SYNTHESIS, PROPERTIES, AND BIOLOGICAL ACTIVITY OF 3-PYRIDYLAMIDES OF 4-ARYL-2-HYDROXY-4-OXO-2-BUTENIC (AROYLPYRUVIC) ACIDS

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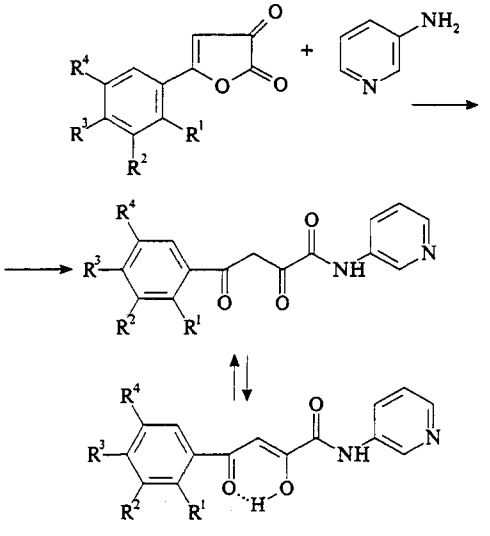
Previously we have demonstrated that 2-pyridylamides of aroylpyruvic acids exhibited antiinflammatory and analgesic properties [1], and their esters and arylamides showed a pronounced antimicrobial effect [2, 3].

In this connection, it was of interest to synthesize 3-pyridylamides of the aroylpyruvic acids and determine the effect of the modified structure of the pyridine fragment on the biological activity of compounds.

The 3-pyridylamides (I – XI) were obtained by interaction of 5-aryl-2,3-dihydrofuran-2,3-diones with β-aminopyridine in an anhydrous dioxane medium [1].



I – XI

$R^1 = R^2 = R^4 = H$, $R^3 = H$ (I), Me (II), OMe (V), OEt (VI), F (VII), Cl (VIII), Br (IX), NO_2 (XI);
$R^2 = R^4 = H$, $R^1 = R^3 = Me$ (III);
$R^2 = R^3 = H$, $R^1 = R^4 = Me$ (IV);
$R^1 = R^3 = R^4 = H$, $R^2 = Br$ (X).

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The target products were obtained with high yields (Table 1). Similarly to other derivatives of the aroylpyruvic acids, these compounds produce cherry-red coloration of the ethanol solution of iron(III) chloride, showing evidence of enolization at the α-carbonyl group [4, 5]. The proposed structures of compounds were confirmed by the results of elemental analyses and the IR and ^1H NMR spectroscopic data. The IR spectra of compounds I – XI display the absorption bands due to stretching vibrations of the amide NH group (3340 – 3312 cm^{-1}), amide carbonyl group (1700 – 1690 cm^{-1}), and γ-carbonyl group (1606 – 1596 cm^{-1}); the latter signal shows that the γ-carbonyl is involved in the cycle [4, 6].

The ^1H NMR spectra of compounds I – XI measured in DMSO-d_6 contain a singlet signal of methine proton (6.78 – 7.20 ppm), a multiplet due to aromatic protons (7.20 – 7.80 ppm), a group of signals due to protons of the pyridine cycle (8.05 – 9.11 ppm), and a broad singlet of the amino group protons (10.55 – 10.90 ppm). The signals of protons from other groups are observed at the expected values of chemical shift, 2.35 – 2.43 ppm (singlet, CH_3) and 3.85 ppm (singlet, OCH_3), in agreement with published data [7].

As is known, derivatives (esters, arylamides, etc.) of the α,γ-diketocarboxylic acids react with hydrazine hydrate to yield derivatives of the pyrazole-3-carboxylic acids [8, 9], with hydroxylamine to form derivatives of the isoxazole-3-carboxylic acids [10], and with *o*-phenylenediamine to give 1,5-benzodiazepines [11, 12]. We have used the synthesized 3-pyridylamides of aroylpyruvic acids in these reactions to find a pathway to new biologically active compounds.

The reactions with hydrazine hydrate and hydroxylamine proceed smoothly and result in the formation of 3-pyridylamides of 5-arylpyrazole-3-carboxylic acids (XII – XIX) and isoxazole-3-carboxylic acids (XX – XXII) with a good yield.

As for the reaction with *o*-phenylenediamine, the 3-pyridylamides of aroylpyruvic acids led predominantly to the formation of 3-aroylmethylene-2-quinoxalones (XXIII –

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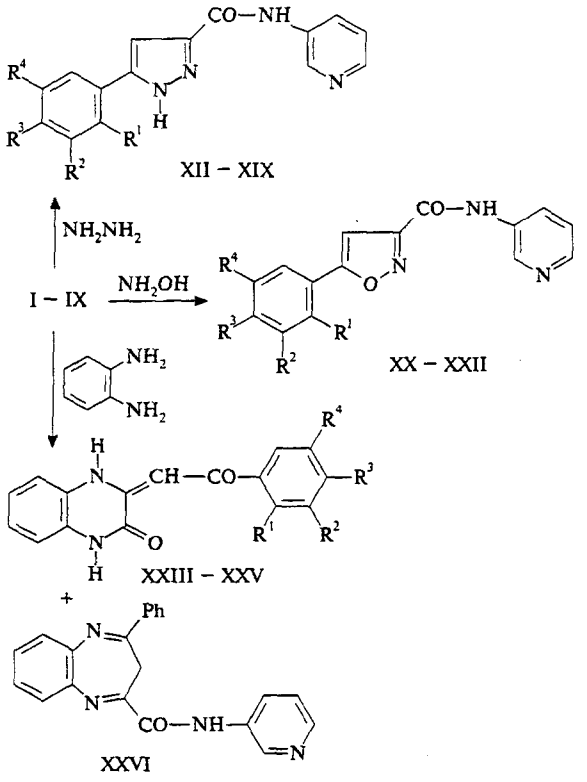
XXV). It was only in the reaction with a derivative of benzoylpyruvic acid that we succeeded in obtaining, besides the quinoxalone, 3-pyridylamide of 4-phenylbenz[b]diazepin-2-carboxylic acid (XXVI) with a 39 % yield.



$R^1 = R^2 = R^3 = R^4 = H$ (XII, XX, XXIII);
$R^1 = R^2 = R^4 = H$, R^3 = Me (XIII, XXI), OMe (XV), OEt (XVI), F (XVIII, XXV), Cl (XVII, XXII);
$R^1 = R^3 = R^4 = H$, R^2 = Br (XIX);
$R^2 = R^4 = H$, $R^1 = R^3$ = Me (XIV);
$R^2 = R^3 = H$, $R^1 = R^4$ = Me (XXIV).

Physicochemical characteristics of the synthesized compounds are listed in Table 1. The products had a white color, except for yellow compounds XXIII – XXV. Compounds XII – XXII (in contrast to I – XI) are poorly soluble in most of the organic solvents; all compounds are insoluble in water.

The structures of compounds XII – XXVI were confirmed by the results of elemental analyses and the IR and ^{1}H NMR spectroscopic data. Unlike the initial amides of aroylpyruvic acids, compounds XII – XXII do not produce coloration on mixing with an iron(III) chloride solution. The IR spectra of samples (measured as vaseline oil suspensions) contain no absorption bands of the γ-ketone carbonyl, but display bands due to the amide NH group (3288 – 3200 cm^{-1}) and amide carbonyl group (1675 – 1660 cm^{-1}). The IR spectra of compounds XII – XIX show a new band in the region of 3370 – 3340 cm^{-1}, which is attributed to stretching vibrations of the NH group of the pyrazole cycle.

The ^{1}H NMR spectra of compounds XII – XXII contain a series of signals due to aromatic and methine protons, centered at 7.46 – 8.15 ppm, and singlets of the amide NH and pyrazole protons in the region of 10.30 – 11.30 and 13.60 – 13.90 ppm, respectively. The signals due to protons of the pyridine ring are manifested in the region of aromatic protons (7.70 – 8.50 ppm).

The appearance of compounds XXIII and XXIV, as well as their IR spectra and melting temperatures (Table 1), correspond to those of 3-aroylmethylene-2-quinoxalones reported in [11].

Compound XXVI reacts with concentrated hydrochloric acid to yield a salt of black color [12]. The ^{1}H NMR spectrum of XXVI contains a singlet due to methylene protons (3.41 ppm), a multiplet due to protons of the pyridine and aromatic rings (centered at 7.65 ppm), and a singlet signal from the amino group protons (8.71 ppm). The IR absorption spectrum exhibits absorption in the region of 3350 cm^{-1} (NH stretching) and 1680 cm^{-1} (amide carbonyl).

EXPERIMENTAL CHEMICAL PART

IR spectra of the synthesized compounds were measured on a Carl Zeiss Specord 80M spectrophotometer (Germany) using samples prepared as nujol mulls. The ^{1}H NMR spectra were recorded on a RYa-2310 (60 MHz) spectrometer (Rus-

TABLE 1. Characteristics of Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula
I	93	178 – 180	$C_{15}H_{12}N_2O_3$
II	90	150 – 151	$C_{16}H_{14}N_2O_3$
III	91	133 – 135	$C_{17}H_{16}N_2O_3$
IV	90	137 – 139	$C_{17}H_{16}N_2O_3$
V	91	163 – 164	$C_{16}H_{14}N_2O_4$
VI	92	160 – 162	$C_{17}H_{16}N_2O_4$
VII	89	177 – 178	$C_{15}H_{11}FN_2O_3$
VIII	85	184 – 185	$C_{15}H_{11}ClN_2O_3$
IX	87	177 – 178	$C_{15}H_{11}BrN_2O_3$
X	68	173 – 175	$C_{15}H_{11}BrN_2O_3$
XI	83	210 – 211	$C_{15}H_{11}N_3O_2$
XII	74	229 – 231	$C_{15}H_{12}N_4O$
XIII	97	165 – 167	$C_{16}H_{14}N_4O$
XIV	56	198 – 200	$C_{17}H_{16}N_4O$
XV	94	220 – 221	$C_{16}H_{14}N_4O_2$
XVI	75	272 – 274	$C_{17}H_{16}N_4O_2$
XVII	93	291 – 293	$C_{15}H_{11}ClN_4O$
XVIII	76	248 – 250	$C_{15}H_{11}FN_4O$
XIX	63	265 – 267	$C_{15}H_{11}BrN_4O$
XX	80	196 – 198	$C_{15}H_{11}N_3O_2$
XXI	98	170 – 172	$C_{16}H_{13}N_3O_2$
XXII	100	209 – 210	$C_{15}H_{10}ClN_3O_2$
XXIII	83	258 – 260*	$C_{16}H_{12}N_2O_2$
XXIV	70	231 – 232**	$C_{18}H_{16}N_2O_2$
XXV	47	265 – 266	$C_{16}H_{12}FN_3O_2$
XXVI	39	196 – 197	$C_{20}H_{16}N_4O$

* Published data, 260 – 261°C [4].
** Published data, 231 – 232°C [4].

sia) using DMSO-d_6 as solvent and HMDS as the internal standard.

3-Pyridylamides of aroylpyruvic acids (I – XI). A solution of 0.94 g (10 mmole) of 3-aminopyridine in 15 ml of absolute dioxane was added to a solution of 10 mmole of the corresponding 5-aryl-2,3-dihydrofuran-2,3-dione [13] in 40 ml of the same solvent and the mixture was allowed to stand for 3 h. Then the solution was diluted with water, the precipitate was separated by filtration and recrystallized from ethanol or acetonitrile.

3-Pyridylamides of 5-arylpyrazole-3-carboxylic acids (XII – XIX). To 5 mmole (1.34 g) of compound I in 35 ml ethanol was added on stirring 5 mmole (0.50 g) of a 50% hydrazine hydrate solution in 10 ml of ethanol and the mixture was allowed to stand for 2 h. Then the precipitate was separated by filtration and recrystallized from a DMF – water mixture (1 : 1). The other compounds were obtained by a similar procedure.

3-Pyridylamides of 5-arylisoxazole-3-carboxylic acids (XX – XXII). To a solution of 1.83 g (0.022 mole) of hydroxylamine hydrochloride in 30 ml of ethanol was added a solution of 1.12 g (0.02 mole) of potassium hydroxide in 20 ml of the same solvent. Precipitated potassium chloride was separated by filtration. To the filtrate was added 0.52 g (0.02 mole) of compound I and the mixture was boiled on a water bath for 3.5 h and cooled. The white precipitate was separated with a filter and the filtrate was evaporated to dryness. The total residue was recrystallized from isopropanol. The other compounds were obtained by a similar procedure.

3-Aroylmethylene-1,2,3,4-tetrahydro-2-quinoxalones (XXIII – XXV) and 3-pyridylamide of 4-phenylbenz[b]diazepin-2-carboxylic acid (XXVI). To 0.54 g (0.002 mole) of compound I was added 30 ml of isopropanol, 4 ml of acetic acid, and 0.24 g (0.002 mole) of *o*-phenylenediamine. The mixture was boiled for 30 min and cooled. The yellow precipitate of compound XXIII was separated by filtering and recrystallized from acetic acid; m.p., 256 – 258°C. The filtrate was evaporated to dryness and the white residue (XXVI) was recrystallized from isopropanol; m.p., 196 – 197°C.

Compounds XXIV and XXV were obtained by an analogous method.

EXPERIMENTAL BIOLOGICAL PART

The synthesized compounds were characterized with respect to antimicrobial, antiinflammatory, analgesic activity and acute toxicity.

The antimicrobial activity was studied with respect to standard strains of *Escherichia coli* M_{17} and *Staphylococcus aureus* P-309 by a conventional method of serial double dilutions in a beef-extract broth [14], using a bacterial load of 250 thousand microbe units per 1 ml solution. The effective dose was evaluated as the minimum inhibiting concentration (MIC) of the test compound (i.e., the maximum dilution) still leading to the complete suppression of the microbial growth. Ethacridine lactate and mercury chloride [15, 16] served as references.

The antiinflammatory effect was studied on a model of acute inflammatory edema induced by subplantar 0.1 ml injections of a 1% aqueous carrageenan solution into the hind paws of white rats weighing 180 – 200 g [17]. The effect was evaluated by the degree of exudation inhibition (determined as % of control) upon the intraperitoneal injection of test compounds at a dose of 50 mg / kg with a 2% starch jelly. Each compound was tested on a group of 5 animals. Ortophen was used as the reference [15].

The analgesic activity was studied on mongrel mice weighing 18 – 22 g using a "hot-plate" thermal irritation

TABLE 2. Toxicity and Biological Activity of Synthesized Compounds

Compound	LD_{50}, mg / kg	Antiinflammatory activity			Analgesic activity: reflex time, sec	Antimicrobial activity (MIC), μg / ml	
		dose, mg / kg	average edema weight gain, % of control	exudation inhibition, % of control		*St. aureus* P-209	*E. coli* M_{17}
I	690	50	79.3	35.4	14.2	125	250
II	690	50	74.2	40.5	14.4	125	125
V	1600	50	74.2	42.5	14.6	125	250
VI	1410	50	74.1	41.0	15.0	125	250
VIII	1350	50	99.8	19.6	15.0	250	500
XI						125	1000
XIII		50	96.2	23.4		250	125
XIV						125	500
XV		50	96.8	23.0	–	–	–
Control (2% starch)		–	125.5	–	11.9	–	–
Ortophen		10	56.0	53.0	26.2	–	–
Mercury dichloride		–	–	–	–	1000	1000
Ethacridine lactate		–	–	–	–	500	2000

technique [18]. The compounds were injected at a dose of 50 mg/kg 0.5 h before placing animals onto the metal plate heated to 53.5°C. A change in the pain reaction was evaluated by the time (measured in sec) an animal stayed on the hot plate before licking the hind paws. Each compound was tested in a group of 10 animals.

The acute toxicity (evaluated as LD_{50}) of the compounds was determined by a conventional method [14] upon intraperitoneal injection (with a 2% starch jelly) into white mice weighing 18 – 22 g.

The antimicrobial tests showed that most of the compounds studied exhibited a higher activity with respect to *St. aureus* than to *E. coli*. The activity was found to depend on the character of substituent in benzene ring of the aroyl fragment (Table 2).

The antiinflammatory activity was also observed in most of the 3-pyridylamides of aroylpyruvic acids. The electron-donor substituents (compounds II, V, VI) favored increasing activity, while the electron-acceptor ones reduced the antiinflammatory effect (compound VIII). The transition from amides of aroylpyruvic acids to amides of pyrazole-3-carboxylic acids also decreased the activity (compounds XIII, XV). A comparison of the antiinflammatory effect of 3-pyridylamides with that of the previously studied 2-pyridylamides of aroylpyruvic acids [1] shows that the former compounds exhibit a much more pronounced activity in this respect.

All the 3-pyridylamides studied in this work have a rather weak analgesic activity. The character of substituents in the *para* position of benzene ring in the aroyl fragment has virtually no effect on this property.

The 3-pyridylamides studied have proved to be virtually nontoxic [16], since their LD_{50} values fall within 690 – 1600 mg/kg.

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