PRODUCTS OF REACTION BETWEEN HETERENO[a]-2,3-DIHYDRO-2,3-PYRROLEDIONES AND ARYL- OR HETERYLAMINES AND THEIR PHARMACOLOGICAL ACTIVITY

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 34, No. 12, pp. 13 – 16, December, 2000.

Original article submitted November 25, 1999.

We established earlier that 3-aroyl-1,2,4,5-tetrahydropyrrolo[1,2-a]quinoxaline- and 3-aroyl-1,2-dihydro-4H-pyrrolo-[5,1-c][1, 4]benzoxazine-1,2,4-triones exhibit antimicrobial and analgesic activity [1]. It seemed of interest to modify their structure by introducing various heterocycles into the molecule for the above-indicated compounds and to study the chemical behavior of the products obtained and their properties, by investigating the effect of the structure of the compounds on their biological activity.

With this objective, we obtained the reaction products for reaction of 3-aroyl-1,2-dihydro-4H-pyrrolo[5,1-c][1,4]benzoxazine-1,2,4-triones (I – II) and 3-aroyl-1,2,4,5-tetrahydropyrrolo[1,2-a]quinoxaline-1,2,4-triones (III – VI) with 2-aminopyridine, 5-aminoisoquinoline, N-phenyl-*o*-phenylenediamine, and 2,5-dichloroaniline.

Compounds I – VI [1] react easily with amines for a 1 : 1 mole ratio of the reagents. They react with most amines at room temperature, but react with N-phenyl-*o*-phenylenediamine with brief (1 – 3 min) heating. Two series of products are formed as a result: N-substituted 3a-amino(heterylamino)-3-aroyl-2-hydroxy-1,3a-dihydro-4H-pyrrolo[2,1-c][1,4] benzoxazine-1,4-diones (VII – X) (direction a) and substituted amides of 4-aryl-2,4-dioxo(Z)-3-(2-oxo-3,4-dihydro-2H-1,4-benzoxazin-3-ylidene)butanoic acids (XI – XXIV) (direction b), which is quite consistent with our data on the reaction products for reaction of 3-aroylpyrrolobenzoxazinetriones with dialkylamines and arylamines [2].

The composition of compounds VII – XXIV was confirmed by elemental analysis data; the structure was confirmed by IR and PMR, spectroscopy.

The compounds formed via "direction a" as a result of nucleophilic addition of amines to the carbon atom in the 5 position of the dihydropyrroledione ring (atom 3a of compounds I – VI) usually are unstable [2, 3] due to the reversibility of the initial addition and the tendency toward further conversions. So although according to TLC data the addition products are present in the reaction mixture in practically all the studied cases, it is only in a certain number of cases that they were isolated in pure form. The isolated compounds VII – IX are colorless, while compound X is a yellow crystalline material. Compounds VII – X are difficultly soluble in conventional organic solvents and give a positive test (cherry red color) for the presence of enol hydroxyl with an alcoholic solution of ferric chloride. The solutions of compounds VII – X in acetone or chloroform are violet or raspberry-colored, and the intensity of the color increases upon heating. This supports [2] the reversibility of addition of the starting amines. In the IR spectra of compounds VII – X, there are broad bands for stretching vibrations of the OH groups in the 3000 – 3150 cm^{-1} region, stretching vibration bands for the lactone C^4=O carbonyl in the 1775 – 1780 cm^{-1} region, the lactam C^1=O and aroyl carbonyls in the 1700 – 1710 cm^{-1} and 1600 – 1610 cm^{-1} region respectively. The spectral characteristics of compounds VII – X correspond to the proposed structure and are similar to the characteristics of the products of addition of alcohols and dialkylamines to compounds I – VI [2]. The spectral properties suggest that they exist in enolized form with an intramolecular hydrogen bond of the H-chelate type between the C^2–OH groups and the carbonyl of the aroyl substituent in the 3 position. Furthermore, in order to theoretically explain the direction of primary nucleophilic attack on compounds I – VI, we calculated the electron density distribution in the 3-benzoyl-1,2,4,5-tetrahydropyrrolo[1,2-a]quinoxaline-1,2,4-trione (III) molecule by the semi-empirical MNDO method using the program GAUSSIAN-94W [4] with full optimization of its geometry. According to the calculation results, the C^1, C^2, C^4, and C^{3a} atoms are the most electron-deficient (the charges on

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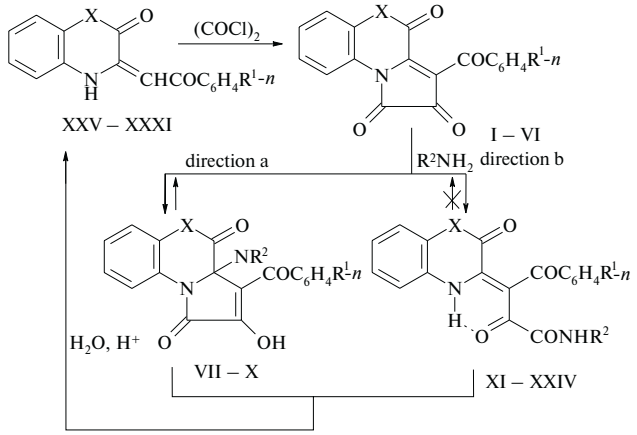
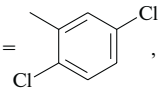
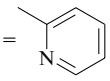
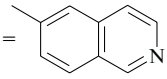
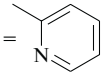
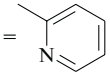
the atoms are respectively equal to: + 0.394, + 0.325, + 0.467, + 0.408) but the greatest contribution to the lowest unoccupied molecular orbital comes from the $2p_z$ orbital of the C^{3a} atom (coefficient 0.551). Earlier [3] we carried out a MNDO calculation for the 3-benzoyl-1,2-dihydro-4H-pyrrolo[5,1-c][1,4]oxazine-1,2,4-trione (I) molecule with partial optimization of the geometry. Comparing the calculations for both structures shows that they are similar with respect to the nature of the electron distribution and differ only in the absolute values of the atomic orbital coefficients, i.e., if the reaction is orbitally-controlled then it is specifically the C^{3a} atom which should be the target for nucleophilic attack, as is observed in the result of the reaction for direction a.



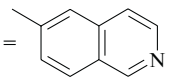
X = O (I, II); R^1 = H (I), CH_3 (II).
X = NH (III – VI); R^1 = H (III), CH_3 (IV), Br (V), NO_2 (VI).

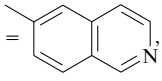
R^1 = H: R^2 =  , X = O (VII, XI), NH (VIII);

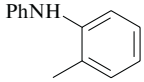
R^2 =  , X = O (IX). R^1 = CH_3: R^2 =  ,

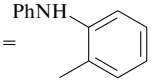
X = O (X); R^2 =  , X = O (XII). R^2 =  ,

X = NH: R^1 = H(XIII), CH_3 (XIV), Br (XV), NO_2 (XVI).

R^2 =  , X = O: R^1 = H(XVII), CH_3 (XVIII).

R^2 =  , X = NH: R^1 = H(XIX), CH_3 (XX),

Br (XXI). R^1 = CH_3, X = O, R^2 =  (XXII).

X = NH, R^2 =  : R^1 = H (XXIII), CH_3 (XXIV)

In the case of a charge-controlled reaction (direction b), the C^1 carbon undergoes nucleophilic attack. Moreover, we should note that the values of the positive charges on the C^{3a} and C^4 atoms of the molecule of compound III are higher than on the C^1 atom, so that the reason for attack at the C^1 carbon atom probably involves thermodynamic factors: the high stability of compounds XI – XXIV compared with the products of nucleophilic attack at the C^{3a} and C^4 atoms of compounds I – VI. Compounds XI – XXIV are formed (like the products for reaction of pyrrolobenzoxazinetriones with arylamines [2]) as a result of nucleophilic attack by the NH group of aryl- and heterylamines on the C^1 carbonyl carbon atom of compounds I – VI and cleavage of the dihydropyrroledione ring along the C^1–N^{10} bond.

Compounds XI – XXIV are more stable than compounds VII – X, although they easily undergo hydrolysis and aminolysis. They are orange crystalline compounds, difficultly soluble in conventional organic solvents, and do not give a positive test for the presence of enol hydroxyl with an alcoholic solution of ferric chloride. In the IR spectra of these compounds, there are stretching vibration bands for the NH amide group of the side chain in the form of a narrow peak in the 3150 – 3200 cm^{-1} region, for the N^4H group of the heterocycle involved in the intramolecular hydrogen bond of the H-chelate type in the 2965 – 3100 cm^{-1} region, for the C^2=O carbonyl of the benzoxazine ring (compounds XI, XII, XVII, XXII) in the 1730 – 1750 cm^{-1} region, for the amide C^1=O and aroyl C^4=O carbonyls in the 1610 – 1710 cm^{-1} region, for the carbonyl group in the 2 position in the form of a broad band in the 1580 – 1610 cm^{-1} region, and the "Amide II" band in the 1510 – 1540 cm^{-1} region.

In the PMR spectra of the compounds (XIII, XIX, XX, XXIV), in addition to signals from protons of the aromatic substituents and the groups bonded to them, we see a singlet from the amide NH group of the side chain in the 7.95 – 8.89 ppm region, a singlet from the amide N^1H group of the heterocycle in the 9.3 – 10.75 ppm region, and a singlet from the N^4H group of the heterocycle involved in the intramolecular hydrogen bond of the H-chelate type in the 12.02 – 12.76 ppm region.

Hydrolysis of compounds VII – XXIV when boiled in a dioxane – 10% HCl mixture for 1 – 5 min leads to cleavage of the pyrroledione ring and formation of benzoxazin-2-ones (XXV, XXVI) and 2-quinoxalones (XXVII – XXXI).

EXPERIMENTAL CHEMICAL PART

The IR spectra of the compounds obtained were taken in vaseline oil on a UR-20 spectrophotometer. The PMR spectra were recorded on an RYa-2310 spectrometer in DMSO-d_6 solution, internal standard HMDS.

3a-(2,5-Dichlorophenylamino)-2-hydroxy-3-benzoyl-3a,4-dihydro-1H-pyrrolo[2,1-c][1, 4]benzoxazine-1,4-dione (VII). A solution of 3.19 g (10 mmoles) of compound I in 50 ml anhydrous dioxane was added with stirring to a solution of 1.48 g (10 mmoles) of 2,5-dichloroaniline in 30 mL

TABLE 1. Characteristics of the Synthesized Compounds

Compound	Yield, %	m.p., °C	Empirical formula
VI	57	163 – 165 (d.)	$C_{24}H_{14}Cl_2N_2O_5$
VIII	75	183 – 185 (d.)	$C_{24}H_{15}Cl_2N_3O_4$
IX	70	176 – 178 (d.)	$C_{28}H_{19}N_3O_5$
X	62	120 – 122 (d.)	$C_{23}H_{15}N_3O_5$
XI	35	260 – 262	$C_{23}H_{15}N_3O_5$
XII	73	230 – 232	$C_{24}H_{17}N_3O_5$
XIII	82	222 – 224	$C_{23}H_{16}N_4O_4$
XIV	84	215 – 217	$C_{24}H_{18}N_4O_4$
XV	72	250 – 252	$C_{23}H_{15}BrN_4O_4$
XVI	79	237 – 239	$C_{23}H_{15}N_5O_6$
XVII	76	147 – 149	$C_{27}H_{17}N_3O_5$
XVIII	83	187 – 189	$C_{28}H_{19}N_3O_5$
XIX	89	212 – 214	$C_{27}H_{18}N_4O_4$
XX	81	227 – 230	$C_{28}H_{20}N_4O_4$
XXI	84	257 – 259	$C_{27}H_{17}BrN_4O_4$
XXII	91	217 – 219	$C_{31}H_{23}N_3O_5$
XXIII	87	292 – 293	$C_{30}H_{22}N_4O_4$
XXIV	79	223 – 226	$C_{31}H_{24}N_4O_4$

anhydrous dioxane. After 24 h, a pale yellow precipitate of compound VII fell out of solution. This was recrystallized from acetonitrile.

Compounds VIII – IX were obtained similarly.

Pyridylamide of 4-phenyl-2,4-dioxo-Z-3-(2-oxo-1,2,3,4-tetrahydro-3-quinoxalinylindene)butanoic acid (XIII). A solution of 3.32 g (10 mmoles) of compound III in 50 ml of anhydrous dioxane was added with stirring to a solution of 0.94 g (10 mmoles) 2-aminopyridine in anhydrous dioxane. The precipitate was filtered off and recrystallized from a 1 : 1 mixture of acetonitrile and dioxane.

Compounds XI, XII, XIV – XXIV were obtained similarly.

EXPERIMENTAL BIOLOGICAL PART

Procedure

We studied the analgesic and antimicrobial activity and the acute toxicity of compounds VII – XXIV.

The acute toxicity (LD_{50}) of the compounds when administered orally was determined in outbred mice weighing 18 – 22 g [5]. Each dose of the compounds was studied using 10 animals. The compounds were administered as a 2% starch mucilage suspension (dosage based on 0.1 ml per 10 g), after which the animals were observed for 10 days.

The analgesic activity was studied on outbred mice weighing 18 – 22 g using the thermal stimulation ("hot plate") method [6]. The test compounds were injected intraperitoneally in a dose of 50 mg/kg 30 min before plac-

TABLE 2. Toxicity and Biological Activity of the Synthesized Compounds

Compound	Dose, mg/kg	Rough acute toxicity LD_{50}, mg/kg	Analgesic activity (latency period of reflex in seconds)	Antimicrobial activity (MIC), μg/ml, minimum bacteriostatic concentration / minimum bactericidal concentration	
				E. coli	*St. aureus*
VII	50	> 1500	20.2 ± 2.36*	1000	1000
VIII				250/1000	250/1000
IX	-"-	-"-	19.6 ± 2.69*	1000	1000
XII				1000/2000	500/2000
XIII	-"-	-"-	23.3 ± 1.44*	250/500	250/500
XIV	-"-	-"-	21.1 ± 4.12*	2000	125/500
XV				> 2000	> 2000
XVI	-"-	-"-	21.2 ± 2.12*	500/1000	500/1000
XVII	-"-	-"-	20.8 ± 2.66*	500	250
XVIII	-"-	-"-	22.8 ± 1.74*	125/500	62.5/125
XIX	-"-	-"-	20.1 ± 2.04*	500/1000	500/1000
XX				1000	1000
XXI				> 2000	> 2000
XXII				> 2000	> 2000
XXIII	-"-	-"-	22.0 ± 1.72*	> 2000	> 2000
XXIV	-"-	-"-	21.0 ± 1.26*	1000	1000
Control (2% starch mucilage)		–	11.9 ± 2.3		
Voltaren	10	380	26.2 ± 0.84*		

* $p < 0.05$ compared with control

ing the animals on a metal plate heated up to 53.5°C. The index of the change in pain sensitivity was the time the animal remained on the "hot plate" before licking its hind paws, measured in seconds. For untreated animals, the latency period for the defensive reflex is no longer than 15 sec.

The antimicrobial action was determined by the two-fold serial dilution method using cultures of two pathogenic reference strains of microorganisms (*Escherichia coli*, strain 675; *Staphylococcus aureus*, strain 209-P) obtained from the A. N. Tarasevich State Institute for Standardization and Control [5]. We prepared the starting dilutions of the microbe bodies from a 24-hour agar culture using an optical standard. The microbial load in determination of the minimum bacteriostatic concentration (MBSC) corresponded to 2.5×10^5 microbe bodies per ml, while in determination of the minimum bactericidal concentration (MBC) the microbial load was 5.0×10^5 microbe bodies per ml. The microbial suspension was added to the prepared dilutions of the drugs in a nutrient medium. The results were counted after thermostatting at 37°C for 20 h (MBSC) and 7 h (MBC).

RESULTS AND DISCUSSION

All the tested compounds have an analgesic effect. The most pronounced effect is exhibited by compounds XIII, XVIII, and XXIII. The analgesic action does not change much when we vary the aryl and heteryl substituents at the nitrogen atom of the side chain for compounds VII – XXIV, and is greatest in the case of the pyridylamide of quinoxalinylidenebutanoic acid (compound XIII). For the products of opening of the dihydropyrroledione ring (compounds VIII, IX, XVI – XIX, XXIII, XXIV), the analgesic activity is somewhat higher than for the addition products (compounds VII and IX).

The antimicrobial tests show that the tested compounds (except for compounds XV, XXI – XXIII) display different degrees of antimicrobial activity and this activity: increases when pyridylamide and quinolineamide moieties are present in the molecule of the compound; is practically nonexistent when the N-phenyl-*o*-phenyleneamine moiety is introduced; and increases when a benzoxazin-2-one ring is present in the molecule of the tested compound, compared with the quinoxalone ring.

Thus the results obtained indicate that the synthesized compounds have low toxicity and exhibit analgesic and antimicrobial action.

REFERENCES

1. A. N. Maslivets, I. V. Mashevskaya, L. I. Smirnova, et al., USSR Inventor's Certificate 1768603. IPC^4 C 07D 498/04, A 61 K 31/535.
2. A. N. Maslivets, I. V. Mashevskaya, and Yu. S. Andreichikov, *Zh. Org. Khim.*, **29**(10), 2056 – 2064 (1993).
3. A. N. Maslivets, I. V. Mashevskaya, O. P. Krasnykh, et al., *Zh. Org. Khim.*, **28**(12), 2545 – 2553 (1992).
4. M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al., *Gaussian 94, Revision E.3*, Gaussian, Inc., Pittsburgh (1995).
5. G. N. Pershin, *Methods in Experimental Chemotherapy* [in Russian], Meditsina, Moscow (1971), pp. 113 – 115.
6. G. Wolfe and A. D. McDonald, *J. Pharmacol. Exp. Ther.*, **80**(3), 300 – 307 (1944).