

## DRUG SYNTHESIS

SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF SOME  
1,2,4-TRIAZOLE DERIVATIVESAYKUT A. IKIZLER<sup>1</sup>, C.B. JOHANSSON<sup>2</sup>, O. BEKIRCAN<sup>1</sup> and C. ÇELİK<sup>2</sup><sup>1</sup> Department of Chemistry, Karadeniz Technical University, 61080-Trabzon, Turkey<sup>2</sup> Department of Microbiology, Faculty of Medicine, University of Marmara, Istanbul, Turkey

**Abstract:** Reactions of 3-amino-1,2,4-triazole with some dicarboxylic acid anhydrides and diketones were studied. The structural assignments of the compounds obtained from the reactions are based on elemental analyses and spectral data. The compounds were screened for their *in vitro* antibacterial activities.

**Keywords:** 1,2,4-triazole, 3-amino-1,2,4-triazole, dicarboxylic acid anhydrides, diketones, synthesis, antibacterial activity.

A number of articles have been devoted to biological and biochemical investigations on 3-amino-1,2,4-triazole (amitrole) [I] and some of its derivatives. On the other hand, several studies involving reactions of I have also been reported (1–14). The present work describes the treatment of I with phthalic [II], cis-hexahydrophthalic [IV], tetrachlorophthalic [VI], glutaric [VIII], and 1,8-naphthalic [X] anhydrides to afford 3-phthalimido-1,2,4-triazole [III], 3-(cis-hexahydrophthalimido)-1,2,4-triazole [V], 3-tetra-chlorophthalimido-1,2,4-triazole [VII], 3-glutrimido-1,2,4-triazole [IX] and 3-(1,8-naphthalimido)-1,2,4-triazole [XI], respectively. The work also involves the reactions of I with *cis*-1,2,3,6-tetrahydrophthalic anhydride [XII], pyromellitic dianhydride [XV], succinic anhydride [XVIII], 2,4-pentandione [XX] and 2,5-hexandione [XXII] to give *N*-(1,2,4-triazol-3-yl)-*cis*-1,2,3,6-tetrahydrophthalic acid monoamide [XIII], *N,N'*-di(1,2,4-triazol-3-yl)-pyromellitic acid *m*-diamide [XVI], *N,N'*-di(1,2,4-triazol-3-yl)-succindiamide [XIX], 4,6-dimethyl-1,2,3-triazolo-[1,5-*a*]pyrimidine [XXI] and 1-(1,2,4-triazol-3-yl)-2,5-dimethylpyrrole [XXIII] respectively.

On heating, compound XVI was cyclized to *N,N'*-di(1,2,4-triazol-3-yl)-pyromellitic diimide [XVII]. But compound XIII could not be converted to compound XIV because it sublimed on heating (Scheme 1).

All the compounds synthesized in the study were tested for their *in vitro* antimicrobial activities against standard strains of some Gram-positive (*Staphylococcus aureus* ATCC 6538 P, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC

29212), some Gram-negative (*Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853) bacteria and yeast (*Candida albicans* ATCC 10231). Moreover, *in vitro* tuberculostatic activities of the some compounds against *Mycobacterium tuberculosis* H37 Rv were also screened.

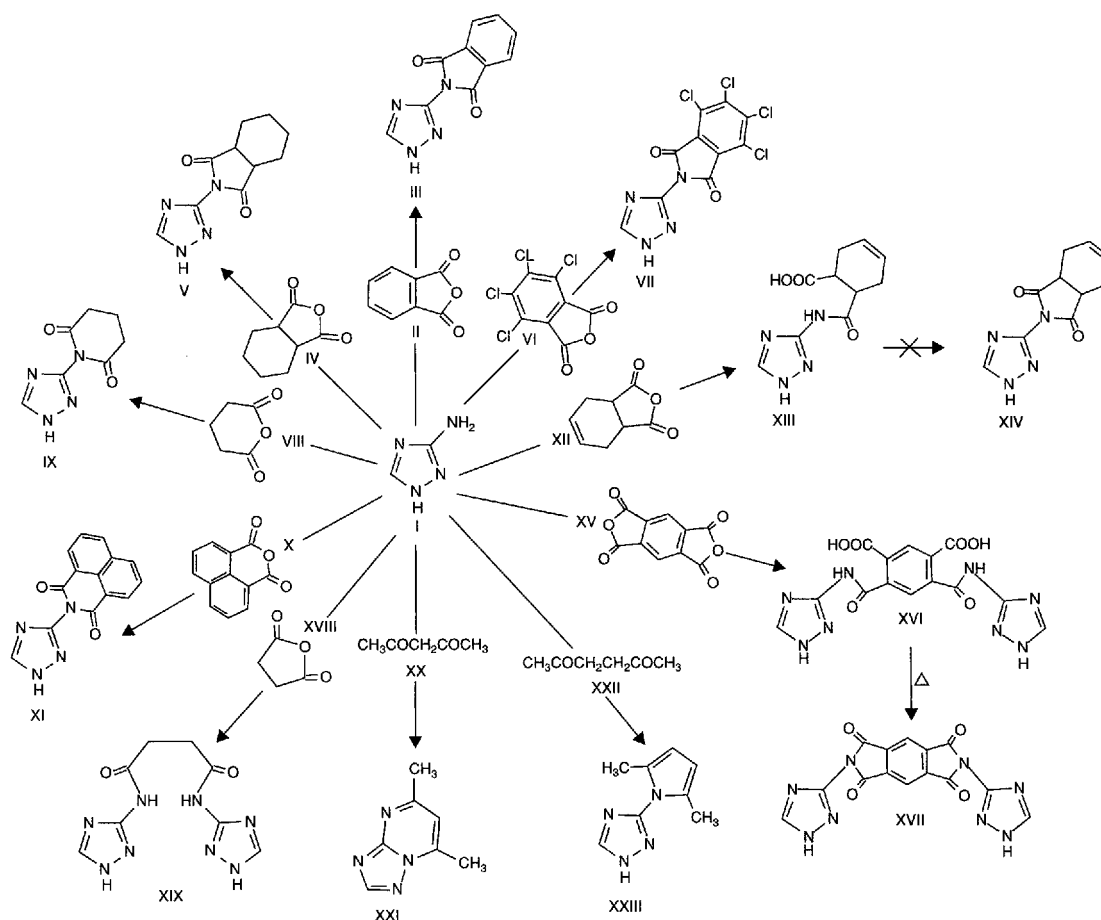
## EXPERIMENTAL

## Synthesis

Melting points were determined on a Büchi oil heated melting point apparatus and are uncorrected. Experimental data of the new compounds are given in Table 1. <sup>1</sup>H-NMR spectra ( $\delta$ , ppm) were obtained on a Varia 60 A spectrometer by using TMS as internal reference (Table 2). IR spectra ( $\nu$ , cm<sup>-1</sup>) were recorded as potassium bromide pellets on a Perkin Elmer 1600 spectrophotometer (Table 3). UV absorption spectra were measured between 200–400 nm with a Shimadzu UV-1201 spectrophotometer by using 10 mm quartz cells. All the measurements were carried out with ethanolic solutions 1.10<sup>-5</sup>–1.10<sup>-4</sup> (Table 3). Microanalyses were performed on a Carlo Erba 1106 elemental analyzer (Table 1). The necessary chemicals were obtained from Fluka. Compound XVI was obtained as the dihydrate form.

General method for the synthesis of compounds III, V, VII, IX, XI, XIII and XVI

3-Amino-1,2,4-triazole [I] (0.01 mol) was heated with 0.01 mol of compound II, IV, VI, VIII, X, XII or XV, at high temperatures (Table 1)



Scheme 1.

for 1.5 h and then cooled. The crude product was recrystallized from an appropriate solvent to afford compound **III**, **V**, **VII**, **IX**, **XI**, **XIII** or **XVI**, respectively (Table 1).

#### Synthesis of compound **XVII**

Compound **XVI** (0.01 mol) was heated at 210–215°C for 0.5 h and then cooled. The solid product was recrystallized from acetic acid to give compound **XVII**.

#### Synthesis of compound **XIX**

3-Amino-1,2,4-triazole [**I**] (0.01 mol) was treated with succinic anhydride [**XVIII**] (0.01 mol) and the mixture was heated at 205–219°C for 1.5 h and then cooled. The solid product was washed with 10 ml of hot dimethylsulfoxide and dried in vacuo. Several recrystallizations of the product from acetic acid gave pure compound **XIX**.

#### Synthesis of compounds **XXI** and **XXIII**

3-Amino-1,2,4-triazole [**I**] (0.01 mol) was heated with the corresponding diketone [**XX** or **XXIII**] at high temperatures (Table 1) for 1.5 h. After cooling, the solid product formed was recrystallized from an appropriate solvent mixture to afford compound **XXI** or **XXIII** (Table 1).

#### Microbiology

Compounds were dissolved in dilute alcohol (1:10 dilution in distilled water) for the preparation of stock solutions of 10,000 µg/ml. Working solutions were prepared from the stock solutions in distilled water.

#### Determination of *in vitro* antimicrobial activity

The paper disc diffusion method (15) was used to determine the antimicrobial effects of the compounds synthesized in the study qualitatively.

Table 1. Experimental data of new compounds

Comp.	Reaction temp. (°C)	M.p. (°C) Yield (%)	Molecular formula (Molecular weight)	Analysis (Calculated/Found)		
				%C	%H	%N
III	215–220	338–340 (dec) <sup>a</sup>	C <sub>10</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>	56.07	2.82	26.16
		75	214.18	55.79	2.83	26.05
V	195–200	265–266 <sup>a</sup>	C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>	54.54	5.49	25.44
		72	220.23	54.84	5.45	25.56
VII	160–165	>365 <sup>b</sup>	C <sub>10</sub> H <sub>2</sub> Cl <sub>4</sub> N <sub>4</sub> O <sub>2</sub>	34.13	0.57	15.92
		83	351.96	34.40	0.61	15.66
IX	165–170	313–315 (dec) <sup>b</sup>	C <sub>7</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>	46.66	4.48	31.10
		73	180.17	6.94	4.77	30.90
XI	230–235	363–365 (dec) <sup>a</sup>	C <sub>14</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>	63.63	3.05	21.20
		82	264.24	63.34	3.07	20.93
XIII	215–220	218–220 subl.	C <sub>10</sub> H <sub>12</sub> N <sub>4</sub> O <sub>3</sub>	50.84	5.12	23.72
		69	236.29	50.79	5.17	23.64
XVI	160–165	* a	C <sub>14</sub> H <sub>8</sub> N <sub>8</sub> O <sub>6</sub> ·2H <sub>2</sub> O	40.00	2.88	26.66
		83	420.30	39.88	3.18	26.43
XVII	210–215	>365 <sup>c</sup>	C <sub>14</sub> H <sub>6</sub> N <sub>8</sub> O <sub>2</sub>	48.01	1.73	31.99
		95	350.26	48.30	1.70	31.85
XIX	205–210	328–330 (dec) <sup>d</sup>	C <sub>8</sub> H <sub>10</sub> N <sub>8</sub> O <sub>2</sub>	38.40	4.03	44.79
		40	250.22	38.15	3.89	45.05
XXI	130–135	134–135 <sup>e</sup>	C <sub>7</sub> H <sub>8</sub> N <sub>4</sub>	56.74	5.44	37.82
		73	148.17	57.00	5.54	37.58
XXIII	160–165	197–198 <sup>f</sup>	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub>	59.24	6.21	34.55
		85	162.19	58.99	6.01	34.38

Crystallized from: <sup>a</sup> ethanol; <sup>b</sup> ethanol-acetic acid (2:1); <sup>c</sup> dimethyl sulfoxide; <sup>d</sup> acetic acid; <sup>e</sup> ethyl acetate-petroleum ether (1:2); <sup>f</sup> ethanol-benzene (1:2). \* The compound changed to 17 on heating.

Table 2. <sup>1</sup>H-NMR data of new compounds (δ, ppm)<sup>a</sup>

Comp.	CH <sub>3</sub>	CH <sub>2</sub>	CH	CH=CH	ArH (Homoaromatic)	NH
III	–	–	8.54 (s, 1H)	–	7.80–8.20	14.20 (s, 1H)
V	–	1.20–2.00	3.00–3.40	–	–	14.12 (s, 1H)
		(m, 8H, 4CH <sub>2</sub> )	(m, 2H, 2CH)			
VII	–	–	8.46 (s, 1H)	–	–	14.60 (s, 1H)
			8.85 (s, 1H)			
IX	–	1.60–2.40	8.30 (s, 1H)	–	–	14.00 (s, 1H)
		(m, 6H, 3CH <sub>2</sub> )				
XI	–	–	8.85 (s, 1H)	–	7.70–8.10 (m, 2H)	14.45 (s, 1H)
			8.40–8.70 (m, 4H)			
XIII <sup>b</sup>	–	2.04–2.50	3.40	5.90 (s, 2H)	–	9.75 (s, 1H)
		(m, 4H, 2CH <sub>2</sub> )	(s, 2H, 2CH)			14.28 (s, 1H)
XVI <sup>c</sup>	–	–	8.56 (s, 1H)	–	8.00 (s, 2H)	9.70 (s, 2H, 2NH)
			(s, 2H, 2CH)			14.10 (s, 2H, 2NH)
XVII <sup>d</sup>	–	–	–	–	–	–
XIX	–	3.00–3.40	9.00	–	–	e
		(m, 4H, 2CH <sub>2</sub> )	(s, 2H, 2CH)			
XXI	3.00 (s, 3H)	–	7.80 (s, 1H)	–	–	–
	3.40 (s, 3H)	–	9.20 (s, 1H)			
XXIII	2.10	–	8.60 (s, 1H)	5.90 (s, 2H)	–	14.20 (s, 1H)
	(s, 6H, 2CH <sub>3</sub> )					

<sup>a</sup> TFA for compound 19 and DMSO-d<sub>6</sub> for the other compounds; <sup>b</sup> δ 11.40 (s, 1H, OH); <sup>c</sup> δ 11.30 (s, 2H, 2OH); <sup>d</sup> <sup>1</sup>H-NMR spectrum could not be obtained because the compound was insoluble in NMR solvents; <sup>e</sup> NH signals could not be obtained in TFA.

Table 3. IR and UV data of new compounds

Comp.	$\nu_{\text{NH}}$	$\nu_{\text{C=O}}$	$\nu_{\text{C=N}}$	$\lambda_{\text{max}}$ , nm ( $\epsilon \times 10^{-3}$ )
III	3050	1735	1530, 1500	294 (0.83), 218 (13.78)
V	3100	1722	1532, 1495	210 (1.59)
VII	3080	1730	1525, 1497	223 (12.09)
IX	3090	1720	1545, 1500	229 (6.46)
XI	3080	1720	1590, 1490	333 (10.37), 231 (23.83)
XIII <sup>a</sup>	3100, 3070	1785, 1720	1525, 1490	210 (1.80)
XVI <sup>b</sup>	3130, 3080	1800, 1750	1545, 1497	295 (2.83), 217 (22.20)
XVII	3100	1705	1550, 1500	321 (25.43), 248 (36.54)
XIX	3150, 3100	1685	1550, 1500	223 (2.17)
XXI	–	–	1640, 1550, 480	271 (4.52), 213 (16.96)
XXIII	3105	–	1548, 1510	226 (19.81), 218 (19.46)

<sup>a</sup> 3140 ( $\nu_{\text{OH}}$ ), <sup>b</sup> 3540, 3170 ( $\nu_{\text{OH}}$ )

Table 4. Antimicrobial activity of selected compounds<sup>a)</sup>

Microorganisms <sup>b)</sup>	V	XI	XVI	XXIII
<i>S. aureus</i> ATCC 6538P	–	–	500	500
<i>S. epidermidis</i> ATCC 12228	–	–	500	250
<i>B. subtilis</i> ATCC 6633	–	–	–	–
<i>E. faecalis</i> ATCC 29212	–	–	500 $\leq$	–
<i>E. coli</i> ATCC 8739	–	–	500 $\leq$	500
<i>P. mirabilis</i> ATCC 14153	–	–	–	250
<i>Kl. pneumoniae</i> ATCC 4352	–	–	500 $\leq$	500 $\leq$
<i>P. aeruginosa</i> ATCC 27853	500 $\leq$	–	–	500
<i>C. albicans</i> ATCC 10231	–	15.6	–	–

a) Values are given as MIC,  $\mu\text{g/ml}$ .

b) The other compounds were found to be inactive toward the microorganisms used in the study.

Table 5. Tuberculostatic effect of the compounds against *M. tuberculosis* H37 Rv.

Compounds	MIC ( $\mu\text{g/ml}$ )	Compounds	MIC ( $\mu\text{g/ml}$ )
III	250	XIII	500 $\leq$
V	500	XVI	500 $\leq$
VII	500 $\leq$	XVII	500 $\leq$
IX	500 $\leq$	XIX	250
		XXIII	250

Overnight cultures of microorganisms were adjusted to approx.  $5 \times 10^6$  c.f.u. / ml according to the Mc Farland turbidity standards No. 0.5 (16) and spread over the appropriate media (Mueller – Hin-

ton agar for bacteria; Sabouraud dextrose agar for the yeast) in petri dishes. Filter paper discs ( $\phi$  5 mm) impregnated with the solutions (each disc containing 750  $\mu\text{g}$  of compound) were placed on the air dried surface of the media inoculated with the respective organisms. Discs containing diluted alcohol were used as control. After overnight incubation at 37°C, the zones of inhibition around the discs were measured. The compounds that produced  $20 \leq$  mm zones of inhibition were further tested in respective broth media by using double dilution and the MIC (Minimal Inhibitory Concentration) values ( $\mu\text{g/ml}$ ) were determined (17). The MIC was defined as the lowest compound concentration for which no visible growth

was observed after overnight incubation. The same test was carried out with 1:10 diluted alcohol as control. The tests were carried out in triplicate.

#### Determination of *in vitro* tuberculostatic activity

Microdilution susceptibility testing was performed in clear 96-well polystyrene microdilution plates (18,19). The compounds were dissolved in alcohol and drug solutions (50  $\mu$ l) were dispensed into the plates containing Middlebrook 7H9 broth with 0.05 % Tween 80. Two-fold dilutions were made for each compound. The inoculum was prepared by subculturing from the 3-week culture of *Mycobacterium tuberculosis* H 37 Rv strain on Löwenstein-Jensen medium into Middlebrook 7H9 broth for 10 days. The culture suspension of the organism was then adjusted to the optical density of a 0.5 Mc Farland turbidity standard and then diluted in fresh broth to yield approximately  $10^5$  bacilli per 50  $\mu$ l. Inoculum was dispensed, 50  $\mu$ l into each well, and the plates were sealed in plastic bags and incubated at 37°C in a humid atmosphere for 21 days. Control wells for alcohol plus inoculum and inoculum without compounds were included. The MIC was defined as the lowest compound concentration for which no visible growth was observed after 21 days. The tests were carried out in triplicate.

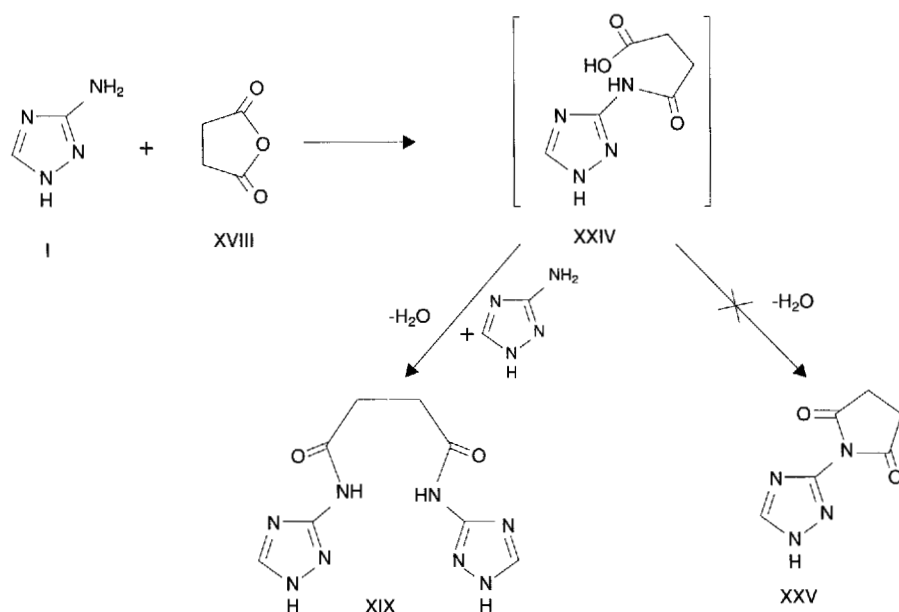
Results of the microbiological test results are shown in Tables 4 and 5.

## RESULTS AND DISCUSSION

In general, the reactions of 3-amino-1,2,4-triazole with dicarboxylic acid anhydrides resulted in the formation of imide type compounds, as seen from Scheme 1. The isolation of compounds **XII** and **XVI** reveals that these reactions of 3-amino-1,2,4-triazol (**I**) generally proceed *via* the formation of intermediate carboxylic acid derivatives similar to **XIII** or **XVI**. However, these type intermediates could not be isolated from the other anhydrides under relatively lower temperatures. This situation can be attributed to the nearness of the formation and cyclization temperatures of the predicted intermediates in these reactions.

The reaction of **I** with succinic anhydride led to the formation of the unexpected compound **XIX** instead of **XXV**. The probable mechanism of this reaction also involves a carboxylic acid derivative **XXIV** as intermediate (Scheme 2).

The formation of a Schiff base from the reaction of **I** with 2,4-pentandione in boiling ethanol has recently been reported (7). However, this reaction resulted in the formation of 4,6-dimethyl-1,2,4-triazolo[1,5-a]pyrimidine [**XXI**] at elevated temperatures. The assignment of the structure [**XXI**] to the fused compound is based mainly on the spectral data and on an assumption that the 1,2,4-triazolo[4,3-a]pyrimidine system is thermodynamically more stable than the isomeric



Scheme 2.

1,2,4-triazolo[4,3-a] pyrimidine system (6, 9, 20).

Microbiological results of the compounds screened in the study are presented in Tables 4 and 5. As seen from Table 4, a slight activity was detected against some bacteria with compounds **V**, **XVI** and **XXIII**. Compound **XI** showed good activity with MIC value of 15.6 against the yeast *Candida albicans*. All the compounds showed slight tuberculostatic activity against the test strain. Alcohol showed no inhibitory effect on the microorganisms.

#### REFERENCES

1. Kreutzberg A., Meyer B.: Eur J. Med. Chem.-Chim. Ther. 14, 13 (1979).
2. Parkanyi C., Abdelhamid A.O., Cheng J.C.S., Shawali A.S.: J. Heterocycl. Chem. 21, 1029 (1984).
3. Hlayka J.J., Bitha P., Lin Y., Strohmeyer T.: J. Heterocycl. Chem. 22, 1317 (1985).
4. Metwally M.A., Yousif M.Y., Ismaiel A.M., Etman H.A.: Heterocycles 23, 2251 (1985).
5. Rusinov V.L., Petrov A. Yu., Pilicheva T.L., Chupakhin O.N., Kovalev G.V., Komina E.R.: Khim.-Harm. Zh. 20, 178 (1986).
6. Stanovik B., Urleb U., Tisler M.: Monatsh. Chem. 118, 601 (1987).
7. El-Borai M., El-Barbary A.A., Fahmy M., El-Naggar H.H.: Delta J. Sci 12, 93 (1988).
8. Ibrahim M., Kamal A.: Ind. J. Chem. B. 27, 478 (1988).
9. Ram V.J.: Ind. J. Chem. B 27, 825 (1988).
10. Desenko S.M., Orlov V.D., Estrada J.: Khim. Geterotsikl. Soedin 999 (1990).
11. Deep A.: Collect. Czech. Chem. Commun. 56, 1560 (1991).
12. Wermann K., Hartmann M.: Synthesis 189 (1991)
13. Desenko S.M., Orlov V.D., Lipson V.V., Shishkin O.V., Lideman S.V., Struchkov Yu.T.: Khim. Geterotsikl. Soedin 1539 (1991).
14. Desenko S.M., Orlov V.D., Getmanskii N.N., Shishkin O.V., Lideman S.V., Struchkov Yu.T.: Dokl. Akd. Nauk 324, 801 (1992).
15. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disc Susceptibility Tests—Sixth Edition; Approved Standard. NCCLS document M2 – A6, Vol. 17, No. 1, Pennsylvania (1997).
16. Lorian V (Ed.): Antibiotics in Laboratory Medicine, 2nd Ed. Williams & Wilkins, London, 116 (1986).
17. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically – Fourth Edition; Approved Standards. NCCLS document M7 – A4, Vol. 17, No. 2, Pennsylvania (1997).
18. Richard J., Wallace R.J.Jr., Donald R., Nash C.L., Steele V., Steingrube V.: J. Clin. Microbiol. 24, 976 (1986).
19. Cooksey R.C., Crawford J.T., Jacobs W.Jr., Shinnick T.M.: Antimicrob. Agents Chemother. 37, 1348 (1993).
20. Allen C.F.H., Beilfuss H.R., Burness D.M., Reynolds G.A., Tinker J.F., Van Allan J.A.: J.Org. Chem. 24, 796 (1959).

Received: 15.03.1999