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Synthesis and diuretic activity of novel 5-amino-1,3,4-thiadiazole-2-thiol derivatives

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Aim. Synthesis, *in vivo* study and characterization of diuretic activity of novel 5-amino-1,3,4-thiadiazole-2-thiol derivatives. **Methods.** Organic synthesis (one-pot reaction). ^{1}H NMR spectroscopy. Biological methods: *in vivo* study on white rats model; urinalysis; saluretic/natriuretic/carbonic anhydrase inhibition indexes; biochemical laboratory tests (ALT, AST, ALP, γ-GGT, total bilirubin and protein levels determination). **Results.** The series of 5-amino-1,3,4-thiadiazole-2-thiol derivatives have been synthesized using convenient one-pot approach. All compounds were evaluated for their diuretic activity by estimation of total urinary output per day and urinalysis profile. The 5-benzylthio-1,3,4-thiadiazol-2-amine derivatives **2a**, **2c** and **2e** have been found the most active and have been studied for their kaliuretic, saluretic, and natriuretic properties and estimated for carbonic anhydrase inhibition ability. The structure – diuretic activity relationship has been formed. **Conclusions.** Series of 1,3,4-thiadiazole-bearing derivatives have been designed and synthesized and some derivatives have demonstrated a high level of diuretic action with satisfactory kaliuretic, saluretic, and natriuretic properties.

K e y w o r d s: 5-amino-1,3,4-thiadiazole-2-thiol derivatives; diuretic activity; saluretic/natriuretic/carbonic anhydrase inhibition indexes; SAR

Introduction

Diuretics are a particularly important group of drugs that are used individually or more often in combination therapy for the treatment of different pathologic states and conditions [1–

4]. The cardiovascular diseases (arterial hypertension, heart failure) and some kinds of edema were and in fact, remain the main pathologies, in which diuretics played a leading therapeutic role. However, nowadays, diuretics are widely used for the treatment of the central nervous system pathologies and diseases (epilepsy, migraine, depression) [5–6], intoxicating conditions in metabolic disorders [7–8], infectious diseases [9–11]. On the other hand all diuretics possess a wide set of side effects (microelements and hemostasis imbalance, hyperglycemia, hyperlipidemia, thiamine deficiency, *etc.* [12–14]), therefore, they have to be explored and optimized.

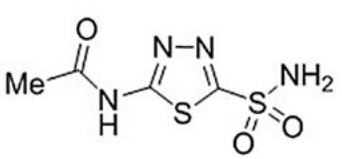
The diuretics approved for use, as well as potent candidates and lead-compounds with mentioned action present wide chemical diversity [1, 15]. The various heterocyclic compounds form the basis of the modern arsenal of the most effective diuretics [1, 15]. The modified 1,3,4-thiadiazole nucleus is known as a scaffold for the design of potent derivatives with diuretic action [16–19]. The acetazolamide and methazolamide (Fig.1) present widely used diuretic drugs with 1,3,4-thiazdiazole core belong to the carbonic anhydrase inhibitors (CAIs) [20–23].

The targeting of CA and search for selective CA modulators are the modern and actual directions in the current medicinal chemistry [24,25]. The CAIs normally were used in therapy as diuretic and antiglaucoma agents. But nowadays some of them are in the clinical development as potential agents for the treatment of obesity [26, 27], some types of cancer [28]. Moreover, the bacterial, fungal and protozoan CAs present in many pathogens have recently been considered as potential targets for the development of inhibitors with therapeutic applications. The currently available CAIs show undesired side effects due to indiscriminate inhibition of CA isoforms. Thus the design of novel selective CAIs still remains an attractive area for studies.

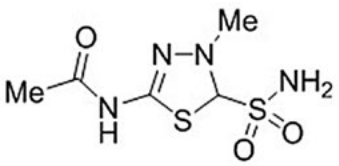
Due to our ongoing interest in the studies of drug-likeness azoles [29–33], in the present work we report the synthesis of novel 5-amino-1,3,4-thiadiazole-2-thiol derivatives, *in vivo* study, and characterization of their diuretic action profile as possible non-sulfonamide CAIs.

Materials and Methods

Chemistry. All starting materials were purchased from Merck and used without purification. NMR spectra were determined with Varian Mercury 400 (400 MHz) spectrometer, in DMSO-d_6. Melting points were measured in open capillary tubes and are uncorrected. The purity of the compounds was checked by thin-layer chromatography per-



Acetazolamide



Methazolamide

Fig. 1. 1,3,4-Thiadiazole-bearing diuretics with carbonic anhydrase inhibition properties.

formed with Merck Silica Gel 60 F_{254} aluminum sheets.

General synthetic procedure for preparation of 5-thio-1,3,4-thiadiazol-2-amine derivatives 2a-f and 3a-j. The suspension of 10 mmol of 5-thio-1,3,4-thiadiazol-2-amine and 10 mmol of potassium hydroxide was slightly heated for 5–10 min in 10 mL of ethanol for preparing potassium salt. Then 9 mmol of appropriate derivative of chloromethylbenzene or 2-bromo-1-phenylethan-1-one with trace amounts of potassium iodide were added to the mixture and reflux for 1 h. After cooling the obtained precipitate was filtered off, washed, dried and crystallized from ethanol.

(2a) 5-(Benzylthio)-1,3,4-thiadiazol-2-amine. Yield 65 %, mp=160–162°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.26–7.36 (m, 7H, NH_2+arom), 4.29 (s, 2H, CH_2. Anal. Calc. for $C_9H_9N_3S_2$: C 48.41 %; H 4.06 %; N 18.82 %. Found: C 48.60 %, H 4.20 %; N 18.90 %.

(2b) 5-((2-Chlorobenzyl)thio)-1,3,4-thiadiazol-2-amine. Yield 72 %, mp=150–152°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.26–7.33 (m, 4H, NH_2+arom), 7.40–7.47 (m, 2H, arom) 4.35 (s, 2H, CH_2). Anal. Calc. for $C_9H_8ClN_3S_2$: C 41.94 %; H 3.13 %; N 16.30 %. Found: C 42.10 %, H 3.30 %; N 16.50 %.

(2c) 5-((4-Chlorobenzyl)thio)-1,3,4-thiadiazol-2-amine. Yield 76 %, mp=159–161°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.25–7.48 (m, 6H, NH_2+arom), 4.28 (s, 2H, CH_2). Anal. Calc. for $C_9H_8ClN_3S_2$: C 41.94 %; H 3.13 %; N 16.30 %. Found: C 42.00 %, H 3.20 %; N 16.50 %.

(2d) 5-((4-Bromobenzyl)thio)-1,3,4-thiadiazol-2-amine. Yield 78 %, mp=172–174°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.50 (d, 2H, arom, J = 8.8 Hz), 7.28–7.32 (m, 4H, NH_2+arom), 4.26 (s, 2H, CH_2). Anal. Calc. for $C_9H_8BrN_3S_2$: C 35.77 %; H 2.67 %; N 13.90 %. Found: C 36.00 %, H 2.80 %; N 14.10 %.

(2e) 5-((2,4-Dichlorobenzyl)thio)-1,3,4-thiadiazol-2-amine. Yield 64 %, mp=132–134°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.63 (s, 1H, arom), 7.34–7.43 (m, 4H, NH_2+ arom), 4.33 (s, 2H, CH_2). Anal. Calc. for $C_9H_7Cl_2N_3S_2$: C 36.99 %; H 2.41 %; N 14.38 %. Found: C 37.10 %, H 2.60 %; N 14.50 %.

(2f) 5-((2,6-Dichlorobenzyl)thio)-1,3,4-thiadiazol-2-amine. Yield 69 %, mp=168–170°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.33–7.49 (m, 5H, NH_2+ arom), 4.44 (s, 2H, CH_2). Anal. Calc. for $C_9H_7Cl_2N_3S_2$: C 36.99 %; H 2.41 %; N 14.38 %. Found: C 37.20 %, H 2.70 %; N 14.60 %.

(3a) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-phenylethan-1-one. Yield 64 %, mp=171–172°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.02 (d, 2H, arom, J = 7.6 Hz), 7.69 (t, 1H, arom , J = 7,3Hz), 7.56 (t, 2H, arom, J = 7,4 Hz), 7.27 (s, 2H, NH_2), 4.81 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_9N_3OS_2$: C 47.79 %; H 3.61 %; N 16.72 %. Found: C 48.00 %, H 3.80 %; N 17.00 %.

(3b) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(o-tolyl)ethan-1-one. Yield 61 %, mp=147–149°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.83 (d, 1H, arom, J = 7,6 Hz), 7.39–7.41 (m, 2H, arom), 7.28 (s, 2H, NH_2), 7.18 (t, 1H, arom, J = 7,1 Hz), 4.77 (s, 2H, CH_2), 2.40 (s, 3H, CH_3) . Anal. Calc. for $C_{11}H_{11}N_3OS_2$: C 49.79 %; H 4.18 %; N 15.84 %. Found: C 50.00 %, H 4.40 %; N 16.00 %.

(3c) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(p-tolyl)ethan-1-one. Yield 54 %,

mp=166–168°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.91 (d, 2H, arom, J = 7,9 Hz), 7.36 (d, 2H, arom, J = 7,8 Hz), 7.27 (s, 2H, NH_2), 4.76 (s, 2H, CH_2), 2.40 (s, 3H, CH_3). Anal. Calc. for $C_{11}H_{11}N_3OS_2$: C 49.79 %; H 4.18 %; N 15.84 %. Found: C 49.90 %, H 4.30 %; N 16.10 %.

(3d) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-methoxyphenyl)ethan-1-one. Yield 59 %, mp=187–189°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.00 (d, 2H, arom, J = 7,5 Hz), 7.26 (s, 2H, NH_2), 7.07 (d, 2H, arom, J = 7,5 Hz), 4.74 (s, 2H, CH_2), 3.86 (s, 3H, CH_3). Anal. Calc. for $C_{11}H_{11}N_3O_2S_2$: C 46.96 %; H 3.94 %; N 14.93 %. Found: C 47.10 %, H 4.10 %; N 15.10 %.

(3e) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-fluorophenyl)ethan-1-one. Yield 64 %, mp=148–150°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.03 (d, 2H, arom, J = 7,6 Hz), 7.63 (t, 2H, arom, J = 7,7 Hz), 7.29 (s, 2H, NH_2), 4.80 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_8FN_3OS_2$: C 44.60 %; H 2.99 %; N 15.60 %. Found: C 44.60 %, H 3.10 %; N 15.80 %.

(3f) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(2,4-difluorophenyl)ethan-1-one. Yield 64 %, mp=148–150°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.98 (d, 1H, arom, J = 8,2 Hz), 7.36 (t, 1H, arom, J = 8.1 Hz), 7.24 (s, 2H, NH_2), 7.17 (t, 1H, arom, J = 8.1 Hz), 4.68 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_7F_2N_3OS_2$: C 44.60 %; H 2.99 %; N 15.60 %. Found: C 44.60 %, H 3.10 %; N 15.80 %.

(3g) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-chlorophenyl)ethan-1-one. Yield 69 %, mp=179–180°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.03 (d, 2H, arom, J = 7.7 Hz), 7.63 (d, 2H, arom, J = 7.7 Hz), 7.28 (s, 2H, NH_2), 4.79 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_8ClN_3OS_2$: C 42.03 %; H 2.82 %; N 14.70 %. Found: C 42.20 %, H 3.00 %; N 14.80 %.

(3h) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(2,4-dichlorophenyl)ethan-1-one. Yield 68 %, mp=128–130°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.82 (d, 1H, arom, J = 8.4 Hz), 7.77 (s, 1H, arom), 7.59 (d, 1H, arom, J = 8.4 Hz), 7.29 (s, 2H, NH_2), 4.65 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_7Cl_2N_3OS_2$: C 35.71 %; H 2.20 %; N 13.12 %. Found: C 35.90 %, H 2.40 %; N 13.30 %.

(3i) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-bromophenyl)ethan-1-one. Yield 71 %, mp=173–175°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.95 (d, 2H, arom, J = 7.8 Hz), 7.78 (d, 2H, arom, J = 7.7 Hz), 7.28 (s, 2H, NH_2), 4.78 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_8BrN_3OS_2$: C 36.37 %; H 2.44 %; N 12.71 %. Found: C 36.60 %, H 2.60 %; N 13.00 %.

(3j) 2-((5-Amino-1,3,4-thiadiazol-2-yl) thio)-1-(4-nitrophenyl)ethan-1-one. Yield 62 %, mp=140–142°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.03 (d, 2H, arom, J = 8.4 Hz), 7.73 (d, 2H, arom, J = 8.4 Hz), 7.29 (s, 2H, NH_2), 4.79 (s, 2H, CH_2). Anal. Calc. for $C_{10}H_8N_4O_3S_2$: C 40.53 %; H 2.72 %; N 18.91 %. Found: C 40.60 %, H 2.90 %; N 19.00 %.

Animals. All the animals used for this study were kept in standard cages and maintained under controlled laboratory conditions of temperature (22±3 °C), humidity and 12 hour day-12 hour night and had free access to food (standard pellet diet) and water ad libitum. The animals were treated humanely

throughout the study period adhering to the guideline for use and care of animals in declaration of Helsinki (National Research Council, 2011). The study protocol (№15 of April 4, 2018) was approved by the Animal Ethics Committee of the Danylo Halytsky Lviv National Medical University.

Biological Activity

Diuretic activity of the compounds **2a-f**, **3a-j** was evaluated on healthy adult albino rats weighing 160–180 g with slightly modified method [34].

The rats were divided into groups, (n=6) and placed in standard metabolic cages. Food/pellet and water were withdrawn 18 hours prior to the experiment session. The compounds **2a-f**, **3a-j**, normal saline and standard drugs were administered based on the animals' weight. The diuretic activity was measured by collecting total excreted urine of the rats kept in metabolic cages designed to separate the urine and faeces. The cages together with the funnel and measuring cylinder used in the studies were coated with liquid paraffin before each experiment to facilitate the collection of urine with minimum loss. Each animal is placed in a metabolic cage provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves are placed in the funnel to retain feaces and to allow the urine to pass. The rats were placed in metabolic cages individually as soon as the treatments started. The urine sample was collected for a total period of 24 h (urine collected initially for 20 min was discarded). All the doses were administered with the aid of an oral dosing needle. The test compounds were administered orally at a 1/10 of LD50 dose ~45 mg/kg body weight in 5 ml of the mixture of 0.5 % carboxy methyl cellulose + 0.9 % NaCl solution. Control group received 5 ml of 0.9 % NaCl solution per kilogram of body weight. The test compounds are compared with two standard diuretics, hydrochlorothiazide (10 mg/kg body weight in 5 ml of 0.5 % carboxy methyl cellulose + 0.9 % NaCl solution) and acetazolamide (45 mg/kg body weight in 5 mL of 0.5 % carboxy methyl cellulose + 0.9 % NaCl solution). For all groups of experimental animals the water loading was carried out. Drinking water in the volume of 5 % of body weight was injected in the stomach using a metal probe.

The excreted urine was collected, measured and studied for cumulative urine output, diuretic action, diuretic activity. Total urine volume was measured after 5 h and 24 h for all rats. The urinary excretion was calculated as the total urinary output divided by total liquid administered. The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic action of the diuretics. The diuretic activity was also calculated as the ratio of diuretic action of the test substances to that of the standard drugs. Prior to the start of the experiment it was decided that diuretic activity will be considered "nil", "little", "moderate", and "good", if the values were < 0.72, 0.72–1.00, 1.00–1.5, and > 1.5, respectively [35].

Urinalysis was performed using Citolab 11 Test and Citolab reader (Pharmasco Ltd., Ukraine). Electrolytes (Na^+, K^+ and Cl^-) levels in rats urine were estimated using Ion Selective Electrode (ISE) analysis (Easylyte plus Na/K/Cl analyzer, Medica Corp., USA). Dilutions of the urine samples were made as required to

bring electrolyte content in the range that can be determined by the electrolyte analyser.

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saliuretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl^-/(Na^+ + K^+)$ was calculated to estimate carbonic anhydrase inhibition [36].

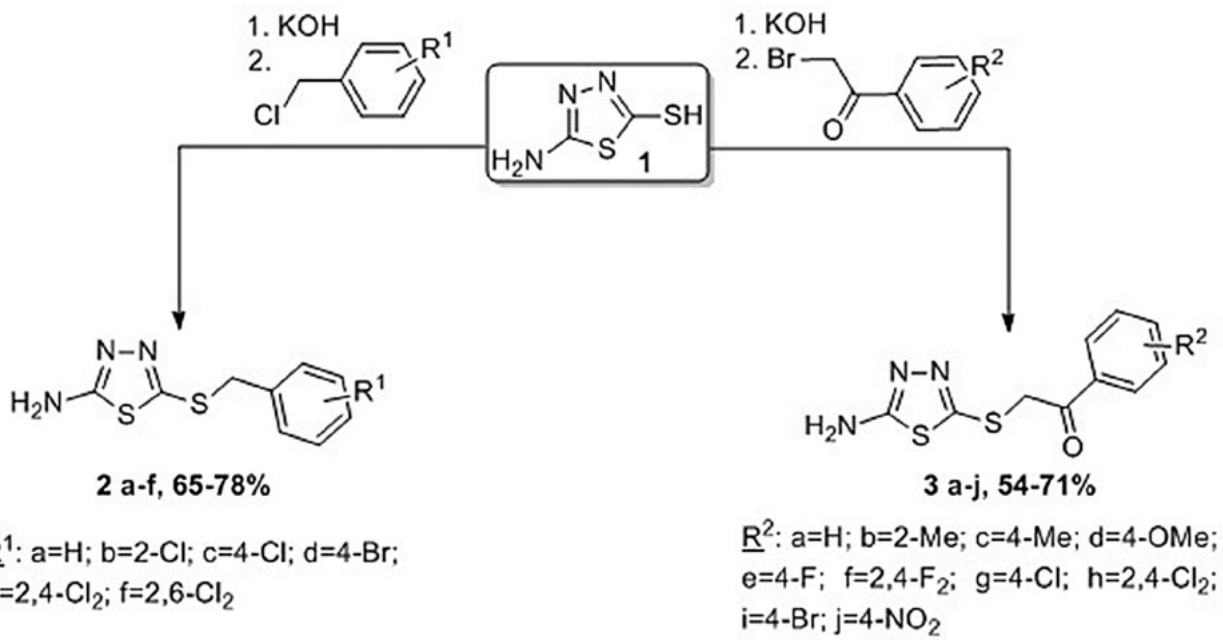
Assessment of liver function. The compounds that demonstrated excellent diuretic profile have been selected for the study. The serum collected from the groups of albino rats was used for the estimation of biochemical parameters to determine the functional state of the liver. The levels of total alkaline phosphatase (ALP), total bilirubine (Bil. total), total protein, gamma glutamyltransferase (γ-GTP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated photometrically according to the reported methods using CORMAY ACCENT-200 automatic analyzer (PZ Cormay, Poland).

Statistical analysis. All values were expressed as mean values ± SEM (standard error of mean) and data were analyzed by applying an analysis of variance (ANOVA) followed by Student's t-test. The results were considered statistically significant if $P < 0.05$.

Results and Discussion

Chemistry

The convenient and straightforward one-pot protocol was used (scheme 1) for synthesis of target derivatives **2a-f** and **3 a-j**. Initially the potassium 5-amino-1,3,4-thiadiazole-2-thiolate



Scheme 1. Synthesis of 1,3,4-thiadiazole-bearing molecules 2 a-f and 3 a-j. Reagents and conditions: 1) 1 (10 mmol), KOH (10 mmol), ethanol (10 mL), slightly heated for 5–10 min; 2) appropriate derivative of chloromethylbenzene or 2-bromo-1-phenylethan-1-one (9 mmol), KI trace, reflux 1 h.

was obtained *in situ* by heating 5-amino-1,3,4-thiadiazole-2-thiol (**1**) with ethanolic solution of potassium hydroxide. Then the appropriate derivative of chloromethylbenzene or 2-bromo-1-phenylethan-1-one was added to the reaction mixture and after reflux during 1 h the derivatives **2a-f** and **3 a-j** were obtained with satisfactory yields and purity level.

The structure of synthesized compounds was confirmed by ^{1}H NMR spectroscopy. In ^{1}H NMR spectra of compounds **2a-f** and **3 a-j** the protons signals of all the structural units were observed in their characteristic ranges.

Diuretic Activity

The *in vivo* diuretic activity and details of urine volume, the diuretic action of the synthesized compounds are summarized and presented in Fig. 2 and Tab. 1. Hydrochlorothiazide ($P<0.05$) and acetazolamide increased the total 24-hour urine volume significantly when compared with intact control rats. Overall eleven compounds (68.75 % from the total group) were more potent than the reference-drug in the parameter "total urine output" and the derivatives **2a**, **2c**, **2e**, **2f**, **3a**, **3g**, and **3h** were the most active. Moreover, the volume of excreted urine under the conditions of treatment with **2a**, **2c**, **2e** was more than two fold (from 2.14 to 2.42) higher as compared to intact control.

The level of the diuretic activity for **2a**, **2c**, **2e** (Tab. 1) could be considered as "good" compare with AAZ (the appropriate coefficient is more than1.5). If compared with HCTZ only **2c** demonstrated "good" activity level (coefficient=1.57) whereas derivatives **2a** and **2e** were less active.

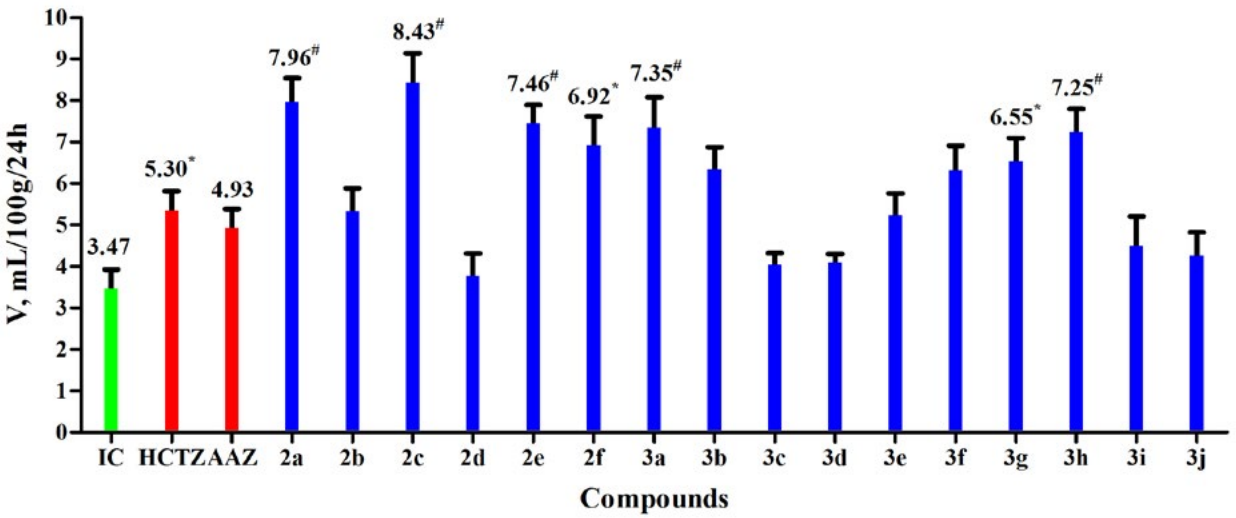


Fig. 2. Total urinary output per day (ml/100g/24h) in white rats after oral administration of compounds **2a-f**, **3a-j**. IC — values for animals from group "intact control". HCTZ — values for animals from the group receiving reference-drug hydrochlorothiazide. AAZ — values for animals from the group receiving reference-drug acetazolamide. Significant change at *P ≤0.05 and # P ≤0.001 compare with group "intact control".

Table 1. **Effect of compounds 2a, 2c, 2e on urine volume in white rats (oral administration, values are expressed as M±m)**

Groups	Urine volume, ml/100g/24h	Diuretic action[a]	Diuretic activity[b]	
			Compared to HCTZ	Compared to AAZ
IC	3.47±0.45	1.0	-	-
HCTZ	5.35±0.46*	1.54	1.00	1.08
AAZ	4.93±0.45	1.42	0.92	1.00
2a	7.96±0.58#	2.29	1.49	1.61
2c	8.43±0.71#	2.42	1.57	1.71
2e	7.46±0.43#	2.14	1.39	1.51

[a] Diuretic action=urine volume of test group/urine volume of intact control group.

[b] Diuretic activity=urine volume of test group/urine volume of reference-drug groups.

Significant change at *P≤0.05 and #P≤0.001 compare with group "intact control".

The shift of pH value to 8.0–8.5 (low alkali) was observed in urinalysis groups treated with **2a**, **2c**, **2e** compared to the intact animals where the pH was 7.1. Such pH shift is a typical pharmacokinetic feature for 1.3.4-thiadiazol-bearing diuretics [21–23]. No negative changes such as appearance of glucose, protein, nitrite, occult blood in urine, or increasing specific gravity value were observed under the treatment with **2a**, **2c**, **2e.**

The administration of the compounds **2a**, **2c**, **2e** led to a statistically significant increase in the urinary excretion of sodium, potassium, and chloride ions compared with the intact animals (Tab. 2). However, the electrolyte excretion values were lower (**2a**, **2c**) or equal to HCTZ (**2e**) in comparison with the reference drugs. Noteworthy, the derivative **2c** possesses a lower kaliuretic property (K^+index=1.68) than the reference drugs and derivatives **2a**, **2e**, which is an important pharmacological feature.

The reference drugs and compounds **2a**, **2c**, **2e** showed potent saluretic activity compared to intact control animals (Tab. 3). However, no impact on the ratio Na^+/K^+ (natriuretic ef-

Table 2. **Effect of compounds 2a, 2c, 2e on urinary electrolyte excretion in white rats (oral administration, values are expressed as M±m)**

Groups	Urinary Na^+(mmol/L)	Urinary K^+(mmol/L)	Urinary Cl^-(mmol/L)	Na^+ index[a]	K^+ index[a]	Cl^- index[a]
IC	98.45±8.79	54.12±5.03	88.46±7.34	1.00	1.00	1.00
HCTZ	191.84±16.35#	105.27±11.41*	129.36±15.71*	1.95	1.95	1.46
AAZ	203.52±29.38#	114.43±18.57*	134.58±21.60	2.07	2.11	1.52
2a	185.16±23.62#	100.39±16.75*	117.93±24.28	1.88	1.85	1.33
2c	179.44±14.89#	91.18±8.30*	103.25±14.91	1.82	1.68	1.16
2e	190.34±26.73#	108.52±19.83*	121.83±26.59	1.93	2.00	1.37

[a] Index=excretion in test group/excretion in intact control group.

Significant change at *P≤0.05 and #P≤0.001 compare with group "intact control"

Table 3. **Impact of compounds 2a, 2c, 2e on saluretic and natriuretic effects, carbonic anhydrase inhibition in white rats (oral administration, values are expressed as M±m)**

Groups	Saluretic effect (Na^++Cl^-)	Natriuretic effect (Na^+/K^+)	CAI[a], Cl^-/ (Na^++K^+)	Saluretic index[b]	Natriuretic index[b]	CAI index[b]
IC	186.91±16.03	1.82±0.23	0.58±0.02	1.00	1.00	1.00
HCTZ	321.20±27.76#	1.83±0.21	0.44±0.02	1.69	1.01	0.76
AAZ	338.10±50.98	1.78±0.20	0.42±0.02	1.80	0.98	0.72
2a	303.09±47.90	1.84±0.21	0.41±0.02	1.62	1.01	0.71
2c	282.69±29.80	1.97±0.26	0.38±0.02	1.51	1.08	0.66
2e	312.17±43.32	1.78±0.22	0.41±0.02	1.67	0.98	0.71

[a] CAI - carbonic anhydrase inhibition.

[b] Index=excretion in test group/excretion in intact control group.

Significant change at *P≤0.05 and #P≤0.001 compared with intact control

Table 4. **The liver enzymes activity in rats treated with compounds 2a, 2c, 2e (oral administration, M±m, n=6 in each group)**

Groups	ALT, U/L	AST, U/ L	ALP, U/L	γ-GGT, IU/L	Bil. total, mg/L	Protein total, g/L
IC	71.3±8.0	164.5±17.3	392.5±25.0	4.0±0.5	5.1±1.1	74.7±6.4
2a	84.6±7.3	187.3±22.7	414.7±30.9	4.2±0.8	5.0±1.0	70.1±5.3
2c	72.1±4.5	161.8±14.7	397.4±17.8	3.9±1.0	5.2±0.8	75.6±6.0
2e	79.1±6.8	177.7±20.9	405.2±28.9	4.0±0.6	5.1±0.9	71.8±4.4

Bil. total - bilirubin total

fect) was observed in the experimental conditions and the natriuretic index was ~1.0 like for the intact control animals as well as for all treated groups. Accordingly, the calculated data of CAI index for compounds **2a**, **2c**, **2e** indicate the equivalent or lower carbonic anhydrase inhibition compared with acetazolamide.

The extent of hepatic damage after administration of compounds **2a**, **2c**, **2e** was assessed by the level of liver function biochemical parameters (Tab. 4). The estimation revealed that there was no significant increase in the ALS, AST, ALP, γ-GGT and total bilirubin levels. There is an insignificant decrease in protein level in serum for **2a** and **2e** as compared with the intact control level. It was clearly indicated that none of the compounds showed any toxicity on the liver as compared with intact control.

The conducted *in vivo* diuretic studies suggested the following structure – diuretic activity relationships (Fig. 3). The 5-benzylthio-1,3,4-thiadiazol-2-amine derivatives **2 a-f** were found more active than appropriate 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one **3 a-j**.

The unsubstituted phenyl group or the substituted one with a chlorine atom at the position 4 was the most favorable for the activity. The isosteric replacement of chlorine atom by the bromine or methyl- and nitro-groups leads to a drastic decrease in the activity. The change of the chlorine atom position from 4 to 2 sim-

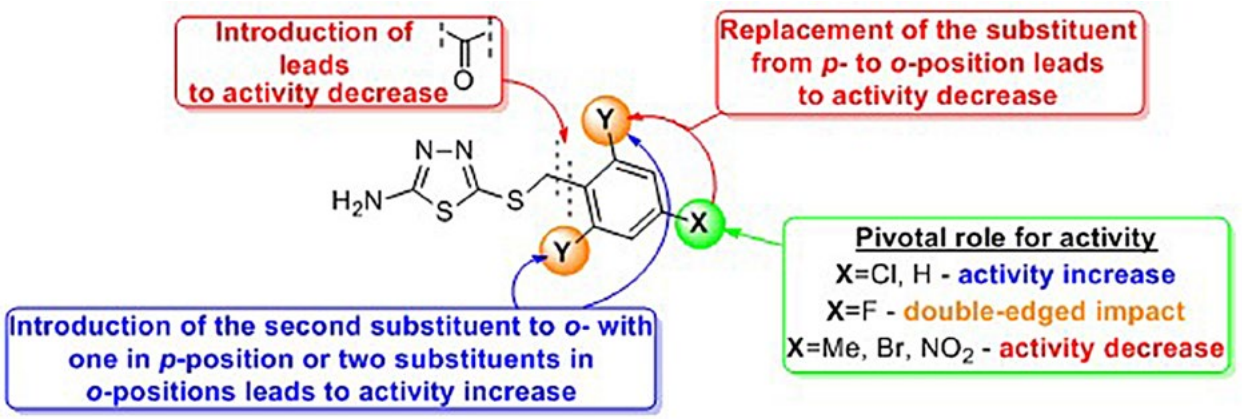


Fig. 3. Structure – diuretic activity relationships for synthesized 1,3,4-thiadiazol-bearing molecules **2 a-f** and **3 a-j.**

ilarly leads to disappearance of the activity. However, the additional introduction of second chlorine atom provides the diuretic activity. The presence of a fluorine atom is ambiguous for the diuretic activity, but in general, its contribution can be assessed as positive and the design of new fluorine-substituted derivatives can be considered as a possible direction for obtaining new potent diuretics among this class of compounds.

Conclusions

To summarize, in an attempt to obtain an efficacious and non-toxic diuretic, we have designed and synthesized a series of 1,3,4-thiadiazole-bearing derivatives. Some derivatives have demonstrated a high level of diuretic action with satisfactory kaliuretic, saluretic, and natriuretic properties. Additional research on the mechanisms of activity of these compounds and modification is underway. The results obtained from the *in vivo* diuretic studies demonstrate the potential of searching for diuretic agents among 5-benzylthio-1,3,4-thiadiazol-2-amine derivatives.

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Синтез та діуретична активність нових похідних 5-аміно-1,3,4-тіадіазол-2-тіолу

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Мета. Синтез, *in vivo* дослідження та характеристика діуретичної активності нових похідних 5-аміно-1,3,4-тіадіазол-2-тіолу. **Методи.** Органічний синтез (однореакторні реакції). ^{1}H ЯМР-спектроскопія. Біологічні методи: *in vivo* дослідження на білих щурах; загальний аналіз сечі; індекси салуретичної/натрійуретичної активності та інгібування карбоангідрази; біохімічні лабораторні дослідження (визначення рівнів АЛТ, АСТ, ЛФ, γ-ГГТ, загального білірубіну та білка). **Результати.** Синтезовано серію похідних 5-аміно-1,3,4-тіадіазол-2-тіолу з використанням ефективного однореакторного підходу. Усі сполуки досліджувались на предмет діуретичної активності шляхом оцінки загального добового діурезу та профілю загального аналізу сечі. Ідентифіковано 5-бензилтіо-1,3,4-тіадіазол-2-аміни 2a, 2c та 2e як потенційні діуретини, для яких були досліджені калійуретичні, салуретичні та натрійуретичні властивості, а також було оцінено їх здатність інгібувати карбоангідразу. Сформульовано взаємозв'язоки структура - діуретична дія. **Висновки.** Синтезовано серію похідних 1,3,4-тіадіазолу, серед яких ідентифіковано сполуки з високим рівнем діуретичної дії та задовільними каліуретичними, салуретичними та натрійуретичними властивостями.

Ключові слова: похідні 5-аміно-1,3,4-тіадіазол-2-тіолу; сечогінна активність; салуретична/натрійуретична активність; інгібування карбоангідрази; залежність структура-активність

Синтез и диуретическая активность новых производных 5-амино-1,3,4-тиадиазол-2-тиола

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Цель. Синтез, *in vivo* исследование и характеристика диуретической активности новых производных 5-амино-1,3,4-тиадиазол-2-тиола. **Методы.** Органический синтез (однореакторные реакции). ^{1}H ЯМР-спектроскопия. Биологические методы: *in vivo* исследование на белых крысах; общий анализ мочи; индексы салуретической, натрийуретической активности и ингибирования карбоангидразы; биохимические лабораторные исследования (определение уровней АЛТ, АСТ, ЩФ, γ-ГГТ, общего билирубина и белка). **Результаты.** Синтезирована серия производных 5-ами-

но-1,3,4-тиадиазол-2-тиола с использованием эффективного однореакторного подхода. Все соединения исследовались на предмет диуретической активности путем оценки общего суточного диуреза и профиля общего анализа мочи. Идентифицированы 5-бензилтио-1,3,4-тиадиазол-2-амины 2a, 2c и 2e как потенциальные диуретики для которых были исследованы при калийуретические, салуретические и натрийуретические свойства, а также было оценена их способность ингибировать карбоангидразу. Сформулировано завсисимости структура - диуретическое действие. **Выводы.** Синтезирована серия производных 1,3,4-тиадиазола, среди которой идентифицированы соединения с высоким уровнем диуретического действия и удовлетворительными калиуретическими, салуретическими и натрийуретическими свойствами.

Ключевые слова: производные 5-амино-1,3,4-тиадиазол-2-тиола; диуретическая активность; салуретическая/натрийуретическая активность; ингибирование карбоангидразы; зависимость структура-активность

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