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SYNTHESIS AND NEUROTROPIC PROPERTIES OF N-ACYLPHENOTHIAZINES

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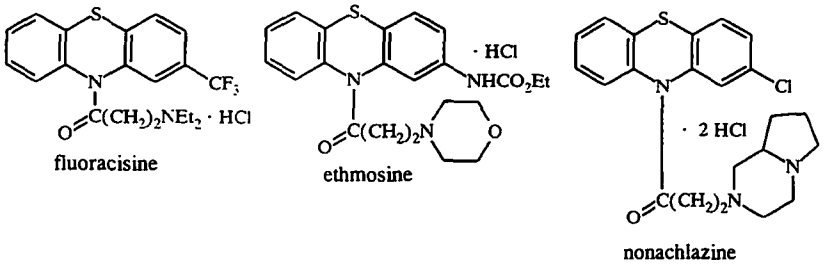
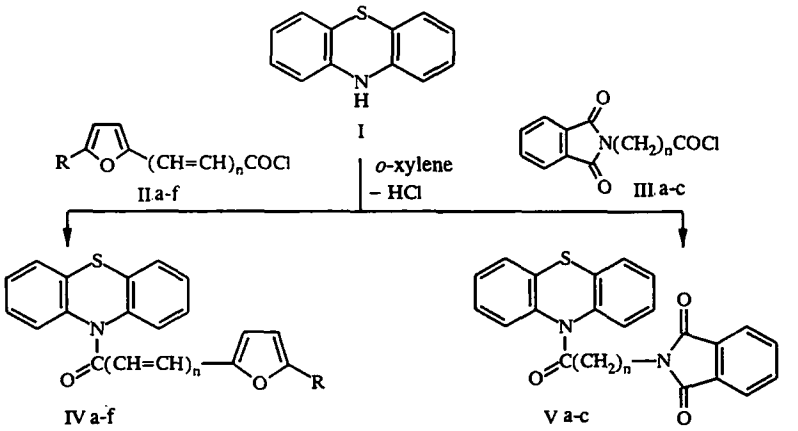
N-Acylphenothiazines (derivatives of furan and phthalylamino acids) were synthesized, and their neurotropic properties were studied. It was established that the derivatives of phthalyl-γ-aminobutyric acid increase the duration of hexenal narcosis (by 1.7 times) and increase the stimulant activity of phenamine (by 2.1 times). The glycine derivative prolongs the life of animals under the conditions of hypoxia by 77%.

Derivatives of phenothiazine have found widespread use in medical practice. Some of them are neuroleptic products. However, antidepressants (fluoracisine), coronary dilating products (nonachlazine), antiarrhythmic agents (ethmosine), and antiemetic agents have also been created on the basis of phenothiazines [1].



In order to study the psychotropic properties of new derivatives of phenothiazine [2] we synthesized the amides of furan (IVa-f) and phthalylamino acids (Va-c) by the reaction of phenothiazine (I) with the chlorides of the respective acids (IIa-f, IIIa-c).



II, IV a n = 0, R = H; b n = 0, R = Br; c n = 0, R = NO_2; d n = 1, R = H; e n = 1, R = Br; f n = 1, R = NO_2; III, V a n = 1; b n = 2; c n = 3

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TABLE 1. Characteristics of N-Acylphenothiazines (IVa-f, Va-c, VIII)

Compound	Molecular formula	mp, °C*	R_f†	Yield, %
IVa	$C_{17}H_{11}NO_2S$	146...148	0,43	77
IVb	$C_{17}H_{10}BrNO_2S$	191...193	0,58	70
IVc	$C_{17}H_{10}N_2O_4S$	194...197	0,34	78
IVd	$C_{19}H_{13}NO_2S$	149...151	0,43	67
IVe	$C_{19}H_{12}BrNO_2S$	159...160	0,49	73
IVf	$C_{19}H_{12}N_2O_4S$	265...267	0,17	78
Va	$C_{22}H_{14}N_2O_3S$	232...236	0,19	79
Vb	$C_{23}H_{16}N_2O_3S$	266...269	0,13	70
Vc	$C_{24}H_{18}N_2O_3S$	185...188	0,14	84
VIII	$C_{20}H_{16}BrN_3O_2S$	259...261		75

*Compounds (IVa-f, Va-c) were purified from *o*-xylene.

†Hexane—ether, 1:1.

TABLE 2. PMR Spectra of Compounds (IVa-f, Va-c) in DMSO-d_6

Compound	Chemical shifts, δ, ppm (SSCC, *J*, Hz)
IVa	7,71 (1H, dd *J* = 1,8 and 0.9, furan $H_{(5)}$); 7,7...7,2 (8H, m, phenothiazine; 6,49 (1H, dd *J* = 3,6 and 1,8, furan $H_{(4)}$); 6,30 (1H, dd, *J* = 3,6 and 0,9, furan $H_{(3)}$)
IVb	7,67...7,25 (8H, m, phenothiazine; 6,24 and 6,60 (2H, doublets *J* = 3,6, furan $H_{(3,4)}$)
IVc	7,77...7,25 (9H, m, phenothiazine + furan $H_{(4)}$); 6,46 (1H, d, *J* = 4,0, furan $H_{(3)}$)
IVd	7,74...7,18 (10H, m, phenothiazine H + furan $H_{(5)}$ + =CH-); 6,88 (1H, d, *J* = 3,3, furan $H_{(3)}$); 6,55 (1H, dd *J* = 3,3 and 1,8, furan $H_{(4)}$); 6,48 (1H, d, *J* = 15,3, -CH=)
IVe	7,73...7,22 (8H, m, phenothiazine 7,22 (1H, d, *J* = 15,6, =CH-); 6,93 (1H, d, *J* = 3,0, furan $H_{(3)}$); 6,71 (1H, d, *J* = 3,0, furan $H_{(4)}$); 6,45 (1H, d, *J* = 15,6, -CH=)
IVf	7,73...7,34 (10H, m, phenothiazine H + furan $H_{(4)}$ + -CH=); 7,20 (1H, d, *J* = 3,9, furan $H_{(3)}$); 6,77 (1H, d, *J* = 15,4, -CH=)
Va	7,84 (4H, s, phenothiazine; 7,84...7,24 (8H, m, phenothiazine; 4,50 (2H, s, CH_2)
Vb	7,82 (4H, s, phenothiazine; 7,62...7,25 (8H, m, phenothiazine; 3,75 (2H, t, *J* = 7,5, CH_2); 2,86 (2H, t, *J* = 7,5, CH_2)
Vc	7,81 (4H, s, phenothiazine; 7,58...7,07 (8H, m, phenothiazine; 3,51 (2H, t, *J* = 6,6, CH_2); 2,48 (2H, t, *J* = 6,6, CH_2); 1,80 (2H, q, *J* = 6,6, CH_2)
VIII	12,5 (1H, s, NOH); 8,96 и 8,22 (2H, doublets *J* = 6,4, pyridine H); 8,37 (1H, s, -CH=); 7,87...7,26 (8H, m, phthalimide); 5,8 (2H, b s CH_2)

1-[2′-(10-Phenothiazinyl)-2′-oxoethyl]-4-hydroxyiminomethylpyridinium bromide (VIII) was obtained by the reaction of N-bromomethylcarbonylphenothiazine (VI) with 4-pyridinaldoxime (VII) in acetonitrile.

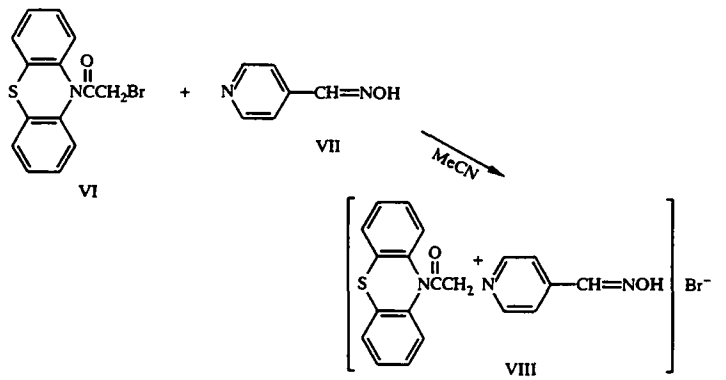

TABLE 3. Neurotropic Activity of Derivatives of Phenothiazine ($M + m$), % of Control (100%)

Compound	LD_{50}, mg/kg	Test					
		Hypoxia	Phenamine hyperactivity	Hexenal narcosis	Ethanol narcosis	Corazol convulsions, ml/sec	CRPA†/ RA‡, %
IVa	4470 (3130...5960)	90,4	35,0*	90,4	118,6	140,3*/132,1	92,5*/83
IVb	>5000	100,0	146,0*	151,5*	98,6	83,9/74,1	19,1/50
IVc	>5000	134,4*	245,8*	116,3*	123,6	146,0*/127,3	63,3*/67
IVd	>5000	104,0	135,5*	126,0*	192,5*	142,5*/133,3	54,1*/33
IVe	4470 (3130...5960)	114,3	130,7	148,3*	114,3	125,3*/132,3	70,8*/83
IVf	7080 (5010...9250)	130,4*	61,4*	164,7*	184,8*	117,3/90,4	50,8/50
Va	1410 (650...2090)	176,9*	56,5*	145,8*	155,4*	82,6/107,9	35,8/50
Vb	8900 (5600...12900	140,5*	131,4*	155,0*	82,7	85,9/113,7	1,9/17
Vc	2050 (1468...2880)	135,0*	210,0*	174,4*	84,7	122,5/176,8*	42,5/83
VIII	355 (249...461)	121,3	37,3*	127,0*	115,5	125,3/243,9*	48/50

*The differences in relation to the control are statistically reliable at $P < 0.05$.
†Conditional reflex of passive avoidance, sec.
‡Retrograde amnesia, %.

With the exception of compound (VIII) (LD_{50} 355 mg/kg) the investigated phenothiazine derivatives are low-toxicity substances, the LD_{50} values of which exceed 1000 mg/kg. In the series of phenothiazinylamides of phthalylamino acids (Va-c) the toxicity depends on the length of the hydrocarbon chain and amounts to 1410 mg/kg for n = 1, 2050 mg/kg for n = 3, and 8900 for n = 2. In the series of derivatives of furan acids LD_{50} is about 5000 mg/kg and higher. Therefore, if clearly defined neurotropic activity is detected the therapeutic index can be fairly high.

Investigations of the neurotropic activity by the "rotating rod," "tube," and "pull up on a cross-bar (trabecula)" method show that only the phenothiazinylamides of 3-(5-nitro-2-furyl)acrylic acid (IVf) and phthalylglycine (Va) exhibit small stimulating (depriming) activity (ED_{50} 69-178 mg/kg). The other investigated N-acylphenothiazines do not possess this activity ($ED_{50} > 500$ mg/kg).

The synthesized compounds exhibit hypothermal activity in approximately the same doses as stimulating (depriming) activity. None of the investigated compounds has analgesic properties.

The amides of 5-nitro-2-furancarboxylic (IVc) and 3-(5-nitro-2-furyl)acrylic (IVf) acids and also the derivatives of glycine (Va), alanine (Vb), and γ-aminobutyric acid (Vc) exhibit antihypoxia activity; the derivatives of glycine prolong the life of animals under the conditions of hypoxia by 77%.

It was established that N-acylphenothiazines prolong hexenal narcosis. The bromine derivatives (IVb, e) are the most active in the series of furan acids, while the derivative of nitrofurylacrylic (IVf) is even more active. In the derivatives of phthalylamino acids the length of the carbon chain plays an important role. The duration of hexenal narcosis increases in the series n = 1 < n = 2 < n = 3 by 1.45, 1.55, and 1.74 times for (Va-b) respectively.

The duration of ethanol narcosis is increased in the presence of the derivatives of furylacrylic acids by 1.92 times for (IVd) and 1.84 times for (IVf) and also in the presence of the phenothiazide of phthalylglycine (Va). However, it is hardly changed at all by the action of furancarboxylic acids (IVa-c).

The anticonvulsive properties of the investigated phenothiazine amides during corazol convulsions are poorly defined. Thus, the clonic phase is reduced by compounds (IVa, c, d), and a lethal outcome is delayed by compounds (Vc) and, particularly, by compound (VIII). None of the phenothiazine amides prevent convulsions due to maximal electric shock.

The stimulating (depriming) activity of the substances is also demonstrated by their antagonistic action on the pharmacological effects of phenamine. The behavior of phenothiazines in this test depends on the type of acid residue. Thus, the phenothiazine amides of furancarboxylic (IVa), nitrofurancarboxylic (IVe), and phthalaminoacetic (Va) acids antagonize

the stimulant action of phenamine. The derivatives of bromofurylacrylic (IVb) and nitrofurylacrylic (IVc), on the other hand, intensify the stimulating activity of phenamine by 1.46 and 2.46 times. Among the derivatives of furylacrylic acid the weak intensifying activity is revealed by compounds (IVd, e). The phenothiazine amides of phthalylaminopropionic (Vb) and phthalylaminobutyric (Vc) acids intensify the phenamine locomotor activity of animals.

Thus, the derivative of γ-aminobutyric acid (Vc) exhibits high activity in three tests; it increases the duration of hexenal narcosis by 1.74 times, intensifies the stimulating activity of phenamine by 2.1 times, and delays lethal outcome in corazol convulsions.

Compounds having components with activating action (increasing the stimulating effects of phenamine and corazol, reducing the duration of hexenal and ethanol narcosis, and exhibiting antihypoxia activity) in the spectrum of pharmacological activity facilitated the healing of experimental animals and reduced or prevented retrograde amnesia due to electric shock (IVa, c, e).

EXPERIMENTAL

The course of the reactions and also the purity of the products were monitored by TLC on Silufol UV-254 plates in the 1:1 ether—hexane system. The elemental analyses for C, H, and N agreed with calculated data.

General Procedure for the Production of Compounds (IVa-f) and (Va-c). A mixture of compounds (IIa-f) or (IIIa-c) and phenothiazine in molar ratios of 1.1-1.2:1 was boiled in *o*-xylene for 4-6 h. After cooling and holding for 12 h the mixture was filtered, and the precipitate was washed with *o*-xylene. The product was recrystallized from *o*-xylene with the addition of activated carbon of grade B. The yields of the products (IVa-f, Va-c), the melting points, and the R_f values are given in Table 1.

1-[2'-(10-Phenothiazinyl)-2'-oxoethyl]-4-hydroxyiminomethylpyridinium Bromide (VIII). A mixture of 3.2 g (0.01 mole) of N-bromomethylcarbonylphenothiazine and 1.2 g (0.01 mole) of 4-pyridinaldoxime in acetonitrile was boiled for 4 h, cooled, and filtered. The product was recrystallized from ethanol with the addition of activated carbon of grade B. We obtained 3.3 g (75%) of the product; mp 259-261°C.

Neurotropic Activity. The neurotropic activity was studied on bisexual mice of the BALB/c strain and of the ICR strain with a mass of 17-23 g in the winter—spring season. The temperature in the laboratory situation and in the vivarium was kept in the range of 22 ± 1.5°C. The investigated substances in the form of suspensions, prepared with Tween 80, were administered intraperitoneally 1 h before the test was set up. A comparative assessment of the activity of the substance on hypoxia, hexenal narcosis, phenamine hyperactivity, corazol convulsions, and the memory process was made on groups of animals, consisting of 6-8 individuals, with administration of the phenothiazine derivatives at a dose of 5 mg/kg. The control animals were injected in the abdominal cavity with the same volume of distilled water.

Action of the Substances on the Central Nervous System. The action of the substances on the central nervous system was assessed by the following tests:

1) By the effect on the coordination of movements and the muscle tone using the "rotating rod" method on Ugo Basile apparatus (Italy) at a frequency of 8 rpm for 2 min, the "tube" test (a 30 × 2-cm glass tube for 30 sec), and the "pull-up on cross-bar" test (a metal wire, 2 mm in diameter, for 5 sec).

2) By the effect on the body temperature, measured in the rectum by means of an electrothermometer; the test criterion was a decrease of the rectal temperature by 3°C or more.

3) By the analgesic effect, determined by the "hot plate" method on Ugo Basile apparatus.

4) By the anticonvulsive activity, investigated by the maximum electric shock test (alternating current, 50 mA, frequency 50 pulses/sec with 0.2 sec stimulation) and by corazol convulsions, brought about by intravenous titration with a 1% solution of corazol at a rate of 0.01 ml/sec.

5) By the effect on hexenal narcosis (a 0.4% solution of hexenal intravenously at a dose of 70 mg/kg).

6) By the effect on the life duration of the animals under the conditions of hypoxic hypoxia, caused by placing the animals (singly) in an airtight chamber with a volume of 220 cm^3 without the absorption of CO_2.

7) By the change in the degree of phenamine hyperactivity (a 0.4% solution of phenamine subcutaneously at a dose of 10 mg/kg).

8) By the effect on the learning process and retrograde amnesia, caused by electric shock. The acute toxicity was also determined, and the mean lethal doses (LD_{50}) were established.

The experimental data were processed statistically. (The average LD_{50} and ED_{50} values from 10-20 observations were established using the express method.) The arithmetical mean values and their standard error ($M \pm m$) were calculated in order to assess the average duration of the narcotic action of hexenal, the protective properties during corazol convulsions, hypoxia, and the degree of phenamine hyperactivity. The Student test was used to determine the significance of the difference between the mean values. The differences were considered reliable at probability level $P \leq 0.05$.

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