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Potential chimeric peptides to block the SARS-CoV-2 Spike RBD

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ABSTRACT

Background: There are no known medicines or vaccines to control the COVID-19 pandemic caused by SARS-CoV-2 (nCoV). Antiviral peptides are superior to conventional drugs and may also be effective against COVID-19. Hence, we investigated the SARS-CoV-2 Spike RBD (nCoV-RBD) that interacts with hACE2 for viral attachment and entry. **Methods:** Three strategies and bioinformatics approaches were employed to design potential nCoV-RBD - hACE2 interaction-blocking peptides that may restrict viral attachment and entry. Firstly, the key residues interacting with nCoV-RBD - hACE2 are identified and hACE2 sequence based peptides are designed. Second, peptides from five antibacterial peptide databases that block nCoV-RBD are identified; finally, a chimeric peptide design approach is used to design peptides that can bind to key nCoV-RBD residues. The final peptides are selected based on their physiochemical properties, numbers and positions of key residues binding, binding energy, and antiviral properties. **Results:** We found (i) three amino acid stretches in hACE2 interact with nCoV-RBD; (ii) effective peptides must bind to three key positions of nCoV-RBD: Gly485/Phe486/Asn487, Gln493, and Gln498/Thr500/Asn501; (iii) Phe486, Gln493, and Asn501 are critical residues; (iv) AC20 and AC23 derived from hACE2 may block two key critical positions; (iv) DBP6 identified from databases can block the three sites of the nCoV-RBD interacting with one critical position Gln498; (v) seven chimeric peptides were considered promising among which cnCoVP-3, cnCoVP-4, and cnCoVP-7 are the top three; and (vi) cnCoVP-4 meets all the criteria and is the best peptide. **Conclusion:** All the ten peptides need experimental validation for their therapeutic efficacy.

Key words: Antiviral peptides, COVID-19, SARS-CoV-2, nCoV-19, peptide design, ACE2, Spike protein

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INTRODUCTION

The world is under the severe COVID 19 pandemic caused by SARS-CoV-2 or novel corona virus (nCoV-19) that originated from the Wuhan city of China [1,2] and spread across the world. So far two million people have been infected and more than 120,000 deaths are recorded across the globe. The death rate is > 20% and the most affected countries are the USA, Italy, Spain, the UK and France that have each recorded more than 10,000 deaths within a couple of weeks (https://www.worldometers.info/coronavirus). The SARS-CoV-2 is highly contagious in humans and so far no medicine or vaccine has been developed to tackle this novel Corona Virus 2019 (nCoV-2019) making it impossible to control its spread across the globe [3] . Although, drugs like Hydroxychloroquine, Remdesivir, and Lopinavir [4] are currently being suggested to treat COVID-19 infection, there is no clinical study so far to prove their efficacy in treating these patients. Therefore, currently there is a global search for appropriate drug and vaccine candidates against SARS-CoV-2.

SARS-CoV-2 has shown 80% genome identity with SARS-CoV, which is the causal agent of the Severe acute respiratory syndrome (SARS) seen in 2002-2003 [5]. SARS-CoV binds to human angiotensin-converting enzyme 2 (hACE2) receptor through its Spike protein (S) to enter into the host cell [6], and it is now reported that SARS-CoV-2 also binds to ACE2 to transmit its genetic material to human [7-9]. Therefore, blocking the Spike protein of SARS-CoV-2 could be an attractive and effective way to prevent the SARS-CoV-2 infection.

The crystal structure of hACE2 receptor and the receptor binding domain (RBD) of SARS-CoV-2 Spike protein (nCoV-RBD) (PDB: 6M17) showed that a total of 8 residues namely, Gln24, Asp30, His34, Tyr41, and Gln42 from α1 helix, residue Met82 in α2, and Lys353 and Arg357 in the β3 and β4 linkers are important for the binding [9]. The important interactions between the nCoV-RBD with ACE2 are Lys417 (Spike)--Asp30 (hACE2), Tyr453 (Spike) --His34 (hACE2), Gln474 (Spike) --Gln24 (hACE2), Phe486 (Spike) --Met82 (hACE2), Gln498 (Spike) --Tyr41 (hACE2), Thr500 (Spike) --Gln42 (hACE2), and Asn501 (Spike) --Lys353 [9].

Peptide-based drugs are of a better choice than conventional drugs due to their higher efficiency, lesser molecular weight, and lower toxicity and side effects [10]. In this regard, antiviral peptides (AVPs), a subset of antimicrobial peptides (AMPs), are of specific interest due to their higher efficacy in inhibiting viral infection by targeting various stages of the viral life cycle. AVPs can directly invoke innate immune response [11] and inhibit viral entry by targeting viral attachment

and entry to host cell, and replication, transcription, translation, multiplication, and release inside the host cell [12,13]. Previously, several AVPs have been reported to inhibit the SARS-CoV Spike protein or viral entry [14-16].

In this report, using bioinformatics strategies, we attempted to design anti-Spike peptides for SARS-CoV-2 towards motivating potential therapeutics against the SARS-CoV-2 infection.

METHODS

We adopted three strategies to predict potential AVPs against the SARS-CoV-2 Spike protein.

Strategy-I: In the first strategy, we re-analysed the SARS-CoV-2 Spike RBD with hACE2 to identify the key interacting residues in both the proteins. A recent report suggests that the B chain of SARS-CoV-2 Spike protein interacts with the B or D homodomain of hACE2 [9]. Therefore, in this analysis, we used individual B chain of SARS-CoV-2 Spike RBD (PDB:6LZG) and the B chain of hACE2 (PDB: 6M18) to dock with each other using AutoDock-4 [17]. Once we identified the key interacting residues, in the next step, we designed a number of AVPs based on the interacting hACE2 residues to the RBD of the Spike protein. Binding to SARS-CoV-2 Spike RBD with the peptides was determined by the HPEPDOCK protein-peptide docking Server [18].

Strategy-II: In the second strategy, we screened the available antiviral peptides against the SARS-CoV-2 Spike RBD. We used Antiviral peptides database (AVPdb) [19], HIV inhibiting peptides database (HIPdb) [12], Antimicrobial peptide database (APD3) [20], dbAMP [21], and FDA approved therapeutic peptides and proteins database (THPdb) [22] and screened the peptides against the SARS-CoV-2 Spike RBD. In this process, the HPEPDOCK protein-peptide docking Server [18] was also used fixing the eight active amino acid binding sites of the Spike protein (Lys417, Tyr453, Gln474, Phe486, Gln493, Gln498, Thr500, and Asn501). The final peptides were selected based on their HPEPDOCK docking energy score, number of binding, number of selected target residue binding, physiochemical properties, and AVPpred prediction [13].

Strategy-III: In the third strategy, we adopted an in-house chimeric peptide design approach where the two fragments of two different peptides selected in our previous two approaches are composed in such a way that the resultant peptide can bind to our given target residues in the SARS-CoV-2 Spike RBD. A total of 500 such chimeric peptides were designed and docked with Spike protein target residues: Lys417, Tyr453, Gln474, Phe486, Gln493, Gln498, Thr500, and Asn501 using the HPEPDOCK protein-peptide docking Server [18]. The final peptides were selected based on similar criteria as adopted in the second strategy.

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Physiochemical analysis of peptides

The peptides are predicted for their antiviral properties using AVPpred [13]. The molecular formula, molecular weight, net charge, grand average hydropathy, total hydrophobic ratio, hydrophobicity, and protein-binding potential (Boman index) are calculated using antimicrobial peptide calculator and predictor [20]. The IC_{50} of the peptides is predicted using AVP-IC_{50}Pred server selecting RSV/INFV/HSV [23]. Hemolytic potency of peptides is determined using HemoPI server [24] where the values tending towards "0" are unlikely to be hemolytic. ToxinPred [25] was used to predict the toxicity of the peptides. The final peptides were selected based on their HPEPDOCK docking energy score, number of binding, number of selected target residue binding, physiochemical properties, and AVPpred prediction [13].

RESULTS AND DISCUSSION

Identification of hACE2 residue-based peptides

In SARS-CoV-2 Spike RBD - hACE2 interaction analysis, similar to Yan et al (2020) [9], we found that three stretches of peptides that harbour the active residues of hACE2 interact with Spike RBD. These stretches have amino acid positions: 21-43 (5 sites), 78-87 (1 site), and 348-361 (2 sites). In the Spike RBD, the key interacting amino acid stretches are 480-489 and 490-505. Previous report suggests that among the Spike residues, the most important residues interacting with hACE2 are Phe486, Gln493, and Asn501 [26]. An in-depth analysis revealed that any peptide that potentially blocks the Spike RBD should bind at least three critical positions of the RBD: (i) Gly485 or Phe486 or Asn487, (ii) Gln493, and (iii) Gln498 or Thr500 or Asn501 among which the Phe486, Gln493, and Asn501 are essential.

We designed three peptides from the first stretch of the hACE2 that shows maximum active binding residues with the Spike RBD. A 26 amino acid peptide binds to Thr500 and Asn501 of the Spike RBD (**Fig-1A**), the 23 amino acid peptide binds to Tyr489 and Thr500 of the Spike RBD (**Fig-1B**), and a third 20 amino acid peptide binds to Gln493 and Asn501 of the Spike RBD (**Fig-1C**). All these peptides show acceptable physiochemical properties to be used as therapeutic peptides (**Table-S1**). However, none of these three peptides are able to block all the key three positions of the Spike RBD. Therefore, these peptides may not be suitable for developing much effective anti-SARS-CoV-2 therapeutics targeting its Spike RBD. However, AC20 and AC23 can be further tested.

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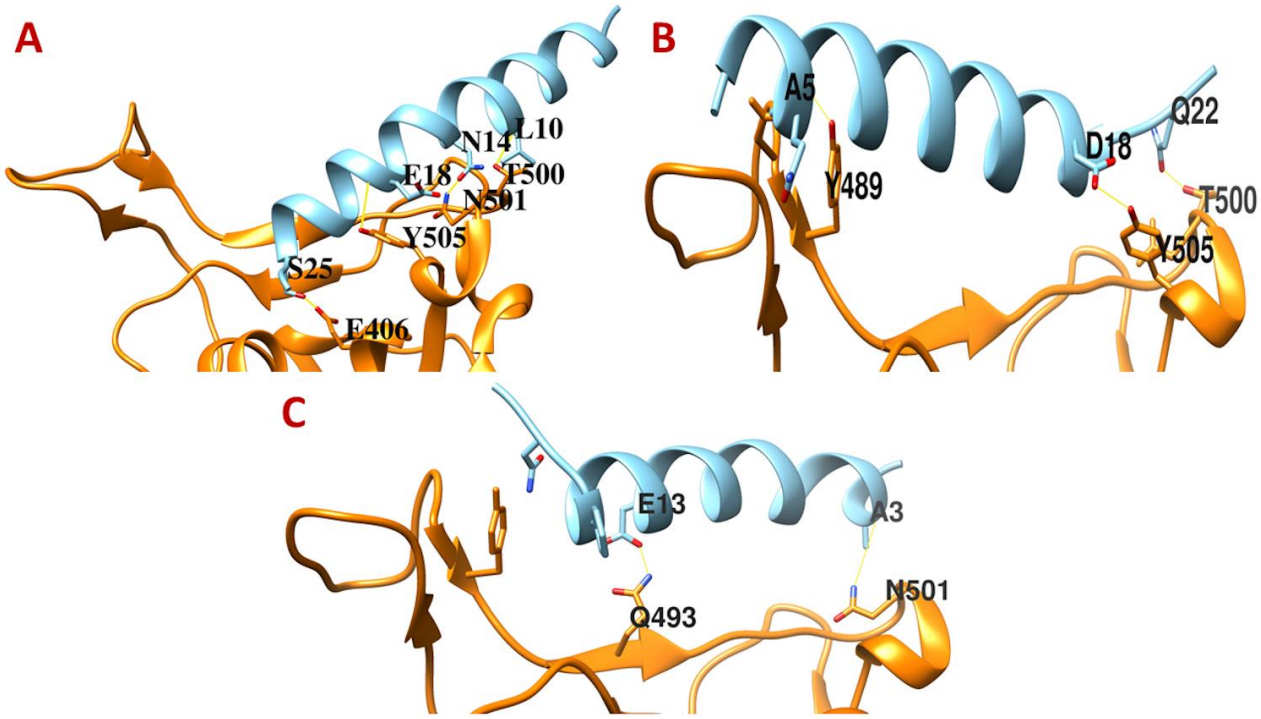

Fig-1: The binding interfaces between SARS-CoV-2 Spike RBD with hACE2 derived peptides. (A) AC26, (B) AC23, and (C) AC20.

Identification of peptides from antimicrobial peptide databases

We identified seven peptides by screening the five different anti- microbial peptide databases. The Spike protein sequence of SARS-CoV-2 is highly similar to SARS-CoV [8] and SARS-related coronaviruses and SARS-CoV directly interacts with hACE2 through their RBD located in the B chain of the Spike protein [27,28]. In our peptide database analysis, we also observed that the peptides that experimentally proved to be effective against SARS-CoV have the potential of being used against the SARS-CoV-2 Spike protein. All the identified seven peptides (**Table-S1**) are of 20 amino acid length and are reported to target the Spike protein of SARS-CoV to exhibit their anti-SARS virus activities (US7491489). Although the peptides show 4 to 11 "H" bonds and form between 2 to 4 bonds with our given 8 target residues, most of these peptides do not bind to all the three positions (i) Gly485 or Phe486 or Asn487, (ii) Gln493, and (iii) Gln498 or Thr500 or Asn501 to effectively block the Spike RBD.

The DBP1, DBP2, and DBP3 peptides bind to only the third position (Gln498 and Asn501) of the Spike RBD without binding to the other two sites (**Table-S1**) (**Fig-2A-C**). DBP4, DBP5, and DBP7 interact with first (Gly485 or Phe486 or Asn487) and third (Gln498 and Asn501) sites of the Spike RBD without binding to the middle or the second site (Gln493) (**Table-S1**) (**Fig-2D, E, G**). DBP6 binds to all the three sites within the range of the target residues but do not interact with

the key residues of the first and third sites (**Table-S1**) (**Fig-2F**). The DBP6 is also predicted as an antiviral peptide by AVPpred [13]. Therefore, DBP6 could be a potential peptide to be tested for SARS-CoV-2 Spike protein-based drug development.

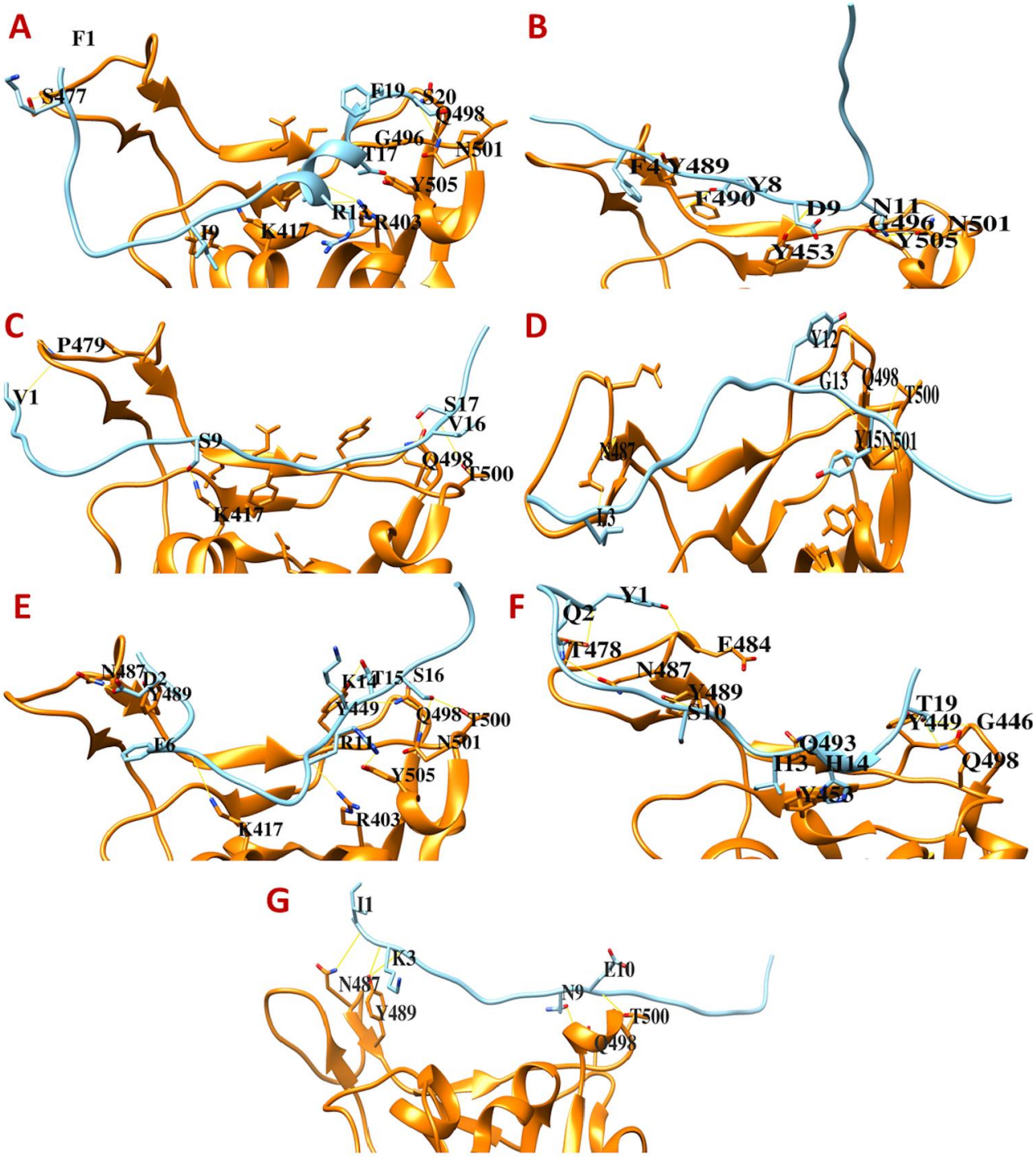

Fig-2: The binding interfaces between SARS-CoV-2 Spike RBD with peptides screened from the antimicrobial peptide database. (A) DBP1, (B) DBP2, (C) DBP3, (D) DBP4, (E) DBP5, (F) DBP6, and (G) DBP7.

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Chimeric peptides against SARS-CoV-2 Spike RBD

Out of 500 chimeric peptides generated, only seven are selected for final analysis. All these peptides are non-hemolytic, non-toxic, and meet all the criteria of a therapeutic peptide (**Table-S1**). Among these seven peptides, cnCoVP-3, cnCoVP-4, and cnCoVP-7 interact with all the three sites and two key residues of the second (Gln493) and third (Asn501). However, these peptides bind one amino acid apart from the key residue (Phe486) in the first site and potentially block the access of SARS-CoV-2 Spike Phe486 to the hACE2 (**Table-S1**) (**Fig-3C, D, G**). AVPpred [13] also predicted cnCoVP-4 as an antiviral peptide. Therefore, these three peptides can be selected for further *in vitro* and *in vivo* testing.

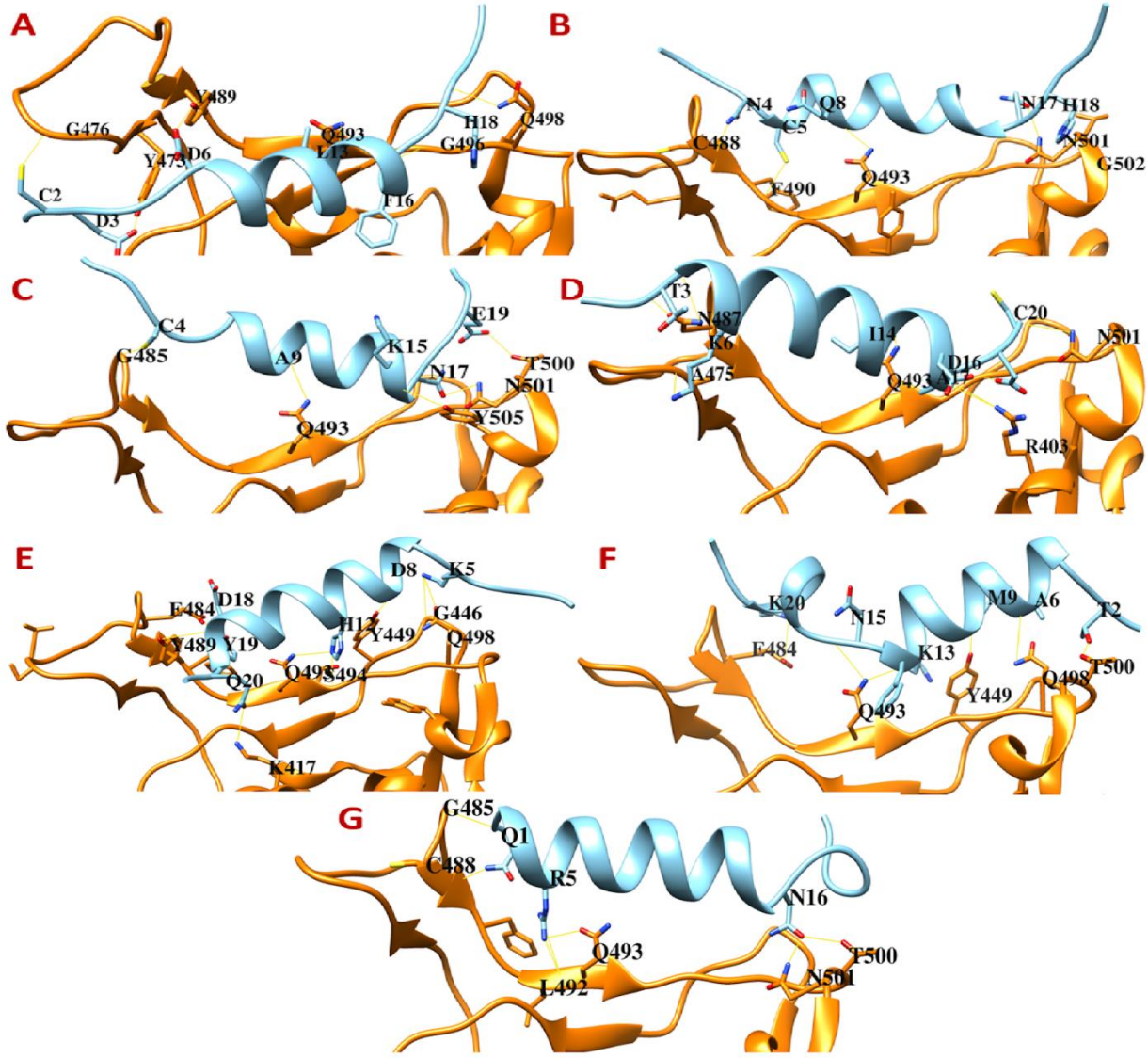

Fig-3: The binding interfaces between SARS-CoV-2 Spike RBD with designed chimeric peptides. (A) cnCoVP-1, (B) cnCoVP-2, (C) cnCoVP-3, (D) cnCoVP-4, (E) cnCoVP-5, (F) cnCoVP-6, and (G) cnCoVP-7.

Although the chimeric peptides cnCoVP-2, cnCoVP-5, and cnCoVP-6 interact with all the three sites, they do not interact with the key residue (Phe486) or the immediate to key residue of the first site. Instead they bind a residue that is 2- 3 amino acid apart from the key residue (Phe486) (**Table-S1**) (**Fig-3B, E, F**). Therefore, these three peptides may not block the first site of the Spike RBD in interacting with hACE2. However, they should also be synthesized and tested for their *in vitro* effects. The last peptide, i.e., cnCoVP-1 was found to interact with all the three sites, however it only interacts with the key residue (Gln493) of the second site. In the other two sites, it interacts at position (Tyr489) of the first site and (Gln498) of the third site (**Table-S1**) (**Fig-3A**). Although the Gln498 is a key residue of the third site Tyr489, it is not an interacting residue in the original interaction between SARS-CoV-2 Spike RBD and hACE2. Thus, this peptide may partially block the access of SARS-CoV-2 Spike RBD to hACE2 and needs further *in vitro* and *in vivo* testing and validation.

CONCLUSION

In this article, we screened and designed several peptides that may potentially block the interaction between SARS-CoV-2 Spike RBD and hACE2. Ten peptides (AC20, AC23, DBP6, and cnCoVP-1- cnCoVP-7) have very high potential to achieve this interaction indicating that these peptides could be attractive therapeutics against SARS-CoV-2. However, peptide synthesis, *in vitro*, and *in vivo* experiments are required to evaluate and ensure their potential therapeutic efficacy.

Author's contribution

DB: conceived and designed the experiment, collected data, did primary analysis, result interpretation, and wrote the paper; ST: collected data, did data analysis and data interpretation; PG, BSA, RK, LCJA, MG, AG-N, VA: did reanalysis and validation.

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