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Antimicrobial activity of some *N*-alkyl substituted of (E)-4-azachalconium and (E)-3′-hydroxy-4-azachalconium bromides

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Abstract

Twelve new *N*-substituted (E)-azachalconium bromides were synthesized and tested for antimicrobial and antifungal activities. Compounds **5c**, **5d** and **5h**–**5l** showed very good antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis* as well as *Bacillus subtilis* and **5h**–**5j** showed moderate activity against *Escherichia coli*. In particular, (E)-*N*-dodecyl-4-azachalconium bromide (**5i**) and (E)-*N*-tetradecyl-4-azachalconium bromide (**5j**) showed the most intensive activity against all tested microorganisms.

Keywords: Antimicrobial activity; *N*-Substituted (E)-Azachalconium bromides

1. Introduction

Over the last twenty years, analogs of azachalcones have been synthetically prepared by several investigators and tested for antimicrobial [1–10], antituberculostatic [11,12] and anti-inflammatory activities [13,14]. The 4-azachalcone and their derivatives [15,16] were the most potent of the chalcone series as inhibitors of myeloperoxidase (MPO) enzyme release from rat polymorphonuclear leukocytes (PMN).

A few years ago we described the antimicrobial activity of several derivatives of (E)-4-azachalcones with a structure characterized by the presence of *N*-bromoalkyl, *ortho*-(*meta*- and *para*-)halobenzyl and alkylthiouracil substituents [2–4]. Among them a few compounds were of particular interest [as (E)-*N*-uracil-(and 6-methyluracil-)thiodecyl substituted 4-azachalconium bromides, (E)-*N*-halobenzyl-4-azachalconium bromides and (E)-*N*-bromodecyl-4-azachalconium bromide], exhibiting a good level of activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Enterococcus faecalis* and *Bacillus subtilis* with minimum inhibitory concentration (MIC) between 1 and 10 μg/ml.

In view of the continuous interest of antimicrobial agents we deemed it worthwhile to investigate other *N*-substituted (E)-azachalconium bromides in this respect, in order to better evaluate the structural requirements for activity. We wanted to determine the influence of the three structural modifications on the antimicrobial activity of (E)-*N*-bromoalkyl-4-azachalconium bromides. These modifications, i.e. the lack of the hydrophilic bromine atom at the end of the *N*-alkyl substituent, the length of the carbon chain in the *N*-alkyl substituent as well as the introduction of the strongly hydrophilic hydroxy group on the 3 positions of the phenyl ring, change both the electronic distribution and the lipophilic–hydrophilic balance of the molecules of *N*-substituted bromides of (E)-chalcones. It ought to be pointed out that according to Tsuchiya et al. [17], the presence of hydroxyl groups at C-2, C-4 and C-2′ in chalcone is essential to inhibit growth of the *Candida* species.

In this paper, we describe the synthesis and characteristics of 12 new (E)-*N*-alkyl-4-azachalconium bromides, and (E)-*N*-alkyl-3′-hydroxy-4-azachalconium bromides, and report the results of microbiological screening.

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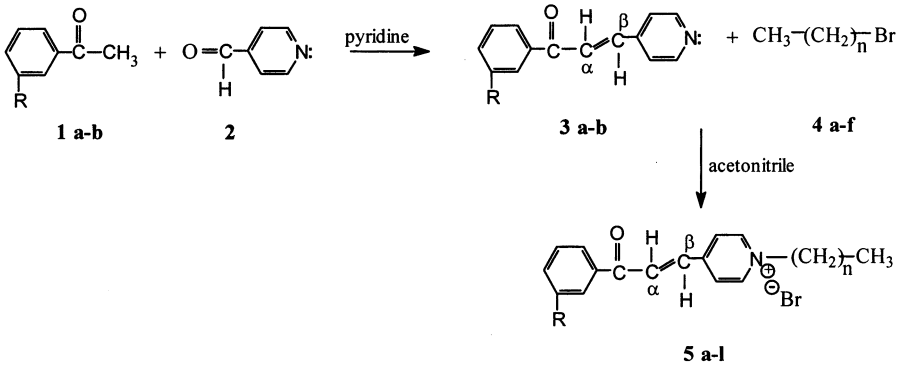
2. Chemistry

Scheme 1 illustrates the synthetic approach chosen for the preparation of (E)-*N*-alkyl-3′-hydroxy-4-azachalconium bromides (**5a**–**5f**) and (E)-*N*-alkyl-4-azachalconium bromides (**5g**–**5l**). (E)-3′-hydroxy-4-azachalcone (**3a**) and (E)-4-azachalcone (**3b**) were selected as the starting materials together with 1-bromohexane (**4a**), 1-bromodecane (**4b**), 1-bromododecane (**4c**), 1-bromotetradecane (**4d**), 1-bromohexadecane (**4e**) and 1-bromooctadecane (**4f**). **3a** and **3b** were synthesized by condensation of corresponding acetophenone (**1a**–**1b**) with 4-pyridinecarboxyaldehyde in pyridine. The reactions of **3a**–**3b** with **4a**–**4f** were carried out in boiling acetonitrile. Twelve new (E)-*N*-alkyl substituted 3′-hydroxy-4-azachalconium bromides (**5a**–**5f**) and (E)-*N*-alkyl-4-azachalconium bromides (**5g**–**5l**) were obtained in these reactions of nucleophylic substitution. The structures of all obtained compounds were determined by examining their UV–Vis, IR, ^{1}H NMR and ^{13}C NMR spectra, as well as by elemental analyses (Tables 1 and 2).

It should be mentioned that analysis of IR spectra revealed (E)-configuration for all obtained compounds due to the presence of strong bonds of out-of-plane *trans* olefinic C–H bending vibrations between 960 and 980 cm^{-1}. The geometry at the ethylene bridge of (E)-*N*-alkyl-4-azachalconium bromides (**5a**–**5l**) was also assigned as E based on the olefin ^{1}H NMR coupling constants ($J = 15.6$ Hz) [18].

3. Results and discussion

The new obtained compounds **5a**–**5l** were assayed against the following nine strains of microorganisms: Gram-positive cocci: *S. aureus*, *E. faecalis*; aerobic bacilli: *B. subtilis*; Gram-negative rods: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*; yeasts: *Candida albicans*; moulds: *Aspergillus fumigatus*; and



Comp. 1	n	R	Comp. 5	n	R
a	-	OH	**a**	5	OH
b	-	H	**b**	9	OH
Comp. 3			**c**	11	OH
a	-	OH	**d**	13	OH
b	-	H	**e**	15	OH
Comp. 4			**f**	17	OH
a	5	-	**g**	5	H
b	9	-	**h**	9	H
c	11	-	**i**	11	H
d	13	-	**j**	13	H
e	15	-	**k**	15	H
f	17	-	**l**	17	H

Scheme 1.

Table 1
Physico-chemical data of compounds **5a**–**5l**

Compound	Formula [a]	Yield (%)	m.p. (°C)	^{13}C NMR (DMSO-d$_6$) δ ppm				TLC (R_f)
				C=O	N–CH$_2$	C$_\alpha$	C$_\beta$	
5a	$C_{20}H_{24}NO_2Br$ (390.32)	65	184–186	188.62	60.37	136.98	132.25	0.31
5b	$C_{24}H_{32}NO_2Br$ (446.43)	41	123–125	188.61	60.37	136.97	132.25	0.31
5c	$C_{26}H_{36}NO_2Br$ (474.48)	59	139–141	188.59	60.35	136.95	132.23	0.32
5d	$C_{28}H_{40}NO_2Br$ (502.54)	55	155–158	188.60	60.35	136.97	132.23	0.31
5e	$C_{30}H_{44}NO_2Br$ (530.59)	62	153–156	188.58	60.37	136.95	132.23	0.32
5f	$C_{32}H_{48}NO_2Br$ (558.64)	65	149–152	188.58	60.35	136.95	132.24	0.31
5g	$C_{20}H_{24}NOBr$ (374.32)	39	141–143	188.73	60.34	136.53	134.13	0.35
5h	$C_{24}H_{32}NOBr$ (430.43)	45	159–161	188.75	60.34	136.54	134.08	0.34
5I	$C_{26}H_{36}NOBr$ (458.48)	49	147–149	188.77	60.33	136.54	134.04	0.35
5j	$C_{28}H_{40}NOBr$ (486.54)	47	152–154	188.77	60.34	136.53	134.06	0.34
5k	$C_{30}H_{44}NOBr$ (514.59)	46	146–149	188.75	60.33	136.52	134.04	0.33
5l	$C_{32}H_{48}NOBr$ (542.64)	49	130–131	188.73	60.34	136.54	134.06	0.33

[a] Analyses for C, H and N are within $\pm$0.4% of the theoretical values.

Table 2
Physico-chemical data of compounds **5a**–**5l**

Compound	IR (KBr) (cm^{-1}, νC=O)	^{1}H NMR (DMSO-d$_6$) δ ppm, J Hz [a]					UV–Vis λ_{nm} (log ε)		
		CH$_3$	CH$_2$–N	OH	C$_\alpha$–H	C$_\beta$–H			
5a	1667.9	0.86 t	4.60 t	9.96 s	7.83 d	8.43 d	291.0 (4.46)		210.0 (4.37)
5b	1667.0	0.85 t	4.59 t	9.94 s	7.82 d	8.43 d	291.0 (4.46)		206.5 (4.38)
5c	1666.8	0.85 t	4.60 t	9.97 s	7.82 d	8.43 d	291.0 (4.44)		208.5 (4.37)
5d	1666.8	0.85 t	4.60 t	9.95 s	7.83 d	8.42 d	290.5 (4.46)		209.5 (4.37)
5e	1666.3	0.85 t	4.59 t	9.93 s	7.82 d	8.42 d	291.0 (4.45)		209.0 (4.34)
5f	1660.9	0.85 t	4.60 t	9.94 s	7.83 d	8.42 d	291.0 (4.45)		206.5 (4.37)
5g	1664.8	0.87 t	4.62 t	–	7.86 d	8.52 d	289.5 (4.37)	222.0 (4.01)	203.0 (4.36)
5h	1661.9	0.87 t	4.61 t	–	7.85 d	8.52 d	289.0 (4.40)	222.0 (4.03)	203.0 (4.37)
5I	1664.7	0.85 t	4.61 t	–	7.86 d	8.53 d	290.0 (4.48)	220.5 (4.05)	203.0 (4.40)
5j	1662.5	0.85 t	4.58 t	–	7.86 d	8.51 d	289.0 (4.47)	220.5 (4.01)	203.0 (4.36)
5k	1661.0	0.85 t	4.59 t	–	7.86 d	8.51 d	290.5 (4.49)	220.0 (4.05)	202.5 (4.39)
5l	1663.2	0.85 t	4.59 t	–	7.87 d	8.52 d	290.5 (4.48)	222.0 (4.04)	203.0 (4.37)

[a] The ^3J (HH) of 15.6 Hz between H-α and H-β.

dermatophytes: *Microsporum gypseum* K$_1$. (Table 3). Many of the obtained compounds showed very good antibacterial and antifungal activity. The effect on Gram-positive cocci was stronger than on Gram-negative rods. The strongest effect on Gram-positive bacteria were observed for (E)-*N*-dodecyl-3′-hydroxy-4-azachalconium bromide (**5c**) (MIC 5.0 μg/ml) and (E)-*N*-tetradecyl-3′-hydroxy-4-azachalconium bromide (**5d**) (MIC 5.0–7.5 μg/ml) as well as for (E)-*N*-dodecyl-4-azachalconium bromide (**5i**), (E)-*N*-tetradecyl-4-azachalconium bromide (**5j**) and (E)-*N*-hexadecyl-4-azachalconium bromide (**5k**) (MIC 5.0 μg/ml). It should be pointed out that all of these compounds are also very effective against *B. subtilis* (MIC 0.25–7.5 μg/ml). Moderate effects on *E. coli* were observed for compounds **5h**–**5j** (MIC 10–25 μg/ml). It is interesting that the all *N*-substituted (E)-4-azachalconium bromides (**5g**–**5l**) discussed herein are effective against *M. gypseum* (MIC 10–100 μg/ml). In the series of the derivatives of 3′-hydroxy-4-azachalcone (**5a**–**5f**), only (E)-*N*-dodecyl-3′-hydroxy-4-azachalconium bromide (**5c**) showed moderate effects on *M. gypseum* (MIC 100 μg/ml). Among the (E)-azachalcone derivatives (**5g**–**5l**) examined, (E)-*N*-dodecyl-4-azachalconium bromide (**5i**) and (E)-*N*-tetradecyl-4-azachalconium bromide (**5j**) showed the strongest activity against all tested microorganisms. It should be noted that **5c** and **5i**–**5k** break the evolution of *S. aureus* and *E. faecalis* in the same range of concentrations as Chloramphenicol. Similarly **5c**, **5j** and **5k** affect *B. subtilis*, but it ought to be pointed out that **5i** is stronger and it is active in lower concentration (MIC 0.25 μg/ml). Only (E)-*N*-octadecyl-3′-hydroxy-4-azachalconium bromide (**5f**) showed no antibacterial or antifungal activity. The comparison of the antibacterial and antifungal activity of (E)-*N*-pentyl-4-azachalconium bromide (**5g**) and (E)-*N*-bromopentyl-4-azachalconium bromide (**3c** lit.4) showed that the lack of the bromine atom at the end of

the alkyl chain increases the activity against *S. aureus*. The comparison of antibacterial and antifungal activity of (E)-*N*-alkyl-3′-hydroxy-4-azachalconium bromides (**5a**–**5f**) and (E)-*N*-alkyl-4-azachalconium bromides (**5g**–**5l**) showed that the presence of hydroxy substituent in the 3′ position of the phenyl ring of the skeleton of (E)-4-azachalcone clearly influences the diminishing of the activity against *M. gypseum* in the series 3′-hydroxy substituted derivatives of chalcone (**5a**–**5f**). The same effect is seen in the cases of *E. coli* (**5a**–**5e**), *K. pneumoniae* (**5d**), *P. aeruginosa* (**5c** and **5d**), *C. albicans* (**5c**), *S. aureus* (**5a**, **5b**, **5e** and **5f**), *E. faecalis* (**5b** and **5d**–**5f**) and *B. subtilis* (**5b**–**5f**).

It is also clear that the length of the alkyl chain influences the broadening of the spectrum of antimicrobial activity and the value of MIC of investigated compounds **5a**–**5l**. The optimum length of the alkyl chain for better and broader activity is situated in the range of 12–16 carbon atoms in the series of **5g**–**5l**, and 12–14 carbon atoms in the series of **5a**–**5f**.

4. Experimental

The melting points were determined on a Melt-Temp II melting point apparatus and are uncorrected. R_f values refer to TLC plates with silica gel F_{254} (Merck) developed with chloroform–methanol (5:1) and observed under UV light ($\lambda = 254$ nm). Infrared spectra were recorded as KBr pellets on a Bruker IFS 113 FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini VT 300 spectrometer at 300.075 MHz, using Me_4Si as an internal standard and DMSO-d_6 as solvent. 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 [19].

1-bromohexane (**4a**), 1-bromodecane (**4b**), 1-bromododecane (**4c**), 1-bromotetradecane (**4d**), 1-bromohexadecane (**4e**) and 1-bromooctadecane (**4f**) were obtained from Aldrich.

4.1. General procedure for synthesis of compounds 5a–5l

A mixture of (E)-3′-hydroxy-4-azachalcone or (E)-4-azachalcone (0.001 mol) and bromoalkanes (1-bromohexane, 1-bromodecane, 1-bromododecane, 1-bromotetradecane, 1-bromohexadecane; 0.003 mol) in acetonitrile (50 ml) was refluxed for 30 h. Then half a volume of acetonitrile was removed using a rotatory evaporator, and the precipitated solid was purified by column chromatography (column, length 30 cm, diameter 2 cm) on silica gel (25 g, Merck, 0.063–0.1 mm). The column was eluted successively with the following solvent mixture: chloroform–methanol, A [50:1, 100 ml] and B [20:1, 150 ml]. Fractions of 20 ml were collected and monitored by analytical TLC. The desired products **5a**–**5l** were obtained from fractions 5–11. The isolated crude product was recrystallized from chloroform–methanol 1:1.

Table 3
Antimicrobial activity of **5a**–**5f** and **5g**–**5l**

Compound	Minimum inhibitory concentration [a] (μg/ml)							
	1	2	3	4	5	6	7	8
5a	100	100	100	– [b]	– [b]	– [b]	– [b]	– [b]
5b	100	100	100	100	100	– [b]	– [b]	– [b]
5c	5	5	5	100	100	– [b]	– [b]	100
5d	5	7.5	7.5	100	– [b]	– [b]	– [b]	– [b]
5e	100	100	100	– [b]	– [b]	– [b]	– [b]	– [b]
5f	– [b]	– [b]	– [b]	– [b]	– [b]	– [b]	– [b]	– [b]
5g	10	100	100	100	– [b]	– [b]	– [b]	100
5h	10	10	10	10	100	– [b]	– [b]	100
5I	5	5	0.25	10	100	100	100	50
5j	5	5	5	25	50	75	– [b]	10
5k	5	5	5	100	– [b]	– [b]	– [b]	50
5l	100	5	5	– [b]	– [b]	– [b]	– [b]	10
Chloramphenicol (Polfa-Łódź)	5	5	5	5	50			
Amfotericin						0.5	10	1

A, Chloramphenicol (Polfa–Łódź); B, Amfotericin. 1, *S. aureus* FDA 209 P; 2, *E. faecalis* ATCC 8040/1; 3, *B. subtilis* ATCC1633; 4, *E. coli* PZH 026B6; 5, *K. pneumoniae* 231; 6, *P. aeruginosa* SR1; 7, *C. albicans* PCM 1409 PZH; 8, *M. gypseum* K1.

[a] MIC, the minimum inhibitory concentration is the lowest value of concentration of the investigated compound which brakes the evolution of the microorganism.

[b] The values of MIC are situated in the range 250–1000 μg/ml.

4.2. Biological test procedures

The activity of the compounds was investigated against the following strains: Gram-positive cocci (*S. aureus* FDA209P, *E. faecalis* ATCC 8040), aerobic bacilli (*B. subtilis* ATCC1633), Gram-negative rods (*E. coli* PZH 026B6, *K. pneumoniae* 231, *P. aeruginosa* S85/2), yeasts (*C. albicans* PCM 1409 PZH), moulds (*A. fumigatus* C1) and dermatophytes (*M. gypseum* K_1).

4.3. Determination of MIC

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 assays both fungi culture sterility and standard fungi growth were checked.

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