SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SUBSTITUTED 4-ARYL-3-PHENYLHYDRAZONO-2,4-DIOXOBUTANOIC ACIDS

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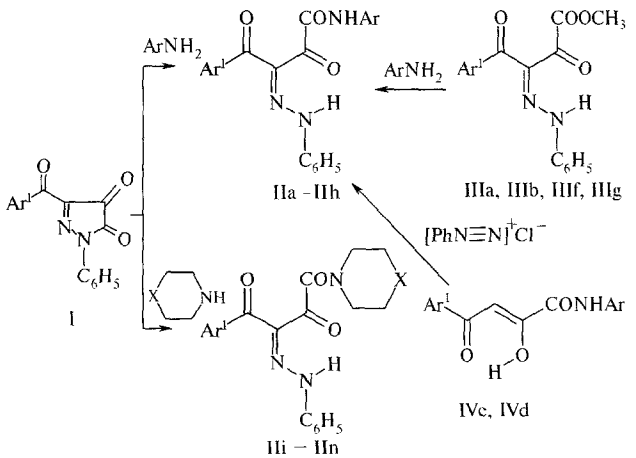
In continuation of the previous investigations into the pharmacological activity of aroylpyruvic acid amides [1], we have synthesized a series of new compounds of this class, containing β-arylhydrazone fragments, and assessed their activity in comparison with that of the other reported amides [2].

Interactions of 1-phenyl-3-aroyl-4,5-dihydro-4,5-pyrazolodiones (I) with primary aromatic amines and secondary cyclic amines (morpholine and piperidine) led to the corresponding 4-aryl-3-phenylhydrazono-2,4-dioxobutanoic acid amides (IIa, IIc – IIe, IIh – IIn) [2] (Table 1, Method A). Amides IIa, IIb, IIf, and IIg were synthesized via interaction of 3-phenylhydrazono-2,4-dioxobutanoic acids (III) with arylamines (Method B). Compounds IIc and IId were also obtained by the reaction of azoaddition of the corresponding amides (IVc, IVd) with phenyldiazonium chloride (Table 1, Method C).



Ar^1 = C_6H_5 (a, b, i), *p*-$CH_3C_6H_4$ (c, d, j, m), *p*-ClC_6H_4 (e, k, n), *p*-BrC_6H_4 (h, l), *p*-$EtOC_6H_4$ (f, g);
Ar = C_6H_5 (a), *p*-$CH_3C_6H_4$ (b, g, n), *p*-$CH_3OC_6H_4$ (c), *p*-ClC_6H_4 (d, f), *m*-$CF_3C_6H_4$ (e, h);
X = CH_2 (i – l), O (m, n).

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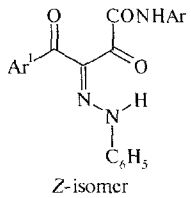
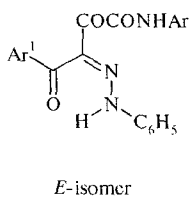
Selection of the particular method of synthesis was determined by availability of the initial pyrazolodiones I. The formation of amides during reactions of compounds I with arylamines was accompanied by side reactions and strongly affected by the reaction conditions [3].

Compounds IIa – IIh appear as yellow crystalline substances well soluble in common organic solvents. The IR spectra of arylamides IIa – IIh contain absorption bands assigned to the stretching vibrations of the ketone and amide carbonyl groups (1640 and 1670 cm^{-1}, respectively) and the amide NH groups (3200 – 3310 cm^{-1}), as well as the "amide II" bands (1515 – 1535 cm^{-1}). Low values of the frequencies of the carbonyl stretching vibrations are explained by participation of these groups in the formation of intramolecular hydrogen bonds of the H-chelate type with the NH groups of hydrazone fragments. The stretching vibration frequency of the latter groups also decreases to fall within the region of absorption of the Vaseline oil [3].

The ^1H NMR spectra of arylamides IIa – IIh show signals due to protons of the aromatic substituents and the related groups, and two to four signals from protons of the amide and hydrazone NH groups (8 – 14 ppm). The splitting of signals indicates that compounds IIa – IIh exist in solution in the form of a mixture of *Z*- and *E*-isomers (as reported previously in [4]), both isomers containing strong intramolecular hydrogen bonds of the H-chelate type.



Z-isomer



E-isomer

The IR spectra of secondary amides IIi – IIn display absorption bands related to the ketone and amide carbonyl groups (1630 – 1650 cm^{-1}) and a broad band assigned to the stretching vibrations of the hydrazone NH group (3090 – 3110 cm^{-1}).

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TABLE 1. Physicochemical Characteristics and Antimicrobial Activity of the Synthesized Compounds

Compound	Yield, %			M.p., °C	Empirical formula	MIC, μg/ml	
	A	B	C			*St. aureus*	*E. coli*
IIa	39	40	–	156 – 157 (methanol)	$C_{22}H_{17}N_3O_3$	1000	2000
IIb	–	40	–	147 – 148 (methanol; with decomp.)	$C_{23}H_{19}N_3O_3$	2000	2000
IIc	73	–	28	157 – 158 (ethanol; with decomp.)	$C_{24}H_{21}N_3O_3$	2000	2000
IId	32	–	30	183 – 184 (ethanol; with decomp.)	$C_{23}H_{18}N_3O_3Cl$	2000	2000
IIe	99	–	–	167 – 168 (ethanol; with decomp.)	$C_{23}H_{15}N_3O_3FCl$	1000	1000
IIf	–	47	–	159 – 160 (toluene)	$C_{24}H_{20}N_3O_4Cl$	2000	2000
IIg	–	63	–	160 – 161 (methanol)	$C_{25}H_{23}N_3O_4$	2000	2000
IIh	76	–	–	164 – 165 (toluene; with decomp.)	$C_{23}H_{15}N_3O_3BrCl$	1000	2000
IIi	52	–	–	130 – 131 (benzene – hexane; with decomp.)	$C_{21}H_{21}N_3O_3$	1000	1000
IIj	53	–	–	167 – 168 (benzene – hexane; with decomp.)	$C_{22}H_{23}N_3O_3$	1000	1000
IIk	56	–	–	158 – 159 (benzene – hexane; with decomp.)	$C_{21}H_{20}N_3O_3Cl$	1000	1000
IIl	54	–	–	164 – 165 (toluene; with decomp.)	$C_{21}H_{20}N_3O_3Br$	1000	1000
IIm	92	–	–	174 – 175 (benzene – hexane; with decomp.)	$C_{21}H_{21}N_3O_4$	1000	1000
IIn	81	–	–	169 – 170 (benzene – hexane; with decomp.)	$C_{20}H_{18}N_3O_4Cl$	1000	1000
Ethacridine lactate						500	200
Furacin						4	16

The ^{1}H NMR spectra of compounds IIi – IIn contain, besides the signals from protons of the aromatic substituents and the related groups, a single signal from protons of the NH groups of hydrazone fragments (14.15 – 14.20 ppm).

EXPERIMENTAL CHEMICAL PART

The IR spectra of the synthesized compounds were measured on an UR-20 spectrophotometer (Carl Zeiss, Germany) using samples prepared as nujol mulls. The ^{1}H NMR spectra were recorded using an RYa-2310 (60 MHz) spectrometer (Russia) and a Bruker AC-300 instrument (USA) using DMSO-d_6 as the solvent and HMDS as the internal standard. The mass spectra were obtained with an RKh-2310 instrument operating at an electron-impact ionization energy of 70 eV.

The course of the synthesis was monitored by TLC on Silufol UV-254 plates eluted with an ether – benzene (1 : 1) mixture or ethyl acetate.

Physicochemical characteristics of the synthesized compounds are given in Table 1. The data of elemental analyses agree with the results of calculations performed according to the empirical formulas.

Compounds I were synthesized by the method described elsewhere [5].

4-Phenyl-3-phenylhydrazono-2,4-dioxobutanoic acid phenylamide (IIa). Method A. A solution of 1.39 g (5 mmole) of 1-phenyl-3-benzoyl-4,5-dihydro-4,5-pyrazolodione and 0.47 g (5 mmole) of aniline in 50 ml of anhydrous toluene was boiled for 1 h. The precipitated compound II was separated by filtration.

A similar procedure was used for the synthesis of compounds IIc – IIe and IIh.

Method B. A solution of 1.55 g (5 mmole) of 4-phenyl-3-phenylhydrazono-2,4-dioxobutanoic acid and 0.47 g (5 mmole) of aniline in 50 ml of anhydrous toluene was boiled for 3 h. Then the mixture was diluted with hexane and cooled. The precipitated compound II was separated by filtration.

An analogous method was used to obtain compounds IIb, IIf, and IIg.

4-*p*-Tolyl-3-phenylhydrazono-2,4-dioxobutanoic acid *p*-methoxyphenylamide (IIc). Method C. To a solution of 3.11 g (10 mmole) of 4-*p*-tolyl-2,4-dioxobutanoic acid (IVc) in 50 ml dioxane was added by small portions with stirring and cooling a solution of phenyldiazonium chloride obtained from 0.93 g (10 mmole) of aniline, 0.85 g (12.3 mmole) of sodium nitrite, 2.5 ml of concentrated hydrochloric acid, and 8 ml of water. To this reaction mass was added 1.5 g (18.3 mmole) of sodium acetate and the mixture was allowed to stand for 24 h, after which the precipitated target compound was filtered.

A similar procedure was used for the synthesis of compound IId.

4-Phenyl-3-phenylhydrazono-2,4-dioxobutanoic acid piperidide (IIi). A mixture of 0.50 g (1.8 mmole) of 1-phenyl-3-benzoyl-4,5-dihydro-4,5-pyrazolodione and 0.15 g (1.8 mmole) of piperidine in 10 ml of anhydrous toluene was kept for 24 h. Then hexane was added and the precipitated compound IIi was separated by filtration.

A similar procedure was used for the synthesis of compounds IIj – IIn.

EXPERIMENTAL BIOLOGICAL PART

The bacteriostatic activity of the synthesized compounds with respect to the standard strains of *E. coli* M_{17} and *St. aureus* P-209 was evaluated by the conventional method of double serial dilutions in a beef-extract broth [6]. The tests were performed using a daily culture of the test microbes

grown on a beef-extract broth, washed-off with a sterile sodium chloride solution and diluted to an initial concentration of 500×10^6 microbial cells/ml according to a bacterial standard. This mixture was diluted with a sterile beef-extract broth to 1/100 of the initial concentration to obtain a working solution containing 5×10^6 microbial cells/ml. The working solution was introduced in an amount of 0.1 ml into 2 ml of the beef-extract broth to obtain a final solution with a bacterial load of 250,000 microbial cells per ml of liquid medium. The results of experiments were determined after incubation of the control and test cultures for 18 – 20 h at 36 – 37°C, as evidenced by the bacterial growth present or absent as a result of the bacteriostatic effect of the drugs studied. The effective dose was determined as the minimum inhibiting concentration (MIC) (in μg/ml) of the test compound with respect to growth of the test microbes. The reference drugs were Furacin [7, 8] and ethacridine lactate [7].

It was found that none of the compounds tested produced a significant bacteriostatic effect with respect to the microbial strains studied, which implies that introduction of an arylhydrazonium fragment into the β-position of aroylpyruvic acid amides does not increase the antimicrobial properties of the initial compounds.

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