IJPSR (2020), Volume 11, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 December 2019; received in revised form, 14 April 2020; accepted, 20 April 2020; published 01 December 2020

MOLECULAR DOCKING STUDY OF IBUPROFEN DERIVATIVES AS SELECTIVE INHIBITORS OF CYCLOOXYGENASE-2

A. Chebrolu [*1] and S. Madhavan [2]

Independence High School [1], 10555 Independence Pkwy, Frisco, TX 75035, USA.
Department of Internal Medicine [2], University of North Dakota School of Medicine and Health Sciences, 1919 N Elm Street, Fargo, ND 58102, USA.

Keywords:

Cyclooxygenase, Ibuprofen, Molecular Docking, Pain, Analgesics

Correspondence to Author:
A. Chebrolu

Independence High School,
10555 Independence Pkwy, Frisco,
TX 75035, USA.

E-mail: adityarchebrolu@gmail.com

ABSTRACT: The inhibition of the protein cyclooxygenase (COX) is a well-known mechanism for achieving analgesia. Selective inhibitors of cyclooxygenase-2 (COX-2) provide excellent analgesia but can have side-effects. In this study, we chose ibuprofen, a non-selective COX inhibitor that has been safely used for a long time, to develop a novel, selective COX-2 inhibitor. The 3-dimensional structure of the drug target, COX-2, was obtained from the RCSB PDB online database and was input in SeeSAR. Three hundred and thirty alterations were made to ibuprofen molecule. Initial docking was performed in SeeSAR and CLC Drug Discovery Workbench to get a docking score. The 216 ligands that bound to COX-2 with the best binding score were then docked to cyclooxygenase-1 protein (COX-1), and a score was generated based on the binding affinity. Twenty-six of these molecules that didn't bind to COX-1 were chosen as the selective inhibitors of COX-2 and these were tested for drug-likeness properties using the DruLiTo software. Twenty-two compounds with good drug-likeness properties were subjected to ADMET verification. 5 compounds with good ADMET properties were then subjected to a slower but extremely accurate binding energy test using the AUTODOCK VINA software. The ligand (2S)-2-[4-(2-oxo-2, 5-dihydro-furan-3-yl)-3-(pyridin-4-yl) phenyl] propanoic acid was chosen as the best selective inhibitor of COX-2 that could be derived from Ibuprofen base structure and could be a potential drug compound that can be further tested for effective treatment of pain.

INTRODUCTION: Non-Steroidal Anti-inflammatory Drugs (NSAIDs) reduce pain by inhibiting the cyclooxygenase (COX) enzyme [1]. There are two cyclooxygenase isoenzymes that have been identified: cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2).

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(12).6526-31

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6526-31

The COX enzyme oxidizes arachidonic acid to prostaglandin G2 (PGG2), which is then per-oxidized to PGH2. PGH2 is then metabolized into structurally related yet different prostaglandins, such as prostaglandin E2 (PGE2), prostaglandin D2 (PGD2), prostaglandin F2 (PGF2), prostaglandin I2 (PGI2), and thromboxane (TXA2). Prostaglandins that are derived from COX-1 are responsible for stimulating bodily functions such as stomach mucous production, regulation of gastric acid, kidney water excretion, and platelet aggregation. On the other hand, COX-2 induces pain and inflammation by increasing the production of PGE2 [1, 2].

Most NSAIDs are non-selective COX inhibitors, meaning they bind to both forms of cyclo-oxygenase [3, 4]. Inhibition of COX-1, however, will increase gastrointestinal irritation and may cause gastric ulcers [5]. Further, COX-1 inhibition decreases the rate of platelet aggregation and increases the risk of bleeding.

The inhibition of COX-2, on the other hand, reduces pain and inflammation, and this is the mechanism of pain control [5, 6]. Selective COX-2 inhibitors, which inhibit only COX-2 and do not inhibit COX-1, were thought to have reduced side effects as a result [6]. However, the currently available selective COX-2 inhibitors, the Coxibs, such as Celecoxib, Rofecoxib, and Valdecoxib, were found to have their own side effects, primarily cardiovascular side effects such as an increased risk of heart attacks and strokes [7, 8]. Only Celecoxib is currently available in the US market after the removal of Rofecoxib and Valdecoxib by the FDA. Ibuprofen is a non-selective NSAID that has been well-researched and has been in use for more than 40 years as an over the counter analgesic [9, 10].

In-silico or computational methods have been widely applied to the subject of pharmacology and have been commonly used to discover and optimize novel compounds by calculating binding capabilities, drug-likeness properties, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) [11].

The aim of the study will be to identify various ligands *in-silico* using the molecular structure of the commonly used and relatively safe ibuprofen as the base and identify molecules that preferentially bind and inhibit the activity of COX-2 selectively. This will eventually pave the way to develop a novel, possibly safer, and more effective analgesic alternative.

MATERIALS AND METHODS:

Retrieval and Preparation of Proteins: The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank will be used to retrieve the 3-dimensional structures of the proteins COX-2 and COX-1, with the PDB codes of 4PH9 and 1EQG, respectively [12, 13, 14]. These files will be downloaded and saved in the protein data bank file format (.pdb).

Preparation of Ligands: For this study, we plan to use the SeeSAR version 9.2 software designed by BioSolveIT. Using the SeeSAR software, various molecules differing in their chemical structure will be derived from the molecular structure of ibuprofen, [(RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid], which will be obtained from the PubChem database [15]. The compounds will be created using randomized edits to the ibuprofen molecular structure while making sure that their atomic structures are compatible. These ligands will be saved in the structure-data file format (.sdf) [15].

Preliminary Molecular Docking: A preliminary docking test will be performed in SeeSAR with all the created molecules to both the proteins COX-1 and COX-2. SeeSAR uses an algorithm known as FlexX [16]. In FlexX, hydrogen bonds and metal and aromatic ring attractions are matched. Then, the remaining components are incrementally built-up in accordance with a set of predefined rotatable torsion angles to account for ligand flexibility. However, FlexX does not provide a binding affinity/docking score [16]. Therefore, we also intend to use Qiagen Bioinformatics' CLC Drug Discovery Workbench version 3.0.2 to generate a docking score, while SeeSAR would be primarily used to show the binding site and docking pose of the ligand [17]. The binding site would be set as the binding site of ibuprofen for both software. The docking score function used in CLC Drug Discovery Workbench is the PLANTSPLP scoring function. A negative score indicates a strong binding while a less negative or a positive score indicates a weak or non-existent binding [17]. The scoring formula used is as follows:

$$\text{Score} = S_{\text{target}} - \text{ligand} + S_{\text{ligand}}$$

An average of 5 trials will be performed for each molecular ligand for both COX-1 and COX-2 in CLC Drug Discovery Workbench. These trials would each docked with default parameters with a population size of 200 with 100 generations and 2 solutions [17]. While searching for COX-2selectivity, all the ligands with a poor binding affinity to COX-1 yet with a good binding affinity to COX-2 according to the CLC Drug Discovery Workbench will be selected to move on to the next stage to demonstrate COX-2 selectivity.

We also intend to use a student's t-test using Microsoft Excel to assess if the lead molecule is significantly different with respect to the binding affinity towards COX-2 when compared to ibuprofen to ensure that the lead molecule in this study was drawn from a different group with a significant difference within their population means.

This would show that the COX-2 values for the lead molecule in this study are statistically significant and not just random data. The null hypothesis in this experiment would be that there is no difference in the binding affinity between the non-selective ibuprofen and the COX-2 selective lead molecule. In this study, a two-sample two-tailed, unpaired t-test, will be performed.

Drug - Likeliness Analysis: The qualifying compounds from the previous step will then be tested for drug-likeliness properties in the next stage. We plan to first test the compounds for drug relevant properties using the DruLiTo software, designed by the National Institute of Pharmaceutical Education and Research (NIPER) based on Chris Lipinski's Rule of 5 and Arup Ghose's Ghose Filter. Lipinski's Rule of 5 is a set of criteria used to determine compounds that have good absorption and permeation in biological systems. The set criteria are: molecular weight under 500 g/mol, the value of logP is lower than 5, and the molecule has the utmost 5 H-donor and 10 H-acceptor atoms [18].

Ghose Filter is another criterion used to define drug-like compounds as calculated log P coefficient is between -0.4 and 5.6, molecular weight is between 160 and 480 g/mol, molar refractivity is between 40 and 130, and the total number of atoms is between 20 and 70. None of these drug-likeness constraints can be violated, or the ligand will not have properties suitable for drug-like compounds and therefore may cause problems in further drug development [19, 20].

Analysis of ADMET Properties: The compounds that satisfied both the set criteria will be advanced to the next stage, and these would then be tested for ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties using the admet SAR 2.0 web tool. Admet SAR is anonline web tool developed by the Shanghai Key Laboratory of New Drug Design that is used to calculate the ADMET testing of a compound, and this program uses 200,000 ADMET annotated data points from roughly 96,000 various compounds to predict ADMET properties.

The properties to be tested include blood-brain barrier (BBB+), human intestinal absorption (HIA+), Caco-2 permeability (Caco-2+), AMES (AMES-), and carcinogenicity (carcinogenicity-) [21]. Using the Open Babel software, the saved molecules in the .sdf format will be converted to the SMILES format as this is the input format that admet SAR requires [22].

Final Docking: The molecules that satisfied the ADMET properties will then be subjected to a more detailed and accurate docking test using the AUTODOCK VINA program in the PyRx software, version 0.823,24. AUTODOCK VINA generates a binding energy score by incorporating Monte Carlo simulated Lamarckian genetic algorithm methods. The AMBER force field, including van der Waals interactions, hydrogen bonding, electrostatic interactions, conformational entropy, and solvation terms, is incorporated into the scoring function [23]. Based on these results, conclusions on proper drug candidates can be made, and a lead molecule would be defined.

RESULTS AND DISCUSSION: The 330 ligands that were developed from ibuprofen structure using See SAR were subjected to CLC Drug Discovery to calculate their affinity to COX-2. The CLC Drug Discovery Workbench generates a binding affinity score from the ligand-protein interactions. Two hundred and sixteen of these 330 ligands were found to bind to COX-2, and these were then subjected to another separate docking test with the new drug target of COX-1.

By selecting ligands that have the best binding energy to COX-2 while showing no binding to COX-1, the selective COX-2 inhibitors were selected from the group being tested. Stronger binding affinity to the protein complex is usually characterized by lower binding affinity measurements. Of the 216 compounds that bound to COX-2 earlier, only 26 ligands did not bind to COX-1, and hence those were identified as the selective COX-2 inhibitors.

TABLE 1: (2S)-2-[4-(2-OXO-2, 5-DIHYDRO-FURAN-3-YL) -3-(PYRIDIN-4-YL) PHENYL] PROPANOIC ACID SCORE OF PRELIMINARY DOCKING FOR COX-2

Trials	Binding Affinity Towards COX-2 (kcal/mol)	Binding Affinity Towards COX-1 (kcal/mol)
Trial 1	-50.21	-28.14
Trial 2	-50.19	-28.10
Trial 3	-50.19	-28.09
Trial 4	-50.19	-28.06
Trial 5	-50.17	-27.93
Average	-50.19	-28.06

Table 1 shows the values of the binding affinity. The lead molecule shows an excellent binding affinity to the COX-2 protein, whereas it shows a poor binding affinity towards the COX-1 protein. This shows that it can effectively selectively bind to the cyclooxygenase-2 protein. Fig. 1 and 2 show the molecule in its binding site.

The 26 COX-2 selective molecules were then tested for drug-likeness properties using DruLiTo (Drug Likeness Tool) software to test for drug-likeness properties. All the 26 compounds passed the criteria set for Lipinski's Rule of 5, while 4 compounds didn't pass the criteria set for the Ghose Filter, leaving 22 compounds chosen to move onto the next portion of this study.

TABLE 2: DRUG-LIKENESS PROPERTIES OF THE (2S)-2-[4- (2-OXO-2, 5- DIHYDROFURAN-3-YL)-3-(PYRIDIN-4- YL)PHENYL]PROPANOIC ACID

Molecular Weight	logP partition coefficient	Hydrogen Bond Acceptors	Hydrogen Bond Donors	Molar Refractivity	Number of Atoms
308.09	0.55	5	0	87.86	37

Table 2 depicts the Lipinski's Rule of 5 and Ghose Filter, which confirms that the molecule has good drug-likeness properties.

These 22 compounds were then subjected to an ADMET verification stage. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties determine the disposition of a compound in the human body [30]. Of the 22 compounds, only 5 COX-2 selective ligands surpassed the set requirements for all these ADMET properties and were moved on to lead molecule declaration phase.

TABLE 3: ADMET PROPERTIES OF THE (2S)-2-[4-(2-OXO-2, 5-DIHYDROFURAN-3-YL)-3-(PYRIDIN-4-YL) PHENYL] PROPANOIC ACID

Blood Brain Barrier	AMES Toxicity	Carcinogenicity	Human Intestinal Absorption	Caco-2 Permeability
Yes	No	No	Yes	Yes

Table 3 depict safe important ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties that determine the disposition of the compound in the human body.

Each one of these 5 ligands is not only selective COX-2 inhibitors of the cohort but also had good drug-likeness and ADMET properties. To further winnow the field and choose the best possible lead molecule, a slower and extremely accurate binding energy test was performed on each of these 5 ligands using the AUTODOCK VINA program in the PyRx software. With the excellent binding affinity of -7.9 kcal/mol and RMSD values of 2.092 (upper bound) and 1.584 (lower bound) that showed a good reproduction of the selected docking pose of the ligand to the receptor and with the analysis of all the previous data collected so far, a single lead molecule, with the structure of (2S)-2-[4-(2-oxo-2,5-dihydrofuran-3-yl)-3-(pyridin -4- yl)phenyl]propanoic acid, was successfully declared as the lead molecule from the final list of the 5 potential drug candidates.

The student's t-test was performed to evaluate if the lead molecule is different from Ibuprofen and revealed a t-statistic of 680.495403223303. This ensures that the difference in the binding affinity towards COX-2 for Ibuprofen and the lead molecule in this study are statistically significant.

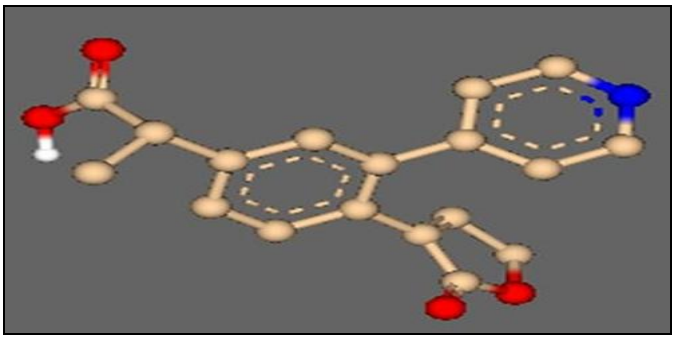

FIG. 1: MOLECULAR STRUCTURE OF THE (2S) -2-[4-(2-OXO-2, 5- DIHYDRO-FURAN-3-YL)-3-(PYRIDIN-4-YL)PHENYL]PROPANOI CACID

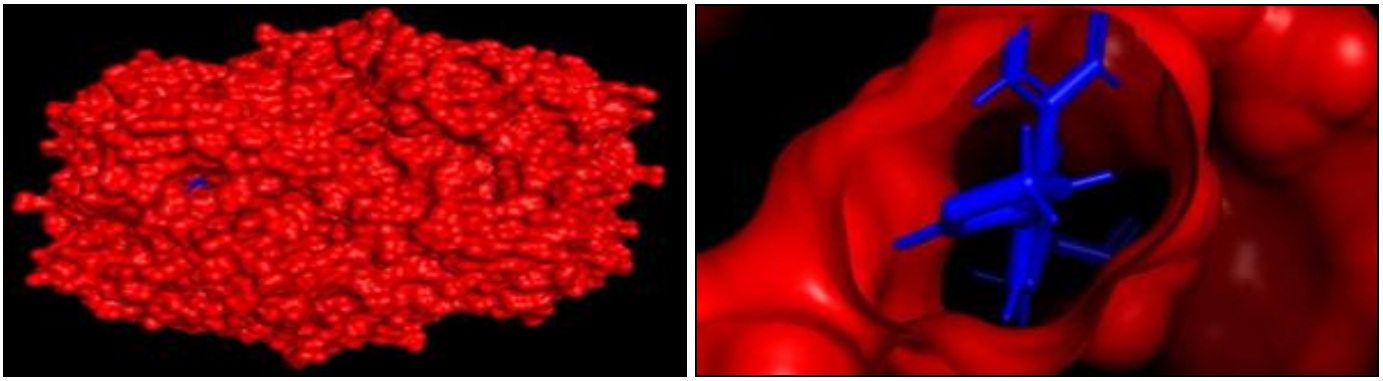

FIG. 2: DOCKING POSE OF THE (2S)-2-[4-(2-OXO-2, 5- DIHYDROFURAN-3-YL)-3-(PYRIDIN-4-YL)PHENYL] PROPANOIC ACID WITH THE DRUG TARGET CYCLOOXYGENASE-2

TABLE 4: AUTODOCK VINA RESULTS

Target Protein	Binding Affinity (kcal/mol)	RMSD Lower Bound	RMSD Upper Bound
Cyclooxygenase-2	-7.9	2.092	1.584

Table 4 presents the binding affinity of the lead molecule to the protein target, cyclooxygenase-2.

Previously, Hizliates *et al.,* performed an *in-silico* and in-vitro molecular docking analysis of ligands derived from Ibuprofen for the inhibition of COX-2. However, they did not perform *in-silico* analysis as thoroughly as done in this study, such as testing for drug-likeliness and ADMET properties [25]. Bitten court *et al.,* have also reported a similar study in which they present a comparison of the properties of ibuprofen to that of two benzoyl propionic acid derivatives. However, they did not derive molecules from NSAIDs themselves, such as ibuprofen, to have a strong foundational chemical structure to improve on [26].

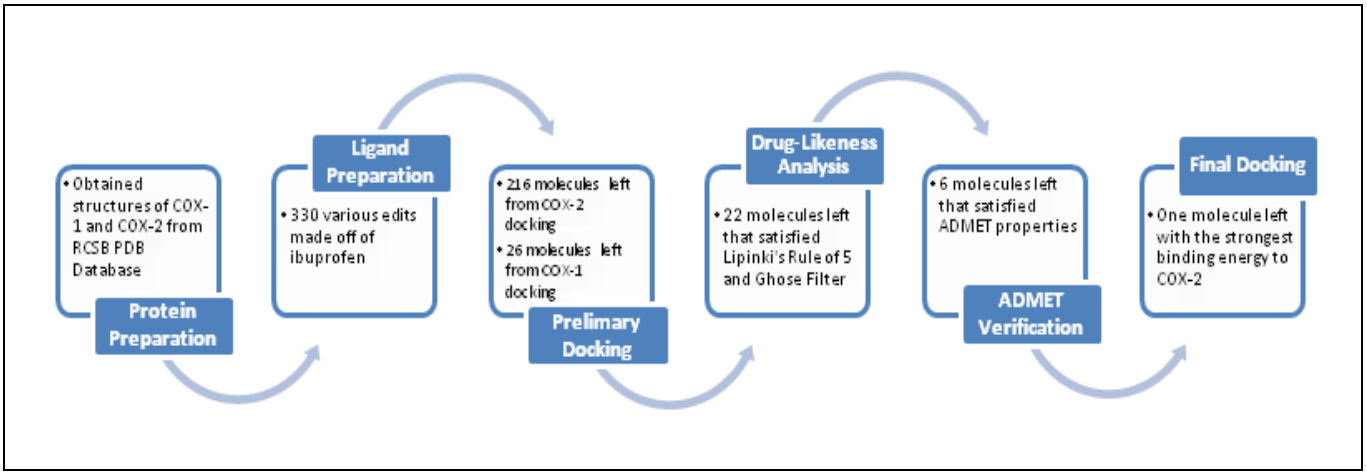

FIG. 3: FLOWCHART OF THE METHODOLOGY AND RESULTS FOR EACH STEP IN THIS STUDY

This study is only as comprehensive as the capabilities of the software used and the computational power available to run these tests. Further, the computer algorithms simply cannot express the immense complexity of the human body, and it's highly intertwined homeostatic systems. In addition, the inaccuracy of protein flexibility, molecule conformation, and promiscuity in these technology-driven methods hinder accurate predictions [27]. However, as technology advances, *in-silico* pharmacologic development and testing is becoming increasingly common as an alternative to lab-like testing due to the reduction in the number of molecules made and tested and increased speed of experiments. Ultimately, large reductions in the cost of drug development are prominent with the increasing use of *in-silico* methods [28].

CONCLUSION: In this study, a successful attempt was made to identify a lead compound with the structure (2S)-2-[4-(2-oxo-2, 5-dihydro-furan -3-yl)-3-(pyridin-4-yl) phenyl] propanoic acid that can successfully and selectively inhibit cyclo-oxygenase-2 using ibuprofen as the base molecule.

This lead molecule has a novel structure, having been derived from the known chemical structure of ibuprofen. Further testing of this compound and *in-vivo* trials will help develop it into a safer drug to alleviate pain and combat the opioid epidemic.

ACKNOWLEDGEMENT: None

CONFLICTS OF INTEREST: None

REFERENCES:

1. Vuilleumier, Pascal H, Schliessbach J and Curatolo M: Current evidence for central analgesic effects of NSAIDs: an overview of the literature. Minerva anestesiologica 2018; 84(7): 865-870.
2. Wongrakpanich S, Wongrakpanich A, Melhado K and Rangaswami J: A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis 2018; 9(1): 143-50.
3. Ornelas A, Zacharias-Millward N and Menter DG: Beyond COX-1: the effects of aspirin on platelet biology and potential mechanisms of chemoprevention. Cancer Metastasis Rev 2017; 36(2): 289-03.
4. Osafo N and Agyare C: Mechanism of action of non-steroidal anti-inflammatory drugs. Nonsteroidal Anti-Inflammatory Drugs 2017; 2.
5. Bjarnason I, Scarpignato C, Holmgren E, Olszewski M, Rainsford KD and Lanas A: Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. Gastroenterology2018; 154(3): 500-14.
6. Ferrer MD, Busquets-Cortés C, Capó X, Tejada S, Tur JA, Pons A and Sureda A: Cyclooxygenase-2 inhibitors as a therapeutic target in inflammatory diseases. Current Medicinal Chemistry 2019; 26(18): 3225-41.
7. Rayar AM, Lagarde N, Ferroud C, Zagury JF, Montes M and Sylla-Iyarreta VM: Update on COX-2 selective inhibitors: Chemical classification, side effects and their use in cancers and neuronal diseases. Current Topics in Medicinal Chemistry 2017; 17(26): 2935-56.
8. Curtis E, Fuggle N and Shaw S: Safety of cyclo-oxygenase-2 inhibitors in osteoarthritis: outcomes of a systematic review and meta-analysis. Drugs Aging. 2019; 36(Suppl 1): 25-44.
9. Poddighe D, Brambilla I, Licari A and Marseglia GL: Ibuprofen for pain control in children: new value for an old molecule. Pediatric Emergency Care 2019; 35(6): 448-53.
10. Kanabar RDJ: A clinical and safety review of paracetamol and ibuprofen in children. Inflammopharmacology 2017; 25(1): 1–9
11. Ekins S and Mestres J: *In-silico* pharmacology for drug discovery: applicationsto targets and beyond. British Journal of Pharmacology 2017; 152(1): 21-37.
12. Berman HM and Westbrook J: The Protein Data Bank Nucleic Acids Research 2000; 28: 235-42.
13. Orlando BJ and Lucido MJ: The structure of ibuprofen bound tocyclooxygenase-2. JSB 2015; 189(1): 62-66.
14. Selinsky BS and Gupta K: Structural analysis of NSAID binding by prostaglandinH2 synthase: time-dependent and time-independent inhibitors elicit identical enzyme conformations. Biochemistry 2001; 1-40(17): 5172-80.
15. SeeSAR version 8.1: Bio SolveI T GmbH, Sankt Augustin, Germany,2018, www.biosolveit.de/SeeSAR
16. Rarey M and Kramer B: A fast flexible docking method using anincremental construction algorithm. Journal of Molecular Biology 1996; 261(3): 470-89.
17. Thomsen R and Christensen MH: MolDock: A New Technique for High-Accuracy Molecular Docking. Medicinal Chemistry 2006; 49(11): 3315-21.
18. Geete and Ankit: Drug Likeness Tool (DruLiTo). National Institute of Pharmaceutical Education and Research, India. www.niper.gov.in/pi_dev_tools/DruLiToWeb/DruLiTo_index.html.
19. Lipinski CA: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advances in Drug Delivery Review 1997; 23: 3-25.
20. Ghose AK and Viswanadhan VN: A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery.1.Aqualitativeand quantitative characterization of known drug databases. Journal of Computational Chemistry1999; 1: 55-68.
21. Yang H, Lou C, Sun L, Li J, Cai Y, Wang Z, Li W, Liu G, Tang Y:admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. Bio-informatics 2019; 35(6): 1067-9.
22. O'Boyle NM and M Banck M: Open Babel: An open chemical toolbox. J of Cheminform 2011; 3(33): 2946-53.
23. Trott O and Olson AJ: Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry 2010; 31:4 55.
24. Dallakyan S, Olson AJ: Small-Molecule Library Screening by Docking with PyRx. Methods of Molecular Biology 2015; 1263: 243-50.
25. Gundogdu-Hizliates, Cevher, Alyuruk H, Gocmenturk M, Ergun Y and L Cavas: Synthesis of New ibuprofen derivatives with their *in-silico* and *in-vitro* cyclo-oxygenase-2 inhibitions. Bioorganic Chem 2014; 52: 8-15.
26. Bittencourt JA and Neto MF: *In-silico* evaluation of ibuprofen and two benzoylpropionic acid derivatives with potential anti-inflammatory activity. Molecules 2018; 24(8): 1476.
27. Piñero J, Furlong LI and Sanz F: *In-silico* models in drug development: where we are. Current Opinion in Pharmacology 2018; 42: 111-21.
28. March-Vila E, Pinzi L, Sturm N, Tinivella A, Engkvist O, Chen H and Rastelli G: On the integration of *in-silico* drug design methods for drug repurposing. Frontiers in Pharmacology 2017; 8:298.
29. The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

How to cite this article:
Chebrolu A and Madhavan S: Molecular docking study of ibuprofen derivatives as selective inhibitors of cyclooxygenase-2. Int J Pharm Sci & Res 2020; 11(12): 6526-31. doi: 10.13040/IJPSR.0975-8232.11(12).6526-31.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)