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IDENTIFICATION AND OPTIMIZATION OF BINDING SITE FOR AN ACTIVE METABOLITE OF CLOPIDOGREL, PRASUGREL AND TICLOPIDINE ON RECEPTOR P2Y12

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Keywords:

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ABSTRACT: In this article, an attempt was made to develop molecular docking studies on active metabolites of Prasugrel, Clopidogrel, and Ticlopidine acting as protein P2Y12 inhibitors. Molecular docking analysis was performing by using autodock version 4.3 adjoin with discovery studio to better understand the interactions between P2Y12 targets and inhibitors in this series. Hydrophobic and hydrogen bond interactions lead to the identification of active binding sites of P2Y12 protein in the docked complex, signifying the affection of active Metabolite of Prasugrel is more than other active metabolites of ticlopidine and clopidrogrel. The present study may lead to the discovery of therapeutically potent agents against clinically very important cardiovascular disorders, including arterial thrombosis, Hypertension, embolism etc. cardiac diseases. Hence the computer-aided drug design docking model proposed in this work can be employed to design the metabolites of Clopidogrel, Prasugrel, and Ticlopidine with specific P2Y12 inhibitory activity and futuristic active metabolites possibilities.

INTRODUCTION: Molecular modeling and computational tools have become a close matching part of experimenting in the understanding of molecular aspects of genetic systems [1-4]. The computational strategies like molecular docking and quantitative structure-activity relationship (QSAR) are utilized to find the new hits for different helpful targets [5-7]. The ongoing report featured the interface between computational methodologies and experiments as an essential tool in the drug discovery technology [8, 9].

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Using the prominent rising interest in the design of ligand-enzyme inhibitors, this present study is to elucidate the molecular docking study of active metabolites of clopidogrel, prasugrel, and ticlopidine as P2Y12 inhibitor using computational tools that can be applied to understand interactions between inhibitors and their target proteins.

P2Y12 Crystal structures of the human P2Y12 receptor shown in figure 1 have as of late been settled. Structures of the receptor in a complex with the agonists 2-methylthio-adenosine diphosphate (2MeS-ADP) and 2-methylthio-adenosine tri-phosphate (2MeS-ATP) [10, 11] and with the non-nucleotide antagonist ethyl 6-(4-[(benzylsulfonyl) carbamoyl] piperidin-1-yl)-5- cyano-2-methyl-nicotinate (AZD1283) were obtained [12, 13]. The P2Y12 receptor represents a successful drug target, with several clinical drugs on the market, including

clopidogrel, prasugrel, ticlopidine, cangrelor, and ticagrelor, and more drugs in clinical trials [14]. Along these lines, the advancement of P2Y12 antagonist has been a functioning region of medication improvement, and endeavors have been founded on dynamic metabolites of clopidogrel, prasugrel, and ticlopidine with the plan to maintain a strategic distance from biotransformation of the prodrug.

Docking considers as the structures of progressively potential medication target are explained the open door for the computer to perform beginning binding studies is expanding. By computationally docking a ligand to a protein, one point of confinement worries about examine intricacy, for example, compound solvency and the requirements to keep up broad physical compound libraries.

The goal of computational docking is to decide how atoms of realized structure will collaborate. The molecule may bind to the receptor and modify its function. The docking considers performed between receptor (P2Y12, PDB code: 4NTJ) and ligands by utilizing PyRx-Python solution (adaptation 0.8). 4NTJ **Fig. 1**, recovered from RCSB and arranged by Discovery Studio visualizer rendition 16.1.01

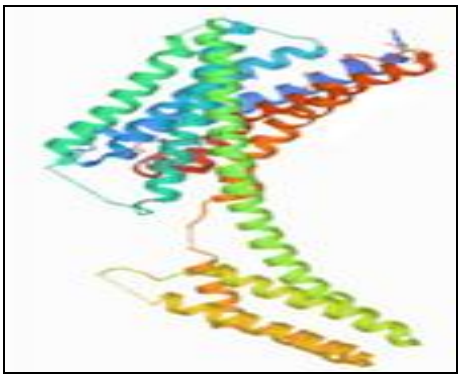

FIG. 1 STRUCTURE OF 4NTJ

Experimental Methods: 3D structure of the enzyme P2Y12 with PDB code: 4NTJ by Zhang *et al.,* and active metabolites are shown in **Fig. 2** of clopidogrel, prasugrel, and ticlopidine were taken from literature [15, 16]. The protein structure was downloaded from the information base online Protein Data Bank (PDB) [17, 18]. Two and three-dimensional structure of metabolites drawn utilizing program package ChemDraw Ultra v12.0.2, 2010.

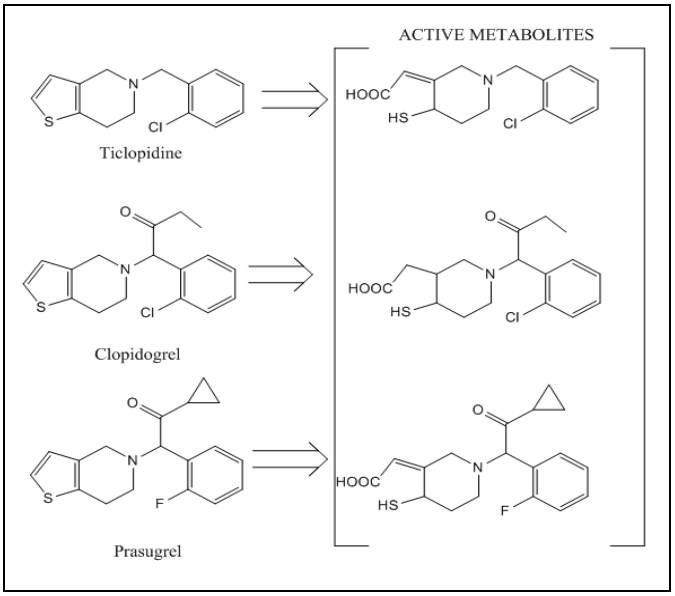

FIG. 2: ACTIVE METABOLITE OF TICLOPIDINE, CLOPIDOGREL AND PRASUGEL

Preparation of Protein Structure: The 3D coordinates of the crystal structure of P2Y12 (PDB ID: 4NTJ) were downloaded from the Protein Data Bank [19–21]. 4NTJ (chains A) were picked for the docking reenactments. Before docking, all water atoms are ousted from protein document 4NTJ. In the wake of ousting the water molecules, H atom was added to protein for right ionization and tautomeric states of amino corrosive, such as ARG, CYS, LYS, PHE TYR, and VAL.

Preparation of Ligand Structures: The ligands used for the docking study were selected from the literature [22–24]. The ligand structures were generated using the tool ChemDraw ultra v12.0.2. Three-dimensional optimizations of the ligand structures were done and saved as 'PDB file'. Geometry optimizations of the ligands were performed using the Steepest descent calculation method using Avagdro software. The compounds included in the study are active metabolites of clopidogrel, prasugrel, and ticlopidine; the bioactive compounds considered for the study are listed in **Fig. 2**.

Protein-ligand Interaction using PyRx (Autodock Vina): The docking studies were conceded by PyRx (Autodock vina) tools [25, 26] version v 0.8 programs. The looking through lattice reached out over the favoured objective proteins; polar hydrogen was added to the ligand moieties. Kollman charges were assigned, and atomic solvation parameters were added. Polar hydrogen

charges of the Gasteiger-type were assigned, and the non-polar hydrogen was merged with the carbons, and the internal degrees of freedom and torsions were set. Active metabolites were docked to target protein complex (4NTJ), with the molecule considered as a rigid body and the ligand being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 Å. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 9 generations. Evaluation of the results was done by sorting the different complexes concerning the predicted binding energy. A cluster analysis based on root mean square deviation values concerning the starting geometry was subsequently performed, and the lowest energy conformation of the more populated cluster was considered as the most trustable solution.

RESULTS AND DISCUSSION: In this present study, to understand the formation of hydrogen bond interactions between the active metabolites and active sites of the crystal structure of P2Y12 (PDB code: 4NTJ) was used to explore their binding mode, and a docking study was performed by using PyRx (Autodock vina) [27, 28]. Three active metabolites of clopidogrel, prasugrel and ticlopidine were retrieved from literature [29]. The 3D structure and energy minimization was done by Avogadro software. All these chemical compounds. To date, three crystal structures of P2Y12 in complex with agonist and antagonist have been reported in the literature. In the present study, we have used X-ray crystallography structure of P2Y12 (PDB code: 4NTJ) **Fig. 1** in ternary complex with the non-nucleotide antagonist ethyl 6-(4-[(benzylsulfonyl) carbamoyl] piperidine-1-yl)-5- cyano-2-methylnicotinate (AZD1283) is used for the docking study.

Binding Site of the Protein: The detection of ligand-binding sites is often the starting point for protein function identification and drug discovery [30, 31]. In our study, PyRx (autodock vina) predicted the active site of the receptor P2Y12 (4NTJ) with higher average precision. P2Y12 (4NTJ) 's active site comprises amino acid residues such as CYS97, VAL102, TYR105, PHE106, TYR109, MET152, LEU155, SER156, ASN159, HIS187, VAL190, ASN191, CYS194, PHE252, ALA255, ARG256, TYR259, LEU276, AND VAL279. As most of the amino acid residues in the active site are hydrophobic, so they are the main contributors to the receptor and ligand-binding interaction. Amino acid interaction of active metabolite of Ticlopidine, Clopidogrel, and Prasugrel shown in **Fig. 3**, **4,** and **5** respectively, and standard drug ethyl 6- (4-((benzylsulfonyl) carbamoyl) piperidin- 1- yl)- 5-cyano-2-methylnicotinate amino acid interaction shown in **Fig. 6**.

Interaction between Active Metabolites and 4ntj:

TABLE 1: DOCKING ENERGY OF ACTIVE METABOLITES OF COLPIDOGREL, PRASUGREL, AND TICLOPIDINE

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Metabolite of Ticlopidine	-7	6.94	1.496
Metabolite of Colpidogrel	-5.7	30.813	29.362
Metabolite of Prasugrel	-7.1	2.378	2.126
Std	-6.8	12.349	11.335

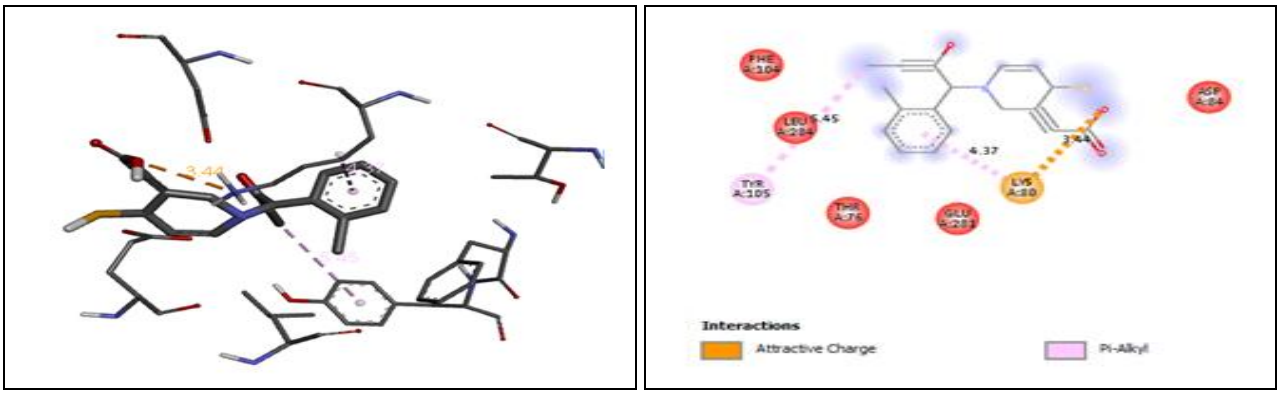

FIG. 3: AMINO ACID INTERACTION WITH TICLODIPINE LIGAND

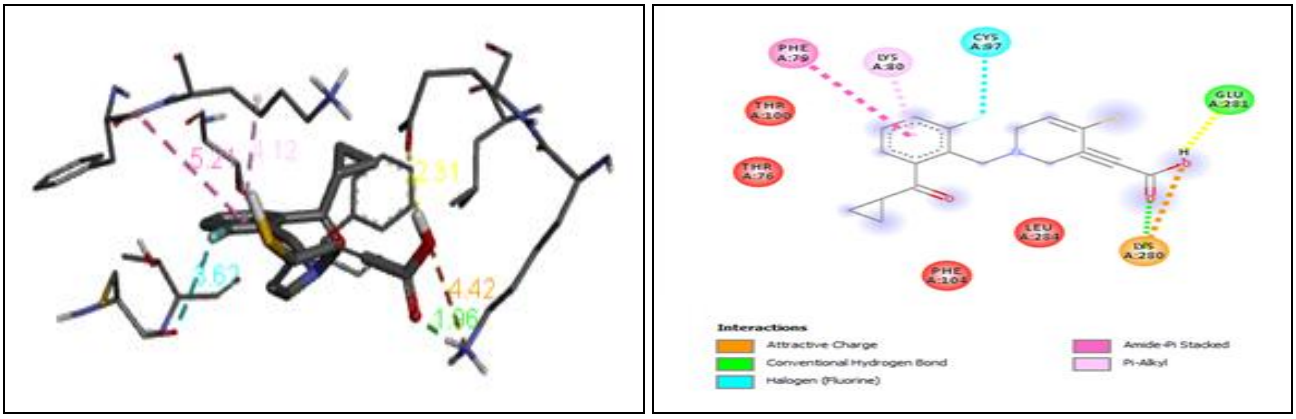

FIG. 4: AMINO ACID INTERACTION WITH COLPIDOGREL LIGAND

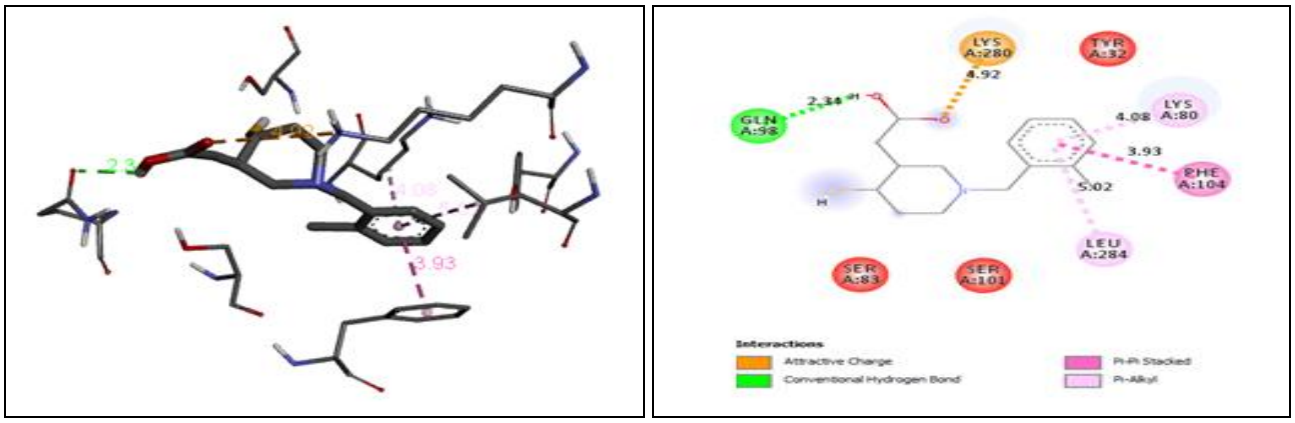

FIG. 5: AMINO ACID INTERACTION WITH PRASUGREL LIGAND

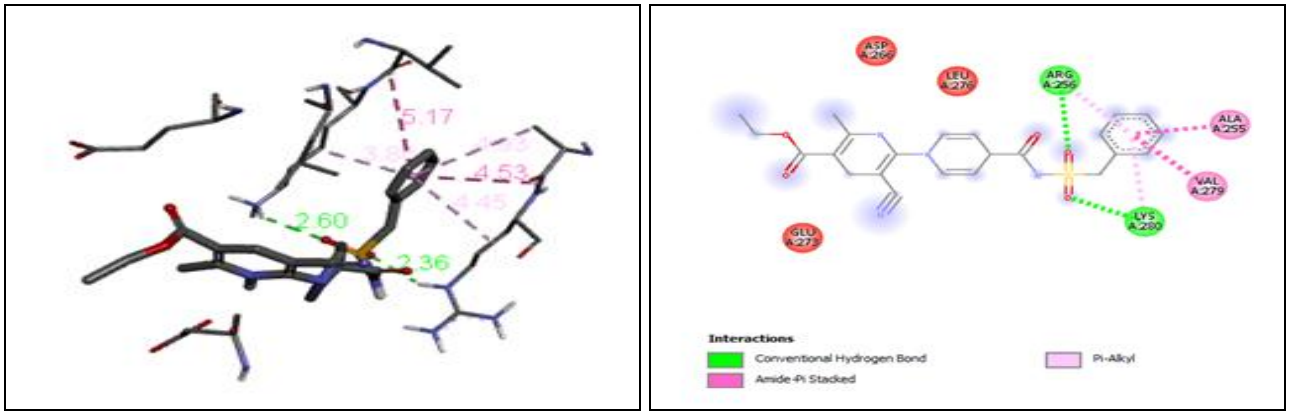

FIG. 6: AMINO ACID INTERACTION WITH STD ETHYL 6-(4-((BENZYLSULFONYL) CARBAMOYL) PIPERIDIN-1-YL)-5-CYANO-2-METHYLNICOTINATE LIGAND

CONCLUSION: The P2Y12 receptor is a significant objective of antithrombotic treatment. In PCI patients, new P2Y12 inhibitors decrease all-cause mortality and major ischemic conditions, specifically in PCI for STEMI patients.

These further backings that a more significant level of platelet inhibition than clopidogrel (600 mg) is required for, by far, most of the patients. This study based on binding energy for receptor P2Y12 (4NTJ) of the metabolite of Prasugrel ligand showed better activity than Metabolite of Ticlopidine Metabolite of Colpidogrel, which may show a better dose regimen with lesser side effects. This research will help for further wetlab Pharmacological investigation of Prasugrel ligand as compared to a metabolite of Ticlopidine and metabolite of Clopidogrel.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Wang R, Hozumi Y, Yin C and Wei GW: Decoding SARS-CoV-2 Transmission and Evolution and Ramifications for COVID-19 Diagnosis, Vaccine and Medicine. Journal of Chemical Information and Modeling 2020; 1(1): 101-12.
2. Villas-Boas GR: The New Coronavirus (SARS-CoV-2): A Comprehensive Review on Immunity and the Application of Bioinformatics and Molecular Modeling to the Discovery of Potential Anti-SARS-CoV-2 Agents. Molecules 2020; 1(1): 121-34.
3. Mir-Artigues P, Twyman RM, Alvarez D, Cerda Bennasser P, Balcells M, Christou P and Capell TA: Simplified Techno-Economic Model for the Molecular Pharming of Antibodies. Biotechnology and Bioengineering 2019; 4(14): 1458-65.
4. Martin RW, Butts CT, Cross TJ, Takahashi GR, Diessner EM, Crosby MG, Farahmand V and Zhuang S: Sequence characterization and molecular modeling of clinically relevant variants of the SARS-CoV-2 main protease. Biochemistry 2020; 2(2): 151-68.
5. De P, Bhayye S, Kumar V and Roy K: *In-silico* modeling for quick prediction of inhibitory activity against 3CLpro enzyme in SARS CoV diseases. Journal of Biomolecular Structure and Dynamics 2020; 2(2): 123-35.
6. Nesměrák K: Medicinal Chemistry meets electro-chemistry: redox potential in the role of endpoint or molecular descriptor in QSAR/QSPR. Mini-Reviews in Medicinal Chemistry 2020; 2(3): 201-13.
7. Chinen K and Malloy T: QSAR use in REACH analyses of alternatives to predict human health and environmental Toxicity of Alternative Chemical Substances. Integrated Environmental Assessment and Management 2020; 2(5): 3-13.
8. Hughes JP, Rees S, Kalindjian SB and Philpott KL: Principles of Early Drug Discovery. British Journal of Pharmacology 2011; 162 (6): 1239-49.
9. Drews J: Drug Discovery: A Historical Perspective. Science (80-.) 2000; 3 (2): 135-45.
10. Shi C and Kim T: crystal clots as therapeutic target in cholesterol crystal embolism. Circ Res 2020; 3 (5): 22-36.
11. Neumann A, Müller CE and Namasivayam V: P2Y1-like nucleotide receptors—structures, molecular modeling, mutagenesis, and oligomerization. wiley interdiscip. Wiley Interdisciplinary Reviews: Computational Molecular Science 2020; 10 (4): 134-46.
12. Kong D, Xue T, Guo B, Cheng J, Liu S, Wei J, Lu Z, Liu H, Gong G, Lan T, Hu W and Yang Y: Optimization of P2Y12 Antagonist Ethyl 6-(4-((Benzylsulfonyl) Carbamoyl) Piperidin-1-Yl)-5-Cyano-2-Methylnicotinate (AZD1283) Led to the Discovery of an Oral Antiplatelet Agent with Improved Druglike Properties. Journal of Medicinal Chemistry 2019; 62 (6): 3088-06.
13. Van Doren, L, Nguyen N, Garzia C, Fletcher E, Stevenson R, Jaramillo D, Kuliopulos A and Covic L: Blockade of lipid receptor gpr31 suppresses platelet reactivity and thrombosis with minimal effect on hemostasis. Blood 2019; 134 (Supplement_1): 1064-64.
14. Kaye AD and Manchikanti L: responsible, safe, and effective use of antithrombotics and anticoagulants in patients undergoing interventional techniques: American society of interventional pain physicians (ASIPP) Guidelines. Pain Physician 2019; 22(1S): S75-S128.
15. Qiu J, Lingna W, Jinghong H and Yongqing Z: Oral administration of leeches (shuizhi): a review of the mechanisms of action on antiplatelet aggregation. Journal of Ethnopharmacology 2019; 1(2): 201-22.
16. Ticagrelol versus aspirin in ischemic stroke. Case Med Res 2019.
17. Bank W: How countries are using edtech (including online learning, radio, television, texting) to support access to remote learning during the COVID-19 Pandemichttps:// Www.Worldbank.Org/En/Topic/Edutech/Brief/How-Countries-Are-Using-Edtech-to-Support-Remote-Learn. Worldbank.org 2020; 1 (1): 1-22.
18. Akbar: Snapshot Perbankan Syariah Indonesia. Snapshoot Perbank. syariah 2018; 1 (1): 1–9.
19. Demirguc-Kunt A, Klapper L, Singer D, Ansar S and Hess J: The global findex database 2017: measuring financial inclusion and the fintech revolution. glob. findex database 2017 meas. Financ Incl Fintech Revolut 2018; 1(1): 121-34.
20. Kurbucz MT: A Joint Dataset of Official COVID-19 Reports and the Governance, Trade and Competitiveness Indicators of World Bank Group Platforms. Data Br 2020; 31(1): 15-17.
21. The Work Bank. Employment in Agriculture (Percentage of Total Employment). Int. Labour Organ. ILOSTAT database. Data Retrieved 2014; 1(1): 19-36.
22. Vardhan S and Sahoo SK: *In-silico* ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. Computers in Biology and Medicine 2020; 124(1): 135-56.
23. Bajgain KT, Badal S, Bajgain BB and Santana MJ: Prevalence of comorbidities among individuals with covid-19: a Rapid Review of Current Literature. American Journal of Infection Control 2020; 1(1): 123-45.
24. Passarelli PC, Lopez MA, Bonaviri GNM, Garcia-Godoy, F and DâAddona A: Taste and Smell as chemosensory dysfunctions in CO VID-19 infection. American Journal of Dentistry 2020; 33(3): 135-37.
25. Solis-Vasquez L, Santos-Martins D, Koch A and Forli S: Evaluating the energy efficiency of opencl-accelerated autodock molecular docking. in proceedings - 2020 28th Euromicro International Conference on Parallel, Distributed and Network-Based Processing, PDP 2020; 162-66.
26. Kumar S, Kashyap P, Chowdhury S, Kumar S, Panwar A and Kumar A: Identification of phytochemicals as potential therapeutic agents that binds to nsp15 protein target of coronavirus (SARS-CoV-2) That Are Capable of Inhibiting Virus Replication. Phytomedicine 2020; 1(1): 145-56.
27. Gandhi AJ, Rupareliya JD, Shukla VJ, Donga SB and Acharya R: An ayurvedic perspective along with *in-silico* study of the drugs for the management of SARS-CoV-2. Journal of Ayurv and Integr Medicine 2020; 1 (1): 145-56.
28. Singh V: Structure based docking of secondary metabolites against drpe1 to Treat Tuberculosis. Int J Res Appl Sci Eng Technol 2020; 8(6): 2515-20.
29. Baqi Y and Müller CE: Antithrombotic P2Y 12 receptor antagonists: recent developments in drug discovery. Drug Discovery Today 2019; 24(1): 325-33.
30. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y, Yu J, Wang L, Yang K, Liu F, Jiang R, Yang X, You T, Liu X, Yang X, Bai F, Liu H, Liu X, Guddat LW, Xu W, Xiao G, Qin C, Shi Z, Jiang H, Rao Z and Yang H: Structure of Mpro from SARS-CoV-2 and Discovery of Its Inhibitors. Nature 2020; 582 (7811): 289-93.

31. Crommelin D, Stolk P, Besançon L, Shah V, Midha K and Leufkens H: Pharmaceutical Sciences in 2020. Nature Reviews Drug Discovery 2010; 9(2): 99-00.

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