3. Ya. L. Gol'dfarb, Ya. L. Danyushevskii, and M. A. Vinogradova, Dokl. Akad. Nauk SSSR, 151, No. 2, 332-335 (1963).
4. P. A. Konstantinov and R. I. Shutik, Zh. Obshch. Khim., 33, No. 4, 1251-1255 (1963).
5. E. Ya. Lukevits, N. P. Erchak, and V. D. Shatts, Chemistry of Heteroorganic Compounds [in Russian], Nauka, Leningrad (1976), pp. 56-63.
6. E. Ya. Lukevits, N. P. Erchak, Yu. Yu. Popelis, and I. V. Dipan, Zh. Obshch. Khim., 47, No. 4, 802-808 (1977).
7. E. Ya. Lukevits, N. P. Erchak, I. Castro, et al., Zh. Obshch. Khim., 55, No. 9, 1315-1323 (1984).
8. E. Ya. Lukevits, N. P. Erchak, I. Castro, et al., Zh. Obshch. 2 Khim., 55, No. 9, 2062-2071 (1985).
9. A. L. Mndzhoyan, V. G. Afrikyan, M. T. Grigoryan, and E. A. Markaryan, Dokl. Akad. Nauk Arm. SSR, 27, 301-304 (1985).
10. V. P. Panov, A. K. Trukhmanov, and R. G. Zhbankov, Zh. Prikl. Spektrosk., 19, No. 6, 1131-1133 (1973).
11. A. A. Ponomarev, Synthesis and Reactions of Furan Compounds [in Russian], Izd. Saratovsk Univ. (1960), pp. 158-159 (1960).
12. M. Bergman and L. Zervas, Ber., 64, 975 (1931).
13. S. F. Thames and H. G. Odom, J. Med. Chem., 3, No. 4, 490-494 (1966).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1,3-DIMETHYL-6-NITRO-7-CARBOXYLALKYL[ARYL]AMINOPYRIDO[2,3-D]PYRIMIDINES

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Developing the work done [1-3] on synthesizing new pyrido [2,3-d]pyrimidine derivatives in order to find biologically active substances, we have studied the reaction of 1,3-dimethyl-2,4,5-trioxo-6-nitro-7-chloro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidine (I) [1] with aliphatic amino acids, anthranilic acid and its ester. When this reaction is carried out in DMSO or N-ethylpyrrolidone, high yields (70-95%) are produced of the 7-carboxyalkyl-(aryl)amino derivatives IIa-g, which are high-melting, crystalline substances, soluble in DMF, DMSO, N-ethylpyrrolidone, and acids, but having low solubility in alcohols, acetone, and $CHCl_3$.

Acids IIb and IIc yield the methyl (III) and heptyl (XI) esters.

Apart from the pyrimidine ring methyl group signals and the aliphatic (compounds IIa-e, III, and XI) and aromatic (compounds IIf and IIg) proton signals, the PMR spectra of pyrido-[2,3-d]pyrimidines IIa-g, III, and XI contain a C(7)—H proton signal which is seen at 8.85-9.2 ppm. In the spectra of IIa-g and III, which were recorded in DMSO-D_6, CF_3COOH, and CF_3COOD due to the low solubility of the substance, no N(8)—H proton signal is observed, while in the readily-soluble ester XI it is registered as a singlet at 14.36 ppm.

Catalytic hydrogenation of compound III produced amino ester IV which, on treatment with MeONa, was converted into 1,3-dimethyl-2,4,5,7-tetraoxo-1,2,3,4,6,10,11-heptahydro[1,4]-benzodiazepino(2',3':5,6)pyrido[2,3-d]pyrimidine (V).

Under similar conditions the methyl ester of the anthranilic acid derivative IIg yielded 1,3-dimethyl-2,4,5,7-tetraoxo-1,2,3,4,5,6,7,13-octahydro-12H-[1,4]benzodiazepino(2',3':5,6)pyrido[2,3-d]pyrimidine (VII).

The structure of the cyclic products V and VII was corroborated by spectral data. The mass spectra of the compounds contain molecular ion peaks with I_0 = 100%. In the IR spectra NH-group vibrations are recorded at 3250 and 3350 cm^{-1} for V and at 3320 and 3400 cm^{-1} for VII. The methyl group ester signals, which could be seen for the starting compounds III, IV, IIg, and VI at 3.6-3.8 ppm, were absent in the PMR spectra. (See scheme on following page.)

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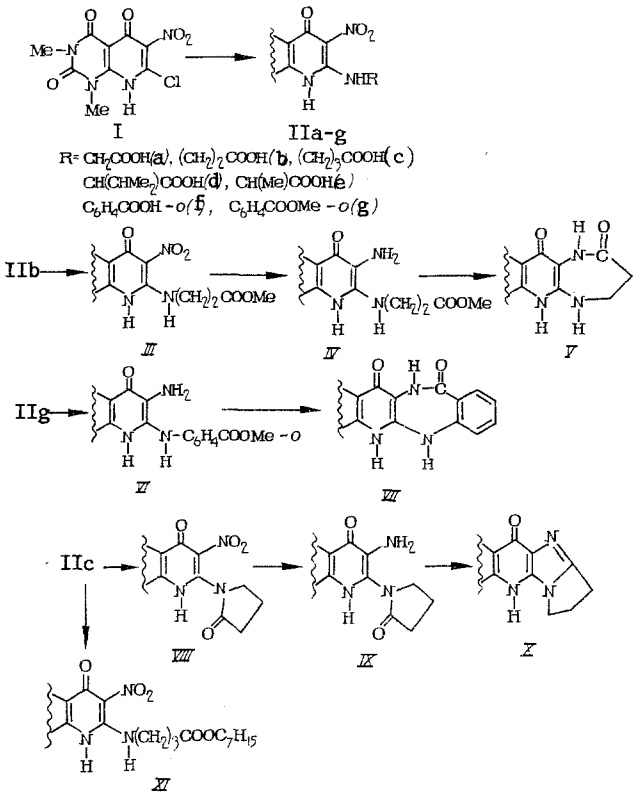

When the γ-aminobutyric acid derivative IIc is treated with $SOCl_2$, lactam VIII is formed, which on catalytic hydrogenation yields 6-aminopyridopyrimidine IX. Heating of IX with PPA produces 1,3-dimehtyl-2,4,5-trioxo-5H-1,2,3,4,7,8,9,10-octahydropyrrolidino[1",2":1,2]-imidazo(4',5':5,6)pyrido[2,3-d]pyrimidine (X).

The mass spectrum of the cyclic compound X contains a maximum intensity molecular ion peak, while no molecular ion peaks are seen in the spectra of the lactams VIII and IX present initially. An M^+-NO_2 peak with I_0 = 100% is recorded for compound VIII. The PMR spectra of pyrido[2,3-d]pyrimidines VIII and IX show no C(7)—NH proton signal, though this can be observed in the starting acid as a triplet with J = 5 Hz at 8.85 ppm.

EXPERIMENTAL (CHEMICAL)

IR spectra were recorded in KBr tablets on a Specord IR-75 spectrometer. PMR spectra were taken on a Tesla BS-407, internal standard HMDS. Mass spectra of the substances were obtained on a Varian MAT-311A instrument with direct sample insertion into the ion chamber.

The properties of the compounds synthesized are given in Table 1. Elemental analysis data on C, H, and N was in line with calculated values.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7) Glycine (IIa). A mixture of 0.5 g (1.74 mmoles) of compound I and 0.32 g (4.26 mmoles) of glycine in 3 ml of DMSO was heated at 140-145°C for 0.5 h. The reaction mass was cooled to 80°C and diluted with 15 ml of water. The precipitate was filtered off. Yield 0.4 g.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-β-Alanine (IIb). A mixture of 1.6 g (5.58 mmoles) of compound I and 2.48g (27.8 mmoles) of β-alanine in 10 ml of DMSO was boiled for 20 min. The reaction mass was cooled to 25°C and diluted with 30 ml of water. The precipitate was filtered off. Yield 1.4 g of IIb. Mass spectrum, m/z (%): 339(100) M+.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-γ-Aminobutyric Acid (IIc). A mixture of 3.0 g (10.5 mmoles) of compound I and 4.3 g (42 mmoles) of γ-aminobutyric acid in 25 ml of DMSO was heated at 110-120°C for 1.5 h. The reaction mass was cooled to 25°C and diluted with 50 ml of water. The precipitate was filtered off. Yield 3.52 g of IIc.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-Valine (IId). A mixture of 0.5 g (1.74 mmoles) of compound I and 0.61 g (5.21 mmoles) of

TABLE 1. Physicochemical Properties of Synthesized Compounds

Compound	Empirical formula	mp*	PMR spectra. Chemical shifts, δ, ppm. (coupling constant, J, Hz)**	Yield, %
IIa	$C_{11}H_{11}N_5O_7$	280—3	3,16 (3H, s, CH_3); 3,32 (3H s, CH_3); 4,08 (2H, d, J=4,5, CH_2); 9,04 (1H, t, J=5 Hz, C (7) — NH)	70,2
IIb	$C_{12}H_{13}N_5O_7$	191—3	2,3—2,7 (2H, m., CH_2); 3,15 (3H, s. CH_3); 3,35 (3H, s, CH_3; 3,5—3,8 (2H, m, CH_2); 8,9—9,0 (1H, broad s C(7)—NH)	74,1
IIc	$C_{13}H_{15}N_5O_7$	206—9	1,65—2,0 (2H, m, CH_2); 2,24 (2H, t, J=7 Hz, CH_2); 3,20 (3H, s., CH_3); 3,4 (3H, s, CH_3); 3,4—3,6 (2H, m, CH_2); 8,85 (1H, t, J=5 Hz, C(7)—NH)	95,2
IId	$C_{14}H_{17}N_5O_7$	249—51	0,95 (3H, s, CH_3); 1,02 (3H, s., CH_3); 2,0—2,5 (1H, m, CH); 3,3 (3H, s, CH_3); 3,48 (3H, s, CH_3); 4,4—4,7 (1H, m., CH); 9,18 (1H, d, J=6 Hz, C(7)—NH)	76,6
IIe	$C_{12}H_{13}N_5O_7$	243—6	1,58 (3H, d, J=7 Hz, CH_3); 3,32 (3H, t, CH_3); 3,50 (3H, s, CH_3); 4,5—4,9 (1H, m, CH); 8,9—9,1 (1H, aromat. C(7)—NH)	77,7
IIf	$C_{16}H_{13}N_5O_7$	290—3	3,34 (3H, s, CH_3); 3,44 (3H, s, CH_3), 7,0—8,2 (4H, m, aromat. H)	84,8
IIg	$C_{17}H_{15}N_5O_7$	254—6	3,0 (3H, s, CH_3); 3,38 (3H, s, CH_3); 3,84 (3H, s, CH_3), 7,08—8,00 (4H, m, aromat. H)	80,6
III	$C_{13}H_{15}N_5O_7$	158—9,5	2,72 (2H, t, J=7 Hz, CH_2); 3,28 (3H, s, CH_3); 3,48 (3H, s, CH_3); 3,68 (3H, s, CH_3); 3,7—3,9 (2H, m, CH_2); 9,0 (1H, t, J=5 Hz, C(7)—NH)	93,8
IV	$C_{13}H_{17}N_5O_5$	203—5	2,62 (2H, t, J=6 Hz, CH_2); 3,18 (3H, s, CH_3); 3,4 (3H, s, CH_3); 3,6 (3H, s., CH_3); 3,4—3,8 (2H, m, CH_2); 6,78 (1H, t, J=6 Hz, C(7)—NH)	53,8
V	$C_{12}H_{13}N_5O_4$	317—9	2,8—3,1 (2H, m, CH_2); 3,32 (3H, s, CH); 3,6 (3H, s, CH_3); 3,7—4,0 (2H, m, CH_2)	52,6
VI	$C_{17}H_{17}N_5O_5$	268—70	3,32 (3H, s, CH_3); 3,48 (3H, s., CH_3); 3,81 (3H, s, CH_3); 6,9—8,2 (4H, m, aromat. H)	81,1
VII	$C_{16}H_{13}N_5O_4$	>300	3,32 (3H, s, CH_3); 3,58 (3H, s, CH_3); 6,5—7,9 (4H, m, aromat. H)	87,9
VIII	$C_{13}H_{13}N_5O_6$	246—7	1,8—2,2 (2H, m, CH_2); 2,3—2,6 (2H, m,) CH_2); 3,22 (3H, s, CH_3); 3,44 (3H, s., CH_3); 4,0 (2H, t, J=7 Hz, CH_2)	89,5
IX	$C_{13}H_{15}N_5O_4$	205—6	1,85—2,2 (2H, m, CH_2); 2,3—2,7 (2H, m, CH_2); 3,22 (3H, s, CH_3); 3,38 (3H, s, CH_3); 3,9 (2H, J=7 Hz, CH_2); 6,7—7,2 (2H, broad s, NH_2)	58,2
X	$C_{13}H_{13}N_5O_3$	248—9,5	2,7—3,4 (4H, m, $2CH_2$); 3,41 (3H, s, CH_3); 3,67 (3H, s., CH_3); 4,38 (2H, t, J=6 Hz, CH_2)	59,6
XI	$C_{20}H_{29}N_5O_7$	100—1	0,7—0,9 (3H, m, CH_3); 1,0—1,4 (8H, m., $4CH_2$); 1,4—1,75 (2H, m., CH_2); 1,8—2,15 (2H, m, CH_2); 2,35 (2H, t, J=7 Hz, CH_2); 3,33 (3H, s, CH_3); 3,52 (3H, s, CH_3); 3,5—3,7 (2H, m, CH_2); 4,0 (2H, t, J=7 Hz, CH_2); 8,7—9,0 (1H, broad s C (7)—NH); 14,36 (1H, s, H(8)—H)	74,1

*Compounds IIa, IIf, IIg, and VI crystallized from DMF: IIb, IV, and IX from MeOH; IIc, IId, IIe, and III from ethanol; V from dioxane; VII and X from aqueous DMF: VIII from acetone; XI from aqueous acetone.

**PMR spectra of compounds IIa-c, III, IV, VIII, and IX recorded in DMSO-D6; compounds IId, IIe, V-VII, and X in CF_3COOH; IIf and IIg in CF_3COOD: X in $CDCl_3$.

valine in 3 ml of DMSO was heated at 150-155°C for 0.5 h. The reaction mass was cooled to 25°C and diluted with 20 ml of water. The precipitate was filtered off. Yield 0.49 g of IId.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-α-Alanine (IIe). A mixture of 0.5 g (1.74 mmoles) of compound I and 0.46 g (5.16 mmoles) of α-alanine in 4 ml of DMSO was heated at 140-145°C for 15 min. The reaction mass was cooled to 25°C and diluted with 20 ml of water. The precipitate was filtered off. Yield 0.46 g of IIe.

N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-Anthranolic Acid (IIf). A mixture of 2.88 g (10.05 mmoles) of compound I and 6.86 g (50.02 mmoles) of anthranilic acid in 15 ml of N-ethylpyrrolidone was heated at 100-105°C for 2h. The reaction mass was cooled to 25°c and diluted with 5 ml of acetone. The precipitate was filtered off and washed with 100 ml of acetone. Yield 3.3 g of IIf. Mass spectrum, m/z (%): 387(100) M^+.

Methyl Ester of N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]-pyrimidinyl-7)Anthranilic Acid (IIg). A mixture of 1.4 g (4.88 mmoles) of compound I and 2 ml (21 mmoles) of methyl anthranilate in 7 ml of DMSO was heated at 140-145°C for 20 min. The reaction mass was cooled to 25°C. The precipitate was filtered off and washed with 50 ml of MeOH. Yield 1.58 g of IIg.

Methyl Ester of N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]-pyrimidinyl-7)-β-Alanine (III). To 2 g (5.9 mmoles) of compound IIb in 20 ml (499 mmoles) of MeOH was added dropwise 0.9 ml (12.6 mmoles) of $SOCl_2$. The reaction mass was boiled for 1.5 h and then cooled to 25°C. The precipitate was filtered off and washed with 25 ml of MeOH. Yield 1.95 g of III.

Methyl Ester of N-(1,3-Dimethyl-2,4,5-trioxo-6-amino-1,2,3,4,5,8-hexahydropyrido[2,3-d]-pyrimidinyl-7)-β-Alanine (IV). A sample of 1.95 g of compound III in 100 ml of ethanol was hydrogenated over 1.5 g of 5% PdO/C. When the hydrogen had been absorbed, the reaction mass was filtered and the slurry washed with 50 ml of hot DMAA. The alcohol was evaporated off from the filtrate and the residue was diluted with 100 ml of water. The precipitate was filtered off. Yield 0.96 g of IV.

1,3-Dimethyl-2,4,5,7-tetraoxo-1,2,3,4,5,6,10,11-octahydro[1,4]diazepine(2',3':5,6)-pyrido[2,3-d]pyrimidine (V). To an alcoholic solution of 21.75 mmoles of MeONa prepared from 0.5 g of Na and 50 ml of MeOH was added 1.9 g (5.88 mmoles) of compound IV. The reaction mass was boiled for 7 h and evaporated to dryness. to the residue was added 25 ml of water; the resulting mass was acidified with AcOH to pH 5. The precipitate was filtered off. Yield 0.9 g of V. mass spectrum, m/z (%): 291(100)M^+.

Methyl Ester of N-(1,3-Dimethyl-2,4,5-trioxo-6-amino-1,2,3,4,5,8-hexahydropyrido[2,3-d]-pyrimidinyl-7)Anthranilic Acid (VI). A sample of 2 g (4.98 mmoles) of compound II g in 100 ml of MeOH was hydrogenated over 1.3 g of 5% PdO/C. When the hydrogen had been absorbed, the reaction mass was filtered and the slurry containing spent catalyst and reaction product was boiled in 50 ml of DMF for 5 min and then filtered. The filtrate was diluted with 50 ml of water and cooled to 5°C. The precipitate was filtered off. Yield 1.5 g of VI.

1,3-Dimethyl-2,4,5,7-tetraoxo-1,2,3,4,5,6,7,13-octahydro-12H-[1,4]benzodiazepino(2'3':5,6)pyrido[2,3-d]pyrimidine (VII). To an alcoholic solution of 8.69 mmoles of MeONa prepared from 0.2 g of Na in 40 ml of MeOH was added 1 g (2.69 mmoles) of compound VI. The reaction mass was kept at boiling point for 1.5 h and evaporated to dryness. To the residue was added 50 ml of water. The resultant suspension was heated at 50-55°C for 10 min and acidified with AcOH to pH 5. The precipitate was filtered off. Yield 0.8 g of VII. Mass spectrum, m/z (%): 339(100)M^+.

1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydro-7-(2-oxopyrrolidinyl)pyrido-[2,3-d]pyrimidine (VIII). A solution of 2 g (5.66 mmoles) of compound IIb in 6 ml of $SOCl_2$ was kept at boiling point for 1.5 h and the solvent was evaporated off to dryness. To the residue was added 100 ml of water. The precipitate was filtered off. Yield 1.7 g of VIII. Mass spectrum, m/z (%): 289(100 M^+–NO_2), 290(17), 40(3), 82(13), 55(10), 41(8), 80(7), 69(6), 81(6), 68(5).

1,3-Dimethyl-2,4,5-trioxo-6-amino-1,2,3,4,5,8-hexahydro-7-(2-oxopyrrolidinyl)pyrido-[2,3-d]pyrimidine (IX). A sample of 0.6 g (1.79 mmoles) of compound VIII in 100 ml of MeOH was hydrogenated over 0.5 g of 5% PdO/C. When the hydrogen had been absorbed, the reaction mass was heated to boiling point and filtered. The filtrate was evaporated to dryness. Yield 0.32 g of IX. Mass spectrum, m/z (%): 80(100), 48(51), 235(38), 64(38), 290(30), 234(8), 207(7), 261(7), 82(6), 236(5).

1,3-Dimethyl-2,4,5-trioxo-5H-1,2,3,4,7,8,9,10-octahydropyrrolidino[1",2":1,2]imidazo-(4'5':5,6)pyrido[2,3-d]pyrimidine (X). A suspension of 0.5 g of compound IX in 50 ml of PPA was heated at 120-125°C for 20 min. The reaction mass was cooled to 25°C, diluted with 50 ml of water, and neutralized with aqueous ammonia to pH 7. The precipitate was filtered off. Yield 0.28 g of X. Mass spectrum, m/z (%): 287(100)M^+.

Heptyl Ester of N-(1,3-Dimethyl-2,4,5-trioxo-6-nitro-1,2,3,4,5,8-hexahydropyrido[2,3-d]pyrimidinyl-7)-γ-Aminobutyric Acid (XI). To 0.6 g (1.7 mmoles) of compound IIb in 10 ml (7 mmoles) of heptanol was added dropwise 0.6 ml (8.5 mmoles) of $SOCl_2$. The reaction mass was heated at 100°C for 1 h, then cooled to 25°C. The precipitate was filtered off. Yield 0.57 g of XI. Mass spectrum, m/z (%): 451(8.9)M^+.

EXPERIMETNAL (BIOLOGICAL)

The toxicity of pyrido[2,3-d]pyrimidine derivatives was evaluated after a single intraperitoneal injection into outbred mice (single lethal dose, LD_{100}, mg/kg). It was also assessed by studying the effect that multiple intraperitoneal administrations had on the body mass of test animals as compared to that of animals in the control group (growth coefficient, GC, %) [4].

The antitumor activity of the compounds was tested on outbred female rats which had a Jensen sarcoma transplanted subcutaneously, and on BDF_1 mice which were given a leukemia

P-388 intraperitoneal transplantation. The compounds were injected into the animals 5 days after transplantation of the sarcoma, and 24 h after transplantation in the case of the leukemia P-388.

All the substances were administered by intraperitoneal injection in the form of a finely divided suspension in 10% polyvinylpyrrolidone solution in dosages of 55-150 mg/kg each day (once per day) for 5-7 days. At the end of the experiment (13-14 days after transplantation) all the animals with the Jensen sarcoma were destroyed and weighed. The tumors were excised, weighed and the tumor growth inhibition index (I_I, %) [4] was determined. In the case of the leukemia P-388 the change in the average life span of the treated animals was measured against the control group. The data was processed using the Student-Fisher method, only statistically reliable indices (α between 0.95 and 0.99) being taken into consideration.

A study of the antiblastic activity of compounds IIa, IIc, IIf, IIg, and VIII showed that they are substances of low toxicity (LD_{100} between 250 and 640 mg/kg), which the test animals tolerated well in therapeutic doses administered in multiple injections (GC > 0 in all tests).

It was found that derivative IIa, which contains the glycine group, had no effect on the growth of the Jensen sarcoma (I_I = 12%). Compounds IIb and VIII, which have the ν-aminobutyric acid group or its lactam at C(7), displayed a slight antitumor effect towards the Jensen sarcoma (I_I = 25-27%). However, the introduction of the anthranilic acid group into position 7 of the pyridopyrimidine ring caused compound IIf to show moderate antiblastic activity (I_I = 58%). Moving from the acid IIf to the ester IIg brought with it a reduction in activity (I_I = 25%).

None of the compounds investigated had any effect on the development of the leukemia P-388.

LITERATURE CITED

1. O. A. Burova, I. D. Bystryakova, N. M. Smirnova, and T. S. Safonova, Khim. Geterotsikl. Soedin., No. 5, 662-666 (1990).
2. O. A. Burova, I. D. Bystryakova, N. M. Smirnova, and T. S. Safonova, Khim. Geterotsikl. Soedin., No. 4, 497-501 (1991).
3. N. M. Smirnova, N. M. Cherdantseva, O. A. Burova, et al., Khim. Geterotsikl. Soedin., No. 7, 971-975 (1990).
4. V. A. Chernov, Methods of Experimental Tumor Chemotherapy [in Russian], Moscow (1971), p. 357.