Lebda Medical Journal
Vol. 1, Jan. 2024, p.16-19

Klefah, Musa,
Received May 12, 2024; accepted, published Jul 09, 2024

Docking of various molecules including two NSAIDs as prototypes into the COX-1 enzyme

Klefah A. K. Musa*
*Department of medicinal chemistry, Pharmacy College, Elmergib University, Libya.

ABSTRACT:
Molecular modeling studies for the cyclo-oxygenase isozyme (COX-1) and docking processes in the COX-1 active site pocket is of an importance of research in Today's world. This is performed in order to study the inhibition effect of these different compounds to the COX-1 enzyme by using molecular operation environment (MOE) program. Teen different chemical molecules were fitted in the active site pocket of the COX-1 enzyme and optimized in both cases before and after molecular dynamic simulations that is after taking the best conformation of each ligand of these molecules depending upon scoring investigation. The energy gain for the whole system is obtained by the subtraction of the complexed systems energies from the separated ones. The teen neutral (protonated) molecules were studied and show that the compound 3, 7 and 9 under re-optimization process after MD simulation show the best fitting among all studied molecules and their energy gains were -34.2, -40.4 and -41.21 kcal/mol, respectively. In order to know the effect of ionization on the system, The possible ionized compounds 1, 2 and 3 were studied in both the neutral and deprotonated forms. The obtained results show that both compound 1 and 2 show relatively small inhibition once they studied as in their neutral species. The effect of ionization is very clear once they re-optimized in their deprotonated species. The significant enzyme inhibition is noted since their energies gain changed in neutral forms from 14.4 and 27.3 Kcal/mol to 39.7 and 29.4 Kcal/mol of the deprotonated species, respectively.

Key wards: COX-1, Docking, NSAIDs, Ketoprofen, Ibuprofen.

* Corresponding author: Klefah, Musa, E. mail: Klefah.musa@gmail.com

Introduction:

The first committed step in the conversion of arachidonic acid into various molecules such as prostaglandins and thromboxanes is catalyzed by an endogenous enzyme named as cyclooxygenase (COX) enzyme. The produced molecules play important functions in the gastric, hematic and renal systems in addition to their use in regulation of different processes such as body temperature and inflammation [1, 2]. The cyclooxygenase (COX) enzyme is inhibited competitively by various drugs that gathered in a group and named as non-steroidal anti-inflammatory drugs (NSAIDs). Aspirin, diclofenac, ibuprofen, indomethacin and naproxen are good examples of this class. Inhibition of cyclooxygenase (COX) enzyme by the NSAIDs results in analgesic, antipyretic, anti-inflammatory and antithrombotic activities [3]. Besides their beneficial action, the use of NSAIDs is also associated with deleterious side effects especially with their continuous administration leading to the nephrotoxicity and gastric ulceration [4, 5]. Designing and producing new NSAIDs for safer treatment with lesser or without side effects are the need of the hour. NSAID aspirin shows an irreversible inhibition mode while other NSAIDs are showing reversible inhibition mode [6]. NSAIDs in this circumstance are divided into two classes, one is a time independent inhibition, in which the ligand competes reversibly with the natural substrate to form a complex enzyme-inhibitor, while the second displays a time dependent inhibition including an initial reversible binding which progresses to a tight irreversible to form a new complex enzyme-inhibitor [7].

Cyclooxygenase (COX) enzyme is of various isozymes mainly cyclooxygenase-1 (COX-1) enzyme and cyclooxygenase-2 (COX-2) enzyme. The first one, COX-1 isozyme plays an important role in the maintenance of the essential normal physiological states in various tissues such as in gastrointestinal tract, kidney and platelets. In gastric mucosa the activation of COX-1 leads to prostaglandin PG-I2 (prostacyclin) production that plays an important role as cyto-protective action. In platelets the thromboxane A2 is synthesized via COX-1 activity that can cause platelets aggregation. In addition, thromboxane A2 is responsible for vasoconstriction and smooth muscle proliferation. Inhibition of COX-1 isozyme using NSAIDs can cause gastrointestinal damage [11].

Cyclo-oxygenase 2 (COX-2) isozyme from other side, is less widely expressed that induced by pro-inflammatory stimuli and catalyses the production of prostaglandins that mediate inflammation. COX-2 isozyme is inhibited by COX-2 inhibitors; the later cannot inhibit COX-1 isozyme at therapeutic doses. This means that the COX-2 inhibitors have the capability to relieve inflammation with less gastrointestinal toxicity than that of conventional NSAIDs. Besides their role in inflammation, the selective COX-2 inhibitors in vascular endothelium they mediate production of prostaglandin PGI2 (prostacyclin), a vasodilator and inhibitor of both platelet's aggregation and proliferation of vascular smooth muscle cells. The highly inducible gene (COX-2) is expressed at the site of inflammation in response to a variety of pro-inflammatory agents such as cytokines, growth factors and tumor promoters [8]. The isozyme COX-2 has been implicated in inflammatory and other chronical diseases including cancer [9]

as well as in rheumatic arthritis [10]. Both (COX-1 and COX-2) isozymes are probably important in both the vascular homeostasis and the regulation of platelets function [11].

Generally, NSAIDs as COX-1 and COX-2 inhibitors work throughout inhibiting the production of prostaglandins. Prostaglandins are nothing except fatty-acids derivatives that are well known for their inflammation and immune response effects and located all over the body organs. Prostaglandins play many important and different roles in the body, for example, in blood clotting, ovulation, renal function, vasomotor tone, wound healing, platelets aggregation, nerve growth, differentiation of immune cells, bone metabolism and initiation of the labor. For instance, prostaglandins are lining the stomach in order to protect it from the effect of the acid, the use of unselective NSAIDs working (e.g., aspirin) causes inhibition of prostaglandins system leading to stomach irritation, digestive tract problems and moreover intestinal and stomach bleeding and lastly death may occurred [12, 18]. Traditional NSAIDs such as aspirin, ibuprofen and naproxen are acting unselectively by locking the action of both COX-1 and COX-2 isozymes whereas, celecoxib is acting as selective for COX-2 isozyme and widely used as COX-2 inhibitor which is of a great importance to treat inflammation in people with arthritis and variety of pain conditions [14, 18]. The COX-2 inhibitors that considered as a healthier and more targeted are treating the inflammation commonly found in inflammatory and immune cells than the COX-1 drugs [12, 24]. In Figure 1 we show the pathway of changing the membrane-bound phospholipids and showing the mechanism of COX-1 and COX-2 inhibitors [24].

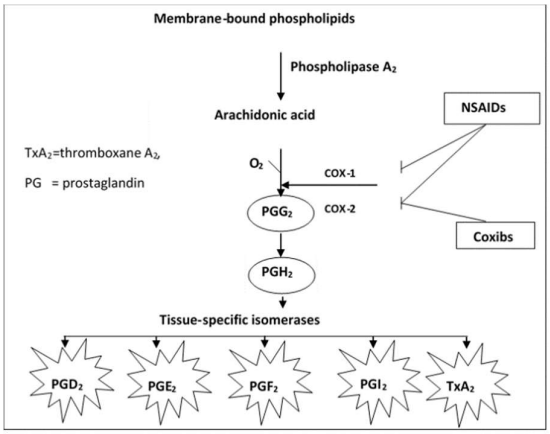

Figure 1. Mechanism of action of COX-1 and COX-2 [25]

From molecular biology point of view, both COX-1 and COX-2 are of similar molecular weight about 67 and 72 kDa, respectively. They have around 65 % amino acids sequence homology and near identical at catalytic sites. The substitution of isoleucine amino acid at position 523 in COX-1 (Ile-523) with valine amino acid residue in COX-2 (Val-523) is the most noted significant difference between the COX-1 and COX-2 isozymes. This difference between these isozymes allows selective inhibition since the Val-523 in COX-2 gives access to the hydrophobic side pocket enzyme whereas, the Ile-523 has sterically hinders. Several drug molecules such as Coxibs are designed depending upon this difference oriented these molecules to be selective inhibitors to the COX-2 [13]. The active site cavity of these isozymes contains larger number of the non-conserved residue, and the COX-1 residues include Thr-89 (Val), Leu-92(Ile), Ile-112 (Leu), Leu-115 (Tyr), Va1-119 (Ser), and Leu-357 (Phe): (residues in parenthesis correspond to COX-2) [15].

Methodology:

In order to investigate different chemical molecules abilities to inhibit the target COX-1 enzyme, the way of fitting of these molecules inside the COX-1 enzyme active site pocket and to know range of ionization effect on the energy gain of various systems under the study. Various compounds shown in Figure 2 were investigated and we calculate the energy gain of each system complex (ligand and COX-1 enzyme complex). Ketoprofen and Ibuprofen, compound 1 and 2, respectively, are taken as prototypes for the series of molecules studied herein.

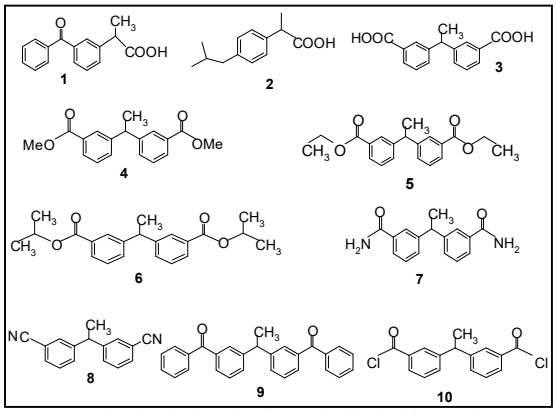

Figure 2. The chemical molecules under the docking studies [19].

The molecules shown in Figure 2 were studied using MOE (molecular operating environment) program [16]; first the isozyme needed for this study (COX-1) was obtained from Protein Data Bank (PDB) [17], then transfer and saved in the MOE program. The COX-1 isozyme consists of two identical chains (two identical subunits) as seen in the Figure 3. Because of the identical subunits of COX-1 isozyme, we have removed one chain (one subunit) in order to simplify the calculations and to save the cost and the time. The active site (Figure 4 (a)) is determined and the database for all chemical molecules to be studied shown in Figure 2 obtained with best scoring, then in turn docked in the target. For instance, docking process of ibuprofen (molecule 2 in Figure 2) is displayed in the Figure 4(b). All systems are obeying to the same circumstances of docking process and optimized to use these systems later in the molecular dynamic (MD) simulations. Molecular dynamic simulations were performed on the optimized docked complexes for 100 ps at constant temperature (298 K) after heating the system from 100 K for 50 ps. All systems were re-optimized in both the complex systems and in the separated forms. For each molecule of those under the study, the process is done in the protonated and deprotonated forms of the molecule if possible. In another word, this process is repeated with each of molecule shown in the Figure 2 (from molecule 1 to 10) in its protonated (neutral) and deprotonated (if possible) forms. The results obtained herein are discussed in details later.

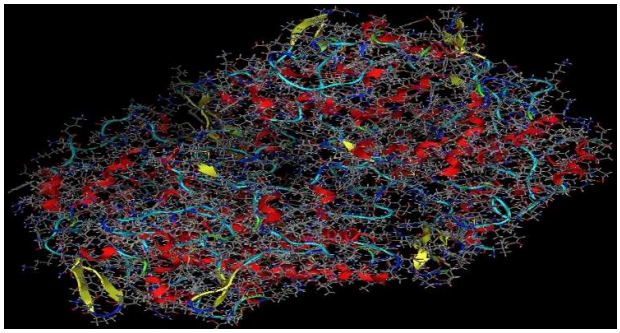

Figure 3. Two identical subunits of COX-1 enzyme.

Corresponding author: Klefah Musa, E. mail: klefah.musa@gmail.com

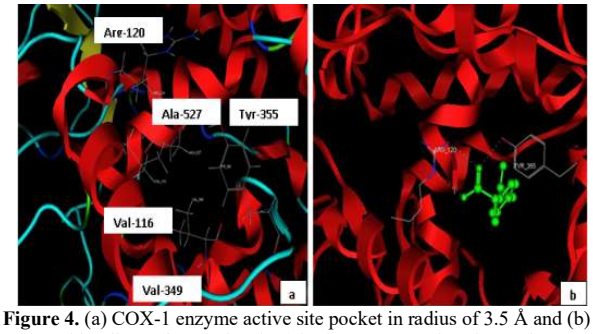

Figure 4. (a) COX-1 enzyme active site pocket in radius of 3.5 Å and (b) Docking ibuprofen (molecule 2) in the COX-1 active site pocket.

Results and Discussion:

I- Study of the protonated (neutral) form of the ligands.

Since we take ibuprofen as prototypes and then followed the study of other molecules by the same manner. Once the compound 2 (ibuprofen) is docked in the COX-1 active site pocket the hydrogen bonds are formed with the catalytic amino acids. There are three hydrogen bonds are formed. The first one is with Tyr-355 and other two hydrogen bonds are with Arg-120. This is in a line with similar studies for the same molecule [15, 20-23]. Beside these hydrogen bonds formed, large Vander Waals interactions between the various ligands and active site pocket amino acids have been noted. After complete docking processes we obtained the following results. (i) Both compound 1 and 2 are relatively small and occupy only fraction of the active site cavity. (ii) Compound 9 gave the best inhibition response among all molecules under the study, as seen in the Table-1, and this is in very agree with previous studies [15, 20-23]. The obtained results from neutral species of compound 1 and 2 show less inhibition response for COX-1 and this disagrees with former studies which could be explained below under an ionization effect. Fitness function of compound 9 is significantly higher from those of known drugs 1 and 2, which attributed to its specific interaction with Ala-527 and water molecules. Due to the long hydrophobic channel-like active site, unfavorable interaction with Arg-120 and Glu-524 can be avoided. The compounds 3, 7 and 9 under re-optimized after MD simulation show the best fitting among all studied molecules and their energy gain were -34.2, -40.4 and -41.21 kcal/mol, respectively, as shown in the Table 1 and Figure 5. Re-optimized systems 4, 5, 6, 8 and 10 after MD simulations show intermediate inhibition and the energy gain of their systems range between -21.7 to -32.7 kcal/mol.

Both molecules 1 and 2 show relatively small inhibition and that may be due to the re-optimization processes were done in their neutral species and that's why the work needed to be extended to consider also the possible deprotonated species of few molecules in the group shown in Figure 2. Once we studied the possible deprotonated compounds in Figure 2, we noted that only molecules 1, 2 and 3 are susceptible to deprotonated and they required further study in their deprotonated form.

II- Study of deprotonated form of the ligands.

Due to the disagree results obtained from the docking processes for compound 1 and 2 in their neutral (protonated) forms, we extended the study of these forms in order to include the deprotonated species. Interestingly, the obtained results herein were in very agree with that obtained theoretical and experimental results of previous works [15, 20-23], once they re-optimized in their deprotonated forms, as seen in the Table 2 and Figure 6.

Table 1. Energy calculation of various molecules shown in Figure 2 in their neutral (protonated) forms after MD simulations (energy in kcal/mol).

Molecule	Energy of complex system after MD calculation	Energy of separated system after MD calculation	Energy gain
1	-7152.70	-7138.32	-14.38
2	-7560.86	-7533.61	-27.25
3	-7533.66	-7499.46	-34.2
4	-7626.22	-7595.32	-30.9
5	-7094.34	-7072.67	-21.67
6	-7549.81	-7521.65	-28.16
7	-7600.98	-7560.63	-40.35
8	-7581.36	-7554.63	-26.73
9	-7538.73	-7497.52	-41.21
10	-7588.00	-7555.30	-32.70

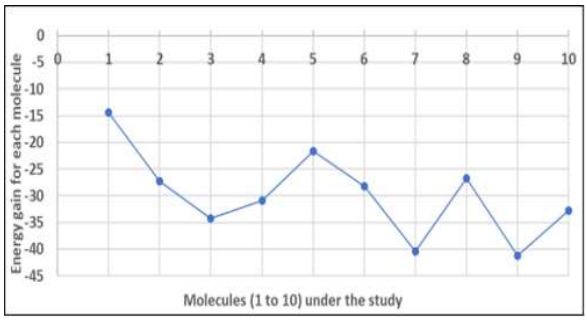

Figure 5. Energy gain of all systems under the study "all ligands are in their neutral forms".

The results obtained from both neutral and deprotonated species studies show that the re-optimized processes after MD simulations for compounds 1 and 2 in their protonated forms (neutral species) were calculated to be -14.4 and -27.3 Kcal/mol. While, once the studies performed upon the deprotonated species in the current work, they dramatically changed and calculated to be -39.7 and -29.4 kcal/mol, respectively. We can herein note that the energy gain of system 1 is very close to that obtained for 7 and 9 systems. Because of molecule size and the way of fitting inside the COX-1 active site the compound 2 show less energy gain by around 10 Kcal/mol to that of the latter two systems (molecule 7 and 9). Overall, the systems 1 and 2 show very significant COX-1 enzyme inhibition depending upon an ionization effect. In addition, other compounds of this study show relatively less enzyme-inhibition effect among all compounds under study.

Table 2. Energy calculation of various molecules shown in Figure 2 in their deprotonated forms after MD simulations (energy in kcal/mol).

Molecule	Energy of complex system after MD calculation	Energy of separated system after MD calculation	Energy gain
1	-7142.39	-7102.72	-39.67
2	-7597.22	-7567.83	-29.39
3	-7544.52	-7521.23	-23.29
4	-7626.22	-7595.32	-30.90
5	-7094.34	-7072.67	-21.67
6	-7549.81	-7521.65	-28.16
7	-7600.98	-7560.63	-40.35
8	-7581.36	-7554.63	-26.73
9	-7538.73	-7497.52	-41.21
10	-7588.00	-7555.30	-32.70

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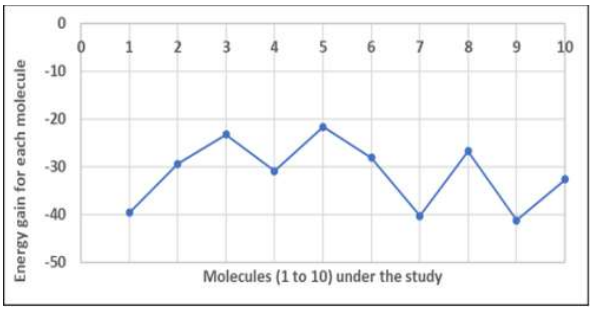

Figure 6. Energy gain of all systems under the study "ligands 1, 2 and 3 are in their deprotonated forms while others studied in their neutral form and the same circumstances of deprotonated species".

Conclusion:

Molecular modeling studies for the cyclo-oxygenase isozyme (COX-1) and docking processes for teen different chemical molecules in the active site pocket of COX-1 were preformed herein, in order to study the enzyme inhibition effect caused by these different molecules using MOE program. The energy gain for the whole system is obtained by the subtraction of the complexed systems energies from the corresponding separated ones. The compounds 1, 2 and 3 were studied in both forms as neutral and deprotonated form in order to see the effect of ionization on the system. The compound 3, 7 and 9 under re-optimized after MD simulation show the best fitting among all molecules studied and their energy gain were -34.2, -40.4 and -41.21 kcal/mol respectively. Re-optimized systems 4, 5, 6, 8 and 10 after MD simulations show intermediate inhibition and the energy gain of their systems range between -21.7 to -32.7 kcal/mol.

The re-optimized processes after MD simulations for compounds 1 and 2 in their protonated forms (neutral species) were calculated to be 14.4 and 27.3 Kcal/mol. Interestingly, this is in very agree with the theoretical and experimental former studies. While, once the studies performed upon the deprotonated species in the present work, they dramatically changed and calculated to be -39.7 and 29.4 kcal/mol, respectively. This significant enzyme inhibition of the molecule 1 is very close to that obtained for compounds 7 and 9, whereas, the molecule 2 has enzyme inhibition effect with about10 kcal/mol less than that of later two compounds which could be explained depending upon the size molecule and its way in fitting inside the active site of COX-1 enzyme.

In general, we can conclude that the studies of enzyme inhibition effect caused by any ligand must be done in both cases as neutral and deprotonated forms of a ligand. That is in order to know a significant effect change between these two species and to clarify the results obtained.

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Corresponding author: Klefah Musa, E. mail: klefah.musa@gmail.com