DESIGN AND MOLECULAR DOCKING VALIDATION OF FOUR DIHYDROPYRAZOLE-BASED MurA ENZYME INHIBITORS

F. Shang[1)], **A. Bakunovich**[1)]

[1)] *International Sakharov Environmental Institute of Belarusian State University, Dolgobrodskaya str., 23/1, 220070, Minsk, Belarus, 497716844@qq.com*

This study examines the mechanism of action of the MurA enzyme and its crucial role in bacterial peptidoglycan synthesis. MurA is recognized as a promising target for the development of novel antibacterial agents. Enterobacter cloacae MurA (PDB: 4E7C) was selected as the target protein. Four novel molecules were designed based on the dihydropyrazole scaffold using structure-based drug design approaches. Molecular docking studies were performed to evaluate their binding affinity and inhibitory potential. The results revealed that compound F2 exhibits high inhibitory activity, making it a promising candidate for further optimization.

Keywords: MurA inhibitors; Molecular docking; Peptidoglycan

ДИЗАЙН И ВАЛИДАЦИЯ МОЛЕКУЛЯРНОГО ДОКИНГА ЧЕТЫРЁХ ИНГИБИТОРОВ ФЕРМЕНТА MurA НА ОСНОВЕ ДИГИДРОПИРАЗОЛА

Ф. Шан[1)], **А. В. Бакунович**[1)]

[1)] *Учреждение образования «Международный государственный экологический институт имени А. Д. Сахарова» Белорусского государственного университета, ул. Долгобродская, 23/1, 220070, г. Минск, Беларусь, 497716844@qq.com*

В данном исследовании рассматривается механизм действия фермента MurA и его ключевая роль в синтезе бактериального пептидогликана. MurA признан перспективной мишенью для разработки новых антибактериальных агентов. В качестве целевого белка был выбран MurA из Enterobacter cloacae (PDB: 4E7C). Разработаны четыре новые молекулы на основе дигидропиразольного скелета с использованием подходов структорно-ориентированного дизайна лекарств. Проведены исследования молекулярного докинга для оценки их сродства к мишени и ингибиторного потенциала. Результаты показали, что соединение F2 обладает высокой ингибиторной активностью, что делает его перспективным кандидатом для дальнейшей оптимизации.

Ключевые слова: ингибиторы MurA; молекулярный докинг; пептидогликан.

https://doi.org/10.46646/SAKH-2025-1-414-417

Peptidoglycan is an essential component of the bacterial cell wall, crucial for bacterial survival. It provides the rigid structure necessary for bacteria to withstand hypotonic environments [1].

The Mur enzymes (MurA-MurF) are primarily involved in the cytoplasmic phase of bacterial peptidoglycan synthesis. MurA, also known as UDP-N-acetylglucosamine enolpyruvate transferase, catalyzes the transfer of the enolpyruvate group from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UNAG), forming UDP-N-acetylglucosamine enolpyruvate (EP-UNAG) and releasing inorganic phosphate (Fig. 1) [2].

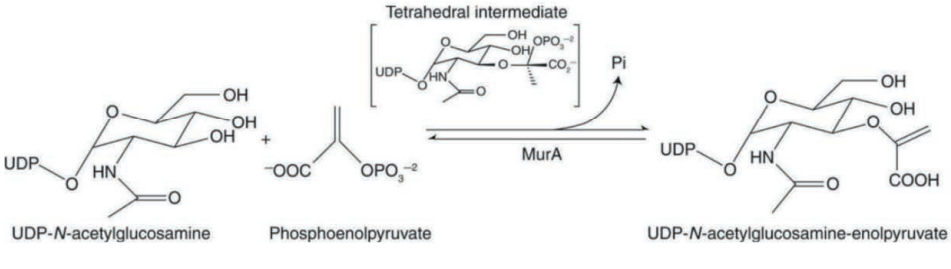


Fig. 1. Mechanism of MurA enzyme action [3]

Since this reaction mechanism is exclusive to the vast majority of bacteria and absent in mammals, MurA has become a significant target for the development of novel antibacterial inhibitors. Numerous studies have reported on antibacterial inhibitors targeting MurA. The naturally occurring broad-spectrum antibiotic fosfomycin acts as a PEP analog by covalently reacting with the active site Cys residue of MurA, making it a unique inhibitor of MurA [4]. Macarena Funes Chabán and colleagues identified compounds such as carnosol (1), rosmanol (2) and carnosic acid (3) from natural products. They also synthesized several derivatives of carnosic acid and dehydroabietic acid, demonstrating antimicrobial activity against both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* and compounds 1, 4 and 5 marked in Fig. 2 showed significant inhibition of MurA enzyme from *E. coli* with IC_{50} values less than 5μM [5]. However, only a few inhibitors can simultaneously inhibit MurA enzymes across different species, highlighting the importance of further research into the development and optimization of MurA inhibitors in terms of structure and bioactivity.

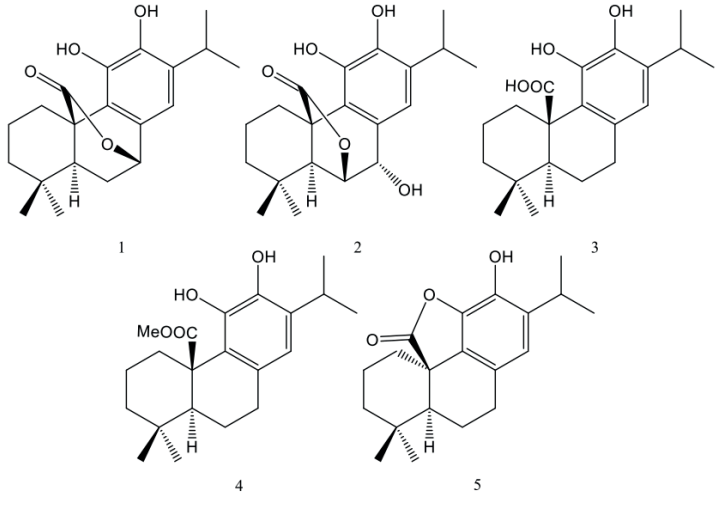


Fig. 2. Chemical structures of carnosol (1), rosmanol (2), carnosic acid (3), carnosic acid-γ-lactone (4), 20-methyl carnosate (5)

In this study, *Enterobacter cloacae* MurA (PDB code: 4E7C) was used as the target protein, and molecular docking methods were employed to analyze the interaction mechanisms between *Enterobacter cloacae* MurA inhibitors and the target protein.

Pyrazole, a five-membered heterocyclic structure composed of three carbon atoms and two nitrogen atoms, exhibits weak basicity. Its derivatives possess a wide range of biological activities. Pyrazole serves as the core structure of many major drugs, with several pyrazole-containing drugs already on the market. Examples include celecoxib, which inhibits prostaglandin synthesis, effectively combating inflammation and providing analgesic effects [6]; tepoxalin, a non-steroidal anti-inflammatory drug (NSAID) used as a veterinary analgesic for treating pain and inflammation associated with osteoarthritis; rimonabant, an anti-obesity drug; and difenamizole, an analgesic. Additionally, numerous investigational drugs with antibacterial, anticancer, antimalarial, antitubercular, and cardiovascular disease treatment potential have been reported [7]. This demonstrates the broad application of pyrazole derivatives in the pharmaceutical field.

Therefore, this study designed four novel pyrazole derivatives based on dihydropyrazole. Molecular docking was used to assist in drug design, and computational simulations were performed to screen compounds with potential antibacterial activity. All protein modifications, molecular

adjustments, and molecular docking were conducted using ChemOffice 20.0, PyMOL 2.3.2, and AutoDock Vina 1.2.6. The designed pyrazole derivatives are represented by the following SMILES notations:

1. F1: O=C(CCCCC)N1N=C(C2=CC=C(F)C=C2)CC1C3=CC=CC=C3
2. F2: O=C(CCCCC)N1N=C(C2=C(F)C=C(F)C=C2F)CC1C3=CC=NC=C3
3. F3: O=C(CCCCC)N1N=C(C2=CC=C(C(F)(F)F)C=C2)CC1C3=CCNC=C3
4. F4: O=C(CCCCC)N1N=C(C2=CCN(Cl)C=C2)CC1C3=CCNC=C3

The structural formulas of the developed molecules, as well as the results of molecular docking, are presented in Table 1.

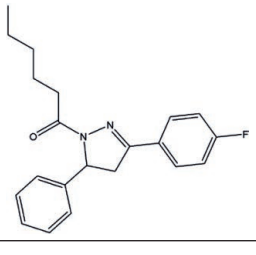
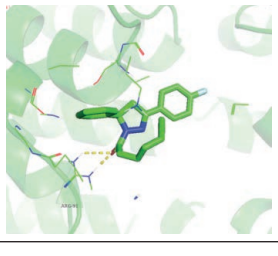
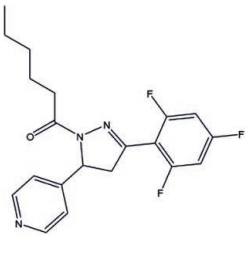
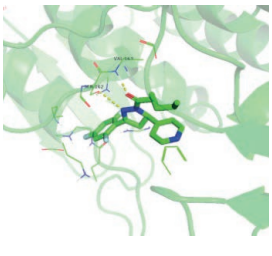
Studies have shown that in compound F1, the oxygen atom of the acyl group forms a hydrogen bond with the amino acid residue ARG-91 of the target protein, contributing to a binding energy of -5.158 kcal/mol. This interaction suggests moderate stability within the binding pocket. In F2, the oxygen atom of the acyl group establishes hydrogen bonds with VAL-163, while the nitrogen atom of the dihydropyrazole forms an additional hydrogen bond with SER-162, leading to a binding energy of -5.541 kcal/mol. These multiple interactions enhance the stability of F2 in the active site.

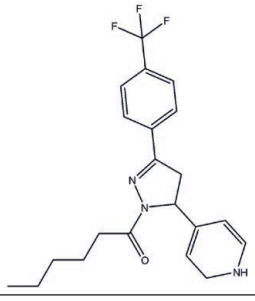
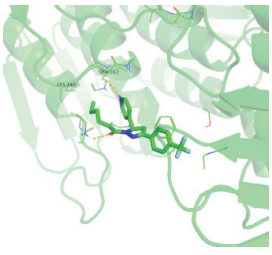
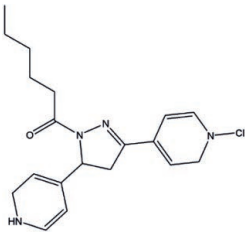
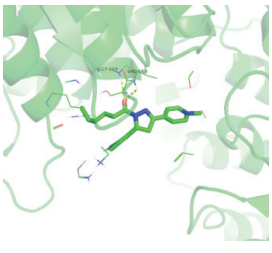
For F3, the oxygen atom of the acyl group forms a hydrogen bond with LYS-160, and the nitrogen atom of the pyrazole moiety, which is linked to the dihydropyrazole core, forms another hydrogen bond with SER-162. This dual interaction results in the highest binding energy among the four compounds, measured at -5.921 kcal/mol, indicating the strongest binding affinity. The oxygen atom of the acyl group in F4 forms two hydrogen bonds with GLY-164 and VAL-163, contributing to a binding energy of -5.226 kcal/mol, suggesting a stable but slightly weaker interaction compared to F3.

Furthermore, structural modifications such as the addition of a benzene ring and a fluorine atom at position 3 of the dihydropyrimidine do not form hydrogen bonds within the UTP binding pocket and have a negligible impact on the overall binding energy. Among the four compounds, F2 exhibits the lowest binding energy relative to the remaining three molecules within the UTP binding pocket, signifying its strongest inhibitory potential against MurA. These findings highlight F2 as the most promising candidate for further optimization and development as a MurA inhibitor.

Table 1

Molecular docking results of four inhibitors

Name	Structures	UTP Pocket Molecular Docking	Binding Energy (kcal/mol)
F1			-5.158
F2			-5.541

Name	Structures	UTP Pocket Molecular Docking	Binding Energy (kcal/mol)
F3			-5.921
F4			-5.226

References

1. *L. Fan., M. Haoyi., S. Zhengyang.* Research progress in targeting bacterial peptidoglycan synthase inhibitors // Journal of Pharmacy. 2017. Vol. 52 №. 3. P. 362–370.

2. Advances in UDP-N-Acetylglucosamine Enolpyruvyl Transferase (MurA) Covalent Inhibition / de Oliveira MVD [et al.] // Front Mol Biosci. 2022. Vol. 9:889825. doi: 10.3389/fmolb.2022.889825.

3. *Silver L. L.*, Fosfomycin: Mechanism and Resistance // Cold Spring Harb Perspect Med. 2017. Vol. 7(2): a025262. doi: 10.1101/cshperspect.a025262.

4. *Mihalovits L. M., Ferenczy G. G., Keserű G. M.,* Catalytic Mechanism and Covalent Inhibition of UDP-N-Acetylglucosamine Enolpyruvyl Transferase (MurA): Implications to the Design of Novel Antibacterials // Journal of Chemical Information and Modeling. 2019. Vol. 59, iss. 12. P. 5161–5173.

5. Antibacterial effects of extracts obtained from plants of Argentina: Bioguided isolation of compounds from the anti-infectious medicinal plant Lepechinia meyenii / Chabán M.F. [et al.] // Journal of ethnopharmacology. 2019. Vol. 239: doi: 10.1016/j.jep.2019.111930.

6. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis / Steinbach G. [et al.] // The New England journal of medicine. 2000. Vol. 342 №. 26 P. 1946–1952.

7. Recent advanced in bioactive systems containing pyrazole fused with a five membered heterocycle / Demetrio Raffa. [et al.] // European journal of medicinal chemistry. 2015. Vol. 97. P. 732–46.