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Research Article

Predictive analysis and prediction of the main molecular targets for the N-acetyl-6-aminohexanoate derivative

Anton P. Danilenko[1], Alexey A. Khentov[1], Alexey V. Kuznetsov[1], Veronika S. Shmigerova[1], Yu V. Stepenko[1], Alla P. Tarasova[1], Vladimir I. Yakushev[1], Elizaveta V. Boeva[1], Albina V. Miller[1], Irina A. Tatarenkova[2], Tatiana V. Puzanova[1], Yana V. Loboda[1], Andrei P. Trashchenko[1], Oleg S. Gudyrev[1], Anna L. Motailo, Vladimir N. Kuzichkin[1], Valentina N. Milashechko[1], Lyudmila M. Danilenko[1]

1 Belgorod State National Research University, 85 Pobedy St., Belgorod 308015 Russia
2 Kursk State Medical University, 3 Karl Marx St., Kursk 305041 Russia

Corresponding author: Lyudmila M. Danilenko (danilenko_L@bsu.edu.ru)

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Abstract

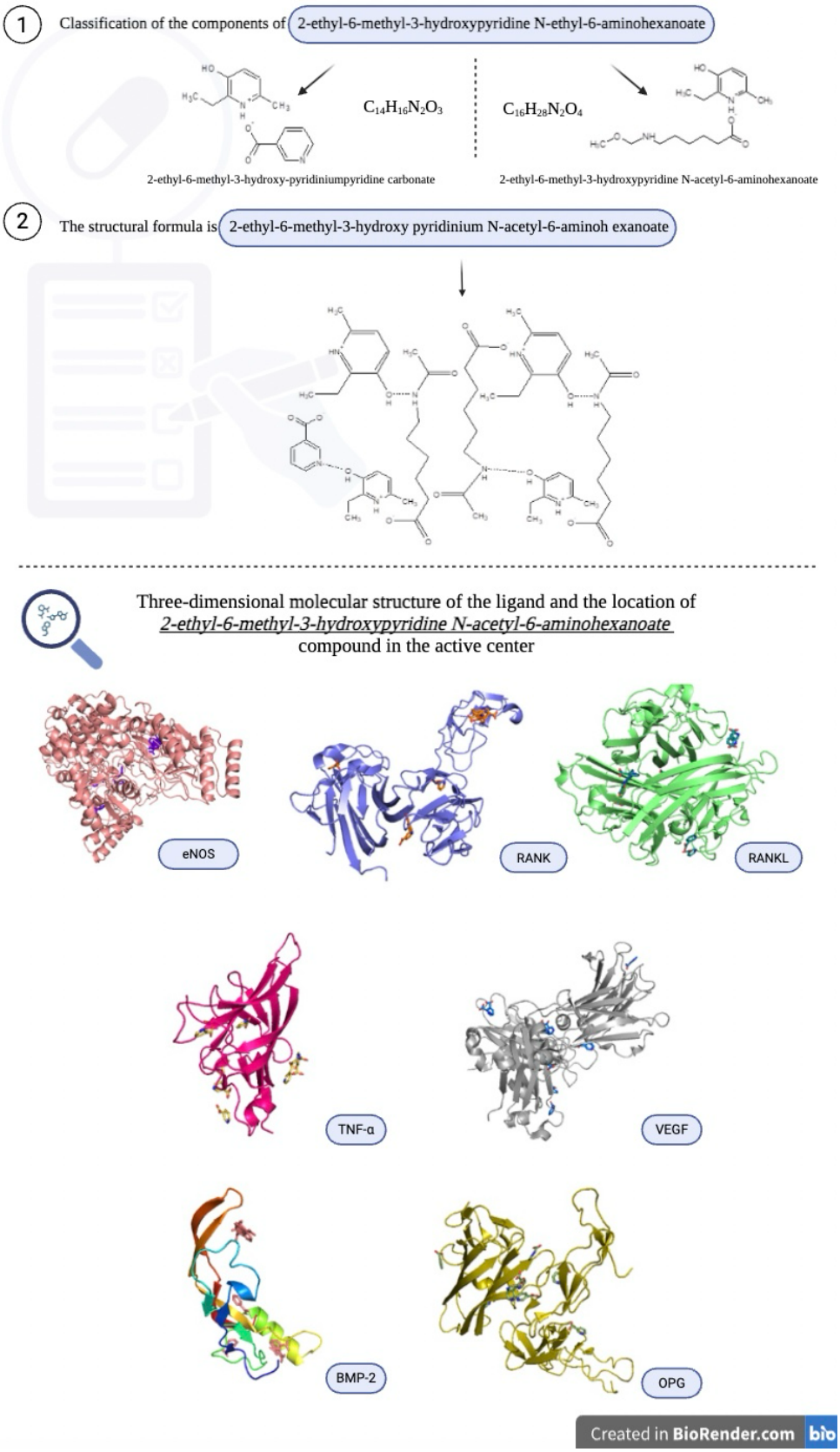
Introduction: N-acetyl-6-aminohexanoate (acexamic acid) and its derivatives are actively studied as promising compounds for the creation of new drugs, but their pharmacokinetic parameters and detailed mechanisms underlying a wide range of biochemical activities are still unclear.

Materials and Methods: PASS Online, Molinspiration Property Calculation Service and OSIRIS Property Explorer were used for predictive analysis. To determine the molecular biomarkers of the N-acetyl-6-aminohexanoate derivative – 2-ethyl-6-methyl-3-hydroxypyridinium N-acetylhexanoic acid, ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) parameters were predicted. Molecular docking was performed using GalaxyWEB Sagittarius service followed by evaluation of the results using RCSB Protein Data Bank and UniProt Consortium databases.

Results: The study of the pharmacokinetic properties of 2-ethyl-6-methyl-3-hydroxypyridine N-acetyl-6-aminohexanoate revealed its potential suitability as a promising drug with high bioavailability. The highest degree of affinity is predicted with the eNOS center – binding energy from -7.2 to -8.3 kcal/mol; the VEGF center – binding energies from -6.1 to -7.7 kcal/mol, and the RANKL centre – binding energies from - 6.0 - 6,9 kcal mol.

Conclusion: The results of predictor analysis and molecular docking suggest that the N-acetyl-6-aminohexanoate-2-ethyl-6-methyl-3-hydroxypyridinium derivative is a safe and promising compound. Potential biotargets include eNOS, VEGF, and RANKL.

Graphical abstract



Keywords molecular docking, drug discovery, 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate, eNOS, VEGF, RANKL

Introduction

Recently, many studies have been aimed to study N-acetyl-6-aminohexanoate (acexamic acid) and its derivatives (Blinova et al. 2021; Alkhatatneh Bashar 2023; Mazov et al. 2023; Skachilova et al. 2023). The results obtained from studying the effectiveness of acexamic acid itself were clinically insufficient for practical use (Andrianova and Egorova 2019). Therefore, numerous new derivatives were synthesized. Some of these derivatives demonstrated high biological efficiency. In Russia, Doctor of Chemical Sciences Professor S.Ya. Skachilova is carrying out a lot of work to synthesize new derivatives of N-Acetyl-6-Aminohexanoic Acid. The biological activity of some of these new derivatives is presented in Table 1.

Table 1. Pharmacological effects of N-acetyl-6-aminohexanoate derivatives

№	Title	Studied pharmacological effects
1.	N-Acetyl-6-aminohexanoate of zinc (zinc salt of e-acetamidocaproic acid)	Renal ischemia; peptic ulcers, including when taking acetylsalicylic acid; bone loss in streptozotocin-induced diabetes in rats in vivo (Hadj Abdallah et al. 2018)
2.	N-Acetyl-6-aminohexanoate of sodium (sodium salt e-acetamidocaproic acid)	Therapeutic potential has not been proven
3.	N-Acetyl-6-aminohexanoate of calcium	Therapeutic potential has not been proven
4.	2-(5-ethyl-1,3,4-thiadiazolyl) amide of N-acetyl-6-aminohexanoic acid	Anti-inflammatory effect (Malygin et al. 2018)
5.	N-Acetyl-6-aminohexanoate of silver, cerium	They stimulate the regeneration and mineralization of bone tissue in osteoporosis, tissue regenerative capacity, cosmetic effects on skin burns, periodontal diseases (Alkhatatneh et al. 2020)
6.	2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate	They stimulate regeneration and mineralization of bone tissue in patients with hypoestrogenic and steroid-induced osteoporosis, as well as anxiolytic and nootropic effects. The tissue regenerative capacity is studied by Danilenko et al.(2023)

The high biological activity of acexamic acid derivatives suggests promising research for the further development of drugs that can be widely used in clinical practice, especially for stimulating, regenerating and mineralizing bone tissue in osteoporosis, as well as for fractures against its background. These drugs can also be used to clean wound surfaces from necrotic masses, reduce exudation, activate the growth of granulation tissue, vascularization, and epithelialization of wounds. They can also help prevent the development of keloid scars.

In addition, despite the enormous therapeutic advances in this area (Perez-Amodio et al. 2021; Bhardwaj et al. 2023; Nguyen et al. 2025), the problem remains open and relevant (Sorg et al. 2023; Klinaku et al. 2025).

From a medical point of view, one of the new derivatives of acetic acid, complex salts – 2-ethyl-6-methyl-3-hydroxypyridine N-acetyl-6-aminohexanate, is of great interest. The method of its preparation using topochemical synthesis allows to improve its pharmacokinetic parameters, increasing oral bioavailability on average by 14 times and the maximum concentration by more than 40 times compared to separately dosed substances (Danilenko et al. 2024). Russian patent RU 2668966 dated 2018 confirms that N-acetyl-6-aminohexanoate-2-ethyl-6 methyl-3 hydroxypyrindium stimulates bone regeneration and mineralization in osteoporosis. The effect of this complex salt on regenerative and cosmetic effects in topical dosage forms for skin burns has been experimentally demonstrated, and its efficacy in diabetes mellitus has also been proven (Blinova et al. 2021; Pakhomov et al. 2020). The effect of N-acetyl-6-N-acetyl-2-ethyl-6-methyl-3-hydroxypyridinium 6-aminohexanoate on osteoprotection processes has been shown in models of hypoestrogenic and steroid-induced osteoporosis. In vivo studies have demonstrated the presence of anxiolytic and nootropic effects of this compound (Bogomolova et al. 2018), owing to its restoration of neurotransmitter balance and its effect on the regulation of receptor function and membrane-bound enzyme activity, as well as its antioxidant properties.

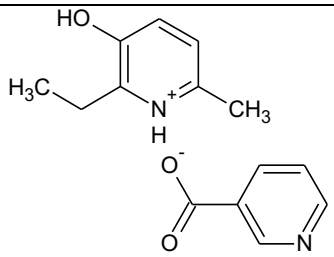
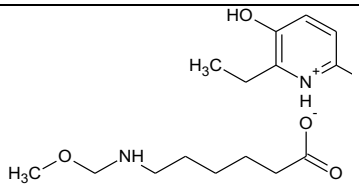
Taking into account the above, it seems timely and relevant to conduct a predictive analysis of a new derivative of acexamic acid – 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate and to study its molecular biomarkers.

Materials and Methods

The compound under investigation

The chemical description of the studied compounds, which was used to synthesize the 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate complex, is presented in Table 2 for predictive analysis and prediction of main molecular targets.

Table 2. Classification of the components of 2-ethyl-6-methyl-3-hydroxypyridine N-ethyl-6-aminohexanoate

№	Molar mass, g/mol	Structural formula	Gross formula	Chemical name
1.	260.29		$C_{14}H_{16}N_2O_3$	2-ethyl-6-methyl-3-hydroxy-pyridinium pyridine carbonate
2.	312.41		$C_{16}H_{28}N_2O_4$	2-ethyl-6-methyl-3-hydroxypyridine N-acetyl-6-aminohexanoate

The synthesis of new pharmaceutical compounds based on toponimic principles is carried out by drying to a constant weight at 100-105 °C for 2 hours, homogenizing and grinding to 5-10 µm. Compounds 2-ethyl-6-methyl-3-hydroxypyridine N-acetyl-6-aminohexanoate and 2-ethyl-6- methyl-3-hydroxy-pyridinium pyridine carboxamide are used.

26.0 g (0.1 g/mol) of 2-ethyl-6-methyl-3-hydroxypyridinium-3-pyridinecarbonate is loaded into a homogenizer and 93.2 g (0,1 g/ mol) of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate is gradually added while stirring. The mixture is homogenized for 10–15 minutes at 300–400 rpm, and the particle size is checked. If necessary, the powder is further homogenized. As a result, 119.0 grams of white crystalline powder with a Tpl of 139–143°C is obtained, which dissolves in water with slight opalescence.

Found, %: C 62.48; H 7.98; N 9.39 $C_{62}H_{94}N_8O_{15}$ м.м. 1191.46

Calculated, %: C 62.50; H 7.95; N 9.41; O 20.14.

IR spectrum (v, cm^{-1}): 3412 (OH) 3290 (NH), 2941 (CH), 2673 (N^+), 1781 (C=N-), 1634 (C=C), 1561 (COO^-).

The mass spectrum of the protonated new pharmaceutical compound in the positive ion scanning mode $[M+H]^+$ is M/z: 1195.46, which corresponds to mm 1191.46. g/mol (Patent of the Russian Federation, Trunov et al. 2023).

The structural formula of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate is shown in Figure 1.

Research in silico

The paper presents the results of an *in-silico* predictor analysis of the drug similarity of compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate. Possible biomarkers and potential biological activity were identified, and ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) parameters were predicted. For this purpose, software services such as PASS Online and Molinspiration Property Calculation Service OSIRIS Property Explorer (Kravchenko et al. 2022) were used, which contain information on the properties of approximately 3,300 drugs and 15,000 commercially available compounds. These services were employed to evaluate the lipophilicity degree of the cLogP compound, solubility (logS), topological polar surface area (Topological Polar Surface Area, TPSA), and several other parameters. The main physical-chemical parameters according to the "rule of five" include the number of hydrogen bond donors and acceptors, molecular weight, and partition coefficient in an octanol-water system. Important descriptors, such as molecular refraction, the number of rotating bonds, polar surface area and others, were also taken into account.

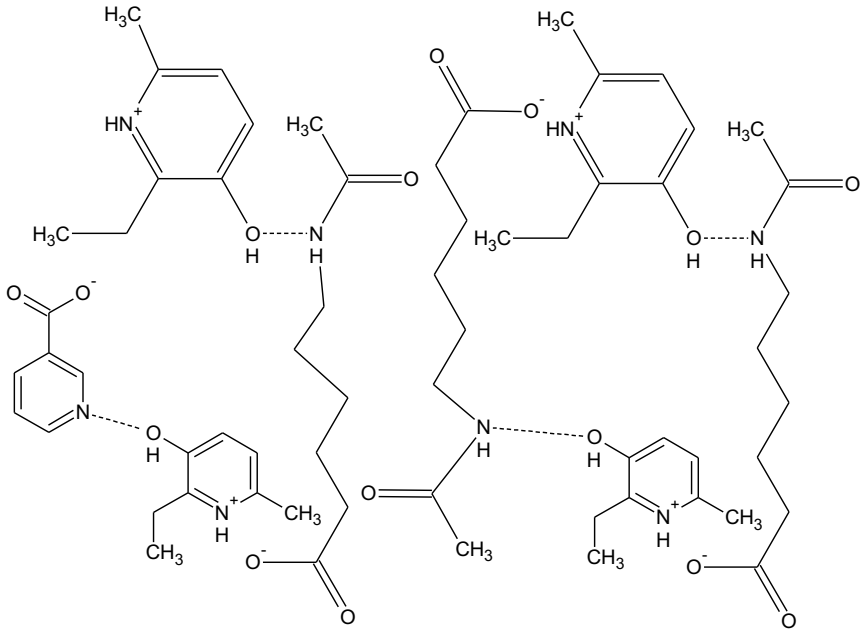


Figure 1. The structural formula is 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate.

Results

Based on the results of *in-silico* analysis of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate, fundamental parameters such as absorption, distribution, metabolism, excretion, and toxicity were determined. This provides valuable information about the similarities to the drug and pharmacokinetic properties of the compound.

The study of the pharmacokinetic properties of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate began with an assessment based on the key criteria of similarity to the drugs developed by Lipinski, Gose, Weber and Egan and Muegge. It was revealed that the studied compound, 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate, clearly meets all of the criteria being evaluated, indicating its very favorable pharmacokinetic characteristics.

According to Lipinski's rule, the molecule has a molecular mass less than 500 grams per mole, a log P value less than five, and no violations of hydrogen bonding donors or acceptors. The Gosse rule is met, as the compounds fall within the acceptable ranges for both lipophilicity and molar refractivity. According to Weaver's law, compounds have limited numbers of rotatable bonds and a total polar surface area (TPSA) of 95.76 angstroms. The results of pharmacokinetic parameters are presented in Figure 2.

Compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate falls within the specified range described by the Egan rule, indicating high solubility and the likelihood of oral bioavailability. Analysis of the absorption abilities of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate showed good absorption in the gastrointestinal tract (GIT) and the ability of the compound to not inhibit five major CYPs isoenzymes including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, which are responsible for more than 80 % metabolism of clinical drugs . Thus, the overall profile of ADME suggests that the compound has potential as a promising drug with high bioavailability, as shown in Table 3.

The acute oral toxicity of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate was evaluated in accordance with the interstate standard GOST RF 32644-2014 (OECD Test No. 423: 2001 IDT). Based on the results of the laboratory experiment, the LD50 for 2-ethyl-6-methyl-3-hydroxypyridine N-acetyl-6-aminohexanoic acid is 2,000 milligrams per kilogram (Fig. 3).

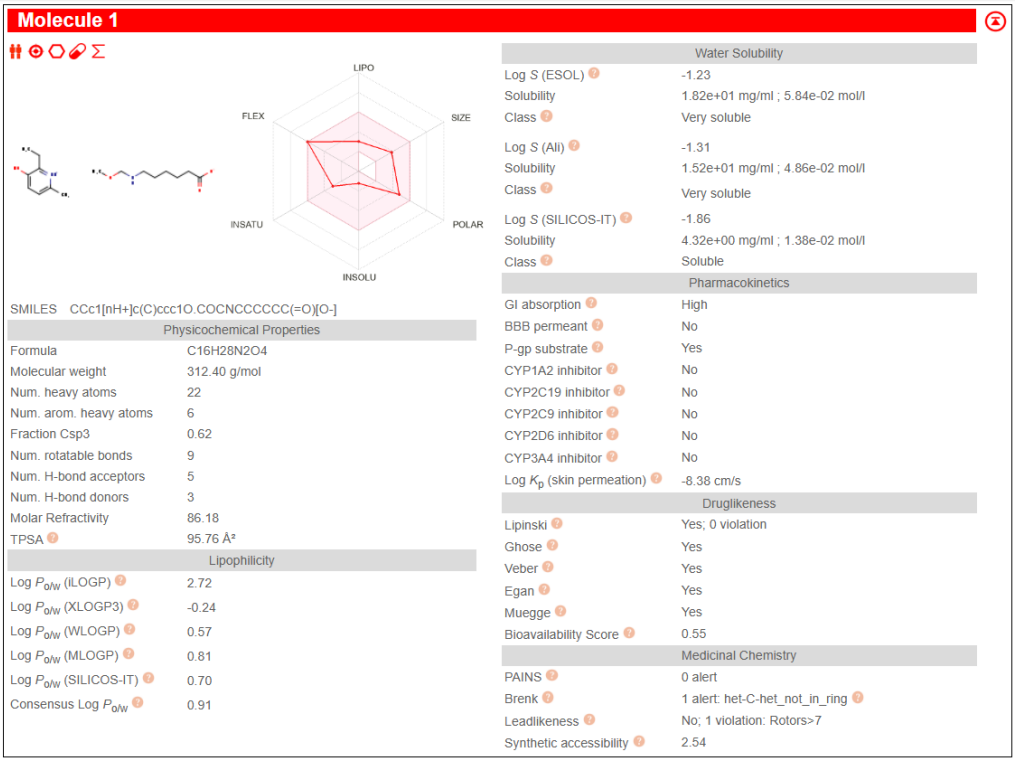


Figure 2. The ADMET profile of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate and Bioavailability radar of compound.

Table 3. Physico-chemical parameters, ADMET parameters and biological activity of a 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate test sample were predicted using OSIRIS Property Explore and software services from Swiss ADME and Swiss Target Prediction

Compound	*logS*	*AlogS*	Molecular weight	Bioavailability index	TPSA	*logP*
2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate	-1,23	-1,31	312,40	0,55	0,947	2,72

Note: logS – solubility in water; logP – lipophilicity; *AlogS* – solubility, *TPSA* – polarity.

As a result of the analysis of the forecast of toxicological characteristics, compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate was assigned to class IV toxicity, which characterized the compound as having low toxicity. It was found that the accuracy of prediction of these properties ranged from 54.26% with an average similarity of 47.87%. Toxicological characteristics such as acute toxicity, mutagenicity, oncogenicity and effect on reproductive functions were taken into account.

Taking into account the results of virtual bioscreening and predictor analysis of the compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate, molecular docking was performed. This method is one of the most widely used approaches to virtual search for pharmacologically active compounds. It is based on calculating the binding energy of a chemical compound molecule to a target protein. Docking is used to predict ADMET characteristics of pharmacologically active molecules by evaluating their affinity for relevant target proteins. A range of biological targets have been identified for 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate acid due to its chemical structure and pharmacological effects. These include eNOS, RANK, RANKL, TNF-α, VEGF, BMP-2, and OPG. The volume of search space is > 27,000 Angstroms. The molecular coupling of key therapeutic targets and active compounds of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate is shown in Figures 4 (A, B, C, D, E, F, G). In 3D interactions, colored ring structures represent active ingredients; colored segments on protein structures represent amino acids that form hydrogen bonds with active ingredients. Yellow dotted lines represent hydrogen bonds between ingredients and proteins. According to receptor-ligand docking theory, docking energy is inversely proportional to binding affinity, with a more negative energy indicating a stronger binding. The energies at 9 best locations, and geometric differences between them, for eNOS, RANK, RANKL, TNF-α, VEGF, BMP-2 and OPG targets, are presented in Table 4 and Figure 4 below.

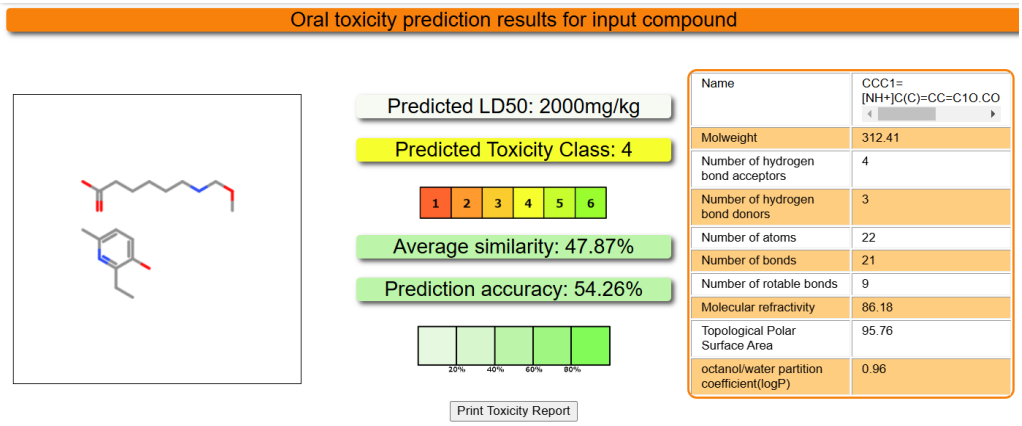


Figure 3. Prediction of the toxicity of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate derivative.

From the docking output, we obtain means that represent the estimated binding affinity from the docking estimate, as well as distances between poses from the best predicted binding mode calculated using various methods. If the affinity value is less than 5 kcal/mol, the binding is considered insignificant. If it is more than 5 kcal/mole, it is effective (Table 4).

In principle, for all ligands, variants of the structure have been found in which the ligand enters the active center. The three-dimensional molecular structure of the ligand, and the location of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate compound in the active center of eNOS, RANK, RANKL, TNF-α, VEGF, BMP-2 and OPG are presented in Figure 4 below.

Generally, docking has shown good results –- the ligand almost always gets into the pocket. However, inside the pocket, the orientation of the ligand turns out to be different. In some cases, the ligand leaves the binding site, eg TNF-α, RANK. Although 2 of these states are located in one place and the other two states are in another, this picture shows that all the states of a ligand molecule move freely inside a protein binding center. Notably, the range of affinity for proteins is most pronounced when they interact with eNOS, RANKL, and VEGF.

Discussion

Modern drug discovery and development is a time-consuming and resource-intensive process. The procedure from the initial synthesis of a drug to the pre-clinical phase can take approximately two to four years and costs hundreds of millions of dollars. Reasons for failures in pre-clinical and clinical trials include the inefficiency of compounds or unpredictable side effects. Therefore, conducting drug development can accelerate drug development projects by prioritizing compounds.

Molecular docking plays an important role in various stages of drug development, acting as a powerful and effective tool that significantly speeds up and reduces the cost of drug creation. Docking not only allows predicting potential side effects and resistance to drugs, but also repurposing already known drugs, opening new areas for research and going beyond traditional experimental approaches. Development of software and hardware, as well as accumulation of data about a new compound are becoming an indispensable part of modern drug discovery and development processes.

Therefore, the information obtained from *in silico* studies of compound 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate confirms the potential for further research. The compound under study has an analysis of properties corresponding to the Lipinski rule: molecular weight less than 500 Da; logP less than 5; fewer than 5 hydrogen bond donors and acceptors, and molar refraction between 40 and 130. Junctions analyzed revealed the strongest binding affinity for 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate eNOS (-8.3 kcal/mol), RANKL (-6.9 kcal/mol), and VEGF (-7.7 kcal/mol), with lower affinity for RANK (-4.2kcal/mol) TNF-alpha (-4.7kcal/mol), BMP -2 (-3.6kcal/m), and OPG (-3.7 cal/m).

One of the promising developments in the search for effective drugs is the creation of drugs based on acexamic acid (AC). The advantage of known and newly synthesized derivatives of AC –salts of silver, cerium, and pyridinium – is their low toxicity and ability to stimulate bone and soft tissue regeneration.

Table 4. Predicted results of the affinity of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate to the active center of eNOS, RANK, RANKL, TNF-α, VEGF, BMP-2 and OPG

Mode	Affinity(kcal/mol)	Dist from (rmsd l.b.)|	Best mode (rmsd u.b.)
		eNOS	
1	-8.3	0.000	0.000
2	-8.2	8.346	8.858
3	-8.2	29.464	29.826
4	-8.0	33.839	34.827
5	-7.9	16.835	17.917
6	-7.7	20.451	21.272
7	-7.3	20.372	21.152
8	-7.3	33.784	34.790
9	-7.2	49.030	49.902
		RANK	
1	-4.2	0.000	0.000
2	-3.4	1.492	2.350
3	-3.4	28.472	29.284
4	-3.3	2.259	2.688
5	-3.2	3.079	3.969
6	-3.1	54.554	55.307
7	-2.9	3.903	4.91
8	-2.9	50.873	51.772
9	-2.9	47.926	49.214
		RANKL	
1	-6,9	0.000	0.000
2	-6,8	23.901	24.246
3	-6.7	4.069	4.907
4	-6.5	35.482	36.575
5	-6.5	35.549	36.553
6	-6.4	5.620	6.327
7	-6.0	31.528	32.491
8	-6.0	46.176	46.920
9	-6.0	40.469	41.414
		TNF-α	
1	-4.7	0.000	0.000
2	-4.1	24.210	24.688
3	-4.0	25.783	26.922
4	-3.9	28.721	29.496
5	-3.9	24.698	25.309
6	-3.8	7.776	8.633
7	-3.8	26.322	27.187
8	-3.8	29.181	29.853
9	-3.7	21.566	22.443
		VEGF	
1	-7.7	0.000	0.000
2	-7.2	2.802	3.501
3	-7.0	1.715	2.270
4	-7.8	17.072	17.818
5	-7.7	3.272	4.118
6	-7.6	2.995	3.964
7	-6.2	32.982	34.141
8	-6.1	38.361	39.542
9	-6.1	32.329	33.304
		BMP-2	
1	-3.6	0.000	0.000
2	-3.5	31.766	32.736
3	-3.3	2.550	3.195
4	-3.3	32.158	33.081
5	-3.2	2.407	3.072
6	-3.0	17.969	19.03
7	-2.9	39.482	40.673
8	-2.9	3.248	4.088
9	-2.8	31.811	32.750
		OPG	
1	-3.7	0.000	0.000
2	-3.5	8.576	8.773
3	-3.4	31.721	32.386
4	-3.4	31.721	32.386
5	-3.4	42.047	42.738
6	-3.2	11.781	12.792
7	-3.2	39.723	40.021
8	-3.2	10.544	11.580
1	-3.7	0.000	0.000

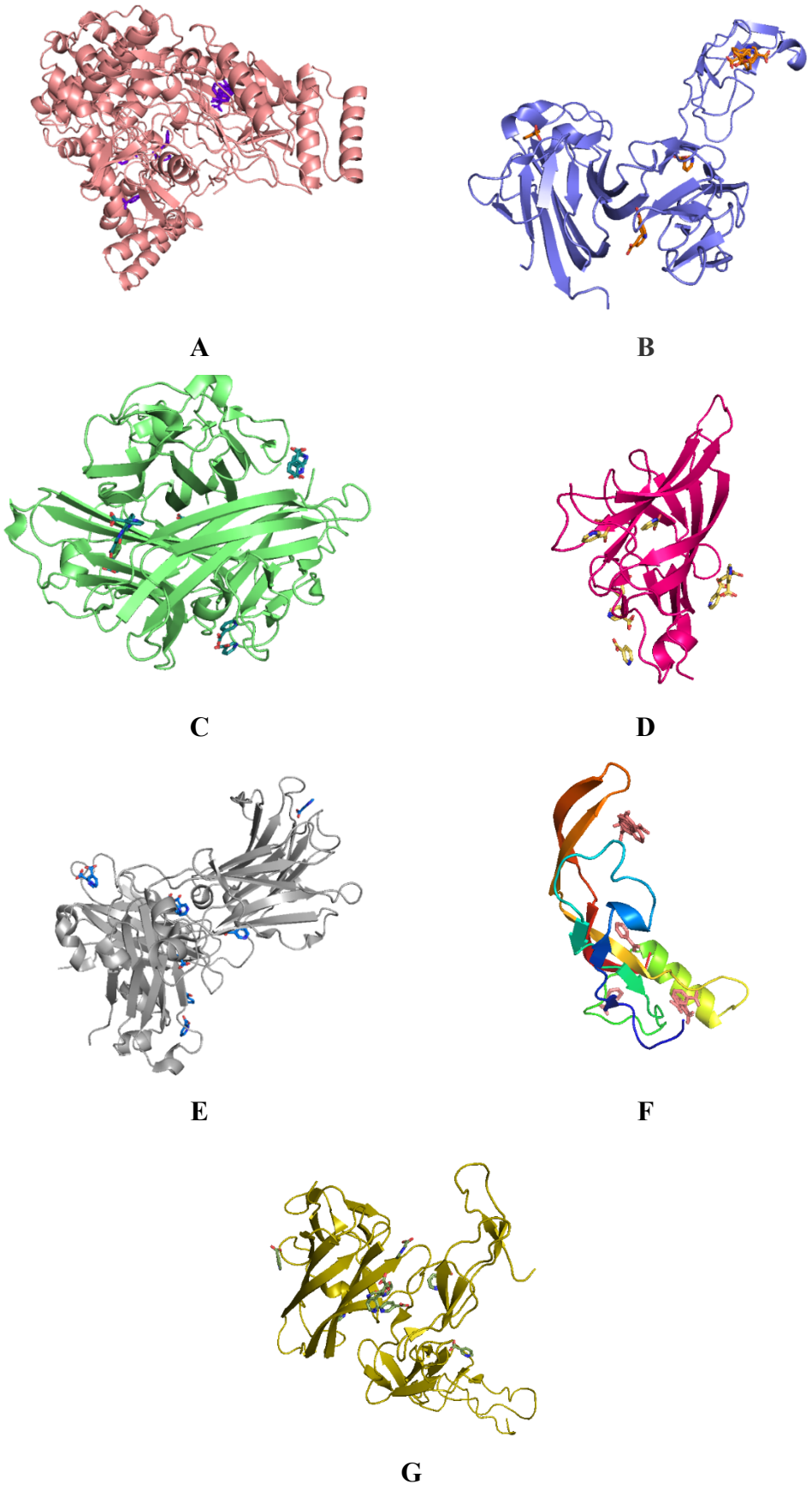


Figure 4. Three-dimensional molecular structure of the ligand and the location of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate compound in the active center of eNOS (**A**), RANK (**B**), RANKL (**C**), TNF-α (**D**), VEGF (**E**), BMP-2 (**F**) and OPG (**G**).

A derivative of 3-hydroxypyridine, 2-ethyl-6-methyl-3 hydroxypyridin, deserves special attention. Among derivatives of 2 ethyl-6-methyl-3-hydroxypyridine, there are a number of drugs with a wide range of biological activities. One of them is succinate 2-ethyl-6-methyl-3-hydroxypyridine (Mexidol), synthesized by Smirnov and Kuzmin at Research Institute of Pharmacology Russian Academy of Medical Science. It restores neurotransmitters balance, regulates receptors and membrane bound enzymes, has antioxidant properties, and has a wide pharmacological activity (Shcheblykina et al. 2022; Danilenko et al. 2018). Numerous studies have shown that mexidol inhibits the process of lipid peroxidation (POL), reacts actively with primary and hydroxyl radicals in peptides, reduces pathologically elevated NO levels in the brain and on the other hand increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, which are responsible for the formation and consumption of lipid peroxides and reactive oxygen species. Additionally, 2-ethyl-6-methyl-3-oxypyridine succinate has also been shown to have a positive effect on microcirculation disorders and endothelial protection (Polozova et al. 2021; Andrianova 2023; Petrovskaya et al. 2024). The results obtained *in silico* for 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate compound open up broad scientific and practical prospects for further research. In scientific terms, they relate to expanding the spectrum of pharmacological activity of the complex studied, because the study showed that the compound has high affinity, target specificity, and low toxicity. In addition, it has good tissue penetration, which makes it a promising candidate for future research.

Conclusion

The complex salt of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate after analysis for ADMET/pharmacokinetic evaluation deserves further analysis as a promising candidate. A series of studies has shown that the binding energy of this complex salt is ranked as follows: eNOS (-8.3 kcal/mol), VEGF (-7.7 kcal/mol), RANKL (-6.9 kcal/mol), TNF-α(-4.7 kcal/mol), RANK(-4.2), OPG(-3.7 kcal/mol), and BMP-2(-3.6 kcal/mol). The data obtained for 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate are promising for further development as a potential therapeutic agent for bone and soft tissue repair processes where endothelial damage makes a significant contribution.

Additional information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The authors have no funding to report.

Data availability

All of the data that support the findings of this study are available in the main text.

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Author Contribution

- **Anton P. Danilenko**, postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: 1190540@bsuedu.ru; **ORCID ID:** https://orcid.org/0009-0001-2419-8109. The author participated in the study design, conducted the experimental work, and analyzed the data.
- **Alexey A. Khentov**, postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: alexeyhentov@gmail.com; **ORCID ID:** https://orcid.org/0009-0006-4315-0828. The author analyzed the results and participated in the experimental work.
- **Alexey V. Kuznetsov**, postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: kav8080@bk.ru; **ORCID ID:** https://orcid.org/0000-0003-1889-1297. The author analyzed the results and participated in the experimental work.
- **Veronika S. Shmigerova**, Assistant Professor, Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: nika.beliaeva@yandex.ru; **ORCID ID:** https://orcid.org/0000-0003-2941-0241. The author took part in writing the article and processing the results obtained.
- **Yu V. Stepenko**, Assistant Professor, Assistant, Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: stepenko@bsu.edu.ru; **ORCID ID:** https://orcid.org/0000-0002-7414-7326. The author took part in writing the article and processing the results obtained.
- **Alla P. Tarasova**, PhD in Medicine , Associate Professor, anesthesiologist resuscitator, St. Joasaph Belgorod Regional Clinical Hospital, Belgorod, Russia; e-mail: tarasova_ap@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-7296-0022. The author analyzed the literature and participated in interpreting the data.
- **Vladimir I. Yakushev**, PhD in Medicine, Associate Professor, Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: vladiyakush@yandex.ru; **ORCID ID:** https://orcid.org/0000-0003-3922-2359. The author participated in the review and analysis of literature sources.
- **Elizaveta V. Boeva**, undergraduate student, Medical Institute, Belgorod State National Research University, Belgorod, Russia; e-mail: liza.boeva31@gmail.com; **ORCID ID:** https://orcid.org/0000-0002-4802-172X. The author took part in writing the article and processing the results obtained.
- **Albina V. Miller**, undergraduate student, Medical Institute, Belgorod State National Research University, Belgorod, Russia; e-mail: cherednichenko.albina@gmail.com; **ORCID ID:** https://orcid.org/0000-0003-0162-9196. The author took part in writing the article and processing the results obtained.
- **Irina A. Tatarenkova,** Assistant Professor, Associate Professor of the Department of Pharmacology, Kursk State Medical University, Kursk, Russia; e-mail: irtalex@yandex.ru; **ORCID ID:** https://orcid.org/0000-0002-6477-1934. The author analyzed the literature and participated in interpreting the data.
- **Tatiana V. Puzanova**, postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: tatius_ktv@mail.ru; **ORCID ID:** https://orcid.org/0009-0003-7926-8447. The author participated in the article writing, analyzed the results and participated in the experimental work.
- **Yana V. Loboda**, MD, postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: loboda.yana0104@yandex.ru; **ORCID ID:** https://orcid.org/0009-0004-0237-5233. The author analyzed the results and participated in the experimental work.
- **Andrei P. Trashchenko**, undergraduate student, Institute of Medicine, Belgorod State National Research University, Belgorod, Russia; e-mail: 1698008@bsuedu.ru; **ORCID ID:** https://orcid.org/0009-0004-5523-5779. The author analyzed the literature and participated in interpreting the data.
- **Oleg S. Gudyrev,** Assistant Professor, Associate Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: gudyrev@mail.ru; **ORCID ID:** https://orcid.org/0000-0003-0097-000X. The author took part in writing the article, developing a research design, and processing the results obtained.

- **Anna L. Motailo**, undergraduate student, Belgorod State National Research University, Belgorod, Russia; e-mail: 1468748@bsuedu.ru; **ORCID ID:** https://orcid.org/0009-0007-5118-5964. The author was engaged in conducting the literature analysis.
- **Vladimir N. Kuzichkin**, neurosurgeon, City Hospital No 2 of Belgorod, Belgorod, Russia; e-mail: kuzichkin_v@bsuedu.ru; **ORCID ID:** https://orcid.org/0009-0006-4612-0851. The author analyzed the literature and participated in interpreting the data.
- **Valentina N. Milashechko**, undergraduate student, Medical Institute, Belgorod State National Research University, Belgorod, Russia; e-mail: 1463347@bsuedu.ru; **ORCID ID:** https://orcid.org/0009-0003-9002-3985. The author analyzed the literature and participated in interpreting the data obtained.
- **Lyudmila M. Danilenko**, Assistant Professor, Full Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: Danilenko_L@bsuedu.ru; **ORCID ID:** https://orcid.org/0000-0001-6101-8712. The author was engaged in collection and interpretation of the data for the paper.