SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

INHIBITORY ACTIVITY OF MACROLIDE ANTIBIOTICS AGAINST INITIAL STAGES OF EGRESS OF PLASMODIUM

Sumeet Gupta[1]
[1] *Researcher, Health & You, Chandigarh, India*

Swati Gupta[2]
[2] *Senior Researcher, Health & You, Chandigarh, India*

Richa Gupta[3*]
[3] *PGIMER, Chandigarh, India*

Rajeev Garg[4]
[4]*Paras Hospital, Panchkula*

Minoti Gupta[5]
[5] *Senior Researcher, Health & You, Chandigarh, India*

ABSTRACT

Background: *Malaria treatment requires new target therapies due to rapidly emerging drug resistance. The need of hour is to find drugs with novel mechanism of action. Our aim of the study was to study the merozoite egress inhibitory potential of macrolide antibiotics.*

Methodology: *To understand the mechanism, Molecular docking study was performed with fourteen drug molecules. 3-dimensional chemical structures of molecules were prepared through UCSF Chimera and Autodock Tools freeware. Molecular docking study was performed using AutoDock Vina software. Discovery studio 4.5 was used to predict the active site of target sites and PyMolwas utilized to visualise the induced fit docking.*

Results: *In the present study, it was demonstrated that Pristinamycin and Solithromycinare potential inhibitors of initial stages, that is when merozoite egress from erythrocytes. These inhibitors, together with their novel mechanisms of action, could complement current antimalarials which generally act on intracellular parasites during their growth phase.*

Conclusion *- Pristinamycin, Solithromycin and Troleandomycin appeared attractive candidates as potential inhibitors of SUB1. However, in vitro, and in vivo studies are necessary to further investigate their therapeutic potential in treating malaria.*

INTRODUCTION

Malaria poses a grave health burden with an estimated 219 million cases worldwide in 2017, resulting in 435,000 deaths (World Health Organization, 2018). A total of four Plasmodium spp. infect humans—P.ovale, P.malariae, P.falciparum and P.vivax. Plasmodium falciparumis one of the deadliest infecting humans. Lately there have been reports of emerging drug resistance in P. falciparum towards Artemisinin combination therapies (ACTs), whereby reports of delayed parasite clearance have been reported in regions of South-eastern Asia.[1,2]With the emergence of resistance to artemisinin and other drugs, it is imperative that an assessment of existing therapeutics be done for their antimalarial potential. To understand the mechanism of emerging anti-malarial resistance it is crucial to

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

understand the pathogenesis and the life cycle of malarial parasite.

Blood stage infection caused by asexual blood stage parasites begins with the intracellular ring stage, which matures to trophozoite stage and finally to DNA-replicative schizont stage. Each mature schizontsrupture to liberate approximately 20 invasive merozoites. This release of merozoites are known to egress from the nutrient-deprived infected RBC (iRBC) after a highly coordinated and sequential enzymatic process involving proteolytic breakdown of the Parasitophorous vacuole membrane (PVM) and rupture of the RBC membrane.[3]Egress appears to be initiated via the activation of cGMP dependent protein kinase G (PKG), which triggers the discharge of the serine subtilisin-like protease (SUB1) from the exonemes[4]afterautoprocessing in the endoplasmic reticulum (ER),[5] followed by a second processing event, requiring the aspartic protease, plasmepsin X (PMX)[6,7]. Release of mature SUB1 into the parasitophorous vacuole (PV) processesa number of proteins like serine rich antigen 5 and 6 proteins (SERA5/6)[8], merozoite surface protein 1 (MSP1)[9]. SERA6 and MSP1 disrupt the RBC membranevia cleavage and binding of spectrinrespectively.[9]With this background about the pathogenesis of the infection by malarial parasite, we aimed to facilitate repurposing of drugsas antimalarialsby using molecular docking.

METHODOLOGY

2.1. Protein preparation

The crystal structure of the molecular targetSUB1 protein (PDBID :4TR2), was retrieved from RCSB protein data bank.[10] The first step in molecular docking process is the preparation of the target. Target protein preparation involves removal of the complexes bound to the protein receptor molecule, removal of the water molecules and finally adding polar hydrogen atoms were added into target. All these processes were carried out in the Auto Dock window execution file.

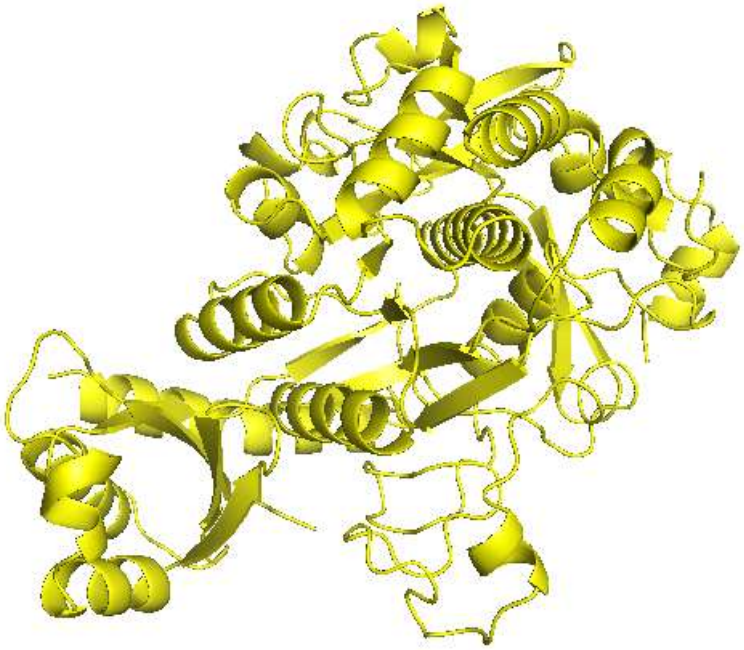

Fig.1 :- Prepared Protein Structure (A chain; PDBID 4TR2)

2.2. Ligand preparation

Investigational ligands were built using canonical smiles obtained from PUBCHEM, saved in.pdb format using UCSF Chimera[11] and subsequently converted into.pdbqt format by Autodock tools.[12] In the current study, identification of binding modes of the investigational ligands with target was done using Auto Dock Vinasoftware program.[13] In order to confirm actual binding interaction with targets, blind docking was performed and the best conformers were represented with lowest binding energy (-kcal/mol). For SUB1 protein (PDBID: 4TR2), the docking parameters were defined as coordinates of the center of binding site with x = 114, y = 106, z = 92 and binding radius = 1 Å. All AutoDock output files (.pdbqt) were analyzed through Biovia Discovery Suiteand PyMol.[14, 15]Top-scoring molecules in the largest cluster were analyzed. Conformers of the ligand were automatically docked to the proteins and most stable conformer in terms of binding affinity (most negative) was used for post-docking analysis.

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

3. RESULT AND DISCUSSION

3.1 Binding energies

Docking scores of Macrolide Antibiotic using Autodock Vina

The binding energies of various ligands with the target proteins has been enlisted in Table 1. The binding energy of Pristinamycin 1, Pristinmaycin2, Solithromycin and Troleandomycin to the SUB1protein (A chain; PDBID: 4TR2) were -7.5 kcal/mol, -8.3 kcal/mol, -8.2 kcal/mol and -8.0 kcal/mol and that of Azithromycin was -7.0 kcal/mol. The binding energy of Pristinamycin1, Pristinmaycin2 and Solithromycin to the active site is even smaller than that of the Azithromycin, indicating that Pristinamycin 1, Pristinmaycin 2, Solithromycin and Troleandomycin have a higher binding activity. From the binding energy values, it can be seen thatPristinamycin shows strong interactions with SUB1 protein.

TABLE 1

S. No.	Investigational Ligand	SUB1 (PDBID: 4TR2)
1	Azithromycin	-7.0
2	Dirithromycin	-6.2
3	Erythromycin	-7.3
4	Flurithromycin	-7.4
5	Josamycin	-6.9
6	Lincomycin	-5.7
7	Midecamycin	-6.4
8	Pristinamycin 1	-7.5
9	Pristinamycin 2	-8.3
10	Rokitamycin	-6.8
11	Solithromycin	-8.2
12	Troleandomycin	-8.0
13	Spiramycin	-6.4
14	Telithromycin	-7.7

MolecularDocking

From Fig.2, it can be observed thatazithromycin forms bonds hydrogen bonds with SER 65, SER 463 and ASN 464 and characteristic Pi-Alkyl bonds with Met 62. From Fig.3, it can be inferred that Pristinamycin1form hydrogen bonds with Ser 65 and Arg485. Arg 485 also forms Pi-cation bonds with Pristinamycin1. Pristinamcyin2 also forms hydrogen bonds with SER 65 as can be inferred from Fig. 4. Pi-alkyl and alkyl bonds are formed with ALA 466 and LYS 469. On other hand Solithromycin forms hydrogen bonds with SER 463, SER 464 and HIS 467. Pi-Anion bonds are formed with Glu 68 amino acid residue. Lastly, it can be seen from figure 6 that troleandomycin forms hydrogen bonds with SER 65 and ASP 438.

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

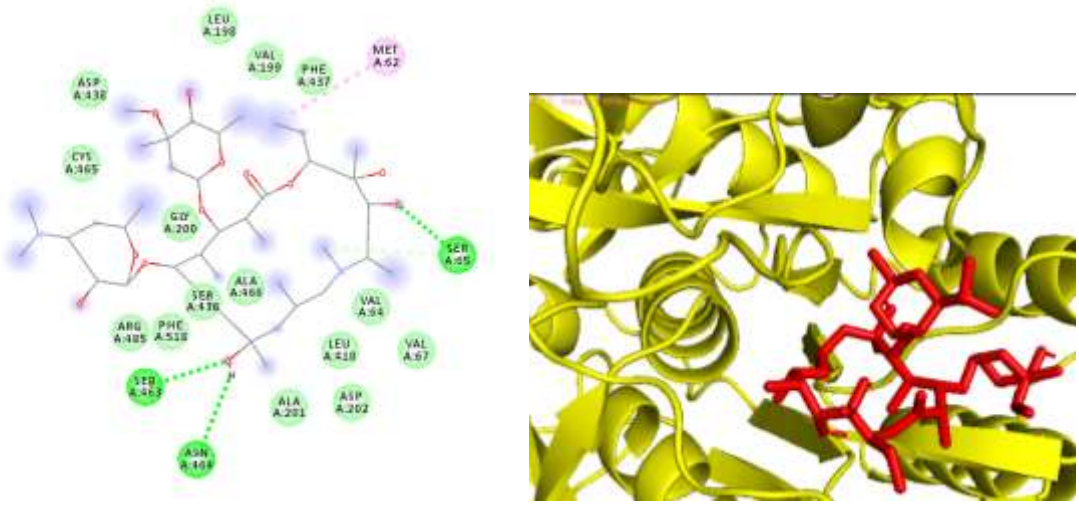

Fig.2: Visualization of interactions and binding region of Azithromycin with 4TR2

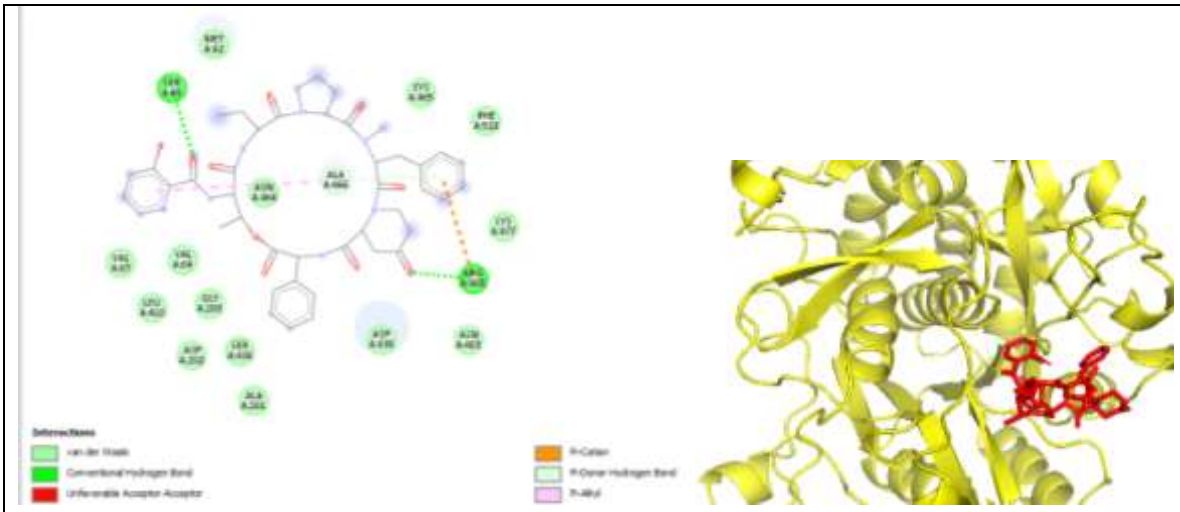

Fig.3:Visualization of interactions and binding region of Pristinamycin1with 4TR2

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

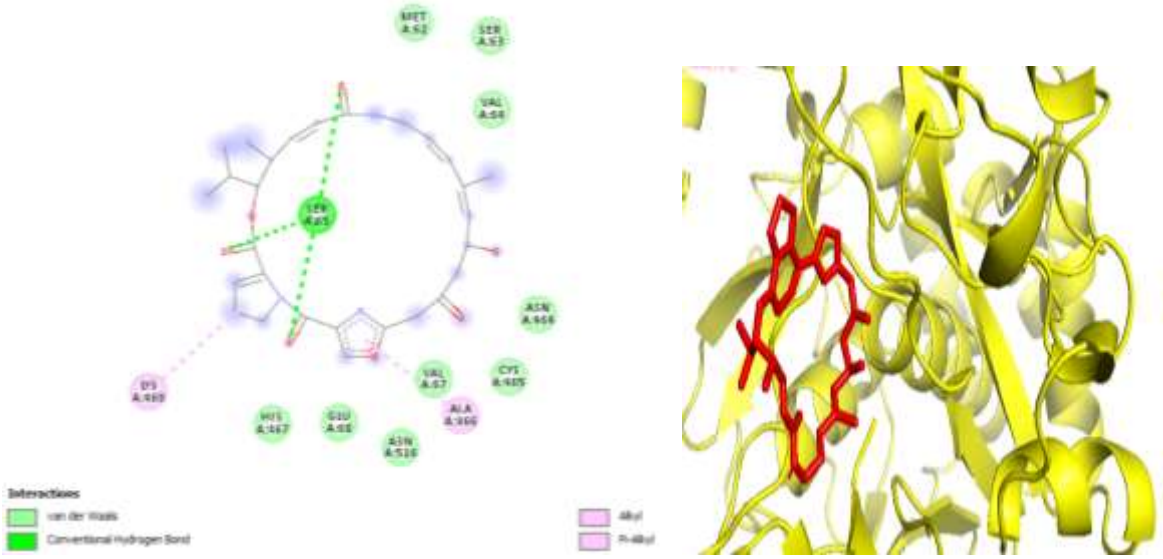

Fig. 4: Visualization of interactions and binding region of Pristinamycin2with 4TR2

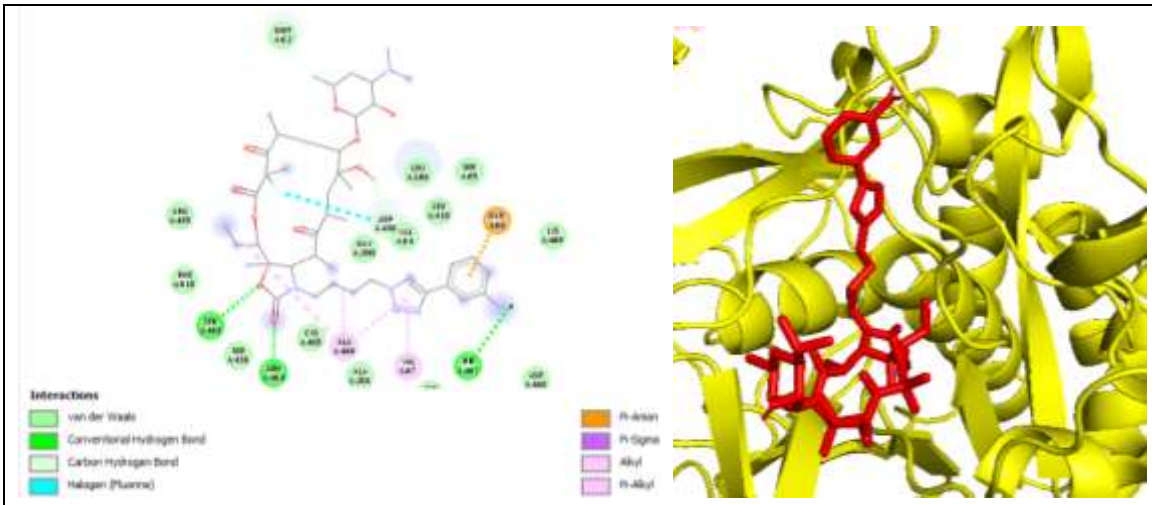

Fig.5: Visualization of interactions and binding region of Soithromycinwith 4TR2

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

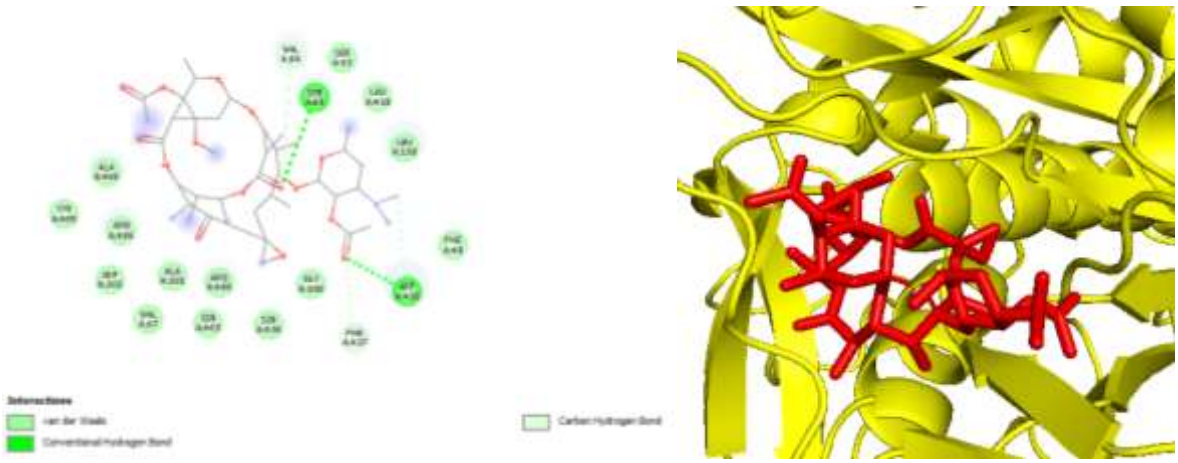

Fig.6: Visualizationof interactions and binding region of Troleandomycin with 4TR2

DISCUSSION

Malaria related fatalities have caused immense human and economic losses fuelling efforts to discover effective cure and therapeutics. In search for such potential therapeutic agents, we used in silico structure-based drug design and drug repurposing strategies. Several groups have targeted SUB1for developing effect drugs against Malaria. It is important to identify the target proteins of the egress inhibitor compounds because this will inform structure-activity relationship-based drug design to improve the potencies of compounds. Once their targets are known, these compounds could also act as useful tools to further dissect molecular details of egress and invasion processes in the parasite.

In this study, we have shown that by using the molecular docking studies it is possible to effectively screen compound libraries for inhibitors of parasite egress of RBCs. Using this technique, we screened the macrolide antibiotics that inhibit parasite egress. These inhibitors, together with their novel mechanisms of action, could complement current antimalarials which generally act on intracellular parasites during their growth phase.

CONCLUSION

To conclude, results obtained in our molecular docking studies, Pristinamycin, Solithromycin and Troleandomycin appeared attractive candidates as potential inhibitors of SUB1. However, in vitro, and in vivo studies are necessary to further investigate their therapeutic potential in treating malaria.

REFERENCES

1. *Fairhurst, R. M., &Dondorp, A. M.Artemisinin-Resistant Plasmodium falciparum Malaria. Microbiology spectrum 2016; 4(3), 10.1128/microbiolspec.EI10-0013-2016.*
2. *Artemisinin resistance and artemisinin-based combination therapy efficacy: status report. World HealthOrganization.2018 https://apps.who.int/iris/handle/10665/274362. License: CC BY-NC-SA 3.0 IGO. Accessed on July 31,2020.*
3. *R. Chandramohanadas, Y. Park, L. Lui, A.Li, D. Quinn, K. Liew, M. DiezSilva, Y. Sung, M. Dao, C.T. Lim,* et al.*Biophysics of malarial parasite exit frominfected erythrocytesPLoS ONE 2011; 6:e20869.*
4. *C.R. Collins, F. Hackett, M. Strath, M.Penzo, C. WithersMartinez, D.A. Baker, M.J. BlackmanMal aria parasite cGMP-dependent protein kinase regulates blood stage merozoite secretory organelle discharge and egressPLoSPathog 2013;9:e1003344.*
5. *Maturation and specificity of Plasmodium falciparum subtilisin-like protease-1, a malaria merozoite subtilisin-like serine protease.Sajid M, Withers-Martinez C, Blackman MJ. J Biol Chem. 2000; 275(1):631-41.*
6. *A.S. Nasamu, S. Glushakova, I. Russo, B.Vaupel, A. Oksman, A.S. Kim, D.H.Fremont, N. Tolia, J.R . Beck, M.J. Meyers,* et al.*Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasionScience 2017;358:518-22*
7. *P. Pino, R. Caldelari, B. Mukherjee, J.Vahokoski, N. Klages, B. Maco, C.R.Collins, M.J. Blackman , I. Kursula, V.Heussler,* et al.*A multistage antimalarial target the plasmepsins IX and X essential for invasion and egress. Science 2017; 358: 522-8.*
8. *Das S, Hertrich N, Perrin AJ, et al. Processing of Plasmodium falciparum Merozoite Surface Protein MSP1 Activates a Spectrin-Binding Function Enabling Parasite Egress from RBCs. Cell Host Microbe. 2015;18(4):433-44.*
9. *www.rcsb.org. Accessed on July 31,2020.*
10. *Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-12.*
11. *Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson,*

SJIF Impact Factor: 7.001| ISI I.F.Value:1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 5 | Issue: 8 | August 2020 - Peer Reviewed Journal

A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry *2009, 16: 2785-91*.

12. *O. Trott, A. J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 2010; 31: 455-61.*
13. *Biovia, D.S.Discovery studio modeling environment. San Diego, Dassault Systems, Release, (2015): 4.*
14. *DeLano, W. L. Pymol: An open-source molecular graphics tool. CCP4 Newsletter On Protein Crystallography 2002; 40:82-92.*