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IN SILICO AND *IN VIVO* SCREENING OF TRIAMTERENE SYNTHETIC ANALOGUES AS PROMISING DIURETICS

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Abstract. In silico and *in vivo* screening of triamterene synthetic analogues as promising diuretics. Sokolova K.V., Stavytskyi V.V., Voskoboinik O.Yu., Podpletnya O.A., Kovalenko S.I. *The modification of lead-compound aimed to the increasing of activity, decrement of toxicity or improvement of selectivity is one of the most important methods used for elaboration of novel medications. Natural compounds, approved or investigational drugs or just compounds with proved biological activity could be the lead-compound. Often the chemical modification of lead compounds is directed at the enhancement of ligand-biological target interactions. Abovementioned approach, namely structural modification of known drug triamterene was used for purposeful search for novel diuretics. The preliminary prognostication of ligand-target interactions and affinity levels allow to reduce quantity of experimental animals, synthesis, and pharmacological studies costs. Conducted studies revealed the series of promising 6,7-disubstituted pteridine-2,4(1H,3H)-diones with diuretic activity that comparable with pharmacological effect of triamterene. Aim – purposeful search for promising diuretics among structural analogues of triamterene that includes preliminary in silico studies, synthesis and in vivo screening of novel compounds for diuretic activity. Methods used: organic synthesis, physicochemical methods of analysis of organic compounds (NMR ^{1}H-spectroscopy, chromato-mass spectrometry, elemental analysis). Prediction of affinity for a biological target, prediction of toxicity and lipophilicity of the combinatorial library, which was created on the basis of the drug triamterene, was carried out using computer services. Studies of compounds that affect the excretory function of the kidneys of rats were performed according to the generally accepted method of E.B. Berkhin with water load. Research of the probable mechanism 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 epithelial sodium channel (ENaC) ((PDB ID – 6WTH). The substantiation of potential diuretics design was conducted by in silico methods (prediction of affinity, ligand-enzyme interactions and pharmacokinetic characteristics). The structural modification of triamterene molecule was carried out by replacing of amino-group in positions 2, 4 and 7 by others "pharmacophore" fragments. Abovementioned transformation is aimed at the changing of ligand-enzyme interactions in active site, lipophility and toxicity. Synthesis of 6,7-disubstituted pteridine-2,4(1H,3H)-diones was conducted by condensation 5,6-diamino-2-oxo-(thioxo-)-2,3-dihydropyrimidin-4(1H)-ones with carbonyl-containing compounds or oxocarboxylic acids. The further modification of obtained compounds was performed by alkylation, hydrazinolysis and nucleophilic addition/elimination. The structure of obtained compounds was proven by elemental analysis, chromato-mass and ^{1}H NMR-spectral analysis. The studies of synthesized compounds effect on excretion function of kidneys allowed to detect series of promising structural analogues of triamterene that exceed it in pharmacological activity by 27.3-99.0%. The "structure-biological activity" relationship was discussed and perspective of the further search of diuretics among abovementioned compounds were shown. The design of new biologically active compounds with diuretic activity was performed using in silico methodologies and realized by structural modification of the well-known diuretic triamterene. Traditional organic synthesis was used for preparation of target compounds, in vivo experiments were*

used to detect compounds with significant biological activity. Several effective compounds were identified among pteridines, which exceed the reference drug triamterene in terms of daily diuresis. The obtained results substantiate further purposeful search, in-depth research on experimental pathologies and study of the mechanism of action of potential diuretics among this class of compounds.

Реферат. In silico та *in vivo* скринінг синтетичних аналогів тріамтерену як перспективних діуретиків. Соколова К.В., Ставицький В.В., Воскобойнік О.Ю., Подплетня О.А., Коваленко С.І. *Модифікація сполуки-лідера, спрямована на підвищення активності, зниження токсичності або покращення селективності, є одним з найважливіших методів, що використовуються для розробки нових ліків. Сполукою лідером можуть бути природні речовини, схвалені або досліджувані ліки або просто сполуки з доведеною біологічною активністю. Часто хімічна модифікація сполуки-лідера спрямована на посилення взаємодії ліганд-біологічна мішень. Вищезазначений підхід, а саме структурну модифікацію відомого препарату тріамтерен, використовували для цілеспрямованого пошуку нових діуретиків. Попереднє прогнозування взаємодій ліганд-біологічна мішень і рівнів афінності дозволяє скоротити кількість дослідних тварин, витрати на синтез і фармакологічні дослідження. Проведені дослідження виявили ряд перспективних 6,7-дизаміщених птеридин-2,4(1H,3H)-діонів з діуретичною активністю, порівняною з фармакологічною дією тріамтерену. Мета – цілеспрямований пошук перспективних діуретиків серед структурних аналогів тріамтерену, що включає попередні in silico дослідження, синтез та in vivo скринінг нових сполук на діуретичну активність. Використовувались методи органічного синтезу, фізико-хімічні методи аналізу органічних сполук (ЯМР ^{1}H-спектроскопія, хроматомасспектрометрія, елементний аналіз). Прогнозування спорідненості до біологічної мішені, прогнозування токсичності та ліпофільності комбінаторної бібліотеки, яку створено на основі препарату тріамтерен, здійснювали за допомогою комп'ютерних сервісів. Дослідження сполук, що впливають на видільну функцію нирок щурів, проводили за загальноприйнятою методикою Е.В. Берхін з водним навантаженням. Дослідження ймовірного механізму проводилося за допомогою гнучкого молекулярного докінгу, як підходу пошуку молекул зі спорідненістю до певної біологічної мішені. Макромолекулярні дані були завантажені з Protein Data Bank (PDB), а саме кристалічні структури епітеліального натрієвого каналу (ENaC) ((PDB ID – 6WTH). Обґрунтування дизайну потенційних діуретиків проводили методами in silico (прогноз афінності, ліганд-ферментних взаємодій та фармакокінетичних характеристик). Здійснено структурну модифікацію молекули тріамтерену шляхом заміни аміногрупи в положеннях 2, 4 і 7 на інші «фармакофорні» фрагменти. Вищезгадана трансформація спрямована на зміну ліганд-ферментних взаємодій в активному центрі, ліпофільності та токсичності. Синтез 6,7-дизаміщених птеридин-2,4(1H,3H)-діонів проводили конденсацією 5,6-діаміно-2-оксо-(тіоксо-)-2,3-дигідро-піримідин-4(1H)-онів з карбонілвмісними сполуками або оксокарбоновими кислотами. Подальшу модифікацію отриманих сполук проводили шляхом алкілування, гідразинолізу та нуклеофільного приєднання/елімінування. Структуру отриманих сполук підтверджено елементним аналізом, хроматомас- та ЯМР-спектральним аналізом. Дослідження впливу синтезованих сполук на видільну функцію нирок дозволили виявити ряд перспективних структурних аналогів тріамтерену, які перевищують його за фармакологічною активністю на 27,3-99,0%. Обговорено взаємозв'язок структура - біологічна активність та показано перспективи подальшого пошуку діуретиків серед зазначених сполук. Розробку нових біологічно активних сполук із діуретичною активністю виконано за методологією in silico та реалізовано шляхом структурної модифікації відомого діуретика тріамтерену. Для отримання цільових сполук використано традиційний органічний синтез, використано експерименти in vivo для виявлення сполук зі значною біологічною активністю. Серед птеридинів виявлено кілька ефективних сполук, які за добовим діурезом перевищують препарат порівняння тріамтерен. Отримані результати обґрунтовують подальший цілеспрямований пошук, поглиблене дослідження експериментальної патології та вивчення механізму дії потенційних діуретиків серед цього класу сполук.*

The process of new drugs developing has been significantly transformed today from *in vivo* models to "de novo design" methods, which are represented by directed design of ligands with high-affinity for biological targets involved in key stages of the pathogenesis of diseases [1]. The effectiveness of medications depends on their affinity for a protein or receptor, and molecules with low affinity will not be able to determine the required biological response. Thus, the affinity of a drug for a biological target is vital for predicting target-drug interactions, and allows a significant number of compounds to be studied prior to a traditional experiment.

One of the important biotargets is the epithelial sodium channels (ENaC), which are responsible for the reabsorption of sodium by the epithelium, lining the distal part of the renal tubules. In addition, ENaCs perform similar functional roles in some other tissues, such as the respiratory tract and the distal part of the colon [2, 3]. Sodium reabsorption is regulated by aldosterone, vasopressin and glucocorticoids and is one of the main mechanisms of regulation of sodium balance, blood volume and blood pressure. Sodium reabsorption is also inhibited by potassium-sparing diuretics: Amiloride and its analogues (Benzamil and Phenamil) and triamterene [4]. However, these drugs

have side effects, namely hyperkalemia and urolithiasis, disorders of the gastrointestinal tract and central nervous system [5].

The choice of heterocyclic matrix, namely pteridine, which is present in the structure of known drug triamterene (6-phenylpteridine-2,4,7-triamine) was substantiated for the development of novel diuretics. Moreover, pteridines and related heterocycles are intensively studied in terms of the search for promising drugs [4, 6]. Our previous studies have shown that for some 1-methyl-3-R-6-(2-hydroxy-(oxo-)-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7 (1*H*, 3*H*, 8*H*)-triones is characterized by a high diuretic effect [7]. Therefore, the design and search for biologically active compounds with diuretic activity among the products of lead-compound triamterene modification is reasonable. The design of abovementioned compounds can be carried out by the replacement of amino groups of positions 2, 4 and 7 with other "pharmacophore" fragments in order to change the ligand-enzyme interactions in the active site of the enzyme; introduction of other structural fragments to positions 2, 6 and 7 in order to change lipophilicity and toxicity (Fig. 1). This structural modification of the "lead-compound" can result in potentiation of the desired pharmacological activity, reduce toxicity and improve the selectivity of action.

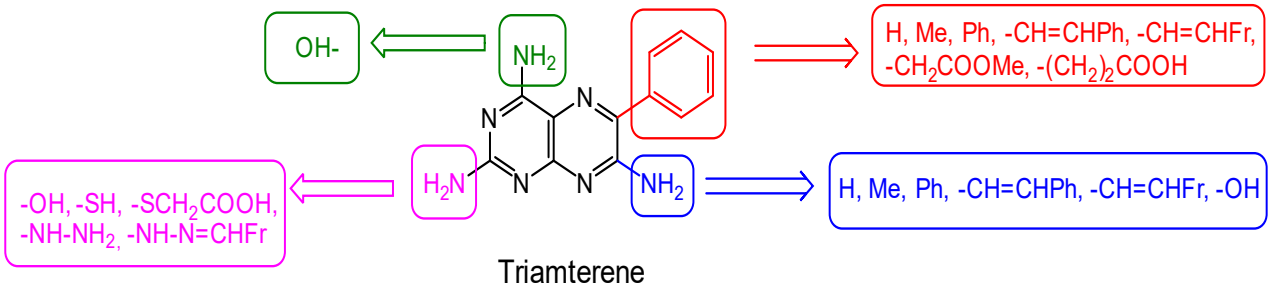

Fig. 1. Directed structural modification of the triamterene molecule to search for new diuretics

Therefore, the aim of present work is to continue our studies on the purposeful search for new biologically active compounds with diuretic activity among pteridines, namely the structural modification of the known drug triamterene using *in silico* methodologies and traditional synthesis and *in vivo* screening.

MATERIALS AND METHODS OF RESEARCH

Melting points were determined in open capillary tubes in a «Mettler Toledo MP 50» apparatus and were not corrected. The elemental analyses (C, H, N) 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. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 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). Mass-spectra of electron impact (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA). The purity of all obtained compounds was checked by ^{1}H-NMR and LC-MS.

5,6-Diaminopyrimidine-2,4(1*H*,3*H*)-dione (**1.1**, CAS: 3240-72-0), 5,6-diamino-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (**1.2**, CAS: 6972-82-3) and 5,6-diamino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (**1.3**, CAS: 1004-76-8) and other starting materials and solvents were obtained from commercially available sources and used without additional purification.

The general methods for the synthesis of substituted 1-R$_1$-6-R$_2$-7-R$_3$-pteridine-2,4(1*H*,3*H*)-diones (2.1-2.12). To a suspension of 10 mmol of compounds **1.1-1.3** in 30 ml of acetic acid 10 mmol of the corresponding dicarbonyl compound or ketocarboxylic acid was added. The reaction mixture was refluxed for 1-3 h and cooled. The formed precipitate was filtered off, washed with water and dried.

1-Methylpteridine-2,4,7(1H,3H,8H)-trione (**2.1**). Yield: 71.0%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 11.70 (s, 1H, 8-NH), 10.78 (s, 1H, 3-NH), 8.74 (s, 1H, H-6), 3.35 (s, 3H, 1-N-CH$_3$); LC-MS: m/z=195 [M+H]; Anal. Calcd. for: $C_7H_6N_4O_3$: C, 43.31; H, 3.12; N, 28.86; Found: C, 43.36; H, 3.19; N, 28.92.

1,6-Dimethylpteridine-2,4,7(1H,3H,8H)-trione (**2.2**). Yield: 69.8%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 12.83 (s, 1H, 8-NH), 11.40 (s, 1H, 3-NH), 3.44 (s, 3H, 1-CH$_3$), 2.42 (s, 3H, 6-CH$_3$); LC-MS: m/z=209 [M+H]; Anal.

Calcd. for: $C_8H_8N_4O_3$: C, 46.16; H, 3.87; N, 26.91, Found: C, 46.21; H, 3.90; N, 26.96.

1,6,7-Trimethylpteridine-2,4(1H,3H)-dione (**2.3**). Yield: 69.8%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.69 (s, 1H, 3-NH), 3.50 (s, 3H, 1-N-CH_3), 2.62 (s, 3H, 6-CH_3), 2.59 (s, 3H, 7-CH_3); LC-MS: m/z=207 [M+H]; Anal. Calcd. for: $C_9H_{10}N_4O_2$: C, 52.42; H, 4.89; N, 27.17, Found: C, 52.44; H, 4.93; N, 27.21.

1-Methyl-6,7-di(styryl)pteridine-2,4(1H,3H)-dione (**2.4**). Yield: 67, 1%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H, 3-NH), 7.99 (d, *J*=15.3 Hz, 1H, 6-CH=CH-Ph), 7.88 (d, *J*=15.5 Hz, 1H, 7-CH=CH-Ph), 7.86 – 7.71 (m, 6H, 6-CH=CH-Ph, 7-CH=CH-Ph, 6-Ar H-2,6, 7-Ar H-2,6), 7.54-7.24 (m, 6H, 6-Ar H-3, 4, 5, 7-Ar H-3,4,5), 3.64 (s, 3H, 1-N-CH_3). LC-MS: m/z=383 [M+H]; Anal. Calcd. for: $C_{23}H_{18}N_4O_2$: C, 72.24; H, 4.74; N, 14.65, Found: C, 72.29; H, 4.77; N, 14.69.

Methyl 2-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl) acetate (**2.5**). Yield: 69.8%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H, 8-NH), 11.49 (s, 1H, 3-NH), 3.80 (s, 2H, CH_2COOCH$_3$), 3.67 (s, 3H, CH$_2$COOCH_3), 3.45 (s, 3H, 1-N-CH_3); LC-MS: m/z=267 [M+H]; Anal. Calcd. for: $C_{10}H_{10}N_4O_5$: C, 45.12; H, 3.79; N, 21.05, Found: C, 45.16; H, 3.83; N, 21.11.

3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid **(2.6)**. Yield: 75.0%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.82 (s, 2H, 8-NH, -COOH), 11.42 (s, 1H, 3-NH), 3.44 (s, 3H, 1-N-CH_3), 2.93 (t, 2H, –CH_2CH$_2$COOH), 2.69 (t, 2H, -CH$_2$CH_2COOH). LC-MS: m/z= 267 [M+H]; Anal. Calcd. for: $C_{13}H_{15}N_5O_6$: C, 45.15; H, 3.82; N, 21.09, Found: C, 45.12; H, 3.79; N, 21.05.

2-Thioxo-2,3-dihydropteridin-4(1H)-one **(2.7)**. Yield: 69.8%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.21 (s, 1H, 1-NH), 12.68 (s, 1H, 3-NH), 8.63 (d, *J*=2.1 Hz, 1H, H-6), 8.52 (d, *J*=2.1 Hz, 1H, H-7), LC-MS: m/z=181 [M+H]; Anal. Calcd. for: $C_6H_4N_4OS$: C, 40.00; H, 2.24; N, 31.09; S, 17.79, Found: C, 40.06; H, 2.29; N, 31.12; S, 17.81.

6-Methyl-2-thioxo-2,3-dihydropteridine-4,7(1H,8H)-dione (**2.8**). Yield: 70.3%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.33-12.46 (m, 2H, 1-NH, 7-OH), 12.34 (s, 1H, 3-NH), 2.38 (s, 3H, 6-CH_3); LC-MS: m/z=211 [M+H]; Anal. Calcd. for: $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.65; S, 15.25; Found: C, 40.07; H, 2.93; N, 26.69; S, 15.29.

6,7-Dimethyl-2-thioxo-2,3-dihydropteridin-4(1H)-one (**2.9**). Yield: 66.4%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00 (s, 1H, 1-NH), 12.53 (s, 1H, 3-NH), 2.60 (s, 3H, 6-CH_3), 2.58 (s, 3H, 7-CH_3); LC-MS: m/z=209 [M+H]; Anal. Calcd. for: $C_8H_8N_4OS$: C, 46.14; H, 3.87; N, 26.91; S, 15.40, Found: C, 46.17; H, 3.91; N, 26.94; S, 15.46.

6,7-Diphenyl-2-thioxo-2,3-dihydropteridin-4(1H)-one (**2.10**). Yield: 72.3%; Mp.: 184-186°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.31 (s, 1H, 1-NH), 12.72 (s, 1H, 3-NH), 7.43 (d, *J*=7.8 Hz, 2H, 7-Ar H-2,6), 7.38 (d, *J*=7.4 Hz, 2H, 6-Ar H-2,6), 7.34-7.23 (m, 6H, 6-Ar H-3, 4, 5, 7-Ar 3, 4, 5). LC-MS: m/z=333 [M+1]; Anal. Calcd. for: $C_{18}H_{12}N_4OS$: C, 65.05; H, 3.64; N, 16.86; S, 9.65; Found: C, 65.09; H, 3.69; N, 16.71; S, 9.69.

6,7-Bis((E)-2-(furan-2-yl)vinyl)-2-thioxo-2,3-dihydropteridin-4(1H)-one (**2.11**). Yield: 54.1%; Mp.: >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (s, 1H, 1-NH), 12.59 (s, 1H, 3-NH), 7.79 – 7.72 (m, 2H, 6-CH=CH-furan, 6-furan H-5), 7.69 – 7.61 (m, 2H, 7-CH=CH-furan, 7-furan H-5), 7.40 (d, *J*=14.6 Hz, 1H, 6-CH=CH-furan), 7.37 (d, *J*=14.9 Hz, 1H, 7-CH=CH-furan), 6.93 – 6.84 (m, 1H, 6-furan H-3), 6.78 – 6.70 (m, 1H, 7-furan H-3), 6.58 (s, 1H, 6-furan H-4), 6.53 (s, 1H, 7-furan H-4). LC-MS: m/z=365 [M+H]; Anal. Calcd. for: $C_{23}H_{18}N_4O_2$: C, 59.33; H, 3.32; N, 15.38; S, 8.80, Found: C, 59.39; H, 3.38; N, 15.42; S, 8.86.

3-(4,7-dioxo-2-thioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid (**2.12**). Yield: 66.7%; Mp.: 293-295°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (s, 1H, 7-OH), 12.24 (s, 1H, 1-NH), 11.79 (s, 1H, COOH), 10.87 (s, 1H, 3-NH), 3.17 (t, *J*=7.3 Hz, 2H, CH_2CH$_2$COOH), 2.78 (t, *J*=7.3 Hz, 2H, CH$_2$CH_2COOH); LC-MS: m/z=269 [M+1]; Anal. Calcd. for: $C_9H_8N_4O_4S$: C, 40.30; H, 3.01; N, 20.89; S, 11.95; Found: C, 40.37; H, 3.08; N, 20.92; S, 12.02.

The general methods for the synthesis of 2-(6-R_2-7-R_3-4-oxo-3,4-dihydropteridin-2-yl)thio)acetic acids (3.1-3.4). An appropriate amount of sodium hydroxide (20 mmol (0.8 g) for compounds **2.9, 2.10**, 30 mmol (0.12 g) for compound **2.8**, 40 mmol (1.6 g) for compound **2.12**) was added to the suspension 10 mmol of 6-R_1-7-R_2-2-thioxo-2,3-dihydropteridin-4(1*H*)-ones (**2.8-2.12**) in 20 ml of a methanol-water mixture (1:1). The resulting mixture was heated to dissolution and 10 mmol of 2-chloroacetic acid was added. The mixture was refluxed until the neutral pH value (around 1 hour) and cooled. The mixture was filtered, and filtrate was acidified up to pH 3-4, the formed precipitate was filtered off, washed with water and dried.

2-((6,7-Dimethyl-4-oxo-3,4-dihydropteridin-2-yl)thio)acetic acid (**3.1**). Yield: 67.3%; Mp. 240-242°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27-12.61 (m, 2H, 3-NH, 7-OH), 4.06 (s, 2H, -SCH$_2$-), 2.62-2.55 (m, 6H, 6-CH_3, 7-CH_3); LC-MS: m/z=267 [M+1]; Anal. Calcd. for: $C_{10}H_{10}N_4O_3S$: C, 45.11; H, 3.79; N, 21.04; S, 12.04; Found: C, 45.16; H, 3.83; N, 21.11; S, 12.09.

2-((6,7-Diphenyl-4-oxo-3,4-dihydropteridin-2-yl)thio)acetic acid (**3.2**). Yield: 68.8%; Mp. 274-276°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s,

1H, 3-NH), 11.64 (s, 1H, COOH), 7.54 – 7.40 (m, 4H, 6-Ar H-2,6, 7-Ar H-2,6), 7.40 – 7.22 (m, 6H, 6-Ar H-3, 4, 5, 7-Ar H-3,4,5), 3.99 (s, 2H, -SCH_2-); LC-MS: m/z=391 [M+1]; Anal. Calcd. for: $C_{20}H_{14}N_4O_3S$: C, 61.53; H, 3.61; N, 14.35; S, 8.21; Found: C, 61.57; H, 3.65; N, 14.42; S, 8.27.

2-((6-methyl-4,7-dioxo-3,4,7,8-tetrahydropteridin-2-yl)thio)acetic acid (**3.3**). Yield: 57.8%; Mp. 289-291°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92-12.03 (m, 3H, COOH, 3-NH, 7-OH), 4.01 (s, 2H, -SCH_2-), 2.33 (s, 3H, 6-CH_3); LC-MS: m/z=269 [M+1]; Anal. Calcd. for: $C_9H_8N_4O_4S$: C, 40.30; H, 3.01; N, 20.89; S, 11.95; Found: C, 40.35; H, 3.08; N, 20.91; S, 11.98.

3-(2-((carboxymethyl)thio)-4,7-dioxo-3,4,7,8-tetrahydropteridin-6-yl)propanoic acid (**3.4**). Yield: 59.2%; Mp. 266-268°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18-11.77 (m, 4H, 2-COOH, 3-NH, 7-NH), 4.01 (s, 2H, -SCH_2-), 2.95-2.88 (m, 2H, C$\underline{H_2}$$CH_2$COOH), 2.66 (t, *J*=7.0 Hz, 2H, CH_2C$\underline{H_2}$COOH); LC-MS: m/z=327 [M+1]; Anal. Calcd. for: $C_{11}H_{10}N_4O_6S$: C, 40.49; H, 3.09; N, 17.17; S, 9.83; Found: C, 40.52; H, 3.11; N, 17.22; S, 9.86.

The general methods for the synthesis of 6-R_2-7-R_3-2-hydrazineyl-pteridin-4(3*H*)-ones (4.1-4.2). To a suspension of 10 mmol of compound **3.1** or **3.4** in 20 ml of ethanol 1 ml (20 mmol) of hydrazine hydrate was added. The reaction mixture was refluxed for 1.5 h until the hydrogen sulfide evolution over. The reaction mixture was cooled, and the formed precipitate was filtered off and died.

2-Hydrazineyl-6,7-dimethylpteridin-4(3H)-one (**4.1**). Yield: 59.2%; Mp. 270-272°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H, 3-NH), 7.22 (s, 1H, N$\underline{H}$$NH_2$), 4.36 (s, 2H, NH$\underline{NH_2}$), 2.48 – 2.43 (m, 6H, 6-CH_3, 7-CH_3), LC-MS: m/z=207 [M+H]; Anal. Calcd. for: $C_8H_{10}N_6O$: C, 46.60; H, 4.89; N, 40.76; S, 17.79, Found: C, 46.60; H, 4.89; N, 40.76.

3-(2-Hydrazineyl-7-hydroxy-4-oxo-3,4-dihydropteridin-6-yl)propanoic acid (**4.2**). Yield: 59.2%; Mp. >300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H, 3-NH), 7.40 (s, 1H, N$\underline{H}$$NH_2$), 4.20 (s, 2H, NH$\underline{NH_2}$), 2.82 (t, 2H, $\underline{CH_2}$$CH_2$COOH), 2.59 (t, 2H, $CH_2\underline{CH_2}$COOH), LC-MS: m/z=267 [M+H]; Calculated for $C_9H_{10}N_6O_4$: C, 40.61; H, 3.79; N, 31.57, Found: C, 40.64; H, 3.82; N, 31.61.

The general methods for the synthesis of 6-R_2-7-R_3-2-((furan-2-ylmethylene)hydrazineylidene)-6,7-dimethyl-2,3-dihydropteridin-4(1*H*)-ones (5.1, 5.2). To a suspension of 10 mmol of compound 4.1 or 4.2 in 20 ml of acetic acid 0.96 g (10 mmol) of furan-2-carbaldehyde was added. The reaction mixture was refluxed for 1.5 h. The reaction mixture was cooled and the formed precipitate was filtered off, washed with water and dried.

2-((Furan-2-ylmethylene)hydrazineylidene)-6,7-dimethyl-2,3-dihydropteridin-4(1H)-one (**5.1**). Yield: 84.2%; Mp. 300°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H, 3-NH), 10.84 (s, 1H, $\underline{1\text{-}NH}$), 8.00 (s, 1H, $\underline{CH}$=N), 7.64 (d, 1H, furan H-5), 7.06 (d, 1H, furan H-3), 6.54 (t, 1H, furan H-4), 2.57 (s, 3H, 6-CH_3), 2.50 (s, 3H, 7-CH_3). LC-MS: m/z=285 [M+H]; Anal. Calcd. for: $C_{13}H_{12}N_6O_2$: C, 54.93; H, 4.25; N, 29.56. Found: C, 54.96; H, 4.29; N, 29.61.

3-(-2-((Furan-2-ylmethylene)hydrazineylidene)-4,7-dioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid (**5.2**). Yield: 79.6%; Mp. 300°C; ^{1}H NMR (400 MHz, DMSO-d6) δ 12.34 (s, 1H, 8-NH), 12.09 (s, 1H, 3-NH), 11.80 (s, 1H, COOH), 10.61 (s, 1H, 3-NH), 8.01 (s, 1H, $\underline{CH}$=N), 7.65 (d, *J*=2.4 Hz, 1H, furan H-5), 7.09 (d, *J*=2.2 Hz, 1H, furan H-3), 6.53 (t, 1H, furan H-4), 2.89 (t, 2H, C$\underline{H_2}$$CH_2$COOH), 2.65 (t, 2H, CH_2C$\underline{H_2}$COOH), LC-MS: m/z=345 [M+H]; Anal. Calcd. for: $C_{14}H_{12}N_6O_5$: C, 48.84; H, 3.51; N, 24.41, Found: C, 48.89; H, 3.58; N, 24.46.

Molecular docking. Research was conducted by flexible molecular docking, as an approach of finding the molecules with affinity to a specific biological target. Macromolecule of full-length human ENaC ECD (PDB ID – 6WTH) from Protein Data Bank (PDB) was used as biological target [8].

Ligand preparation. The substances were drawn using MarvinSketch 20.20.0 and saved in mol format [9]. 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, the pdb-files were converted into PDBQT, number of active torsions was set as default [10].

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 protein were saved as pdb-files [11]. In AutoDockTools- 1.5.6 polar hydrogens were added and saved as PDBQT. Grid box was set as following: center_x=161.722, center_y=163.028, center_z=189.222, size_x=30, size_y=30, size_z=30 for epithelial sodium channel (ENaC) (6WTH). Vina was used to carry docking. For visualization Discovery Studio v 19.1.0.18287 was used.

Toxicity prognosis. Prediction of acute toxicity and calculation of octanol/water partition coefficient (logP) was made *in silico* using the service ProTox-II [12].

Study of the effect of compounds on the excretory function of the kidneys. The initial screening was performed on 174 white male Wistar rats weighing 120-170 g. The in-depth experiment was performed on 24 white male Wistar rats weighing 100-140 g, which

were kept in standard conditions of the vivarium of the Dnipro State Medical University. 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) [13]. Screening of the new synthesized compounds, in order to identify diuretic properties in a few pteridine derivatives, was carried out according to the generally accepted method of E.B. Berkhin [14, 15]. 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 and without. 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 and 24 hours. triamterene in equivalent doses for rats was selected as the reference drug [15].

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 $p \leq 0.05$ [16].

RESULTS AND DISCUSSION

According to the design of the present study the calculation of predicted affinity for biological target, predicted toxicity and lipophilicity were conducted for more then 50 compounds from combinatorial library based on triamterene structure. (Fig. 1). Molecular docking revealed that in most of cases structural modification of triamterene resulted the decreasing of studied compounds affinity for ENaC (Table. 1). At the same time according to the calculations studied compounds have the higher predicted hydrophilicity (exceptions are **2.4**, **2.10**, **2.11** and **3.2**) and correspondingly lower predicted toxicity (exceptions are **2.3**, **2.5**, **2.6, 2.8, 2.9**).

Table 1

Results of molecular docking and predicted toxicometric parameters of compounds according to TEST data

Compd.*	R_1	R_2	R_3	X	Affinity (kcal/mol) to ENaC (6WTH)	LD_{50}, mg/kg (Toxicity Class)**	LogP
2.1	**Me**	**H**	**OH**	**O**	**-6.8**	**811 (IV)**	**-1.69**
2.2	**Me**	**Me**	**OH**	**O**	**-7.1**	**304 (III)**	**-1.38**
2.3	**Me**	**Me**	**Me**	**O**	**-7.2**	**19 (II)**	**-0.37**
2.4	**Me**	**-CH=CH-Ph**	**-CH=CH-Ph**	**O**	**-6.9**	**450 (IV)**	**3.36**
2.5	**Me**	**$-CH_2COOMe$**	**OH**	**O**	**-6.3**	**19 (II)**	**-1.97**
2.6	**Me**	**$-(CH_2)_2COOH$**	**OH**	**O**	**-7.7**	**61 (III)**	**-1.67**
2.7	**H**	**H**	**H**	**S**	**-6.9**	**811 (IV)**	**0.38**
2.8	**H**	**Me**	**OH**	**S**	**-7.0**	**61 (III)**	**-0.02**
2.9	**H**	**Me**	**Me**	**S**	**-6.8**	**19 (II)**	**0.99**
2.10	**H**	**Ph**	**Ph**	**S**	**-7.9**	**1800 (III)**	**3.71**
2.11	**H**	**(furan-2-yl)-vinyl**	**(furan-2-yl)-vinyl**	**S**	**-6.5**	**2500 (V)**	**3.90**
2.12	**H**	**$-(CH_2)_2COOH$**	**OH**	**S**	**-6.2**	**1000 (IV)**	**0.1**
3.1	**–**	**Me**	**Me**	**–**	**-7.0**	**1711 (IV)**	**0.51**
3.2	**–**	**Ph**	**Ph**	**–**	**-6.5**	**1800 (III)**	**3.22**
3.3	**–**	**Me**	**OH**	**–**	**-7.5**	**1000 (IV)**	**-0.1**
3.4	**–**	**$-(CH_2)_2COOH$**	**OH**	**–**	**-7.1**	**300 (III)**	**-0.39**
4.1	**–**	**Me**	**Me**	**–**	**-7.7**	**1800 (III)**	**0.39**
4.2	**–**	**$-(CH_2)_2COOH$**	**OH**	**–**	**-7.7**	**1900 (III)**	**-0.51**
5.1	**–**	**Me**	**Me**	**–**	**-6.3**	**500 (IV)**	**0.79**
5.2	**–**	**$-(CH_2)_2COOH$**	**OH**	**–**	**-6.5**	**520 (IV)**	**-0.52**
Triamterene					**-8.2**	**285 (III)**	**2.58**

Notes: * – table shows data for compounds whose affinity is >6.0 kcal/mol; ** Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$); Class VI: non-toxic ($LD_{50} > 5000$).

Additionally, visualization of interactions between triamterene and active site of epithelial sodium channels (ENaC) was conducted for estimation of possible mechanism of their biological activity. The analysis of the docking of triamterene to ENaC (Table 2, Fig. 2, A) revealed the interaction of amino-group in positions 2, 4 and 7 of pteridine system with amino-acids moieties ASN185 (2,33Å), ASP268 (2,22Å) and GLU254 (2,52Å), LEU257 (2,56Å) *via* four conventional hydrogen bond. Besides, weak electrostatic (π-anion) and hydrophobic (amide-π-stacked, and π-alkyl) interactions of phenyl fragment of molecule with ASP268 (3,64Å), ALA269 (4,48Å), LEU257 (4,66Å).

Table 2

The main types of interactions of synthesized compounds and pharmacological standards with amino acid residues of epithelial sodium channel (ENaC)

Compd.	The main interaction types between compounds, pharmacological standards and amino acid residues of enzymes*
Triamterene	ASP268[a], GLU254[a], LEU257[a], ASN185[a], ASP268[b], ASP268[c], ALA269[c], LEU257[d], ALA269[d]
2.1	CYS267[a], SER210[h], THR259[h], LEU257[h], ASP268[b], ASP268[b], ALA269[e], LEU257[e]
2.2	LEU257[h], ASP268[b], ASP268[b], ALA269[e], LEU257[e]
2.3	CYS267[a], SER210[h], LEU257[h], ASP268[b], ASP268[b], ALA269[e], LEU257[e], CYS260[f]
2.4	HIS299[a], HIS299[a], PRO300[a], PHE297[g], PHE297[g], PRO437[e], ALA360[f]
2.5	GLN213[a], SER312[a], GLN213[a], THR216[a], SER312[a], LYS503[g], LYS503[i,g], THR216[i], GLN307[i], THR216[d], LEU480[e], TRP471[d]
2.6	THR209[a], CYS267[a], ALA253[h], LEU257[h], ASP268[b], ASP268[b], ALA269[e], LEU257[e]
2.7	ASN185[a], ASP487[a], THR209[a], SER210[h], LEU257[h], GLU254[h], ASP268[b]
2.8	THR209[a], ASP487[a], ASP268[b], ALA269[e], LEU257[e]
2.9	ASP487[a], THR209[a], ASN185[a], SER210[h], ASP268[b], ALA269[e], LEU257[e], CYS260[e]
2.10	ALA360[a], HIS299[a], PRO300[h], HIS299[g], PHE297[g], PHE297[l], PHE297[f], ALA360[f]
2.11	MET432[a], SER296[i], VAL265[k], VAL265[k], CYS267[l], LEU295[d], VAL434[d], LEU295[d]
2.12	CYS260[a], VAL258[a], ASP268[b], ASP268[b], LEU257[c], VAL258[c], ALA269[d]
3.1	ALA269[a], LEU257[a], ARG490[e]
3.2	GLN491[a], HIS187[h], HIS187[h], HIS187[h], ARG490[j], GLU254[b], GLN491[i], GLU254[k], HIS187[g], ARG490[f]
3.3	ASN185[a], VAL258[a], ASP268[b]
3.4	ASN185[a], ALA186[a], LEU257[a], THR209[a], HIS187[h], ASP268[b]
4.1	ASP268[j], ASP268[j], CYS260[a], CYS267[a], CYS260[a], SER266[a], ASP268[b], ASP268[b]
4.2	ALA186[a], LEU257[a], ASN185[a], ALA186[a], CYS189[a], THR209[a], HIS187[h], ASP268[h]
5.1	ARG330[a], TYR335[a], TYR335[a], GLU303[h], ARG206[j], ARG330[j], GLU217[b], GLU303[k], HIS268[g], HIS268[g], ALA100[e], ALA311[e], TYR269[d]
5.2	GLN334[a], GLN334[a], PRO338[h], ASN288[i], ASN288[i], ALA387[f]

Notes: * – a – Conventional Hydrogen Bond; b – Electrostatic (π-Anion), c – Hydrophobic (Amide-π-Stacked), d – Hydrophobic (π-Alkyl); e – Hydrophobic (Alk); f – Hydrophobic (π-Alk), g – Hydrophobic (π-π-T-shaped), h – Carbon Hydrogen Bond, i – π-Donor Hydrogen Bond, j – Electrostatic (π-Cation), k – Hydrophobic (π-Sigma), l – π-Sulfur.

The studied compounds have various interaction with amino acid moieties in active site of ENaC (Table 2). Hence, visualization of interaction of compound **2.1** with ENaC (Fig. 2, B) revealed the presence

of one conventional hydrogen bond between NH-group in position with amino-acid moiety CYS267 (2,84Å). Besides, compound **2.1** forms three weaker carbon hydrogen bond between long pairs of N-5, O-4, NH-group of pteridine cycle and SER210 (3,68Å), THR259 (3,52Å) and LEU257 (3,37Å), correspondingly. Additionally for compound **2.1** were predicted electrostatic (π-anion) and hydrophobic (Alk) interactions between electron deficient cycle and ASP268 (3,78Å, 3,20Å), ALA269 (3,84Å) and LEU257 (3,37Å). Abovementioned interaction provide the placing of **2.1** molecule (LogP = -1.68) in lipophilic part of ENaC active site. It should be noted that placing of compound **2.1** is different from placing of triamterene molecule that despite higher lipophilicity (LogP = 2.58) located in more hydrophilic part of the ENaC pocket.

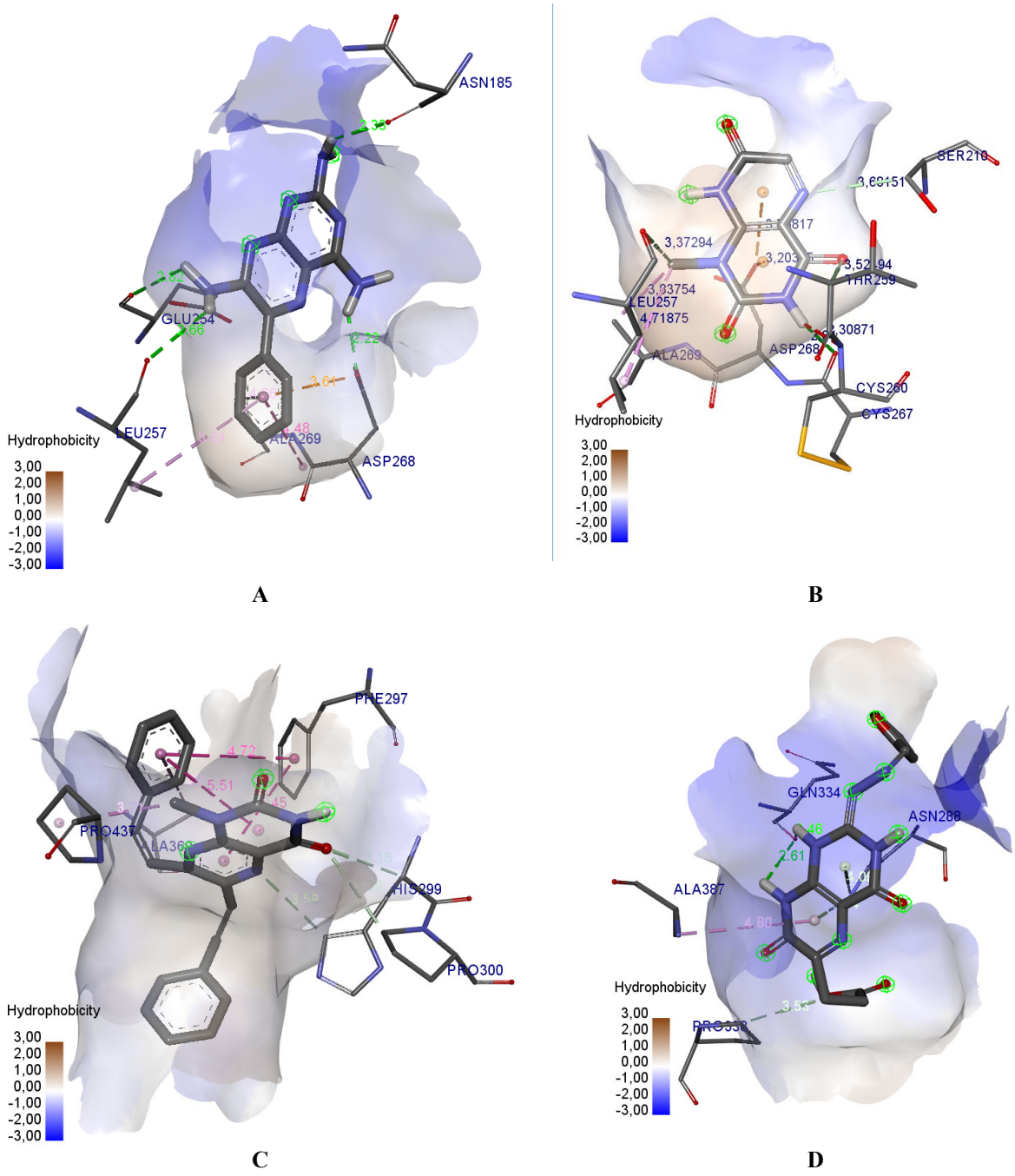

Fig. 2. Types of ligand-enzyme interactions according to the visualization of the docking study: A) triamterene with ENaC 3D; B) compound 2.1 with ENaC 3D; C) compound 2.4 with ENaC 3D; D) compound 5.2 with ENaC 3D

Molecule of compound **2.4** (LogP =3.36) is placed in lipophilic part of active site of enzyme as well (Fig. 2, C). At the same time molecule of abovementioned compound form interaction with other amino acid moieties. (Table 2). Hence, molecular docking of compound **2.4** toward ENaC revealed (Fig. 2, C) that ligand-enzyme interactions are formed by conventional hydrogen bond between lone pair of O-4 and N-5 of pteridine cycle and amino acids moieties HIS299 (3,16Å), PRO300 (3,39Å) and HIS299 (3,59Å) correspondingly. The other bindings of compound **2.4** are weak electrostatic interactions (Alk, π-Alk, π-π-T-shaped) with PRO437 (3,71Å), ALA360 (5,08Å), PHE297 (5,45Å), PHE297 (4,72Å). The listed interactions are decisive for placing of molecule in active site of molecular target.

Molecule of compound **5.2** unlike compounds **2.1** and **2.5** is placed in hydrophilic part of ENaC active center (Fig. 2, D). Abovementioned placing is provided by two conventional hydrogen bonds of *NH*-fragment in 1st and 8th position of the cycle and carbon hydrogen bond of carboxylic group with GLN334 (2,46Å and 2.41Å) and PRO338 (3,53 Å) correspondingly. Additionally placing of ligand in active site of enzyme is facilitated by π-donor hydrogen bond and hydrophobic (π-Alk) interaction of pteridine cycle with ASN288 (4,09Å and 3,54Å) and ALA387 (4,80Å), correspondingly.

In this way, conducted molecular docking of studied compounds toward biological target showed, that most of the compounds formed different from the triamterene ligand-enzyme interactions. At that time compound **5.2** has similar to triamterene position in active site of ENaC. Despite the obvious benefits, computer-aided drug design methods have some limitations. Hence, computer modelling methods do not consider diversity of the drug's effects on living organism of experimental animals. Traditional organic synthesis methods and their *in vivo* screening for biological activity should be conducted to estimate tha value of compounds as potential diuretics.

Hence, the following step of the present study included synthesis of compounds **2** via condensation of 5,6-diamino-2-oxo-(thioxo-)-2,3-dihydropyrimidin-4(1*H*)-ones (**1.1-1.3**) with carbonyl containing compounds (Fig. 3) [17, 18]. The formation of the pteridine system (compounds **2**) was proven by the presence of characteristic signals that correspond to the substituents in positions 6 and 7. Additionally compounds **2** were characterized by the singlet signals of exchangeable protons at the N_8-, N_3- and N_1-atoms of pteridine cycle at the 13.72-10.78 ppm and singlet signals of COOH-group protons (compound **2.6**, **2.12**) at the11.82-11.79 ppm. It should be mentioned that signals of exchangeable protons underwent significant paramagnetic shift in case of thio-containing derivatives (**2.7-2.12**) [19].

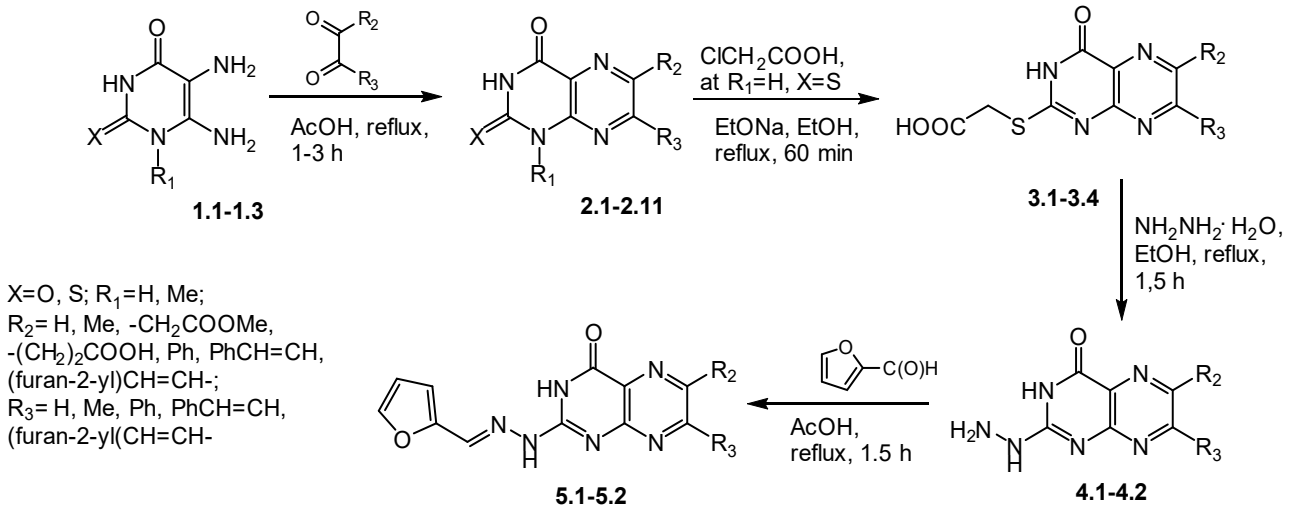

Fig. 3. Approaches to the synthesis of triamterene synthetic analogue's

The following step of chemical modification was the S-alkylation of compounds **2.8**, **2.9, 2.10** and **2.12** with halogen-containing carboxylic acid. Abovementioned reaction was conducted according to standard procedure [20]. The ^{1}H NMR spectra of compounds **3.1-3.4**, unlike spectra of compounds **2**, were characterized by the presence of the signals that associated with substituents at sulfur atom, namely two proton singlet of SCH_2-group at the 3.99-4,06 ppm. Besides, spectra of abovementioned compounds were characterized by the signals of exchangeable protons and set of the signals correspond to substituents at the positions 6 and 7 [19].

It should be mentioned, that replacement of carboxyethylthiol fragment in position 2 of pteridine cycle of compound **3** by hydrazino-group proceeded easily. Abovementioned reaction required addition of 2-fold excess of hydrazine hydrate and short-term refluxing (Fig. 3). This reaction resulted in the formation of compounds **4** with satisfactory yields. Reaction of compounds **4** with furfural yielded corresponding hydrazones **5**. Signals of hydrazine-group protons were registered at the 7.40-7.22 ppm and 4.36-4.20 ppm in ^{1}H NMR spectra of compounds **4**. At the same time spectra of compounds **5** were characterized by the singlet of azomethine proton at the 8.01-8.00 ppm. Presented data definitely prove the structure of obtained compounds.

The results of biological studies showed (Table 3), that most of the investigated compounds by the 2 hour of experiment revealed moderate diuretic effect or inhibited the diuresis of experimental animals that is characteristic for pteridine derivatives [4]. However, compounds **2.3** and **5.1** with diuretic effect comparable with activity of triamterene were found among the studied compounds. (Table 3). More interesting were the results of the study of synthesized compounds effect on day diuresis. It was found that compounds **2.2**, **2.4**, **2.7, 2.11** and **5.2** increased day diuresis by 76.4-148.1%, that exceeded activity of triamterene (49,1%, Table 3).

Table 3

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)*

No.	No. of compound	Diuresis, ml/100 g/2 h	% related to control	Diuresis, ml/100 g/24 h	Of changes % related to control
	control	3.48±0.09	–	2.16±0.05	–
2.1	1К-152АА	3.44±0.09	-1.1	4.34±0.30	100.9
2.2	1К-2	3.41±0.11	-2.0	3.06±0.14*	41.7
2.3	1К-133	4.11±0.12	18.1	1.21±0.19*	-44.0
2.4	1К-202	3.25±0.09	-6.6	5.36±0.71*	148.1
2.5	1К-140	3.60±0.15	3.4	2.53±0.15	17.1
2.7	1К-267	2.94±0.08	-15.5	3.95±0.08	82.9
2.8	1К-152АВ	3.59±0.12	3.2	1.73±0.09	-19.9
2.9	1К-152	3.23±0.09	-7.2	1.31±0.11*	-39.4
2.10	1К-265	2.64±0.11	-24.1	1.20±0.05*	-44.4
2.11	1К-202С	3.16±0.08	-9.2	3.81±0.13	76.4
3.2	1К-265А	2.37±0.08	-31.9	1.44±0.06*	-33.3
4.1	1К-268В	3.10±0.09	-10.9	1.53±0.07	-29.2
5.1	1К-280	4.05±0.05	16.4	2.63±0.10	21.8
5.2	1К-289	3.00±0.09	-13.8	4.85±0.30*	124.5
	Control	3.39±0.30	–	2.20±0.28	–
	Triamterene	4.00±0.23*	18.0	3.28±0.17*	49.1

Notes: # – significant changes in control ($p<0.05$); n – is the number of animals in the group.

Conducted QSAR studies showed that structural modification of triamterene *via* replacing of amino group in 2nd and 4th position by oxo-groups in most of cases was reasonable (compounds **2.1**, **2.2** and **2.4**). Replacing of oxo-group in position 2 of pteridine system by thioxo-group (**2.8-2.10**), hydrazino-group

(**4.1** and **4.2**) led to the loss of diuretic activity. Transformation of thioxo-group at the 2nd position into mercapthomethylcarboxylic fragment (compound **3.2**) resulted in the decreasing of activity as well. Introduction of furylhydrazone fragment to position 2 of pteridine system (**5.1** and **5.2**) enhances diuresis. The nature of substituents in positions 6 and 7 have a significant impact on diuretic activity level.

Therefore, compounds **2.4** and **2.11** that contain arylvinyl fragments in positions 6 and 7 reveal significant diuretic activity. Hence, the purposeful search of biologically active compounds with diuretic activity among the structural analogues of triamterene is reasonable and opens prospects for further design, synthesis and advanced studies of their effect on excretion system.

CONCLUSIONS

The design of new biologically active compounds with diuretic activity was performed using *in silico* methodologies and realized by structural modification of the well-known diuretic triamterene. Condensation of 5,6-diamino-2-oxo-(thioxo-)-2,3-dihydropyrimidin-4(1*H*)-ones with carbonyl containing compounds is convenient method for synthesis of promising diuretics that can be used for preparation of various by structure compounds. Several effective substances were identified among synthesized pteridines, which exceed the reference drug triamterene by the level of diuretic activity. Replacing of amino group in 2nd and 4th position by oxo-groups, introduction of furylhydrazone fragment to position 2 of pteridine system and arylvinyl fragments to positions 6 and 7 are reasonable in scope of novel diuretics synthesis while replacing oxo-group in position 2 of pteridine system by thioxo-group hydrazino-group, as well, as transformation of thioxo-group in the 2nd position into mercapthomethylcarboxylic fragment lead to the loss of diuretic activity. The obtained results substantiate further purposeful search, in-depth research on experimental pathologies and study of the mechanism of action of potential diuretics among this class of compounds.

Recommendations.

The results of studies confirmed the diuretic effect of some 6-substituted pteridine-2,4,7 (1*H*, 3*H*, 8*H*) - triones and open up prospects for further study of their effects on the urinary system. *In vitro* study of synthesized compounds effect on molecular targets associated with diuretic activity, structural modification of active compounds by introducing of additional pharmacophore groups or their "bioisosteric" substitutions are among reasonable directions of further studies.

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