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

I. V. Drapak¹, B. S. Zimenkovsky¹, M. V. Slabyy¹, S. M. Holota¹,², L. O. Perekhoda³, R. V. Yaremkevych¹, I. O. Nektegayev ¹

¹ Danylo Halytsky Lviv National Medical University
69, Pekarska Str., Lviv, Ukraine, 79010
² Lesya Ukrainka Volyn National University
13, Voli Ave., Lutsk, Ukraine, 43025
³ National University of Pharmacy
53, Pushkinska Str., Kharkiv, Ukraine, 61002
iradrapak@ukr.net

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). ¹H 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.

Keywords: 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–
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-thiadiazole 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-aminoo-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-<em>d</em><sub>6</sub>. Melting points were measured in open capillary tubes and are uncorrected. The purity of the compounds was checked by thin-layer chromatography per-
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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.26–7.36 (m, 7Н, NH\textsubscript{2}+arom), 4.29 (s, 2Н, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{9}N\textsubscript{3}S\textsubscript{2}: С 48.41 %; H 4.06 %; N 18.82 %. Found: С 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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.26–7.33 (m, 4Н, NH\textsubscript{2}+arom), 7.40–7.47 (m, 2Н, arom) 4.35 (s, 2Н, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{8}ClN\textsubscript{3}S\textsubscript{2}: С 41.94 %; H 3.13 %; N 16.30 %. Found: С 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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.25–7.48 (m, 4Н, NH\textsubscript{2}+arom), 4.28 (s, 2Н, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{8}ClN\textsubscript{3}S\textsubscript{2}: С 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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.50 (d, 2Н, arom, J = 8.8 Hz), 7.28–7.32 (m, 4Н, NH\textsubscript{2}+arom), 4.26 (s, 2H, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{8}BrN\textsubscript{3}S\textsubscript{2}: С 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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.63 (s, 1H, arom), 7.34–7.43 (m, 4Н, NH\textsubscript{2}+ arom), 4.33 (s, 2H, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{7}Cl\textsubscript{2}N\textsubscript{3}S\textsubscript{2}: С 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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.33–7.49 (m, 5H, NH\textsubscript{2}+ arom), 4.44 (s, 2H, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{9}H\textsubscript{7}Cl\textsubscript{2}N\textsubscript{3}S\textsubscript{2}: С 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=168–172°C. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 8.02 (d, 2Н, arom, J = 7.6 Hz), 7.69 (t, 1Н, arom , J = 7,3Hz), 7.56 (t, 2H, arom, J = 7,4 Hz), 7.27 (s, 2H, NH\textsubscript{2}), 4.81 (s, 2Н, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{10}H\textsubscript{9}N\textsubscript{3}S\textsubscript{2}O\textsubscript{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. 1H NMR (400 MHz, DMSO-\textit{d}_6, \delta): 7.83 (d, 1Н, 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\textsubscript{2}), 4.81 (s, 2H, CH\textsubscript{2}). Anal. Calc. for C\textsubscript{11}H\textsubscript{11}N\textsubscript{3}S\textsubscript{2}O\textsubscript{2}: C 47.79 %; H 3.61 %; N 16.72 %. Found: C 48.00 %, H 3.80 %; N 17.00 %.

(3c) 2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-(p-tolyl)ethan-1-one. Yield 54 %,
mp=166–168°C. ¹H 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$_3$OS$_2$: 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. ¹H 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$_3$O$_2$S$_2$: 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. ¹H NMR (400 MHz, DMSO-$_d_6$, δ): 8.03 (d, 2H, arom, $J = 7.6$ Hz), 7.63 (t, 2H, arom, $J = 8.1$ Hz), 7.29 (s, 2H, NH$_2$), 4.80 (s, 2H, CH$_2$). Anal. Calc. for C$_{10}$H$_8$FN$_3$OS$_2$: C 44.60 %; H 3.94 %; N 14.93 %. Found: C 44.70 %, H 4.10 %; N 15.10 %.

(3f) 2-((5-Amino-1,3,4-thiadiazol-2-yl)thio)-1-(2,4-difluorophenyl)ethan-1-one. Yield 64 %, mp=148–150°C. ¹H 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$_7$F$_2$N$_3$OS$_2$: 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. ¹H 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$_8$ClN$_3$OS$_2$: 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. ¹H 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$_7$Cl$_2$N$_3$OS$_2$: 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. ¹H NMR (400 MHz, DMSO-$_d_6$, δ): 7.95 (d, 2H, arom, $J = 7.7$ Hz), 7.78 (d, 2H, arom, $J = 7.7$ Hz), 7.29 (s, 2H, NH$_2$), 4.79 (s, 2H, CH$_2$). Anal. Calc. for C$_{10}$H$_8$BrN$_3$OS$_2$: C 36.37 %; H 2.44 %; N 12.71 %. Found: C 36.60 %, H 2.60 %; N 13.00 %.

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

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 faeces 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 (Easylite 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 \( \text{Na}^+ \) and \( \text{Cl}^- \) urinary excretion was calculated as a parameter of saluretic activity. The ratio \( \text{Na}^+ / \text{K}^+ \) was calculated for natriuretic activity. The ratio \( \text{Cl}^-/(\text{Na}^++\text{K}^+) \) 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 (\( \gamma \)-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 3a-j. Initially the potassium 5-amino-1,3,4-thiadiazole-2-thiolate (Scheme 1). Synthesis of 1,3,4-thiadiazole-bearing molecules 2a-f and 3a-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.

\[
\begin{align*}
&1. \text{KOH} \\
&2. \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^1 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^2 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^3 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^4 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^5 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^6 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^7 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^8 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^9 \quad \text{Cl} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{N} \quad \text{S} \\
&\text{H}_2\text{N} \quad \text{S} \\
&\text{R}^{10} \quad \text{Cl} \\
\end{align*}
\]

**Scheme 1.** Synthesis of 1,3,4-thiadiazole-bearing molecules 2a-f and 3a-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 3a-j were obtained with satisfactory yields and purity level.

The structure of synthesized compounds was confirmed by 1H NMR spectroscopy. In 1H NMR spectra of compounds 2a-f and 3a-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”.](image-url)
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 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 actiona | Diuretic activityb 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”.

### 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) | \(Na^+\) indexa | \(K^+\) indexa | \(Cl^-\) indexa |
|--------|--------------------------|-------------------------|--------------------------|----------------|---------------|----------------|
| 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”.
Synthesis and diuretic activity of novel 5-amino-1,3,4-thiadiazole-2-thiol derivatives

...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 2a-f were found more active than appropriate 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one 3a-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-

---

**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<sup>+</sup>Cl<sup>-</sup>) | Natriuretic effect (Na<sup>+</sup>/K<sup>-</sup>) | CAI<sub>a</sub>, Cl<sup>-</sup>/(Na<sup>+</sup>)/K<sup>-</sup>) | Saluretic index<sub>b</sub> | Natriuretic index<sub>b</sub> | CAI index<sub>b</sub> |
|--------|---------------------------------------------|-----------------------------------------------|---------------------------------|------------------------|------------------------|---------------------|
| IC     | 186.91±16.03                               | 1.82±0.23                                    | 0.58±0.02                       | 1.00                   | 1.00                   | 1.00                |
| HCTZ   | 321.20±27.76<sup>#</sup>                  | 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                |

<sup>a</sup> CAI - carbonic anhydrase inhibition.<br><sup>b</sup> 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
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.

**REFERENCES**

1. Titko T, Perekhoda L, Drapak I, Tsapko Y. Modern trends in diuretics development. Eur J Med Chem. 2020;208:112855.
2. Williams B. Drug discovery in renin-angiotensin system intervention: past and future. Ther Adv Cardiovasc Dis. 2016;10(3):118–25.
3. Blowey DL. Diuretics in the treatment of hypertension. Pediatr Nephrol. 2016;31(12):2223–2233.
4. Islam MS. The Art and Science of Using Diuretics in the Treatment of Heart Failure in Diverse Clinical Settings. Adv Exp Med Biol. 2018;1067:47–65.
5. Maa EH, Kahle KT, Walcott BP, Spitz MC, Stanley KJ. Diuretics and epilepsy: will the past and present meet? Epilepsia. 2011;52(9):1559–69.
6. Supuran CT. Applications of carbonic anhydrases inhibitors in renal and central nervous system diseases. Expert Opin Ther Pat. 2018;28(10):713–721.
7. Cabandugama PK, Gardner MJ, Sowers JR. The renin angiotensin aldosterone system in obesity and hypertension: roles in the cardiorenal metabolic syndrome. Med Clin North Am. 2017;101(1):129–137.
8. Cherkas A, Golota S. An intermittent exhaustion of the pool of glycogen in the human organism as a simple universal health promoting mechanism. Med Hypotheses. 2014;82(3):387–9.
9. Pericleous M, Sarnowski A, Moore A, Fijten R, Zaman M. The clinical management of abdominal

---

**Fig. 3.** Structure – diuretic activity relationships for synthesized 1,3,4-thiadiazol-bearing molecules 2 a-f and 3 a-j.
ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: a review of current guidelines and recommendations. Eur J Gastroenterol Hepatol. 2016;28(3):e10–8.

10. Cherkas A, Golota S, Guéraud F, Abrahamovych O, Pichler C, Nersesyan A, Krupak V, Bugiichyk V, Yatskevych O, Pliatsko M, Eckl P, Knasmüller S. A Helicobacter pylori-associated insulin resistance in asymptomatic sedentary young men does not correlate with inflammatory markers and urine levels of 8-iso-PGF2α or 1,4-dihydroxynonane mercapturic acid. Arch Physiol Biochem. 2018;124(3): 275–285.

11. Cherkas A, Eckl P, Gueraud F, Abrahamovych O, Serhiyenko V, Yatskevych O, Pliatsko M, Golota S. Helicobacter pylori in sedentary men is linked to higher heart rate, sympathetic activity, and insulin resistance but not inflammation or oxidative stress. Croat Med J. 2016;57(2):141–9.

12. Bartoli E, Rossi L, Sola D, Castello L, Sainaghi PP, Smirne C. Use, misuse and abuse of diuretics. Eur J Intern Med. 2017;39:9–17.

13. Wile D. Diuretics: a review. Ann Clin Biochem. 2012;49(Pt 5):419–31.

14. Sister PM. Vergessene metabolische Nebenwirkungen der Diuretika: Lipid-, Glukose- und Vitamin B1-Stoffwechsel [Forgotten metabolic side effects of diuretics: lipids, glucose and vitamin B1 (thiamin) metabolism]. Praxis (Bern 1994). 2004;93(20): 857–63.

15. Ukrainets IV, Bereznyakova NL. Heterocyclic diuretics. Chem Heterocycl Comp. 2012;48: 155–65.

16. Li Y, Geng J, Liu Y, Yu S, Zhao G. Thiadiazole-a promising structure in medicinal chemistry. ChemMedChem. 2013;8(1):27–41.

17. Haider S, Alan MS, Hamid H. 1,3,4-Thiadiazoles: a potent multi targeted pharmacological scaffold. Eur J Med Chem. 2015;92:156–77.

18. Hu Y, Li CY, Wang XM, Yang YH, Zhu HL. 1,3,4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry. Chem Rev. 2014;114(10):5572–610.

19. Matysiak J. Biological and pharmacological activities of 1,3,4-thiadiazole based compounds. Mini Rev Med Chem. 2015;15(9):762–75.

20. Wongboonsin J, Thongprayoon C, Bathini T, Ungprasert P, Aeedulla NR, Mao MA, Cheungpasitporn W. Acetazolamide therapy in patients with heart failure: a meta-analysis. J Clin Med. 2019;8(3):349.

21. Fossati S, Giannoni P, Ssolesio ME, Cocklin SL, Cabrera E, Ghiso J, Rostagno A. The carbonic anhydrase inhibitor methazolamide prevents amyloid beta-induced mitochondrial dysfunction and caspase activation protecting neuronal and glial cells in vitro and in the mouse brain. Neurobiol Dis. 2016;86:29–40.

22. Diaz JR, Cami GE, Liu-González M, Vega DR, Vullo D, Juárez A, Pedregosa JC, Supuran CT. Salts of 5-amino-2-sulfonamide-1,3,4-thiadiazole, a structural and analog of acetazolamide, show interesting carbonic anhydrase inhibitory properties, diuretic, and anticonvulsant action. J Enzyme Inhib Med Chem. 2016;31(6):1102–10.

23. Biswas S, Carta F, Scozzafava A, McKenna R, Supuran CT. Structural effect of phenyl ring compared to thiadiazole based adamantyl-sulfonamides on carbonic anhydrase inhibition. Bioorg Med Chem. 2013;21(8):2314–2318.

24. Iqbal J, Al-Rashida M, Durdagi S, Alterio V, Di Fiore A. Recent developments of carbonic anhydrase inhibitors as potential drugs. Biomed Res Int. 2015;2015:174178.

25. Lomelino C, McKenna R. Carbonic anhydrase inhibitors: a review on the progress of patent literature (2011-2016). Expert Opin Ther Pat. 2016;26(8):947–56.

26. Supuran CT. Carbonic anhydrase activators. Future Med Chem. 2018;10(5):561-573. doi: 10.4155/fmc-2017-0223.

27. Das Mahapatra A, Queen A, Yousuf M, Khan P, Hussain A, Rehman MT, Alajmi MF, Datta B, Hassan MI. Design and development of 5-(4H)-oxazolones as potential inhibitors of human carbonic anhydrase VA: towards therapeutic management of diabetes and obesity. J Biomol Struct Dyn. 2020:1–11.

28. Supuran CT, Winum JY. Designing carbonic anhydrase inhibitors for the treatment of breast cancer. Expert Opin Drug Discov. 2015;10(6):591–7.

29. Sych IV, Drapak IV, Suleiman MM, Rakhimova MV, Kobzar NP, Sych IA, Perekhoda LO. Search for
Biologically Active Substances with Antimicrobial and Antifungal Action in the series of 2,5-disubstituted 1,3,4-tdiazoles. Res J Pharm Tech, 2019; 12(6):1–6.

30. Drapak IV, Perekhoda LO, Vynogradova O, Protopopov MV, Sych IA, Kiz OV. The use of the docking studies with the purpose of searching potential cardioprotectors among derivatives of 2-imino-1,3-thiazoline and 2,5-disubstituted derivatives of 1,3,4-thiadiazole. Pharmacia. 2018; 65(2):40–6.

31. Drapak IV. Pharmacophore modeling of diuretic activity of 1,3-tiazole and 1,3,4-thiadiazole derivatives. Pharmacol Drug Toxicol. 2019; 13(3): 197–202.

32. Holota SM, Derkach GO, Zasidko VV, Trokhymchuk VV, Furdychko LO, Demchuk IL, Semenciv GM, Soronovych II, Kutsyk RV, Lesyk RB. Features of antimicrobial activity of some 5-aminemethylene-2-thioxo-4-thiazolidinones. Biopolym Cell. 2019; 35(5):371–80.

33. Golota S, Sydorenko I, Surma R, Karpenko O, Gzelala A, Lesyk R. Facile one-pot synthesis of 5-aryl/heterylidene-2-(2-hydroxyethyl- and 3-hydroxypropy lamino)-thiazol-4-ones via catalytic aminolysis. Synth Comm. 2017; 47(11):1071–6.

34. Berkhin EB. Methods for studying the effect of new chemical compounds on renal function. Chem Pharm J. 1977;17(5):3–11.

35. Hailu W, Engidawork E. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of Ajuga remota Benth (Lamiaceae) leaves in mice. BMC Complement Altern Med. 2014; 14:135.

36. Somova LI, Shode FO, Ramnanan P, Nadar A. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies africana leaves. J Ethnopharmacol. 2003;84(2–3):299–305.

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

І. В. Драпак, Б. С. Зіменковський, М. В. Слабій, С. Н. Голота, Л. А. Перехода, Р. В. Яремкевич, І. О Нектегаев

**Мета.** Синтез, in vivo дослідження та характеристика діуретичної активності нових похідних 5-аміно-1,3,4-тиадіазол-2-тіолу. Методи. Органічний синтез (однореакторні реакції). ЯМР-спектроскопія. Біологічні методи: in vivo дослідження на білих щурах; загальний аналіз сечі; індекси салуретичної та натрійуретичної активності та інгібування карбоангідрази; біохімічні лабораторні дослідження (визначення рівнів АЛТ, АСТ, ЩФ, γ-ГТТ, загального білірубіну та білка). Результати. Синтезовано серію похідних 5-аміно-1,3,4-тиадіазол-2-тіолу використанням ефективного однореакторного підходу. Усі сполуки досліджувались на предмет діуретичної активності шляхом визначення загального добового діурезу та профілю загального аналізу сечі. Ідентифікувався значний вплив карбоангідрази на карбоангідразу. Сформульовано взаємозв’язок структура - діуретична дія. Висновки. Синтезовано серію похідних 1,3,4-тиадіазолу, серед яких ідентифіковано сполуки з високим рівнем діуретичної дії та задовільними каліуретичними, салуретичними та натрійуретичними властивостями.

**Ключові слова:** похідні 5-аміно-1,3,4-тиадіазол-2-тіолу; сечогінна активність; салуретична/натрійуретична активність; інгібування карбоангідрази; залежність структура-активність
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; ингибитор карбоангидразы; зависимость структура-активность

Received 20.11.2020