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Synthesis and antimicrobial properties of *N*-substituted derivatives of (*E*)-4-azachalcones

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Abstract

Syntheses of 11 new *N*-bromoalkyl substituted bromides of (*E*)-4-azachalcone and *N*-*o*-(*m*- and *p*-) halobenzyl substituted halides of (*E*)-3′-hydroxy-4-azachalcone of antimicrobial activity are reported. Compounds **3d**, **3e**, **6b**, **6c**, and **6e** reveal good antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis* as well as moderate activity against *Escherichia coli* and *Klebsiella pneumoniae*.

Keywords: (*E*)-4-Azachalcone; (*E*)-3′-Hydroxy-4-azachalcone; Antibacterial and antifungal activity

1. Introduction

Chalcones constitute a class of naturally occurring substances of great biological interest [1]. They are regarded [2] as precursors in the biosynthesis of all flavanoid-type natural products. Chalcones are reported to show insecticidal [3], antimicrobial [4], antichinovirus and antipicornivirus [5,6] and bacteriostatic activity [7], they also act as antibiotic [8].

The derivatives of chalcones with an annular nitrogen atom in the phenyl ring, i.e. azachalcones, have been also reported to display a wide variety of biological activities [9] especially they have shown antibacterial [10–15], antituberculostatic [16,17] and anti-inflammatory activities [18,19]. The azachalcones were the most potent of the chalcone series as inhibitors of MPO (myeloperoxidase) release from rat PMN (polymorphonuclear leukocytes) and microtubule polymerization inhibitors which bind to the colchicine-binding site of microtubules [9,20].

As a continuation of our programme on the investigation of the synthesis and physico-chemical as well as antimicrobial properties of azastilbenes [21–24] and azachalcones [15], our attention was drawn to bromides of (*E*)-*N*-bromoalkyl-4-azachalcones and halides of (*E*)-*N*-halobenzyl-3′-hydroxy-4-azachalcones.

The present paper deals with the synthesis, spectral characterization and results of a microbiological screening of unreported bromides of (*E*)-*N*-bromoethyl(bromopropyl, bromopentyl, bromohexyl and bromodecyl)-4-azachalcones **3a**–**3e** and chlorides of (*E*)-*N*-*o*-(*m*- and *p*-)chlorobenzyl-3′-hydroxy-4-azachalcones **6a**–**6c** as well as bromides of (*E*)-*N*-*o*-(*m*- and *p*-)bromobenzyl-3′-hydroxy-4-azachalcones **6d**–**6f** Scheme 1.

2. Chemistry

The syntheses of (*E*)-4-azachalcone **1** and (*E*)-3′-hydroxy-4-azachalcone **4** as substrates for the compounds tested (**3a**–**3e** and **6a**–**6f**) were performed using the Claisen–Schmidt condensation of an appropriate aromatic ketone with 4-pyridinecarboxaldehyde [25].

A series of five new bromides of (*E*)-*N*-bromoalkyl substituted derivatives of (*E*)-4-azachalcone **3a**–**3e** have been synthesized by reaction of (*E*)-4-azachalcone **1** with dibromoalkanes **2a**–**2e** (1,2-dibromoethane, 1,3-dibromopropane, 1,5-dibromopentane, 1,6-dibromohexane and 1,10-dibromodecane) in boiling acetonitrile.

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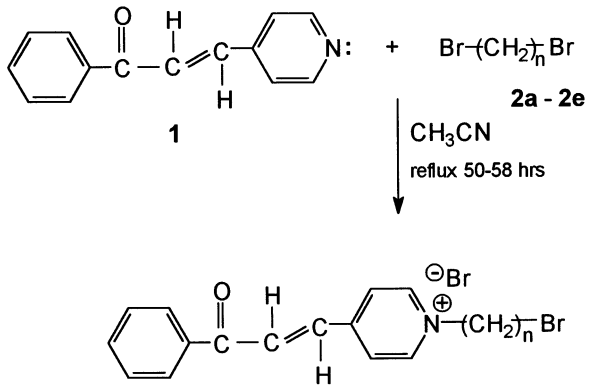
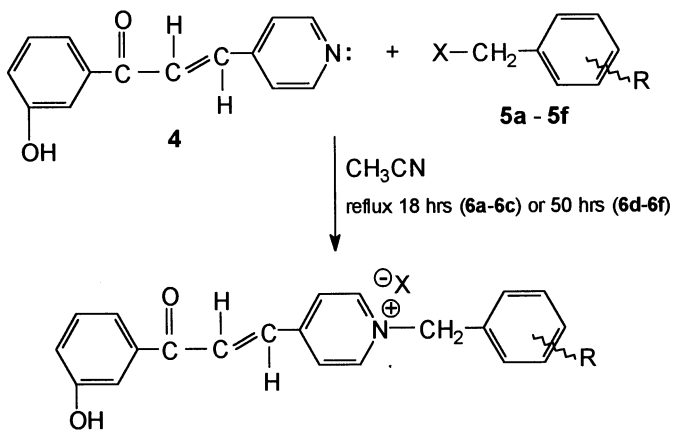
Treatment of (*E*)-3′-hydroxy-4-azachalcone **4** with the corresponding benzyl halide **5a**–**5f** [*o*-(*m*- or *p*-)chlorobenzyl chloride and *o*-(*m*- or *p*-)bromobenzyl bromide], in boiling acetonitrile as a solvent, afforded (*E*)-*N*-benzyl-3′-hydroxy-4-azachalcone halides **6a**–**6f** Scheme 1.

All compounds have been characterized on the basis of spectral studies (IR, ^{1}H NMR [26], UV–Vis) and elemental analysis, whose results are in harmony with the proposed structure (Table 1). The antimicrobial activity of the new compounds (**3a**–**3e**, **6a**–**6f**) has been determined (Table 2).

3. Results and discussion

The new compounds obtained **3a**–**3e** and **6a**–**6f** were assayed against the following nine strains of microorganisms: Gram-positive cocci (*Staphylococcus aureus* 209PFDA, *Enterococcus faecalis* ATCC 8040), aerobic bacilli (*Bacillus subtilis* ATCC1633), Gram-negative rods (*Escherichia coli* PZH 026B6, *Klebsiella pneumoniae* 231, *Pseudomonas aeruginosa* SR1), yeasts (*Candida albicans* PCM 1409 PZH), moulds (*Aspergillus fumigatus* C1) and dermatophytes (*Microsporum gypseum* K_1).





Comp. **2**	n	Comp. **3**	n	Comp. **5**	X	R	Comp. **6**	X	R
2a	2	**3a**	2	**5a**	Br	o-Br	**6a**	Br	o-Br
2b	3	**3b**	3	**5b**	Br	m-Br	**6b**	Br	m-Br
2c	5	**3c**	5	**5c**	Br	p-Br	**6c**	Br	p-Br
2d	6	**3d**	6	**5d**	Cl	o-Cl	**6d**	Cl	o-Cl
2e	10	**3e**	10	**5e**	Cl	m-Cl	**6e**	Cl	m-Cl
				5f	Cl	p-Cl	**6f**	Cl	p-Cl

Scheme 1.

Table 1
Physico-chemical data of compounds **3a**–**3e** and **6a**–**6f**

Comp.	Yield (%)	m.p. (°C)	IR (KBr, cm^{-1}) νC=O	TLC, R_f	^{1}H NMR (DMSO-d_6)			δ (ppm)	J (Hz)	UV–Vis		Analyses (%), calcd./found		
					CH_2–Br	CH_2–N	OH	C_α	C_β	λ_{nm}	(log ε)	C	H	N
3a	29	122–124 [a]	1655	0.35	4.1 t	5.1 t		7.8 d	8.4 d	293.0	(4.44)	48.11	3.76	3.50
										205.0	(4.35)	48.39	3.81	3.53
3b	48	119–121 [a]	1659	0.34	3.6 t	4.8 t		7.8 d	8.3 d	290.5	(4.39)	49.49	4.04	3.38
										204.5	(4.29)	49.66	4.17	3.41
3c	48	166–168 [a]	1662	0.35	3.5 t	4.7 t		7.9 d	8.4 d	290.5	(4.42)	51.96	4.82	3.19
										204.5	(4.29)	51.80	4.79	3.10
3d	49	132–134 [a]	1660	0.34	3.5 t	4.6 t		7.9 d	8.4 d	290.0	(4.43)	53.00	5.12	3.09
										204.5	(4.29)	53.09	5.12	3.13
3e	80	140–142 [a]	1664	0.33	3.4 t	4.6 t		7.8 d	8.4 d	290.0	(4.42)	56.60	6.13	2.75
										204.5	(4.29)	56.71	6.33	2.72
6a	75	229–230 [b]	1667	0.39		6.0 s	10.0 s	7.9 d	8.5 d	293.0	(4.32)	53.08	3.61	2.95
										213.5	(4.47)	52.98	3.57	2.91
6b	65	216–218 [b]	1659	0.40		5.9 s	10.0 s	7.9 d	8.4 d	293.5	(4.31)	53.08	3.61	2.95
										214.0	(4.48)	53.10	3.64	2.93
6c	45	147–148 [b]	1662	0.41		5.9 s	10.0 s	7.8 d	8.4 d	299.0	(4.31)	53.08	3.61	2.95
										213.5	(4.46)	52.45	3.82	2.81
6d	55	191–194 [b]	1660	0.28		6.0 s	10.2 s	7.8 d	8.5 d	293.0	(4.30)	65.30	4.44	3.63
										214.0	(4.46)	65.21	4.38	3.55
6e	47	222–224 [b]	1659	0.27		5.9 s	10.1 s	7.8 d	8.4 d	293.0	(4.33)	65.30	4.44	3.63
										214.0	(4.47)	65.19	4.37	3.53
6f	40	165–168 [b]	1661	0.28		5.9 s	10.1 s	7.9 d	8.4 d	292.0	(4.38)	65.30	4.44	3.63
										216.0	(4.45)	65.27	4.39	3.54

[a] Crystallized from chloroform–methanol 5:1.
[b] Crystallized from chloroform–methanol 1:1.

Table 2
Antimicrobial activity of **3a**–**3e** and **6a**–**6f**

Comp.	Minimum inhibitory concentration (μg/cm^3) [a]							
	1 [b]	2 [c]	3 [d]	4 [e]	5 [f]	6 [g]	7 [h]	8 [i]
3a			100					<100
3b	100		100					<100
3c	100	100	100					
3d	10	10	100	100				
3e	1	1	5	100	100			<100
6a	100	100	100					
6b	7.5	10	100	100				
6c	7.5	10	100	100				
6d	100	100	100					
6e	7.5	100	100	100				
6f	100	100	100	100	100			
A [j]	5	5	5	5	50			
B [k]						0.5	10	1

[a] MIC — the minimum inhibitory concentration is the lowest value of concentration of the investigated compound which brakes the evolution of the microorganism.
[b] *Staphylococcus aureus* FDA 209 P.
[c] *Enterococcus faecalis* ATCC 8040/1.
[d] *Bacillus subtilis* ATCC1633.
[e] *Escherichia coli* PZH 026B6.
[f] *Klebsiella pneumoniae* 231.
[g] *Candida albicans* PCM 1409 PZH.
[h] *Aspergillus fumigatus* C1.
[i] *Microsporum gypseum* K1.
[j] Chloramphenicole (Polfa-Łódź).
[k] Amphoterricine.

Table 2 shows the antimicrobial activity (MIC, minimum inhibitory concentration, values) of the compounds tested as well as the reference antibacterial drug (chloramphenicol) and reference antifungal drug (amphoterricine B). The data obtained in this study indicate that in the series of the compounds investigated, the effects on Gram-positive cocci and aerobic bacilli were stronger than on Gram-negative rods. It should be noted that **3d**, **3e**, **6b**, **6c**, **6e** and **6f** are broad-spectrum antibacterial agents.

The strongest effects on Gram-positive bacteria were observed for bromides of (*E*)-*N*-bromohexyl-4-azachalcone (**3d**), bromide of (*E*)-*N*-bromodecyl-4-azachalcone (**3e**), bromide of (*E*)-*N*-*m*-bromobenzyl-3′-hydroxy-4-azachalcone (**6b**), bromide of (*E*)-*N*-*p*-bromobenzyl-3′-hydroxy-4-azachalcone (**6c**) and chloride of (*E*)-*N*-*m*-chlorobenzyl-3′-hydroxy-4-azachalcone (**6e**).

It should also be pointed out that the bromide of (*E*)-*N*-bromodecyl-4-azachalcone (**3e**) is simultaneously active against dermatophytes.

The data shown in Table 2 suggest that the length of the *N*-bromoalkyl chain influences the antimicrobial activity in the series **3a**–**3e**. The best activity is connected with the presence of 10 carbon atoms in the bromo alkyl chain of the molecule of **3e**. A comparison of the activities of halides of (*E*)-*N*-*o*-(*m*- and *p*-)halobenzyl-3′-hydroxy-4-azachalcones (**6a**–**6f**) indicates that the nature of the atom of the halogen present in the benzyl group, as well as the halide part of the molecules of **6a**–**6f** significantly influence the activity. The presence of Br in the *m*- or *p*-positions of the phenyl ring of the benzyl group and simultaneously in the anionic part of the molecules gives derivatives with stronger antibacterial activity (**6b**, **6c** against **6e**,**6f**).

4. Chemical experimental section

4.1. Chemistry

The purity of all compounds described was investigated with the use of melting points, TLC and elemental analyses. The melting points (uncorrected) were determined on a Boetius apparatus. R_f values refer to TLC plates with silica gel 60 F_{254} (E. Merck) developed with chloroform–methanol (5:1) and observed under UV light ($\lambda = 254$ nm). IR spectra were recorded on a Perkin–Elmer M 180 spectrophotometer in KBr pellets. ^{1}H NMR spectra were recorded on a Varian Gemini VT 300 spectrometer at 300.075 MHz in DMSO-d_6 solution with TMS as internal standard. The standard resolution was 0.2 Hz per point for ^{1}H spectra. All chemical shifts are quoted in δ (ppm) values. UV–Vis spectra were recorded on a Specord UV–Vis spectrophotometer in methanol solution.

(*E*)-4-Azachalcone and (*E*)-3′-hydroxy-4-azachalcone were prepared according to the literature [25].

4.1.1. General procedure for synthesis of compounds 3a–3e

A mixture of 0.42 g (0.002 mol) of (*E*)-4-azachalkone (**1**) and 0.006 mol of the corresponding dibromoalkane **2a**–**2e** (1,2-dibromoethane, 1,3-dibromopropane, 1,5-dibromopentane, 1,6-dibromohexane or 1,10-dibromodecane) was heated at reflux for 50–58 h in 50 ml of acetonitrile. The precipitated solids were filtered off while still hot. After filtration the solvent was evaporated on a rotatory evaporator and the residue was separated on a silica gel column (63–100 mesh) using as eluents chloroform–methanol 50:1 (200 ml), and then chloroform–methanol 20:1 (150 ml) to give the crude products of **3a**–**3e**. Recrystallization from the chloroform–methanol (5:1) mixture afforded the analytical samples of compounds **3a**–**3e**.

4.1.2. General procedure for synthesis of compounds 6a–6f

A mixture of 0.45 g (0.002 mol) of (*E*)-3′-hydroxy-4-azachalcone (**4**) and 0.002 mol of the corresponding benzyl halides **5a**–**5f** [*o*-(*m*- or *p*-)bromobenzyl bromide and *o*-(*m*- or *p*-)chlorobenzyl chloride] was heated at reflux in 50 ml of acetonitrile for 18 h (**6a**–**6c**) or 50 h (**6d**–**6f**). The precipitated solids of **6a**–**6f** were filtered off while still hot. Then the solvent was evaporated on a rotatory evaporator. The residue was separated on a silica gel column by eluting with chloroform–methanol 50:1 (150 ml), then chloroform–methanol 20:1 (150 ml) to give the crude products of **6a**–**6f**. Recrystallization from the chloroform–methanol (1:1) mixture afforded the analytical samples of compounds **6a**–**6f**.

4.2. Biological test procedures

The antimicrobial activity of the compounds was investigated against the following strains: Gram-positive cocci (*Staphylococcus aureus* FDA209P, *Enterococcus faecalis* ATCC 8040), aerobic bacilli (*Bacillus subtilis* ATCC1633), Gram-negative rods (*Escherichia coli* PZH 026B6, *Klebsiella pneumoniae* 231, *Pseudomonas aeruginosa* S85/2), yeasts (*Candida albicans* PCM 1409 PZH), moulds (*Aspergillus fumigatus* C1) and dermatophytes (*Microsporum gypseum* K_1).

4.3. Determination of minimum inhibitory concentration

Compounds were dissolved using DMSO (Serva); concentration was 1000 μg/ml. The MIC values of the compounds were determined, with reference to standard microorganisms, by introducing 1 ml of the corresponding solutions at various concentrations into a series of tubes (each 12 × 100 mm), then 0.1 ml of a standardized 1:1000 diluted suspension of a microorganism was added. The MIC values were determined after 18 h of incubation at 37°C. As a test medium for bacteria the fluid medium Penassay Broth (Difco) was used. In each assay the control of both the bacterial culture sterility and standard bacteria growth was performed. Sabouraud dextrise broth (Difco) was used as a test medium for fungi; MIC values were determined after 3–7 days of incubation at 25°C. In all assay both fungi culture sterility and standard fungi growth were checked.

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