

Research article

***In silico* molecular docking studies of drugs Donepezil and Galantamine towards SARS-CoV-2 main protease (M^{Pro})**Sumitha A.¹, Parthiban Brindha Devi², Vidhya Subramanian³, B. Geetha⁴, G. Vaishnavi⁵¹Department of Pharmacology, ³Department of Pathology, ACS Medical College and Hospital, Chennai, 600077, Tamil Nadu, India²Vels Institute of Science, Technology and Advanced Studies, Chennai, 600117, Tamil Nadu, India⁴Faculty of Pharmacy, ⁵Faculty of Physiotherapy, Dr. M.G.R. Educational and Research Institute, Velappanchavadi, Chennai, 600077, Tamil Nadu, India

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ABSTRACT

Introduction and Aim: COVID-19 (Corona viral disease) has caused morbidity and mortality across the globe. In spite of some repurposed drugs and vaccines, researchers are in search of effective treatment against SARS-CoV-2 infection. Donepezil and Galantamine are acetylcholinesterase inhibitor drugs with pharmacological benefit in Alzheimer's therapy. An effort has been made in this study for *in silico* evaluation of drugs Donepezil and Galantamine for its antiviral activity against Coronavirus.

Materials and Methods: Molecular docking studies of ligands Donepezil and Galantamine were executed by using corona viral Protease (M^{Pro}) protein as a target. Docking study was performed using Autodock 4.2.6 software. The activity of ligands depends on hydrogen bond interactions with active sites of protein of M^{Pro} protein and docking score.

Results: Docking studies reveal that the compound Donepezil is well interacted with nonpolar amino acids. The Galantamine molecule has good affinity towards the protein and reveals the docking score of -7.3 kcal/mol. The tight binding of ligands Donepezil and Galantamine to the protein of M^{Pro} will stop further replication and transcription of viral proteins.

Conclusion: Ligands Donepezil and Galantamine are well engaged with the active site of the main protease (M^{Pro}) of SARS-CoV-2. These compounds can help in novel drug identification towards Covid-19.

Keywords: Molecular docking; M^{Pro}; Covid-19; Donepezil; Galantamine.

INTRODUCTION

Corona viral disease (COVID-19) is an infectious disease caused by SARS-CoV-2 virus. India's economic and medical infrastructure has been challenged by corona viral outbreak (1-3). There were more than 100 million subjects globally with confirmed Covid-19 infection based on the molecular assay (4). Symptoms of corona viral disease vary from mild illness in 80% patients to severe illness in 14% of patients. People with hypertension, diabetes, heart disease and elderly people have more risk of severe illness (5,6). Chest radiograph of patients are characterized by peripheral and multifocal airspace opacities (7). FDA approved an antiviral drug remdesivir, molnupiravir and emergency use authorization of monoclonal antibodies like bamlanivimab against COVID-19 infection.

Despite available medications and vaccines, researchers are in search of a viable cure for SARS-CoV-2 infection. In Corona viral replication, nonstructural proteins like main protease (M^{Pro}), Papain like protease (PL^{Pro}), RNA-dependent RNA polymerase (RdRp) play a significant role. M^{Pro} catalyzes nonstructural protein formation which is necessary for the corona viral transcription process (8). Computational methods of drug designing and

drug repurposing together make the drug discovery process more effective. Molecular docking method predicts conformation of molecules into binding site of target, which is also analyzed using docking scores (9). Drugs like Doxycycline, Quinine which are used for treatment of malarial infections have shown good binding affinity against nonstructural proteins of coronavirus in docking studies (10). Similarly, Donepezil is a reversible acetylcholinesterase inhibitor used as a drug therapy for Alzheimer's disease.

Donepezil is used off label to reduce behavioral symptoms in people with thought problems following a stroke, vascular dementia (11). Vesicular stomatitis virus differentiation in leukocytes of healthy blood donors is inhibited by Donepezil. TNF- α and Interferon production is reduced in a dose dependent manner in human leukocytes by this drug. Donepezil also reduces NF- κ B activation (12). So in this study, an attempt has been made for molecular docking study with drug Donepezil against M^{Pro} (main protease) enzyme.

Galantamine, alkaloid from Amaryllidaceae plant family is a reversible inhibitor of acetylcholinesterase. Galantamine possesses pharmacological activities like antibacterial, antiviral, and anti-inflammatory effects (13,14). So, in this study an effort has been made for

in silico study with drugs Donepezil and Galantamine against active site of Mpro enzyme of COVID-19.

MATERIALS AND METHODS

Computer information for docking analysis

The computer configuration used for the software was processing with Intel® i5 processor, 1.60 GHz 2.11 GHz processor with system memory of 8 GB RAM and 64-bit window 10 operating system.

Preparation of ligands

The ligands Donepezil and Galantamine were selected for our study. The compounds were retrieved from the PubChem database and the structure was drawn using Chemdraw and the canonical smiles were generated. The canonical smiles were converted to protein data bank form for the docking analysis throughout the study.

Preparation of protein

The 3-Dimensional X-Ray crystallographic structure of SARS-CoV-2 M^{pro} protein with PDB ID 7RM2 was retrieved from the Protein Data bank. The protein M^{pro} was in complex with the molecule CSR-494190-S1. The structure of protein with co-crystallized ligand was depicted in Fig. 1.

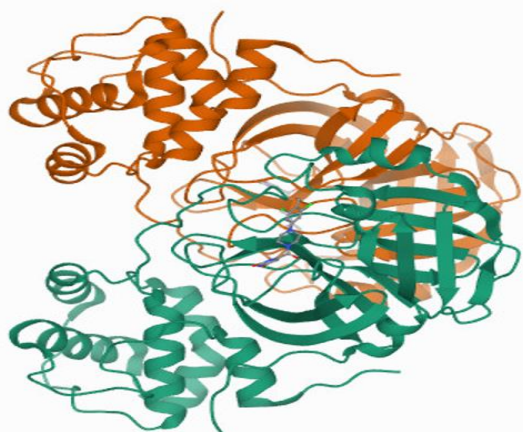


Fig. 1: The retrieved co-crystallized main protease with Ligand (PDB Id: 7RM2)

The active site determination for docking analysis

The active site of the co-crystallized M^{pro} protein was predicted using the Online Server Metapocket 2.0 (15,16). The active site of the protein plays a vital role in binding of ligands with the protein. Active site contains the important amino acid residues which are needed for effective binding of the ligand to the protein. The prepared co-crystallized protein was uploaded in the online server and based on the Z-score ligand binding sites were generated for the ideal docking analysis. The predicted active site of the M^{pro} protein was tabulated in Table 1.

Table 1: The predicted active site of M^{pro} protein with its residue number

S. No	Amino acid	Residue number
1	Alanine	17
2	Arginine	11
3	Asparagine	21
4	Aspartic acid	17
5	Cysteine	12
6	Glutamine	14
7	Glutamic acid	09
8	Glycine	26
9	Histidine	07
10	Isoleucine	11
11	Leucine	29
12	Lysine	11
13	Methionine	10
14	Phenylalanine	17
15	Proline	13
16	Serine	16
17	Threonine	24
18	Tryptophan	03
19	Tyrosine	11
20	Valine	27

The docking analysis for the co-crystallized ligand using AutoDock software

To perform docking studies for the co-crystallized protein with the selected ligand, the AutoDock 4.2.6 software was used. The software along with the MGL Tools were downloaded from their official web page “The Scripps Research Institute”. The docking study for the co-crystallized protein with the predicted active site residues were selected for the compounds Donepezil and Galantamine.

Grid generation for docking analysis

The generation of Grid plays a major role in docking analysis. The grid is a rectangular box which determines the active site residues of the co-crystallized protein. The default parameters have been chosen to design the grid with 0.375 Å⁰, Middle grid box values were set to x= -13.677, y = 12.737 and z= 70.782 with dimensions as 70×76×78.

Discovery Studio Visualizer 3.5

Discover Studio Visualizer 3.5 and PyMOL 2.3 were used to visualize the interactions of protein with the ligands. Both 2-D and 3-D interactions were visualized.

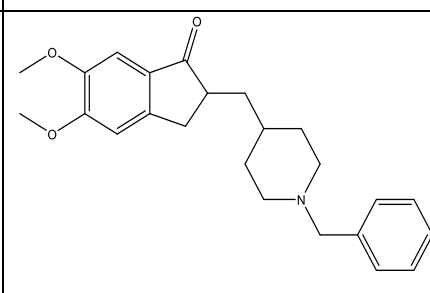
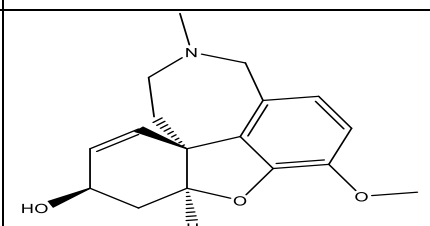
RESULTS

Molecular docking studies of ligands Donepezil and Galantamine were executed with the co-crystallized protein Main Protease. The Autodock 4.2.6 software was used for the entire Molecular docking studies. The Docking score of the compounds mainly depends upon the interaction of the ligand with the protein molecule. The structure of Donepezil and Galantamine are listed in Table 2.

The structure activity of the compound Donepezil reveals that it is well interacted with non-polar amino acids Thr25, Thr24, Ser46 and Thr45. The Binding

interaction and ligand interaction of compound Donepezil with the active site of protein of Mpro enzyme is depicted in Fig. 2.

Table 2: Details of structure of ligands donepezil and galantamine

S. No	Compound	Chemical formula	Structure
1	Donepezil	C ₂₄ H ₂₉ NO ₃	
2	Galantamine	C ₁₇ H ₂₁ NO ₃	

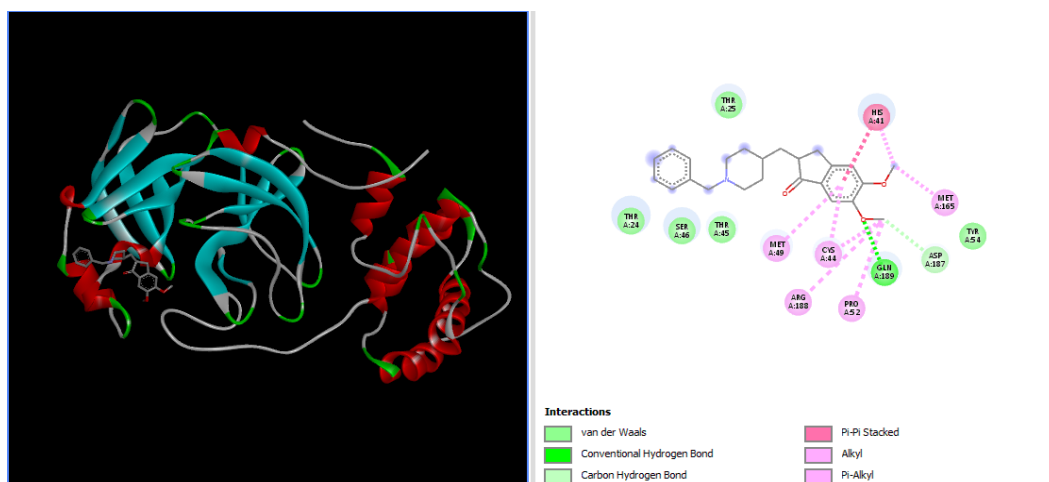


Fig. 2: Binding Interaction and ligand interaction of compound Donepezil with active site of M^{PRO}

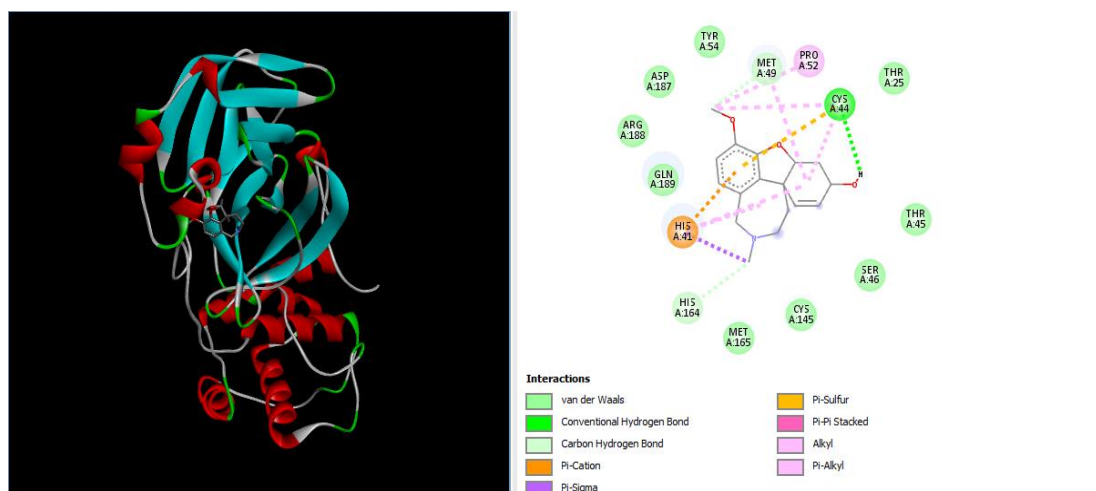


Fig. 3: Binding Interaction and ligand interaction of compound Galantamine with active site of M^{PRO} enzyme

Table 3: Interactions and binding scores of ligands donepezil and galantamine with active site of SARS M^{PRO}

S. No	Compound	Binding Score (kcal/mol)	Hydrogen bond interactions	No. of hydrogen bonds
1	Donepezil	-7.78	Asp 187, Gln 189	2
2	Galantamine	-7.3	Cys 44, His 164, Met 49	3

The galantamine is wholly surrounded with nonpolar amino acids like tyrosine, arginine, and serine. The compound is well associated with hydrogen bond interaction with amino acids Met49, Pro52 and His164 as depicted in Fig.3. The compounds were docked into the co-crystallized protein and the docked score, and interaction of the ligands are tabulated in Table 3.

DISCUSSION

SARS-CoV infection due to their rapid transmission and high mortality rate in humans has gained worldwide attention (17). Once SARS-CoV-2 virus enter the host cells, two polyproteins pp1a and pp1ab are synthesized by translation of viral RNA. Polyproteins pp1a and pp1ab peptide bonds are cleaved by M^{pro}- main protease enzyme of COVID-19 virus. M^{pro} enzyme is responsible for cutting viral polyproteins into functional units (18). Thus, M^{pro} enzyme catalyzes nonstructural protein formation which is necessary for COVID-19 virus transcription process to synthesize new RNA. Mpro enzyme because of its involvement in the viral life cycle has become an important target for development of inhibitor compounds in both *in silico* and *in vitro* studies (19,20). Donepezil and galantamine are anticholine esterase, which are used in Alzheimer's disease. *In vitro* studies have shown antiviral effects for these drug molecules (11). So, an attempt has been made in this study to do molecular docking studies with donepezil and galantamine as ligand molecules against M^{pro} enzyme of SARS-Cov 2 virus.

Molecular docking studies show that compound donepezil is well interacted with non-polar amino acids Thr25, Thr24, Ser46 and Thr45. The benzyl group in the compound donepezil has good stacking interaction with the amino acids His41. The carbonyl moiety in the donepezil has good hydrogen bond interaction with Met49, Cys44, Arg188, Pro52 and Met165. On closer look, the compound reveals that donepezil is well occupied with the active site of the protein of M^{pro} enzyme as depicted in Fig. 2. This makes the compound stable and the docking score of the compound was found to be -7.78kcal/mol as shown in Table 3. The ligand donepezil tightly binds to the M^{pro} enzyme protein, preventing further transcription and replication of viral proteins. This helps in complete arresting of transcription and production of spike proteins.

Molecular docking study on galantamine molecules shows that the phenyl group present in the moieties of galantamine has good stacking interactions with

amino acids His41 and Cys44. The compound is well associated with hydrogen bond interaction with amino acids Met49, Pro52 and His164. The galantamine is wholly surrounded with non-polar amino acids like tyrosine, arginine and serine as depicted in Fig. 3. The galantamine molecule has good affinity towards the protein and reveals the docking score of -7.3kcal/mol as shown in Table 3. Based on the *in-silico* study, galantamine plays a vital role in interacting with M^{pro} of COVID-19. This leads to destruction of binding of enzymes responsible for viral replication. Thus, the moieties present around the ligand galantamine plays an important role in blocking the enzyme activity. This molecular docking study shows that ligands donepezil and galantamine have significant activity against main the protease enzyme of coronavirus.

CONCLUSION

At present there are repurposed drugs in the market to treat SARS-CoV-2 infection. Research is vigorously going on to develop lead molecules and precursors that could actively inhibit the infection with less adverse effects. Donepezil and galantamine are well engaged with the active site of the main protease of SARS CoV-2. These compounds can help in novel drug identification towards Covid-19. Still further *in vitro* studies and clinical studies in COVID-19 patients are needed to confirm its beneficial effects.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Abbas, M. S., Ahmed, A. G., Ali, S. Q., AL-Rubaii, B. A. Immunological inflammatory factors in patients diagnosed with COVID-19. *Biomedicine*. 2023 Feb 26;43(1):230-235.
2. Kumar, N., Mathew, G., Huchchannavar, R. Home-based care programme (HBCP) for patients with COVID-19: Boon or burden during pandemic management?. *Biomedicine*. 2023 May 4;43(02):649-654.
3. Jabaris, S., Sugin, L., Ananthalakshmi, V. The current situation of COVID-19 in India. *Brain Behav Immun Health*. 2021;11: 1-5.
4. Wang, C., Wang, Z., Wang, G. COVID-19 in early 2021: Current status and looking forward. *Signal Transduct Target Ther*. 2021; 6:114.
5. Jayaswal, S.K., Singh, S., Malik, P.S., Venigalla, S.K., Gupta, P., Samaga, S.N., *et al.*, Detrimental effect of diabetes and hypertension on the severity and mortality of COVID-19 infection: A multi-center case-control study

- from India. *Diabetes & Metab Syndr*. 2021; 15 (5): e102248.
6. Wang, D., Cai, J., Shi, T., Xiao, Y., Feng, X., Yang, M., *et al.*, Epidemiological characteristics and the entire evolution of coronavirus disease 2019 in Wuhan, China. *Respir Res*. 2020; 21: 257.
 7. Rio, D., Carlos, Malani, P.N. COVID-19-New Insights on a Rapidly Changing Epidemic. *JAMA*. 2020; 323(14):1339-1340.
 8. Hosseini, M., Chen, W., Xiao, D., Wang, C. Computational molecular docking and virtual screening revealed promising SARS-CoV-2 drugs. *Precis Clin Med*. 2021; 4(1):1-16.
 9. Schrabby, F., Bagheri, M., Aryapour, H. Performing an *in-silico* repurposing of existing drugs by combining virtual screening and molecular dynamics simulation. *Methods Mol Biol*. 2019; 1903:23-43.
 10. Sumitha, A., Brindha Devi, P., Soumya, H., Dhanasekaran, R. Covid-19 *In silico* structure prediction and molecular docking studies with Doxycycline and Quinine. *Biomed Pharmacol J*. 2020;13(3):1185-1193.
 11. Seltzer, B. Donepezil: a review. *Expert Opin Drug Metab Toxicol*. 2005;1(3):527-536.
 12. Sochocka, M., Zaczynska, E., Leszek, J., Siemieniec, I. Effect of Donepezil on innate antiviral immunity of human leukocytes. *J Neurol Sci*. 2008;273(1-2):75-80.
 13. Jahn, S., Seiwert, B., Kretzing, S., Abraham, G., Regenthal, R., Karst, U. Metabolic studies of the Amaryllidaceae alkaloids galantamine and lycorine based on electrochemical simulation in addition to in vivo and in vitro models. *Anal Chim Acta*. 2012;756:60-72.
 14. Mazumder, M.K., Paul, R., Phukan, B.C., Dutta, A., Chakrabarty, J., Bhattacharya, P., *et al.*, Garcinol, an effective monoamine oxidase-B inhibitor for the treatment of Parkinson's disease. *Med Hypotheses*. 2018;117:54-58.
 15. Huang, B. Meta pocket: a meta-approach to improve protein ligand binding site prediction. *OMICS*. 2009;13(4):325-330.
 16. Zhang, Z., Li, Y., Lin, B., Schroeder, M., Huang, B. Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction. *Bioinformatics*. 2011;27(15):2083-2088.
 17. Roy, H.S., Singh, R., Ghosh, D. SARS-CoV-2 and tissue damage: current insights and biomaterial-based therapeutic strategies. *Biomater Sci*. 2021 ;9: 2804-2824.
 18. Koudelka, T., Boger, J., Henkel, A., Schonherr, R., Krantz, S., Fuchs, S., *et al.*, N-terminomics for the identification of in vitro substrates and cleavage site specificity of the SARS-CoV-2 main protease. *Proteomics*. 2021;21(2): e2000246.
 19. Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., *et al.*, Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature*, 2020; 582:289-293.
 20. Antonopoulou, I., Sapountzaki, E., Rova, U., Christakopoulos, P., Inhibition of the main protease of SARS-CoV-2 (Mpro) by repurposing/designing drug-like substances and utilizing nature's toolbox of bioactive compounds. *Comput Struct Biotechnol J*. 2022; 20:1306-1344.