ISSN 2582-788X RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF
APPLIED CHEMICAL AND BIOLOGICAL SCIENCES

BIOSTATISTICAL APPROACH TO AVAILABLE ANTIVIRAL TARGETS FOR CONQUERING COVID-19

Jasdev Singh Tuteja*, Shilpa Elsa Mathew, Priti Patidar, Riya Sarkar, Anil Prajapati

School of Pharmacy, Devi Ahilya Vishwavidyalaya, Takshashila Campus, Indore, India

ABSTRACT

Corona virus is one of the significant pathogens which damages the human respiratory functioning. Deaths and casualties caused by coronaviruses (CoVs) include the Severe Acute Respiratory Syndrome (SARS)-CoV and the Middle East Respiratory Syndrome (MERS)-CoV. The aim of the work was to compare several antiviral drugs and find out which is the most active drug against MERS, SARS and COVID -19 and also to determine correlation coefficient (r) for binding affinity of these compounds between MERS, COVID-19 targets and SARS, COVID-19 targets. In this study Molecular Docking approach was used to determine the binding affinities of 62 antiviral molecules. The study was carried out using Molegro Virtual Docker 6.0 with PDB 5DUS, 3V3M, 6W63 procured from RCSB Protein Data Bank (PDB). Indinavir, Saquinavir, Simeprevir were discovered to be most potent having MolDock scores -190.337, -206.847, -239.807 when docked with PDB 5DUS, 3V3M, 6W63 respectively. The correlation coefficient (r) was calculated to be 0.97 for binding affinity of compounds between MERS and COVID-19 targets whereas 0.94 for relating SARS and COVID-19 targets. Further studies may be conducted to design more potent analogue and defeat COVID-19.

Keywords: Molecular Docking, Molegro Virtual Docker 6.0, Correlation Coefficient, Corona Virus

INTRODUCTION

Corona virus is one of the critical pathogens that essentially centers on the human respiratory system [1]. Past flare-ups of coronaviruses (CoVs) join the Severe Acute Respiratory Disorder (SARS)- CoV and the Middle East Respiratory Syndrome (MERS)-CoV which have been already depicted as administrators which are dangerous to mankind [2]. A batch of patients was admitted to emergency clinics with a fundamental finding of pneumonia of an unknown etiology in late December 2019. These patients were epidemiologically associated to a sea food and wet animal wholesale market in Hubei Region, Wuhan, China [3].

In December 2019, the primary cases were reported [4] and from then within 10 days five patients were hospitalized with serious respiratory difficulty condition, out of which one died [5]. By January 2, 2020, 41 admitted emergency clinic patients had been perceived as having lab certified COVID-19 contamination, not actually half of these patients had fundamental diseases, including diabetes, hypertension, and cardiovascular disorder [6]. These patients were presumed to be debased in that clinical

facility, likely in case of nosocomial disease. It was assumed that the COVID-19 is certainly not a super-hot spreading disease (spread by one patient to various others), yet rather likely spread as a result of various patients getting contaminated at various zones all via emergency clinic through unclear segments. Furthermore, just patients that turned out to be clinically sick were attempted, in like manner there were likely significantly more patients that were clearly contaminated.

By January 22, 2020 a total of 571 instances of the COVID-19 were represented in 25 regions in China [7]. China's National Health Commission uncovered the subtleties of the underlying 17 deaths up to January 22, 2020. On January 25, 2020, a total of 1975 cases were certified to be tainted with the COVID-19 in the region of China with a whole of 56 deaths [8] but an another report as per January 24, 2020 assessed the consolidated event in China to be 5502 cases [9]. By January 30, 2020, total of 7734 cases have been stated by China and 90 distinct cases have moreover been represented from different countries that fuse Taiwan, Thailand, Vietnam, Malaysia, Nepal, Sri Lanka, Cambodia, Japan, Singapore, Republic of Korea, United Arab Emirates, US, The Philippines, India, Australia, Canada, Finland, France, and Germany. The case causality rate was resolved to be 2.2% (170/7824) [10].

At the hour of setting up this manuscript (June 02, 2020), the World Health Organization announced 6 194 533 as total confirmed cases with 113 198 new cases in 24 hours and 376 320 total deaths with 4 242 deaths in 24 hours [11].

The period from the earliest starting point of COVID-19 signs to death went from 6 to 41 days with a median of 14 days. This period is dependent upon the age of the patient and status of the patient's immune system. It was observed that patients above the age of 70 are at higher risk than those underneath this age [8]. The most widely recognized symptoms of COVID-19 at early stage of infection were fever, cough, and weakness, while other symptoms incorporate sputum production, headache, hemoptysis [6], diarrhea [6], dyspnea [5], and lymphopenia [6].

Clinical highlights by a chest CT scan indicated pneumonia, nonetheless, there were unpredictable highlights, for instance, RNAaemia, acute respiratory distress syndrome, acute cardiac injury, and incidence of grand-glass opacities that prompted death [6]. A few cases revealed various fringe ground-glass opacities in subpleural regions of both lungs [12] that possible actuated both systemic and localized immune response that incited to expanded aggravation. Unfortunately, treatment of certain cases with interferon inhalation indicated no clinical effect and rather appeared to worsen the condition by advancing pulmonary opacities [12].

Likewise, taking into account results from chest radiographs upon affirmation, a segment of the cases shows an attack in the upper flap of the lung that is connected with growing dyspnea with hypoxemia [13]. However patients tainted with COVID-19 demonstrated gastrointestinal side effects like diarrhea, then again a low degree of MERS-CoV or SARS-CoV patients experienced comparable GI trouble [14]. Consequently, it is basic to test fecal and urine samples to dismiss a potential elective course of transmission, expressly through health care workers, patients, etc. [15]

Patients infected with COVID-19 exhibited higher leukocyte numbers, irregular respiratory revelations, and extended degrees of plasma pro-inflammatory cytokines [12]. One of the COVID-19 case reports showed a patient at 5 days of fever gave a cough, coarse breathing sounds of the two lungs, and a bit

higher body temperature (39 °C). The patient's sputum revealed positive result with real time polymerase chain reaction (rtPCR) that certified COVID-19 disease. The laboratory examinations demonstrated leucopenia with leukocyte counted up to 2.91 × 109 cells/L of which 70.0% were neutrophils. Also, an estimation of 16.16 mg/L of blood C-reactive protein was noted which is over the common range (0–10 mg/L). High erythrocyte sedimentation rate and D-dimer were likewise observed [12].

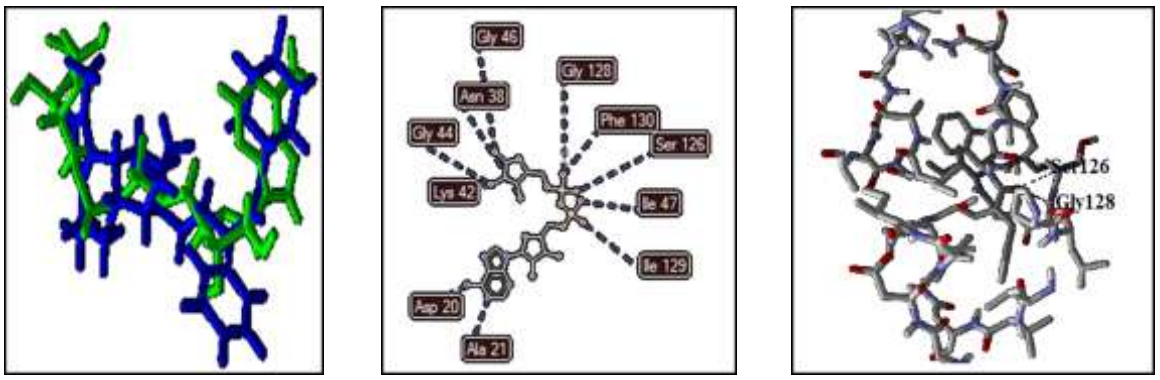
Genomic sequence analysis of COVID-19 exhibited 88% identity with two bat-derived severe acute respiratory syndrome (SARS)- like coronaviruses, [16] [17] demonstrating that mammals are the most plausible association between COVID-19 and individuals. In a report, it is stated that women in their third trimester who were certified to be tainted with the coronavirus, there was no evidence that there is transmission from mother to child. In any case, every single pregnant lady experienced cesarean sections, so it remains ill-defined whether transmission can occur during vaginal birth. This is huge in light of the fact that pregnant ladies are decently more vulnerable to disease by respiratory pathogens and serious pneumonia [18].

The binding of a receptor communicated by host cells is the underlying advance of viral infection followed by fusion with the cell membrane. It is observed that the lung epithelial cells are the primary target of the disease. Subsequently, it has been represented that human-to-human transmissions of SARS-CoV occurs by the binding between the receptor-binding domain of virus spikes and the cell receptor which has been recognized as Angiotensin-Converting Enzyme 2 (ACE2) receptor [17,19]. Critically, the sequence of the receptor-binding domain of COVID-19 spikes is like that of SARS-CoV. This data unequivocally suggests that passage into the host cells is without a doubt by methods for the ACE2 receptor [17].

At present, there are no specific antiviral medications or vaccine against COVID-19 ailment for potential treatment of individuals. The primary decision accessible is using broad-spectrum antiviral drugs like Nucleoside analogs and furthermore HIV-protease inhibitors that could decrease infection disease until the specific antiviral becomes available [7]. The treatment that have so far been tried shown that 75 patients were administrated existing antiviral medications. The course of treatment included two times every day oral administration of 75 mg oseltamivir, 500 mg lopinavir, 500 mg ritonavir and the intravenous organization of 0.25 g ganciclovir for 3–14 days [20]. Another report demonstrated that the broad spectrum antiviral remdesivir and chloroquine are significantly convincing in the control of 2019-nCoV infection in-vitro. These antiviral drugs have been used in human patients with a safety track record. In this way, these remedial experts can be considered to treat COVID-19 infections [21].

MATERIALS AND METHODS

The study begins with carrying out the literature survey on COVID-19. Research and review papers were explored for available compounds that have antiviral property [22] [23]. These compounds were drawn using software Chem Draw Ultra 8.0. These 2D structures were transformed into the 3D structures using the Chem 3D Ultra 8.0 followed by energy minimization via MM2 (Molecular Mechanics) force field and re-optimized with Molecular Orbital PACkage (MOPAC) having Root Mean Square (RMS) gradient value of 0.01 Kcal/ mol Aº.



(a) (b) (c)

Fig 1. Docking analysis of MERS-CoV

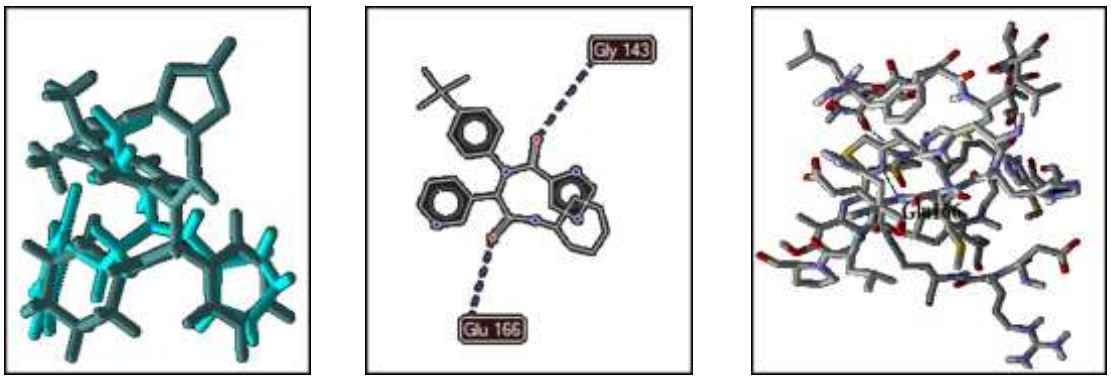
(a) Validation Showing superimposition,

(b) 2-D Ligand H bond interaction in the PDB,

(c) Dock pose of most active compound

To conduct a target based molecular docking study, three PDB's (Protein Data Bank) were selected on the basis of minimum resolution and literature review of MERS (PDB ID: 5DUS) [24], SARS-1 (PDB ID: 3V3M) [25] and COVID-19 (PDB ID: 6W63) [26] that had resolutions of 1.43Aº, 1.96Aº, 2.10Aº . The crystal structures were obtained from the RCSB Protein Data Bank. All the structures were imported to Molegro Virtual Docker 6.0. The protein preparation was carried out and bonds, bond orders, hydrogen atoms and charges were assigned whereas water molecules were removed.

The binding cavities were detected by the software, of which the largest one was selected out of five cavities for further process. The docking took place via the docking suite of the software. The poses generated were rearranged by MolDock score. Generally, the more negative the MolDock score, the better is the binding efficiency. These MolDock scores were used to calculate correlation coefficient using Karl-Pearson approach via the formula given



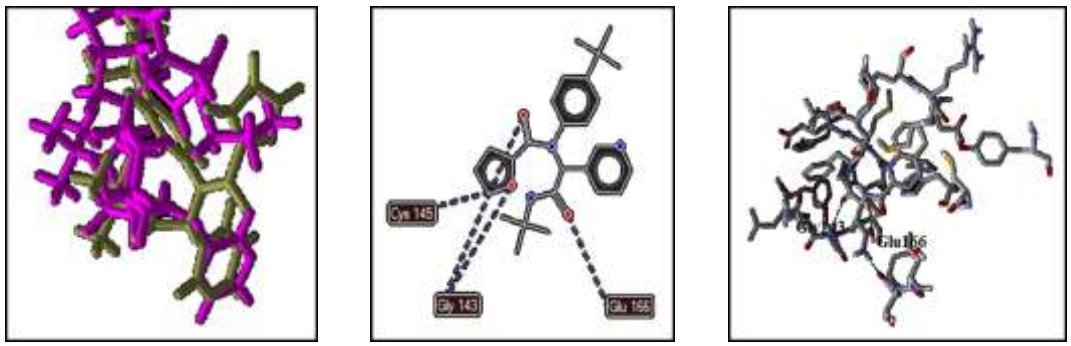
(a) (b) (c)

Fig 2. Docking analysis of COVID-19

(a) Validation Showing superimposition

(b) 2-D Ligand H bond interaction in the PDB

(c) Dock pose of most active compound



(a) (b) (c)

Fig 3. Docking analysis of SARS-CoV

(a) Validation Showing superimposition

(b) 2-D Ligand H bond interaction in the PDB

(c) Dock pose of most active compound

below:

$$r = \Sigma\, a*b \,/\, \sqrt{(\Sigma a^2.\Sigma b^2)}$$

$$r = \Sigma\, c*b \,/\, \sqrt{(\Sigma c^2.\Sigma b^2)}$$

Where, r = coefficient of correlation, a = deviation of "A" variable, b = deviation of "B" variable, c =deviation of "C" variable.

RESULTS AND DISCUSSION

For validation of the study, the cognate ligand and the co crystallized ligand were docked together to check for the superimposition. It was revealed that all PDBs used in the study were superimposed making the process authentic [Fig. 1(a), 2(a) and 3(a)]. The molecule with the highest MolDock score with respect to the PDB 5DUS, 3V3M, 6W63 were found to be Indinavir, Saquinavir, Simeprevir having MolDock scores - 190.337, -206.847 and - 239.807 respectively. Furthermore, the correlation coefficient (r) was calculated to be 0.97 for binding affinity of compounds between MERS and COVID-19 targets whereas 0.94 for relating SARS and COVID-19 targets. The correlation coefficient value clearly demonstrates higher degree of correlation between the MolDock scores of MERS and COVID-19 target i.e. r = 0.97 than the MolDock scores of SARS and COVID-19 target i.e. r = 0.94 that implicates more resemblance of COVID-19 target to MERS to that of SARS.

Table 1. Moldock score, Rerank score of top 10 antiviral compounds when docked with PDB 5DUS, 6W63, 3V3M with their H-bond values and interactions.

Name of PDB	Name of Compound	Moldock Score	Rerank Score	H-Bond	H-bond Interactions
5DUS	Indinavir	-190.337	-145.934	-3.3544	Gly128, Ser126
	Raltegravir	-178.48	-105.519	-7.4155	Gly128, Ser126, Phe130
	Sofosbuvir	-177.477	-125.787	-10.806	lle47, Phe130
	Amprenavir	-164.01	-1.27393	-10.533	Gly128, Ala21, lle129, Phe130
	Delavirdine	-163.192	-100.409	-1.7997	lle47
	Dipivoxil	-163.041	-132.301	-8.2934	lle47, lle129
	Telaprevir	-162.848	207.834	-5.9953	Gly128, Phe130
	Rilpivirine	-161.595	-123.668	-5.3732	Asp20
	Valganciclovir	-150.496	-124.013	-8.2318	Gly128, Ser126,
	Fosamprenavir	-148.301	73.0223	-7.3635	Ser126
6W63	Simeprevir	-239.807	-154.963	-1.5102	Glu166
	Saquinavir	-211.987	-134.803	-3.3124	Glu166
	Lopinavir	-205.705	-126.43	-8.0716	Glu166
	Fosamprenavir	-195.898	-136.792	-7.0349	Glu166
	Amprenavir	-189.426	-132.195	-9.7126	Gly143
	Ritonavir	-187.033	-117.307	-7.1773	Glu166
	Atazanavir	-185.598	-96.9726	-8.4373	Gly143
	Darunavir	-185.108	-142.138	-5.2521	Glu166
	Boceprevir	-175.8	-124.334	-4.1985	His163
	Sofosbuvir	-171.497	-132.722	-11.176	Glu166
3V3M	Saquinavir	-206.847	-156.499	-5.2102	Glu166, Gly143
	Indinavir	-176.056	-133.755	-7.3563	Glu166
	Fosamprenavir	-172.097	-57.7634	-6.163	Glu166
	Darunavir	-172.018	-135.423	-2.846	Gly143
	Tipranavir	-171.438	-112.92	-6.3176	Glu166
	Maraviroc	-169.41	-115.639	-0.7954	Glu166
	Boceprevir	-169.154	-114.418	-11.421	Gly143, His163
	Sofosbuvir	-163.867	-112.536	-10.655	Gly143, His163
	Amprenavir	-159.524	-110.585	-8.2811	His163
	Tenofovir	-159.293	-112.74	-5.5791	Glu166

Table 2. Calculations to determine correlation coefficient (r) for binding affinity of compounds between MERS and COVID-19 targets. The MolDock score obtained from PDB: 5DUS was considered as "A" and MolDock score obtained from PDB: 6W63 as "B".

Sr. No.	Moldock Score (A)	$\bar{A}$ = ΣA/N where N = total number of observations	a = A - $\bar{A}$	a^2	Moldock Score (B)	$\bar{B}$ = ΣB/N where N = total number of observations	b = B - $\bar{B}$	b^2	a*b
1	190.337		24.25	587.9	239.807		45.02	2027	1091.6
2	178.48		12.39	153.5	211.987		17.2	295.9	213.11
3	177.477		11.39	129.65	205.705		10.92	119.2	124.33
4	164.01		-2.08	4.33	195.898		1.11	1.24	-2.31
5	163.192		-2.9	8.4	189.426		-5.36	28.73	15.54
6	163.041		-3.05	9.3	187.033		-7.75	60.11	23.64
7	162.848		-3.24	10.51	185.598		-9.19	84.42	29.79
8	161.595		-4.5	20.21	185.108		-9.68	93.66	43.51
9	150.496		-15.59	243.19	175.8		-18.99	360.5	296.07
10	149.428		-16.66	277.64	171.497		-23.29	542.4	388.05
Total	ΣA = 1660.904	166.09		Σa^2 = 1444.62	ΣB = 1947.859	194.786		Σb^2 = 3613.0	Σa*b = 2223.34

The values of Σa^2, Σb^2 and Σa*b were applied in the formula to determine correlation coefficient which was found to be 0.97.

Table 3. Calculations to determine correlation coefficient (r) for binding affinity of compounds between SARS and COVID-19 targets. The MolDock score obtained from PDB: 3V3M was considered as "C" and MolDock score obtained from PDB: 6W63 as "B".

Sr. No.	Moldock Score (C)	$\bar{C}$ = ΣC/N where N = total number of observations	c = C - $\bar{C}$	c^2	Moldock Score (B)	$\bar{B}$ = ΣB/N where N = total number of observations	b = B - $\bar{B}$	b^2	c*b
1	206.847		34.877	1216.4	239.807		45.027	2027	1570.4
2	176.056		4.086	16.69	211.987		17.207	296.1	70.3
3	172.097		0.127	0.01	205.705		10.925	119.4	1.38
4	172.018		0.048	0	195.898		1.118	1.24	0.053
5	171.438		-0.532	0.28	189.426		-5.354	28.66	2.84
6	169.41		-2.56	6.55	187.033		-7.747	60.01	19.83
7	169.154		-2.816	7.92	185.598		-9.182	84.3	25.85
8	163.867		-8.103	65.65	185.108		-9.672	93.54	78.37
9	159.524		-12.446	154.9	175.8		-18.98	360.2	236.22
10	159.293		-12.677	160.7	171.497		-23.283	542.1	295.15
Total	ΣC = 1719.704	171.97		Σc^2 = 1629.15	ΣB = 1947.859	194.786		Σb^2 = 3612.99	Σc*b = 2300.44

The values of Σc^2, Σb^2 and Σc*b were applied in the formula to determine correlation coefficient which was found to be 0.94.

CONCLUSION

On the basis of docking study, it can be concluded that drugs like Indinavir, Saquinavir, Simeprevir were discovered to be most potent having MolDock scores -190.337, -206.847, -239.807 when docked with PDB 5DUS, 3V3M, 6W63 respectively. The correlation coefficient (r) was calculated to be 0.97 for binding affinity of compounds between MERS and COVID-19 targets whereas 0.94 for relating SARS and COVID-19 targets. Further in-vitro and in-vivo studies may be conducted to design more potent analogues and the clinical trials may be conducted to evaluate its efficacy.

ACKNOWLEDGEMENT

We would like to thank Dr. Rajesh Sharma, Professor & Head, School of Pharmacy, D.A.V.V., Indore for providing the facility for this work.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

SOURCE/S OF FUNDING

No source of funding

REFERENCES

1. Rothan HA, Byrareddy SN (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak, *Journal of autoimmunity*; **102433**: 1-4.

2. Watts BA, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. (2020). Pneumonia of unknown etiology in Wuhan, China: potential for international spread via commercial air travel, *Journal of Travel Medicine*: 1-3.

3. Lu H, Stratton CW, Tang YW (2020). Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle, *Journal of Medicine Virology*; **92(4)**: 401–402.

4. Toit Du. A (2020). Outbreak of a novel coronavirus, *Natural Review Microbiology*; **18(3)**: 123.

5. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T, and Li H (2020). Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study, *Chinese Medicine Journal*: 1-10.

6. Huang C. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, *Lancet*; **395(10223)**: 497–506.

7. Lu H. (2020). Drug treatment options for the 2019-new coronavirus (2019-nCoV), *Bio science Trends*; **14(1)**: 69-71.

8. Wang W, Tang J, Wei F (2020). Updated understanding of the outbreak of 2019 novel coron6avirus (2019-nCoV) in Wuhan, China, *Journal of Medicine Virology*; **92(4)**: 441–447.

9. Nishiura H, Jung SM, Linton NM, Kinoshita R, Yang Y, Hayashi K & Akhmetzhanov AR(2020). The extent of transmission of novel coronavirus in Wuhan, China, 2020, *Journal of Clinical Medicine*; **330(9)**: 1-5.

10. Bassetti M, Vena A, Giacobbe DR. (2020). The Novel Chinese Coronavirus (2019-nCoV) Infections: challenges for fighting the storm, *European Journal of Clinical Investivagation*; **(13209)**:1-4.

11. WHO (2020). Coronavirus disease (COVID-2019) situation reports, retrieved from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

12. Lei J, Li J, Li X, Qi X, (2020). CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia, *Radiology*; **295(1)**: 18.

13. Phan LT, Nguyen TV, Luong QC, Nguyen TV, Nguyen HT, Le HQ, & Pham QD (2020). Importation and human-to-human transmission of a novel coronavirus in Vietnam, *New England Journal of Medicine*; **382(9)**: 872-874.

14. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, & Makhdoom HQ (2013). Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *The Lancet infectious diseases*; **13(9):** 752-761.

15. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, & Lui SF. (2003). A major outbreak of severe acute respiratory syndrome in Hong Kong. *New England Journal of Medicine*; **348(20):** 1986-1994.

16. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, & Bi Y. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*; **395(10224)**: 565-574.

17. Wan Y, Shang J, Graham R, Baric RS, Li F. (2020). Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS, *Journal of Virology*; **94(7)**: 1-9

18. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, & Liao J. (2020). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *The Lancet*; **395(10226):** 809-815.

19. Jaimes JA, Millet JK, Stout AE, Andre NM, Whittaker GR. (2020). A tale of two viruses: the distinct spike glycoproteins of feline coronaviruses, *Viruses*; **12(1)**, 83 : 1-14.

20. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, & Yu T. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet;* **395(10223)**: 507-513.

21. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, & Xiao G. (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell research*; **30(3)**: 269-271.

22. De Clercq E, Li G. (2016). Approved antiviral drugs over the past 50 years, *Clinical microbiology reviews*; **29(3)**: 695-747.

23. Chaudhuri S, Symons JA, Deval J. (2018). Innovation and trends in the development and approval of antiviral medicines: 1987–2017 and beyond, *Antiviral research*; **155**: 76-88.

24. Cho CC, Lin MH, Chuang CY, and Hsu CH, (2016). Macro domain from Middle East respiratory syndrome coronavirus (MERS-CoV) is an efficient ADP-ribose binding module crystal structure and biochemical studies. *Journal of Biological Chemistry*; **291(10)**: 4894-4902.

25. Jacobs J, Grum-Tokars V, Zhou Y, Turlington M, Saldanha SA, Chase P, & Mielech, AM. (2013). Discovery, synthesis, and structure-based optimization of a series of N-(tert-butyl)-2-(N-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease. *Journal of medicinal chemistry*; **56(2)**: 534-546.

26. Estrada E, (2020). Topological analysis of SARS CoV-2 main protease, Chaos: *An Interdisciplinary Journal of Nonlinear Science*; **30(6)**: 061102.