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In-Silico Study of N-Hydroxysuccinimide Folate and GAPDH as Targeting Agents for Tuberculosis Treatment

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Abstract
Tuberculosis remains a major public health concern due to emerging cases of drug resistance. To overcome this issue, the effectiveness of antitubercular medications must be reinforced. One of the methods that can be used is using active targeting, which involves small molecules that bind to a specific receptor. In this research, we study the potential of N-hydroxysuccinimide (NHS) folate to be used as the targeting agent against the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) cell-surface receptor, a common virulence factor found on many pathogenic bacteria, including *Mycobacterium tuberculosis*. We conducted this research using computational methods, specifically molecular docking and molecular dynamics simulation. Based on the evaluation of molecular docking and molecular dynamics simulation results, such as binding affinities during docking, RMSD, RMSF, and MM-PBSA analysis results, it can be concluded that NHS-folate strongly interacts with GAPDH receptor and is predicted to have a huge potential to be an active targeting agent against the GAPDH receptor of *Mycobacterium tuberculosis*.

Keywords: Antitubercular medications, Molecular docking, Molecular dynamics simulation, glyceraldehyde-3-phosphate dehydrogenase, N-hydroxysuccinimide folate.

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Studi *In-Silico* N-Hydroxysuccinimide Folate dan GAPDH sebagai Agen Penarget Pengobatan Tuberkulosis

Abstrak
Tuberkulosis masih menjadi masalah kesehatan global, yang dikarenakan oleh munculnya kasus terkait resistensi obat. Untuk mengatasi masalah ini, efektivitas obat antituberkular harus ditingkatkan, salah satunya dengan menggunakan penargetan aktif, yang biasanya melibatkan molekul kecil yang dapat berikatan dengan reseptor yang spesifik. Pada penelitian ini, dipelajari potensial dari N-hydroxysuccinimide (NHS) folat untuk dapat diguanakan sebagai agen penarget dari GAPDH (glyceraldehyde-3-phospate dehydrogenase) yang merupakan salah satu reseptor pada permukaan sel, yang juga merupakan salah satu faktor virulensi yang umum ditemui pada bakteri pathogen, salah satunya adalah *Mycobacterium tuberculosis*. Riset ini dilakukan dengan menggunakan metode komputasi, yang terdiri dari metode penambatan molekul disertai dengan simulasi dinamika molekuler. Berdasarkan evaluasi hasil simulasi penambatan molekul dan dinamika molekuler, NHS-folate diprediksi memiliki potensi besar untuk dapat dijadikan senyawa penarget aktif terhadap reseptor GAPDH yang terdapat pada *Mycobacterium tuberculosis*.

Kata Kunci: Obat antituberkular, Penambatan molekul, Simulasi dinamika molekular, Glyceraldehyde-3-phospate dehydrogenase, N-hydroxysuccinimide folat

1. Introduction

Tuberculosis, commonly known as TB, is an infectious disease caused by a bacterium from the *Mycobacterium* genus called *Mycobacterium tuberculosis*. It mainly exists as pulmonary TB that affects the lungs but can also spread to and affect other organs, such as bones, joints, and nervous systems.[1] TB has shown remarkable survivability due to its unique characteristics, which can change its viability from active to dormant and vice versa. It also has a mycolic acid cell wall that could inhibit the penetration of antibiotics into its nucleus.[2] Due to these characteristics, TB continues to coexist with humans despite many medications and TB eradication strategies that have been implemented, bringing significant healthcare problems and economic woes, especially in developing countries. One of the major factors that leads to the exacerbation of the TB cases in these countries is due to the low access to anti-TB drugs.[3,4]

In most circumstances, TB can be treated with first-line anti-TB drugs, such as isoniazid, pyrazinamide, ethambutol, and rifampicin, with a minimum total treatment duration of 6 months.[5] Despite their effectiveness as anti-TB drugs, resistance is still a major problem in TB treatment that is mainly due to non-adherence of patients. TB cases can be considered drug-resistant if they are resistant to either rifampicin or isoniazid.[1,5] If these cases happen, patients are required to take second-line or third-line anti-TB medications which are usually more expensive and have more severe side effects.[6]

Thanks to the advancements of technology, there are ways to improve the therapeutic outcome of anti-TB medications, by localising and increasing the drug concentration on the target sites and concurrently decreasing the drug uptake on the nontarget sites. As a result, the side effects and the required dose can be reduced while the drug's efficacy is increased.[7,8] There are two types of targeted drug delivery: active and passive drug targeting. Passive targeting usually involves nanoparticles that exploit the microenvironment of the target, such as if the cells or target have a leaky environment. On the other hand, active targeting usually utilises a small molecule or ligand that is chosen to bind to specific receptors that expressed or overexpressed by a specific cell.[9]

To date, there are limited reports on the targeting of M.TB cells. The majority of the studies on the targeted drug delivery towards TB are focused on the passive drug targeting using nanoparticles. However, a work by Malhotra et al. showed that a surface receptor called GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is discovered on the surface of M.TB cells.[10] GAPDH is a common virulence factor found in pathogenic bacteria that was reported to interact with and internalise lactoferrin and transferrin to increase the iron intake into M.TB cells are essential to its survival.[11] GAPDH has also been reported to promote immune cell migration, tissue invasion, and cell adhesion by sequestering and capturing plasminogens. A report by Noh and coworkers also reported that a folic acid derivative is predicted to be a potential small molecule that can be a targeting agent against GAPDH.[12] However, other studies have not yet reported the dynamic interaction between folic acid derivatives and GAPDH. This research uses the molecular dynamics simulation to discover the potential interaction dynamics between GAPDH and N-hydroxysuccinimide (NHS) folate, a folic acid derivative.

2. Materials and Methods

We used Autodock Vina 1.2.3 to predict the optimal conformation of NHS-folate after docking with the GAPDH receptor.[13,14] We treated the receptor as a rigid body and allowed the ligand structure to rotate freely. The grid box used in the docking process is set on the dimension of 60 × 60 × 60 units along the x, y, and z axes and centred on the predicted active site of the GAPDH receptor. The active site prediction was carried out using DeepSite server.[15] We executed the docking processes in 90 search runs, resulting in 90 binding poses.

GAPDH macromolecule was obtained from the Protein Data Bank (PDB ID: 6IEP). All water molecules and unrelated ions were removed using BIOVIA Discovery Studio 2024. Hydrogen atoms were added, and the structure of the macromolecule was corrected using the Protein Repair and Analysis server to fix any missing atoms in all amino acid residues.[16] Subsequently, Kollman charges were added using Autodock Tools (ADT) 1.5.7. We then saved the structure in PDBQT format.

The ligand was constructed from scratch. After the structure was obtained, energy minimisation was carried out using Avogadro software.[17] The structure was then prepared in ADT by adding Gasteiger charges and specifying torsional centres needed in the docking processes. The structure was also saved in PDBQT format.

Following the whole docking processes, the structure with the lowest binding energy was then chosen to be run using MD simulation. The entire preparation and MD simulation were carried out using GROMACS 2022.04.[18] The required simulations were performed using CHARMM and CGenFF potentials, which govern all molecular behaviour in the protein-ligand complex.[19]

All short-range nonbonded interactions were cut off at 1.2 nm and long-range electrostatics were calculated using the Particle Mesh Ewald (PME) method. Before the MD simulation, energy minimisation was carried out using the steepest descent algorithm until the maximum force of the system was below 1000 kJ/mol/nm. The system was also treated with restrained NVT and NPT equilibration with 100 ps timeframe for each ensemble. We employed C-rescale pressure coupling (set to 1 bar) and V-rescale temperature coupling (set to 300K) in the simulation. The production step of the simulations was carried out in a 200 ns timeframe. After the simulation, an MM-PBSA analysis was also conducted using GMX_MMPBSA software, in which all PBC conditions within the GROMACS trajectory file were removed before any calculations were made.[20,21] The components of van der Waals energy (ΔVDW), electrostatics (Δelectrostatics) and Poisson-Boltzmann energy (ΔPB) were used in the calculation.

3. Results

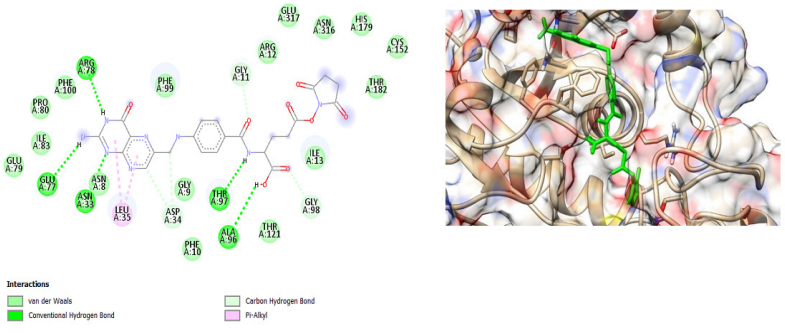
Because the cocrystal of the protein has no ligand, the active site was predicted using the DeepSite server. To ensure strong interaction between the receptor and the ligand, the ligand was docked into the active site using the centre point coordinate of -14.583 × 23.843 × -15.356 along the x, y, and z axes. The docking processes resulted in the best binding affinity of 9.0 kcal/mol. It was evident that NHS-folate interacted with the residues of Asn-33, Glu-77, Arg-78, Ala-96, and Thr-97 through hydrogen bonding, Leu-35 through pi-alkyl interaction, and Asn-8, Gly-9, Phe-10, Gly-11, Arg-12, Ile-13, Asp-34, Glu-79, Pro-80, Ile-83, Gly-98, Phe-99, Phe-100, Thr-121, Cys-152, Thr-182, Asn-316, and Glu-317 through van der Waals interaction, with one unfavourable donor-donor interaction. These interactions are represented in Figure 1A, while the interaction between NHS-folate and GAPDH is also described in Figure 1B.

After the molecular docking process, molecular dynamics simulation was conducted to study the interaction between GAPDH receptor and NHS-folate ligand in a 200 ns timeframe. After simulation, the simulation results can be analysed, which consists of the RMSF of the residues in the receptor during simulation (Figure 2A), the RMSD of the ligand during simulation (Figure 2B), and the receptor's gyration radius during simulation (Figure 2C). It can be concluded that the RMSD value during run is relatively high, with the peak value of 2.66 nm and the average value during run is 1.84 nm. From the simulation result, the radius of gyration can also be extracted, represented in Figure 2C. Throughout the simulation, the radius of gyration reached a maximum value of 2.19 nm, with an average of 2.09 nm.

The interaction stability over time can also be observed in the data of the predicted interaction energy. From the simulation results, the electrostatic and van der Waals interaction energy data can be extracted and the results are represented in Figure 3. We also conducted an MM-PBSA calculation to calculate the change in end-state energies in the simulation. Because there were two different complex states, two different calculations were performed. The calculations were performed within the two phases, consisting of the calculation before and after the deviation of the position of NHS-folate, described in Table 1.

4. Discussion

Based on the docking results, the binding affinity value was 9.0 kcal/mol. Although the number of hydrogen bonds was smaller compared to the study conducted using the same ligand conducted by Noh and coworkers, which resulted in the binding affinity value of 9.22 kcal/mol.[12] The lack of hydrogen interaction may be compensated with more van der Waals interaction, contributing to the interaction between NHS-folate and the GAPDH receptor. The binding position of the NHS-folate ligand is represented in Figure 2.



a b

Figure 1. (a) Graphical representation of interactions between GAPDH receptor and NHS-Folate in docking, and (b) 3D representation of the interaction between NHS-Folate and GAPDH during docking Simulation. The NHS-Folate ligand was indicated in green.

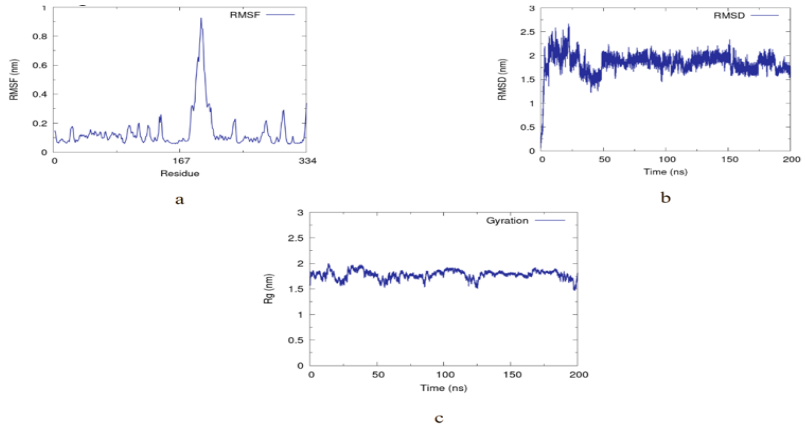


Figure 2. Representation of the (a) RMSF, (b) RMSD of the NHS-folate, and (c) radius of gyration during simulation.

Based on the MD simulation results, the value of RMSD in the MD simulation was relatively high, which was caused by the change of shape of the binding pocket of the GAPDH receptor, where some residues are moving and deviating from their initial position. The movement can be explained further based on the RMSF value, where some residues deviate from their original position.

In the MD simulation, after the 50 ns timeframe, the ligand repositioned itself into a more favourable position during simulation. After finding a more favourable position, the ligand stayed in its position without further movement. The radius of gyration, which represents the compactness of the receptor's structure and is consistently high during simulation, also explains the deviation of the protein structure from its original state. The radius of gyration during simulation can reach the highest value of 2.19 nm with the average value of 2.09.

These results suggest that the deviation of some residues during simulation also affected the interaction between the GAPDH receptor and NHS-folate. However, the overall interactions remain intact. In the simulation, the NHS-folate ligand still interacted with the receptor, although not in its original position. However, after a 50 ns timeframe, the ligand repositioned itself into a more favourable position during simulation. After finding a more favourable position, the ligand stayed without further movement. This can be explained further in the interaction energy between NHS-folate and GAPDH receptor, which is relatively stable after the initial 50 ns.

Based on the MM-PBSA calculation results, we also concluded that in the MD simulation, the interaction became stronger after the deviation of the position of the NHS-folate. Based on the total end-state free energy, the interaction of the complex is -16.54 kcal/mol before the deviation and -24.35 kcal/mol after the deviation. The hydrophobic interactions were also stronger after the deviation (-31.2 kcal/mol) than before (-16.39 kcal/mol). However, the electrostatic interactions were higher in total before the deviation (-39.73 kcal/mol) than after (31.63 kcal/mol). From these results, it could also be inferred that the main driving force of the interactions in the complex was the electrostatic interactions, and the hydrophobic interactions became the main driving force after the deviation of the position of NHS-folate.

However, with all precautions taken, the deviation of NHS-folate initial structure before MD simulation may result from the initial position of NHS-folate before MD

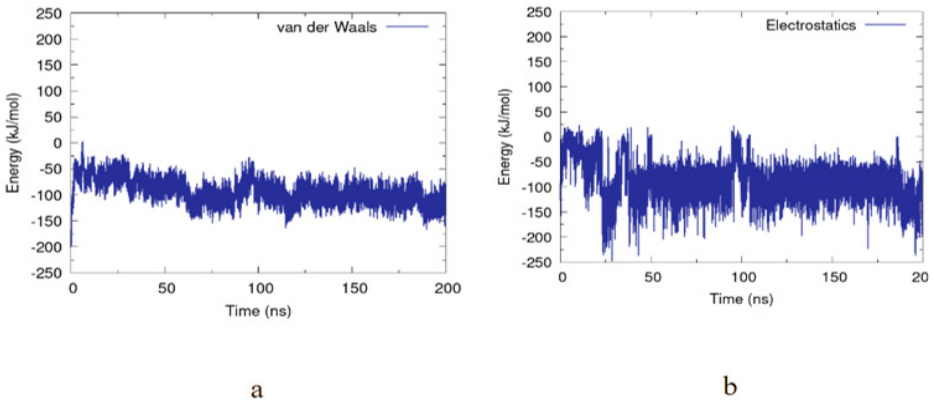


Figure 3. Representation of electrostatics and van der Waals interaction during simulation consists of (a) van der Waals Interaction and (b) electrostatics interaction.

Table 1. Calculation Before and After the Deviation of the Position of NHS-Folate

Energies (kcal/mol)	Before deviation	After deviation
ΔVDW	-16.69	-31.2
ΔElectrostatics	-39.73	-31.63
ΔPB	42.73	32,34
Total	-16.54	-24.35

simulations despite good binding affinity results in the docking processes. Lack of native ligand in the initial structure made it difficult to pinpoint the best spot in docking, despite already having an ample search space applied during the docking processes.

In addition, further study is necessary to understand how the NHS-folate binds to and releases from a carrier molecule, such as a nanocarrier, as well as to explore the potential of NHS-folate to be used as an active targeting agent against TB in a more realistic environment, such as with the presence of physiological pH and temperature, either using MD simulation or lab experimentation. Future studies also need to provide any comparison to other potential ligands or native ligands to provide a more robust understanding of the applications of NHS-folate to target GAPDH of M.TB.

5. Conclusion

We conducted the molecular docking studies to investigate the interaction between the GAPDH receptor and NHS-folate. From 90 search runs that we performed, we obtained a complex with the optimum binding affinity value of 9,0 kcal/mol. At the same time, we ran the molecular dynamics simulation to look into how the GAPDH receptor and NHS-folate interact with each other. Based on the MD simulation results which consist of the RMSD of the ligand, the RMSF of the receptor, the radius of gyration of the receptor, and the interaction energy between the GAPDH receptor and NHS-folate, it can be predicted that NHS-folate can be a candidate for the targeting agents against TB using the mechanism of targeting of GAPDH. Based on the results of MM-PBSA analysis, we also conclude that NHS-folate interacts strongly with GAPDH receptor and is predicted to be an active targeting agent against the GAPDH of M.TB.

Conflict of Interest

We declare no conflict of interest within this work.

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