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Linezolid as Potent Inhibitor of SARS-CoV-2 Nsp13 Helicase: Grid Based Docking Approach

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ABSTRACT: A diversified originator in humans and wildlife, the corona virus (COVID-19) is an enveloped RNA virus. Six different species have been shown to be the root of human sickness. Human diseases are greatly influenced by viral infections, and one of the most recent global epidemics is the appearance of the new corona. The SARS (Severe Acute Respiratory Syndrome) virus, a potentially fatal viral infection, was caused by the SS-RNA virus from the enveloped corona virus family. In many nations around the world, disease is rapidly expanding. 462,684 confirmed cases and 20,834 fatalities had been reported as of March 26, 2020, internationally. On March 11, 2020, COVID-19 was declared a pandemic by the World Health Organization (WHO). There are numerous medication trials ongoing, and some of the outcomes are encouraging. However, since there is no vaccine, the only approach to fight the virus is through preventative measures. Patients with bacterial nosocomial pneumonia were successfully treated with the antibiotic "Linezolid" by receiving an intravenous dose of 600 mg of linezolid every 12 hours for 7 to 10 days. All of the patients made a full recovery and were allowed to leave the hospital. Additionally, previous studies have shown that linezolid is more clinically and microbiologically effective than other antibiotics (vancomycin). The goal of the current study was to use a molecular docking approach to evaluate linezolid's potential against SAR-CoV-2 infection.

Keywords: SARS-CoV-2 Nsp13 Helicase, Linezolid, Molecular Docking & Prevention measures.

RESEARCH PAPER

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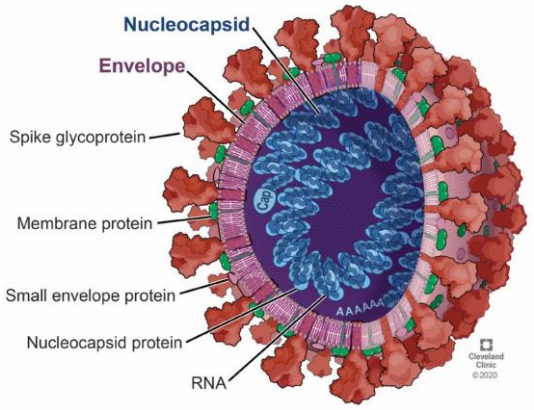
INTRODUCTION

The first significant global epidemic of the new millennium has been caused by a novel corona virus (SARS-CoV). CoV outbreaks and recurrent emergence point to a risk to the public's health. This raises the prospect that recently discovered CoVs could be transmitted from animal to human and human to human. Future outbreaks of these illnesses are increasingly likely due to the ongoing ecological and climatic changes. Around the world, 188 countries and territories are in difficulty because of the COVID-19 corona virus. According to a survey, the corona virus was responsible for 13071 recorded deaths and 31 2002 total cases [1]. Since there is no consensus on the best treatment for corona virus-associated SARS, the condition is still being studied. Antiviral drugs, supportive care, immune-modulation therapy, and broad-spectrum antibiotics are among the predictable therapeutic options for SARS [2]. This time, almost ten years after SARS, the Middle East countries have seen the emergence of the extremely dangerous Middle East Respiratory Syndrome Corona virus (MERS-CoV) [3]. The order Nidovirales, which contains the families Coronaviridae, Arteriviridae, and Roniviridae, is mostly comprised of viruses known as corona viruses (CoVs) [4]. A corona virus is an envelope, single-stranded RNA with surface spikes that are between 9 and 12 nm long. Fever, coughing, and shortness of breath are just a few of the symptoms [5-6].



Structure of CoV

Mode of transmission [6]

- Person-to-person extend of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is thought to occur mainly via respiratory droplets**.**
- Infection can also transpire if a person touches an infected surface and then touches his or her eyes, nose, or mouth.

Co-morbidity associated with CO-V 19

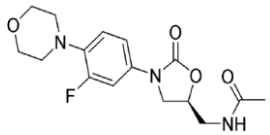
- A logical analysis of CoV cases suggests that diabetes and hypertension are equally ubiquitous in approximately 50% of the patients. CHD are present in 30% and obesity in 16% of the cases. These circumstances down-regulate the synthesis of pro-inflammatory cytokines and impair the host's innate and humoral immune systems [6].

Prevention of COVID-19 [7]

- Wash your hands; use a hand sanitizer that contains at least 60% alcohol
- keep away from touching your eyes, nose, and mouth with unwashed hands
- Avoid close contact
- Cover your mouth and nose
- Wear a facemask
- Clean and disinfect frequently touched surfaces daily

Linezolid belongs to oxazolidinones class and is a first official member of the synthetic antibiotic. Oxazolidinone class of compounds was first discovered in 1978 and was used first to treat plant diseases. A few years later, some new compounds with relatively improved antimicrobial property and safety were documented [8]. Linezolid discovered in 1996 and was a first candidate to enter and completed the human studies. This drug got approval in 2000 from US- Food and Drug Administration (US-FDA) [9].

Description of Drug [10]

Structure	
IUPAC name	Linezolid is N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl]-2-oxo-1, 3-oxazolidin-5-yl] methyl} acetamide.
Mol. wt.	337.351 g/mol
Molecular Formula	$C_{16}H_{20}FN_3O_4$
Therapeutic Category	Antibacterial
Physical Characteristics	It is white to cream color, odorless, crystalline powder
Melting Point	179-181°C
Half-life	4.5-5 Hrs.
Storage	LNZ should be kept in airtight container. It should be protected from light and stored at a room temperature.

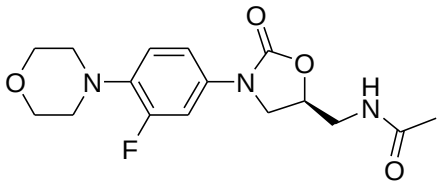
"Linezolid" was a good treatment for bacterial nosocomial pneumonia patients who were suffering from bacterial pneumonia with intravenous dose of 600 mg of linezolid every 12 hours for 7 to 10 days and they all recovered and discharged from hospital. In addition, old researches have confirmed better clinical and microbiological efficacy of linezolid compared to other antibiotics (vancomycin). SARS-CoV-2 Nsp13 Helicase protein from severe acute respiratory syndrome coronavirus (SARS-CoV) is postulated to play a number of crucial roles in the viral life cycle, making it an attractive target for anti-SARS therapy [11]. Present work was designed for assessment of linezolid potential against SAR-CoV-2 infection via molecular docking approach.

EXPERIMENTAL WORKS

Molecular docking studies of SARS-CoV-2 Helicase

Ligand Preparation:

2D Structure of ligand linezolid was drawn by using Chem Draw [12].The two-dimensional structures of ligand was converted into 3-D structures with optimized 3D geometry by using Chem3D software. The optimized structure was saved in PDB format for AutoDock compatibility [13].



Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is given in table 6 [14].

Table 1: The grid-coordinate of the grid-box used in the current study.

Proteins	x-D	y-D	z-D	Spacing (Å)	x center	y center	z center
6zsl	50	50	50	0.458	-13.606	25.925	-70.215

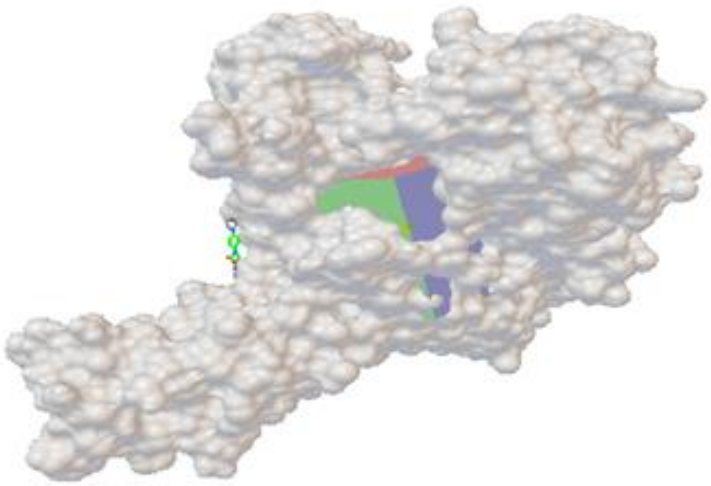


Figure 1: Grid box covering all active sites in helicase enzyme (6zsl) of SARS-CoV2.

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus[15].

Macromolecular structure

Helicase Enzyme of SARS-CoV-2

The crystal structure of the Helicase enzyme consisting of macromolecular receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6zsl.pdb) registered in the Protein data bank was used [16].

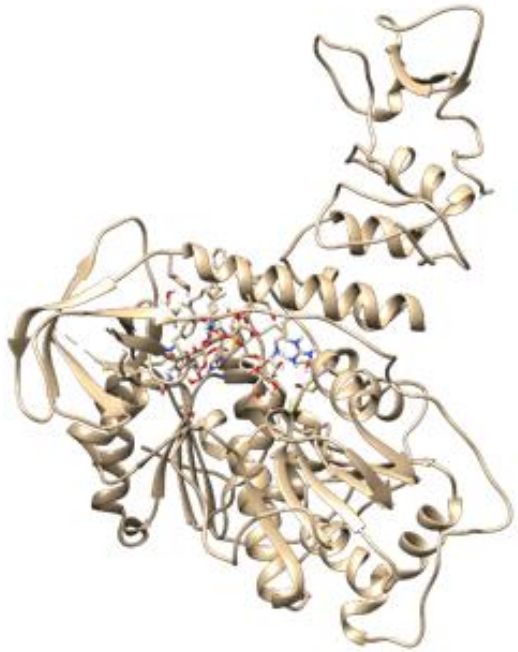


Figure 2: Crystal structure of Helicase enzyme. (PDB ID-6zsl)

Molecular Docking Simulation Studies

Docking of ligand linezolid was performed against Helicase enzyme was performed by Autodock to establish its probable mechanism of action for their antiviral effect. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible[17].

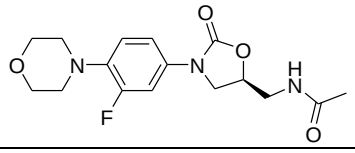
RESULT AND DISCUSSION

The novel corona virus (SARS-CoV) virus is the first major epidemic of the new millennium in various countries around the world. CoV outbreaks and recurring outbreaks represent a threat to public health. This suggests the potential for animal-to-human and human-to-human transmission of the emerging CoV. Ongoing changes in ecosystems and climate make such infections more likely in the future. The novel corona virus COVID-19 afflicts 188 countries and territories around the world. A total of 312,002 cases and 13,071 deaths from corona virus were reported, according to the reported survey. Treatment of corona virus-associated SARS is evolving and there is no consensus on the optimal regimen. Prospected therapeutic interventions for SARS include broad-spectrum antibiotics and supportive care, antiviral and Immunomodulators therapy. About 10 years after SARS, another highly pathogenic CoV, Middle East respiratory syndrome corona virus (MERS-CoV), now emerged in the Middle East, in the Arteriviridae and Roniviridae countries. Coronaviruses are enveloped and single-stranded ribonucleic acids with surface spikes up to 9–12 nm long. Various symptoms include: Fever, cough, shortness of breath. Linezolid can be considered the first member of the class of oxazolidinone antibiotics. This compound is a synthetic antibiotic that inhibits bacterial protein synthesis by binding to rRNA. It also inhibits the formation of initiation complexes during protein synthesis. This shortens the length of the developed peptide chain and slows down the translation elongation reaction rate. Linezolid is recommended for infections with vancomycin-resistant *Enterococcus faecium*, nosocomial pneumonia with *Staphylococcus aureus*, complicated skin and skin structure infections (SSSI), uncomplicated SSSI with methicillin-susceptible *Staphylococcus aureus* or *Streptococcus pyogenes* and *Streptococcus pneumoniae* with Community-acquired pneumonia. Molecular docking is becoming an increasingly important tool in drug discovery. Molecular docking approaches for flexible receptors, especially receptors that involve the flexibility of the receptor backbone, are a challenge to available docking methods. Molecular docking approaches can be used to model interactions between small molecules and proteins at the atomic level, characterize the behavior of small molecules at their target protein binding sites, and elucidate basic biochemical processes can be elucidated. The docking process involves two basic steps: predicting the conformation of the ligand and their position and orientation within their sites (commonly called poses), and assessing their binding affinities.

As per literature survey Moghadam VD *et al*; 2021 this antibiotic named linezolid was a good treatment for bacterial nosocomial pneumonia with better clinical and microbiological efficacy as compared to other popular antibiotic (vancomycin) [18]. The superiority of linezolid is based on better penetration of respiratory secretions compared to vancomycin. Spinoni *et al*; 2021. also, linezolid was used to treat COVID-19 patients who were initially treated with ticoplanin and ceftazidime/avibactam. They then replaced ticoplanin with linezolid but exact mechanism against SARS-CoV will not clear. With intention to propose the most probable mechanism of action of linezolid the docking based computational analysis has been performed against SARS-CoV Helicase as targeted protein. The molecular docking of linezolid with SARS-CoV Nsp13 Helicase showed binding energy (Kcal/mol) -5.83 having molecular interaction Asn381, Ala407, Pro408, Lys139, Thr380, Arg409 and Thr120. The result was tabulated in table 2 and fig.3-5.The outcome of investigation showed that Linezolid binding effectively with SARS-CoV Helicase and act as potent inhibitor of SARS-CoV Nsp13 Helicase. The pharmacokinetic profiling of the linezolid ligand has revealed that it is having good pharmacokinetic profile associated without

the presence of major toxic effects like reproductive effects, irritant effect, and tumorogenic properties, but shows the presence of some mutagenicity. The pharmacokinetic and toxicity profiling results of Linezolid was shown in figure 6.

Table 2: Result of docking study of Helicase enzyme

S.No.	Compound Name	Structure	Binding Energy (kcal/mol)	Interacting Residues
1	Linezolid		-5.83	Asn381, Ala407, Pro408, Lys139, Thr380, Arg409, and Thr120

Interactions

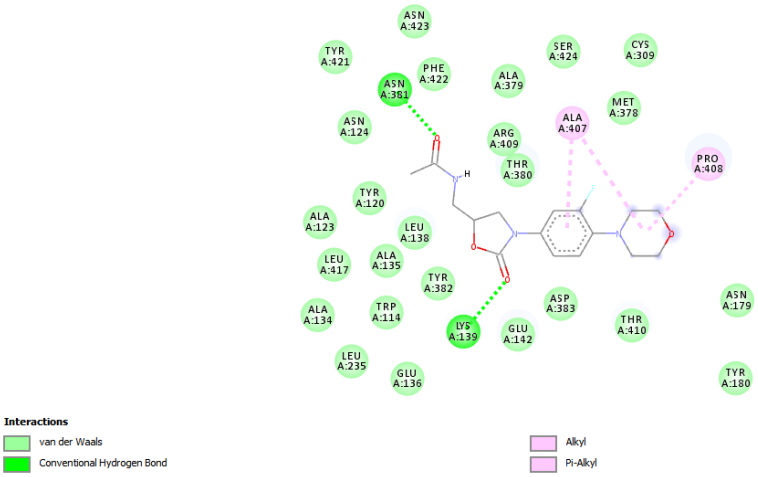


Figure 3: Two-dimensional binding interaction of linezolid with Helicase enzyme.

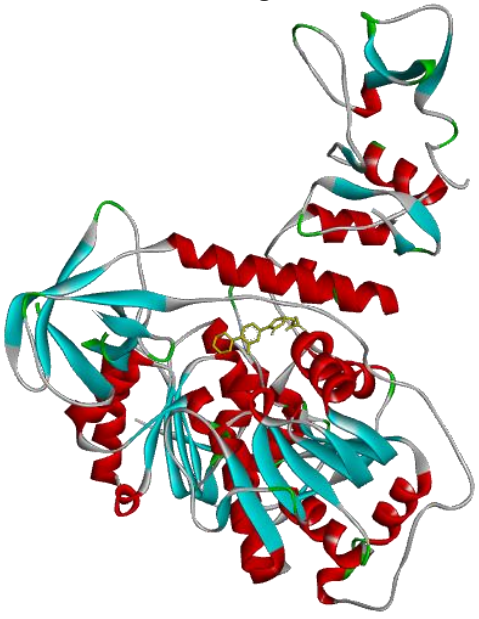


Figure 4: Three-dimensional binding interaction of Linezolid with Helicase enzyme

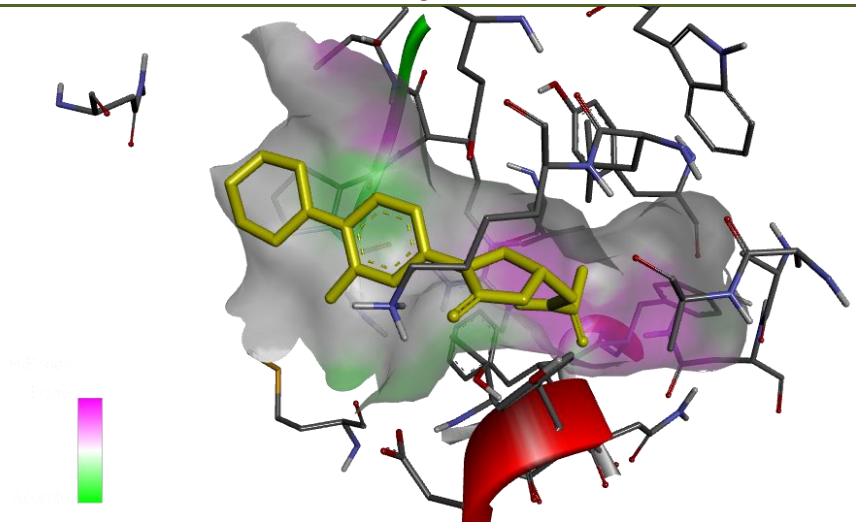


Figure 5: Binding conformation of ligand linezolid with Helicase enzyme

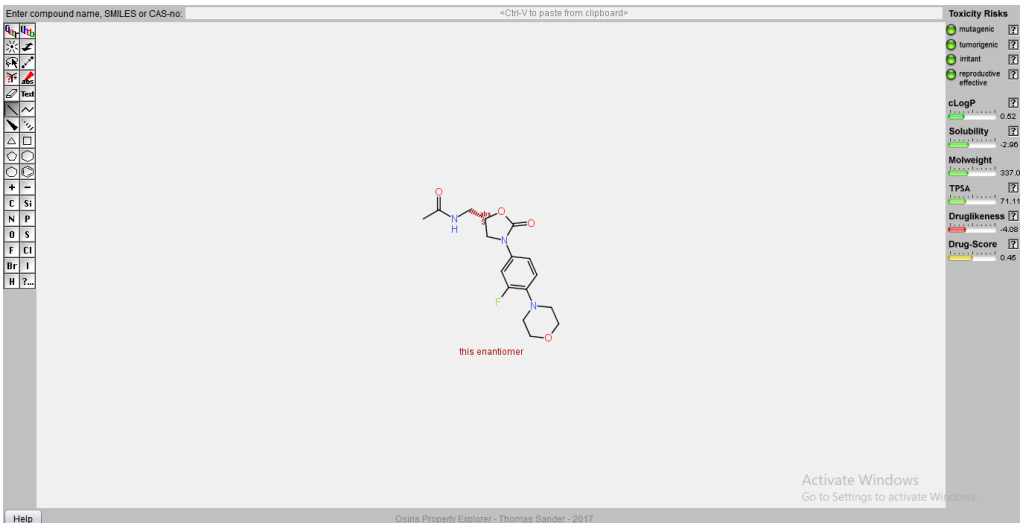


Figure 6: Pharmacokinetic and toxicity profiling of linezolid

CONCLUSION

The 2019 novel corona virus (nCoV) is the leading cause of disaster in the 21st century. However, at present there is a lack of specific drugs to treat attack, which is highly needed. Drug discovery against SAR CoV is a challenging task due to repeated recombination events. Linezolid was a good treatment for bacterial nosocomial pneumonia with better clinical and microbiological efficacy as compared to other popular antibiotic (vancomycin).The superiority of linezolid is based on better penetration of respiratory secretions compared to vancomycin. The linezolid is traditionally utilized for the tubercular drug regimen from the immortal time. In present work an attempt had been made for assessment of efficacy of linezolid along with elucidation of proposed mechanism of action linezolid against SAR CoV infection. The exact mechanism of action for the antiviral action of linezolid was still not revealed. With intent to propose the most probable mechanism of action of linezolid the docking based computational analysis has been performed against the antiviral drug targets like SAR CoV Helicase. SARS-CoV-2 helicase Nsp13 has both ATPase and helicase activity, as it unwinds the RNA helices in an ATP-dependent manner. Remarkably, due to its high sequence conservation across the corona virus family, Nsp13 is considered an attractive target for the development of antiviral drugs. Also, it was shown that SARS-CoV-2 helicase Nsp13 can hydrolyze all types of NTPs including ATP to unwind the RNA helices. Therefore, the known ATP-binding site of the helicase Nsp13 is a promising target for effective inhibition. The outcome of investigation of docking analysis, chemical interactions, followed by the physicochemical based pharmacokinetic profiling has revealed that the linezolid is executing its antiviral

action *via* inhibiting SAR CoV Helicase thereby hindered the ATPase and helicase activity, as it unwinds the RNA helices in an ATP-dependent pattern.

REFERENCE

1. Sarvesh Sharma, Himesh Soni, Jitender K. Malik, Sanjay Khare, Vimal Kumar. CORONA: A REVIEW ON CURRENT CLINICAL SYMPATHETIC. *Sch J App Med Sci*, March., 2020; 8(3): 1054-1061.
2. Himesh Soni, Sarvesh Sharma and Jitender K.Malik Synergistic Prophylaxis on COVID-19 by Nature Golden Heart (Piper betle) & Swarna Bhasma. Asian Journal of Research in Dermatological Science. 3(2): 21-27, 2020.
3. Himesh Soni, Satish Sarankar, Sarvesh Sharma & Jitender K Malik. Hydroxychloroquine as Potent Inhibitor of COVID -19 Main Protease : Grid Based Docking Approach. EJMO 2020;4(3):219–226.
4. Himesh Soni, Dr. V.K. Gautam, Sarvesh Sharma, Jitender K Malik. Rifampicin as Potent Inhibitor of COVID - 19 Main Protease: In-Silico Docking Approach. Saudi J Med Pharm Sci, September, 2020; 6(9): 588-593.
5. Himesh Soni, Sarvesh Sharma & Jitender K Malik. Swarna Bhasma: A Hypothetical Approach to Fight against Corona Virus. South Asian Res J Pharm Sci; Vol-3, Iss-1 (Jan-Feb, 2021): 6-11.
6. Himesh Soni *et al* (2021). Corona: Impact of Non-Living Virus to Living World. Saudi J Med Pharm Sci,7(10): 496-503.
7. Himesh Soni etal. (2022). Silibin as Potent Inhibitor of COVID -19 Main Protease: In-Silico Docking Approach. Journal of Volume-4, Issue-1 (January-June, 2022)Molecular Pharmaceuticals and Regulatory Affairs.1-7.
8. C. Ford, G. Zurenko, M. Barbachyn, The Discovery of Linezolid, the First Oxazolidinone Antibacterial Agent, Curr. Drug Target -Infectious Disord. 1 (2001) 181–199. https://doi.org/10.2174/1568005014606099.
9. S.M. Hashemian, T. Farhadi, M. Ganjparvar, Linezolid: a review of its properties, function, and use in critical care, Drug Des. Devel. Ther. Volume 12 (2018) 1759–1767. https://doi.org/10.2147/DDDT.S164515.
10. https://go.drugbank.com/drugs/DB00601.
11. Vivek-Ananth RP, Krishnaswamy S, Samal A. Potential phytochemical inhibitors of SARS-CoV-2 helicase Nsp13: a molecular docking and dynamic simulation study. Mol Divers. 2022 Feb;26(1):429-442.
12. K.R. Cousins, ChemDraw Ultra 9.0. CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft. com. See Web site for pricing options, ACS Publications, 2005.
13. Jain, R., & Mujwar, S. (2020). Repurposing metocurine as main protease inhibitor to develop novel antiviral therapy for COVID-19. *Structural chemistry*, *31*(6), 2487-2499.
14. Mujwar, S. (2021). Computational repurposing of tamibarotene against triple mutant variant of SARS-CoV-2. *Computers in Biology and Medicine*, *136*, 104748.
15. Mujwar, S., Deshmukh, R., Harwansh, R. K., Gupta, J. K., & Gour, A. (2019). Drug repurposing approach for developing novel therapy against mupirocin-resistant Staphylococcus aureus. *ASSAY and Drug Development Technologies*, *17*(7), 298-309.
16. Kaur, A., Mujwar, S., & Adlakha, N. (2016). In-silico analysis of riboswitch of Nocardia farcinica for design of its inhibitors and pharmacophores. *International Journal of Computational Biology and Drug Design*, *9*(3), 261-276.
17. Saurabh Soni , Jitender K Malik , Satish K. Sarankar & Himesh Soni. Rutin as a c Potent Inhibitor of Dihydrofolate Reductase: A Computational Design and Docking. EASJ Pharm & Pharmacol; Vol-1, Iss-6 (Nov-Dec, 2019): 130-134.
18. Moghadam VD, Momenimovahed Z, Ghorbani M, Khodadadi J. Linezolid a potential treatment for COVID-19 coinfections. Braz J Anesthesiol. 2021 Mar-Apr;71(2):198.