PHARMACOLOGICAL INVESTIGATIONS BASED ON NOVEL TRIAZOLE COMPOUNDS: MOLECULAR MODELING AND ADMET ANALYSIS

Bekmurodova Malika Ruzimurod qizi
" Samarkand State Medical University, Faculty of "
General Medicine-2"5th-year student
Abdullayev Ma'rufjon Ismoil o'g'li
Samarkand State Medical University, Faculty of "
General Medicine-1", 4th-year student
Beknazarov Shahobiddin Fazliddin o'g'li
Samarkand State Medical University, Faculty of Pediatrics, 4th-year student
Scientific Advisor: Doston Rustamovich Toirov
Associate Professor, Department of Propaedeutics of
Internal Diseases,Samarkand State Medical University, PhD
bekmurodovamalika15@gmail.com

ANNOTATION: *In the treatment of hyperuricemia and gout, inhibition of xanthine oxidase enzyme activity is considered a key therapeutic approach. In this study, the xanthine oxidase inhibitory activity of 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives is evaluated based on molecular modeling. QSAR models were developed using CoMFA and CoMSIA methods, and their reliability was confirmed by internal and external validation. Molecular docking and dynamic analyses revealed that the most active molecules — No. 8 and No. 22 — form stable interactions with the xanthine oxidase enzyme. Additionally, the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the newly proposed compounds Pred 4 and Pred 5 were studied, showing favorable pharmacokinetic and toxicological profiles for drug use. The obtained results indicate that the Pred 4 and Pred 5 molecules may be promising candidates for the treatment of hyperuricemia and gout.*

KEYWORDS: *Hyperuricemia, gout, xanthine oxidase, CoMFA, CoMSIA, molecular docking, ADMET, allopurinol, febuxostat, topiroxostat, 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives.*

INTRODUCTION: Gout is the most common cause of inflammatory arthritis worldwide (Dalbeth et al., 2021). It is a chronic disease characterized by hyperuricemia, tophi, joint damage, and kidney stones (Ragab et al., 2017). Uric acid, formed as a result of purine metabolism, is the final metabolite in the human body and is excreted through the kidneys and gastrointestinal tract. In this process, the enzyme xanthine oxidase (XO) plays a crucial role by converting hypoxanthine and xanthine into uric acid (Furuhashi, 2020). Therefore, XO inhibitors are considered effective in the treatment of hyperuricemia and gout. For instance, allopurinol is often prescribed as the first-line urate-lowering therapy (Linani et al., 2022). According to epidemiological data, the

prevalence of gout is 0.3%–3% in Europe, 3.9% in the United States (6.6% among Asian Americans), and 4.9% in Taiwan (Pascart et al., 2024). Currently, three XO inhibitors — allopurinol, febuxostat, and topiroxostat — are widely used in clinical practice. Allopurinol has been in use since 1966 and remains the most frequently prescribed drug (Sato et al., 2018). Nevertheless, there is a need for the development of next-generation drugs with higher efficacy and improved target specificity (Cicero et al., 2021). The 4-(phenoxymethyl)-1H-1,2,3-triazole compounds synthesized by Zhang and co-authors (2022) have been biologically evaluated and identified as promising XO inhibitors. Molecular modeling studies have been conducted on these compounds, and novel drug candidates have been identified using 3D-QSAR, molecular docking, molecular dynamics, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) analyses (Er-Rajy et al., 2022–2024). The 3D-QSAR methodology determines the relationship between the molecular structure and biological activity (pIC50). The most widely used methods are CoMFA and CoMSIA, and the quality of the models is assessed through internal and external validation. Additionally, molecular docking is performed to determine how the molecules interact with the target enzyme, and molecular dynamics simulation over 100 ns is carried out to evaluate their stability. Based on the results, novel XO inhibitors are proposed. These analyses reveal the nature and intensity of molecular interactions such as hydrogen bonding, van der Waals forces, and steric effects.

MATERIALS AND METHODS: In this study, the experimental data set based on 26 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives synthesized by Zhang et al. (2022) was used to construct CoMFA and CoMSIA models. These compounds were evaluated based on their inhibitory activity against the xanthine oxidase (XO) enzyme, and IC_{50} (μM) values were determined. For the purpose of model building and result evaluation, the entire data set was divided into a training set and a test set (Er-Rajy et al., 2023c).

Model construction and performance evaluation: The SYBYL-X 2.1 software was used to generate CoMFA and CoMSIA fields, as well as to perform molecular minimization (Bringmann and Rummey, 2003). For each grid, CoMFA descriptors were calculated within a grid extending up to 4 units in three-dimensional coordinates with a spacing of 1 unit (Bohm et al., 1999). Steric and electrostatic field energies were determined using the van der Waals potential, Coulombic forces, and a +1 charged sp^3 hybridized carbon atom as a probe. Energy values were capped at a maximum of 30 kcal/mol (Er-Rajy et al., 2023a). For the CoMSIA field, Gaussian-type distance-dependent physicochemical properties were selected to avoid singularities between atoms. Steric, electrostatic, hydrogen bond acceptor, hydrogen bond donor, and hydrophobic effects were included in the CoMSIA calculation using uniform standard parameters (Guo et al., 2005; Baidya et al., 2023).

The properties related to the inhibitory activity of the compounds were evaluated using the partial least squares (PLS) approach. Model reliability was assessed using the cross-validated correlation coefficient (Q^2), the determination coefficient (R^2), the number of components (NOC), and the standard error of estimate (SEE). Subsequently, non-

validated versions of PLS were used to develop various versions of the CoMFA and CoMSIA models (Aloui et al., 2024; Er-Rajy et al., 2024b). Model stability was defined by $Q^2 > 0.5$ and NOC values ranging from 1 to 6. Once optimal values for Q^2 and NOC were identified, the overall statistical significance of the model was assessed by the R^2 coefficient and SEE. A 3D-QSAR model is considered reliable if $R^2 > 0.6$ and SEE is low; under these conditions, the model can provide accurate results for pIC_{50} values based on the structural features of the molecules (Roy and Pratim Roy, 2009; Nour et al., 2022a; Er-Rajy et al., 2023d).

Molecular docking study: Before the docking study, the two most active compounds were drawn using ChemDraw 16.0 and their geometries were optimized to a stable state using MM2 optimization (Allinger, 1977). At this stage, the protonation states of the compounds and polar hydrogen atoms in aqueous medium were determined. In the next step, the main structures of the protein and ligands were prepared (Er-Rajy et al., 2025). During protein preparation, water molecules and non-protein elements were removed, polar hydrogens were added, and Gasteiger charges were assigned (Nour et al., 2022b). Both compounds were tested for anti-gout activity. For this purpose, the xanthine oxidase receptor obtained from the Protein Data Bank (PDB ID: 3NVY, 2.00 Å resolution) was used (Cao et al., 2014). Grid coordinates were set as X = 39.948, Y = −17.942, and Z = 24.367 Å. Molecular docking was performed using AutoDockTools software (Holt et al., 2008). The lowest binding free energy (ΔG) of the protein-ligand complex was determined using the Lamarckian Genetic Algorithm (LGA). A grid of 40×40×40 points was used along each dimension (X, Y, Z). For each docking case, 100 solutions were computed, with a population size of 350. After preparing the molecules and removing water molecules from the complex, the docking protocol was fully executed (Hjouji et al., 2025). Discovery Studio 2021 was used to visualize ligand–protein interactions, correct missing side chains, and merge non-polar hydrogens (Pinzi and Rastelli, 2019).

Molecular dynamics analysis: According to the molecular docking results, the two most active ligands were selected, and a 100-nanosecond molecular dynamics simulation was performed to assess their binding stability with the target protein. This simulation was carried out using the Desmond software and Schrodinger Suite (Maestro interface) based on the OPLS3e force field. The complexes were placed in an aqueous environment with a 10 Å buffer, and water molecules were simulated using the TIP3P model. To neutralize the system, Na^+ and Cl^- ions were added. Electrostatic interactions were computed using the Particle Mesh Ewald (PME) algorithm. Simulations were conducted at 300 K temperature and 1 bar pressure using the Nose–Hoover thermostat and barostat. The specific interactions between the ligand and the protein were analyzed using the Desmond interaction diagram module.

ADMET property analysis: The pharmacokinetic and toxicological properties of the newly synthesized molecules were evaluated. The following parameters were analyzed using the SwissADME and pkCSM online servers: lipophilicity, water solubility, saturation level, molecular flexibility, and bioavailability. Additionally, pharmacokinetic factors such as metabolism, excretion, and potential toxicity were assessed. The "drug-

likeness" of the compounds was also evaluated. This assessment was based on Lipinski's rules, determining the probability of effective and safe oral administration.

RESULTS AND DISCUSSION: ***Construction and analysis of 3D-QSAR models:*** To evaluate the potential of 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives against xanthine oxidase enzyme activity, two 3D-QSAR models — CoMFA and CoMSIA — were constructed. The molecules were aligned in the Sybyl X-2.1 software based on the most active compound (compound 22) as a common template. Using these models, the difference between the theoretical activity and experimental values for each compound was calculated. According to the results, the residual values were mostly close to zero, reaching up to 0.3 in some cases. This indicates that the models are highly stable and reliable, and they can serve as effective tools for predicting XO enzyme inhibition.

Model evaluation and validation: To assess the reliability and statistical performance of the model, the PLS (Partial Least Squares) cross-validation method was applied to the training set. As a result, the CoMFA model showed $Q^2 = 0.652$, $R^2 = 0.859$, with an optimal number of components of 2 and a standard error of 0.195. In this model, it was determined that steric factors contributed 68.4% and electrostatic factors 31.6% to the biological activity of the molecules. Additionally, in the CoMSIA/SEA model, $Q^2 = 0.676$, $R^2 = 0.805$, with a standard error of 0.229 and an optimal number of components of 2. This model demonstrated that steric, electrostatic, and hydrogen bond acceptor properties collectively contributed to the highest predictive power. The robustness of the models was also confirmed through external validation: $R^2\_ext = 0.683$ for the CoMFA model and $R^2\_ext = 0.767$ for the CoMSIA model. These values indicate that the models can provide accurate predictions even for external molecules.

Molecular analysis based on the model: During the study, the shape of the molecules, steric and electrostatic fields affecting their active regions, and hydrogen bond acceptor zones were analyzed based on the models. The most biologically active compound was taken as the reference. Through this analysis, the structural features of the molecules that may enhance or reduce their activity were identified.

ADMET properties – pharmacokinetic and toxicological evaluation: The main objective of this study is to predict the favorable drug-like properties of the newly proposed molecules and to assess their safety in the organism. This allows the identification of compounds suitable for therapeutic use without toxic effects. The evaluation considered the following parameters:

- LIPO (Lipophilicity): Solubility of the molecule in a lipid environment (acceptable range: −0.7 to 5);
- SIZE (Size): Molecular weight of the compound (should be between 150–500 g/mol);
- POLAR (Polarity): TPSA (topological polar surface area) in the range of 20–130 $Å^2$;
- INSOLU (Insolubility): logS < 6 (a lower logS indicates better solubility);

- INSATU (Unsaturation): Fraction of sp^3-hybridized carbon atoms should be ≥ 0.25;
- FLEX (Flexibility): Number of rotatable bonds should not exceed 9.

Based on these parameters, the oral suitability, bioavailability, and potential for drug application of the molecules were evaluated.

The ADMET analysis was conducted to assess the potential of the newly proposed compounds for drug use. During this process, their lipophilicity, molecular weight, polarity, solubility, degree of unsaturation, and flexibility were taken into account. According to oral bioavailability indicators, compounds N°22 and N°8 are not fully compliant, as some of their properties fall outside the optimal range. The Pred 5 molecule shows excessive flexibility and thus does not fall within the optimal range. Pred 4, on the other hand, meets nearly all required parameters. All molecules were evaluated for drug-likeness and toxicity and subjected to ADMET analysis.

The newly proposed compounds Pred 4 and Pred 5, as well as the active molecules N°8 and N°22, were evaluated for absorption, distribution, metabolism, excretion, and toxicity:

- Intestinal absorption was very high in all molecules (96%–98%);
- Blood–brain barrier (BBB) permeability was low, indicating limited effects on the central nervous system;
- CYP450 enzyme interactions were detected: all molecules inhibit CYP3A4 and CYP2C19 enzymes;
- Compound N°22 was evaluated as toxic according to the AMES test, while the others were non-toxic;
- Pred 4 exhibited good metabolic stability and high excretion rates;
- Pred 5 had the lowest water solubility and is not a CYP2D6 substrate.

Based on drug-likeness assessment and ADMET property evaluation, the proposed compounds Pred 4 and Pred 5 are considered promising candidates as xanthine oxidase (XO) inhibitors. They show good absorption, distribution, and metabolism profiles, low excretion levels, and are distinguished by their non-toxicity.

CONCLUSION: In this study, 4-(phenoxymethyl)-1H-1,2,3-triazole derivatives were investigated for the treatment of gout. Their activity was predicted using two models — CoMFA and CoMSIA — while molecular docking and dynamics demonstrated stable binding with the enzyme. Two newly proposed compounds — Pred 4 and Pred 5 — are considered promising as drugs, possess favorable ADMET properties, and effectively inhibit xanthine oxidase.

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