SYNTHESIS AND PSYCHOTROPIC ACTIVITY OF TRIAZOLES AND TETRAZOLES CONDENSED WITH SPYRO(BENZO[H]QUINAZOLINE-5,1′-CYCLOALKANES)

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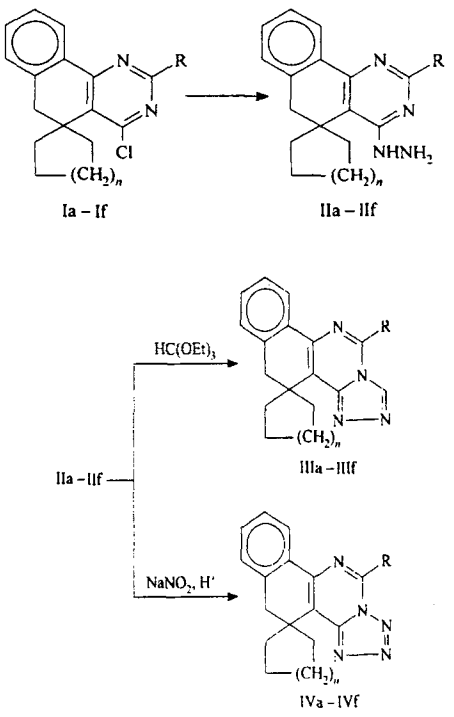
Atypical anxiolytics (derivatives of triazolodiazepine and -quinazoline) recently came into use in medical practice, extending the class of tranquilizers. However, these drugs, like all benzodiazepine tranquilizers, exhibit certain side effects such as myorelaxation, negative action upon the cognitive function and memory, sleep perturbation, development of drug dependence, and teratogenic manifestations [1 – 6]. At the same time, anxiolytic doses of buspirone, which is an atypical tranquilizer of the nonbenzodiazepine series, lead to virtually no undesired side effects typical of benzodiazepines. However, this drug is toxic and insufficiently active as a tranquilizer [1, 2, 4].

The purpose of this work was to synthesize triazolo and tetrazolo derivatives of benzo[h]quinazoline, spyro-conjugated to carbocycles (cyclopentane or cyclohexane), and study their neurotropic properties using a new method developed for the assessment of tranquilizer activity.

We have synthesized 2-substituted 4-hydrazino-5,6-dihydrospyro(benzo[h]quinazoline-5,1′-cycloalcanes) (IIa – IIf) by condensation of the corresponding 4-chloro derivatives (Ia – If) with hydrazine hydrate. Note that the reaction could be performed only using a 99 – 100% hydrazine hydrate, whereas a higher water content leads to hydrolysis of chlorine-containing compounds (Ia – If) with the formation of 4-oxo derivatives of benzo[h]quinazoline.

The IR spectra of compounds IIa – IIf show absorption bands attributed to hydrazine groups in the region of 3150 – 3350 cm^{-1}. The ^1H NMR spectra of these compounds contain signals due to protons of the hydrazine group, represented by broad lines with a 3H intensity in the region of 3.5 – 6.0 ppm.

The condensation of hydrazines IIa – IIf with an orthoformic acid ether leads to the formation of 2-substituted 7,8-dihydrospyro(benzo[h]triazolo[4, 3-c]quinazoline-7,1′-cycloalkanes) (IIIa – IIIf). Reactions of the same hydrazides with an aqueous solution of sodium nitrite under acidic conditions yields 2-substituted 7,8-dihydrospyro(benzo[h]tetrazolo[4, 5-c]quinazoline-7,1′-cycloalkanes) (IVa – IVf).



I – IV	a	b	c	d	e	f
R	Et	Ph	CH_2Ph	Et	Ph	CH_2Ph
n	1	1	1	2	2	2

The IR absorption spectra of compounds IIIa – IIIf display characteristic bands due to aromatic and triazole rings in the regions of 1580 – 1600 and 1610 – 1640 cm^{-1}, respectively. The ^1H NMR spectra of IIIa – IIIf contain singlet signals of the methine proton with a 1H intensity in the region of 8.78 – 9.03 ppm. The IR spectra of compounds IVa – IVf contain characteristic absorption bands of aromatic and

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triazole rings in the regions of 1590 – 1595 and 1610 – 1630 cm^{-1}, respectively, and show no bands in the region of 2200 cm^{-1} that would be indicative of the azide group.

EXPERIMENTAL CHEMICAL PART

The IR spectra were measured on an UR-20 spectrophotometer (Germany) using samples prepared as nujol mulls. The ^{1}N NMR spectra were obtained on a Varian T-60 spectrometer operated at 60 MHz, using appropriate deuterated solvents and TMS or HMDS as internal standards. The mass spectra were recorded on a MX-1320 spectrometer (Russia) equipped with a system of direct sample injection into the ion source. The purity of compounds was checked by TLC on Silufol UV-254 plates developed with iodine vapors. The melting temperatures were determined using a heating stage of the Boethius type.

2-Substituted 4-hydrazino-5,6-dihydrospyro(benzo[h]quinazoline-5,1′-cycloalkanes) (IIa – IIf). A mixture of 0.01 mole chloroquinazoline Ia – If [7, 8] and 5 ml hydrazine hydrate was boiled with reflux for 7 h. Upon cooling, the precipitate was separated by filtering, thoroughly washed with water, and recrystallized from a water – isopropanol (2 : 1) mixture (see Table 1).

2-Substituted 7,8-dihydrospyro(benzo[h]triazolo[4,3-c]-quinazoline-7,1′-cycloalkanes) (IIIa – IIIf). A mixture of 0.01 mole hydrazinoquinazoline IIa – IIf and 10 ml of triethyl ether of orthoformic acid was boiled with reflux for 15 h. Upon cooling, the precipitate was separated by filtering and recrystallized from butanol. The yields and characteristics of triazoloquinazolines IIIa – IIIf are given in Table 2.

2-Substituted 7,8-dihydrospyro(benzo[h]tetrazolo-[4,5-c]quinazoline-7,1′-cycloalkanes) (IVa – IVf). To a mixture of 0.01 mole hydrazinoquinazoline IIa – IIf in 50 ml acetic acid was added on stirring at room temperature 0.9 g (0.013 mole) of sodium nitrite. After stirring for 3 h, the precipitate was separated by filtering, washed with water, and and recrystallized from butanol. The yields and charac-

TABLE 1. Yields and Characteristics of Compounds IIa – IIf

Compound	Yield, %	M.p., °C	R_f*	IR spectrum ν_{NHNH_2}, cm^{-1}	Empirical formula
IIa	68	81 – 83	0.54	3265	$C_{18}H_{22}N_4$
IIb	51	195 – 197	0.63	3290	$C_{22}H_{22}N_4$
IIc	88	95 – 97	0.62	3300	$C_{23}H_{24}N_4$
IId	75	187 – 189	0.52	3290	$C_{19}H_{24}N_4$
IIe	49	197 – 199	0.63	3270	$C_{23}H_{24}N_4$
IIf	75	159 – 161	0.60	3310	$C_{24}H_{26}N_4$

* TLC in a nonane – ethyl acetate (1 : 1) system.

TABLE 2. Yields and Characteristics of Triazoloquinazolines IIIa – IIIf

Compound	Yield, %	M.p., °C	R_f*	IR spectrum ($\nu_{C=N}$), cm^{-1}	^{1}H NMR spectrum ($CDCl_3$, $\delta_{4\text{-}CH}$), ppm	Mass spectrum, m / z (%)	Empirical formula
IIIa	79	161 – 163	0.48	1630	8.86s	304 (51), 289 (8), 275 (32), 263 (100), 249 (7), 234 (3), 220 (4)	$C_{19}H_{20}N_4$
IIIb	52	201 – 203	0.61	1645	9.0s	352 (63), 337 (7), 328 (30), 311 (100), 299 (23), 286 (29), 285 (12)	$C_{23}H_{20}N_4$
IIIc	69	186 – 187	0.60	1630	8.78s	366 (88), 351 (8), 338 (18), 337 (47), 326 (29), 325 (100), 311 (6)	$C_{24}H_{22}N_4$
IIId	65	206 – 208	0.36	1650	8.93s	318 (100), 303 (19), 289 (31), 275 (80), 263 (90), 262 (38), 249 (53), 234 (9)	$C_{20}H_{22}N_4$
IIIe	64	224 – 226	0.61	1660	9.03s	366 (100), 351 (8), 342 (32), 323 (40), 311 (60), 297 (24), 286 (20), 263 (16)	$C_{24}H_{22}N_4$
IIIf	77	209 – 211	0.54	1660	8.80s	380 (100), 379 (34), 365 (5), 351 (11), 338 (15), 337 (37), 325 (56), 311 (21)	$C_{25}H_{24}N_4$

* TLC in a nonane – ethyl acetate (1 : 2) system.

TABLE 3. Yields and Characteristics of Tetrazoloquinazolines IVa – IVf

Compound	Yield, %	M.p., °C	R_f*	^{1}H NMR spectrum ($CDCl_3$), $\delta_{8\text{-}CH_2}$, ppm	Mass spectrum, m / z (%)	Empirical formula
IVa	92	167 – 169	0.59	3.03s	305 (51), 276 (13), 262 (2), 248 (22), 236 (70), 234 (100), 220 (12), 207 (13)	$C_{18}H_{19}N_5$
IVb	77	188 – 190	0.59	3.03s	353 (65), 346 (10), 325 (28), 296 (31), 284 (72), 282 (100), 255 (11), 243 (24)	$C_{22}H_{19}N_5$
IVc	84	164 – 166	0.55	2.96s	367 (78), 339 (26), 338 (24), 310 (23), 296 (100), 248 (10), 206 (20)	$C_{23}H_{21}N_5$
IVd	97	224 – 226	0.57	3.20s	319 (100), 291 (23), 276 (4), 262 (17), 248 (50), 235 (66), 234 (96), 207 (17)	$C_{19}H_{21}N_5$
IVe	73	181 – 182	0.58	3.23s	367 (65), 339 (71), 310 (16), 298 (34), 296 (71), 282 (100), 255 (12), 243 (20)	$C_{23}H_{21}N_5$
IVf	84	183 – 185	0.58	3.20s	381 (100), 353 (33), 352 (24), 324 (12), 312 (41), 310 (38), 296 (87), 193 (26)	$C_{24}H_{23}N_5$

* TLC in a nonane – ethyl acetate (2 : 1) system.

teristics of tetrazoloquinazolines IVa – IVf are given in Table 3.

EXPERIMENTAL PHARMACOLOGICAL PART

The anxiolytic properties of synthesized compounds were studied using a model of the alarm state specially developed for the screening of atypical tranquilizers [9]. The experiments were performed on male mice weighing 22 ± 2 g; each control or test group contained 5 – 10 animals. The model is based on the innate reflex of avoiding illuminated sites in the dark – light chamber and the "visible cliff" effect, consisting in the apparent lack of support for the paws in the light compartment with transparent floor overhanging above the table. On stepping onto the transparent floor in the light compartment, mice fell into a conflict (disagreement) between visible and tactile percept, enhanced by an innate fear of altitude. A conflict between the orientative-trying reaction directed to examination of the entire chamber and the hole reflex (runaway to the preferred dark compartment) served as a stable alarm background. We measured the total time of mice staying in the light compartment and the number of runs between dark and light compartments. An increase in the time of staying in the light compartment (against the control) was indicative of the anxiolytic activity of a compound tested. A change in the number of runs between compartments allowed us to preliminarily differentiate between the compounds belonging to the classical tranquilizers (increasing the number of runs) and atypical tranquilizers (decreasing the number of runs) of the benzodiazepine class.

TABLE 4. Effect of Spyrobenzo[h]quinazoline Derivatives on the Behavior of Mice in the Model of Alarm

Compound	Dose, mg / kg	Time of stay in the light, sec ($M \pm m$)	Number of runs between compartments, ($M \pm m$)
Control		46.63 ± 4.95	13.75 ± 1.37
IIIa	50	95.0 ± 10.67*	6.13 ± 1.3*
Control		51.8 ± 7.28	10.4 ± 1.22
IIIb	50	87.5 ± 13.31*	9.4 ± 1.75
Control		57.13 ± 5.29	11.13 ± 1.56
IIIc	50	103.63 ± 3.63*	8.5 ± 1.36
Control		51.8 ± 7.28	10.4 ± 1.22
IIId	50	63.3 ± 8.92	8.8 ± 1.16
Control		46.63 ± 4.95	13.75 ± 1.37
IIIe	50	59.5 ± 9.55	6.75 ± 0.56*
Control		51.8 ± 7.28	10.4 ± 1.22
IIIf	50	73.7 ± 14.43	8.6 ± 1.36
Control		58.4 ± 3.89	16.5 ± 0.81
IVa	50	59.2 ± 3.34	15.8 ± 0.76
Control		50.6 ± 3.13	13.9 ± 0.99
IVb	50	44.4 ± 5.05	13.8 ± 1.9
Control		57.13 ± 5.29	11.13 ± 1.56
IVc	50	66.25 ± 16.22	11.88 ± 0.79
IVd	50	56.13 ± 4.18	12.75 ± 1.29
Control		46.63 ± 4.95	13.75 ± 1.37
IVe	50	122.25 ± 12.89*	5.13 ± 0.77*
Control		57.13 ± 5.29	11.13 ± 1.56
IVf	50	67.13 ± 6.59	9.63 ± 1.03
Control		50.0 ± 5.06	11.6 ± 0.45
Diazepam	1	67.8 ± 6.95	21.2 ± 1.48*
	2.5	72.5 ± 6.48*	17.8 ± 1.14*
Control		60.5 ± 4.75	5.6 ± 0.27
Chlordiazepoxide	10	83.9 ± 3.73*	8.3 ± 0.73*
Control		51.5 ± 3.18	15.4 ± 0.43
Buspirone	5	83.5 ± 3.58*	3.8 ± 0.36*

* $p < 0.05$.

In order to finally judge on the presence of anxiolytic properties (tranquilizer activity) in the most active substances, preselected according to the model of the alarm, we used a specially developed method of "conflict situation" [10]. These experiments were performed on male rats weighing 180 ± 20 g; each control or test group contained 5 – 10 animals. The conflict was created by the collision of two reactions, thirst and defense. The anxiolytic effect was assessed by the increase in the number of water takes despite obtaining additional electrical shocks. Also monitored were the total number of attempts to approach the drinking bowl and the general motor activity of the test animals.

The sedative properties of the compounds were studied by monitoring perturbations in the orientative-trying reaction of mice in the "grid climbing" test [11].

The anticonvulsive activity of substances was determined in experiments on mice by the ability to prevent the clonic component of convulsions induced by subcutaneous injections of Corazole (85 mg / kg) [12] and the tonic extension in the maximum electroshock test (50 mA, 0.2 sec) [13].

The neurotoxic and myorelaxant action of substances (as manifested by perturbed coordination of movements and ataxia) was studied using the "rotating rod" test [14].

The anxiolytic activity of benzo[h]quinazoline derivatives, spyroconjugated to carbocycles, was investigated in comparison with the effects of diazepam, chlordiazepoxide, and buspirone.

All substances were injected intraperitoneally as suspensions with Tween-80 and carboxymethyl cellulose at a dose of 50 mg / kg. The injections were made 45 min before the beginning of tests. The reference preparations were introduced at anxiolytic doses. The control animals were injected with emulsifier.

The experimental data were processed on an IBM PC / XT (Intel 8088) computer using a program package for the statistical treatment according to the Student's t-criterion for independent series and pair sampling. The differences were considered reliable for a bilateral significance level of $p < 0.05$. The roles of individual fragments in molecules of the test compounds were assessed with the aid of a special computer program calculating a mean quantity W (the sum of

TABLE 5. Anxiolytic Activity of Spyrobenzo[h]quinazoline Derivatives on the Behavior of Rats under Conflict Conditions

Compound	Dose, mg/kg	Number of water takes ($M \pm m$)	Number of trials to approach drinking bowl ($M \pm m$)	Motor activity ($M \pm m$)
Control		3.0 ± 0.6	1.38 ± 0.26	12.88 ± 1.61
IIIa	50	18.63 ± 2.49*	4.5 ± 0.53*	14.0 ± 2.24
Control		2.88 ± 0.29	1.38 ± 0.18	24.38 ± 4.96
IIIb	50	9.5 ± 1.16*	2.5 ± 0.19*	15.88 ± 1.69
IIIc	50	16.88 ± 3.7*	4.0 ± 0.53*	16.5 ± 1.86
Control		3.0 ± 0.6	1.38 ± 0.26	12.88 ± 1.61
IVe	50	8.75 ± 0.82*	4.13 ± 0.44*	41.88 ± 3.16*
Control		1.86 ± 0.25	6.57 ± 0.75	18.0 ± 3.75
Diazepam	1	6.3 ± 0.97*	20.3 ± 4.65*	39.5 ± 3.69*
	2.5	19.1 ± 0.57*	20.8 ± 0.71*	32.7 ± 4.33*
Control		3.5 ± 0.62	3.0 ± 0.76	36.25 ± 6.08
Chlordiazepoxide	10	16.0 ± 3.67*	14.1 ± 3.36*	160.6 ± 39.12*
Control		3.2 ± 0.7	2.4 ± 0.51	12.2 ± 2.24
Buspirone	5	9.57 ± 2.23*	5.57 ± 1.04*	40.43 ± 4.15*
Control		4.17 ± 0.4	2.67 ± 0.42	18.67 ± 3.18
Buspirone	10	14.2 ± 2.03*	3.3 ± 0.45	8.8 ± 0.68*

* $p < 0.05$.

rank numbers of the experimental values) by the Wilcoxon method. The W values, determined for a parameter characterizing the anxiolytic activity of compounds in the model of the alarm (percentage with respect to control), allowed us to assess both the activity of individual compounds and that of the groups with different chemical structures [15].

Data gained for the model of the alarm (Table 4) show that triazolo derivatives of benzo[h]quinazoline (IIIa – IIIf) increase the time of mice staying in the light compartment and decrease the number of runs between dark and light compartments, the most efficient being the compounds with spyroconjugated cyclopentane (IIIa – IIIc). A similar effect was observed under the same conditions for buspirone. The classical benzodiazepine tranquilizers (diazepam and chlordiazepoxide) increased both the time of mice staying in the light compartment and the number of runs between compartments. Unlike the triazolo derivatives, the tetrazolo compounds (except for compound IVe) produced no significant effect on the behavior of mice.

As for the "conflict situation" test on rats, the most active compounds IIIa – IIIc and IVe increased the number of "punished" water takes (3 – 6 times against the control) and the number of attempts to approach the drinking bowl (Table 5).

The motor activity of animals increased only upon the introduction of compound IVe. Diazepam, chlordiazepoxide, and buspirone also produced a tranquilizing effect with respect to the conflict situation test.

All the compounds studied virtually did not prevent the appearance of clonic Corazole-induced convulsions and tonic extension for the maximum electroshock at doses from 10 to 200 mg/kg, but violated the orientative reflexes and exhibited sedative action (in 40 – 80% of animals). Anxiolytic doses of buspirone (5 and 10 mg/kg) produced the sedative effect in 100% of mice, while no such effects were observed for diazepam (1 and 2.5 mg/kg) and chlordiazepoxide (10 mg/kg).

Nor did the compounds studied induce myorelaxation and perturb the coordination of movements in animals at doses between 50 and 200 mg/kg.

Thus, using a new model of alarm in mice allowed us to select compounds possessing anxiolytic activity among the triazolo and tetrazolo derivatives of benzo[h]quinazoline, spyroconjugated to carbocycles.

A computer-aided analysis of the role of molecular fragments showed evidence for the maximum activity of triazoles, which was most pronounced in compounds spyroconjugated to cyclopentane. With respect to the substituent in position 2, these compounds can be arranged in the following sequence in order of decreasing anxiolytic activity: IIIa (R = ethyl) ≥ IIIc (R = benzyl) > IIIb (R = phenyl). Note that a reverse sequence was found in the triazolo derivatives of benzo[h]quinazoline, spyroconjugated to cyclohexane.

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