International Journal of Pharmacy and Biological Sciences-IJPBS™ (2021) 11 (4): 175-180
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

In-silico Study of Baricitinib as Inhibitor of AP2-Associated Protein Kinase 1 (AAK1) for the treatment of SARS-CoV-2

Monideepa Guha[1] and Aditya Narayan Singh[2*]
[1]Department of Genetics, Indian Academy Degree College-Autonomous, Bangalore, Karnataka-560043
[2]School of Forensic Science, National Forensic Sciences University, Sector-9, Gandhinagar, Gujarat-382007

Received: 02 Jul 2021/ Accepted: 9 Aug 2021 / Published online: 1 Oct 2021
***Corresponding Author Email:** ans41574@gmail.com

Abstract
Baricitinib is an effective disease-modifying anti-rheumatic drug which has gained importance in treatment of SARS-CoV-2 by targeting the Adapter Protein-2 Associated protein Kinase 1 (AAK1) which is involved in clathrin-mediated endocytosis. Using the available protein data, it was found that Lys74 residue in the protein is involved in ATP-binding. In the present study, we aimed at visualizing the binding of baricitinib within the active site AAK1. The amino acids of AAK1 invoalved in the active site were visualized using Caver Web tools and molecular docking of baricitinib showed that it interacted with three amino acids of the active site namely Gly55, Cys129 and Asp194 with polar bonds and binding energy of -10.76 kcal/mol. Lys74 was found to interact with the drug using an alkyl bond of length 4.96Å which shows that the drug serves as a competitive kinase inhibitor for the enzyme by competing with ATP in the active site of the protein. ADME analysis was also performed for the drug to analyse its lipophilicity, topographical polar surface area and intestinal permeability when administered to a patient.

Keywords
AAK1, Baricitinib, competitive inhibition, endocytosis, molecular docking

*****

INTRODUCTION
Baricitinib is an oral drug, which was initially utilized as an effective disease- modifying anti-rheumatic drug (DMARD) against Rheumatoid Arthritis [1,2]. It shows reversible inhibitory effects on Janus Kinases (JAK1 and JAK2), and activates the JAK-STAT (Signal Transducer and Activator of Transcription proteins) signalling pathway [3,4]. An artificial intelligence study predicted the anti-cytokine and anti-viral properties of the drug, by its inhibition of the human-numb associated kinase (hNAK) members, showing a great affinity towards AP2-associated protein kinase 1 (AAK1) of (8.2 nM) and of 120 nM towards GAK1[5]. The role of AAK1 and GAK has been reported to be essential for viral infections and their inhibition as one of the important anti-viral mechanisms [4,6]. In a study, to identify novel targets for neuropathic pain, AAK1 inhibitors were constructed which showed relief and suppression from pain[7], therefore proving their efficacy as pain relief drugs.

AAK1 belongs to the Ark1/Prk1 family of serine/threonine kinases which are important in controlling the endocytic pathways through phosphorylation [8]. It is activated in clathrin-coated pits, and directly binds to the ear domain of α-adaptin. The Adapter Protein-2 (AP-2) consists of four subunits namely α, β2, μ2 and σ2. AAK1 acts as endogenous μ2 kinase, phosphorylating the μ2 subunit of AP-2, which is then activated and acts as a bridge between the membrane cargo and clathrin

coat. µ2 subunit binds to sorting signals which are present in the cytoplasmic side of membrane proteins and binding affinity of the subunit to the sorting signals is around 25-fold higher when the subunit is phosphorylated when compared to the dephosphorylated state [9]. AAK1 and GAK1 are exploited by viruses such as Filoviruses (EBOV) [10], Hepatitis C virus [11], Rabies virus [12] and potentially SARS-CoV-2 [6] for replication, by utilizing their endocytic machinery and infecting the cells.

The SARS-CoV-2 virus binds to the ACE2 (Angiotensin converting enzyme 2) receptor on the cell, followed by its endocytosis, a phenomenon promoted by AAK1 and GAK [13,14]. Studies have shown that Baricitinib shows the highest affinity towards AAK1 and inhibits it. It also binds to GAK, and is therefore, proposed to be a potential treatment against SARS CoV-2 [14]. Baricitinib in combination with Remdesivir (Veklury) showed a greater recovery rate at a shorter duration of time in hospitalized Covid-19 patients [15]. Baricitinib has been studied extensively by researchers as a potential drug against SARS CoV-2 as it acts as the substrate for a variety of drug transporter molecules, such as AAK1 and GAK1. It has shown various positives aspects as a drug utilized against SARS CoV-2, due to its anti-inflammatory, anti-viral and immunosuppressive properties, but there may also be a risk in using the drug since it might cause secondary infections, due to delayed viral clearance [16]. Thus further research on calibrating the dosage for the drug should be done for achieving higher efficacy and minimal side effects.

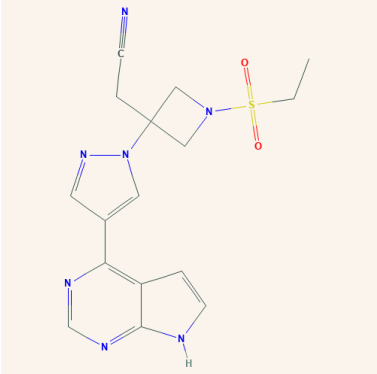
This study aims to dock Baricitinib to AAK1, visualize its binding within the active site of the target protein using molecular docking software and tools such as Caver Web, MGL Tools, PyMOL and Discovery Studio and assess its ability to act as an inhibitor of AAK1. As accessed from UniProtKB (ID Q2M2I8) the active site of the AAK1 consists of Lys74 (ATP Binding site) and Asp176 (proton acceptor) while the amino acids Leu52, Ala53, Glu54, Gly55, Gly56, Phe57, Ala58, Ile59 and Val60 constitute the nucleotide binding site.

MATERIALS AND METHODS

1. Obtaining the X-ray crystallography structure of AAK1:
   The X-ray crystallography structure of AAK1 was obtained from RCSB Protein Data Bank (PDB) database using the PDB ID – 4WSQ [17]. The protein was obtained as a dimer with each subunit bound to three zinc atoms and one molecule each of 1,2-Ethanedoil and an alkaloid inhibitor K-252A.

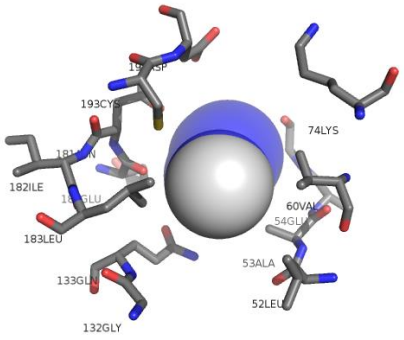
2. Obtaining the ligand structure:
   The 3D structure of Baricitinib was obtained using PubChem data base (PubChem CID – 44205240) in spatial data file (.sdf) format. It was then converted to AutoDock structure file (.pdbqt) format using Open Babel software (Version 3.1.1 developed by Open Babel development team) [18] to be used for docking. The 2-D structure of ligand as obtained from PubChem can be observed in figure 1.

Figure-1 – 2-D structure of Baricitinib obtained from PubChem



3. Scanning of binding pockets –
   In order to look out for binding pockets within AAK1, Caver Web tool (Version 1.1 developed by Loschmidt Laboratories) [19] was used. The PDB id of the protein was entered in the search bar while keeping the search parameters at default settings. A tunnel of bottleneck radius 2.9 Å and length 3.7 Å was selected for further analysis. The bottleneck consisted of the following amino acids – Leu52, Ala53, Glu54, Gly55, Val60, Ala72, Lys74, Met126, Asp127, Phe128, Cys129, Gly132, Gln133, Lys176, Glu180, Asn181, Ile182, Leu183, Cys193 and Asp194. The bottle neck can be visualized in Figure 2.

Figure-2 – Amino acids constituting the bottleneck



4. Docking of Baricitinib to AAK1:
   Baricitinib was docked to AAK1 using AutoDock tools (Version 4.2 developed by The Scripps Research Institute) [20] Prior to docking, first subunit B was removed entirely followed by removing three zinc atoms, 1,2-ethanediol and K-252A inhibitor bound to subunit A. This was followed by removing all water molecules, adding polar hydrogens and computing Gasteiger charge for subunit A.
   The binding sites within the protein were determined using UniProtKB (ID Q2M2I8) and a grid box of dimensions 56Å x 56Å x 60Å with x, y and z centre coordinates as 2.928, -16.56 and -50.314 respectively was chosen assuming the binding pocket to be present within the grid. All aforementioned modifications to the protein and drawing of gird box were done using Python Molecular Viewer (v1.5.6 included in the MGL tools v1.5.6 package developed by The Scripps Research Institute). AutoGrid4 programme was allowed to run keeping all parameters at default to generate a grid log file. This was followed by setting the parameters for docking. Number of runs was restricted to 10 while keeping all other parameters at default. A Docking Parameter File (.dpf) was generated selecting the output to follow Lamarckian Genetic Algorithm. AutoDock4 programme was initiated using the DPF file and all configurations of docking were obtained in a Docking Log File (.dlg) post-operation.
5. Visualization of Docking –
   The docking configurations were first observed using the Python Molecular Viewer (Version 1.5.6) and the best output file having the highest binding energy was exported as .pdb file to be visualized using PyMOL Molecular Graphics System (Version 2.3.4 developed by Schrödinger LLC). The binding sites were displayed in a pre-set cartoon display style to ease the visualization of docking. The whole protein, amino acids in the active site and the ligand was colored distinctly and visualized in pre-set ribbon and surface display formats within PyMOL. All images were exported in portable network graphics (.png) format.
   The docking file was also visualized using LigPlot+ (Version 2.2.4 developed by European Bioinformatics Institute) [21] and Discovery Studio (Version 21.1.0.20298 developed by Dassault Systems Biovia) to obtain a 2-D image of docking.

RESULTS AND DISCUSSION

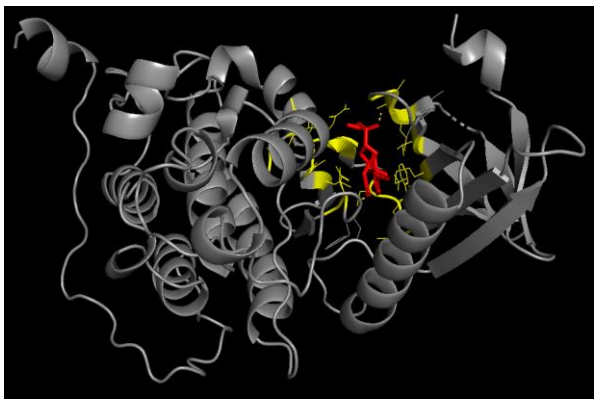
The docking output file when observed in PyMOL showed baricitinib fitting perfectly inside the binding pocket of AAK1 which was visualized using Caver Web. Figures 3 and 4 show the cartoon and surface representation of baricitinib positioned inside binding region of AAK1.

A binding affinity of -10.76 kcal/mol and an estimated inhibition constant (at 298.15K) of 12.97 nM (nanomolar) was reported in the docking log. Baricitinib formed four polar bonds – one with Gly55 of length 2.1Å, one with Asp194 of length 2.0Å and two with Cys129 of lengths 1.8Å and 2.3Å (figure 5). The other amino acids of the binding site were present in the vicinity of the ligand as shown in figure 6 (2D representation using Discovery Studio).

It can be inferred from the work of Zhang et al that Baricitinib serves as a reversible ATP competitive kinase inhibitor of AAK1.[22] Based on the reactive residue data available from UniProtKB (ID – Q2M2I8) it was the Lys74 residue which was involved in ATP binding. In our results, we observed the presence of Lys74 at around 4.96Å from baricitinib interacting with the ligand using alkyl hydrophobic interaction (figure 7) thereby confirming the ability of baricitinib to successfully bind to AAK1 and act as its inhibitor. In the absence of ATP, the µ2 subunit of AP-2 will not be phosphorylated and therefore the entire bridging process between membrane cargo and clathrin vesicle mediated by AP-2 will not occur. This will therefore inhibit/reduce the endocytosis of the cargo, in our case – SARS-CoV-2 bound to its surface receptor.

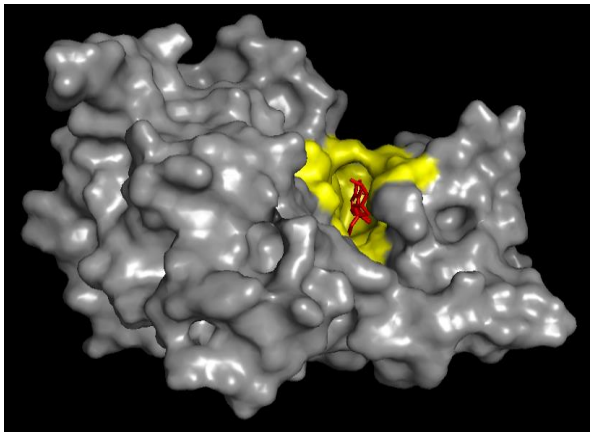
The ADME analysis of Baricitinib as a drug using the Swiss ADME server [23] showed high GI-Absorption and no violations for Lipinski, Ghose, Veber, Egan, and Muegge filters. The drug was found to have a fragmental lipophilicity (WLOGP) value of 1.80 with a topographical polar surface area (TPSA) of 128.94Å. The relatively smaller TPSA enables the drug to be absorbed in the GI tract but it still remains impermeable to the blood-brain barrier which has a maximum TPSA limit of 90Å. [24] Drugs having TPSA above 140Å usually have lower intestinal absorption [25]. Baricitinib also acts as a substrate for p-glycoprotein (PGP+ in BOILED Egg). The absorption results of ADME can be observed using BOILED-Egg (figure 8).

Figure-3 – Cartoon representation of baricitinib bound in the active site of AAK1



Legends – In gray – Amino acid residues of AAK1; In yellow – Amino acid residues of active site; In red – Baricitinib

Figure-4 – Surface representation of baricitinib bound in the active site of AAK1



Legends – In gray – Amino acid of AAK1; In yellow – Amino acid residues of active site; In red – Baricitinib

Figure-5 – Amino acids of active site interacting with baricitinib using hydrogen bonds

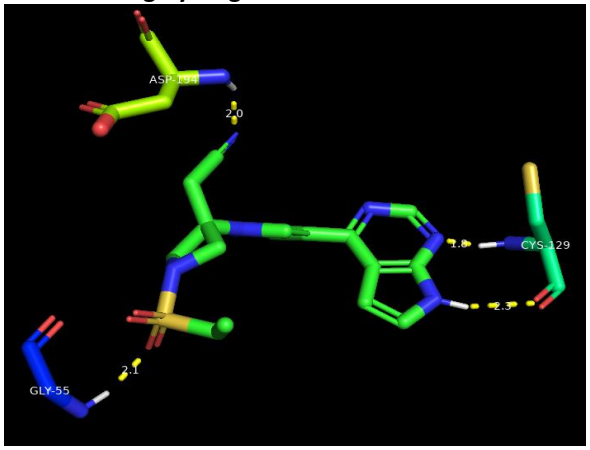


Figure-6 – 2-D representation of other amino acids of active site

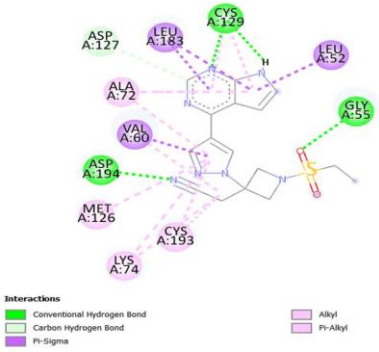


Figure-7 – Presence of Lys74 (coloured olive) near baricitinib (at centre)

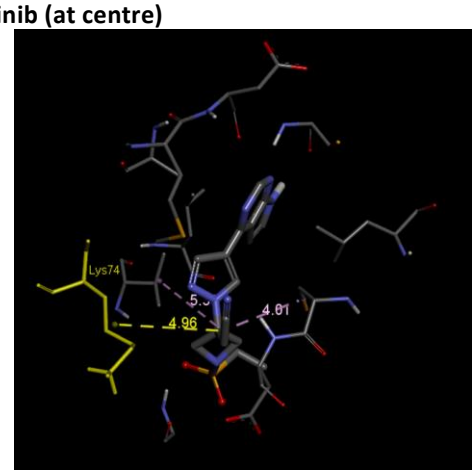
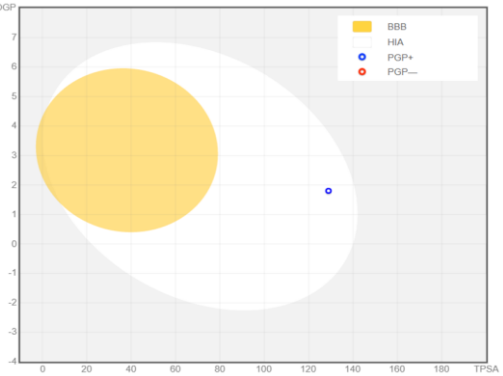


Figure-8 – BOILED-Egg ADME analysis of baricitinib



In November 2020, Baricitinib was approved by FDA for emergency use authorization, along with Remdesivir for adults and children above the age of 2, who are hospitalized with severe COVID-19 pneumonia [26]. The Adaptive COVID-19 Treatment Trial (ACTT-2) results showed that Baricitinib given along with Remdesivir proved to be more superior to Remdesivir alone, in reducing the recovery time and morbidity rate of severe cases of COVID-19 [15]. Baricitinib is sold as 2 mg per tablets under the brand

name Olumiant. The recommended dosage of Baricitinib for adults is 4 mg daily (oral) for 14 days, or until hospital discharge [26]. Pharmacokinetics of the drug suggests, that at this dosage the baricitinib free C_{max} (peak serum concentration achieved by the drug) values exceed the IC_{50} values required for inhibiting the cytokine-induced JAK/STAT signalling pathway, as well as exceeding the dissociation constant (K_d) for AAK1 but showing that a higher dose may be required for inhibition of GAK. After oral administration, the drug reaches its peak plasma concentration within 60 minutes, with a plasma protein binding percentage of 50% (independent of concentration) [16].

Baricitinib has an absolute bioavailability of 79% and is excreted out of the body mainly through urine (75%), out of which 69% remains unchanged, and (20%) is excreted out through faeces or stool, with 15% being unchanged. The remaining (5-6%), which is a very small fraction of the total drug present in the body, undergoes hepatic metabolism predominantly by CYP3A4. The metabolism of the drug is unaffected by either of the CYP inhibitors or inducers, such as ketoconazole (a strong CYP3A4 inhibitor) or Rifampin (a strong CYP3A4 inducer), but its renal excretion can get affected by Probenecid (Probalan) [16,26] . Baricitinib undergoes oxidation via CYP3A4, although a very less amount undergoes this biotransformation and thus there are no quantifiable metabolites found in the plasma [27].

CONCLUSION

This study aimed to visualize the interaction of baricitinib, a potential SARS-CoV-2 drug to its target protein AP2- Associated protein Kinase 1 (AAK1) which is involved in clathrin-mediated endocytosis of substances inside the cell. Baricitinib was found to be bound in the binding pocket of AAK1 which was visualized using Caver Web tool. The drug interacted with three amino acids Gly55, Cys129 and Asp194 via polar bonds. The target amino acid Lys74 was found to interact with the drug using hydrophobic (alkyl) linkage. Thus, when ATP fails to bind to lys74 due to presence of baricitinib, clathrin-mediated vesicle formation is hindered, and the endocytosis of the virus is greatly affected. Also, the drug had a high GI absorption as observed by ADME analysis. Through our study, we conclude that Baricitinib serves perfectly as an inhibitor or AAK1. However, further studies can be carried out to find ways of reducing the side effects caused by Baricitinib and develop it as a potential risk-free drug against SARS CoV-2, for future usage.

REFERENCES

1. Genovese, M. C., Kremer, J., Zamani, O., Ludivico, C., Krogulec, M., Xie, L., ... & Smolen, J. S., Baricitinib in patients with refractory rheumatoid arthritis. N Engl J Med, 374:1243-1252, (2016)
2. Al-Salama, Z. T., & Scott, L. J., Baricitinib: a review in rheumatoid arthritis. Drugs, 78(7):761-772, (2018)
3. Kubo, S., Nakayamada, S., Sakata, K., Kitanaga, Y., Ma, X., Lee, S., ... & Tanaka, Y., Janus kinase inhibitor baricitinib modulates human innate and adaptive immune system. Frontiers in immunology, 9:1510, (2018)
4. Al-Humaidhi, A. M., A review study of targeting of AAK1 and JAK1/2 using baricitinib in COVID-19 infected human cells. AL-Kindy College Medical Journal, 16(supplement):9-15, (2020)
5. Stebbing, J., Krishnan, V., Sd, B., Ottaviani, S., Casalini, G., Richardson, P. J., ... & Corbellino, M., Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients.Research Square, (2020)
6. Bekerman, E., Neveu, G., Shulla, A., Brannan, J., Pu, S. Y., Wang, S., ... & Einav, S., Anticancer kinase inhibitors impair intracellular viral trafficking and exert broad-spectrum antiviral effects. The Journal of clinical investigation, 127(4):1338-1352, (2017)
7. Kostich, W., Hamman, B. D., Li, Y. W., Naidu, S., Dandapani, K., Feng, J., ... & Albright, C. F., Inhibition of AAK1 kinase as a novel therapeutic approach to treat neuropathic pain. Journal of Pharmacology and Experimental Therapeutics, 358(3):371-386, (2016)
8. Smythe, E., & Ayscough, K. R., The Ark1/Prk1 family of protein kinases: Regulators of endocytosis and the actin cytoskeleton. EMBO reports. 4(3):246-251, (2003)
9. Ricotta, D., Conner, S. D., Schmid, S. L., Von Figura, K., & Höning, S., Phosphorylation of the AP2 μ subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. The Journal of cell biology. 156(5):791-795, (2002)
10. Brannan, J., Bekerman, E., Neveu, G., Shulla, A., Pu, S. Y., Wang, S., ... & Einav, S., Investigating AAK1-and GAK-Regulated Virus-Host Interactions Uncovers Broad-Spectrum Antivirals. USAMRIID Ft Detrick United States, (2017)
11. Neveu, G., Ziv-Av, A., Barouch-Bentov, R., Bekerman, E., Mulholland, J., & Einav, S., AAK1 and GAK regulate hepatitis C virus entry and are potential drug targets. J Virology, 89(8):4387-4404, (2015)
12. Wang, C., Wang, J., Shuai, L., Ma, X., Zhang, H., Liu, R., ... & Bu, Z., The serine/threonine kinase AP2-associated kinase 1 plays an important role in rabies virus entry. Viruses, 12(1): pp45, (2020)
13. Xiao, L., Sakagami, H., & Miwa, N., ACE2: the key molecule for understanding the pathophysiology of severe and critical conditions of COVID-19: demon or angel? Viruses, 12(5):pp491, (2020)
14. Richardson, P., Griffin, I., Tucker, C., Smith, D., Oechsle, O., Phelan, A., ... & Stebbing, J., Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet (London, England), 395(10223):e30, (2020)
15. Kalil, A. C., Patterson, T. F., Mehta, A. K., Tomashek, K. M., Wolfe, C. R., Ghazaryan, V., ... & Beigel, J. H., Baricitinib plus remdesivir for hospitalized adults with Covid-19. New England Journal of Medicine, 384(9):795-807, (2021)
16. Jorgensen, S. C., Tse, C. L., Burry, L., & Dresser, L. D., Baricitinib: a review of pharmacology, safety, and emerging clinical experience in COVID-19. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 40(8):843-856, (2020)
17. Sorrell, F. J., Szklarz, M., Azeez, K. R. A., Elkins, J. M., & Knapp, S., Family-wide structural analysis of human numb-

associated protein kinases. Structure. 24(3):401-411, (2016)
18. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. Open Babel: An open chemical toolbox. Journal of cheminformatics, 3(1):1-14, (2011)
19. Stourac, J., Vavra, O., Kokkonen, P., Filipovic, J., Pinto, G., Brezovsky, J., ... & Bednar, D., Caver Web 1.0: identification of tunnels and channels in proteins and analysis of ligand transport. Nucleic acids research, 47:414-422, (2019)
20. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry, 30(16):2785-2791, (2009)
21. Wallace, A. C., Laskowski, R. A., & Thornton, J. M., LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein engineering, design and selection, 8(2):127-134, (1995)
22. Zhang, X., Zhang, Y., Qiao, W., Zhang, J., & Qi, Z., Baricitinib, a drug with potential effect to prevent SARS-COV-2 from entering target cells and control cytokine storm induced by COVID-19. International immunopharmacology, 106749, (2020)
23. Daina, A., Michielin, O., & Zoete, V., SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7(1):-13, (2017)
24. Pajouhesh, H., & Lenz, G. R., Medicinal chemical properties of successful central nervous system drugs. NeuroRx, 2(4):541-553, (2005)
25. Clark, D. E., Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. Journal of pharmaceutical sciences, 88(8):807-814, (1999)
26. LiverTox. Baricitinib. Clinical and Research Information on Drug-Induced Liver Injury; Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. 2012.
27. Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., ... & Woolsey, J. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic acids research, 34(suppl_1):668-672, (2006)