# Identification of Novel Hits Against SARS-CoV-2 Main Protease through Virtual Screening of Human Metabolites

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A joint project by SGSITS Indore and IIT Indore

Presented by **Dr. Omprakash Tanwar**

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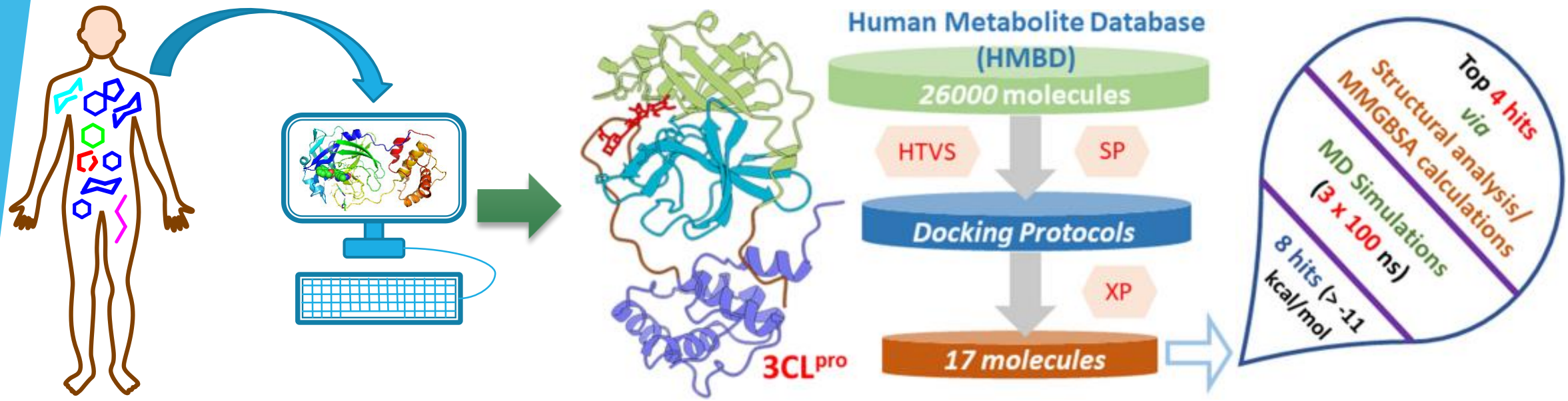
# Graphical abstract

Title: Identification of Novel Hits Against SARS-CoV-2 Main Protease through Virtual Screening of Human Metabolites




Human Metabolites → In-silico Metabolomics screening → Best Hits

# Abstract

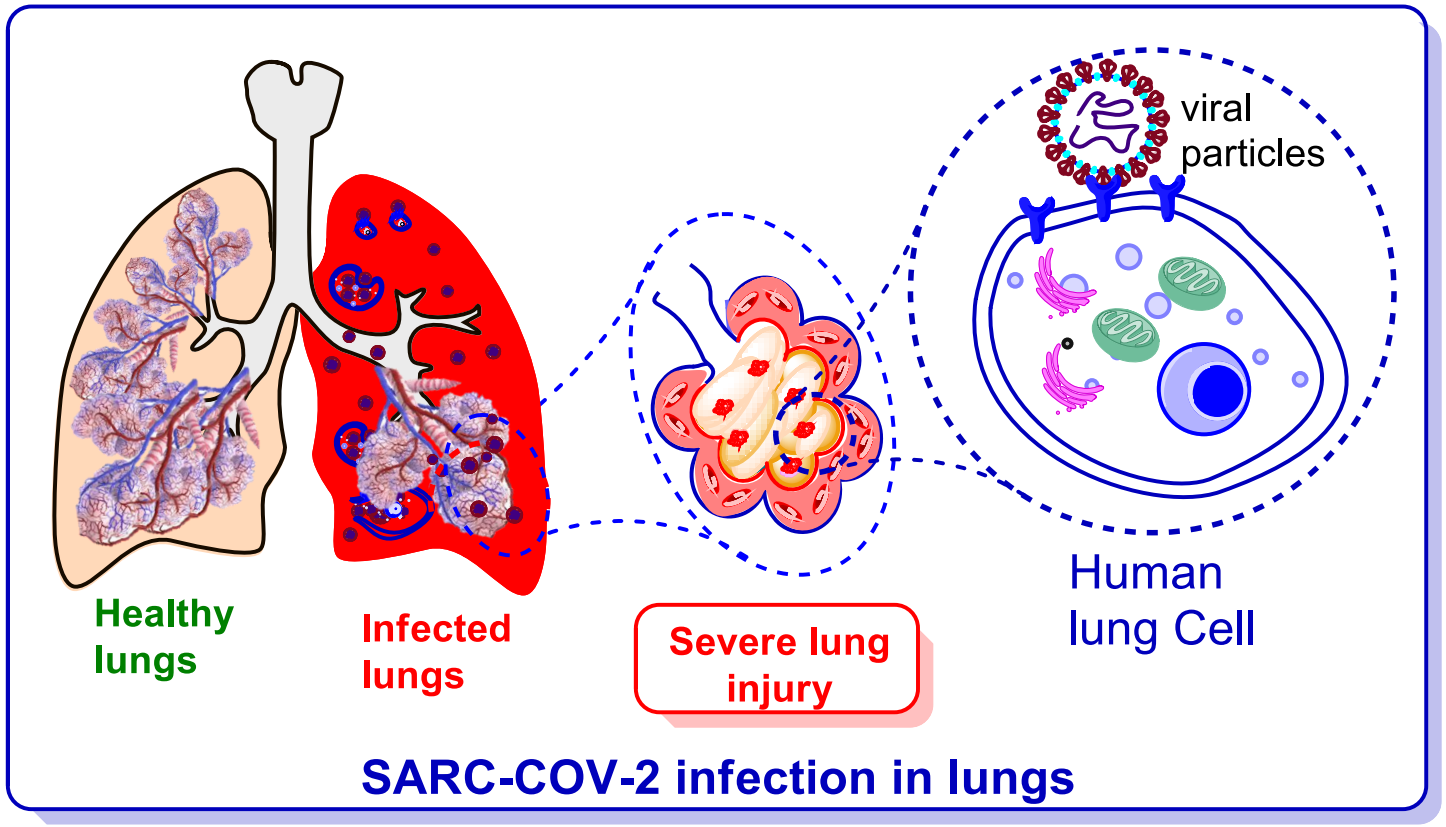

- The present research covers the identification of novel hits against the 3-chymotrypsin-like proteases (3CLpro) protein of SARS-CoV-2 using virtual screening (VS) and Molecular Dynamic (MD) simulations. After VS and MD simulations, the top 8 virtual hits displayed a docking score varying between ~ -11 to ~ -14 kcal/mol. MS simulations further confirmed their binding modes and supported the results. The best hit (HMDB0132640) possesses the docking score (glide score) of -14.06 kcal/mol, and MM-GBSA binding free energy, of -18.08 kcal/mol. The other compounds were selected and found to possess drug-like ADME properties. The other virtual hits include HMDB0127868, HMDB0134119, and HMDB0125821, and they showed favorable contributions from the intermolecular electrostatic and van der Waals interactions. To the best of our knowledge, we are the first to identify novel compounds from a pool of human metabolite databases against SARS-CoV-2 Main Protease that was unexplored before about developing novel antiviral drugs



- **Keywords**: SARS-COV-2; Main Protease; Human metabolites; Virtual Screening; Molecular Dynamics; Free Energy.

# Introduction

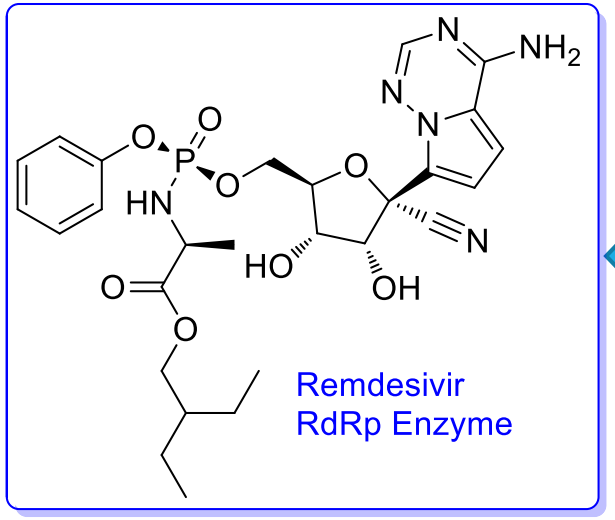
- Novel coronaviruses (SARS-CoV-2) infection leads to the development of COVID-19 which if severe, results in death due to cytokine storm in lungs and other tissues.



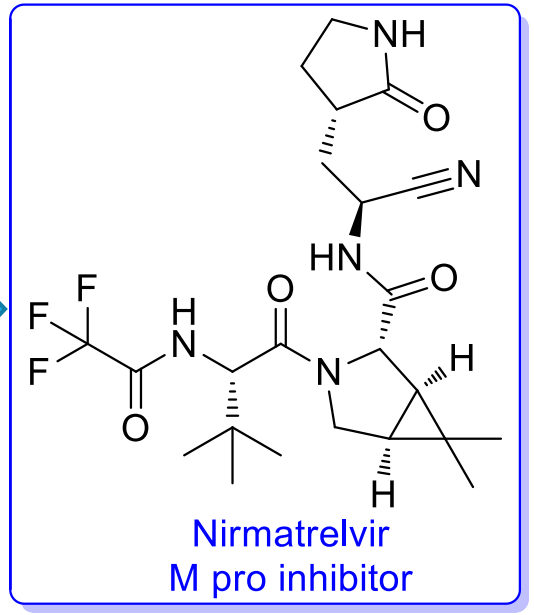

Hussman JP., Front Pharmacol. 2020 Jul 29;11:1169.

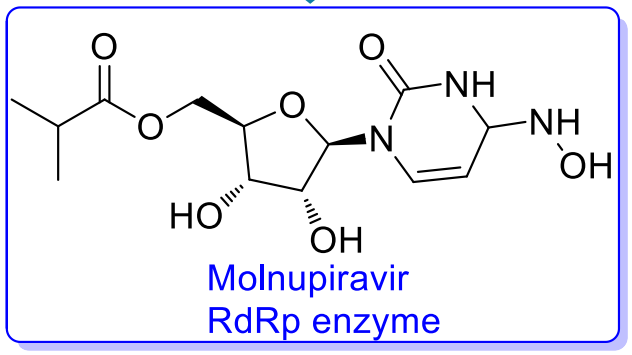
# Introduction

- According to National Health Service (NHS) UK, there are three small molecules available for the treatment of COVID-19, however they have many side effects which limits their use.




**Approved Small molecules for the treatment of COVID-19**




Ref: https://www.nhs.uk/conditions/coronavirus-covid-19/self-care-and-treatments-for-coronavirus/treatments-for-coronavirus/

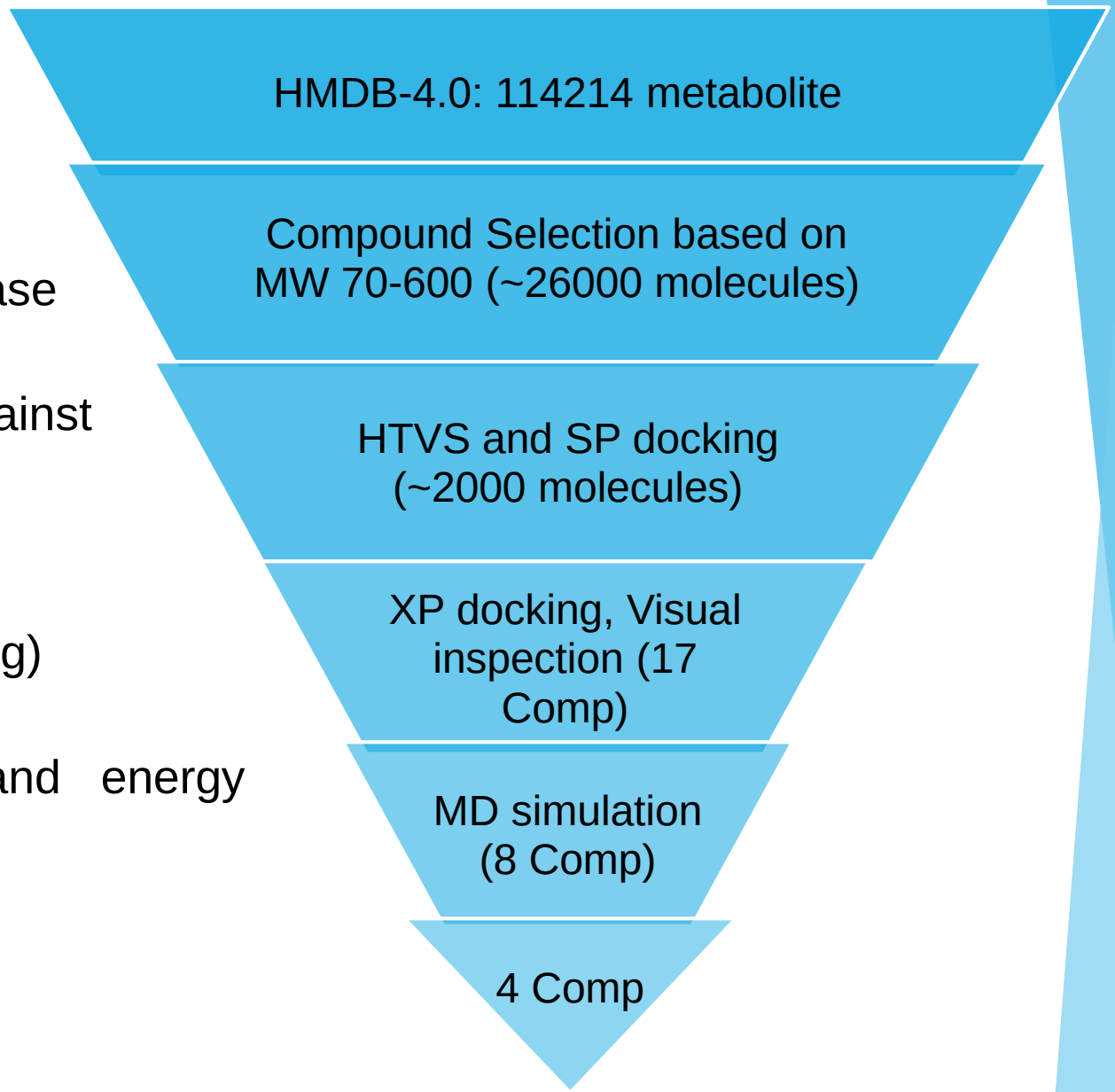
# Introduction

- Thus there is a need of effective small molecules that can reduce the viral load effectively and do less harm to the human body.
- **The metabolomics** has been shown to be one of the promising techniques in drug discovery [1].
- The metabolomics is an emerging technology, and very soon, it will be an essential part of precision medicine [2].
- The Human Metabolome Database (**HMDB**) is a freely downloadable database of indigenous metabolites, narutal compound metabolites and drug metabolites.
- Herein, we have screened the molecules available at HMDB against 3CLpro (main protease, or **Mpro**) of SARS-CoV-2 to find some potential metabolites and to develop new therapies to tackle viral infections.


1. Cuperlovic-Culf M, Culf AS., Expert Opin Drug Discov. 2016 Aug;11(8):759-70.
2. Wishart, D., Nat Rev Drug Discov 15, 473–484 (2016).

# Methodology and Work Flow

- Database collection and virtual screening setup
- Molecular Weight based filtering of database
- Coarse Screening (HTVS/SP docking) against 3CLpro of SARS-COV-2 (**PDB ID- 6LU7)**
- Extensive sampling Screening (XP docking)
- Hit validation using MD simulation and energy calculation
- Final Selection of best compounds.



# Methodology: MD simulation & Binding Energy

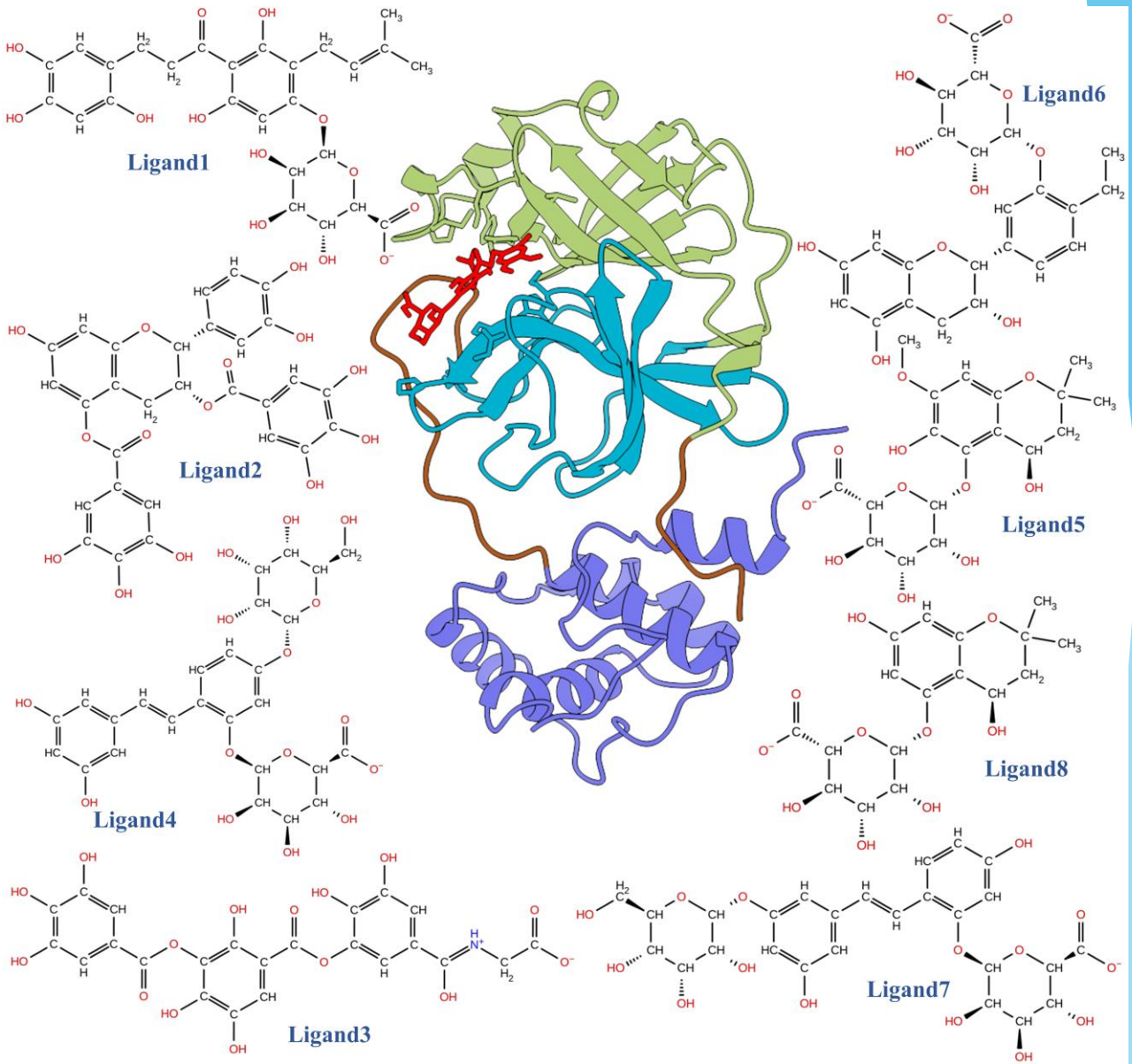
- **Molecular dynamics (MD) simulation**:
- Molecular dynamics (MD) simulation studies were performed using the LEaP module of AmberTools19 [1] with the TIP3P [2]. All complex structures were subjected to 3 x 100 ns production runs under the NPT ensemble. Trajectories were analyzed using the cpptraj module of AmberTools19, and the last 50 ns trajectories were used for the binding free energy calculation.
- To estimate the binding free energy, 2500 frames were selected uniformly from the last 50 ns trajectories, and calculation was done with the help of the MMPBSA.py script available on AmberTools19. The entropic contribution was estimated using the normal mode analysis, and the **MM-GBSA** pair-wise decomposition scheme also assessed the contribution from each amino acid

1. D. A. Case et al., “AMBER 2018,” University of California, San Francisco, 2018, Price DJ, Brooks CL 3rd, J Chem Phys. 2004 Nov 22;121(20):10096-103.

# Results and Discussion

**Figure 1: Structures of Best 8 hits**

Ribbon representation of COVID19 $3CL^{pro}$ complexed with the inhibitor, shown in ball and stick representation. The different part of $3CL^{pro}$ is shown in different color, i.e., Green: Domain I, Cyan: Domain II, Blue: Domain III, Brown: Inter-domain connecting loop, and Red: Ligand molecules. The top 8 molecules which are screened by the virtual screening workflow are shown in 2D illustration



# Results and Discussion

- Energetics: Different components of docking scores obtained from the Glide-XP docking scheme.

| Lead molecule | Molecular Weight | G-Score[a] | Glide-lipo[b] | Glide-hbond[c] | Glide-evdw[d] |
|---|---|---|---|---|---|
| Ligand1 (HMDB0132640) | 568.657 | **-14.060** | -4.160 | 0.000 | -39.543 |
| Ligand2 (HMDB0030665) | 622.706 | -12.399 | -2.859 | -0.339 | -61.108 |
| Ligand3 (HMDB0128347) | 573.59 | -12.151 | -2.359 | -0.800 | -48.830 |
| Ligand4 (HMDB0134117) | 598.64 | -11.724 | -2.228 | -0.769 | -36.536 |
| Ligand5 (HMDB0125819) | 424.444 | -11.534 | -3.702 | -0.160 | -47.989 |
| Ligand6 (HMDB0127868) | 492.564 | -11.134 | -2.904 | -0.887 | -29.007 |
| Ligand7 (HMDB0134119) | 598.64 | -11.119 | -3.060 | -0.430 | -40.603 |
| Ligand8 (HMDB0125821) | 394.418 | -11.051 | -3.369 | -0.480 | -44.484 |

[a] Glide Score (kcal/mol), [b] Lipophilic term derived from hydrophobic grid potential, [c] hydrogen bonding term in GlideScore, [d] Van der Waals energy

# Results and Discussion

- Eight best hits were selected based on their interaction pattern, Molecular Dynamic simulation stability pattern of complexes and binding energies.

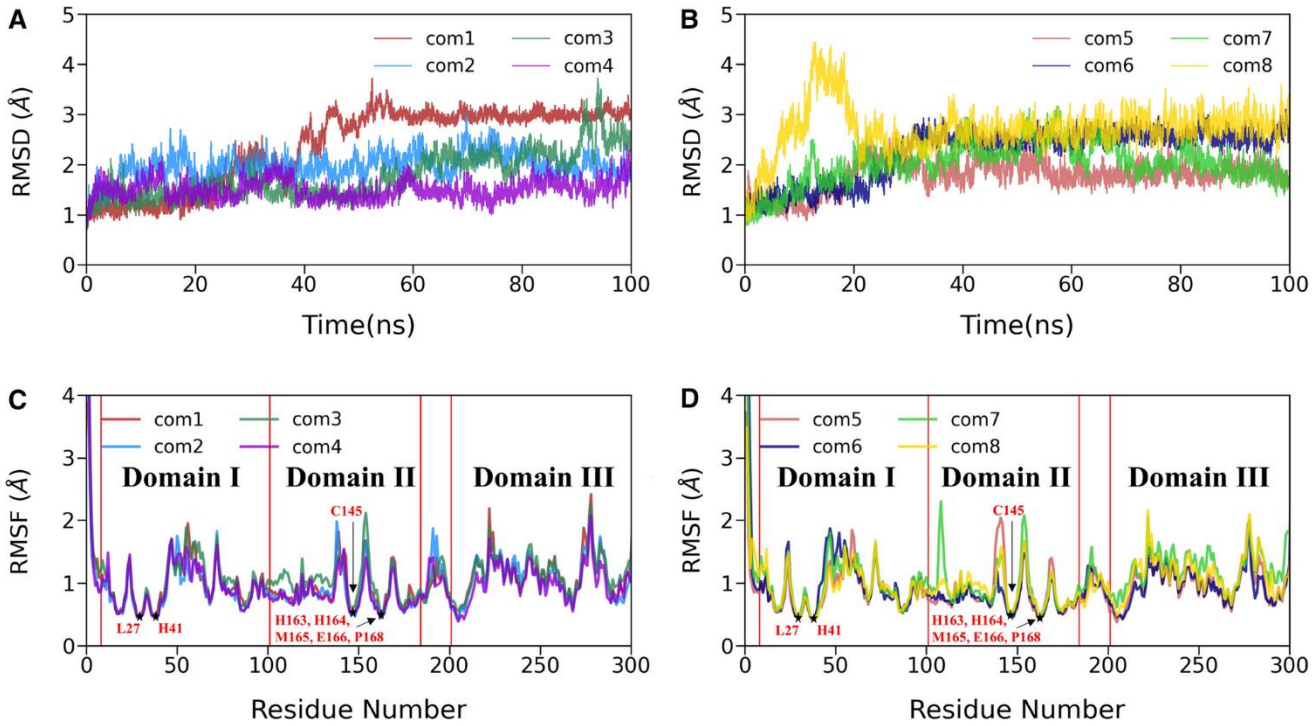



**Figure 2;** A, B The time evolution of root means square deviation (RMSD) of backbone atoms of 3CLpro-complex relative to their respective initial coordinates. **C, D** The root mean square fluctuations (RMSFs) of Cα atoms for all eight 3CLpro-ligand complexes

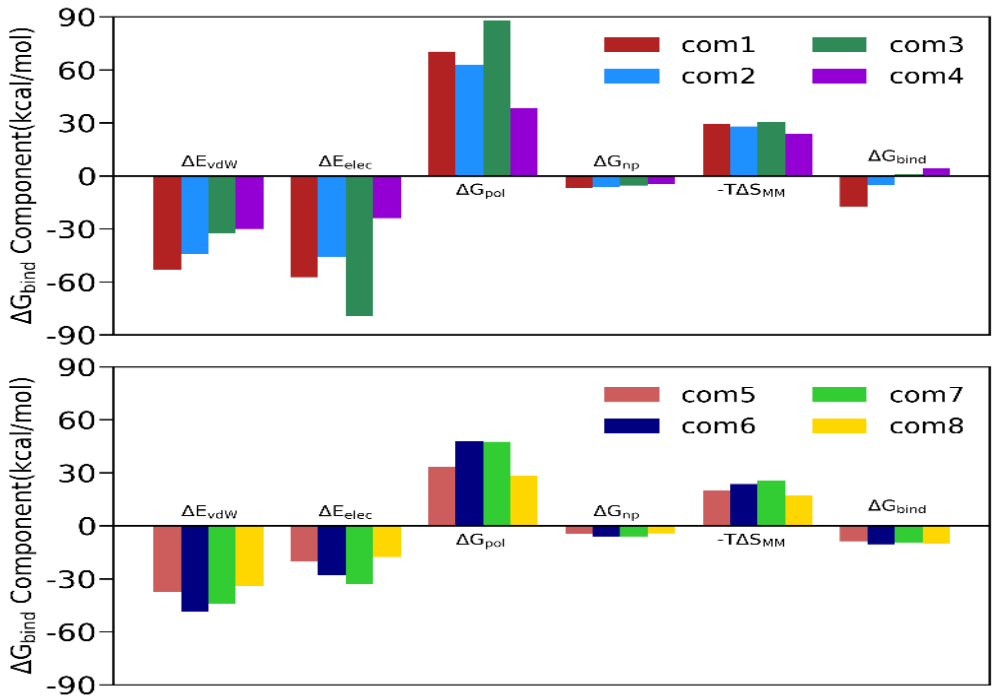
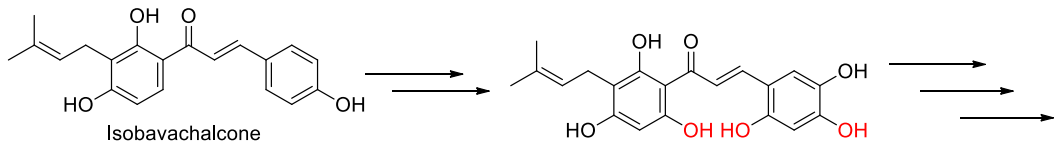
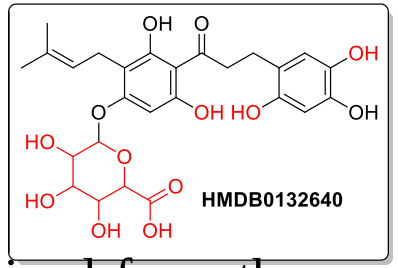



**Figure 2:** Energy components (kcal/mol) for human metabolites' binding affinity against 3CL$^{pro}$. $\Delta E_{vdW}$ and $\Delta E_{elec}$ are change in van der Waals and electrostatic energy components in the gas phase; $\Delta G_{pol}$ and $\Delta G_{np}$ are changes in polar and non-polar solvation energy components. $T\Delta S_{MM}$, configurational entropy contribution and $\Delta G_{bind}$, total affinity.

# Results and Discussion: More about Hit-1

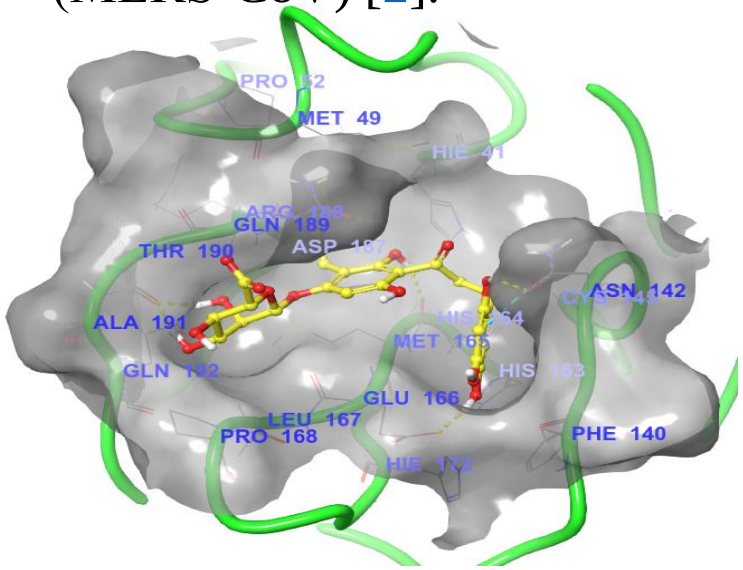
- **Hit-1 (HMDB0132640)**:



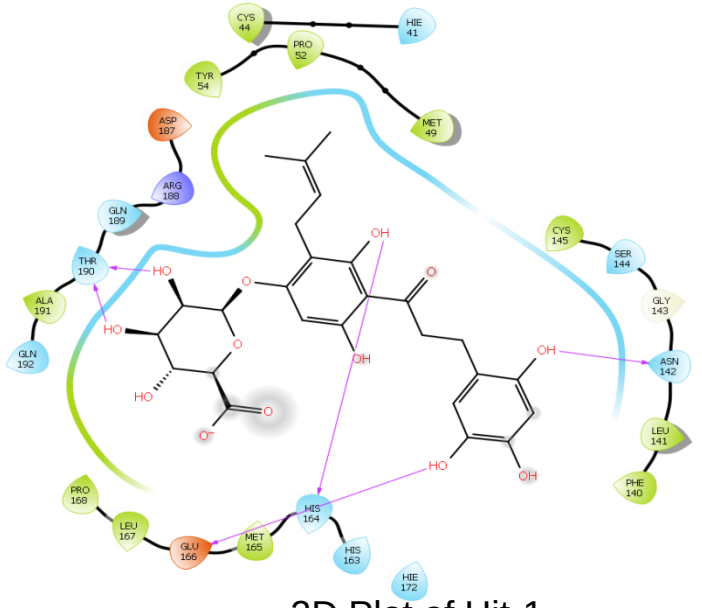




Babchi

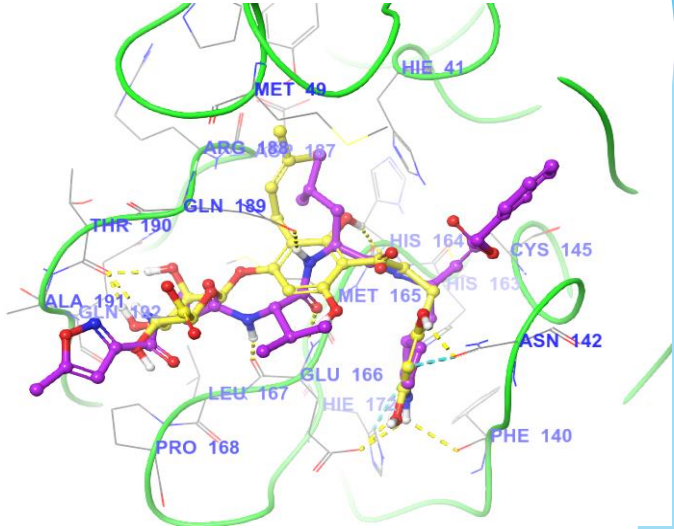
- **Hit-1** is one of the predicted metabolites of Isobavachalcone which is obtained from the seeds of Psoralea corylifolia (Babchi). Isobavachalcone has a proven effective against **papain-like protease** (PLpro) of SARS-CoV [1] and mPro of Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) [2].




Docking pose of Hit-1




2D Plot of Hit-1




Superimposed Hit-1 and Reference Ligand


1. Kim DW, Seo KH, Curtis-Long MJ, Oh KY, Oh JW, Cho JK, Lee KH, Park KH, J Enzyme Inhib Med Chem. 2014 Feb;29(1):59-63
2. Jo S, Kim H, Kim S, Shin DH, Kim MS. Chem Biol Drug Des. 2019 Dec;94(6):2023-2030. .

# Conclusions and Future Plans

- This study concludes that the natural compound metabolites can play a promising role in managing the SARS-COV-2 infection.
- We screened the human metabolite database-4.0 against main protease ($M^{pro}$) of SARS-COV-2 and selected the top 17 lead molecules for further validation.
- Finally 8 compounds were selected based on 100ns long molecular dynamics simulations and the free energy calculation using the MM/GBSA scheme.
- We found that one of the metabolites of **isobavachalcone** (Ligand1: HMDB0132640) binds very well (-18.08 kcal/mol) to 3CLpro of SARS-COV-2.
- Overall, these metabolites have a good chance of being developed as possible COVID19 protease inhibitors.
- In future we plan to synthesize and test these derivatives for further validation

# Publication and Acknowledgments


- **This article has been published as**; Roy, R., Sk, M.F., Tanwar, O. et al. Computational studies indicated the effectiveness of human metabolites against SARS-Cov-2 main protease. ***Mol Divers*** (2022). https://doi.org/10.1007/s11030-022-10513-6.
- This work was partially supported by the Department of Science and Technology, Govt. of India (grant number DST/NSM/R&D_HPC_Applications/2021/03.18). RR thanks the Indian Institute of Technology Indore for financial assistance. MFS would like to thank the Department of Science and Technology, Govt. of India, for providing a doctoral fellowship under the INSPIRE fellowship scheme (DST/INSPIRE Fellowship/2017/IF170145).
- **Dr. Omprakash Tanwar** from wish to acknowledge the support of **Dr. Rakesh Saksena**, Director Shri G.S. Institute of Technology and Science, Indore, Madhya Pradesh, India for providing sufficient computational facilities to accomplish this work.