doi.org/10.36721/PJPS.2020.33.5.REG.2187-2191.1

QadirVID-19: A fusion inhibitor against Novel Coronavirus-2019 for specific management of COVID-19

Muhammad Imran Qadir
Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

Abstract: With the emergence of COVID-19 (Corona virus Disease 19), the workers tried to search the drug/s for its management. Chloroquine, hydroxychloroquine and protease inhibitors including Ritonavir have been tested. But the safety and efficacy of these drugs for the treatment of COVID-19 were not very strong. Fusion inhibitors may be used for the management or prophylaxis of viral diseases. Objective of the present study was to design a fusion inhibitor for specific management of COVID-19. A series of BLAST was performed to get an optimized and conservative region of the spike protein of the virus which is used in fusion process. Based on the recent data available, residue 1160-1189 of spike protein of Novel coronavirus-2019 (2019-nCoV) was found highly conserved region with 100% similarity, with maximum score of 99.5 and with an E-value of $5e^{-24}$. Thus, this sequence was selected as a drug. This drug is a synthetic peptide composed of 30 amino acids of *Heptad Repeat 2* (HR2) region of spike protein of 2019-nCoV and was named as QadirVID-19 (Qadir, name of the scientist who developed it and VID-19 from COVID-19).

Keywords: Coronavirus, peptide, QadirVID-19

INTRODUCTION

Novel coronavirus-2019 (2019-nCoV) or Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative agent of COVID-19 (Corona Virus Disease 19), representing a pandemic threat and has been declared an international public health emergency (WHO, 2020). Therefore, objective of the present study was to develop a candidate for the management of COVID-19.

The life cycle coronavirus is initiated by fusion of the viral envelope with human cell membrane (specifically the lung cells). This fusion is maintained by the corona viral spike protein (S Protein). The S protein is divided into two subunits: S1 subunit responsible for receptor recognition, and S2 subunit is responsible for membrane fusion. The S2 subunit contains two lucine zipper like amino acid sequences: HR1 (heptad repeat 1) and HR2 (heptad repeat 2). HR1 is a heptad repeat region present on the amino-terminus (N-HR), while HR2 is a heptad repeat region present on the carboxy-terminus (C-HR) (Hulswit *et al*., 2016). HR1 and HR2 domains in the SARS-CoV-2 S protein S2 subunit are conformed as six-helical bundle (6-HB) and most of the residues located at the "a" and "d" positions of the helical structures are hydrophobic and more conserved than those in the "b", "c", and "f" positions, which are hydrophilic. This feature suggests that these helices form homologous oligomers by hydrophobic association through "knobs-into-holes" packing interactions at the "a" and "d" positions. The residues at the "b", "c" and "f" positions form a hydrophilic face towards the solution, making the oligomeric bundles water soluble (fig. 1) (Xia *et al*., 2019; Xia *et al*., 2020).

**Corresponding author:* e-mail: mrimranqadir@hotmail.com

Oligomerization of three HR1 and three HR2 to make 6-helical bundle, is an essential component of the fusion process. It causes the closeness of the two membranes, viral envelope and human cell membrane, resulting in the fusion of the two membranes. Fusion of the membranes leads to form a pore for viral material to enter into human cell (Hulswit *et al*., 2016).

Fusion inhibitors block the entry of viral genome into human cells. They bind to HR regions of spike protein on the virus and inhibit the joining of HR2 with HR1. So the virus can not come close to the human cell membrane and ultimately fusion of the viral envelope with human cell membrane is prohibited (Qadir and Malik, 2010; Qadir and Malik, 2011). HR2 of spike protein of 2019-nCoV is the shorter region than HR1, therefore, component of this part can be used as effective inhibitor as in the case of HIV fusion inhibitors (Qadir, 2011). The antiviral activity of the fusion inhibitors depends on their optimum peptide sequences and conformations. Changes in their sequences or conformations could substantially affect their antiviral activity and the stability of the complexes they form (Liu *et al*., 2004).

MATERIALS AND METHODS

BLAST (Basic Local Alignment Search Tool) was used to get an optimized and conservative region of HR2. Predict Protein software was further used to study the peptide using its online resources available at https://www.predictprotein.org/ (Predict Protein software). Transmembrane prediction, disulfide bridges prediction, functions of the molecule, effects of point mutations were predicted by using the software.

Table 1: Summary of optimization using BLAST with 100% similar sequences

Important Selected Sequence	No. of Amino Acids	Reference ID	Score	E-Value
INASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	36	EEXKGA22014	70.5	$5e^{-15}$
Removal of amino acids from amino-terminus				
NASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	35	EEXUDMW4014	68.9	$2e^{-14}$
ASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	34	EEXZX47J016	67.0	$1e^{-13}$
SVVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	33	EEYAA783016	65.1	$7e^{-13}$
VVNIQKEIDRLNEVAKNLNESLIDLQELGKYE	32	EEYGDBK0014	63.2	$3e^{-12}$
VNIQKEIDRLNEVAKNLNESLIDLQELGKYE	31	EEYMCUHE014	61.6	$1e^{-11}$
NIQKEIDRLNEVAKNLNESLIDLQELGKYE	30(QadirVID-19)	EEYSCAGP016	99.5*	$5e^{-24}$ **
IQKEIDRLNEVAKNLNESLIDLQELGKYE	29	EEYX6XFS014	96.1	$9e^{-23}$
QKEIDRLNEVAKNLNESLIDLQELGKYE	28	EEZ240CD014	92.7	$2e^{-21}$
Removal of amino acids from carboxy-terminus				
INASVVNIQKEIDRLNEVAKNLNESLIDLQELGKY	35	EV8KH8MX01R	68.6	$2e^{-14}$
INASVVNIQKEIDRLNEVAKNLNESLIDLQELGK	34	EV8TSNRP016	65.9	$3e^{-13}$
INASVVNIQKEIDRLNEVAKNLNESLIDLQELG	33	EV8Z899E016	63.9	$2e^{-12}$
INASVVNIQKEIDRLNEVAKNLNESLIDLQEL	32	EV93Y8WG016	61.6	$1e^{-11}$
INASVVNIQKEIDRLNEVAKNLNESLIDLQE	31	EV98ENCH016	60.1	$6e^{-11}$
INASVVNIQKEIDRLNEVAKNLNESLIDLQ	30	EV9C7ZWW016	97.8	$1e^{-23}$
INASVVNIQKEIDRLNEVAKNLNESLIDL	29	EV9G7217014	94.4	$2e^{-22}$
INASVVNIQKEIDRLNEVAKNLNESLID	28	EV9K299B016	91.4	$2e^{-21}$
Moving through the Sequence with 30 Amino Acids				
VNIQKEIDRLNEVAKNLNESLIDLQELGKY	30	EYYS6UXK01R	99.0	$8e^{-24}$
VVNIQKEIDRLNEVAKNLNESLIDLQELGK	30	EYZ20VTS016	97.8	$2e^{-23}$
SVVNIQKEIDRLNEVAKNLNESLIDLQELG	30	EYZ64ERJ01R	97.3	$4e^{-23}$
ASVVNIQKEIDRLNEVAKNLNESLIDLQEL	30	EYZ9ECEH014	97.3	$1e^{-23}$
NASVVNIQKEIDRLNEVAKNLNESLIDLQE	30	EYZDMZWC01R	97.8	$1e^{-23}$

**More the score, more the good result, **Less the E-value, more the good results*

Table 2: ADMET Predicted Profile of QadirVID-19

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB-	0.9698
Human Intestinal Absorption	HIA+	0.7784
Renal Organic Cation Transporter	Non-inhibitor	0.8690
Distribution		
Subcellular localization	Mitochondria	0.7597
Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.7996
CYP450 2D6 Substrate	Non-substrate	0.7737
CYP450 3A4 Substrate	Non-substrate	0.5861
CYP450 1A2 Inhibitor	Non-inhibitor	0.8281
CYP450 2C9 Inhibitor	Non-inhibitor	0.8692
CYP450 2D6 Inhibitor	Non-inhibitor	0.8669
CYP450 2C19 Inhibitor	Non-inhibitor	0.7942
CYP450 3A4 Inhibitor	Non-inhibitor	0.8910
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9691
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Non-inhibitor	0.8667
AMES Toxicity	Non AMES toxic	0.7440
Carcinogens	Non-carcinogens	0.8494
Honey Bee Toxicity	Low HBT	0.6995
Biodegradation	Not ready biodegradable	0.8547

Translation of our peptide sequence into SMILES (simplified molecular-input line-entry system) code were completed by using the BIOPEP-UWM database (formerly BIOPEP) available at: http://www.uwm.edu.pl/biochemia/index.php/pl/biopep.

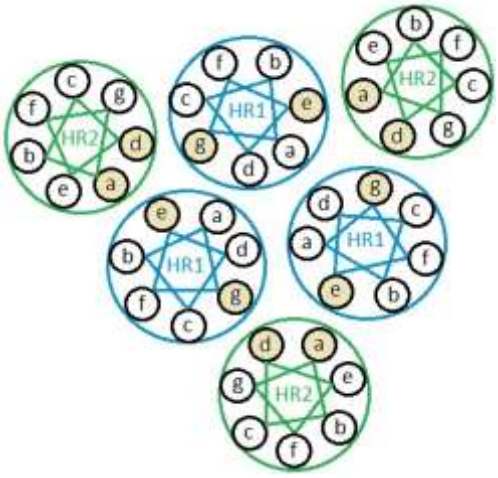

Fig. 1: Wheel projection of heptad repeats, HR1 and HR2, of S2 subunit in S protein of SARS-CoV-2. The helical wheel consists of seven corners, corresponding to the fit of seven amino acid residues into every two helical turns. The positions "a" and "d" of the helical structures are hydrophobic and more conserved.

d a d a d a d a d
NIQKEIDRLNEVAKNLNESLIDLQELGKYE

Fig. 2: Amino acid sequence of QadirVID-19 indicating the positions "a" and "d", which mostly are hydrophobic to make successive attachment with its target.

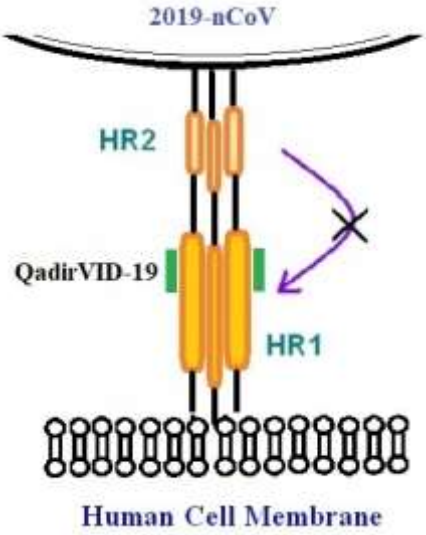

Fig. 3: Mechanism of action of QadirVID-19 – binding of the drug to HR1 and halting the fusion of the viral envelope with human cell membrane, ultimately preventing the entry of 2019-nCoV genome into human cells.

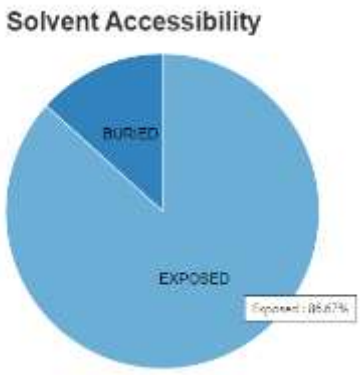

Fig. 4: Solvent accessibility of QadirVID-19

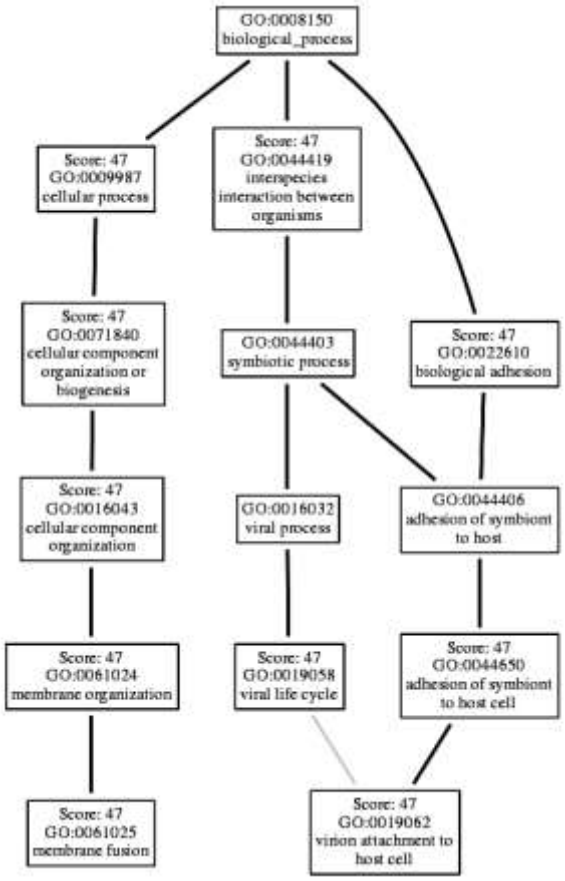

Fig. 5: Biological functions of QadirVID-19 using PredictProtein software

Briefly clicked on the "Bioactive peptides" tab, then "Analysis" tab and then "SMILES" icon. Pasted the sequence into the window and clicked the icon "Show SMILES". The SMILES code was also converted into sdf file using Online SMILES Translator and Structure File Generator, available at https://cactus.nci.nih.gov/translate/. SMILES code and sdf file were needed for further study.

PAINS (pan assay interference compounds) was performed by PAINS-Remover to prove the candidate as an effective drug (Jonathan and Georgina, 2010).

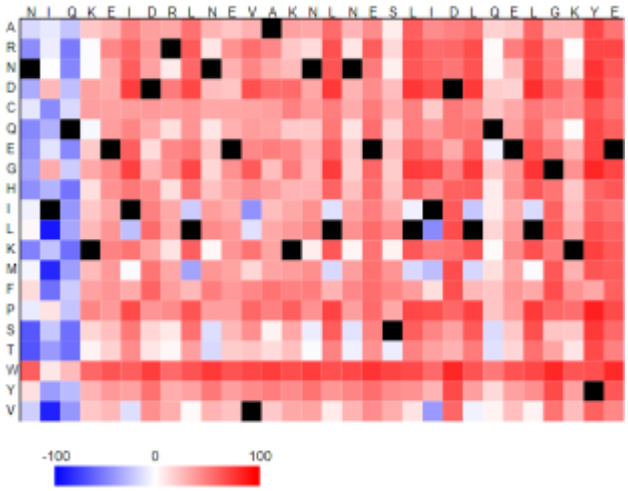

Fig. 6: Functional effects of mutations (Black is wild type)

Pharmacokinetic studies were performed by using admet SAR (http://lmmd.ecust.edu.cn/admetsar1/home/) and Swiss ADME (http://www.swissadme.ch/index.php).

STATISTICAL ANALYSIS

BLAST score was calculated from a formula that takes into account the alignment of similar or identical residues, as well as any gaps introduced to align the sequences. The BLOSUM matrix was used to calculate the probability. To calculate the BLOSUM matrix, the following equation is used:

$$S_{ij} = \frac{1}{\lambda} \log \frac{p_{ij}}{q_i q_j}$$

Here, p_{ij} is the probability of two amino acids i and j replacing each other in a homologous sequence, and q_i and q_i are the background probabilities of finding the amino acids i and j in any protein sequence. The factor λ is a scaling factor, set such that the matrix contains easily computable integer values. The higher the score, the better the alignment is!

The E-value gives an indication of the statistical significance of a given pairwise alignment and reflects the size of the database and the scoring system used. The lower the E-value, the more significant the results are. A sequence alignment that has an E-value of 0.05, it means that this similarity has a 5 in 100 (1 in 20) chance of occurring by chance (Altschul *et al.*, 1990; Altschul *et al.*, 1997).

RESULTS

Based on the recent HR2 sequence data available, residue 1160-1189 of spike protein of 2019-nCoV was found highly conserved region as per BLAST with 100% similarity with maximum score of 99.5 with E-value of $5e^{-24}$ (table 1). Thus, this sequence is suggested as a fusion inhibitor for COVID-19. Describing precisely, it is a synthetic peptide composed of 30 amino acids of *Heptad Repeat 2* (HR2) regions of S protein of 2019-nCoV, and is named as QadirVID-19 (fig. 2). As the molecule is a part of viral components, therefore, it has 100% affinity with its target i.e. HR1, logically (fig. 3).

Further, assessing the molecule on Predict Protein software, the peptide showed promising characteristics. In solvent accessibility of QadirVID-19, 87.67% exposed region (fig. 4) indicates the high percentage of the molecule available to interact with its target. Trans-membrane prediction showed that the molecule will not be part of any membrane and has no signaling capacity. Similarly, disulfide bridges prediction predicted no disulfide bridge, endorsing its stability as an effective drug molecule. The software confirmed functions of the molecule and its involvement in membrane fusion and viral attachment to host cell (fig. 5). Functional effects of point mutations were also predicted (fig. 6). If the point mutation is occurring inside the virus corresponding to our molecule, the function was affected by mutation in first three amino acids only (blue region) while the remaining 27 residues remained functional by point mutation. It strongly recommend functionality of our molecule.

PAINS (pan assay interference compounds) is likely to interfere in screening technologies via a number of means but particularly through protein reactivity. They represent poor choices for drug development. But, QadirVID-19 passed the filter established by PAINS-Remover Online Server and confirmed to be an effective drug. Pharmacokinetic studies were performed by using admet SAR and Swiss ADME and are elaborated in table 2. QadirVID-19 is not easily metabolized, so the drug will maintain its serum concentration for a long time.

DISCUSSION

With the emergence of the disease, the workers tried to search the drug/s for its management, but, unfortunately were unable to get an effective targeted therapy. Although, chloroquine and hydroxychloroquine elicit antiviral potential but showed efficacy for COVID-19 induced pneumonia-related complications, and have restricted use if there is no pre-existing contraindication (Gao *et al*., 2020; Guo *et al*., 2020; McKee *et al*., 2020). The protease inhibitor drugs including Ritonavir, Darunavir and Cobicistat, have also been tested. But the

safety and efficacy of these drugs for the treatment of COVID-19 infection were not strong (Dorward *et al*., 2020). However, some workers were able to get a fusion inhibitor (EK1-derived) for COVID-19 (Xia *et al*., 2019) but the amino acid sequence of EK1 corresponding to SARS-CoV-2 are not conservative and enormous mutations are there in this region, and it is more relative to bovine and rodent coronavirus, not to SARS-CoV-2. Liu *et al*., (2004) gave CP-1 molecule for the treatment of SARS-associated coronavirus. But, this molecule is also useless for COVID-19 because its amino acid sequence does not concede to the sequence of the novel coronavirus-2019. However, the sequence optimized by the present study gave 100% similarity to atleast 100 sequences with a score of 99.5 with an E-value of $5e^{-24}$, which was a very good with reference to all other molecules.

The efficacy of the peptide may be increased by different ways, for example, by attaching GSGSG as linker, polyethylene glycol-4 (PEG4) and cholesterol (Chol) to increase the bioavailability of the peptide; the attachments have dramatically increased antiviral potency of the fusion inhibitors (Xia *et al*., 2020; Quinn *et al*., 2017). Therefore, employing the same strategies, the bioavailability of QadirVID-19 may also be increased by attaching GSGSG as linker, polyethylene glycol-4 (PEG4) and cholesterol (Chol). Thus it becomes as NIQKEIDRLNEVAKNLNESLIDLQELGKYE-GSGSG-PEG4-Chol.

Fusion inhibitors have been proved as long-acting antiviral preparations with low toxicity and absence of drug-drug interactions (Quinn *et al*., 2017). Therefore, the said molecule (QadirVID-19) may be undertaken to the trials for further investigation, to save the millions of lives at this emergency situation.

CONCLUSION

The study concluded with the development of a formula of a fusion inhibitor against Novel Coronavirus-2019 for specific management of COVID-19 that was named as QadirVID-19.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic Local Alignment Search Tool. *J. Mol. Biol.*, **215**(3): 403-410.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.,* **25**(17):3389-3402.

Dorward J and Gbinigie K (2020). Lopinavir/ritonavir: A rapid review of effectiveness in COVID-19. The Centre for Evidence-Based Medicine. https://www.cebm.net/ retrieved on 20th April, 2020

Gao J, Tian Z and Yang X (2020). Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci. Trends,* (Advance Publication)

Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY and Yan Y (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. *Military Med. Res.*, **7**(1): 1-10.

Hulswit RJG, de Haan CAM and Bosch BJ (2016). Chapter Two Coronavirus Spike Protein and Tropism Changes, Editor(s): John Ziebuhr, Advances in Virus Research, Academic Press, **96**: 29-57.

Jonathan B (2010). Baell and Georgina A. Holloway. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.*, **53**(7): 2719-2740.

Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P and Jiang S (2004). Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors. *Lancet* **363**(9413): 938-947.

McKee DL, Sternberg A, Stange U, Laufer S and Naujokat C (2020). Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol. Res.*, **157**: 104859.

PredictProtein software: available at https://www.predictprotein.org/

Qadir MI (2011). Qadirvirtide. *Pak. J. Pharm. Sci.*, **24**(4): 593-595.

Qadir MI and Malik SA (2010). HIV Fusion Inhibitors. *Rev. Med. Virol.*, **20**(1): 23-33.

Qadir MI and Malik SA (2011). Genetic Variation in HR Region of env gene of HIV: A Perspective for Resistance to HIV Fusion Inhibitors. *AIDS Res. Hum. Retrovir.*, **27**(1): 57-63.

Quinn K, Traboni C and Penchala SD (2017). A first-in-human study of the novel HIV-fusion inhibitor C34-PEG4-Chol. *Sci. Rep.*, **7**(1): 9447.

World Health Organization. https://www.who.int/ retrieved on 20th April, 2020.

Xia S, Liu M and Wang C (2020). Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res.*, **30**(4): 343-355.

Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng CT, Wang Q, Du L, Tan W, Wilson IA and Jiang S (2019). A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Science Adv.*, **5**(4): eaav4580.