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Identification of Small Molecular SARS-CoV-2 Cell Entry Inhibitors

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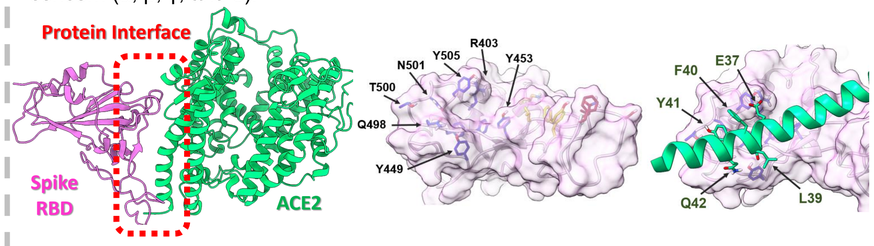
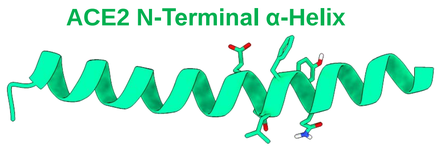
• Introduction

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) is a novel and highly pathogenic coronavirus that caused the coronavirus disease 2019 (COVID-19) - an ongoing pandemic that has posed a serious threat to public health and global economy. The high morbidity and mortality associated with COVID-19 and the severe bottlenecks in vaccine production/distribution underscores the urgent need for developing effective antiviral therapies. Similar to all coronaviruses, SARS-CoV-2 virions display the characteristic club-shaped projections formed by trimers of viral Spike glycoprotein on their surface. To invade the host cell, the **R**eceptor-**B**inding **D**omain (**RBD**) of viral Spike protein binds to the host cell's **A**ngiotensin-**C**onverting **E**nzyme **2** (**ACE2**) receptor, followed by several cleavage events that allow the viral Spike protein to fuse with the host cell membrane. Thus, the essential role of Spike protein in ACE2 receptor binding and viral fusion makes it a promising target. It is challenging to block the Spike-RBD/ACE2 protein-protein interaction since the protein interface is too large for a drug-like small molecule to fully occupy. However, as revealed by others' research[2-5], not all the interface residues are equally important. It is feasible to only focus on the most critical residues (or "hot-spots") at Spike-RBD and take the advantage of the minimum interface area to disrupt the dominant contacts between the two proteins. Based on our recent study[1] and others' publication[2-5], E35, E37, D38, Q42, Y41, and K353 of ACE2 N-terminal helix as well as the adjacent β-sheet play a critical role in forming effective contacts with Spike RBD. The corresponding interacting residues of Spike RBD surface mainly consists of Y505, R503, Y495, N501, Y449, and Q493. Importantly, these residues formed a small and shallow pocket which is favorable for accommodating a drug-like small molecule. In this work, we performed a virtual screening against this site and identified "**SAI4**" as the promising hit compound which displayed promising inhibitory activities against not only ancestral SARS-CoV-2 but also four variants of concern (α, β, γ, and δ).

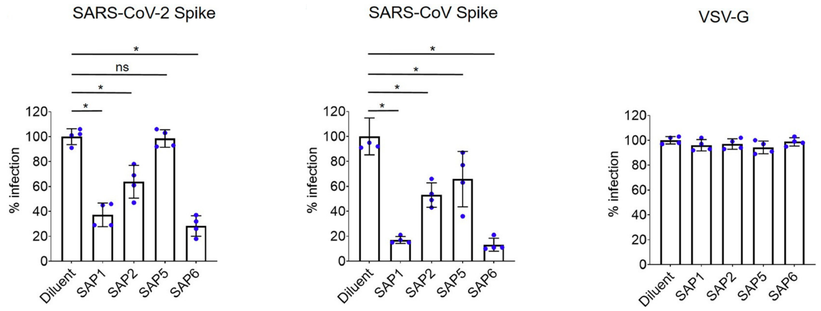


ACE2 N-Terminal α-Helix

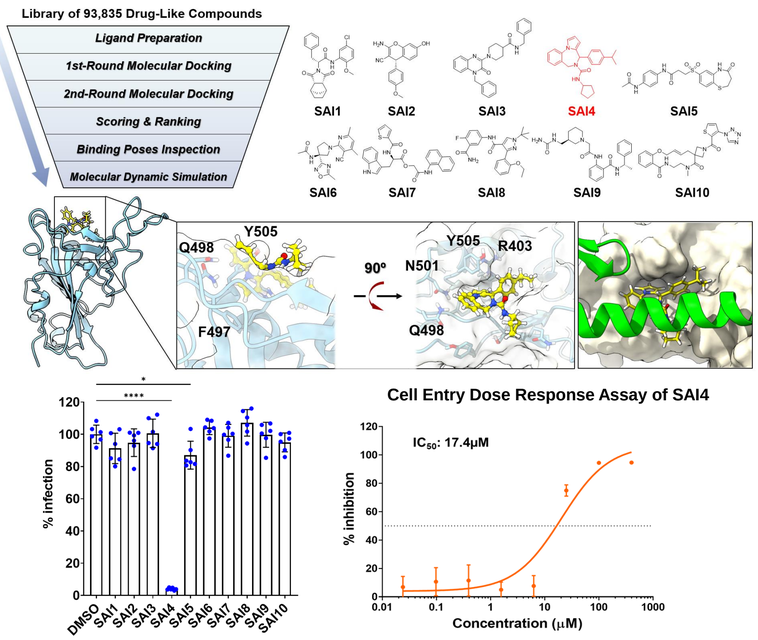


^{19}STIEEQAKTFLDKFNHEAEDLFYQSSLASWNYN^{51}

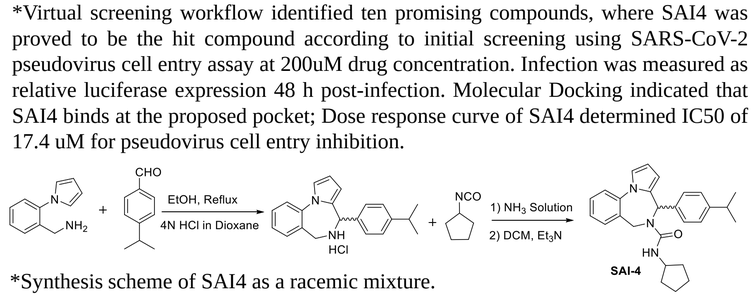
Peptide	Amino Acid Sequence
SAP1	27-TFLDKFNHEAEDLFYQ-42
SAP2	37-EDLFYQSSL-45
SAP3	79-LAQMYPL-85
SAP4	352-GKGDFRIL-359
SAP5	24-QAKTFLDKFNHEA-36
SAP6	37-EDLFYQ-42



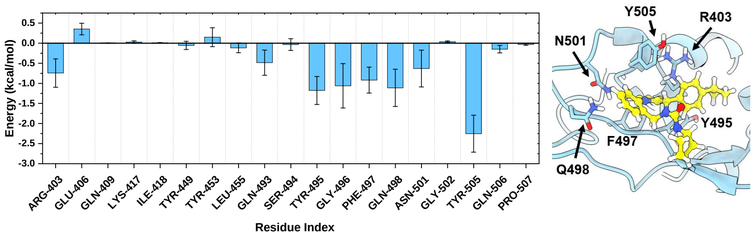
*Inhibition of Spike- and VSV-G-pseudotyped lentivirus infection by (**S**pike-targeting **A**CE2-derived **P**eptides) **SAP**s. Luciferase-encoding lentiviruses pseudotyped with indicated viral glycoprotein were incubated with 3 mM of SAPs indicated or diluent control for 1 h prior to infection of 293T-ACE2 cells. Infection was measured as relative luciferase expression 48 h post-infection.



*Virtual screening workflow identified ten promising compounds, where SAI4 was proved to be the hit compound according to initial screening using SARS-CoV-2 pseudovirus cell entry assay at 200uM drug concentration. Infection was measured as relative luciferase expression 48 h post-infection. Molecular Docking indicated that SAI4 binds at the proposed pocket; Dose response curve of SAI4 determined IC50 of 17.4 uM for pseudovirus cell entry inhibition.



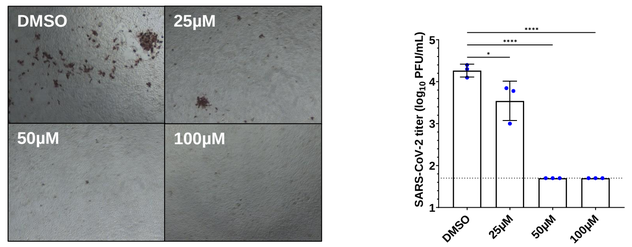
*Synthesis scheme of SAI4 as a racemic mixture.



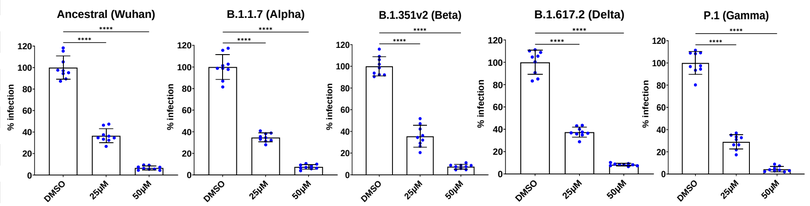
*Molecular Dynamic simulation and MM/GBSA per-residue energy decomposition profile of SAI4 bound with SARS-CoV-2 Spike-RBD.

• Results and Discussion

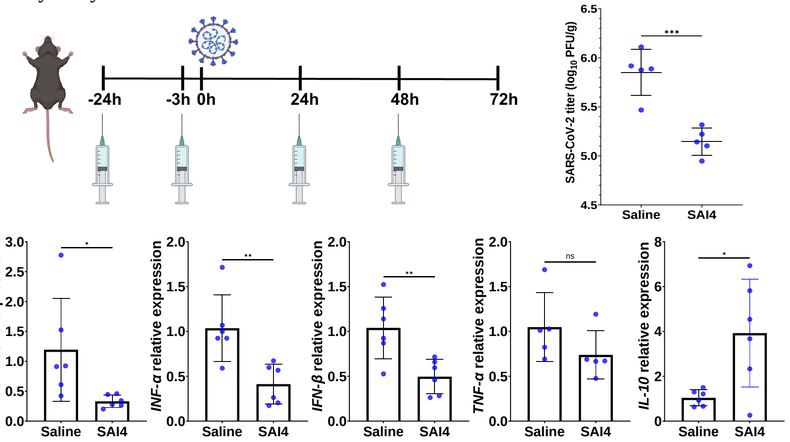
We used virtual screening to identify potential compounds from commercial drug-like libraries (Enamine™ and ChemDIV™). Ten promising compounds have been identified and SAI4 was the hit molecule according to the initial pseudovirus cell entry assay. Molecular docking predicted that SAI4 can bind at the proposed pocket right beneath the ACE2 N-terminal helix. A 50ns molecular dynamic simulation of RBD/SAI4 complex was performed in water and MM/GBSA per-residue energy decomposition profile indicated the contribution of the critical "hot-spot" residues to the protein-ligand interaction. Further evaluation supported that SAI4 inhibits genuine SARS-CoV-2 infection and cell entry of four variants of concern. Besides, SAI4 also decreased pro-inflammatory cytokines and increase anti-inflammatory cytokines *in vivo* at the dose of 5mg/kg.



*SAI4-mediated inhibition of genuine SARS-CoV-2 infection measured by N-protein immunostaining.



*SAI4-mediated inhibition of SARS-CoV-2 variants of concern by pseudovirus cell entry assay.



*Four-week-old SPF C57BL/6 female mice were intraperitoneally delivered **5 mg/kg** SA14 or PBS (control) 24h and 3 h before intranasal infection with 5 × 104 PFU of MA SARS-CoV-2 strain in 20 μl of DMEM. PBS or SAI4 was administered every 24hr for the remainder of the study. Body weight were measured daily. On three days after infection, mice were euthanized, and left lung were collected and homogenized for virus titration.

• Conclusion

In this work, we identified a small molecule named SAI4 that has promising inhibitory activity against SARS-CoV-2 from virtual screening, which will pave a way for the further therapeutic development and contribute to the battle against COVID19 pandemic.

• Acknowledgement

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• Reference

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