RADIOPROTECTOR AND ANTINEOPLASTIC ACTIVITY OF CERTAIN TETRAZOLE DERIVATIVES

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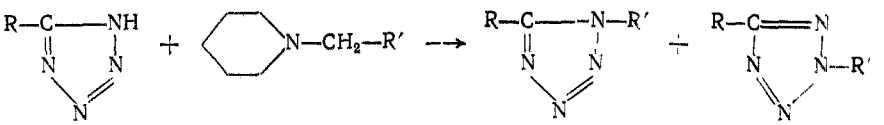
Earlier [1, 2, 4] we synthesized and investigated certain 5-substituted tetrazoles and their salts with organic bases as potential biologically active compounds.

A study of the radioprotective activity of 5-substituted tetrazoles showed that it is low in these compounds — they protect up to 42% of laboratory animals (CBA and BALB mice) irradiated in a dose of 680-710 R [1]. The activity of salts of 5-substituted tetrazoles with organic bases is significantly higher; they protect up to 70% of the animals (C57BL/6 mice) irradiated in a dose of $LD_{95-99/30}$ [4].

For a modification of the biological activity of the tetrazoles that we studied earlier, we synthesized products of their alkylation by Mannich bases from phenols, since substances with good radioprotective properties have been detected among the phenols [5].

As alkylating agents we used N-(3,5-dimethyl-4-hydroxybenzyl)piperidine and N-(2-hydroxy-3-methoxy-5-formylbenzyl)piperidine, produced from 2,6-dimethylphenol or vanillin, formaldehyde, and piperidine.

The alkylation products (I-IX) were synthesized according to the scheme:



I, III, VI—VIII I—VI, IX

R' = 3,5-dimethyl-4-hydroxybenzyl (A), 2-hydroxy-3-methoxy-5-formylbenzyl (B); I: R = benzyl, R' = A; II) R = phenyl, R' = A; III: R = phenyl, R' = B; IV: R = 4-methoxyphenyl, R' = A; V: R = 3,4-dimethoxyphenyl, R' = A; VI: R = 3,4-dimethoxyphenyl, R' = B; VII: R = 4-bromophenyl, R' = A; VIII: R = 4-pyridyl, R' = A; IX: R = 3-pyridyl, R' = A.

As a result of alkylation of tetrazoles (I-IX, R = H) with the Mannich base obtained from vanillin, we isolated mixtures of N_1- and N_2-isomers (III, VI); in alkylation with the Mannich base from 2,6-dimethylphenol, however, only one of the two possible N_1- or N_2-isomers was isolated, with the exception of compound I, which represented a mixture of N_1- and N_2-isomers (Table 1).

The structure of compounds I-IX was confirmed by IR and PMR spectroscopy and the dipole moments [3].

Tetrazoles I-IX are colorless, difficultly water-soluble substances. In organic solvents ethanol, dimethyl sulfoxide — these compounds dissolve better than in water.

The toxic action, radioprotective and antineoplastic activity of compounds I-IX were investigated.

The toxicity of compounds I-IX was lower than in the original 5-substituted tetrazoles [1]. Administered in doses of 850-2000 mg/kg, they did not cause death of the animals. For compounds I, II, and VIII, LD_{50} could not be determined on account of the great viscosity of the solution.

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TABLE 1. Products of Alkylation of 5-Substituted Tetrazoles by Mannich Bases

Compound	mp, °C	Yield, %	Found, %				Gross formula	Calculated, %			PMR spectra, δ, ppm
			R_f	C	H	N		C	H	N	
I	87—8	50,0	0,95	69,27	5,94	18,91	$C_{17}H_{18}N_4O$	69,37	6,16	19,03	4,19; 4,35 (C—CH_2); 5,41; 5,64 (N—CH_2)[a]
II	128—9	76,6	0,97	68,92	6,06	19,96	$C_{16}H_{16}N_4O$	68,55	5,75	19,99	5,79 (CH_2)
III	163—5	60,7	0,93	61,97	4,67	18,24	$C_{16}H_{14}N_4O_3$	61,93	4,55	18,05	5,92; 6,01 (CH_2)[6]
IV	124—6	74,8	0,95	65,69	6,00	18,00	$C_{17}H_{18}N_4O_2$	65,79	5,85	18,05	5,75 (CH_2)
V	166—8	66,6	0,93	63,73	5,93	16,91	$C_{18}H_{20}N_4O_3$	63,52	5,93	16,46	5,75 (CH_2)
VI	191—3	56,5	0,85	58,14	4,78	15,29	$C_{18}H_{18}N_4O_5$	58,37	4,90	15,13	5,96; 5,83 (CH_2)[B]
VII	146—7	65,0	0,63	53,74	4,61	15,66	$C_{16}H_{15}BrN_4O$	53,50	4,21	15,60	5,78 (CH_2)
VIII	174—5	58,8	0,94	64,18	5,12	25,16	$C_{15}H_{15}N_5O$	64,05	5,37	24,89	5,84 (CH_2)
IX	156—7	59,9	0,71	64,14	5,37	24,96	$C_{15}H_{15}N_5O$	64,05	5,37	24,89	5,83 (CH_2)

[a]Mixture of isomers N_2: N_1 = 1:4.
[b]Mixutre of isomers N_2: N_1 = 7:1.
[c]Mixture of isomers N_2: N_1 = 1:3.

TABLE 2. Radioprotective Activity of Tetrazole Derivatives I-IX

Compound	LD_{50}		Dose introduced		Survival, %	ALT, days
	mg/kg	mM/kg	mg/kg	mM/kg		
I	Не определена		330	1,1	0	5,2
II	» »		500	1,8	14,6	9,6
III	1250	4,0	416	1,4	0	7,3
IV	850	2,7	385	1,2	0	6,4
V	1500	4,4	250	0,7	5	5,2
VI	Not determined		500	1,3	0	7,4
VII	1000	2,8	330	0,9	0	7,6
VIII	Not determined		750	2,5	46,5	10,8
IX	2000	7,1	666	2,3	10	13,1
Control					0	6,5

TABLE 3. Antineoplastic Activity of Certain Tetrazole Derivatives

Compound	Dose, mg/kg	Tumor strain				
		AC-755	S-37	S-180	La	LT
I	150	+18	40	7	4	3
	70	+68	15	2	4	3
II	150	+10	29	+17	4	Death
	100	+89	15	+28	5	16
IV	200	+87	+28	8	4	14
	150	+43	+17	12	0	17
V	250	11	19	16	1	6
	200	11	1	+9	6	3
VI	200	12	14	10	1	+27
	150	30	35	+87	1	+19
VIII	100	+18	+56	+56	10	+7
	50	+11	+45	+45	8	+1
IX	200	21	65	+3	13	3
	150	12	32	+18	1	7

Note. LT represents a Lewis tumor, La stands for leukemia. Compounds marked by the symbol "+" stimulate tumor growth; compounds not marked by the symbol "+" inhibit tumor growth.

Directly after administration, the compounds induced excitation, which was replaced by adynamia after 3 h. Death occurred after 2-3 days.

The radioprotective activity was estimated according to the test of survival of the animals for 30 days and the average lifetime (ALT) of the animals that died.

The results obtained (Table 2) are evidence that most of the compounds studied proved inactive according to the test of 30-day survival of lethally irradiated animals. An exception is compound VIII, which exhibited substantial radioprotective activity: it provides for the survival of 46.5% of lethally irradiated animals.

Compounds II, VII, and IX are characterized by a higher ALT in comparison with the control.

A study of tetrazole derivatives as antineoplastic agents showed that they do not possess high antineoplastic activity, with the exception of IX, which inhibits the growth of sarcoma 37 (S-37) by 65% (Table 3). Compounds I, II, IV, and VIII stipulate the growth of adenocarcinoma 755 (AC-755), sometimes extremely substantially (87-89%). Compound VIII appreciably stimulates the growth of the tumors S-37 and sarcoma 180 (S-180) — up to 56%. This suggests that radioprotective action of this compound is based on the stimulation of the proliferation of hemopoietic stem cells.

Thus, the low toxicity in the series of new tetrazole derivatives I-IX and the data obtained on their activity are evidence of the advisability of the further study of this class of compounds as potential biologically active agents.

EXPERIMENTAL (CHEMICAL)

The IR spectra were recorded on a UR-20 spectrophotometer (German Democratic Republic). The PMR spectra were recorded in DMSO-d_6 on a Tesla BS 567 A instrument (100 mHz).

The course of the reaction of alkylation of 5-substituted tetrazoles was monitored by thin-layer chromatography on a plate of Silufol UV-254 according to the disappearance of the spot of the Mannich base and the appearance of a new spot, belonging to the alkylation product. An ethyl acetate–acetone–acetic acid–water mixture, 8:2:2:1 (I-VI, VIII-IX) and a 2:1 hexane–acetone mixture (VII) were used for the separation.

The initial 5-substituted tetrazoles were produced according to the data of [7]. 3,5-Dimethyl-4-hydroxy-N-benzylpiperidine was produced according to the data of [6]. 2-Hydroxy-3-methoxy-5-formyl-N-benzylpiperidine was produced analogously.

2-(3,5-Dimethyl-4-hydroxy-benzyl-5-phenyltetrazole (II). A 2 g (14 mmoles) portion of 5-phenyltetrazole and 2.04 g (6 mmoles) 3,5-dimethyl-4-hydroxy-N-benzylpiperidine were dissolved in 30 ml of dimethylformamide, 0.02 g (0.14 mmole) potash was added, and the reaction mixture was heated in a stream of argon or nitrogen at 130°C for 6 h. The solution was cooled to room temperature, 200 ml of ice water was added, and it was acidified with concentrated hydrochloric acid to pH 5.5-6.0. The precipitate formed was filtered off and dried in air. After recrystallization from aqueous ethanol, the end product was obtained.

The constants of the synthesized alkylation products I-IX are presented in Table 1.

Other alkylation products were produced analogously (see Table 1).

EXPERIMENTAL (BIOLOGICAL)

The acute toxicity was determined on C57BL/6 mice and estimated both according to the direct manifestation of the action on animals after the injection and according to the death of the animals in three days.

The preparations were injected intraperitoneally in a volume of 0.2 ml in a 20% solution of Tween-80. Treatment of the data on the toxicity of the compounds studied was performed according to the method of Litchfield and Wilcoxon, and LD_{16}, LD_{50}, and LD_{84} were determined.

The radioprotective activity was studied at doses of irradiation 9 and 9.5 Gy, constituting LD_{95-99} for males and females of the C57BL/6 strain, respectively.

The solutions were administered 15 min before irradiation in a dose of 1/2 LD_{16} or 1/3 LD_{50}.

The antineoplastic activity of the compounds was studied in experiments on transplantable mouse tumors, taken from the tumor bank of the All-Union Oncological Science Center, Academy of Medical Sciences of the USSR: sarcoma 37, sarcoma 180, Lewis tumor, adenocarcinoma 755, and leukemia (see Table 3).

The experiments were conducted on noninbred mice and inbred C57BL/6 mice, acquired from the Stolbovaya Laboratory Animals Nursery.

The investigated compounds were injected intraperitoneally in the form of a starch suspension, five times at 24 h intervals. The animals were sacrificed a week after the end of the administration of the preparation. The effectiveness of the antitumor action was estimated according to the percent inhibition of tumor growth, which was calculated according to the formula:

$$I = \frac{P_c - P_e}{P_c} \cdot 100\%,$$

where I is the % inhibition; P_c is the average tumor mass in the control; P_e is the average tumor mass in the experimental group.

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SYNTHESIS OF (3-PYRIDYL)GLYOXYLIC ACID DERIVATIVES AND THEIR ANTIMICROBIAL PROPERTIES

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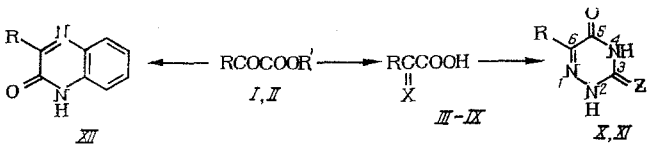
The synthesis and properties of alkyl and arylglyoxylic acids have been fairly widely investigated [2]. Compounds with different biological activity were detected [1, 8] among their transformation products. The preparation of several heteroarylglyoxylic acids has also been described [1], but their properties have practically not been investigated. It was interesting to carry out a comparative study of chemical and biological characteristics of these structurally related compounds, to determine the influence of the heteroaromatic and, in particular, the basic pyridine ring on the properties of the α-oxo-acids.

To solve the problem, we synthesized a representative of the heteroaromatic α-oxo-acids, (3-pyridyl)glyoxylic acid (I), and studied its chemical transformations. Acid I was prepared by oxidizing 3-ethylpyridine by $KMnO_4$ in an aqueous–alkaline medium and by the method described in [4]. Acid I is a fairly stable compound. Only when it is heated to melting point (170-180°C), decarboxylation is observed, with resinification of the products formed, possibly due to their polymerization. Acid I was esterified in a yield of 47% by heating twice with an ethanolic solution of HCl. When H_2SO_4 was used as the esterification catalyst, the yield of ethyl (3-pyridyl)glyoxylate (II) decreased to 18%. It was found that when ester II is boiled in an aqueous solution for 1 h, it hydrolyzes to acid I.

Acid I readily forms derivatives at the oxo group: hydrazone (III), oxime (IV), semicarbazone (V), thiosemicarbazone (VI), 4-phenylsemicarbazone (VII), 4-phenylthiosemicarbazone (VIII), phenylhydrazone (IX), While the reactions of the aliphatic and aromatic α-oxo-acids



I: R' = H; II: R' = Et; III: X = NNH_2; IV: X = NOH; V: X = $NNHCONH_2$; VI: X = $NNHCSNH_2$; VII: X = NNHCONHPh;; VIII X = NNHCSNHPh; IX:X = NNHPh; X:Z = O; XI:Z = S; I-XII: R = 3-pyridyl

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