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Synthesis and Antimicrobial Activity of New Thiazole-2(3*H*)-thiones Containing 1,1,3-Trisubstituted Cyclobutane

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ABSTRACT

The reaction of potassium salts of RNHCSSK with 2-chloro-1-(3-methyl-3-phenylcyclobutyl)ethan-1-one in ethanol at 78–80°C afforded new 1,3-thiazole-2(3*H*)-thiones containing 1,1,3-trisubstituted cyclobutane rings at C-4. The antimicrobial activities of these compounds were also investigated against seven different microorganisms, and some of them were found to be active against several of the microorganisms at higher concentrations.

KEYWORDS

α-Haloketone, cyclobutane, dithiocarbamate, 1,3-thiazole-2(3*H*)-thiones, antimicrobial activity.

1. Introduction

α-Haloketones are frequently used in the synthesis of thiazole, oxazole and thiazolidine-2-thiones or 1,3-thiazole-2(3*H*)-thiones. 1,3-Thiazole-2(3*H*)-thiones have found many uses in recent years in such diverse fields as photography, agrochemistry and radiochemistry.[1–12] In agrochemistry in particular, different derivatives of 1,3-thiazole-2(3*H*)-thiones are used as potential plant protecting compounds, e.g. as fungicides, herbicides, nematicides and insecticides.[13] It is well known that 3-substituted cyclobutanecarboxylic acid derivatives exhibit anti-inflammatory and antidepressant activities.[14] Cukurovali *et al.* have reported the synthesis and characterization of cyclobutane-substituted thiazolecarbamate ligands. They used these ligands to complex with some first-row transition metals, and investigated their antimicrobial activities.[15] It has been reported that several thiazoles and thiazolidin-4-ones are of biological importance especially as antimetabolites and schistosomicides.[16]

In the present paper, we report the synthesis, characterization and antibacterial activity of new 4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thiones. It is possible that compounds bearing both cyclobutane and 4-thiazoline-2-thione moieties may display very interesting bioactivities. However, the synthesis and physicochemical properties of 4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thiones have not been reported so far. These compounds containing cyclobutane and 1,3-thiazole-2(3*H*)-thione units seem to be suitable candidates for further chemical modifications and may be pharmacologically active as well as useful ligands in coordination chemistry.

2. Results and Discussion

Treatment of potassium salts RNH(C=S)SK 1 (R = benzyl, phenyl, anilino, cyclohexyl, *n*-butyl and piperidin-1-yl) in ethanol with 2-chloro-1-(3-methyl-3-phenylcyclobutyl)ethanone **2** at 25–30°C afforded 4-hydroxy-1,3-thiazolidine-2-thiones **3**.[17,18] Increasing the temperature from 25–30°C to 80°C in the reaction between **1** and **2** resulted in the elimination of water to give the expected 4-substituted 1,3-thiazole-2(3*H*)-thiones **4–9** (Scheme 1). The mechanism of formation for compounds **4–9** is proposed to be as shown in Scheme 1.

The cyclic structures **4–9** were characterized by means of IR, ^{1}H and ^{13}C NMR spectroscopy as well as by elemental analysis. Melting points for all these compounds were determined by conventional methods and checked by the DSC technique, but are uncorrected.

Since the substituent in the thiazole-2-thione ring at C-4 (3-phenyl-3-methycyclobut-1-yl) is the same in all the compounds, similar IR characteristics were observed for this component of the products. The absence of OH absorption bands and the presence of the [NC(=S)S-] absorption band in the IR spectrum for **4–9** indicate the formation of the expected compounds. The series of products **4–9** shows absorption bands (KBr; cm^{-1}) at 1586, 1585, 1586, 1570, 1575, 1576, respectively, which are characteristic for the [NC(=S)S-] function of the 1,3-thiazole-2(3*H*)-thione ring. Additionally, in the IR spectra of compound **6**, there is a band at 3313 cm^{-1} which is characteristic for the N–H stretching of the *N*-anilino substituent.

The ^{1}H NMR spectra of compounds **4–9** showed the methine proton (>C-*H* in the cyclobutane ring) at δ 3.30, 3.18, 3.25, 3.51, 3.55 and 3.57, respectively; methylene protons (CH_2 in the cyclobutane ring) at δ 2.33 2.38, 2.00-2.39, 2.00-2.44, 2.15 2.59, 2.40 2.62 and 2.17 2.58, respectively; and methyl protons (CH_3) at δ 1.44, 1.32, 1.38, 1.32, 1.59, and 1.58, respectively. These chemical shifts are characteristic for cyclobutane rings.[18] The 1,3-thiazole-2(3*H*)-thione protons (=C*H*-S) appear as singlets at δ 6.20, 6.27, 6.52, 6.14, 6.17 and 6.01, respectively for **4–9**. As expected, aromatic protons appear as multiplets in the range δ 6.26–7.65. The D_2O exchangeable N*H* proton of the compound **6** is a broad singlet at δ 7.88. The ^{13}C NMR spectral data of the compounds, the numbering system for which is shown in Scheme 2, corroborated the ^{1}H NMR spectral results. Additional signals were observed in the ^{13}C NMR spectrum of compound **7**, for which two conformations of the cyclohexane ring (i.e., with the substituent either axial or equatorial) are possible. Detailed ^{1}H NMR and ^{13}C NMR data for the compounds are given in the experimental section.

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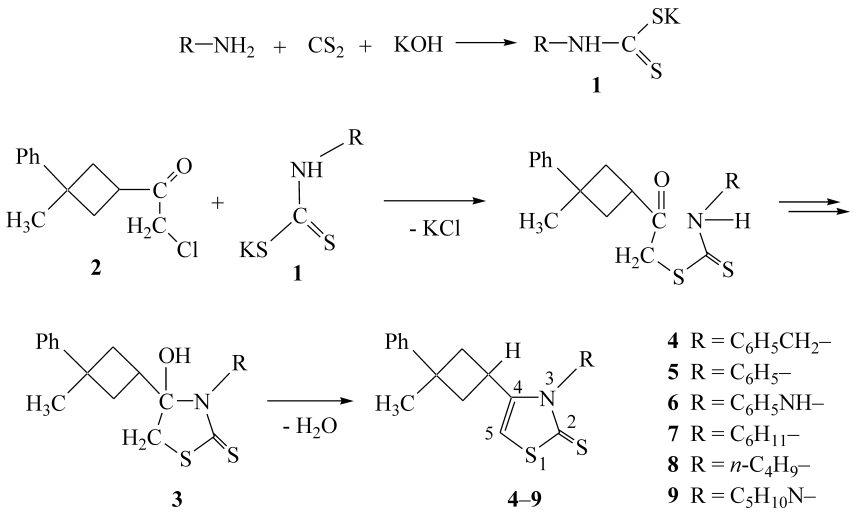


Scheme 1
Suggested reaction path for the formation of 4-substituted thiazole-2(3*H*)-thiones **4–9.**

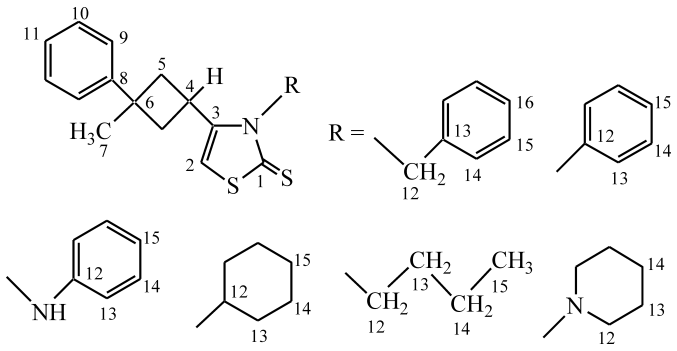
3. Antimicrobial Results

For the biological evaluation of the compounds synthesized in this work, screening against seven different microorganisms was carried out. The test results obtained are listed in Table 1. Antimicrobial data for the drugs amikacin (AMK), ampicillin (AMP), chloramphenicol (CHL) and penicillin G (PEN) are also included in this Table for purposes of comparison.

There was no significant effect of compounds **4, 8** and control (DMSO) on the growth of the microorganisms used in the screen. The inhibition zone was significantly increased on culture with antibiotic disks dosed with compounds **5, 6, 7** and **9**, the observed effects depending on disk concentration and the specific microorganism used (Table 1). Compound **6** in particular was found to be very effective on all the microorganisms tested, except for *P. aeruginosa.* As can clearly be seen from Table 1, compound **6** has the greatest antimicrobial efficiency, followed by compound **9**. *E. coli* was found to be the most resistant to the compounds in the study, while the fungus *C. albicans* was resistant to the antibiotic drugs penicillin G, ampicillin, chloramphenicol and amikacin. These results indicate the efficacy of compounds **5, 6, 7** and **9** in the biological systems.

4. Experimental

2-Chloro-1-(3-methyl-3-phenylcyclobutyl)ethan-1-one 2 was synthesized by well-established methods described in the literature.[19,20] All other starting materials were obtained from commercial suppliers and used without purification. IR spectra (KBr, cm^{-1}) were recorded on a Mattson 1000 FT-IR spectrometer.



Scheme 2
The numbering system of the compounds for ^{13}C NMR spectra.

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 200-MHz spectrometer in DMSO-d_6. Chemical shifts values (δ) are reported in parts per million (ppm) relative to TMS, and coupling constants (*J*) are in Hertz (Hz). The elemental analyses were performed in the TUBITAK (Centre of Science and Technology, Research Council of Turkey) laboratory. Thin-layer chromatography (TLC) analyses were performed on silica gel plates (PolyGram SILG/UV 254). Melting points were determined on a Thomas Hovver melting point apparatus and are uncorrected, but checked by differential scanning calorimetry (DSC). The microorganism strains used were provided from the Culture Collection of the Biotechnology Laboratory, Department of Biology, Faculty of Arts and Sciences, Firat University, Elazig, Turkey. In addition, standard antibiotic discs were obtained from Sigma Chemical Co. (St. Louis, USA).

Potassium benzylcarbamodithioate. Representative procedure

Potassium hydroxide (2.80 g, 0.05 mol) was added in portions to a stirred solution of benzylamine (5.35 g, 0.05 mol) in diethyl ether (20 ml) at 0°C. After stirring for 30 min, carbon disulphide (4.6 g, 0.06 mol) was added dropwise at 0–10°C over 1 h. The reaction mixture was stirred at 25–30°C for 24 h. The solid thus formed was collected by filtration, washed with diethyl ether (50 ml) and dried in air at 25–30°C. *Potassium benzylcarbamodithioate,* [$C_6H_5CH_2NHC(=S)SK$], was obtained in 93% yield and was used without further purification.

*3-Benzyl-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **4**. *General procedure*

2-Chloro-1-(3-methyl-3-phenylcyclobutyl)ethan-1-one **2** (4.45 g, 0.02 mol) was added in portions to a stirred slurry containing the above potassium salt (4.4 g, 0.02 mol) in ethanol (40 ml). After stirring for 50 min the temperature had risen from 20 to 40°C. The stirred reaction mixture was heated at reflux (78–80°C) for 4 h and then allowed to cool. Stirring was maintained at 25–30°C for 24 h. After cooling to 5°C, water (60 ml) was added. The solid was collected by filtration, washed with water and dried in air at 25–30°C. After recrystallization from ethanol (32%), *3-benzyl-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **4** melted at 151–152°C. IR (KBr, cm^{-1}) 1363 and 1302 [NC(=S)S mode of 1,3-thiazole-2(3*H*)-thione ring] and 1577 [mode of 1,3-thiazole-2(3*H*)-thione ring],[17,18] 1024 (C=S). ^{1}H NMR (200 MHz, DMSO-d_6, ppm): δ 1.44 (s, 3H, CH_3), 2.33 2.38 (m,

Table 1 Antimicrobial effects of compounds **4–9**. [a]

Compound	Concent. μg/disk	Microorganisms/inhibition zones (mm)						
		E. coli	*K. pneumoniae*	*S. aureus*	*Streptococcus*	*B. cereus*	*P. aeruginosa*	*C. albicans*
4	200	–	–	–	–	–	–	–
	400	–	–	–	–	–	–	–
5	200	–	–	14	14	14	–	14
	400	–	17	17	16	17	15	16
6	200	15	16	17	15	15	14	16
	400	18	21	24	19	21	17	23
7	200	–	–	14	14	14	15	–
	400	16	19	17	17	18	18	16
8	200	–	–	–	–	14	–	–
	400	–	–	–	–	15	–	–
9	200	14	–	16	14	14	15	–
	400	16	18	20	17	16	19	16
AMK		27	28	32	26	24	32	–
AMP		20	24	33	23	–	14	–
CHL		28	15	30	25	17	–	–
PEN		15	16	33	22	14	14	–
DMSO		–	–	–	–	–	–	–

[a] AMK = amikacin; AMP = ampicillin; CHL = chloramphenicol; PEN = penicillin G, DMSO = dimethyl sulphoxide (control). Inactive (–); moderately active (14–17); highly active (>17).

4H, CH_2), 3.30 (quint, 1H, *J* 9 Hz, C*H* of cyclobutane), 5.52 (s, 2H, C$H_2C_6H_5$), 6.20 (s, 1H, =*CH* of thiazolethione ring), 7.04–7.43 (m, 10H, aromatics). ^{13}C NMR (50.34 MHz, DMSO-d_6, ppm): δ 191.82 (C_1), 107.13 (C_2), 149.94 (C_3), 31.87 (C_4), 40.97 (C_5), 41.44 (C_6), 30.40 (C_7), 152.87 (C_8), 126.41 (C_9), 130.95 (C_{10}), 128.34 (C_{11}), 55.13 (C_{12}), 137.11 (C_{13}), 129.87 (C_{14}), 130.44 (C_{15}), 127.82 (C_{16}). Anal. Calcd. for $C_{21}H_{21}NS_2$: C, 71.79; H, 5.98; N, 3.99; S, 18.23. Found: C, 72.10; H, 6.08; N, 4.22; S, 18.39%.

The following compounds were prepared in similar fashion:

*3-Phenyl-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **5**: white crystals (50%), m.p. 179–180°C. IR (KBr, cm^{-1}) 1338 and 1298 [NC(=S)S mode of 1,3-thiazole-2(3*H*)-thione ring] and 1574 [mode of 1,3-thiazole-2(3*H*)-thione ring],[17,18] 1064 (C=S). ^{1}H NMR (200 MHz, DMSO-d_6, ppm): δ 1.32 (3H, CH_3), 2.00 2.39 (m, 4H, CH_2), 3.18 (quint, 1H, *J* 9 Hz, C*H* of cyclobutane), 6.26 (s, 1H, =*CH* of thiazolethione ring), 6.26 7.65 (m, 10H, aromatics). ^{13}C NMR (50.34 MHz, DMSO-$d_{6,}$ ppm): δ 192.64 (C_1), 107.22 (C_2), 139.88 (C_3), 31.84 (C_4), 41.04 (C_5), 41.50 (C_6), 30.96 (C_7), 150.30 (C_8), 130.40 (C_9), 131.72 (C_{10}), 130.40 (C_{11}), 153.05 (C_{12}), 126.40 (C_{13}), 131.79 (C_{14}), 127.74 (C_{15}). Anal. Calcd. for $C_{20}H_{19}NS_2$: C, 71.23; H, 5.64; N, 4.15; S, 18.99. Found: C, 71.55; H, 5.73; N, 4.40; S, 19.39%.

*3-Anilino-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **6**: white crystals (60%), m.p. 145–146°C. IR (KBr, cm^{-1}) 1346 and 1303 (NC(=S)S- mode of 1,3-thiazole-2(3*H*)-thione ring) , and 1602 (mode of 1,3-thiazole-2(3*H*)-thione ring)[17,18], 1029 (C=S), 3313 (NH). ^{1}H NMR (200 MHz, DMSO-$d_{6,}$ ppm): δ 1.38 (s, 3H, CH_3), 2.00–2.44 (m, 4H, CH_2), 3.25 (quint, 1H, *J* 9, C*H* of cyclobutane), 6.52 (s, 1H, =C*H* of thiazolethione ring), 6.90–7.39 (m, 10H, aromatics), 7.34 (s, 1H, N*H*). ^{13}C NMR (50.34 MHz, DMSO-$d_{6,}$ ppm): δ 191.82 (C_1), 107.13 (C_2), 149.94 (C_3), 36.74 (C_4), 37.22 (C_5), 40.58 (C_6), 32.43 (C_7), 147.79 (C_8),), 126.52 (C_9), 130.38 (C_{10}), 127.66 (C_{11}), 153.24 (C_{12}), 117.10 (C_{13}), 131.18 (C_{14}), 124.58 (C_{15}). Anal. Calcd. for $C_{20}H_{20}N_2S_2$: C, 68.18; H, 5.68; N, 7.95; S, 18.18. Found: C, 68.35; H, 5.77; N, 8.15; S, 18.51%.

*3-Cyclohexyl-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **7**: white crystals (50%), m.p. 179–180°C. IR (KBr, cm^{-1}) 1344 and 1284 [NC(=S)S- mode of 1,3-thiazole-2(3*H*)-thione ring] , and 1573 [mode of 1,3-thiazole-2(3*H*)-thione ring],[17,18] 1079 (C=S). ^{1}H NMR (200 MHz, DMSO-$d_{6,}$ ppm): δ 1.19–1.25 (m, 5H, CH_2 of cyclohexane), 1.32 (s, 3H, CH_3), 1.43–1.95 (m, 5H, CH_2 of cyclohexane), 2.15–2.59 (m, 4H, CH_2 of cyclobutane), 3.51 (quint, 1H, *J* 9, C*H* of cyclobutane), 3.87 (m, 1H, NC*H* of cyclohexane), 6.14 (s, 1H, =C*H* of thiazolethione ring), 7.12–7.35 (m, 5H, aromatics). ^{13}C NMR (50.34 MHz, DMSO-$d_{6,}$ ppm): δ 190.65 (C_1), 108.77 (C_2), 150.59 (C_3), 32.18 (C_4), 401.18 (C_5), 41.53 (C_6), 28.94 (C_7), 152.92 (C_8),), 126.49 (C_9), 130.47 (C_{10}), 127.84 (C_{11}), 63.81 (C_{12}), 30.88 (C_{13}), 28.60 (C_{14}), 26.00 (C_{15}), 127.82 (C_{16}). Anal. Calcd. for $C_{20}H_{24}NS_2$: C, 70.17; H, 7.02; N, 4.09; S, 18.71. Found: C, 70.52; H, 7.12; N, 4.34; S, 19.03%.

*3-Butyl-4-(3-methyl-3-phenylcyclobut-1-yl)-1,3-thiazole-2(3*H*)-thione* **8**: white crystals (53%), m.p. 93–94°C. IR (KBr, cm^{-1}): 1352 and 1278 [NC(=S)S- mode of 1,3-thiazole-2(3*H*)-thione ring] and 1576 [mode of 1,3-thiazole-2(3*H*)-thione ring],[17,18] 1134 (C=S). ^{1}H NMR (200 MHz, DMSO-$d_{6,}$ ppm): δ 1.02 (t, 3H, CH_3CH$_2$), 1.41–1.84 (m, 4H, CH_2), 1.59 (s, 3H, CH_3), 2.40–2.62 (m, 4H, CH_2 of cyclobutane), 3.55 (quint, C*H* of cyclobutane), 4.10 (t, 2H, NCH_2), 6.17 (s, =C*H* of thiazolethione ring), 7.12–7.37 (m, 5H, aromatics). ^{13}C NMR (50.34 MHz, DMSO-$d_{6,}$ ppm): δ 190.24 (C_1), 107.33 (C_2), 149.49 (C_3), 32.11 (C_4), 41.16 (C_5), 41.64 (C_6), 30.38 (C_7), 152.90 (C_8),), 126.46 (C_9), 130.49 (C_{10}), 127.86 (C_{11}), 49.38 (C_{12}), 31.56 (C_{13}), 2224 (C_{14}), 15.76 (C_{15}). Anal. Calcd. for $C_{18}H_{23}NS_2$: C, 68.14; H, 7.25; N, 4.42; S, 20.19. Found: C, 68.42; H, 7.37; N, 4.54; S, 20.44%.

*4-(3-Methyl-3-phenylcyclobut-1-yl)-3-(piperidin-1-yl)-1,3-thiazole-2(3*H*)-thione* **9**: white crystals (35%), m.p. 175–176°C. IR (KBr, cm^{-1}): 1355 and 1281 [NC(=S)S- mode of 1,3-thiazole-2(3*H*)-thione ring] and 1578 [mode of 1,3-thiazole-2(3*H*)-thione ring],[17,18] 1011 (C=S). ^{1}H NMR (200 MHz, DMSO-$d_{6,}$ ppm): δ 1.53 (s, 3H, CH_3CH$_2$-, 1.63–1.83 (m, CH_2), 2.17–2.58 (m, 4H, CH_2 of cyclobutane), 2.89–2.95 (m, CH_2), 3.58 (quint, 1H, C*H* of cyclobutane), 4.63 (t, 2H, CH_2N), 6.01 (s, 1H, =C*H* of thiazolethione ring), 7.12–7.36 (m, 5H, aromatics). ^{13}C NMR (50.34 MHz, DMSO-$d_{6,}$ ppm): δ 189.27 (C_1), 105.29 (C_2), 151.58 (C_3), 32.21 (C_4), 41.29 (C_5), 41.44 (C_6), 28.66 (C_7), 153.41 (C_8),), 126.55 (C_9), 130.38 (C_{10}), 127.66 (C_{11}), 52.14 (C_{12}), 30.58 (C_{13}), 25.13 (C_{14}). Anal. Calcd. for $C_{19}H_{24}NS_2$: C, 69.09; H, 7.29; N, 4.24; S, 19.39. Found: C, 69.45; H, 7.35; N, 4.40; S, 19.62%.

Preparation of microbial cultures

The antimicrobial activities of the compounds **4–9** were tested against *E. coli* DH5α, *Klebsiella pneumonia* FMC 5, *Staphylococcus aureus* COWAN 1, *Streptococcus, Bacillus cereus* FMC 39, *Pseudomonas aeruginosa* DSM 50071 and *Candida albicans* CCM 314 using

DMSO as the solvent at 37°C. The sample concentrations were 200 μg and 400 μg. Standard antibiotic discs containing penicillin G, ampicillin, chloramphenicol and amikacin purchased from Sigma Chemical Co. (St. Louis, USA) were used for comparison. The strain of *E. coli* used was purchased from Bethesda Research Laboratories (Gibco-BRL, Paisley, UK). All other microorganism strains were obtained from the Culture Collection of the Biotechnology Laboratory of Firat University, Elazig, Turkey. The YEPD medium used [1% (w/v) yeast extract, 2% (w/v) bactopeptone and 2% (w/v) glucose] was from Difco. Cell cultures were prepared as described by Connerton.[21] YEPD medium (5 ml) was inoculated with each cell from the plate cultures. Cells were incubated overnight with shaking. These overnight stationary phase cultures (1 ml) were inoculated into YEPD (200 ml) and incubated at 30°C with shaking until the OD_{600} reached 0.5. The antibiotic sensitivities of the compounds **4–9** were tested by using the antibiotic disk assay as described by Chan *et al.*[22] Mueller-Hinton Agar [0.5% (w/v) beef extract, 1.75% (w/v) bactopeptone, 0.5% (w/v) glucose, 1.7% (w/v) agar] was purchased from Difco. Each cell culture (1 ml) was transferred into Muller Hinton Agar (MHA) (15 ml) and mixed gently. The mixture was inoculated into the sterile plate. The plates were rotated firmly and allowed to solidify at room temperature for 5 min. Prepared antibiotic disks (200 μg and 400 μg) were placed on the surface of the agar medium. The discs injected with DMSO only were used as a control. Plates were kept at 4°C for 1 h, then incubated at 30°C for 24–48 h. The inhibition zones were measured with a millimetre ruler at the end of the incubation period.

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References

1 G.M. Haist, W.J. Hamphlett and J.R. King, Fr. Demande, 1970, 2,019, 578, July 3 (*Chem. Abstr.,* 1971, **74**, 81769k).

2 J.D. Bass, Ger. Offen., 1949, 418, Oct. 29, 1970 (*Chem. Abstr.,* 1971, **75**, 14622x).

3 Fuji Photo Film Co. Ltd., Brit. Patent, 1,086,657, Oct. 11, 1967 (*Chem. Abstr.,* 1968, **68**, 174219).

4 Fuji Photo Film Co. Ltd., Brit. Patent, 1,086,613, Oct. 11, 1967 (*Chem. Abstr.,* 1968, **68**, 17432u).

5 Y. Nakazawa, K. Nasu and F. Nishio, U.S. Patent, 3,367,779, Feb. 6, 1968 (*Chem. Abstr.,* 1968, **68**, 115716j).

6 A.H. Herz, Fr. Demande, 2, 019, 603, July 3, 1970 (*Chem. Abstr.,* 1971, **74**, 70255p).

7 M.F. Sullivan, R.M. Cole and W.J. Humphlett, Ger. Offen., 2,329,170, Dec. 20, 1973 (*Chem. Abstr.,* 1974, **80**, 76665t).

8 I. Saikawa and S. Takano, Jap. Patent, 7.027,976, Sept. 12, 1970 (*Chem. Abstr.,* 1971, **74**, 3606r).

9 Y. Usui, T. Iwatani and I. Aoki, Jap. Patent, 7,420,309, May. 23, 1974 (*Chem. Abstr.,* 1975, **82**, 155838w).

10 K. Arakawa, Jap. Patent, 7,391,065, Nov. 27, 1973 (*Chem. Abstr.,* 1974, **80**, 146144y).

11 E. Hiraoka, Y. Tamura, H. Kishi, M. Shimizu and H. Nishimura, Jap. Patent, 7,385,715, Nov. 13, 1973 (*Chem. Abstr.,* 1974, **80**, 104168d).

12 I. Ito, Y. Kurayanbayi, K. Suzuki and M. Washinoc, Jap. Patent, 7,126, 490, July 31, 1971 (*Chem. Abstr.,* 1972, **76**, 3839k).

13 W. Hanefeld and S. Wurdz, *J. Prakt. Chem.,* 2000, **342**, 355–370.

14 P. Vergnon, J.P. Girard, J. Legheand, R. Granger and B. Drevon, *Eur. J. Med. Chem.,* 1975, **10**, 65–71.

15 A. Cukurovali, I. Yilmaz, M. Ahmedzade and S. Kirbag, *Heteroatom Chem.,* 2001, **12**, 665–670.

16 M.I. El-Zahar, M.M. Kamal and M.M. Anwar, *Pharmazie,* 1994, **49**, 616–617.

17 J.J. Damico, F.G. Bollinger and J.J. Freeman, *J. Heterocyclic Chem.*, 1986, **23**, 101–104.

18 J.J. Damico, F.G. Bollinger, J.J. Freeman and W.E. Dahl, *J. Heterocyclic Chem.*, 1986, **23**, 105–112.

19 M.A. Akhmedov, I.K. Sardarov, I.M. Akhmedov, R.R. Kostikov, A.V. Kisin and N.M. Babaev, *J. Org. Chem. USSR (Engl. Trans.*) 1991, **27**, 1254–1260 (*Chem. Abstr.,* 1992, **116**, 807).

20 A. Cukurovali and I. Yilmaz, *Polish J. Chem.,* 2000, **74**, 147–152.

21 I.F. Connerton, in *Analysis of Membrane Proteins* (G.W. Gould, ed.), Portland Press, London, 1994, pp. 177–242.

22 E.C.S. Chan, M.J. Pelczar and N.R. Krieg, in *Laboratory Exercises in Microbiology* (E.C.S. Chan *et al.,* eds.), McGraw-Hill, New York, USA, 1993, pp. 225–232.