SEARCH FOR NEW DRUGS

SYNTHESIS AND NEUROTROPIC ACTIVITY OF NEW PYRANO[4′,3′:4,5]THIENO[2,3:4″,5″]PYRIMIDO[2,3-c]-(1,2,4)TRIAZINES

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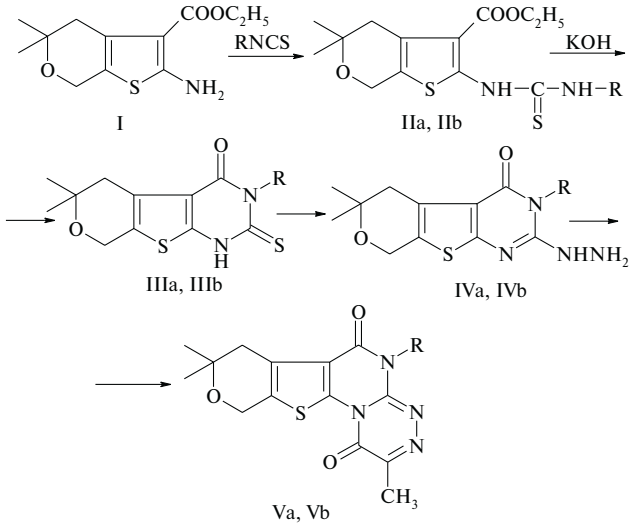
Previously we reported that some derivatives of condensed thienopyrimidines possess neurotropic properties [1, 2]. To our knowledge, the synthesis and biological properties of pyranothienopyrimidino(1,2,4)triazines were not described so far. We have developed a method for the synthesis of new thienopyrimidinotriazines, which are condensed with a tetrahydropyran ring, and studied their anticonvulsant activity.

In the first stage, we used the reactions of 2-aminothiophene (I) [1] with isothiocyanates to obtain thioureido derivatives IIa and IIb. These products exhibit cyclization in the presence of potassium hydroxide with the formation of the corresponding 2-thio-4-oxothienopyrimidines (IIIa, IIIb). Boiled with hydrazine hydrate, compounds IIIa and IIIb yielded hydrazino derivatives IVa and IVb.



II – V: R = CH_3 (a), CH_2CH_2 (b).

Finally, the condensation of compounds IVa and IVb with pyruvic acid in a mixture of acetic acid and ethyl alcohol led to the formation of tetracyclic compounds (Va, Vb) containing the pyrimido[2,3-c]-(1,2,4)triazine fragment.

EXPERIMENTAL PHARMACOLOGICAL PART

The anticonvulsant activity of compounds Va and Vb was studied on rats with model convulsions induced by corazole (90 mg/kg, s.c.) and characterized by the effective dose (ED_{50}) preventing the clonic convulsion component in 50% of test animals [3].

The tranquilizer effect was studied using the "conflict situation" test. The model conflict between drinking and defense motivations was created in rats with preliminarily developed drinking reflex. During an attempt at taking water the animal received electric irritation and, hence, could not satisfy the dominating need in drinking. The conflict of two motivations was manifested by anxiety and emotional stress. The experimental setup provided automated determination of the amount of water taken (despite the electric irritation), the number of approaches to the drinking bowl, and the motor activity. The anxiolytic activity of the synthesized compounds was characterized by an increase in the number of water takes [4].

The effect of the synthesized compounds upon the mnemonic functions was studied in rats with a passive avoidance conditioned reflex (PACR) developed as described in [5] using electric shock as the amnesic factor. During the test, the duration of stay in the dark and light compartments of the experimental setup was measured over a time period of 3 min. Then the PACR was developed (learned) by a single electric irritation (via the electrode floor) of an animal visiting the dark compartment. The memory trace was erased by a single electroshock applied immediately after learning. A test for PACR reproduction was performed 24 h after learning. An

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TABLE 1. Neurotropic Activity of Compounds Va and Vb

Compound	Anticorazole activity: ED_{50}, mg/kg	Anxiolytic activity (number of water takes)	Antiamnesic effect (time of stay in light compartment, sec)	
			1st day	2nd day
Control	–	3.4 (2.3 – 4.5)	2.6 (2.3 – 3.0)	2.4 (2.0 – 2.3)
Compound Va	70 (28 – 175)	12 (6.7 – 17.3)	2.7 (2.3 – 3.1)	72.1 (28.7 – 115.4)
Control	–	3.4 (2.3 – 4.5)	2.6 (2.2 – 3.0)	2.4 (2.0 – 2.7)
Compound Vb	200	3.3 (2.4 – 4.3)	2.4 (1.9 – 2.9)	2.6 (1.3 – 3.9)
Control	–	1.6 (1.1 – 2.1)	4.5 (3.0 – 6.0)	5.0 (2.9 – 7.0)
Phenazepam	0.037 (0.026 – 0.052)	7.3 (5.1 – 9.1)	5.5 (4.1 – 6.9)	2.3 (0.6 – 4.0)
Control	–	1.6 (1.1 – 2.1)	4.5 (3.0 – 6.0)	5.0 (2.9 – 7.0)
Pyracetam	n/a	2.7 (2.0 – 3.4)	33.6 (22.7 – 44.5)	133.2 (86.2 – 180.2)

Notes: Data for anxiolytic effect of compounds Va, Vb, phenazepam, and pyracetam are given for doses 25, 25, 0.5, and 1000 mg/kg, respectively; for antiamnesic affect, 25, 25, 1, and 1450 mg/kg, respectively; values in parentheses indicate the confidence interval for $P = 0.05$.

increase in the duration of stay in the light compartment (relative to control) on the next day was evidence that the drug possesses antiamnesic properties.

The substances to be tested were introduced by intraperitoneal injections in the form of suspensions with Tween-80 in a dose of 10, 25, 50, 100, 200, 1000, or 2000 mg/kg. The injections were made 45 min before the administration of the convulsant (corazole) or the test for tranquilizer (anxiolytic) activity, neurotoxicity, and antiamnesic activity. Animals in the control group were injected with pure emulsifier. The reference drugs were phenazepam and pyracetam [6 – 8].

The study of the anticonvulsant activity showed that compound Va (beginning with a dose of 25 mg/kg) is capable of preventing corazole convulsions (see Table 1). Compound Vb produced a comparable effect in a dose of 200 mg/kg. In the "conflict situation" test, injections of compound Va (beginning with 10 mg/kg) led to a significant increase in the number of water takes, which is evidence of a tranquilizer activity. Under the same conditions, phenazepam was effective in a dose of 0.5 mg/kg, while compound Vb (25 mg/kg) and pyracetam (1000 mg/kg) were ineffective. In the model of electroshock amnesia, compound Va (25 mg/kg) produced a more than 30-fold increase in the time of PACR reproduction, which was comparable with the antiamnesic effect of pyracetam in a dose of 1450 mg/kg (see Table 1). Administered under the same conditions, phenazepam (up to 1 mg/kg) and compound Vb did not significantly change the behavior of test animals.

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were measured on a UR-20 spectrophotometer (Germany) using samples prepared as nujol mulls. The IR spectra of the synthesized compounds exhibit characteristic absorption bands in the regions of 1665 – 1670 cm^{-1} (amide C=O bonds) and 3100 – 3400 (NH, NH_2).

The ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer using TMS as the internal standard. The mass spectra were obtained with an MX-1320 spectrometer equipped with a system of direct sample introduction into the ion source. TLC was performed on Silufol UV-254 plates; the spots were visualized by exposure to iodine vapor. The data of elemental analyses agree with the results of calculations performed using the empirical formulas.

2-(N′-Methylthioureido)-3-ethoxycarbonyl-5,5-dimethyl-4,5-dihydro-7H-thieno[2,3-c]pyran (IIa). A mixture of 2.55 g (0.01 mole) of compound I and 0.73 g (0.01 mole) of methyl isocyanate in 50 ml of butanol was boiled for 10 h and allowed to stand overnight. The precipitated crystals were separated by filtration and washed with diethyl ether to obtain 2.14 g (65.4%) of compound IIa; m.p., 194 – 195°C; R_f, 0.67 (CCl_4 – acetone, 2 : 1); $C_{14}H_{20}N_2O_3S_2$.

^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 12.06 (s, 1H, NH), 6.90 (s, 1H, NH–CH_3), 4.65 (t, 2H, 7-CH_2), 4.52 (q, O$CH_2$$CH_3$), 3.06 (d, NH–$CH_3$), 2.96 (t, 2H, 4-$CH_2$), 2.72 (t, 3H, O$CH_2$$CH_3$), 1.32 (s, 6H, $C(CH_3)_2$).

2-(N′-Benzylthioureido)-3-ethoxycarbonyl-5,5-dimethyl-4,5-dihydro-7H-thieno[2,3-c]pyran (IIb). Compound IIb was obtained by a procedure analogous to that described above, proceeding from 2.55 g (0.01 mole) of compound I and 1.5 g (0.01 mole) of benzyl isothiocyanate; yield, 1.7 g (42.1%); m.p., 148 – 149°C; R_f, 0.72 (chloroform – benzene – acetone, 4 : 4 : 1); $C_{20}H_{24}N_2O_3S_2$.

^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 11.90 (s, 1H, NH), 7.17 (s, 5H, C_6H_5), 6.87 (m, 1H, NH–CH_2–C_6H_5), 4.60 (d, 2H, $CH_2C_6H_5$), 4.53 (t, 2H, 7-CH_2), 4.13 (q, O$CH_2$$CH_3$), 2.70 (t, 3H, O$CH_2$$CH_3$), 1.20 (s, 6H, $C(CH_3)_2$).

3,6,6-Trimethyl-4-oxo-2-thio-5,6-dihydro-8H-pyrano[4′,3′:4,5]thieno[2,3-d]pyrimidine (IIIa). A mixture of 3.28 g (0.01 mole) of compound IIa and 1.12 g (0.02 mole) of potassium hydroxide in 50 ml of 50% aqueous ethanol

was boiled for 2 h. Upon cooling, the reaction mixture was acidified with a 10% aqueous hydrochloric acid solution to obtain a weak acid reaction. The precipitated crystals were separated by filtration, washed with water, and dried to obtain 2.70 g (95.8%) of compound IIIa; m.p., 273 – 275°C (ethanol); R_f, 0.71 (acetone – carbon tetrachloride, 2 : 1); $C_{12}H_{14}N_2O_2S_2$.

^{1}H NMR spectrum in C_5D_5N (δ, ppm): 10.2 (s, 1H, NH), 4.70 (t, 2H, 8-CH_2), 3.46 (s, 3H, 3-N–CH_3), 3.10 (s, 2H, 5-$\underline{CH_2}$), 1.30 (s, 6H, $C(CH_3)_2$).

3-Benzyl-4-oxo-2-thio-5,6-dihydro-8H-pyrano[4′,3′:4,5]thieno[2,3-d]pyrimidine (IIIb). Compound IIIb was obtained by a procedure analogous to that described above, proceeding from 4.04 g (0.01 mole) of compound IIb; yield, 3.23 g (90.3%); m.p., 252 – 253°C (ethanol); R_f, 0.66 (chloroform – benzene – acetone, 2 : 2 : 1); $C_{18}H_{18}N_2O_2S_2$.

^{1}H NMR spectrum in C_5D_5N (δ, ppm): 10.76 (s, 1H, NH), 8.0 – 7.05, (s, 5H, C_6H_5), 6.0 (s, 2H, $\underline{CH_2}C_6H_5$), 4.73 (t, 2H, 8-CH_2), 2.98 (s, 2H, 5-CH_2), 1.27 (s, 6H, $C(CH_3)_2$).

2-Hydrazino-3,6,6-trimethyl-4-oxo-5,6-dihydro-8H-pyrano[4′,3′:4,5]thieno[2,3-d]pyrimidine (IVa). A mixture of 2.82 g (0.01 mole) of compound IIIa and 5 ml of hydrazine hydrate in 25 ml of butanol was boiled for 8 h and allowed to stand overnight. Upon cooling, the precipitated crystals were separated by filtration, washed with water, and dried to obtain 2.0 g (72.5%) of compound IVa; m.p., 252 – 254°C (pyridine); R_f, 0.56 (hexane – ethyl acetate, 1 : 2); $C_{12}H_{16}N_4O_2S$.

^{1}H NMR spectrum in C_5D_5N (δ, ppm): 5.70 (s, 1H, $\underline{NH}NH_2$), 4.63 (bs, 4H, NH–$\underline{NH_2}$, 8-CH_2), 3.40 (s, N=CH_3), 3.15 (t, 2H, 5-CH_2), 1.28 (s, 6H, $C(CH_3)_2$).

3-Benzyl-2-hydrazino-6,6-dimethyl-4-oxo-5,6-dihydro-8H-pyrano[4′,3′:4,5]thieno[2,3-d]pyrimidine (IVb).
Compound IVb was obtained by a procedure analogous to that described above, proceeding from 3.58 g (0.01 mole) of compound IIIb; yield, 2.3 g (64.5%); m.p., 209 – 210°C (pyridine); R_f, 0.62 (chloroform – benzene – acetone, 2 : 1 : 1); $C_{18}H_{20}N_4O_2S$.

^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 5.60 (s, 1H, $\underline{CH_2}C_6H_5$), 5.68 (s, 1H, $\underline{NH}NH_2$), 4.58 (bs, 4H, $NH\underline{NH_2}$, 8-CH_2), 2.97 (s, 2H, 5-CH_2), 1.23 (s, 6H, $C(CH_3)_2$).

2,5,8,8-Tetramethyl-2,6-dioxo-7,8-dihydro-10H-pyrano[4′,3′:4,5]thieno[2,3:4″,5″]pyrimido[2,3-c]-(1,2,4)triazine (Va). A mixture of 2.8 g (0.01 mole) of compound IVa and 0.88 g (0.01 mole) of pyruvic acid in 50 ml of ethanol was boiled for 10 h. Upon cooling, the precipitated crystals were separated by filtration, washed with water, and dried to obtain 2.0 g (62.3%) of compound Va; m.p., 264 – 266°C (DMSO); R_f, 0.60 (ethanol – chloroform, 1 : 2); $C_{15}H_{16}N_4O_3S$.

^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 4.82 (s, 2H, 10-CH_2), 3.48 (s, 3H, 5-N–CH_3), 3.02 (t, 2H, 7-CH_2), 2.54 (s, 3H, –N=C–CH_3), 1.28 (s, 6H, $C(CH_3)_2$); mass spectrum, m/z (I_{rel}, %): M^+ 332(53), 303(19), 274(89), 246(100), 177(18).

5-Benzyl-2,8,8-trimethyl-1,6-dioxo-7,8-dihydro-10H-pyrano[4′,3′:4,5]thieno[2,3:4″,5″]pyrimido[2,3-c]-(1,2,4)-triazine (Vb). Compound Vb was obtained by a procedure analogous to that described above, proceeding from 3.42 g (0.01 mole) of compound IVb; yield, 2.6 g (65.0%); m.p., 230 – 231°C (DMSO); R_f, 0.58 (chloroform–benzene–acetone, 2 : 1 : 1); $C_{21}H_{20}N_4O_3S$.

^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.64 (bs, 5H, $\underline{C_6H_5}$), 5.60 (s, 2H, $\underline{CH_2}$–C_6H_5), 4.80 (t, 2H, 10-CH_2), 3.00 (t, 2H, 7-CH_2), 2.57 (s, 3H, –N=C–$\underline{CH_3}$), 1.32 (s, 6H, $C(CH_3)_2$).

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