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DIRECTED SEARCH FOR COMPOUNDS THAT AFFECT THE EXCRETORY FUNCTION OF RAT KIDNEYS, AMONG NEW CYCLOALKYLCARBONYL THIOUREAS AND THIOSEMICARBAZIDES DERIVATIVES

Introduction. *Prolonged usage of diuretics, especially in large doses, the number and severity of side effects (water-electrolyte and metabolic disorders), as well as the relatively limited range of existing diuretics dictate the need to find new compounds that would have diuretic effects, simple production technology and would be safer.*

The aim of the study *– directed search for diuretics among unknown disubstituted thioureas and thiosemicarbazides using the molecular docking methodology as the way to explain the probable mechanism of action.*

Research Methods. *The structures of the target compounds have been proposed using drug-design approaches, namely the introduction of structural fragments into thioureas and thiosemicarbazides, which are characteristic of known diuretics. The synthesis of substituted cycloalkylcarbonylthioureas or thiosemicarbazides was carried out by a single-reactor method using cycloalkylcarbonyl chlorides, ammonium isothiocyanate and substituted anilines or carboxylic acid hydrazides. The structure of the synthesized compounds was proved by IR, ^{1}H NMR spectroscopy, chromato-mass spectrometry and elemental analysis. Probable molecular mechanisms of activity were predicted due to molecular docking. Directed search for compounds that affect the excretory function of the kidneys of rats was conducted by the conventional method of E.B. Berkhin with water load.*

Results and Discussion. *It has been found that the single-reaction of cycloalkylcarbonyl chlorides with an equimolecular amount of ammonium isothiocyanate and substituted anilines or carboxylic acid hydrazides resulted in formation of substituted cycloalkylcarbonylthioureas or thiosemicarbazides. The structure of the synthesized compounds was discussed using IR, ^{1}H NMR and chromato-mass spectra. Studies of the effect of the synthesized compounds on the excretory function of rats kidneys under water load have revealed several compounds that exceed the diuretic effect of "Furosemide" and compete with "Hydrochlorothiazide". Molecular docking results have shown that the test compounds demonstrated a high affinity for carbonic anhydrase II and similar binding sites to reference drugs. This indicates their probable mechanism of action.*

Conclusion. *The developed and implemented strategy for searching of diuretics among cycloalkylcarbonyl thioureas and thiosemicarbazides derivatives has allowed to identify an effective compound (**3.2**), which is close to the reference drug "Hydrochlorothiazide" in terms of diuretic effect. Importantly, according to the results of molecular docking, the synthesized compounds as well as the reference drugs have a similar mechanism of action (carbonic anhydrase II inhibitors). It is likely, that the expressed diuretic effect of several compounds is related to the ability of substituted thioureas to form coordination bonds with the zinc cation in the active site of CA II. The obtained results substantiate the further purposeful search for potential diuretics among this class of compounds.*

KEY WORDS: **synthesis; cycloalkylcarbonyl thioureas and thiosemicarbazides; spectral data; molecular docking; carbonic anhydrase II; diuretic activity.**

INTRODUCTION. Therapy with sulfur-containing drugs plays an important role in the development of the pharmaceutical industry [1]. Sulfur-containing components are a part of medicines and native products. More than 350 drugs containing sulfur are known in international practice. For example, the most common are sulfonamides, thioesters, sulfones and penicillins, which have been deeply studied and used for several decades [2]. Sulfur-containing derivatives include several diuretics, namely thiazide diuretics (hydrochlorothiazide, cyclomethiazide), loop diuretics (furosemide, clopamide), carbonic anhydrase inhibitors (diacarb), which contain sulfamide group. And they are widely used in medical practice in case of hypertension, chronic heart failure, chronic kidney disease, glaucoma, emergencies (swelling of the brain, lungs etc.) [3]. Recently, their mechanism of

action as nonselective carbonic anhydrase inhibitors has been discussed [3, 4]. For example, thiazide and loop diuretics catalyze the conversion reaction between carbon dioxide and bicarbonate ions, which reduces the availability of hydrogen ions for active transport in the lumen of the renal tubules, alkaline urine and increases the excretion of bicarbonate, sodium ions, potassium, water, ammonia reabsorption. However, long-term usage of diuretics, especially in large doses, the number and severity of side effects (water-electrolyte and metabolic disorders) [3, 5], as well as the relatively limited range of existing diuretics dictate the need to find new compounds that would have diuretic effects, simple production technology and would be safer.

In turn, we paid attention to thiourea derivatives, as they are the starting compounds for the synthesis of various sulfur-containing heterocycles [6, 7], which are used as an effective drugs [8], and show high biological activity (antibacterial, antitumoral, antithyroid, anticonvulsant, anti-inflammatory, antiallergic, antioxidant and antihypertensive activity, etc.) [9–12]. Our strategy for the targeted search for compounds that would affect the excretory function of the kidneys was as following: the synthesis of substituted cycloalkylcarbonylisothiocyan ates with different cycle sizes (cyclopropane, cyclobutane, cyclopentane and cyclohexane) and the introduction to specified molecules of functional fragments that are characteristic of known diuretics (sulfamide, carboxyl groups, chlorine and others). It is also important, that thioureas and its derivatives are characterized by prototropic tautomerism and the ability to complexation [13]. Therefore, the ability to complexation may be one of the aspects of the manifestation of diuretic activity, because for some drugs complexes involving the zinc cation of carbonic anhydrase (CA II) were described [4, 14].

The aim of the study. Thus, the aim of the work is the search of diuretics among unknown thioureas and thiosemicarbazides using molecular docking methodology to explain the probable mechanism of action.

RESEARCH METHODS. Melting points were determined in open capillary tubes in a “Mettler Toledo MP 50” apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ^{1}H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) spectrometers with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high-performance liquid chromatography “Agilent 1100 Series” (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector “Agilent LC/MSD SL” (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA).

Cycloalkylcarbonyl chlorides (**1.1–1.3**) were synthesized by known method [15]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

*The general method of N-(R-phenylcarbamothioyl)cycloalkylcarboxamides **(2.1–2.10)** synthesis.* To the solution of corresponding 0.01 mol of cycloalkylcarbonyl chlorides *(**1.1–1.3**)* in 20 mL of acetonitrile 0.76 g (0.01 mol) of ammonium isothiocyanate was added and stirred at 80 °C for 30 min. The mixture was cooled down to r.t. and 0.01 mol of corresponding aniline was added and stirred at 80 °C for 90 min. The solution was cooled down, poured into the water and formed precipitate is filtrated, dried and recrystallized from methanol.

*N-((2-Methoxyphenyl)carbamothioyl)cyclopropanecarboxamide **(2.1).*** Yield: 80.0 %; Mp.:186-190 °C; IR (cm^{-1}): 3744 (ν_{NH}), 3255 (ν_{NH}), 1666 (ν_{CO}), 1530 (δ_{NH}), 1397, 1226, 1168, 1026, 925, 745, 678; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H, -N*H*C(S)-), 11.48 (s, 1H, -N*H*C(O)-), 8.63 (d, *J* = 8.0 Hz, 1H, Ar H-6), 7.13 (t, *J* = 7.9 Hz, 1H, Ar H-4), 6.98 (d, *J* = 8.2 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.8 Hz, 1H, Ar H-5), 3.89 (s, 3H, CH_3),), 2.13 (tt, *J* = 7.9, 4.5 Hz, 1H, cyclopropyl H-1), 1.01-0.88 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 251 [M+1]; Anal. Calcd. for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19; S, 12.81; Found: C, 57.62; H, 5.69; N, 11.24; S, 12.85.

*N-((2-Chlorophenyl)carbamothioyl)cyclopropanecarboxamide **(2.2).*** Yield: 77.2 %; Mp.:160-161 °C; IR (cm^{-1}): 3233 (ν_{NH}), 3144 (ν_{NH}), 1659 (ν_{CO}), 1515 (δ_{NH}), 1396, 1316, 1239, 1176, 1097, 1031, 927, 741, 672; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H, -N*H*C(S)-), 11.74 (s, 1H, -N*H*C(O)-), 8.22 (dd, *J* = 8.2, 1.5 Hz, 1H, Ar H-6), 7.41 (d, *J* = 7.9 Hz, 1H, Ar H-3), 7.33-7.24 (m, 1H, Ar H-5), 7.18 (t, *J* = 9.3 Hz, 1H, Ar H-4), 2.11 (tt, *J* = 7.9, 4.5 Hz, 1H, cyclopropyl H-1), 1.03-0.77 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 255 [M+1]; Anal. Calcd. for $C_{11}H_{11}ClN_2OS$: C, 51.87; H, 4.35; N, 11.00; S, 12.59; Found: C, 51.92; H, 4.41; N, 11.07; S, 12.63.

*3-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid **(2.3).*** Yield: 66.x0 %; Mp.:223-228 °C; IR (cm^{-1}): 3120 (ν_{NH}), 3007 (ν_{NH}), 1681 (ν_{CO}), 1524 (δ_{NH}),

1452, 1298, 1250, 1164, 942, 696, 666, 619; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H, -C(S)N<u>H</u>-), 11.67 (s, 1H, -C(O)N<u>H</u>-), 8.14 (t, J = 1.9 Hz, 1H, Ar H-2), 7.85-7.74 (m, 2H, Ar H-4, 6), 7.41 (t, J = 7.9 Hz, 1H, Ar H-5), 2.14-2.05 (m, 1H, cyclopropyl H-1), 1.00-0.86 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13; Found: C, 54.59; H, 4.67; N, 10.69; S, 12.18.

4-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid ***(2.4).*** Yield: 73.8 %; Mp.:230-233 °C; IR (cm^{-1}): 3121 (ν_{NH}), 3004 (ν_{NH}), 2986 (ν_{CH2}), 1679 (ν_{CO}), 1512 (δ_{NH}), 1288, 1252, 1157, 860, 778, 742, 728, 694; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H, -C(S)N<u>H</u>-), 11.69 (s, 1H, -C(O)N<u>H</u>-), 7.91 (d, J = 8.4 Hz, 2H, Ar H-2,6), 7.77 (d, J = 8.4 Hz, 2H, Ar H-3,5), 2.14-2.03 (m, 1H, cyclopropyl H-1), 1.00-0.86 (m, 4H, cyclopropyl H-2eq, 2ax, 3eq, 3ax); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13; Found: C, 54.58; H, 4.62; N, 10.65; S, 12.15.

Dimethyl 2-(3-(Cyclopropanecarbonyl)thioureido)terephthalate ***(2.5).*** Yield: 74.4 %; Mp.: 208-211°C; IR (cm^{-1}): 3121 (ν_{NH}), 3004 (ν_{NH}), 2986 (ν_{CH2}), 1715 (ν_{CO}), 1686(ν_{CO}), 1525 (δ_{NH}), 1432, 1390, 1281, 1241, 1222, 1192, 1158, 1130, 1098, 1065, 949, 937, 883, 755, 709, 674, 613; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H, -C(S)N<u>H</u>-), 11.71 (s, 1H, -C(O)N<u>H</u>-), 8.71 (s, 1H, Ar H-3), 7.95 (d, J = 8.1 Hz, 1H, Ar H-6), 7.82 (dd, J = 8.2, 1.7 Hz, 1H, Ar H-5), 2.16-2.07 (m, 1H, cyclopropyl H-1), 1.00 – 0.88 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 337 [M+1]; Anal. Calcd. for $C_{15}H_{16}N_2O_5S$: C, 53.56; H, 4.79; N, 8.33; S, 9.53; Found: C, 53.62; H, 4.84; N, 8.39; S, 9.61.

N-((4-Sulfamoylphenyl)carbamothioyl)cyclopropanecarboxamide ***(2.6).*** Yield: 61.7 %; Mp.: 213-216 °C; IR (cm^{-1}): 3357 (ν_{NH}), 3286 (ν_{NH}), 3143 (ν_{NH}), 3000 (ν_{CH2}), 1673 (ν_{CO}), 1526 (δ_{NH}), 1329, 1147, 767, 724, 687; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H, -C(S)N<u>H</u>-), 11.74 (s, 1H, -C(O)N<u>H</u>-), 7.84-7.75 (m, 4H, Ar H-2,3,5,6), 7.12 (s, 2H, -SO_2N<u>H_2</u>-), 2.15-2.05 (m, 1H, cyclopropyl H-1), 1.00- 0.88 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 301 [M+2]; Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; O, 16.03; S, 21.42; Found: C, 44.19; H, 4.41; N, 14.09; S, 21.48.

4-Bromo-3-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid ***(2.7).*** Yield: 30.6 %; Mp.: 163-166 °C; IR (cm^{-1}): 3191 (ν_{NH}), 3012 (ν_{CH2}), 1694 (ν_{CO}), 1527 (δ_{NH}), 1417, 1243, 1169, 1097, 937, 823, 763, 716, 673; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H, -C(S)N<u>H</u>-), 11.60 (s, 1H, -C(O)N<u>H</u>-), 8.53 (s, 1H, Ar-6), 7.80 (d, J = 8.4 Hz, 1H, Ar-3), 7.37 (dd, J = 8.5, 2.0 Hz, 1H, Ar-4), 2.14-2.05 (m, 1H, cyclopropyl H-1), 0.98-0.80 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 344 [M+1]; Anal. Calcd. for $C_{12}H_{11}BrN_2O_3S$: C, 42.00; H, 3.23; Br, 23.28; N, 8.16; S, 9.34; Found: C, 42.09; H, 3.31; Br, 23.34; N, 8.23; S, 9.38.

N-((2-Methoxyphenyl)carbamothioyl)cyclobutanecarboxamide ***(2.8).*** Yield: 52.5 %; Mp.: 153-156 °C; IR (cm^{-1}): 3257 (ν_{NH}), 2984 (ν_{CH2}), 1691 (ν_{CO}), 1599 (ν_{CO}), 1519 (δ_{NH}), 1445, 1359, 1310, 1244, 1222, 1179, 1145, 1025, 848, 746; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.89 (s, 1H, -C(S)N<u>H</u>-), 11.02 (s, 1H, -C(O)N<u>H</u>-), 8.64 (d, *J* = 8.1 Hz, 1H, Ar H-6), 7.23-7.07 (m, 1H, Ar H-4), 6.99 (d, *J* = 8.1 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.7 Hz, 1H, Ar H-5), 3.93 (s, 3H, CH_3), 3.43 (p, *J* = 8.3 Hz, 1H, cyclobutyl H-1), 2.30-2.17 (m, 2H, cyclobutyl H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax},), 2.05-1.82 (m, 2H, cyclobutyl H-3_{eq}, 3_{ax}); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60; S, 12.13; Found: C, 59.13; H, 6.15; N, 10.66; S, 12.18.

N-((2-Methoxyphenyl)carbamothioyl)cyclopentanecarboxamide ***(2.9).*** Yield: 62.1 %; Mp.: 141-143 °C; IR (cm^{-1}): 3195 (ν_{NH}), 2934 (ν_{CH2}), 1682 (ν_{CO}), 1601 (ν_{CO}), 1537 (δ_{NH}), 1350, 1315, 1223, 1146, 1025, 843, 744; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.87 (s, 1H, -C(S)N<u>H</u>-), 11.14 (s, 1H, -C(O)N<u>H</u>-), 8.65 (d, *J* = 8.0 Hz, 1H, Ar H-6), 7.14 (t, *J* = 7.7 Hz, 1H, Ar H-4), 6.99 (d, *J* = 8.1 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.7 Hz, 1H, Ar H-5), 3.92 (s, 3H, CH_3), 3.00 (p, *J* = 8.1 Hz, 1H, cyclopentyl H-1), 1.95-1.44 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, m/z = 279 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06; S, 11.52; Found: C, 60.47; H, 6.57; N, 10.21; S, 11.58.

N-((2-Chlorophenyl)carbamothioyl)cyclopentanecarboxamide ***(2.10).*** Yield: 67.6 %; Mp.: 113-115 °C; IR (cm^{-1}): 3189 (ν_{NH}), 2949 (ν_{asCH2}), 1688 (ν_{CO}), 1587 (δ_{NH}), 1529, 1329, 1237, 1154, 728; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.76 (s, 1H, -C(S)N<u>H</u>-), 11.41 (s, 1H, -C(O)N<u>H</u>-), 8.25 (d, *J* = 8.1 Hz, 1H, Ar H-6), 7.42 (d, *J* = 8.0 Hz, 1H, Ar H-3), 7.28 (t, *J* = 7.7 Hz, 1H, Ar H-5), 7.18 (t, *J* = 7.7 Hz, 1H, Ar H-4), 2.98 (p, *J* = 7.8 Hz, 1H, cyclopentyl H-1), 1.92-1.49 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, m/z = 283 [M+1]; Anal. Calcd. for $C_{13}H_{15}ClN_2OS$: C, 55.22; H, 5.35; N, 9.91; S, 11.34;

The general method of N-(2-aroyl-(hetaroyl-) hydrazine-1-carbonothioyl)cycloalkylcarboxamides ***(3.1–3.8)*** *synthesis.* To the solution of corresponding 0.01 mol of cycloalkylcarbonyl chlorides (**1.1–1.3**) in 20 mL of acetonitrile 0.76 g (0.01 mol) of ammonium isothiocyanate was added and stirred at 80 °C for 30 min. The mixture was cooled down to r.t. and 0.01 mol of corresponding hydrazides was added and stirred at 80 °C for 90 min. The solution was cooled down, poured into the water and formed precipitate was filtrated, dried and recrystallized from methanol.

N-(2-(2-Phenoxyacetyl)hydrazine-1-carbonothioyl)cyclopropanecarboxamide ***(3.1).*** Yield: 70.3 %; Mp.: 194-195 °C; IR (cm^{-1}): 3286 (ν_{NH}), 3143 (ν_{NH}), 3000 (ν_{CH2}), 1650 (ν_{CO}), 1549 (δ_{NH}), 1472, 1441, 1394, 1224, 1158, 1036, 935, 885, 836, 749, 668, 628; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (br.s, 1H, -C(S)N*H*-), 11.77 (s, 1H, -C(O)N*H*-), 10.73 (br.s. 1H, -HNN*H*C(O)-), 7.27 (t, J = 7.8 Hz, 2H, Ph H-3,5), 6.99-6.91 (m, 3H, Ph H-2,4,6), 4.65 (s, 2H, -CH_2OPh), 2.12-2.03 (m, 1H, 1H, cyclopropyl H-1), 1.01-0. 88 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 294 [M+1]; Anal. Calcd. for $C_{13}H_{15}N_3O_3S$: C, 53.23; H, 5.15; N, 14.32; S, 10.93; Found: C, 53.27; H, 5.19; N, 14.35; S, 10.99.

N-(2-(2-(Phenylthio)acetyl)hydrazine-1-carbonothioyl)cyclopropanecarboxamide ***(3.2).*** Yield: 76.5 %; Mp.: 187-188 °C; IR (cm^{-1}): 3485 (ν_{NH}), 3308 (ν_{NH}), 2931 (ν_{CH2}), 1680 (ν_{CO}), 1643 (δ_{NH}), 1436, 1386, 1213, 1156, 873, 738, 670; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (br.s, 1H, -C(S)N*H*-), 11.68 (s, 1H, -C(O)N*H*-), 10.99 (br.s, 1H, -HNN*H*C(O)-), 7.40 (d, J = 7.7 Hz, 2H, Ph H-2,6), 7.28 (t, J = 7.7 Hz, 2H, Ph H-3,5), 7.17 (t, J = 7.4 Hz, 1H, Ph H-4), 3.76 (s, 2H, -CH_2SPh), 2.10-2.01 (m, 1H, cyclopropyl H-1), 0.99-0. 86 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 310 [M+1]; Anal. Calcd. for $C_{13}H_{15}N_3O_2S_2$: C, 50.47; H, 4.89; N, 13.58; S, 20.72; Found: C, 50.51; H, 4.91; N, 13.54; S, 20.76.

N-(2-Isonicotinoylhydrazine-1-carbonothioyl) cyclopropanecarboxamide ***(3.3).*** Yield: 72.1 %; Mp.: 182-186 °C; IR (cm^{-1}): 3486 (ν_{NH}), 3234 (ν_{NH}), 3121 (ν_{NH}), 2846 (ν_{CH2}), 1655 (ν_{CO}), 1472 (δ_{NH}), 1217, 1162, 1113, 944, 910, 870, 734, 674; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H, -C(S)N*H*-), 11.80 (s, 1H, -C(O)N*H*-), 11.20 (s, 1H, -HNN*H*-C(O)-), 8.70 (d, J = 5.3 Hz, 2H, Pyr H-3,5), 7.80 (d, J = 5.1 Hz, 2H, Pyr H-2,6), 2.15-2.06 (m, 1H, cyclopropyl H-1), 1.03-0.90 (m, 4H, cyclopropyl H-2_{eq}, 2_{ax}, 3_{eq}, 3_{ax}); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{11}H_{12}N_4O_2S$: C, 49.99; H, 4.58; N, 21.20; S, 12.13; Found: C, 50.03; H, 4.60; N, 21.16; S, 12.18.

N-(2-(2-Aminobenzoyl)hydrazine-1-carbonothioyl)cyclobutanecarboxamide ***(3.4).*** Yield: 32.9 %; Mp.: 201-202 °C; IR (cm^{-1}): 3492 (ν_{NH}), 3352 (ν_{NH}), 3193 (ν_{NH}), 2931 (ν_{CH2}), 1677 (ν_{CO}), 1618 (δ_{NH}), 1497, 1417, 1246, 1153, 734, 694; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H, -C(S)N*H*-), 11.32-11.16 (br.s, 2H, -C(O)N*H*-, -HNN*H*-C(O)-), 7.54 (d, *J* = 7.9 Hz, 1H, Ar H-6), 7.16 (t, J = 7.7 Hz, 1H, Ar H-4), 6.73 (d, J = 8.3 Hz, 1H, Ar H-3), 6.54 (t, J = 7.5 Hz, 1H, Ar H-5), 5.31 (d, *J* = 4.5 Hz, 2H, -NH_2), 3.41 (p, *J* = 8.4 Hz, 1H, cyclobutyl H-1), 2.37-2.09 (m, 4H, cyclobutyl H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax}), 2.07-1.83 (m, 2H, cyclobutyl H-3_{eq}, 3_{ax}); LC-MS, m/z = 293 [M+1]; Anal. Calcd. for $C_{13}H_{16}N_4O_2S$: C, 53.41; H, 5.52; N, 19.16; S, 10.97; Found: C, 53.47; H, 5.59; N, 19.21; S, 11.03.

N-(2-(2-Aminobenzoyl)hydrazine-1-carbonothioyl)cyclopentanecarboxamide ***(3.5).*** Yield: 55.6 %; Mp.: 195-197 °C; IR (cm^{-1}): 3487 (ν_{NH}), 3349 (ν_{NH}), 3203 (ν_{NH}), 2941(ν_{CH2}), 1679 (ν_{CO}), 1493 (δ_{NH}), 1427, 1252, 1217, 1157, 1048, 970, 932, 901, 870, 842, 734, 692, 661, 643, 608; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H, -C(S)N*H*-), 11.44-11.28 (br.s, 2H, -C(O)N*H*-, -HNN*H*-C(O)-), 7.54 (d, J = 8.0 Hz, 1H, Ar H-6), 7.15 (t, J = 7.7 Hz, 1H, Ar H-4), 6.72 (d, J = 8.3 Hz, 1H, Ar H-3), 6.53 (t, J = 7.5 Hz, 1H, Ar H-5), 5.38 (d, *J* = 4.5 Hz, 2H, -NH_2), 3.03-2.93 (m, 1H, cyclopentyl H-1), 1.96-1.55 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, m/z = 307 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_4O_2S$: C, 54.88; H, 5.92; N, 18.29; S, 10.46; Found: C, 54.96; H, 5.98; N, 18.32; S, 10.38.

N-(2-(2-Aminobenzoyl)hydrazine-1-carbonothioyl)cyclohexanecarboxamide ***(3.6).*** Yield: 44.9 %; Mp.: 213-216 °C; IR (cm^{-1}): 3225 (ν_{NH}), 3009 (ν_{CH2}), 1694 (ν_{CO}), 1527 (δ_{NH}), 1417, 1243, 1169, 1097, 937, 823, 763, 716, 673; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (s, 1H, -C(S)N*H*-), 11.34-11.21 (br.s, 2H, -C(O)N*H*-, -HNN*H*-C(O)-), 7.52 (d, *J* = 8.0 Hz, 1H, Ar H-6), 7.15 (t, *J* = 7.7 Hz, 1H, Ar H-4), 6.72 (d, *J* = 8.3 Hz, 1H, Ar H-3), 6.53 (t, *J* = 7.5 Hz, 1H, Ar H-5), 5.30 (d, *J* = 4.5 Hz, 2H, -NH_2), 2.60-2.46 (m, 1H, cyclohexyl H-1), 1.92-1.74 (m, 4H, cyclohexyl H-2_{eq}, 6_{eq}, 2_{ax}, 6_{ax}), 1.51-1.14 (m, 6H, cyclohexyl H-3_{eq}, 5_{eq}, 3_{ax}, 5_{ax},4_{eq}, 4_{ax}); LC-MS, m/z = 321 [M+1]; Anal. Calcd. for $C_{15}H_{20}N_4O_2S$: C, 56.23; H, 6.29; N, 17.49; S, 10.01; Found: C, 56.20; H, 6.31; N, 17.42; S, 9.97.

N-(2-(2-Methylfuran-3-carbonyl)hydrazine-1-carbonothioyl)cyclohexanecarboxamide ***(3.7).*** Yield: 84.3 %; Mp.: 183-187 °C; IR (cm^{-1}): 3176 (ν_{NH}), 3030 (ν_{NH}), 2926 (ν_{CH2}), 2851, 1538 (ν_{CO}), 1519 (δ_{NH}), 1445, 1209, 1184, 1157, 1136, 1074, 1026, 941, 900, 875, 751, 737, 716, 688; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36-12.30 (br.s, 1H, -C(S) N*H*-), 11.29 (s, 1H, -C(O)N*H*-), 10.32 (br.s, 1H, -HNN*H*C(O)-), 7.32 (d, *J* = 2.0 Hz, 1H, Furyl H-5), 6.83 (d, *J* = 2.1 Hz, 1H, , Furyl H-4), 2.53 (s, 3H, -CH_3), 1.85-1.60 (m, 5H, cyclohexyl H-1, 2_{eq}, 6_{eq}, 2_{ax}, 6_{ax}), 1.42-1.14 (m, 6H, cyclohexyl H-3_{eq}, 5_{eq}, 3_{ax}, 5_{ax},4_{eq}, 4_{ax});); LC-MS, m/z = 310 [M+1]; Anal. Calcd. for $C_{14}H_{19}N_3O_3S$: C, 54.35; H, 6.19; N, 13.58; S, 10.36; Found: C, 54.29; H, 6.12; N, 13.54; S, 10.40.

Molecular docking. Research was conducted by flexible molecular docking, as an approach of finding molecules with affinity to a specific biological target. Macromolecular data were downloaded from the Protein Data Bank (PDB) namely, the crystal structures of human carbonic anhydrase II (PDB ID – 3HS4) [16].

Ligand preparation. Substances were drawn using MarvinSketch 20.6.0 and saved in mol format

[17]. After that they were optimized by program Chem3D, using molecular mechanical MM2 algorithm and saved as pdb-files. Molecular mechanics was used to produce more realistic geometry values for most organic molecules, owing to the fact of being highly parameterized. Using AutoDockTools-1.5.6 pdb-files were converted into PDBQT, number of active torsions was set as default [18].

Protein preparation. PDB files were downloaded from the protein data bank. Discovery Studio v 19.1.0.18287 was used to delete water molecules and ligands. Structures of proteins were saved as pdf-files [19]. In AutoDockTools-1.5.6 polar hydrogens were added and saved as PDBQT. Grid box was set as following: center_x = -5.75, center_y = 3.36, center_z = 13.47, size_x = 20, size_y = 20, size_z = 20 for human carbonic anhydrase II; Vina was used to carry docking [18]. For visualization Discovery Studio v 19.1.0.18287 was used.

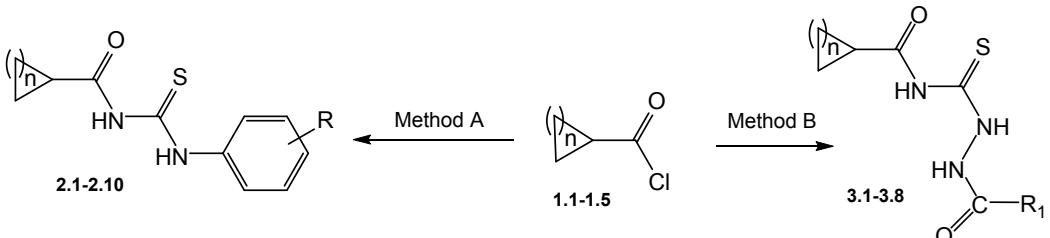
Study of the effect of compounds on the excretory function of the kidneys. The experiment was performed on 126 white male Wistar rats weighing 120–170 g, which were kept in standard conditions of the vivarium of the State Medical Institution of the Ministry of Health of Ukraine. Experimental studies were performed in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001), the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [20]. Screening of the new synthesized compounds, in order to identify diuretic properties in a few disubstituted derivatives of thioureas and thiosemicarbazides, was carried out according to the generally accepted method of E. B. Berkhin [21, 22]. Prior to the experiment, the animals were kept without food for three hours. The diuretic effect of the compounds was studied under liquid load at the rate of 5 ml per 100 g of animal weight. The test compounds were administered to rats once intragastrically at a doses of 2.6 mg/kg body weight as an aqueous suspension simultaneously with the water load. Animals were placed in individual cages for urine collection during three hours. "Hypothiazide" and "Furosemide" in equivalent doses for rats were selected as the reference drugs [23].

The obtained data were statistically processed using the software package Statistica 6.1 (StatSoft Inc., serial number AGAR909E415822FA). The arithmetic mean values (M) and their errors (±m) were calculated. The probability of intergroup differences was determined using Student's parametric t-test and one-way analysis of variance (ANOVA). The differences were considered statistically significant at a value of $p \leq 0.05$ [24].

RESULTS AND DISCUSSION. Unknown substituted acylthioureas (**2.1–2.10**) were obtained by the method [25], which consisted in the sequential addition to cycloalkylcarbonyl chlorides (**1.1–1.3**) of equimolecular amounts of ammonium isothiocyanate and substituted anilines (Scheme). Substituted acylthiosemicarbazides (**3.1–3.7**) were also synthezised by a similar method, namely by the interaction of the starting compounds **1.1–1.4** with carboxylic acid hydrazides. The reaction was carried out through intermediate cycloalkylcarbonylisothio cyanates, which easily undergo the nucleophilic addition reaction with anilines or hydrazides. In this case, the target products were formed with a yield of 30.6–84.3 %.

N-(R-phenylcarbamothioyl)cycloalkylcarboxamides (**2.1–2.10**) and N-(2-aryl-(hetaryl-)hydrazine-1-carbonothioyl)cycloalkylcarboxamides (**3.1–3.8**) are light yellow crystalline substances, practically insoluble in water, soluble in aqueous solutions of sodium (potassium) hydroxides, alcohol, dioxane and DMF.

The LC-MS using positive-ion atmospheric pressure chemical ionization (APCI) show the appropriately molecular ions, which confirms the expected molecular weights of compounds **2.1–2.10** and **3.1–3.8**. In addition, the data of ^{1}H NMR spectra, show the characteristic singlet signals of protons of thioamide (-NHC(S)-) and amide (-NHC(O)-) groups



2.1-2.10 **1.1-1.5** **3.1-3.8**

n=1-3, R=2-MeO, 2-Cl, 4-COOH, 3-COOH, 2,5-$(COOMe)_2$, 4-SO_2NH_2, 6-Br-3-COOH

n=1-4; R_1=$PhOCH_2$-, $PhSCH_2$-, 2-$NH_2C_6H_4$, 2-Me-furyl-3, pyridyl-4

Method A: NH_4SCN; MeCN, 80 °C, 30 min; R-$C_6H_4NH_2$, MeCN, 80 °C, 1,5 h;
Method B: NH_4SCN; MeCN, 80 °C, 30 min; R-$CONHNH_2$, MeCN, 80 °C, 1,5 h

Scheme. One-pot synthesis of N-substituted cycloalcylcarbonylthioureas and thiosemicarbazides.

at the 13.13–12.71 ppm and 11.74–11.02 ppm, respectively, that indicate in favor of the formationof substituted cycloalkylcarbonylthioureas (**2**). Whereas, signals of three proton signals of thioamide (-NHC(S)-), amide (-C(O)NH-) and hydrazide (-HNNHC(O)-) groups were recorded in the weak field part of the spectrum, namely at the 12.69–12.27 ppm, at the 11.80–11.29 ppm and at the 11.20–10.32 ppm, respectively in case of the substituted cycloalkylcarbonylthiosemicarbazides (**3**). The last two proton signals for compounds 3.4–3.6 in most cases appear together in the form of expanded singlets at the 11.44–11.16 ppm. The ^{1}H NMR spectra of the compounds the signals of the methine proton (H-1) of cyclopropane (compounds **2.1–2.7, 3.1–3.3**) and cyclohexane **(3.6, 3.7**) fragment were also as a triplet of triplet or multiplet at the 2.16-2.03 ppm and multiplet at the 2.6–1.86 ppm, respectively. Whereas, the signals of methine protons (H-1) of cyclobutane (**2.8, 3.4**) and cyclopentane (**2.9, 2.10, 3.5**) appear in the form of a pentene at the 3.43 ppm, 3.41 ppm and 3.0 ppm, 2.98 and 3.00 ppm, respectively. Signals of equatorial and axial protons of methylene groups in all cases were shown as multiples in a wide range of strong magnetic field. In addition, proton signals were present in the 1H NMR spectra of compounds **2** and **3**, which characterize the nature of anilide and aryl (hetaryl) hydrazide fragments [26].

In the IR spectra of compounds 2 and 3 bands of valence oscillations of the associated NH groups in the region 3744–3004 cm^{-1}, were characteristic which indicate the presence of amides in the molecule. In addition, the compounds were characterized by oscillations of νCO (S)-groups (band "Amide I") at the 1694–1538 cm^{-1} and mixed valence-strain oscillations of NH and CN ("Amide II") bonds at the 1643–1472 cm^{-1}. Along with the key oscillation bands, compounds **2** and **3** were characterized by low-intensity oscillations νC = C-bond of the aromatic ring at the 1486–1424 cm^{-1}, nonplanar oscillations γ (=C-H) at the 850–666 cm^{-1} and intense bands of symmetrical and antisymmetric oscillations of νCH_2 groups and indicate the presence of cycloalkyl fragments at the 3012–2846 cm^{-1} [27].

The results of the biological experiment showed, (Table 1) that *N*-substituted cycloalkylcarbonylureas (**2.1**–**2.10**) have different effects on the excretory function of the kidneys. Thus, compounds **2.3** and **2.6** containing "pharmacophore" groups characteristic of diuretics (carboxyl and sulfamide groups, respectively) inhibit the diuretic effect under water load. As for compounds **2.4, 2.7** and **2.8**, they were also characterized by a slight diuretic effect (3.40–17.00 %), despite the fact, that their molecules include a carboxyl group. Higher diuretic activity was characteristic for compounds **2.2, 2.5, 2.8**–**2.10**, which increase diuresis by 17.00–33.60 %.

Urinary indicators on the background of these compounds exceeded the indicators of the comparison drug Furosemide by 2.30–16.30 %. Interestingly, the most active compounds (**2.2, 2.5**) were cyclopropane-carbonylthioureides and contain 2-chlorophenyl (**2.2**) and 2,5-dimethylcarboxyl (**2.5**) fragments in the molecule. Compound **2.10**, which

Table 1 – **The effect of the synthesized compounds and reference drugs on the process of urination in intact rats under water load with a single injection (M±m, n=6) and molecular docking results**

№	Groups	Diuresis, mL/100 g	Influence on the process of urination, %	Affinity (kcal/mol) to human carbonic anhydrase II (PDB ID – 3HS4)
1.	Control	3.48±0.09	–	–
2.	Furosemide	4.09±0.11*	**17.50**	-6.6
3.	Hydrochlorothiazide	5.39±0.07*	**54.90**	-6.6
4.	**2.1**	3.66±0.19	5.17	-6.7
5.	**2.2**	4.65±0.09*	**33.60**	-7.2
6.	**2.3**	2.87±0.08*	–17.50	-6.6
7.	**2.4**	3.60±0.20	3.40	-6.9
8.	**2.5**	4.47±0.29*	**28.40**	-6.1
9.	**2.6**	2.79±0.08*	–19.80	-6.4
10.	**2.7**	3.82±0.08*	9.80	-7.0
11.	**2.8**	4.07±0.02*	**17.00**	-6.4
12.	**2.9**	4.17±0.04*	**19.80**	-6.5
13.	**2.10**	4.24±0.11*	**21.80**	-6.7
14.	**3.1**	3.87±0.39	11.20	-6.6
15.	**3.2**	5.17±0.08*	**48.60**	-6.9
16.	**3.3**	3.66±0.20	5.20	-6.5
17.	**3.4**	3.99±0.10*	14.00	-7.5
18.	**3.5**	3.48±0.10	0	-7.5
19.	**3.6**	3.56±0.11	2.30	-7.9
20.	**3.7**	4.07±0.14*	**17.00**	-6.5

Note. Significant changes in control ($p<0.05$); n is the number of animals in the group.

was a cyclopentanecarbonylthioureide derivative and contains a 2-chlorophenyl substituent in its structure, also has a significant diuretic effect (21.80 %).

Speaking about *N*-substituted cycloalkylcarbo nylsemicarbazides (**3.1**–**3.8**). Most of them (**3.1, 3.3, 3.4**–**3.7**) showed a slight diuretic effect (0–17.0 %), while inferior to furosemide. While compound **3.2** increased diuresis by 48.6 %, approaching the effect of Hydrochlorothiazide (54.90 %). Importantly, compound **3.2** was also a cyclopropanecarbonylthiose micarbazide with a phenylthioacetyl moiety in the molecule.

It is known that thiazide and loop diuretics with a sulfamide moiety in the molecule act as excellent zinc-complexing groups in the active site of CA II [4]. This is associated with responsibility for all physiological effects of this group of diuretics. With this in mind, we performed a molecular docking of this enzyme to elucidate the probable mechanism of action of compounds **2** and **3**. The results of the studies showed (Table 1) that most compounds are not inferior to CA II in the level of affinity, and in some cases exceed (compounds **2.2, 2.4, 2.7. 3.2, 3.4–3.6**) reference drugs. This fact, as well as the structural features (ability to complex) of the studied compounds served as a basis for a more detailed definition of the main types of interactions with amino acid residues of CA II.

The main types of interactions of synthesized compounds **2**, **3** and pharmacological standards with amino acid residues of carbonic anhydrase II are presented in table 2. Data analysis showed that the studied compounds and standard drugs have a large number of hydrogen bonds, π-halogen, π-sulfur, hydrophobic bonds with amino acid residues and coordination with the zinc cation CA II. Thus, the visualization of Hydrochlorothiazide with

Table 2 – **The main types of interactions of synthesized compounds and pharmacologic standards with amino acid residues of carboanhydrase II**

Compd.	The main interactions types between compounds, pharmacological standards and amino acid residues of enzymes
Hydrochlorothiazide	THR200^{A}, THR199^{A},ZN301^{E}, GLN92^{B}, HIS96^{C}, HIS119^{C}, VAL121^{B}, VAL143^{B}, LEU198^{B}, HIS94^{B}, VAL121^{C}, LEU198 ^{B}
Furosemide	THR199^{A}, THR199^{A}, LEU198^{A}, GLN92^{A}, HIS94^{C}, PHE131^{B}, VAL121^{B}, LEU141^{B}, VAL143^{B}, LEU198^{B}, VAL121^{B}, LEU198^{B}, ILE91^{B}
2.1	THR199^{A}, THR200^{A}, THR200^{A}, THR200^{B}, HIS94^{B}, VAL12^{B}, VAL14 ^{B}, LEU198^{B}, HIS94^{B}, HIS119^{B}, TRP209^{B}, ALA6 ^{B}
2.2	THR200^{A}, HIS94^{A}, HIS94^{B}, ALA65^{B}, VAL121^{B}, LEU198^{B}, HIS94^{B}, HIS96^{B}, PHE131^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}
2.3	THR199^{A}, THR200^{A}, HIS94^{D}, LEU198^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}
2.4	ASN62^{A}, ASN67^{A}, THR200^{A}, THR200^{A}, THR199^{A}, ZN301^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}, LEU198^{B}
2.5	THR200^{A}, PRO201^{A}, ASN62^{A}, THR200^{A}, PHE131^{C}, VAL121^{B}, VAL143^{B}, LEU198^{B}, HIS94^{B}, HIS119^{B}, TRP209^{B}
2.6	THR200^{A}, THR199^{A}, THR199^{A}, HIS94^{A}, LEU198^{A}, LEU198^{B}, HIS94^{C}, HIS94^{B}, VAL121^{B}
2.7	ASN62^{A}, THR199^{A}, THR200^{A}, THR200^{A}, THR200^{A}, LEU198^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}
2.8	THR199^{A}, THR200^{A}, THR200^{A}, THR200^{A}, ASN62^{A}, THR200^{A}, VAL121^{B}, VAL143^{B}, LEU198^{B}, HIS94^{B}, HIS119^{B}, TRP209^{B}
2.9	ASN62^{A}, THR200^{A}, THR200^{B}, HIS94^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}, HIS94^{B}, HIS119^{B}, ALA65^{B}
2.10	GLN92^{A}, HIS94^{A}, ASN62^{A}, THR200^{A}, HIS94^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}, TRP5^{B}, HIS64^{B}, HIS94^{B}, HIS119^{B}, ALA65^{B},
3.1	THR200^{A}, THR200^{A}, ZN301^{D}, TRP5^{C}, TRP5^{C}, HIS94^{B}, ALA65^{B}, HIS94^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}
3.2	GLN92^{A}, THR199^{A}, THR200^{A}, THR200^{A}, ZN301^{E}, PHE131^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}, HIS94^{B}, HIS119^{B}, TRP209^{B}, ILE9^{1}B
3.3	THR199^{A}, ZN301^{D}, LEU198^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}, VAL143^{B}
3.4	GLN92^{A}, THR200^{A}, THR200^{A}, THR200^{A}, THR199^{A}, LEU198^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}, VAL143^{B}
3.5	GLN92^{A}, THR200^{A}, THR199^{A}, THR200^{A}, ZN301^{D}, LEU198^{B}, HIS94^{B}, ALA65^{B}, HIS94^{B}, HIS96^{B}, VAL121^{B}, VAL143^{B}
3.6	THR200^{A}, THR200^{A}, ASN67^{A}, THR199 ^{A}, THR200 ^{A}, ZN301^{D}, HIS94$^{A;}$ LEU198^{B}, HIS94^{B}, HIS64^{B}, VAL121^{B}, VAL143^{B}
3.7	GLN92^{A}, GLN92^{A}, ASN62^{A}, THR200^{B}, HIS94^{B}, VAL121^{B}, LEU198^{B}, TRP5^{B}, HIS64^{B}, ALA65^{B}

Notes. A – Hydrogen Bond; B – Hydrophobic, C – Halogen (π-sulfur), D – Electrostatic, E – Metal-Acceptor.

the active site of CA II (Fig. 1, A) revealed the presence of two hydrogen bonds of the sulfamide group with amino acid residues THR200A (2.14Å), THR199A (2.39Å), hydrophobic π-interactions of the aromatic fragment with amino acid residues GLN92B (4.17Å), VAL121B (4.96Å), π-interactions of Oxygen and Sulfur of sulfamide group and Chlorine with VAL143B (4.24Å), VAL143B (5.07Å), HIS96C (5.37Å), HIS94B (5.48Å), HIS119C (5.07Å), LEU198B (4.67Å), VAL121B (3.84Å), LEU198B (4.67Å), VAL121B (4.96Å), LEU198B (4.67Å). In addition, the active site has a coordination link of the sulfamide group Hydrochlorothiazide with the zinc cation CA II (ZN301E 2.62Å).

Visualization of the results of the molecular docking of compound **2.2** indicates, that it has a similar docking with CA II (Fig. 1, B). First, due to hydrogen bonds of amide groups with amino acid residues THR200A (2.24Å and 3.26Å), HIS94A (2.24Å), hydrophobic π-interactions of the aromatic moiety with LEU198B (4.78Å), VAL143B (5.16Å), HIS94B (4.77Å), π-interactions of the Chlorine atom with LEU198B (4.69Å), VAL121B (3.63Å), PHE131B (5.38Å). Second, compound **2.2** is characterized by π-alkyl bonds (hydrophobic interactions of the cyclopropyl moiety) with ALA65B (3.56Å) and HIS96B (5.21Å).

At that time, the analysis of the molecular docking of CA II with compound **3.2** (Fig. 1, C, D) showed better results. Thus, in this case, more interactions were predicted: four hydrogen bonds of amide groups with amino acid residues: GLN92A (3.61Å), THR199A (3.08Å), THR200A (2.80Å), THR200A (1.90Å), hydrophobic π-interactions of the aromatic moiety with ILE91B (5.28Å) and PHE131B (4.03Å). π-alkyl bonds of the cyclopropyl moiety with VAL143B (3.98Å), HIS94B (5.21Å), HIS119B (5.38Å) and TRP209B (5.36Å) were also observed. In addition, compound **3.2**, as well as Hydrochlorothiazide, was characterized by a coordination bond of the amide group of the molecule with the zinc cation CA II (ZN301E 2.22Å). In our opinion, the presence of similar interactions of CA II with compound **3.2** and the pharmacological standard and provides high diuretic activity.

CONCLUSIONS. The developed and implemented strategy of searching for diuretics among

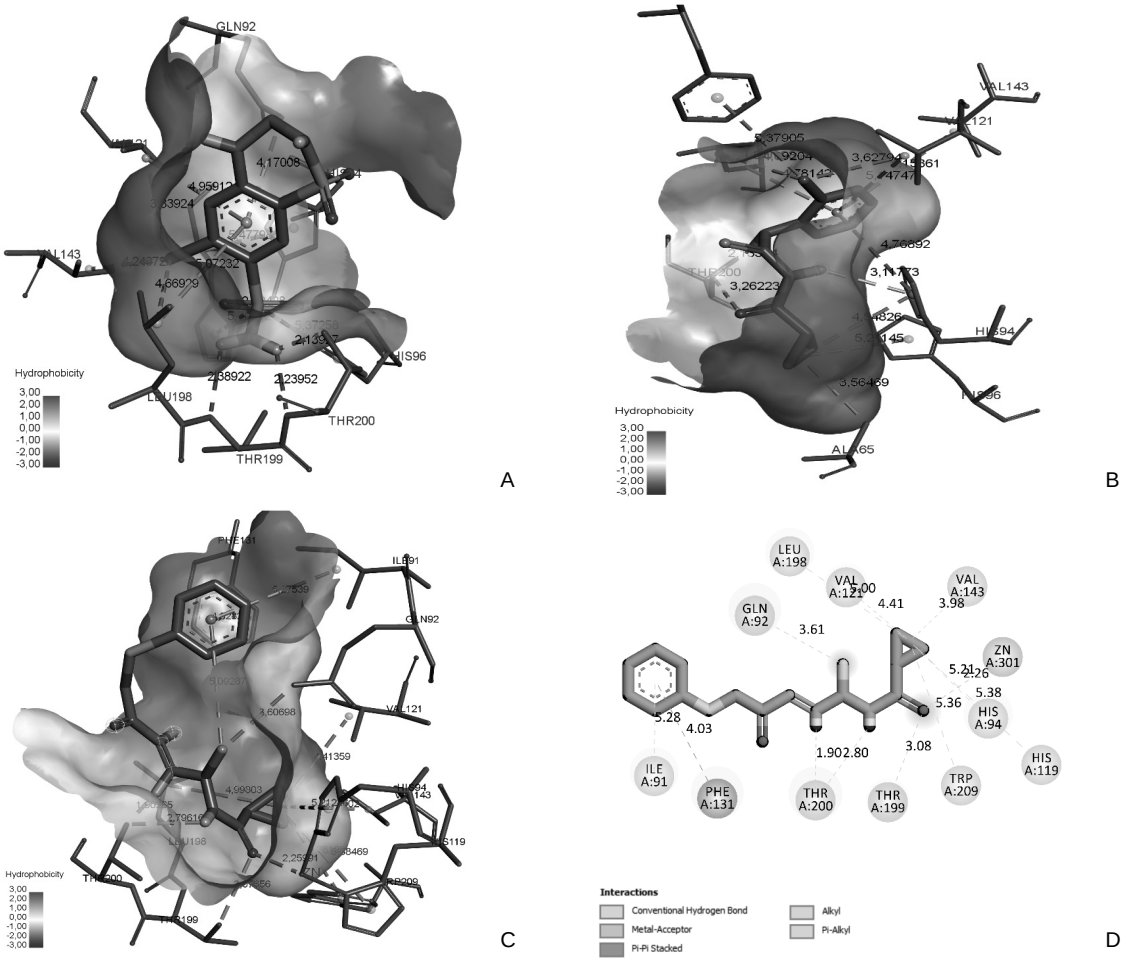


Fig. 1. Types of the ligand – enzyme interactions according to visualization of docking study: A) Hydrochlorothiazide with CA II 3D; B) compound 2.2 with CA II 3D, C) compound 3.2 with CA II 3D; D) compound 3.2 with CA II 2D.

cycloalkylcarbonyl thioureas and thiosemicarbazides derivatives has revealed an effective compound (**3.2**), which is close to the reference drug "Hydrochlorothiazide" in terms of diuretic effect. Importantly, according to the results of molecular docking, the synthesized compounds as well as the reference drugs have a similar mechanism of action (carbonic anhydrase II inhibitors). Importantly, the results of molecular docking synthesized compounds, as well as reference drugs have a similar mechanism of action (carbon dioxide anhydrase II inhibitors). It is likely, that the pronounced diuretic effect of a few compounds is associated with the ability of substituted thioureas to form coordination bonds with the zinc cation in the active site of CA II. The obtained results substantiate the further purposeful search for potential diuretics among this class of compounds.

Pospects of further research. The results of the studies have confirmed the presence of a diuretic effect in substituted thioureas and reveal prospects for further study of their effects on the urinary system. First, it is a further structural modification of the active compounds by the introduction of additional pharmacophore groups or their heterocyclization in the corresponding sulfur-containing heterocyclic systems. Second, conducting in vitro studies on the ability of the synthesized compounds to inhibit carbonic anhydrase II, which will serve as a method of selection for their further in vivo studies.

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ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ[1]
ДНІПРОПЕТРОВСЬКА МЕДИЧНА АКАДЕМІЯ МОЗ УКРАЇНИ[2]

СПРЯМОВАНИЙ ПОШУК СПОЛУК, ЩО ВПЛИВАЮТЬ НА ВИДІЛЬНУ ФУНКЦІЮ НИРОК ЩУРІВ, СЕРЕД НОВИХ ПОХІДНИХ ЦИКЛОАЛКІЛКАРБОНІЛТІОСЕЧОВИН І ТІОСЕМІКАРБАЗИДІВ

Резюме

Вступ. *Тривале застосування діуретиків, особливо у великих дозах, численність і тяжкість побічних ефектів (водно-електролітні й метаболічні порушення), а також відносно обмежена номенклатура існуючих діуретичних засобів диктують необхідність пошуку нових сполук, які б проявляли діуретичну дію, мали нескладну технологію виробництва та були більш безпечними.*

Мета дослідження *– здійснити спрямований пошук діуретиків серед невідомих дизаміщених тіосечовин і тіосемікарбазидів з використанням методології молекулярного докінгу для пояснення імовірного механізму дії.*

Методи дослідження. *Структури цільових сполук запропоновано із застосуванням підходів "drug-design", а саме введенням до тіосечовин і тіосемікарбазидів структурних фрагментів, характерних для відомих діуретиків. Заміщені циклоалкілкарбонілтіосечовини чи тіосемікарбазиди синтезовано одно-реакторним методом з використанням циклоалкілкарбонілхлоридів, амонію ізотіоціанату та заміщених анілінів або гідразидів карбонових кислот. Будову синтезованих сполук доведено методами ІЧ-, ^{1}Н ЯМР-спектроскопії, хроматомас-спектрометрії та елементним аналізом. Спрямований пошук сполук, що впливають на видільну функцію нирок щурів, здійснено за загальноприйнятим методом Є. Б. Бєрхіна з водним навантаженням. Імовірний молекулярний механізм дії передбачено завдяки молекулярному докінгу.*

Результати й обговорення. *Одно-реакторна реакція циклоалкілкарбонілхлоридів з еквімолекулярною кількістю амонію ізотіоціанату та заміщених анілінів або гідразидів карбонових кислот приводить до заміщених циклоалкілкарбонілтіосечовин чи тіосемікарбазидів. Обговорено будову синтезованих сполук з використанням даних ІЧ-, ^{1}Н ЯМР- і хроматомас-спектрів. Дослідження впливу синтезованих сполук на видільну функцію нирок щурів при водному навантаженні дозволили виявити ряд сполук, які за діуретичною дією перевищують фуросемід та конкурують з гідрохлортіазидом. Результати молекулярного докінгу показали, що досліджувані сполуки проявляли високу спорідненість до карбоангідрази ІІ і мали подібні сайти зв'язування з референс-препаратами. Зазначене вказує на ймовірний їх механізм дії.*

Висновки. *Розроблена та реалізована стратегія пошуку діуретиків серед заміщених циклоалкілкарбонілтіосечовин і тіосемікарбазидів дозволила виявити ефективну сполуку **(3.2)**, яка за силою сечогінного ефекту наближається до референс-препарату "Гідрохлортіазид". Важливо, що, згідно з результатами молекулярного докінгу, синтезовані сполуки, як і референс-препарати, мають подібний механізм дії (інгібітори карбоангідрази ІІ). Імовірно, виражений діуретичний ефект досліджуваних сполук пов'язаний зі здатністю заміщених тіосечовин утворювати координаційні зв'язки з катіоном цинку в активній ділянці СА ІІ. Отримані результати обґрунтовують подальший цілеспрямований пошук потенційних діуретиків серед цього класу сполук.*

КЛЮЧОВІ СЛОВА: **синтез; циклоалкілкарбонілтіосечовини; тіосемікарбазиди; спектральні дані; молекулярний докінг; карбоангідраза ІІ; діуретична дія.**

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ЗАПОРОЖСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ[1]
ДНЕПРОПЕТРОВСКАЯ МЕДИЦИНСКАЯ АКАДЕМИЯ МОЗ УКРАИНЫ[2]

НАПРАВЛЕННЫЙ ПОИСК СОЕДИНЕНИЙ, ВЛИЯЮЩИХ НА ВЫДЕЛИТЕЛЬНУЮ ФУНКЦИЮ ПОЧЕК КРЫС, СРЕДИ НОВЫХ ПРОИЗВОДНЫХ ЦИКЛОАЛКИЛКАРБОНИЛТИОМОЧЕВИН И ТИОСЕМИКАРБАЗИДОВ

Резюме

Вступление. *Длительное применение диуретиков, особенно в больших дозах, численность и тяжесть побочных эффектов (водно-электролитные и метаболические нарушения), а также относительно ограниченная номенклатура существующих диуретических средств диктуют необходимость поиска новых соединений, которые бы проявляли диуретическое действие, имели несложную технологию производства и были более безопасными.*

Цель исследования *– осуществить направленный поиск диуретиков среди неизвестных дизамещенных тиомочевин и тиосемикарбазидов с использованием методологии молекулярного докинга для объяснения возможного механизма действия.*

Методы исследования. *Структуры целевых соединений предложены с применением подходов "drug-design", а именно введением в тиомочевины и тиосемикарбазиды структурных фрагментов, характерных для известных диуретиков. Замещенные циклоалкилкарбонилтиомочевины или тиосемикарбазиды синтезированы однореакторным методом с использованием циклоалкилкарбонилхлоридов, аммония изотиоцианата и замещенных анилинов или гидразидов карбоновых кислот. Строение синтезированных соединений доказано методами ИК-, ^{1}Н ЯМР-спектроскопии, хромато-масс-спектрометрии и элементным анализом. Направленный поиск соединений, влияющих на выделительную функцию почек крыс, осуществлен по общепринятому методу Е. Б. Берхина с водной нагрузкой. Вероятный молекулярный механизм действия предусмотрен благодаря молекулярному докингу.*

Результаты и обсуждение. *Однореакторная реакция циклоалкилкарбонилхлоридов с эквимолекулярным количеством аммония изотиоцианата и замещенных анилинов или гидразидов карбоновых кислот приводит к замещенным циклоалкилкарбонилтиомочевинам или тиосемикарбазидам. Обсуждено строение синтезированных соединений с использованием данных ИК-, ^{1}Н ЯМР- и хроматомасс-спектров. Исследования влияния синтезированных соединений на выделительную функцию почек крыс при водной нагрузке позволили выявить ряд соединений, которые по диуретическому действию превышают фуросемид и конкурируют с гидрохлортиазидом. Результаты молекулярного докинга показали, что исследуемые соединения проявляли высокое сродство к карбоангидразе II и имели подобные сайты связывания с референс-препаратами. Отмеченное указывает на вероятный их механизм действия.*

Выводы. *Разработанная и реализованная стратегия поиска диуретиков среди замещенных циклоалкилкарбонилтиомочевин и тиосемикарбазидов позволила выявить эффективное соединение* ***(3.2)****, которое по силе мочегонного эффекта приближается к референс-препарату "Гидрохлортиазид". Важно то, что, согласно результатам молекулярного докинга, синтезированные соединения, как и референс-препараты, имеют сходный механизм действия (ингибиторы карбоангидразы II). Вероятно, выраженный диуретический эффект исследуемых соединений связан со способностью замещенных тиомочевин образовывать координационные связи с катионом цинка в активном участке CA II. Полученные результаты обосновывают дальнейший целенаправленный поиск потенциальных диуретиков среди этого класса соединений.*

КЛЮЧЕВЫЕ СЛОВА: **синтез; циклоалкилкарбонилтиомочевины; тиосемикарбазиды; спектральные данные; молекулярный докинг; карбоангидраза II; диуретическое действие.**

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