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Synthesis and diuretic activity of 2-(5-(phenoxymethyl)-4-r$_1$-1,2,4-triazole-3-ylthio)acetic acids and their salts

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Key words: *1,2,4-triazole, Diuretic, Organic synthesis.*

Aim. It is known that drugs based on the 1,2,4-triazole have a wide spectrum of biological activities.They are effective cardioprotectors, hepatoprotectors, anti-oxidants, which are widely used in cardiology practice. So this class of heterocyclic compounds has all the attributes of relevance.

Methods and results. We have synthesized new 2-(5-(phenoxymethyl)-4-R$_1$-1,2,4-triazol-3-ylthio) acetate acid and its salts. We have used complex physical and chemical methods of analysis to establish the structure of the synthesized compounds. The chemical properties and diuretic activity of synthesized compounds have been studied. The relation between the structure of obtained salts and their biological effect has been set. Compounds with strong diuretic activity have been found among the newly synthesized compounds.

Conclusion. This fact testifies to further study and introduction into medical practice as the original drugs.

Синтез та діуретична дія 2-(5-(феноксиметил)-4-R$_1$-1,2,4-тріазол-3-ілтіо)ацетатних кислот та їх солей

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Відомо, що лікарські препарати на основі 1,2,4-тріазолу володіють широким спектром біологічної дії, зокрема – це ефективні кардіопротектори, гепатопротектори, антиоксиданти, що широко застосовуються в кардіологічній практиці. Тому цей клас гетероциклічних сполук володіє усіма ознаками актуальності.

Нами синтезовано нові 2-(5-(феноксиметил)-4-R$_1$-1,2,4-тріазол-3-ілтіо)ацетатні кислоти та їх солі. З метою встановлення будови синтезованих сполук був використаний комплекс сучасних фізико-хімічних методів аналізу. А також вивчено їхні хімічні властивості та діуретичну активність. Встановлено взаємозв'язок між будовою отриманих солей та їх досліджуваною дією. Серед нових синтезованих речовин знайдені сполуки з вираженою діуретичною активністю, що свідчить про подальші дослідження з метою впровадження в медичну практику як оригінальні лікарські засоби.

Ключові слова: *1,2,4-тріазол, діуретик, органічний синтез.*

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Синтез и диуретическое действие 2-(5-(феноксиметил)-4-R$_1$-1,2,4-триазол-3-илтио)ацетатных кислот, а также их солей

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Известно, что лекарственные препараты на основе 1,2,4-триазола обладают широким спектром биологического действия, в частности – это эффективные кардиопротекторы, гепатопротекторы, антиоксиданты, которые широко применяются в кардиологической практике. Поэтому данный класс гетероциклических соединений обладает всеми признаками актуальности.

Нами синтезированы новые 2-(5-(феноксиметил)-4-R$_1$-1,2,4-триазол-3-илтио)ацетатные кислоты, а также их соли. С целью установления строения синтезированных соединений был использован комплекс современных физико-химических методов анализа. А также изучены их химические свойства и диуретическая активность. Установлена взаимосвязь между строением полученных солей и их исследуемым действием. Среди новых синтезированных веществ найдены соединения с выраженной диуретической активностью, что свидетельствует о дальнейших исследованиях с целью внедрения в медицинскую практику в качестве оригинальных лекарственных средств.

***Ключевые слова*:** *1,2,4-триазол, диуретик, органический синтез.*

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The delay of water and salts in the body is an often side effect in many pathological conditions, such as congestive heart failure, kidney disease, liver cirrhosis, etc. This leads to swelling and fluid accumulation in body cavities, particularly in the abdominal cavity (ascites), pleural one, and other. Modern pharmaceutical market has 5 pharmacological groups of drugs that exhibit diuretic effect.

It is known that drugs based on 1,2,4-triazole have wide range of biological effects, in particular it is effective cardioprotective and hepatoprotective drugs, antioxidants, which are widely used in cardiology practice[2]. However, there are currently no drugs of this heterocyclic system with high diuretic indicators. Therefore, the study of diuretic properties of 2-(5-(phenoxymethyl)-4-R$_1$-1,2,4-triazole-3-ylthio)acetic acids and its derivatives is an important task for all scientists around the world.

The aim of our study was the purposeful search for new highly efficient and low-toxic, biologically active substances among the number of 2-(5-(phenoxymethyl)-4-R$_1$-1,2,4-triazole-3-ylthio) acetic acids and its derivatives, which show diuretic effect, and also the determination of communication between biological activity and chemical structure.

Materials and methods

The feasibility of the synthesis of 2-(5-(phenoxymethyl)-4-R$_1$-1,2,4-triazole-3-ylthio)acetic acids has been drawn based on the literature review[3, 6, 7].

The above mentioned acids have been obtained by reacting 5-(phenoxymethyl)-4-R$_1$-1,2,4-triazole-3-thiones[5] with monochloroacetic acid and the addition of equimolar amount of sodium hydroxide in dimethylformamide medium.

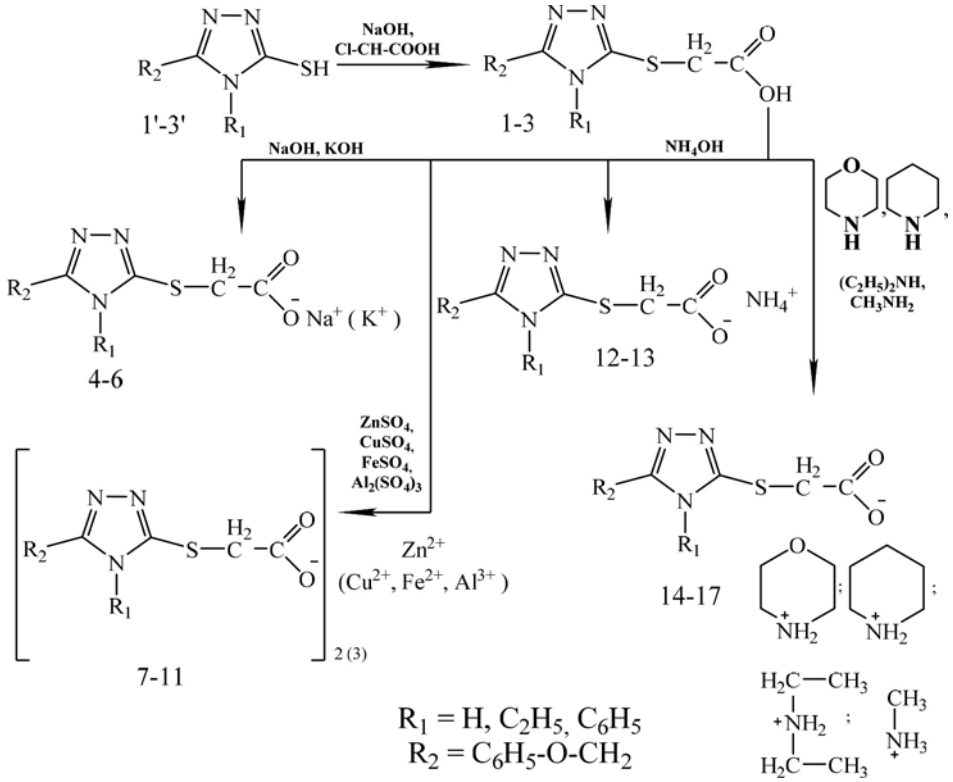


Figure 1. Scheme of synthesis of 2-(5-R_2-4-R_1-1,2,4-triazole-3-ylthio)acetic acids and its salts.

Sodium, potassium and ammonium salts of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acids (4-6, 12-13, *Tab. 1*) have been synthesized by reacting the corresponding acids with ammonia, sodium or potassium hydroxide in an aqueous medium followed by evaporation of the solvent *(Fig. 1)*. The dry residue has been crystallized from ethanol.

Aluminum, zinc, copper and iron (II) salts of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetic acids (7-11) have been obtained by reaction of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acids with corresponding sulfates (Fig. 1).

Salts of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetic acids with organic bases (12-17, Table. 1) (morpholine, piperidine, methylamine and diethylamine) have been synthesized by reacting starting materials in the ethanol medium, followed by evaporation of the solvent. Physical and chemical constants of salts (4-17) are shown in Table 1. [6].

Study of some physical and chemical properties of the synthesized compounds has been carried out according to the methods listed in the State Pharmacopoeia of Ukraine (SPU, edition 1). The melting point has been determined by capillary method (2.2.14) with instrument PTP-m.

Elemental composition of new compounds has been found in elemental analyzer ELEMENTAR vario EL cube (standard is sulfonamide).

IR spectra have been recorded in potassium bromide tablets (substance concentration is 1%) with spectrophotometer Specord M-80 in the region of 4000-500 cm^{-1} (scanning conditions: program 3.0, the time constant – $\tau = 3$ s, scan time 33 min).

NMR spectra have been recorded with spectrophotometer of nuclear magnetic resonance «Varian VXR-300», the solvent is DMSO-D6, an internal standard is tetramethylsilane.The data has been deciphered with computer program ADVASP 143.

2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetic acids (1-3)

0.01 mol of sodium hydroxide has been added to 0.01 mol solution of corresponding 5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-thiones in 30 ml of DMF. The solid components have been dissolved by heating and then 0.01 mol of monochloroacetic acidhas been added. The mixture has been heated for 5 hours, cooled, the precipitate has been filtered off, the filtrate has been evaporated to the dry residue which has been recrystallized from ethanol. They are white crystalline substances which are sparingly soluble in water, soluble in organic solvents, alkaline solutions and solutions of alkali metals hydrogen carbonates.

Sodium and potassium 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetates (4-6)

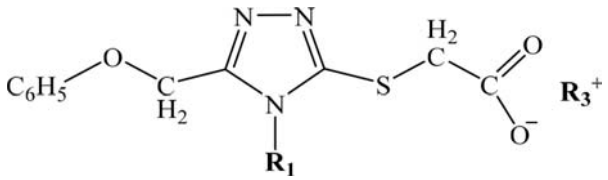
A mixture of 0.01 mol corresponding 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazoles-3-ylthio)acetic acid and 0.01 mol of potassium or sodium hydroxide in 30 ml of water have been evaporated in a water bath. The dry residue has been crystallized from ethanol. They are yellow (4.6) or red (5) crystalline substances which are easily soluble in water, slightly soluble in organic solvents.

Ammonium 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetates (12-13)

A solution of 0.01 mol corresponding 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acid has been evaporated in 30 ml of 25% ammonia solution. Obtained 12-13 compounds are white crystalline precipitate which is soluble in water, sparingly

Table 1

Physical and chemical constants of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetic acids and its salts



№	R_1	R_3^+	T_m, °C	Gross formula	Yield, %	Found, %		Calculated, %		^{1}H NMR (δ, ppm, TMS); $\nu_{COOH}(\nu \frac{as/s}{coo^-})$, cm^{-1}	Diuresis after			
						C	H	C	H		2 h, ml	% to contr	4 h, ml	% to contr
1.	H	H	112-114	$C_{11}H_{11}N_3O_3S$	72	49,75	4,19	49,80	4,18	12,81(1H,c,N^2), 6,86-7,29 (5H, м, $-C_6H_5$), 4,19-5,15 (2H, м, $-CH_2$-); 12,68(1H,c,-OH); 1715	1,25±0,25	98,71	2,09±0,21	91,39
2.	C_2H_5	H	122-124	$C_{13}H_{15}N_3O_3S$	96	53,13	5,16	53,23	5,15	12,81(1H,c,N^2), 7,08-7,30(5H, м, $-C_6H_5$), 4,20-5,35 (2H, м, $-CH_2$-); 12,75(1H,c,-OH), 1,33 (3H, т, $-CH_3$); 1708	1,29±0,12	101,62	2,12±0,28	92,41
3.	C_6H_5	H	115-117	$C_{17}H_{15}N_3O_3S$	93	59,65	4,44	59,81	4,43	12,81(1H,c,N^2), 7,09-7,65(5H, м, $-C_6H_5$), 4,09-5,39 (2H, м, $-CH_2$-); 12,79(1H,c,-OH); 1721	1,32±0,29	104,12	2,24±0,19	97,60
4.	C_6H_5	Na	190-192	$C_{17}H_{14}N_3NaO_3S$	95	56,21	3,87	56,19	3,88	7,09-7,67(5H, м, $-C_6H_5$), 4,03-5,27 (2H, м, $-CH_2$-); 1340/1555	1,44±0,09	113,36	2,32±0,16	101,45
5.	C_6H_5	K	265-267	$C_{17}H_{14}KN_3O_3S$	86	54,01	3,71	53,81	3,72	6,89-7,63(5H, м, $-C_6H_5$), 4,02-5,35 (2H, м, $-CH_2$-); 1360/1535	1,40±0,15	110,12	2,28±0,23	99,36
6.	C_2H_5	K	300-302	$C_{13}H_{14}KN_3O_3S$	89	47,01	4,25	47,11	4,26	6,95-7,31(5H, м, $-C_6H_5$), 4,02-5,34 (2H, м, $-CH_2$-); 1,29 (3H, т, $-CH_3$); 1370/1545	1,47±0,19	115,89	2,55±0,26	111,20
7.	C_6H_5	Fe	188-190	$C_{34}H_{28}FeN_6O_6S_2$	91	55,22	3,81	55,44	3,83	7,12-7,65(5H, м, $-C_6H_5$), 4,11-5,32 (2H, м, $-CH_2$-); 1375/1580	2,19±0,09	172,16	3,84±0,12	167,52
8.	C_6H_5	Zn	198-200	$C_{34}H_{28}N_6O_6S_2Zn$	97	54,89	3,76	54,73	3,78	7,10-7,61(5H, м, $-C_6H_5$), 4,22-5,40 (2H, м, $-CH_2$-); 1340/1592	1,89±0,15	149,10	3,05±0,19	133,19
9.	C_6H_5	Al	183-185	$C_{51}H_{42}AlN_9O_9S_3$	94	58,39	4,05	58,44	4,04	6,90-7,61(5H, м, $-C_6H_5$), 4,14-5,31 (2H, м, $-CH_2$-); 1310/1550	1,59±0,21	125,53	2,57±0,32	112,33
10.	H	Cu	230-232	$C_{22}H_{20}CuN_6O_6S_2$	92	44,24	3,41	44,63	3,40	12,87(1H,c,N^2); 7,01-7,34(5H, м, $-C_6H_5$), 4,20-5,17 (2H, м, $-CH_2$-); 1325/1590	1,85±0,24	145,51	3,23±0,36	141,12
11.	C_2H_5	Zn	255-258	$C_{26}H_{28}N_6O_6S_2Zn$	95	48,54	4,35	48,04	4,34	7,90-7,32(5H, м, $-C_6H_5$), 4,10-5,34 (2H, м, $-CH_2$-); 1,32 (3H, т, $-CH_3$); 1330/1515	2,26±0,13	178,23	3,95±0,17	172,69
12.	H	NH_4	161-163	$C_{11}H_{14}N_4O_3S$	88	46,87	5,02	46,80	5,00	12,93(1H,c,N^2); 7,13-7,29(5H, м, $-C_6H_5$), 4,02-5,14 (2H, м, $-CH_2$-); 1340/1595	2,06±0,27	162,46	3,66±0,37	159,82
13.	C_6H_5	NH_4	138-140	$C_{17}H_{18}N_4O_3S$	91	56,90	5,04	56,97	5,06	6,92-7,63(5H, м, $-C_6H_5$), 4,05-5,34 (2H, м, others $-CH_2$-); 1345/1605	1,91±0,32	150,12	3,30±0,21	143,97
14.	H	CH_3NH_2	173-175	$C_{12}H_{16}N_4O_3S$	92	47,94	5,43	48,64	5,44	12,88(1H,c,N^2), 6,89-7,34(5H, м, $-C_6H_5$), 4,06-5,17 (2H, м, $-CH_2$-); 2,87 (3H, т, $-CH_3$); 8,29 (2H,c, $-NH_2$);1350/1630	2,10±0,16	165,44	3,65±0,25	159,21
15.	H	mor-phol.	182-184	$C_{15}H_{20}N_4O_4S$	89	52,23	5,71	51,12	5,72	12,90(1H,c,N^2); 6,91-7,36(5H, м, $-C_6H_5$), 4,03-5,15 (2H, м,$-CH_2$-); 1372/1595	2,58±0,22	202,98	4,50±0,20	196,53
16.	C_2H_5	piperid.	188-191	$C_{18}H_{26}N_4O_3S$	93	57,55	6,94	57,12	6,92	6,90-7,34(5H, м, $-C_6H_5$), 3,32-5,34(2H, м, $-CH_2$-); 1,34 (3H, т, $-CH_3$); 1340/1605	1,85±0,11	145,87	3,19±0,15	139,25
17.	C_2H_5	diethyla mm.	185-187	$C_{17}H_{26}N_4O_3S$	90	54,74	7,14	55,71	7,15	6,95-7,29(5H, м, $-C_6H_5$), 3,36-5,30 (2H, м, $-CH_2$-); 1,32-1,63 (3H, т, $-CH_3$); 1335/1640	2,31±0,08	181,58	4,11±0,16	179,69
18.	Control										1,27±0,08	100,00	2,29±0,15	100,00
19.	Hydrochlorothiazide										2,32±0,17	182,40	3,91±0,16	171,00
20.	Furosemide										3,34±0,38	263,20	6,63±0,42	289,60

soluble in ethanol. Compounds 12-13 have been recrystallized for the analysis from ethanol.

Aluminum, zinc, copper (II), iron (II) tris or bis(2-(5-(phenoxymethyl)-4-R1-1,2,4-triazole-3-ylthio) acetates) (7-11)

0.03 mol of potassium (sodium) hydroxide has been added to 0.03 mol solution of corresponding 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acid in 30 ml of water, dissolved with heating and then 0.01 mol of $Al_2(SO_4)_3$ or 0.02 mol $ZnSO_4$, $CuSO_4$, $FeSO_4$ have been added in reaction mixture. The white formed (Al^{3+}, Zn^{2+}), green (Cu^{2+}), brown (Fe^{2+}) color precipitates have been filtered off and washed with ethanol, dried (7-11, Table 1).

Morpholin-4-ium, piperidinium,methanaminium and dietylammonium 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio) acetates (14-17)

A solution of 0.01 mol corresponding 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acid, 0.01 mol of the appropriate organic base (morpholine, piperidine, monoethanolamine) and 50 ml of methanol was left for 24 hours, the reaction products have been filtrated. They are yellow crystalline compounds soluble in water, slightly soluble in ether, chloroform. Synthesized compound have been recrystallized for the analysis from ethanol.

The experimental biological part

The study of synthesized compounds' effects on renal function has been carried out on outbred white rats weighing 129-197 g by the method of E. B. Berkhin[1]. Compounds have been administered at a dose of 1/10 LD50. The amount of urine has been taken into account every hour for 4 h. The amount of urine which provided a control group of animals (they didn't receive the compounds) has been taken as 100%. Research and analysis of the experimental data have been performed in comparison with standard diuretics - hydrochlorothiazide and furosemide. The results of experimental studies are presented in *Table 1.*

Results and discussion

In the IR spectra of the synthesized compounds asymmetric and symmetric accumulations of carboxyl group are present in the 1310-1375 cm^{-1} and 1535-1630 cm^{-1} wavenumbers' intervals and accumulations of carbonyl groups are in the 1708-1721 cm^{-1}. NMR spectra are characterized by the presence of 5 proton signals (multiplet) of the phenyl substituent at 6,86-7,65 ppm, the protons signals of hydroxyl radicals (singlet) at 12,68-12,79 ppm and the proton signals of methylene groups are at 3,32-5,40 ppm [4].

From the experimental results of the biological activity, we see that there is a dependency of the pharmacological action on the structure. The increasingof diuretic effect occurs with the movement from salts of inorganic bases to the salts with cation of organic nature.

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 from the most active compounds.

Conclusions

The 17 new compounds have been synthesized. Their structure has been confirmed by complex use of modern physical and chemical methods of analysis.

The studied derivatives of 2-(5-(phenoxymethyl)-4-R_1-1,2,4-triazole-3-ylthio)acetic acids show diuretic activity.

The most pronounced diuretic effect has morpholin-4-ium 2-(5-(phenoxymethyl)-1,2,4-triazole-3-ylthio)acetate which exceeds the result of a reference drug hydrochlorothiazide.

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