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Antimicrobial activity of some *N*-alkyl and *N*-nitrobenzyl substituted halides of (*E*)-4′-hydroxy-3′-methoxystilbazoles-4

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Abstract

The synthesis of 11 new *N*-substituted derivatives of (*E*)-4′-hydroxy-3′-methoxystilbazoles-4 and their antimicrobial activity are reported. The relationships between structure and antibacterial activity are studied. In particular, compounds **5**, **7** and **8** showed good antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis* and *Bacillus subtilis*, while compound **6** did so against *S. aureus* and *S. faecalis*. © 1999 Elsevier Science S.A. All rights reserved.

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1. Introduction

(*E*)-Azastilbenes have been known from their biological activities [1–6]. Cavallito et al. [7–9] and later Grifantini and co-workers [10] demonstrated a high inhibition of choline acetyltransferase (ChA) by *N*-substituted derivatives of *trans*-styrylpyridines. Many of *N*-substituted stilbazoles have shown good antimicrobial activity [11–13].

In previous communications [14–16] we reported the synthesis and antimicrobial activity of some (*E*)-*N*-alkyl (or benzyl) substituted halides of α-(or γ-)-azastilbenols-2′(3′ and 4′). To improve antimicrobial activity of stilbazoles we introduced a methoxy group to their phenyl ring.

For this reason we decided to synthesize 11 new *N*-bromoalkyl, *N*-alkyl, *N*-benzyl and *N*-nitrobenzyl substituted derivatives of (*E*)-4′-hydroxy-3′-methoxystilbazoles-4 having a methoxy residue in the 3′ position. In this paper we also describe their physicochemical data and microbiological screening.

2. Chemistry

The synthetic approach to obtaining bromides of (*E*)-*N*-bromoalkyl-4′-hydroxy-3′-methoxystilbazoles-4 (**2**–**8**), bromide of (*E*)-*N*-benzyl-4′-hydroxy-3′-methoxystilbazole-4 (**9**), and halides of (*E*)-*N*-*o*-(*m*- or *p*-)nitrobenzyl-4′-hydroxy-3′-methoxystilbazoles-4 (**10**–**12**) followed the reactions shown in Scheme 1.

(*E*)-4′-Hydroxy-3′-methoxystilbazole-4 (**1**) as the starting material was synthesized by condensation of 4-picoline with 4-hydroxy-3-methoxybenzaldehyde in refluxing acetic anhydride [17].

A series of 11 new halides of *N*-bromoalkyl, *N*-alkyl, *N*-benzyl and *N*-nitrobenzyl substituted derivatives of (*E*)-4′-hydroxy-3′-methoxystilbazoles-4 were synthesized by reaction of (*E*)-4′-hydroxy-3′-methoxystilbazole-4 (**1**) with bromoalkanes (1-bromoethane, 1-bromopentane, 1-bromodecane, 1-bromododecane, 1-bromohexadecane), dibromoalkanes (1,5-dibromopropane, 1,10-dibromodecane) as well as benzyl bromide or (*m*- or *p*-)nitrobenzyl halides in boiling nitromethane.

The structures of all obtained compounds **1**–**12** were confirmed by elemental analysis and spectral data. It should be mentioned that analysis of IR and UV–Vis spectra revealed (*E*) configuration for all obtained compounds (Table 1) [18–21].

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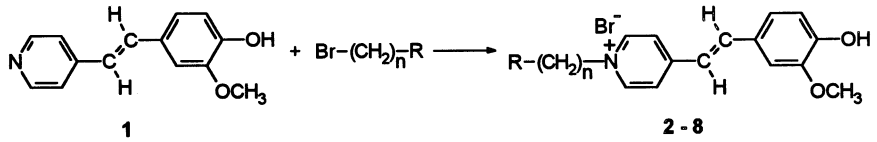

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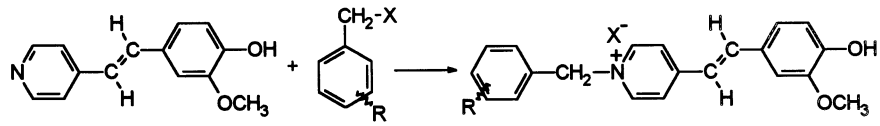

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Compound	n	R	Compound	R	X
2	1	CH_3	**9**	H	Br
3	4	CH_3	**10**	o-NO_2	Cl
4	5	Br	**11**	m-NO_2	Br
5	9	CH_3	**12**	p-NO_2	Br
6	10	Br			
7	11	CH_3			
8	15	CH_3			

Scheme 1.

The antimicrobial activity of the investigated compounds **1**–**12** was measured as minimum inhibitory concentrations (MICs) (Table 2).

3. Results and discussion

The obtained compounds **1**–**12** were assayed against the following nine strains of microorganisms: Gram-positive cocci (*Staphylococcus aureus* FDA209P, *Streptococcus faecalis* ATCC 8040), aerobic bacilli (*Bacillus subtilis* ATCC 6633), Gram-negative rods (*Escherichia coli* PZH 026B6, *Klebsiella pneumoniae* 231, *Pseudomonas aeruginosa* S 85/2), yeasts (*Candida albicans* PCM 1409 PZH), dermatophytes (*Microsporum gypseum* K_1) and moulds (*Aspergillus fumigatus* C1). Table 2 shows the antibacterial activity (MIC values) of compounds **1**–**12**.

(*E*)-4′-Hydroxy-3′-methoxystilbazole-4 (**1**) and chloride of (*E*)-*N*-(*o*-nitrobenzyl)-4′-hydroxy-3′-methoxystilbazole-4 (**10**) showed no antibacterial or fungicidal activity (Table 2).

The strongest effects on Gram-positive *S. aureus*, *S. faecalis* and *B. subtilis* were observed for bromides of (*E*)-*N*-decyl (dodecyl, hexadecyl)-4′-hydroxy-3′-methoxystilbazoles-4 (**5**, **7**, **8**). Compounds **5** and **7** also showed low antimicrobial activity against *E. coli*, *K. pneumoniae* and *C. albicans*.

The bromide of (*E*)-*N*-(*n*-bromodecyl)-4′-hydroxy-3′-methoxystilbazole-4 (**6**) revealed strong antibacterial activity against Gram-positive cocci (*S. aureus*, *S. faecalis*), and low antibacterial activity against aerobic bacilli (*B. subtilis*) and Gram-negitive rods (*E. coli*, *K. pneumoniae*, *P. aeruginosa*).

Table 1
Chemical and physical data of compounds **1**–**12**

Comp.	Yield (%)	M.p. (°C)	TLC (R_f)	IR (KBr) (cm^{-1}) $\delta_{CH=CH}$	Analysis	^1H NMR δ (ppm) $-CH_2-^+N$
1	63.1	204–207	0.85	955	$(C_{14}H_{13}NO_2)$C,H,N	
2	89.0	271–274	0.16	970	$(C_{16}H_{18}BrNO_2)$C,H,N	4.53
3	83.9	205–207	0.46	985	$(C_{19}H_{24}BrNO_2)$C,H,N	4.50
4	67.7	147–150	0.39	960	$(C_{19}H_{23}Br_2NO_2)$C,H,N	4.51
5	61.9	186–189	0.58	970	$(C_{24}H_{34}BrNO_2)$C,H,N	4.49
6	77.2	122–125	0.58	955	$(C_{24}H_{33}Br_2NO_2)$C,H,N	4.47
7	88.7	160–162	0.60	970	$(C_{26}H_{38}BrNO_2)$C,H,N	4.49
8	90.0	157–160	0.62	970	$(C_{30}H_{46}BrNO_2)$C,H,N	4.48
9	92.0	243–246	0.42	960	$(C_{21}H_{20}BrNO_2)$C,H,N	5.79
10	77.3	142–145	0.35	960	$(C_{21}H_{19}ClN_2O_4)$C,H,N	6.17
11	93.7	237–240	0.29	970	$(C_{21}H_{19}BrN_2O_4)$C,H,N	5.93
12	87.0	146–149	0.21	960	$(C_{21}H_{19}BrN_2O_4)$C,H,N	5.96

Table 2
Antimicrobial activity of compounds **1**–**12**

Comp.	Minimal inhibitory concentration (MIC) μg/ml [a]								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	1000	1000	>1000	1000	1000	1000	>500	>500	>500
2	>1000	500	>1000	>1000	>1000	>1000	250	100	1000
3	500	500	1000	1000	>1000	1000	500	100	1000
4	100	100	1000	1000	1000	1000	>500	100	1000
5	7.5	10	10	100	100	500	100	500	>500
6	7.5	10	100	100	100	100	500	500	>500
7	10	7.5	5	100	100	500	250	500	>500
8	5	5	5	500	1000	500	500	>500	>500
9	100	1000	>1000	1000	1000	1000	500	500	>500
10	500	>1000	>1000	1000	>1000	1000	>500	250	1000
11	500	>1000	1000	1000	>1000	1000	>500	100	1000
12	500	100	1000	500	1000	1000	>500	250	1000

[a] 1, *S. aureus* FDA209P; 2, *S. faecalis* ATCC 8040; 3, *B. subtilis* ATCC 6633; 4, *E. coli* PZH 026B6; 5, *K. pneumoniae* 231; 6, *P. aeruginosa* S 85/2; 7, *C. albicans* PCM 1409 PZH; 8, *M. gypseum* K1; 9, *A. fumigatus* C1.

The bromides of *N*-alkyl short-chain substituted (*E*)-4′-hydroxy-3′-methoxystilbazoles-4 (**2**, **3**, **4**) and the bromides of *N*-nitrobenzyl substituted (*E*)-4′-hydroxy-3′-methoxystilbazoles-4 (**10**, **11**, **12**) were characterized by low antifungal activity against *M. gypseum* (Table 2). Compound **2** also had low antifungal activity against *C. albicans*. The bromide of (*E*)-*N*-(*n*-bromopentyl)-4′-hydroxy-3′-methoxystilbazole-4 (**4**) had low antibacterial activity against *S. aureus* and *S. faecalis*, as did the bromide of (*E*)-*N*-benzyl-4′-hydroxy-3′-methoxystilbazole-4 (**9**) against *S. aureus* and the bromide of (*E*)-*N*(*p*-nitrobenzyl)-4′-hydroxy-3′-methoxystilbazole-4 (**12**) against *S. faecalis*.

The data obtained in this study indicate that, in the series of investigated compounds, the effects on Gram-positive cocci (compounds **4**, **5**, **6**, **7**, **8**) and aerobic bacilli (compounds **5**, **7**, **8**) were stronger than on Gram-negative rods (Table 2).

4. Experimental

4.1. Chemistry

The purity of all compounds described was monitored by melting points, TLC and elemental analyses. Melting points (uncorrected) were determined on a Böetius apparatus. R_f values refer to TLC plates with Silica Gel 60 F_{254} (E. Merck) developed with 2:1 chloroform:methanol and observed under UV light ($\lambda = 254$ nm, and $\lambda = 360$ nm). IR spectra were recorded on a Perkin–Elmer M180 spectrophotometer in KBr pellets.

^{1}H NMR spectra were determined on a Varian Gemini 300 (300 MHz) in DMSO-d_6 solution with TMS as internal standard. All chemical shifts are quoted in δ values. UV–Vis spectra were recorded on a Shimadzu UV-160 spectrophotometer in methanol solution.

(*E*)-4′-Hydroxy-3′-methoxystilbazole-4 (**1**) was prepared according to the literature [17].

4.1.1. General procedure for synthesis of compounds **2**–**12**

(*E*)-4′-Hydroxy-3′-methoxystilbazole-4 (**1**, 5 mmol) was dissolved in 50 ml of boiling nitromethane. Upon dissolution, 25 mmol of corresponding bromoalkanes (dibromoalkanes or benzyl halides) were added. The reaction mixture was refluxed for 8 h (or 5 h for **2**, **4**, **11** or 1.5 h for **9**, **12**). The precipitated solids of **2**, **4**, **9** and **12** were filtered off while still hot. After cooling the reaction mixtures of **3**, **5**–**8**, **10**, **11** for 5 h in the refrigerator, the precipitated solids were filtered off, washed with nitromethane and dried. Then half a volume of nitromethane from the filtrates of **2**–**12** was removed using a rotatory evaporator. The residues were cooled for 24 h in the refrigerator and the precipitated second fractions of solids were filtered off, washed with nitromethane and dried.

The desired products were obtained by combining both fractions of solids and recrystallizing them from nitromethane.

4.2. Biological test procedures

The antimicrobial activity of the compounds was investigated against the following strains: Gram-positive cocci (*S. aureus* FDA209P, *S. faecalis* ATCC 8040), aerobic bacilli (*B. subtilis* ATCC 6633), Gram-negative rods (*E. coli* PZH 026B6, *K. pneumoniae* 231, *P. aeruginosa* S 85/2), yeasts (*C. albicans* PCM 1409 PZH), dermatophytes (*M. gypseum* K_1) and moulds (*A. fumigatus* C1).

5. Determination of minimum inhibitory concentration

Compounds were dissolved using DMSO (Serva); concentration was 1000 μg/ml. A series of dilutions with concentrations ranging from 1 to 1000 μg/ml were prepared for each compound. 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 microorganism was added. MIC values were determined after 18 h of incubation at 37°C. The fluid medium Penassay broth (Difco) was used as a test medium for bacteria; in all assays both bacterial culture sterility and standard bacterial growth were checked. Sabouraud dextrose 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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