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Topotecan and (22S)-Budesonide as potential Drug candidates against ORF3a in SARS-CoV-2 virus

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Abstract

SARS-CoV-2 is a positive-sense single-stranded RNA virus covered in a spiked glycoprotein envelope which acts as a causative agent for COVID-19. SARS-CoV-2 ORF3 gene encodes a novel structural protein ORF3a whose actual mechanism and functions are still unclear. According to the recent findings, ORF3a protein forms an ion channel and modulates viral release. Not only the mode of viral entry but its replication and release play a major role in increasing the copy number of the virus. The study involves finding an effective drug that can bind to the active pocket of ORF3a protein to reduce the viral load. In our work, we have used in silico techniques to screen FDA-approved drugs. 164 screened molecules that obey the screening filters were further ranked based on their binding affinity to ORF3a protein. Topotecan and (22S)-Budesonide showed favourable binding energy of -11.85 and -9.4 kcal/mol with the target protein ORF3a. For both the top-scoring compounds the clustering RMSD was found to be 0.00 and an estimated Inhibition Constant (K_i) was found to be 3.90 nM and 242.88 nM.

Keywords: Glycoprotein, Replication, Insilco, Lipinski, Toxicity, Grid.

Introduction

Covid-19 has brought a severe threat to humanity throughout the globe. This pandemic started at the end of the year 2019 in Wuhan city, China and gradually it spread all over the world (Chen *et al*., 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent for coronavirus disease (COVID-19) (Hui *et al*., 2019). Based on the viral genetic constitution coronaviruses are classified into four main subgroups- alpha, beta, gamma, and delta (Harvey *et al*., 2021). A novel strain of SARS-CoV-2 belongs to the β-coronavirus family which includes a large class of viruses that are prevalent (Guo *et al*., 2020). SARS-CoV-2 are diverse and can find a wide range of hosts as primary, intermediate and final hosts for their multiplication, which make it very difficult to control the disease transmission rate. The propensity of this virus to

transmit and replicate across species can be one of the reasons for the outbreak of the diseases (Cui *et al*., 2019). The global mortality rate for SARS-CoV-2 is much higher as compared to SARS and Middle East respiratory syndrome coronaviruses (MERS-CoV) respectively (Banerjee *et al*., 2020). According to recent studies in China SARS-CoV-2 has a relatively high transmissibility rate, 87% of the cases were aged between 30–79 years (Wang *et al*., 2020).

SARS-CoV-2 contains as many as 14 open reading frames (Orfs) The 5' Orf1a / Orf1ab encodes polyproteins, which are auto-proteolytically processed into 16 non-structural proteins (Nsp1-16) which form the replicase / transcriptase complex (RTC) (Vlachakis *et al*., 2020). The RTC consists of multiple enzymes, including the papain-like protease (Nsp3), the main protease (Nsp5), the Nsp7-Nsp8 primase complex, the primary RNA-dependent RNA polymerase (Nsp12), a helicase/triphosphatase (Nsp13), an exoribonuclease (Nsp14), an endonuclease (Nsp15), and N7- and 2'O-methyltransferases (Nsp10/Nsp16)1,16,17 (Kangarshahi *et al*., 2021). At the 3' end of the viral genome 13 Orfs are expressed from nine predicted sub-genomic RNAs which includes four structural protein as Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) 17, and nine putative accessory factors (Fehr & Perlman 2015). There are lots of similarities in the genomic sequences between SARS-CoV and SARS-CoV-2 but the detectable changes in the Orf3b and Orf10 with limited detectable protein homology to SARS-CoV (Jungreis *et al*., 2021), and its Orf8 is intact while SARS-CoV encodes Orf8a and Orf8b (Zandi, M. 2021). The earlier study reported that ORF3a encoded by SARS-CoV induce apoptosis in the host cells (law *et al*., 2005). Host cells in response to the viral infection use apoptosis as a defence mechanism to control the viral transmission from one cell to another. A similar comparative experiment done in ORF3a from SARS-CoV-2 showed that it can induce apoptosis however the magnitude of apoptosis is reduced in comparison to ORF3a from SARS CoV (Bianchi *et al*., 2021). Since the antiviral defence from the infected host cell is reduced, infection is either asymptomatic or mild, thus allowing the deadly SARS-CoV-2 to spread rapidly (Yujie *et al*., 2020). ORF3a is a viroporin ion channel that has N-terminal, transmembrane & 8-stranded β barrel C-terminal domain (Issa *et al*., 2020). The study showed that this ion channel may promote the release of the virus and regulate the viral cycle (Padhan *et al*., 2007). To treat the covid infected individual instantly, the National Institute of Health (NIH) has recommended a few drugs such as etesevimab & bamlanivimab etc. FDA has approved remdesivir (Wang *et al*., 2020). However, remdesivir cannot be used in patients with severe hypoxia conditions (Beigel *et al*., 2020), as consequence research for new drugs and an effective mode of drug delivery system is the prime need in the current situation. In this research work, attention has been mainly focused on the drug (22S)-Budesonide (a non-halogenated glucocorticoid generally used

as an anti-inflammatory drug to treat asthma and pulmonary diseases) (Zetterstrom *et al*., 2001) and its interactions specifically with the target protein ORF3a. The study conducted by Ramakrishnan *et al*., in the year 2021 showed that after inhaling 1.6 mg of budesonide in moderately infected covid-19 patients, recovery time has been reduced significantly. A non-covalent reversible inhibitor against ORF3a protein has the potential to become a new drug with few or no side effects for the control of the devastating effect of SARS-CoV-2.

Materials and methods

Retrieval and preparation of ORF3a protein

The structure of SARS-CoV-2 ORF3a solved by cryo-electron microscopy was obtained from the Protein Data Bank (PDB) with a resolution of 2.90 Å. The three-dimensional (3-D) structure was used to test for the presence of water molecules, ions, and heteroatoms in the structure before they were removed. Simulated Annealing algorithm was used and the optimal amount of hydrogen atoms and Kolmann charges were applied to the ORF3a protein including the atomic coordinates, partial charges, and solvation parameters for all atoms in the macromolecule.

Preparation of the FDA approved drugs

A drug server was used to retrieve all 1930 FDA-approved medicinal compounds, and ChemSketch was used to construct three-dimensional structures for the ligands available in the two-dimensional structure, and the structures were minimized using Avogadro algorithm, version 1.2.0.

Screening of the FDA approved drugs

All the 1930 compounds were screened based on their chemical properties as well as their affinity towards the protein receptor ORF3a based on the Lipinski rule of five and toxicity parameters (Mutagenic, Tumerogenic, Reproductive effects, and irritants).

Molecular interaction studies based on binding energy

Molecular interaction studies were carried out using Lamarkian, Simulated Annealing algorithm. The protein complexes were visualized using Discovery studio visualizer. Interacting residues were determined using LigPLOT^{+} algorithm ,version 2.2.

Results and discussion

Screening of the ligand molecules based on ligand affinity

Out of 1930 FDA-approved drugs the discontinued drugs were removed, 164 molecules

passed through the Lipinski's filters and none of them was found positive for toxicity characteristics. Broyden-Fletcher-Goldfarb-Shanno algorithm was used for virtual screening which was performed blindly considering the whole protein molecule in a grid with both the chains (Grid dimensions: Center X=143.8427, Y=143.0602, Z=154.1643; Dimensions (Å): X=52.35362, Y=51.5386, Z=96.8385). The highest ligand affinity was found to be -8.2 kcal/mol with a clustering RMSD value of 0.00 for Ezetimibe, -8 kcal/mol for Meloxicam, -7.7 kcal/mol for topotecan, -7.6 kcal/mol for (22S)-Budesonide.

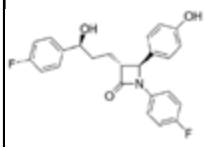
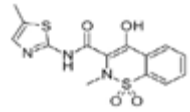
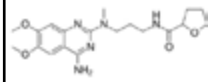
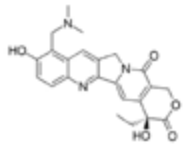
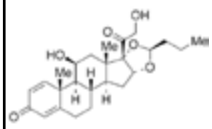
Molecular interaction studies using Binding energy

A new pocket specific gridbox was defined from the ORF3a and Ezetimibe complex using AutoDock Toos-1.5.6 (Grid dimensions: number of points in dimension X=36, Y=40, Z=38; Center (Å): X=150.046, Y=136.174, Z=153.078; Spacing (Å): 0.375) from the previously achieved highest score from Broyden-Fletcher-Goldfarb-Shanno algorithm(autodockvina, Trott, O. and Olson, A.J. 2010) screening of Ezetimibe. The best-scored compound was re-docked using Lamarkian, Simulated Annealing algorithm(autodock) which showed binding energy as -8.2 kcal/mol with an estimated Inhibition Constant (K_i) of 982.85 nM, for Meloxicam the binding energy was found to be -5.9 kcal/mol, -5.9 kcal/mol for Alfuzosin, for topotecan the binding energy was found to be -11.85 kcal/mol and for Budesonide it was found to be -9.4 kcal/mol. The scores for top lead compounds are shown in Table 1.

The propensities and the positioning of the different amino acid residues contribute to the overall stability and the architecture of the protein structure. Several different amino acid residues which are involved in the formation of the molecular pore and its stabilization are illustrated in Fig.1A. Amino acid residues which are important as a clipper of the dimeric chains and their stability are shown in Fig. 1B and 1C. The active pocket with all the interacting residues is shown in Fig. 1 D. The Ramachandran plot analysis shows that 89.2 % of the overall structure falls under the most favoured region providing a major topology for the small molecule to interact with the protein ORF3a (Fig. 1E).

In the topetican ORF3a complex, there were two major hydrogen-bond interactions involving Arg87 and His39 respectively from the chain A of ORF3a protein (Fig. 2C). The non-bonded interactions that further stabilize the receptor-ligand complex involve Arg83, Asp103, Tyr161, Ser214, Lys215, Ile217, Thr218, Leu219, and His232 of ORF3a (Fig. 2C). Similarly, amino acid residue Lys36 of ORF3a was found to form a hydrogen bond with the compound Budesonide (Fig. 3C). The non-bonded interactions that further stabilize the receptor-ligand complex involve Lys22, His39, Asp103, Ile217, and His232 of ORF3a (Fig. 3 C). The active pocket with all the interacting residues is shown in Fig. 2 B & 3B respectively.

Table.1. Showing top 5 screened molecules that are carried further for molecular interaction studies

Sl No	Drug name	Binding affinity (kcal/ mol)	Binding energy (kcal/ mol)	Clustering RMSD	Inhibition Constant (Ki)	Pose for Binding energy	2-D Structure
1.	Ezetimibe	-8.2	-8.2	0.00	982.85 nM	5th	
2.	Meloxicam	-8	-7.81	0.00	1.87 uM	1st	
3.	(R)-Alfuzosin	-7.9	-5.9	0.00	259.03 uM	8th	
4.	Topotecan	-7.7	-11.85	0.00	3.90 nM	2nd	
5.	(22S)-Budesonide	-7.6	-9.4	0.00	242.88 nM	5th	

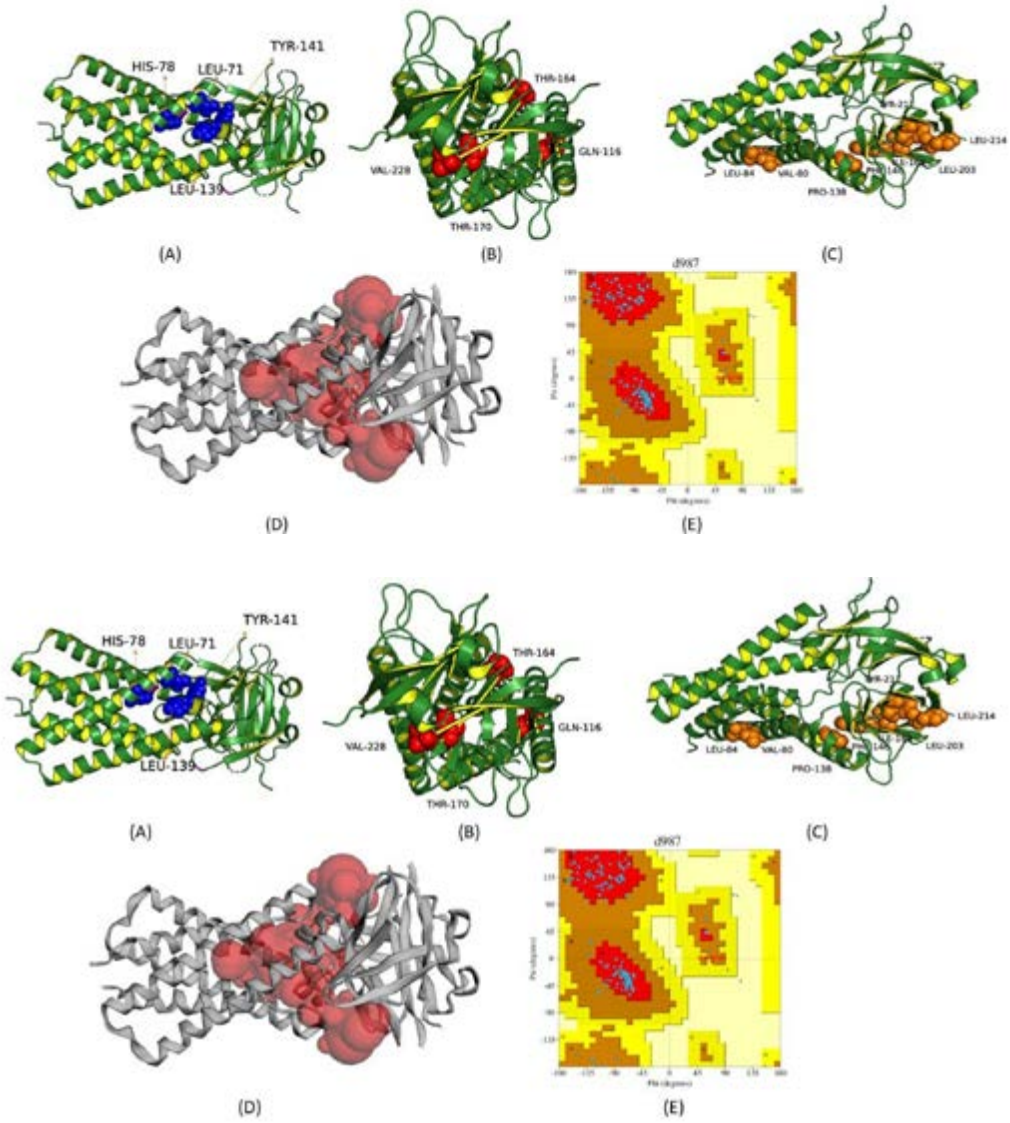


Fig. 1. **(A)** Showing the amino acid residues (Leu71, His 78, Leu 139 and Tyr 141) that are responsible for the pore and tunnel stabilization **(B)** The amino acid residues (Gln 116, Thr 164, Thr 170 and Val 228) which are highly conserved and help in the dimer formation between chain A and B **(C)** The amino acid residues (Gln 80, Leu 84, Pro 138, Phe 146, Ile 169, Leu 203, Tyr 212, and Leu 214) which contribute to the structural stability and uniformity supported by Intra-monomer interactions **(D)** Amino acid residues that form the active site for ORF3a protein determined by Computed Atlas of Surface Topography of proteins (CASTp) **(E)** Representing the Ramachandran plot for the ORF3a protein with 89.2% of Most favoured regions and 10.2% of Additional allowed regions.

Fig. 2. (A) Structure of ORF3a bound to Topotecan. (B) The binding pocket of ORF3a with the interacting compound topotecan. (C) A 2-dimensional representation of topotecan with the interacting residues of ORF3a.

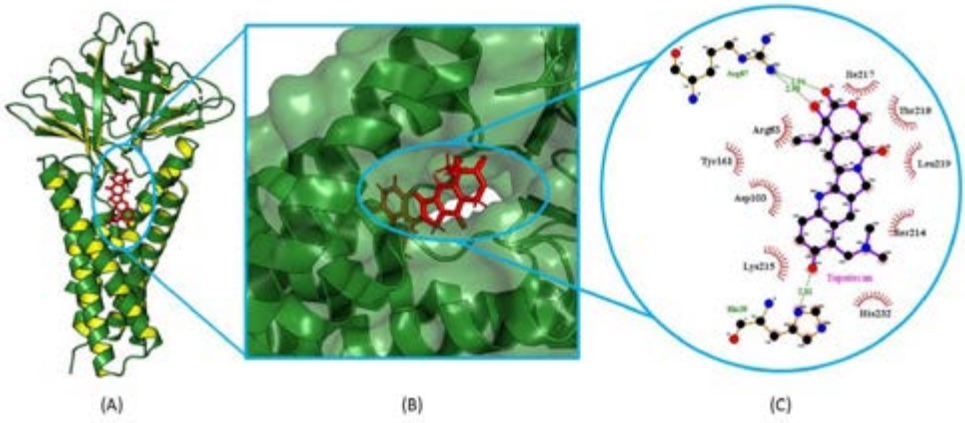
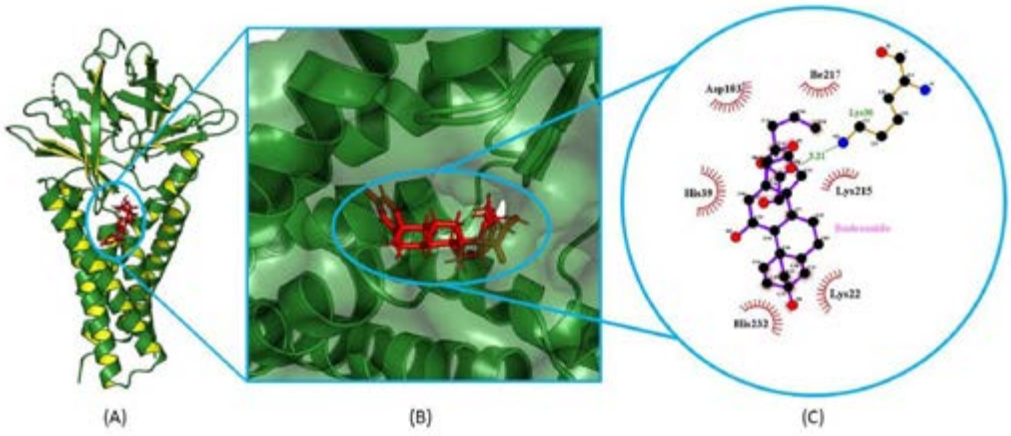


Fig.3. (A) Structure of ORF3a bound Budesonide. (B) The binding pocket of ORF3a with the interacting compound Budesonide. (C) A 2-dimensional representation of Budesonide with the interacting residues of ORF3a.



Conclusion

The main purpose of this study was to establish a strong binding of an applied drug with the ORF3a protein of SARS-CoV-2. (22S)-Budesonide which was previously used as inhalers for the treatment of asthma and chronic obstructive pulmonary disease (COPD) and Topotecan which was known as the anti-cancerous drug has shown promising interaction scores. According to WHO, list of essential medicines, (22S)-Budesonide has been listed as one of the safest and the most effective medicine. The inhibition constant (K_i) of this drug was found to be 242.88 nM which shows the effectiveness of the drug for its administration. It is therefore necessary to take these studies further for wet bench experimentation to check the effectiveness of these drugs for the treatment of COVID-19.

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References

Banerjee, A., Doxey, A. C., Tremblay, B. J. M., Mansfield, M. J., Subudhi, S., Hirota, J. A., ... and Mossman, K. 2020. Predicting the recombination potential of severe acute respiratory syndrome coronavirus 2 and Middle East respiratory syndrome coronavirus. *The Journal of General Virology*, *101*(12): 1251.

Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., ... and Lane, H. C. 2020. Remdesivir for the treatment of Covid-19. *New England Journal of Medicine*, *383*(19): 1813-1826.

Bianchi, M., Borsetti, A., Ciccozzi, M. and Pascarella, S. 2021. SARS-Cov-2 ORF3a: mutability and function. *International Journal of Biological Macromolecules*, *170*: 820-826.

Chen, Y., Liu, Q. and Guo, D. 2020). Emerging coronaviruses: genome structure, replication, and pathogenesis. *Journal of Medical Virology*, *92*(4): 418-423.

Cui, J., Li, F. and Shi, Z. L. 2019. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, *17*(3): 181-192.

Fehr, A. R. and Perlman, S. 2015. Coronaviruses: an overview of their replication and pathogenesis. *Coronaviruses*, *1282*:1-23.

Harvey, W. T., Carabelli, A. M., Jackson, B., Gupta, R. K., Thomson, E. C., Harrison, E. M., ... and Robertson, D. L. 2021. SARS-CoV-2 variants, spike mutations and immune escape. *Nature Reviews Microbiology*, *19*(7): 409-424.

Hui, D. S., Azhar, E. I., Madani, T. A., Ntoumi, F., Kock, R., Dar, O. and Petersen, E. 2020. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health.The latest 2019 novel coronavirus outbreak in Wuhan, China. *International Journal of Infectious Diseases*, *91*: 264-266.

Issa, E., Merhi, G., Panossian, B., Salloum, T. and Tokajian, S. 2020. SARS-CoV-2 and ORF3a: nonsynonymous mutations, functional domains, and viral pathogenesis. *Msystems*, *5*(3): e00266-20.

Jungreis, I., Sealfon, R. and Kellis, M. 2021. SARS-CoV-2 gene content and COVID-19 mutation impact by comparing 44 Sarbecovirus genomes. *Nature Communications*, *12*(1): 1-20.

Kangarshahi, Z. T., Lak, S., Ghadam, M., Motamed, N., Sardari, S. and Rahimi, S. 2021. The proteins of sars-cov-2 and their functions. *Military Medical Science Letters (Vojenske Zdravotnicke Listy)*, *90(4):*172-190.

Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J. and Hsueh, P. R. 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *International Journal of Antimicrobial Agents*, *55*(3): 105924.

Law, P. T., Wong, C. H., Au, T. C., Chuck, C. P., Kong, S. K., Chan, P. K. and Tsui, S. K. 2005. The 3a protein of severe acute respiratory syndrome-associated coronavirus induces apoptosis in Vero E6 cells. *Journal of General Virology*, *86*(7): 1921-1930.

Neher, R. A., Dyrdak, R., Druelle, V., Hodcroft, E. B., & Albert, J. (2020). Potential impact of seasonal forcing on a SARS-CoV-2 pandemic. *Swiss medical weekly*, (11):1.

Padhan, K., Tanwar, C., Hussain, A., Hui, P. Y., Lee, M. Y., Cheung, C. Y. and Jameel, S. 2007. Severe acute respiratory syndrome coronavirus Orf3a protein interacts with caveolin. *Journal of General Virology*, *88*(11): 3067-3077.

Park, M., Cook, A. R., Lim, J. T., Sun, Y. and Dickens, B. L. 2020. A systematic review of COVID-19 epidemiology based on current evidence. *Journal of Clinical Medicine*, *9*(4): 967.

Ramakrishnan, S., Nicolau Jr, D. V., Langford, B., Mahdi, M., Jeffers, H., Mwasuku, C. and Bafadhel, M. 2021. Inhaled budesonide in the treatment of early COVID-19 (STOIC): a phase 2, open-label, randomised controlled trial. *The Lancet Respiratory Medicine*, *9*(7):763-772.

Ren, Y., Shu, T., Wu, D., Mu, J., Wang, C., Huang, M. and Zhou, X. 2020. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cellular & molecular immunology*, *17*(8): 881-883.

Scott, C. and Griffin, S. 2015. Viroporins: structure, function and potential as antiviral targets. *Journal of General Virology*, *96*(8): 2000-2027.

Trott, O. and Olson, A.J. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*(2):455-461.

Vlachakis, D., Papakonstantinou, E., Mitsis, T., Pierouli, K., Diakou, I., Chrousos, G. and Bacopoulou, F. 2020. Molecular mechanisms of the novel coronavirus SARS-CoV-2 and potential anti-COVID19 pharmacological targets since the outbreak of the pandemic. *Food and Chemical Toxicology*, *146*:111805.

Wang, L., Wang, Y., Ye, D. and Liu, Q. 2020. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. *International Journal of Antimicrobial Agents*, *55*(6): 105948.

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y. and Wang, C. 2020. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet*, *395*(10236): 1569-1578.

Zandi, M. 2021. ORF8a as a viroporin in SARS-CoV-2 infection. *Cytokine and Growth Factor Reviews*, *61*:1.

Zetterström, O., Buhl, R., Mellem, H., Perpina, M., Hedman, J., O'Neill, S., & Ekström, T. (2001). Improved asthma control with budesonide/formoterol in a single inhaler, compared with budesonide alone. *European Respiratory Journal*, *18*(2), 262-268.