SYNTHESIS AND BIOLOGICAL ACTIVITY OF SUBSTITUTED PYRIDYL AMIDES OF AROYLPYRUVIC ACID

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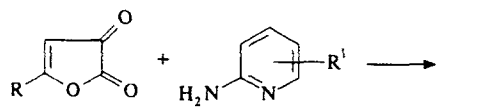
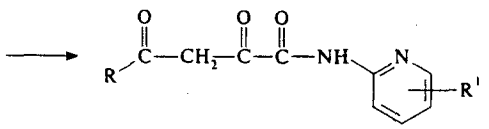
2-Aroylpyridyl amides of aroylpyruvic acids were found to exhibit the antiinflammatory and analgesic activity at rather low toxicity [1].

Of interest is the study of pharmacological effect of 2-pyridyl amides as a function of the substituent nature in the pyridine moiety. Thus we synthesized 2-(6-methyl)- and 2-(5-bromo)pyridyl amides of aroylpyruvic acids (IV – XXVI) and studied their acute toxicity and antiinflammatory, analgesic, and antimicrobial activity. Compounds IV – XXVI were obtained by the reaction of 5-aryl-2,3-dihydrofuran-2,3-diones (I) with 6-methyl- and 5-bromo-2-aminopyridines (II and III) carried out in anhydrous dioxane at room temperature.



I

II, III



IV – XXVI

R = Ph(IV, XVII),
4-MeC_6H_4(V, XVIII),
2,4-$Me_2C_6H_3$(VI, XIX),
2,5-$Me_2C_6H_3$(VII),
4-$MeOC_6H_4$(VIII, XX),
4-$EtOC_6H_4$(IX, XXI),
4-BrC_6H_4(X, XXII),
3-BrC_6H_4(XI),
4-ClC_6H_4(XII, XXIII),
4-FC_6H_4(XIII, XXIV),
4-IC_6H_4(XIV),
4-$O_2NC_6H_4$(XV, XXV),
α-naphthyl (XVI, XXVI);

R^1 = 6-Me(II, IV – XVI),
5-Br(III, XVII – XXVI).

The synthesized compounds are white crystalline solids, soluble in DMF and DMSO, poorly soluble in ethanol and benzene, and insoluble in water. Ethanol solutions of these compounds give a cherry color with an ethanol solution of $FeCl_3$, which indicates the presence of the enol form in the solutions [2] due to enolization of one of ketone carbonyls.

The IR spectra of the synthesized compounds contain the absorption bands ranging from 3370 to 3270 cm^{-1} (assigned to the stretching vibrations of the NH bond in the amide groups), from 1700 to 1680 cm^{-1} (assigned to the amide carbonyl), and from 1610 to 1590 cm^{-1} (assigned to the γ-ketone carbonyl involved in the intramolecular hydrogen bond) [3].

The ^1H NMR spectra of the studied compounds contain a singlet of the proton of the methine group at 6.75 to 7.25 ppm, a group of signals at 7.58 – 7.90 ppm assigned to protons of the aromatic and pyridine rings, and a broadened signal of the amino group at 9.30 to 10.11 ppm. The signals of the methyl group in the spectra of compounds IV – XVI fall within the region ranging from 2.31 to 2.45 ppm. The signals of the other functional groups are recorded within the expected regions. The nitrogen content is consistent with the calculated values.

It is well known that condensation of β-dicarbonyl compounds with *o*-phenylenediamine results in formation of 3(H)-1,5-benzdiazepines [4], and aroylpyruvic acids and their esters react with *o*-phenylenediamine to form 3-phenacylidene-3-quinoxalones [5]. Aryl amides of aroylpyruvic acids react with *o*-phenylenediamine to give 2-arylbenz[b]diazepin-4-carboxylic acids in a high yield and, at the same time, 3-phenacylidene-2-quinoxalones [6]. The presence of the α, γ-dicarbonyl moiety in the structure of pyridyl amides of aroylpyruvic acids makes it possible to expect that the condensation of these compounds with *o*-phenylenediamine proceeds in two directions.

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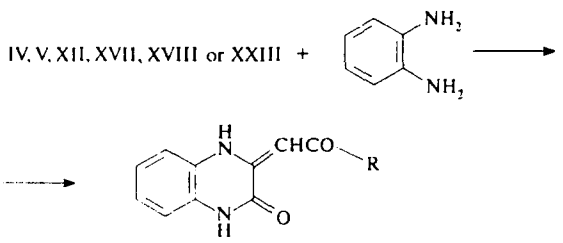
However, it turned out that in the case of 6-methyl- and 5-bromopyridylamides the condensation results only in formation of 3-phenalidene-2-quinoxalones.



IVa, Va, XIIa, XVIIa, XVIIIa, XXIIIa

The visual appearance, melting points, and spectral parameters of the synthesized compounds are consistent with the published data [6, 7].

EXPERIMENTAL CHEMICAL PART

IR spectra were recorded on UR-20 and Zeiss Specord M-80 (Germany) spectrophotometers for pastes in Vaseline oil. ^{1}H NMR spectra were recorded on a RYa-2310 spectrometer (60 MHz) (Russia) in DMSO-d_6 with HMDS used as the internal standard.

2-(6-Methyl)pyridyl amide of benzoylpyruvic acid (IV). A solution of 1.08 g (10 mmole) of 2-amino-6-methylpyridine in 15 ml of dry dioxane was added to a solution of 1.74 g (10 mmole) of 5-phenyl-2,3-dihydrofuran-2,3-dione in 20 ml of the same solvent, and the resulting mixture was allowed to stand until the solvent was evaporated. The dry residue was recrystallized from isopropanol. Compound IV (2.51 g) was obtained, m.p. 135 – 136°C. Yield 89%. IR (ν_{max}, cm^{-1}): 3360 (NH), 1700 (amide carbonyl) 1600 (ketone carbonyl). ^{1}H NMR δ, ppm: 2.39 s (3H, CH_3 of pyridine ring); 7.25 s (1H, methine); 7.78 (8H, aromatic and pyridine rings); 9.30 s (1H, amino group).

Compounds V – XXVI were synthesized by the same procedure.

3-Phenacylidene-2-quinoxalone (IVa). *o*-Phenylenediamine (1 mmole, 0.108 g) was added to 0.29 g (1 mmole) of compound IV, and the resulting mixture was allowed to stand for 15 min on a metal bath at 90°C. A yellow solid precipitate that formed from the melt was then recrystallized from glacial acetic acid to yield compound IVa (0.097 g, 36%), m.p. 261 – 263°C. IR (ν_{max}, cm^{-1}): 3058 (=CH groups), 1691 (CO amide), 1617 (CO ketone). ^{1}H NMR (DMSO-d_6), δ, ppm: 6.80 s (1H, methine); 7.55 m (10H, aromatic), 10.65 s (1H, amino groups).

Compounds Va, XIIa, XVIIa, XVIIIa, and XXIIIa were synthesized by the same procedure.

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity against standard strains of fungi *E. coli* M17 and *St. aureus* P-209 was determined by the standard serial twofold dilution technique in meat-infusion broth (MIB) [8]. The bacterial load was 250,000 microbial bodies per 1 ml of the liquid. The minimum inhibitory concentration (MIC) of the drug (the lowest concentration (maximum dilution)), which completely suppresses the growth of test-microbes, was taken as an effective dose. Mercuric chloride and ethacridine were used as reference drugs [9].

The antiinflammatory effect was studied on a model of acute inflammatory edema induced by the subplantar injection of 0.1 ml of a 1% carrageenan solution into the hind paw of albino rats (weighing 180 to 200 g) [10]. The antiinflammatory effect was evaluated by the inhibition of exudation (% with respect to the control), the compounds were injected intraperitoneally in a dose of 50 mg/kg in the form of suspension in a 2% starch mucus, the activity was compared to that of ortofen [11]. Each compound was tested on five animals.

We studied the analgesic activity using the "hot plate" assay on mongrel albino mice weighing 18 to 20 g [12]. The tested compounds were administered in a dose of 50 mg/kg 30 min prior to placing the animals on the metal plate heated to 53.5°C. The duration of stay on the hot plate till the moment at which mice start to lick their hind paws was taken as the nociception index. In intact animals, the latent period of defensive reflex did not exceed 15 sec. Each compound was studied on ten animals.

TABLE 1. Characteristics of Compounds IV – XXVI

Compound	Yield, %	M.p., °C	Empirical formula
IV	92	135 – 136	$C_{16}H_{14}N_2O_3$
V	90	138 – 140	$C_{17}H_{16}N_2O_3$
VI	87	130 – 131	$C_{18}H_{18}N_2O_3$
VII	84	118 – 119	$C_{18}H_{18}N_2O_3$
VIII	79	126 – 127	$C_{17}H_{16}N_2O_4$
IX	80	154 – 155	$C_{18}H_{18}N_2O_4$
X	85	143 – 144	$C_{16}H_{13}BrN_2O_3$
XI	80	146 – 147	$C_{16}H_{13}BrN_2O_3$
XII	73	165 – 166	$C_{16}H_{13}ClN_2O_3$
XIII	88	162 – 163	$C_{16}H_{13}FN_2O_3$
XIV	83	188	$C_{16}H_{13}IN_2O_3$
XV	84	158 – 159	$C_{16}H_{13}N_3O_5$
XVI	86	137 – 138	$C_{20}H_{19}N_2O_3$
XVII	94	221 – 222	$C_{15}H_{11}BrN_2O_3$
XVIII	70	223	$C_{16}H_{13}BrN_2O_3$
XIX	75	206 – 208	$C_{17}H_{15}BrN_2O_3$
XX	82	205 – 207	$C_{16}H_{13}BrN_2O_4$
XXI	78	208 – 209	$C_{17}H_{15}BrN_2O_4$
XXII	82	241 – 242	$C_{15}H_{10}Br_2N_2O_3$
XXIII	83	243 – 244	$C_{15}H_{10}BrClN_2O_3$
XXIV	78	222 – 223	$C_{15}H_{10}BrFN_2O_3$
XXV	88	246	$C_{15}H_{10}BrN_3O_5$
XXVI	80	155 – 156	$C_{19}H_{13}BrN_2O_3$

TABLE 2. Acute Toxicity, Antiinflammatory, Analgesic, and Antimicrobial Activities of 2-(6-Methyl)- and 2-(6-Bromo)pyridylamides of Aroylpyruvic Acids (IV – XXIV).

Compound	Acute toxicity, LD_{50} mg/kg	Growth of rat paw volume with respect to control, %	Percent of exudation inhibition, %	Defensive reflex period, sec	MIC, μg/ml	
					E. Coli	*St.aureus* P-209
IV	1600.0	135.2	– 31.6	14.4	500	500
V	1600.0	97.6	+ 5.0	17.2	500	125
VII	...	41.2	+ 60.0	22	...	...
VIII	178.0	60.6	+ 41.0	...	...	...
X	...	...	...	...	125	62
XII	815.0	84.0	+ 18.3	...	250	125
XIII	...	...	...	...	500	250
XVII	...	39.6	+ 47.5	14.4	125	125
XVIII	2820	36.9	+ 51.1	13.0	250	125
XIX	1600	64.3	+ 37.8	14.2	...	...
XXI	...	78.2	+ 23.9	...	...	...
XXII	2050	69.6	+ 32.3	...	...	...
XXIII	2580	32.8	+ 56.5	...	500	500
XXIV	...	79.2	+ 21.8		...	...
Control (2% starch mucus)	...	102.7	...	12.7	...	...
Ortofen	380	45.5	+ 55.7			...
Mercuric chloride			...	...	1000	1000
Ethacridine lactate					2000	500

The acute toxicity of the tested compounds was evaluated on mongrel mice weighing 18 – 22 g in oral administration [8]. Each dose was studied on ten animals. The compounds were given as a suspension in 2% starch mucus in a dose of 0.1 ml per 10 g, and the animals were under observation for 10 h. A 50% end point lethal dose (LD_{50}) was used as the toxicity index.

The study of antimicrobial activity of compounds IV – XXVI (Table 2) showed that they exhibit a weak bacteriostatic effect. Compound X containing bromine in the aroyl moiety exhibits the highest activity. It is of interest that introduction of the bromine atom into the pyridyl substituent decreases its activity.

The compounds tested exhibit weak analgesic activity regardless of the nature of the substituents both in the *para*-position of the benzene ring and in the amide moiety.

The compounds of both pyridylamide series exhibit antiinflammatory activity. It is of interest that 2-(6-methylpyridyl)amide IV, which has no substituents in the benzoyl moiety, exhibits the inflammatory instead of antiinflammatory effect. At the same time, in both series the antiinflammatory activity substantially changes as the nature of the substituents in the aroyl group varies.

Thus, the studied pyridylamides are assigned to moderate-toxicity substances [13].

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