Molecular Dynamics IN PROTO-NOOS:

Comparative Analysis of EcDHFR-NADPH-Ligand Complex Dynamics for Selected Trimethoprim Analogues

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PUT Drug Discovery

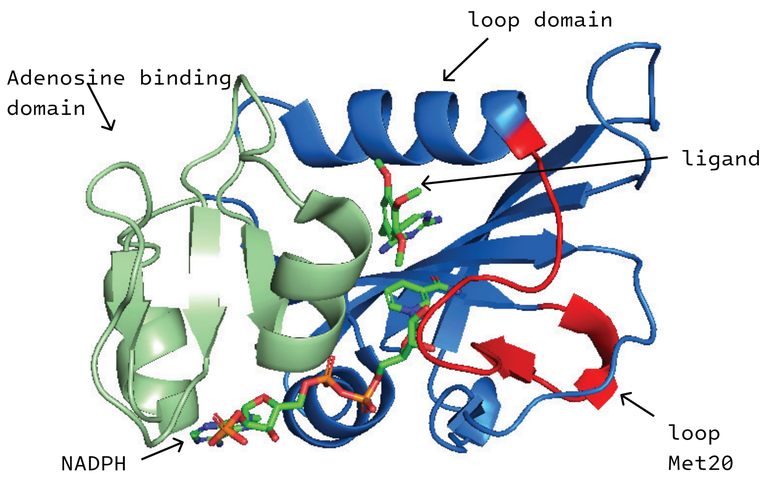
KNBz

01 MOTIVATION

The primary challenge in designing ecDHFR inhibitors is moving from docking to a lasting biological effect. While PROTO-NOOS excels at proposing candidate molecules, molecular dynamics determines their actual therapeutic potential.

Our motivation is to answer critical questions:

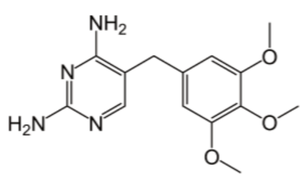
1. Is the binding stable? We use GROMACS to verify if docking poses survive thermal fluctuations and protein vibrations.
2. Are there steric clashes? We investigate whether the hydrophobic "tails" of novel compounds create structural tensions that would hinder efficacy.
3. How does the M20 loop respond? We analyze whether the candidates can maintain the M20 loop in a closed conformation, which is essential for effective enzyme inhibition.



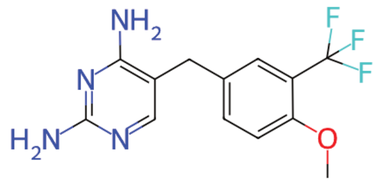
02 Structural overview of the EcDHFR–ligand–NADPH complex.

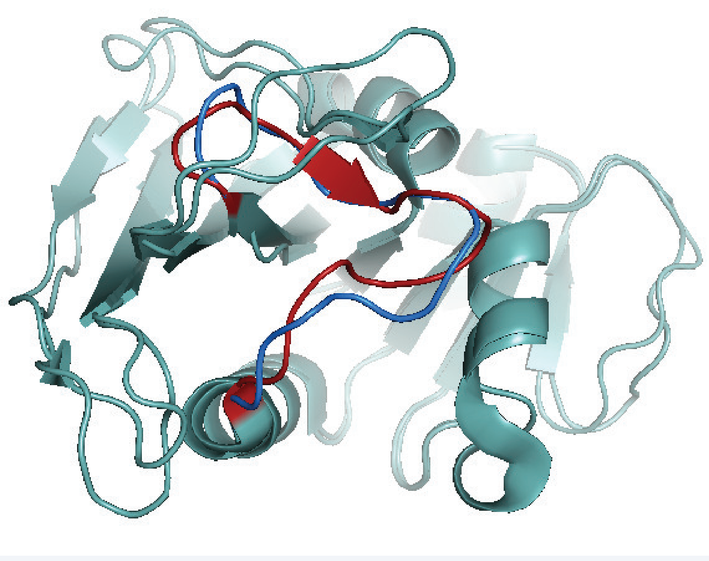
The figure presents Escherichia coli dihydrofolate reductase in cartoon representation, with the ligand and NADPH cofactor shown as sticks. The labeled regions indicate the spatial organization of the complex: the ligand occupies the active-site pocket close to NADPH, while the M20 loop is positioned near the catalytic region and may influence active-site closure and ligand recognition. The loop domain and adenosine-binding domain form the main structural framework surrounding the binding site and contribute to cofactor positioning and inhibitor stabilization.

Trimethoprim(TMP)



CHEMBL277391



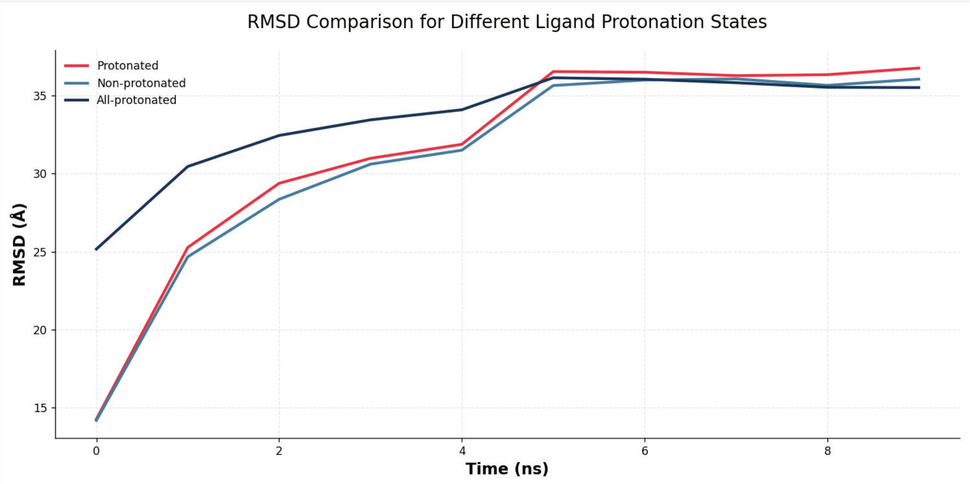


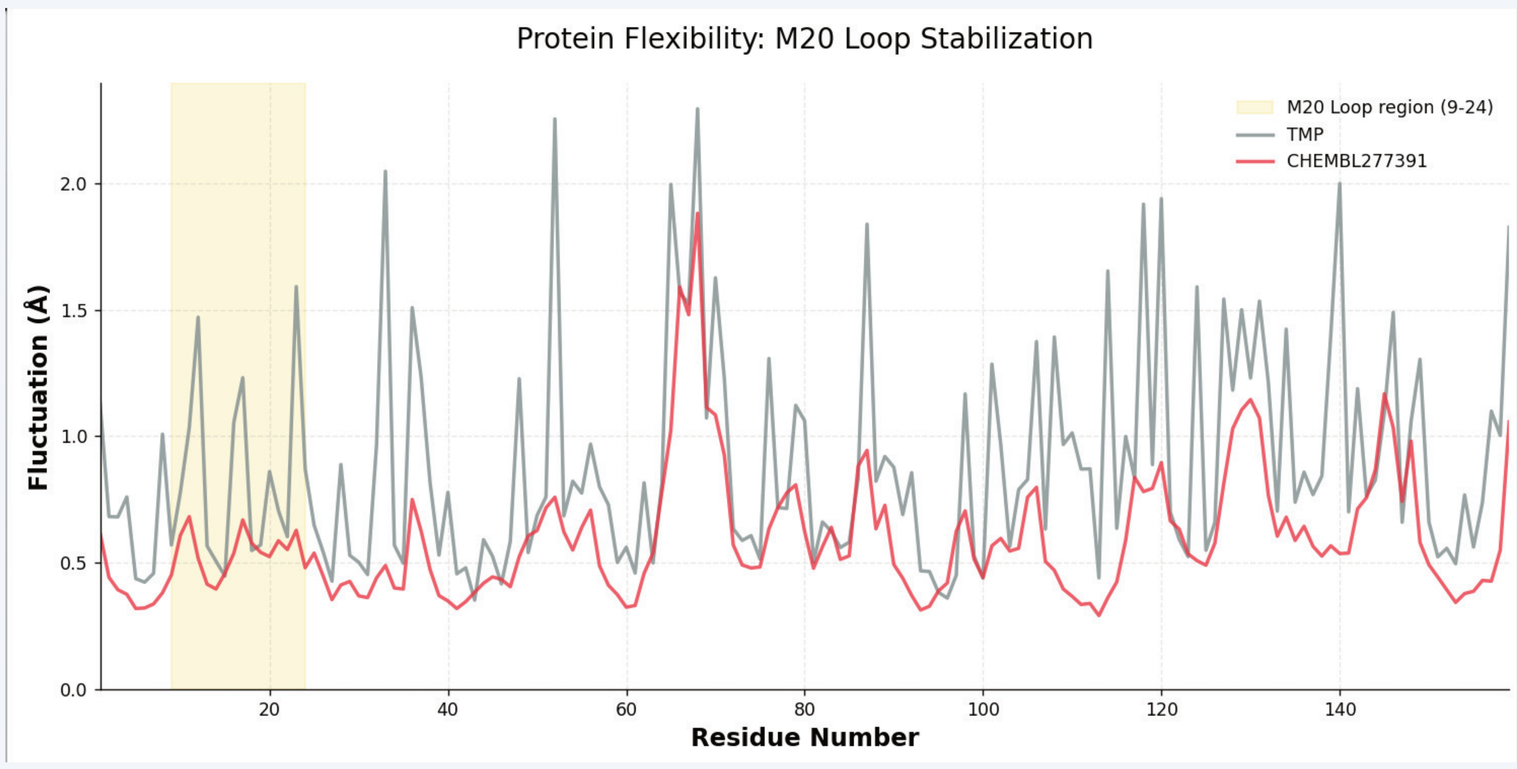
03 OUTLINING THE DISADVANTAGES OF STATIC DOCKING

M20 loop dynamics in EcDHFR are highly relevant for ligand recognition and binding-site organization. Static docking captures only a single structural pose and does not account for loop rearrangements, thermal fluctuations, or cofactor-dependent active-site plasticity. Using GROMACS molecular dynamics, we assess whether candidate ligands maintain a closed-like M20-loop geometry or whether they promote increased loop mobility and reduced binding-site retention.

04 CHEMBL277391 - TMP-like EcDHFR inhibitor candidate

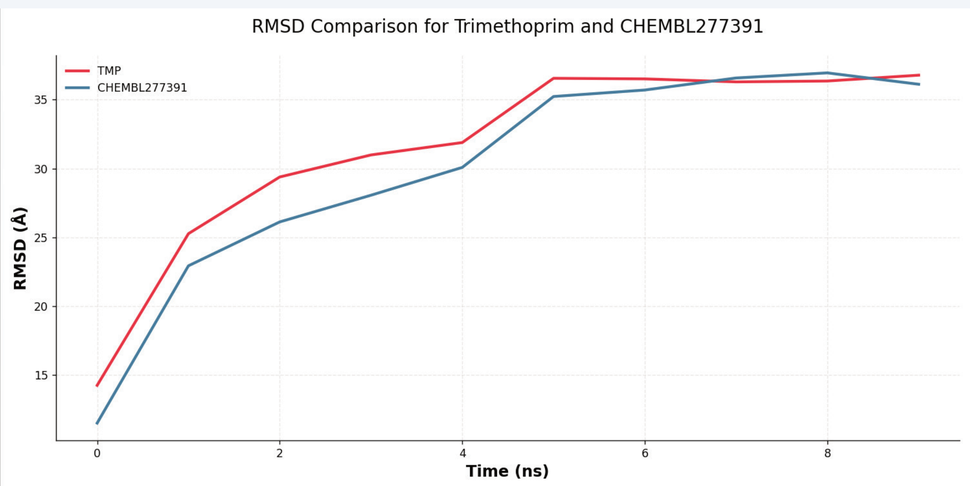
- CHEMBL277391 is a trimethoprim-like candidate containing a 2,4-diaminopyrimidine pharmacophore. This polar ring was analyzed as the key recognition motif for Asp27 in the EcDHFR active site.
- The substituted aromatic tail, including the CF3 group, was evaluated as a hydrophobic/steric element that may influence pocket fitting, ligand stability, and M20-loop response during MD simulation.





05 Impact of Protonation States on Binding Dynamics

- Protonation-Dependent Conformational Shifts: The simulation reveals that the protonation state of the inhibitor significantly dictates its initial stability within the binding pocket. The All-protonated variant exhibits a delayed increase in RMSD, suggesting that the additional positive charges may facilitate transient stabilizing interactions with acidic residues (e.g., Asp27).
- Electrostatic Sensitivity: The rapid separation of the Non-protonated and Protonated curves within the first 4 ns indicates high sensitivity of the ecDHFR binding site to the ligand's electrostatic surface potential.
- Kinetic Differences: While all states eventually reach a similar RMSD plateau, the "All-protonated" state shows a more gradual transition, suggesting a slower or distinct binding-pose relaxation pattern compared with the non-protonated form.



06 RMSF Analysis & M20 Loop Dynamics:

- CHEMBL277391 shows lower local RMSF than TMP in the M20-loop region, suggesting reduced loop mobility in the simulated complex.
- The M20 loop remains flexible; however, these fluctuations are interpreted as local motions rather than a full transition to open/occluded conformations.
- This interpretation is supported by the Asn18–His45 distance, which remains mainly within the closed-like range (~7–8 Å) during the 10 ns MD simulation.

07 Comparative RMSD analysis of CHEMBL277391 and Trimethoprim

The simulation demonstrates that CHEMBL277391 maintains a more constrained conformation during the equilibration phase (first 5 ns) compared to TMP, ultimately reaching a stable structural plateau consistent with the reference inhibitor.

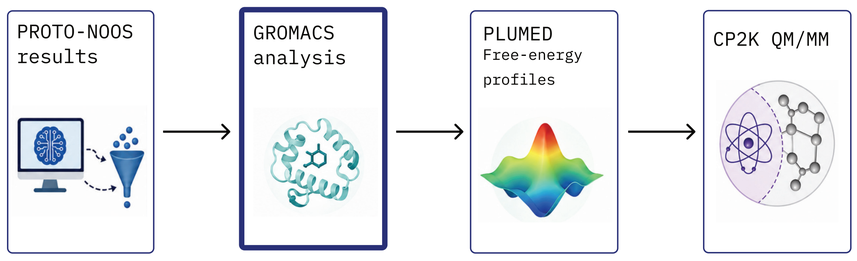
08 Solvent Accessible Surface Area (SASA) Analysis:

- CHEMBL277391 consistently exhibits lower SASA values (avg. 470Å^2) compared to Trimethoprim (avg. 555Å2), indicating a more compact protein-ligand complex.
- The significant reduction in surface exposure (approx. 85Å^2) suggests that the candidate ligand buries deeper into the binding pocket, effectively displacing solvent molecules and enhancing hydrophobic stabilization.
- Together with RMSF analysis, this supports good structural compatibility of CHEMBL277391 with the active-site environment during the simulated timescale.

09 Ligand–Protein Hydrogen-Bond Persistence

- Both ligands establish a robust and stable hydrogen-bonding network within the ecDHFR active site, maintaining an average of ~4.0–4.2 bonds throughout the 10 ns trajectory.
- LIGP (CHEMBL277391) demonstrates comparable binding persistence to the reference TMP, effectively mimicking its essential interaction pattern.
- The persistence of these interactions, together with reduced local M20-loop flexibility and lower ligand solvent exposure, supports stable binding-pocket retention of CHEMBL277391 during the 10 ns simulation.

Planned workflow for EcDHFR inhibitor development



PROTO-NOOS candidates will be filtered by MD-based stability analysis in GROMACS, followed by PLUMED enhanced sampling to explore free-energy landscapes of binding and loop rearrangements. Selected conformations will then be used in CP2K QM/MM calculations to connect inhibitor-induced structural effects with EcDHFR catalytic chemistry.

10 CONCLUSIONS

01 CHEMBL277391 remained compatible with the EcDHFR–NADPH active-site architecture during the 10 ns MD simulation, supporting its use as a TMP-like candidate for further in silico validation.

02 The 2,4-diaminopyrimidine-like pharmacophore provides a plausible motif for Asp27-directed polar recognition, while the CF3-substituted aromatic tail appears tolerated in the binding pocket on the simulated timescale.

03 M20-loop analysis indicates reduced local mobility relative to TMP, but not rigid locking. The loop retains local fluctuations while remaining predominantly within a closed-like conformational regime.

04 Protonation state strongly affected binding-pose stability. While the singly protonated variant remained compatible with the active site, the all-protonated state showed partial displacement from the binding pocket, suggesting that excessive ligand charge may destabilize pocket retention.

We also performed a preliminary analysis of the protonated CHEMBL346871 variant, with protonation assigned to the 2,4-diaminopyrimidine ring. The additional cyclic element at the hydrophobic tail may introduce steric strain near NADPH and adjacent active-site residues, potentially contributing to reduced pocket retention. This observation requires further validation in longer simulations.

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PREPRINT

https://komputerowe-projektowanie-lekow.github.io/webpage/

CODE

https://komputerowe-projektowanie-lekow.github.io/webpage/proto-noos_bca.html