Identifying and repurposing drugs capable of stabilizing multiple variants of the SARS-Cov-2 S-glycoprotein in a closed conformation, using *in silico* techniques

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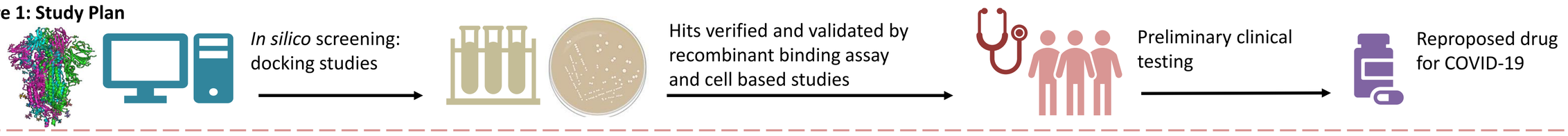
INTRODUCTION: The binding interaction between the surface S-glycoprotein (spike protein) and angiotensin converting enzyme 2 (ACE2) membrane receptor is vital to the infectious nature of the novel SARS-Cov-2 virus. Viral variants have caused much concern as studies have associated a number of mutations with both increased viral binding affinity [1] and antibody resistance [2]. The need for novel antiviral approaches effective across all viral variants remains essential. The aim of this study is to identify drugs that bind to and maintain the S-glycoprotein in a closed conformational state thereby inhibiting viral binding to host targets. These drugs may potentially be re-purposed for the therapeutic management of COVID-19.

METHODS: *In silico* docking studies verified by a detailed study of the structural and molecular interaction dynamics are used to identify drugs that bind across variants of concern: B.1.1.7 (U.K. variant), B.1.351 (South African variant), and the common strain. Verified hits will be further validated for repurposing in pilot clinical studies. (Figure 1. for study plan)

Figure 1: Study Plan



RESULTS: We have identified a number of drugs that have been shown to bind to the S-glycoprotein in a closed conformational state. This was further verified and characterized across multiple variants and mutations (see figure 2 for significant mutations) of the S-glycoprotein (B.1.1.7: PDB:7LWS, B.1.351 PDB:7LYL, and common strain PDB:6VXX) (table 1). Of the identified drugs Ivermectin was shown to have the best binding score across all variants (figure 3.)

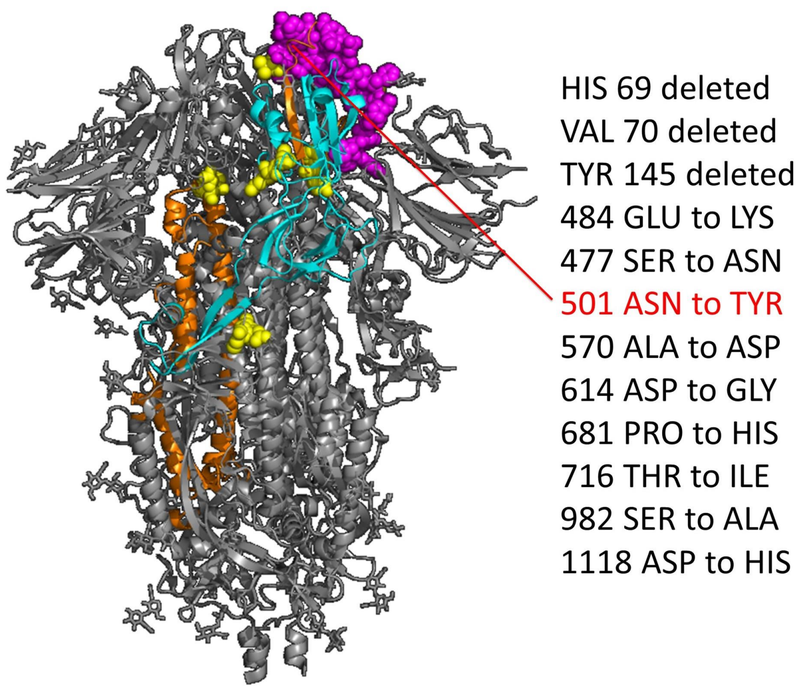


Figure 2. *Trimeric S-glycoprotein key binding residues highlighted as magenta spheres allosteric hotspot residues in yellow. Cyan and orange ribbons highlight regions of allosteric potential. Significant mutations across variants listed to the left.*

Table 1. *Binding scores of hit drugs across viral variants. AutoDock Vina [3] was used for docking studies. We identified drugs binding to key binding residues and highlighted allosteric residues corresponding to color scheme on figure 2. NSAID: non-steroidal anti-inflammatory drug.*

Drug (Class)	Binding Score (kcal/mol)			Drug Class	Suitability for COVID Clinical setting
	6VXX	7LWS	7LYL		
Ivermectin	-15	-15.7	-14.7	Antiparasitic	Ongoing clinical trials
Ribavarin	-6.5	-6.9	-6.6	Antiviral	Ongoing clinical trials
Remdesivir	-8.1	-7.5	-8.5	Antiviral	Approved for treatment
Indinavir	-9	-8.8.	-9.2	Antiviral	Promising
Fosamprenavir	-7.4	-7.7	-6.3	Antiviral	Promising
Ketoprofen	-7.8	-6.6	-7.5	NSAID	Promising
Dexketoprofen	-6.2	-6.3	-7.3	NSAID	Promising
Pelubiprofen	-6.8	-6.7	-6.8	NSAID	Promising
Diflumidone	-7.5	-6.9	-6.7	NSAID	Promising
Paromomycin	-7.5	-8.6	-7.4	Antibiotic	Promising
Kanamycin	-7.0	-7.0	-6.8	Antibiotic	Promising
Glycyrrhizin	-10.0	-7.2	-7.2	Herbal/mucolytic	Ongoing clinical trials
Punicalagin	-10.6	-10.6	-11.1	Herbal	Promising

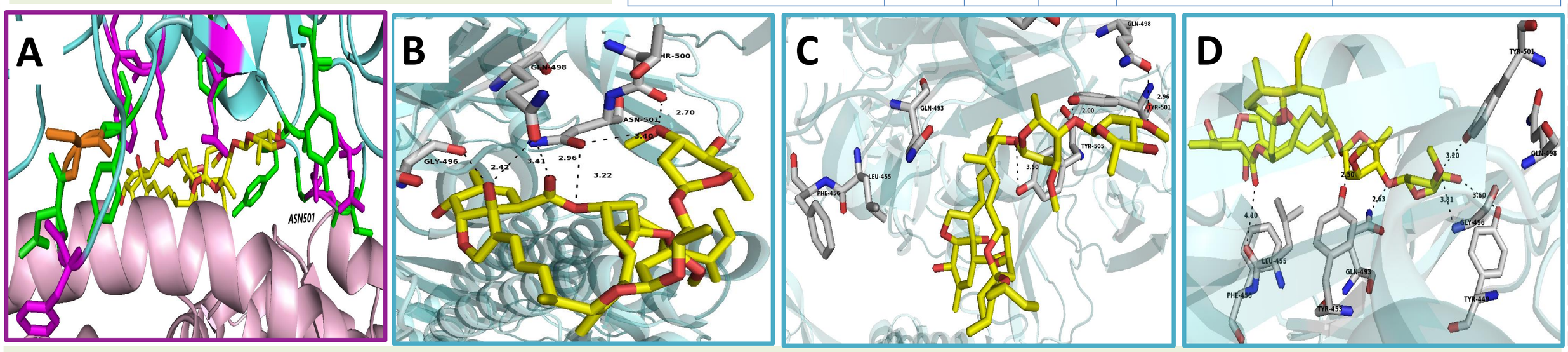


Figure 3: (A) *Binding of Ivermectin at the interface of SARS-2 spike protein (cyan) and ACE-2 (pink). Binding residues in spike protein are shown in green, key residues are shown in magenta, allosteric hot spots are shown in brown and ivermectin is shown in yellow and red. Ivermectin lies at the protein interface and interacts with key residues (H-bond with ASN 501) of the spike protein in its closed conformation.* ***(B)*** *Docking of ivermectin in SARS-2 spike protein* ***(6VXX variant)*** *binding site showing potential electrostatic interactions with key residues ASN 501, GLN 498, and GLY 496, and THR 500.* ***(C) (7LWS variant)*** *binding site. H-bond with the mutated residue TYR 501 and key residue GLN 498. Van der walls interactions with key residues; LEU 455, PHE 456, and GLN 493.* ***(D) (7LYL variant)*** *binding site. H-bonds with the key residues mutated TYR 501 and GLN 493, and LEU 455, PHE 456, TYR 449, and TYR 453. Interactions between ivermectin (yellow and red) and binding residues (grey, blue and red). Distances are represented as black dotted lines and are in Angstrom.*

CONCLUSION AND FURTHER WORK: We utilize a number of targeted *in silico* drug discovery approaches to identify drugs that bind the S-glycoprotein and may be repurposed to inhibit the viral-host interaction. Our study targets the viral protein in its closed conformational state and agents identified have shown binding capacity across major viral variants. Successful hits identified will be further verified using recombinant protein binding assays and may represent potential targets for preliminary clinical investigations.

REFERENCES:
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