STUDYING THE CRITICAL ASSOCIATION OF OMICRON SPIKE PROTEIN WITH HUMAN ACE2 PROTEIN: APPROACH TO DEVELOP QUINOLINE DERIVATIVES AND NOVEL PEPTIDES AS ANTIMICROBIAL AGENT

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Omicron Spike Protein, Peptide design, Novel Quinoline Derivatives, Protein-protein Interactions, in silico, hACE2

Introduction:

The novel Betacoronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of coronavirus disease-19 (COVID-19). The pandemic has caused havoc worldwide, and there has not been any specific therapy till date.The main reasons for the lack of treatment is the mutations of the virus. One such latest mutation which high transmissibility is Omicron Variant.

Peptides are easy to develop both in terms of time, and technology; and the peptides are cost-effective. Peptides are small fragments of proteins consisting typically of 2-50 amino acid residues bound together by amide bonds. Peptide-based drug candidates or structurally modified peptides have several advantages such as easy availability, low production cost, better bioavailability, low immunogenic responses, high specificity, high biological activity, low intrinsic toxicity, convenient purification, and storage

This project is a two way approach to design peptides that can block the interactions between Omicron Spike protein and human ACE2 that causes infection, the second approach is to prove the radical scavenging and antimicrobial activities of novel quinolone compounds that can serve as an effective drug for aftermath of COVID 19 which results in weaker immune system in humans.

Objectives:

1. To Study the protein-protein interactions of Omicron spike protein with human ACE2 protein.
2. To design peptides that can act as inhibitors to the binding of Omicron Spike protein with human ACE2 protein.

3. To gaining an insight on the therapeutic value of Quinoline derivatives through antimicrobial and scavenging assessment.

Methodology:

PEPTIDE DESIGN TO INHIBIT THE BINDING OF OMICRON VIRUS TO HUMAN ACE 2 (insilico studies):

1. For this study, the Omicron-hACE2 protein complex was retrieved from RCSB Site (https://www.rcsb.org/structure/7t9k) and the sequence was saved in FASTA sequence for further queries.
2. The protein complex was then visualised in Biovia Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer) where only the chains A and D were saved by deleting the rest of the chains which were not solely responsible for the viral infection. The knowledge about the chains were obtained in PDBsum (https://bio.tools/pdbsum_generate) which illustrated the chains interacting alongside the number of hydrogen bonds present for each binding chain.
3. The interacting residues responsible for this critical association was identified using SPPIDER tool (https://sppider.cchmc.org/). The results displayed the residues which was utilized to design peptides.
4. The saved protein complex structure was viewed in sequence format using Biovia tool and the peptides were designed and saved in .pdb format.
5. The saved peptides were docked against the human ACE2 protein using HPEPDOCK (http://huanglab.phys.hust.edu.cn/hpepdock/) which provided the docking scores that confirmed these peptides to be a good inhibitor to the Omicron Spike protein.
6. The toxicity of the peptides along with its physicochemical properties were obtained using ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/).
7. The peptide with best properties was finalised to be a good drug candidate.

THERAUEPTIC PROPERTIES OF QUINOLINE DERIVATIVES:

1. Novel quinolone derivatives pre-synthesized was obtained and checked for the radical scavenging properties using hydrogen peroxide scavenging assay as described by Ruch et al (1989), the percentage of inhibition was calculated using the results.
2. Absorbance at 230 nm was registered to decide the relative H_2O_2 percentage decrease of compounds. H_2O_2 dissolved in phosphate buffer read at 230 nm was used as control OD. Ascorbic acid was used as positive control. The percentage scavenging of H_2O_2 and ascorbic acid were determined by the succeeding formula:
   % H_2O_2 reducing activity = $[OD_0 - OD_1/OD_0] \times 100$
   Where, OD_0 is the optical density (OD) of the control and OD_1 is the OD of target compounds.

3. The antimicrobial activity of the derivatives against Gram positive bacterial strains such as *Streptococcus pneumoniae* (MTCC 1936), *Staphylococcus aureus* (MTCC 737) and Gram-negative bacterial strains *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) were procured from the MTCC (Chandigarh, India) was tested. All bacterial strains were preserved in nutrient broth (NB) at -80 ºC supplemented with 15% (v/v) glycerol. Before using the frozen stock, the bacteria was cultured in 3ml nutrient broth incubated at 37 ºC overnight.
4. The compounds were serially diluted 5 folds (100 µL -500 µL) and bacterial samples were added After that, 6 h attained mid-log phase bacterial culture (~10^8 CFU/mL) were diluted to ~10^5 CFU/mL in nutrient broth. From this, 150 µL of bacterial suspensions were added to all wells that containing compounds. The plates were allowed to incubate for 24 h at 37 °C under shaking condition. After that, the optical density (OD) of the plates were measured at 600 nm using UV Spectrophotometer. The MIC was eventually calculated.

Results:

A D : R403 K440 V445 Y449 Y453 L455 F456 Y473 A475 G476 N477 F486 N487 Y489 R493 S494 S496 R498 P499 T500 Y501 G502 H505 SPPIDER results showing the critical interactions between omicron spike protein and hACE2 The following shows the peptides designed in Biovia software.

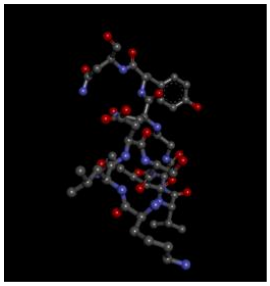

Fig1: Peptide 1
Sequence:
KLDSKVSGNYN

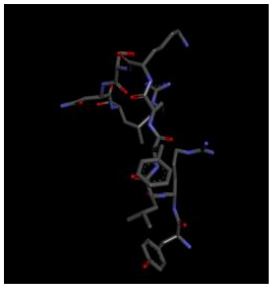

Fig2: Peptide 2
Sequence :
YRLFRKSN

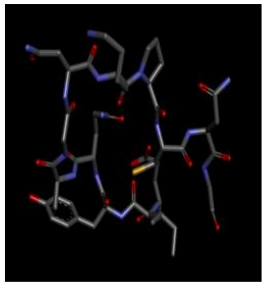

Fig3: Peptide 3
Sequence:
EIYQAGNKPCNG

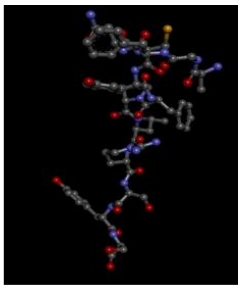

Fig 4: Peptide 4
Sequence:
AGFNCYFPLRSYS

The peptide-protein docking results were obtained from HPEPDOCK as shown below:

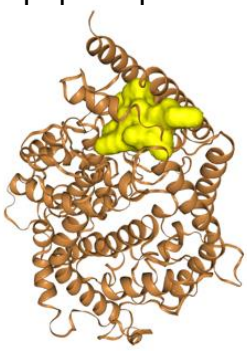

Fig5: Peptide 1
Docking Score: -88.204

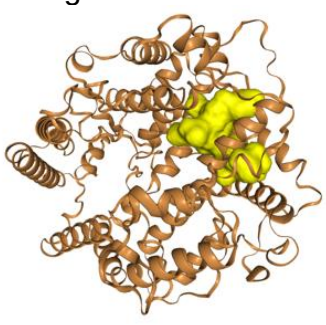

Fig6: Peptide 2
Docking Score: -128.185

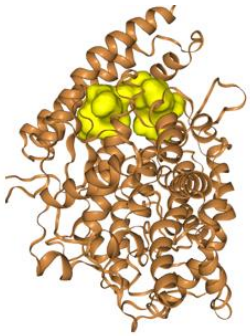

Fig7: Peptide 3
Docking Score: -93.601

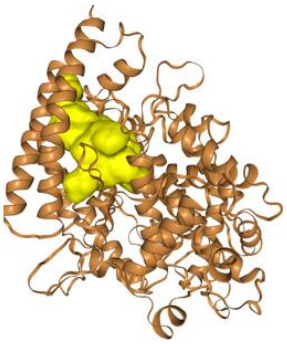

Fig8: Peptide 4
Docking Score: -145.152

The ToxinPred results proved the peptides to be non-toxic.

Table 1

PEPTIDE SEQUENCE	MOLECULAR WT.	TOXICITY
KLDSKVSGNYN	1224.49	Non-Toxic
YRLFRKSNL	1196.54	Non-Toxic
EIYQAGNKPCN G	1293.59	Non-Toxic
AGFNCYFPLRS YS	1524.88	Non-Toxic

Radical Scavenging Results:

Compounds	% inhibition
5a	96.84
5b	94.08
5c	80.14
5d	91.18
5e	83.15
5f	93.24
5g	86.24
Ascorbic acid	92.24

MIC results:

Compounds	**Percentage of Inhibition (%)**			
	Gram-Positive Bacteria		Gram-Negative Bacteria	
	S. aureus	*S. pneumoniae*	*E. coli*	*P.*
5a	90.91	91.5	91.08	90.912
5b	89.66	85.25	90.083	90
5f	90.32	90.4	89.41	89.16

Conclusion:

Host receptor recognition and attachment by virion is facilitated by interface of omicron RBD with h-ACE-2. Thus, design of peptides that have resemblance with h-ACE2 can inhibit the protein-protein interaction and be a potential therapeutic in order to combat the virus.

This study based on the above statement promises a peptide as a drug candidate for omicron virus. The properties of quinoline derivatives is an effective measure as a therapeutic for aftermath of COVID 19 to fight against different diseases that is caused by the loss of immunity due to previous attack of COVID 19.

Scope For Future Work:

The peptides proposed in this project should be further subjected to molecular dynamic simulations to study the structural changes with time.

The insilico studies can be utilized to amplify the peptides and conduct clinical trials after evident invitro studies.