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Synthesis, physical-chemical properties and the study of anti-hypoxemic activity of 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-thion alkylderivatives

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***Key words**: Derivatives, 1,2,4-triazole, Physical-chemical Properties, Anti-hypoxemic Activity.*

Heterocyclic systems, in particular, 1,2,4-triazole derivatives cause great interest in the development of new medicinal substances for the production of highly effective and safe antihypoxants. This fact has created favorable basis for the synthesis of new alkylderivatives 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-thion, where R is methyl, phenyl.

Aim. In order to identify the most promising active compounds the pharmacological screening of anti-hypoxemic activity of synthesized compounds has been carried out.

Results. Due to the results of the study a number of compounds with anti-hypoxemic activity have been revealed. Some regularities between structure and pharmacological effect have been established.

Conclusion. This demonstrates the prospects of further studies of the obtained compounds.

Синтез, фізико-хімічні властивості та вивчення антигіпоксичної активності алкілпохідних 5-(адамантан-1-іл)-4-R-1,2,4-тріазол-3-тіону

В. М. Одинцова, Є. С. Пругло

Значний інтерес у процесі розробки нових лікарських субстанцій для отримання високоефективних і безпечних антигіпоксантів викликають гетероциклічні системи, зокрема похідні 1,2,4-тріазолу. Це створило сприятливе підґрунтя для синтезу нових алкілпохідних 5-(адамантан-1-іл)-4-R-1,2,4-тріазол-3-тіону, де R – метил, феніл. З метою виявлення найбільш активних перспективних сполук здійснили фармакологічний скринінг антигіпоксичної активності речовин, які синтезували. У результаті дослідження виявили ряд сполук, що характеризуються антигіпоксичною дією. Встановили окремі закономірності між структурою та фармакологічним ефектом. Це свідчить про перспективність дослідження отриманих сполук.

***Ключові слова:** похідні 1,2,4-тріазолу, фізико-хімічні властивості, антигіпоксична активність.*

Запорізький медичний журнал. – 2015. – №2 (89). – С. 93–96

Синтез, физико-химические свойства и изучение антигипоксической активности алкилпроизводных 5-(адамантан-1-ил)-4-R-1,2,4-триазол-3-тиона

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Большой интерес в разработке новых лекарственных субстанций для получения высокоэффективных и безопасных антигипоксантов вызывают гетероциклические системы, в частности производные 1,2,4-триазола. Это создало благоприятную почву для синтеза новых алкилпроизводных 5-(адамантан-1-ил)-4-R-1,2,4-триазол-3-тиона, где R – метил, фенил. С целью установления наиболее активных перспективных соединений проведен фармакологический скрининг антигипоксической активности синтезированных веществ. В результате исследования установлен ряд соединений, обладающих антигипоксическим действием. Отмечены некоторые закономерности между структурой и фармакологическим эффектом. Это свидетельствует о перспективности дальнейшего исследования полученных соединений.

***Ключевые слова**: производные 1,2,4-триазола, физико-химические свойства, антигипоксическая активность.*

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Hypoxia is a typical pathological process, which occurs as a result of the failure of biological oxidation and due to its energy insecurity of life processes [1]. There is acute and chronic oxygen starvation of cells. Acute hypoxia develops in all types of shock, blood loss, and physical overload. Chronic hypoxia is observed in several pathological conditions: diseases of the respiratory system, cardiovascular system, diseases of the blood, liver, kidneys, and endocrine system [2,3,4].

Various forms of hypoxia, including ischemia are major cause of cerebral stroke and coronary heart disease [5].

To reduce hypoxia, pharmacological means (antihypoxants and antioxidants) and methods are used to enhance the oxygen's delivery into the body and improve the utilization of the body's circulating oxygen, reducing the oxygen demand in the organs and tissues. Drugs contribute to more economical oxygen's expenditure by the tissues, its best utilization and reducing of hypoxia and they also increase the body's resistance to oxygen deficiency [6,7].

Mexidol, pyracetam, amoxyne, pentoxifilline are most commonly used antihypoxants, which have numerous side effects and they limit their use. Therefore, the development of new medicinal substances for the production of highly effective and safe antihypoxants is one of the most important areas of modern biological, pharmaceutical and medicinal chemistry. Heterocyclic systems call great interest in this direction, in particular, 1,2,4-triazole derivatives.

The aim of this work was the synthesis of s-derivatives of 3-alkyl-5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole, where R is methyl, phenyl and pharmacological screening of anti-hypoxemic activity of synthesized compounds.

Materials and methods of the study

The objects of study are alkylderivatives of 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-thions, where R is methyl, phenyl. As an initial matter, we have used 2-(adamantane-1-yl)-N-phenylhydrazincarbothioamide and 2-(adamantane-1-yl)-N-methylhydrazincarbothioamide, which were synthesized by the reaction of hydrazide adamantane-1-carboxylic acid with phenylisothiocyanate and methylisothiocyanate, respectively, in the environment of methyl alcohol. Synthesis of 5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole-3-thiones (comp. I, II) was conducted by the cyclization of 2-(adamantane-1-yl)-N-phenylhydrazincarbothioamide and 2-(adamantane-1-yl)-N-methylhydrazincarbothioamide in an alkaline media. The obtaining of 3-alkylthio-5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole (comp. Ia-Ig, IIa-IIg) was carried out by adding α-halogenoalkanes to the corresponding 5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole-3-thiones in the environment of n-butanol.

The obtained compounds are crystalline substances of white color, which were recrystallized from n-butanol for analysis. Physical-chemical constants of the obtained compounds are given in the *table 1*.

5-(adamantane-1-yl)-4-methyl-4H-1,2,4-triazole-3-thion and 5-(adamantane-1-yl)-4-phenyl-4H-1,2,4-triazole-3-thion (I, II)

0.1 mol of KOH is added to water solution of 0.1 mol of 2-(adamantane-1-yl)-N-methylhydrazincarbothioamide or 2-(adamantane-1-yl)-N-phenylhydrazincarbothioamide and boiled for 1 hour. It is neutralized by the acetic acid. White precipitate is filtered off and recrystallized (dioxane : water, 20:1).

3-alkylthio-5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole (Ia-Ig, IIa-IIg)

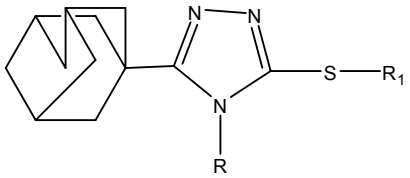
0.01 mol of NaOH is added to 0.01 mol of 5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole-3-thion, where R is phenyl or methyl, in butanol media. It is heated to the dissolving of the precipitate. 0.01 mol is added to haloalkane (1-brombutane or 1-brompentane, or 1-bromhexane, or 1-bromheptane, or 1-bromo ctane, or 1-bromnonane, or 1-bromdecane). Boiled and evaporated to neutral pH.

Antihypoxic activity of 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-thion alkylderivatives has been studied [8] to simulate hypoxia with hypercapnia, which was reproduced by placing rats in glass jars of the same size (1330 ml) closed hermetically and turned upside down and placed in a cuvette with water to prevent the admission of air. Mexidol in a dose of 100 mg/kg was used as a comparative drug in the research [9].

Comparative drug Mexidol was injected in aqueous solution. The action of each substance was studied in 7 animals. The control group received isotonic sodium chloride solution. The investigated compounds were administered in a dose of 1/10 of LD50.

Table 1

Physical-chemical constants of 3-R_1thio-5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole



№ compounds	R	R_1	T of melting., °C	gross formula	Output, %	Found, %				Calculated, %			
I	CH_3	H	235-237	$C_{13}H_{19}N_3S$	73,40	C	H	N	S	C	H	N	S
II	C_6H_5	H	132-133	$C_{18}H_{21}N_3S$	78,67	62,45	7,70	16,82	12,83	62,61	7,68	16,85	12,86
Ia	CH_3	н-C_4H_9	>230	$C_{17}H_{27}N_3S$	73,77	69,21	6,82	13,53	10,31	69,42	6,80	13,49	10,30
Ib	CH_3	н-C_5H_{11}	>230	$C_{18}H_{29}N_3S$	74,50	67,01	8,93	13,72	10,48	66,84	8,91	13,76	10,50
Ic	CH_3	н-C_6H_{13}	>230	$C_{19}H_{31}N_3S$	80,52	67,82	9,12	13,17	10,07	67,66	9,15	13,15	10,04
Id	CH_3	н-C_7H_{15}	>230	$C_{20}H_{33}N_3S$	75,54	68,21	9,39	12,62	9,58	68,42	9,37	12,60	9,61
Ie	CH_3	н-C_8H_{17}	178-180	$C_{21}H_{35}N_3S$	76,12	69,30	9,54	12,13	9,26	69,11	9,57	12,09	9,23
If	CH_3	н-C_9H_{19}	>230	$C_{22}H_{37}N_3S$	73,33	69,54	9,78	11,63	8,90	69,75	9,76	11,62	8,87
Ig	CH_3	н-$C_{10}H_{21}$	>230	$C_{23}H_{39}N_3S$	77,17	70,17	9,90	11,21	8,55	70,35	9,93	11,19	8,54
IIa	C_6H_5	н-C_4H_9	>230	$C_{22}H_{29}N_3S$	69,72	70,72	10,07	10,81	8,24	70,90	10,09	10,78	8,23
IIb	C_6H_5	н-C_5H_{11}	105-108	$C_{23}H_{31}N_3S$	65,57	72,06	7,92	11,40	8,69	71,89	7,95	11,43	8,72
IIc	C_6H_5	н-C_6H_{13}	>230	$C_{24}H_{33}N_3S$	66,45	72,25	8,17	11,04	8,43	72,40	8,19	11,01	8,40
IId	C_6H_5	н-C_7H_{15}	>230	$C_{25}H_{35}N_3S$	72,51	73,05	8,39	10,59	8,13	72,87	8,41	10,62	8,11
IIe	C_6H_5	н-C_8H_{17}	150-152	$C_{26}H_{37}N_3S$	70,11	73,08	8,60	10,28	7,81	73,30	8,61	10,26	7,83
IIf	C_6H_5	н-C_9H_{19}	>230	$C_{27}H_{39}N_3S$	71,42	73,93	8,78	9,95	7,54	73,71	8,80	9,92	7,57
IIg	C_6H_5	н-$C_{10}H_{21}$	>230	$C_{28}H_{41}N_3S$	65,74	73,91	8,97	9,63	7,34	74,09	8,98	9,60	7,33

The research results are processed by modern statistical methods of analysis on a personal computer using standard software package Microsoft Office 2010 (Microsoft Excel) and «STATISTICA® for Windows 6.0». Average arithmetics (M) and standard errors of (± m) were calculated. Reliability of inter groups differences according to the experiments were established by using t-Student criterion. 3 levels of statistical significance of differences in the results of the research – $p<0.05$; $p<0.01$; and $p<0.001$ were used [10, 11].

The results and their discussion

The structure of all synthesized compounds has been confirmed by the integrated use of modern physical-chemical methods of analysis: elemental, IR spectroscopy and their individuality is detected by thin layer chromatography.

Band oscillations groups characteristic for the nucleus of 1,2,4-triazole: NH– within 3400–3100 cm^{-1}, – C=N– 1690-1620 cm^{-1} are present in the IR spectra of compounds I, II. There are also present band oscillations groups –C–S in 705-570 cm^{-1}. There are band oscillations within 2600–2550 cm^{-1}, which may indicate to the presence of –SH groups in the molecule.

The band oscillations groups characteristic for the nucleus of 1,2,4-triazole: NH– within 3400–3100 cm^{-1}, –C=N– 1690–1620 $cm^{-1}$are available in the study of the IR spectra of Ia–Ig, IIa–IIg compounds. There are also present band oscillations groups –C–S in 705–570 cm^{-1}. There are band oscillations characteristic for a group $–CH_3$ within 2975–2950 cm^{-1} and for a group $–CH_2$ – 2940–2915 cm^{-1}.

The signals of methyl group are present in the ^{1}H NMR spectra of 3-(adamantane-1-yl)-5-(butylthio)-4-phenyl-4H-1,2,4-triole which are recorded in the spectrum in the form of a triplet multiplets at 0,89 m. h. (3H), protons of adamant cycle [12] are recorded in the form of 21,59 m. h. (6N), 1,69 m. h. (3H) and deplete, 82 m.h. (6N), the signals of methine group at 1,28 (2N), 1,59 (2N), the signals of the protons of a methyl group, associated with sulphur whithin 2,93 m. h. (2N) and the protons of the aromatic cycle, which are fixed in the form of 2 triplets at 6,80 m. h. (1H), 7,18 m. h. (2N) and the multiplet of 7,48 m. h. (2N).

The most active was compound II, which is the derivative for the synthesis of the investigated alkylderivatives, its anti-hypoxemic activity exceeded the control on 169,10 % ($p<0.001$) and Mexidol on 30,90 % ($p<0.001$).

It is established that anti-hypoxemic activity in a series of alkylderivatives 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-tion (comp. I, II) due to the nature of the substituent at the N_4 atom nitrogen of the nucleus of 1,2,4-triazole and the length of the

Table 2

The characteristic of ^{1}H NMR spectra of the obtained compounds

№ comp.	^{1}H NMR (DMSO-d^{6}δppm)
I	1,61 (t, 6H, CH_2), 1,73 (t, 3H, CH), 1,94 (d, 6H, CH_2), 3,61 (s, 3H, CH_3), 13,15 (s, 1H, SH)
II	1,59 (s, 6H, CH_2), 1,68 (t, 3H, CH), 1,85 (d, 6H, CH_2), 7,21 (t, 1H, Ar-H), 7,52 (t, 2H, Ar-H), 7,78 (t, 2H, Ar-H), 13,15 (s, 1H, SH)
IIa	0,89 (t, 3H, CH_3), 1,28 (m, 2H, CH_2), 1,59 (m, 8H, CH_2), 1,69 (m, 3H, CH), 1,82 (m, 6H, CH_2), 2,93 (t, 2H, CH_2), 6,80 (t, 1H, Ar-H), 7,18 (t, 2H, Ar-H), 7,48 (m, 2H, Ar-H)
Ib	1,60 (t, 3H, CH_3), 1,70 (m, 10H, CH_2), 1,92 (m, 2H, CH_2; 3H, CH), 2,68 (d, 6H, CH_2), 3,12 (s, 3H, CH_3), 3,28 (t, 2H, CH_2)
Ic	0,82 (t, 3H, CH_3), 1,21 (m, 6H, CH_2), 1,60 (m, 8H, CH_2), 1,79 (m, 3H, CH), 2,00 (d, 6H, CH_2), 3,35 (s, 3H, CH_3), 3,62 (t, 3H, CH_3)
If	0,82 (t, 3H, CH_3), 1,22 (m, 12H, CH_2), 1,60 (m, 8H, CH_2), 1,79 (m, 3H, CH), 2,00 (d, 6H, CH_2), 3,35 (s, 3H, CH_3), 3,62 (t, 3H, CH_3)
Ig	0,82 (t, 3H, CH_3), 1,22 (m, 14H, CH_2), 1,60 (m, 8H, CH_2), 1,79 (m, 3H, CH), 2,00 (d, 6H, CH_2), 3,35 (s, 3H, CH_3), 3,62 (t, 3H, CH_3)

Table 3

Anti-hypoxemic activity of alkylderivatives 5-(adamantane-1-yl)-4-R-1,2,4-triazole-3-thion (n=7)

Compound / group	Dose compounds, mg/kg	The average life span of rats min.	Activity accordingly to contro l%	Activity accordingly to Mexidol, Δ%
control	-	66,57±1,86	-	-22,59
Mexidol	100	86,00±2,26*	129,18	-
I	84,2	73,71±2,71	110,73	-14,29
II	357	112,57±3,69*	169,10	30,90
IIa	56,5	80,43±6,17**	120,82	-6,48
control	-	54,29±4,45	-	-30,15
Mexidol	100	77,71±9,4*	143,16	-
Ib	122	78,00±5,18*	143,68	0,37
Ic	62,4	82,43±5,55*	151,84	6,07
If	125	56,57±4,43	104,21	-27,21
Ig	182	61,43±5,98	113,16	-30,86
IIb	153	75,29±4,98*	138,68	-3,12
IIc	132	100,14±14,95*	184,47	28,86
IId	166	69,57±5,39	128,16	-10,48
IIe	174	55,88±6,99	102,93	-28,10
IIg	179	98,14±13,64*	180,79	26,29

Notes: * – $p<0.05$ relatively to control; ** – $p<0.05$ relatively to Mexidol; n – the number of animals in each group of the research.

carbon chain alkyl residue with the sulfur atom.

The compounds IIc and IIg showed antihypoxic activity, which exceeds the control on 184,47 % ($p<0.01$) and 180,79 % ($p<0.05$) and were higher than the activity of the Mexidol on 28,86 and 26,29 %, respectively.

Ib and Ic compounds are close to Mexidol activity, but well worth to note the IIb compound, which 3,12 % less efficient than Mexidol.

The analysis of the results indicates high antihypoxic activity due to the life expectancy indicator of rats of all studied substances in comparison with the control.

As a result of the studies (II, IIc, IIg) compounds were identified, which antihypoxic activity higher than Mexidol the comparison product.

Analyzing the dependence of the chemical structure from antihypoxic activity certain patterns were established between pharmacological activity and chemical structure. Thus, the increase of the alkyl substituent on the sulfur atom to six carbon atoms in the presence, as a methylic (comp. Ic) and phenolic (comp. IIc) radicals at N_4 atoms causes the growth of antihypoxic effect.

It was found that the methyl radical on phenyl substituent replacing at N_4 atom of nitrogen enhances antihypoxic activity of (II, IIc, IIg comp.).

The significant intensification of antihypoxic activity in the transition from aliling to hexylene substituents in the molecules of 3-alkylthio-5-(adamantane-1-yl)-4-methyl(phenyl)-1,2,4-triazole, the degree of which depends on the substituent at the N_4 atom of nitrogen (Ib, Ic, IIb, IIc comp.).

A significant reduction of antihypoxic activity is observed with increasing carbon chain to heptile, octile and nonile substituents in the case, as with methyl and phenyl substituents (IIe, IId, If). But the growth up to ten carbon atoms promotes the growth of antihypoxic activity (comp. IIg).

Conclusions

1. A number of new compounds, derivatives of 5-(adamantane-1-yl)-4-R-4H-1,2,4-triazole-3-thiones, have been synthesized the structure of these compounds has been confirmed by the integrated use of modern physical-chemical methods of analysis.

2. The most active among the investigated compounds are 5-(adamantane-1-yl)-4-phenyl-1,2,4-triazole-3-thion, its antihypoxic activity exceeds the control on 169,10 % ($p<0.001$) and Mexidol on 30,90 % ($p<0.001$).

3. IIg IIc compounds exceed the activity of Mexidol on 28,86 % ($p<0.01$) and on 26,29 % ($p<0.01$), respectively.

4.The significant intensification of antihypoxic activity in the transition from alylic to hexilene substituents in the molecules of 3-alkylthio-5-(adamantane-1-yl)-4-methyl(phenyl)-1,2,4-triazole, the degree of which depends on the substituent at the N_4 atom of nitrogen.

5. It was established that the methyl radical replacing on phenyl substituent at N_4 atom of nitrogen enhances antihypoxic activity.

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