ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 151–157

IL FARMACO

Short Communication

Synthesis and antimicrobial activity of (*E*)-acetoxystilbenes and α,α′-dibromoacetoxybibenzyls

Elżbieta Wyrzykiewicz [a,*], Alfred Błaszczyk [a], Bogdan Kędzia [b]

[a] *Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*
[b] *Institute of Medicinal Plants, Libelta 27,61-707 Poznań, Poland*

Received 25 June 1999; accepted 8 November 1999

Abstract

The synthesis of 13 new (*E*)-acetoxystilbenes and α,α′-dibromoacetoxybibenzyls and their antimicrobial activity are reported. The results of microbiological screening of 17 (*E*)-stilbenols and (*E*)-acetoxystilbenes, unknown in the literature, have also been discussed. In particular, coumpounds **1c**, **1g**, **2a**, **3a**, **3b**, **4a**, **6a**, **6b** showed good antibacterial activity against *Staphylococcus aureus* and **1c** also against *Bacillus subtilis*. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Antimicrobial activity; (*E*)-Acetoxystilbenes; α,α′-Dibromoacetoxybibenzyls

Natural (*E*)-stilbenes, hydroxylated in two to five positions, are produced by woody plants and exhibit a broad spectrum of antimicrobial activity [1–5]. Bibenzyls (dihydrostilbenes) are structurally similar to stilbenes and many derivatives of bibenzyls, isolated from a number of plants, are bioactive [6–9]. Owing to the biological properties of (*E*)-stilbenols, (*E*)-azastilbenols and their derivatives, the reactions of the modifications of these compounds and physicochemical, as well as biological behaviour, have been studied previously in our laboratory [10–13]. In 1990 Schultz et al. [2] investigated the role of stilbenes in the natural durability of wood. These authors measured the fungicidal bioactivity of a number of (*E*)-4-hydroxy-3′ and/or 4′Me, OMe, F, Cl and OH substituted stilbenes, 4-hydroxybibenzyl, 4-hydroxy-4′-methoxybibenzyl and related analogs. A quantitative structure–activity relationship study found that the fungicidal activity against the brown-rot fungi *Gleophyllum trabeum* and *Poria placenta* of investigated stilbenes was linearly related to hydrophobicity. On the other hand, 4-hydroxybibenzyl with 4′ hydrogen showed fungicidal activity against the white-rot fungus *Coriolus versicolor*, and against the two brown-rot fungi mentioned above.

However, little is known as yet about the antimicrobial activity of (*E*)-acetoxystilbens and related acetoxybibenzyls. It has been reported that the antifungal activities of (*E*)-3,3′,4,5′-tetraacetoxystilbene and 3,3′,4,5′-tetrahydroxybibenzyl were weaker than that of natural (*E*)-3,3′,4,5′-tetrahydroxystilbene, a constituent of *Cassia garretiana Craib* (*Leguminosae*) [14]. In view of the fact that the substitution of an acetyl group into a hydroxy substituent and introduction of the flexibility of the ethane bridge in bibenzyl moieties instead of the rigid ethylene bridge of the molecules of (*E*)-stilbenes influences the antifungal activity, it may be of interest to direct our further synthetic work towards new (*E*)-acetoxystilbenes and acetoxybibenzyls unknown in the literature.

The purpose of this investigation was to elucidate the influence of the presence of the chloro, nitro and dinitro substituents in the phenyl ring, as well as bromo substituents in the ethane bridge of the skeleton of bibenzyl on the antimicrobial activity of these compounds in order to acquire further information on the structural characteristic enhancing this activity. This paper presents the synthesis and characteristics of 13 new (*E*)-2-acetoxystilbenes and α,α′-dibromo-2-(3-and 4-)acetoxybibenzyls.

* Corresponding author.

0014-827X/00/$ - see front matter © 2000 Elsevier Science S.A. All rights reserved.
PII: S0014-827X(99)00127-5

We also report for comparative purposes the results of microbiological screening of the analogs of natural (*E*)-stilbenol-4 (**1a**), a constituent of *Pinus griffiti* [15], i.e. (*E*)-stilbenols-2 (-3 and -4) (**1b**–**1g**; **4a**–**4c**) and (*E*)-2-(3- and 4-) acetoxystilbenes (**2a**, **2b**, **2d**, **2e**,**2g**, **5b**, **5c**). Data dealing with the antimicrobial activity of these compounds have not been published in the literature.

1. Chemistry

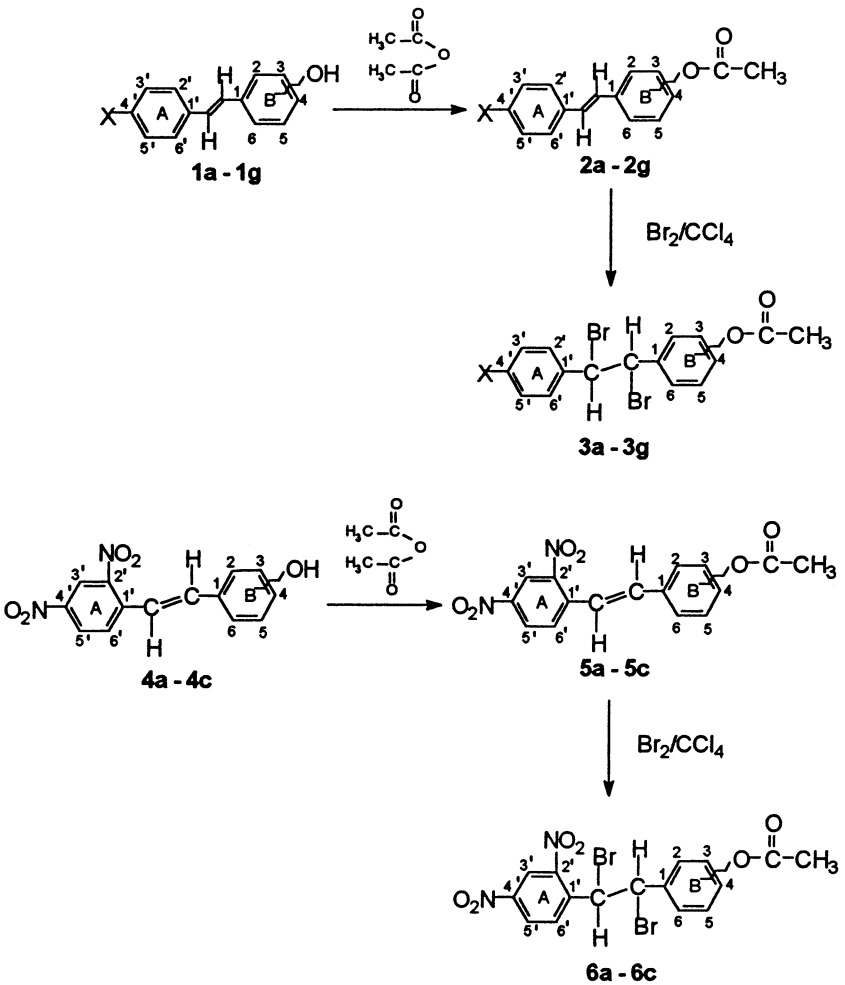
The synthetic approach to obtaining α,α′-dibromo-2-(and 4-)acetoxybibenzyls (**3a**, **3b**), α,α′-dibromo-4′-chloro-2-(and 4-)acetoxybibenzyls (**3c**, **3d**), α,α′-dibromo-4′-nitro-2-(3- and 4-)acetoxybibenzyls (**3e**–**3g**), α,α′-dibromo-2′,4′-dinitro-2-(3- and 4-) acetoxybibenzyls (**6a**–**6c**) followed the reactions in Scheme 1. It should be pointed out that no reports have been published to date concerning (*E*)-4′-chloro-2-acetoxystilbene (**2c**), (*E*)-4′-nitro-3-acetoxystilbene (**2f**) and (*E*)-2′,4′-dinitro-2-acetoxystilbene (**5a**). We accomplished the synthesis of these compounds by the reaction of the corresponding (*E*)-stilbenols (**1c**, **1f** and **4a**) with the boiling anhydride of acetic acid. In the synthesis of α,α′-dibromoacetoxybibenzyls, the starting materials were (*E*)-2-(3- and 4-)acetoxystilbenes (**2a**–**2f**, **5a**–**5c**) together with the solution of bromine in the aprotic solvent, i.e. carbon tetrachloride. These compounds were reacted at room temperature. Ten new racemic α,α′-dibromo-2-(and 4-)acetoxybibenzyls (**3a**, **3b**), α,α′-dibromo-4′chloro-2-(and 4-)acetoxybibenzyls (**3c**, **3d**) α,α′-dibromo-4′-nitro-2-(3-and 4-)acetoxybibenzyls (**3e**–**3g**), as well as α,α′-dibromo-2′,4′-dinitro-2-(3-and 4-) acetoxybibenzyls (**6a**–**6c**) were obtained in these electrophylic addition reactions.

The structures of all the compounds obtained were determined by elemental analyses and by examination of their IR and ^{1}H NMR spectra. All new compounds (**2c**, **2f**, **5a**, **3a**–**3g**, **6a**–**6c**) gave satisfactory microanalyses for C, H and N (Table 1). The infrared spectra of these compounds showed strong carbonyl stretching absorption bands in the region 1700–1750 cm^{-1}. The infrared spectra of α,α′-dibromo substituted derivatives of acetoxybibenzyls (**3a**–**3g**, **6a**–**6c**) also showed strong absorption between 590 and 610 cm^{-1} arising from the stretching vibrations of the carbon–bromine bonds. It should be mentioned that analysis of the IR spectra revealed (*E*)-configuration for **2c**, **2f** and **5a** [16–18] due to the presence of strong bands of out-of-plane *trans* olefinic C–H bending vibrations between 950 and 980 cm^{-1} (Table 1). The geometry at the ethylene bridge of acetoxystilbenes (**2c**, **2f** and **5a**) was also assigned as *E* based on the olefin ^{1}H NMR coupling constants. The 300 MHz ^{1}H NMR spectra of these compounds showed two sets of signals. The former between δ 2.31 and 2.39 was assigned to the methyl protons of the acetyl group. The latter set, between δ 8.21 and 7.13, exhibited signals integrating for ten (**2c**, **2f**) and nine (**5a**) protons in the olefinic and aromatic region (rings A,B) (Table 2). In this set the AB spin system; dealing with *trans* olefinic protons, showed two doublets with large coupling constants ($J = 16.1$ Hz) situated at δ 7.85 (**2c**), 7.19 (**2f**), 7.54 (**5a**) and δ 7.14 (**2c**), 7.13 (**2f**), 7.16 (**5a**), respectively (Table 2).

The 300 MHz ^{1}H NMR spectra of α,α′-dibromoacetoxybibenzyls (**3a**–**3g**, **6a**–**6c**) revealed the absence of an olefinic proton system; instead they exhibited two doublets ($J = 9$ Hz) integrating for two methine groups protons in the range δ 5.36–6.41. Two sets of signals have also been seen in these spectra. The former between δ 2.29 and 2.44 was assigned to methyl protons of the acetyl group. The latter set between δ 8.82 and

Table 1
Chemical and physical data of compounds **2c**, **2f**, **5a**, **3a**–**3g** and **6a**–**6c**

Comp.	Yield (%)	M.p. (°C)	TLC (R_f)	IR (KBr) (cm^{-1})			*Anal.*
				δ(CH=CH)	ν(C–Br)	ν(C=O)	
2c	87.8	92–94	0.87	950		1750	($C_{16}H_{13}O_2Cl$) C,H
2f	89.5	126–128	0.68	960		1750	($C_{16}H_{13}O_4N$) C,H,N
5a	82.7	185–187	0.61	980		1710	($C_{16}H_{12}O_6N_2$) C,H,N
3a	69.4	156–158	0.70		610	1750	($C_{16}H_{14}O_2Br_2$) C,H
3b	62.8	179–181	0.68		610	1750	($C_{16}H_{14}O_2Br_2$) C,H
3c	65.3	178–180	0.84		600	1700	($C_{16}H_{13}O_2ClBr_2$) C,H
3d	79.1	191–193	0.48		590	1700	($C_{16}H_{13}O_2ClBr_2$) C,H
3e	73.2	160–162	0.68		600	1710	($C_{16}H_{13}O_4NBr_2$) C,H,N
3f	73.8	149–151	0.56		600	1710	($C_{16}H_{13}O_4NBr_2$) C,H,N
3g	78.6	212–214	0.61		600	1700	($C_{16}H_{13}O_4NBr_2$) C,H,N
6a	83.3	127–129	0.59		610	1700	($C_{16}H_{12}O_6N_2Br_2$) C,H,N
6b	87.2	117–119	0.43		610	1700	($C_{16}H_{12}O_6N_2Br_2$) C,H,N
6c	71.3	185–187	0.39		610	1700	($C_{16}H_{12}O_6N_2Br_2$) C,H,N



Compound 1	X	OH
a	H	2
b	H	4
c	Cl	2
d	Cl	4
e	NO_2	2
f	NO_2	3
g	NO_2	4
Compound 2	**X**	**$OCOCH_3$**
a	H	2
b	H	4
c	Cl	2
d	Cl	4
e	NO_2	2
f	NO_2	3
g	NO_2	4
Compound 3	**X**	**$OCOCH_3$**
a	H	2
b	H	4
c	Cl	2
d	Cl	4
e	NO_2	2
f	NO_2	3
g	NO_2	4

Compound 4	OH
a	2
b	3
c	4
Compound 5	**$OCOCH_3$**
a	2
b	3
c	4
Compound 6	**$OCOCH_3$**
a	2
b	3
c	4

Scheme 1.

Table 2
^{1}H NMR spectral data (δ)of **2c**, **2f**, **5a**, **3a**–**g** and **6a**–**c**

Comp.	^{1}H-NMR, δ (DMSO-d_6)
2c	7.86 (d, 2H, arom. ring A $J = 9$ Hz) 7.65 (d, 2H arom. ring A, $J = 9$ Hz), 7.45–7.13 (m, 4H, arom. ring B) 7.65 (d,1H, olefinic trans system, $J = 17$ Hz), 7.14 (d, 1H, olefinic trans system, $J = 17$ Hz) 2.39 (s, 3H, CH_3)
2f	8.21 (d, 2H, arom. ring A, $J = 10$ Hz 7.59 (d, 2H, arom. ring A, $J = 10$ Hz), 7.67–7.14 (m, 4H, arom. ring A) 7.19 (d, 2H,olefinic trans system, $J = 16$ Hz) 7.13 (d, 2H,olefinic trans system, $J = 16$ Hz) 2.33 (s,3H, CH_3)
5a	8.72 (s,1H, arom. ring A) 8.51 (d, 2H, arom. ring A, $J = 9$ Hz), 8.22 (d, H arom. ring A, $J = 9$ Hz), 7.14–7.67 (m, 4H, arom. ring B), 7.54 (d,2H,olefinic trans system, $J = 16$ Hz)7.6 (d, 2H, olefinic trans system $J = 15$ Hz) 2.31 (s, 3H, CH_3)
3a	7.55–7.42 (m, 5H, arom. ring A) 7.40–7.18 (m, 4H, arom. ring B), 5.50 (d, 1H, CHBr, $J = 11$ Hz), 5.43 (d,1H, CHBr, $J = 11$ Hz), 2.30 (s, 3H, CH_3)
3b	7.53–7.42 (m, 5H, arom. ring A) 7.40 (d, 2H, arom-ring B, $J = 9$ Hz), 7.14 (d, 2H, arom. ring B, $J = 9$ Hz), 5.48 (d,1H,CHBr, $J = 11$ Hz), 5.42 (d,1H, CHBr, $J = 11$ Hz), 2.31 (s, 3H, CH_3)
3c	7.78 (d, 2H, arom. ring A, $J = 9$ Hz) 7.51 (d, 2H arom. ring A, $J = 9$ Hz), 7.46–7.21 (m, 4H, arom. ring B), 6.20 (d,1H, CHBr, $J = 11$ Hz), 6.08 (d,1H, CHBr,$J = 11$ Hz), 2.44 (s, 3H, CH_3)
3d	7.75 (d, 4H, arom. ring A, arom. ring B, $J = 9$ Hz) 7.51 (d, 2H arom. ring A, $J = 9$ Hz), 7.21 (d, 2H, arom. ring B, $J = 9$ Hz), 6.20 (s, 2H,CHBr), 2.31 (s, 3H, CH_3)
3e	8.31 (d, 2H, arom. ring A, $J = 9$ Hz) 8.06 (d, 2H, arom. ring A, $J = 9$ Hz), 7.91–7.23 (m, 4H, arom. ring B), 6.36 (d, 1H, CHBr, $J = 11$ Hz), 6.13 (d, 1H, CHBr, $J = 11$ Hz), 2.45 (s, 3H, CH_3)
3f	8.33 (d, 2H, arom. ring A, $J = 9$ Hz) 8.01 (d, 2H, arom. ring A, $J = 9$ Hz), 7.98–7.15 (m, 4H, arom. ring B), 6.39 (d, 1H, CHBr, $J = 11$ Hz), 6.30 (d, 1H, CHBr $J = 11$ Hz), 2.32 (s, 3H, CH_3)
3g	8.34 (d, 2H, arom. ring A, $J = 9$ Hz) 8.01 (d, 2H arom. ring A, $J = 9$ Hz), 7.77 (d, 2H, arom. ring B, $J = 9$ Hz), 7.24 (d, 2H, arom. ring B, $J = 9$ Hz), 6.41 (d, 1H, CHBr, $J = 11$ Hz), 6.34 (d, 1H, CHBr,$J = 11$ Hz), 2.31 (s, 3H, CH_3)
6a	8.82 (s, 1H, arom. ring A) 8.52 (d, 1H, arom. ring A, $J = 9$ Hz), 8.09 (d, 1H, arom. ring A, $J = 9$ Hz), 7.47–7.15 (m, 4H, arom. ring B), 5.79 (d, 1H, CHBr, $J = 11$ Hz), 5.63 (d, 1H, CHBr, $J = 11$ Hz), 2.31 (s, 3H, CH_3)
6b	8.82 (s, 1H, arom. ring A) 8.53 (d, 1H arom-ring A, $J = 9$ Hz), 8.09 (d, 1H, arom. ring A, $J = 9$ Hz), 7.48–7.15 (m, 4H, arom. ring B), 6.37 (d, 1H, CHBr, $J = 11$ Hz), 5.63 (d, 1H, CHBr, $J = 11$ Hz), 2.35 (s, 3H, CH_3)
6c	8.82 (s, 1H, arom. ring A) 8.53 (d, 1H arom. ring A, $J = 9$ Hz), 8.10 (d, 1H, arom. ring A, $J = 9$ Hz), 7.52 (d, 2H, arom. ring B, $J = 9$ Hz), 7.18 (d, 2H, arom. ring B, $J = 9$ Hz), 6.40 (d, 1H, CHBr, $J = 11$ Hz), 5.40 (d, 1H, CHBr, $J = 11$ Hz), 2.33 (s, 3H, CH_3)

7.14 exhibited signals integrating for nine (**3a**,**3b**), eight (**3c**–**3g**) and eight (**6a**–**6c**) protons in the phenyl rings A and B of the skeleton of bibenzyl (Table 2).

2. Results and discussion

The new compounds, racemic α,α′-dibromo-2-(3- and 4-)acetoxybibenzyls (**3a**–**3g**; **6a**–**6c**), obtained, as well as the starting materials (*E*)-2-(3- and 4)-acetoxystilbenes (**2a**–**2g**; **5a**–**5c**) and corresponding (*E*)-stilbenols-2(-3 and -4) (**1a**–**1g**; **4a**–**4c**) were assayed against the following nine strains of microorganism: Gram-positive cocci, aerobic bacilli, Gram-negative rods, yeasts, dermatophytes and moulds. Compounds **1a**–**1g**, **2a**–**2g** and **4a**–**4c**, as well as **5a**–**5c** were included in the study for comparative purposes and because the results of such a microbiological screening of these compounds are unknown in the literature. Table 3 shows the antimicrobial activity of compounds with the values of minimum inhibitory concentration (MIC) in the range from 0.5 to 100 μg/cm^3 of **1b**–**g**, **2a**, **2d**, **2f**, **2g**, **3a**, **3b**, **3d**, **3e**, **3g**, **4a**–**c**, **5b**, **5c** and **6a**–**c** as well as the reference antibacterial drug (chloramphenicole) and reference antifungal drug (amphoterricine B). Compounds **1a**, **2b**, **2c**, **2e**, **3c**, **3f** and **5a** showed very weak antibacterial and fungicidal activity. All compounds investigated also showed very weak activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The effect on Gram-positive cocci was stronger than on Gram-negative rods. The strongest effects on Gram-positive bacteria were observed for (*E*)-4′-chlorostilbenol-2 (**1c**), (*E*)-4′-nitrostilbenol-4 (**1g**), (*E*)-2′,4′-dinitrostilbenol-2 (**4a**), (*E*)-2-acetoxystilbene (**2a**), α,α′-dibromo-2-acetoxybibenzyl (**3a**), α,α′-dibromo-4-acetoxybibenzyl (**3b**), α,α′-dibromo-2′,4′-dinitro-2-acetoxybibenzyl (**6a**) and α,α′-dibromo-2′,4′-dinitro-3-acetoxybibenzyl (**6b**). It should be pointed out that **1c** and **6b** are also effective against *Candida albicans* and *Aspergillus fumigatus*. Medium effects on *A. fumigatus* were also observed for compounds **1b**, **1d**–**1g**, **2a**, **2d**, **2g**, **3d**, **3g**, **3g**, **4a**–**4c**, **5b**, **5c** and **6a**–**6c**. The data obtained in this study indicate that **1b**, **1c**, **4b**, **4c**, **6b** and **6c** are antibacterial and also antifungal agents. According to the data shown in Table 3, the nature of the substitution of hydrogen in positions 2′ and 4′ of the phenyl ring A of the skeleton of (*E*)-stilbenols-2(-3 and -4) influenced activity. In the series of investigated (*E*)-stilbenols (**1a**–**1g**; **4a**–**4b**) the presence of chloro and nitro substituents in the 4-position of the phenyl ring A of the skeleton of (*E*)-stilbenol, as well as two nitro substituents in the 2′,4′-positions of the same ring, led to derivatives with better antibacterial and antifungal activity (**1c**, **1g**, **4a**–**4c**), especially against

Table 3
Antimicrobial activity of **1b–g**, **2a**, **2d**, **2f**, **2g**, **3a**, **3b**, **3d**, **3e**, **3g**, **4a–c**, **5b**, **5c** and **6a–c**

Comp.	Minimal inhibitory concentration (MIC) (μg/cm^3)								
	S. aureus 209P FDA	*S. faecalis* ATCC 8040	*B. subtilis* ATCC 1633	*Escherichia coli* PZH 026 B6	*K. pneumoniae* 231	*P. aeruginosa* SR1	*C. albicans* PCM 1409 PZH	*M. gypseum* K1	*A. fumigatus* C1
1b	100	100	100						<50
1c	7.5	100	7.5				100	100	<50
1d	100	100					25		100
1e	100								<50
1f		100							<50
1g	7.5	100	100						<50
2a	7.5								<50
2d									100
2f	100								
2g									100
3a	5								
3b	<10	100							
3d									100
3e			100						
3g									100
4a	7.5								<50
4b	100	100	100	100					<50
4c	100	100	100						<50
5b				100			100		100
5c									100
6a	7.5								150
6b	2.5	100	100				75		100
6c	100	100			>100		100		100
Chloroamphenicole	5.0	5.0	5.0	5.0	50.0	50.0			
Amphoterricin B							0.5	10.0	1.0

S. aureus and *A. fumigatus* (Table 3). The comparison of the activities of (*E*)-stilbenols (**1a**–**1g**; **4a**–**4c**) and (*E*)-acetoxystilbenes (**2a**–**2g**; **5a**–**5c**) showed that the change of the hydroxy groups on the acetoxy groups in the skeleton of (*E*)-stilbene reduced the activity (Table 3).

On the other hand, the comparison of the activity of (*E*)-acetoxystilbenes (**2a**–**2g**; **5a**–**5c**) and α,α′-dibromoacetoxybibenzyls (**3a**–**3g**; **6a**–**6c**) does not show a clear-cut correlation with the structures. The different influence on the antimicrobial activity of the presence of the α,α′-dibromoethane bridge in the skeleton of investigated molecules is noted in the cases of **3a**–**3g** and **6a**–**6c**. It should be pointed out that in the cases of unsubstituted and 4′-monochloro (or nitro)-substituted ring A (*E*)-acetoxystilbenes (**2a**–**2g**) and α,α′-dibromoacetoxybibenzyls (**3a**–**3g**) the antibacterial and antifungal activities are almost the same. The only exception is α,α′-dibromo-4-acetoxybibenzyl (**3b**). This compound has much better antibacterial activity against *S. aureus* and *Streptococcus faecalis* than (*E*)-4-acetoxystilbene (**2b**). The comparison of the antibacterial and antifungal activities of (*E*)-2′,4′-dinitro-2(-3 and -4)acetoxystilbenes (**5a**–**5c**) and 2′,4′-dinitro-α,α′-dibromo-2(-3 and -4) acetoxybibenzyls (**6a**–**6c**) showed that the saturation of the ethylene bridge and introduction of α,α′-dibromosubstituents increase the activity although not always to the same extent. In fact the presence of two nitro substituents in the 2′ and 4′ positions of phenyl ring A of the skeleton of (*E*)-stilbenol -2(-3 and -4), as well as α,α′-dibromo-2(-3 and -4)acetoxybibenzyl led to derivatives with interesting antibacterial and antifungal activity. As a matter of fact our observations showed that the presence of acetoxy group and α,α′-dibromoethane bridge in the molecules of α,α′-dibromoacetoxybibenzyls scarcely affected the antimicrobial activity, when the two nitro groups are present in the positions 2′ and 4′ of the phenyl ring A. These observations indicate the possibility of obtaining new antimicrobial agents in the series of derivatives of 2′,4′-dinitro substituted α,α′-dibromobibenzyls.

3. Experimental

3.1. Chemistry

The purity of the described compounds was monitored by melting point, TLC and elemental analyses. Melting points (uncorrected) were determined on Böetuis apparatus. R_f values refer to TLC plates with silica gel F_{254} (E. Merck) developed with chloroform and observed under UV light ($\lambda = 254$ and 366 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) spectrophotometer in dimethyl-d_6 sulfoxide solution with tetramethylsilane as internal standard. All chemical shifts are quoted in δ values. Elemental analyses were performed on a Perkin–Elmer 240 C-CHN analyser.

(*E*)-stilbenol-2 (**1a**) [19,20], (*E*)-stilbenol-4 (**1b**) [21,22], (*E*)-4′-chlorostilbenol-2 (**1c**) [20], (*E*)-4′-chlorostilbenol-4 (**1d**) [21,22], (*E*)-4′-nitrostilbenol-2 (**1e**) [29], (*E*)-4′-nitrostilbenol-3 (**1f**) [23–25], (*E*)-4′-nitrostilbenol-4 (**1g**) [24], (*E*)-2′,4′-dinitrostilbenol-2 (**4a**) [21–30], (*E*)-2′,4′-dinitrostilbenol-3 (**4b**) [23–29], (*E*)-2′,4′-dinitrostilbenol-4 (**4c**) [23–25], (*E*)-2-acetoxystilbene (**2a**) [26], (*E*)-4-acetoxystilbene (**2b**) [28], (*E*)-4′-chloroacetoxystilbene (**2d**) [23], (*E*)-4′-nitro-2-acetoxystilbene (**2e**) [23], (*E*)-4′-nitro-4-acetoxystilbene (**2g**), (*E*)-2′,4′-dinitro-3-acetoxystilbene (**5b**) [23–25] and (*E*)-2′,4′-dinitro-4-acetoxystilbene (**5c**) [23–25] were prepared according to the literature.

3.1.1. General procedure for synthesis of compounds *2e*, *2f*, *5a*

A mixture of 0.5 g of the corresponding (*E*)-stilbenol (**1c**, **1f**, **4a**) (i.e. 0.0021 M of **1c**, 0.0020 M of **1c**, 0.0020 M of **1f** and 0.0017 M of **4a**) and 50 ml of anhydride of acetic acid was heated (100°C) under reflux for 3 h. The reaction mixture was then concentrated to ca. half the original volume on a rotatory evaporator. The residue was cooled, the precipitated solid was filtered off, dried and recrystallized from ethanol.

3.1.2. General procedure for synthesis of compounds *3a–3g*, *6a–6c*

A solution consisting of 0.5 g of the corresponding (*E*)-acetoxystilbene (**2a**–**2g**, **5a**–**5c**) (i.e. 0.0021 M of **2a**,**2b**; 0.0018 M of **2c**,**2d**, 0.0017 M of **2e**–**2g**, 0.0016 M of **5a**–**5c**) and 0.671 g (0.0042 M) of bromine in 50 ml of carbon tetrachloride was stirred at room temperature for 1 h. The precipitated solids of **3a**–**3g** and **6a**–**6c** were then filtered off, washed with carbon tetrachloride, dried and recrystallized from ethanol.

3.2. Biological test procedures

The activity of the compounds was investigated against the following strains: Gram-positive cocci (*S. aureus* 209P FDA, *S. faecalis* ATCC 8040), aerobic bacilli (*Bacillus subtilis* ATCC 1633), Gram-negative rods (*Escherichia coli* PZH 026 B6, *K. pneumoniae* 231, *P. aeruginosa* SR1), yeasts (*C. albicans* PCM 1409 PZH), dermatophytes (*Microsporum gypseum* K1), moulds (*A. fumigatus* C1).

4. Determination of minimum inhibitory concentration (MIC)

Compounds were dissolved using DMSO (Serva); concentration 1000 μg/ml. A series of dilutions with

concentrations ranging from 10 to 1000 μg/ml was 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); 0.1 ml of standardized 1:1000 diluted suspension of microorganism was then added. MIC values were determined after 18 h of incubation at 37°C.

The fluid medium Penassay broth (Difco) was used as the 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.

The MIC values determined were compared with those of the standards, chloramphenicole (the reference antibacterial drug) and amphoterricine B (the reference antifungal drug).

References

[1] H. Kindl, in: T. Higuchi (Ed.), Biosynthesis and Biodegradation of Wood Components, Academic Press, New York, 1985 (Chapter 14).
[2] T.P. Schultz, T.F. Hubbard Jr., L. Jin, T.H. Fisher, D.D. Nicholas, Phytochemistry 29 (1990) 1501.
[3] T.P. Schultz, Q. Cheng, W. Boldin, T.F. Hubbard Jr., L. Jin, T.H. Fisher, D.D. Nicholas, Phytochemistry 30 (1991) 2939.
[4] T.P. Schultz, W. Boldin, T.H. Fisher, D.D. Nicholas, K.D. McMurtrey, K. Pobanz, Phytochemistry 31 (1992) 3801.
[5] E.N. Frankel, A.L. Waterhouse, J.E. Kinsella, Lancet 341 (1993) 1103.
[6] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W.W. Beecher, H.H.S. Fong, N.R. Fornsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzutto, Science 275 (1997) 218.
[7] K. Hata, K. Baba, M. Kozawa, Chem. Pharm. Bull. 27 (1979) 984.
[8] The Merck Index, 10th ed., Merck, 1983, p. 3112, 8676.
[9] T. Hashimoto, M. Tori, Y. Asakawa, Phytochemistry 27 (1988) 109.
[10] E. Wyrzykiewicz, A. Łapucha, K. Golankiewicz, S. Kucharski, J. Krysiński, Pharmazie 36 (1981) 407.
[11] E. Wyrzykiewicz, W. Prukała, B. Kêdzia, Pharmazie 45 (1990) 790.
[12] E. Wyrzykiewicz, W. Prukała, B. Kêdzia, Farmaco 49 (1994) 127.
[13] W. Prukała, E. Wyrzykiewicz, B. Kêdzia, Farmaco 50 (1995) 779.
[14] Y. Inamori, Y. Kato, M. Kobo, M. Yasuda, K. Baba, M. Kozawa, Chem. Pharm. Bull. 32 (1984) 213.
[15] W.B. Mahesh, T.R. Seshadri, Sci. Ind. Res. 13B (1984) 835.
[16] D.F. Detar, L.A. Cerpino, J. Am. Chem. Soc. 78 (1956) 475.
[17] H.W. Thompson, W. Sheppard, J. Chem. Soc. (1945) 640.
[18] M. Oki, H. Kunimoto, Spectrochem. Acta 19 (1963) 1463.
[19] (a) H. Gusten, M. Salzwedel, Tetrahedron 23 (1967) 173. (b) H. Gusten, M. Salzwedel, Tetrahedron 23 (1967) 187.
[20] R. Kikumoto, A. Tobe, H. Fukami, K. Ninomiya, M. Agawa, J. Med. Chem. 275 (1984) 645.
[21] G. Cavallini, E. Massarani, Pat. Ital. 635 (1962) 951.
[22] E. Massarani, Farmaco 12 (1957) 380.
[23] J. Cullinane, J. Chem. Soc. 123 (1923) 2055.
[24] H. Veschambre, G. Dauphin, A. Kergomard, Bull. Soc. Chim. Fr. 8 (1967) 2846.
[25] P. Pfeiffer, Ann. 411 (1915) 136.
[26] S.T. Kostanecki, J. Tambor, Chem. Ber. 42 (1909) 826.
[27] P. Ruggli, O. Schmid, Helv.Chim. Acta 18 (1935) 247.
[28] T. Zincke, A. Geibel, Justus Liebigs Ann. Chem. 349 (1906) 112.
[29] W.E. Solodar, M. Green, J. Org. Chem. 23 (1958) 103.
[30] H. Hoyer, B. Vogel, Monatsh. Chem. 93 (1962) 766.