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Synthesis and antimicrobial activity of methoxy azachalcones and N-alkyl substituted methoxy azachalconium bromides

Canan ALBAY[1], Nuran KAHRİMAN[1], Nagihan YILMAZ İSKENDER[1], Şengül ALPAY KARAOĞLU[2], Nurettin YAYLI[1,*]

[1] *Department of Chemistry, Faculty of Sciences, Karadeniz Technical University, 61080, Trabzon-TURKEY*

e-mail: yayli@ktu.edu.tr

[2] *Department of Biology, Faculty of Arts and Sciences, Rize University, 53100, Rize-TURKEY*

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In this study, 18 new N-octyl, N-decyl, and N-dodecyl substituted *o*-, *m*-, and *p*-methoxy (*E*)-3- and 4-azachalcones, {4- or 3-[(1*E*)-3-(4-, 3-, or 2-methoxyphenyl)-3-oxoprop-1-en-1-yl]-1-alkyl ($C_{8,10,12}$) pyridinium bromides} (**1a-6a**, **1b-1b**, and **1c-6c**), and 4 new *o*-, and *m*-methoxy (*E*)-3- and 4-azachalcones (**2**, **3**, **5**, and **6**) were synthesized and tested for antimicrobial activities against *Escherichia coli*, *Yersinia pseudotuberculosis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, and *Candida albicans*. N-Alkyl substituted azachalconium bromides showed good antimicrobial activity against all tested microorganisms with minimal inhibitory concentration (MIC) values in the range of 0.42-58.7 μg/mL in most cases. Nonalkylated compounds **1-9** were not as effective as the alkylated compounds. They showed only antimicrobial activity against gram-positive bacteria and yeast in the range of 1.77-123.7 μg/mL. The optimum length of the alkyl chain for better activity is situated with 12 carbon atoms in the series of compounds **1a-c**, **2a-c**, **3a-c**, **4a-c**, **5a-c**, and **6a-c**. N-Alkyl derivatives of *m*-methoxy (*E*)-3-azachalcone (**4a-c**, **5a-c**, and **6a-c**) showed better activity in comparison to those of *o*- and *p*-methoxy (*E*)-4-azachalcones (**1a-c**, **2a-c**, and **3a-c**).

Key Words: N-Alkyl *p*-methyl-(*E*)-3- and 4-azachalconium bromides, antimicrobial activity

Introduction

A range of synthetic chalcones and azachalcones were prepared and the structure-activity relationships were explored.[1–4] Chalcones (1,3-diphenyl-2-propen-1-ones) are key precursors in the synthesis of a large array of

*Corresponding author

biologically important heterocycles (azachalcones).[5,6] Thus, the synthesis of azachalcones has generated vast interest among organic and medicinal chemists. Chalcones and their heterocyclic analogs (azachalcones) display a wide range of biological activities, such as anticancer,[7] antimitotic,[8] antiinflammatory,[9] antituberculosis,[10] antimalarial,[11,12] antileishmanial,[13,14] nitric oxide regulation modulatory,[15] cardiovascular,[16] cell differentiation-inducing[17] and antihyperglycemic,[18] activities.

N-alkyl derivatives of azachalcones have also been synthesized by several researchers[5,6,19–25] and were shown to possess a wide variety of biological activities, such as antiinflammatory, antimicrobial, antituberculostatic, and antibacterial potentials.[19–33] In our previous works, nitro- and methyl-substituted (*E*)-3- and 4-azachalcones and their N-alkyl derivatives were synthesized and showed very good antimicrobial activities, especially against gram-positive bacteria.[25–28] Many of the more active chalcone and azachalcone compounds are substituted with methoxy and/or hydroxyl groups.[1,2] In view of the continuing interest in new antimicrobial agents, we prepared other N-alkyl($C_{8,10,12}$) substituted *m*- and *p*-methoxy-(*E*)-3- and 4-azachalconium bromides in this respect. We aimed to determine the influence of the methoxy substitution, the length of the carbon chain in the N-alkyl substituent, and the position of N in the pyridyl ring on antimicrobial activity. The attempts to synthesize N-alkylated *o*-, *m*-, and *p*-methoxy (*E*)-2-azachalcones were unsuccessful. The present work deals with the synthesis, spectral characterization, and antimicrobial activity of 18 new N-alkyl substituted *m*- and *p*-methoxy (*E*)-3- and 4-azachalcones (**1a-6a**, **1b-6b**, and **1c-6c**) and 4 new *o*- and *m*-methoxy (*E*)-3- and 4-azachalcones (**2**, **3**, **5**, and **6**) (Scheme).

Experimental

General

NMR spectra were recorded on a Varian Mercury NMR at 200 MHz in $CDCl_3$. 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 PerkinElmer 1600 FT-IR (4000-400 cm^{-1}) spectrophotometer. Melting points were determined by using a Thermovar 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 F_{254} analytical aluminum plates. Column chromatography studies were carried out on silica gel.

Materials and methods

o−, *m*−, and *p*-Methoxy acetophenone and 2-, 3-, and 4-pyridine carboxaldehydes were purchased from Sigma-Aldrich and Merck and were used without further purification. The analytical grade or bulk solvents used (chloroform, *n*-hexane, ethanol, methanol, acetonitrile, and diethyl ether) were distilled before use.

Synthesis of compounds 1-9

Compounds **1-9** were prepared according to the literature.[5,18–36] See the physicochemical and ^{1}H- and ^{13}C-NMR data of compounds **1-9** in Tables 1-3.

Table 1. Physicochemical data of compounds **1-9**.

Comp.	IR ($CHCl_3$, cm^{-1})				Formula[a]	LC-MS (%)	Yield (%)	Mp (°C)	UV-Vis λ_{nm} (log ε)		TLC (R_f)
	C=O	$-OCH_3$	=CH	−CH		$[M+1]^+$					
1	1664	1263	3030, 3066	2835, 2967	$C_{15}H_{13}NO_2$	240 (100)	96	121-123	286 (4.4)	316 (4.3)	0.47[b]
2	1667	1259	3022, 3071	2836, 2940	$C_{15}H_{13}NO_2$	240 (048)	68	79-81	284 (4.4)	340 (3.6)	0.56[c]
3	1663	1244	3026, 3066	2830, 2940	$C_{15}H_{13}NO_2$	240 (100)	79	71-75	284 (4.2)	328 (3.5)	0.41[c]
4	1664	1263	3038, 3071	2841, 2974	$C_{15}H_{13}NO_2$	240 (100)	82	93-96	282 (4.0)	314 (4.2)	0.37[b]
5	1665	1258	3033, 3066	2830, 2940	$C_{15}H_{13}NO_2$	240 (100)	56	82-85	283 (4.3)	302 (4.4)	0.52[c]
6	1660	1244	3028, 3077	2841, 2943	$C_{15}H_{13}NO_2$	240 (100)	64	60-63	282 (4.3)	302 (4.4)	0.46[b]
7	1661	1260	3015, 3054	2840, 2935	$C_{15}H_{13}NO_2$	240 (068)	68	49-52	276 (3.5)	318 (3.9)	0.63[b]
8	1665	1254	3020, 3066	2835, 2938	$C_{15}H_{13}NO_2$	240 (100)	61	65-68	280 (4.1)	308 (4.3)	0.67[b]
9	1660	1244	3016, 3071	2835, 2940	$C_{15}H_{13}NO_2$	240 (100)	75	70-74	280 (3.7)	304 (3.9)	0.72[c]

[a]Elemental analyses of compounds **1-9** for C, H, O, and N are within ±0.4% of the theoretical values.
[b]Ether.
[c]Ethyl acetate.

Table 2. ^{1}H-NMR (δ ppm, J Hz) data of compounds **1-9** in $CDCl_3$.

H No.	Compounds*								
	1	2	3	4	5	6	7	8	9
2	7.6, d, 15.6	7.5, d, 16.8	7.5, d, 16.8	7.7, d, 16.0	7.5, d, 15.6	7.4, d ,16.0	7.7, d, 15.6	7.8, d, 15.4	7.6, d, 15.6
3	7.7, d, 15.6	7.6, d, 16.8	7.6, d, 16.8	7.6, d, 16.0	7.7, d, 15.6	7.6, d, 16.0	8.1, d, 15.6	8.1, d, 15.4	7.8, d, 15.6
2'	8.0, d, 8.2	7.6, s	-	8.0, d, 8.6	7.6, s	-	8.1, d, 9.0	7.6, s	-
3'	7.0, d, 8.6	-	7.0, d, 8.2	6.9, d, 8.6	-	7.0, d, 8.6	7.0, d, 9.0	-	7.0, d, 8.6
4'	-	7.1, d, 8.0	7.5, d, 8.2	-	7.1, d, 8.0	7.4, d, 8.8	-	7.1, d, 7.8	7.4, m
5'	7.0, d, 8.6	7.3, d, 7.8	7.0, t, 7.4	6.9, d, 8.6	7.4, d, 8.2	7.0, t, 7.4	7.0, d, 9.0	7.4, t, 7.8	7.0, d, 7.6
6'	8.0, d, 8.2	7.6, d, 7.8	7.6, d, 7.8	8.0, d, 8.6	7.5, d, 8.2	7.6, d, 7.4	8.1, d, 9.0	7.7, d, 6.6	7.6, d, 8.0
2"	7.4, d, 5.4	7.4, d, 5.4	7.4, d, 5.8	8.8, s	8.8, s	8.8, s	-	-	-
3"	8.6, d, 5.0	8.6, d, 5.4	8.6, d, 6.0	-	-	-	8.7, d, 4.4	8. 7, d, 4.8	8.6, d, 4.4
4"	-	-	-	8.6, d, 6.6	8.6, d, 5.4	8.6, d, 4.6	7.3, t, 6.6	7.4, d, 6.6	7.2, t, 6.6
5"	8.6, d, 5.0	8.6, d, 5.4	8.6, d, 6.0	7.3, t, 6.8	7.3, m	7.3, m	7.7, t , 6.6	7.8, t, 5.8	7.7, t, 5.8
6"	7.4, d, 5.4	7.4, d, 5.4	7.4, d, 5.8	7.9, d, 6.2	7.9, d, 6.8	7.9, d, 6.2	7.4, d ,7.8	7.5, d, 7.8	7.4, d ,7.4
-OCH_3	3.9, s	3.8, s	3.9, s	3.8, s	3.8, s	3.9, s	3.9, s	3.9, s	3.9, s

*Assignment based on ^{1}H, ^{1}H-^{1}H.

Table 3. ^{13}C-NMR (δ ppm) data of compounds **1-9** in $CDCl_3$.

H No.	Compounds*								
	1	2	3	4	5	6	7	8	9
1	187.8	189.2	191.7	187.7	189.3	192.9	188.5	190.1	193.0
2	125.7	125.8	130.5	123.6	123.6	128.6	125.4	125.4	124.5
3	140.5	141.3	139.0	139.8	140.7	138.7	141.9	142.7	141.4
1'	130.2	138.6	128.2	130.6	138.9	128. 5	130.7	139.1	128.8
2'	130.9	112.7	158.3	130.7	112.7	158.1	131.0	112.6	158.1
3'	113.9	159.8	111.5	113.8	159.7	111.5	113.8	159.8	111.4
4'	163.7	121.0	133.6	163.5	119.5	134.5	163.6	119.8	133.1
5'	113.9	129.6	120.7	113.8	129.5	120.7	113.8	129.6	120.5
6'	130.9	121.9	130.7	130.7	123.7	130.4	131.0	124.4	130.2
1"	142.1	141.8	142.3	130.4	130.4	130.8	153.2	153.0	153.4
2"	121.9	119.6	121.9	149.7	149.9	149.7	-	-	-
3"	150.4	150.4	150.3	-	-	-	150.0	150.1	150.0
4"	-	-	-	150.7	150.9	150.6	125.3	121.4	124.0
5"	150.4	150.4	150.3	123.4	120.9	123.6	136.9	136.9	136.6
6"	121.9	119.6	121.9	134.4	134.4	133.3	124.3	119.8	124.0
-OCH_3	55.4	55.3	55.6	55.4	55.3	55.6	55.4	55.4	55.6

*Assignment based on APT and comparison with ACD-NMR program.

Synthesis of compounds 1a-6a, 1b-6b, and 1c-6c

Compounds **1a-6a**, **1b-6b**, and **1c-6c** were prepared according to the general procedure given below. See the physicochemical and ^{1}H- and ^{13}C-NMR data of compounds **1a-6a** in Tables 4-8.

General procedure for synthesis of compounds 1a-6a, 1b-6b, and 1c-6c

m− and *p*-Methoxy (*E*)-3- or 4-azachalcones (0.02 mol each) and n-bromoalkanes (1-bromooctane, 1-bromodecane, and 1-bromododecane (0.05 mol each)) in acetonitrile (30 mL) were refluxed separately for 12-24 h.[25] The reactions were followed and monitored by TLC. After the reactions were complete, the acetonitrile was removed using a rotary evaporator and the residues were separated by column chromatography on silica gel (30 × 2 cm, approximately 25 g each, Merck, 230-400 mesh), first with ethyl acetate (30 mL) and then with ethyl acetate-methanol (3:1, 20 mL; 3:2, 20 mL), methanol (30 mL), and finally methanol-water (4:1, 30 mL). Fractions (6-12 mL each) were collected and monitored by analytical TLC. The desired dark red amorphous solids (**1a-6a**, **1b-6b**, and **1c-6c**) were obtained from fractions 6-13 (yields are shown in Table 1).

Table 4. Physicochemical data of compounds **1a-6a**, **1b-6b**, and **1c-6c**.

Comp.	IR ($CHCl_3$, cm^{-1})				Formula[a]	LC MS/MS (%)				Yield (%)	Mp (°C)	UV-Vis λ_{nm} (log ε)		TLC (R_f)[b]
	C=O	OCH_3	=CH	−CH		$[M(^{79}Br)]^+$	$[M(^{81}Br)]^+$	$[M-^{79}Br]^+$	$[M(^{79}Br)-RH]^+$					
1a	1661	1234	3030	2926	$C_{23}H_{30}BrNO_2$	431 (5)	433 (18)	352 (88)	239 (100)	63	177-180	290 (4.1)	356 (3.7)	0.16
2a	1667	1261	3026	2927	$C_{23}H_{30}BrNO_2$	431 (8)	433 (10)	352 (63)	239 (100)	83	137-140	292 (4.4)	360 (3.5)	0.18
3a	1657	1245	3030	2927	$C_{23}H_{30}BrNO_2$	431 (6)	433 (12)	352 (90)	239 (100)	88	99-101	294 (4.4)	358 (3.5)	0.13
4a	1663	1241	3031	2928	$C_{23}H_{30}BrNO_2$	431 (5)	433 (22)	352 (50)	239 (100)	39	55-58	280 (4.3)	336 (4.0)	0.15
5a	1668	1263	3020	2928	$C_{23}H_{30}BrNO_2$	431 (3)	433 (20)	352 (60)	239 (100)	46	98-101	284 (4.2)	354 (3.4)	0.16
6a	1662	1247	3025	2927	$C_{23}H_{30}BrNO_2$	431 (12)	433 (9)	352 (78)	239 (100)	65	oily	278 (4.2)	350 (3.6)	0.13
1b	1661	1234	3024	2922	$C_{25}H_{34}BrNO_2$	459 (5)	461 (11)	380 (85)	239 (100)	54	166-169	290 (4.4)	356 (4.0)	0.15
2b	1667	1262	3020	2926	$C_{25}H_{34}BrNO_2$	459 (10)	461 (35)	380 (100)	239 (78)	78	146-148	292 (4.1)	354 (3.1)	0.16
3b	1657	1246	3021	2926	$C_{25}H_{34}BrNO_2$	459 (3)	461 (10)	380 (100)	239 (65)	65	107-110	294 (4.3)	356 (3.4)	0.15
4b	1663	1241	3040	2926	$C_{25}H_{34}BrNO_2$	459 (3)	461 (2)	380 (82)	239 (100)	44	57-60	282 (4.4)	336 (4.1)	0.17
5b	1668	1263	3032	2926	$C_{25}H_{34}BrNO_2$	459 (4)	461 (8)	380 (72)	239 (100)	52	113-115	284 (4.1)	352 (3.3)	0.20
6b	1662	1247	3020	2926	$C_{25}H_{34}BrNO_2$	459 (8)	461 (5)	380 (100)	239 (100)	50	oily	276 (4.3)	354 (3.6)	0.20
1c	1661	1236	3030	2923	$C_{27}H_{38}BrNO_2$	487 (12)	489 (11)	408 (100)	239 (80)	76	170-172	288 (4.4)	356 (4.0)	0.10
2c	1666	1261	3045	2925	$C_{27}H_{38}BrNO_2$	487 (10)	489 (10)	408 (85)	239 (100)	84	128-131	294 (4.4)	356 (3.5)	0.20
3c	1660	1246	3025	2925	$C_{27}H_{38}BrNO_2$	487 (22)	489 (15)	408 (88)	239 (100)	76	115-118	292 (4.4)	358 (3.5)	0.18
4c	1663	1241	3020	2926	$C_{27}H_{38}BrNO_2$	487 (15)	489 (10)	408 (100)	239 (100)	43	61-64	280 (4.3)	338 (4.1)	0.21
5c	1668	1262	3025	2924	$C_{27}H_{38}BrNO_2$	487 (2)	489 (22)	408 (61)	239 (100)	43	106-108	284 (4.2)	348 (3.3)	0.21
6c	1662	1247	3026	2925	$C_{27}H_{38}BrNO_2$	487 (12)	489 (5)	408 (95)	239 (100)	49	oily	276 (4.3)	354 (3.6)	0.17

[a]Elemental analyses of compounds **1a-6a, 1b-6b**, and **1c-6c** for C, H, O, Br, and N are within ±0.4% of the theoretical values.
[b]Methanol.

Table 5. ^{1}H-NMR (δ ppm, J Hz) data of compounds **1a-6a** in $CDCl_3$.

H No.	Compounds*					
	1a	2a	3a	4a	5a	6a
2	7.7, d, 15.8	7.7, d, 15.6	7.6, d, 15.8	7.7, d, 15.8	7.7, d, 15.8	7.7, d, 15.8
3	8.3, d, 15.8	8.3, d, 15.6	7.9, d, 15.8	8.6, d, 15.8	8.5, d, 15.8	7.9, d, 15.8
2'	8.2, d, 9.0	7.6, s	-	8.4, d, 8.8	7.7, s	-
3'	7.0, d, 9.0	-	7.0, d, 9.0	7.0, d, 8.8	-	7.0, d, 8.4
4'	-	7.8, d, 7.8	7.6, t, 8.4	-	7.1, t, 8.0	7.5, t, 8.4
5'	7.0, d, 9.0	7.4, t, 8.0	7.1, t, 8.4	7.0, d, 8.8	7.4, t, 8.0	7.0, t, 8.4
6'	8.2, d,9.0	7.2, t, 8.0	7.5, t, 8.4	8.4, d, 8.8	7.9, d, 7.8	7.7, d, 8.2
2"	8.5, d, 6.4	8.5, d, 6.8	8.2, d, 6.4	10.5, s	10.5, s	10.0, s
3"	9.4, d, 6.4	9.3, d, 6.8	9.5, d, 6.4	-	-	-
4"	-	-	-	9.1, d, 6.0	9.2, d, 5.6	9.4, d, 5.8
5"	9.4, d, 6.4	9.3, d, 6.8	9.5, d, 6.4	8.1, t, 7.0	8.2, t, 6.4	8.3, t, 6.4
6"	8.5, d, 6.4	8.5, d, 6.8	8.2, d, 6.4	8.7, d, 6.8	8.7, d, 8.0	8.7, d, 7.6
NCH_2-	4.9, t, 7.0	4.9, t, 7.4	5.0, t, 7.4	5.1, t, 7.4	5.0, t, 7.4	5.1, t, 7.4
-$(CH_2)_6$-	2.0, m, 2H 1.3, m, 10H	2.0, m, 2H 1.3, m, 10H	2.0, m, 2H 1.3, m, 10H	2.1, m, 2H 1.3, m, 10H	2.1, m, 2H 1.3, m, 10H	2.1, m, 2H, 1.3, m, 10H
-CH_3	0.8, t, 6.6	0.9, t, 7.0	0.9, t, 6.2	0.8, t, 6.8	0.8, t, 7.2	0.8, t, 6.8
-OCH_3	3.9, s	3.9, s	4.0, s	3.9, s	3.9, s	4.0, s

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

Table 6. ^{1}H-NMR (δ ppm, JHz) data of compounds **1b-6b** in $CDCl_3$.

H No.	Compounds*					
	1b	2b	3b	4b	5b	6b
2	7.7, d, 15.4	7.7, d, 15.4	7.6, d, 15.8	7.7, d, 15.8	7.7, d, 15.8	7.7, d, 15.8
3	8.4, d, 15.4	8.3, d, 15.4	7.9, d, 15.8	8.7, d, 15.8	8.5, d, 15.8	7.9, d, 15.8
2'	8.2, d, 8.8	7.6, s	-	8.4, d, 9.0	7.7, s	-
3'	7.0, d, 8.8	-	7.0, d, 8.2	7.0, d, 9.0	-	7.0, d, 8.0
4'	-	7.2, d, 8.2	7.6, t, 8.2	-	7.1, d, 8.0	7.5, t, 8.0
5'	7.0, d, 8.8	7.4, t, 8.0	7.1, t, 8.0	7.0, d, 9.0	7.4, t, 8.0	7.0, t, 8.0
6'	8.2, d, 8.8	7.8, d, 7.8	7.8, d, 8.0	8.4, d, 9.0	8.0, d, 7.8	7.7, d, 7.8
2"	8.5, d, 6.8	8.5, d, 6.4	8.2, d, 6.8	10.6, s	10.4, s	9.9, s
3"	9.4, d, 6.8	9.4, d, 6.4	9.5, d, 6.8	-	-	-
4"	-	-	-	9.1, d, 5.8	9.2, d, 6.2	9.4, d, 5.6
5"	9.4, d, 6.8	9.4, d, 6.4	9.5, d, 6.8	8.1, t, 6.2	8.1, t, 6.4	8.3, t, 6.4
6"	8.5, d, 6.8	8.5, d, 6.4	8.2, d, 6.8	8.7, d, 8.4	8.7, d, 8.0	8.7, d, 8.2
NCH_2-	4.9, t, 7.0	4.9, t, 7.8	5.0, t, 7.4	5.1, t, 7.4	5.1, t, 7.4	5.1, t, 7.4
-$(CH_2)_8$-	2.0, m, 2H 1.3, m, 14H	2.1, m, 2H 1.3, m, 14H	2.0, m, 2H 1.3, m, 14H	2.2, m, 2H 1.2, m, 14H	2.1, m, 2H 1.3, m, 14H	2.1, m, 2H 1.3, m, 14H
-CH_3	0.9, t, 6.6	0.9, t, 6.4	0.9, t, 6.8	0.9, t, 6.8	0.9, t, 7.0	0.9, t, 6.8
-OCH_3	3.9, s	3.9, s	4.0, s	3.9, s	3.9, s	4.0, s

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

Table 7. ^{1}H-NMR (δ ppm, J Hz) data of compounds **1c-6c** in $CDCl_3$.

H No.	Compounds*					
	1c	2c	3c	4c	5c	6c
2	7.7, d, 15.8	7.7, d, 15.4	7.6, d, 15.8	7.6, d, 15.8	7.7, d, 15.8	7.6, d, 16.2
3	8.3, d, 15.8	8.2, d, 15.4	7.9, d, 15.8	8.7, d, 15.8	8.5, d, 15.8	7.9, d, 16.2
2'	8.2, d, 8.8	7.6, s	-	8.4, d, 8.0	7.7, s	-
3'	7.0, d, 8.8	-	7.0, d, 8.8	7.0, d, 8.0	-	7.0, d, 7.8
4'	-	7.8, d, 7.8	7.6, t, 8.4	-	7.1, d, 8.4	7.5, t, 8.4
5'	7.0, d, 8.8	7.4, t, 8.0	7.1, t, 8.6	7.0, d, 8.0	7.4, t, 8.0	7.0, t, 7.8
6'	8.2, d, 8.8	7.2, t, 8.0	7.8, d, 8.4	8.4, d, 8.0	7.9, d, 7.8	7.7, d, 7.8
2"	8.5, d, 6.8	8.5, d, 6.8	8.2, d, 6.4	10.6, s	10.5, s	9.9, s
3"	9.3, d, 6.8	9.3, d, 6.8	9.5, d, 6.4	-	-	-
4"	-	-	-	8.9, d, 5.8	9.2, d, 6.0	9.4, d, 6.0
5"	9.3, d, 6.8	9.3, d, 6.8	9.5, d, 6.4	8.0, t, 6.2	8.1, t, 6.2	8.3, d, 6.0
6"	8.5, d, 6.8	8.5, d, 6.8	8.2, d, 6.4	8.6, d, 8.8	8.7, d, 8.0	8.7, d, 8.0
NCH_2-	4.9, t, 7.2	4.9, t, 7.4	5.0, t, 7.4	5.1, t, 7.4	5.1, t, 7.0	5.1, t, 7.4
-$(CH_2)_{10}$-	2.1, m, 2H 1.2, m, 18H	2.0, m, 2H 1.3, m, 18H	2.0, m, 2H 1.3, m, 18H	2.2, m, 2H 1.3, m, 18H	2.1, m, 2H 1.3, m, 18H	2.1, m, 2H 1.3, m, 18H
-CH_3	0.9, t, J 6.6	0.9, t, 7.0	0.9, t, 6.8	0.9, t, 6.8	0.9, t, 7.2	0.9, t, 6.8
-OCH_3	3.9, s	3.9, s	4.0, s	3.8, s	3.9, s	4.0, s

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

Antimicrobial activity assessment

All test microorganisms were obtained from the Refik Saydam National Public Health Agency (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 702 Roma, and *Candida albicans* ATCC 60193. All of the newly synthesized compounds were weighed and dissolved in dimethylsulphoxide (DMSO) to prepare an extract stock solution of milligrams per milliliter.

The antimicrobial effects of the substances were tested quantitatively by the microdilution technique in Mueller Hinton broth. The minimal inhibition concentration (MIC) of the extracts was also determined using a 2-fold dilution method.[37] The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and a buffered yeast nitrogen base (Difco) at pH 7.0, respectively. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (1.2 mg/mL) and fluconazole (2 mg/mL) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide (DMSO) was used as the solvent control. The results are shown in Table 9.

Table 8. ^{13}C-NMR (δ ppm) data of compounds **1a-6a**, **1b-6b**, and **1c-6c** in $CDCl_3$.

++	Compounds*																	
	1a	2a	3a	4a	5a	6a	1b	2b	3b	4b	5b	6b	1c	2c	3c	4c	6c	5c
1	186.7	188.6	189.8	186.9	188.4	190.4	186.7	188.2	189.8	186.9	188.6	190.3	186.6	188.2	189.7	186.9	188.4	190.3
2	133.4	133.3	133.5	130.4	129.8	128.7	133.4	133.1	133.5	130.3	130.2	128.7	133.6	133.1	133.4	130.7	129.9	128.7
3	135.5	136.5	137.1	133.8	134.9	132.8	135.5	136.2	137.0	133.7	134.8	132.6	135.3	136.1	137.2	133.6	134.8	132.6
1'	129.5	138.0	127.1	129.6	137.9	127.3	129.5	137.7	127.0	129.6	138.0	127.3	129.5	137.7	127.1	129.7	137.9	127.3
2'	131.9	113.0	159.0	132.2	112.8	158.8	131.9	112.7	158.9	132.1	112.9	158.8	131.9	112.7	159.1	132.3	112.8	158.7
3'	114.3	160.3	111.8	114.2	159.8	111.8	114.3	160.0	111.7	114.1	160.1	111.7	114.4	160.0	111.8	114.3	159.9	111.7
4'	164.4	121.3	134.9	164.2	120.6	134.3	164.4	121.1	134.9	164.1	120.9	134.4	164.5	121.1	135.0	164.3	120.7	134.4
5'	114.3	130.3	121.0	114.2	129.7	120.7	114.3	130.1	121.0	114.1	130.0	120.7	114.4	130.1	121.1	114.3	129.8	120.7
6'	131.9	127.1	131.1	132.2	122.3	133.6	131.9	126.7	131.0	132.1	122.5	133.6	131.9	126.7	131.1	132.3	122.3	133.6
1''	151.2	151.1	151.3	136.4	135.9	136.0	151.2	150.9	151.3	136.4	136.1	136.0	151.4	150.9	151.4	136.7	135.9	136.0
2''	126.8	122.3	126.1	144.3	144.2	144.6	126.8	122.1	126.1	145.0	144.5	144.6	126.7	122.1	126.1	144.4	144.3	144.6
3''	145.1	145.4	145.3	-	-	-	145. 0	145.1	145.3	-	-	-	144.9	145.1	145.3	-	-	-
4''	-	-	-	144.2	144.0	144.2	-	-	-	144.1	144.2	144.1	-	-	-	144.2	144.0	144.1
5''	145.1	145.4	145.3	128.1	128.3	130.7	145.0	145.1	145.3	128.1	128.3	130.8	144.9	145.1	145.3	127.9	128.3	130.8
6''	126.8	122.3	126.1	143.3	144.0	142.5	126.8	122.1	126.1	143.3	143.9	142.5	126.7	122.1	126.1	142.9	143.8	142.5
NCH_2-	61.7	62.0	61.5	62.0	61.9	62.0	61.7	61.8	61.4	61.9	62.1	62.0	61.9	61.8	61.6	62.1	61.9	61.9
-$(CH_2)_n$-	31.9 31.6 29.0 26.1 22.6	32.1 31.9 29.2 26.3 22.8	31.9 31.6 28.9 26.0 22.5	32.1 31.7 29.0 26.1 22.7	31.9 31.5 28.9 26.0 22.4	31.8 31.4 28.9 25.9 22.4	31.8 29.4 29.2 29.1 26.1 22. 7	31.8 29.4 29.3 29.2 29.0 26.1 22.6	31.8 31.7 29.3 29.2 29.1 29.0 26.0 22.5	32.1 31.7 29.4 29.6 29.1 29.0 26.0 22.6	32.1 31.8 29.5 29.4 29.2 29.1 26.1 22.6	31.9 31.7 29.3 29.2 29.1 29.0 25.9 22.5	31.9 29.6 29.5 29.3 29.1 26.1 22.7	31.8 29.5 29.5 29.3 29.0 26.1 22.6	31.9 31.8 29.5 29.4 29.3 29.0 26.1 22.6	32.2 31.9 29.6 29.5 29.3 29.1 26.1 22.7	32.0 31.8 29.5 29.4 29.3 29.2 29.0 26.0 22.6	31.9 31.7 29.4 29.4 29.2 29.2 29.0 25.9 22.5
-CH_3	14.1	14.3	14.0	14.1	13.9	13.9	14.1	14.1	14.0	14.0	14.1	14.0	14.1	14.1	14.1	14.1	14.0	14.0
-OCH_3	55.7	56.0	56.1	55.6	55.8	56.1	55.7	55.7	56.0	55.5	56.0	56.1	55.7	55.7	56.1	55.6	55.8	56.1

*Assignment based on APT and comparison with ACD-NMR program; n: 6, 8, 10.

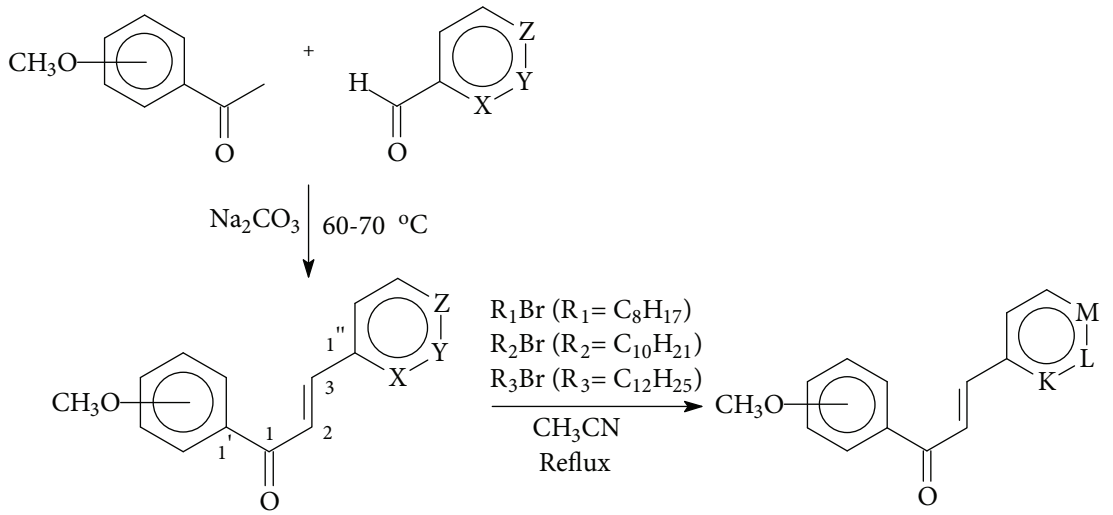
Table 9. Minimum inhibitory concentrations (MIC, μg/mL) of compounds **1-9**, **1a-6a**, **1b-6b**, and **1c-6c**.

Comp.	Stock conc.	Minimal inhibition concentration values (μg/mL)							
	(μg/100 μL)	*Ec* (-)	*Yp* (-)	*En* (-)	*Pa* (-)	*Sa* (+)	*Ef* (+)	*Bc* (+)	*Ca*
1	9900	> 495	> 495	> 495	> 495	61.9	123.7	61.9	15.5
2	11,300	> 283	> 283	> 283	> 283	2.20	8.83	8.83	17.6
3	9900	> 247	> 247	> 247	> 247	7.72	30.9	15.5	15.5
4	10,400	> 260	> 260	> 260	> 260	65.0	30.0	65.0	32.5
5	12,100	> 303	> 303	> 303	> 303	4.72	37.8	9.45	37.8
6	9600	> 240	> 240	> 240	> 240	30.0	120	30.0	15.0
7	10,200	> 510	> 510	> 510	> 260	7.96	31.8	15.9	15.9
8	9100	> 227	> 227	> 227	> 227	1.77	14.7	7.10	< 7.10
9	9100	> 227	> 227	> 227	> 227	7.10	28.4	14.2	7.10
1a	8600	6.72	13.5	26.8	53.7	0.42	1.67	1.67	< 6.72
1b	8800	3.44	3.44	3.44	13.7	0.42	0.42	0.42	< 6.87
1c	9300	> 233	> 233	> 233	116.2	0.45	0.45	0.45	58.1
2a	9200	3.59	7.18	14.4	28.7	0.45	0.90	0.90	< 7.18
2b	9600	0.94	3.75	3.75	15.0	< 0.47	< 0.47	< 0.47	< 7.50
2c	9800	0.47	0.96	0.45	7.65	< 0.47	< 0.47	< 0.47	< 7.65
3a	1040	8.12	16.2	16.2	32.5	0.50	1.01	2.03	< 8.12
3b	8700	3.40	6.80	3.40	13.6	< 0.43	< 0.43	< 0.43	< 6.80
3c	9800	0.96	3.82	1.92	3.82	< 0.46	< 0.46	< 0.46	< 7.65
4a	9300	3.63	7.26	29.0	29.0	0.45	0.90	0.90	< 7.26
4b	8800	0.84	3.44	6.8	13.7	< 0.44	< 0.44	< 0.44	< 6.8
4c	9900	0.48	1.93	0.96	7.73	< 0.48	< 0.48	< 0.48	< 7.73
5a	9500	3.71	7.42	7.42	14.8	< 0.46	< 0.46	< 0.46	< 7.42
5b	9600	1.87	7.50	3.75	7.50	< 0.46	< 0.46	< 0.46	< 7.50
5c	8700	1.70	3.40	1.70	13.8	< 0.43	< 0.43	< 0.43	< 6.80
6a	9400	7.34	14.7	14.7	58.7	< 0.45	< 0.45	< 0.45	< 7.34
6b	9400	0.90	3.67	1.83	3.67	< 0.45	< 0.45	< 0.45	< 7.34
6c	9300	0.90	1.81	1.81	14.5	< 0.45	< 0.45	< 0.45	< 7.25
Amp.	1200	8	32	> 64	> 128	2	2	< 1	
Flu.	2000								< 8

a: C_8, b: C_{10}, c: C_{12}; *Ec*: *Escherichia coli*, *Yp*: *Yersinia pseudotuberculosis*, *En*: *Enterobacter aerogenes*, *Pa*: *Pseudomonas aeruginosa*, *Sa*: *Staphylococcus aureus*, *Ef*: *Enterococcus faecalis*, *Bc*: *Bacillus cereus* 702 Roma, *Ca*: *Candida albicans*, Amp: ampicillin, Flu: fluconazole.

Results and discussion

The convenient approach for the synthesis of *o*-, *m*-, and *p*-methoxy (*E*)-2-, -3-, and 4-azachalcones (**1-9**) involves the Claisen-Schmidt condensation of methoxy-substituted aryl methyl ketones with pyridine carboxaldehydes with 3 equivalents of Na_2CO_3 solution (EtOH, 95%), which yields the *trans* isomer of the corresponding α,β-unsaturated (J = 15.4/16.8 Hz, respectively) compounds (**1-9**) (Scheme, Tables 2-3).[5,25–28]



1-9 1a-6a,1b-6b, and 1c-6c

1	X,Y= CH,	Z= N,	4-OCH_3
2	X,Y= CH,	Z= N,	3-OCH_3
3	X,Y= CH,	Z= N,	2-OCH_3
4	X, Z= CH,	Y= N,	4-OCH_3
5	X, Z= CH,	Y= N,	3-OCH_3
6	X, Z= CH,	Y= N,	2-OCH_3
7	Y, Z= CH,	X= N,	4-OCH_3
8	Y, Z= CH,	X= N,	3-OCH_3
9	Y, Z= CH,	X= N,	2-OCH_3

1a	K, L= CH,	M= N^+R_1 / Br^-,	4-OCH_3
2a	K, L = CH,	M= N^+R_1 / Br^-,	3-OCH_3
3a	K, L = CH ,	M= N^+R_1 / Br^-,	2-OCH_3
4a	K, M = CH,	L= N^+R_1 / Br^-,	4-OCH_3
5a	K, M= CH,	L= N^+R_1 / Br^-,	3-OCH_3
6a	K, M = CH,	L= N^+R_1 / Br^-,	2-OCH_3
1b	K, L = CH,	M= N^+R_2 / Br^-,	4-OCH_3
2b	K, L = CH,	M= N^+R_2 / Br^-,	3-OCH_3
3b	K, L = CH,	M= N^+R_2 / Br^-,	2-OCH_3
4b	K, M = CH,	L= N^+R_2 / Br^-,	4-OCH_3
5b	K, M = CH,	L= N^+R_2 / Br^-,	3-OCH_3
6b	K, M = CH,	L= N^+R_2 / Br^-,	2-OCH_3
1c	K, L = CH,	M= N^+R_3 / Br^-,	4-OCH_3
2c	K, L = CH,	M= N^+R_3 / Br^-,	3-OCH_3
3c	K, L = CH,	M= N^+R_3 / Br^-,	2-OCH_3
4c	K, M = CH,	L= N^+R_3 / Br^-,	4-OCH_3
5c	K, M = CH,	L= N^+R_3 / Br^-,	3-OCH_3
6c	K, M = CH,	L= N^+R_3 / Br^-,	2-OCH_3

Scheme

N-alkyl derivatives of *m*- and *p*-methoxy (*E*)-3- or 4-azachalcones were synthesized from the corresponding azachalcones with n-bromoalkanes (1-bromooctane, 1-bromodecane, and 1-bromododecane) in acetonitrile

solution by reflux.[19,20,25–27] In the ^{1}H-NMR spectra of **1a-6a**, **1b-6b**, and **1c-6c**, the characteristic -CH_2- signal of the N-alkyl groups was exhibited at δ 4.9-5.1 ppm (2H, t, J = 7.0-7.4 Hz) (Tables 5-7) due to pyridinium salt.[19,20,25]

N-Alkyl derivatives of azachalcones attract widespread interest because many of them have exhibited a wide variety of biological activities.[25–33] All of the synthesized compounds (**1-9**, **1a-6a**, **1b-6b**, and **1c-6c**) were characterized on the basis of spectral data analyses (^{1}H-NMR, ^{13}C-NMR, APT, ^{1}H-^{1}H COSY-NMR, ACD-NMR, FT-IR, UV, LC-MS/MS, and elemental analysis), whose results were in agreement with the proposed structures (Tables 1-8). The LC mass spectra of **1a-6a**, **1b-6b**, and **1c-6c** exhibited molecular ion peaks for $[M(^{79}Br)]^+$ and $[M(^{81}Br)]^+$, and the base ion peaks were $[M(^{79}Br)\text{-}79]^+$ and $[M(^{81}Br)\text{-}81]^+$ with other fragment ions such as $[M(^{79}Br)\text{-}R]^+$ and $[M(^{81}Br)\text{-}R]^+$ (Table 4).

The antimicrobial activity of compounds **1-9**, **1a-6a**, **1b-6b**, and **1c-6c** was determined against *E. coli*, *Y. pseudotuberculosis*, *E. aerogenes*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *B. cereus*, and *C. albicans* (Table 9). The activities of compounds **1-9**, **1a-6a**, **1b-6b**, and **1c-6c** were investigated by the dilution method.[37] Some compounds showed from slight to pronounced antimicrobial activity against gram-positive and gram-negative bacteria. Nonalkylated compounds **1-9** were not as effective as the alkylated compounds with 8, 10, or 12 C alkyl groups (**1a-6a**, **1b-6b**, and **1c-6c**). The N-alkyl derivatives of compounds **1-9** were more active against the gram-positive bacteria (*S. aureus*, *E. faecalis*, and *B. cereus)* in the range of 0.42-2.03 μg/mL compared to the gram-negative bacteria (*E. coli*, *Y. pseudotuberculosis*, *E. aerogenes*, and *P. aeruginosa*) in the range of 0.47-233 μg/mL. *o*-, *m*-, and *p*-Methoxy substituted 3-azachalcones displayed better activity against gram-negative bacteria in the range of 0.48-58.7 μg/mL compared to *o*-, *m*-, and *p*-methoxy substituted 4-azachalcones in the range of 0.47-233 μg/mL. *m*-Methoxy 4- and 3-azachalcones showed better activity against *E. coli*, *Y. pseudotuberculosis*, *E. aerogenes*, and *P. aeruginosa* in the ranges of 0.47-28.7 and 1.70-14.8 μg/mL, as opposed to *p*-methoxy 4- and 3-azachalcones (3.44-233 and 0.48-29.0 μg/mL) and *o*-methoxy 4- and 3-azachalcones (0.96-32.5 and 0.90-58.7 μg/mL), respectively. Our previous works and research by Nowakowska et al. have shown a similar trend in activity with respect to bacterial type with methyl-, nitro-, and hydroxy-substituted and unsubstituted N-alkyl derivatives of azachalcones.[20,26–31] The antimicrobial activity increased as the length of the alkyl substitution increased from 8 to 12, and similar results have been observed in the literature for N-bromoalkyl derivatives of azachalcones.[20,26–31] The solvent control DMSO showed no inhibition effect on any test microorganisms.

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