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Tetracyclines as Bioactive SARS-CoV-2 Main Protease Inhibitors: Insights into their Mechanism of Action using Free Energy Calculations and Molecular Dynamics Simulation

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ABSTRACT

The coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains an extant threat against public health on a global scale. The SARS-CoV-2 main protease (M^{pro}) was identified as a key enzyme for processing the viral polyproteins to produce functional non-structural proteins. Thus, inhibiting M^{pro} activity could stop the spread of infection. In the following study, we investigated the M^{pro} inhibitory potential of clinically approved Tetracyclines, using free energy calculations and a molecular dynamics approach. In this study, 13 approved Tetracyclines were docked against the main protease of SARS CoV-2. Among the studied Tetracyclines, Lymecycline appear as potential inhibitor of this protease. When docked against M^{pro} crystal structure 6lu7, this compound revealed a minimum binding free energy of -8.87 kcal/mol with 168 binding modes detected in the binding substrate pocket. Further, molecular dynamics simulation and binding free energy calculations were performed to evaluate the dynamic behavior and stability of protein-ligand complex. The results obtained herein highlight the enhanced stability and good binding affinity of Lymecycline towards the target protein.

Keywords: Tetracyclines, SARS-CoV-2, Free energy, Molecular Dynamics Simulations.

1. Introduction

Coronavirus disease (COVID-19) has become an important public emergency across the globe since December 2019. The development of potent anti-SARS-COV-2 agents is still of high necessity for COVID-19 disease treatment. The SARS-CoV-2 main protease (M^{pro}) was identified as a key enzyme for processing the viral polyproteins to produce functional non-structural proteins. Thus, inhibiting M^{pro} activity could stop the spread of infection [1]. Repurposing strategies of known small molecules seems to be a very efficient way in order to develop potent drugs to combat Coronavirus. Tetracyclines are broad-spectrum antibiotics relatively safe, synthesized by modifying natural tetracycline to form several new compounds. Besides their antibacterial function, Tetracyclines have a number of non-antibiotic proprieties such as antiviral activity, which has been demonstrated both clinically and experimentally [2]. In the following study, we investigated the M^{pro} inhibitory potential of clinically approved Tetracyclines, using free energy calculations and a molecular dynamics approach.

2. Experimental

In this study, a set of 13 approved Tetracyclines, was docked against the main protease of SARS CoV-2 (6LU7) using the SwissDock [3] under the accurate mode with no flexibility of the side chain of any amino acid of the target protein. SwissDock generates all possible binding modes for each ligand and the most favorable binding modes at a given pocket were clustered. All ligand clusters were saved in an output file called "prediction file". The prediction file provided; Cluster rank, Element, Fulfitness and estimated binding free energy (ΔG). A cluster corresponds to a predicted binding pocket on the target protein and the cluster rank represents the different conformations. After docking Chimera, Pymol and Biovia Drug Discovery Studio softwares were used to visualize the receptor ligand interactions for the lowest energy model of the clusters obtained from the previous step. To assess the stability and dynamics of the

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interactions between the best interacting drug and the target protease, a molecular dynamics simulation was performed (120ns). The estimation of the binding free energy of the best M^{pro} inhibitor was carried out using PMEMD cuda module implemented in AMBER 18 with explicit water model under periodic boundary conditions [4]. The system was solvated using the TIP3P water model in a periodic box, with a minimum spacing of 10 Å from the solute/protein atoms. After that neutralization of the system with counter ions (Na^+) replacing solvent molecules at the position of electrostatic favorable potential. To remove bad contacts and possible steric clashes Ligand-protein complex was subjected to energy minimization.

3. Results and Discussion

Among the studied Tetracyclines, Lymecycline appear as potential inhibitor of this protease. When docked against M^{pro} crystal structure, this compound revealed a minimum binding free energy of -8.87 kcal/mol with 168 binding modes detected in the binding substrate pocket. A significant number of non-covalent interactions such as hydrogen bonding and electrostatic interactions were detected in M^{pro}-Lymecycline complex. Further, molecular dynamics simulation and binding free energy calculations were performed to evaluate the dynamic behavior and stability of Lymecycline-M^{pro} complex. The MD trajectories were examined based on various parameters including Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (Rg) and binding free energy calculations. RMSD monitors the deviations in displacement experienced by the backbone atoms of proteins with respect to the initial conformations towards the final position. The RMSD during the whole run was <4 Å which is in acceptable range. The RMSF is an essential parameter to determine the protein's flexibility. The average RMSF values were 1.46 ± 0.93 Å indicating lower structural mobility. The Rg was calculated to determine whether the tested drugs maintained the folding of the system. As evident, the Lymecycline complex attained more compacted form as the MD simulation progressed, indicating a well converged system. Further, free energy calculations envisage ligand binding affinities by considering several atomic level interactions that could be responsible for the affinity of ligand toward targeted protein. The binding of Lymecycline to the target protein M^{pro} was found to be 22.19 ± 5.23 kcal/mol and is favored mostly by intermolecular electrostatic interactions, which is evident from the maximum number of hydrogen bond with various M^{pro} active site residues. The results obtained herein highlight the enhanced stability and good binding affinity of Lymecycline towards the target protease M^{pro}.

4. Conclusion

In silico drug repositioning results could be of interest to assist the design of a treatment for SARS-CoV-2 infection. This study showed Lymecycline as a potential approved drug to act as an inhibitor for the M^{pro} of SARS-CoV-2. This approved drug might be used either as COVID-19 therapeutic or as starting point to develop novel anti-SARS-CoV-2 drugs. Starting from approved drugs for which numerous information is available such as pharmacokinetics, toxicity and adverse drug reactions in human or animal models, constitute an interesting approach to combat COVID-19.

References

[1]: Li X., Song Y., Structure and function of SARS-CoV and SARS-CoV-2 main proteases and their inhibition: A comprehensive review. European Journal of Medicinal Chemistry, 260 (2023), 115772.

[2]: Mosquera-Sulbaran J. A., Hernández-Fonseca H., Tetracycline and viruses: a possible treatment for COVID-19?. Archives of virology, 166 (2021), 1-7.

[3]: Grosdidier A., Zoete V., Michielin O., SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic acids research, 39(2011), W270-W277.

[4]: Case D. A., Cheatham III T. E., Darden T., Gohlke H., Luo R., Merz Jr, K. M., Onufriev A., Simmerling C., Wang B., & Woods R. J., The Amber biomolecular simulation programs. Journal of computational chemistry. 26(2005), 1668-1688.

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