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K.V. Sokolova [1 *],
V.V. Stavytskyi [2],
S.I. Kovalenko [2],
O.A. Podpletnya [1]

DIRECTED SEARCH FOR DIURETICS AMONG 6-SUBSTITUTED PTERIDINE-2,4,7(1*H*,3*H*,8*H*)-TRIONES

Dnipro State Medical University [1]
V. Vernadskyi str., 9, Dnipro, 49044, Ukraine
Zaporizhzhia State Medical University [2]
Mayakovskyi av., 26, Zaporizhzhia, 69035, Ukraine
Дніпровський державний медичний університет [1]
вул. В. Вернадського, 9, Дніпро, 49044, Україна
Запорізький державний медичний університет [2]
пр. Маяковського, 26, Запоріжжя, 69035, Україна
**e-mail: cat@dma.dp.ua*

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Ключові слова: *6-заміщені птеридин-2,4,7(1H,3H,8H)-тріони, діуретики, молекулярний докінг, SAR-аналіз, механізм дії*
Кючевые слова: *6-замещенные птеридин-2,4,7(1H,3H,8H)-трионы, диуретики, молекулярный докинг, SAR-анализ, механизм действия*

Abstract. Directed search for diuretics among 6-substituted pteridine-2,4,7(1H,3H,8H)-triones. Sokolova K.V., Stavytskyi V.V., Kovalenko S.I., Podpletnya O.A. *Directed search for biologically active compounds among heterocycles still remains a relevant area of medical chemistry. Among the significant number of heterocyclic compounds, pteridines deserve special attention. Among the above-mentioned ones the drugs with antitumor, antimicrobial, antiviral, diuretic and other types of biological action are known. Nevertheless, 6-substituted pteridine-2,4,7(1H,3H,8H)-triones, which are structurally similar to triamterene (6-phenylpteridine-2,4,7-triamine) – a diuretic with potassium-sparing action are interesting objects for search for diuretics. All the more, they are characterized by prototropic tautomerism, able to form hydrogen and donor-acceptor bonds with various ligands, and it is likely that these structural features will provide their diuretic effect. The aim of the study is the directed search for diuretics among 6-substituted pteridine-2,4,7(1H,3H,8H)-triones using in silico and in vivo methodology and elucidation of the probable mechanism of action. 1-methyl-3-R-6- (2-oxo-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones were selected to study the effect on renal excretory function. and 1-methyl-3-R-6- (2-hydroxy-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones. 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. The content of creatinine, sodium, potassium and chlorides in blood and urine plasma was determined by biochemical methods using standard test kits of NPV "Philisit-Diagnostics" (Ukraine) and calculations were performed according to generally accepted methods. 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 Human carbonic anhydrase II (PDB ID – 3HS4) and epithelial sodium channel (ENaC) (PDB ID – 4NTX). Studies of the effect of the synthesized compounds on the excretory function of the kidneys of rats showed that 1-methyl-3-R-6- (2-oxo-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones containing 4-fluorophenyl, 2,4-difluorophenyl, 4-chlorophenyl fragments in the molecule increase diuresis by the second hour by 27.3-70.1% compared with the control group. According to the results of the impact on daily diuresis, it was found that the most active was 1-methyl-6- (2-oxo-2-phenyl) ethyl) pteridine-2,4,7(1H,3H,8H)-triones, which increased daily diuresis by 168.1%, exceeding the effect of Hydrochlorothiazide (41.8%) and Triamterene (49.1%). However, substituted 1-methyl-3-R-6- (2-hydroxy-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones are inactive compounds. In-depth studies using biological tests and molecular docking have suggested that 1-methyl-6- (2-oxo-2-aryl) ethyl) pteridine-2,4,7(1H,3H,8H)-triones 2.1, 2.5 and 2.6) probable mechanisms of diuretic action are disruption of sodium transport in the distal convoluted tubules, causing sodium excretion and water loss and possibly inhibition of epithelial sodium channels that promote sodium uptake and*

potassium secretion in the distal convolutions and tubules, which implements potassium-sparing action. A well-founded and developed strategy for the search for diuretics among 6-substituted pteridine-2,4,7(1H,3H,8H)-triones has identified a number of effective compounds that by diuretic effect are superior to the reference drugs "Hydrochlorothiazide" and "Triamterene". Importantly, the results of molecular docking suggested a mechanism of action of the compounds under study, similar to thiazide diuretics. This action may be related to the tautomerism of these compounds and, as a consequence, their ability to form coordination bonds with the zinc cation and the additional interaction of halogens in the active site of CA II. It was possible to detect the presence of potassium-sparing action, probably due to the ability to inhibit epithelial sodium channels (ENaC). The obtained results substantiate the further purposeful search for potential diuretics among this class of compounds.

Реферат. Спрямований пошук діуретиків серед 6-заміщених птеридин-2,4,7(1H,3H,8H)-тріонів. Соколова К.В., Ставицький В.В., Коваленко С.І., Подплетня О.А. *Спрямований пошук біологічно активних сполук серед гетероциклів і на сьогодні залишається актуальним напрямом медичної хімії. Серед значної кількості гетероциклічних сполук певної уваги заслуговують птеридини. Серед них відомі лікарські засоби з протипухлинною, протимікробною, противірусною, діуретичною та іншими видами біологічної дії. Незважаючи на це, цікавими об'єктами для пошуку діуретичних засобів є 6-заміщені птеридин-2,4,7(1H,3H,8H)-тріонів, які за структурою подібні до тріамтерену (6-phenylpteridine-2,4,7-triamine) – діуретика з калійзберігаючою дією. Тим більше, що для них характерна прототропна таутомерія, вони здатні до утворення водневих та донорно-акцепторних зв'язків з різноманітними лігандами, і вірогідно, що саме зазначені особливості будови й будуть забезпечувати їхню діуретичну дію. Метою роботи став спрямований пошук діуретичних засобів серед 6-заміщених птеридин-2,4,7(1H,3H,8H)-тріонів з використанням методології методами in silico й in vivo та з'ясування ймовірного механізму дії. Для дослідження впливу на видільну функцію нирок були відібрані 1-methyl-3-R-6-(2-oxo-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7(1H,3H,8H)-triones and 1-methyl-3-R-6-(2-hydroxy-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7(1H,3H,8H)-triones. Спрямований пошук сполук, що впливають на видільну функцію нирок щурів, проводили за загальноприйнятою методикою Є.Б. Берхіна з водним навантаженням. У плазмі крові та сечі біохімічними методами визначали вміст креатиніну, натрію, калію та хлоридів за допомогою стандартних тест-наборів НПВ «Філісіт-Діагностика» (Україна) та проводили розрахунки згідно із загальноприйнятими методиками. Дослідження ймовірного механізму проводилося за допомогою гнучкого молекулярного докінгу, як підходу пошуку молекул, які мають спорідненість до певної біологічної мішені. Макромолекулярні дані були завантажені з банку білкових даних (PDB), а саме: кристалічні структури карбоангідрази II людини (PDB ID - 3HS4) та епітеліального натрієвого каналу (ENaC) (PDB ID – 4NTX). Дослідження впливу синтезованих сполук на видільну функцію нирок щурів показали, що 1-methyl-3-R-6-(2-oxo-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7(1H,3H,8H)-triones, які містять у молекулі 4-фторфенільний, 2,4-дифторфенільний, 4-хлорфенільний фрагменти, посилюють діурез на другу годину на 27,3-70,1% порівняно з контрольною групою. За результатами впливу на добовий діурез установлено, що найбільш активним виявився 1-methyl-6-(2-oxo-2-phenyl)ethyl)pteridine-2,4,7(1H,3H,8H)-triones, який підвищував добовий діурез на 168,1%, перевищуючи при цьому ефект Hydrochlorothiazide (41,8%) та Triamterene (49,1%). У той же час заміщені 1-methyl-3-R-6-(2-hydroxy-2-aryl-(hetaryl-)ethyl)pteridine-2,4,7(1H,3H,8H)-triones малоактивні сполуки. Проведені поглиблені дослідження з використанням біологічних тестів та молекулярного докінгу надали змогу припустити, що для 1-methyl-6-(2-oxo-2-aryl)ethyl)pteridine-2,4,7(1H,3H,8H)-triones (2.1, 2.5 та 2.6) імовірними механізмами діуретичної дії є порушення транспорту натрію в дистальних звивистих канальцях, що викликає виведення натрію та втрату води організмом та, ймовірно, інгібування епітеліальних натрієвих каналів, що сприяють поглинанню натрію та секреції калію, у дистальних звивистих канальцях і збиральних трубочках, що реалізує калійзберігаючу дію. Обґрунтована та розроблена стратегія пошуку діуретиків серед 6-заміщених птеридин-2,4,7(1H,3H,8H)-тріонів дозволила виділити ряд ефективних сполук, які за діуретичною дією перевищують референс-препарати «Гідрохлоротіазид» та «Тріамтерен». Важливо, що результати молекулярного докінгу дали змогу припустити механізм дії досліджуваних сполук, подібний до тіазидних діуретиків. Зазначена дія може бути пов'язана з таутомерією цих сполук і, як наслідок, їх здатністю утворювати координаційні зв'язки з катіоном цинку та додатковою взаємодією галогенів в активній ділянці CA II. Також вдалось виявити наявність калійзберігаючої дії, певно, за рахунок здатності інгібувати епітеліальні натрієві канали (ENaC). Отримані результати обґрунтовують подальший цілеспрямований пошук потенційних діуретиків серед цього класу сполук.*

The urgency of the problem of rational use of diuretics is due to the widespread use of this class of drugs in clinical practice, and water-electrolyte and metabolic disorders accompanying their use. Also a problem is resistance to diuretics, and as a consequence – increasing their dose to obtain the appropriate therapeutic effect [1]. Thus, increasing the dose of thiazides and thiazide-like diuretics leads

to a number of side effects (hypokalemia, hypomagnesemia, hyponatremia, hypochloremic alkalosis), causes ventricular ectopia and sudden death [2, 3]. To eliminate the side effects and enhance the action of thiazides and thiazide-like diuretics, their combinations with potassium-sparing diuretics (triamterene, spirolactone, eplerenone, amiloride) are currently used. For example, triamterene has such an advantage, a weak diuretic with potassium-sparing action, which in combination with hydrochlorothiazide reduces hypokalemia and enhances its diuretic effect [4]. The mechanism of action of this drug is to reduce the permeability of the cell membranes of the distal tubules for sodium ions, while preserving potassium ions, it enhances their excretion with the urine. These properties allow the safe use of triamterene (triampur) in the treatment of edema of various origins, hypertension and use as a prevention of hypokalemia on the background of the use of saluretics. However, potassium-sparing diuretics themselves do not always meet safety requirements. The most dangerous side effect of sodium channel blockers is hyperkalemia, that is why triamterene is contraindicated in the presence of this condition. No less common adverse effect is the formation of kidney stones, due to the low solubility of triamterene a precipitate in the urine may by formed [5]. In addition, taking potassium-sparing drugs is accompanied by disorders of the gastrointestinal tract and central nervous system. Given the above, the search for new diuretics among heterocyclic compounds, namely 6-substituted pteridine-2,4,7(1H,3H,8H)-triones, is undeniably relevant [6, 7]. First, they are structurally similar to substituted pteridine-2,4,7-triamine (triamterene), they are also characterized by prototropic tautomerism, capable of forming hydrogen and donor-acceptor bonds with various ligands, and secondly, probably that these features of the structure will provide their diuretic effect.

Therefore, *the aim of the work* is to search for diuretics among 6-substituted pteridine-2,4,7(1H,3H,8H)-triones using *in silico* and *in vivo* methodology and to elucidate the probable mechanism of action.

MATERIALS AND METHODS OF RESEARCH

To study the effect on renal excretory function 1-methyl-3-R-6- (2-oxo-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones there were selected (2) and 1-methyl-3-R-6- (2-hydroxy-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones (3). Synthesis and physicochemical data of the studied compounds were described earlier [8, 9], and their structure is shown in Table 1.

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 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) and the conclusion of the commission on issues of biomedical ethics of DSMU (protocol No. 3 16.02.2022) [10]. 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 [11, 12]. 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. "Hydrochlorothiazide" and "Triamterene" in equivalent doses for rats were selected as the reference drugs [13]. The content of creatinine, sodium, potassium and chlorides in blood and urine was determined by biochemical methods using standard test kits of NPV "Philisit-Diagnostics" (Ukraine) and performed calculations according to generally accepted methods and formulas [14].

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) and epithelial sodium channel (ENaC) (PDB ID – 4NTX) [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.

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 $p \leq 0.05$ [15].

RESULTS AND DISCUSSION

Diuretics by localization and mechanism of action are divided into agents that act at the level of the apical and basement membranes, carbonic anhydrase inhibitors, osmotic diuretics and extrarenal diuretics. To date, a number of key enzymes have been identified that are responsible for these mechanisms of action [16]. Thus, thiazide and loop diuretics act as zinc-complexing groups on the active site of CA II, potassium-sparing diuretics directly block ENaC on the side of the lumen of the kidney [4]. With this in mind, we molecularly docked more than 50 synthesized 6-substituted pteridine-2,4,7(1H,3H,8H)-triones to calculate affinity scores and select the most active ones to further study their effects on diuresis.

The results of the research showed that the majority of compounds in terms of level and affinity to CA II and ENaC in almost all cases exceed the reference drugs "Hydrochlorothiazide" and "Triamterene" (Table 1). Importantly, 1-methyl-3-R-6- (2-oxo-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones (2), for which characteristic keto-enol tautomerism [8], have a greater affinity for enzymes in ketone form (B).

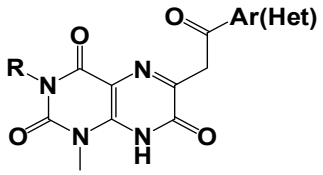
Biological assay

The results of the biological experiment showed (Table 2) that most of the studied compounds for 2 hours of the experiment inhibit the diuresis of experimental animals. However, among 1-methyl-3-R-6- (2-oxo-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones (2.1-2.13) found compounds and with significant diuretic action. Thus, compounds containing in the molecule 4-fluorophenyl (2.5), 2,4-difluorophenyl (2.6), 4-chlorophenyl (2.8) or 3,4,5-trimethoxyphenyl (2.10) fragments increase diuresis by 27,3-70, 1% compared to the control group. Concerning substituted 1-methyl-3-R-6- (2-hydroxy-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones (3.1-3.13), then almost all test compounds have antidiuretic effect. Exceptions are compounds with 4-methylphenyl (3.3), 4-fluorophenyl (3.5) and 2-thienyl (3.13) substituents in the molecule, which increase diuresis by 6.0-10.3% for 2 hours of experiment.

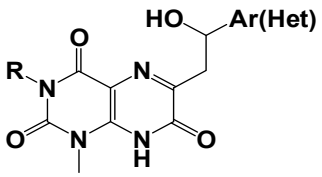
According to the results of the effect of compounds 2.1-2.13 on daily diuresis (Table 2), it was found that the most active was 1-methyl-6- (2-oxo-2-phenyl) ethyl) pteridine-2,4,7(1H, 3H,8H)-triones (2.1), which increased daily diuresis by 168.1%, exceeding the effect of “Hydrochlorothiazide” (41.8%) and “Triamterene” (49.1%). It was found that the introduction of the phenyl substituent of the methyl group (2.3) in the para-position leads to an insignificant decrease in diuretic action (36.6% compared to the control). Compounds 2.5, 2.8, which were effective for 2 hours of the experiment, in contrast inhibited diuresis by 31.5 and 30.6% compared to control. However, compounds 2.6 and 2.10 retain the diuretic effect for 24 hours, but it is quite weak (up to 12.0% compared to control). Substituted 1-methyl-3-R-6- (2-hydroxy-2-aryl- (hetaryl-) ethyl) pteridine-2,4,7(1H,3H,8H)-triones (3.1-3.13) are also ineffective diuretics (Table 1). However, among them are compounds 3.2-3.4, 3.6, 3.7, 3.10 and 3.11, whose daily diuresis exceeds the control group by 4.6-28.7%. In this case, they are all inversely dependent, and only compound 3.3 increases daily diuresis by 26.4% compared with the 2-hour experiment. Thus, the data of the biological experiment allow us to state that the introduction of the corresponding pteridines (2.5, 2.6 and 2.8) of electron-accepting substituents (fluorine or chlorine) into the phenacyl residue leads to a pronounced diuretic effect for 2 hours.

Table 1

Fundamental structure of synthesized compounds and molecular docking of key enzymes



2.1-2.13



3.1-3.13

Groups*	R	Ar(Het)	Affinity (kcal/mol) to CA II (PDB ID - 3HS4)#	Affinity (kcal/mol) to ENaC (PDB ID - 4NTX) #
2.1	H	Ph	-7.9/-8.4	-7.6/-7.6
2.2	Me	Ph	-7.9/-8.2	-7.2/-7.5
2.3	H	4-MeC_6H_4	-8.0/-7.6	-7.3/-7.9
2.4	H	2-FC_6H_4	-8.2/-8.0	-7.7/-8.2
2.5	H	4-FC_6H_4	-8.1/-8.4	-8.0/-7.9
2.6	H	2,4-$F_2C_6H_3$	-8.3/-8.0	-7.5/-7.9
2.7	H	2-ClC_6H_4	-7.9/-7.9	-7.6/-8.4
2.8	H	4-ClC_6H_4	-8.1/-8.0	-7.8/-7.4
2.9	H	4-$MeOC_6H_4$	-7.4/-7.3	-7.4/-7.6
2.10	H	3,4,5-$(MeO)_3C_6H_2$	-7.1/-8.1	-7.0/-6.7
2.11	H	3-$NO_2C_6H_4$	-7.7/-7.6	-7.8/-7.9
2.12	H	furyl-2	-7.3/-7.1	-7.3/-7.3
2.13	H	thienyl-2	-7.1/-7.1	-6.9/-7.4
3.1	H	Ph	-7.6	-6.7
3.2	Me	Ph	-7.2	-7.3
3.3	H	4-MeC_6H_4	-7.9	-7.2
3.4	H	2-FC_6H_4	-7.8	-7.7
3.5	H	4-FC_6H_4	-7.8	-7.2
3.6	H	2,4-$F_2C_6H_3$	-8.2	-7.2
3.7	H	2-ClC_6H_4	-7.8	-7.4
3.8	H	4-ClC_6H_4	-7.7	-7.2
3.9	H	4-$MeOC_6H_4$	-6.9	-7.4
3.10	H	3,4,5-$(MeO)_3C_6H_2$	-6.7	-6.7
3.11	H	3-$NO_2C_6H_4$	-7.7	-7.9
3.12	H	furyl-2	-6.9	-7.3
3.13	H	thienyl-2	-7.1	-6.9
Hydro-chlorothiazide		–	-7.3	-5.9
Triamterene	–	–	-6.7	-6.6

Notes: * – the table shows the compounds that exceed the reference drugs in terms of affinity; # - affinity calculations of compounds 2.1-2.13 were performed for tautomeric forms (A -enol / B -ketone) [8].

Table 2

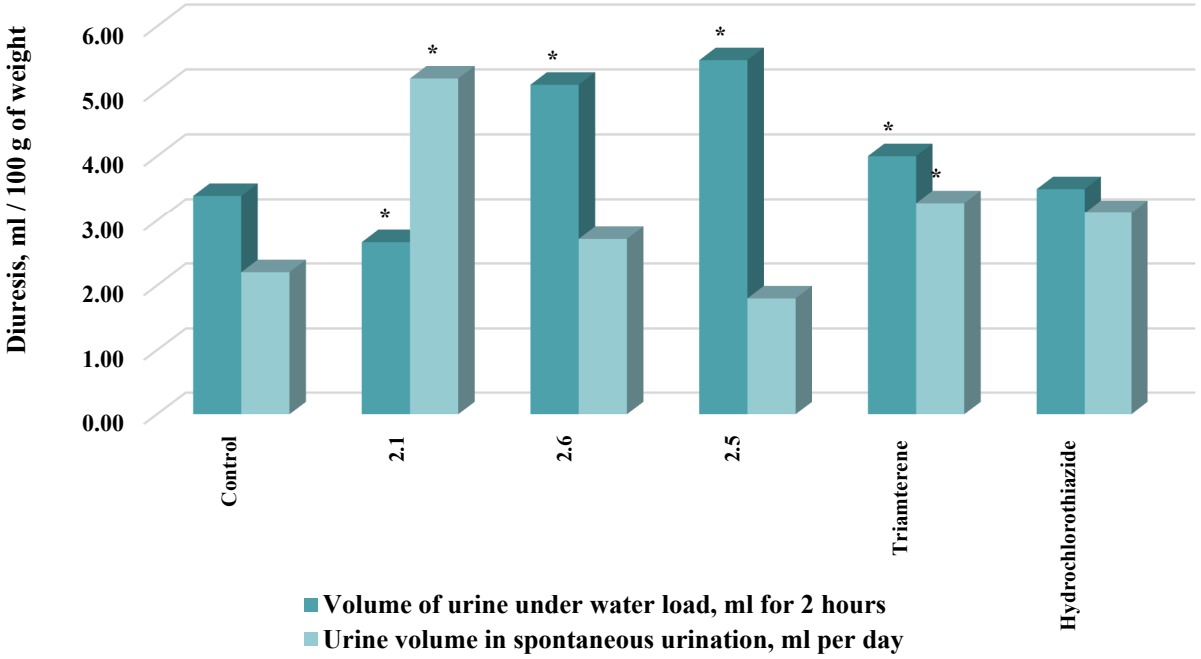
The effect of the synthesized compounds and reference drugs on the process of urination in intact rats under water load with a single injection (M±m, n=6) and molecular docking results

Groups	Diuresis, mL/100 g/2 h	Influence on the process of urination, %	Diuresis, mL/100 g/24 h	Influence on the process of urination, %
Control	3.39±0.30	–	2.20±0.28	–
2.1	2.63±0.08	-24.4	5.79±0.53*	163.2
2.2	3.35±0.11	-3.7	1.49±0.12	-31.0
2.3	2.28±0.12*	-34.5	2.95±0.20*	36.6
2.4	3.46±0.18	-0.6	2.37±0.21	9.7
2.5	5.92±0.24*	70.1	1.48±0.15	-31.5
2.6	5.40±0.21*	55.2	2.42±0.22	12.0
2.7	2.49±0.09	-28.4	1.94±0.27	-10.2
2.8	4.57±0.19*	31.3	1.50±0.09	-30.6
2.9	1.25±0.08*	-64.1	1.93±0.15	-10.6
2.10	4.43±0.17	27.3	2.40±0.28	11.1
2.11	3.52±0.05	1.1	2.29±0.18	6.0
2.12	2.44±0.13	-29.9	2.24±0.13	3.7
2.13	3.19±0.12	-8.3	1.31±0.11*	-39.4
3.1	2.60±0.05	-25.3	1.77±0.10	-18.1
3.2	3.19±0.19	-8.3	2.63±0.19	21.8
3.3	3.69±0.05	6.0	2.78±0.29	26.4
3.4	3.30±0.04	-5.2	2.57±0.14	19.0
3.5	3.83±0.11	10.1	1.78±0.29	-17.6
3.6	3.46±0.10	-0.6	2.36±0.21	9.3
3.7	2.75±0.08	-21.0	2.31±0.26	6.9
3.8	3.45±0.15	-0.9	1.81±0.06	-16.2
3.9	2.27±0.10	-34.8	1.61±0.06	-25.5
3.10	3.28±0.16	-5.7	2.26±0.15	4.6
3.11	2.60±0.14	-25.3	2.28±0.17	5.6
3.12	2.37±0.12	-31.9	1.75±0.17	-19.0
3.13	3.84±0.10	10.3	1.67±0.13	-22.7
Hydrochlorothiazide	3.49±0.38	2.9	3.12±0.27	41.8
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.

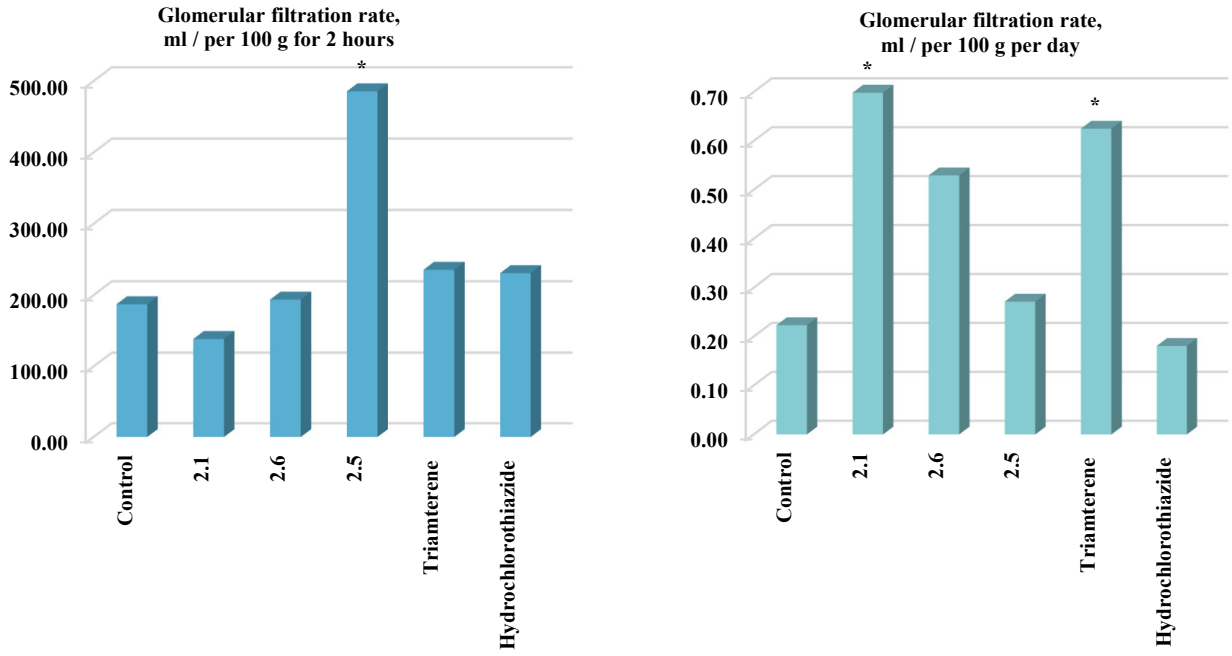
To determine the probable mechanism of action of compounds 2.1, 2.6, 2.5 on the processes of excretory function of the kidneys in biological systems, biochemical markers and the composition of electrolytes in the blood plasma and urine of rats were determined (Fig. 1, 2). In-depth study of excretory function of the kidneys on the background of the introduction of substances 2.6, 2.5 indicates an

increase in diuresis under water load by 50.4 and 61.7%, respectively, compared with controls. For compound 2.6 GFR almost did not differ from the control, while 2.5 was 159.1% higher than the control values. In the conditions of spontaneous urination, against the background of the introduction of compound 2.6, diuresis is increased by 23.2% and GFR – by 140.9%. Under the influence of compound 2.1, diuresis is increased by 136.4% and GFR – by 218.2%, respectively, compared to the control.



* – significant changes in control ($p<0.05$).

Fig. 1. Changes in diuresis in rats on the background of the introduction of compounds 2.1, 2.6, 2.5 under water load and spontaneous urination



* – significant changes in control ($p<0.05$).

Fig. 2. Changes in glomerular filtration rate under the influence of compounds under water load and spontaneous urination

The effect of potentially effective compounds was evaluated by changes in ion-osmotic parameters of rat blood plasma (Table 3). Analyzing the data in the tables, it should be noted that of the studied objects, substances 2.1 and 2.5 under water load conditions somewhat prevent a significant drop in plasma potassium levels, but have significant changes in control. In the conditions of spontaneous urination, substances 2.6 and 2.5 have a similar tendency.

Table 3

Changes in ionic-osmotic parameters of blood plasma and urine of animals under different modes of administration against the background of the compounds under study (M±m)

Indexes	Control	2.1	2.6	2.5	Triamterene	Hydrochloro-thiazide
Under water load						
Plasma sodium, mmol/l	132.55±4.85	106.81±2.86*	73.39±2.38*	172.33±1.96*	109.15±8.85*	107.69±8.51*
Sodium in the urine, mmol/l	67.66±6.63	57.97±3.48	62.45±2.13	118.77±3.51*	67.40±2.96	58.67±2.36
Excretory faction of sodium, %	1.06±0.31	1.10±0.29	2.35±0.58*	0.79±0.10	1.11±0.12	0.83±0.11
Plasma potassium, mmol/l	7.05±0.57	4.03±0.14*	3.44±0.13*	5.33±0.07*	7.38±0.42	5.57±0.37*
Potassium of urine, mmol/l	9.43±0.24	6.64±0.06*	10.72±0.29*	16.45±0.65*	9.56±0.38	13.94±1.10*
Excretory faction of potassium, %	2.79±0.85	3.39±1.02	8.58±1.98*	3.53±0.41	2.33±0.30	3.84±0.66
Urine chlorides, mmol/2 hours	126.00±17.17	134.82±19.75	232.05±18.09*	405.36±21.13*	64.12±14.12*	96.75±13.32
Plasma chlorides, mmol/l	93.06±3.97	82.97±4.10*	90.55±4.39	93.97±3.02	77.73±9.08	88.45±6.36
Excretory fraction of chlorine, %	2.75±0.59	3.34±1.13	7.01±1.32*	4.96±0.81	1.52±0.53	1.69±0.39
Excretory fraction of water, %	2.08±0.65	2.04±0.58	2.76±0.70	1.15±0.15	1.71±0.15	1.52±0.10
In conditions of spontaneous urination						
Plasma sodium, mmol/l	144.83±8.48	61.50±14.10*	65.43±11.20*	112.28±39.13	128.06±13.41	122.40±8.88*
Sodium in the urine, mmol/l	130.16±9.48	109.89±8.32	131.80±8.30	115.18±14.90	214.77±31.92*	202.36±24.47*
Excretory faction of sodium, %	0.93±0.25	1.42±0.45	1.08±0.26	0.77±0.23	0.88±0.07	2.85±0.30*
Plasma potassium, mmol/l	6.02±0.17	4.05±0.77*	7.23±0.92	4.44±0.65*	5.67±0.39	4.40±0.50*
Potassium of urine, mmol/l	23.07±1.94	28.43±3.31	29.05±0.94*	28.68±0.74*	20.71±0.65	20.52±0.61
Excretory faction of potassium, %	3.92±0.96	5.44±1.37	2.13±0.44	4.54±1.23	1.92±0.09	8.09±0.62*
Urine chlorides, mmol/2 hours	205.76±32.72	168.47±60.14	197.11±32.70	31.05±8.28*	1275.69±210.87*	1286.09±174.31*
Plasma chlorides, mmol/l	92.05±6.80	54.07±2.98*	69.26±2.79*	75.39±6.10*	108.15±6.70	95.89±3.71
Excretory fraction of chlorine, %	2.34±0.82	2.30±0.69	1.50±0.37	0.29±0.13*	6.32±1.69*	23.37±4.89*
Excretory fraction of water, %	1.02±0.23	0.75±0.11	0.53±0.09	0.69±0.17	0.53±0.06	1.73±0.12*

Note. * – significant changes in control ($p<0.05$).

Saluretic and natriuretic indices allowed to predict probable mechanisms of action of the studied compounds. The index is the ratio of excretion in the study group to excretion in the control. Figure 3 shows that compounds 2.1, 2.6 have a potential saluretic effect and exceed the control values by 1.59 and 1.56 times, respectively. Compound 2.6 has a pronounced natriuretic effect and is 2.13 times higher than control values. The obtained effects are characteristic of thiazide and loop diuretics, which is confirmed in the literature [20].

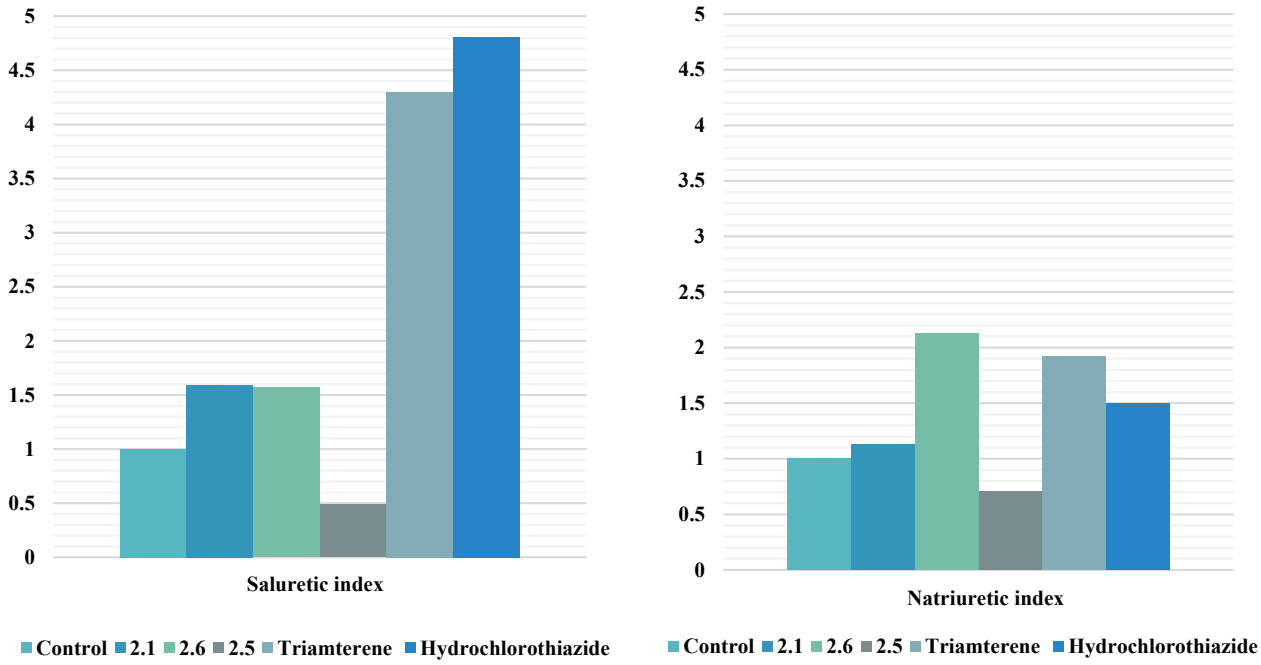


Fig. 3. The effect of the compounds under study on the natriuretic and saluretic effects in rats under conditions of spontaneous urination

To explain the above fact, in the next step we visualized the molecular coupling of compounds 2.1, 2.5 and 2.6 to key enzymes and receptors. Thus, the main types of interactions of compounds 2.1, 2.5 and 2.6 and pharmacological standards with amino acid residues CA II and ENaC are given in Table 4. Analysis of the data showed that the test compounds and standard drugs are characterized by hydrogen, π-halogen and hydrophobic bonds with amino acid residues, as well as coordination with the zinc cation CA II.

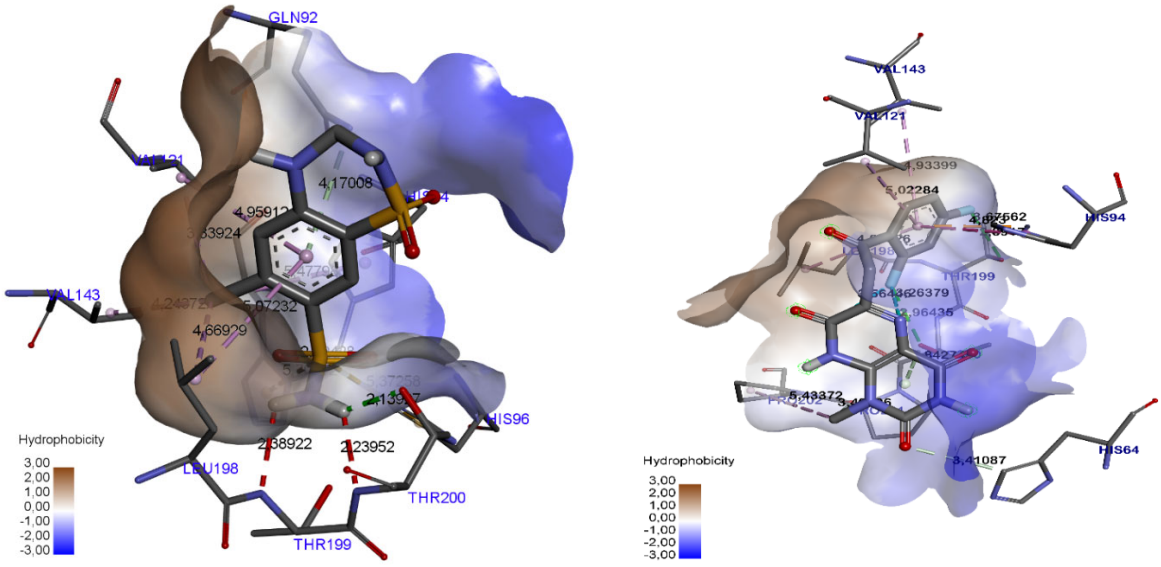
Table 4

The main types of interactions of synthesized compounds and pharmacological standards with amino acid residues of enzimes

Compounds	The main interactions types between compounds, pharmacological standards and amino acid residues of enzimes*	
	CA II (PDB ID - 3HS4) #	ENaC (PDB ID - 4NTX) #
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}	–
Triamterene	–	ARG87^{A}, ARG87^{A}, ASP238^{D}, ASP350^{D}, ASN83^{A}, ASN83^{A}, PRO347^{B}
2.1	HIS64^{A}, Zn301^{D}, THR200^{A}, HIS94^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}	GLU98^{A}, SER241^{A}, NA505^{F}, NA505^{D}, NA505^{D}, PHE242^{B}
2.5	THR199^{A}, THR199^{A}, PRO201^{A}, Zn301^{D}, THR200^{A}, LEU198^{B}, HIS94^{B}, PRO202^{B}, VAL121^{B}, VAL143^{B}	SER67^{A}, ARG87^{A}, ASN82^{C}, ARG87^{A}, ARG87^{D}, ASP350^{D}, PHE351^{B}
2.6	THR199^{A}, THR200^{A}, HIS64^{A}, PRO201^{A}, Zn301^{D}, THR200^{A}, HIS94^{A}, PRO202^{B}, VAL121^{B}, VAL143^{B}, LEU198^{B}	GLU98^{A}, SER241^{A}, GLU236^{C}, NA505^{D}, NA505^{D}

Notes: * – A – Hydrogen Bond; B – Hydrophobic, C – Halogen, D – Electrostatic, E – Metal-Acceptor, F – Other; # – data for tautomeric form B (ketone).

Imaging of "Hydrochlorothiazide" with active site CA II (Fig. 4, A) revealed the presence of two hydrogen bonds of the sulfamide group with amino acid residues THR200^{A} (2,14Å), THR199^{A} (2,39Å), hydrophobic π-interactions of the aromatic moiety with amino acid residues GLN92^{B} (4.17Å), VAL121^{B} (4.96Å), π- interactions of oxygen and sulfur of the sulfamide group and chlorine with VAL143^{B} (4,24Å), VAL143^{B} (5.07Å), HIS96^{C} (5,37Å), HIS94^{B} (5.48Å), HIS119^{C} (5,07Å), LEU198^{B} (4.67Å), VAL121^{B} (3.84Å), LEU198^{B} (4.67Å), VAL121^{B} (4,96Å), LEU198^{B} (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). Interestingly, compounds 2.1, 2.5 and 2.6 have similar types of interactions with CA II amino acid residues, and as a consequence, similar placement in the active site of the enzyme. This is primarily due to the presence of fragments in the molecules, which are characterized by the formation of hydrogen bonds. Thus, the molecular coupling of compound 2.6 with CA II shows (Fig. 4, B) that it occurs due to hydrogen bonds of imide fragments of the molecule with amino acid residues HIS64^{A} (3,41Å), HIS94^{A} (2,96Å), PRO200^{A} (3,43Å), PRO201^{A} (5,43Å), keto groups with THR200^{A} (4,68Å), π- fluorine interactions with THR200^{A} (3.56Å) and THR199^{A} (2.85Å), as well as hydrophobic interactions of the phenyl moiety with VAL121^{B} (4.93Å), VAL143^{B} (5,02Å), LEU198^{B} (4.69Å). In the active site of the enzyme, as in the reference inhibitor, there is a coordination relationship of the imide group with the zinc cation CA II (ZN301^{E} 3.84Å).



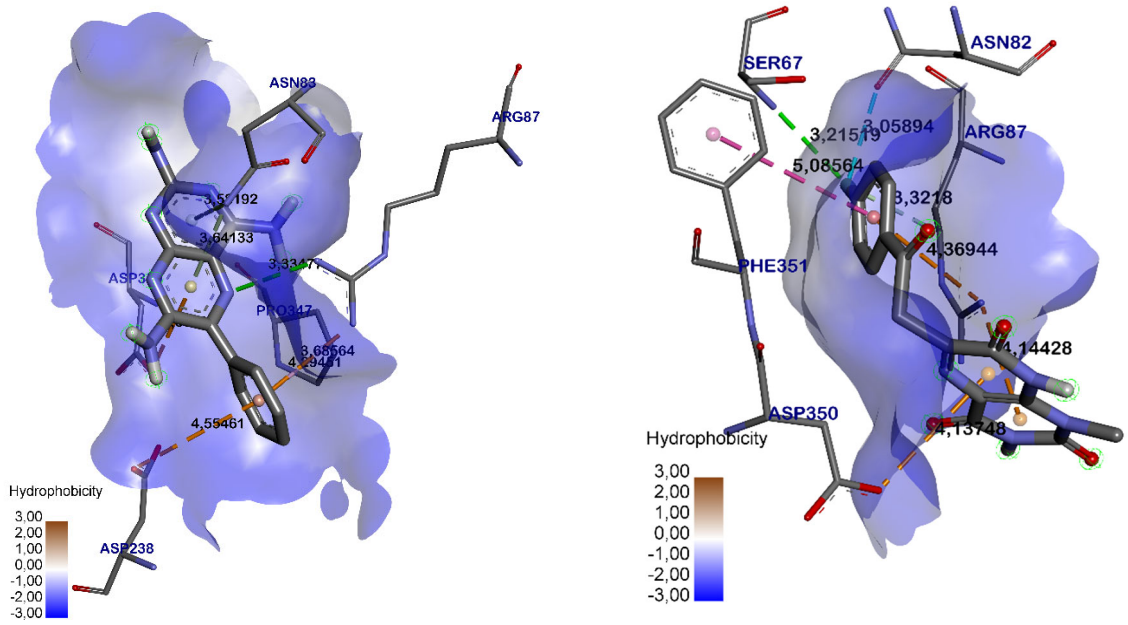
A **B**

Fig. 4. Types of the ligand – enzyme interactions according to visualization of docking study: A) "Hydrochlorothiazide" with CA II 3D, B) compound 2.6 with CA II 3D

At the same time, the analysis of the molecular coupling of "Triamterene" with ENaC (Fig. 5, A) showed that in this case the interaction of nitrogens of nominal fragments of the molecule through four hydrogen bonds with amino acid residues is predicted ARG87^{A} (3,33Å та 3,68Å), ASN83^{A} (3,51Å and 3,64Å), as well as weak electrostatic and hydrophobic interactions of phenyl and pteridine cycles with ASP238^{D} (4,55Å), ASP350^{D} (4,68Å), PRO347^{B} (4,29Å). Importantly, compounds 2.1, 2.5 and 2.6 are somewhat differently located in the active site of the enzyme and, as a consequence, interact with other amino acid residues. For example, compound 2.5 predicts the interaction of imide fragments through hydrogen bonds with ARG87^{A} (3,32Å та 4.36Å), keto groups with SER67^{A} (3,21Å), π- fluorine interaction with ASN82^{C} (3.05Å), as well as hydrophobic and electrostatic interactions of pteridine and phenyl cycles with ARG87^{D} (4.36Å), ASP350^{D} (4,14Å) and PHE351^{B} (5.08Å, Fig. 5, B).

Thus, studies have shown the presence of diuretic action in a number of derivatives of 1-methyl-6- (2-oxo-2-aryl) ethyl) pteridine-2,4,7 (1H, 3H, 8H) –triones and suggested that that compounds 2.1, 2.5, 2.6 have an effect similar in mechanism to thiazide diuretics, which is confirmed in the literature [21-25]. The mechanism of diuretic action is a violation of sodium transport in the distal convoluted tubules, which causes natriuresis and concomitant water loss. This action may be related to the tautomerism of these

compounds [8] and, as a consequence, their ability to form coordination bonds with the zinc cation and the additional interaction of halogens in the active site of CA II (Table 1, Fig. 1). However, in addition to the pronounced natriuretic and saluretic action characteristic of thiazides [26-28], a significant advantage is the presence of potassium-sparing action, probably due to the ability to inhibit epithelial sodium channels (ENaC) located on the luminal side in distal convoluted convoluted tubules, which are transmembrane channels that typically promote sodium uptake and potassium secretion, ions are actively reabsorbed via ENaC on the luminal membrane and displaced from the cell into the peritubular medium by the sodium-potassium exchange pump, Na-K-ATPase. However, these assumptions need further refinement, namely the study of their ability to inhibit CA II and ENaC.



A **B**

Fig. 5. Types of the ligand – enzyme interactions according to visualization of docking study: A) "Triamterene" with ENaC 3D, B) compound 2.5 with ENaC 3D.

CONCLUSIONS

1. A well-founded and developed strategy for the search for diuretics among 6-substituted pteridine-2,4,7(1H,3H,8H)-triones has identified a number of effective compounds that are more diuretic than the reference drugs "Hydrochlorothiazide" and "Triamterene". In-depth studies using biological tests and molecular docking have suggested that for 1-methyl-6- (2-oxo-2-aryl) ethyl) pteridine-2,4,7(1H,3H,8H)-triones 2.1, 2.5 and 2.6) probable mechanisms of diuretic action are disruption of sodium transport in the distal convoluted tubules, causing sodium excretion and water loss and possibly inhibition of epithelial sodium channels that promote sodium uptake and potassium secretion in the distal convolutions and tubules, which implements potassium-sparing action.

2. The results of studies confirmed the presence of a diuretic effect in some 6-substituted pteridine-2,4,7(1H,3H,8H)-triones and open up prospects for further study of their effects on the urinary system. First, it is an *in vitro* study of the ability of synthesized compounds to inhibit enzymes (eg, CA II), and secondly, structural modification of active compounds by introducing additional pharmacophore groups or their "bioisosteric" substitutions for other fragments.

Contributors:

Sokolova K.V. – visualization, writing – original draft, resources, investigation;

Stavytskyi V.V. – Software;

Kovalenko S.I. – project administration, methodology, conceptualization, writing – review & editing

Podpletnya O.A. – writing – review & editing

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