IJPSR (2020), Volume 11, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 June 2020; received in revised form, 26 September 2020; accepted, 29 September 2020; published 01 October 2020

IN-SILICO MODELLING STUDIES ON RITONAVIR AND LOPINAVIR TO COMBAT COVID-19

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Keywords:

Molecular docking, Corona virus, New drug design, *In-silico* study

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ABSTRACT: The coronavirus is a group of families that cause respiratory problems in mammals, which consists of MERS (Middle East Respiratory Syndrome), SARS (Severe Acute Respiratory Syndrome), and COVID-19. The virus attaches to the ACE-2 receptor present in the epithelial cell of the lungs in the infected patients, and the infection may lead to fibrosis if not treated. The size of the genome may vary from 27-34 kilobases, which is the largest size of RNA found till now. The research was carried out to find a potential drug that could inhibit the action of coronavirus, and drugs such as Lopinavir, and Ritonavir derivatives were taken as standards. In the experiment, 21 derivatives were designed by varying substitutions in the structures of Lopinavir and Ritonavir. Further, the designed molecules were subjected to docking for analyzing the binding interactions of the derivative with the active site using the PDB: 2GTB *via* Molegro Virtual Docker 6.0. The docking showed that the top 09 compounds had a higher score than the marketed drugs, while R10 being the most potent, had a Mol Dock score of -225.851 and gave interactions His 164, Asn 142, Glu 166. The marketed drugs Ritonavir and Lopinavir had a MolDock score of -185.386 and -178.251, respectively.

INTRODUCTION: Viruses are the smallest agents that are responsible for causing diseases, which include chickenpox, ebola, hepatitis, herpes, and the most common cough and cold. They are cellular, ultramicroscopic organisms that are made up of nucleic acid covered by a protein sheath known a capsid. These agents remain non-viable in the atmosphere, and when they invade a host, they become active. Viruses are not affected by antibiotics because they lack enzymes. The host may be bacteria, plants, animals, and humans.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(10).5247-53

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5247-53

They contain RNA or DNA as their genome. The size of the RNA/DNA ranges from 20 nm to 300 nm in diameter. The nucleic acid penetrates the host cell, whereas the protein remains outside the cell body. The genome overpowers the genetic machinery of the host cell and uses it for synthesizing viral proteins. It uses the cellular biochemicals from the host for its existence and replication in the host [1, 2].

Viruses are thermolabile *i.e.*, they are temperature resistant and are generally transmitted by a vector. They are host specific. The viruses are regarded as connecting links or intermediate between the living organisms and non-living organisms. The virus contains an envelope, capsid, and nucleoid, which form the ultra-structure of the virus. Bacteriophage attacks or infects the bacteria, and it is also present

in the holy river Ganges which is responsible for purification [3]. Viruses can be segregated on the basis of features like the type of Nucleic acid, Morphology of Virus, Replication of site in the cell, Coating, Serological typing, and cell types infected [4]. The MERS (Middle East Respiratory Syndrome), SARS, and COVID-19 are considered as lethal viruses. One of the viruses which have turned into pandemic from the epidemic is the SARS (Severe Acute Respiratory Syndrome) COVID-19 or novel coronavirus from the family of the corona virus.

The first case of COVID-19 was identified in the city of Wuhan (China) on the 31st December 2019. The symptoms were quite similar to pneumonia-like shortness of breath, cough, fever, dyspnea, tiredness, and frost glass-like symptoms in the lungs. In India, it was first reported on 30th January 2020 in Kerala when students of Indian origin came back from China. As of September 25, 2020, more than 58,13,861 people were infected from the corona.[5]

What is Corona: A coronavirus is a group of family which cause diseases in the mammals. In humans, they are responsible for causing respiratory disorders, which include common cough and lethal viruses such asCOVID–19, SARS, and MERS. Which have an envelope that consists of the nucleocapsid and single-strand RNA genome of helical symmetry. The genome's size may vary from 27 to 34 kilobases, which is the largest size of RNA found till now. The first case of coronavirus was discovered in the 1930's in chickens. The chickens showed respiratory problems, but the human coronavirus was discovered in 1960's, and the first case showed common cold as one of the symptoms [6].

Morphology: The viruses consist of a sizeable particle that is spherical in shape with bulbous like projections, and the diameter was found to be 120 nm. The envelope consists of a lipid membrane that is bilayer in nature, and the membrane, envelope, and spike-like structures are attached to it. In the envelope, there is a nucleocapsid protein that is bound to a positive single-stranded RNA gene in a continuous conformation. The virus is protected by the nucleocapsid, lipid bilayer, and a lipid bilayer envelope [7].

Many cells that were infected by coronavirus were seen within BAL (Bronchial Alveolar Lavage) specimen from a SARS infected patient. The features of the virus are congruent to other viruses present in the family of Corona viridae, and syncytial cells Multinucleated) were infrequently existing. The virons grab an envelope by prospecting into cisternae and formed spherical, occasionally pleomorphic, particles with an average of 78 nm in diameter [3, 8].

Genome: The virus consists of the largest genomes, the RNA virus family, thus giving surplus plasticity in accommodating and improved genes. The G+C (guanine and cytosine) contents in coronaviruses has a range of 32% to 43%. In coding regions, the coronaviruses contain similar genome organization with a unique gene order 5'-replicas ORF (Open Reading Frame)1ab, spike, envelope, membrane, nucleocapsid, 3'- there is a variable number of additional ORF's available in a subgroup of each coronavirus [9].

Mechanism: There are single-stranded RNA viruses that encode 4 essential enzymes to the viral life cycle. The virus attacks on the epithelial cells of lungs by attaching with the ACE-2 receptor with the help of a glycoprotein which is in the form of spike.

The SARS COV-1 has also shown the same attachment with the ACE-2 receptor, like the COVID-19, which occurred in 2003, China. The attachment causes the transfer of the genetic material into the specific cell on which the virus has attacked; the genetic material is responsible for the replication of the virus. When it critical order, viruses are formed, and the cell melts, which causes the infection to proliferate. In its incubation time, in multiples rapidly to millions, which infects the complete respiratory tract [10]. After this, when the immune cells suppress the virus, it causes over action of immune cells as these cells communicate with the help of cytokines. These agents then cause overproduction of immune cells, which end up using a lot of resources. When the neutrophils and killer T-cells try to hinder the action of the corona virus, their (neutrophils and T-cells) action of fighting against the infected cells and the virus is inhibited and thus causing confusion between them.

This may lead to fibrosis in the lungs. The mechanism of the virus is complicated and differs greatly in virus families and strains. The fusion may happen directly at the cell surface after it binds with the receptor or in endocytosis [11].

Causative Agents: The coronavirus family is said to be present in people and many animals such as bats, camels, rats, and cattles. It has also been discovered that dromedary camels harbor different Human Coronaviruses. The transmission of the virus from animals into a human is quite rare. The family of coronavirus is considered a large family [12, 13].

Why Lopinavir and Ritonavir: The combination of these drugs are given to the patients who are suffering from HIV- AIDS, and its combination is called as kaletra but has been found to be effective in COVID-19 treatment. If this drug is given in the early stage of COVID-19, then a treatment regimen consisting of the above drugs may be effective. The drug as found to be less effective in the patients in later stages. In a trial that was conducted with 199 patients who were SARS-COV-2 positive, 99 were assigned in the lopinavir-ritonavir group and 100 to standard treatment.

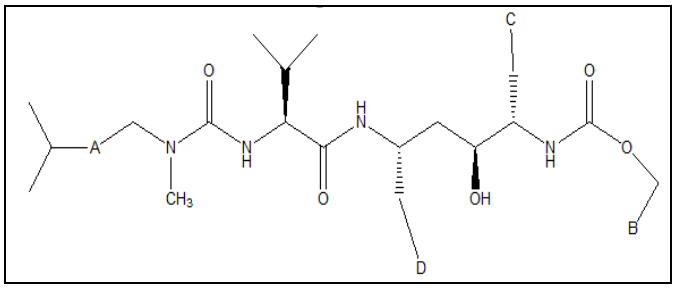
The adverse effects in GIT were common in the lopinavir-ritonavir group, but serious adverse effects were common in standard treatment. In the course of the trial, 33% of patients were administered systemic glucocorticoids in lopinavir-ritonavir group, and in standard treatment, 35.7% was administered. The old-age patients who were infected by the virus, any kind of benefit were not observed in lopinavir-ritonavir treatment [14].

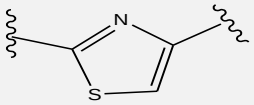
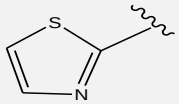
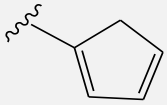
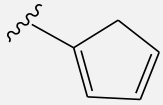
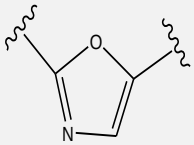
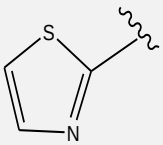
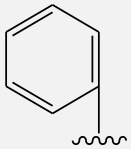
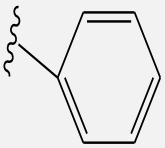
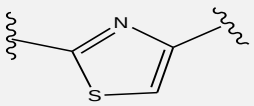
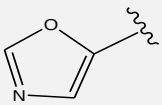
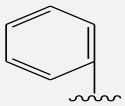
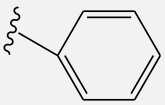
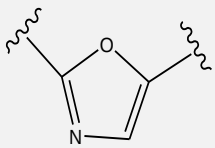
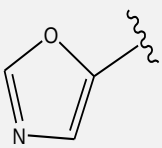
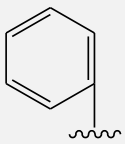
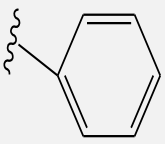
Objective of Research: The objective of the research was to find a novel efficacious drug that could inhibit the action of the corona virus that would be helpful in fighting against COVID-19 *via* using rational drug design tools like Molecular Docking.

MATERIALS AND METHOD:

Designing of Compounds: Eleven derivatives were designed based on the varying the substitutions in the structure of Lopinavir, and likewise, ten derivatives were designed on changing the substitutions in Ritonavir structure as shown in **Table 1** and **2**. All the designed compounds were subjected to a docking study using the available software for the analysis of binding interactions in the active site.

TABLE 1: STRUCTURE OF DESIGNED RITONAVIR BASED DERIVATIVES



Derivative	A	B	C	D
01				
02				
03				
04				

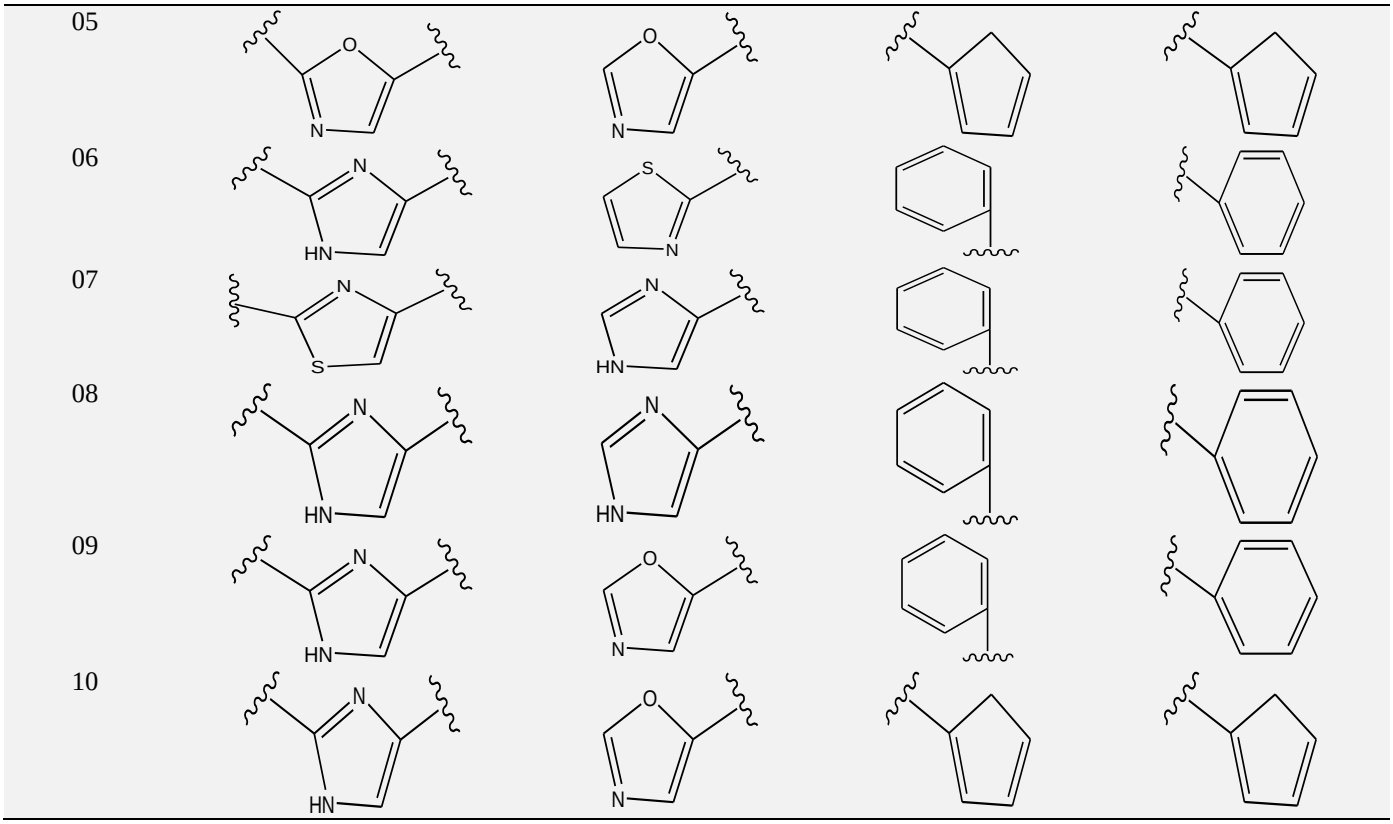
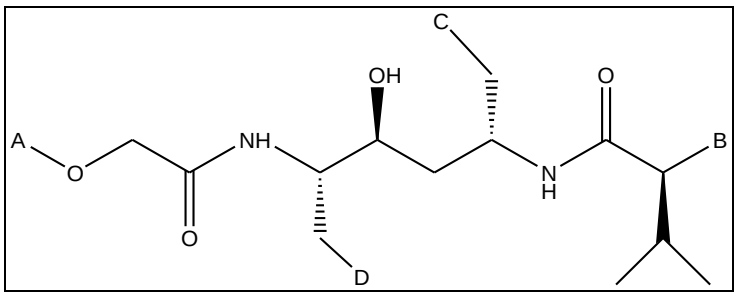
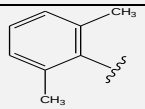
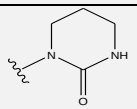
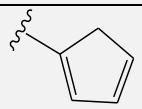
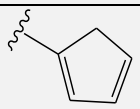
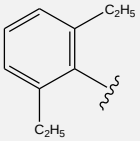
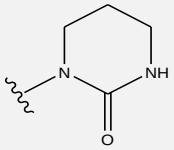
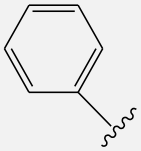
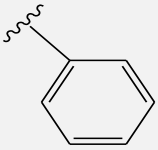
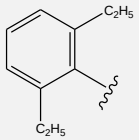
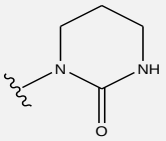
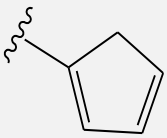
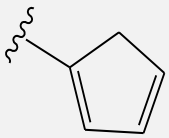
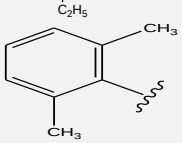
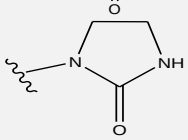
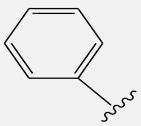
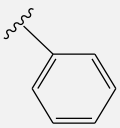
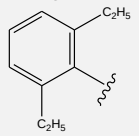
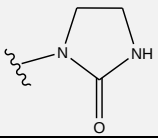
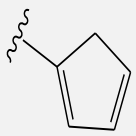
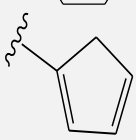
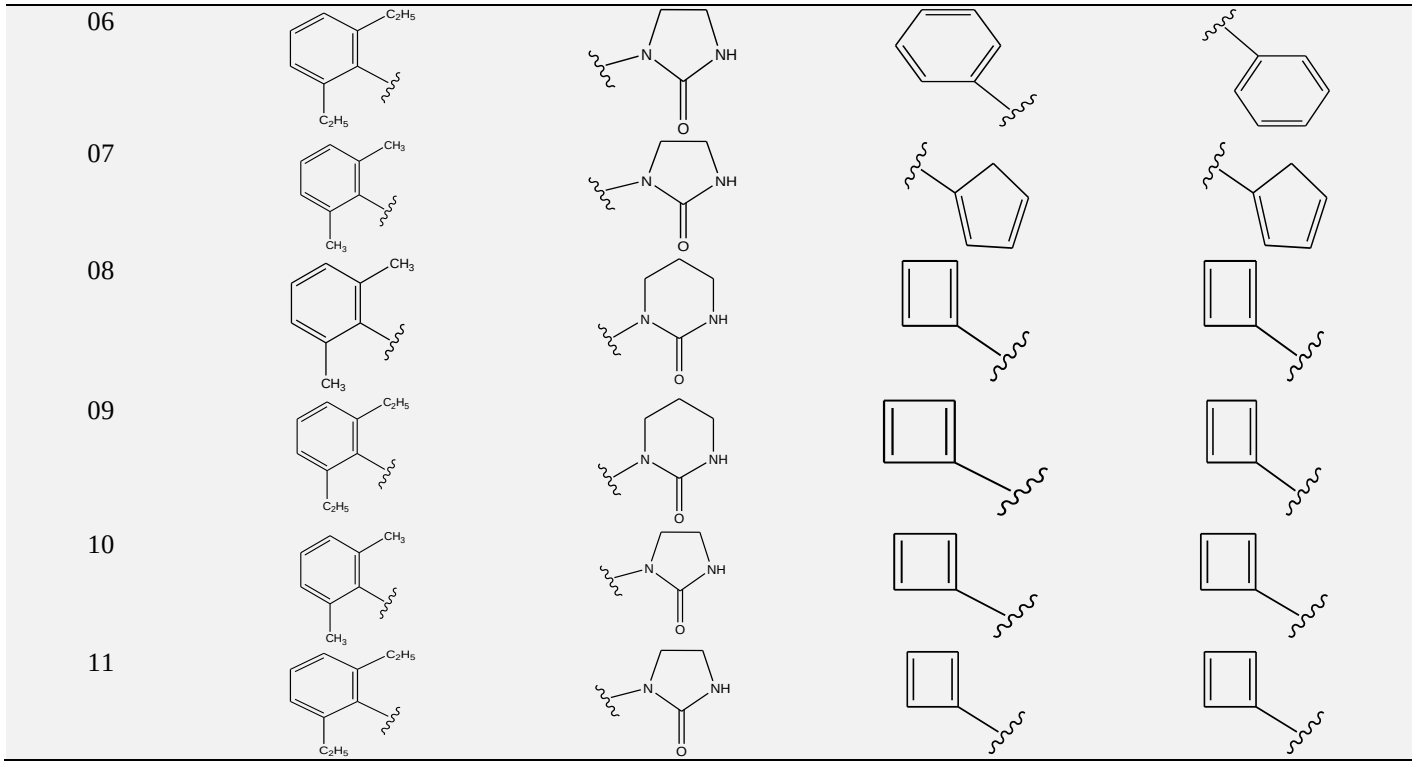


TABLE 2: STRUCTUREOF DESIGNED LOPINAVIR BASED DERIVATIVES



Derivative	A	B	C	D
01				
02				
03				
04				
05				



Drawing and Energy Minimization: The two-dimensional (2D) structures of 21 molecules were drawn using Chem Draw Ultra 8.0 [15]. These 2D structures were transformed into 3D structures using the Chem 3D Ultra 8.0, followed by energy minimization via MM2 (Molecular Mechanics) force field and re-optimized with MOPAC (Molecular Orbital Package) having RMS (Root Mean Square) gradient value of 0.01 Kcal/ mol Aº.

Selection of PDB: As found in the literature review, the structure of the novel coronavirus resembles quite to the SARS-CoV and MERS-CoV [16]. To conduct a target and structure-based molecular docking study, PDB: 2GTB was selected that had a resolution of 2Aº [17].

Docking Studies: SARS Corona Virus crystal structure (PDB 2GTB) was obtained from the RCSB Protein Data Bank. All the designed structures and reference compounds ritonavir and lopinavir were imported in workspace of Molegro Virtual Docker 6.0 [18]. The Protein preparation was carried out and bonds, bond orders, hydrogen atoms and charges were assigned whereas water molecules were removed. The crystal structure of SARS corona virus with PDB code 2GTB having a resolution of 2.0Aº was used for docking study. The binding cavities were detected through an automated process. A maximum of five cavities were detected, the grid resolution and probe size were fixed individually. The docking calculations were performed using Mol Dock SE as search algorithm. The poses generated were categorized by Mol Dock score and Rerank score. Generally, the more negative the Mol Dock score, the better the binding occurred.

RESULTS AND DISCUSSION: To validate the docking study, the crystal structure of the protein (PDB: 2GTB) and the cognate ligand were docked together. As is shown in **Fig. 1**, it can be seen that the re-docked ligand and the crystal structure are almost superimposed together. This means that the docking method is quite reliable.

Table 3 shows the docking scores of top 10 compounds along with the standard drugs Ritonavir and Lopinavir. The compound R10 showed the highest MolDock score of -225.851 and Rerank Score of -100.893, having interactions that can be seen in **Fig. 2** His164, Asn 142, Glu 166 that matched along with that one's reported in PDB as in **Fig. 3,** which was quite higher than the current drugs Ritonavir and Lopinavir being used for the treatment of COVID-19. **Fig. 4** shows the secondary structure arrangement for compound R-10.

TABLE 3: DOCKING SCORES OF TOP 10 MOLECULES

S. no.	Compound Number	MolDock Score	Rerank Score	Interactions
01	R10	-225.851	-100.893	His164, Asn142, Glu166
02	R4	-220.249	-158.300	His164, Gln189
03	R3	-215.839	-118.685	His164, Asn142, Glu166, Gly143,Gln189
04	R8	-215.307	-101.956	Glu166,Gln189
05	R6	-207.639	-115.870	His164,Gln189
06	R1	-202.339	-15.205	His164,Asn142
07	L5	-196.493	-132.005	His164,Glu166
08	L1	-194.197	-118.161	Glu166,Asn142, Gly143
09	R5	-193.296	-70.154	His164,Gly143
10	R7	-185.080	-99.263	His163,Gly143
11	Ritonavir (R)	-185.386	-2.415	Glu166,Gly143
12	Lopinavir (L)	-178.251	-106.178	His164,Asn142

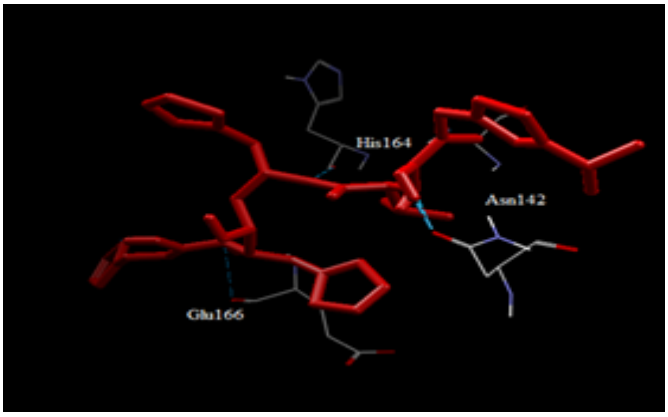


FIG. 1: HYDROGEN BONDING INTERACTIONS- R10

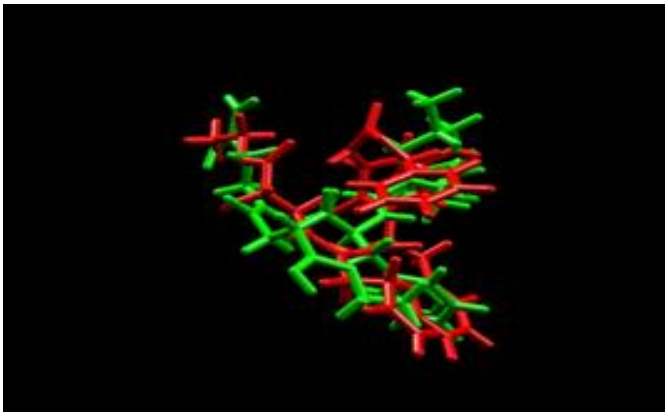


FIG. 2: SUPERIMPOSITION SHOWING VALIDATION

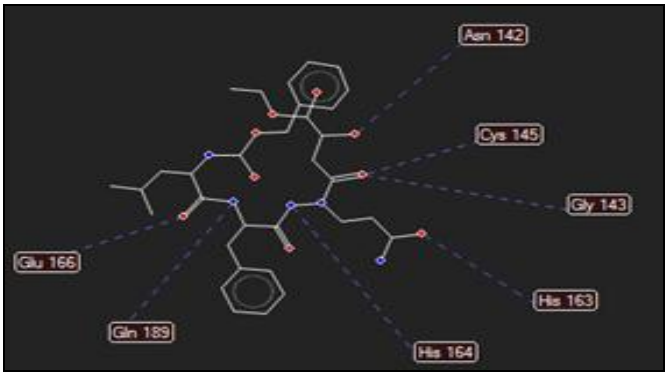


FIG. 3: INTERACTIONS REPORTED IN PDB

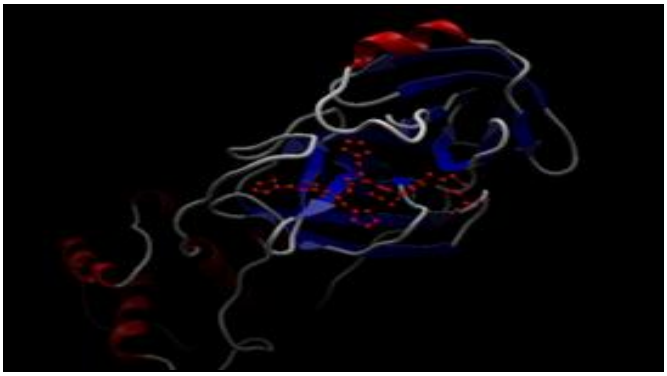


FIG. 4: SECONDARY STRUCTURE IMAGE- R10

CONCLUSION: Molecular docking study was applied to designed derivatives to find a more potent drug for the treatment of COVID-19. The docking results showed that compound R-10 was most active, having a high MolDock score and Rerank Score of -225.851 and -100.893, respectively. The entire compounds designed had good binding affinity, while the top 09 compounds had a higher score than the drug Ritonavir and Lopinavir. Further, *in-vitro* and *in-vivo* studies may be done that may be helpful in designing more potent analogues to fight against this pandemic COVID-19.

ACKNOWLEDGEMENT: The authors would like to thank Dr. Rajesh Sharma, Head, School of Pharmacy, D.A.V.V., Indore, for providing the facility for the work.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Lemke TL, Williams DA, Roche VF and Zito WS: Foye's principles of medicinal chemistry. Wolters Kluwer (India) Pvt Ltd, Eighth Edition 2019.
2. Tripathi KD: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd Eighth Edition 2018.

3. Pelczar JM, Chan ECS and Krieg RN: Microbiology. McGraw Hill Education Fifth Edition 2018.
4. Beale MJ and Block HJ: Wilson and Giswold's Organic Medicinal and Pharmaceutical Chemistry. Lippincott Williams and Wilkins, Twelfth Edition 2011.
5. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R and Niu P: A novel corona virus from patients with pneumonia in China. The New England Journal of Medicine 2020; 1-7.
6. Peng Z, Yi F, Kai Z and Zheng SL: Bat corona viruses in China .Viruses 2019; 11: 1-14.
7. Su S, Wong G, Shi W, Liu J, Lai AC, Zhou J, Liu W, Bi Y and Gao GY : Epidemiology, genetic recombination, and pathogenesis of corona viruses. Trends in Microbiology 2016; 24: 490-02.
8. Goldsmith CS, Tatti KM, Ksiazek TG, Rollin PE, Comer JA, Lee WW, Rota PA, Bankamp B, Bellini WJ and Zaki SR: Ultra structural characterization of SARS corona virus. Emerging Infectious Diseases 2004; 10: 320-26.
9. Patrick CYW, Huang YI, Susanna KPL and Kwok YY: Corona virus Genomics and Bioinformatics Analysis. Viruses 2010; 2: 1806-20.
10. Renhong Y, Yuanyuan Z, Yaning LI, Lu X, Yingying G and Qiang Z: Structural basis for the recognition of SARS-COV-2 by full-length human ACE. Science 2020; 367: 1444-48.
11. Belouzard S, Millet KJ, Licitra NB and Whittaker RG: Mechanisms of Corona virus Cell Entry Mediated by the Viral Spike Protein. Viruses 2012; 4: 1020.
12. Corman MV, Muth D, Niemeyer D and Drosten C: Host Sources of Endemic Human Corona viruses. Advances in Virus Research 2018; 100: 170-77.
13. Fehr RA and Perlman S: Corona viruses- An overview of their Replication and Pathogenesis. Methods in Molecular Biology 2015; 1282: 1-23.
14. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei W and Li: A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. The New England Journal of Medicine 2020; 1-13.
15. CS Chem Office, Version 8.0, Cambridge Soft Corporation, Software Publishers Association, Washin-gton, United States of America 2011.
16. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K and Hilgenfeld : Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Scie 2020; 368: 409-12.
17. Salim B and Noureddine M: Identification of Compounds from *Nigella sativa* as New Potential Inhibitors of 2019 Novel Corona virus (COVID-19): Molecular Docking Study. Chem Rxiv 2020: 1-12.
18. Molegro Virtual Docker, Version 7.0, Molegro ApS, CLC Bio Software Arhus Denmark 2019.

How to cite this article:
Tuteja JS and Chitnis K: *In-silico* modelling studies on ritonavir and lopinavir to combat Covid-19. Int J Pharm Sci & Res 2020; 11(10): 5247-53. doi: 10.13040/IJPSR.0975-8232.11(10).5247-53.