Synthesis, Configuration, and Antimicrobial Properties of Novel Substituted and Cyclized '2′,3″-Thiazachalcones'

by **Asu Usta**[a]), **Ahmet Yaşar**[a]), **Nagihan Yılmaz**[a]), **Canan Güleç**[a]), **Nuran Yaylı**[a]), **Şengül Alpay Karaoğlu**[b]), and **Nurettin Yaylı***[a])

[a]) Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey (phone: +90-462-377-2486; fax: +90-462-325-3196; e-mail: yayli@ktu.edu.tr)
[b]) Department of Biology, Faculty of Arts and Sciences, Rize University, 53100 Rize, Turkey

Nine new thiazachalcone-based drugs, compounds **1**–**9**, were prepared and fully characterized. The configurations of the photochemical-dimerization products **7**–**9** were rationalized by semi-empirical calculations. Both the experimental data and the theoretical calculations showed that the δ-truxinic acid type dimer is the most stable isomer of all. All compounds were tested for their antibacterial and antifungal activities. The *N*-alkylated congeners **4**–**6** showed strong antimicrobial activities against various bacteria and a yeast-like fungus. The *MIC* and *MBC* values were as low as 0.1 μg/ml. All the compounds were active against the *Gram*-positive bacterium *Staphylococcus aureus*.

Introduction. – The so-called chalcones, *i.e.*, derivatives of 1,3-diphenylprop-2-enone, are natural products known to act as precursors of flavonoids [1] and cyclobutane-containing dimeric products [2][3]. Azachalcones contain an annular N-atom in the phenyl ring, giving rise to a pyridyl moiety. In the past years, the syntheses of azachalcones [4–10] and their *N*-alkyl-substituted derivatives [4–8] have been studied, as well as their photochemistry [6–7][11]. Further, the preparation of furan and thiophene analogues of azachalcones have been described [12–18], and some of them have been found to possess a wide variety of biological activities, including antituberculosis, antimicrobial, antioxidant, anti-inflammatory, and antibacterial properties [4–9].

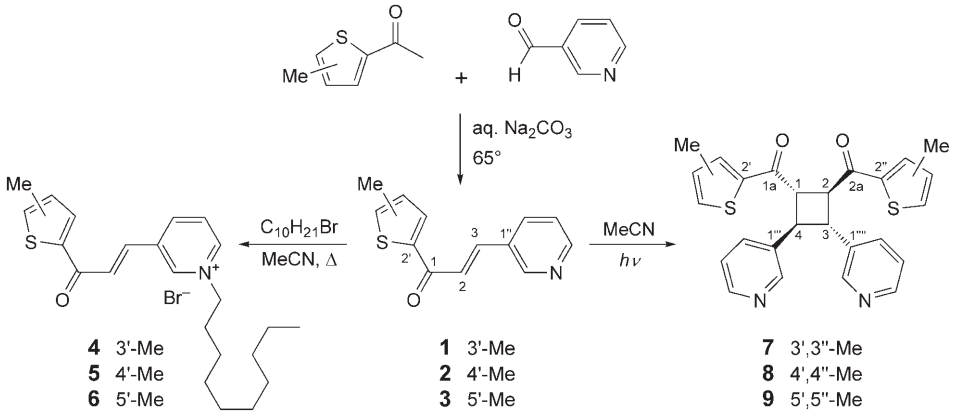
In consideration of the antimicrobial properties of azachalcones and its derivatives, we recently prepared a series of new methyl-substituted 2′,3″-thiazachalcones, and performed some structure–activity-relationship (SAR) studies [7]. The present investigation deals with the synthesis, spectroscopic characterization, and biological activities of a series of new '2′,3″-thiazachalcone' derivatives, compounds **1**–**9** (see *Scheme* below)[1]).

Results and Discussion. – 1. *Synthesis.* The synthesis of compounds **1**–**9**, performed by standard procedures [6–9], is shown in the *Scheme*. All compounds were fully characterized (see *Exper. Part*). The most noticeable feature of the structural characterization of compounds **1**–**3** was the assignment of the ^{1}H-NMR resonances

[1]) For systematic names, see *Exper. Part.*

of the α,β-unsaturated C=C bond. Based on $^3J(\alpha,\beta)$ values of 15.8, 15.8, and 15.4 Hz, respectively, the (*E*)-configuration was assigned to **1**–**3** [4–12].

Scheme



Arbitrary atom numbering

N-Alkyl derivatives of (*E*)-3-azachalcones have attracted widespread interest because of their antimicrobial activities [4–6][10]. We, thus, prepared the *N*-alkylated congeners **4**–**6** from **1**–**3** by reaction with 1-bromodecane in boiling MeCN (*Scheme*). Thereby, the (*E*)-configuration of the conjugated, α,β-unsaturated C=C bond was retained, as indicated by the corresponding ^3J values.

When compounds **1**–**3** were exposed to UV light (400-W high-pressure Hg lamp) in MeCN, the respective cyclobutanes **7**–**9** were obtained in yields of 26, 25, and 17%, respectively, after purification by preparative thin-layer chromatography. Their structures were elucidated from their ^{1}H- and ^{13}C-NMR spectra (*Table 1*), which showed two symmetrical, highly shielded *multiplet*s for the cyclobutane methine groups ($AA'BB'$ system), resonating at δ(H) 4.34 and 3.96 [δ(C) 47.7 and 44.1)], respectively, for compound **7**, with similar chemical shifts for **8** and **9**. Simulation of these NMR patterns allowed the calculation of the following coupling constants for **7**–**9**, respectively: $J(A,A')$ = 8.8, 9.0, 9.0; $J(A,B)$ = 5.4, 5.8, 5.8; $J(A,B')$ = 3.4, 3.2, 3.4; and $J(B,B')$ = 8.6, 9.2, 9.2 Hz. The magnitude of these coupling constants, and the ^{1}H- and ^{13}C-NMR signal patterns for the cyclobutane rings of **7**–**9**, suggest that these compounds were formed by *trans*-type 'head-to-head' junction of the δ-truxinic acid type [6–9][15–18].

2. *Molecular-Orbital Calculations.* For the photochemical reaction of compounds **1**–**3**, we have examined the possibility of frontier-orbital control on the stereochemical outcome, and some theoretical calculations were performed to derive their optimized structures. We calculated the ground-state HOMO–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 [19–21] and PM3-RHF-CI 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

Table 1. *^{1}H- and ^{13}C-NMR Data of* **7**–**9**. Recorded at 200 and 50 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

Position	**7**		**8**		**9**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1, 2	4.34 ($AA'BB'$, J = 8.8, 5.4, 3.4, 2.0)	47.7	4.38 ($AA'BB'$, J = 9.0, 5.8, 3.2, 2.0)	48.3	4.38 ($AA'BB'$, J = 9.0, 5.8, 3.4, 2.4)	50.1
3, 4	3.96 ($AA'BB'$, J = 8.6, 5.4, 3.2, 1.6)	44.1	4.04 ($AA'BB'$, J = 9.2, 5.4, 3.4, 2.0)	44.0	3.95 ($AA'BB'$, J = 9.2, 5.8, 3.4, 2.4)	44.9
1a, 2a		189.7		190.2		190.9
1′, 1″		140.4		142.1		147.3
3′, 3″		151.7	7.25 (br. *s*)	131.3	6.93 (*d*, *J* = 5.0)	131.1
4′, 4″	6.61 (*d*, *J* = 3.6)	127.2		139.2	7.34 (*d*, *J* = 5.0)	132.9
5′, 5″	7.24 (*d*, *J* = 3.6)	134.1	7.25 (br. *s*)	135.4		136.2
Me	2.43 (*s*)	16.0	2.14 (*s*)	15.4	2.61 (*s*)	17.1
1‴, 1⁗		135.9		135.9		133.8
2‴, 2⁗	8.45 (*d*, *J* = 1.6)	148.8	8.53 (br. *s*)	148.9	8.51 (br. *s*)	149.0
4‴, 4⁗	8.47 (*d*, *J* = 6.0)	148.8	8.55 (*d*, *J* = 5.0)	148.9	8.23 (*d*, *J* = 4.0)	148.9
5‴, 5⁗	7.25–7.30 (*m*)	123.6	7.32 (*d*, *J* = 8.0)	123.7	7.28–7.34 (*m*)	123.7
6‴, 6⁗	7.69 (*dt*, *J* = 7.6, 1.6)	134.5	7.76 (*dt*, *J* = 8.0, 1.6)	134.6	7.79 (*dt*, *J* = 8.0, 1.8)	134.7

dimerization through orbital-symmetry and electron-density control. The best interaction occurs between the S_0 and S_1 frontier orbitals due to a low energy gap. In the case of compound **1** and **3**, the relative energies of the HOMO and LUMO S_0 are -9.633 and 9.546 *vs.* -1.141 and -1.100 eV, respectively. In the first-excited singlet state, the HSOMO and LSOMO S_1 orbitals had energies of -4.106 and -3.929 *vs.* -6.663 and -6.618 eV, respectively. Therefore, the best interaction between the above frontier orbitals for **1** and **3** is that between HSOMO S_1 and LUMO S_0.

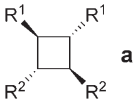
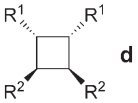
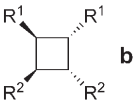
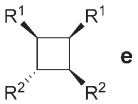
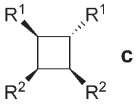
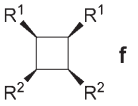
The best superposition of frontier orbitals for compound **2** occurs by interaction of HOMO S_0 and LSOMO S_1. However, the electron density of the HOMO S_0 orbital of **2** is too low to enable dimerization. Thus, the dimerization reaction takes place through the LUMO S_0 and HSOMO S_1 frontier orbitals (*Table 2*).

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_0	S_1	S_0	S_1	S_0	S_1
HOMO	−9.633		−9.547		−9.546	
α-C	−0.48		−0.15		−0.05	
β-C	−0.32		−0.11		−0.04	
LUMO	−1.141		−1.139		−1.100	
α-C	−0.31		−0.32		−0.32	
β-C	0.36		0.37		0.37	
HSOMO		−4.106		−4.116		−3.929
α-C		−0.48		0.48		0.46
β-C		0.38		−0.38		−0.31
LSOMO		−6.663		−6.676		−6.618
α-C		−0.61		−0.61		0.02
β-C		−0.26		−0.26		−0.12

In the dimerization reactions of compounds **1**–**3**, theoretically eleven different isomers can be obtained according to kinetics theory [7][19–21]. We calculated the heat of formation of six 'head-to-head' isomers (**a**–**f**) of compounds **7**–**9** by means of the PM3 semi-empirical method (*Table 3*). Our results indicate that the most stable of the dimers, having the lowest strain energy and heat of formation, is the 'head-to-head' isomer that carries the substituents R^1, R^1, R^2, and R^2 at the cyclobutane ring in a *trans*–*cis*–*trans*–*cis* relation. Thus, compounds **7a**, **8a**, and **9a** were, indeed, identified as the most-stable isomers.

Table 3. *Calculated Heats of Formation* (in kcal/mol) *for Different Configurations of the 'Head-to-Head' Dimers* **7**–**9**

Isomer[a])	$\Delta H^{\neq}$			Isomer[a])	$\Delta H^{\neq}$		
	7	**8**	**9**		**7**	**8**	**9**
 **a**	59.79	56.00	55.82	 **d**	66.24	61.43	64.58
 **b**	64.73	59.33	62.58	 **e**	66.58	63.39	65.01
 **c**	65.07	62.09	61.92	 **f**	76.71	75.54	76.80

[a]) R^1 = 3-, 4-, or 5-methyl-2-thienoyl, R^2 = 3-pyridyl.

3. *Antimicrobial Screening*. The antimicrobial activities of the new compounds **1**–**9** were determined by the broth-microdilution method [22], and the results are collected in *Table 4*. Compounds **1**–**6** showed antimicrobial activities against *Gram*-positive and *Gram*-negative bacteria, and against a yeast-like fungus. Compounds **7**–**9** only showed antimicrobial activities against the *Gram*-positive bacterium *Staphylococcus aureus*. In general, the compounds were more active towards *Gram*-positive bacteria compared to the *Gram*-negative strains. Compounds **4**–**6** exhibited broad-spectrum antimicrobial activities. These three compounds were active against all the test organisms, except for *Pseudomonas aeruginosa*, with minimal-inhibitory concentration (*MIC*) values of 0.1–0.45 μg/ml against the food-bound *Bacillus cereus*, and the same concentration was found to be bactericidal. Compounds **4**–**6** were also highly active against the yeast-like fungus *Candida tropicalis*, and against the bacteria with non-sporicidal bacillus (*Listeria monocytogenes*) and coccus (*Staphylococcus aureus*) morphology, with *MIC* values of 0.2–1.9 μg/ml and minimal-bactericidal concentration (*MBC*) values of 0.8–2.0 μg/ml, respectively. Although the *MIC* and *MBC* values of **4**–**6** were the same for the *Gram*-negative bacteria *Escherichia coli* and *Yersinia pseudotuberculosis*, the *MIC* values for *Enterococcus faecalis* were in the range of 0.9–30 μg/ml, while the corresponding *MBC* values were in the range 3.6–90 μg/ml.

Table 4. *Antimicrobial Activities of* **1**–**9**[a]).

Compound	Stock soln. [μg/ml]	*MIC* [μg/ml][b])							
		Ec	Yp	Pa	Bc	Li	Sa	Ef	Ct
1	2600	n.a.[c])	n.a.	n.a.	n.a.	60	4.0	n.a.	16.2
2	2400	n.a.	n.a.	n.a.	n.a.	60	7.5	n.a.	120
3	3000	n.a.	n.a.	n.a.	4.6	9.3	4.6	n.a.	4.6
4	2400	3.8	120	n.a.	0.45	0.23	0.23	30	1.9
5	2600	2.0	4.0	n.a.	0.25	0.12	0.6	0.3	0.5
6	2500	2.0	7.8	n.a.	0.1	0.2	0.2	0.9	0.4
7	3200	n.a.	n.a.	n.a.	n.a.	n.a.	160	n.a.	n.a.
8	3000	n.a.	n.a.	n.a.	n.a.	n.a.	150	n.a.	n.a.
9	3000	n.a.	n.a.	n.a.	n.a.	n.a.	75	n.a.	n.a.
Ampicillin	100	8	32	32	>128	2	2	<1	
Triflucan	50								8
DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a]) Abbreviations: Ec, *Escherichia coli*; Yp, *Yersinia pseudotuberculosis*; Pa, *Pseudomonas aeruginosa*; *Bc*, *Bacillus cereus*; Li, *Listeria monocytogenes*; Sa, *Staphylococcus aureus*; Ef, *Enterococcus faecalis*; Ct, *Candida tropicalis*. [b]) Minimal-inhibitory activity. [c]) Stock soln. not active.

Compounds **1**–**9** did not show any activity against the *Pseudomonas aeruginosa*. Compared to the findings of an earlier study with analogous azachalcones lacking a thiophenyl ring, compounds **1**–**6** exhibited better antimicrobial activities, especially against *Gram*-negative bacteria [7]. Further, compounds **4**–**6** were active against *Yersinia pseudotuberculosis*, in contrast to previous analogues lacking the thiophenyl ring [7]. Similarly, compounds **1** and **2** were active against *Staphylococcus aureus*, while their analogues without a thiophenyl ring were previously shown to be inactive [7]. The solvent control (DMSO) showed no inhibition effect on all test microorganisms under the experimental conditions.

Conclusions. – A series of new '2′,3″-thiazachalcones' and some dimerized derivatives thereof, compounds **1**–**9**, were synthesized and tested against seven different bacteria and one fungal strain. Most compounds showed significant antimicrobial activities, better than those observed for some previously studied congeners lacking a thiophenyl ring [7]. *N*-Alkylation of the pyridyl ring with a decanyl chain was found to be essential for activity, which, otherwise, was either low or even non-existing, indicating that *N*-alkylation increases the drugs' permeability of the bacterial cell wall. Thus, the *Gram*-positive bacteria were more susceptible to the *N*-alkylated compounds **4**–**6**, probably because of their more-structured cell wall peptidoglycan network and the lack of a lipopolysaccharide layer.

The cyclized congeners **7**–**9** exhibited strongly decreased activities against the *Gram*-positive bacteria and the tested fungal strain, as in the case of the analogues lacking a thiophenyl moiety [7]. There was an effect of the position of the Me group at the thiophenyl ring: methylation at the 3′-position gave rise to a reduced activity in general. Compounds **4**–**6** exhibited broad-spectrum antimicrobial activity.

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Experimental Part

General. 2-Acetyl-5-methylthiophene, 2-acetyl-4-methylthiophene, 2-acetyl-3-methylthiophene, and pyridine-3-carbaldehyde were purchased from *Fluka* or *Merck*, and used without further purification. The solvents $CHCl_3$, hexane, EtOH, MeOH, AcOEt, MeCN, and Et_2O were either of anal. grade or bulk solvents distilled before use. Anal. TLC: *Merck* precoated *Kieselgel 60 F_{254}* aluminum plates. Prep. TLC: *Merck* precoated *Kieselgel 60 F_{254}* plates ($20 \times 20 \times 0.25$ mm). Melting points (m.p.): *Thermo-var* apparatus fitted to a microscope; uncorrected. UV/VIS Spectra: *Unicam UV2-100* spectrophotometer; at 25°; λ (log ε) in nm. IR Spectra: *Perkin-Elmer 1600* FT-IR apparatus; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian Mercury* apparatus, at 200 and 50 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. NMR Assignments are based on ^1H-, ^{13}C-, APT, and $^1H,^1H$-COSY experiments, in combination with spectra simulation using the *ACD* software. Mass spectra (MS): *Micromass Quattro* LC-MS/MS apparatus; in m/z. Elemental analyses: *Costech 4010 CHNS* instrument.

General Procedure (*GP 1*) *for the Synthesis of* **1**–**3**. The heterocyclic thiazachalcones **1**–**3** were readily prepared by condensation of the appropriate pyridinecarboxaldehyde with methyl thiophene ketones, as described in the literature [6–12]. Briefly, to a soln. of Na_2CO_3 (0.53 g, 10 mmol) in dist. H_2O (50 ml) was added at 65° pyridine-3-carbaldehyde (1.07 g, 10 mmol) drop by drop. The resulting mixture was stirred for 15 min. Then, a soln. of the corresponding 2-acetyl-*n*-methylthiophene ($n = 3–5$; 1.40 g, 10 mmol) in EtOH (3 ml) was added drop by drop. When the addition was complete, the mixture was stirred at 65° for 40 min (TLC control). The aq. phase was extracted with $CHCl_3$ (3×30 ml), the combined org. phases were dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was purified by column chromatography (CC) (35 g *Merck* SiO_2, 230–400 mesh; 30×2 cm column), eluting successively with hexane (50 ml); hexane/Et_2O 8:1 (50 ml), 7:2 (75 ml), 6:3 (75 ml), 4:5 (75 ml), 2:7 (50 ml), 1:8 (50 ml); and Et_2O (50 ml). Fractions (15–20 ml each) were collected and monitored by anal. TLC. The desired products were obtained from fractions *3–9*.

*(2*E*)-1-(3-Methylthiophen-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one* (**1**). Light-yellowish solid. Yield: 81%. M.p. 103–105°. TLC (Et_2O/AcOEt 4:1): R_f 0.38. UV ($CHCl_3$): λ_{max} 322 (4.81), 286 (4.83); λ_{min} 312 (4.79). FT-IR: 3052, 2919, 2853, 1651, 1597, 1587, 1567, 1478, 1424, 1343, 1306 1237, 1065, 998, 798, 768, 692, 569. ^1H-NMR ($CDCl_3$, 200 MHz): 8.81 (*d*, $J = 1.6$, H–C(2″)); 8.59 (*dd*, $J = 5.0$, 1.6, H–C(4″)); 7.92 (*dt*, $J = 7.8$, 1.6, H–C(6″)); 7.74 (*AB*, $J = 15.8$, H–C(3)); 7.71 (*d*, $J = 4.0$, H–C(5′)); 7.44 (*AB*, $J = 15.8$, H–C(2)); 7.33 (*dd*, $J = 7.8$, 4.8, H–C(5″)); 6.84 (*dd*, $J = 4.0$, 1.0, H–C(4′)); 2.53 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz): 180.5 (C(1)); 150.6 (C(2″)); 150.5 (C(3′)); 149.6 (C(4″)); 142.7 (C(1′)); 139.0 (C(3)); 134.2 (C(6″)); 132.7 (C(2)); 130.2 (C(1″)); 126.8 (C(4′)); 123.4 (C(5″)); 122.9 (C(5′)); 15.8 (Me). MS (pos.): 230 (100, $[M+H]^+$), 166 (5), 149 (12), 132 (8), 105 (9). Anal. calc. for $C_{13}H_{11}NOS$ (229.30): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.27, H 4.52, N 6.44, O 6.57, S 14.20.

*(2*E*)-1-(4-Methylthiophen-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one* (**2**). Yield: 82%. Light-yellowish solid. M.p. 98–100°. TLC (Et_2O/AcOEt 4:1): R_f 0.44. UV ($CHCl_3$): λ_{max} 326 (4.48), 280 (4.80); λ_{min} 321 (4.44). FT-IR: 3040, 2957, 2925, 1650, 1592, 1582, 1563, 1458, 1421, 1329, 1305 1208, 1151, 989, 977, 862, 805, 760, 690, 626, 577. ^1H-NMR ($CDCl_3$, 200 MHz): 8.86 (*d*, $J = 1.2$, H–C(2″)); 8.63 (*dd*, $J = 4.6$, 1.2, H–C(4″)); 7.95 (*dt*, $J = 7.8$, 1.2, H–C(6″)); 7.81 (*AB*, $J = 15.8$, H–C(3)); 7.70 (*d*, $J = 1.0$, H–C(5′)); 7.45 (*AB*, $J = 15.8$, H–C(2)); 7.37 (*dd*, $J = 7.8$, 4.6, H–C(5″)); 7.32 (br. *s*, H–C(3′)); 2.34 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz): 181.3 (C(1)); 150.1 (C(2″)); 149.9 (C(4″)); 144.5 (C(1′)); 139.9 (C(3)); 139.2 (C(4′)); 134.6 (C(6″)); 134.2 (C(2)); 130.5 (C(1″)); 130.4 (C(5′)); 123.8 (C(5″)); 123.5 (C(3′)); 15.6 (Me). MS (pos.): 252 (95, $[M+Na]^+$), 229 (5, M^+), 215 (100, $[M-Me+H]^+$), 197 (15), 138 (17), 120 (18), 107 (5). Anal. calc. for $C_{13}H_{11}NOS$ (229.30): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.11, H 4.87, N 6.05, O 6.90, S 14.07.

*(2*E*)-1-(5-Methylthiophen-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one* (**3**). Yield: 76%. Light-yellowish solid. M.p. 73–75°. TLC (Et_2O/AcOEt 4:1): R_f 0.48. UV ($CHCl_3$): λ_{max} 328 (4.36), 286 (4.34); λ_{min} 303 (4.32). FT-IR: 3042, 2967, 2921, 1653, 1598, 1565, 1515, 1473, 1401, 1378, 1303, 1206, 1038, 997, 936, 804,

776, 714, 693, 586. ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.83 (br. *s*, H–C(2″)); 8.61 (br. *d*, $J = 5$, H–C(4″)); 7.92 (*dt*, $J = 7.6, 1.8$, H–C(6″)); 7.75 (*AB*, $J = 15.4$, H–C(3)); 7.48 (*d*, $J = 5.0$, H–C(4′)); 7.38 (*AB*, $J = 15.4$, H–C(2)); 7.35 (*dd*, $J = 7.6, 5.0$, H–C(5″)); 7.00 (*d*, $J = 5.0$, H–C(3′)); 2.64 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 50 MHz): 182.3 (C(1)); 151.0 (C(2″)); 150.0 (C(4″)); 146.6 (C(1′)); 139.6 (C(3)); 135.8 (C(5′)); 134.5 (C(6″)); 133.1 (C(2)); 130.6 (C(1″)); 130.1 (C(4′)); 126.0 (C(3′)); 123.8 (C(5″)); 17.1 (Me). MS (pos.): 230 (38, $[M+H]^+$), 212 (22), 193 (25), 168 (22), 132 (100), 125 (68), 108 (26). Anal. calc. for $C_{13}H_{11}NOS$ (229.30): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.16, H 4.97, N 6.13, O 7.13, S 13.61.

General Procedure (*GP 2*) *for the Synthesis of Compounds* **4**–**6**. A soln. of the respective starting material (**1**–**3**; 0.5 mmol) and 1-bromodecane (0.52 mmol) in MeCN (15 ml) was heated at reflux for 12–36 h (TLC control). The solvent was removed *in vacuo*, and the residue was purified by CC (25 g basic Al_2O_3 (*Panreac*), 30 × 2 cm column), eluting successively with hexane (30 ml), hexane/AcOEt 8:1 (45 ml) and 7:2 (45 ml), and AcOEt/MeOH 3:1 (120 ml). Fractions (10–15 ml each) were collected and monitored by TLC. The desired products were obtained from fractions *9*–*15*.

*1-Decyl-3-[(1*E*)-3-(3-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide* (**4**). Yield: 77%. Light-brown, amorphous solid. M.p. 60–62°. TLC (Al_2O_3; AcOEt/MeOH 3:1): R_f 0.20. UV ($CHCl_3$): λ_{max} 338 (4.15), 276 (4.90); λ_{min} 329 (4.03). FT-IR: 3435, 3078, 2925, 2854, 1654, 1602, 1506, 1452, 1343, 1249, 1105, 970, 806. ^{1}H-NMR ($CDCl_3$, 200 MHz): 10.42 (br. *s*, H–C(2″)); 9.13 (*d*, $J = 6.8$, H–C(4″)); 8.72 (*d*, $J = 7.6$, H–C(6″)); 8.36 (*AB*, $J = 15.8$, H–C(3)); 8.36 (*d*, $J = 3.6$, H–C(5′)); 8.05 (*dd*, $J = 7.4, 6.8$, H–C(5″)); 7.59 (*AB*, $J = 15.8$, H–C(2)); 6.72 (*d*, $J = 3.6$, H–C(4′)); 4.96 (*t*, $J = 7.2$, H–C(1‴)); 2.43 (*s*, Me); 1.99, 1.24–1.11 (series of *m*, CH_2(2‴) to CH_2(9‴)); 0.75 (*t*, $J = 6.4$, Me(10‴)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 180.2 (C(1)); 152.1 (C(3′)); 144.1 (C(2″)); 144.1 (C(6″)); 143.5 (C(4″)); 142.5 (C(1′)); 136.5 (C(2)); 135.8 (C(1″)); 133.4 (C(3)); 129.3 (C(5′)); 128.1 (C(5″)); 127.7 (C(4′)); 61.7 (C(1‴)); 31.9, 31.6, 29.2, 29.1, 29.0, 28.9, 25.9, 22.4 (C(2‴) to C(9‴)); 16.1 (Me); 13.9 (C(10‴)). MS (pos.): 452 (18, $[M+2\,(^{81}Br)]^+$), 450 (22, $[M\,(^{79}Br)]^+$), 402 (100, $[M-71+Na]^+$), 403 (28), 404 (8), 370 (100, $[M\,(^{79}Br)-79-H]^+$ or $[M\,(^{81}Br)-81-H]^+$), 371 (92), 372 (26), 262 (4), 248 (3), 230 (12), 125 (27). Anal. calc. for $C_{23}H_{32}BrNOS$ (450.48): C 61.32, H 7.16, N 3.11, S 7.12; found: C 61.28, H 7.40, N 3.08, S 7.48.

*1-Decyl-3-[(1*E*)-3-(4-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide* (**5**). Yield: 74%. Light-brown, amorphous solid. M.p. 71–73°. TLC (Al_2O_3; AcOEt/MeOH 3:1): R_f 0.18. UV ($CHCl_3$): λ_{max} 354 (3.90), 276 (5.08); λ_{min} 339 (3.56). FT-IR: 3434, 3048, 2923, 2851, 1651, 1601, 1506, 1419, 1301, 1223, 1105, 979, 816. ^{1}H-NMR ($CDCl_3$, 200 MHz): 10.50 (br. *s*, H–C(2″)); 9.18 (*d*, $J = 6.2$, H–C(4″)); 8.80 (*d*, $J = 8.2$, H–C(6″)); 8.46 (*d*, $J = 1.0$, H–C(5′)); 8.45 (*AB*, $J = 15.6$, H–C(3)); 8.13 (*dd*, $J = 8.2, 6.2$, H–C(5″)); 7.70 (*AB*, $J = 15.6$, H–C(2)); 7.31 (*d*, $J = 1.0$, H–C(3′)); 5.05 (*t*, $J = 7.0$, H–C(1‴)); 2.29 (*s*, Me); 2.05, 1.38–1.20 (series of *m*, CH_2(2‴) to CH_2(9‴)); 0.84 (*t*, $J = 6.4$, Me(10‴)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 180.8 (C(1)); 144.2 (C(2″)); 144.1 (C(6″)); 144.0 (C(1′)); 143.6 (C(4″)); 139.0 (C(4′)); 137.7 (C(2)); 135.8 (C(1″)); 133.7 (C(3)); 131.7 (C(5′)); 129.7 (C(3′)); 128.2 (C(5″)); 61.9 (C(1‴)); 32.0, 31.7, 29.3, 29.2, 29.1, 29.0, 26.0, 22.5 (C(2‴) to C(9‴)); 15.5 (Me); 13.9 (C(10‴)). MS (pos.): 452 (22, $[M+2\,(^{81}Br)]^+$), 450 (48, $[M\,(^{79}Br)]^+$), 402 (100, $[M-71+Na]^+$), 403 (42), 404 (13), 370 (100, $[M\,(^{79}Br)-79-H]^+$ or $[M\,(^{81}Br)-81-H]^+$), 371 (81), 372 (22), 262 (8), 248 (9), 230 (72), 125 (5). Anal. calc. for $C_{23}H_{32}BrNOS$ (450.48): C 61.32, H 7.16, N 3.11, S 7.12; found: C 61.19, H 7.31, N 3.24, S 7.39.

*1-Decyl-3-[(1*E*)-3-(5-methylthiophen-2-yl)-3-oxoprop-1-en-1-yl]pyridinium Bromide* (**6**). Yield: 86%. Light-brown, amorphous solid. M.p. 55–57°. TLC (Al_2O_3; AcOEt/MeOH 3:1): R_f 0.16. UV ($CHCl_3$): λ_{max} 354 (4.26), 274 (5.17); λ_{mim} 334 (4.11). FT-IR: 3428, 3028, 2924, 2851, 1654, 1604, 1516, 1454, 1401, 1311, 1225, 1105, 982, 818. ^{1}H-NMR ($CDCl_3$, 200 MHz): 10.16 (br. *s*, H–C(2″)); 9.33 (*d*, $J = 6.0$, H–C(4″)); 8.61 (*d*, $J = 8.0$, H–C(6″)); 8.16 (*dd*, $J = 8.0, 6.2$, H–C(5″)); 7.86 (*AB*, $J = 15.6$, H–C(3)); 7.64 (*AB*, $J = 15.6$, H–C(2)); 7.47 (*d*, $J = 5.0$, H–C(4′)); 6.91 (*d*, $J = 5.0$, H–C(3′)); 5.02 (*t*, $J = 7.2$, H–C(1‴)); 2.54 (*s*, Me); 1.99, 1.35–1.26 (series of *m*, CH_2(2‴) to CH_2(9‴)); 0.78 (*t*, $J = 6.4$, Me(10‴)). ^{13}C-NMR ($CDCl_3$, 50 MHz): 181.2 (C(1)); 147.3 (C(1′)); 144.5 (C(2″)); 144.2 (C(6″)); 143.1 (C(4″)); 135.6 (C(5′)); 135.4 (C(1″)); 134.0 (C(2)); 133.0 (C(3)); 131.9 (C(3′)); 131.7 (C(4′)); 128.4 (C(5″)); 61.9 (C(1‴)); 32.0, 31.7, 29.4, 29.3, 29.1, 29.0, 26.0, 22.5 (C(2‴) to C(9‴)); 17.3 (Me); 14.0 (C(10‴)). MS (pos.): 452 (18, $[M+2\,(^{81}Br)]^+$), 450 (26, $[M\,(^{79}Br)]^+$), 402 (100, $[M-71+Na]^+$), 403 (28), 404 (8), 370 (100, $[M\,(^{79}Br)-79-H]^+$ or $[M\,(^{81}Br)-81-H]^+$), 371 (65), 372 (18), 262 (24), 248 (28), 230 (100), 125 (18). Anal. calc. for $C_{23}H_{32}BrNOS$ (450.48): C 61.32, H 7.16, N 3.11, S 7.12; found: C 61.37 H 7.27, N 3.30, S 7.51.

General Procedure (*GP 3*) *for the Synthesis of Compounds* **7**–**9** *by Photodimerization.* A soln. of the respective compound (0.43, 0.44, or 0.48 g, resp., of **1**–**3**) in MeCN (20–25 ml) in a quartz tube was exposed to UV light (400-W high-pressure Hg lamp). The progress of the reactions was followed by TLC (SiO_2; MeCN/AcOEt 1:2). The reactions were stopped after *ca.* 8–10 h. The solvent was evaporated, and a portion of the residue (0.285, 0.221, and 0.283 g, resp.) was purified by prep. TLC (SiO_2) to afford the desired products.

*[(1*S**,2*S**,3*R**,4*R**)-3,4-(Dipyridin-3-yl)cyclobutane-1,2-diyl]bis[(3-methylthiophen-2-yl)methanone]* (**7**). Yield: 26%. Oily substance. TLC (SiO_2; MeCN/AcOEt 1:2): R_f 0.23. UV ($CHCl_3$): λ_{max} 276 (4.60). FT-IR: 3032, 2922, 2840, 1644, 1573, 1531, 1478, 1453, 1305, 1250, 1166, 1066, 1025, 805, 756, 713, 616. ^{1}H- and ^{13}C-NMR: see *Table 1*. MS (pos.): 461 (12, $[M+2+H]^+$), 460 (28, $[M+1+H]^+$), 459 (92, $[M+H]^+$), 389 (3), 331 (24), 273 (12), 230 (100), 183 (36), 125 (20). Anal. calc. for $C_{26}H_{22}N_2O_2S_2$ (458.60): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.46, H 5.01, N 5.95, O 7.01, S 13.57.

*[(1*S**,2*S**,3*R**,4*R**)-3,4-(Dipyridin-3-yl)cyclobutane-1,2-diyl]bis[(4-methylthiophen-2-yl)methanone]* (**8**). Yield: 25%. Amorphous solid. M.p. 93° (dec.). TLC (SiO_2; MeCN/AcOEt 1:2): R_f 0.26. UV ($CHCl_3$): λ_{max} 306 (4.29), 270 (4.56); λ_{min} 296 (4.25). FT-IR: 3082, 2924, 2857, 1637, 1573, 1531, 1429, 1374, 1242, 1160, 1060, 808, 758, 712. ^{1}H- and ^{13}C-NMR: see *Table 1*. MS (pos.): 461 (13, $[M+2+H]^+$), 460 (26, $[M+1+H]^+$), 459 (72, $[M+H]^+$), 389 (15), 331 (26), 273 (18), 230 (100), 183 (52), 132 (28), 125 (33). Anal. calc. for $C_{26}H_{22}N_2O_2S_2$ (458.60): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.13, H 4.80, N 5.96, O 7.25, S 13.86.

*[(1*S**,2*S**,3*R**,4*R**)-3,4-(Dipyridin-3-yl)cyclobutane-1,2-diyl]bis[(5-methylthiophen-2-yl)methanone]* (**9**). Yield: 17%. Oily substance. TLC (SiO_2; MeCN/AcOEt 1:2): R_f 0.29. UV ($CHCl_3$): λ_{max} 306 (4.28), 268 (4.27); λ_{min} 332 (4.18). FT-IR: 3035, 2924, 2846, 1645, 1572, 1518, 1401, 1222, 1024, 839, 758, 712. ^{1}H- and ^{13}C-NMR: see *Table 1*. MS (pos.): 461 (13, $[M+2+H]^+$), 460 (26, $[M+1+H]^+$), 459 (100, $[M+H]^+$), 389 (2), 331 (12), 273 (6), 230 (84), 183 (36), 125 (9). Anal. calc. for $C_{26}H_{22}N_2O_2S_2$ (458.60): C 68.10, H 4.84, N 6.11, O 6.98, S 13.98; found: C 68.40, H 4.73, N 5.92, O 6.93, S 14.02.

Theoretical Calculations. All calculations were performed with the Hyperchem 7.5 software. The HOMO and LUMO energies in the ground state, and the HSOMO and LSOMO energies in the exited state were calculated with the PM3 and PM3-RHF-CI semi-empirical methods as described in the literature [19–21].

Biological Assay. All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey), including *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC 911), *Pseudomonas aeruginosa* (ATCC 10145), *Bacillus cereus* 709 Roma, *Listeria monocytogenes* (ATCC 43251), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Candida tropicalis* (ATCC 13803). All the newly synthesized compounds were dissolved in DMSO to prepare stock solns. (2.4–3 mg/ml). Antimicrobial effects were tested quantitatively in the respective broth media by means of the double-dilution method, and minimal-inhibitory concentrations (*MIC*; in μg/ml) were determined [22]. The antibacterial and antifungal assays were performed in *Mueller–Hinton* broth (MH; *Difco*, Detroit, MI) at pH 7.3, and in buffered Yeast Nitrogen Base (YNB; *Difco*, Detroit, MI) at pH 7.0, resp. Each test substance was diluted to 0.1 ml in sterile MH and YNB broth in a concentration range of 300–0.05 μg/ml. One drop (20 μl) of test-organism suspension in MH and YNB broth (*ca.* 10^6 organisms per milliliter) was added to the extract/broth dilution. After 18 h at 35° incubation, the tubes were examined for growth. The *MIC* value was defined as the lowest concentration that gave rise to no growth. The dilutions without visible growth were used for minimal-bactericidal concentration (*MBC*) determination; the samples (100 μl) were spread across the surface of dried MH and YNB agar with sterile, bent glass rods, and then incubated for 18 h at 35°. The *MBC* of each extract was taken as the lowest concentration that showed no growth on the agar plate. Ampicillin and fluconazole were used as positive antibacterial and antifungal controls, resp., and DMSO was used as solvent control.

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