ANTIMICROBIAL AND TUBERCULOSTATIC ACTIVITY OF 5-ARYL(HETARYL)-1,3,4-OXADIAZOLE-2-THIONES AND THEIR DERIVATIVES

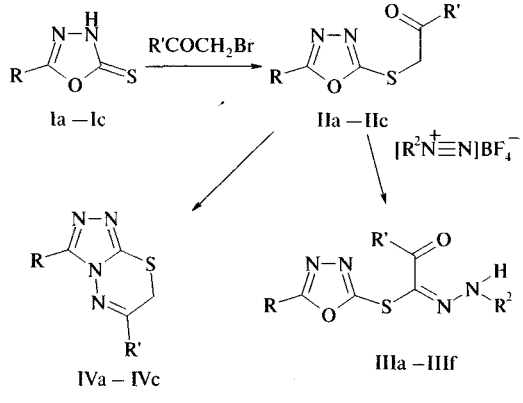
A. N. Krasovskii,[1] A. K. Bulgakov,[1] A. P. Andrushko,[1] I. A. Krasovskii,[1] A. M. Dyachenko,[2] A. A. Bokun,[3] N. A. Kravchenko,[1] and A. M. Demchenko[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 34, No. 3, pp. 13 – 14, March, 2000.

Original article submitted May 24, 1999.

As is known, some 5-R-1,3,4-oxadiazole-2-thione derivatives possess antimicrobial properties [1 – 3] and some 1,3,4-oxadiazole derivatives are used as antituberculous drugs [4 – 10].

In the search for new antimicrobial and tuberculostatic agents, we have employed the interaction of 5-aryl(hetaryl)-1,3,4-oxadiazole-2-thiones (Ia – Ic) [11] with substituted phenacyl bromides to obtain a series of new 5-aryl(hetaryl)-2-(phenacylthio)-1,3,4-oxadiazoles (IIa – IIc).



I: R = 3-BrC_6H_4 (a), 4-pyridyl (b), $C_6H_5CH_2$ (c). II: R = 3-BrC_6H_4 (a, b), 4-pyridyl (c); R' = C_6H_5 (a), 4-$O_2NC_6H_4$ (b), 4-ClC_6H_4 (c). III: R = 3-BrC_6H_4 (a, b), 4-pyridyl (c, d, e), $C_6H_5CH_2$ (f); R' = C_6H_5 (a), 4-$O_2NC_6H_4$ (b), 4-ClC_6H_4 (c, d, e, f); R^2 = 4-$O_2NC_6H_4$ (a, d), 4-$CH_3OC_6H_4$ (b, e, f), 4-BrC_6H_4 (c). IV: R = 3-BrC_6H_4 (a, b), 4-pyridyl (c); R' = C_6H_5 (a), 4-$O_2NC_6H_4$ (b), 4-ClC_6H_4 (c).

We have established for the first time that compounds IIa – IIc exhibit the properties of CH-acids and readily react with active methylene groups in aryldiazonium borofluorides to form the corresponding arylhydrazoes (IIIa – IIIf). At the same time, reactions of compounds IIa – IIc with a hydrazine hydrate solution according to [11] led to 7H-s-triazolo-[3,4-b][1,3,4]thiadiazines (IVa – IVc) [12, 13].

Purity and identity of the synthesized compounds were checked and the proposed structures were confirmed by data of elemental analyses and the results of IR and ^1H NMR spectroscopic measurements (Table 1).

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on an UR-20 spectrophotometer (Germany) using samples pelletized with KBr. The ^1H NMR spectra were recorded on a Bruker-300 (300 MHz) spectrometer using DMSO-d_6 as the solvent and TMS as the internal standard.

The initial 5-aryl(hetaryl)-1,3,4-oxadiazole-2-thiones (Ia – Ic) were synthesized as described in [14, 15].

5-Aryl(hetaryl)-2-(phenacylthio)-1,3,4-oxadiazoles (IIa – IIc). To a solution of 0.01 mole of compound I(a – c) in 30 ml of a 80% aqueous ethanol solution with 0.6 g KOH was added 0.01 mole of the corresponding α-halogenoketone in 30 ml of ethanol and the mixture was kept at 18 – 20°C for 15 – 18 h, after which the precipitated product was filtered.

5-Aryl(hetaryl)-2-(α-arylhydrazonophenacylthio)-1,3,4-oxadiazoles (IIIa – IIIf). To a solution of 0.01 mole of compound II(a – c) and 1 g of anhydrous sodium acetate in a mixture of 30 ml of CH_3COOH and 5 ml of acetic anhydride was added a suspension of 0.01 mole of aryldiazonium borofluorides in a mixture of 20 ml of CH_3COOH and 5 ml of acetic anhydride. The reaction mixture was kept at 18 – 20°C for 20 – 24 h in the dark, after which the precipitated product was filtered.

[1] Technological Institute, Chernigov, Ukraine.
[2] Bashkir State Medical University, Ufa, Bashkortostan, Russian Federation.
[3] Institute of Agricultural Microbiology, Ukrainian Academy of Agricultural Technology and Science, Chernigov, Ukraine.

TABLE 1. Yields and Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula	IR spectrum: λ_{max}, cm^{-1}	Proton chemical shift δ, ppm		
					S–CH_2– (s)	=N–NH– (s)	H_{arom} (m)
IIa	84	118.5 – 119	$C_{16}H_{11}BrN_2O_2S$	1670	5.22	–	7.50 – 8.08
IIb	87	147 – 148.5	$C_{16}H_{10}BrN_3O_4S$	1672	5.26	–	7.53 – 8.42
IIc	85	150 – 151	$C_{15}H_{10}ClN_3O_2S$	1678	5.20	–	7.65 – 8.81
IIIa	73	267 – 268	$C_{22}H_{14}BrN_5O_4S$	1650, 3126, 2985	–	11.55	7.49 – 8.41
IIIb*	67	210 – 210.5	$C_{23}H_{16}BrN_5O_5S$	1650, 3130, 2990	–	11.58	7.10 – 8.40
IIIc	74	245 – 245.5	$C_{21}H_{13}BrClN_5O_2S$	1650, 3210, 2980	–	11.65	7.68 – 8.77
IIId	76	262 – 262.5	$C_{21}H_{13}ClN_6O_4S$	1660, 3126, 2986	–	11.76	7.73 – 8.79
IIIe*	67	227 – 227.5	$C_{22}H_{16}ClN_5O_3S$	1660, 3168, 3000	–	11.56	7.11 – 8.75
IIIf*	65	204 – 205	$C_{24}H_{16}ClN_4O_3S$	1660, 3184, 3030	–	10.78	7.09 – 8.18
IVa	79	224 – 225	$C_{16}H_{11}BrN_4S$		4.45	–	7.53 – 8.22
IVb	78	242 – 243	$C_{16}H_{10}BrN_5O_2S$		4.51	–	7.55 – 8.40
IVc	82	246 – 247	$C_{15}H_{10}ClN_5S$		4.32	–	7.56 – 8.75

* Signal of OCH_3 protons, 3.83 ppm (s).
** Signal of OCH_3 protons, 3.81 ppm (s) and signal of CH_2 protons, 3.53 ppm (s).

7H-s-Triazolo[3,4-b][1,3,4]thiadiazines (IVa – IVc). To a solution of 0.01 mole of compound II(a – c) in 30 ml of acetic acid was added 0.02 mole of 98.9% hydrazine hydrate. The mixture was boiled for 4 h and cooled, after which the precipitated product was filtered.

Compounds IIa – IIc, IIIe – IIIf, and IVa – IVc appear as crystalline substances insoluble in water and soluble in most of the common organic solvents (acetone, acetic acid, DMF, etc.). Compounds IIa – IIc and IVa – IVc are colorless, while compound IIIb is red and compounds IIIa and IIIc – IIIf and IVa – IVc have a yellow-orange color.

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity of the synthesized compounds was determined by the conventional method of double serial dilutions in a meat-extract broth. The stock solutions, prepared by dissolving 100 μg of each compound in 1 ml of the plain broth, were diluted to an initial working concentration of 12.5 μg/ml. Then, sequential half-diluted solutions were placed into tubes and test microbe cultures were added to a load of 2.5×10^5 CFU/ml. The tests were performed with clinical strains of *E. coli*, *P. vulgaris*, *Anthracoides*, *St. aureus*, *Citrobacter*, *Enterobacter*, and *Ps. aeruginosa*. The cultures were incubated for 72 h at 37°C and for 48 h at 25°C.

The antimicrobial activity was judged by inhibition of the test culture growth after incubation for 1, 2, 3, or 5 days. The bactericidal activity was determined by additional tests, whereby the media with no visible growth were inoculated onto dishes with a meat-extract agar and into tubes with the meat-extract broth. These samples were incubated over three days and the results were evaluated after 24, 48, or 72 h [16].

The tuberculostatic activity of compounds IIa, IIIc – IIIf, and IVc *in vitro* was studied by the method of serial dilutions using the Levenstein – Jensen dense egg culture medium. Prior to coagulation of the medium, the test compounds were added to a concentration of 25, 5, 1, and 0.2 μg/ml [17]. The tests were performed with the following cultures: *M. tuberculosis* (strain 192), *M. bovinus* (strain Vallee), and *M. Avium* (strain 14141). The initial culture suspensions were referenced to the bacterial turbidity standard corresponding to 500×10^6 BCG microbial bodies per ml, diluted 1 : 10 with a physiological solution, and introduced (0.2 ml) into each test tube containing the culture medium with (or without, for the control) substances studied. The samples were incubated at 37°C for a time period corresponding to the optimum incubation time for each culture studied (7 to 30 days).

It was found that the compounds studied exhibited no significant antibacterial activity (MTD > 100 – 200 μg/ml). Some inhibition of the growth of tuberculosis mycobacteria was observed in the presence of compound IVc.

REFERENCES

1. K. Misra Hemant, *Arch. Pharm.*, **316**(6), 487 – 493 (1983).
2. M. Y. Yousif, A. M. Ismael, A. A. El-Emam, and M. M. El-Kerdavy, *J. Chem. Soc. Pac.*, **8**(2), 183 – 190 (1986).
3. S. G. Donia, M. M. H. Arief, and A. A. El-Savy, *J. Chem. Soc. Pac.*, **7**(3), 225 – 230 (1985).
4. E. P. Nesynov and A. P. Grekov, *Usp. Khim.*, **33**(10), 1184 – 1197 (1964).
5. J. Brooks, P. Charlton, P. Macey, et al., *J. Chem. Soc.*, 452 (1950).
6. D. Peak and T. Watkins, *J. Chem. Soc.*, 3292 (1951).
7. F. Milan, F. Leonard, R. Meltzer, and J. King, *J. Am. Pharm. Assoc.*, **42**, 457 (1953).
8. H. Konig, W. Seifken, and H. Offe, *Ber.*, **87**, 825 (1954).

9. M. Movrin and D. Maysinger, *Pharmazie*, **38**(8), 561 (1983).
10. D. Pancechowska-Ksepko, H. Foks, E. Landowska, et al., *Acta Pol. Pharm.*, **43**(2), 116 – 123 (1986).
11. Tadashi Sasaki, Eikoh Ito, and Ikuo Shimizu, *J. Org. Chem.*, **47**, 2757 – 2760 (1982).
12. N. Ergenc, N. Ulusoy, G. Capan, et al., *Arch. Pharm.*, **329**(8/9), 427 – 430 (1996).
13. T. N. Yanborosov, N. N. Kasimova, A. V. Milyutin, et al., *Khim.- Farm. Zh.*, **29**(7), 29 – 31 (1995).
14. A. Weisberger (ed.), *Five and Six-Membered Compounds with Nitrogen and Oxygen*, in: *The Chemistry of Heterocyclic Compounds*, Interscience, New York (1962).
15. G. Mekushkene, P. Vainilavichus, A. Getukhaim, and R. Shematovich, *Khim. Geterotsikl. Soedin.*, No. 5, 700 – 705 (1993).
16. G. N. Pershin, *Methods of Experimental Chemotherapy* [in Russian], Meditsina, Moscow (1971).
17. T. N. Yashchenko and I. S. Mecheva, *Practical Guide to the Laboratory Investigation of Tuberculosis* [in Russian], Meditsina, Moscow (1973).