenes*) and coccus (*S. aureus*) morphology with the MIC values of $>$0.24-1.95 μg/mL. The compounds **(1-9)** did not show any activity against the *P. aeruginosa*. The solvent control (DMSO) showed no inhibition effect on all test microorganisms.

The newly synthesized compounds, even though at high concentrations, showed antimicrobial activities towards the microorganisms *B. cereus*, *L. monocitogenes*, *E. faecalis*, and *C. tropicalis*, against which no activity was observed in the methyl (*E*)-2′,3″-thiazachalcone series compounds tested in the previous work.[14]

The compounds **1-3** and **7-9** showed better activity against *B. cereus*, *L. monocitogenes*, *E. Faecalis*, and *C. tropicalis* in comparison to their analogues in methyl (*E*)-2′,3″-thiazachalcone series. The N-alkyl derivatives (**4-6**), in comparison to monomers (**1-3**) and dimers (**7-9**), showed better antimicrobial activity against 7 of the tested microorganisms, excluding *P. aeruginosa* against which no test compound was active, a similar trend seen in the previously published work.[14]

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