Eur J Med Chem (1993) 28, 91–96

Potential anti-microbials. I. Synthesis and structure–activity studies of some new thiazolo[4,5-*d*]pyrimidine derivatives

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(Received 23 May 1991; accepted 23 July 1992)

Summary — The synthesis of several 2,3-dihydro-3,6-diaryl-5-mercapto-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones and related compounds are discussed. Some members of the series displayed broad *in vitro* anti-bacterial and anti-fungal activities. Three compounds were screened for anti-HIV potency but were inactive.

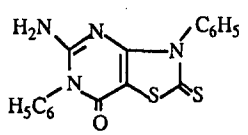
thiazolo[4,5-*d*]pyrimidines / anti-bacterial / anti-fungal

Introduction

The concept of modifying purine bases found naturally in DNA and RNA has often been utilized to obtain biologically active compounds. Replacing a nitrogen atom with sulfur to obtain the corresponding thia analogues has been the subject of a number of current research efforts [1–7]. In view of this fact, we have synthesized several 2,3-dihydro-3,6-diaryl-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones having either a mercapto **2**, alkylmercapto **3**, chloro **4**, azido **5**, triphenylphosphoranylideneamino **6** or amino **7** group at position-5 (scheme 1). Special emphasis was given to studying the influence of such structural modifications upon the anti-microbial potency of the prepared compounds. Among these, compound **7**, namely, 5-amino-2,3-dihydro-3,6-diphenyl-2-thioxo-thiazolo[4,5-*d*]-pyrimidin-7(6*H*)-one, was prepared for its structural similarity to the potent anti-viral purine antagonist, acyclovir [8] (fig 1).

Chemistry

Scheme 1 shows the results of the reaction of the aminothiazoles **1a–d** with isothiocyanates yielding the anticipated 2,3-dihydro-3,6-diaryl-5-mercapto-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **2a–i**. The basic structures of these compounds were confirmed by IR and ^{1}H-NMR spectral data. In addition, the ^{13}C-NMR of compound **2c** revealed two signals corresponding to the 2-thioxo and 7-oxo at 189.88 and 154.64, respectively; and a C-5 signal at 158.88 δ ppm which might indicate its presence mainly in the form of C-SH and not as C=S. The aminothiazoles necessary for our purpose were prepared using a successful procedure described by Gewald [9] and reproduced in scheme 1. The 5-alkylmercapto-2,3-dihydro-3,6-diaryl-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **3a, b** were obtained in good yield by reacting **2a** or **2d** with the appropriate alkyl iodide in the presence of anhydrous potassium carbonate in boiling acetone. Chlorinating **2a** with phosphorus oxychloride in the presence of phosphorus pentachloride afforded the 5-chloro-2,3-dihydro-3,6-diphenyl-2-thioxothia-



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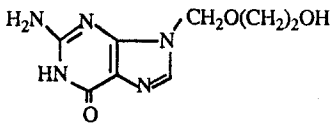

Acyclovir

Fig 1. Structures of compound **7** and acyclovir.

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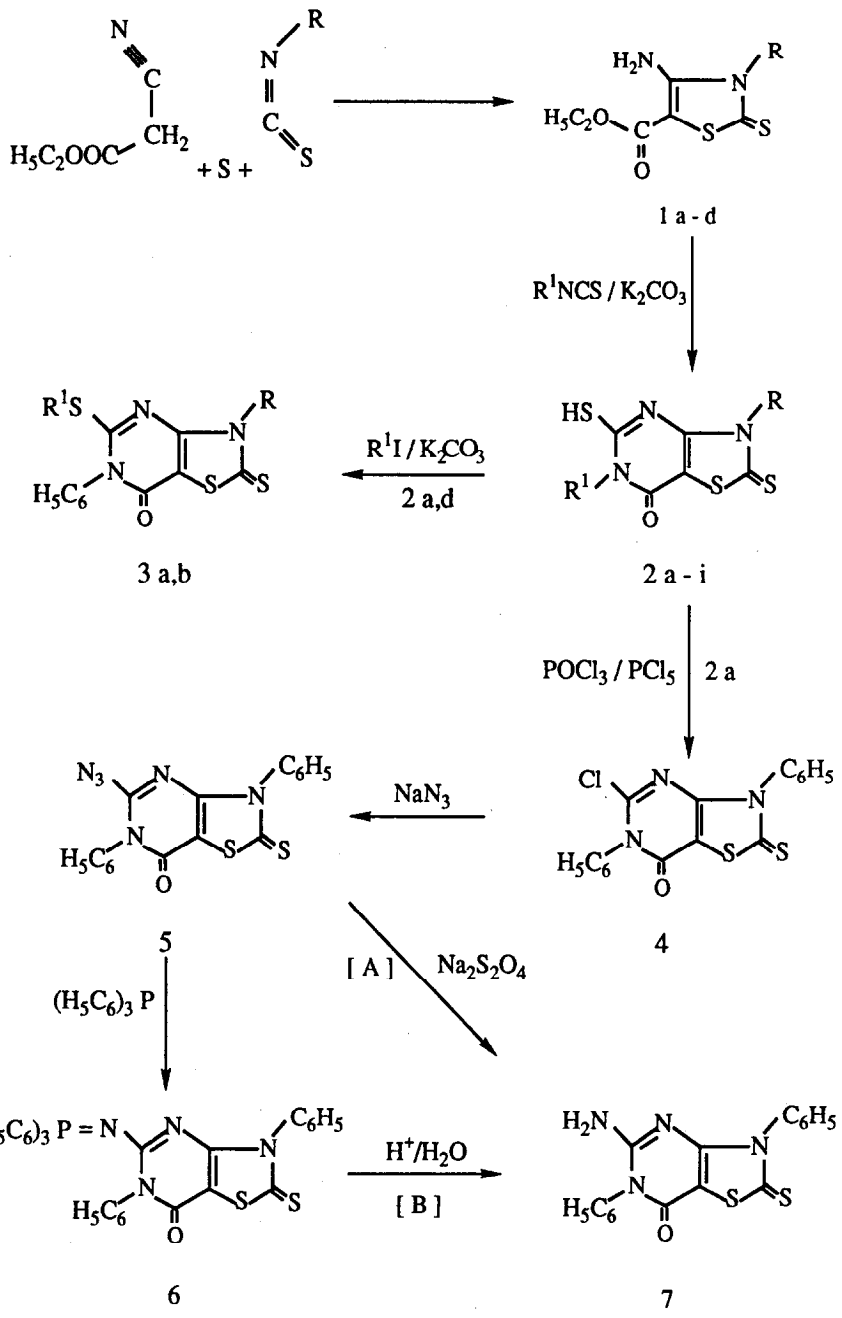

Scheme 1. **1a**: R = C_6H_5; **1b**: R = 2-$CH_3C_6H_4$; **1c**: R = 3-$CH_3C_6H_4$, **1d**: R = 4-ClC_6H_4; **2a–i**: for R,R^1-key see table III; **3a**: R = C_6H_5, R^1 = CH_3; **3b**: R = 2-$CH_3C_6H_4$; R^1 = C_2H_5.

zolo[4,5-*d*]pyrimidin-7(6*H*)-one **4**. Its ^{13}C-NMR spectrum showed the characteristic signals of the 2-thioxo at 191.13, the 7-oxo at 155.99 and C-5 at 156.28 δ ppm. Displacement of the 5-chloro atom in the latter with sodium azide gave the 5-azido derivative **5**, which was converted into the iminophosphorane **6** upon treatment with triphenylphosphine. The 5-amino compound **7** was prepared either by hydrolyzing **6** with hydrochloric acid or by reducing the 5-azido analogue **5** with sodium dithionite.

Biological investigation and discussion

Anti-microbial activity

The anti-microbial activity of the prepared compounds was tested against Gram-positive and Gram-negative bacteria, and against fungi using the cup-diffusion technique [10]. Only the compounds which showed inhibition zones > 10 mm in diameter are recorded in (table I). The data indicate a negligible activity of the compounds against *Sarcina lutea* and *Aspergillus flavus*. The compounds were further evaluated for their minimal inhibitory concentrations (MICs; table II) against the other test organisms using the broth dilution technique [11]. As revealed by the results, most of the 3,6-diarylthiazolo[4,5-*d*]pyrimidines **2a–i** were inactive against *Staphylococcus aureus*, moderately active against *Candida albicans* (MIC = 70–90 μg/ml) and some of them showed slight-to-moderate activity against *Bacillus subtilis* (MIC = 50–

Table I. Inhibition zone measurements (mm)*.

Cmpd	*S aureus*	*B subtilis*	*E coli*	*C albicans*	*S cerevisiae*
2a	–	–	–	14	–
2b	–	11	13	14	–
2c	–	–	–	12	–
2d	–	–	16	13	–
2f	–	11	15	16	–
2g	–	–	–	11	–
2h	24	12	20	15	11
2i	–	16	–	–	19
3a	–	15	16	20	11
3b	21	–	16	–	–
4	20	20	20	18	11
5	25	13	17	27	11
6	24	–	15	–	–
7	20	15	–	23	–

**Sarcina lutea* and *Aspergillus flavus* records were negative.

Table II. Minimal inhibitory concentration (μg/ml).

Cmpd	*S aureus*	*B subtilis*	*E coli*	*C albicans*	*S cerevisiae*
2a	>200	>200	>200	80	140
2b	>200	100	60	70	>200
2c	>200	>200	>200	90	160
2d	>200	>200	50	90	160
2f	>200	80	50	80	>200
2g	>200	>200	>200	90	160
2h	20	50	40	70	90
2i	140	50	>200	100	40
3a	>200	80	40	60	100
3b	60	>200	50	160	>200
4	>200	50	30	70	90
5	20	70	40	40	180
6	20	>200	60	>200	>200
7	30	80	160	50	140
A[a]	1	1	–	–	–
S	4	–	3	–	–
N	–	–	–	2	–

[a]A: ampicillin; S: streptomycin; N: nystatin.

100 μg/ml), *Escherichia coli* (MIC = 40–60 μg/ml) and *Saccharomyces cerevisiae* (MIC = 40–160 μg/ml). In this series of compounds, it seems that the 3,6-diphenyl compound **2a** lacked activity against the Gram-positive and Gram-negative bacteria, but was active against the test fungi. Similar activity was recorded for **2c, g**, in which one of the phenyl groups is replaced by a 3-tolyl moiety; its replacement by a 2-tolyl group (**2b, d**) favored the anti-Gram-negative activity, while substitution by a 4-chlorophenyl group (**2i**) resulted only in the loss of the anti-Gram-negative activity. Replacement of both phenyl groups by 3-tolyl (**2h**) resulted in potentiation of the anti-microbial activity associated with broad-spectrum properties. The 5-alkylmercapto compounds **3a, b** were mostly active against *E coli* (MIC = 40–50 μg/ml). A marked change in activity was observed among the derivatives with chloro (**4**), azido (**5**), triphenylphosphoranylideneamino (**6**) and amino (**7**) substituents at position-5. These compounds exhibited strong activity against *S aureus* (MIC = 20–30 μg/ml), except compound **4**, and a moderate activity against *B subtilis* (MIC = 50–80 μg/ml), except compound **6**. In addition, good anti-Gram-negative activity was recorded for **4** and **5** (MIC = 30–40 μg/ml) and slight-to-moderate activity for **6** and **7** (MIC = 60–160 μg/ml). Moreover, the compounds showed anti-fungal activity, while the organophosphorus compound **6** was devoid of such activity.

None of the tested compounds were found to be superior to the reference antibiotics.

Anti-HIV (human immunodeficiency virus) activity

Compounds **2d**, **2e** and **2f** were screened for their *in vitro* anti-HIV activity according to the National Cancer Institute's *in vitro* Anti-AIDS Discovery Program (conducted by the National Cancer Institute, Bethesda, MD, USA). The assay basically involves the killing of T_4 lymphocytes by HIV. The viral cytopathic effect on the cells in the absence of treatment was used as a quality control parameter. Agents that interfere with the virus's activities will protect the cells from cytolysis and survival values less than 50% were considered acceptable in the current protocol. None of the tested compounds reached this value at the adopted levels.

Experimental protocols

Chemistry

The melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded on a Perkin–Elmer 1430 ratio recording IR spectrophotometer, using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Varian EM-390 at 90 MHz using (DMSO–d_6) (unless otherwise stated) with tetramethylsilane (TMS) as the internal standard. ^{13}C-NMR spectra were measured on a Varian XL-200 using DMSO as the solvent and internal standard. Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values and were performed by the Microanalytical Unit, University of Cairo, Egypt.

*Ethyl 3-aryl-4-amino-2,3-dihydro-2-thioxothiazole-5-carboxylates **1a–d***

These compounds were obtained using the same procedure previously described by Gewald [9]. Compounds **1b–d** are reported here for the first time and were recrystallized from dimethylformamide.

1a (R = phenyl), yield: 70%, mp: 221–223°C, as reported [9].

1b (R = 2-tolyl), yield: 68%, mp: 200°C. IR ν cm^{-1}: 3400, 3200, 1650, 1610, 1500, 1300, 1100, 1070. ^{1}H-NMR: δ 1.4 (t, 3H, CH_3–CH_2), 2.2 (s, 3H, $CH_3C_6H_4$), 4.5 (q, 2H, CH_3–CH_2), 7.2–7.7 (m, 6H, 4 Ar*H* + NH_2). Anal ($C_{13}H_{14}N_2O_2S_2$) C, H, N, S.

1c (R = 3-tolyl), yield: 67%, mp: 187°C. IR ν cm^{-1}: 3450, 3300, 1690, 1600, 1250, 1130, 1050. ^{1}H-NMR (CF_3COOH): δ 1.5 (t, 3H, CH_3–CH_2), 2.5 (s, 3H, CH_3–C_6H_4), 4.5 (q, 2H, CH_3–CH_2), 7.2–7.8 (m, 6H, 4 Ar*H* + NH_2). Anal ($C_{13}H_{14}N_2O_2S_2$) C, H, N, S.

1d (R = 4-chlorophenyl), yield: 70%, mp: 222°C. IR ν cm^{-1}: 3460, 3290, 1690, 1620, 1240, 1140, 1070. ^{1}H-NMR (CF_3COOH): δ 1.4 (t, 3H, CH_2–CH_3), 4.4 (q, 2H, CH_2–CH_3), 7.4 (d, 2 Ar*H*), 7.7 (d, 2 Ar*H*) Anal ($C_{12}H_{11}ClN_2O_2S_2$) C, H, Cl, N, S.

*2,3-Dihydro-3,6-diaryl-5-mercapto-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-ones **2a–i** (table III)*

A mixture of **1a–d** (10 mmol) and the appropriate isothiocyanate (10 mmol) in acetonitrile (30 ml) was heated under reflux for 15 h in the presence of anhydrous potassium carbonate (1.4 g). The reaction mixture was filtered and, after the addition of water and neutralization with hydrochloric acid (2 N), the product was obtained filtered, washed with water, dried and recrystallized from the proper solvents. IR ν cm^{-1}: 3060–2990; 1660–1650; 1550–1500; 1320–1300; 1240–1210; 1100–1050. ^{13}C-NMR of **2c**: δ (ppm) = 20.82 (CH_3); 103.55 (C-7a); 126.12, 129.06, 129.51, 129.90, 130.11, 139.79 (6 Ar*C* at N-3); 130.25, 131.51, 132.12, 133.50, 135.73, 139.95 (6 Ar*C* at N-6); 139.95; 154.13 (C-3a); 154.64 (C=O); 158.88 (C-5); 189.88 (C=S). The ^{1}H-NMR spectral data are recorded in table IV.

*2,3-Dihydro-3,6-diphenyl-5-methylmercapto-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-one **3a***

Methyl iodide (0.62 ml, 10 mmol) was gradually added to a suspension of **2a** (3.69 g, 10 mmol) and anhydrous potassium carbonate (1.4 g) in dry acetone (20 ml). The reaction mixture was refluxed for 4 h, filtered while hot, concentrated and cooled. The yellowish crystalline product obtained was filtered, washed with ethanol, dried and recrystallized from acetone, yield: 85%, mp: 220–222°C. IR ν cm^{-1}: 3060, 1690, 1590, 1200, 1030. ^{1}H-NMR: δ 2.2 (s, 3H, SCH_3), 7.2–7.6 (m, 10 Ar*H*). Anal ($C_{18}H_{13}N_3OS_3$) C, H, N, S.

*2,3-Dihydro-5-ethylmercapto-6-phenyl-2-thioxo-3-(2-tolyl)-thiazolo[4,5-d]pyrimidin-7(6H)-one **3b***

It was similarly prepared from ethyl iodide (0.8 ml, 10 mmol) and **2d** (3.83 g, 10 mmol), yield: 83%, mp: 195–197°C (acetone). IR ν cm^{-1}: 3050, 1690, 1600, 1570, 1250, 1070. ^{1}H- NMR (CF_3COOH): δ 1.1 (s, 3H, CH_3–CH_2), 2.2 (s, 3H, CH_3–C_6H_4), 2.7 (q, 2H, CH_3–CH_2), 7.2–7.7 (m, 9 Ar*H*). Anal ($C_{20}H_{17}N_3OS_3$) C, H, N, S.

Table III. 2,3-Dihydro-3,6-diaryl-5-mercapto-2-thioxothiazolo[4,5-*d*]pyrimidin-7(6*H*)-ones **2a–i**.

Cpd	*R*	*R¹*	*Solvent* [a]	*mp (°C)*	*Yield (%)*	*Molecular formula*	*Elemental analysis*
2a	C_6H_5	C_6H_5	E	> 350	60	$C_{17}H_{11}N_3OS_3$	C, H, N, S
2b	C_6H_5	2-$CH_3C_6H_4$	D	275–277	62	$C_{18}H_{13}N_3OS_3$	C, H, N
2c	C_6H_5	3-$CH_3C_6H_4$	D	281–283	65	$C_{18}H_{13}N_3OS_3$	C, H, N, S
2d	2-$CH_3C_6H_4$	C_6H_5	D	> 350	63	$C_{18}H_{13}N_3OS_3$	C, H, N, S
2e	2-$CH_3C_6H_4$	2-$CH_3C_6H_4$	A	275–278	65	$C_{19}H_{15}N_3OS_3$	C, H, N, S
2f	2-$CH_3C_6H_4$	3-$CH_3C_6H_4$	A	315–318	64	$C_{19}H_{15}N_3OS_3$	C, H, N
2g	3-$CH_3C_6H_4$	C_6H_5	E	> 350	68	$C_{18}H_{13}N_3OS_3$	C, H, N, S
2h	3-$CH_3C_6H_4$	3-$CH_3C_6H_4$	E	180–182	64	$C_{19}H_{15}N_3OS_3$	C, H, N
2i	4-ClC_6H_4	4-$CH_3C_6H_4$	D	255–258	65	$C_{18}H_{12}ClN_3OS_3$	C, H, Cl, N

[a]E: ethanol; D: dimethyiformamide; A: acetone.

5-Chloro-2,3-dihydro-3,6-diphenyl-2-thioxothiazolo[4,5-d]-pyrimidin-7(6H)-one ***4***

A solution of **2a** (3.69 g, 10 mmol) and an equimolar amount of phosphorus pentachloride (2.08 g) in phosphorus oxychloride (20 ml) was heated under reflux for 4 h and the reaction mixture was worked up as previously described [13]; yield: 59%, mp: 255–258°C (acetone). IR ν cm^{-1}: 1700, 1590, 1560, 1500, 1290, 1190. ^{13}C-NMR: δ 107.35 (C-7a); 129.84, 130.11, 130.43, 130.73, 130.86, 139.21 (6 Ar*C* at N-3); 131.06, 131.26, 131.49, 131.75, 131.80, 139.44 (6 Ar*C* at N-6); 152.09 (C-3a); 155.99 (C=O); 156.28 (C-5); 191.13 (C=S). Anal ($C_{17}H_{10}ClN_3OS_2$) C, H, N, S.

5-Azido-2,3-dihydro-3,6-diphenyl-2-thioxothiazolo[4,5-d]pyrimidin-7(6H)-one ***5***

It was prepared by refluxing **4** (3.72 g, 10 mmol) with sodium azide (0.65 g, 10 mmol) in acetone (20 ml) for 1 h. After cooling and addition of water, the product was filtered, dried and recrystallized from ethanol; yield: 66%, mp: 195–198°C dec; IR ν cm^{-1}: 2220, 2140, 1700, 1570, 1500, 1315, 1210, 1060. ^{1}H-NMR: δ 7.0–7.6 (m, 10 ArH). Anal ($C_{17}H_{10}N_6OS_2$) C, H, N, S.

Table IV. ^{1}H-NMR data (δ ppm) of compounds **2a–i**.

Cmpd	*$CH_3C_6H_4$ at N-3 (s)*	*$CH_3C_6H_4$ at N-6 (s)*	*ArH (R^1 + R^2) (m)*
2a	–	–	7.0–7.6 (10 H)
2b	–	2.3 (1-CH_3–)	7.2–7.9 (9 H)[a]
2c	–	2.4 (2-CH_3–)	7.0–7.6 (9 H)
2d	2.2 (1-CH_3–)	–	7.0–7.6 (9 H)
2e	2.2 (1-CH_3–)	2.3 (1-CH_3–)	7.0–7.7 (8 H)
2f	2.2 (1-CH_3–)	2.5 (2-CH_3–)	7.1–7.6 (8 H)
2g	2.5 (2-CH_3–)	–	7.0–7.6 (9 H)
2h	2.5 (2-CH_3–)	2.6 (2-CH_3–)	7.1–7.7 (8 H)[a]
2i	–	2.4 (4-CH_3–)	7.1–7.7 (9 H)

[a]Trifuoroacetic acid was used as solvent.

2,3-Dihydro-3,6-diphenyl-2-thioxo-5-(triphenylphosphoranylideneamino)-thiazolo[4,5-d]pyrimidin-7(6H)-one ***6***

The compound was prepared from **5** (1.51 g, 4 mmol) and triphenylphosphine (1.31 g, 5 mmol) according to our reported procedure [13]; yield: 65%, mp: 335–338°C (dimethylformamide). IR ν cm^{-1}: 1660, 1590, 1560, 1300, 1250, 1100. ^{1}H-NMR: δ 7.0–7.6 (m, 10 Ar*H* at N-3 and N-6), 7.7–7.9 (m, 15 Ar*H* of –N= P(C_6H_5)$_3$). Anal ($C_{35}H_{25}N_4OS_2P$) C, H, N, S.

5-Amino-2,3-dihydro-3,6-diphenyl-2-thioxothiazolo[4,5-d]-pyrimidin-7(6H)-one ***7***

Method A. To a solution of **5** (0.76 g, 2 mmol) in ethanol (20 ml), a solution of sodium dithionite (1.0 g) in water (15 ml) was added. After refluxing for 1 h, excess ethanol was evaporated and the separated product was filtered, washed with water, dried and recrystallized from ethanol, yield: 50%, mp: 275–278°C. IR ν cm^{-1}: 3400, 3200, 1690, 1630, 1570, 1240, 1120, 1060. ^{1}H-NMR: δ 7.3–7.8 (m, 10 Ar*H*), 7.9 (s, 2H, NH_2). Anal ($C_{17}H_{12}N_4OS_2$) C, H, N, S.

Method B. Compound **6** (1.0 g) was hydrolyzed with a hydrochloric acid–methanol mixture according to our reported procedure [12] to give **7** in 30% yield.

Microbiological methods

Test organisms and culture media

Staphylococcus aureus ATCC 25923, *Bacillus subtilis* DSM 347b, *Sarcina lutea* (local isolate) and *Escherichia coli* ATCC 25922 were cultivated in nutrient agar and nutrient broth, while *Candida albicans* DSM 70443, *Saccharomyces cerevisiae* IMG 70014 and *Aspergillus flavus* (local isolate) were grown in Sabouraud agar and liquid Sabouraud.

Inhibition zone measurements

The compounds were dissolved in propylene glycol at a concentration of 1 mg/ml. The suitable medium (nutrient agar

for bacteria and Sabouraud agar for fungi) was inoculated with the test organisms. A volume of the solution of each the test compounds equivalent to 100 μg was placed separately in cups (8 mm in diameter, 5 mm in height), cut in the agar. The plates were incubated at 37°C for 18–24 h for bacteria, 48 h for yeast fungi (*C albicans* and *S cerevisiae*) and 3–4 days for *A flavus*, and the resulting inhibition zones were measured (table I). Propylene glycol, which exhibited no anti-microbial activity against the test organisms, was used as a negative control.

Minimal inhibitory concentration (MIC) measurements

The substances dissolved in propylene glycol at 1 mg/ml were diluted in broth in the range 200–10 μg/ml. Inocula were prepared from well-growing overnight cultures of each test organism such that the final inoculum size was *ca* 10^6 cells/ml. The tubes were then inoculated with 0.1 ml of inoculum and incubated at 37°C for 24 h for bateria and 48 h for fungi. All the results are presented as μg/ml and the lowest concentration of the anti-microbial agent that resulted in the complete inhibition of the visible growth of the microorganisms represents the minimal inhibitory concentration (MIC, table II). Ampicillin, streptomycin and nystatin were used during the test procedure as reference antibiotics.

Acknowledgments

The authors are grateful to T Kappe and H Sterk, Institut für Organische Chemie, Karl-Franzens Universität, Graz, Austria, for providing the ^{13}C-NMR spectral data.

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