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Synthesis of 5-R-4-R_1-3-alkylthio-1,2,4-triazoles and study influence of their adsorption ability on the results of toxicity and anti-hypoxic activity

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During our research of biologically active substances we have synthesized series of new 5-R-4-R_1-3-alkylthio-1,2,4-triazoles for original drugs creation. 14 new compounds have been synthesized in total. The structure of it has been confirmed by a complex of modern physical and chemical methods of analysis. For these compounds the data of toxicity, anti-hypoxic activity, surface activity and adsorption ability has been set. The obtained results of biological activity have been compared with data of adsorption properties of 1,2,4-triazoles derivatives to establish structure-action regularity.

Keyword: 1,2,4-triazoles, physical and chemical properties, susceptibility to adsorption, toxicity, anti-hypoxic activity.

1. Introduction

One of the main problems of the cardiovascular diseases' treatment is the lack of highly efficient drugs with minimal side effects, on the modern pharmaceutical market of Ukraine. The search for new low-toxic biologically active substances requires the use of complex physical and chemical methods of analysis. It is necessary to consider the relation between pharmacological activity and the structure of compound, when a molecular model of a potential new drug is created. The bioavailability and therefore toxicity, biological activity of a substance depend on its adsorption ability.

2. Aim

The aim of our study was a synthesis of series of new pharmacologically active compounds 5-(phenoxymethyl)-4-R_1-3-alkylthio-1,2,4-triazoles and 5-(benzyloxy)-4-R_1-3-alkylthio-1,2,4-triazoles. On the next stage we have determined the data of toxicity, biological activity and have set the dependence of obtained results with surface activity and adsorption ability.

3. Materials and Methods

We have used obtained 5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-thiones and 5-(benzyloxy)-4-R_1-1,2,4-triazole-3-thiones (R_1 = H, C_6H_5) as starting

substances for synthesis of 5-R-4-R1-3-alkylthio-1,2,4-triazoles [1].

Alkylation of thiones 1-4 has been carried out using haloalkanes in alcohol medium with brief heating (neutral pH) of equimolar amounts of starting materials. The reaction has been done by adding sodium hydroxide to the equimolar amounts of initial compounds 1-4. The reaction takes place through an intermediate stage of obtaining corresponding sodium salts of thiones 5-8 which are not given out from the reaction mixture (Fig. 1).

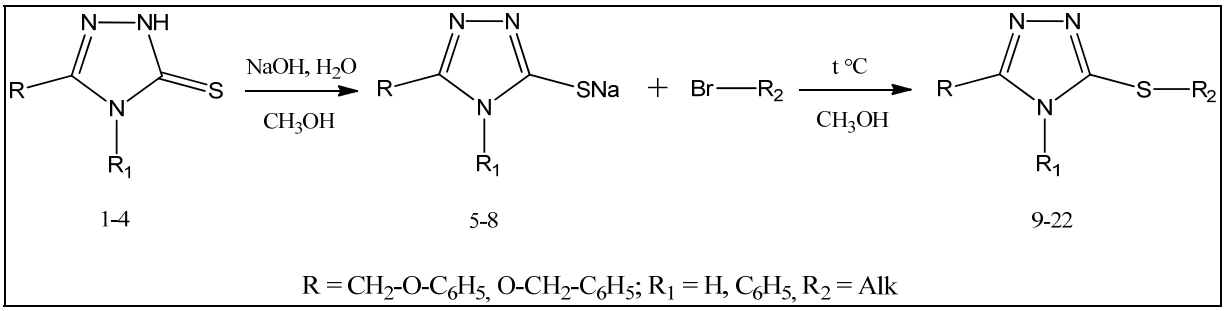

Fig 1: Scheme of 5-R-4-R1-3-alkylthio-1,2,4-triazoles synthesis

Synthesized 3-alkylthio-5-R-4-R1-1,2,4-triazoles (9-22) are light yellow (9, 12, 16-17) or yellow (10-11, 13-15, 18-22) amorphous substances which are insoluble in water and soluble in organic solvents. For analysis synthesized compounds (9-22) have been recrystallized from methanol and acetic acid.

The structure of synthesized substances has been confirmed with complex of physical and chemical methods of analysis such as elemental analysis (table 1), IR-spectrometry and ^{1}H NMR-spectroscopy (table 2).

The study of toxicity and anti-hypoxic activity has been carried out at the Department of Clinical Pharmacy, Pharmacotherapy and MFE of FPE of Zaporozhye State Medical University (responsible performer, candidate of pharmaceutical sciences, Pruglo E. S.).

Toxicity has been determined using express method of V. B. Prozorovsky [2] on nonlinear white laboratory rats. The results are shown in Table 1.

Table 1: Physical and chemical constants, the results of biological activity of 5-R-4-R1-3-alkylthio-1,2,4-triazoles (Fig. 1)

№	R	R1	R2	Tm, °C	Gross formula	Yield , %	Calculated, %		Found, %		Γ ·10^{-6}, mol/m^2	LD50	Activity,%
							C	H	C	H			
9	CH_2-O-C_6H_5	H	C_3H_7	60-64	$C_{12}H_{15}N_3OS$	81	57,81	6,06	57,79	6,01	4,67	536	137,3
10	CH_2-O-C_6H_5	H	C_6H_{13}	58-60	$C_{15}H_{21}N_3OS$	79	61,82	7,26	61,75	7,16	4,78	484	132,3
11	CH_2-O-C_6H_5	H	C_7H_{15}	64-66	$C_{16}H_{23}N_3OS$	67	62,92	7,59	62,88	7,50	4,83	470	129,31
12	CH_2-O-C_6H_5	H	C_9H_{19}	80-83	$C_{18}H_{27}N_3OS$	63	64,83	8,16	64,76	8,14	4,86	414	125,5
13	CH_2-O-C_6H_5	H	$C_{10}H_{21}$	64-67	$C_{19}H_{29}N_3OS$	69	65,67	8,41	65,58	8,37	4,93	376	120,4
14	CH_2-O-C_6H_5	C_6H_5	C_3H_7	50-53	$C_{18}H_{19}N_3OS$	77	66,43	5,88	66,36	5,77	4,3	435	130,8
15	CH_2-O-C_6H_5	C_6H_5	C_6H_{13}	63-64	$C_{21}H_{25}N_3OS$	69	68,63	6,86	68,55	6,79	4,62	465	126,3
16	CH_2-O-C_6H_5	C_6H_5	C_8H_{17}	71-73	$C_{23}H_{29}N_3OS$	75	69,84	7,39	69,72	7,31	4,8	432	122,4
17	CH_2-O-C_6H_5	C_6H_5	C_5H_{11}	58-60	$C_{20}H_{23}N_3OS$	76	67,96	6,56	67,85	6,47	4,53	479	132,1
18	CH_2-O-C_6H_5	C_6H_5	C_9H_{19}	61-62	$C_{24}H_{31}N_4OS$	71	70,38	7,63	70,32	7,60	4,76	397	119,4
19	O-CH_2-C_6H_5	H	C_3H_7	57-58	$C_{12}H_{15}N_3OS$	74	57,81	6,06	57,76	6,02	4,56	523	135,4
20	O-CH_2-C_6H_5	H	C_9H_{19}	76-78	$C_{18}H_{27}N_3OS$	69	64,83	8,16	64,78	8,02	4,79	402	123,7
21	O-CH_2-C_6H_5	C_6H_5	C_3H_7	64-65	$C_{18}H_{19}N_3OS$	76	66,43	5,88	66,37	5,75	4,43	434	128,2
22	O-CH_2-C_6H_5	C_6H_5	C_9H_{19}	54-55	$C_{24}H_{31}N_3OS$	67	70,38	7,63	70,32	7,58	4,68	367	115,4

We have selected next experimental model to determine anti-hypoxic activity of 5-R-4-R$_1$-3-alkylthio-1,2,4-triazoles. It is based on pathological process of hypoxic hypoxia which develops by placing the rats in an enclosed space [3, 4]. The activity of the compounds has been calculated by life expectancy of rats. It was compared with the effect of the reference drug pentoxifylline. The high activity level of it has been previously set [4]. The studied results of anti-hypoxic activity are shown in Table 1.

We have used the experimental data of the surface tension of substances' solutions at the liquid-gas interface to study adsorption properties and next correlation of pharmacological results. Surface tension has been determined by the method of Rebinder [5]. Adsorption has been calculated with the Gibbs equation [5]. Researches have been carried out at the Department of Physical and Colloidal Chemistry of Zaporozhye State Medical University. The studied results are shown in Table 1.

Table 2: Proton signals of 5-R-4-R$_1$-3-alkylthio-1,2,4-triazoles (Fig. 1)

S. №	δ, ppm
9	0,87 (3H, т, -CH$_3$); 1,36 (2H, м, **-CH$_2$-**CH$_3$); 3,02-5,07 (2H, м, others -CH$_2$-); 6,83-7,29 (5H, м, -C$_6$H$_5$)
10	0,85 (3H, т, -CH$_3$); 1,29 (2H, м, **-CH$_2$-**CH$_3$); 1,28-5,10 (2H, м, others -CH$_2$-); 6,94-7,30 (5H, м, -C$_6$H$_5$)
11	0,86 (3H, т, -CH$_3$); 1,3 (2H, м, **-CH$_2$-**CH$_3$); 1,29-5,14 (2H, м, others -CH$_2$-); 6,97-7,36 (5H, м, -C$_6$H$_5$); 13,30 (1H, с, -NH-)
12	0,89 (3H, т, -CH$_3$); 1,28 (2H, м, **-CH$_2$-**CH$_3$); 1,26-5,17 (2H, м, others -CH$_2$-); 6,93-7,15 (5H, м, -C$_6$H$_5$)
13	0,80 (3H, т, -CH$_3$); 1,31 (2H, м, **-CH$_2$-**CH$_3$); 1,27-5,01 (2H, м, others -CH$_2$-); 6,89-7,26 (5H, м, -C$_6$H$_5$)
14	0,90 (3H, т, -CH$_3$); 1,38 (2H, м, **-CH$_2$-**CH$_3$); 3,07-5,27 (2H, м, others -CH$_2$-); 6,96-7,22 (5H, м, -C$_6$H$_5$)
15	0,81 (3H, т, -CH$_3$); 1,24 (2H, м, **-CH$_2$-**CH$_3$); 1,28-5,26 (2H, м, others -CH$_2$-); 6,90-7,50 (5H, м, -C$_6$H$_5$)
16	0,88 (3H, т, -CH$_3$); 1,32 (2H, м, **-CH$_2$-**CH$_3$); 1,27-5,31 (2H, м, others -CH$_2$-); 6,93-7,54 (5H, м, -C$_6$H$_5$)
17	0,84 (3H, т, -CH$_3$); 1,35 (2H, м, **-CH$_2$-**CH$_3$); 0,86-5,32 (2H, м, others -CH$_2$-); 6,97-7,55 (5H, м, -C$_6$H$_5$)
18	0,87 (3H, т, -CH$_3$); 1,29 (2H, м, **-CH$_2$-**CH$_3$); 1,31-5,29 (2H, м, others -CH$_2$-); 6,91-7,56 (5H, м, -C$_6$H$_5$)
19	0,90 (3H, т, -CH$_3$); 1,40 (2H, м, **-CH$_2$-**CH$_3$); 3,05-5,06 (2H, м, others -CH$_2$-); 7,29-7,45 (5H, м, -C$_6$H$_5$)
20	0,88 (3H, т, -CH$_3$); 1,27 (2H, м, **-CH$_2$-**CH$_3$); 1,29-5,13 (2H, м, others -CH$_2$-); 7,38-7,42 (5H, м, -C$_6$H$_5$)
21	0,89 (3H, т, -CH$_3$); 1,38 (2H, м, **-CH$_2$-**CH$_3$); 3,08-5,12 (2H, м, others -CH$_2$-); 7,32-7,57 (5H, м, -C$_6$H$_5$)
22	0,86 (3H, т, -CH$_3$); 1,25 (2H, м, **-CH$_2$-**CH$_3$); 1,26-5,04 (2H, м, others -CH$_2$-); 7,36-7,55 (5H, м, -C$_6$H$_5$)

4. Experimental synthetic part

5-R-4-R$_1$-3-alkylthio-1,2,4-triazoles (9-22*)*

Add 0,01 mol of (5-(phenoxymethyl)-1,2,4-triazole-3-thione (1), 5-(phenoxymethyl)-4-phenyl-1,2,4- triazole-3-thione (2), 5-(benzyloxy)-1,2,4- triazole-3-thione (3) or 5-(benzyloxy)-4- phenyl-1,2,4- triazole-3-thione (4) in a 100 ml round-bottom flask equipped with chemical returning condenser, add methanol in amount 30 ml and NaOH in amount 0,01 mol dissolved in 10 ml of water. The reaction mixture has been heated until thione dissolving with further cooling, then add 0.01 mole of corresponding haloalkane (1-bromopropane, 1-bromopentane, 1-bromohexane, 1-bromoheptane, 1-bromooctane, 1-bromononane, 1-bromodecane). The reaction mixture has been boiled for 2 hours and cooled. The solvent has been evaporated.

5. Results and Discussion

The previously unknown 3-alkyl derivatives of 3-thio-5-R-4-R$_1$-1,2,4-triazoles have been obtained as a result of our synthetic work. We have synthesized fourteen new compounds, the structure of which has been confirmed by the complex usage of modern physical and chemical methods of analysis. In the IR spectra of the synthesized compounds symmetric and asymmetric accumulations of aliphatic methyl radicals present in the 1383-1370 cm^{-1} and 1465-1440 cm^{-1} wavenumbers intervals, scissor accumulations of methylene groups 1464-1446 cm^{-1}, accumulations of SH-groups in the 2350 - 2300 cm^{-1} wavenumbers intervals are absent.

NMR spectra are characterized by the presence of 5 proton signals (multiplet) of the phenyl substituent at 6,83-7,56 ppm, the protons signals of methyl radicals (triplet) at 0,80-0,90 ppm and the proton signals of methylene groups 1,24-1,40 ppm [6].

LD_{50} of synthesized class lie in the range 320-817 mg/kg according to results of toxicity. It belongs to III and IV classes of toxicity (moderate-and low-toxic substances) according to classification of K. K. Sidorov [2].

Researches of anti-hypoxic pharmacological activity of the synthesized compounds have shown that given class of compounds is perspective in further studies of actoprotective, antioxidant, cardioprotective and other activities. We have found a substance that exceed the activity of the reference drug pentoxifylline among the synthesized compounds (9, 14, 17, 19) [7].

From the obtained data of adsorption properties you can follow the dependence that adsorption ability increases both with addition of CH_2-groups in molecule structure and with increasing of substance concentration. But the value of adsorption decreases with increasing molecular weight of the synthesized compounds. This increasing of absorption ability of compounds leads to increasing of toxicity and biological activity.

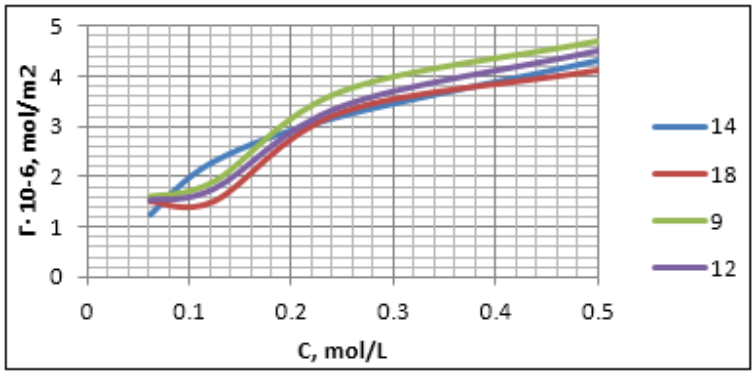

Fig 2: The dependence of adsorption from the concentration of compounds 9, 12, 14,18

6. Prospects for further research

The extension of substances biological activity by obtaining new compounds is one of the main prospects for further researches. A special problem of the further study is the performance of chemical, analytical, pharmacological and technological research to create potential dosage forms of the most active compounds.

7. Conclusions

1. A series of new 5-R-4-R_1-3-alkylthio-1,2,4-triazoles has been synthesized. The structure of their has been confirmed with a set of modern physical and chemical methods of analysis.
2. Research of toxicity and anti-hypoxic activity of obtained substances has shown that given class of compounds is perspective in creation of new potential medicines.
3. Increasing the length of the alkyl radical leads to an increasing the adsorption ability of substances, which in turn increases the toxicity and values of the anti-hypoxic action.

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